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## Biotechnologies used in wastewater treatment

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**Abstract.** One of the most efficient biological wastewater treatment process is based on the biofilm carriers' utilization, on which the microorganisms attaches to form biofilm communities. The biological technology that uses artificial mobile supports is known as the Moving Bed Biofilm Reactor (MBBR) process. To have an efficient attachment of the biomass on the carriers, hydrophilic substrates are needed. To promote biofilm formation and development, the chemical nature of the biofilm carriers should be carefully selected. Most of the biofilm carriers are made from polyethylene. This material has hydrophobic properties. The authors propose a new material with more hydrophilic properties. Talcum is combined with polyethylene resulting a more hydrophilic material. The results regarding the biofilm adhesion on the new material are presented by the authors. Also, the paper presents a laboratory installation for wastewater treatment, on which can be compared different biological treatment processes.

### 1. Introduction

Biotechnologies are widely used for municipal and industrial wastewater treatment plants (WWTPs). The first biological treatment used in WWTPs was based on the activated sludge process, where consortia of microorganisms floated freely inside the wastewater mass. During the last 4 decades this technology had evolved, the biofilm being attached by fixed or mobile supports. Moving Bed Biofilm Reactor (MBBR) technology is based on the principle of biofilm fixation on different solid unfixed surfaces [1]. The central elements of the MBBR process are represented by the biofilm carriers that are floating inside the wastewater treatment reactors. The biological treatment can take place in two conditions for a proper removal of different pollutants: anoxic or aerobic conditions [2].

During the last decades, the technology has evolved, nowadays there are many types of biofilm carriers with different sizes and shapes being realised from a multitude of materials. The most common materials used for their realization are based on plastic materials (polyethylene-based mixtures of materials) [1]. MBBRs are mainly used to reduce the demand for biological and chemical oxygen (BOD and COD) nitrogen and phosphorus compound from streams of municipal and industrial wastewater [3].

Among other biological processes used in wastewater treatment can be mentioned algae [4]-[6] and different types of plants, of which *Lemna Minor* (Duckweed) [7]-[9]. *Lemna Minor* is among the smallest aquatic flowering plants. It has a fast reproduction rate, being capable of doubling its quantity in 4 days [10]. Beside the benefits showed in wastewater treatment few disadvantages are known; this technology requires a high land area and problems can occur during the winter periods [11].

As a part of an international research grant, the authors have investigated the treatment efficiency of different types of biological treatment. Also, improvements of the existing technologies were proposed, developed and tested in laboratory conditions.



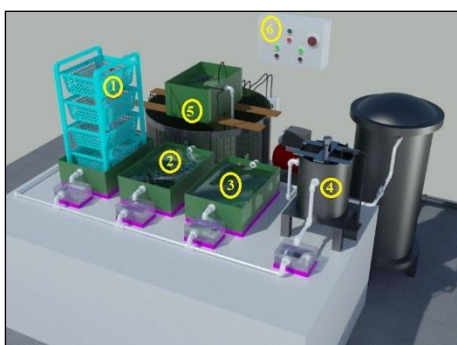
## 2. Material and methods

### 2.1. Laboratory installation to compare different biological wastewater treatment technologies

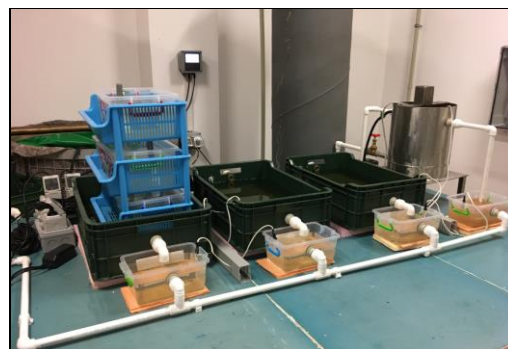
To compare 4 different biological treatment a laboratory installation was conceived, designed (Figure 1) and realized (Figure 2). The installation has the biological lines in parallel to permit a correct comparison between the efficiencies of the 4 different processes. From the main tank the wastewater is equally distributed into the 4 treatment lines. The 4-biological treatment processes evaluated are:

- Line 1 – Tower installation with *Lemna* sp. Four tanks are superposed, wastewater flowing from one tank to another, starting from the upper tank. The high of wastewater inside the tanks is of max 100 mm. At the wastewater surface of each tank, Duckweed (*Lemna Minor*) was introduced.
- Line 2 – Biological treatment with algae and *Lemna Minor*
- Line 3 – Moving Bed Biofilm Reactor with algae. Inside the biological tank, beside the biofilm carriers, algae were introduced.
- Line 4 – Moving Bed Biofilm Reactor with dissolved air. The air quantity needed for the aeration process was assured by a dissolved air flotation system.

