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(Article begins on next page)

1 **Microplastics in real wastewater treatment schemes: comparative**

2 **assessment and relevant inhibition effects on anaerobic processes**

3

4 Lucia Pittura ^a, Alessia Foglia ^{b,*}, Çağrı Akyol ^{b,*}, Giulia Cipolletta ^b, Maura Benedetti ^a,

5 Francesco Regoli ^a, Anna Laura Eusebi ^b, Simona Sabbatini ^b, Linda Y. Tseng ^c, Evina Katsou

6 ^d, Stefania Gorbi ^a, Francesco Fatone ^b

7

8 ^a *Department of Life and Environmental Sciences, Marche Polytechnic University, 60131,*

9 *Ancona, Italy*

10 ^b *Department of Science and Engineering of Materials, Environment and Urban Planning-*

11 *SIMAU, Marche Polytechnic University, 60131, Ancona, Italy*

12 ^c *Department of Environmental Studies and Physics, Colgate University, 13346 New York,*

13 *United States*

14 ^d *Department of Civil Engineering and Environmental Engineering, Institute of Environment,*

15 *Health and Societies, Brunel University London, Middlesex, UB8 3PH Uxbridge, United*

16 *Kingdom*

17

18

19 * Corresponding authors (A. Foglia; Ç. Akyol)

20 E-mail: a.foglia@staff.univpm.it; c.akyol@staff.univpm.it

21 Tel: +39 071 2204911 Fax: +39 071 2204729

22 ORCID ID: 0000-0002-9907-6616 (Ç. Akyol)

23

24

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26 **ABSTRACT**

27 The occurrence, fate and removal of microplastics (MPs) in a wastewater treatment plant
28 (WWTP) in Central Italy were investigated together with their potential adverse effects on
29 anaerobic processes. In the influent of the WWTP, 3.6 MPs·L⁻¹ were detected that mostly
30 comprised polyester fibers and particles in the shape of films, ranging 0.1-0.5 mm and made
31 of polyethylene and polypropylene (PP). The full-scale conventional activated sludge scheme
32 removed 86% of MPs, with the main reduction in the primary and secondary settling. MPs
33 particles bigger than 1 mm were not detected in the final effluent and some loss of polymers
34 types were observed. In comparison, the pilot-scale upflow granular anaerobic sludge blanket
35 (UASB) + anaerobic membrane bioreactor (AnMBR) configuration achieved 94% MPs
36 removal with the abatement of 87% of fibers and 100% of particles. The results highlighted
37 an accumulation phenomenon of MPs in the sludge and suggested the need to further
38 investigate the effects of MPs on anaerobic processes. Accordingly, PP-MPs at concentrations
39 from 5 PP-MPs·gTS⁻¹ to 50 PP-MPs·gTS⁻¹ were spiked in the pilot-scale UASB reactor that
40 was fed with real municipal wastewater, where up to 58% decrease in methanogenic activity
41 was observed at the exposure of 50 PP-MPs·gTS⁻¹. To the best of our knowledge, the
42 presented results will be the first to report of PP-MPs inhibition on anaerobic processes.

43 **Keywords:** microplastics; municipal wastewater; polypropylene; sewage sludge; UASB

44

45 **1. Introduction**

46 Plastic pollution is a high-priority problem due to wide range of applications of plastic
47 materials (Hahladakis et al., 2018; Mason et al., 2016), and their production is expected to
48 increase up to 33 billion tons by 2050 (Fu et al., 2018). Since plastics can enter the
49 environment throughout their life cycle and through multiple pathways (Möller et al., 2020),
50 millions of tons are estimated to enter aquatic and terrestrial ecosystems each year with a

51 wide size distribution, ranging from micrometers to meters (Estahbanati and Fahrenfeld,
52 2016).

53 Microplastics (MPs) are commonly defined as plastic items with sizes below 5 mm (de Sá et
54 al., 2018; Lares et al., 2018), which can be categorized into fibers and particles, as well as
55 primary or secondary, depending on the way they are produced (Sun et al., 2019; Xu et al.,
56 2020) . Current understanding suggests that MPs enter wastewater treatment plants (WWTPs)
57 daily at varying levels of concentration (Blair et al., 2019) and of different polymer typologies
58 (Gatidou et al., 2019; Magni et al., 2019).

59 Although conventional WWTPs are reported to be effective on the removal of MPs up to
60 99.9% (Sun et al., 2019) they may actually be a significant source of MPs given the large
61 volumes of discharged effluents (Bayo et al., 2020): it was estimated that a WWTP (650,000
62 population equivalent) releases 65 million MPs into the receiving water every day (Murphy et
63 al., 2016). Thus, the quality of the final effluents has to be and can be enhanced by innovative
64 treatment configurations (Talvitie et al., 2017), in order to limit the discharge of MPs into the
65 receiving environment. Some of the greatest concerns include the ingestion and accumulation
66 of MPs that could induce toxicity related to a physical disturbance (Seidensticker et al., 2017),
67 as well as, to the leaching of plastic-associated chemicals and microbial pathogens in biofilms
68 that can grow on MPs (Koelmans et al., 2019). Hence, MPs have the potential to act as
69 vectors for other contaminants, especially in WWTPs (Raju et al., 2018), such as endocrine-
70 disrupting compounds, pharmaceuticals, heavy metals, persistent organic pollutants and
71 pathogens (Carr et al., 2016; Hidalgo-Ruz et al., 2012). Besides, the processes themselves can
72 be negatively affected by the presence of MPs resulting in a reduction of treatment
73 performance (Zhang and Chen, 2019). Moreover, most of MPs retain in sewage sludge
74 (Gatidou et al., 2019; Li et al., 2018). This limits and affects the further routes of sewage
75 sludge (i.e. anaerobic digestion (AD), land application) (Peng et al., 2017; Wei et al., 2019a).

