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Identification and Quantification of Microplastics in Aquaculture Environment

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The existence of microplastics (MPs) poses a potential threat to the entire ecosystem and has gained wide public attention. As an essential source of aquatic products, aquaculture industries are inevitably subjected to the pollution of MPs, particularly when the plastic products are widely used in aquaculture. Even so, the identification of MPs in aquaculture is rarely reported. Hence, high-efficient analytical methods for accurate detection of MPs in the aquaculture environment are of utmost significance. This review comprehensively summarizes the analytical methods for MPs in aquaculture, including sampling, extraction, and qualitative and quantitative analyses of MPs. MPs are identified and quantified mainly by visual inspection, spectroscopy, or thermal analysis. In addition, this review also points out the limitations of these methods and the accuracy of quality control. Finally, the need for establishing standard methods is emphasized, and suggestions for future research are also proposed.

Keywords: microplastics, analytical method, sampling, identification, aquaculture environment

INTRODUCTION

The ever-increasing human population has generated a remarkable amount of plastic waste. According to the statistical analysis, the global production of plastics has reached 370 million tons by 2019 (PlasticsEurope, 2020). Plastics are widely utilized in all aspects of our daily life due to their low cost, durability, good ductility, and lightweight, resulting in the accumulation of plastic wastes in the environment, which are inevitably introduced into the ocean through various pathways. Therefore, the ocean may become an immense reservoir for plastic wastes. The natural decomposition of plastics is extremely slow. The plastic waste will be broken into small plastic pieces after physical, chemical, and/or biological action (Wright et al., 2013). Among these, plastics with particle size <5 mm are defined as "microplastics" (MPs) (Erni-Cassola et al., 2017), which was first proposed by Thompson et al. (2004).

In general, MPs are classified into primary MPs and secondary MPs (**Figure 1**). The primary MPs are derived from microbeads in cosmetics, cleaning products, and air-blasting media, which can directly enter the environment (Du and Wang, 2021). The secondary MPs are derived from the

decomposition of larger plastic pieces. Pollution of MPs has gained significantly more research attention in recent years due to its persistence in the natural environment and potential adverse impacts on organisms. MPs can migrate in various environmental compartments, such as air (Dris et al., 2016), soil (Blaesing and Amelung, 2018), oceans (Andrady, 2011), and freshwater (Dris et al., 2015; **Figure 2**). They are easily ingested by organisms and translocated to higher trophic levels through food web. The exposure of MPs to humans aroused severe health concerns, encouraging to explore the removal techniques of MPs. At present, we have been plagued by the pollution of MPs. Therefore, techniques with high sensitivity and selectivity should be developed for the detection, identification, and localization of MPs. MPs can be detected either directly in environmental samples (e.g., aerosols, sediments, soils, and water) or within organisms originated from various habitats. So far, research on the detection of MPs in the aquatic environment has been conducted in some typical farms (**Supplementary Table 1**). These results exhibited that MPs have become a potential source of pollutants in the aquatic environment. Aquatic products are a significant source of human food. In recent years, world aquaculture production of cultured aquatic animals has grown steadily. To achieve the goal of sustainable aquaculture, some countries intend to produce more aquatic animals from aquaculture. Thus, aquaculture environments, such as ponds, lakes, rivers, and oceans, were inevitably contaminated by a plethora of pollutants including MPs that can be detrimental to the growth and development of aquatic organisms and, finally, end up in the human body through food chain, posing a serious threat to human health (**Figure 3**). In contrast, the review regarding the pollution of MPs in aquaculture systems is limited.

To better map the abundance of MPs in the aquaculture environment and potential damage to organisms, future research should focus on the identification and quantification of MPs across the world. The analysis of MPs, including the sampling, preparation, and identification, has been reported (**Figure 4**). Nonetheless, to date, the standardized protocols for sample collection and subsequent analysis have not been proposed. At present, the approaches utilized to detect MPs in the aquaculture environment are rarely reported.

In summary, the goal of this review is to (1) present the most common method for the identification and quantification of MPs in the aquaculture system, (2) discuss the limitations, quality control, and quality assurance of these methodologies, and (3) propose the existing knowledge gaps and recommendations for future direction on the detection of MPs in the aquaculture environment.

