Microplastics Removal from Municipal Wastewater Through Oxide-Biological Processes. Phase 1: Preliminary Fragmentation of Microplastics from Wastewater and Aerobic Pre-conditioning of Wastewater with Activated Sludge

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Abstract. Environmental pollution with microplastic waste is a pressing problem of high importance world-wide. This paper is aiming at testing, on an experimental basis, by using a synthetic wastewater, a combined technology to remove the microplastic waste from wastewaters. The method involves a preliminary fragmentation of microplastics followed by the aerobic pre-conditioning of synthetic wastewater with aerobic activated sludge. The results obtained indicate that the ozonation followed by the pre-conditioning of wastewater with activated sludge improves the biodegradability of microplastics from wastewater. As proved in this paper, results in an improved removal efficiency in comparison with the simple biological treatment. An additional advantage of such a combined technology is their great operational variability, being easily changeable and adaptable to a broad range of operating parameters values (e.g.: ozone concentration, pH, retention time, dissolved oxygen, etc.).

1 Introduction

Currently, both nationally and internationally, most micro-structured substances such as microplastics waste cannot be removed from water even in modernized treatment plants are used. This reality has been highlighted in recent years, with the help of modern analytical methods, which can detect even the smallest amounts of chemicals in water, on the order of micro-, nano- or picograms.

The presence of micro-structured substances (namely MPW) in wastewater treatment plants makes their removal by classical methods (physical, chemical, and biological) problematic and incomplete. Many of the studies published in the specialized literature [1, 2], conclude that these substances basically transit the wastewater treatment plants and end up

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in surface waters and then in our drinking water. For example, the application of conventional treatment can only remove a maximum of 60% of the total microplastics, while the anaerobic biological treatment with adapted microorganisms can lead to an increase in removal efficiencies up to a maximum of 70% [1, 2]. This means that in the end, significant and out of regulations concentrations of emerging pollutants such as microplastics are discharged into the receiving aquatic environment.

The micro-structured pollutants relevant to our study have different anthropogenic origins: packaging, industrial chemicals, pharmaceuticals, personal care products, household chemicals, water from washing clothes made of synthetic materials (e.g., polyester, nylon [3, 4]), etc. The dangerousness for the aquatic environment of these pollutants derives both from their molecular structure and from their physico-chemical properties such as: low solubility in water, low chemical reactivity, high bioaccumulation in different aquatic species, low biodegradability, high toxicity, and absorption capacity in soil and sediments, and a high persistence in the environment, etc. [5, 6].

In addition to their high toxicity, these hazardous substances are characterized by a long persistence in the environment, by requiring hundreds of years to be naturally degraded. Because of their high longevity, they tend to bioaccumulate in the living organisms of that come into contact with that water. Specialized studies published in recent years have demonstrated that due to their very small size and presence in pelagic and benthic ecosystems, microplastics can easily enter the food chain where they are ingested by a number of living things (birds, crustaceans and fish). Moreover, during self-degradation, plastics release additives from their composition. These additives are extremely dangerous chemicals for humans and environment (e.g., Bisphenol A disrupts the endocrine system, has an estrogen-like effect, and cannot be removed from water by classical treatment processes [7-11]). As another example negative effect of MPW, methane and carbon dioxide resulting from the biodegradation of microplastics are greenhouse gases that contribute to global warming [12].

Currently, both nationally and internationally, there is no consensus regarding the standardization of methods for identifying and quantifying microplastics in wastewater, which makes difficult establishing a procedure to obtain credible data concerning their presence and spatial and temporal distribution in the environment aquatic receiver. Also, there are no effective technologies for removing these types of pollutants from surface or ground waters. For this reason, the present paper is aiming at proposing a technology for the removal of microplastics from municipal wastewater treatment plants effluents by using a combined technology involving (i) a chemical polishment (ozonation) pre-treatment of the polluted wastewaters with MPW, followed by (ii) a biological treatment step by using an activated sludge. A synthetic wastewater (SWW) was used to test the efficiency of this novel technology.

Generally, to improve the microplastics biodegradability, the possible methods usually include those by which the biological systems can be acclimated well enough to effectively decompose the influent components, or those by which the originally resistant compounds can be converted into more easily degradable forms prior to entering the biological treatment stage [13]. In this context, this work is presenting the positive results obtained in the first stage of our project, namely, the above mentioned novel combined technology including the preliminary fragmentation of microplastics from wastewater by using strong oxidative agents (O_3 and UV radiation), followed by the adsorption on an activated carbon of the resulted free radicals, and, then, the biological aerobic treatment by using an activated sludge of the wastewater enriched with easily biodegradable organic substances.

