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REVIEW

Large Scale Microplastic Fibre Analysis in Wastewater: A Comprehensive Review and Recommendations

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Abstract

Purpose of Review This review provides a critical analysis of current and emerging methods for identifying and quantifying microplastic fibres (MPF) in wastewater, covering all key steps of sample collection, pretreatment, and analytical analysis. There are currently no universally accepted standards for collecting and analysing MPF. This review aims to provide new insights to develop appropriate processes for collecting and analysing MPF in wastewater through a critical analysis of the literature.

Recent Findings Previous studies have used non-selective grab sampling and stacked sieving apparatuses to collect and/or sort microplastics, but very few have been specifically applied to MPF. Hydrogen peroxide (H_2O_2) digestion is the most widely used for sample preparation prior to MPF analysis. MPF quantification by manual counting under an optical microscope is possible but is inefficient and unable to meet the required level of accuracy. Either micro–Fourier Transport Infrared (μFTIR) or μ-Raman is suitable for polymer identification. They each have distinctive reported strengths and weaknesses, and μFTIR is more appropriate for MPF analysis.

Summary Fast and scalable analysis can be achieved with grab sampling for collection, H_2O_2 digestion for pretreatment, filtration using glass fibre or alumina oxide membranes, and then microscopic imaging with fluorene tagging for automated counting. Transmittance μFTIR is the most appropriate tool for polymer identification. Density separation, extensive sample digestion, manual counting, and Raman spectroscopy are not required or incompatible for MPF analysis.

Keywords Microplastic fibres (MPF) · Sample collection · Hydrogen peroxide digestion · Fluorescence microscopy · Micro-Fourier transform infrared spectroscopy (μFTIR) · Automated counting · Quality control and quality assurance (QA/QC)

Introduction

Microplastic fibres (MPF) are an emerging group of contaminants increasingly recognised for potential health and environmental effects [1, 2]. MPF are distinct from other microplastics (MP) such as fragments and particles due to their physical characteristics, typically elongated in shape, structurally flexible and similar in density to water. Additionally, spectroscopic analysis on environmental MPF reveals polyester as the dominant material, as well as other synthetic fabric blends like polyamide and acrylic [3–5]. Both the physical and chemical properties of MPF detected in the environment indicate that textile laundering and their consequent shedding of fibres are likely the origin of MPF pollution, and this is widely agreed upon in the literature [3, 6, 7]. Furthermore, recent studies have shown that MPF are more frequently detected than other types of MP in both

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water and sediment samples, suggesting there is a disproportionately higher rate of discharge from urban sources, particularly wastewater treatment plants (WWTP), which process textiles [8]. Thus, it is urgent to fully understand the pathways by which WWTP containing highly concentrated quantities of MPF are reaching the environment, and how to effectively eliminate accidental contamination. The principal way to achieve this is through the design and implementation of a standardised protocol for rapid, high-throughput analysis of MPF, which are widespread in wastewater effluent.

General MP analysis procedures are advantageous for several reasons, including ease of use and suitability for a broad range of polymers, sizes, shapes and sample matrices [9–11]. One caveat to general, 'all-rounder' methods, however, is their propensity to contain steps otherwise unnecessary for samples with known characteristics or an expected mix of contaminants. For example, it would be inefficient to apply a generalised MP analysis method to tap water or relatively clean wastewater containing a homogeneous mix of MPF, as these conditions do not necessitate many of the procedures commonly used to clean organic-rich matrices or separate heavy sediments. Thus, non-essential steps must be identified and eliminated from MPF analysis workflows to streamline quantification and increase scalability.

Furthermore, the expansion in MPF analysis studies has generated a wide variety of experimental techniques which seek to increase efficiency, speed, and accuracy of analytical workflows; however, this emergence and diversification of techniques and studies have simultaneously limited the formation of fully standardized procedures or harmony between methods [12]. Some common steps which are prone to minor or major variation between studies include, but are not limited to sample collection, pre-treatment steps like digestion, density separation and filtration, and finally the method of analysis. It is, therefore, vital that MPF analysis be distinct from general MP protocols whilst maintaining a desirable level of simplicity and reliability. To address this need, the following study will discuss several noteworthy techniques used to measure MPF in wastewater and assess their suitability for an optimised, but accessible workflow for use by industry, government and advocacy groups.

Previous reviews have generally focused on the sources, presence, fate, and analysis of a wide range of MP in sludge, water, and sediment [13, 14]; however, few currently exist which are optimised for the specific measurement of MPF. Furthermore, the subtleties of MPF analysis protocols and specific parameters are seldom discussed in enough detail to sufficiently guide future researchers [15]. As MPF are now recognised as the most widespread class of MP entering the environment mostly from WWTP [3], this review focuses primarily on methods by which MPF have been measured in wastewater. Only studies which identified MPF in wastewater samples are included in the analysis. Additionally, this review also discusses the appropriateness of these methods specifically for MPF rather than MP as a whole. First, methods of wastewater collection are discussed, then the primary pre-treatment steps, digestion, density separation and filtration. Finally, the review examines relevant methods for quantifying and enumerating MPF number in the sample. To aid in future decision making for MPF analysis in wastewater, each section is concluded with a recommendation on the most appropriate method.

Methodology

As background research for this study, the Science Direct and Google Scholar databases were used. Recent papers from 2016 to 2024 were prioritised, with only a few exceptions made for key, foundational studies between 2010 and 2017. The search terms ("microplastic fibre" OR "fibrous microplastic" OR "microfiber" OR "synthetic fibre") AND ("wastewater treatment plant" OR "laundry wastewater" OR "textiles wastewater") formed the preliminary search, which returned 73 results (Fig. 1); however, some studies from this search were omitted due to their focus on sludge rather than wastewater. The addition of the terms "analysis", "methods", "quantify", and finally "microplastic" were gradually added to increase search results, given that MPF are still a niche topic. If articles did not contain sufficient information relating specifically to MPF, either in methods or results sections, they were also omitted unless showcasing a post processing technique of particular interest.