76 In fact, it was recently reported that sewage sludge application in agriculture results in a
77 further accumulation of MPs in soils (van den Berg et al., 2020).
78 So far, most of the available information on the MPs in wastewater and/or sewage sludge is
79 limited to monitoring studies in full-scale WWTPs and further data is therefore urgently
80 required from lab-/pilot-scale experiments to better understand the behavior of MPs in
81 WWTPs (Gatidou et al., 2019; Magni et al., 2019). In fact, the study on MPs effect on AD is
82 still very limited (X. Zhang et al., 2020). Up to date, several studies have reported the adverse
83 effects of MPs on AD systems such as polyethylene (PE) (Wei et al., 2019a), polyvinyl
84 chloride (PVC) (Wei et al., 2019b), polyester (PESTs) (Li et al., 2020) and polyethylene
85 terephthalate (PET) (Y.T. Zhang et al., 2020). It is evident that the inhibition of
86 methanogenesis depends on the type and concentration of MPs; and furthermore, to the best
87 of our knowledge, the role of polypropylene (PP) MPs in AD have not been reported. Based
88 on afore-listed reasons, the motivation of this study was to provide relevant information on
89 MPs at different treatment schemes and further evaluate the effect of MPs on anaerobic
90 treatment systems. MPs were characterized in wastewater and sludge lines of a full-scale
91 municipal WWTP, in parallel, the fate and transport pathways of MPs in a pilot-scale
92 anaerobic configuration (upflow granular anaerobic sludge blanket (UASB) + anaerobic
93 membrane bioreactor (AnMBR)) was also monitored. Given the site-specific characterization
94 and quantification of MPs demonstrating PE and PP as the most frequent MPs in the samples.
95 We further investigated the influence of PP-MPs on anaerobic treatment systems since the
96 role of PE-MPs in methanogenesis inhibition has already been demonstrated (Wei et al.,
97 2019a). Accordingly, experiments through continuous operation were carried out and effects
98 of PP-MPs on the performance of the pilot-scale UASB reactor were explored.

99 **2. Materials and methods**

100 **2.1. Full- and pilot-scale field study**

101 The monitored WWTPs are in the Central Italy (Falconara Marittima, AN) on the Adriatic
102 coastline. The full-scale plant receives municipal wastewater with a design treatment capacity
103 of 80,000 population equivalent (PE) at an average dry weather flow rate of 18,000 m³·d⁻¹.
104 After preliminary treatments and primary settling, the pretreated influent is treated in the CAS
105 system that applies Modified Ludzack-Ettinger scheme (Shah, 2018).
106 The pilot-scale plant consists of a UASB reactor coupled with an ultrafiltration hollow fiber
107 membrane with 0.03 µm of pore-size and 0.5 m² of surface area (Foglia et al., 2020). It works
108 in submerged-side-stream AnMBR configuration with low hydraulic retention time (6 h) and
109 temperature at 30°C. The UASB is a cylindrical Plexiglas reactor (16 L) with an internal
110 diameter of 15 cm and a total height of 136 cm. The reactor is divided into two compartments:
111 the first is the real reaction chamber at the bottom (85 cm, 12.4 L), while the second is a tri-
112 phase separator with 21.9 cm height and connected to a hydraulic guard which creates
113 backpressure for biogas release. A completely stirred influent tank was placed right before the
114 UASB reactor that collects the pre-treated influent wastewater.

115 2.2. Wastewater and sludge sampling

116 The sampling was performed in April 2018 during dry weather. Wastewater and sludge
117 samples were collected using automatic samplers that allowed to take an average sample over
118 a 24-hour period. A steel bucket was used only for sludge sampling after the dewatering unit
119 of the WWTP. The flow-schemes of wastewater treatment and sampling points for MPs are
120 shown in **Fig. 1**. Urban wastewater influent of 25 L was sampled after grit chamber (6 mm)
121 and sand removal (PRE-TREAT IN); the effluents (25 L each) was gathered after primary
122 settling (I EFF), after the aerobic biological treatment and the secondary settling (II EFF) and
123 after disinfection (FINAL EFF), along with 5 L of excess sludge (I SLUDGE), 5L of waste
124 activated sludge (AerWAS) and 160 g of final sludge after dewatering unit (DEWAT SLUD).
125 Meanwhile, 18 L of effluent after the UASB (UASB EFF), 25 L of effluent after the AnMBR

126 (PERMEATE) and 300 mL of granular sludge (AnaEXC SLUD) were collected. The
127 characteristics of the raw municipal wastewater during the sampling period are presented in
128 the **e-Supplementary file**. Total solids (TS) were 0.8%, 2.4% and 27.5% in AerWAS, I
129 SLUDGE and DEWAT SLUD, respectively, while total sludge production in the WWTP was
130 approximately 3,419 kgTS·d⁻¹.

