The Recovery of Microplastics from Rock Oysters Using Digestion Method

Che Nan, Siti Nursyamsulbahria; Razali, Azaima*+

Department of Chemistry, Kulliyyah of Science, International Islamic University Malaysia (IIUM), Kuantan Campus, Kuantan, Pahang, MALAYSIA

Miskon, Mohd Fuad

Department of Marine Science, Kulliyyah of Science, International Islamic University Malaysia (IIUM), Kuantan Campus, Kuantan, Pahang, MALAYSIA

ABSTRACT: *The widespread deposition of microplastics (<5.0 mm) in the marine environment has appeared to be pervasive across the globe. It has led to the major attention of many researchers to study this problem. Despite the amount of work conducted to understand these infamous microplastics, there is still no standard procedure for microplastic extraction from marine organism samples. This study investigated three types of digestion treatments; (1) KOH, (2) KOH/H_2O_2, and (3) KOH/NaClO, followed by density separation using 50% KI to extract the spiked microplastics from the rock oyster. Each treatment was tested to study the digestion effectiveness of the organic soft tissue materials while preserving the microplastic particles. Aside from recovering the spiked microplastics, other small contaminants have been detected in each treatment. All the spiked microplastics and the contaminants obtained were analyzed using a microscope and FT-IR for characterization. From this study, it was observed that each treatment resulted in high microplastic recovery. Among the three treatments, using 10% KOH alone provided the highest digestion rate, but it required more time to digest the oyster soft tissue. The contaminants detected in the oyster suggested the possibility of microplastic accumulation in non-digestion organs through adherence.*

KEYWORDS: Microplastics; Digestion; Comparison; Oyster; Adherence.

INTRODUCTION

Plastic is very convenient, thus making it the highest manufactured product in the world. However, this convenience is the reason why plastic poses a serious environmental hazard [1]. Mass plastic production leads to an abundance of plastic debris in the ocean and along the coastline [2]. At sea, plastic debris continuously breaks down into small fragments when exposed to heat, light, weathering, and mechanical wave action, eventually becoming microplastics – small solid particles consisting of polymer mixture <5.0 mm size [3]. Due to this phenomenon, the number of microplastics polluting the world's oceans increases every year [4, 5]. A large number of marine organisms are being threatened or silently killed by these tiny plastics through chemical leaching, entanglement, and ingestion

* *To whom correspondence should be addressed.*
+ *E-mail: azaimarazali@iium.edu.my*
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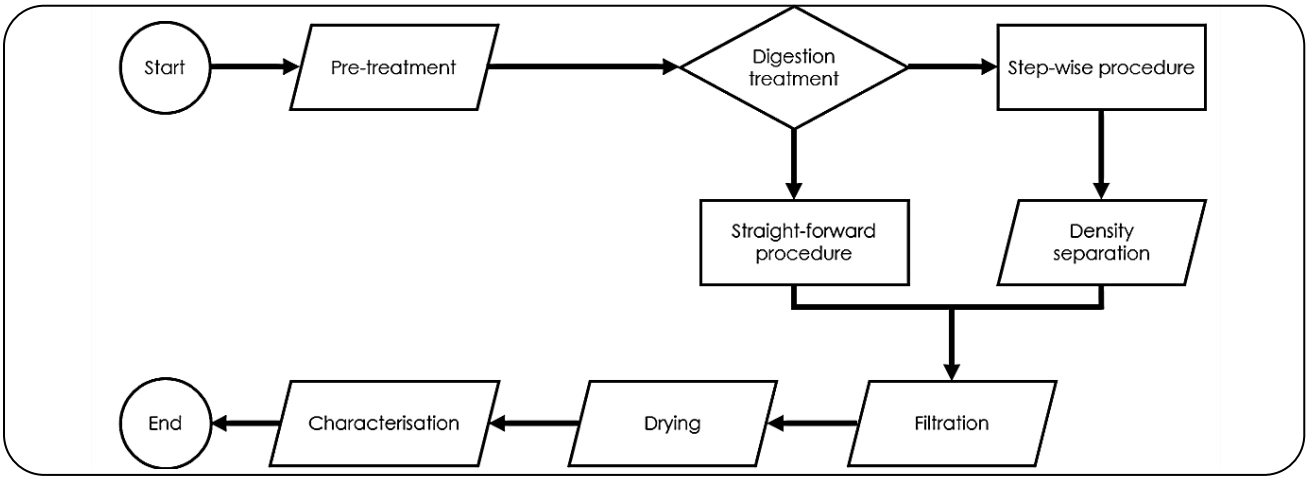

Fig. 1: General procedure of microplastic extraction from marine biota samples.

plastic litter [1, 2, 6, 7]. Microplastics that are being ingested unintentionally by marine organisms may affect food safety and human health via bioaccumulation and biomagnification [1]. Edible marine biota such as oysters that are contaminated by microplastics might carry a significant amount of hazardous chemicals which could risk the health of seafood consumers [8]. In addition, the situation is expected to become direr as plastic debris may persist in marine habitats for centuries [9].

Oysters are bivalve filter feeders that filter large quantities of seawater to sustain themselves. In this process, oysters are particularly exposed to the accumulation of microplastics, chemical pollutants like metals, and marine microorganisms like pea crabs [10–12]. Oysters have been widely used as sentinel organisms or bioindicators to monitor contaminant levels in marine environments. This is because oysters are easily accessible; it has a broad geographical distribution and oysters are able to tolerate high salinity of a substantial range [10].

The quantification of microplastics in marine biota is crucial for assessing its devastating impacts and investigating its possible pathways through the food web [13,14]. With this, various methodologies have been proposed to develop the most efficient technique for microplastic extraction from marine biota samples. However, the abundance of methodologies makes it difficult to conduct comparison studies. Therefore, a standard methodology regarding this matter is needed to obtain comparable results [1, 15].

The general procedure for microplastic extraction is summarised in Fig. 1. In recent years, among all existing methods, the digestion method is the most commonly used for microplastics extraction from marine biota samples [16, 17]. The digestion method can be used in a stand-alone procedure or in combination with the density separation method [6, 18]. Some marine biota samples require a step-wise procedure to further optimize plastics isolation from the sample [16]. Based on Fig. 1, pre-treatment is involved as it can reduce the complexity of the biota samples for digestion [19–21]. For example, the shell is removed from the mussel's soft tissue [18] and the bones are removed from the fish [6]. After the digestion treatment has successfully extracted the microplastics from the marine biota samples, filtration will be conducted followed by drying the microplastics at optimum temperature. All the extracted microplastics will then be characterized physically and chemically.