LITERATURE SEARCH STRATEGY

A systematic literature review was performed to retrieve literature regarding the detection of MPs in aquaculture environment resulting from Google Scholar, Science Direct, Web of Science, PubMed, and other commonly used databases using the combination of keywords, including MPs, detection, identification, quantification, and aquaculture system. The obtained items were further refined to peer-reviewed research articles. The special keywords "microplastic and aquaculture"

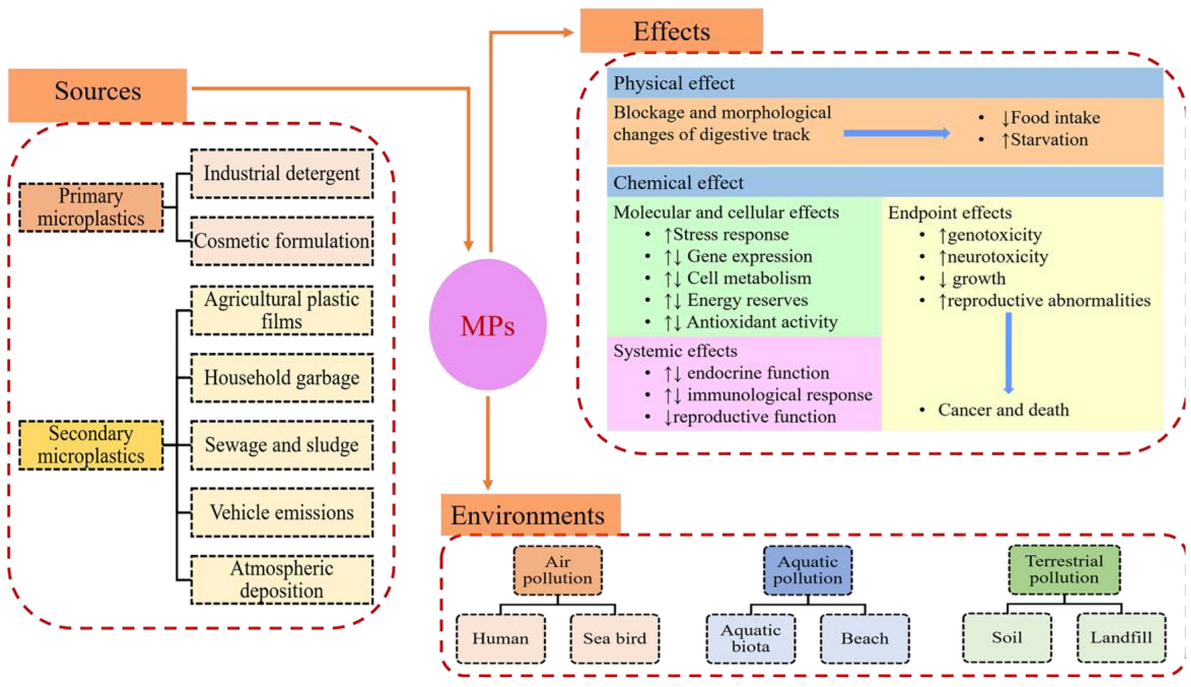

FIGURE 1 | The source and fate of microplastics (MPs).

