# An innovative approach to the application of ultrasounds to remove polyethylene microspheres from activated sludge

Authors – C.Bretas Alvim, M.A Bes-Piá, J.A. Mendoza-Roca

Instituto de Seguridad Industrial, Radiofísica y Medioambiental, Universitat Politècnica de València, Camino de Vera, s/n, Valencia 46022, Spain

* Corresponding author: *claal@posgrado.upv.es*

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## Abstract

During the secondary treatment of Wastewater Treatment Plants (WWTP) previous studies have reported that microplastics (MPs), which correspond to plastic fragments smaller than 5mm, are mostly retained in the activated sludge. Since the sludge can be applied as a fertilizer in agricultural land, the presence of MPs in this matrix can lead to soil contamination. In this study, the application of ultrasounds at a frequency of 80 kHz was tested to remove polyethylene (PE) microspheres from activated sludge samples in order to reduce their concentration in this organic matrix. The application of ultrasounds at a power of 50 W and 8 min 45 sec promoted the removal of 48 ± 4% of MPs from the mixed liquor (2,820 ± 51 mg/L of TSS). However, under the same operating conditions, 12 ± 4% of MPs were recovery from the concentrated mixed liquor (9,395 ± 164 mg/L of TSS). For the concentrated sludge, a higher power and a longer sonication time (80 kHz; 90 W; 30 min) was necessary to improve the MP recovery. Under these conditions 38 ±

4% of MPs were removed from the sludge. This is the first study applying ultrasounds to remove MPs from activated sludge and the results indicate that ultrasonication can be a suitable instrument in minimizing the concentration of PE microspheres in activated sludge, and thereby minimizing soil contamination when biosolids are applied to agriculture.

## 1. Introduction

A great interest has been shown in the applications of ultrasounds (US) in wastewater treatment plants (WWTPs) since the beginning of this century. Focusing on municipal wastewater management, the US irradiation has been studied for four main purposes: elimination of compounds that are not biodegraded in the biological treatment, disinfection, reduction of sludge production and enhancement of methane production in anaerobic digesters [1-12]. Cavitation generated by US can concentrate energy in microreactors leading to the removal of a wide range of organic compounds like surfactants [1] and polycyclic aromatic hydrocarbons, among others [2]. The removal mechanism is based on the collapse of the bubbles created by the cavitation phenomenon, which causes changes in the liquid phase leading to oxidation or to breaking of the chemical bonds and pyrolysis [3]. US can be applied to assist other techniques such as electrochemical treatment [4] or oxidation processes with ozone or hydrogen peroxide [5]. US can be also used for water disinfection as reported by [6], also in combination with conventional oxidation techniques like chlorination [7]. In addition, the combination of US and chlorine reduces the concentration of disinfection by-products as trichloromethane and trichloroacetic acid, which are compounds of great concern for the environment [8].

Concerning to the applications focused on the sludge, US has been applied to produce cell lysis in activated sludge reactors. In this way, the sludge production is reduced, with the consequent saving in the sludge management. In this way, Lin et al. [9] combined oxidation with chlorine dioxide and US irradiation to achieve the reduction of the sludge production and Romero-Pareja et al. [10] assisted the OSA (Oxic-settling-anaerobic) process with US for optimize the reduction of the sludge production. Finally, the application of US for the enhancement of methane production is a well-known application that is based on the effect of US on dissolving organic matter of the waste sludge, which means that the hydrolysis phase of the anaerobic digestion is accelerated, and methane production is enhanced [11,12].

The aforementioned applications have been hardly implemented in WWTPs due to the investment and operating costs. However, the increasingly concern about microplastics (MPs) and emerging compounds can modify this scenario. MPs are plastic particles smaller than 5mm, that can be classified as primary (microfibers and those intentionally added in the formulation of products) and secondary (originated from the fragmentation of larger plastics) [13,14]. Despite the small dimensions of MPs, their numerous harmful effects to living organisms have been reported. For instance, animals are not able to distinguish between a plastic fragment and suitable food, and its ingestion can lead to blockage of the animals' trachea, causing starvation and even death [15]. The harmful effects of MPs on the human body are still unclear. However, due to their hydrophobic surface and high surface area, MPs can act as a vector for large quantities of persistent organic contaminants [16,17,18] which can increase the risk of cancer in humans [18] and can also result in endocrine dysregulation [17].

Previous studies have also reported the high retention of MPs in the activated sludge during the secondary treatment [19,20,21]. During the secondary treatment, the

73 microorganisms can use the MPs' surface as support for their growth and the formation
74 of complex structures of microorganisms on the MPs can lead to the reduction of the
75 MPs' buoyancy and thereby promote their accumulation in the sludge [22-25]. Since this
76 biological material, also named 'biosolid', can be applied as a fertilizer on agricultural
77 land, the retention of MPs in this organic matrix can lead to soil contamination [26,27].
78 Nizzetto et al. [28] estimated that between 63,000-430,000 and 44,000-300,000 tons per
79 year of MPs are added to farmlands in Europe and North-America, respectively. In
80 Australia it was estimated that 2,800-19,000 tons per year of MPs enter agroecosystems
81 due to the application of biosolids [29]. The impacts of the presence of MPs in soils still
82 have many gaps, but recent research has proved that these small particles can alter the
83 germination and growth processes by preventing the uptake of water by plants due to
84 physical blockage [30]. Furthermore, plants can also uptake these particles [31,32]. A
85 recent study by Oliveri Conti et al. [32] reports the presence of MPs in edible fruits and
86 vegetables. Therefore, MPs can be ingested by humans when contaminated plant matter
87 is consumed. Due to their capacity to adsorb and leach toxic substances, such as
88 Bisphenol A (BPA) and polycyclic aromatic hydrocarbons (PAHs) [18,33], the ingestion
89 of MPs can lead to health issues for humans and animals.

90 There is still no process to remove the MPs from the activated sludge to minimize
91 terrestrial contamination. Therefore, this study is focused on using the US process to
92 remove PE microspheres retained in the activated sludge flocs. With the use of
93 ultrasounds, the sludge floc could be disrupted, releasing the MPs to the liquid phase.
94 Once the MPs were displaced to the liquid phase, these particles could be collected and
95 removed from the sludge. As a result, the concentration of MPs in biosolids and the
96 terrestrial contamination would be reduced. Furthermore, to determine the capacity of the
97 US process to remove MPs from sludge with different concentrations of suspended solids,

samples from the aeration basin (mixed liquor) and from the secondary settling (concentrated mixed liquor also named secondary sludge), were studied.