Microplastics study is challenging due to its small size and the complexity of the tissues in which it is accumulated. The presence of natural debris like sediment, seashells, and parts of marine organisms and plants could often be mistaken as microplastics during the visual examination under the microscope. Its presence is due to the low digestion efficiency of the soft tissue. It is likely to float on the surface of the supernatant during the sedimentation process since it usually has low density. Consequently, natural debris is highly likely to be filtered together with microplastics and can lead to misidentification. Previous work reported difficulties in distinguishing plastics from natural debris when analyzing microplastics [22], so it is suggested that samples with high natural debris content require prior removal of natural debris. This is known as matrix removal This technique has also been reported by *Enders et al.*, 2017 [13].

Table 1: List of digestion solutions used to digest the marine biota soft tissue.

	Digestion solution	References
Acid	Hydrochloric acid (HCl)	*Claessens et al.,* 2013 [25]
	Nitric acid (HNO_3)	*Claessens et al.,* 2013 [25]
Base	Sodium hydroxide (NaOH)	*Claessens et al.,* 2013; *Cole et al.,* 2014; *Dehaut et al.,* 2016 [6, 25, 26]
	Potassium hydroxide (KOH)	*Dehaut et al.,* 2016; Foekema et al., 2013; *Phuong et al.,* 2018 [18, 26, 27]
Oxidant	Hydrogen peroxide (H_2O_2)	*Avio et al.,* 2015; *Mathalon & Hill,* 2014; *Nuelle et al.,* 2014 [11, 28, 29]
Enzyme	Proteinase K	*Cole et al.,* 2014 [6]
	Trypsin	*Catarino et al.,* 2017 [30]
Catalyst	Ferrous sulphate ($FeSO_4$)	*Dyachenko et al.,* 2017 [31]
Digestate mix	$HNO_3/HClO_4$	*De Witte et al.,* 2014 [23]
	$NaClO/HNO_3$	*Collard et al.,* 2015 [24]
	KOH/NaClO	*Enders et al.,* 2017 [13]

Various approaches have been developed including acid, base, oxidant, enzyme, catalyst, and digested mix as detailed in Table 1.

The idea of the digested mix is to increase digestion efficiency. The use of KOH or NaClO as a singular digestion solution is not as effective as using a combination of KOH/NaClO [13]. It was reported that a combination of 30% KOH and 30% NaClO with a ratio of 1:1 is capable of offering full digestion of fish stomachs. Meanwhile, a combination solution consisting of 65% HNO_3 and 9% NaClO with a ratio of 1:10 was used to enhance the degradation of fish stomachs [24]. They have reported that after filtration, their samples were free from organic matter except for microplastics on the filter membrane whereby the remaining microplastics were not degraded by NaClO. However, none of the above-mentioned works reported a digestion treatment carried out in oysters.

The paper is organized by first introducing the method that we used. In this study, 3 types of digestion solutions (KOH, KOH/H_2O_2, and KOH/NaClO) for oyster digestion were used to compare the digestion effectiveness of oysters and recovery rate of microplastics. The controlled tests aimed to ensure a plastic-free environment. This was followed by the results and discussion of the experiment.

EXPERIMENTAL SECTION

The oyster samples for the experiment were obtained through sampling at Pantai Teluk Chempedak, Pahang, Malaysia. The samples were digested and mixed with extraction solutions to derive a microplastic extract from the density separation process. The microplastics were then isolated using vacuum filtration. Consequently, the extracted microplastics were taken for imaging by using a microscope. The types of polymers for each detected microplastic were determined using FT-IR.

Materials

In this research, various types of chemicals were used. The chemicals and solvents used were analytical grade and used without any further purification. Sodium hydroxide (NaOH) and potassium iodide (KI) were purchased from Qrec (Asia) Pvt. Ltd., 30 % hydrogen peroxide (H_2O_2) from Classic Chemicals Pvt. Ltd., and 10 % sodium hypochlorite (NaClO) was obtained from Gouden Pvt. Ltd.

Instruments

Two instruments were used in this research; Leica EZ4 stereo microscope for physical characterization and a Perkin Elmer Frontier ATR-FT-IR spectrometer for chemical characterization. All data analyses were performed using Microsoft Excel 2016, and R3.6.3 software was used for data visualization.

Sample collection

Rock oysters were collected from Pantai Teluk Chempedak (3°48’43” N, 103°22’21” E) located in Kuantan, Pahang, Malaysia. The latitude and longitude coordinates were obtained from Google Maps. The samples were taken from the rocky shore at the low tide line. The collected oysters were placed inside a box of seawater with mild aeration. Dead oysters or the ones with broken shells were discarded.

Table 2: List of polymers used to spike the oyster.

Plastic	Source	RIC	Colour	Density, ρ (g/mL)
Polyethylene (PP)	Food container	5	Brown	0.86 – 0.95
High Density Polyethylene (HDPE)	Bottle cap Plastic bag	2	Dark blue Light blue	0.94 – 0.97
Polystyrene (PS)	Plastic lid	6	White	0.96 – 1.04
Polyamide (NY66)	Cable tie	7	Red	1.13 – 1.45
Polyethylene Terephthalate (PET)	Plastic bottle	1	Green	1.38

Marine sample preparation for microplastic spiking

Only live rock oysters with unbroken shells ranging about the same size were used in this experiment. These oyster samples underwent a depuration process for 24 hours with filtered seawater (salinity = 30 ppt) under mild aeration conditions at room temperature. After depuration, all the oyster samples were kept inside the freezer at -20 ºC for at least 24 hours before microplastics spiking.

Microplastics spiking

After defrosting for about an hour, the soft tissue of the oysters was completely removed from their shells using a dissecting spatula. The wet weight of each soft tissue was then recorded.

Each oyster sample was spiked with microplastics using micro dissecting forceps or syringes. For this, we spiked individual oysters with 20 pieces of one type of polymer. The spiked oysters were then dried in an oven at 60 ºC for 24 hours.

The five different types of plastic polymers that were used in this work were taken from various post-consumer products. Table 2 shows the polymers used to spike the oysters with their Resin Identification Code (RIC), color, and reference density. The plastic pieces were broken down into microplastics by using scissors and a cutter. The size class of the microplastics used in this experiment ranged between 1.0-3.0 mm and their shape was categorized as fragments. The color and reference density were noted for each plastic sample. Additionally, FT-IR was used to verify their polymer types.

Sample extraction from rock oysters: Digestion step

Three types of digestion solutions were used for the digestion of oysters; (i) KOH, (ii) KOH/H_2O_2 (1:1), and (iii) KOH/NaClO (1:1). Each solution was labeled D1, D2, and D3, respectively. All the concentrations used for each chemical in the digestion solution were kept at 10%.