Generic Workflow for MPF Analysis

The analysis of MPF in wastewater and sludge samples generally consists of three steps, namely sample collection, pretreatment and preparation, and then identification and quantitative analysis (Fig. 2). Obtaining representative samples is a crucial first step to ensure reliable and accurate analysis, and researchers may want to sample from a variety of locations within and around a WWTP and across multiple timeframes to effectively capture the full range of MPF types and changes in concentrations. To prepare MPF for identification and quantification, organic and inorganic impurities must be removed to prevent interference. Digestion is applied to remove organic substances that may interfere with the fluorescent signal. Density separation is used to remove non-plastic objects that are similar to MPF in size and shape. After sample preparation, MPF are collected on filter discs for identification and quantification. It is noteworthy that the actual workflow for MPF analysis varies based on the sample type and matrix, and steps in Fig. 2 may not be required for all samples.

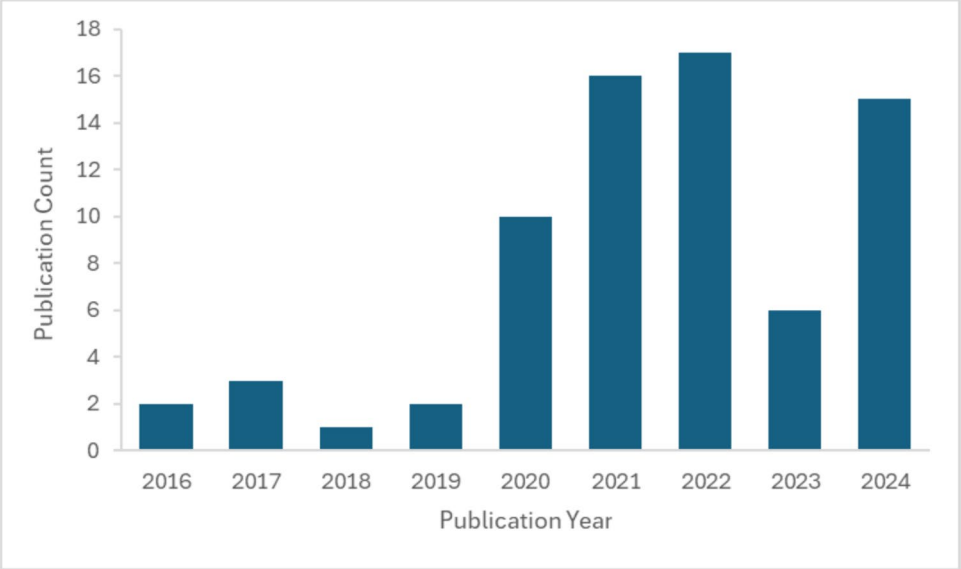

Fig. 1 Count of recent publications (articles and reviews) which have included a discussion of or directly quantified MPF in wastewater

Tarte et al. [16] analysed industrial textile wastewater containing a relatively homogeneous mixture of MPF. The liquid sample matrix did not contain any heavy solids, nor was it heavily contaminated with organic matter, and so a density separation step was not required, nor were extensive digestion protocols. For cleaner water samples designed for use or reuse by humans, such as recycled or drinking water, where even the presence of MPF is unknown, researchers may choose to bypass pre-treatment altogether.

Although widely accepted as the generic workflow for MPF analysis, there has not been any established standard protocol for sampling, preparation, and analysis of MPF in wastewater. A thorough plan for sample collection, pretreatment, and analysis should be created to analyse MPF from different sample matrices per the study goal. As a result, subsequent sections are devoted to an in-depth review of sample collection, pretreatment, and analysis.

Sample Collection

MPF sampling of wastewater in the literature occurs at both small (e.g. individual households) and large (e.g. municipal and industrial treatment plants and commercial laundromats) scales. Both of these scales are important for understanding the origin and fate of MPF in the environment.

Firstly, small scale studies involve domestic washing machines and often standardised, fabric abrasion testing machines, which are used in a controlled laboratory environment. Studies at this scale focus on the shedding characteristics of certain types of materials and fabrics relative to common, realistic washing conditions. Purpose built abrasion-testing machines are conveniently equipped with metal containers for capturing outflow samples of wastewater for further analysis [17, 18]. Through the use of a micro-Deval apparatus, Akyildiz et al. [19] observed that pre-washed and heavier fabrics, as well as those made of recycled plastic materials, were more prone to MPF production from shedding. Zambrano et al. [20] used a Launder-Ometer machine to determine that detergents promote a higher release of MPF during washing. Although highly useful and thorough, many institutions are not equipped with the knowledge or equipment to test fabric abrasion, given that they require highly specialised machinery. In contrast, Zambrano et al. [20] measured MPF release on a commercial washing machine simply by placing a 25 cm nylon mesh screen over the single outflow pipe of the machine to capture outgoing MPF in wastewater for later analysis. At a domestic scale, the use of cheap ceramic, mesh or paper filters presents a cheaper and simpler option for collecting a sample of particles for analysis or simply disposal by researchers or citizen scientists [21, 22]. The degree to which small household filters can effectively remove MPF, however, is still relatively low compared to industrial treatment solutions [23].

The methods by which wastewater samples are collected at WWTP vary. Generally, there are several key methods which are widely adopted [6]. Firstly, grab sampling, either directly from the water source or a dedicated outflow tap, is the most common and simplistic method and requires next to no equipment apart from a non-plastic container, typically < 2.5 L, and protective gear [14]. For wastewater containing a stable, homogenous mix of contaminants like MPF < 1 mm in textile wastewater [16, 24], grab sampling is sufficient to capture the full range of plastic types and concentrations. Grab sampling does, however, require a human to collect wastewater, and when sampling intervals are frequent due to predicted fluctuations in MPF load, the process can be labour intensive. Auto sampling provides