## 2. Materials and methods

### 2.1. PE Microspheres and mixed liquor

In this study, the mixed liquor was collected from the aeration basin of a WWTP located in Comunitat Valenciana (Spain). The mixed liquor (also named activated sludge) was used in a sequencing batch reactor (SBR), which was monitored for five days. Sludge characteristics were presented in Table 3. The SBR was operated with a hydraulic retention time of 24 h, and 3 cycles per day. In each cycle 2 L of feed entered the system and 2 L of effluent (supernatant) were withdrawn (Table 1). During the test, the SBR was fed with a synthetic feed. The synthetic feed was prepared with peptone, meat extract and $K_2HPO_4$ (supplied by Panreac) diluted in tap water, in proportions that guaranteed 500 mg/L of COD and nutrients (nitrogen and phosphorous) following the ratio of COD:N:P of 100:5:1, as reported by Ferrer-Polonio et al. [34]. Regarding to the PE microsphere dosage, a concentration of 253MP per liter of feed was prepared. The MPs used in this research was polyethylene microspheres (from COSPHERIC) with a diameter of 150-180 µm. PE microspheres concentration was calculated considering the diameter of the microspheres and the density of the polymer (around 1 g/cm$^3$). The volume of one sphere was estimated from the diameter value and the mass from the estimated volume and the density. Based on these calculations, the mass required to provide a concentration of 253 MP per liter was added in the feed. This method was already applied in a previous study [24].

Table 1 - SBR operational conditions and parameters

| | |
|---|---|
| Cycles/day | 3 |
| Hydraulic retention time (HRT) | 24h |
| Filling and aerobic reaction | 6h |
| Sedimentation | 90min |
| Draw and idle | 30min |

To measure the concentration of the MPs in the mixed liquor, 50mL of sample were chemically digested with 100 mL of hydrogen peroxide (35%wt) (AcrosOrganics) for 4 hours at 60 ± 2ºC. The efficiency of this method was reported in a previous study [35]. After that, the sample was passed through a 150µm aperture sieve, and the retained material was rinsed with distilled water and filtered on glass fiber filter (1 µm). The number of microspheres was counted visually in a stereomicroscope (LEICA MZ APO).

### 2.2. Mechanism of extraction process via US

When MPs are dispersed in the activated sludge, these particles may have their surface colonized by microbial communities, which increases their density and promotes their settlement in the sludge (Fig. 1.a) [19,23,24]. When the sludge is submitted to US, the cavitation process promotes the rupture of the sludge floc [36], and consequently MPs are released to the liquid phase (supernatant). Therefore, this study aims to evaluate different operational conditions to remove via US the PE microspheres retained in the activated sludge floc. As colloids are generated from the disruption of the sludge floc, they will remain in suspension due to their low density, which increases the supernatant turbidity (Fig. 1.b) [36,37].

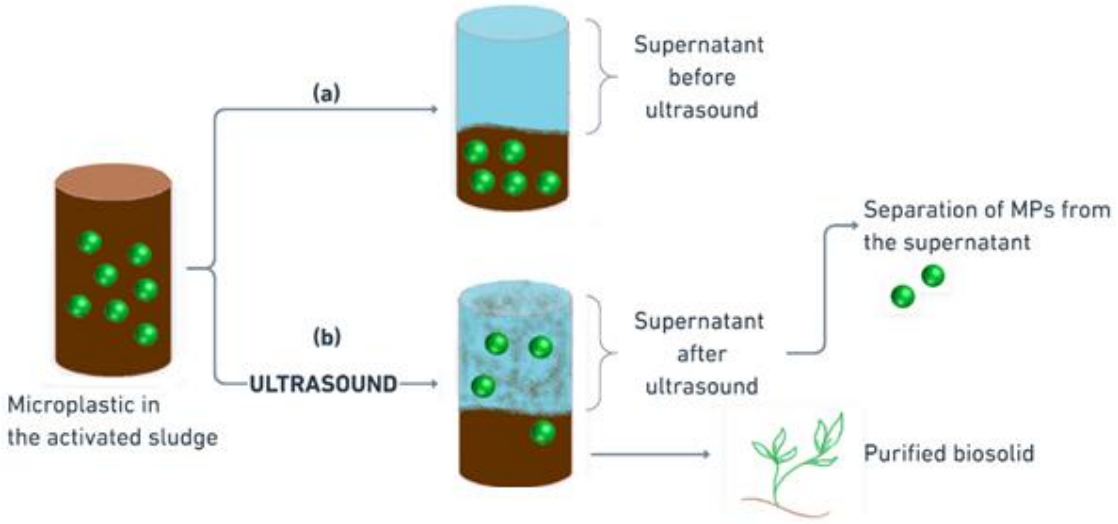



Fig. 1. *Extraction of PE microspheres from activated sludge via US*

### 2.3. Mixed liquor – Extraction of MPs by ultrasound

The ultrasound tests for the mixed liquor were performed at a frequency of 80kHz, and varying the power input (30 W, 60 W and 90 W) and sonication time (5, 10 and 15 minutes). For each test, 100mL of sample were collected and poured into a beaker (250mL) for the extraction process, including an initial sample that was not submitted to US. The US tests were operated under sweep mode controlling the temperature of the mixed liquor. Since the cavitation could result in a heat increase, it was key to maintain the temperature at 25 ± 5ºC. At the end of the US, the samples were settled for 90 min and 25 mL of supernatant was extracted and filtered through a 1 µm glass fiber filter. The experiments were replicated to check their reproducibility. The MPs that were attached on the beaker wall were carefully collected using high precision tweezers. The mixed liquor after ultrasonication was centrifuged at 12,000 rpm during 15 min at 4 ºC to extract the soluble microbial products (SMP). The supernatant was filtered through a 0.45 µm cellulose acetate filter for SCOD, SMP (SMPp - proteins and SMPc - carbohydrates)

161 analysis. SMPp were measured by BCA method [38] and SMPc by anthrone method [39].
162 The SCOD was measured with a kit of the provider Merck Milipore.
163
164 **2.3.1. Experimental Design**
165 An experimental design was carried out with the software Statgraphics Centurion XVII
166 to predict the optimum point of maximum recovery of MPs and minimum turbidity of the
167 supernatant when the mixed liquor was submitted to US. Specifically, a three-level
168 factorial design with response surface method (RSM) was performed. For this, the
169 independent variables were power input (W) and sonication time (min) (Table 2), whereas
170 the response variables were the percentage of microspheres recovered from the mixed
171 liquor and turbidity. In this work, 18 experimental runs were performed and executed in
172 two blocs. In addition, Statgraphics Centurion XVII was used for data analysis. Thus,
173 based on the results of RSM it was possible to better visualize the behavior of a single
174 variable response under the operational conditions proposed, and the interaction of the
175 variables in the optimum condition to obtain the highest percentual removal of MPs from
176 the mixed liquor. The analysis of variance (ANOVA) was applied in the experimental
177 models to assess if the independent variables had significance, and to determinate the
178 degree of the correlation between the predicted and real responses.
179
180 Table 2 – Values of variables for the experimental design.

