Microplastics in real wastewater treatment schemes: Comparative assessment and relevant inhibition effects on anaerobic processes

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ABSTRACT

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 anaerobic processes. In the influent of the WWTP, 3.6 $MPs \cdot L^{-1}$ were detected that mostly comprised polyester fibers and particles in the shape of films, ranging 0.1-0.5 mm and made of polyethylene and polypropylene (PP). The full-scale conventional activated sludge scheme 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 types were observed. In comparison, the pilot-scale upflow granular anaerobic sludge blanket (UASB) + anaerobic membrane bioreactor (AnMBR) configuration achieved 94% MPs removal with the abatement of 87% of fibers and 100% of particles. The results highlighted an accumulation phenomenon of MPs in the sludge and suggested the need to further investigate the effects of MPs on anaerobic processes. Accordingly, PP-MPs at concentrations from 5 $PP\text{-}MPs \cdot gTS^{-1}$ to 50 $PP\text{-}MPs \cdot gTS^{-1}$ were spiked in the pilot-scale UASB reactor that was fed with real municipal wastewater, where up to 58% decrease in methanogenic activity was observed at the exposure of 50 $PP\text{-}MPs \cdot gTS^{-1}$. To the best of our knowledge, the presented results will be the first to report of PP-MPs inhibition on anaerobic processes.

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

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 (Estahbanati 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).

Although conventional WWTPs are reported to be effective on the removal of MPs up to 99.9% (Sun et al., 2019) they may actually be a significant source of MPs given the large volumes of discharged effluents (Bayo et al., 2020): it was estimated that a WWTP (650,000 population equivalent) releases 65 million MPs into the receiving water every day (Murphy et 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 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), as well as, to the leaching of plastic-associated chemicals and microbial pathogens in biofilms that can grow on MPs (Koelmans et al., 2019). Hence, MPs have the potential to act as vectors for other contaminants, especially in WWTPs (Raju et al., 2018), such as endocrine-disrupting compounds, pharmaceuticals, heavy metals, persistent organic pollutants and pathogens (Carr et al., 2016; Hidalgo-Ruz et al., 2012). Besides, the processes themselves can be negatively affected by the presence of MPs resulting in a reduction of treatment performance (Zhang and Chen, 2019). Moreover, most of MPs retain in sewage sludge (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).

In fact, it was recently reported that sewage sludge application in agriculture results in a further 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 limited to monitoring studies in full-scale WWTPs and further data is therefore urgently required from lab-/pilot-scale experiments to better understand the behavior of MPs in WWTPs (Gatidou et al., 2019; Magni et al., 2019). In fact, the study on MPs effect on AD is 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 chloride (PVC) (Wei et al., 2019b), polyester (PESTs) (Li et al., 2020) and polyethylene terephthalate (PET) (Y.T. Zhang et al., 2020). It is evident that the inhibition of methanogenesis depends on the type and concentration of MPs; and furthermore, to the best of our knowledge, the role of polypropylene (PP) MPs in AD have not been reported. Based 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 treatment systems. MPs were characterized in wastewater and sludge lines of a full-scale municipal WWTP, in parallel, the fate and transport pathways of MPs in a pilot-scale anaerobic configuration (upflow granular anaerobic sludge blanket (UASB) + anaerobic membrane bioreactor (AnMBR)) was also monitored. Given the site-specific characterization 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 role of PE-MPs in methanogenesis inhibition has already been demonstrated (Wei et al., 2019a). Accordingly, experiments through continuous operation were carried out and effects of PP-MPs on the performance of the pilot-scale UASB reactor were explored.

2. Materials and methods

2.1. Full- and pilot-scale field study

The monitored WWTPs are in the Central Italy (Falconara Marittima, AN) on the Adriatic coastline. The full-scale plant receives municipal wastewater with a design treatment capacity of 80,000 population equivalent (PE) at an average dry weather flow rate of 18,000 $m^3 \cdot d^{-1}$. After preliminary treatments and primary settling, the pretreated influent is treated in the CAS system that applies Modified Ludzack-Ettinger scheme (Shah, 2018).

The pilot-scale plant consists of a UASB reactor coupled with an ultrafiltration hollow fiber membrane with 0.03 µm of pore-size and 0.5 m^2 of surface area (Foglia et al., 2020). It works in submerged-side-stream AnMBR configuration with low hydraulic retention time (6 h) and temperature at 30°C. The UASB is a cylindrical Plexiglas reactor (16 L) with an internal diameter of 15 cm and a total height of 136 cm. The reactor is divided into two compartments: the first is the real reaction chamber at the bottom (85 cm, 12.4 L), while the second is a tri-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 UASB reactor that collects the pre-treated influent wastewater.

