

# A review of methods for the isolation of microplastics in municipal wastewater treatment

**Paulina Ormaniec**

paulina.ormaniec@pk.edu.pl |  <https://orcid.org/0000-0003-3434-4517>

Department of Environmental Technologies,  
Faculty of Environmental and Power Engineering,  
Cracow University of Technology

**Jerzy Mikosz**

jerzy.mikosz@pk.edu.pl |  <https://orcid.org/0000-0002-1464-2675>

Department of Environmental Technologies,  
Faculty of Environmental and Power Engineering,  
Cracow University of Technology

**Scientific Editor:** Michał Zielina,  
Cracow University of Technology

**Technical Editor:** Aleksandra Urzędowska,  
Cracow University of Technology Press

**Language Verification:** Timothy Churcher,  
Merlin Language Services

**Typesetting:** Anna Pawlik, Cracow  
University of Technology Press

**Received:** February 10, 2022

**Accepted:** July 8, 2022

**Copyright:** © 2022 Ormaniec, Mikosz.

This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Competing interests:** The authors have declared that no competing interests exist.

**Citation:** Ormaniec, P., Mikosz, J. (2022). A review of methods for the isolation of microplastics in municipal wastewater treatment. *Technical Transactions*, e2022010. <https://doi.org/10.37705/TechTrans/e2022010>

**Abstract**

Wastewater treatment plants are considered to be one of the largest sources of microplastics in the natural environment. The problem of microplastics has been widely studied in many environments. It remains a subject of the growing interest for researchers. By definition, microplastic is plastic that does not exceed 5 mm in size. There are three stages in the study of microplastics in wastewater: sampling, treatment, and the identification of microplastics. This paper aims to review the strategies for sampling microplastics in wastewater treatment plants and their laboratory treatment to isolate microplastics. The collection of samples from the wastewater treatment plant is based on two mechanisms, namely a continuous filtering and pumping system and instantaneous sampling (steel bucket, glass jar, telescopic sampling). The removal of organic and inorganic matter is carried out with the use of physical and chemical analyses. The subject of this article is the compilation of the generally available research methods on microplastics. Based on the literature analysis, conclusions were drawn regarding the recommended methods of microplastic detection.

**Keywords:** microplastics, wastewater treatment plant, wastewater, sewage sludge

## 1. Introduction

The Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) define MPs as a group of plastic particles with a diameter of 1 nm to 5 mm (GESAMP, 2015). Pollution of the environment by microplastic (MP) is currently one of the major environmental problems all over the world. Plastics, due to their unique properties and relatively low production cost, have become an inseparable element of today's world. The new Water Framework Directive on the quality of water intended for human consumption in conjunction with the operation of the European Green Deal assumes the raising of water quality standards by introducing micropollutants such as pharmaceuticals, endocrine disruptors, or MP onto the watch list (Directive (EU) 2020/2184). The strategy assumes that by 2030, all plastic packaging available on the European Union market will be recycled. Despite efforts to combat plastic, wastewater treatment plants (WWTPs) are reported to be one of the most important routes for MPs to enter the aquatic environment. MPs have been identified in wastewater samples and sewage sludge across practically the whole world (Hamidian et al., 2021).

Recently published studies show that plastic in the environment is slowly degrading and fragmenting into smaller pieces. This is directly influenced by environmental conditions, including solar radiation. Human actions and the continuous development of technology have a great impact on the amount of MPs in the environment.

The isolation of MP in wastewater and sewage sludge involves sample collection and removal of matter interfering with its further determination. However, this methodology is not fully developed. The lack of standardization of the MP testing methods in the WWTPs samples causes difficulties in comparing the currently available and developed methods. This work focuses on the methods of sampling from WWTPs and isolating MPs from them.

## 2. The occurrence of MP in the environment

Taking into account the variety of sources of MPs, their occurrence has been noticed in all aquatic ecosystems around the world: on the surface of the sea, in deep-sea locations, in coastal and marine sediments, in freshwater, and in river sediments (He et al., 2020; Isobe et al., 2015; Kane et al., 2019; Phuong et al., 2021; Rakesh et al., 2021; Zeri et al., 2021). The presence of MPs in the arctic snowcap has also been noticed, which indicates significant air pollution with MPs and thus the quick and effective transport of MPs throughout the globe (Bergmann et al., 2019). Although most studies focus on MPs in the aquatic environment, it has been demonstrated that soils also contain large concentrations of MPs (Rillig et al., 2017).

