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NEW BIOFILM CARRIERS FOR WASTEWATER TREATMENT

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Abstract

The present study was done to improve the wastewater treatment efficiency of the wastewater treatment plants which are found within the recirculated aquaculture systems. Initially, the research experiments were done in laboratory conditions with synthetic wastewater. The mobile bed biofilm reactor (MBBR) technology was improved by some of the authors. A new material and a new shape for biofilm carriers were proposed, realized and tested. Improvements in both wastewater treatment efficiency and the quantity of attached biofilm on the improved biofilm carriers were obtained. The capability of the MBBR to reduce the carbon and nitrogen compounds was also investigated.

***Keywords**: Wastewater treatment; Ammonia; Biological oxygen demand; Photosynthetic microorganisms; High density polyethylene; Biofilm carriers; Nitrification; Denitrification*

Introduction

Wastewater treatment within Mobile Bed Biofilm Reactors

The Moving Bed Biofilm Reactor (MBBR) process is based on the principle of biofilm attachment on solid mobile surfaces [1]. This technology has the advantages of activated sludge wastewater treatment process but in the same time exceeds the disadvantages of activated sludge technology. The MBBR process is based on the existence of biofilm carriers that are introduced inside the wastewater treatment tanks. The biological processes can take place in different conditions: aerobic or anoxic [2]. The biofilm carriers have different shapes and sizes (Fig. 1a) being made from a variety of materials, but most of them are from high density polyethylene (HDPE) made from a variety of polyethylene-based mixtures of materials [1]. The biofilm carriers are selected based on several criteria such as: size, porosity, density (biofilm carriers having a density slightly smaller than the water density), specific area per $1.0m^3$, resistance to erosion etc. [1].

By using a relatively small volume of reactor, biofilm carriers with a large specific surface area (m^2/m^3) can maintain high biological activity. With the help of bubbles produced by the aeration system diffusers, the biofilm carriers are mixed within the wastewater tanks. Inside the anoxic tanks a mixer is necessary to maintain the biofilm carriers in motion [3-5].

This type of biological treatment, using mobile biofilm carriers, is the most efficient and the clogging of the systems is not possible. There is an internal zone for the biofilm carriers where the biofilm is attached, developed and protected. These internal surfaces provide the

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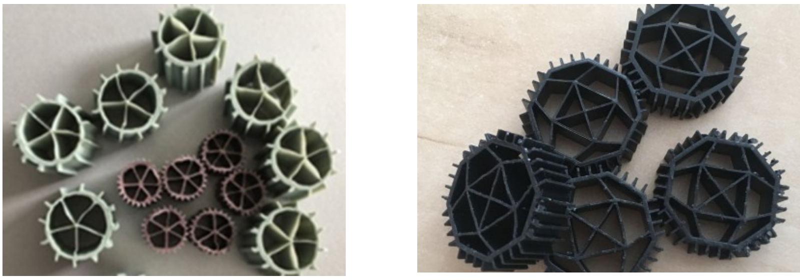
bacterial culture protected areas with optimal conditions to thrive and develop. With fluctuations in the discharge of pollutants, the biofilm inside each carrier element protects individual bacteria against industrial processes. Compared to the activated sludge process, the free biofilm carriers represent a stable place and a large area for the microorganisms to grow, so less tank volume is needed [1]. The only control aspects for the process operation are essentially the nutrient and dissolved oxygen levels. MBBRs are mainly used to eliminate the demand for biological and chemical oxygen (BOD and COD) from streams of wastewater [6]. The removal of nitrogen in MBBRs is also effective. In order to achieve higher efficiencies for BOD, COD, nitrogen and phosphorus removal, existing activated wastewater treatment plants can be upgraded using biofilm carriers. Thousands of municipal and industrial beneficiaries worldwide were satisfied with the MBBR technology. A new type of biofilm carrier [7] (Fig. 1b) was developed based on the Kaldness process [1], which evolved from existing models and was modified to obtain a higher strength and a larger surface for biofilm development.



a b

Fig. 1. Models of biofilm carriers: a - existing biofilm carriers [8]; b - new biofilm carriers [7])

