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

# 2 assessment and relevant inhibition effects on anaerobic processes

- 3
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### 26 ABSTRACT

27 The occurrence, fate and removal of microplastics (MPs) in a wastewater treatment plant (WWTP) in Central Italy were investigated together with their potential adverse effects on 28 anaerobic processes. In the influent of the WWTP, 3.6 MPs<sup>-1</sup> were detected that mostly 29 comprised polyester fibers and particles in the shape of films, ranging 0.1-0.5 mm and made 30 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 particles bigger than 1 mm were not detected in the final effluent and some loss of polymers 33 34 types were observed. In comparison, the pilot-scale upflow granular anaerobic sludge blanket 35 (UASB) + anaerobic membrane bioreactor (AnMBR) configuration achieved 94% MPs removal with the abatement of 87% of fibers and 100% of particles. The results highlighted 36 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 from 5 PP-MPs<sup>-</sup>gTS<sup>-1</sup> to 50 PP-MPs<sup>-</sup>gTS<sup>-1</sup> were spiked in the pilot-scale UASB reactor that 39 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<sup>-1</sup>. To the best of our knowledge, the presented results will be the first to report of PP-MPs inhibition on anaerobic processes. 42 43 **Keywords:** microplastics; municipal wastewater; polypropylene; sewage sludge; UASB 44

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#### 1. Introduction

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

wide size distribution, ranging from micrometers to meters (Establanati and Fahrenfeld,
2016).

Microplastics (MPs) are commonly defined as plastic items with sizes below 5 mm (de Sá et
al., 2018; Lares et al., 2018), which can be categorized into fibers and particles, as well as
primary or secondary, depending on the way they are produced (Sun et al., 2019; Xu et al.,
2020). Current understanding suggests that MPs enter wastewater treatment plants (WWTPs)
daily at varying levels of concentration (Blair et al., 2019) and of different polymer typologies
(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 treatment configurations (Talvitie et al., 2017), in order to limit the discharge of MPs into the 64 65 receiving environment. Some of the greatest concerns include the ingestion and accumulation of MPs that could induce toxicity related to a physical disturbance (Seidensticker et al., 2017), 66 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 sludge (i.e. anaerobic digestion (AD), land application) (Peng et al., 2017; Wei et al., 2019a). 75

In fact, it was recently reported that sewage sludge application in agriculture results in afurther accumulation of MPs in soils (van den Berg et al., 2020).

So far, most of the available information on the MPs in wastewater and/or sewage sludge is 78 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 effects of MPs on AD systems such as polyethylene (PE) (Wei et al., 2019a), polyvinyl 83 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 MPs at different treatment schemes and further evaluate the effect of MPs on anaerobic 89 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. We further investigated the influence of PP-MPs on anaerobic treatment systems since the 95 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

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 m<sup>3</sup>·d<sup>-1</sup>. 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 membrane with 0.03  $\mu$ m of pore-size and 0.5 m<sup>2</sup> of surface area (Foglia et al., 2020). It works 107 108 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:

The monitored WWTPs are in the Central Italy (Falconara Marittima, AN) on the Adriatic

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

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

101

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). Meanwhile, 18 L of effluent after the UASB (UASB EFF), 25 L of effluent after the AnMBR 125

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

131 2.3.Extraction, quantification and identification of microplastics

132 2.3.1. Microplastics extraction from wastewater

The collected wastewater samples were *in loco* passed through a steel sieves battery of 5 mm,
2 mm and 63 µm mesh size (ISO 3310-1:2000): solids retained on 2 mm and 63 µm sieves
were rinsed into glass jars with ultrapure water and subsequently filtered onto cellulose nitrate
filters (Sartorius Stedim Biotech, Ø 47 mm, 8 µm pore size) using a vacuum pump. Filters
were recovered in petri dishes, covered with 15% H<sub>2</sub>O<sub>2</sub> and maintained at 50°C overnight (or
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 H<sub>2</sub>O<sub>2</sub> and maintaining sludge samples in stove at 50°C for two days. This step was followed by the density separation procedure, carried out in 250 mL cylinders, stirring the samples with 142 high-density saturated NaBr salt solution (1.4 g cm<sup>3-1</sup>) for 30 min (Frias et al., 2018) and 143 144 leaving to settle the mixture overnight. The supernatant was then vacuum filtered and filters were treated with 15% H<sub>2</sub>O<sub>2</sub>. To evaluate the potential for loss during the density separation 145 146 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