The dry weight of each oyster was initially recorded, before placing the oysters into a conical flask containing 20 mL of D1. The mixture underwent a digestion process at 60 ºC for 24 hours. The same steps were repeated with D2 and D3.

Microplastic extraction from digested oysters was carried out as proposed by *Phuong et al.* (2018) [18] with some modifications. Generally, there are 2 steps in the extraction of microplastics: density separation and filtration steps. Both of these methods are elaborated on in the following section.

Sample extraction from rock oysters: density separation and filtration

After the oysters have been completely digested, the remaining microplastics were easily distinguishable from the oyster soft tissue. Each digestion mixture was transferred into a separating funnel respectively to undergo a density separation process for 4 hours. The terms “density separation” and “floatation” used are interchangeable. In this section, density separation is used to highlight the density differences of the polymer. The density separation was carried out twice. The first density separation allowed the separation of lighter microplastics (e.g.; PP, PE, PS) to become the upper layer and the denser particles (e.g.; PVC, PET) to remain at the bottom layer, mixed with undigested parts of the oyster. The upper layer was then transferred into a conical flask and labeled as fraction A. Meanwhile, the bottom layer (labeled as fraction B) was taken into another separating funnel. 20 mL of 50% KI solution (ρ= 1.55 g/mL) was added into fraction B. 50% KI was added in second density separation with the intention to increase the total density of the mixture solution. We aimed to ensure the density of the mixture solution was higher than the density of all possible microplastics so that all the microplastics could float on top of the mixture solution. The mixture of fraction B and 50% KI was shaken vigorously and then the mixture underwent a second-density separation for another 4 hours.

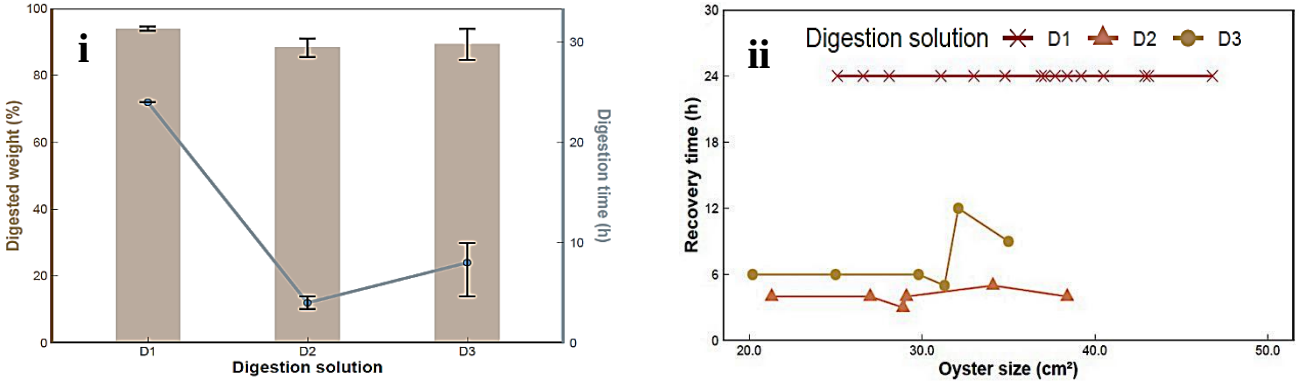

Fig. 2: Degree of oyster digestion via; (i) digested weight and (ii) oyster size.

The second density separation allowed all remaining microplastics to float and left the unwanted particles at the bottom layer. Next, the upper layer from this second-density separation was taken and mixed with fraction A. The bottom part of the mixture was also taken and labeled as fraction C. A filtration of fractions A and the upper layer from the second density separation mixture was performed, labeled as filter paper (i). The bottom layer from the second density separation (fraction C) also went through a filtration, labeled as filter paper (ii). The filtration steps were carried out using Whatman Grade 1 filter paper with a pore size of 1.2 µm to filter out microplastics from the solutions. The filter papers speckled with filtered microplastics were dried at 60 ºC for 24 hours.

Sample characterization: Physical characterization of microplastics

The number of microplastics in each oyster was accounted for using a Leica EZ4 stereo microscope (35x magnification). The microscope was equipped with a camera linked to a computer. This allowed images of microplastics on the filter paper to be recorded and was further analyzed using ImageJ software to determine the size of microplastics.

Sample characterization: Chemical characterization of microplastics

The type of polymers of the microplastics was identified using the Perkin Elmer Frontier ATR-FT-IR spectrometer at wavelengths between 500 cm^{-1} to 4000 cm^{-1}.

Contamination mitigation

Throughout the experimental procedure, stringent contamination control was done to reduce the intrusion of microplastics from the surroundings of the working area. This contamination control protocol was carried out as described in detail by *Murphy et al.* (2016) [32]. Therefore, in this experiment, only equipment made from either aluminum or glass was used. The equipment and the working area were kept clean and dry before experimental conduct. During the experiment, filter papers containing the microplastics were kept in sealed Petri dishes at room temperature. Additionally, an outer lab coat made of cotton was worn at all times to reduce the contamination of fiber from synthetic clothing.

RESULTS AND DISCUSSION

Digestion oyster

The digestion efficiency of oyster soft tissues by the three digestion solutions; D1: KOH, D2: KOH/H_2O_2 (1:1), and D3: KOH/NaClO (1:1) are reported in Fig. 2 (i). Based on the figure, the presence of KOH in combination with bleaching solutions (H_2O_2 or NaClO) played a significant impact on the duration of oysters' digestion. The oyster soft tissue is considered to be completely digested when there are no particles visible to the naked eye in the digestate.