Due to the origin of MPs in the natural environment, primary and secondary MPs can be distinguished (Cole et al., 2011; GESAMP, 2015). Regardless of their source these pollutants from both urban and peri-urban areas find their way through run-off to rivers, lakes, seas, and oceans. Primary MPs are those that are deliberately produced in a microscopic size and they are found in personal care cosmetics such as facial products and body scrubs (Derraik, 2002; UNEP, 2015; Zitko et al., 1991). Primary MPs found in the environment are often by-products and waste generated during production processes, for example in the production of granular plastic or industrial abrasive materials (GESAMP, 2015). Moreover, primary MPs are found in medical products and pharmaceuticals (Duis et al., 2016). Secondary MPs are one of the specific sources of plastic contamination of the environment and come from the disintegration of larger pieces of plastic. This disintegration is caused by a number of processes, including physical, chemical, and biological interactions. As a result, plastic loses its structural integrity and becomes fragmented. Among them, the washing of synthetic

clothes is of great significance as the MPs released during washing may pass through WWTPs to surface water, posing a serious hazard to the environment (Andrady et al., 2011; De Falco et al., 2019). Plastic wastes left on the beach are exposed to direct sunlight and high access to oxygen quickly degrades them so they become fragile and breakable, and any mechanical force can crush plastic pieces, creating MPs (Andrady et al., 2011; GESAMP, 2015; Moore, 2008). Emission from road traffic, specifically dust from car tires and horizontal road signs is another source of environmental pollution from secondary MPs (Sommer et al., 2018; Tamis et al., 2021). Other sources include the abrasion of polymer paints or emissions from landfills (Rakesh et al., 2021).

### 3. Review of strategies for the treatment of wastewater and sewage sludge samples for MP detection

WWTPs are one of the main sources of environmental pollution with MPs. Gatidou et al. reported that MP concentrations can range up to 3160 particles/dm<sup>3</sup> in raw wastewater and 125 particles/dm<sup>3</sup> in treated effluent. From 72% to 99.4% by mass of MPs are removed during wastewater treatment in municipal WWTPs (Gatidou et al., 2019). However, they are not biodegradable in typical wastewater treatment processes, and thus over 90% are accumulated in the sewage sludge, where they reach concentrations of up to  $170.9 \times 10^3$  particles/kg dry solids (ds) (Gatidou et al., 2019).

The detection of MPs in wastewater treatment plants is comprised of three stages: sample collection, treatment and characterisation of MPs (Hamidian et al., 2021). There is not a comprehensive methodology for the sampling, treatment, and identification of MPs in wastewater and sewage sludge. The lack of standardised reference methods for the analysis of MPs in samples from WWTPs causes difficulties in the evaluation of the methods presented by different authors. This work focuses on methods of sampling from WWTPs and isolating MPs from them.

#### 3.1. Sample collection

Sampling to identify MPs in WWTPs can be performed in different ways. Samples can be taken from both the inlet and outlet channels, as well as from biological reactors and settling tanks. This collection can take place in both instantaneous (vessel) and continuous (pumping and filtering system) forms. The sampling of MPs in the liquid column requires a decision on the size ranges to be analysed and then the appropriate equipment is selected. The collection of solid samples such as sewage sludge for the detection of MPs requires the selection of appropriate equipment.

Tagg et al. in his research used a telescopic sampling pole to collect 10 l of wastewater from the surface of the activated sludge at its aerobic stage. The samples were then transported in sterilized low-density polyethylene containers under aerobic conditions through a continuous supply of air (Tagg et al., 2015). Additionally, Lares et al. used a simple steel bucket with a capacity of 10 l to collect samples of various volumes (4–10 dm<sup>3</sup>) from the WWTP. Attaching the bucket to a metal rod allowed more wastewater to be collected in a shorter time. The isolation of MPs was performed by straining the sample through sieves with a mesh size of 0.25 mm and 5.0 mm (Lares et al., 2018). The collection of smaller volumes of wastewater (1–5 dm<sup>3</sup>) can be done with the use of glass jars, tightly closed and stored in the dark at a reduced temperature until the time of analysis (Leslie et al., 2017). Instantaneous sampling, despite its wide range of applications which is influenced by the wide availability of used vessels and their relatively low price, is less efficient than continuous sampling. In addition, sampling a large volume requires a lot of time. In order to minimize these drawbacks continuous sampling with pumps is used.

The most common method is the use of pumping and filtering devices, which guarantees continuous sampling. For example, Sutton et al. pumped treated wastewater through 8-inch stacked Tyler stainless steel sieves with mesh diameters of 0.355 mm and 0.125 mm for 2 hours (Sutton et al., 2016). Lv et al. separated MPs from wastewater flowing into the plant with the help of a tool specifically constructed by his team, consisting of 5 sieves (20 cm in diameter) made of stainless steel with appropriate mesh sizes: 0.500; 0.250; 0.125; 0.0625; 0.025 mm. The collection was performed by the pumping system and ended when the eyelets were blocked by material isolated on the sieve or after reaching a wastewater volume of 200 l (Lv et al., 2019). Mintening et al. used a custom-made mobile pumping and filtration device to collect a 1000 l wastewater sample. The device consisted of a flexible polyvinyl chloride hose with a weighted end connected to a diaphragm pump and a filtration unit in the form of a stainless-steel filter with a diameter of 10 mm and a flow meter (Mintening et al., 2017).