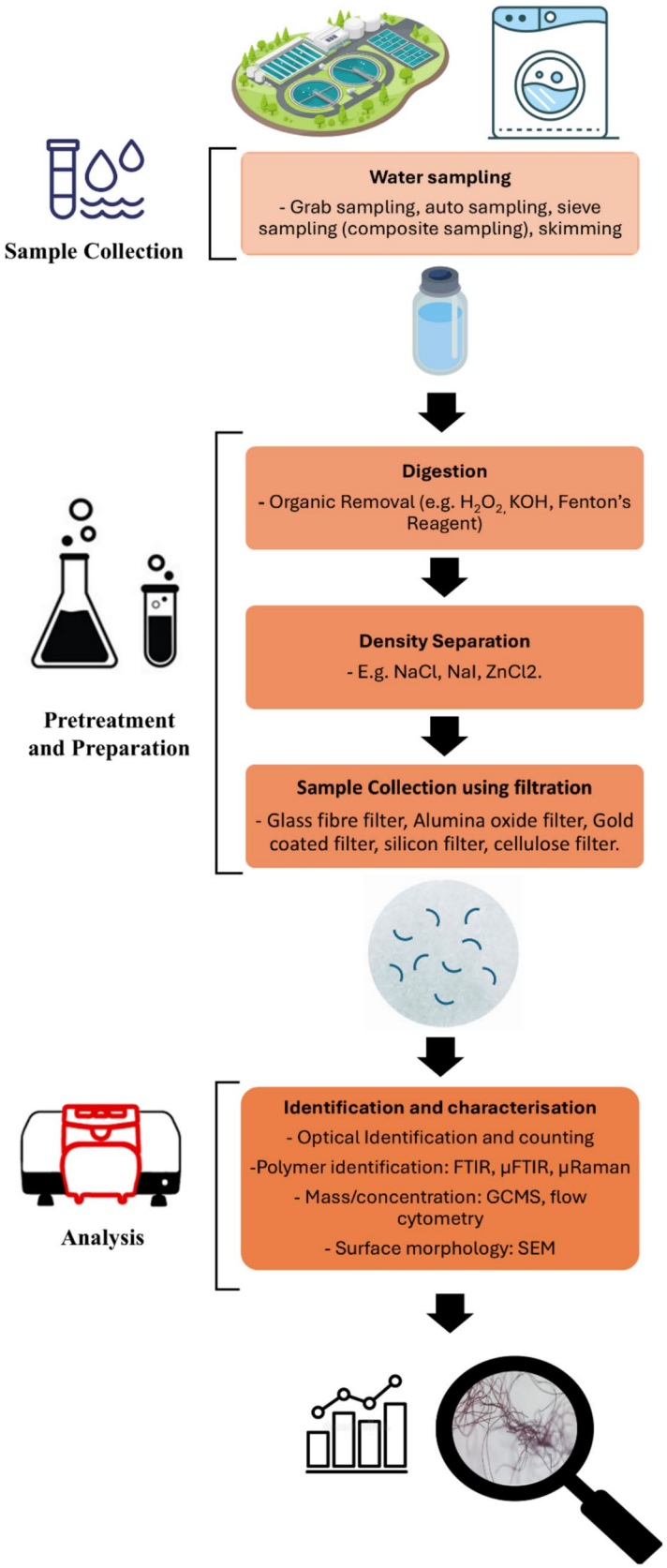

Fig. 2 Diagram highlighting the key steps in MPF analysis in wastewater and the techniques/equipment recommended for each step. Density separation, as discussed in the text, is not a necessary step, but is included for clarity (FTIR: Fourier Transform Infrared Spectroscopy; GC–MS: Gas Chromatography Mass Spectrometry; SEM: Scanning Electron Microscopy)

one solution to this problem, whereby a dedicated outflow point is programmed to systematically collect a sample at predefined times and volumes. This can be combined with a stack of mesh sieves of graduating pore size to automatically separate MPF into size classes as wastewater is filtered or pumped through, avoiding later manual sorting [25]. Sieving can be particularly useful for wastewater containing a broad range of MP types, sizes and shapes, however, care must be taken when sampling from fibre dominated wastewater, as MPF have a well-documented propensity to clog filters quickly due to clumping [26]. Skimming or surface filtration is an important method for sampling buoyant MPF across a large surface area, such as a wastewater vat, but again, the irregular and elongated shape allows MPF to easily pass through or be retained lengthways on filter pores, leading to under- or overestimation. Vojnovic et al. [27] elaborated on this issue and highlighted that surface sampling of wastewater by towing a net is unsuitable for MPF, and instead, sampling high volumes with a pump may be more sufficient to capture MPF. Additionally, combinations of the above methods are widely practised, such as the use of an electric pump connected to a multi-level sieving system, a method increasing in popularity due to its capacity to sample very high volumes of wastewater and sort solids into size classes [25, 28–30].

Another factor to consider when sampling from wastewater is the location where samples are derived from within the treatment plant. For example, a traditional WWTP may include a bioreactor, coagulation, sand filtration and chlorination components, and Na et al. [31] in an experimental study identified that MPF can persist through most of these components until sand filtration, where a 98% removal efficiency was recorded. In summary, grab sampling is labour-intensive but suitable for homogeneous mixes of pollutants, such as MPF < 1 mm. Seiving, on the other hand, is a suitable technique for sampling water with a wide variety of MP types, sizes, and forms. Pump-assisted sampling is strongly advised for surface water.

Sample Pretreatment

After sample collection, the process of analysing MP from the environmental sample begins with sample preparation. A careful review of the sample preparation of wastewater will be conducted in this part. Particular focus is given to digestion, density separation and filtration.

Digestion

Digestion is used in most studies examining MP in wastewater. However, very few studies have investigated the specific effects of digestion solutions on MPF. Cunsolo et al. [32] identified four factors to consider when determining the best digestion method. They include (1) the technique's total organic carbon (TOC) removal efficiency and digestion time; (2) the possibility of damage to MP particles, including spectral changes before and after the treatment; (3) cost; and (4) the recovery rate in spiking experiments. Currently, a hydrogen peroxide (H_2O_2) solution typically at v/v 30% concentration, diluted with distilled water is most commonly applied to wastewater, often with the addition of iron sulphate as a catalyst (becoming Fentons Reagent). The conditions surrounding the application of H_2O_2, however, remain variable between studies, despite several articles reporting on "optimal" conditions [33–35].

Akyildiz et al. [33] compared the effectiveness of the four most common treatment chemicals, including 15% H_2O_2, Fentons Reagent, 20% HCl, 10% KOH, and 20% NaOH, on lab-prepared wastewater containing only MPF of different types. Using two treatment conditions: 25 °C for 5 days or 60 °C for 6 h, they reported that H_2O_2, Fenton's reagent and HCl were far more effective than NaOH and KOH at removing organic material while retaining MPF structure. They also reported that the use of heat accelerated digestion time, but did not result in altered MPF, indicating that, should heating equipment be available, optimized MPF analysis of textile wastewater may benefit from heated digestion. This study, however, did not describe the volume of digestion chemicals, nor the size of MPF used, limiting its comparability to other studies.