131 2.3.Extraction, quantification and identification of microplastics

132 2.3.1. Microplastics extraction from wastewater

133 The collected wastewater samples were *in loco* passed through a steel sieves battery of 5 mm,
134 2 mm and 63 µm mesh size (ISO 3310-1:2000): solids retained on 2 mm and 63 µm sieves
135 were rinsed into glass jars with ultrapure water and subsequently filtered onto cellulose nitrate
136 filters (Sartorius Stedim Biotech, Ø 47 mm, 8 µm pore size) using a vacuum pump. Filters
137 were recovered in petri dishes, covered with 15% H₂O₂ and maintained at 50°C overnight (or
138 more if necessary) to remove organics.

139 2.3.2. Microplastics extraction from sludge

140 After collection, a first organic matter digestion was performed in glass beakers adding 15%
141 H₂O₂ and maintaining sludge samples in stove at 50°C for two days. This step was followed
142 by the density separation procedure, carried out in 250 mL cylinders, stirring the samples with
143 high-density saturated NaBr salt solution (1.4 g cm³⁻¹) for 30 min (Frias et al., 2018) and
144 leaving to settle the mixture overnight. The supernatant was then vacuum filtered and filters
145 were treated with 15% H₂O₂. To evaluate the potential for loss during the density separation
146 procedure for sludge fraction and to calculate the extraction yield of microplastics, a total of
147 12 particles, 2 for each representative polymer (polyethylene, polypropylene, polystyrene,
148 polyethylene terephthalate, nylon, polyisoprene rubber) in the size range of 0.5-1.5 mm, were
149 spiked into samples and blanks, starting from the first organic matter digestion step. The
150 particles of polyethylene, polypropylene and polystyrene were standard materials purchased

151 from a plastic company (Fainplast, Italy), while those of polyethylene terephthalate, nylon
152 and polyisoprene were obtained by cutting a plastic bottle, a fishing wire and an elastic band,
153 respectively. All of them were photographed and measured, and IR spectra were acquired
154 before and after the test, showing no appreciable changing in shape, size and polymer
155 characteristics of recovered particles. The resulted extraction yield of spiked MPs was 100%
156 in blank samples, 95% in I SLUDGE, 92% in AerWAS, 96% in DEWAT SLUD and 98% in
157 AnaEXC SLUD.

158 2.3.3. Microplastics quantification and characterization

159 Wastewater and sludge filters resulted from the extraction procedure were observed using a
160 stereomicroscope (Optika SZM-D equipped with OPTIKAMB5 digital camera), with
161 maximum magnification of 45X. All items resembling plastic and fibers were manually
162 collected using a tweezer, transferred onto a clean cellulose acetate membrane (Sartorius
163 Stedim Biotech, Ø 47 mm, 0.45 µm pore size) located on a microscope slide (subsequently
164 used as support for the µFT-IR analyses), quantified and categorized based on shape, size and
165 polymer type. In terms of their shape, MPs were categorized in fiber-shaped (MPFs)
166 according to the definition proposed elsewhere (Liu et al., 2019) and particle-shaped (MPPs),
167 which included five main typologies: lines, fragments, films, spheres, glitters identified
168 according to characteristics given in (Hartmann et al., 2019; Lusher et al., 2017; Magni et al.,
169 2019; Yurtsever, 2019). MPPs were measured on the basis of the largest dimension
170 (Hartmann et al., 2019), using an image analysis software (Optika Vision Lite 2.1) and
171 classified in four size classes in the range of 1-5 mm, 0.5-1 mm, 0.1-0.5 mm and 0.03-0.1
172 mm. To confirm the synthetic nature and gain the abundance of MPs, all the collected
173 particles and fibers were characterized by µFTIR spectroscopy in attenuated total reflectance
174 mode, using a Spotlight 200i FT-IR microscope system (Perkin Elmer) equipped with
175 Spectrum Two and driven by Spectrum 10 software. After background scans, each sample

176 spectrum was recorded performing 32 accumulations, ranging from 600 to 4000 cm^{-1} with the
177 resolution at 4 cm^{-1} . When the spectrum was not resolved at first acquisition, more than one
178 measurement was conducted per samples. IR spectrum of the cellulose acetate membrane was
179 aquired and substracted to that of each sample in order to avoid the overlay of spectra. The
180 output spectra were subsequently subjected to a spectral search against reference libraries of
181 polymer spectra represented by Perkin Elmer database (ATRPolymer, polyATR, FIBERS3,
182 plast1, RP, POLIMERI, PIGMENTI, resin and PERKIN1 libraries were selected), by the
183 database compiled within the framework of the JPI-OCEANS project BASEMAN (Primpke
184 et al., 2018) and by personal created ones. For accurate identification, the match factor
185 threshold was calculated as 0.70 and a lower level (0.60-0.70) was accepted after careful
186 examination of peaks characteristics. Based on the recommendations by (Hartmann et al.,
187 2019), synthetic polymers (petroleum-based, biobased and hybrid polymers), modified natural
188 ones (e.g. rayon), copolymers and composites were considered as plastic. Details of the MPs
189 shapes, reference polymer libraries, matching factor and examples of the output IR spectra are
190 given in the **e-Supplementary file**.

191 2.3.4. Quality control

192 Special care was taken during sampling, sieving and sample treatments in laboratory to
193 prevent external contamination, especially by synthetic fibers released from clothing or from
194 atmospheric fallout. Cotton laboratory coats were worn during the entire sampling and
195 laboratory processes; glassware and metal equipment were preferred, and they were rinsed
196 with ultrapure water before use; working benches were cleaned with ethanol. Steel sieves
197 were carefully washed first with tap water and then three times with pre-filtered deionized
198 water between samples to avoid cross-contamination. One blank control sample, consisting of
199 10 L of deionized water pre-filtered onto cellulose acetate membranes, was processed like and
200 for each kind of wastewater and sludge sample (10 total blanks).