Samples for analysis should be collected in a representative manner. For example, buckets made of stainless steel with a capacity of 10 dm<sup>3</sup> or smaller can be used for the sampling of activated sludge or digested sludge (Lares et al., 2018). Leslie et al. collected the sewage sludge samples using glass jars with a capacity from 1 to 5 dm<sup>3</sup> (Leslie et al., 2017). Half a kilogram of sewage sludge samples was collected with shovels and then stored at a reduced temperature in a polyvinyl chloride container protected from light (Mintening et al., 2017). Lv et al. used an MP trapper for the sampling of sewage sludge. Samples passed through the MP trapper by gravity to separate and collect different sized MP particles from sewage sludge until the moment when the mesh size was clogged by the solid matter (Lv et al., 2019).

Table 1 presents examples of methods used for collecting wastewater and sewage sludge to identify MPs.

**Table 1.** Summary of sampling tests from the WWTPs

No.	Subject of research	Sampling volume/mass	Type of sampling	Method of collection	Source
1	wastewater	10 [dm <sup>3</sup> ]	instantaneous	telescopic sampling pole	Tagg et al., 2015
2	wastewater	4–10 [dm <sup>3</sup> ]	instantaneous	steel bucket (10 l)	Lares et al., 2018
	sewage sludge		instantaneous	stainless steel vessel (0.25 l) and steel bucket (10 l)	
3	wastewater, sewage sludge	1–5 [dm <sup>3</sup> ]	instantaneous	glass jars	Leslie et al., 2017
4	wastewater	2-hour sewage flow	continuous	pumping device + stainless steel sieves (0.355 mm; 0.125 mm)	Sutton et al., 2016
5	wastewater, sewage sludge	200 [dm <sup>3</sup> ] / mesh clogging in sieves	continuous	pumping device + stainless steel sieves (0.500, 0.250; 0.125; 0.0625; 0.025 mm)	Lv et al., 2019
6	wastewater	1000 [dm <sup>3</sup> ]	continuous	pumping and filtration device	Mintening et al., 2017
	sewage sludge	0.5 [kg]	instantaneous	steel shovels	

### 3.2. Treatment of samples

Samples taken from WWTPs cans contain a significant amount of organic and inorganic substances, which makes it difficult to identify MPs. For the subsequent quantitative and qualitative analysis of MPs, a sample must be processed by isolating the MPs from other contaminants.

#### 3.2.1. Inorganic matter

The removal of inorganic matter from wastewater and sewage sludge samples is usually performed through density separation with the use of an appropriate salt solution. Polymers differ from each other in structure and chemical composition, and thus each polymer in a different environment behaves especially with respect to its properties. The density of the primary polymers ranges from 0.90 to 1.60 g/cm<sup>3</sup>, with the typical density of inorganic pollutants (e.g. sand and rust) being around 2.6 g/cm<sup>3</sup>. The separation is conducted as a result of the higher density material falling to the bottom of the tank, while the fraction of lower density remains on the surface of the liquid. A tank filled with a liquid with a density greater than that of isolated polymers allows the isolation of lighter materials (MPs) on the surface of the liquid. Due to its slow cost and non-toxicity, the most frequently used solution for this purpose is a saturated solution of sodium chloride (NaCl). It is widely accepted that separation based on the difference in density may be a convenient and effective technique to apply (Alvim et al., 2020; Leslie et al., 2017).

For example, Bayo et al. isolated MPs from the analysed wastewater samples using an aqueous solution of 120 g of NaCl dissolved in a litre of the sample (solution density equal 1.08 g/cm<sup>3</sup>). The mixture was placed in a 2 litre beaker and mixed with a mechanical stirrer for 20 minutes. The supernatant was filtered and washed with distilled water, and the isolated MPs were dried in a Petri dish overnight (Bayo et al., 2020). In a study by Mintening et al., in order to isolate MPs from sewage sludge, a solution of 125 mg of the sludge in 825 cm<sup>3</sup> of distilled water was mixed with 400 g of solid sodium hydroxide and stirred for 24 hours at 60°C. The mixture was then neutralised with 37% hydrochloric acid. The resulting sodium chloride solution with a density of 1.14 g/cm<sup>3</sup> enabled the separation of polyethylene, polypropylene, and polystyrene after 96 hours (Mintening et al., 2017).

A similar method of density separation in wastewater and sewage sludge samples was applied by Vermaire et al. They used sodium chloride containing 6 g salt per 20 cm<sup>3</sup> of the analysed solution, which was sewage sludge diluted with water. In order to isolate the separated MPs from inorganic matter, the solution was placed in a separating funnel and left for 30 minutes. After this time the sediment from the bottom of the vessel was removed and the fraction collected from the liquid surface was dried and further analysed to identify MPs (Vermaire et al., 2017).