| **Independent variables** | **Coded values** | | |
|---|---|---|---|
| | -1 | 0 | 1 |
| (Factor_A) Time (min) | 5 | 10 | 15 |
| (Factor_B) Power (W) | 30 | 60 | 90 |

181

182

183 **2.4. Concentrated mixed liquor**

184 Concentrated mixed liquor (simulating sludge from secondary settling) was achieved
185 concentrating the mixed liquor 3 times to reach a concentration of around 9,000mg/L. US
186 tests for this sample were performed using a frequency of 80 kHz and a power of 90 W.
187 Longer US times, compared to the tests with mixed liquor, were applied (30 min and 60
188 min). The temperature was also controlled and maintained at 25 ± 5ºC. 100 mL of
189 concentrated mixed liquor were used for each experiment, which were replicated to
190 evaluate the reproducibility of the method.

191 Unlike the procedure applied for the mixed liquor (Section 2.3), the supernatant of the
192 concentrated mixed liquor was not drained due to the small column of water in this
193 sample. Therefore, after US, the sample was left to stand for 90 min and then the MPs
194 were carefully removed from the sample surface with high precision tweezers and placed
195 in other beaker with distilled water. This final sample was filtered through a glass fiber
196 filter, similarly to the process adopted in section 2.3.

197

198 **2.5. Sonochemical effects**

199 The sonochemical effects, which correspond to the production of hydroxyl radicals due
200 to the abrupt collapse of the microbubbles, were determined by KI dosimetry method
201 (Weissler reaction). This method is based on the oxidation of $I^-$ when free radicals are
202 generated during the ultrasonication, and the final product is the ion $I_3^-$, which can be
203 measured at wavelength of 355 nm. A solution of KI 1% was prepared for the tests.
204 Quantification of sonochemical effects was carry out applying the same conditions used
205 for the activated sludge. 100 mL of KI solution were placed in a 250 mL beaker and
206 exposed to ultrasonication. The power values used were 30 W, 60 W and 90 W. An aliquot

of KI solution was collected at the beginning of the test (initial sample) and after 5 min, 10 min, 15 min and 20 min of reaction, and its absorbance was measure at λ=355 nm by DR6000 (Hach) spectrophotometer. As suggested by Wang et al. [40], the concentration of free radicals (HO•) in the system is related to the absorption of the KI solution after US treatment and can be estimated by Eq.1.

$$C(\mathrm{HO}\bullet) = \frac{2A}{\varepsilon b} \times 10^6 \times 2 \quad (1)$$

Where the concentration of hydroxyl radical C(HO•) is obtained in μmol/L, A is the absorption of the KI solution after US, ε is the molar absorptivity of $I_3^-$ (26,303 $dm^3mol^{-1}cm^{-1}$) and b is the thickness of quartz cuvette (10 mm).

### 2.6. Particle size distribution of MPs after ultrasonication

Since MPs could be downsized to nanoplastics (NPs) due to water shear forces [41] the particle size distribution of the supernatant was determined by Dynamic Light Scattering (Zetasizer Nano ZS90) to assess the eventual fragmentation of PE microspheres into smaller fragments. The experiments were performed with distilled water because the high presence of suspended solids from the activated sludge after US could make the analysis unfeasible. After the US, the sample was passed through a 150 μm aperture sieve to remove the initial MPs size. The removal of the initial MPs of 150 μm was required since the equipment identified particles in a range size of 0.3 nm and 5 μm and bigger particles could interfere in the results. Moreover, the analysis of the distilled water blank was also required to minimize the influence of contamination.

## 3. Results

### 3.1. Microspheres concentration in the mixed liquor

When the supernatant of the initial mixed liquor (without US application) was collected, only 2 ± 0.5% of the microspheres were recovered, i.e. 98 ± 0.5% were accumulated in the sludge (Fig. 2). Due to the high deposition of MPs in the biomass the final concentration of microspheres in the mixed liquor was 1,400 MP/L (Table 3). The concentrated mixed liquor had a TSS concentration of 9,395 ± 164mg/L and a concentration of microspheres approximately 3 times higher than the mixed liquor (4,480 MP/L). Likewise, for this sample 2 ± 0.5% of MPs were recovered from the supernatant before the US.

Table 3 – Sludge samples characterization (mean±S.D)

| Parameter | Sample | |
|---|---|---|
| | Mixed liquor | Concentrated mixed liquor |
| TSS (mg/L) | 2,820 ± 51 | 9,395 ± 164 |
| VSS/TSS | 0.86 | 0.81 |
| MPs/L | 1,400 | 4,480 |

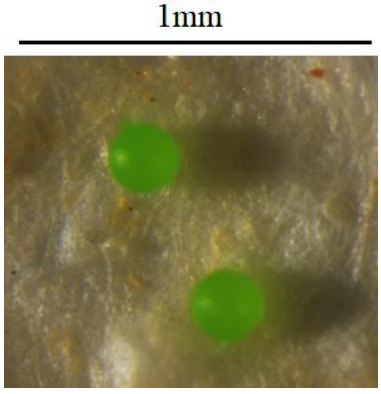



Fig.2. PE microspheres removed from the activated sludge

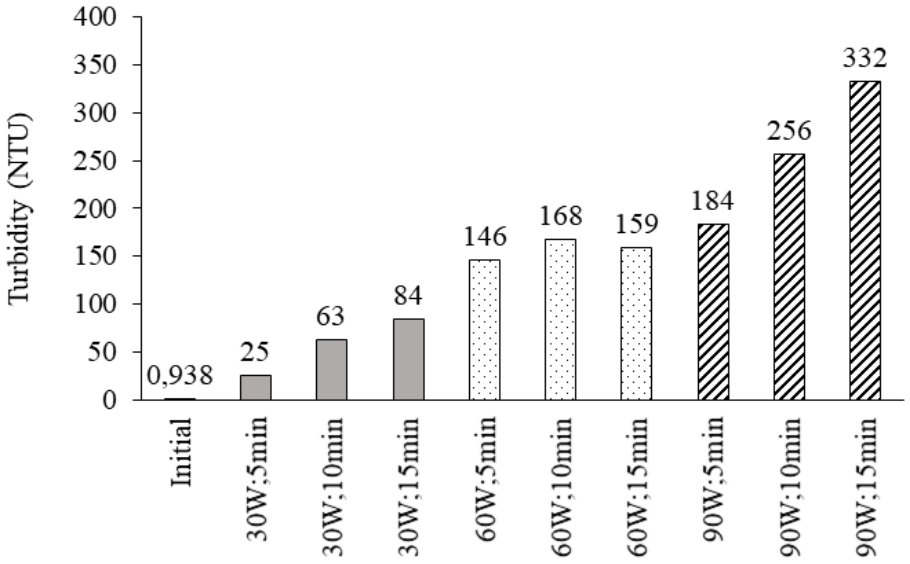
## 3.2. Influence of power and time on MP extraction by ultrasounds