The biological substrate (Duckweed and photosynthetic microorganisms/algae) were provided by Aquaterra and by the Institute of Biology Bucharest (IBB). IBB also provided the synthetic wastewater that was used during the experiments. The wastewater composition (g/L) used during the experiments was: 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 [12].



**Figure 1.** Laboratory installation - 3D design; 1 - 4: 4 parallel lines of different biological treatment; 5 - wastewater tank; 6 - electric panel.



**Figure 2.** Laboratory installation.

### 2.2. Determination of the treatment efficiency

Analyses of the water quality were performed at an external Romanian accredited laboratory. The determinations of ammonium, nitrites, nitrates, and Biological Oxygen Demand (BOD) were realised according to the following standards:

- SR 1899-1 / 2003 Determination of biochemical oxygen demand after n days (CBOn). Part 1: Dilution and sowing method with allylthiourea intake. This standard applies to waters having BOD greater than or equal to 3 mg/l  $O_2$  and not exceeding 6 000 mg/l  $O_2$ .
- SR ISO 7890-3: Water quality. Determination of nitrate content. Part 3: Spectrometric method with sulfosalicylic acid. The method is suitable for application to both waste and potable waters samples.
- SR ISO 7150-1: Water quality. Determination of ammonium content. Part 1: Manual spectrometric method. The principle of this method consists in spectrometric measurement (at 655 nm) of the blue compound resulted from the ammonium reaction with hypochlorite and salicylate ions in the presence of sodium nitroprusside.
- SR EN 26777 ISO 6777: Water quality. Determination of nitrite content. Molecular absorption spectrometry method.

### 2.3. Biofilm carriers – shapes and materials

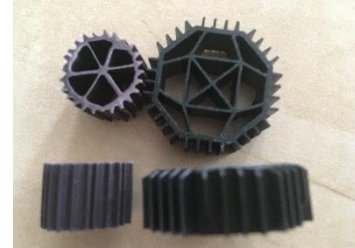
Two types of biofilm carriers were used during the experiments (Figure 3-5). In the experimental installation were introduced small biofilm carriers (Figure 3) immobilized by IBB with heterotrophic denitrifying microbial consortia [12,14-15].



**Figure 3.** Known biofilm carriers.



**Figure 4.** Biofilm carrier according to Patent request A/01053/2017.



**Figure 5.** Comparison between known and new biofilm carriers.

The innovative biofilm carriers (Patent request A/01053/2017) beside the new shape were realized from a new material. High density polyethylene was mixed with talcum to increase the hydrophilic property of the material.

### 2.4. Methodology used for biomass quantity determination

The biomass quantity determination was determined with the help of the IBB's team and infrastructure. The biofilm attachment and growth on the new biofilm carriers was investigated by crystal violet method [13]. Four main phases were realised:

- biofilm attachment on the carriers – new, clean carriers were introduced in wastewater tanks containing selected microbial populations;
- biofilm development on the new carriers – at 7 days periods artificial biofilm carriers were extracted from the experimental tanks and introduced into special vessels containing formalin solution;
- colouring the attached biomass - the unattached microorganisms were removed from the carriers by delicate washing using physiological serum. After washing, a solution of crystal violet was used to colour the amount of attached biofilm;
- quantifying the biofilm - 30% acetic acid in water was used to solubilize the crystal violet and using a spectrophotometer the biomass quantity was evidenced.

Different conditions (aerobic and anoxic) and different types of carriers were use, that is why 4 tanks were necessary, as can be observed in Figure 6.



**Figure 6.** Tanks and biofilm carriers used to determine the efficiency of the new material.

### 3. Results and discussions

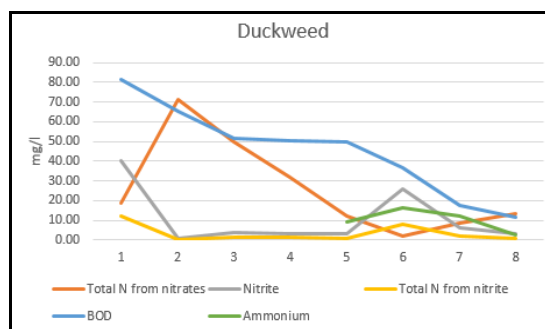
#### 3.1. Results of the biological wastewater treatment with *Lemna Sp*

Figure 7 presents photos from different moments of experimentation. The main results obtained are presented in Figure 8 and in table 1.