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

spectrum was recorded performing 32 accumulations, ranging from 600 to 4000 cm<sup>-1</sup> with the 176 resolution at 4 cm<sup>-1</sup>. When the spectrum was not resolved at first acquisition, more than one 177 measurement was conducted per samples. IR spectrum of the cellulose acetate membrane was 178 179 aquired and substracted to that of each sample in order to avoid the overlay of spectra. The output spectra were subsequently subjected to a spectral search against reference libraries of 180 181 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-MPs gTS<sup>-1</sup> and then increased to 18 PP-MPs gTS<sup>-1</sup> and finally to 50 PP-MPs gTS<sup>-1</sup> at the same 204 205 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<sub>4</sub> gVSS<sup>-1</sup>d<sup>-1</sup>.

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 fibers, made of polyesters, were found and 0.3 MPFs/L were detected in the blank related to I 218 219 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**.

**Table 1.** Number of detected MPs and distribution of MPs (including MPPs and MFs) in the

full-scale CAS configuration and in the pilot-scale UASB+AnMBR configuration.

Treatment scheme	Sampling point	Concentration	Load	Distribution
		$(MPs L^{-1})$	(MPs <sup>·</sup> h <sup>1</sup> 10000 <sup>-1</sup> )	(%)
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	Load	Distribution
		$(MPs^{-1}L^{-1})$	$(MPs h^{-1})$	(%)
UASB+AnMBR	UASB EFF	1.72	5.1	47
	PERMEATE	0.2	0.6	6

227 3.1.1. Microplastics in the full-scale CAS configuration

The full-scale CAS configuration removed 86% of MPs from the influent with a remarkableaccumulation 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

concentrations in the sludge were 1.67 MPs gTS<sup>-1</sup> in the I SLUDGE, 5.3 MPs gTS<sup>-1</sup> in the

AerWAS and 4.74 MPs<sup>-</sup>gTS<sup>-1</sup> in the DEWAT SLUD.

The mass balance showed that 12,170,000 MPs<sup>-h<sup>-1</sup></sup> entered the full-scale plant, and after the

primary treatment, only 6,390,000 MPs<sup>-1</sup> remained in the wastewater line. After the

secondary treatment, the MPs reduced to 2,530,000 MPs h<sup>-1</sup>. Finally, after the disinfection, the

236 MPs being discharged into the water body was about 1,730,000 MPs  $h^{-1}$ .

237

The identification of MPs is shown in Fig. 2. MPs in PRE-TREAT IN were represented by 239 240 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 DEWAT SLUD (Fig. 2A). The MPPs extracted from the PRE-TREAT IN were only films 243 244 (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). Most of the MPPs characterized in the PRE-TREAT IN fell in the 0.1-0.5 mm size class 252 253 (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 µFT-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); low frequencies were observed for polystyrene (PS) (4%), for polyamide (PA), 263

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 MPs gTS <sup>-1</sup> .
286	In this configuration, 10.9 MPs·h <sup>-1</sup> entered the system and 5.1 MPs <sup>-</sup> h <sup>-1</sup> were detected in the
287	UASB effluent. After the AnMBR unit, only 0.6 MPs <sup>-h<sup>-1</sup></sup> were discharged with the permeate.

288 The identification of MPs in the pilot-plant is presented in Fig. 3. In UASB EFF, the relative

contribution of MPPs and MPFs was similar to that of PRE-TREAT IN, with a frequency of

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

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,

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

relative contribution of PRE-TREAT IN; however, in comparison to the influent, a higher

contribution (27%) of smaller particles (between 0.03-0.1 mm) were recorded and no particles

bigger than 1 mm were extracted. Conversely, particles between 1 and 5 mm in size were

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

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%

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 <sup>-1</sup> 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 <sup>-1</sup> ). 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 <sup>-1</sup> d <sup>-1</sup> during the first phase at
317	the concentration of 18 PP-MPs gTS <sup>-1</sup> compared to the operating period without PP-MPs
318	(around $40\pm7$ mLCH <sub>4</sub> ·kgVSS <sup>-1</sup> d <sup>-1</sup> ). When the PP-MPs concentration was increased to 50 PP-
319	MPs <sup>·</sup> gTS <sup>-1</sup> in the second phase, a sharp decrease to 17±14.3 mLCH <sub>4</sub> ·kgVSS <sup>-1</sup> d <sup>-1</sup> 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 <sup>-1</sup> , 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).