Mixing KOH with bleaching agents reduced the digestion time to less than 24 hours. On the other hand, solution D1 (only KOH) treatment was found to have the highest digestion effectiveness where 95%±1.44 out of the dried individual oysters have been digested. It is followed by D2 and D3 with a recovery rate of 89%±4.63 and 88%±2.80 respectively. Fig. 2(ii) compares the size of the oyster and the duration of complete digestion. Looking at this figure, it is apparent that the time needed for the oyster to be completely digested by solution D1 was relatively constant across oyster sizes. Meanwhile, the results for solutions mixed with bleaching agents, D2 (average digestion time: 4 hours±0.63) and D3 (average digestion time: 8 hours±2.65) are inconsistent to draw a significant conclusion. Therefore, more

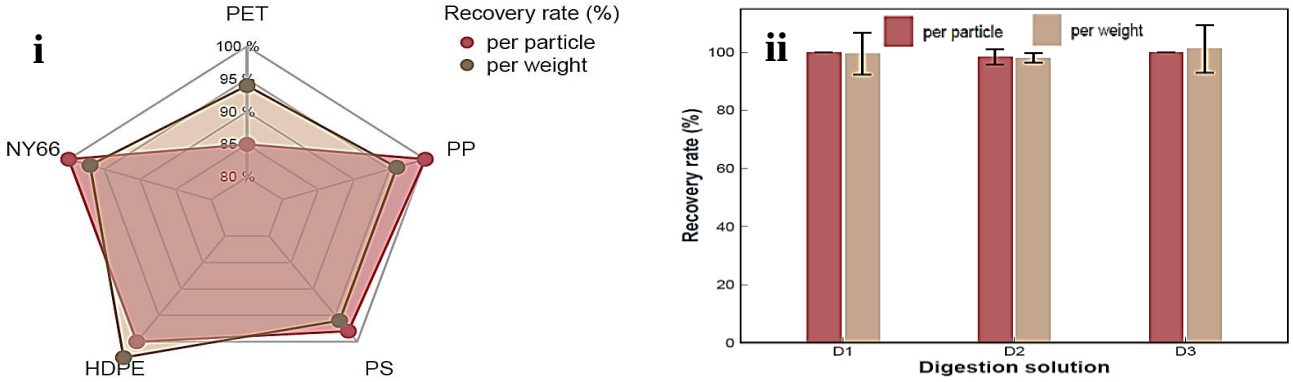

Fig. 3: Microplastic recovery (per recovery weight and per particle) via; (i) various polymer types (using D1 treatment), and (ii) various digestion solutions for PP and HDPE polymers.

experiments need to be carried out by these mixed reagents to obtain the optimum time for complete digestion.

Microplastics recovery

The extraction efficiencies for the spiked microplastic particle associated with different polymer types and different digestion methods are shown in Fig. 3. The recovery rates obtained were calculated per weight and per particle of the recovered microplastics. The former is calculated by measuring the dry weight of the recovered microplastics. Calculation of the recovered microplastics was done manually alone under the microscope for the latter.

In Fig. 3(i), the recovery rate for each polymer using D1 treatment was observed. The overall recovery rates for different types of microplastics were all above 95% except for PET. PET showed the lowest recovery rate of 94%±17.67 per weight and 85%±13.23 per particle.

Next, the spiked microplastic recovery rate using D1, D2, and D3 digestion solutions is shown in Fig. 3(ii). Based on this, it can be recommended that all three types of digestion solutions are suitable for microplastic recovery from oyster soft tissue. There was no significant difference between these three digestion solutions. This was because D1, D2 and D3 have a high recovery rate of about 100% for both per particle and per weight.

During the filtration step, the surface color of the filter papers (i) and (ii) from each digestion solution, D1, D2 and D3 were observed. It was found that the addition of a bleaching agent to the digestion solution produced a cleaner surface. These observations were elaborated on in the following section.

Almost all of the spiked microplastics were successfully recovered without any physical or chemical degradation at the temperature of 60 ºC, with the recovery rates being above 95%. These results are comparable to the extraction efficiencies observed by *Phuong et al*. (2018) [18]. It has been reported that temperatures of 50 ºC and 60 ºC could negatively affect the recovery rates of some microplastics such as NY66, PET, PVC, and rayon [15, 26, 33]. Although adverse effects on the polymers due to the 60 ºC temperature were not observed in this work, it is recommended that 40 ºC be used for future works. This digestion temperature was also recommended by *Thiele et al*. (2019) for microplastic recovery [15].

The search score and the transmittance intensity of the FT-IR spectrum for each recovered polymer (used as spiked microplastics) from each treatment were analyzed, as shown in Fig. 4. This analysis was conducted to observe the accuracy of the polymer identification measured by the FT-IR after being extracted using the digestion solution. The search score of each polymer determines the correlation coefficient between the major peak maxima in the spectrum of the sample and the standard reference (which is available in the FT-IR library). The search score is a value between 0 to 1; where 1 indicates a higher correlation to the standard reference. It is calculated by comparing the peak intensity values at the same wavenumber from the two spectra. From Fig. 4 (i), it was observed that the average search score obtained for each recovered microplastic was 0.97±0.01, except for PET. The average search score obtained for PET was 0.73±0.03 and, for this study, any polymer identification with a search score value above 0.7 is considered an acceptable value. Next, the transmittance intensity of major peak maxima for each recovered microplastic also was analyzed for three repetitions. The transmittance intensity in Fig. 4 (ii) was chosen based on the highest intensity peak value

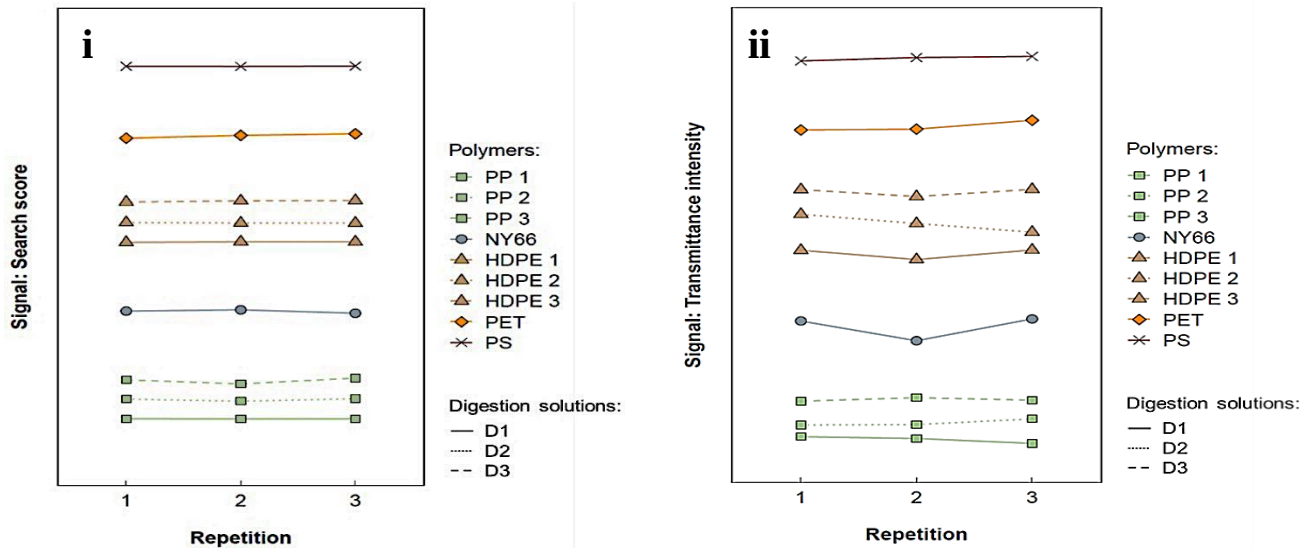

Fig. 4: Analytical figures of merit for search score and transmittance intensity of each recovered microplastics with three repetition.