The separation of the inorganic part from the MPs in the sewage sludge samples with NaCl solution was also used by Li et al. A volume of 300 cm<sup>3</sup> of distilled water and a NaCl solution (1.2 g/cm<sup>3</sup>) were added to the flask with 20 g of a homogenised sludge sample. The solution was stirred for 15 minutes and left for 2 hours for separation of the appropriate parts (Li et al., 2018).

Even though the NaCl solution is the most popular, there are also methods of density separation using other salt solutions. For example, inorganic matter from the wastewater sample has also been removed using a zinc chloride solution (ZnCl<sub>2</sub>). Mintening et al. removed the inorganic parts with a ZnCl<sub>2</sub> solution with a density of 1.6 g/cm<sup>3</sup>. After 24 hours of density separation, the material deposited on the bottom was removed and the MPs fraction was subjected to further analysis (Mintening et al., 2017). He et al. used a solution of zinc chloride dissolved in distilled water to obtain a solution with a density of 1.6 g/cm<sup>3</sup>. The dried solid (50 g) was placed in a glass beaker and then 500 cm<sup>3</sup>

of  $\text{ZnCl}_2$  solution was added. After 15 minutes of stirring, the solution was left for 40 minutes. The collected supernatant containing MP was then filtered, dried, and subjected to further analyses (He et al., 2020).

Another solution used for density separation is an aqueous solution of sodium iodide (NaI). Lv et al. isolated MPs from wastewater samples with a NaI solution with a density of around  $1.46 \text{ g/cm}^3$ . In the tested sample, they detected the presence of polymers such as poly(ethylene terephthalate), polystyrene, polypropylene, and polyethylene (Lv et al., 2019).

Quinn et al. proved that it is possible to recover MPs with sizes of  $800\text{--}1000 \text{ }\mu\text{m}$  and  $200\text{--}400 \text{ }\mu\text{m}$  as a result of density separation using water and aqueous solutions of sodium chloride (NaCl), sodium bromide (NaBr), sodium iodide (NaI) and zinc bromide ( $\text{ZnBr}_2$ ). Particles with a size of  $800\text{--}1000 \text{ }\mu\text{m}$  contained in their composition polypropylene, high-density polypropylene, poly(vinyl chloride), poly(ethylene terephthalate), and polystyrene. Research with smaller particles has shown a different composition of the polymers (polyethylene, high-density polyethylene, polystyrene, nylon, poly(ethylene terephthalate), poly(vinyl chloride)). It was noticed that the density separation of MPs using saturated solutions of sodium iodide with a density of  $1.6 \text{ g/cm}^3$  and zinc bromide with a density of  $1.7 \text{ g/cm}^3$  showed a higher extraction efficiency of polymers compared to solutions of sodium chloride with a density of  $1.2 \text{ g/cm}^3$  and sodium bromide with a density of  $1.4 \text{ g/cm}^3$ . The lowest recovery of polymers was observed with water. Moreover, with the increase in the density of the solutions used, the extraction efficiency of high-density plastics increases (Quinn et al., 2017). The assessment of the efficiency of the removal of inorganic parts supported by the research by Quinn et al. is summarised in Table 2.

### 3.2.2. Organic matter

In order to remove organic matter from samples from WWTPs, the method of wet oxidation with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) can be used. It is able to oxidise a significant amount of organic matter at a moderate temperature and at normal pressure. The oxidising effect of the Fenton reaction depends on the pH of the solution, the ratio of hydrogen peroxide concentrations to iron(II) ions, the temperature used during the reaction, the amount of hydrogen peroxide used in relation to the organic pollutants, and the initial concentration of iron(II) ions. The catalytic reaction does not affect the quantity and quality of MPs in the analysed samples, which is why it is very popular. The dose of the Fenton reagent depends on the ds of the analysed sample. Lares et al., in order to determine the required amount of reagents to carry out the Fenton reaction, determined the dry mass of the test sample. Based on this, they oxidised the dry sample by adding  $20 \text{ cm}^3$  of a 30% hydrogen peroxide solution together with  $20 \text{ cm}^3$  of a 0.05M aqueous solution of iron(II) ions. After sedimentation of the sludge, the beaker with the solution was mixed on a magnetic stirrer and heated to a temperature of  $75^\circ\text{C}$ . After cooling and filtering the obtained mixtures using a cellulose nitrate filter with a porosity of 0.8 mm, the retained residue together with the filters was air-dried for 24 hours in Petri dishes and subjected to further analysis (Lares et al., 2018). In the scientific literature, it is the most used method to remove organic matter from a sample. This was based on the method of the National Oceanic and Atmospheric Administration (NOAA), and its greatest advantage is the lack of impact on plastics to be determined in the further stages of the analysis (Sutton et al., 2016; Masura et al., 2015). Depending on the specific requirements, the concentrations of the used reagents are modified. For example, in their research, Lv et al. used salt of iron(II) ions at a concentration of 20 mM as a catalyst for the reaction (Lv et al., 2019).