The MPs in the PRE-TREAT IN highlighted the prevalence of fibers and films. The 337 338 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 ( $< 63 \,\mu m$  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.,

360 via gravity separation that can further be favored by the adherence of MPs to TSS (Long et

2019; Ngo et al., 2019). During the primary treatment, sinking of MPs in the tank can occur

al., 2019). Based on the MP characterization in I EFF, the primary settling caused the

359

sedimentation of MPPs of larger dimensions, confirming that this stage had the biggest impact
on the removal of larger MPs (Long et al., 2019). In particular, MPPs between 1 and 0.5 mm
were reduced by 82% and those of 1-5 mm were completely removed, that is consistent with
Dris and co-authors (Dris et al., 2015) reporting a decrease from 45% to 7% of particles
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 prevalent component in the final effluent as already highlighted by other studies (Carr et al., 375 376 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 CAS process (98.3%) (Lares et al., 2018). However, it should be noted that AnMBR has 386

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 size 1-5 mm in favor of those between 0.1-0.5 mm and a lower frequency of PE with respect 400 401 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 possible sign of tolerance to PP-MPs was observed up the concentration of 18 PP-MPs gTS<sup>-1</sup> 417 418 since the methane production was merely affected. The third exposure of 50 PP-MPs<sup>-</sup>gTS<sup>-1</sup>; 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 varying concentrations between 1,000-200,000 PESTs-MPs<sup>-</sup>kgWAS<sup>-1</sup> (Li et al., 2020). In 435

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 <sup>-1</sup> ) 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
445	<sup>1</sup> 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 <sup>-1</sup> (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.

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

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

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

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 MPs466 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.
- PE and PP were the most detected MPs in the samples and their concentrations
   decreased gradually in the effluents, while they remained and accumulated in the
   sludge in considerable amounts, which pose issues on the final sludge disposal or
   valorization.
- The pilot-scale experiments highlighted that the methanogenic activity of the UASB
  reactor can tolerate up to a concentration of 18 PP-MPs·gTS<sup>-1</sup> while a further elevated
  concentration of 50 PP-MPs·gTS<sup>-1</sup> 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

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.

## 499 **References**

- 500 AccuStandard, 2018. Plastic Additive Standards Guide, Catalog.
- 501 https://doi.org/10.1111/poms.12209
- 502 Akyol, Ç., Foglia, A., Ozbayram, E.G., Frison, N., Katsou, E., Eusebi, A.L., Fatone, F., 2020.
- 503 Validated innovative approaches for energy-efficient resource recovery and re-use from
- 504 municipal wastewater: From anaerobic treatment systems to a biorefinery concept. Crit.
- 505 Rev. Environ. Sci. Technol. 50, 869–902.
- 506 https://doi.org/10.1080/10643389.2019.1634456
- 507 APHA, 2012. Standard methods for the examination of water and wastewater, 22nd edn.
- 508 American Public Health Association, Washington, DC. https://doi.org/ISBN
- **509** 9780875532356
- 510 Bayo, J., Olmos, S., López-Castellanos, J., 2020. Microplastics in an urban wastewater
- 511 treatment plant: The influence of physicochemical parameters and environmental factors.
- 512 Chemosphere 238. https://doi.org/10.1016/j.chemosphere.2019.124593
- 513 Blair, R.M., Waldron, S., Gauchotte-Lindsay, C., 2019. Average daily flow of microplastics
- through a tertiary wastewater treatment plant over a ten-month period. Water Res. 163,
- 515 114909. https://doi.org/10.1016/j.watres.2019.114909
- 516 Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in