obtained from the FT-IR spectra of each recovered microplastic. It was found that the highest intensity peak value represents the major functional group of the sample. The average highest transmittance intensity for PP, NY66, HDPE, PET, and PS belonged to the wavenumber of 2918 (C–H stretching vibration of alkane chain), 1635±0.58 (N–H bending vibration of amine), 2901±13.28 (C–H stretching vibration of alkane chain), 723 (C=C conjugated alkene in benzene ring), and 695 cm^{-1} (C=C conjugated alkene in benzene ring), respectively. Overall, based on the shape of the graphs in this work show the potential for repeatability.

Floatation or density separation

A floatation or density separation step is required in this experiment due to the presence of undigested oyster soft tissue and other biological contaminants. This caused the solution to become heterogeneous and the filter paper darker and dirtier, making it difficult to analyze the microplastics directly from these filter papers. This technique is supported by *Hurley et al*. (2018) [34]. The first floatation was performed to separate the supernatant from the heterogeneous mixture of oyster-content sediment. It is expected that the supernatant contained all the lighter microplastics such as PP and PE. Next, in the second floatation, 50% KI solution was added to the sediment of the heterogeneous mixture to extract the denser microplastics that might be possibly trapped within it.

Fig. 5 displays the color characteristics of the filter membrane (i) and filter membrane (ii) after D1, D2 and D3

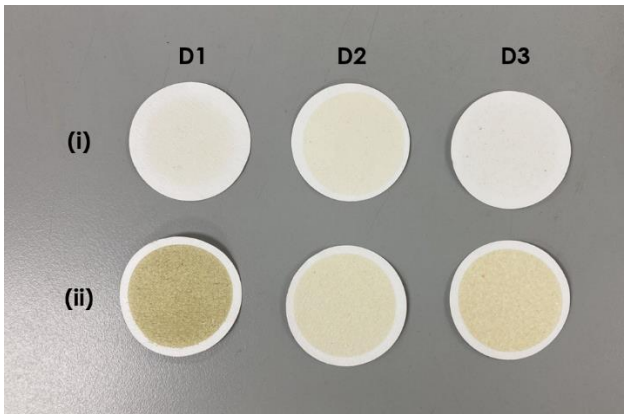

Fig. 5: Surface of filter membrane for different digestion treatments; (i) filtration from fractions A and B, and (ii) filtration from fraction C.

digestion solutions treatments. Filter paper (i) is expected to have all the microplastics extracted from the oyster sample. Filter paper (ii) is expected to have all the denser unwanted particles such as biological matters. According to the method by *Phuong et al.* (2018) [18], digested from filter paper (ii) was discarded, but in this experiment, filter paper (ii) was taken into characterization because of the possibility for denser microplastics that are not able to float into fraction A or B during the density separation step.

Interestingly, the use of potassium salts 10% KOH (ρ = 1.08 g/mL) and 50% KI (ρ = 1.55 g/mL) in the floatation step was unable to separate the spiked PET. This might be due to the density of PET (ρ = 1.30-1.50 g/mL) being higher than the density of 50% KI. Therefore,

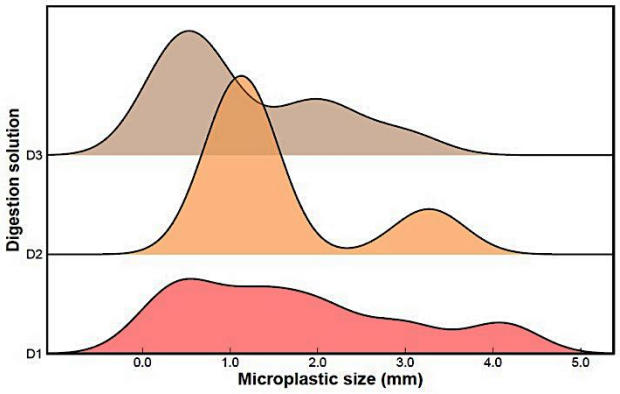

Fig. 6: Size distribution of contaminants. Each size distribution is separated based on a digestion solution to make it easy for the eye.

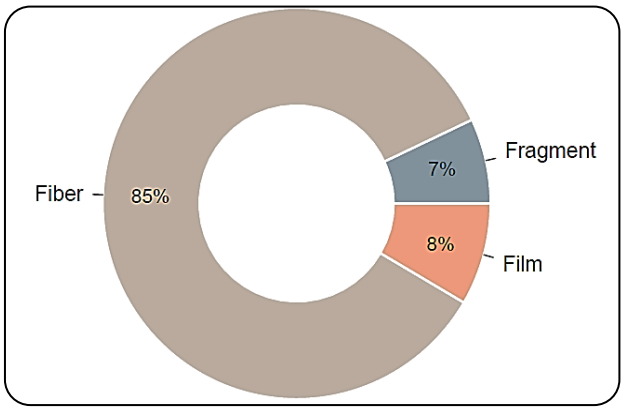

Fig. 7: Shape of contaminants.

it is recommended to use high-density salt solution which has been previously used for microplastic recoveries such as $ZnCl_2$ (ρ = 1.70 g/mL) [35], NaI (ρ = 1.57 g/mL) [33] and $ZnBr_2$ (ρ = 1.71 g/mL) [36] or increase the density of the current salt solution (KI). *Scott* and *Green* (2020) have suggested that KI with a density of 1.80 g/mL, was the best salt solution for density separation [37].

A filter membrane with a clear surface, as in filter paper (i), is preferred so that it is easier to examine the microplastics under the microscope. Based on Fig. 5, a double separation process is recommended for digestion solution D1 treatment. Meanwhile, for digestion solutions that contain bleaching agents such as H_2O_2 and NaClO, both of their filter papers (i) and (ii) showed a cleaner appearance on the surface of the filter membrane. The combination of KOH with bleaching solutions to digest the oyster soft tissue resulted in fewer biological organic particles while the remaining organic particles altered to pale cream or white color. This observation was consistent with the past study conducted by *Nuelle et al*. (2014) [29]. This alludes that the first floatation step (fraction A) is not necessarily required for this type of digestion solutions treatment and it is quite possible to proceed with the addition of salt solution for density separation (second floatation). The possibility of skipping one of the procedure steps gives an additional advantage of short time consumption to conduct the whole experiment.

