Influence of Six Digestion Methods on the Determination of Polystyrene Microplastics in Organisms Using the Fluorescence Intensity

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Abstract: Microplastic pollution has become a global environmental problem and is a cause of great concern. To evaluate the biological effects of microplastics, microplastics in organisms need to be accurately quantified. The quantification of microplastics in organisms using the fluorescence intensity is common; the digestion of biological samples is an important pretreatment method. However, the microplastics may be destroyed by digestion, which affects the fluorescence intensity of the microplastics and results in large deviations between measured and true values. In this study, six commonly used digestive agents were studied: KOH, NaOH, H_2O_2, HNO_3, HNO_3: hcl, and HNO_3: $HClO_4$. The effect of different digestion methods on the fluorescence intensity and surface morphology of microplastics was studied and the most suitable protocol was selected. The results show that, among the six different digestion methods, KOH digestion(100 g·L^{-1}, 60°C)has the least influence on the fluorescence intensity of the microplastics and does not affect their surface morphology. The other five digestion methods lead to different degrees of reduction of the fluorescence intensity of microplastics and damage the microplastics' surface(aggregation, bubbles, scratches, and depressions). In addition, the KOH digestion method was used to extract microplastics from biological samples. The recovery rate was≥96.3%±0.5%, indicating that the KOH digestion method is suitable for fluorescent microplastics in biological samples.

In recent years, the pollution of microplastics has become more and more serious, which has attracted widespread attention all over the world. Plastic waste is discarded into the environment, cracked into smaller fragments under various natural and human actions, and formed plastic fragments or particles with a particle size of less than 5mm through continuous weathering, which is defined as microplastics [1]. The micro plastics

in the environment have fragments. Fibrous and granular forms mainly come from the weathering of large plastics [2, 3] and plastic beads added to some personal toiletries [4]. These plastic products are discharged into various water environments, including rivers, with the tail water of the wastewater treatment plant after use. Lakes and oceans [5, 6]. Due to its small particle size and wide distribution, microplastics are easy to be ingested by many organisms as food. Naidu et al. [7] found that there are microplastics in benthic invertebrates on the southwest coast of the Indian Ocean. Studies have sampled and analyzed chironomid larvae in urban rivers in South Africa [8], and found that more than 75% of chironomid larvae samples contain microplastics. In addition to the intake of microplastics found in invertebrates, microplastics are also found in some vertebrates such as fish. Alomar et al. [9] sampled and analyzed the black mouth saw tail shark in the Mediterranean Sea and found that 16.8% of the samples contained microplastics. Studies have shown that microplastics may be transferred between organisms through the food chain and enriched in organisms [10~12], so it is necessary to study the distribution and pollution level of microplastics in organisms.

Fluorescent microplastics are often used in previous studies to study the intake of microplastics by organisms and the distribution and transfer of microplastics in organisms [13~15]. The microplastics in the organism can be quantified through the combination of tissue sectioning and fluorescence microscope observation [16], but this observation method is complex, easy to be affected by the subjective influence of the observer, and the quantification is inaccurate. Some studies have shown that [14], quantification by fluorescence intensity is a simple and feasible method, and the fluorescence intensity of microplastics can be used as a basis for quantitative research in experiments. Biological tissues need to be removed before measuring fluorescence intensity, so digestion of biological samples before fluorescence quantitative analysis is an important pretreatment step. At present, the main pretreatment methods are acid digestion (such as HCl, HNO_3 and $HClO_4$)[17, 18]. Alkali digestion (such as naoh and KOH)[18] and oxidant digestion (H_2O_2)[17, 19].

Due to the different pretreatment methods for the detection of microplastics in biological samples, the research results vary greatly. For example, Cole et al. [18] found that the recovery rate of microplastics using acid and alkali digestion varies greatly, among which the recovery rate of hcl digestion is nearly 72.1%±9.2%, while the recovery rate of naoh can reach 91.3%±0.4%. Avio et al. [20] found that the recovery rate of microplastics using nitric acid digestion is only 4.0%±3.0%, Phuong et al. [21] used KOH to digest mussel tissue, the extraction efficiency of the obtained microplastics is as high as 99.9%. In order to explore the influence of different digestion methods on the extraction efficiency of microplastics in biological samples, six different digestion methods used in previous studies are selected to digest the fluorescent polystyrene microspheres commonly used in the experiment. The influence of each method on the fluorescence intensity and surface morphology of fluorescent polystyrene microspheres is evaluated, and the best digestion method is selected to extract microplastics in biological samples. The optimized biological tissue has good digestion effect. A digestion method with little influence on the fluorescence of microplastics, in order to provide method support for subsequent research.