Hydrogen peroxide solution can also be used alone. In order to remove organic matter, Zeri et al. used  $40 \text{ cm}^3$  of 15% hydrogen peroxide per 3 grams of ds and heated the mixture to  $40^\circ\text{C}$  for 24 hours (Zeri, 2021). When analysing



the efficiency of organic matter removal using 30% or 35%  $H_2O_2$  solution, the samples were immersed in the liquid for 3, 5 and 7 days. This showed that most of the organic matter can be effectively eliminated within 7 days of exposing the sample to hydrogen peroxide. The contact time of MPs with  $H_2O_2$  does not affect the degradation of polymers (Tagg et al., 2015).

The removal of organic matter from wastewater and sewage sludge samples can also be performed with acid and alkaline treatment. This requires the use of strong bases and acids at elevated temperatures, which however may affect the degradation of MPs present in the analysed sample (Hurley et al., 2018). Strong organic acids (e.g. concentrated  $H_2SO_4$ ) are used to remove organic material, but there is a risk that at low pH, the acids damage the structure of polymers with low pH tolerance (Van Cauwenberghe et al., 2015). The research shows that the removal of organic matter with 1M and 2M hydrochloric acid (HCl) is not very effective. The purification efficiency of the sample is  $82.6 \pm 3.7\%$  for 1M HCl and  $72.1 \pm 9.2\%$  for 2M HCl. Better results can be obtained with sodium hydroxide solution (NaOH 1M and 2M), the organic matter was eliminated at the levels of  $90.0 \pm 2.9\%$  and  $85.0 \pm 5.0\%$ , respectively. In order to achieve around 90% efficiency in the removal of organic matter, it is required to use  $40 \text{ cm}^3$  of 10M NaOH per 0.2 grams of ds and keep it at a high temperature for 48 hours (Cole et al., 2014). The assessment of the efficiency of the removal of organic parts supported by the research of Cole et al. is summarised in Table 2.

Another approach to eliminate organic matter from MPs samples is enzymatic purification. During this process, the samples are immersed in mixtures of technical enzymes and incubated at the appropriate temperature for a sufficient period of time. In the first purification step, Löder et al. proposed the lipase enzyme (Lipase FE-01,  $1300 \text{ U/cm}^3$  in PBS pH = 9.0) and incubated the sample for 24 hours at  $40^\circ\text{C}$ . A sample with the amylase enzyme (Amylase  $40 \text{ U/cm}^3$  in PBS pH = 5) was then subjected to incubation for 24 hours at  $50^\circ\text{C}$ . However, in the case of samples with a high content of organic matter (such as wastewater and sewage sludge), it is suggested to use additional enzymes in the purification process (for example protease or cellulose), although this further extends the possibility of mistakes and loss of the analysed sample (Löder et al., 2017).

**Table 2.** Assessment of the effectiveness of the removal of pollutants

Method of removing pollutants	Isolated MPs and effectiveness assessment	Source
<b>evaluation of the effectiveness of the removal of inorganic parts</b>		
density separation $H_2O$	lowest polymer recovery	Quinn et al., 2016
density separation with an aqueous solution of NaCl ( $\rho = 1.2 \text{ g/cm}^3$ )	average polymer recovery	
density separation with aqueous solution NaBr ( $\rho = 1.4 \text{ g/cm}^3$ )		
density separation with aqueous solution NaI ( $\rho = 1.6 \text{ g/cm}^3$ )	highest recovery of polymers (polypropylene, high-density polypropylene, poly(vinyl chloride), poly(ethylene terephthalate), polystyrene, nylon)	
density separation with aqueous solution $ZnBr_2$ ( $\rho = 1.7 \text{ g/cm}^3$ )		
<b>evaluation of the effectiveness of the removal of organic parts</b>		
1M HCl, 2M HCl	low effectiveness of the method	Cole et al., 2014
1M NaOH, 2M NaOH		
10M NaOH	degradation of polymers – nylon, polyethylene, poly(vinyl chloride)	
enzymatic digestion	highest recovery of polymers (polyamide, polypropylene, polyurethane, polyethylene, acrylic)	