Contaminant

The depuration step was performed to eliminate all biological and physical contaminants including transient microplastics inside the oyster gut through the expulsion of intestinal contents. Many studies have reported the potential reduction of microplastics during the depuration period [5,12,38]. Therefore, depuration is one of the important steps for microplastic recovery from biological organisms. However, even though all of the oyster samples used in this study were subjected to the depuration procedure, contaminants of below 5.0 mm were detected in each treatment as presented in Fig. 6.

These contaminants might come from the surroundings, also known as airborne contamination. Other than that, it can also be expected that the contaminants originated from the soft tissue (other than digestion organs such as gill, stomach, and intestine) of the oyster itself. The findings of the possibility for microplastics to accumulate in non-digestion organs through adherence were agreed by *Kolandhasamy et al*. (2018) [10]. It is reported that in the adherence process, these two factors might play important roles in gathering microplastics; (1) the surface area with the sticking ability of non-digestion organ [10] and (2) the translocation of smaller microplastics from the gut of bivalve into their circulatory system which are consequently transferred to those non-digestion organs [39]. Also, adherence of microplastics to animals would provide a pathway for microplastics to be transferred into the food web [40].

Fig. 7 illustrates that the contaminants obtained mainly occurred as fibers and were detected in most of the samples.

During the visual inspection of the oyster samples, the shape and the color of the particles play an important role since their conspicuous appearance makes it easier to isolate and often misrepresented as microplastic. According to *Willis et al*. (2017), colored microfibers were likely easier to detect and more visible under the microscope compared to colored particles (such as fragment, film or bead) due to their unique irregular bent thread shape and their tendency to commonly settle on top the filtered particles [41]. Fragment, film and bead

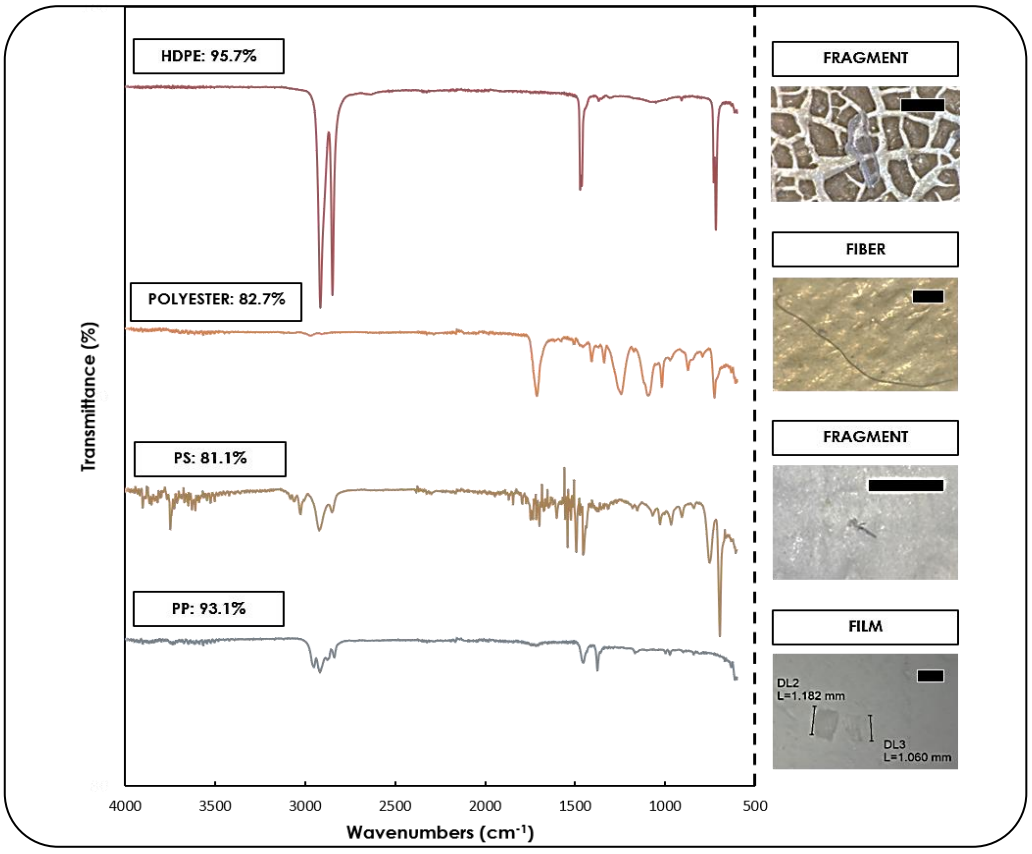

Fig. 8: FT-IR spectra and microscope images of contaminants in the samples. The scale bars in the images represent 1.0 mm.

microplastics were harder to detect as their shapes were more similar to those of undigested natural debris. These shapes could be misidentified in counts from the samples. In the study conducted by *Foekema et al.* (2013), they excluded contaminants in the form of small fibers from their analysis [27]. This was because these fibers could potentially bias the results since it was speculated to be airborne contamination. However, in our work, the detected contaminants including small fibers were analyzed using a digital microscope and ATR-FT-IR (refer to Fig. 8).

Fig. 8 shows the FT-IR spectra of microplastic contaminants that were detected and their image under a microscope. It was found that the polymer types of the detected fibers were polyester, rayon, and polystyrene. High-density polyethylene and polypropylene were also detected by ATR-FT-IR in the form of fragments and film.

CONCLUSIONS

Overall, this study showed that each treatment gave positive results for the digestion rate and the microplastic recovery from rock oysters. It would give more satisfactory results with some improvement in the choice of chemicals used and its concentration for the digestion and microplastic extraction from the bivalve soft tissue. More samples and polymer types are needed for a better evaluation of the optimized protocol. In terms of efficiency, treatment with a bleaching solution is suggested since it is less time-consuming and offers high microplastic recovery. This also supports the observation reported in the previous study. Meanwhile, as for consistency, using 10% KOH alone is suggested since it provides a high digestion rate of oyster soft tissue and high microplastic recovery through the digestion process. A list of previous work (but not exhaustive) that used similar digestion solutions as reported in this work is shown in Table 3.

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Table 3. Comparison studies of digestion method used for microplastic extraction.