### 3.2.1. Results of the experiment design for mixed liquor

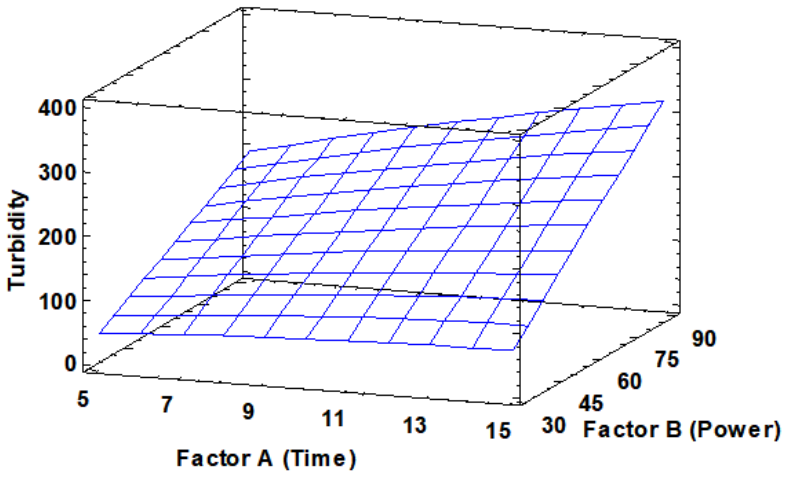
In the Fig.3.a, it was observed that the increase in the power and the exposure time contributed to the increase of the supernatant turbidity, reaching a maximum value of 332 NTU when the sludge was exposed to 90 W and 15 min. The increase of the turbidity indicates that the sludge flocs were disrupted by the hydro-mechanical effects of the US, leading to a higher concentration of suspended solids in the supernatant. The contribution of the power and the sonication time to the disintegration of the sludge floc and therefore the rise of the turbidity, is clearly noted with the surface response (Fig.3.b). The following equation (Eq.2) corresponds to the regression model established according to the results of turbidity response in the experimental design, where A is the time and B the power.

$$Turbidity(NTU) = -34{,}44 + 1.78A + 1.70B - 0.23A^2 + 0.17AB - 0.00092B^2$$ (Eq.2)

The analysis of variance for the regression model also corroborated that the variables A and B and the interactive model terms (AB) are significant (p-Value < 0.05), at the 95% confidence level, for the increment in the turbidity value. Moreover, a high squared regression coefficient ($R^2$) and adjusted squared regression coefficient ($R^2_{adj}$) were achieved (0.95 and 0.93, respectively), indicating a great correlation between the predicted and real responses.




(a)




(b)

269

270 Fig.3. Turbidity of the supernatant after US tests at different operating conditions (a);

271 Response surface plot for turbidity (b)

272

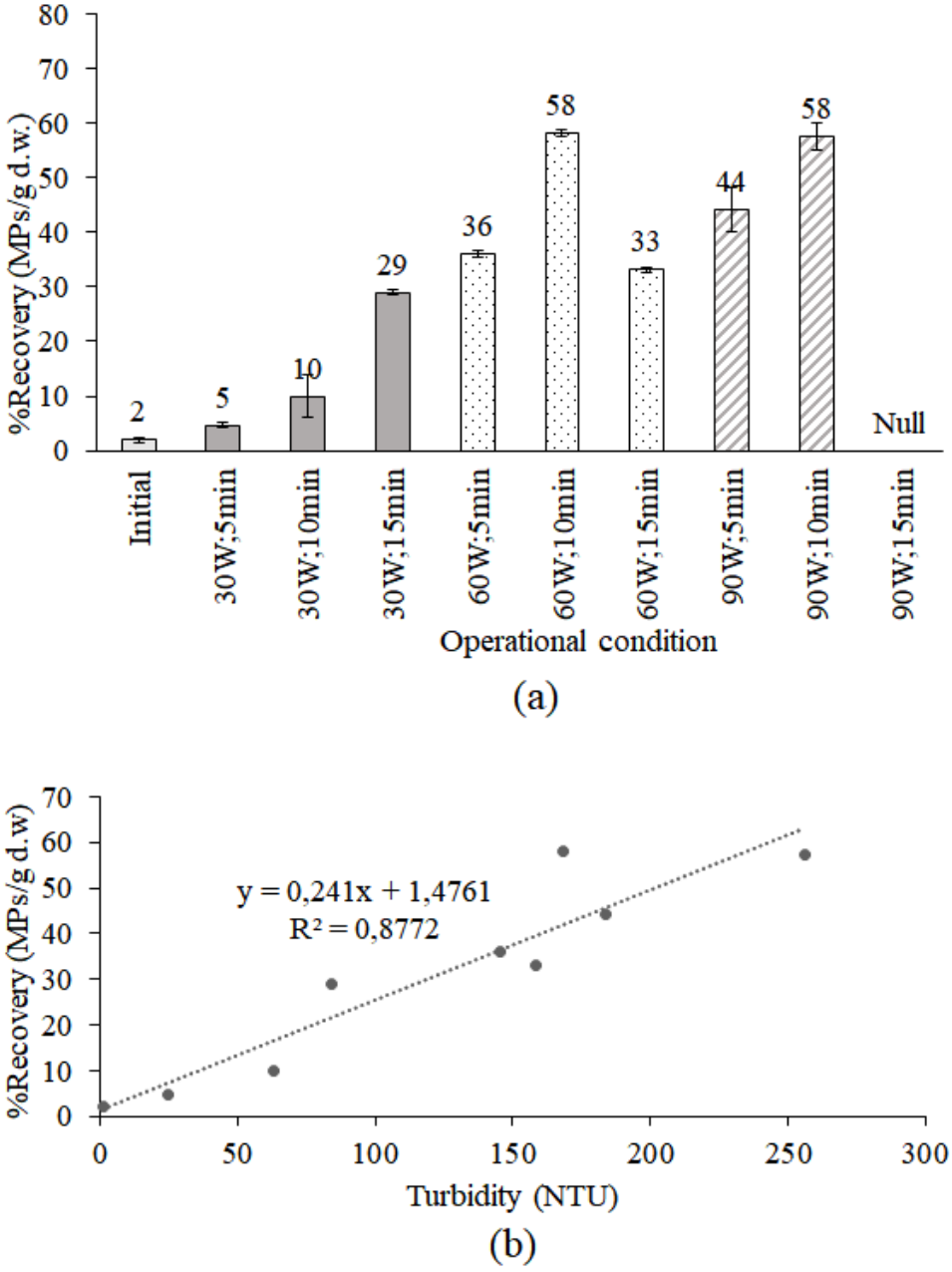
273 The percentage of recovery of MPs as a function of the power and time is represented in