1 Materials and methods

1.1 Micro plastic treatment

Fluorescent polystyrene microspheres (FMP)used in the experiment were purchased from Tianjin Bessler chromatographic technology development center. The microplastics used in the experiment were ultrasonicized in an ultrasonic oscillator for 10 minutes (120 W)to ensure that the microplastics were better dispersed in the system. Take 1.0 mg fluorescent micro plastic and put it into a 10mLor 100mLglass bottle with a lid. Add a certain volume of digestion solution, cover it to prevent pollution, and then put the sample into a constant temperature water bath for digestion. See Table 1 for specific experimental conditions. The raw fluorescent polystyrene microspheres without any treatment and the samples of the digested fluorescent polystyrene microspheres were diluted with ultra pure water to a final volume of 100 ml, and the fluorescence intensity was measured with a fluorescence spectrophotometer (F-4600, Hitachi, Japan).

Table 1 Details about the six selected digestion protocols for the extraction of fmps

Digester	Concentration	Volume/ml	Temperature/°C	Digestion time/h	Literature
KOH	100 g·L^{-1}	40.0	60	24	[22]
NaOH	10 mol·L^{-1}	40.0	60	24	[18]
H_2O_2	30% (mass fraction)	40.0	65	24	[19]
HNO_3	69% (mass fraction)	1.0	70	2	[14, 23]
HNO_3: hcl	1: 1 (volume ratio)	1.0	80	1/2	[24]
HNO_3: $HClO_4$	4: 1 (volume ratio)	1.0	20/90[1]	12/(1/6)[1]	[25]

1)Digest at 20°C for 12 hours, and then increase the temperature to 90°C for 10 minutes.

1.2 Characterization of fluorescent polystyrene microsphere samples

After digestion, take a part of the fluorescent polystyrene microsphere solution and use the blood cell counting plate to place it under the fluorescence microscope (FEICA DM- 2500)20 × the fluorescence intensity of the digested fluorescent polystyrene microspheres was observed at magnification. The remaining fluorescent polystyrene microsphere solution is over 0.45 μM mix the fiber membrane, and freeze dry the filter membrane with micro plastic after filtration for 3 days. The surface morphology of the dried micro plastic samples was determined by scanning electron microscope (SEM, JSM- 6390LV, JEOL, Japan).

1.3 Determination of optimal digestion conditions

According to the change of fluorescence intensity and the results of scanning electron microscope, a kind of digestion agent with the least effect on the fluorescence intensity and surface morphology of microplastics was selected to study the volume of digestion solution. The effects of digestion temperature and digestion time on the fluorescence intensity of microplastics were studied, and the best digestion conditions were selected. The micro plastic processing method is described in Section 1.1. After digestion, the fluorescence intensity of the digested fluorescent polystyrene microspheres was measured by the above method. The digestion conditions are shown in Table 2. The specific experimental settings are: a. The effect of the volume of the digestion agent on the fluorescence intensity: the volume of the digestion agent is 10~60 ml, and it is digested at 60°C for 24 hours; b. Effect of digestion temperature on fluorescence intensity: add 60mL KOH solution, set the temperature at

20~90℃, and digest for 24 hours; c. Effect of digestion time on fluorescence intensity: add 60mLKOH solution, the digestion temperature is 60℃, and the digestion time is 1/6~72 h.

Table **2** Details of the conditions used for the fmps digestion

Digester	Concentration/g·L^{-1}	Volume/ml	Temperature/℃	Digestion time/h
KOH	100	10, 20, 40, 60	20, 40, 50, 60, 70, 80, 90	1/6, 1/2, 2, 24, 48, 60, 72

1.4 Recovery rate of microplastics in biological samples

According to the previous experiments, the fluorescence intensity of a kind of fluorescent polystyrene microspheres is obtained. The digestion method with the least influence on morphological structure is determined as the best digestion method. This method is used to digest the microplastics in biological samples, and the reliability of this method is verified by calculating the recovery rate of microplastics.