Development and utilization of biofilms in process engineering

Biofilms are assemblies of different microorganisms contained in a matrix that function as a cooperative consortium. This biofilm lifestyle is a common feature of most microorganisms existing in natural, engineered and medical systems, including those involved in biological wastewater treatment. Many bacteria may adhere to different surfaces, some microorganisms adhering best to hydrophobic substrates, while others adhere best to hydrophilic substrates or intermediate materials. Interestingly, the growing conditions can affect this preference in case of a single bacterium. To promote biofilm formation and activity, the chemical nature of the biofilm carriers to be used in wastewater treatment plants should be carefully selected. It is known that the HDPE has hydrophobic properties. For this reason, some of the authors proposed a new material with more hydrophilic properties. In this paper the obtained results regarding the biofilm adhesion on the new material are presented.

Microbial communities used in wastewater treatment

The ability of photosynthetic microorganisms to use inorganic nitrogen (NO_3^-, NH_4^+ or ammonia) and orthophosphate as nutrients to sustain their growth has gained attention in the last six decades for the use of these cells as biocatalysts in wastewater treatment [9-17] but their use in RAS is a rather new topic with promising results [13, 17-19].

The purpose of this paper is to present the results concerning the use of previously selected consortia of denitrifying microorganisms immobilized on artificial mobile biofilm carriers and photosynthetic microorganisms to work now at pilot level as a model for RAS but with significance for treatment of outlet waters from municipal wastewater plants.

The present study was done to improve the wastewater treatment efficiency within the wastewater treatment plants which are found within the recirculated aquaculture systems (RAS). Initially, the research experiments were done in laboratory conditions with synthetic wastewater (SWW).

Materials and Methods

Analytical methods

During the experimental investigations several methods were used by the authors. Part of the analyses were performed at the laboratory of Institute of Biology Bucharest, and others were performed by DFR Systems.

Ammonium, nitrate and phosphorus determinations realized at the Institute of Biology Bucharest were performed using the Spectroquant® Nitrate test kit 1.09713.0001 (Merck) and Spectroquant® Phosphate Test 1.14842.0001 (Merck). All the NO_3^- and PO_4^{3-} concentrations were calculated using a standard curve prepared with Nitrate IC-STD solution 119811 (Merck) and Phosphate IC-STD solution 11989.

Analyses performed at DFR Systems, for the determination of nitrates, nitrites, ammonium and BOD were performed according to the standards:

- SR ISO 7890-3: *Water quality. Determination of nitrate content*, Part 3: Spectrometric method with sulfosalicylic acid;

- SR EN 26777 ISO 6777: *Water quality. Determination of nitrite content,* Molecular absorption spectrometry method;

- SR ISO 7150-1: *Water quality. Determination of ammonium content,* Part 1: Manual spectrometric method;

- SR 1899-1/2003: *Determination of biochemical oxygen demand after n days* (CBOn). Part 1: Dilution and sowing method with allylthiourea intake;

- SR EN 25813/2000: *Water quality. Determination of dissolved oxygen content,* The iodometric method.

Microorganisms and culture media

Twelve heterotrophic denitrifying microbial consortia were previously isolated and tested for their abilities to perform aerobic denitrification [20]. Of all these, the most two efficient microbial consortia were selected based not only on their capacity to remove NO_3^- as well as on their ability to remove other nitrogen compounds (i.e. NH_4^+ and/or NO_2^-) and carbon [21]. In the present study, these two denitrifying consortia named BOD and Raja3 were further used to create a new and improved microbial consortium, by combining them with ammonium oxidizing microorganisms (AOM). This mixed microbial consortium was immobilized on biocompatible biofilm carriers and used in all subsequent experiments.

The diversity of starting biological material for further selection was increased as much as possible by mixing different photosynthetic assemblages of populations already existing in the Microbiology Department of Institute of Biology Bucharest as well as mixtures of populations from the Plutonița fish farm and from domestic aquaria. The mixture of consortia was currently grown in BG11 medium at 20ºC under continuous white fluorescent illumination. The selection of photosynthetic microorganisms for RAS was done by growing them in a modified BG_{11} medium, a medium with low nitrate concentration (40mg nitrate/L), model for synthetic wastewater. The cells were grown either immobilized or free in solution. Immobilization was done by growing them in the presence of a solid support (hydrophilic (e.g. cotton) or hydrophobic). Selection of free photosynthetic microorganisms was focused predominantly on filamentous ones which can grow and form a network in which unicellular photosynthetic microorganisms can reside. Cell morphology was investigated microscopically in bright field without any added dye or treated with alkaline methylene blue which, in the presence of polyphosphate inclusions, becomes reddish.