274 Fig.4.a. In general terms, the higher time and power, the more MPs were recovered from

275 the mixed liquor supernatant. For 90 W and 15 min the turbidity was so high that it made

276 impossible the separation of the supernatant and the MPs. Because of that, the value for

277 this operational condition of MPs recovery was registered as null. Appling 60 W and 15
278 min, the MPs recovery decreased compared to 10 min, which does not follow the
279 aforementioned trend. In order to better understand the relation between the recovery of
280 PE microspheres and the sludge disintegration, the Fig.4.b shows that the percentage of
281 microspheres recovered from the mixed liquor can be directly related to the degree of
282 sludge floc disintegration (express in terms of turbidity). As the rupture of the sludge is
283 accompanied by increased turbidity in the supernatant, the lower recovery of PE
284 microspheres under 60 W and 15 min compared with the recovery under 60W and 10 min
285 can be justified due to the lower sludge disintegration in the first operational condition
286 (lower turbidity in the supernatant).
287
288




289

290 Fig.4. Recovery of MPs after US tests at different operating conditions (a); Recovery of

291 MPs in turbidity function (b)

292

293 The following equation (Eq.3) corresponds to the regression model established according

294 to the results of percentual MPs recovery response in the experimental design. The

295 analysis of variance conduced for the regression model, showed that the factor B, the

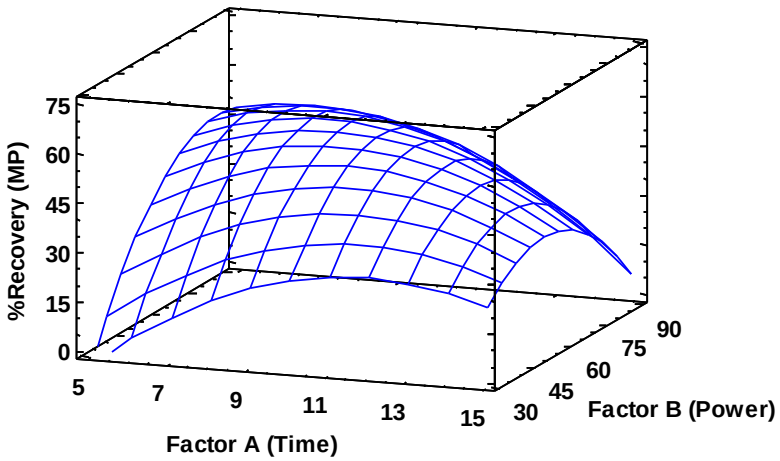
296 quadratic models ($A^2$ and $B^2$), and the interactive model terms (AB), were all significative
297 (p-Value< 0.05), at the 95% confidence level.

298

299 $$\%Recovery\ (MP) = -164.22 + 19.35A + 3.82B - 0.66A^2 - 0.11AB - 0.019B^2$$
300 (Eq.3)

301

302 The squared regression coefficient ($R^2$) and the adjusted squared regression coefficient
303 ($R^2_{adj}$) (0.82 and 0.73, respectively) indicate that there is a correlation between the
304 predicted and real responses. The recovery of MPs from mixed liquor was influenced by
305 power and time (Fig.5), but the extent of this dependence should be further evaluated in
306 future studies to improve the extraction process and reach a higher correlation between
307 the model and real responses. The contribution of the frequency can also be an important
308 parameter to understand better the role of US in the recovery of MPs from activated
309 sludge.




310

311 Fig.5. Response surface plot for %Recovery (MP)

### 3.2.2. Mixed liquor disintegration by ultrasonication

As stated above, the sonication power and time are parameters that played an important role in the degree of sludge disintegration. Thus, the higher power input, the more effective the sound pressure was, and therefore, a greater disintegration of the sludge was obtained. Similarly, with a longer ultrasonication time, the rupture of the sludge floc and cell membranes was more effective. In section 3.2.1 turbidity was reported as a measurement of sludge disintegration. In this section, results are focused on reporting the quality of the supernatant (final effluent from WWTP) in terms of SCOD and of its main components (SMP).

According to the SCOD results (Fig.6.a), it was observed an increment in SCOD value with the increase of the power input. In addition, an increment in SCOD with 10 minutes of ultrasonication, compared to 5min, was also observed for all experiments. The increase in SCOD value is related to the organic matter released into water due to the microorganism's cell lysis. As the microorganism's cells are mostly composed by protein, their lysis result in more soluble microbial proteins (SMPp) released in the water comparing with soluble microbial carbohydrates (SMPc) [42,43]. The increase in SMPp concentration was observed after all the US experiments (Fig.6.b), suggesting that the hydro-mechanical shear forces, during the US, were sufficient to promote the rupture of the sludge floc.