In contrast, Munno et al. [35] cautioned against the use of high temperatures > 60 °C for H_2O_2-based oxidation due to the potential for structural damage to some MP like cosmetic microbeads, but also promoted the use of KOH at room temperature and in combination with H_2O_2. It is noteworthy that artificial MPF preparation was not described in detail beyond cutting fibres from nylon carpet, and digestion efficiency was measured using FT-IR spectral library comparisons, so this study may be limited in its capacity to inform real wastewater sample analysis.

Furthermore, it is important to consider the resistance of filter membranes to solutions, as these frequently serve as the medium for MPF digestion in studies. Prata et al. [34] evaluated the resistance of several types of filter membranes to digestion solutions, revealing that glass fibre and quartz filters are relatively safe to use with a range of digestion solutions, losing less than 10% of their weight following a 1 h digestion with KOH and Fentons reagent, however, cellulose based filter membranes were heavily degraded after 1 h KOH treatment at 50 °C.

For laundry and textile wastewater samples containing low total organic carbon (TOC), users can determine whether digestion is required at all through simple visual assessment of water clarity, but a minor treatment of 30% H_2O_2 to aliquoted samples is sufficient to eliminate any

dissolved chemicals such as trace detergents that could impede fluorescence analysis or staining protocols in the wastewater [16]. For samples with higher TOC, consider adding a small amount of iron sulphate at ~ 50 °C to accelerate and strengthen oxidation.

Density Separation

Density separation is used to isolate MPF from a complex sample matrix of plastic-like particulate matter and occurs either following digestion or between repeated digestion stages. High-density salts such as sodium chloride (NaCl) [24, 36, 37], sodium iodide (NaI) [25] and zinc chloride ($ZnCl_2$) [38] are dissolved into the aqueous sample matrix, which increases the density of the sample and allows it to settle for multiple hours or days while plastics of low density float to the surface for extraction [39]. In some cases, centrifugation can be used for density separation without any salt addition [26]. There are, however, few studies currently available which directly compare the effectiveness of a given solution on wastewater containing specifically MPF. Additionally, MPF, typically made of polyethylene terephthalate (PET, also known as polyester), possess a similar density to water, as well as a tendency to adhere to the surfaces of their containers, which was highlighted by Hurley et al. [37]. This behaviour increases the difficulty of extracting MPF from water matrices, however, the previous study identified that consecutive separation steps and the use of smaller glass containers like test tubes increase over recovery efficiency of MPF.

Although density separation is a ubiquitous and simple step in the general MP extraction process (Fig. 2), it may be unnecessary for sample matrices not containing sediment or heavy non-plastic particles. For relatively clean samples such as textile wastewater, users may opt to skip density separation to achieve greater speeds and increase the scalability of analysis.

Filtration

Filtration is usually the final step in sample preparation before analysis commences and involves the isolation of MPF from a clean sample matrix onto a sterile membrane surface. The importance of an appropriate filtration procedure cannot be understated as this membrane surface often becomes the medium on which MP are viewed under a microscope and/or spectroscopically analysed, so it is vital that non-plastic particles are either distinguished from MP or separated before analysis. Some key elements in this step, which users should consider, are the qualities of the filter itself, such as its diameter, pore size, material and resistance against chemicals or high loads of suspended solids, as well as the type of filtration apparatus and volume of sample matrix passing through the filter.

Arguably, filter membrane material is the most important feature. Glass fibre (GF) filters are amongst the most common types used by researchers (Table 1). The utility of glass fibre filters as a cheap, robust and accessible membrane makes them a popular choice for a variety of microplastics studies sampling from many environments. Given the range of pore sizes that glass fibre filters can be constructed with, they are also widely used in wastewater studies and are particularly hard against extremely complex sample matrices. Na et al. [31] measured the persistence of MPF throughout the wastewater treatment process. In their lab-controlled study, they used GF filters to isolate wastewater from a municipal WWTP to create a realistic sample matrix for combination with spiked MP and MPF, rather than for measuring solids retained on the filter itself. From their experiments, they discovered that despite effective removal efficiencies in sludge bioreactors and coagulation chambers, MPF persisted through treatment until sand filtration, where it was effectively retained in the sand column (~ 98% removal efficiency) before chlorination. This study showcases a novel way to employ GF filters to retain the liquid sample matrix rather than remove it. In another study, Akyildiz et al. [24] employed more traditional techniques using GF filters to directly examine MP retained on the filter surface, which derived from wastewater effluent of a WWTP servicing the textiles industry. These GF filters were the medium for both optical microscopy as well as μFTIR analysis. Furthermore, GF filters are frequently used as intermediate filters on which MPF are subjected to chemical pre-treatment or staining before final filtration due to their robustness against harsh conditions.

Another prominent filter used in MPF sampling from wastewater is the stainless-steel mesh (Table 1). Steel meshes may be a desirable choice for a number of reasons, including high resistance to chemical pre-treatment or digestion, low risk of cross-contamination, and potential for the same filter used in initial sample retrieval and fractionation to undergo further pre-treatment and analysis. Steel meshes are commonly employed to analyse samples which contain a non-homogenous distribution of MPF of different sizes, for example, Ziajahromi et al. [25] used stainless steel mesh filters both to fractionate samples in situ at the WWTP sampling site, as well as following density separation, becoming the final medium for MP microscopic analysis. The final mesh used in filtration had a pore size of 25 μm; however, it must be noted that MPF have been recorded with diameters close to or less than 25 μm, indicating that in this study, some MPF concentrations may have been underestimated due to the mesh size. Similarly, Park et al. [38] used a 45 μm pore size steel mesh filter and recorded higher proportions of MP fragments than MPF, and went on to theorize that this

Table 1 Summary of sample origin, digestion methods, density separation methods, filter types and maximum MPF concentrations in recent studies of wastewater (with standardised units). Results highlight the diversity of methods and parameters in the literature, as well as the lack of large scale (high concentration) MPF analysis