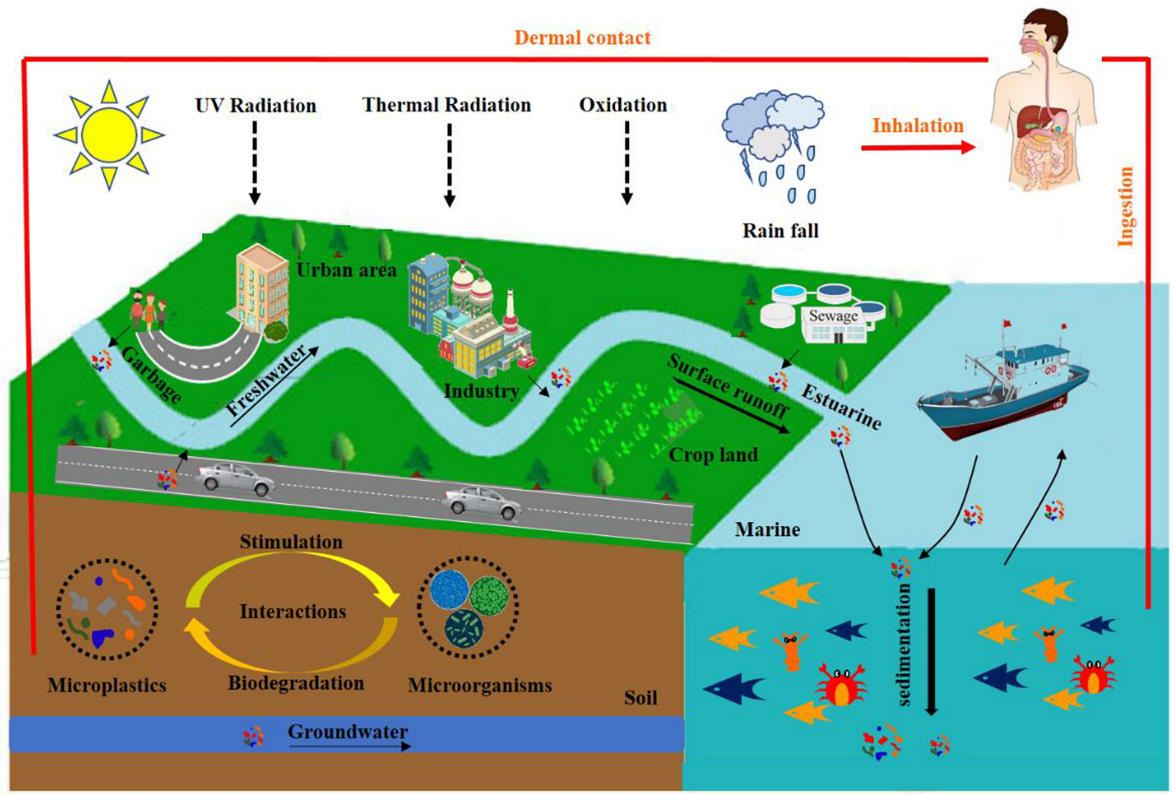

FIGURE 2 | The migration of microplastics (MPs) in the natural environment.

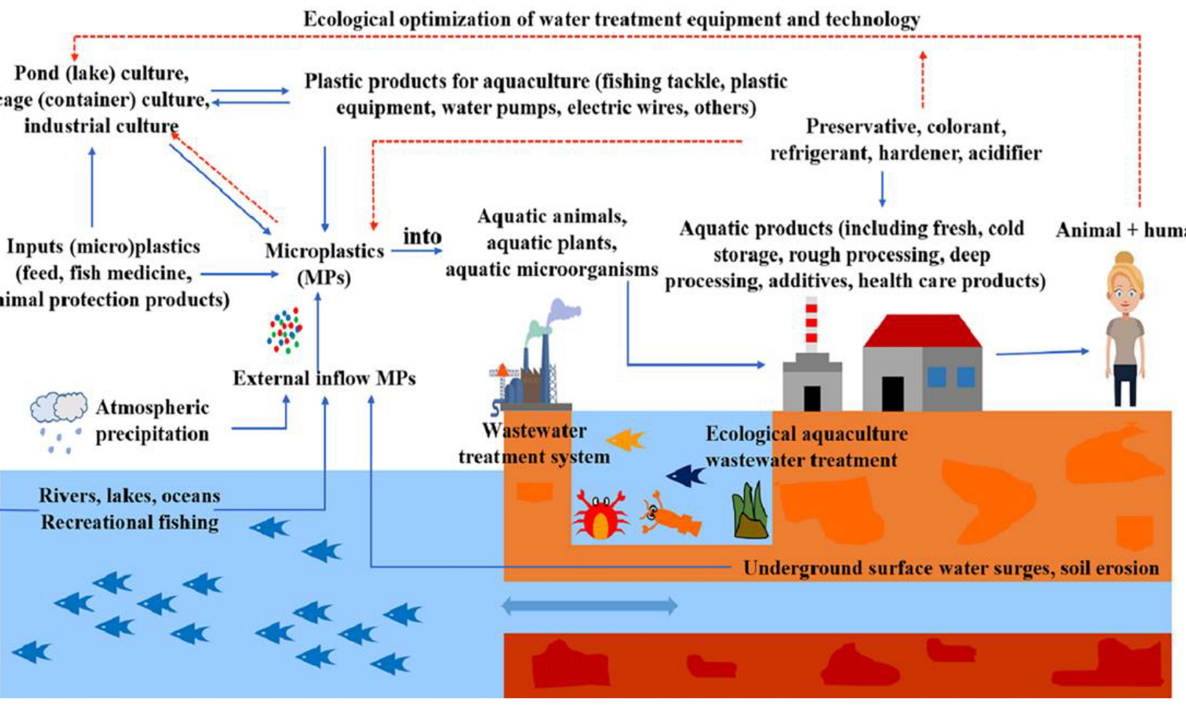

FIGURE 3 | The migration of microplastics (MPs) in aquaculture system (reproduced with permission from Zhou et al., 2021).