Daphnia magna and zebrafish were selected as test organisms, and they were not fed 24 hours before the beginning of the experiment. The recovery rate experiment was divided into two groups: a. The recovery rate was determined by direct mixing of the tested organisms with the original fluorescent polystyrene microspheres. The main treatment method is to kill 100 Daphnia magna or 1 zebrafish (20 mg±5 mg)and homogenize the tissue, add it to a glass beaker containing 2 mg of original fluorescent polystyrene microspheres, digest it with KOH method (100 g·L^{-1}, 60℃), three parallel in each group, measure the fluorescence intensity with a fluorescence spectrophotometer, calculate the content through the standard curve, record it as Q_m, and calculate the recovery rate of fluorescent polystyrene microspheres R_m; b. Determination of the recovery rate of fluorescent microplastics in vivo. 100 Daphnia magna or a zebrafish were randomly added to a 100mLbeaker containing 40mLof ultra pure water and 2 mg of fluorescent polystyrene microspheres after 24 hours of starvation. Each group was set up with 3 parallel ones and placed in a constant temperature light incubator (25℃, light: dark = 12: 12). After 24 hours of culture, the biological individuals who ingested fluorescent polystyrene microspheres were taken out, washed with ultra pure water for 3 times to remove the microplastics adhered to the surface, killed and homogenized, the biological samples were treated by KOH digestion method, and the fluorescence intensity was measured by fluorescence spectrophotometer. The calculated content of polystyrene microspheres was recorded as Q_{i-1}. At the same time, the fluorescence intensity of the remaining undigested fluorescent polystyrene microspheres in the beaker was measured, and the calculated content was recorded as Q_{i-2}. The recovery rate of fluorescent polystyrene microspheres R_i was calculated by the following formula.

The recovery rate (%)of fluorescent polystyrene microspheres in biological samples is calculated as follows:

$$R_m = Q_m / Q_s \times 100\% \qquad (1)$$

$$Q_i = Q_{i-1} + Q_{i-2} \qquad (2)$$

$$R_i = Q_i / Q_s \times 100\% \qquad (3)$$

Where, R_m(%)is the recovery rate of fluorescent polystyrene microspheres during direct mixing, and Q_m is the content of fluorescent polystyrene microspheres measured during direct mixing; R_i(%)is the recovery rate of fluorescent polystyrene microspheres during exposure culture, Q_i is the content of fluorescent

polystyrene microspheres measured during exposure culture, Q_{i-1} is the content of fluorescent polystyrene microspheres ingested in the organism, Q_{i-2} is the content of residual fluorescent polystyrene microspheres in the culture system, and Q_s is the content of fluorescent polystyrene microspheres initially added.

1.5 Data processing

SPSS 20.0 was used for data analysis and comparison, and one-way analysis of variance (ANOVA)was used to analyze the significant differences between the influencing factors. $P<$ 0.05 indicates that there are significant differences between the treatment groups, and excel 2016 was used for mapping.

2 Results and analysis

2.1 Effect of six digestion methods on fluorescence intensity of fluorescent polystyrene microplastics

Figure 1 shows the change of fluorescence intensity after six digestion methods were used to digest fluorescent polystyrene microspheres. It can be seen that HNO_3. The fluorescence intensity of microplastics digested by HNO_3: HCl and HNO_3: $HClO_4$ decreased significantly, among which the fluorescence intensity decreased by 77.1% when HNO_3 digested high concentration microplastics (12 mg·L^{-1}), and the fluorescence of microplastics digested by hno: hcl and hno: HClO almost disappeared. When the concentration of microplastics is high ($\geq$ 7 mg·L^{-1}), the fluorescence intensity decreases by 94.0%~97.4% [Figure 1 (a)~1 (c)]. Figure 1 (d)and Figure 1 (f)show that H_2O_2 and KOH treatment have little effect on the fluorescence intensity of fluorescent polystyrene microspheres. The fluorescence intensity of fluorescent polystyrene microspheres has almost no change when H_2O_2 is treated with low