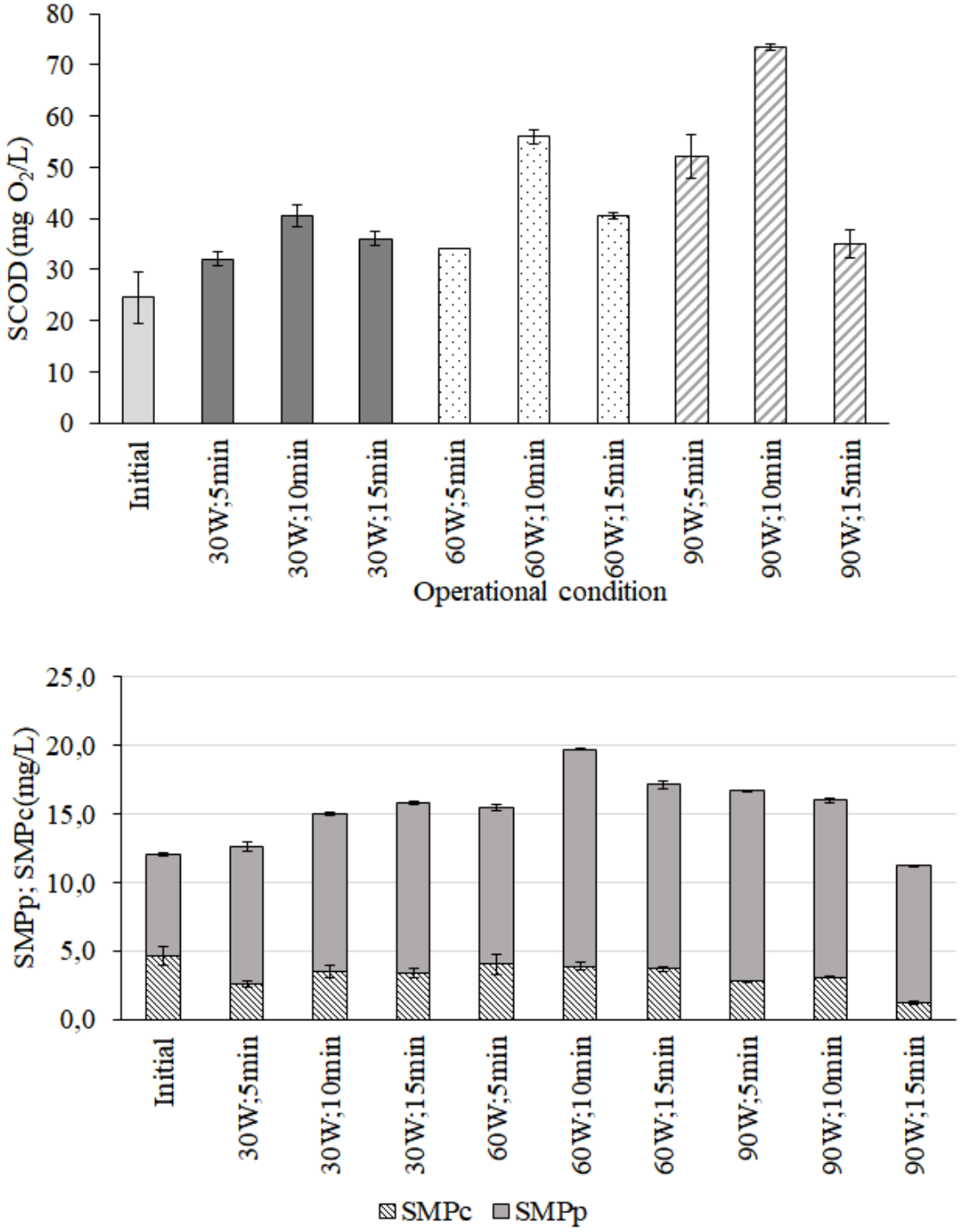
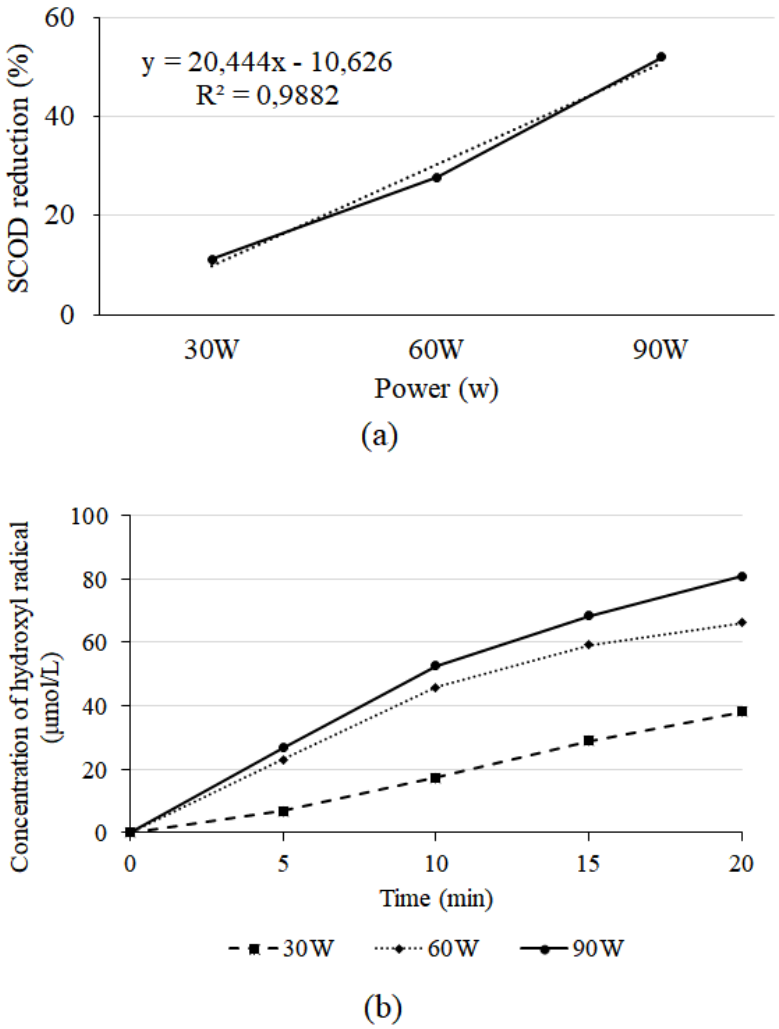



Fig.6. Mixed liquor disintegration in terms of SCOD released concentration (a) and carbohydrates and proteins concentrations (b)

However, for all powers tested it was observed that, when the time was increased from 10 minutes up to 15 minutes, a decrease of SCOD occurred. This reduction can be justified by the sonochemical effects. In this process, due to the acoustic cavitation, microbubbles are formed and violently collapsed, resulting in high temperature and pressure points. The result of this mechanism is the sonochemical effect, which results in the generation of hydroxyl radicals [44]. Hydroxyl radicals have a high oxidant capacity and react with organic species fast and in a nonspecific way [45]. The reaction of •OH with the extracellular polymeric substances (EPS) - present in the sludge floc - can also promote its rupture and the release of soluble organics as SMP, or even its mineralization into $CO_2$ and $H_2O$ [42]. When the mixed liquor was submitted to 15 minutes of US at any tested power, the oxidation of soluble organic matter due to the sonochemical effects, was more evident, resulting in a final value of SCOD lower than 10min. Analyzing the SMPc results after the ultrasonication, a reduction in its concentration was observed compared to the initial value. This can be another evidence of the oxidation of soluble organic matter by the generated hydroxyl radicals. Based on these results, it was established a hypothesis that a maximum cell lysis was achieved at 10min. After that, cell lysis still occurred, but the mineralization of SCOD seemed to be more intense. Wang et al. [45] reported that the sludge disintegration during the ultrasonication is performed by both hydro-mechanical shear forces and oxidizing effect. To mask the oxidizing effect of radical •OH, the authors proposed the addition of $NaHCO_3$ in the sludge before the US. Al-Juboori et al. [46] investigated the removal of dissolved organic carbon (DOC) present in Narda lagoon (Autralia) applying a 20khz frequency, power input of 93W and 143W and treatment time of 5 and 15min. The authors used real water, which contains a high concentration of microbes, to assess the influence of the microbe's presence in the ultrasonication process.