In all subsequent experiments, SWW with the following composition (g/L) was used: peptone 0.16, meat extract 0.11, urea 0.030, K_2HPO_4 0.028, NaCl 0.007, $CaCl_2$ 0.004, $MgSO_4$ 0.002.

For microscopic investigations, live/dead protocol [22] and volutine protocol [23] were performed as described in literature.

The formation of microbial biofilm on artificial mobile biofilm carriers was monitored by crystal violet method [24] in 4 main stages: (i) growing a biofilm - "clean" biofilm carriers were introduced in synthetic wastewater containing selected microbial populations; (ii) biofilm immobilization on biofilm carriers - biofilm carrier were removed from the experimental tank at different time intervals and introduced into formalin solution; (iii) staining the attached biofilm - the unattached cells were removed by gentle washing with physiological serum and a solution of crystal violet was used to highlight the amount of attached biomass; (iv) quantifying the biofilm - 30% acetic acid in water was used to solubilize the crystal violet; the biomass quantity was evidenced.

Results and Discussions

Wastewater treatment with mixed microorganisms consortia and biofilm carriers

In the laboratory tests, the BOD consortium showed an efficiency of 88% in NH_4^+ removal, 100% in NO_3^-, 18% in reduction total organic carbon (COT), and 96% reduction in total nitrogen (AT) (data not shown). The Raja3 consortium showed an efficiency of 94.69% in NH_4 removal, 100% in NO_3^-, 28.24% in COT reduction, and 100% in AT reduction [21]. Both consortia (BOD and Raja3) recorded an NH_4^+ reduction rate of 0.1mg NH_4^+/L/day.

Thelaboratory experimental data obtained have shown that the two selected bacterial consortia can effectively remove the nitrogen and organic carbon compounds from the culture medium whose composition can be assimilated to that of the synthetic residual water resulting from RAS.

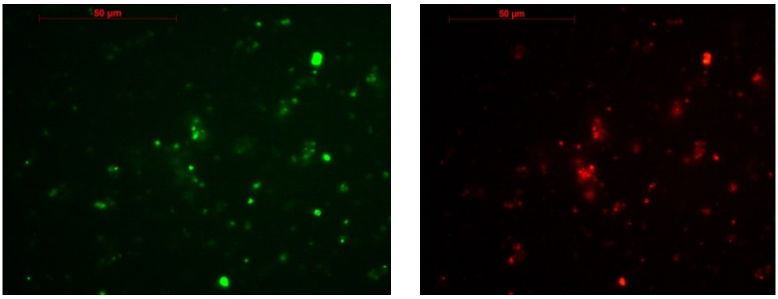
In Table 1 the evolution of NH_4^+, NO_3^-, and PO_4^{3-} concentrations over time are presented in experiments conducted with the mixed consortia of denitrifying microorganisms and AOM in the laboratory wastewater treatment stage.

Table 1. Evolution over time of the NH_4^+, NO_3^-, and PO_4^{3-}, concentrations [mg/L] in experiments using mixed consortia of denitrifying microorganisms and AOM.

	NH_4^+	NO_3^-	PO_4^{3-}
0 h	0.68	90.79	666.67
24 h	3.13	62.55	82.79
48 h	4.54	42.84	83.88
69 h	4.14	38.07	91.67

The results obtained show a good efficiency in nitrate (58%) and phosphorus (86%) removal, while the ammonium concentration showed an increase in the first 48h, its value starting to drop only at the end of the experimental time. The increase in the NH_4^+ concentration can be explained by the decomposition of the urea from the synthetic wastewater composition. The AOM populations which are slow growing microorganisms and are inhibited by the organic compounds, started to consume the ammonium only close to the end of the experimental time therefore, these results mitigate for a longer retention time when wastewaters rich in organics are used. Similar results were obtained with photosynthetic microorganisms (results not shown).