2.2.Wastewater and sludge sampling

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

(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 $kgTS \cdot d^{-1}$.

2.3. Extraction, quantification and identification of microplastics

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_2O_2 and maintained at 50°C overnight (or more if necessary) to remove organics.

2.3.2. Microplastics extraction from sludge

After collection, a first organic matter digestion was performed in glass beakers adding 15% H_2O_2 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 high-density saturated NaBr salt solution (1.4 g cm^{3-1}) for 30 min (Frias et al., 2018) and leaving to settle the mixture overnight. The supernatant was then vacuum filtered and filters were treated with 15% H_2O_2. To evaluate the potential for loss during the density separation procedure for sludge fraction and to calculate the extraction yield of microplastics, a total of 12 particles, 2 for each representative polymer (polyethylene, polypropylene, polystyrene, polyethylene terephthalate, nylon, polyisoprene rubber) in the size range of 0.5-1.5 mm, were spiked into samples and blanks, starting from the first organic matter digestion step. The 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.

2.3.3. Microplastics quantification and characterization

Wastewater and sludge filters resulted from the extraction procedure were observed using a stereomicroscope (Optika SZM-D equipped with OPTIKAMB5 digital camera), with maximum magnification of 45X. All items resembling plastic and fibers were manually collected using a tweezer, transferred onto a clean cellulose acetate membrane (Sartorius Stedim Biotech, Ø 47 mm, 0.45 μm pore size) located on a microscope slide (subsequently used as support for the μFT-IR analyses), quantified and categorized based on shape, size and polymer type. In terms of their shape, MPs were categorized in fiber-shaped (MPFs) according to the definition proposed elsewhere (Liu et al., 2019) and particle-shaped (MPPs), which included five main typologies: lines, fragments, films, spheres, glitters identified according to characteristics given in (Hartmann et al., 2019; Lusher et al., 2017; Magni et al., 2019; Yurtsever, 2019). MPPs were measured on the basis of the largest dimension (Hartmann et al., 2019), using an image analysis software (Optika Vision Lite 2.1) and 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 mm. To confirm the synthetic nature and gain the abundance of MPs, all the collected particles and fibers were characterized by μFTIR spectroscopy in attenuated total reflectance mode, using a Spotlight 200i FT-IR microscope system (Perkin Elmer) equipped with 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^{-1} with the resolution at 4 cm^{-1}. When the spectrum was not resolved at first acquisition, more than one measurement was conducted per samples. IR spectrum of the cellulose acetate membrane was 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 polymer spectra represented by Perkin Elmer database (ATRPolymer, polyATR, FIBERS3, plast1, RP, POLIMERI, PIGMENTI, resin and PERKIN1 libraries were selected), by the database compiled within the framework of the JPI-OCEANS project BASEMAN (Primpke et al., 2018) and by personal created ones. For accurate identification, the match factor threshold was calculated as 0.70 and a lower level (0.60-0.70) was accepted after careful examination of peaks characteristics. Based on the recommendations by (Hartmann et al., 2019), synthetic polymers (petroleum-based, biobased and hybrid polymers), modified natural ones (e.g. rayon), copolymers and composites were considered as plastic. Details of the MPs shapes, reference polymer libraries, matching factor and examples of the output IR spectra are given in the **e-Supplementary file**.