Day 2 – *Lemna Minor* at the water surface      Day 2 – *Lemna Minor* blocks the passing filter from the tower installations      Day 13 – Superior tank      Day 13 – Inactive *Lemna Minor* on the botom of the tank      Day 13 – Duckweed from a lower tank

**Figure 7.** *Lemna Minor* utilization for wastewater treatment. Line 1 of the laboratory installation.



**Figure 8.** Evolution of water quality indicators.

**Table 1.** Treatment efficiency.

Duckweed	Max. value measured [mg/l]	Min. value measured [mg/l]	Treatment efficiency [%]
Nitrate	314.70	54.80	82.59
Nitrite	40.34	2.92	92.76
BOD	812.40	498.20	38.68
Ammonia	9.27	2.47	73.35

#### 3.2. Results of the biological wastewater treatment with *Lemna Minor* and algae

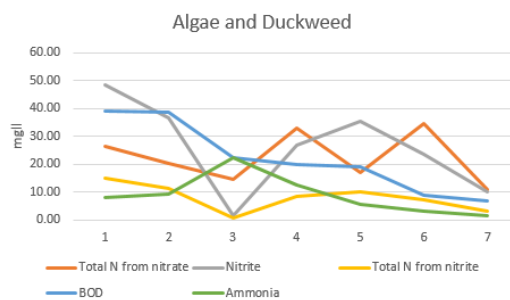
Figure 9 presents photos from different moments of experimentation. The main results obtained are presented in Figure 10 and in table 2.



Day 2 – Testing tank      Day 3 – Algae development      Day 8 – Testing tank      Day 9 – Testing tank      Day 10 – Algae embedded Duckweed

**Figure 9.** Duckweed and algae utilization for wastewater treatment. Line 2 of the laboratory installation.





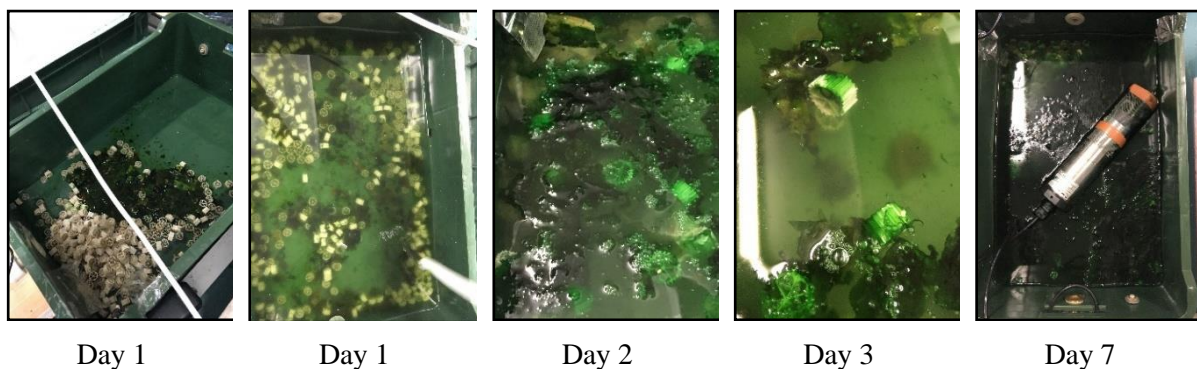
**Figure 10.** Evolution of water quality indicators.

**Table 2.** Treatment efficiency.

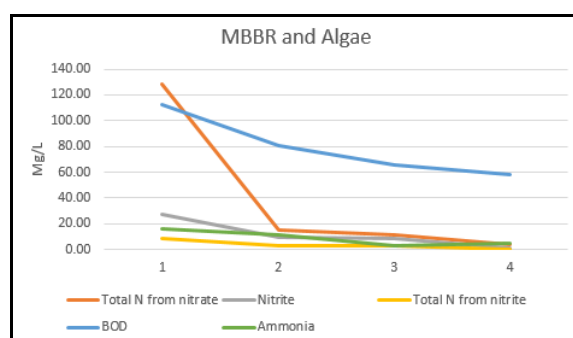
<i>Lemna Minor</i> and algae	Max. value measured [mg/l]	Min. value measured [mg/l]	Treatment efficiency [%]
Nitrate	115.18	65.06	43.51
Nitrite	48.54	1.58	96.74
BOD	390.60	190.14	51.32
Ammonia	7.88	12.47	-58.25

### 3.3. Results of the biological wastewater treatment with MBBR and algae

Figure 11 presents photos from different moments of experimentation. The main results obtained are presented in Figure 12 and in table 3.