concentration microplastics solution (< 3 mg·L^{-1}), while the fluorescence intensity decreases by 11.2% when high concentration microplastics solution ($\geq$ 5 mg·L^{-1})is digested, which has a certain impact on the fluorescence intensity. After naoh treatment, the fluorescence intensity of fluorescent polystyrene microspheres still showed a good linear correlation with the concentration of microplastics, but the fluorescence intensity decreased to a certain extent, ranging from 0.3% to 21.0%, of which the lowest fluorescence intensity appeared in the microplastics concentration group of 7 mg·L^{-1}. When the microplastics concentration was low ($\leq$ 5 mg·L^{-1}), the fluorescence intensity decreased by 5.8% to 21.0%, and when the microplastics concentration was $\geq$ 10 mg·L^{-1}, the fluorescence intensity decreased by 8.3% to 16.7% [figure 1 (e)] The fluorescent polystyrene microspheres treated with KOH have no significant difference compared with those before digestion, and the fluorescence intensity is very close to that of the microplastics before digestion, with a reduction of less than 5.4%. Compared with acid digestion, hydrogen peroxide and alkali digestion have less effect on the fluorescence intensity of microplastics, and KOH digestion has the least effect on the fluorescence intensity of fluorescent polystyrene microspheres.

2.2 Effects of six digestion methods on the surface morphology of fluorescent polystyrene microspheres

The effects of different digestion methods on the surface fluorescence and morphology of fluorescent polystyrene microspheres are shown in Figure 2 and Figure 3. Figure 2 shows the photos of fluorescent polystyrene microspheres taken under the fluorescence microscope, and Figure 2 (a)shows the micro plastics without any treatment. It can be seen that the surface

fluorescence is very uniform, and the micro plastics can be well dispersed. After HNO₃[Figure 2 (b)]. The surface fluorescence intensity of hno₃: hcl [Figure 2 (C₁)~2 (C₂)] and hno₃: HClO₄ [Figure 2 (D₁)~(D₂)] after acid digestion is significantly lower than that of the original microplastics. Among them, it is difficult to observe the fluorescence of hno₃: HClO₄ digested microplastics under the fluorescence microscope, and the microplastics have obvious agglomeration (shown by the yellow arrow), and the fluorescence of some microplastics has fallen off (shown by the red arrow). On the contrary, the strong oxidant H₂O₂ [Figure 2 (E)] and two alkali naoh[Figure 2 (f)], Koh [Figure 2 (g)] digestion method have little effect on the fluorescence intensity of microplastics, and the fluorescence on the surface of fluorescent polystyrene microspheres is still relatively uniform, and can be well dispersed, without obvious agglomeration.
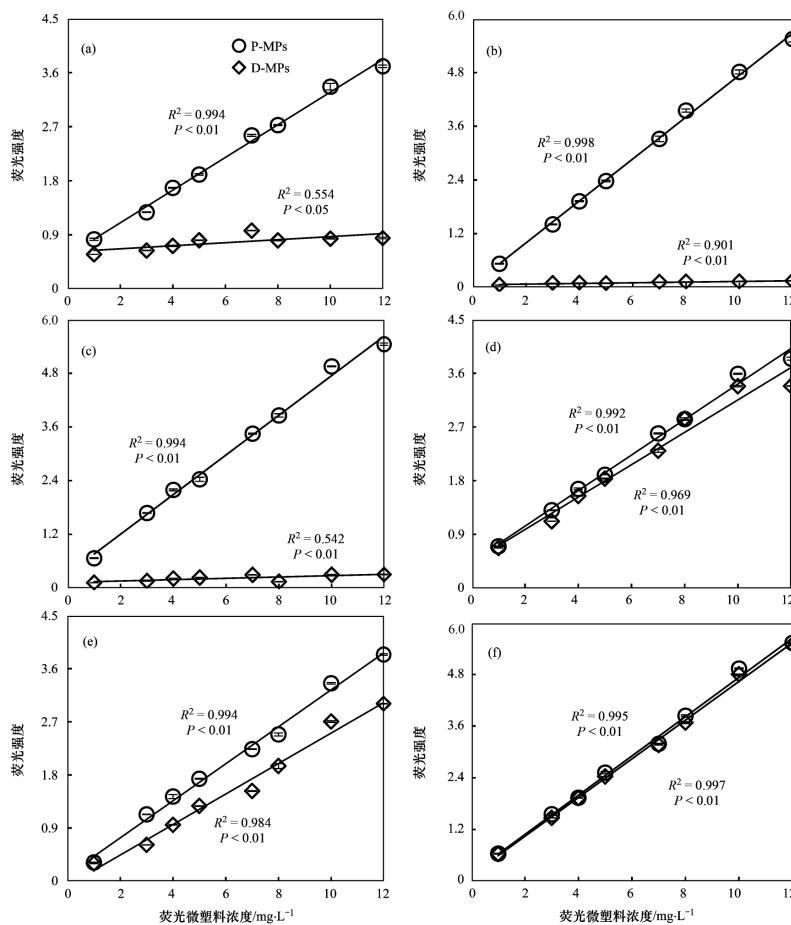
P-MPs and D-MPs are initial microplastics and digested microplastics, respectively; (a)HNO₃, (b)HNO₃: HCl, (c)HNO₃: HClO₄, (d)H₂O₂, (e)NaOH, (f)KOH