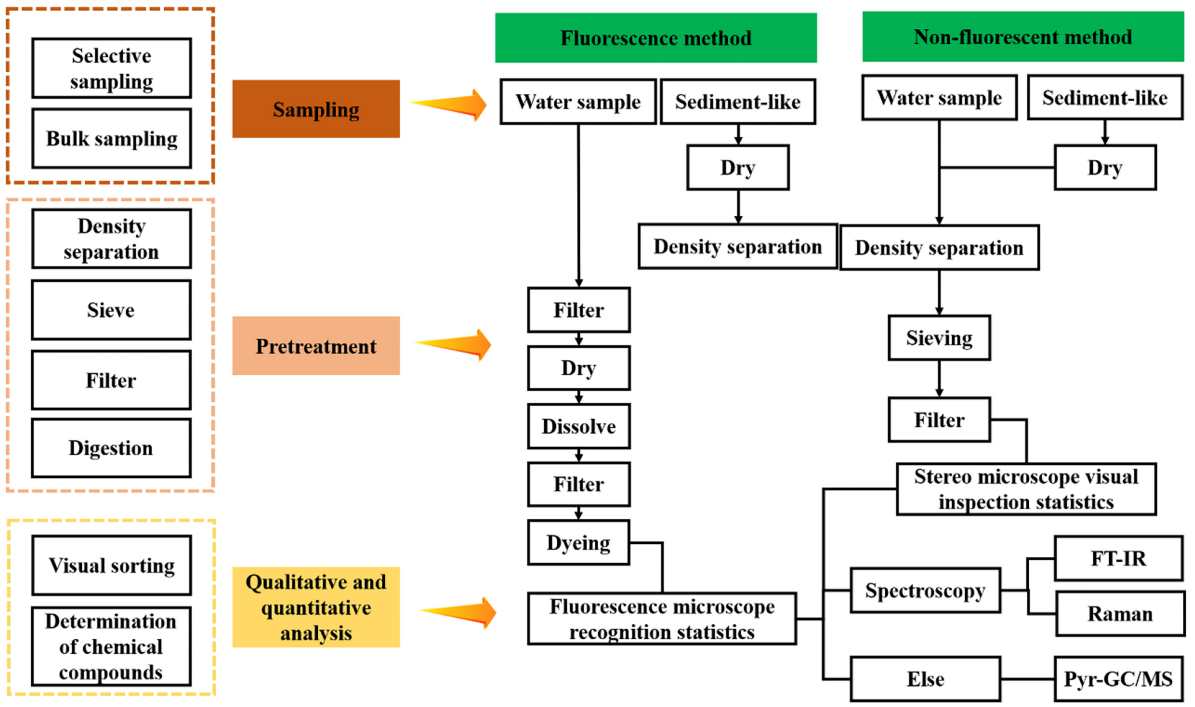

FIGURE 4 | Typical treatment and analysis process of microplastics (MPs).

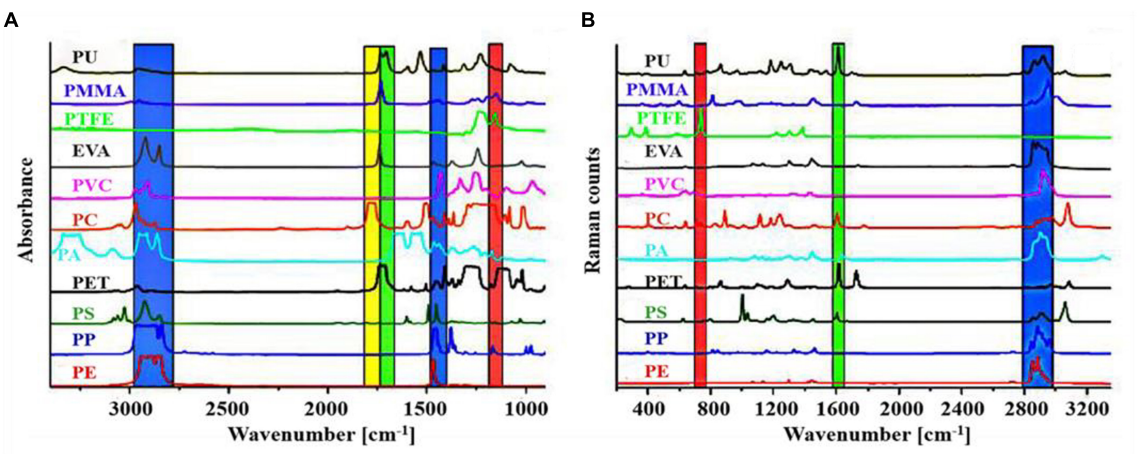

FIGURE 5 | (A) Fourier transform infrared (FTIR) (transmission mode) and **(B)** Raman spectra of various kinds of microplastics (MPs) (reproduced with permission from Käppler et al., 2016).

were applied as the key research criteria. We also examined and retrieved the reference list of each retrieved paper when it is necessary. Overall, research articles about the detection of MPs in the aquaculture environment were involved in this review.