201 2.4. Pilot-scale UASB reactor operation with spiked polypropylene microplastics
202 Fate and effects of PP-MPs in continuous operating mode were investigated in the pilot-scale
203 UASB reactor. The spiking of PP-MPs in a completely stirred influent tank started from 5 PP-
204 MP \cdot gTS $^{-1}$ and then increased to 18 PP-MP \cdot gTS $^{-1}$ and finally to 50 PP-MP \cdot gTS $^{-1}$ at the same
205 operating conditions as stated previously. PP-MPs were counted in the influent and in the
206 effluent as well as in the granular sludge. The operating conditions and influent-effluent
207 characteristics of the UASB reactor are given in the **e-Supplementary file**. All physical-
208 chemical analyses of wastewater and sludge samples were done according to Standard
209 Methods (APHA, 2012). Biogas production in the UASB reactor was measured by milligas
210 counter (Ritter, Germany). Methane content was analyzed by Brüel and Kjaer Multi-gas
211 Monitor Type 1302. Specific methane production rate was expressed as mLCH $_4$ ·gVSS $^{-1}$ d $^{-1}$.

212 3. Results

213 3.1. Occurrence, fate, removal and identification of microplastics in full and pilot plants
214 A total of 1342 items were potentially recognized as MPs by the visual sorting of filters of
215 samples collected both from full and pilot-scale plants, 1024 of them were confirmed as
216 synthetic nature, comprising both MPPs and MPFs. The number of extracted MPs was further
217 corrected after the subtraction of synthetic items quantified in the respective blanks: only
218 fibers, made of polyesters, were found and 0.3 MPFs/L were detected in the blank related to I
219 EFF; 0.2 MPFs/L for blank of the PRE-TREAT IN, FINAL EFF, AerWAS, DEWAT SLUD
220 and UASB EFF; 0.1 MPFs/L for blank of II EFF and I SLUDGE; none MPFs in blank
221 samples for PERMEATE and AnaEXC SLUD. A mass balance of MPs was additionally
222 performed in the full-scale and pilot-scale with respect to the number of particles and fibers
223 that entered the system per hour as given in **Table 1**.
224 **Table 1.** Number of detected MPs and distribution of MPs (including MPPs and MFs) in the
225 full-scale CAS configuration and in the pilot-scale UASB+AnMBR configuration.

Treatment scheme	Sampling point	Concentration (MPs·L ⁻¹)	Load (MPs·h ⁻¹ 10000 ⁻¹)	Distribution (%)
CAS	UWW IN	3.64	1217	100
	PRE-TREATED	3.64	1217	100
	IN			
	I EFF	1.9	639	53
	II EFF	0.76	253	21
	FINAL EFF	0.52	173	14
Treatment scheme	Sampling point	Concentration (MPs·L ⁻¹)	Load (MPs·h ⁻¹)	Distribution (%)
UASB+AnMBR	UASB EFF	1.72	5.1	47
	PERMEATE	0.2	0.6	6

226

227 3.1.1. Microplastics in the full-scale CAS configuration

228 The full-scale CAS configuration removed 86% of MPs from the influent with a remarkable
229 accumulation in the sludge. 47.3% of MPs were removed in the primary settling, 32% in the
230 biological treatment and secondary settling and 6.5% after the disinfection. In fact, MPs
231 concentrations in the sludge were 1.67 MPs·gTS⁻¹ in the I SLUDGE, 5.3 MPs·gTS⁻¹ in the
232 AerWAS and 4.74 MPs·gTS⁻¹ in the DEWAT SLUD.

233 The mass balance showed that 12,170,000 MPs·h⁻¹ entered the full-scale plant, and after the
234 primary treatment, only 6,390,000 MPs·h⁻¹ remained in the wastewater line. After the
235 secondary treatment, the MPs reduced to 2,530,000 MPs·h⁻¹. Finally, after the disinfection, the
236 MPs being discharged into the water body was about 1,730,000 MPs·h⁻¹.

237

238

239 The identification of MPs is shown in **Fig. 2**. MPs in PRE-TREAT IN were represented by
240 fibers and particles at the same percentage. The relative contribution of MPFs and MPPs
241 changed in I EFF in favor of MPFs, representing 65% of MPFs, while in the sludge more
242 MPPs were observed in all samples with 70% in I SLUDGE and around 80% in AerWAS and
243 DEWAT SLUD (**Fig. 2A**). The MPPs extracted from the PRE-TREAT IN were only films
244 (55%), fragments (36%) and lines (9%). Films and fragments remained as the most frequently
245 detected types in II EFF (33% and 44% respectively) and the only typologies of particles
246 extracted from I EFF (53% and 47%) and FINAL EFF, in the latter, films prevailing on
247 fragments were 67% and 33%, respectively. Fragments represented the predominant
248 component in the sludge, where also spheres and glitters were found unlike wastewater
249 samples; however, they contributed to a minimal percentage on the total MPPs abundance:
250 spheres were extracted from I SLUDGE (0.7%) and glitters from AerWAS (4.4%) and
251 DEWAT SLUD (2.5%) (**Fig. 2B**).