**Figure 11.** MBBR and algae utilization for wastewater treatment. Line 3 of the laboratory installation.



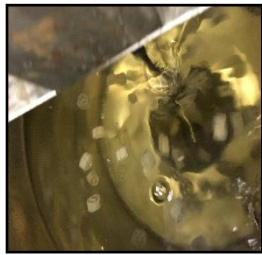
**Figure 12.** Evolution of water quality indicators.

**Table 3.** Treatment efficiency.

MBBR and algae	Max. value measured [mg/l]	Min. value measured [mg/l]	Treatment efficiency [%]
Nitrate	569.30	16.80	97.05
Nitrite	27.16	0.69	97.46
BOD	112.20	58.42	47.93
Ammonia	15.97	4.89	69.38

### 3.4. Results of the biological wastewater treatment with biofilm carriers and dissolved oxygen

Figure 13 presents photos from different moments of experimentation. The main results obtained are presented in Figure 14 and in table 4.



Biofilm carriers inside the tank



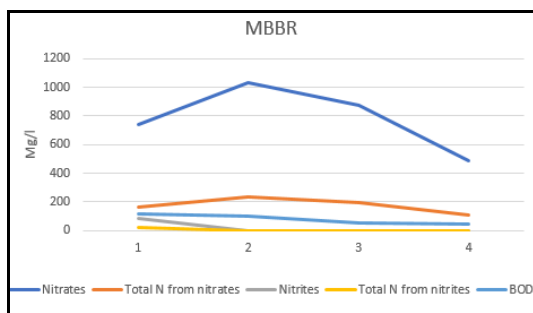
MBBR equipped with sensors for dissolved oxygen and ammonia



MBBR



Pressurizing capsule for dissolving oxygen in water

**Figure 13.** MBBR utilization for wastewater treatment. Line 4 of the laboratory installation.**Figure 14.** Evolution of water quality indicators.**Table 4.** Treatment efficiency.

MBBR	Max. value measured [mg/l]	Min. value measured [mg/l]	Treatment efficiency [%]
Nitrate	1031.63	490.18	52.48
Nitrite	85.90	0.70	99.19
BOD	1182.40	496.20	58.03

### 3.5. Results of the biofilm attachment on the new biofilm carrier

The results that presents the biofilm cuntification on the different carriers in aerobic and anozic conditions are presented in Table 5. The experimental tanks had the following types of biofilm carriers:

- Aerobic tank no. 1 containing common biofilm carriers (Figure 3) made from the new material;
- Aerobic tank no. 2 containing common biofilm carriers (Figure 3) made of 100% HDPE;
- Aerobic tank no. 3 containing new biofilm carriers (Figure 4) made of 100% HDPE;
- Anoxic tank no. 4 containing new biofilm carriers (Figure 4) made of 100% HDPE.

**Table 5.** The amount of biomass attached to different biofilm carriers under different conditions.

Time	Common biofilm carriers		Improved biofilm carriers	
	100% HDPE (Tank 1)	HDPE + Talcum (Tank 2)	Aerobic tank (Tank 3)	Anoxic tank (Tank 4)
Day 8	0.617	1.342	0.545	0.564
Day 15	0.441	1.85	1.127	0.405
Day 21	0.757	2.315	1.634	0.206

## 4. Conclusions

The most efficient process has been found by the authors to be the one that used biofilm carriers and algae, but also were identified some deficiencies caused by the use of algae (putrefaction of algae, excessive algae propagation, increased BOD values due to the algae presence, difficult removal of dead algae).

Regarding the experiments with algae and *Lemna Minor*, the authors identified some problems in using *Duckweed*, namely: it is hard to separate the living plants from the inactive ones; the plants can clog the filters situated between the biological tanks; algae tend to grow excessively, attaching

themselves to any solid surface, including the *Lemna Minor* plants; although *Lemna Minor* has a rapid multiplication, in combination with algae populations the development of *Lemna Minor* was stopped (the plants rapidly became inactive).

From the authors' point of view, the most efficient and robust process capable of purifying water under non-linear conditions is the improved MBBR.

The researches will continue using real wastewater from recirculated aquaculture systems. During the next experiments the MBBR utilizations will be further tested.

The quantity of biofilm developed on the new material increased with 217% in the first week, 419% in the second week and with 305% in the third week of experiments compared with the biofilm attached by the carriers realised from HDPE. The new material proved its efficiency in biomass attachment, showing a great potential of its utilization in real wastewater treatment plants.

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.

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