Sample type	Digestion protocol	Density separation protocol	Filter type	Maximum reported fibre concentration	Ref
Sewerage + washing machine effluent	Not listed	Saturated NaCl solution	Paper filter	> 100 fibres/L	[3]
100 mL of ultrapure water with MPF washed from a mesh screen	0–50 mL 30% H_2O_2 at 60 °C until evaporated with automated stirring	10 mL NaI at 1.49 mg/L and 3500G centrifugation for 5 min	≥ 25 µm pore size, stainless steel mesh	3.3 fibres/L	[25]
50 mL of WWTP effluent combined with MiliQ water	50 mL 30% H_2O_2 at 24 °C for 2 days on a vibrating plate	Not listed	8 µm pore size, paper filters	1.65 fibres/L	[40]
1 L Untreated inflow and clarified outflow of WWTP	1L sample with 15% H_2O_2 for 5 days	Centrifuging at 6000 rpm for 20 min, then 150 g of NaCl was added to the sample for settlement over 1 day	0.7 µm pore size, glass fibre; 47 mm diameter	4452 fibres/L	[24]
5 L effluent from WWTP	20 mL 30% H_2O_2 and 20 mL 0.05 M Fe(II) at 75 °C until clear	10 mL of 1.8 g mL^{-1} NaI then centrifuged at 3500 rpm for 5 min	55 µm pore size mesh sieve	14.5 fibres/L	[41]
Wastewater effluent from a municipal WWTP	30% H_2O_2 at room temperature for 7 days	Not listed	0.7 µm pore size, glass fibre	6.79 fibres/L	[42]
WWTP samples from grit/grease removal, Primary Clarifier, activated sludge bioreactor and effluent from secondary clarifier	Not listed	120 g/L NaCl (1 Vol salt to 1 Vol sample) mechanically stirred for 20 min at 300 rpm, then 45 min of settling	0.45 µm pore size, paper filters, 110 mm diameter	< 1 fibre/L	[36]
210 mL of WWTP influent	210 mL of 30% H_2O_2 for 3 h or until the solution became clear at 60 °C	20 mL $ZnCl_2$ was added to the sample for 3 h	45 µm pore size, stainless steel mesh, 25 mm diameter	< 1 fibre/L	[38]
Grab sampling of influent, effluent, sludge and sediment in WWTP. continuous centrifuging of suspended solids	Invertebrate soft tissues treated with NaOH and 30% H_2O_2	1.2 kg NaCl/L for wastewater 1.2 g/mL NaCl for suspended solids, sediment and sludge	0.7 um pore size, glass fibre filter, 0.2 um pore size Anodisc membrane, 25 mm diameter	566 fibres/L	[43]
Secondary and Tertiary effluent from a WWTP	2–5 mL 8.25% NaClO (bleach) added to 5 mL sample	Residue from filtering centrifuged at 4000 rpm for 20 min in 15 mL tubes	Not listed	< 1 fibre/L	[26]

pore size could have resulted in many MPF passing through during vacuum filtration of wastewater samples.

There is a need to standardize the use of filters with pore sizes < 20 µm, or closer to 1 µm, to prevent the issue of underestimating MPF concentrations in non-homogenous wastewater samples. It must be emphasised, however, that using smaller pore size filters usually results in slower filtration time, so researchers must ensure samples are as clean and free of organics as possible prior to using these types of filters to prevent clogging. Alumina oxide (Anodisc) membranes are one such filter that reaches minimum pore sizes of 0.1 µm (Merk) and can effectively retain very small MP and MPF, as well as sub-micron nanoplastics. Anodisc filters are also a popular choice for studies involving the direct inspection of MP and MPF in µFTIR spectroscopy due to their rigid and semi-transparent surface that enables both attenuated total reflection (ATR) and transmittance FTIR. Leslie et al. [43] used anodisc membranes to conduct MP analysis on riverine, sediment and wastewater effluent samples, discovering that MPF < 300 µm made up the bulk of MP found in wastewater and sludge samples. They also went on to note that FTIR was unable to create spectra for very thin and colourless fibres due to the constraints and lower size detection limits of the equipment.

In conclusion, GF filters are advised for use in a variety of filtration processes; nevertheless, alumina-based filters are much more suited for spectroscopic examination.

Detection and Quantification

Commonly used techniques for MPF identification and quantification are summarised in Table 2. Also included are equipment and any additional treatment steps to facilitate MPF detection, as well as emerging methods that may increase the accuracy and efficiency of MP analysis. This section also discusses the appropriateness of available analytical techniques for identifying and quantifying MPF in wastewater.

Quantification

The counting of MPF is an essential step in the analysis procedure, and from only a small aliquot of a larger sample, researchers have been able to produce accurate and reliable measurements of MPF concentrations in wastewater. Low-cost visual counting with the aid of stereo, light microscopes with around 40 times magnification can be used to quantify submillimeter MPF (Table 2) [30, 44, 70–72]. However, they are labour-intensive and prone to human errors. Thus, most recent studies opt to inspect just a section of the filter under a microscope (such as Galvão et al. [72] or with the guidance of a gridded overlay, as with Shim et al. [11]). The use of stereo microscopes may also increase the risk of false positive identification, as well as underestimation of transparent or small particles < 100 µm, particularly when examining white filter membranes [14]. Overall, it is more reliable to utilise high-powered compound microscopes equipped with greater magnification and alternate light sources to prevent the underestimation of small and/or transparent MPF.

The use of high-end microscopes, although not as widely used nor accessible as the former (Table 2), also allows for the development of more advanced techniques in MP analysis, such as automation and/or fluorescence. Automated techniques serve to increase the efficiency, scalability and consistency of analysis by reducing human bias and input, allowing for one or multiple processes to run simultaneously. During the imaging step, for example, fine-scale, highly magnified images of filter membranes containing MPF can be automatically stitched together through the construction of a custom microscope stage, such as with Maes et al. [73] or by utilising the advanced programming and built-in stage controls of high-end microscopes such as with Tarte et al. [16]. Both with the outcome of producing detailed images for further post-processing. Automation may also occur during post-processing and data analysis to aid in MPF enumeration, as well as size/shape descriptions. ImageJ FIJI [74] has emerged as the gold standard of image-based analysis and is prevalent throughout many general MP studies as a primary means of obtaining MP concentrations and morphological data [16, 45, 75–77]. Typically, a set of customised instructions is created in the ImageJ coding language, which can perform a variety of tasks, notably binary recreation of images based on particle brightness or contrast thresholds, and listing of each particle and its morphological characteristics. Prata et al. [45], for example, designed a macro that would not only count MP from environmental water samples on stitched filter images but automatically organise particle shapes into fragments, fibres and particles based on elongation and roundness measurements, preparing an exportable report for further analysis. This method could prevent hours of human labour and potential bias and enables high-throughput, scalable analysis, particularly when combined with batch instructions, which allows for numerous images to be analysed in sequence with no human input required. Despite the obvious advantages of using ImageJ for particle analysis, there are no studies which have used ImageJ on MPF samples deriving from textiles wastewater apart from one recent study by Tarte et al. [16]. As such, further research is needed to assess how high concentrations of small MPF can be better quantified with greater time efficiency using ImageJ (Table 2).