SAMPLING COLLECTION

Collection of samples is the first step of sampling methods of MPs. The most common sampling methods of MPs include selective sampling, volume-reduced sampling, and bulk sampling (Hidalgo-Ruz et al., 2012). MPs are directly extracted from samples by visual identification, which is defined as selective sampling. Volume-reduced sampling indicates that samples are filtered and sieved, and thus the target components can be used for additional analysis at the sampling location. Bulk sampling does not separate components on-site and keeps all samples. For large water body areas, static sampling is carried out at each sampling point, and filtering sample collection is generally selected. For example, Wang et al. (2017) used a precleaned 12 V

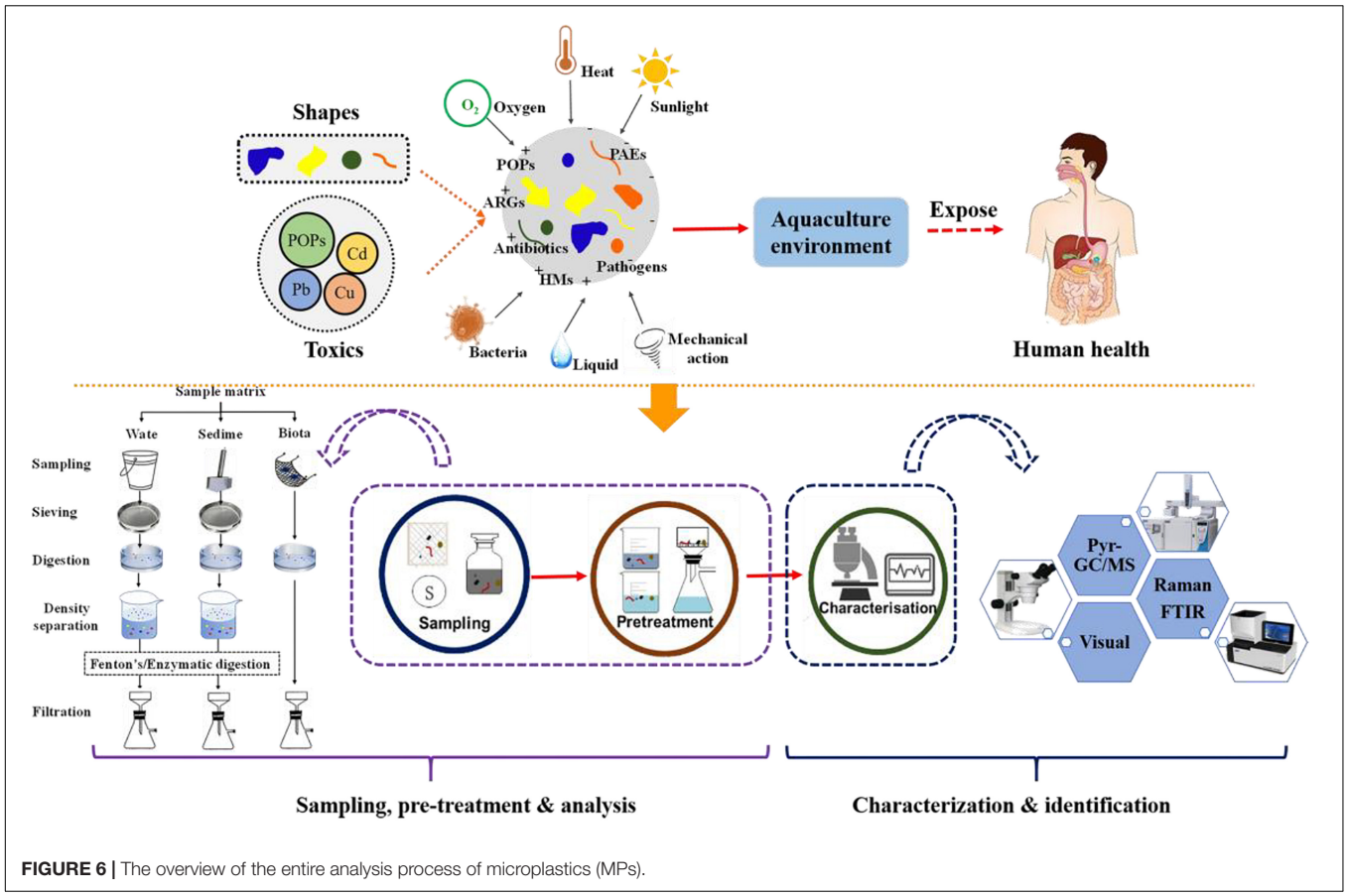

FIGURE 6 | The overview of the entire analysis process of microplastics (MPs).