2 Experimental

2.1 Experimental plant description

The experimental setup used for continuous treatment of wastewater containing microplastics consists of the following five modules:

(1) an oxidative module in which a preliminary fragmentation of microplastics takes place under the action of oxidative agents such as ozone and UV-C radiation.

(2) a module for retention by adsorption on activated carbon of unreacted free radicals that once reached the next module would negatively affect the activity of the aerobic activated sludge.

(3) an intermediate wastewater aeration module for the residual carbon oxidation and the wastewater enrichment with macro- and micronutrients necessary for cellular metabolism.

(4) an advanced aerobic biological treatment module that uses biomass specialized in the microplastics degradation, immobilized on porous, natural, and biodegradable solid supports.

(5) a module for separation, recirculation and reconditioning of excess biomass.

The schematic configuration of the experimental plant is shown in Figure 1.

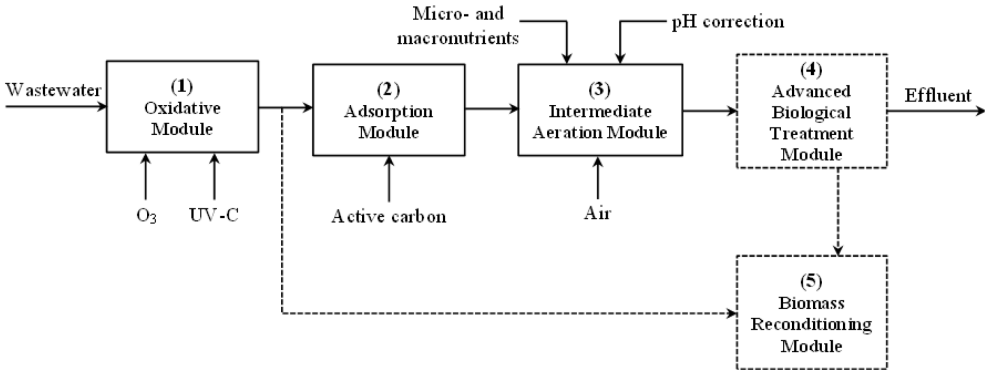

Figure 1. The experimental treatment plant schema.

Within the oxidative module (1), the ozonation of wastewater takes place in counter-current in two successive columns equipped with foam separators. The effluent saturated in free radicals and dissolved ozone is passed through a UV-C type photoreactor to intensify the action of free radicals on refractory microplastics.

In the adsorption module (2) the unreacted free radicals are neutralized and retained by passing through two activated carbon columns.

In the intermediate aeration module (3), the effluent from the activated carbon adsorption column is pre-conditioned with activated sludge for the oxidation and metabolism by microorganisms of the easily biodegradable organic substances formed in the oxidative module (1). Also, in this stage, the pH value, and the proportion of macro- and micro-nutrients necessary for the development of the bacterial mass are checked and adjusted.

In the advanced biological degradation of microplastics module (4), bacterial cultures adapted for the degradation of the most frequently encountered microplastics in the aquatic environment are used (e.g.: polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polystyrene (PS), etc.). These specialized bacterial cultures are immobilized on porous, natural, and biodegradable solid supports.

In the reconditioning module (5), the excess specialized biomass formed, and the microporous solid supports are subjected to washing/cleaning and sterilization operations for

reuse by exposure to the ozone- and free radical-rich effluent from the oxidative module (1), before passes through the activated carbon column (2).

The advanced biological degradation module (4) and the excess biomass reconditioning module (5) are not the subject of this paper.

2.2 Operating conditions

The ozone reactor (bubble column type) was made of glass with 4.5 L volume, 1 m height and 0.045 m internal diameter. The gas stream was fed through a porous glass plate of 10 to 25 μm pore diameter, to enhance the transfer of gas (air enriched with ozone) to the liquid phase (wastewater). The reaction temperature inside the ozone reactor was controlled and maintained at 21±1°C.

The ozone generation unit provided the reaction gas by ionizing oxygen from the air using Corona-type electric discharges. The generated ozone flowrate was about 4.0 g/h and was determined by the iodometric method.

The unreacted ozone retention unit has the role to retain the excess of ozone and to prevent its release into the atmosphere, as it is a toxic, irritating and greenhouse effect gas. This unit consists of two absorption columns filled with activated carbon and two columns with potassium iodide indicator solution (c = 10 mg KI/L).