They concluded that the release of microbial cell lysis products and the reduction of DOC occurred simultaneously when applying US to water with a high microbial load.

With the application of 30W there was a decrease of SCOD in 12% between 10 and 15min. For 60W and 90W a reduction of 28% and 58% were obtained, respectively (Fig.7.a). The explanation of the relation between the reduction of SCOD and the power input is that the power intensity is linked to the size of the cavitation bubbles, the temperature, and the internal pressure of the bubbles during their collapse. Increasing the power intensity results in a greater number of collapsed bubbles, higher temperature and greater sonochemical effects [47]. This may be the reason why the increase in the power produced a proportional SCOD reduction between 10 and 15min test. The results of the sonochemical effects measured at 5, 10, 15 and 20 min, were plotted in Fig.7(b) in order to assess the hydroxyl radical production in the system. As expected, the sonochemical effects, increased with both exposition time and power applied.




373

374 Fig.7. SCOD reduction with power at 10min and 15min of US (a); Production of free

375 radicals in function of time and power (b)

376

377 **3.2.3. Optimized extraction model**

378 From the results obtained in experimental design (section 3.2.1), the optimum point of

379 maximum recovery of MPs and minimum turbidity of the supernatant was predicted.

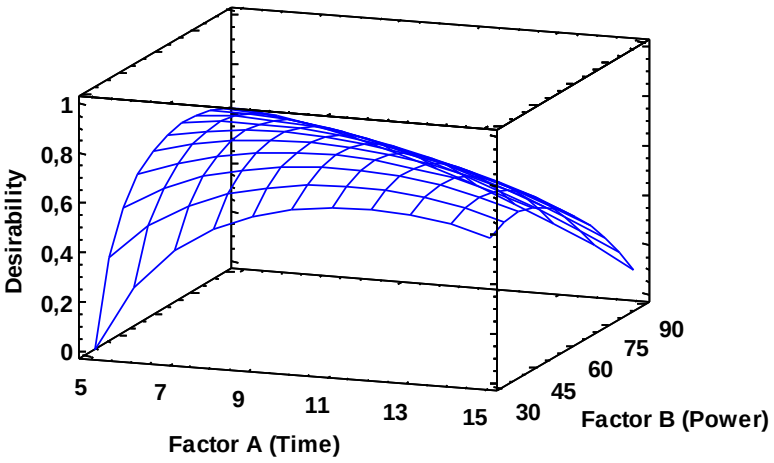
380 According to the response surface established by the software (Fig.8), a test time of 8 min

381 45 sec at a power of 54 W was ideal. These operational conditions would theoretically

382 result in a recovery percentage of 50 MPs and a turbidity of 134 NTU (Table 4). Due to

383 the power adjustment possibilities in the US equipment, it was not possible to apply 54

384 W. Therefore, 50 W was established as the power for the optimal point test. According to
385 the established model, the expected response for the percentage of MPs recovery was
386 50% and for the turbidity 136 NTU. When carrying out the extraction process, 48 ± 4%
387 of MPs were recovered and a turbidity of 179 ± 6 NTU was produced. Moreover, an
388 increase in SCOD was observed (45 ± 3 mg$O_2$/L after US). The results obtained under
389 the optimal condition showed a high correlation between the expected values and the real
390 ones, and therefore, it showed a relevant step in the possible application of US to remove
391 MPs from activated sludge.
392




393
394 Fig.8. Response surface plot for optimization
395
396 Table 4 - Ultrasound optimized operational conditions for mixed liquor.

| **Factor** | **Value** | **Parameter** | |
|---|---|---|---|
| Factor_A (min) | 8 min 45 sec | %Recovery (MPs) | Maximize |
| Factor_B (W) | 54 | Turbidity (NTU) | Minimize |
| | **Predicted response** | **Real response** | |

| | | |
|---|---|---|
| %Recovery (MPs) | 50 | 48±4 |
| Turbidity (NTU) | 136 | 179±6 |

397

398

399 ### 3.2.4. Concentrated mixed liquor

400 The extraction of MPs from WWTP sludge may be influenced by the concentration of
401 total solids (TS) in the sludge. A high concentration of TS can absorb acoustic energy and
402 reduce the efficiency of the sludge floc disintegration [48], and consequently decrease the
403 release of MPs. Applying the optimum point established in section 3.2.3. for the mixed
404 liquor, 48 ± 4% of PE microspheres were recovered by the supernatant. However, when
405 the same experimental conditions were applied to the concentrated mixed liquor, only 12
406 ± 4% recovery was achieved. The high concentration of TS, compromised the cavitation
407 process, making difficult to detach the MPs retained in the sludge. However, submitting
408 the sludge to a higher power and time (90 W; 30 min) the recovery of MPs reached 38 ±
409 4% (Table 5).

410

411 Table 5 – MPs recovery (%) and SCOD concentrations achieved at different ultrasound
412 operational conditions for concentrated mixed liquor.

| **Operational condition** | **%Recovery (MPs)** | **SCOD (mg/L)** |
|---|---|---|
| Initial | 2 ± 1 | 39 ± 1 |
| 80 kHz; 50 W; 8 min 45 sec | 12 ± 4 | 61 ± 4 |
| 80 kHz; 90 W; 30 min | 38 ± 4 | 188 ± 6 |
| 80 kHz; 90 W; 60 min | 29 ± 1 | 310 ± 4 |