| Fluorescence intensity |
| --- |
| Fluorescent microplastics concentration |

Figure 1 Effect of the six digestion methods on the fluorescence intensities of the FMPs

Scanning electron microscope (SEM)was used to observe the surface morphology of microplastics treated by different digestion methods. The results are shown in Figure 3. It can be seen that the untreated microplastics (initial fluorescent polystyrene

microspheres)have good dispersion, and the surface of plastic microspheres is very smooth [Figure 3 (a)]. Compared with the initial fluorescent polystyrene microspheres, HNO$_3$ [Figure 3 (b)]. Hno$_3$: hcl [Figure 3 (c)] and hno$_3$: HClO$_4$ [Figure 3 (d)] after acid digestion, the surface of the microplastics was damaged to varying degrees, including bubbles (shown by the red arrow). Slight scratches and deep surface dents (shown by the blue arrow), in addition, there was obvious adhesion between polystyrene microspheres (shown by the yellow arrow). Among them, the micro plastic after HNO$_3$ digestion has the least damage, and only a small amount of bubbles appear on the surface. The micro plastics after hno$_3$: HClO$_4$ digestion are the most severely damaged. As can be seen from Figure 3 (d), the micro plastics after

digestion have obvious melting and bonding, resulting in large-area agglomeration between micro plastics and obvious shrinkage on the surface of microspheres. A small amount of bubbles appeared on the surface of the microplastics digested by strong oxidant H$_2$O$_2$ [Figure 3 (E)and 3 (E)], and no obvious agglomeration and scratches were found. However, naoh digestion leads to obvious agglomeration of microplastics [Figure 3 (F$_1$)and 3 (F$_1$)] and slight scratches [Figure 3 (F$_2$)and 3 (F$_2$)]. In contrast, the polystyrene fluorescent microspheres after KOH digestion are still in a uniform dispersion state, and there is no obvious surface damage. Agglomeration and other phenomena are not significantly different from the untreated microplastics in Figure 3 (a).
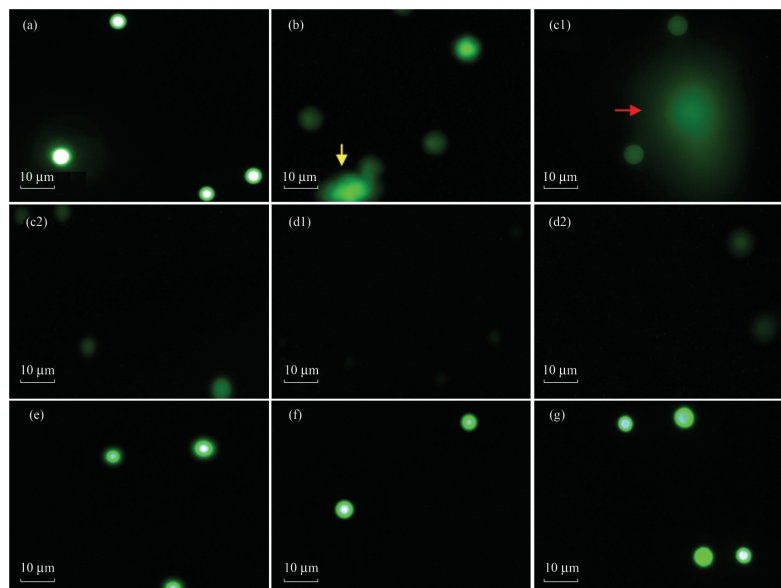
(a)Initial fluorescent polystyrene microspheres; (b)HNO$_3$; (c)HNO$_3$: hcl; (d)HNO$_3$: HClO$_4$; (e)H$_2$O$_2$; (f)naoh; (g)KOH