Although the presented enzymatic method is effective, it can be further modified by adding a sample oxidation step. Mintening et al. used multi-stage oxidation-enzymatic treatment in their research. In the first step, the sample with the sodium dodecyl sulphate (5% w/vol) was incubated at 70°C for 24 hours. In the second step, protease (Protease A-01, 1800 U/dm<sup>3</sup> in PBS pH = 9) was added and the samples were incubated for 48 hours at 50°C. In the next step, the sample with cellulose (Cellulose TXL, 44 U/dm<sup>3</sup> in PBS pH = 4.5) and lipase (Lipase FE-01, 2320 U/dm<sup>3</sup> in PBS pH = 10.5) was incubated for 96 hours at 40°C, and then for 6 days at 50°C. The obtained material was rinsed with ethanol (30%), sonicated for 3 minutes and again rinsed with water and ethanol. The material was isolated by filtration. Fractions <500 µm on the sieves were flooded with H<sub>2</sub>O<sub>2</sub> (30 cm<sup>3</sup>, 35%) and incubated for 24 hours at 50°C. After filtration (removal of H<sub>2</sub>O<sub>2</sub>) the sample was incubated for 48 hours at 37°C in the presence of chitinase (Chitodextrins, 96 U/dm<sup>3</sup> in PBS pH = 5.6) (Mintening et al., 2017). The application of enzymatic-oxidative purification prevents the risk of decay and even the loss of polymers that can occur from the use of aggressive chemicals, but this process is time consuming. In addition, there is a risk of contamination of the analysed sample and its loss due to multiple incubation steps at an individual enzymatic pH.

The summary of the selected methods of removing organic and non-organic pollutants from wastewater and sewage sludge samples for analysis of MPs is presented in Table 3.

**Table 3.** Summary of selected studies on the removal of organic and inorganic impurities for MP separation

No.	Method of removing organic matter	Method of removing inorganic matter	Isolated MPs	Source
1	Fenton reagent (H <sub>2</sub> O <sub>2</sub> + Fe(II))	–	polystyrene, polyethylene, polyamide, polypropylene	Lares et al., 2018
2	Fenton reagent (H <sub>2</sub> O <sub>2</sub> + Fe(II))	density separation with aqueous solution NaI (ρ ~ 1.46 g/cm <sup>3</sup> )	poly(ethylene tetra phthalate), polystyrene, polypropylene, polyethylene	Lv et al., 2019
3	hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	visual assessment	polyester, polypropylene, polyethylene, poly(ethylene terephthalate), acrylic trawls: nylon-6, polyurethane	Zeri, 2021
4	enzymatic-oxidative digestion	density separation with an aqueous solution of NaCl (ρ = 1.14 g/cm <sup>3</sup> ) – sewage sludge density separation with aqueous solution ZnCl <sub>2</sub> (ρ = 1.6 g/cm <sup>3</sup> ) – wastewater	polyethylene, polypropylene, polystyrene	Mintening et al., 2017
5	–	density separation with aqueous solution of NaCl (ρ = 1.08 g/cm <sup>3</sup> )	polyethylene, acrylate, polypropylene, polystyrene, polyamide, methacrylate, polyester, poly(phenylene vinylene), polyisobutylene, poly(tetrafluoroethylene)	Bayo, et al., 2020
6	hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	density separation with an aqueous solution of NaCl (ρ = 0.3 g/cm <sup>3</sup> )	microfibers	Vermaire et al., 2017
7	hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	Density separation with an aqueous solution of NaCl (ρ = 1.2 g/cm <sup>3</sup> )	polyolefins, acrylic fibres, polyethylene, polyamide, alkyl resin, polystyrene	Li et al., 2018



#### 4. Conclusion

WWTPs are considered one of the main routes of MPs entering the natural environment, which in turn poses a huge threat to the ecosystem as well as to all living organisms including humans. The lack of standardised reference methods for the sampling and treatment of MPs in WWTPs does not allow a clear comparison of different methodologies. As a result, sampling from WWTPs is performed in various ways and is based on two mechanisms, namely continuous and instantaneous sampling. The method of continuous sampling is definitely faster and requires less human work. For this purpose, a diaphragm pump is used, which takes a large volume of the sample and goes directly to metal sieves with an appropriate mesh size. This enables the preliminary isolation of the appropriate size fractions. Environmental samples, apart from MPs, contain many inorganic and organic pollutants. In order to identify microplastics, it is necessary to isolate them. To this end, methods of removing inorganic and organic matter are used.

Chemical treatment of samples performed in the laboratory is a time-consuming process requiring knowledge of various analytical techniques. The most common method of removing inorganic parts from samples obtained from WWTPs is density separation. Density separation can be performed using different aqueous salt solutions. However, taking into account the economic and environmental aspect, the use of an aqueous solution of sodium chloride seems to be one of the best methods of removing inorganic parts from environmental samples.

Although several methods for the removal of organic matter from wastewater and sludge samples are presented in the literature, the most popular seem to be those based on the Fenton method. The mixture of hydrogen peroxide and iron(II) ions does not significantly affect the quantity and quality of MPs in the tested samples, therefore, this approach is more popular than acid or alkaline treatment. Enzymatic digestion and enzymatic-oxidative digestion, as with the Fenton reaction, are effective methods in the removal of organic matter, however, these methods are multi-step and are therefore very time-consuming. Considering the time, cost and recovery of polymers, the best available method seems to be the Fenton reaction.