Microscopic investigations of these selected consortia of microorganisms show the occurrence of cells with healthy cell membrane, permeable to Syber green but also dead cells with unhealthy cell membrane (permeable to Syber green but also to propidium homodimer). In figure 2 is shown the same microscopic field both the cells labelled with Syber green (all cells-alive and dead) and with propidium homodimer.



a b

Fig. 2. Total cells:
a - total cells labelled with Syber green - Syber green penetrate both alive and dead cells membrane;
b - total dead cells, the membrane is permeable to propidium homodimer

Knowing that some of selected microbiota should be involved in the phosphorous cycle, being able to develop intracellular inclusions of polyphosphate (volutine or *Babes-Ernst granules*), the mixed populations were labelled with alkaline methylene blue.

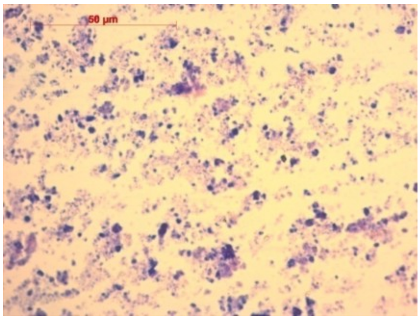


Fig. 3. Cells labelled with alkaline methylene blue - those cells which contains volutine are coloured in pink (metachromatic coloration) those without volume are simply coloured in blue. These results argue that intracellular deposition of polyphosphate is one mechanism involved in decreasing extracellular concentration of orto-phosphate.

Wastewater treatment improved biofilm carriers

Biofilm carriers with immobilized micro-organisms introduced in synthetic wastewater were used during the laboratory experiments (Fig. 4). An improved material for the biofilm carrier's realization was used. The material was a mixture between high density polyethylene and an inorganic material with hydrophilic properties. Three sets of experiments were performed, all in 18L of synthetic wastewater.

Fig. 4. Experimental MBBR stage

The three experiments can be found in the diagrams called SAM 1, SAM 2 and SAM 3.

The main quality indicators of wastewater are presented in the diagrams shown in Figs. 5 and 6 with the indication that the values obtained for BOD in reality are 10 times higher. If the real values were mentioned, it was not possible to draw up the graphs with all the indicators of the quality of the wastewater (the scale of values would have been too high and the low values, such as total nitrite nitrogen could not be observed).

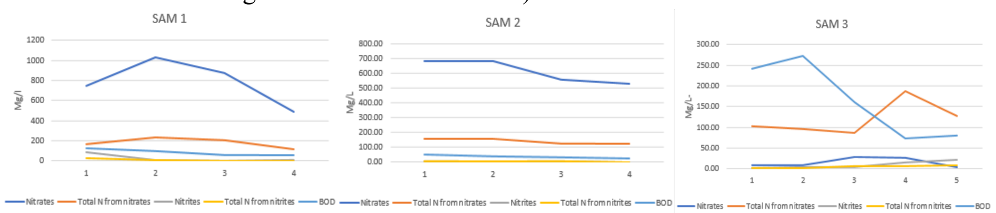


Fig. 5. Diagrams for each experiment with total wastewater quality indicators

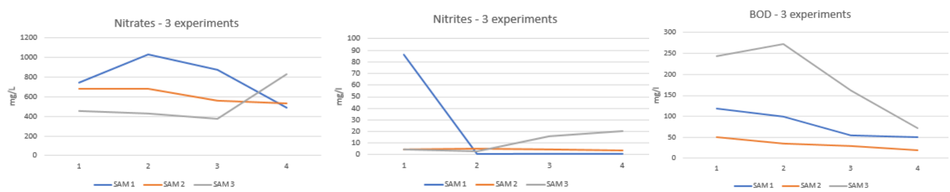


Fig. 6. Diagrams for each quality indicator for the 3 experiments to highlight the effectiveness of the process

The process efficiency is shown in Table 2.