Figure 2 Fluorescence microscope photographs of microplastics treated with different digestion methods

2.3 Digestion solution volume. Effect of temperature and time on fluorescence intensity of fluorescent polystyrene microspheres

Through the above experimental results, it is concluded that KOH digestion method is the best digestion method, which has the least impact on the fluorescence intensity and surface morphology of polystyrene fluorescent microspheres. The optimal conditions are screened by setting different digestion solution volumes, digestion temperatures and digestion times. The experimental results are shown in

Figure 4 and FIGURE 5. It can be seen from Figure 4 that when the concentration of microplastics is low (≤ 8 mg·L^{-1}), the fluorescence intensity of fluorescent polystyrene microspheres decreases slightly with the increase of the volume of digestion solution. When the concentration of microplastics is high (≥ 10 mg·L^{-1}), there is no significant difference in the influence of different digestion solution volumes on the fluorescence intensity. With the increase of digestion temperature, the fluorescence intensity remains basically unchanged at first, while when the temperature is higher than 70℃, the fluorescence intensity decreases [Figure 5 (a)]. When the temperature is 50~60℃, the fluorescence intensity of the digested polystyrene fluorescent microspheres decreases by only 0.3%, which is almost unchanged from the initial fluorescence intensity of the polystyrene fluorescent microspheres. When the temperature is higher than 70℃, the fluorescence intensity decreases rapidly, which is 3.2%~33.7% less than the initial fluorescence intensity. The digestion time has no significant effect on the fluorescence intensity of fluorescent polystyrene microspheres [Figure 5 (b)]. With the extension of digestion time, the fluorescence intensity does not change significantly, which is close to the fluorescence intensity of the initial fluorescent polystyrene microspheres, and the reduction of fluorescence intensity is less than 4.9%. In the process of digestion, the higher the temperature is, the faster the reaction is. At the same time, in order to digest other biomass in the actual sample completely, the volume of digestion solution is 60mLand the digestion temperature is 60℃ is the best digestion condition.
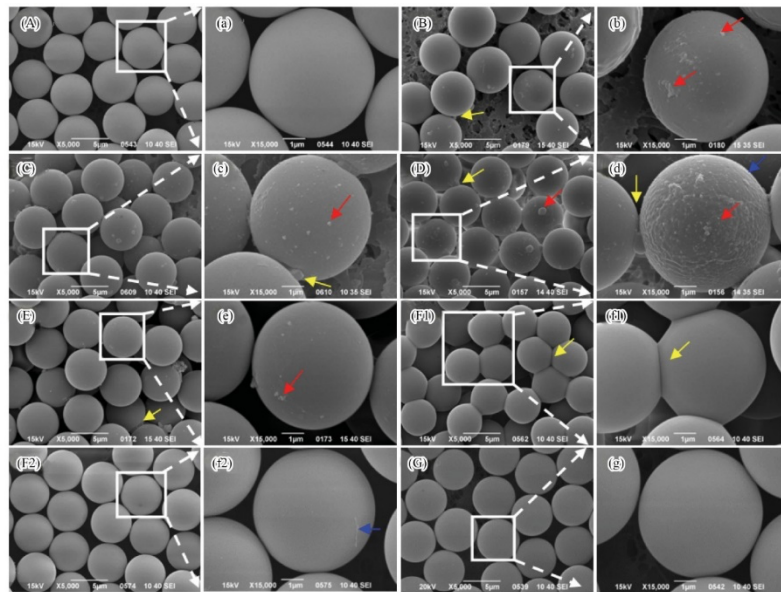

(A)Initial fluorescent polystyrene microspheres; (B)HNO$_3$; (C)HNO$_3$: HCl; (D)HNO$_3$: HClO$_4$; (E)H$_2$O$_2$; (F)NaOH; (G)KOH

Figure 3 SEM images of the microplastics treated with different digestion methods

2.4 Recovery rate of fluorescent polystyrene microspheres in biological samples by KOH digestion method

Among the six digestion methods, the best KOH digestion method (60℃, 72 h)was used to extract fluorescent microplastics from zebrafish and Daphnia magna cultured in the laboratory.