252 Most of the MPPs characterized in the PRE-TREAT IN fell in the 0.1-0.5 mm size class
253 (57%), 17% were in the dimensional range of 0.5-1 mm, 15% in that of 1-5 mm and 11%
254 belonged to the smallest size class of 0.03-0.1 mm. The size class of 0.1-0.5 mm were the
255 most frequently found also in the effluents, especially after primary settling (70% of MPPs);
256 no particles between 5 and 1 mm were detected in I and FINAL EFF and no ones in the range
257 of 0.03-0.1 mm in the II EFF. In all sludge samples MPPs of all sizes were observed;
258 however, most of particles were between 5 and 0.5 mm in I SLUDGE, while in AerWAS and
259 DEWAT SLUD they were mainly between 1 and 0.1 mm (**Fig. 2C**).

260 The μ FT-IR characterization of MPPs in the PRE-TREAT IN identified 12 different
261 polymers: PE was the predominant component (43%) followed by PP (13%),
262 ethylene/propylene (EPM) (11%), polyesters (PESTs) and polyurethane (PUR) (9% each);
263 low frequencies were observed for polystyrene (PS) (4%), for polyamide (PA),

264 polyacrilamide (PAM), polyvinyl-acetate (PVAC), ethylene-vinyl- acetate (EVA), polyvinyl
265 chloride/polyvinyl alcohol/polyethylene (PVC/PVAC/PE) and for polyesters based copolymer
266 (2% each). A stepwise reduction in the number of polymer types was observed after
267 subsequent treatments until detection of only PE, polyurethane (PUR), PESTs and EPM in the
268 FINAL EFF. PE remained as the predominant polymer in I EFF (41%), while its relative
269 contribution decreased in II EFF (33%) and further in FINAL EFF (17%), EPM and PUR
270 prevailed (33% each). A major number of polymers types were observed in the sludge
271 compared to the wastewater, especially in AerWAS and DEWAT SLUD; however, the main
272 contribution was given by PE, particularly in I SLUDGE (52%), and by PP (**Fig. 2D** and
273 **Table S4**).

274 The characterization of fibers highlighted a higher frequency of synthetic polymers than
275 natural ones (i.e. cellulose, kapok) both in wastewater (74.5% of MPFs in PRE TREAT IN,
276 90% in I EFF, 50% in II EFF, 100% in FINAL EFF) and in sludge (72% of MPFs in I
277 SLUDGE and DEWAT SLUD, 76% in AerWAS). MPFs in the wastewater were all made of
278 PESTs; meanwhile they were mainly of polyesters in the sludge (around 80% for each
279 sample), with the occurrence of other polymers at variable frequencies (i.e. PE, PP, EVA, PA,
280 polyacrylate (PAK) and rayon).

281 3.1.2. Microplastics in the pilot-scale UASB+AnMBR configuration

282 The number of extracted MPs in the water line of the pilot-scale treatment system is reported
283 in **Table 1**. Overall, the innovative configuration removed 94% of influent MPs and 52.6% of
284 the overall removal was made by the UASB and further 41.4% by the AnMBR. The
285 accumulation of MPs in AnaEXC SLUD was $1.04 \text{ MPs} \cdot \text{gTS}^{-1}$.

286 In this configuration, $10.9 \text{ MPs} \cdot \text{h}^{-1}$ entered the system and $5.1 \text{ MPs} \cdot \text{h}^{-1}$ were detected in the
287 UASB effluent. After the AnMBR unit, only $0.6 \text{ MPs} \cdot \text{h}^{-1}$ were discharged with the permeate.

288 The identification of MPs in the pilot-plant is presented in **Fig. 3**. In UASB EFF, the relative
289 contribution of MPPs and MPFs was similar to that of PRE-TREAT IN, with a frequency of
290 45% and 55%, respectively. No MPPs were extracted from the PERMEATE and thus 0.2
291 MPs/L was only represented by MPFs. In AnaEXC SLUD, more MPPs (79%) were found
292 than MPFs (21%) (**Fig. 3A**). Among MPPs typologies, only films (73%) and fragments (27%)
293 were found in UASB EFF and a similar composition was observed in AnaEXC SLUD,
294 together with small contribution of glitters (2%) (**Fig. 3B**).

295 Most of the MPPs in UASB EFF were in the range of 0.1-0.5 mm, maintaining the same
296 relative contribution of PRE-TREAT IN; however, in comparison to the influent, a higher
297 contribution (27%) of smaller particles (between 0.03-0.1 mm) were recorded and no particles
298 bigger than 1 mm were extracted. Conversely, particles between 1 and 5 mm in size were
299 found in the AnaEXC SLUD, though in a little percentage (8%); in addition, a greater relative
300 contribution of the smaller size class (0.03-0.1 mm) was observed (42%) in respect to what
301 found in the PRE-TREAT IN and UASB EFF (**Fig. 3C**). A loss of polymers types from the
302 PRE-TREAT IN to the UASB EFF was observed; however, PE maintained as the highest
303 frequency of occurrence (53%). More polymers typologies were detected in AnaEXC SLUD
304 compared to PRE-TREAT IN and UASB EFF, without a clear prevalence of a polymer: in
305 particular, PE, that in wastewater fraction dominated, was present with an only 18%
306 frequency in the sludge (**Fig. 3D** and **Table S4**). Concerning results on fibers: the synthetic
307 ones (MPFs) represented the 82% of the total extracted from both UASB EFF and
308 PERMEATE and they were all made of PESTs. In AnaEXC SLUD, MPFs were only 22%
309 and made mainly of PESTs (92%), while the remaining part was represented by PP (8%).