At start-up, the intermediate wastewater aeration tank was inoculated with a consortium of microorganisms from a municipal wastewater treatment plant. The aeration tank was continuously operated to adapt the activated sludge to the degradation of micro-structured organic pollutants. Finally, after six months of continuously running experimental, the activated sludge was able to remove about 40% to 50% of initial COD. The temperature inside the aeration tank was controlled and maintained at 30±1°C. The pH-value of the aeration tank was controlled by 1 M NaOH and 0.1 M H_3PO_4 solutions. The aeration tank (volume 2.5 L) was used for saturating the wastewater with oxygen (by air) necessary for the respiration of the activated sludge microorganisms, for residual carbon oxidation and to enrich the wastewater with macro and micronutrients necessary for cellular metabolism, too.

The experimental plant was operated with the operating parameters displayed in Table 1.

Table 1. Operating conditions for the continuous experimental plant.

Parameter	Value
Feed flow rate	8.6 L/day
COD feed	425 mg O_2/L
Air flow rate	1.0 L/min
Organic loading of aeration tank	4.6 g COD/L·day
Biomass concentration	19.3 g dw/L
Organic loading of activated sludge[a]	0.23-0.27 g COD/g dw·day
Dissolved ozone concentrations in the reactor influent	0.05 - 0.2 mg O_3/L
Residence time[b]	3.06 hrs

[a] Organic loading of biomass is defined as the ratio between the organic impurities, expressed as the daily fed of COD, and the overall biomass quantity, expressed as dried material.

[b] Residence time in the system is defined as the ratio between the volume of liquid in the system and the liquid feed flow rate.

2.3 Wastewater composition

In order to perform the experiment, a synthetic wastewater was prepared, e.g., a mixture of organic and inorganic compounds. These substrates are necessary for microorganisms to synthesize the cell material and to obtain the essential energy. In a referenced experiment, a mixture of organic acid salts: natrium acetate and glucose, was used as source of carbon (see the composition in Table 2). In the continuous experiments, only the microplastics (of maximum 1 mg/L concentration) was used as source of carbon. Before feeding, the wastewater pH was adjusted to the value 7.0 by using a sodium hydroxide (NaOH) solution or a sulphuric acid (H_2SO_4) solution according to the case.

The microplastic particles used in the experiments were obtained in the laboratory by crushing a mineral water bottle (PET) and a liquid detergent bottle (HDPE). The diameter of the obtained particles varied between 20 μm and 2 mm. Before use in the experiments, the microplastic particles obtained were washed with bleach and rinsed few times with ultrapure water (Millipore).

The wastewater used in the experiments was prepared in the laboratory and had the composition shown in Table 2. During the referenced experiment the carbon sources were sodium acetate and glucose, while in the continuous experiment the mixture of particles of microplastics PET and HDPE in a ratio of 1:1 (weight ratio) was the only carbon source.

Table 2. The synthetic wastewater composition.

Compound	Concentration (mM/L)	
	Referenced experiment	Continuous experiment
CH_3COONa	0.25	-
$C_6H_{12}O_6$	0.25	-
NaH_2PO_4	0.07	0.16
NH_4Cl	0.35	0.81
$MgCl_2 \times 6H_2O$	15×10^{-3}	35×10^{-3}
$FeCl_2 \times 4H_2O$	0.668×10^{-3}	1.5×10^{-3}
$CaCl_2 \times 2H_2O$	1.26×10^{-3}	2.9×10^{-3}
	Amount (mg dw/L)	
Microplastics	-	1.00

2.4 Analytical methods

During the experiments, the adaptation of the microorganisms to the fed microplastics substrate was continuously studied and measured in the previously described experimental plant. Samples of plant influent and effluent were taken daily in order to determine the degree of substrate consumption. The samples were immediately analyzed or after a short storage under 4°C temperature. At various experiment stages, bacteria numbers, bacterial mass, and biomass activity were evaluated.

The chemical oxygen demand (COD) is determined titrimetric based on the decreasing in the chromate concentration resulted after 2 hrs. of oxidation at 148°C with an oxidative mixture: potassium dichromate, sulphuric acid, and silver sulphate. The excess of potassium dichromate is titrated with a solution of iron and ammonium sulfate and the COD value is calculated starting from the amount of reduced potassium dichromate according to the provisions of the standardized method SR ISO 6060:1996 [14].

The concentration of microplastics was determined using an Infrared spectrophotometer with Fourier transform System (Jasco FT/IR-4100). The principle of the method consists in the determination of polymeric materials by the "cast film" technique. The microplastics separated from the sample based on the density are dissolved in a non-hygroscopic solvent. The resulting solution is deposited on the surface of a NaCl cell and then evaporated to complete dryness. The film formed on the cell is analyzed directly [15, 16, 17].