Digestion solution	Previous study	Reference	Our study
KOH	Low recovery rate of microplastics.	(*Enders et al*., 2017)	High recovery rate of microplastics.
	Physical or chemical degradation on the tested microplastics with saturated digestate solution and at high digestion temperature.	(*Enders et al*., 2017; *Karami et al*., 2017)	No physical or chemical degradation on the tested microplastics.
	Require long consumption of digestion time to digest the biological matrices.	(*Karami et al*., 2017)	Require long consumption of digestion time to digest the biological matrices.
	High digestion rate of biotic soft tissue.	(*Karami et al*., 2017)	High digestion rate of biotic soft tissue.
H_2O_2	Low amount undigested biological matrices.	(*Karami et al*., 2017; *Nuelle et al*., 2014)	-
	Foam formation.	(*Nuelle et al*., 2014)	Foam formation with addition of bleaching solution.
	Discoloration of the tested microplastics.	(*Karami et al*., 2017; *Nuelle et al*., 2014)	-
NaOCl	High digestion rate of biotic soft tissue.	(*Collard et al*., 2015)	-
	Low digestion rate of biotic soft tissue.	(*Karami et al*., 2017)	-
HNO_3/NaClO	High digestion rate of biotic soft tissue and biological matrices.	(*Collard et al*., 2015)	-
$FeSO_4/H_2O_2$	No physical or chemical changes to the tested polymer.	(*Pfohl et al*., 2021)	-
	Ferrous residue of FeSO4 remains on the filter paper membrane.	(*Pfohl et al*., 2021)	-
	Foam formation.	(*Pfohl et al*., 2021)	-
NH_4OH/H_2O_2	High digestion rate of biological matrices.	(*Pfohl et al*., 2021)	-
	Physical damage on the tested polymer.	(*Pfohl et al*., 2021)	-
	Foam formation.	(*Pfohl et al*., 2021)	-
KOH/NaClO	High recovery rate of microplastics.	(*Enders et al*., 2017)	High recovery rate of microplastics.
	Foam formation.	(*Enders et al*., 2017; *Karami et al*., 2017; *Nuelle et al*., 2014)	Foam formation with the addition of bleaching solution.
	No physical changes to the tested polymer.	(*Enders et al*., 2017)	No physical changes to the tested polymer.
	High digestion rate of biotic soft tissue.	(*Enders et al*., 2017)	High digestion rate of biotic soft tissue.
	Fast and effective protocol.	(*Enders et al*., 2017)	Fast and effective protocol.

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References

[1] Shahul Hamid F., Bhatti M.S., Anuar N., Anuar N., Mohan P., Periathamby A., Worldwide Distribution and Abundance of Microplastic: How Dire Is the Situation?, *Waste Manag. Res.*, **36(10):** 873-897 (2018).

[2] Miller M.E., Kroon F.J., Motti C.A., Recovering Microplastics from Marine Samples: A Review of Current Practices, *Mar. Pol. Bul.*, **123(1-2):** 6-18 (2017).

[3] Besley A., Vijver M.G., Behrens P., Bosker T., A Standardized Method for Sampling and Extraction Methods for Quantifying Microplastics in Beach Sand, *Mar. Pollut. Bull.*, **114(1):** 77-83 (2017).

[4] Crichton E.M., Noël M., Gies E.A., Ross P.S., A Novel, Density-Independent and FT-IR-Compatible Approach for the Rapid Extraction of Microplastics from Aquatic Sediments, *Anal. Meth.*, **9:** 1419-1428 (2017).

[5] Hantoro I., Löhr A.J., Van Belleghem F.G.A.J., Widianarko B., Ragas A.M.J., Microplastics in Coastal Areas and Seafood: Implications for Food Safety, *Food Add. Conta. - Part A Chem. Anal. Control. Expo. Risk Assess.*, **36(5):** 674-711 (2019).

[6] Cole M., Webb H., Lindeque P.K., Fileman E.S., Halsband C., Galloway T.S., Isolation of Microplastics in Biota-Rich Seawater Samples and Marine Organisms, *Sci. Rep.*, **4(1):** 1-8 (2014).

[7] Vandermeersch G., Van Cauwenberghe L., Janssen C.R., Marques A., Granby K., Fait G., Kotterman M.J.J., Diogène J., Bekaert K., Robbens J., Devriese L., A Critical View on Microplastic Quantification in Aquatic Organisms, *Envir. Res.*, **143:** 46-55 (2015).

[8] Karami A., Golieskardi A., Keong Choo C., Larat V., Galloway T.S., Salamatinia B., The Presence of Microplastics in Commercial Salts from Different Countries, *Sci. Rep.*, **7(1):** 1-9 (2017).

[9] Costa M.F., Barletta M., Microplastics in Coastal and Marine Environments of the Western Tropical and Sub-Tropical Atlantic Ocean, *Environ. Sci. Process. Impacts*, **17(11):** 1868-1879 (2015).

[10] Kolandhasamy P., Su L., Li J., Qu X., Jabeen K., Shi H., Adherence of Microplastics to Soft Tissue of Mussels: A Novel Way to Uptake Microplastics beyond Ingestion, *Sci. Total Environ.*, **610:** 635-640 (2018).

[11] Mathalon A., Hill P., Microplastic Fibers in the Intertidal Ecosystem Surrounding Halifax Harbor, Nova Scotia, *Mar. Pollut. Bull.*, **81:** 69-79 (2014).

[12] Van Cauwenberghe L., Janssen C.R., Microplastics in Bivalves Cultured for Human Consumption, *Environ. Pollut.*, **193:** 65-70 (2014).

[13] Enders K., Lenz R., Beer S., Stedmon C.A., Extraction of Microplastic from Biota: Recommended Acidic Digestion Destroys Common Plastic Polymers, *ICES J. Mar. Sci.*, **74:** 326-331 (2017).

[14] Setälä O., Fleming-Lehtinen V., Lehtiniemi M., Ingestion and Transfer of Microplastics in the Planktonic Food Web, *Environ. Pollut.*, **185:** 77-83 (2014).

[15] Thiele C.J., Hudson M.D., Russell A.E., Evaluation of Existing Methods to Extract Microplastics from Bivalve Tissue: Adapted KOH Digestion Protocol Improves Filtration at Single-Digit Pore Size, *Mar. Pollut. Bull.*, **142:** 384-393 (2019).

[16] Lusher A.L., Munno K., Hermabessiere L., Carr S., Isolation and Extraction of Microplastics from Environmental Samples: An Evaluation of Practical Approaches and Recommendations for Further Harmonization, *Applied Spectroscopy*, **74:** 1049-1065 (2020).