310 3.2.Effects of spiked polypropylene microplastics during the operation of the pilot-scale
311 UASB reactor

312 The initial exposure of 5 PP-MPs:gTS⁻¹ did not influence the performance of the UASB in
313 terms of methane production. At this point, the following two phases were further evaluated
314 for each PP-MPs concentration (i.e. 18 and 50 PP-MPs:gTS⁻¹). Specific methane production
315 rate in the UASB reactor corresponding to spiked PP-MPs is given in **Fig. 4**. Methane
316 production slightly decreased by 4% to 38.5±7.7 mLCH₄:kgVSS⁻¹d⁻¹ during the first phase at
317 the concentration of 18 PP-MPs:gTS⁻¹ compared to the operating period without PP-MPs
318 (around 40±7 mLCH₄:kgVSS⁻¹d⁻¹). When the PP-MPs concentration was increased to 50 PP-
319 MPs:gTS⁻¹ in the second phase, a sharp decrease to 17±14.3 mLCH₄:kgVSS⁻¹d⁻¹ was observed.
320 This decrease remarked approximately 58% of inhibition on the methanogenic activity.
321 Meanwhile, the average methane content of the biogas in each phase was slightly affected by
322 the exposure of PP-MPs (30% in no PP-MPs period, 29% and 27% in the spiking of 18 and
323 50 PP-MPs:gTS⁻¹, respectively). Furthermore, no change in the structure of PP-MPs during
324 the UASB operation was observed (data not shown).

325 4. Discussion

326 4.1. Microplastics in real municipal wastewater parallel treatment schemes

327 There are currently no policies or regulations requiring the removal of MPs during wastewater
328 treatment, and the potential of wastewater technologies to eliminate these particles before
329 they reach surface waters has attracted quite attention in recent years (Freeman et al., 2020).
330 Based on our results, the full-scale WWTP of Falconara Marittima receives 3.6 MPs/L, which
331 is comparable to those reported for a bigger WWTP in Northern Italy (2.5 MPs/L) (Magni et
332 al., 2019). This suggests that the size of WWTPs might not directly affect the number of
333 MPs/L in wastewater, as already highlighted in a survey in 12 WWTPs in Germany (Mintenig
334 et al., 2017). Instead, other factors could be more influential such as wastewater sources,
335 typology of sewer systems, waters infiltrations, sampling periods and human activities (Sun et
336 al., 2019).

337 The MPs in the PRE-TREAT IN highlighted the prevalence of fibers and films. The
338 predominant contribution of fibers in WWTPs was already reported (Ngo et al., 2019; Raju et
339 al., 2018; Sun et al., 2019). In addition, the highest percentage of MPFs was polyesters
340 originating from laundering (Hu et al., 2019). Among MPPs, the prevalence of films was also
341 reported in the influent of a Northern Italian WWTP (Magni et al., 2019), while other studies
342 found films at low frequencies regarding fragments (Blair et al., 2019; Gies et al., 2018;
343 Michielssen et al., 2016). Films could be mainly originated from the breakage of plastic bag
344 and packaging products (Nizzetto et al., 2016). The exposure of these materials to sunlight
345 and high temperature may lead to their rapid fragmentation and transport to WWTPs by run-
346 off in case of combined sewer system (Ziajahromi et al., 2017). Although most of the MPPs
347 had dimensions between 0.5 and 0.1 mm, smaller particles ($< 63 \mu\text{m}$ sieving mesh) were also
348 extracted. It depends on the aggregation of particles with other materials in wastewater and on
349 the potential occlusion of the sieve during filtration (Magni et al., 2019). The most prominent
350 polymer at the WWTP influent was PE, in accordance with earlier studies (Carr et al., 2016;
351 Mintenig et al., 2017; Ziajahromi et al., 2017). PE is commonly used in personal care
352 products, water bottles and food packaging films (Ngo et al., 2019; Sun et al., 2019). To date,
353 MPs in Italian WWTPs were only analyzed by (Magni et al., 2019) together with this study.
354 Further investigations in other Italian facilities could be interesting to better understand the
355 phenomenon on geographical basis and to support policymakers for a national-base plastic
356 regulation.

357 The abatement of MPs in I and II EFF compared to PRE-TREAT IN confirmed the
358 importance of physical processes in the removal of MPs (Conley et al., 2019; Gatidou et al.,
359 2019; Ngo et al., 2019). During the primary treatment, sinking of MPs in the tank can occur
360 via gravity separation that can further be favored by the adherence of MPs to TSS (Long et
361 al., 2019). Based on the MP characterization in I EFF, the primary settling caused the

362 sedimentation of MPPs of larger dimensions, confirming that this stage had the biggest impact
363 on the removal of larger MPs (Long et al., 2019). In particular, MPPs between 1 and 0.5 mm
364 were reduced by 82% and those of 1-5 mm were completely removed, that is consistent with
365 Dris and co-authors (Dris et al., 2015) reporting a decrease from 45% to 7% of particles
366 between 1001 and 5000 μm after primary treatment.

367 Conversely, the reduction of MPPs of the lowest size class in the II EFF was probably due to
368 their aggregation with activated sludge flocs and/or interaction with microorganisms and to
369 the subsequent secondary separation (Ngo et al., 2019). At the same time, the degradation of
370 plastic was reported to happen after long-term contact with chlorinated water, that might
371 explain the complete removal of MPPs in the 1-5 mm size class and the recurrence of those
372 between 0.1 and 0.03 mm after the disinfection with sodium hypochlorite. However, the
373 degradation level eventually depends on the contact time, temperature, and chemical
374 concentration (Dris et al., 2015). MPPs in the dimensional range 500-30 μm still remain the
375 prevalent component in the final effluent as already highlighted by other studies (Carr et al.,
376 2016; Conley et al., 2019; Mintenig et al., 2017; Talvitie et al., 2017), emphasizing the
377 importance of monitoring smaller size classes.