The oxygen uptake rate (OUR) is considered as an important indicator of the activated sludge activity, together with the rate of substrate utilization. The OUR gives information about the degree of adaptability of an activated sludge and can be considered as a possible control variable for the biological system. For OUR determination in the aeration tank a membrane covered oxygen electrodes were used together with appropriate measuring units. The oxygen concentrations are determined simultaneously at the input and at the output of the aeration tank. The biomass OUR were determined in a micro-respiration chamber (Clark-type electrode, Rank Brothers, UK) fitted with oxygen electrodes. The variation of the oxygen concentration was continuously recorded, and the OUR value was every time evaluated from the obtained curve slope [18].

The activated sludge concentration was determined gravimetrically, after filtration of homogeneous biomass sample through a polycarbonate membrane filter of 0.2 μm pore size. The cells collected on the filter were washed with distilled water and then dried overnight at 105°C to constant weight [19].

3 Results and discussions

The comparative evolution of COD concentrations during continuous and reference experiments is presented in Figure 2. It is to observe that the presence of ozone leads to an increase in the reaction rate for the microplastics degradation in organic compounds which are less refractory to the biological degradation.

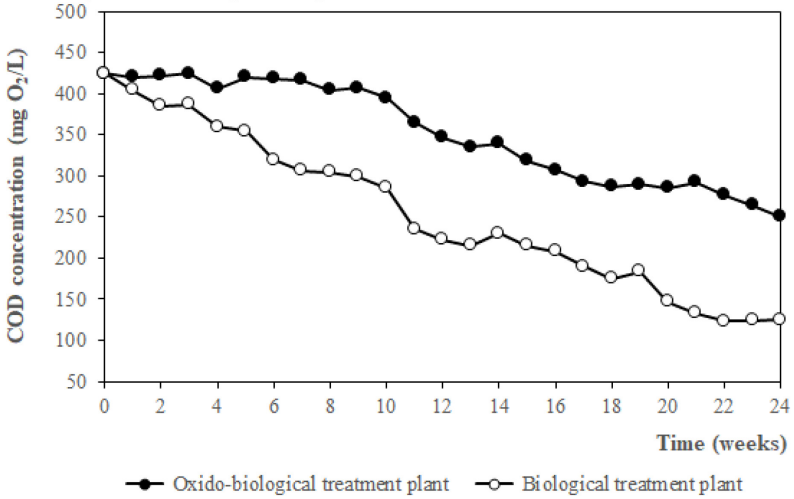

Figure 2. Comparative evolution of the COD concentrations in the two experimental plants: oxido-biological treatment plant and solely biological treatment plant (witness/reference experimental plant).

The microplastics removal realized (see figure 3) is due to the chemical oxidation with ozone, and less to the biological oxidation. Such a result was also confirmed by measuring the oxygen uptake rate (OUR) in respirometry cells. The OUR measurements were performed by using two set of experiments: 1) one set by using a biomass adapted to the

acetic acid and glucose degradation; this biomass was inoculated in the biological reactor of the reference experimental plant; 2) a second set of experiments by using the biomass from the oxido-biological treatment plant after 24 weeks of continuous running. The obtained net OUR values, by using microplastics and sodium acetate are presented in Table 3.

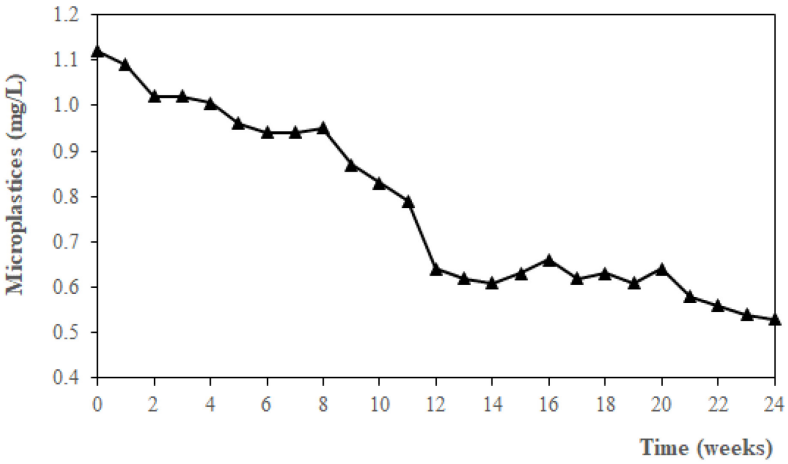

Figure 3. Dynamics of the microplastics concentrations in the oxido-biological treatment plant.