The results show that KOH can completely digest the tissues of zebrafish and Daphnia magna, and has a high extraction efficiency for fluorescent microplastics in organisms, with the extraction efficiency as high as 96.3%±0.5% and 95.6%±0.7% respectively. It shows that KOH digestion method can be well applied to the extraction of microplastics in biological tissues, and it is an efficient and feasible digestion method.
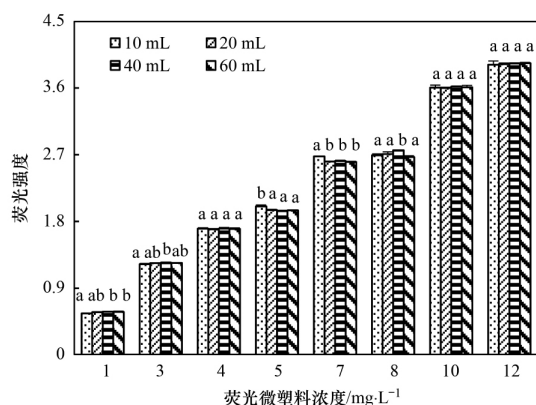
Small letters indicate significant differences between different treatment groups, $P < 0.05$

| Fluorescence intensity |
| Fluorescent microplastics concentration |

Figure 4 Effect of the amount of the digestion reagent on the fluorescence intensities of the fmps
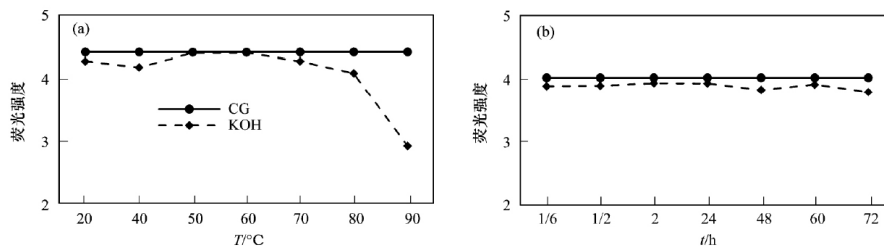
3 Discussion

3.1 Effect of different digestion methods on the surface morphology of fluorescent polystyrene microspheres

In this study, six digestion methods were selected to digest polystyrene fluorescent microspheres. Among them, Koh digestion method had the least effect on the surface morphology of microplastics [Figure 3 (g)], while the other five digestion methods caused plastic surface damage to a certain extent [Figure 3 (b)- 3 (f)], especially under the condition of acid digestion, bubbles appeared on the surface of polystyrene fluorescent microspheres. Slight scratches and folds [Figure 3 (b)- 3 (d)], and obvious adhesion between particles may be due to the strong oxidation and corrosion of strong acids [26] Avio et al. [20] digested fish tissue with nitric acid and the extraction rate of microplastics was only 4%±3%. Dehaut et al. [22] studied the extraction of microplastics in seafood by different digestion methods and found that the use of HNO₃ would lead to significant degradation of microplastics; claessens et al. [27] found that when polystyrene microplastics were directly put into HNO₃ digestion solution, there was obvious fusion of microplastics, which was consistent with the experimental phenomenon of this study [Figure 3 (b)~3 (d)]. Therefore, when digesting biological samples with acid, the content of microplastics measured will be low, indicating that acid digestion is not conducive to the accurate evaluation of microplastics pollution in biological samples. In previous studies, alkaline solutions including naoh and KOH have been used to recover the microplastics in biological samples [22, 28], but naoh digestion method will still cause certain damage to the microplastics. For example, Cole et al. [18] found that naoh digestion leads to partial damage of nylon fibers and melting deformation of polystyrene ethylene, which may be caused by the strong corrosion of naoh; rist et al. [29] used naoh (1 mol·L⁻¹)to digest at 60°C for 24 hours and found that a large amount of agglomeration occurred in polystyrene microspheres. This is consistent with the experimental results of this study [Figure 3 (F₁)]. On the contrary, Koh digestion has no obvious effect on the surface morphology of microplastics, which further proves the feasibility and reliability of KOH digestion method in the qualitative and quantitative determination of microplastics in biological samples. The experimental results of dehaut et al. [22] also confirm this point. They found that

100 g·L⁻¹ KOH digested at 60°C for 24 hours can effectively remove biomass without significant impact on microplastics. In this study, the experimental results of the recovery rate of microplastics in biological samples also show that KOH digestion method is the best digestion method for extracting and quantifying fluorescent polystyrene microspheres from biological samples, and the recovery rate is as high as 96.3%±0.5%.