378 It was demonstrated that the performance of WWTPs in removing MPs can be enhanced if
379 advanced treatments are employed (Hu et al., 2019). Even in this study, the overall MPs
380 removal in the pilot-scale UASB+AnMBR configuration was greater than that of in the full-
381 scale CAS scheme. The ultrafiltration unit was mostly responsible for the total abatement of
382 MPPs from the PRE-TREAT IN, while no change on MPPs/MPFs ratio was observed
383 compared to the final effluent of CAS scheme. The superiority of MBR technology to remove
384 MPs was also highlighted by other authors (Mahon et al., 2017; Talvitie et al., 2017), such as
385 the MBR process had a slightly better removal efficiency of MPs (99.4%) than the overall
386 CAS process (98.3%) (Lares et al., 2018). However, it should be noted that AnMBR has

387 different fouling and permeation characteristics (Foglia et al., 2020) compared to aerobic
388 MBR. Even if only 5 MPFs were found in the PERMEATE, the detection of fibers was
389 initially surprising considering the small size of membrane pores; however, other studies also
390 reported fibers, as well as, MPPs in the permeate (Lares et al., 2018; Michielssen et al., 2016;
391 Talvitie et al., 2017; Ziajahromi et al., 2017). This could be due to occasional breakthroughs
392 of filters from small leaks or to airborne contamination in open tanks where permeate is
393 collected (Talvitie et al., 2017). While AnMBRs have come forward as the core of water
394 resource recovery facilities (Akyol et al., 2020), one of the advantages of AnMBRs is to
395 guarantee a high effluent (permeate) quality, and our results supported the efficiency of MPs
396 removal in AnMBRs that can affirm the possible reuse of the permeate for fertigation.
397 Regarding the fate of MPs in the sludge line, our results on I SLUDGE and DEWAT SLUD
398 suggested that possible modifications might occur on MPs during sludge treatments, since,
399 DEWAT SLUD had a higher abundance of MPs, a lower contribution of MPPs in the range
400 size 1-5 mm in favor of those between 0.1-0.5 mm and a lower frequency of PE with respect
401 to I SLUDGE. To date, quite a number of studies have characterized MPs in treated sludge
402 (Edo et al., 2020; Lares et al., 2018; Mahon et al., 2017; Murphy et al., 2016; Talvitie et al.,
403 2017; Xu et al., 2020).

404 Noteworthy that higher contribution of MPPs of the lower size class (0.03-0.1 mm) and of
405 natural fibers (78%) were recorded in AnaEXC SLUDGE than in other samples, especially
406 when compared to the composition of MPPs and fibers found in I SLUDGE, where the
407 smaller particles and natural fibers were present with a frequency of only 2% and 22%,
408 respectively. This leads to the necessity for better understanding the behavior of MPs in
409 anaerobic processes since it is still largely unknown (X. Zhang et al., 2020) and it may serve
410 as a promising approach to control MPs contamination in sludge (Mahon et al., 2017) in
411 alternative to the aerobic processes.

412 4.2.Effects of polypropylene microplastics on anaerobic processes

413 PP is commonly used in variety of applications including packaging products and plastic parts

414 for industries. Consequently, PP-MPs are among the most-detected MPs in WWTPs, which is

415 also confirmed with the results of our survey in Falconara Marittima WWTP. During the

416 continuous operation of the pilot-scale UASB reactor with the external spiking of PP-MPs, a

417 possible sign of tolerance to PP-MPs was observed up the concentration of 18 PP-MPs:gTS⁻¹;

418 since the methane production was merely affected. The third exposure of 50 PP-MPs:gTS⁻¹;

419 however, had a drastic decrease on the methanogenic activity as mentioned earlier. The main

420 reason for MPs to effect the performance of AD processes is the desorption behavior of the

421 toxic substances in sludge such as antibiotics, persistent organic pollutants and heavy metals

422 during digestion conditions (X. Zhang et al., 2020). MPs inhibition on the sludge

423 methanogenesis was recently addressed (Zhang and Chen, 2019) and the toxicity of MPs was

424 strongly associated with their leachates, which are mostly plastic additives. For instance, in

425 the case of hydrocarbon polymers such as PP and PS, antioxidants are widely used as

426 additives (AccuStandard, 2018). In fact, Müller and colleagues (Müller et al., 2018)

427 demonstrated that PP-MPs had high sorption capacity for ethyl benzene and xylene. In the

428 study of Suhrhoff and colleagues (Suhrhoff and Scholz-Böttcher, 2016), acetyl tri-n-butyl

429 citrate (ATBC) was reported as the most dominant additive leaching from PE-MPs. Another

430 possible reason for the methanogenesis inhibition due to MPs was proposed by demonstrating

431 that MPs may act as significant vectors for metal pollutants in sewage sludge due to their

432 adsorption property (Li et al., 2019).