The use of ImageJ brightness thresholding tools does require significant contrast between MP and the background medium, and one way this is achieved is through the use of staining protocols paired with fluorescence imaging.

Table 2 A summary of quantification and identification methods used to measure microplastic fibres in wastewater, their descriptions, advantages and disadvantages

Analysis Goals	Specific Technique	Description	Advantages (+) and Disadvantages (−)	Ref
Quantification or characterisation	Manual counting with a stereomicroscope	Users extract an aliquoted sub-sample and isolate it on a stable surface like a sectioned membrane filter. Recording is aided by a clicker or counter	+ Cheap and straightforward + Little technical expertise required − Extensive human labour required − Particle size detection is limited by both the microscope and human − Susceptible to human error (underestimation/overestimation) from missed fibres	[30, 44]
	Staining and Fluorescence Microscopy	Applying a fluorescent stain like Nile Red that binds to plastic polymers and is excited under wavelengths 365 nm – 495 nm	+ Enhanced detectability of MPF and quicker counting + Enables fast, contrast-based automated detection + Fluorescence microscopes often have image stitching capabilities for whole-filter, high-resolution capturing − Potential co-staining of non-plastics leading to overestimation − More costly as it requires more advanced microscopes or external fluorescent light sources	[16, 24]
	FIJI ImageJ automated counting	Importing still images containing microplastics into a publicly available software for further manipulation and application of built-in particle counting tools. Custom macro instructions can be written, allowing ImageJ to conduct a set of tasks automatically to achieve	+ Software is freely accessible + Highly powerful image manipulation and analysis tools + Customisable macro instructions can fully automate particle counting, characterisation and data organisation + Low human labour − Best utilised with sufficient training − Particle counting relies on high contrast of colour or particle brightness − Cannot differentiate between similar plastic and non-plastic particles/fibres	[16, 45, 46]
	GC–MS	Particles are heated up to very high temperatures > 600 °C, breaking down polymers into source elements for precise chemical and mass characterisation	+ High levels of chemical compositional detail, both plastic and organic + Mass measurements are very precise − Analysis destroys the particle itself − Not thoroughly assessed on MPF − Lengthy and expensive	[47–49]

Table 2 (continued)

Analysis Goals	Specific Technique	Description	Advantages (+) and Disadvantages (−)	Ref
	SEM	A single electron beam is directed at the surface of a particle, interacting with its atoms and generating signals that are read by various detectors to produce a 3D image	+ Generates extremely high-resolution images of particle surface morphology, even at the nanometre scale + Ideal for investigating shedding dynamics, durability and microscopic physical characteristics of fibres − Very time-consuming and expensive − Unsuitable for high throughput quantification − Requires ample training to achieve well-prepared images	[50–52]
	Flow Cytometry	A suspension of particles is directed down a narrow fluid stream, from which a laser beam hits and illuminates particles as they pass, scattering the light and enabling detection	+ High throughput analysis of particles < 20 µm can be achieved + Can be used in combination with fluorescence and staining protocols + Relatively little human input after sample preparation − More testing required for high concentrations of MPF − Not widely adopted in the literature	[53, 54]
Polymer Identification & Quantification	Reflectance µFTIR	The FTIR beam is directed at the particle, and the sensor detects energy that is reflected from the particle's surface. Background scans are taken from the reflective filter surface	+ No contact with particles + High resolution > 10 µm fibres + unaffected by large particle size − Gold-coated filters are expensive, although steel filters have been used as substitutes − For small particles, reliable spectra will take more time to obtain	[55–57]
	Transmission µFTIR	FTIR beam passes through the entire particle and filter, and a sensor beneath the stage detects the altered signal. Background scans taken from a transparent filter membrane	+ No contact with particles + Can be used in tandem with a vacuum filtration apparatus for easy transfer of fibres from the clean sample matrix + Alumina Oxide filters are inexpensive and easy to handle + high resolution > 20 µm − Full spectra 4000–600 cm^{-1} can only be achieved with high-expensive silicon membrane − Particles must be somewhat transparent; thick particles absorb the full spectrum − Transparent colour can be difficult to differentiate from MPF	[16, 30, 58, 59]

Table 2 (continued)

Analysis Goals	Specific Technique	Description	Advantages (+) and Disadvantages (−)	Ref
	ATR μFTIR	The FTIR beam passes through and is scattered by a tiny diamond which contacts the particle. The change in scattered beam direction upon contact is detected by the sensor	+ Higher resolution spectra in shorter time + No specific filter medium required − Contact between sensor and particle risks cross-contamination − Resolution limited to > 100 μm particles − Can be difficult to set up depending on the spectroscope model	[60]
	FPA detection FTIR	Multiple pixels within an image are scanned simultaneously to obtain potentially thousands of individual FTIR spectra	+ very little chance of misidentification + Very high resolution + Compatible with automated programming − Still takes several hours to scan a full filter membrane − Requires a μFTIR detector fitted with FPA capability	[61–65]
	μRaman spectrometry	Measures the scattering of Raman Stokes waves from a sample particle suspended in a liquid medium, or on a hard surface	+ Obtains excellent spectral resolution of small particles < 20 μm + Less affected by water presence in samples + Ideal for unclean samples with mixed matrices, meaning less sample preparation required − Limited compatibility with automated techniques − Smaller spectral database/library compared to FTIR − Fluorescence of particles leads to spectral interference	[11, 40, 51, 66–69]

Fluorescent tagging promotes easier and more consistent human or software detection of MP, which may otherwise be indistinguishable from their background, or from a mixed matrix of organic matter. Dyes such as rhodamine B, fluorescein isophosphate, Safranine T and Nile Red dye (NR) are amongst the most notable fluorescent stains, however, NR has stood out and quickly become the most commonly used in MP studies, due to its' ability to bind to plastic polymers consistently [73, 78–81]. Currently, there are a wide variety of methods used to prepare and analyse MP stained with NR, such as the concentration of and solvent used with raw NR dye, stain incubation length, and excitation wavelength used to fluoresce particles [78]. Although information on the best fluorescent procedures for use specifically with MPF in wastewater are scant, some studies have identified wavelengths of 365 nm in the UV range and minimal use of NR stain are sufficient to fluoresce polyester particles [16], as opposed to the more common 495 nm wavelength [77]. A combination of NR staining, fluorescence microscopy and automated ImageJ counting may present problems associated with inconsistent brightness and thus partial detection of fluorescent MPF [76]. However, with appropriate adjustments to programmed minimum threshold settings, these problems can be easily addressed [16, 77]. Again, as with use of ImageJ, use of NR staining protocols with MPF in textiles wastewater or with general MP is uncommon (Table 2). Going forward, users may want to consider the addition of this step for heterogenous samples containing several types of MP and MPF or to enhance detectability of plastics in samples.