Fluorescence intensity

Figure 5 Effect of the digestion temperature and duration time on the fluorescence intensities of the fmps

3.2 Effect of different digestion methods on fluorescence intensity of fluorescent polystyrene microspheres

In the experiment, the fluorescent dye of polystyrene fluorescent microspheres is 4-chloro⁻⁷-nitrobenzo⁻²-oxa⁻¹, 3-diazole (NBD CL), which is a commonly used fluorescent dye. It does not fluoresce itself, but it will fluoresce after reacting with amines. In order to maintain the stability of fluorescence, fluorescent dyes are usually wrapped inside the plastic shell in industrial production to prevent the dyes from falling off. El EMAM et al. [30] found that NBD Cl and lisinopril [n-{n-[(s)- 1-carboxyl⁻³-phenylpropyl] -l-lysine} -l-proline, containing amino] will produce yellow fluorescent substances in alkaline medium. Therefore, there are two possible reasons for the fluorescence quenching of fluorescent polystyrene microspheres after digestion: a. It is due to strong acids (HNO_3, hcl and $HClO_4$). Strong oxidant (H_2O_2)and strong alkali (naoh)digestion methods destroy the surface structure of the micro plastic [Figure 3 (b)- 3 (f)], making the fluorescent substances wrapped under the micro plastic shell leak, thereby reducing the fluorescent intensity; b. NBD CL reacts with amines to form C-N bond, which is the basis of fluorescence generation. Some studies have shown that C-N bond is easy to be damaged by strong acid, and it is easy to break under the action of HNO_3, resulting in the reduction or quenching of fluorescence intensity [31]. Therefore, when HNO_3, hno₃: hcl and hno₃: $HClO_4$ are used for digestion, it can react with C-N bond in fluorescent substances on the surface of micro plastics, making the fluorescence weak.

3.3 Effect of digestion temperature and time on fluorescence intensity of fluorescent polystyrene microspheres

Temperature plays an important role in chemical experiments. With the increase of temperature, the properties of reactants will change, which will affect the experimental results [32]. In this study, when the temperature is lower than 80°C, Koh digestion has little effect on the fluorescence intensity of polystyrene fluorescent microspheres (Figure 5). Previous studies have also shown that the use of alkali can completely digest biological tissues at 60°C, and has little effect on microplastics [22], which is consistent with the results of this experiment. However, when the temperature exceeds 70°C,

the fluorescence intensity of fluorescent polystyrene microspheres decreases sharply, which may be due to the high digestion temperature, which is close to the glass transition temperature of plastic (95°C), resulting in the softening of plastic particles and the fusion phenomenon [17], which makes the fluorescent dyes wrapped inside flow out and the fluorescence intensity decreases. The experimental results show that the digestion time has no significant effect on the fluorescence intensity of KOH digested fluorescent polystyrene microspheres, indicating that KOH digestion is not affected by time. Therefore, in practical application, the time of digestion is mainly selected according to whether biological tissues are completely digested.

4 Conclusion

Six different digestion agents were selected to digest fluorescent polystyrene microplastics. By analyzing and comparing the changes of fluorescence intensity and surface morphology of fluorescent polystyrene microspheres after digestion, it was found that KOH digestion method had the best effect and had the least impact on polystyrene fluorescent microspheres. The other five digestion methods reduced the fluorescence intensity of polystyrene fluorescent microspheres to varying degrees, which was not conducive to the detection and analysis of microplastics in biological samples. The experimental conditions of KOH digestion method were explored by controlling variables, and the effects of different influencing factors on digestion were analyzed. The results showed that KOH digestion method had the least effect on the fluorescence intensity and surface morphology of microplastics. The volume of digestion solution and digestion time had no significant effect on Koh digestion of fluorescent polystyrene microspheres. Too high

digestion temperature would reduce the fluorescence intensity of fluorescent polystyrene microspheres, and 60°C was the best digestion temperature. Among the six digestion methods studied in this paper, Koh digestion method is the most suitable digestion method for quantitative analysis of microplastics in biological samples, with an extraction efficiency of 96.3%±0.5%.

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