DC Teflon pump to collect 20 L of surface water sample (0–20 cm in depth) and then the samples obtained were passed through a 50-μm stainless steel sieve.

Microplastics in aquaculture environment can be collected from the water surface or the water column at certain depths (Wang and Wang, 2018). For surface water sampling, neuston nets, and manta trawls are the most commonly utilized tools, but for water column sampling, near-bottom trawls, multiple opening–closing nets, continuous plankton recorders, bongo nets, and plankton nets are the main tools. Some alternative equipment are occasionally applied in surface water or water column sampling for MPs, such as water intake pumps, water collection bottles, or plankton traps. The smaller the size of the mesh, the smaller the particle size of the collected MPs (Uddin et al., 2020). The mesh can be blocked by algae or organisms if the aperture size becomes small. However, when the aperture size was increased, the toxic and small plastic particles may not be collected. In the aquatic environment, the depth of the water should be considered for sampling, and it is generally acknowledged that the abundance of MP surface is higher than that from water depth of 1–2 m. The used trawl nets varied between sampling depths. Samples from surface water used trawl-type sampling devices such as Manta trawl (Ta and Babel, 2020a,b). Bongo nets for mid-water samples and benthic trawls for deeper water from the bottom layer were applied. Typically, the mesh apertures most commonly used for the net are 333–335 μm (Du et al., 2021; Tirkey and Upadhyay, 2021).

SEPARATION AND PURIFICATION

Density Separation

Microplastics tend to float on the water surface due to their lower density than water. The target component and impurities can be separated by density flotation according to their density differences. To be specific, for density separation, the flotation solution was added to the sample, and then MPs were collected through a series of processes such as stirring, mixing, standing, and settling, and finally, the supernatant was separated. The density of most MPs is in the range of 0.80–1.40 g/cm^3 (Ivleva et al., 2017). Generally speaking, MPs with a density of 1.40 g/cm^3 can be obtained using a flotation solution.

The solution of NaCl is used extensively for the separation of MPs because it is cheap, readily available, green, and non-toxic (Eerkes-Medrano et al., 2015; Ma et al., 2020). Other flotation solutions are more efficient but limited to their expensive (SPT, NaI, etc.) or may pose a threat to the environment ($ZnCl_2$, etc.) (Ivleva et al., 2017). In recent years, researchers have developed several MP separators based on density separation to improve their recovery rate by optimizing flotation patterns (Imhof et al., 2012; Nuelle et al., 2014; Karlsson et al., 2017). Moreover,

Imhof et al. (2012) successfully developed a new device that can separate MPs <20 μm. Studies on the salt solutions for polymer separation are summarized in **Supplementary Table 2**.

Filtration and Sieving

Filtering or sieving is the most commonly used approach for separating the supernatant containing MPs from sediment samples and MPs from density separation of water samples. However, there exist some differences. For example, for filtration, the MPs onto filter membrane are obtained using a vacuum pump (Su et al., 2016), and sieving is performed directly onto screens with different pore sizes through gravity (Baldwin et al., 2016). The particle size of MPs collected depends on the size of the sieve and filter apertures. Generally speaking, the pore size (0.45–2 μm) of the filter membrane is smaller than that of the screen (Desforges et al., 2015; Fok and Cheung, 2015; Kim et al., 2015).

However, for the screen, some disadvantages, including severe blockage, its inapplicability to a wide range of sizes, and time-consuming (Mai et al., 2018), still exist. The separation efficiency of MPs can be improved by screens with various aperture sizes. However, the filtration may face the disadvantage that MPs may adhere tightly to the filter membrane, and it is difficult to remove the MPs. To address this issue, Hoffman and Turner (2015) found that C_3H_8O (50%, V/V) was a suitable detergent for the removal of components onto the filter membrane.