2.3.4. Quality control

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

2.4. Pilot-scale UASB reactor operation with spiked polypropylene microplastics

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

3. Results

3.1. Occurrence, fate, removal and identification of microplastics in full and pilot plants

A total of 1342 items were potentially recognized as MPs by the visual sorting of filters of samples collected both from full and pilot-scale plants, 1024 of them were confirmed as synthetic nature, comprising both MPPs and MPFs. The number of extracted MPs was further 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 EFF; 0.2 MPFs/L for blank of the PRE-TREAT IN, FINAL EFF, AerWAS, DEWAT SLUD and UASB EFF; 0.1 MPFs/L for blank of II EFF and I SLUDGE; none MPFs in blank samples for PERMEATE and AnaEXC SLUD. A mass balance of MPs was additionally performed in the full-scale and pilot-scale with respect to the number of particles and fibers 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 ($MPs \cdot L^{-1}$)	Load ($MPs \cdot h^{-1} 10000^{-1}$)	Distribution (%)
CAS	UWW IN	3.64	1217	100
	PRE-TREATED IN	3.64	1217	100
	I EFF	1.9	639	53
	II EFF	0.76	253	21
	FINAL EFF	0.52	173	14
Treatment scheme	Sampling point	Concentration ($MPs \cdot L^{-1}$)	Load ($MPs \cdot h^{-1}$)	Distribution (%)
UASB+AnMBR	UASB EFF	1.72	5.1	47
	PERMEATE	0.2	0.6	6

3.1.1. Microplastics in the full-scale CAS configuration

The full-scale CAS configuration removed 86% of MPs from the influent with a remarkable accumulation in the sludge. 47.3% of MPs were removed in the primary settling, 32% in the biological treatment and secondary settling and 6.5% after the disinfection. In fact, MPs concentrations in the sludge were 1.67 $MPs \cdot gTS^{-1}$ in the I SLUDGE, 5.3 $MPs \cdot gTS^{-1}$ in the AerWAS and 4.74 $MPs \cdot gTS^{-1}$ in the DEWAT SLUD.

The mass balance showed that 12,170,000 $MPs \cdot h^{-1}$ entered the full-scale plant, and after the primary treatment, only 6,390,000 $MPs \cdot h^{-1}$ remained in the wastewater line. After the secondary treatment, the MPs reduced to 2,530,000 $MPs \cdot h^{-1}$. Finally, after the disinfection, the MPs being discharged into the water body was about 1,730,000 $MPs \cdot h^{-1}$.

The identification of MPs is shown in **Fig. 2**. MPs in PRE-TREAT IN were represented by fibers and particles at the same percentage. The relative contribution of MPFs and MPPs changed in I EFF in favor of MPFs, representing 65% of MPFs, while in the sludge more 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 (55%), fragments (36%) and lines (9%). Films and fragments remained as the most frequently detected types in II EFF (33% and 44% respectively) and the only typologies of particles extracted from I EFF (53% and 47%) and FINAL EFF, in the latter, films prevailing on fragments were 67% and 33%, respectively. Fragments represented the predominant component in the sludge, where also spheres and glitters were found unlike wastewater samples; however, they contributed to a minimal percentage on the total MPPs abundance: spheres were extracted from I SLUDGE (0.7%) and glitters from AerWAS (4.4%) and 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 (57%), 17% were in the dimensional range of 0.5-1 mm, 15% in that of 1-5 mm and 11% belonged to the smallest size class of 0.03-0.1 mm. The size class of 0.1-0.5 mm were the most frequently found also in the effluents, especially after primary settling (70% of MPPs); no particles between 5 and 1 mm were detected in I and FINAL EFF and no ones in the range of 0.03-0.1 mm in the II EFF. In all sludge samples MPPs of all sizes were observed; however, most of particles were between 5 and 0.5 mm in I SLUDGE, while in AerWAS and DEWAT SLUD they were mainly between 1 and 0.1 mm (**Fig. 2C**).

The μFT-IR characterization of MPPs in the PRE-TREAT IN identified 12 different polymers: PE was the predominant component (43%) followed by PP (13%), ethylene/propylene (EPM) (11%), polyesters (PESTs) and polyurethane (PUR) (9% each); low frequencies were observed for polystyrene (PS) (4%), for polyamide (PA), polyacrilamide (PAM), polyvinyl-acetate (PVAC), ethylene-vinyl- acetate (EVA), polyvinyl chloride/polyvinyl alcohol/polyethylene (PVC/PVAC/PE) and for polyesters based copolymer (2% each). A stepwise reduction in the number of polymer types was observed after subsequent treatments until detection of only PE, polyurethane (PUR), PESTs and EPM in the FINAL EFF. PE remained as the predominant polymer in I EFF (41%), while its relative contribution decreased in II EFF (33%) and further in FINAL EFF (17%), EPM and PUR prevailed (33% each). A major number of polymers types were observed in the sludge compared to the wastewater, especially in AerWAS and DEWAT SLUD; however, the main contribution was given by PE, particularly in I SLUDGE (52%), and by PP (**Fig. 2D** and **Table S4**).

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

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

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

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

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 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%) were found in UASB EFF and a similar composition was observed in AnaEXC SLUD, together with small contribution of glitters (2%) **(Fig. 3B**).