413

On the contrary, upon being subjected to US, the SCOD value increased instead of decreasing due to the sludge fragmentation, that caused a release of more organic products. In addition, a longer US time (comparing the same power of 90 W) resulted in a greater disintegration of the sludge. Although the US process produces free radicals (extremely oxidizing), results suggested that the sludge disintegration process by hydro-mechanical was higher than the sonochemical effects. Tian et al. [49] studied the ultrasonication effects in the activated sludge (16.2–17.2 g/L of TS), under 20 kHz frequency, and they also observed a low influence of free radicals in the ultrasonication process and the predominance of hydro-mechanical shear forces in the sludge disintegration. The results of SMPp and SMPc for concentrated mixed liquor (Fig.9) corroborate that. At the optimum conditions for the mixed liquor (50 W; 8 min 45 sec), the concentrated mixed liquor suffered a low disintegration. However, when 90W;60min was applied, an increment of more than 450% and 390% of SMPp and SMPc were achieved, respectively. According to these results and the ones regarding the recovery of MPs, the degree of MPs recovery seems to be related to the extent of floc disintegration, as it was also concluded in the section 3.2.1 for the mixed liquor. Nevertheless, an intense sludge fragmentation can be negative for the recovery of MPs. After 90 W; 60 min of ultrasonication a reduction in the recovery of MPs compared to 30 min was observed. Our hypothesis is that, under extreme sludge disintegration conditions, the sample are not able to decant, and the consequence is that the sample is more homogeneous with a more intense mixture of sludge and MPs. Therefore, without a separation of water from the sludge, the buoyancy of MPs is seriously compromised.

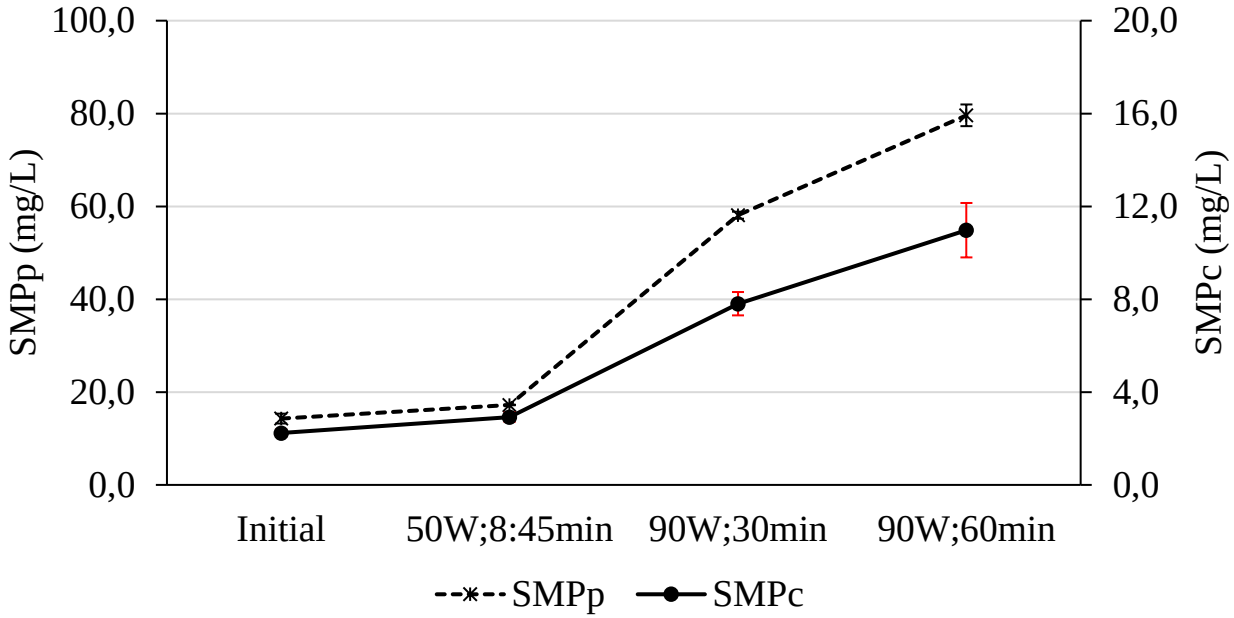



Fig.9. Released SMPp and SMPc after ultrasonication of concentrated mixed liquor

This is the first investigation into how the US can accomplish the extraction of MPs from activated sludge, and therefore the results obtained in this study cannot be compared with previous ones. However, this research provides important information about the viability of treating the activated sludge for the removal PE microspheres. Several microplastics can be found in activated sludge (microfibers, fragments and spheres), of different sizes and compositions, thus further research must be carried out to evaluate the potential for removing these MPs through US.

### 3.3. Particle size distribution of PE microspheres after ultrasonication

According to the results of the particle size distribution in the supernatant for the optimum point (80 kHz; 50 W; 8 min 45 sec) applied to the mixed liquor, and for the best conditions applied to the concentrated sludge (80 kHz; 90 W; 30 min), the fragmentation of the PE

452 microspheres into smaller fragments was not observed. This result would demonstrate
453 that PE microspheres fragmentation should be caused by factors like biodegradation, UV
454 irradiation, and not just mechanical processes such as US [41].

455

456 **4. Conclusions**

457 The main conclusion of this work is that ultrasonication can become an excellent tool to
458 assist in the separation of PE microspheres, a type of MPs, from the activated sludge. It
459 was possible to remove 48 ± 4% of MPs that were retained in the mixed liquor by applying
460 of ultrasonication optimal conditions at a frequency of 80 kHz (power input 50 W and
461 time 8 min 45 sec). After the US, the PE microspheres were transferred to the water
462 column phase due to the disruption of the sludge floc by cavitation process, which allowed
463 its extraction and therefore, the reduction of the concentration of MPs in the sludge. In
464 addition, the quality of the final effluent was not compromised as showed the SCOD and
465 SMP measured values.
466 Regarding the concentrated mixed liquor, when these optimal operational conditions were
467 applied, only 12 ± 4% of MPs could be recovered from the activated sludge. The higher
468 total solid concentration compromised the cavitation process, leading to a poor sludge
469 disintegration compared to that of the mixed liquor. Since the sludge was insufficiently
470 disintegrated, only a small amount of MPs were recovered. However, when the
471 concentrated mixed liquor was exposed to a higher power and a longer US time, (80 kHz;
472 90 W; 30 min) 38 ± 4% of MPs were detached from the sludge floc and moved to the
473 sample surface. Moreover, the fragmentation of the MPs into NPs after the US for both
474 mixed liquor and concentrated mixed liquor was not observed. Based on these results, the
475 US process has an interesting potential for the extraction of MPs from a biomass matrix.
476 Further studies should be developed to better understand the process of extracting MPs

via US and to study the feasibility of its application on an industrial scale. The reduction of the concentration of MPs in the activated sludge deserves attention, since mostly MPs that enter the secondary treatment are retained in the sludge, and this material is commonly applied as fertilizers in agricultural lands leading to their contamination by these synthetic polymers.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