Digestion

Environmental samples contain biological materials that are often confused with MPs, resulting in the overestimation of environmental concentrations and increasing the number of MPs subjected to further analysis. The objective of digestion is to remove organic impurities that interfere with the identification of MPs. It is widely used in the preparation of biological, sewage, and sludge samples. Three common methods are used for the sample pretreatment, such as enzymatic digestion, alkaline digestion, and acid digestion. In particular, for enzymatic digestion, which is a time-consuming process, each enzyme works under its optimal temperature and pH condition, which must be monitored and retained through the experiment (Tirkey and Upadhyay, 2021). Wang et al. (2019) first extracted the MPs, then treated the dried sample with 30% H_2O_2 to digest the organic matter, and finally suspended and filtered the MPs by adding a saturated NaCl solution. Anderson et al. (2017) found that the solution containing H_2O_2 and Fe^{2+} was more beneficial to the oxidation of organic compounds. Researchers applied different kinds of digestion solutions including 100 g/L KOH, 10 mol/L NaOH, 30% H_2O_2, 69% HNO_3, HNO_3:HCl (1:1, V/V), and HNO_3:$HClO_4$ (4:1, V/V) to digest MPs, and they concluded that KOH (100 g/L, 60°C) posed severe damage to MPs (Zou et al., 2019).

MICROPLASTIC IDENTIFICATION

Visual Observation

Visual observation is suitable for plastic particles with large size (>1 mm) (Song et al., 2015). MPs are manually identified and sorted before being counted according to their physical characteristics (e.g., color, shape, and hardness). With the help of optical microscopes, electron microscopes, and scanning probe microscopes, it is possible to obtain more information on MPs. For example, scanning electron microscopy (SEM) can provide extremely high magnification and clear images with a resolution of up to 0.1 μm, which is capable of distinguishing MPs from organic particles. Ribeiro-Claro et al. (2017) believed that particles of MPs with various shapes (e.g., irregular polyhedral, hexagons, spheres, and fibers) and sizes can be accurately determined by SEM. Patterson et al. (2019) initially identified MPs from the Tuticorin coast, Gulf of Mannar, Southeastern India, by using stereomicroscope and then verified the composition of the polymers by Fourier transform infrared (FTIR)-attenuated total reflection (ATR). These results showed that polyethylene (PE) fiber (0.25–0.5 mm) is most common in oysters and seawater. Zhu et al. (2019) characterized the morphology of MPs with the assist of a stereomicroscope and then identified some plastics-like using μ-FTIR. They found that MPs were abundant in the Maowei Sea, a typical mariculture bay.

This method has the advantages of simple operation, low cost, and non-toxic. However, there are many substances similar to MPs in the environment, which readily cause misjudgment (Eriksen et al., 2013). Nile red (NR) can enhance the recognition efficiency. For example, Shim et al. (2016) demonstrated that MPs can be readily identified using an NR solution (5 mg/L) in *n*-hexane that can effectively dye plastics.

Fourier Transform Infrared

Three modes including ATR (Klein et al., 2015; Mani et al., 2015; Imhof et al., 2017), reflection (Harrison et al., 2012; Vianello et al., 2013; Ter Halle et al., 2017), and transmission (Frias et al., 2014; Löder et al., 2015) modes are applied for FTIR. FTIR mainly provides chemical bond information of compounds. The generation of peak types and specific spectrum rely on the bond structure. In comparison with the standard library, MPs can be distinguished from other organic and inorganic substances. The composition of MPs can be identified if the matching degree of MPs detected by infrared spectroscopy reaches more than 70% with the standard library.

The FTIR method was also widely used for the characterization of MPs because of simple operation and accurate identification. However, this method is time-consuming (Käppler et al., 2018), and it is easily affected by plastic inhomogeneity and material aging (Zhou et al., 2015). In addition, some plastic particles with size <20 μm cannot be detected. Micro-FTIR not only improved spatial resolution but also enabled the detection of smaller plastic particles. Garcia et al. (2020) used micro-FTIR to identify MPs isolated from fish tissues and found that the main polymers are PET, PES, and PE. Käppler et al. (2016) identified MPs in the environment using Raman spectroscopy and FTIR and compared and analyzed their advantages and disadvantages. They found that the samples were capable of being detected in different spectral ranges for synthetic polymers, as shown in **Figure 5**.

Raman Spectroscopy

The vibrational spectroscopy technique based on the inelastic scattering of light is defined as Raman spectroscopy. Based on the scattering spectra of different frequencies of the incident light, it is possible to obtain the molecular structure of substances (Chen et al., 2020). Characteristic spectral fingerprints can be achieved by detecting the molecular vibration of a sample through Raman spectroscopy, and the composition of the sample is identified by comparing it with a known reference spectrum.