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 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 PRE-TREAT IN to the UASB EFF was observed; however, PE maintained as the highest frequency of occurrence (53%). More polymers typologies were detected in AnaEXC SLUD compared to PRE-TREAT IN and UASB EFF, without a clear prevalence of a polymer: in particular, PE, that in wastewater fraction dominated, was present with an only 18% frequency in the sludge (**Fig. 3D** and **Table S4**). Concerning results on fibers: the synthetic ones (MPFs) represented the 82% of the total extracted from both UASB EFF and 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%).

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

The initial exposure of 5 PP-MPs$\cdot$gTS^{-1} did not influence the performance of the UASB in terms of methane production. At this point, the following two phases were further evaluated for each PP-MPs concentration (i.e. 18 and 50 PP-MPs$\cdot$gTS^{-1}). Specific methane production rate in the UASB reactor corresponding to spiked PP-MPs is given in **Fig. 4**. Methane production slightly decreased by 4% to 38.5±7.7 mLCH$_4\cdot$kgVSS^{-1}d^{-1} during the first phase at the concentration of 18 PP-MPs$\cdot$gTS^{-1} compared to the operating period without PP-MPs (around 40±7 mLCH$_4\cdot$kgVSS^{-1}d^{-1}). When the PP-MPs concentration was increased to 50 PP-MPs$\cdot$gTS^{-1} in the second phase, a sharp decrease to 17±14.3 mLCH$_4\cdot$kgVSS^{-1}d^{-1} was observed. This decrease remarked approximately 58% of inhibition on the methanogenic activity. Meanwhile, the average methane content of the biogas in each phase was slightly affected by the exposure of PP-MPs (30% in no PP-MPs period, 29% and 27% in the spiking of 18 and 50 PP-MPs$\cdot$gTS^{-1}, respectively). Furthermore, no change in the structure of PP-MPs during the UASB operation was observed (data not shown).

4. Discussion

4.1. Microplastics in real municipal wastewater parallel treatment schemes

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

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

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

Conversely, the reduction of MPPs of the lowest size class in the II EFF was probably due to their aggregation with activated sludge flocs and/or interaction with microorganisms and to the subsequent secondary separation (Ngo et al., 2019). At the same time, the degradation of plastic was reported to happen after long-term contact with chlorinated water, that might explain the complete removal of MPPs in the 1-5 mm size class and the recurrence of those between 0.1 and 0.03 mm after the disinfection with sodium hypochlorite. However, the degradation level eventually depends on the contact time, temperature, and chemical 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., 2016; Conley et al., 2019; Mintenig et al., 2017; Talvitie et al., 2017), emphasizing the importance of monitoring smaller size classes.

It was demonstrated that the performance of WWTPs in removing MPs can be enhanced if advanced treatments are employed (Hu et al., 2019). Even in this study, the overall MPs removal in the pilot-scale UASB+AnMBR configuration was greater than that of in the full-scale CAS scheme. The ultrafiltration unit was mostly responsible for the total abatement of MPPs from the PRE-TREAT IN, while no change on MPPs/MPFs ratio was observed compared to the final effluent of CAS scheme. The superiority of MBR technology to remove MPs was also highlighted by other authors (Mahon et al., 2017; Talvitie et al., 2017), such as 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 different fouling and permeation characteristics (Foglia et al., 2020) compared to aerobic MBR. Even if only 5 MPFs were found in the PERMEATE, the detection of fibers was initially surprising considering the small size of membrane pores; however, other studies also reported fibers, as well as, MPPs in the permeate (Lares et al., 2018; Michielssen et al., 2016; Talvitie et al., 2017; Ziajahromi et al., 2017). This could be due to occasional breakthroughs of filters from small leaks or to airborne contamination in open tanks where permeate is collected (Talvitie et al., 2017). While AnMBRs have come forward as the core of water resource recovery facilities (Akyol et al., 2020), one of the advantages of AnMBRs is to guarantee a high effluent (permeate) quality, and our results supported the efficiency of MPs removal in AnMBRs that can affirm the possible reuse of the permeate for fertigation.

Regarding the fate of MPs in the sludge line, our results on I SLUDGE and DEWAT SLUD suggested that possible modifications might occur on MPs during sludge treatments, since, 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 to I SLUDGE. To date, quite a number of studies have characterized MPs in treated sludge (Edo et al., 2020; Lares et al., 2018; Mahon et al., 2017; Murphy et al., 2016; Talvitie et al., 2017; Xu et al., 2020).