## References

- Alvim, C.B., Mendoza-Roca, J.A., Bes-Pia, A. (2020). Wastewater treatment plant as microplastics release source – Quantification and identification techniques. *Journal of Environmental Management*, 255. <https://doi.org/10.1016/j.jenvman.2019.109739>
- Andrady, A.L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, 62, 1596–1605. <https://doi.org/10.1016/j.marpolbul.2011.05.030>
- Bayo, J., López-Castellanos, J., Olmos, S. (2020). Abatement of microplastic from municipal effluents by two different wastewater treatment Technologies. *Water Pollution XV*, 15-26. <https://doi.org/10.2495/wp200021>
- Bergmann, M., Mützel, S., Primpke, S., Tekaman, M.B., Trachsel, J., Gerdts, G. (2019). White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. *Science Advances*, 5(8). <https://doi.org/10.1126/sciadv.aax1157>
- Cole, M., Lindeque, P., Halsband, C., Galloway, T.S. (2011). Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin*, 62, 2588–2597. <https://doi.org/10.1016/j.marpolbul.2011.09.025>
- Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S. (2014). Isolation of microplastics in biota-rich seawater samples and marine organism. *Scientific Reports*, 4, 2231–2236. <https://doi.org/10.1038/srep04528>
- De Falco, F., Di Pace, E., Cocca, M., Avella, M. (2019). The contribution of washing processes of synthetic clothes to microplastic pollution. *Scientific Reports*, 9(6633). <https://doi.org/10.1038/s41598-019-43023-x>
- Derraik, J.G.B. (2002). The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin*, 44(9), 842–852. [https://doi.org/10.1016/S0025-326X\(02\)00220-5](https://doi.org/10.1016/S0025-326X(02)00220-5)
- Directive (EU) 2020/2184 of the European Parliament and of the council of 16 December 2020 on the quality of water intended for human consumption.
- Duis, K., Coors, A. (2016). Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Environmental Sciences Europe*, 28(2). <https://doi.org/10.1186/s12302-015-0069-y>
- Gatidou, G., Arvaniti, O.S., Stasinakis, A.S. (2019). Review on the occurrence and fate of microplastics in Sewage Treatment Plant. *Journal of Hazardous Materials*, 367, 504–512. <https://doi.org/10.1016/j.jhazmat.2018.12.081>
- GESAMP (2015). Sources, fate and effects of microplastics in the marine environment: a global assessment (Kershaw, P. J., ed.). (IMO/FAO/UNESCOIOC/UNIDO/WMO/IAEA/UN//UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP Vol. 90, 2015, 96 p.
- Hamidian, A.H., Ozumchelouei, E.J., Feizi, F., Wu, C., Zhang, Y., Yang, M. (2021). A review on the characteristics of microplastics in wastewater treatment plants: A source for toxic chemicals. *Journal of Cleaner Production*, 295, 12658. <https://doi.org/10.1016/j.jclepro.2021.126480>
- He, B., Goonetilleke, A., Ayoko, G.A., Rintoul, L. (2020). Abundance, distribution patterns, and identification of microplastics in Brisbane River sediments, Australia. *Science of the Total Environment*, 700, 134467. <https://doi.org/10.1016/j.scitotenv.2019.134467>
- Hurley, R.R., Lusher, A.L., Olsen, M., Nizzetto, L. (2018). Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices. *Environmental Science and Technology*, 52(13), 7409–7417. <https://doi.org/10.1021/acs.est.8b01517>