Tabel 3. The net OUR values obtained by using microplastics (1 mg dw/L) and sodium acetate (1 mg/L).

Experimental set	OUR* (nmol O_2/min g dw)	
	Sodium acetate	Microplastics
1(**)	352	5.5
2(***)	124	82

* The net oxygen uptake value was calculated from the total uptake by correcting for endogenous respiration (endogenous OUR was measured without source of carbon).
** Set 1) biomass adapted to the acetic acid and glucose degradation by using the witness biological treatment plant, after 24 weeks of continuous running.
*** Set 2) biomass from the oxido-biological treatment plant after 24 weeks of continuous running.

Preozonization of MPW for a short time was found to be enough effective in converting a part of the microstructurated pollutants to fragments and organic compounds more easily biodegradable. The conversion occurred in the early stage of oxidation and was reflected in the decline of the pH values of the solutions. For this reason, before the further treatment of water in the biological module with specialized biomass and immobilized on solid supports (this part is beyond the scope of this paper), the pH values need readjustment.

Regarding the evolution of active sludge concentration, similar or comparable increases in both experimental installations have been observed (see Figure 4).

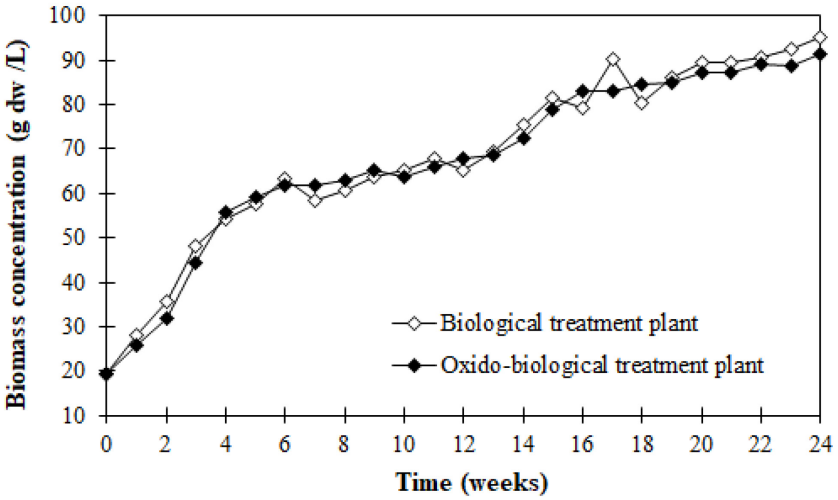

Figure 4. Biomass concentration evolution in the two experimental plants: oxido-biological treatment plant, and the witness / reference biological treatment plant.

These results provided that the metabolic activity of microorganisms was increased in time in both the laboratory treatment plants, but more significantly in the biological treatment plant. The microorganisms ability to use the microplastics was constrained by the operating conditions imposed in the laboratory plants. The biological treatment plant was continuously operated with a dissolved oxygen concentration in influent of 5 mg/L, that is less than the equilibrium saturation value. The combined ozonation-biological treatment plant was operated at various estimated dissolved ozone concentrations in the reactor influent, i.e., in the range of 0.05-0.2 mg/L (estimated based on ozone concentration in the feed and influent flow rate). A more rigorous control of the dissolved oxygen and ozone concentrations could lead to a better microplastics biodegradation efficiency.

At the present stage of research, for the operating conditions of the oxido-biological treatment plant, discussed in this paper, the obtained removal degrees for COD and microplastics are relatively close: about 40-50% for COD and 30-40% for microplastics.

4 Conclusions

As proved in this paper, the combined ozonation - biological treatment of the wastewater containing MPW as pollutant at the beginning of the microorganism adaptation process results in an improved removal efficiency in comparison with the simple biological treatment.

An additional advantage of such a combined technology is their great operational variability, being easily changeable and adaptable to a broad range of operating parameters.

Increase ozone requirements may easily be satisfied by increasing the voltage of the ozone generator. On the other hand, biofilm systems can usually tolerate significant changes in organic load to the system.

If the wastewater is first feed to the biological reactor, adaptation of the microorganisms is stimulated by extended periods of contact with pollutants. This could then further decrease the required amount of ozone, and thus will reduce the operating cost. In principle, the system could also flexibly react to new compounds by an increased ozone feeding rate.

Acknowledgments

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