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

4.2. Effects of polypropylene microplastics on anaerobic processes

PP is commonly used in variety of applications including packaging products and plastic parts for industries. Consequently, PP-MPs are among the most-detected MPs in WWTPs, which is also confirmed with the results of our survey in Falconara Marittima WWTP. During the 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$\cdot$gTS^{-1} since the methane production was merely affected. The third exposure of 50 PP-MPs$\cdot$gTS^{-1}; however, had a drastic decrease on the methanogenic activity as mentioned earlier. The main reason for MPs to effect the performance of AD processes is the desorption behavior of the toxic substances in sludge such as antibiotics, persistent organic pollutants and heavy metals during digestion conditions (X. Zhang et al., 2020). MPs inhibition on the sludge methanogenesis was recently addressed (Zhang and Chen, 2019) and the toxicity of MPs was strongly associated with their leachates, which are mostly plastic additives. For instance, in the case of hydrocarbon polymers such as PP and PS, antioxidants are widely used as additives (AccuStandard, 2018). In fact, Müller and colleagues (Müller et al., 2018) demonstrated that PP-MPs had high sorption capacity for ethyl benzene and xylene. In the study of Suhrhoff and colleagues (Suhrhoff and Scholz-Böttcher, 2016), acetyl tri-n-butyl citrate (ATBC) was reported as the most dominant additive leaching from PE-MPs. Another possible reason for the methanogenesis inhibition due to MPs was proposed by demonstrating that MPs may act as significant vectors for metal pollutants in sewage sludge due to their adsorption property (Li et al., 2019).

Comparable results of the inhibitory effects of MPs on anaerobic systems are summarized in **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$\cdot$kgWAS^{-1} (Li et al., 2020). In other studies reported for PE-MPs (Wei et al., 2019a) and PVC-MPs (Wei et al., 2019b), the authors observed no significant effect of PE-MPs at lower concentrations whereas higher levels of PE-MPs decreased methane production by 12.4%-27.5% (Wei et al., 2019a). The adverse effects of PE-MPs were attributed to the induction of reactive oxygen species rather than the released ATBC. Similarly, higher levels of PVC-MPs (i.e., 20, 40 and 60 particles$\cdot$gTS^{-1}) inhibited methane production in the range of 75%-90% compared to the control (Wei et al., 2019b), which was attributed to bisphenol-A (BPA) leaching. The potential impacts of polyethylene terephthalate MPs (PET-MPs) were recently addressed by (Y. T. Zhang et al., 2020) on anaerobic granular sludge in a UASB reactor and 75-300 MPs$\cdot$L^{-1} caused decreases of COD removal and methane yield by 17.4%-30.4% and 17.2%-28.4%, respectively. In another study, methane production rate was found to be decreased by 40.7% at the PS nanoparticles (NPs) concentration of 0.2 g$\cdot$L^{-1} (Fu et al., 2018). A reduction in the abundances of MPs was also found during the AD of sewage sludge (Mahon et al., 2017), while no evidence was reported to prove the breakdown of MPs in AD. Hence, possible 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 MPs/NPs	Concentration	Process	Inhibition on methanogenesis	Inhibition note	Reference
PESTs-MPs	1,000-200,000 MPs$\cdot$kg WAS^{-1}	Lab-scale AD of WAS	88.53%-95.08%	-	(Li et al., 2020)
PET-MPs	15-300 MPs$\cdot$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$\cdot$gTS^{-}	Lab-scale AD of	12.4%-27.5%	Induction of	(Wei et al.,

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

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

5. Conclusion

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

- The configuration of UASB+AnMBR provided a noteworthy removal of MPs (94%) 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$\cdot$gTS^{-1} while a further elevated concentration of 50 PP-MPs$\cdot$gTS^{-1} caused a remarkable inhibition (58%).

As water resource recovery facilities gain more attention in recent years within the water reuse-energy-carbon nexus, high removal rates of MPs create another positive perspective for 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 inhibition may depend on both, concentration of PP-MPs and operating parameters. In order to avoid any possible performance losses in biological treatment systems, in particular anaerobic processes, primary treatment units of WWTPs should be well-designed and upgraded for enhanced MPs removal.

Credit authorship contribution statement

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

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

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

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