Spectroscopic Analysis

Spectroscopic analysis is essential in MPF studies as the primary means of confirming or identifying polymer types isolated from a sample matrix, either on a filter membrane or mechanically transferred to a specialised slide.

When examining MPF, spectroscopic analysis will distinguish synthetic polymers like polyester, nylon and acrylic from organic fibres such as cotton, cellulose and rayon, preventing false positives, and so it is critical that — unless proper justification is provided for omitting it — this step is incorporated into every MPF study of wastewater or other environmental matrix.

Currently, two methods for polymer identification are used: Fourier Transport Infrared Spectroscopy (FTIR) and Raman spectroscopy. Raman spectroscopy is recognised as the method which enables more fine-scale analysis of MP ~ 1 µm, which can be useful for MPF with a particularly thin diameter unable to be scanned with FTIR [11, 66, 82]. Additionally, Raman spectroscopy can provide accurate spectra from particles containing water, potentially removing the need to dry samples following vacuum filtration [67]. On the other hand, Raman is vulnerable to error as a result of additional pigmentation to the polymer surface, limiting the combination of this method with staining procedures like with NR [61, 83]; however, novel solutions such as systematic photo-bleaching are emerging to solve this problem [84].

The sensitivity and resolution achievable by FTIR and micro-FTIR (µFTIR) are generally less than µRaman at > 20 µm; however, with the mean size of MPF exceeding 20 µm, particularly those shedding from clothing and textiles, this resolution is adequate, resulting in more widespread use of µFTIR in the literature than µRaman (Table 2) [67, 68, 85]. There are several methods in which µFTIR can be used to analyse MPF, all presenting different advantages and disadvantages. Firstly, Attenuated Total Reflectance mode (ATR) involves the physical contact of the sample particle with a specialised crystal attached to the FTIR machine, resulting in the highest quality and consistent spectra. However, this mode relies on MPF of large enough size, often ≥ 100 µm in width, to make adequate contact with the crystal, as well as placement on a firm, stable substrate, leading many researchers to transfer MPF to a specialised plate. So, while ATR and ATR FTIR may be suitable for fibres large enough to see and handle, it will not be appropriate for a bulk of MPF which are < 100 µm wide, often transparent, and accumulating in high concentrations on the filter membrane [62]. For very small MPF, which cannot be easily removed from the filter membrane or mesh, transmittance and reflectance modes are more suitable, as they enable fine scale < 100 µm measurements of MPF directly on the filter membrane without any physical contact or handling. For reflectance mode, specialised gold-coated membranes are the best option to achieve high quality spectra and matches, however, stainless steel meshes are also viable and much cheaper than the former [86]. Finally, µFTIR in transmittance mode also offers high quality spectral analysis of MPF > 10 µm and is commonly used in the literature. Due to the nature of transmittance µFTIR requiring transparent or semi-transparent particles and substrate, this method is often most effective with MPF not heavily laden with coating or which are too thick, as well as a filter membrane which allows the µFTIR to pass through it. Alumina oxide (Anodisc) membranes are cheaper than gold-coated membranes and enable the collection of high-quality spectra in transmittance mode, however, studies have identified that Anodisc membranes prevent the measurement of spectra 1250—600 cm^{-1}, which is a known fingerprint region of spectral signatures [16, 87]. Thus, key differences between fibres of synthetic and organic origin may not be distinguishable on the same filter membrane. Although researchers have continued to yield positive results using transmittance mode with Anodisc membranes, silicon membranes enable transmission scanning whilst gathering the full IR spectrum; however are less accessible due to the significantly greater cost per filter [58].

Scanning individual MPF in point mode is susceptible to human detection bias and has been described as slow and inefficient [55] and despite novel techniques existing to abate this, such as Renner et al. [63] using Python code to prevent scanning of blank filters across a whole filter, automation is greatly needed. Focal Plane Array (FPA) detector based μFTIR was first used as a means of analysing MP in wastewater by Tagg et al. [61] and has since been used in numerous studies to significantly reduce the time taken to scan entire filters and capture spectroscopic information on all present MP [61, 62, 64, 65, 87]. With FPA, there is minimal risk of underestimating even transparent and thin MPF on the filter surface, and it can be used in either reflectance or transmittance mode, however, further work is needed to test its effectiveness specifically with heavily concentrated MPF samples (Table 2). Additionally, while significantly reducing the time spent analysing whole filter membranes, FPA-μFTIR still takes several hours to complete [61, 64], and currently, there are no FPA-based methods which can be achieved in less than an hour. Thus, the use of this method depends on the time constraints and priorities of individual research projects. For non-time-restricted projects, FPA may be a desirable technique to incorporate given the correct training and equipment are available, however for projects with very high sample loads and minimal time, methods like random quadrat surveying in point mode, as with Tarte et al. [16], maybe a more suitable option that has already been proven to work with MPF.

Non-spectroscopic Methods

Several novel and lesser-used methods exist which may complement or validate the quantification and spectroscopic techniques previously described in the "Digestion" and "Density separation" sections. Pyrolysis gas Chromatography/Mass spectrometry (GC–MS) is one such technique that can provide highly detailed information on the chemical composition and mass measurements of MP particles simultaneously with organic contaminants, reducing the need for digestion protocols [47]. On the other hand, calibration of GC–MS is lengthy, and its use does not apply to several types of plastics such as polyvinyl chloride (commonly known as PVC) [48], nor has it been sufficiently tested on MPF. Additionally, GC–MS is a destructive technique, preventing further analysis of particles, so this technique may be more appropriate when in combination with μFTIR or μRaman rather than as the primary means of polymer identification [47, 49].