433 Comparable results of the inhibitory effects of MPs on anaerobic systems are summarized in

434 **Table 2**. In a recent study, an inhibition up to 95.08% on methane production were found at

435 varying concentrations between 1,000-200,000 PESTs-MPs:kgWAS⁻¹ (Li et al., 2020). In

436 other studies reported for PE-MPs (Wei et al., 2019a) and PVC-MPs (Wei et al., 2019b), the

437 authors observed no significant effect of PE-MPs at lower concentrations whereas higher
 438 levels of PE-MPs decreased methane production by 12.4%-27.5% (Wei et al., 2019a). The
 439 adverse effects of PE-MPs were attributed to the induction of reactive oxygen species rather
 440 than the released ATBC. Similarly, higher levels of PVC-MPs (i.e., 20, 40 and 60
 441 particles·gTS⁻¹) inhibited methane production in the range of 75%-90% compared to the
 442 control (Wei et al., 2019b), which was attributed to bisphenol-A (BPA) leaching. The
 443 potential impacts of polyethylene terephthalate MPs (PET-MPs) were recently addressed by
 444 (Y. T. Zhang et al., 2020) on anaerobic granular sludge in a UASB reactor and 75-300 MPs·L⁻¹
 445 ¹ caused decreases of COD removal and methane yield by 17.4%-30.4% and 17.2%-28.4%,
 446 respectively. In another study, methane production rate was found to be decreased by 40.7%
 447 at the PS nanoparticles (NPs) concentration of 0.2 g·L⁻¹ (Fu et al., 2018). A reduction in the
 448 abundances of MPs was also found during the AD of sewage sludge (Mahon et al., 2017),
 449 while no evidence was reported to prove the breakdown of MPs in AD. Hence, possible
 450 degradation patterns of MPs in anaerobic processes still hold a big potential to explore.

451 **Table 2.** A summary of reported MPs and NPs inhibition on methanogenesis.

Type of MPs/NPs	Concentration	Process	Inhibition on methanogenesis	Inhibition note	Reference
PESTs-MPs	1,000-200,000 MPs·kg WAS ⁻¹	Lab-scale AD of WAS	88.53%-95.08%	-	(Li et al., 2020)
PET-MPs	15-300 MPs·L ⁻¹	Lab-scale UASB treating simulated wastewater	17.2%-28.4%	Suppression of the production of extracellular polymeric substances	(Y. T. Zhang et al., 2020)
PE-MPs	10-200 MPs·gTS ⁻¹	Lab-scale AD of	12.4%-27.5%	Induction of	(Wei et al.,

	1	WAS		reactive oxygen species	2019a)
PVC-MPs	10-60 MP _s gTS ⁻¹	Lab-scale AD of WAS	75.8%-90.6%	BPA leaching	(Wei et al., 2019b)
PS-NPs	0.2 NP _s gTS ⁻¹	Lab-scale AD of sewage sludge	14.4%-40.7%	-	(Fu et al., 2018)
PP-MPs	18-50 MP _s gTS ⁻¹	Pilot-scale UASB treating municipal wastewater	4%-58%	-	This study

452

453 Overall, there are two key differences between this particular study and the previously-
454 reported ones: scale of application and reactor type/configuration. **Table 2** clearly shows that
455 the literature has focused on lab-scale anaerobic digesters so far. However, in addition to
456 presenting the impact of PP-MPs on anaerobic treatment systems for the first time, we also
457 provided further novel information on the efficiency of methane production in the presence of
458 elevated PP-MPs concentrations in a pilot-scale UASB reactor. Depending on the operating
459 conditions and the type and concentration of MPs, a remarkable adverse effect on the
460 performance of anaerobic reactors has been observed. In fact, other than anaerobic processes,
461 the inhibition effect of polyamide 66 MPs on aerobic granular sludge was recently reported
462 (Zhao et al., 2020). At this point, primary treatment units of the existing WWTP should be
463 upgraded considering the enhanced removal of MPs before ending up in secondary treatment.

464 5. Conclusion

465 This paper presented the results of the occurrence, fate, removal and inhibition effects of MPs
466 in real wastewater treatment schemes, leading to the following conclusions:

- 467 • The configuration of UASB+AnMBR provided a noteworthy removal of MPs (94%)
468 compared to CAS (86%) thanks to the ultrafiltration.
- 469 • PE and PP were the most detected MPs in the samples and their concentrations
470 decreased gradually in the effluents, while they remained and accumulated in the
471 sludge in considerable amounts, which pose issues on the final sludge disposal or
472 valorization.
- 473 • The pilot-scale experiments highlighted that the methanogenic activity of the UASB
474 reactor can tolerate up to a concentration of 18 PP-MPs·gTS⁻¹ while a further elevated
475 concentration of 50 PP-MPs·gTS⁻¹ caused a remarkable inhibition (58%).

476 As water resource recovery facilities gain more attention in recent years within the water
477 reuse-energy-carbon nexus, high removal rates of MPs create another positive perspective for
478 the re-use of AnMBR effluents for fertigation. On the other hand, the accumulation of MPs in
479 the sludge line affects the performance of biological processes in the long run. The degree of
480 inhibition may depend on both, concentration of PP-MPs and operating parameters. In order
481 to avoid any possible performance losses in biological treatment systems, in particular
482 anaerobic processes, primary treatment units of WWTPs should be well-designed and
483 upgraded for enhanced MPs removal.

484 **Credit authorship contribution statement**

485 The manuscript was written through contributions of all authors. All authors have given
486 approval to the final version of the manuscript.

487 **Declaration of competing interest**

488 The authors declare no competing financial interest.

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497 **Appendix A. Supplementary data**

498 Supplementary data related to this article can be found in the online version.

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