- 517 wastewater treatment plants. Water Res. 91, 174–182.
- 518 https://doi.org/10.1016/j.watres.2016.01.002
- 519 Conley, K., Clum, A., Deepe, J., Lane, H., Beckingham, B., 2019. Wastewater treatment
- 520 plants as a source of microplastics to an urban estuary: Removal efficiencies and loading
- 521 per capita over one year. Water Res. X 3, 100030.
- 522 https://doi.org/10.1016/j.wroa.2019.100030
- de Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L., Futter, M.N., 2018. Studies of the effects
- 524 of microplastics on aquatic organisms: What do we know and where should we focus our
- efforts in the future? Sci. Total Environ. 645, 1029–1039.
- 526 https://doi.org/10.1016/j.scitotenv.2018.07.207
- 527 Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic
- 528 contamination in an urban area: A case study in Greater Paris. Environ. Chem. 12, 592–
- 529 599. https://doi.org/10.1071/EN14167
- 530 Edo, C., González-Pleiter, M., Leganés, F., Fernández-Piñas, F., Rosal, R., 2020. Fate of
- 531 microplastics in wastewater treatment plants and their environmental dispersion with
- effluent and sludge. Environ. Pollut. 259. https://doi.org/10.1016/j.envpol.2019.113837
- 533 Estabbanati, S., Fahrenfeld, N.L., 2016. Influence of wastewater treatment plant discharges on
- 534 microplastic concentrations in surface water. Chemosphere 162, 277–284.
- 535 https://doi.org/10.1016/j.chemosphere.2016.07.083
- 536 Foglia, A., Akyol, Ç., Frison, N., Katsou, E., Laura, A., Fatone, F., 2020. Long-term
- 537 operation of a pilot-scale anaerobic membrane bioreactor (AnMBR) treating high salinity
- low loaded municipal wastewater in real environment. Sep. Purif. Technol. 236, 116279.
- 539 https://doi.org/10.1016/j.seppur.2019.116279
- 540 Freeman, S., Booth, A.M., Sabbah, I., Tiller, R., Dierking, J., Klun, K., Rotter, A., Ben-
- 541 David, E., Javidpour, J., Angel, D.L., 2020. Between source and sea: The role of

- 542 wastewater treatment in reducing marine microplastics. J. Environ. Manage. 266,
- 543 110642. https://doi.org/10.1016/j.jenvman.2020.110642
- 544 Frias, J., Pagter, E., Nash, R., O'Connor, I., Carretero, O., Filgueiras, A., Viñas, L., Gago, J.,
- 545 Antunes, J., Bessa, F., Sobral, P., Goruppi, A., Tirelli, V., Pedrotti, M.L., Suaria, G.,
- 546 Aliani, S., Lopes, C., Raimundo, J., Caetano, M., Palazzo, L., de Lucia, Giuseppe
- 547 Andrea Camedda, A., Muniategui, S., Grueiro, G., Fernandez, V., Andrade, J., Dris, R.,
- 548 Laforsch, C., Scholz-Böttcher, B.M., Gerdts, G., 2018. Standardised protocol for
- 549 monitoring microplastics in sediments. JPI-Oceans BASEMAN Proj. 33.
- 550 https://doi.org/10.13140/RG.2.2.36256.89601/1
- 551 Fu, S.F., Ding, J.N., Zhang, Y., Li, Y.F., Zhu, R., Yuan, X.Z., Zou, H., 2018. Exposure to
- polystyrene nanoplastic leads to inhibition of anaerobic digestion system. Sci. Total
  Environ. 625, 64–70. https://doi.org/10.1016/j.scitotenv.2017.12.158
- 554 Gatidou, G., Arvaniti, O.S., Stasinakis, A.S., 2019. Review on the occurrence and fate of
- microplastics in Sewage Treatment Plants. J. Hazard. Mater. 367, 504–512.
- 556 https://doi.org/10.1016/j.jhazmat.2018.12.081
- 557 Gies, E.A., LeNoble, J.L., Noël, M., Etemadifar, A., Bishay, F., Hall, E.R., Ross, P.S., 2018.
- 558 Retention of microplastics in a major secondary wastewater treatment plant in
- 559 Vancouver, Canada. Mar. Pollut. Bull. 133, 553–561.
- 560 https://doi.org/10.1016/j.marpolbul.2018.06.006
- 561 Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of
- 562 chemical additives present in plastics: Migration, release, fate and environmental impact
- 563 during their use, disposal and recycling. J. Hazard. Mater. 344, 179–199.
- 564 https://doi.org/10.1016/j.jhazmat.2017.10.014
- 565 Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E.,
- 566 Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P.,