Scanning Electron Microscopy (SEM) provides extremely detailed imagery of MP and MPF surface topography and can serve a variety of purposes, including: measuring the influence of laundry chemicals on fibre structure [50], to distinguish MPF from a sediment substrate [51] or simply as a means of reiterating fibre texture and shape [52]. Currently, SEM is limited in its ability to quantify multiple MPF across a large surface area, as this would take a very long time, but researchers with the necessary resources may benefit from including SEM imagery in their respective MPF studies.

Finally, flow cytometry, although not widespread in the literature for MPF studies, has presented several advantages over other techniques, namely the speed and resolution at which MP can be quantified and characterised [88]. Hyeon et al. [53] for example, were able to compare the effectiveness of two different membrane filters, alumina oxide and silica carbide to establish that MPF morphology had a significant impact on retention rates, and generally, alumina oxide membranes retained more MPF and organic matter than silicon. Bianco et al. [54] combined flow cytometry with NR staining protocols to quantify PE particles < 16 μm in a matter of seconds; however, further research is needed to assess whether this method is transferable to other types of plastics common for MPF like polyester.

To reiterate, the aforementioned methods have much potential to accelerate scalable and efficient MPF analysis, however, researchers should avoid relying solely on these methods for analysis as they are currently not standardised or widely adopted, and instead they may be used as complementary methods to enhance data collected by general microscopic imaging and/or μFTIR.

Quality Control and Quality Assurance

Quality control and quality assurance (QA/QC) is a vital element in all MP analysis, but especially MPF workflows, where even a few external fibres can heavily skew results, leading to overestimation of measurements [89]. Sources of cross-contamination include: fibres retained within a container from a previous sample, or suspended airborne fibres which are prevalent [90] and may settle on exposed filter membranes. To prevent cross-contamination, it is commonplace to thoroughly wash any jars, beakers or other glass apparatuses that have contacted the wastewater sample before moving to the next sample. Using a combination of MiliQ or ultrapure water from a squirt bottle frequently with ethanol spray and antistatic wipes can effectively remove any sticky particles adhering to the surfaces of equipment [89]. Covering sample containers with a non-plastic lid and handling them in secure vacuum hoods are also highly recommended. During microscopy and spectroscopy, longer processing times leave filter membranes vulnerable to atmospheric particle settling, so the use of freshly wiped glass cover slips will prevent accidental inclusion of unwanted particles in images. The donning of 100% cotton lab coats will assist in preventing the shedding of fibres from

polyester or plastic-related clothing from affecting sample analysis [59].

Additionally, the sample may become contaminated at various points, thus, it is also crucial to prepare the blank samples in a laboratory setting. Therefore, while reporting the results, a blank correction should be considered. Another element of QA/QC that is often overlooked is the inclusion of recovery tests to ensure the accuracy and reliability of real sampling, and determine the level of over/underestimation occurring [91]. Recovery rate tests normally involve a lab-prepared clean water matrix spiked with lab-grade MP standard particles of a known weight. A stock dispersion of PA particles, for instance, with a diameter of 63–90 µm and a particle count of 1131–198 particles/mL, was made using RO water and 0.025 percent Tween. Three to five recoveries of this stock were conducted for various waterways [59]. Additionally, while employing different analytical tools for the detection and quantification of the MPF, the limits of detection and quantification should be considered.

Implications and Future Outlook

The purpose of this review, on top of detailing how MPF are currently being extracted and analysed for a scientific audience, is to inform agencies, industries and the wider public on the most appropriate, reliable and accessible methods for quick and appropriate sample preparation and measurement of MPF in wastewater samples. Similar reviews on this topic have either neglected or omitted important elements of the analysis procedure that may be of particular concern to professionals not previously versed in MP handling from wastewater, such as decisions regarding equipment purchases at each step or the likely length of time certain techniques may take. Non-scientific readers may wish to extract only small pieces of information from this review; however, one must consider that the goal of the present research is to describe the process of isolating and analysing only microplastic fibres from relatively clear wastewater. The recommendations in this review should *not* be used to inform decisions regarding the analysis of sediment, sludge or heavily soiled samples; however, for liquid samples, the recommendations are made with cost and time efficiency in mind, aligning with common industry goals.

One of the main sources of microplastic pollution is MPF, which is mostly released from synthetic textiles during washing. Although wastewater systems serve as important MPF conduits, their release cannot be adequately managed or monitored with the current treatment infrastructure and analytical techniques. Research on preservation techniques (such as refrigeration and chemical stabilisation) to stop fibre degradation between collection and analysis, as well as mandatory and standardised protocols for sampling MPF, are among the innovations in sampling, separation, detection, and policy that are required to progress the field. Additionally, research is needed on improved automatic image recognition systems for classifying plastics based on shape and colour. Research on AI-assisted analysis pipelines to automate polymer classification and fibre counts is also advised. Ultimately, future studies must discuss methodologies in greater detail to better highlight minor differences in specific parameters and treatment/quantification conditions, promoting replication of experiments and the standardisation of analysis protocols.

Conclusion

This review examines various methods for collecting, preparing, and analysing microplastic fibres in wastewater samples as well as their benefits and drawbacks. We suggest the following MPF analysis workflow: Grab sampling 1–2 L once or twice daily from a designated tap or outflow point throughout the WWTP. Avoid using sieves or nets if leaving sample collection unattended for too long, as clogging can occur. With sample preparation, digestion with a 30% H_2O_2 solution in a wastewater aliquot at 100 °C for a few hours should sufficiently dissolve trace chemicals and detergents present in the water. Density separation can be omitted unless heavy sediments are present. The prepared samples (~ 10–20 mL) must be vacuum-filtered using glass filters for quantification and alumina oxide filters for spectroscopic analysis. After filtering, glass filters are visualised with a powerful microscope at 365 nm under UV light while a high-resolution photo (~ 40 × magnification) is taken. For particle counting, exporting the image to ImageJ programme and converting it to binary is advised. Lastly, the use of micro-FTIR is strongly advised for the identification of the type of polymer in MPFs of over 20 µm in length.

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- Akyildiz et al. (2022) is a recent study of MPF release from a WWTP servicing the textiles industry and is the culmination of several previous studies of the same author which describe optimal methods for MPF pre-treatment.

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Declarations

Competing Interests The authors declare no competing interests.

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