Table 2. Process efficiency

SAM 1	Maximum value [mg/L]	Minimum value [mg/L]	Process efficiency [%]
Nitrates	1031.63	490.18	52.48
Nitrites	85.90	0.70	99.19
BOD	1182.40	496.20	58.03

SAM 2	Maximum value [mg/L]	Minimum value [mg/L]	Process efficiency [%]
Nitrates	682.45	532.17	22.02
Nitrites	4.85	3.67	24.33
BOD	500.20	198.40	60.34

SAM 3	Maximum value [mg/L]	Minimum value [mg/L]	Process efficiency [%]
Nitrates	833.00	654.60	21.42
Nitrites	4.20	2.76	34.29
BOD	272.60	65.40	76.01

The authors have conducted biofilm development analysis on mobile artificial support. Independently of the MBBR experiments, four basins (Fig. 7) were used. Also, SWW, SWW with microorganisms (selected by IBB) and mobile artificial supports made of different materials and shapes were added into these 4 tanks. The tanks had the following types of biofilm carriers:

- Aerobic tank no. 1 containing new biofilm carriers (Fig. 1b) made of 100% HDPE;

- Aerobic tank no. 2 containing old biofilm carriers (Fig. 1a) made from the new material with hydrophilic properties;
- Aerobic tank no. 3 containing old biofilm carriers (Fig. 1a) made of 100% HDPE;
- Anoxic tank no. 4 containing new biofilm carriers (Fig. 1b) made of 100% HDPE.

Fig. 7. Experimental tanks to highlight the biofilm attachment on biofilm carriers

At three-time intervals, biofilm carriers were selected from the 4 tanks and placed in 3.5% formalin solution until measurements were performed. The amount of biomass with the crystal violet substance and a spectrophotometer was highlighted. The procedure for measuring the amount of biofilm formed was in line with that in the literature. In figure 8 are shown pictures taken during the measurements. In order to obtain conclusive results, the samples were diluted 2 times and the results are presented in table 3.

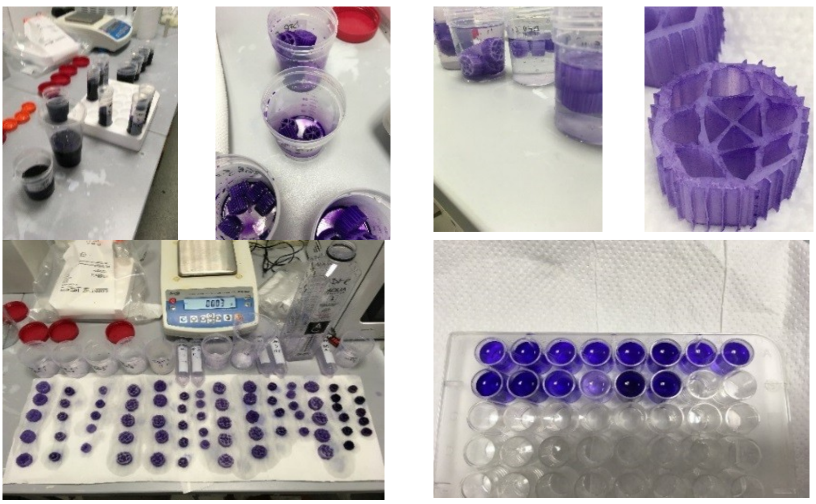


Fig. 8. Experimental tanks to highlight the attachment of biofilm on different biofilm carriers

The results in basins 1 and 4 cannot be compared to those in basins 2 and 3 because a different amount was used for detachment of biomass from the biofilm carriers with crystal violet. For the carriers in tanks 1 and 4, 50mL of acetic acid solution was used, and 30 mL of solution was used for the carriers in basins 1 and 4.

Table 3. The amount of biomass attached to different biofilm carriers under different conditions

Time	**Basin no. 1**	**Basin no. 2**	**Basin no. 3**	**Basin no. 4**
Day 8	0.545	1.342	0.617	0.564
Day 15	1.127	1.85	0.441	0.405
Day 21	1.634	2.315	0.757	0.206

For this reason only comparisons between the carriers of the same shape are relevant. Thus, the amount of biomass attached to the carriers in the new material (basin 2) relative to the 100% HDPE carriers is highlighted. Regarding biomass attached to carriers under aerobic and anoxic conditions it was found that under anoxic conditions the amount of biomass attached is lower than the aerobic case - here are also different species of microorganisms. This fact is also revealed in literature [1].

Conclusions

The new biofilm carriers have proven their effectiveness in the treatment of wastewater under laboratory conditions. The improved material used for the realization of the biofilm carriers turned out to be more affective than 100% HDPE. On the new material the biomass quantity increased by over 200% compared to the 100% HDPE biofilm carriers.

The researchers will continue the experiments, but with real wastewater generated by a recirculated aquaculture system.

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