567	Lusher, A.L., Wagner, M., 2019. Are We Speaking the Same Language?
568	Recommendations for a Definition and Categorization Framework for Plastic Debris.
569	Environ. Sci. Technol. 53, 1039–1047. https://doi.org/10.1021/acs.est.8b05297
570	Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine
571	environment: A review of the methods used for identification and quantification.
572	Environ. Sci. Technol. 46, 3060–3075. https://doi.org/10.1021/es2031505
573	Hu, Y., Gong, M., Wang, J., Bassi, A., 2019. Current research trends on microplastic
574	pollution from wastewater systems: a critical review. Rev. Environ. Sci. Biotechnol. 18,
575	207-230. https://doi.org/10.1007/s11157-019-09498-w
576	Koelmans, A.A., Mohamed Nor, N.H., Hermsen, E., Kooi, M., Mintenig, S.M., De France, J.,
577	2019. Microplastics in freshwaters and drinking water: Critical review and assessment of
578	data quality. Water Res. 155, 410-422. https://doi.org/10.1016/j.watres.2019.02.054
579	Lares, M., Ncibi, M.C., Sillanpää, Markus, Sillanpää, Mika, 2018. Occurrence, identification
580	and removal of microplastic particles and fibers in conventional activated sludge process
581	and advanced MBR technology. Water Res. 133, 236–246.
582	https://doi.org/10.1016/j.watres.2018.01.049
583	Li, L., Geng, S., Li, Z., Song, K., 2020. Effect of microplastic on anaerobic digestion of
584	wasted activated sludge. Chemosphere 247, 125874.
585	https://doi.org/10.1016/j.chemosphere.2020.125874
586	Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., Zeng, E.Y., 2018. Microplastics in
587	sewage sludge from the wastewater treatment plants in China. Water Res. 142, 75-85.
588	https://doi.org/10.1016/j.watres.2018.05.034
589	Li, X., Mei, Q., Chen, L., Zhang, H., Dong, B., Dai, X., He, C., Zhou, J., 2019. Enhancement
590	in adsorption potential of microplastics in sewage sludge for metal pollutants after the
591	wastewater treatment process. Water Res. 157, 228–237.

- 592 https://doi.org/10.1016/j.watres.2019.03.069
- Liu, J., Yang, Y., Ding, J., Zhu, B., Gao, W., 2019. Microfibers: a preliminary discussion on
  their definition and sources. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-
- 595 019-06265-w
- 596 Long, Z., Pan, Z., Wang, W., Ren, J., Yu, X., Lin, L., Lin, H., Chen, H., Jin, X., 2019.
- 597 Microplastic abundance, characteristics, and removal in wastewater treatment plants in a
- 598 coastal city of China. Water Res. 155, 255–265.
- 599 https://doi.org/10.1016/j.watres.2019.02.028
- 600 Lusher, A.L., Hurley, R.R., Vogelsang, C., Nizzetto, L., Olsen, M., 2017. Mapping
- 601 microplastics in sludge. https://doi.org/10.13140/RG.2.2.25277.56804
- 602 Magni, S., Binelli, A., Pittura, L., Avio, C.G., Della Torre, C., Parenti, C.C., Gorbi, S.,
- Regoli, F., 2019. The fate of microplastics in an Italian Wastewater Treatment Plant. Sci.
- 604
   Total Environ. 652, 602–610. https://doi.org/10.1016/j.scitotenv.2018.10.269
- Mahon, A.M., O'Connell, B., Healy, M.G., O'Connor, I., Officer, R., Nash, R., Morrison, L.,
- 606 2017. Microplastics in sewage sludge: Effects of treatment. Environ. Sci. Technol. 51,
- 607 810–818. https://doi.org/10.1021/acs.est.6b04048
- Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P.,
- 609 Papazissimos, D., Rogers, D.L., 2016. Microplastic pollution is widely detected in US
- 610 municipal wastewater treatment plant effluent. Environ. Pollut. 218, 1045–1054.
- 611 https://doi.org/10.1016/j.envpol.2016.08.056
- 612 Michielssen, M.R., Michielssen, E.R., Ni, J., Duhaime, M.B., 2016. Fate of microplastics and
- 613 other small anthropogenic litter (SAL) in wastewater treatment plants depends on unit
- 614 processes employed. Environ. Sci. Water Res. Technol. 2, 1064–1073.
- 615 https://doi.org/10.1039/c6ew00207b
- 616 Mintenig, S.M., Int-Veen, I., Löder, M.G.J., Primpke, S., Gerdts, G., 2017. Identification of