- Isobe, A., Uchida, K., Tokai, T., Iwasaki, S. (2015). East Asian seas: A hot spot of pelagic microplastics. *Marine Pollution Bulletin*, 101(2), 618–623. <https://doi.org/10.1016/j.marpolbul.2015.10.042>
- Kane, I.A., Clare, M.A. (2019). Dispersion, Accumulation, and the Ultimate Fate of Microplastics in Deep-Marine Environments: A Review and Future Directions. *Frontiers Earth Science*, 7(80). <https://doi.org/10.3389/feart.2019.00080>
- Lares, M., Ncibi, M.C., Sillanpää, M., Sillanpää, M. (2018). Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. *Water Research*, 133, 236–246. <https://doi.org/10.1016/j.watres.2018.01.049>
- Leslie, H.A., Brandsma, S.H., Van Velzen, M.J.M., Vethaak, A.D. (2017). Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. *Environment International*, 101, 133–142. <https://doi.org/10.1016/j.envint.2017.01.018>
- Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., Zeng, E.Y. (2018). Microplastics in sewage sludge from the wastewater treatment plants in China. *Water Research*, 142, 75–85. <https://doi.org/10.1016/j.watres.2018.05.034>
- Löder, M.G.J., Imhof, K.H., Ladehoff, M., Löschel, L.A., Lorenz, C., Mintenig, S., Piehl, S., Prompke, S., Schrank, I., Laforsch, C., Gerdt, G. (2017). Enzymatic purification of microplastics in environmental samples. *Environmental Science and Technology*, 51, 14283–14292. <https://doi.org/10.1021/acs.est.7b03055>
- Lv, X., Dong, Q., Zuo, Z., Liu, Y., Huang, X., Wu, W-M. (2019). Microplastics in a municipal wastewater treatment plant: Fate, dynamic distribution, removal efficiencies, and control strategies. *Journal of Cleaner Production*, 225, 579–586. <https://doi.org/10.1016/j.jclepro.2019.03.321>
- Masura, J., Baker, J., Foster, G., Arthur, C. (2015). Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. *NOAA Technical Memorandum NOS-OR&R-48*.
- Mintenig, S.M., Int-Veen, I., Löder, M.G.J., Primpke, S., Gerdt, G. (2017). Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. *Water Research*, 108, 365–372. <https://doi.org/10.1016/j.watres.2016.11.015>
- Moore, C.J. (2008). Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. *Environmental Research*, 108(2), 131–139. <https://doi.org/10.1016/j.envres.2008.07.025>
- Phuong, N.N., Fauvelle, V., Grenz, C., Ourgaud, M. (2021). Highlights from a review of microplastics in marine sediments. *Science of the Total Environment*, 777, 146225. <https://doi.org/10.1016/j.scitotenv.2021.146225>
- Quinn, B., Murphy, F., Ewins, C. (2016). Validation of density separation for the rapid recovery of microplastics from sediment. *Analytical Methods*, 9, 1491–1498. <https://doi.org/10.1039/C6AY02542K>
- Rakesh, S.S., Davamani, V., Murugaragavan, R., Ramesh, P.T., Shrirangasami, S.R. (2021). Microplastics Contamination in the Environment. *The Pharma Innovation Journal*, 10(8), 1412–1417.
- Rillig, M.C., Ingraffa, R., de Souza Nachadi, A.A. (2017). Microplastic Incorporation into Soil in Agroecosystems. *Frontiers in Plant Science*, 8, 1805. <https://doi.org/10.3389/fpls.2017.01805>
- Sommer, F., Dietze, V., Baum, A., Sauer, J., Gilge, S., Maschowski, C., Gieré, R. (2018). Tire Abrasion as a Major Source of Microplastics in the Environment. *Aerosol and Air Quality Research*, 18(8), 2014–2028. <https://doi.org/10.4209/aaqr.2018.03.0099>

- Sutton, R., Mason, S.A., Stanek, S.K., Willis-Norton, E., Wren, I.F., Box, C. (2016). Microplastic contamination in the San Francisco Bay, California, USA. *Marine Pollution Bulletin*, 109(1), 230–235. <https://doi.org/10.1016/j.marpolbul.2016.05.077>
- Tagg, A.S., Sapp, M., Harrison, J.P., Ojeda, J.J. (2015). Identification and Quantification of Microplastics in Wastewater Using Focal Plane Array-Based Reflectance Micro-FT-IR Imaging. *American Chemical Society*, 87, 6032–6040. <https://doi.org/10.1021/acs.analchem.5b00495>
- Tamis, J. E., Koelmans, A.A., Dröge, R., Kaag, N.H.B, Keur, M.C., Tromp P.C., Jongbloed, R.H. (2021). Environmental risks of car tire microplastic particles and other road runoff pollutant. *Microplastics and Nanoplastics*, 1(10). <https://doi.org/10.1186/s43591-021-00008-w>
- UNEP (2015). Plastic in Cosmetics.
- Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R. (2015). Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environmental Pollution*, 199, 10–17. <https://doi.org/10.1016/j.envpol.2015.01.008>
- Vermaire, J.C., Pomeroy, C., Herczegh, S.M., Haggart, O., Murphy, M. (2017). Microplastic abundance and distribution in the open water and sediment of the Ottawa River, Canada, and its tributaries. *FACETS*, 2, 301–314. <https://doi.org/10.1139/facets-2016-0070>
- Zeri, C., Adamopoulou, A., Koi, A., Koutsikos, N., Lytras, E., Dimitriou, E. (2021). Rivers and Wastewater-Treatment Plants as Microplastic Pathways to Eastern Mediterranean Waters: First Records for the Aegean Sea, Greece. *Sustainability*, 13(10), 5328. <https://doi.org/10.3390/su13105328>
- Zitko, V., Hanlon, M. (1991). Another source of pollution by plastics: skin cleansers with plastic scrubbers. *Marine Pollution Bulletin*, 22, 41–42. [https://doi.org/10.1016/0025-326X\(91\)90444-W](https://doi.org/10.1016/0025-326X(91)90444-W)