[17] Markic A., Gaertner J.C., Gaertner-Mazouni N., Koelmans A.A., Plastic Ingestion by Marine Fish in the Wild, *Crit. Rev. Environ. Sci. Technol.*, **50:** 657-697 (2020).

[18] Phuong N.N., Zalouk-Vergnoux A., Kamari A., Mouneyrac C., Amiard F., Poirier L., Lagarde F., Quantification and Characterization of Microplastics in Blue Mussels (Mytilus Edulis): Protocol Setup and Preliminary Data on the Contamination of the French Atlantic Coast, *Environ. Sci. Pollut. Res.*, **25:** 6135-6144 (2018).

[19] Courtene-Jones W., Quinn B., Murphy F., Gary S.F., Narayanaswamy B.E., Optimisation of Enzymatic Digestion and Validation of Specimen Preservation Methods for the Analysis of Ingested Microplastics, *Anal. Methods*, **9:** 1437-1445 (2017).

[20] von Friesen L.W., Granberg M.E., Hassellöv M., Gabrielsen G.W., Magnusson K., An Efficient and Gentle Enzymatic Digestion Protocol for the Extraction of Microplastics from Bivalve Tissue, *Mar. Pollut. Bull.*, **142:** 129-134 (2019).

[21] Yu Z., Peng B., Liu L.Y., Wong C.S., Zeng E.Y., Development and Validation of an Efficient Method for Processing Microplastics in Biota Samples, *Environ. Toxicol. Chem.*, **38:** 1400-1408 (2019).

[22] Wang Z., Taylor S.E., Sharma P., Flury M., Poor Extraction Efficiencies of Polystyrene Nano- and Microplastics from Biosolids and Soil, *PLoS One*, **13:** 1-13 (2018).

[23] De Witte B., Devriese L., Bekaert K., Hoffman S., Vandermeersch G., Cooreman K., Robbens J., Quality Assessment of the Blue Mussel (Mytilus Edulis): Comparison between Commercial and Wild Types, *Mar. Pollut. Bull.*, **85:** 146-155 (2014).

[24] Collard F., Gilbert B., Eppe G., Parmentier E., Das K., Detection of Anthropogenic Particles in Fish Stomachs: An Isolation Method Adapted to Identification by Raman Spectroscopy, *Arch. Environ. Contam. Toxicol.*, **69:** 331-339 (2015).

[25] Claessens M., Van Cauwenberghe L., Vandegehuchte M.B., Janssen C.R., New Techniques for the Detection of Microplastics in Sediments and Field Collected Organisms, *Mar. Pollut. Bull.*, **70:** 227-233 (2013).

[26] Dehaut A., Cassone A.L., Frère L., Hermabessiere L., Himber C., Rinnert E., Rivière G., Lambert C., Soudant P., Huvet A., Duflos G., Paul-Pont I., Microplastics in Seafood: Benchmark Protocol for Their Extraction and Characterization, *EnviMicroplastics in Seafood: Benchmark Protocol for Their Extraction and Characterizationron. Pollut.*, **215:** 223-233 (2016).

[27] Foekema E.M., De Gruijter C., Mergia M.T., Van Franeker J.A., Murk A.J., Koelmans A.A., Plastic in North Sea Fish, *Environ. Sci. Technol.*, **47:** 8818-8824 (2013).

[28] Avio C.G., Gorbi S., Regoli F., Experimental Development of a New Protocol for Extraction and Characterization of Microplastics in Fish Tissues: First Observations in Commercial Species from Adriatic Sea, *Mar. Environ. Res.*, **111:** 18-26 (2015).

[29] Nuelle M.T., Dekiff J.H., Remy D., Fries E., A New Analytical Approach for Monitoring Microplastics in Marine Sediments, *Environ. Pollut.*, **184:** 161-169 (2014).

[30] Catarino A.I., Thompson R., Sanderson W., Henry T.B., Development and Optimization of a Standard Method for Extraction of Microplastics in Mussels by Enzyme Digestion of Soft Tissues, *Environ. Toxicol. Chem.*, **36:** 947-957 (2017).

[31] Dyachenko A., Mitchell J., Arsem N., Extraction and Identification of Microplastic Particles from Secondary Wastewater Treatment Plant (WWTP) Effluent, *Anal. Methods*, **9:** 1412-1418 (2017).

[32] Murphy F., Ewins C., Carbonnier F., Quinn B., Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment, *Environ. Sci. Technol.*, **50:** 5800-5808 (2016).

[33] Karami A., Golieskardi A., Choo C.K., Romano N., Ho Y.B, Salamatinia, B., A High-Performance Protocol for Extraction of Microplastics in Fish, *Sci. Total Environ.*, **578:** 485-494 (2017).

[34] Hurley R.R., Lusher A.L., Olsen M., Nizzetto L., Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices, *Environ. Sci. Technol.*, **52:** 7409-7417 (2018).

[35] Imhof H.K., Schmid J., Niessner R., Ivleva N.P., Laforsch C., A Novel, Highly Efficient Method for the Separation and Quantification of Plastic Particles in Sediments of Aquatic Environments, *Limnol. Oceanogr. Methods*, **10:** 524-537 (2012).

[36] Quinn B., Murphy F., Ewins C., Validation of Density Separation for the Rapid Recovery of Microplastics from Sediment, *Anal. Methods*, **9:** 1491-1498 (2017).

[37] Scott J.W., Green L., "Development and Demonstration of a Superior Method for Microplastics Analysis: Improved Size Detection Limits, Greater Density Limits, and More Informative Reporting", *TR Ser. (Illinois Sustain. Technol. Center) TR-078*, (2020).

[38] Xu X.Y., Lee W.T., Chan A.K.Y., Lo H.S., Shin P.K.S., Cheung S.G., Microplastic Ingestion Reduces Energy Intake in the Clam Atactodea Striata, *Mar. Pollut. Bull.*, **124:** 798-802 (2017).

[39] Browne M.A., Galloway T.S., Thompson R.C., Spatial Patterns of Plastic Debris along Estuarine Shorelines, *Environ. Sci. TechnSpatial Patterns of Plastic Debris along Estuarine Shorelinesol.*, **44:** 3404-3409 (2010).

[40] Gutow L., Eckerlebe A., Giménez L., Saborowski R., Experimental Evaluation of Seaweeds as a Vector for Microplastics into Marine Food Webs, *Environ. Sci. Technol.*, **50:** 915-923 (2016).

[41] Willis K.A., Eriksen R., Wilcox C., Hardesty B.D., Microplastic Distribution at Different Sediment Depths in an Urban Estuary, *Front. Mar. Sci.*, **4:** 1-8 (2017).