- 617 microplastic in effluents of waste water treatment plants using focal plane array-based
- 618 micro-Fourier-transform infrared imaging. Water Res. 108, 365–372.
- 619 https://doi.org/10.1016/j.watres.2016.11.015
- 620 Möller, J.N., Löder, M.G.J., Laforsch, C., 2020. Finding Microplastics in Soils: A Review of
- 621 Analytical Methods. Environ. Sci. Technol. 54, 2978–2090.
- 622 https://doi.org/10.1021/acs.est.9b04618
- 623 Müller, A., Becker, R., Dorgerloh, U., Simon, F.G., Braun, U., 2018. The effect of polymer
- aging on the uptake of fuel aromatics and ethers by microplastics. Environ. Pollut. 240,
- 625 639–646. https://doi.org/10.1016/j.envpol.2018.04.127
- 626 Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater Treatment Works
- 627 (WwTW) as a Source of Microplastics in the Aquatic Environment. Environ. Sci.
- 628 Technol. 50, 5800–5808. https://doi.org/10.1021/acs.est.5b05416
- 629 Ngo, P.L., Pramanik, B.K., Shah, K., Roychand, R., 2019. Pathway, classification and
- removal efficiency of microplastics in wastewater treatment plants. Environ. Pollut. 255,
- 631 113326. https://doi.org/10.1016/j.envpol.2019.113326
- 632 Nizzetto, L., Futter, M., Langaas, S., 2016. Are Agricultural Soils Dumps for Microplastics of
- 633 Urban Origin? Environ. Sci. Technol. 50, 10777–10779.
- 634 https://doi.org/10.1021/acs.est.6b04140
- 635 Peng, J., Wang, J., Cai, L., 2017. Current understanding of microplastics in the environment:
- 636 Occurrence, fate, risks, and what we should do. Integr. Environ. Assess. Manag. 13,
- 637 476–482. https://doi.org/10.1002/ieam.1912
- 638 Primpke, S., Wirth, M., Lorenz, C., Gerdts, G., 2018. Reference database design for the
- automated analysis of microplastic samples based on Fourier transform infrared (FTIR)
- 640 spectroscopy. Anal. Bioanal. Chem. 410, 5131–5141. https://doi.org/10.1007/s00216-
- 641 018-1156-x