Not only Raman spectroscopy can obtain information on the functional groups on the surface of MPs, but it also allows the observation of local microscopic features (Collard et al., 2015). Raman spectroscopy with a high spatial resolution (<l μm) (Oßmann et al., 2018) is not sensitive to interference signals from water and atmospheric carbon dioxide. Moreover, the fluorescence of the sample influences the Raman spectroscopy signal. Zhao et al. (2015) identified the MPs (polypropylene and PE) in the three estuaries of China using Raman spectroscopy. Collard et al. (2015) proposed a new extraction method based on hypochlorite digestion and ultrasonic treatment for the separation of MPs from membranes. This method is appropriate for the subsequent analysis of Raman spectra. It can avoid fluorescence and allows better identification of artificial particles in fish stomachs.

Raman spectroscopy has the advantages of higher spatial resolution and no interference from water, and some information can be obtained using Raman spectroscopy rather than infrared spectroscopy. However, the fluorescence effect from the pigment, additive, or contaminant in the environmental sample affects the measurements of sample with Raman spectroscopy, and the detection time of Raman imaging is remarkably higher than FTIR imaging.

Pyrolysis Gas Chromatography/Mass Spectrometry

Currently, pyrolysis gas chromatography/mass spectrometry (Pyr-GC/MS) is the most commonly applied method for the characterization of polymers. In the Pyr-GC/MS technique, the polymer was pyrolyzed under an inert atmosphere, and then the pyrolyzed products were fed to GC-MS, where GC separates them and pyrogram is formed. The pyrogram of the unknown samples is compared with developed or available reference pyrogram to know the composition of the polymer mass under investigation (Käppler et al., 2016). This technique has the advantages of small sample amount, qualitative and quantitative analyses, no additional reagents, and so on, but it requires stringent experimental conditions (Dekiff et al., 2014). Fischer and Scholz-Böttcher (2017) used the Pyr-GC-MS method to simultaneously identify and quantify many typical MPs including PE, PP, PS, PVC, PA6, PMMA, PET, and PC. Hermabessiere et al. (2018) characterized MPs with Pyr-GC/MS by optimizing the experimental conditions, and thus the detection signal was enhanced and detection time was shortened greatly.

TDS (thermodesorption)-GC/MS first heats the sample up to 800°C under inert conditions such as N_2 and then detects the sample composition by GC-MS (Li et al., 2017). It analyzes sample volumes of up to 100 mg, but it is limited to qualitative analysis. Dümichen et al. (2015) identified and quantified the characteristic decomposition products of spiked PE in complex environmental samples by combining thermogravimetric analysis coupled to solid-phase extraction (TGA-SPE) and TDS-GC/MS. This method with fast sample cleanup does not require any visual classification. However, it is only suitable for the samples that can be easily cleaned and for known polymers. In summary, the identification and quantification of MPs in the aquaculture environment by these analytical techniques are exhibited in **Supplementary Table 3**.

QUALITY ASSURANCE AND QUANTITY CONTROL

During the entire sampling process, quality assurance and quantity control are vital for data accuracy. Researchers should wear 100% cotton clothes and latex gloves. The collected samples are sealed in polythene bags and aluminum foil to avoid the interference of atmospheric MPs (Noik and Tuah, 2015). Since MPs are ubiquitous in the air, a series of blank tests must be conducted to minimize the impact of environmental pollution. The MPs collected on-site should be compared with those in the standards library (Ng and Obbard, 2006).

During the on-site sampling process, a procedure blank and standard addition blank should be prepared (Hanke et al., 2013). Non-plastic materials such as glass bottles should be used during the detection process. The glass bottle should be prewashed in a nitric acid bath and then rinsed with ultrapure water three times.

CONCLUSION AND FUTURE RECOMMENDATIONS

This review briefly summarized the sampling, separation, and purification of MPs in the aquaculture environment (**Figure 6**). In addition, we also compare the superiority and limitations of various characterization techniques. Nonetheless, some knowledge gaps still exist. Future recommendations for the detection of MPs are as follows.

a) During the sampling process, for surface water in lakes or oceans, manta trawls or nets are suitable. For the separation of MPs, density separation is recommended. For the purification of MPs, the Fenton reaction that can effectively oxidize the organic compounds was recommended.
b) It is very difficult to quantify and identify MPs with a single method. The combination of various techniques is recommended for the identification of MPs.
c) MPs can be detected using electrochemical method because surface of MPs with the similar properties to colloid is easy to be charged in the aqueous environment.
d) Establishing standards for quantitative and qualitative analyses of MPs is extremely essential.

AUTHOR CONTRIBUTIONS

SX and YX: conceptualization. XS: resources. SX and HD: data curation. SX and XS: writing—original draft preparation. JW and SX: writing—review and editing. JW: visualization, supervision, project administration, and funding acquisition. All authors have read and agreed to the published version of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.804208/full#supplementary-material

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