The small stuff matters – establishing the most suitable method to extract microplastics from bivalves

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1. Introduction

Microplastics (100 nm - 5 mm) are ingested by marine organisms(1), including bivalves for human consumption(2). The Pacific oyster *Magallana gigas*, for example, mainly retains particles <25 µm(3,4). Evidence is scarce but potential biological risks may be related to particle size in humans(5) and bivalves(6) alike. Microplastics are extracted from water, sediment and biota with different techniques(7,8). Even for the latter various methods exist hindering comparability of results. The aim of this study is to establish a standardised method for those organisms by comparing existing techniques using two type of bivalve.

2. Materials & methods

Fig.1: Pacific oyster *Magallana gigas*

Fig.2: Manila clam *Ruditapes philippinarum*

Comparison of 4 digestion methods:
- 30 % Hydrogen peroxide (H_2O_2)(2)
- Proteinase-K(9)
- Trypsin(10)
- 10 % Potassium hydroxide (KOH)(11)

Evaluating
- Filtration capacity
- Digestion efficacy
- Recovery rates

3. Filtration to 25 µm?

Optimisation? Excessive foaming!

Optimisation? Too expensive!

Optimisation? Filtration at 63 µm only.

Neutralising digestate with citric acid allowed filtering over 1.2 µm.

→ KOH was selected for further steps.

4. Digestion efficacy

Fig.3: Tissue digestates (n = 3) filtered over 1.2 µm. Left: *M. gigas*, right: *R. philippinarum*

KOH

M. gigas
98.0 ±0.5 %
79.1 ±37.3 mg

R. philippinarum
91.2 ±0.5 %
20.7 ±1.9 mg

5. Recovery rates (RR)

KOH

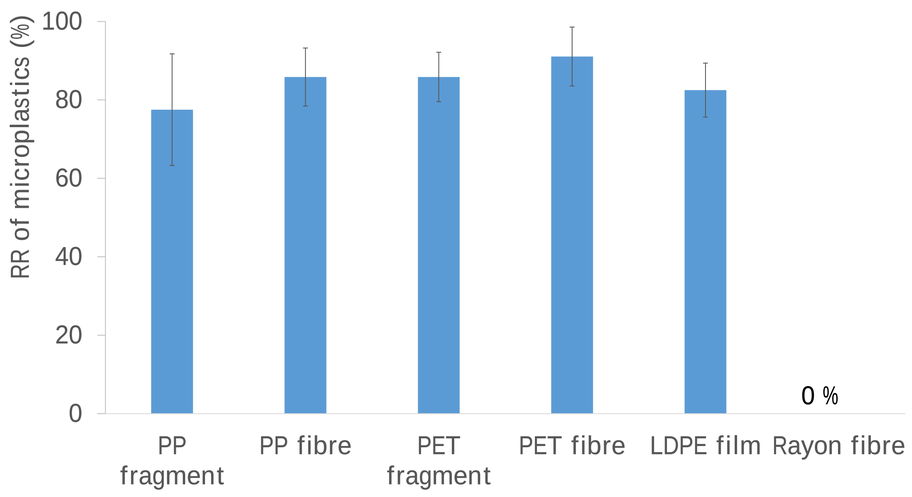

Fig.4: RR of dosed microplastics from oyster tissue after 10 % KOH exposure at 60° C for 2 days (n = 4). PP: Polypropylene, PET: Polyethylene terephthalate, LDPE: Low-density polyethylene.

Table 1: RR of rayon after KOH exposure at 40° C for 2 days (n = 4)

KOH strength	Recovered fibres
5 % KOH	87.5 ±37.7 %
10 % KOH	80.0 ±8.2 %

6. Discussion & Conclusion

Potassium hydroxide has previously been used to dissolve a range of tissues to extract microplastics, but often only filtered to >200 µm(11-14). We show that neutralised KOH allows recovery of particles to 1 µm. Previous studies showed negligible effect of KOH on a range of microplastics(14,15). In this present study, rayon was destroyed at 60°C, but not at 40° C.

It is recommended that 10 % KOH at 40° C for 2 days, neutralised with citric acid is used for extracting microplastics from biota to allow for comparability between studies and greatest scope to answer research questions.

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