- 642 Raju, S., Carbery, M., Kuttykattil, A., Senathirajah, K., Subashchandrabose, S.R., Evans, G.,
- 643 Thavamani, P., 2018. Transport and fate of microplastics in wastewater treatment plants:
- 644 implications to environmental health. Rev. Environ. Sci. Biotechnol. 17, 637–653.
- 645 https://doi.org/10.1007/s11157-018-9480-3
- 646 Seidensticker, S., Zarfl, C., Cirpka, O.A., Fellenberg, G., Grathwohl, P., 2017. Shift in Mass
- 647 Transfer of Wastewater Contaminants from Microplastics in the Presence of Dissolved
- 648 Substances. Environ. Sci. Technol. 51, 12254–12263.
- 649 https://doi.org/10.1021/acs.est.7b02664
- 650 Shah, M.P., 2018. Modified Ludzack Ettinger Process An Innovation for Removal of
- 651 Biological Nitrogen. Austin J Biotechnol Bioeng. 5(2), 1094.
- 652 Suhrhoff, T.J., Scholz-Böttcher, B.M., 2016. Qualitative impact of salinity, UV radiation and
- turbulence on leaching of organic plastic additives from four common plastics A lab
- experiment. Mar. Pollut. Bull. 102, 84–94.
- 655 https://doi.org/10.1016/j.marpolbul.2015.11.054
- 656 Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C.M., Ni, B.J., 2019. Microplastics in
- wastewater treatment plants: Detection, occurrence and removal. Water Res. 152, 21–37.
  https://doi.org/10.1016/j.watres.2018.12.050
- 659 Talvitie, J., Mikola, A., Koistinen, A., Setälä, O., 2017. Solutions to microplastic pollution –
- 660 Removal of microplastics from wastewater effluent with advanced wastewater treatment
- 661 technologies. Water Res. 123, 401–407. https://doi.org/10.1016/j.watres.2017.07.005
- van den Berg, P., Huerta-Lwanga, E., Corradini, F., Geissen, V., 2020. Sewage sludge
- application as a vehicle for microplastics in eastern Spanish agricultural soils. Environ.
- 664 Pollut. 261, 114198. https://doi.org/10.1016/j.envpol.2020.114198
- 665 Wei, W., Huang, Q.-S., Sun, J., Dai, X., Ni, B.-J., 2019a. Revealing the Mechanisms of
- 666 Polyethylene Microplastics Affecting Anaerobic Digestion of Waste Activated Sludge.

- 667 Environ. Sci. Technol. 53, 9604–9613. https://doi.org/10.1021/acs.est.9b02971
- 668 Wei, W., Huang, Q.S., Sun, J., Wang, J.Y., Wu, S.L., Ni, B.J., 2019b. Polyvinyl Chloride
- 669 Microplastics Affect Methane Production from the Anaerobic Digestion of Waste
- 670 Activated Sludge through Leaching Toxic Bisphenol-A. Environ. Sci. Technol. 53,
- 671 2509–2517. https://doi.org/10.1021/acs.est.8b07069
- 672 Xu, Q., Gao, Y., Xu, L., Shi, W., Wang, F., LeBlanc, G.A., Cui, S., An, L., Lei, K., 2020.
- 673 Investigation of the microplastics profile in sludge from China's largest Water
- 674 reclamation plant using a feasible isolation device. J. Hazard. Mater. 388.
- 675 https://doi.org/10.1016/j.jhazmat.2020.122067
- 676 Yurtsever, M., 2019. Tiny, shiny, and colorful microplastics: Are regular glitters a significant
- 677 source of microplastics? Mar. Pollut. Bull. 146, 678–682.
- 678 https://doi.org/10.1016/j.marpolbul.2019.07.009
- 679 Zhang, X., Chen, J., Li, J., 2020. The removal of microplastics in the wastewater treatment
- 680 process and their potential impact on anaerobic digestion due to pollutants association.
- 681 Chemosphere 251. https://doi.org/10.1016/j.chemosphere.2020.126360
- Kang, Y.T., Wei, W., Huang, Q.S., Wang, C., Wang, Y., Ni, B.J., 2020. Insights into the
- 683 microbial response of anaerobic granular sludge during long-term exposure to
- 684 polyethylene terephthalate microplastics. Water Res. 179, 115898.
- 685 https://doi.org/10.1016/j.watres.2020.115898
- 586 Zhang, Z., Chen, Y., 2019. Effects of microplastics on wastewater and sewage sludge
- treatment and their removal: A review. Chem. Eng. J. 122955.
- 688 https://doi.org/10.1016/j.cej.2019.122955
- 689 Zhao, L., Su, C., Liu, W., Qin, R., Tang, L., Deng, X., Wu, S., Chen, M., 2020. Exposure to
- 690 polyamide 66 microplastic leads to effects performance and T microbial community
- 691 structure of aerobic granular sludge. Ecotoxicol. Environ. Saf. 190, 110070.

- 692 Ziajahromi, S., Neale, P.A., Rintoul, L., Leusch, F.D.L., 2017. Wastewater treatment plants as
- a pathway for microplastics: Development of a new approach to sample wastewater-
- based microplastics. Water Res. 112, 93–99.
- 695 https://doi.org/10.1016/j.watres.2017.01.042
- 696