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Peer Review File

Advanced Nanobubble Flotation for Enhanced Removal of Sub-10 µm Microplastics from Wastewater

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Parts of this Peer Review File have been redacted as indicated to remove third-party material.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Comments for NCOMMS-24-07378
The issue of small-sized microplastic pollution has garnered significant attention in recent years. This manuscript explores the potential of micro-nanobubble technology in effectively removing these particles. By shedding light on both the removal efficiency of microplastics across four different sizes and potential supplementary benefits for wastewater treatment, this study highlights the potential of nanobubble-assisted flotation as a promising approach to tackle the challenges associated with eliminating small-sized microplastics. While the topic is intriguing and likely to interest the broad audience of Nature Communications, the overall quality of the manuscript falls short, and the study lacks novelty to some extent. Specific suggestions and comments for enhancement are provided below:

1. The primary concern revolves around the novelty of this work as a whole. Several studies, e.g., 10.1016/j.watres.2017.07.005 and 10.1016/j.chemosphere.2023.139011, have previously proposed DAF processes for microplastic removal. Many of these studies have reported removal efficiency exceeding 90% even without the addition of coagulants. The performance of MB-F and MNB-F in fine microplastic removal in the present study did not appear compelling compared to other existing studies utilizing conventional DAF methods. In addition, some studies have reported that NBs could assist the MBs during the flotation process (e.g., 10.1016/j.seppur.2017.06.007), and facilitate the removal of organics (e.g., 10.1016/j.seppur.2009.12.021). These prior findings may diminish the perceived novelty of the presented study. The authors should also further strengthen the discussion, explicitly highlighting what sets their work apart from existing research and emphasizing the unique advancements introduced by their study.

2. Another major concern pertains to the implications and practical significance of this study. For instance, the study exclusively focuses on spherical polystyrene microbeads, whereas real wastewater contains various types and shapes of microplastics. If MNB-F exerts varying effects on these diverse particles, the relevance of the study to real-world scenarios may be diminished, potentially limiting the value of the findings. And in practical applications, the complex water matrix present in real wastewater may pose challenges to micro-nanobubble generation devices. While upgrading conventional DAF systems to MNB-F may offer potential benefits in terms of improved microplastic/organic removal, it is essential to carefully evaluate the associated costs and weigh them against the expected benefits to determine the feasibility and cost-effectiveness of such upgrades in practical applications.

3. On the other hand, the injection of MBs- or MNBs-enriched deionized water into the flotation jar testers at a flow rate of 240-300 mL/min raises concerns regarding the adequacy of this flow rate to transport microbeads to the top space of the tester due to their small mass. This scenario could inadvertently lead to an overestimation of the effectiveness of MBs/MNBs-F due to flow-induced movement rather than the action of bubbles alone. Without comparative tests against a standard method like conventional DAF, it becomes challenging to accurately gauge the efficacy of MNB-F and MB-F. The addition of MBs- or MNBs-enriched deionized water may further dilute the concentrations of microplastics (also TOC), potentially affecting the accuracy of the results.

4. L55, why is the average removal stated to reach 72% ±61%?

5. There are many small mistakes scattered throughout. For instance, Figure 1 (A) and (B) are incorrectly labeled. Additionally, the current Figure 1(A) appears blurry. Furthermore, the unit of nanobubble concentration in Figure 1(C) is also incorrect. The caption for Figure 3(D) is missing…

6. L135-136: it is noted that there was no significant difference between MB-F and MNB-F regarding the removal efficiency of both sizes of microplastics. This observation raises questions about the necessity of subsequent work.

7. L292-293: the standard curve for the calibration of the APF methods may not be necessary, as indicated by Figure 6(B), given that it is a well-established determination method.

8. L317-318: In fact, it is possible to determine the concentration of naturally occurring microplastics in wastewater. However, the background concentrations in the real wastewater were not provided in this study.

9. L408-409, What do the authors mean by setting the recycle ratio at 20%? Did the wastewater containing microplastics flow through the micro-nanobubble generation devices? Is there a possibility that the devices intercepted a proportion of microplastics due to their small size?

10. Multiple methods exist for generating both microbubbles and nanobubbles. Did the choice of generation method have an impact on the removal efficiency of microplastics?

Reviewer #2

(Remarks to the Author)
In this study Jia et al investigate the removal of various sizes of microplastics by microbubble flotation with and without nanobubbles present. The results explain particles characterization using different techniques. The removal results are rationalized using a mathematical model. In a final section the authors also look at radical generation and TOC removal by microbubbles and explain the removal enhancing effect of nanobubbles in a schematic overview. Overall, the article is well written and structured. The results are interesting and will make a relevant contribution to this field of research. The methods description is mostly adequate, I made a few remarks. The authors struggle a bit with a consistent approach of particle and bubble determination across the different size ranges, but tried their best to overcome this issue in the study. Below is a list of comments that require the authors attention. In addition, due to the high impact factor of the target journal I think the authors may want to elaborate stronger on the novelty and significance of this research by making a stronger literature related discussion both in the latter part of the introduction and in the results and discussion section.

45: Meanwhile, the abundance of small-size MPs exists in high abundance, circular sentence check.
46: A previous study investigating the particle size distribution of MPs between 10-400 μm in the Atlantic Ocean found that the abundance of MPs increased with decreasing particle size, with 10μm MPs existing in the highest abundance, could use a more general overview reference, generally particles fragment and their number increases.
60: Therefore, the development of effective treatment techniques to enhance the removal of small-sized MPs has raised great interest, requires reference(s).
64: believed?
87: delete ‘as a simple and facile’
100-101: The consistency of the size of MPs was confirmed under a microscope, which revealed the presence of MPs with sizes of 40-50 μm, 20 μm, 10 μm and 1 μm. Change phrasing here, it needs to be clear that these are added commercial microplastics.
101: The zeta potential of the MPs at neutral pH was around -34.58 mV. Is this important, expected? Do you use Zeta potential later?
103: ‘the first task is’, keep use of past/present consistent.
104-107: Phrasing could be improved here. Is the size range and bubble concentrations mentioned what is also used in flotation practice, or are these parameters determined by the experimental methods used to produce such bubbles?
114: Delete ‘Remarkably’.
111-121: Section requires comparison to results of others.
Figure 1 (a) requires more explanation, what are green, red dots, and yellow areas, the size legend needs to be increased to be readable.
142-143: The model utilized the commonly reported bubble size distribution of NBs and MBs. This requires giving the ranges used and references.
146-147: clearer visualization compared to?
172-174: Check this statement, this contrasts with the one in 171, and graph as well as numbers in (b) clearly shows that turbidity levels off. For this section also you need a comparison to literature in which size ranges turbidity measurements usually apply. For 1um provide linear regression analysis results. Figure 3a and b use non-linear x-axis, making it slightly difficult to see linearity, though values indicate 1um does not level off. Is the caption in (b) correct? In text 173, you indicate image analysis method used. I believe something is not correct here, have you assigned Figure in text accordingly because Figure 3d does not appear in neither caption nor text.
Figure 3c, although methods description appears later, you need to add a remark in text or figure caption on how to understand MPs area coverage, so reader can follow.
Figure 4: caption needs to indicate that lower row in Figure is the coagulant dosage used.
194-197 vs 227-230, watch repetitive statements.
Figure 5: the value on the left-hand side of (d) requires explanation or should be removed.

365: replace ‘delve’ by better term. Review term ‘diminutive’.
367-369: The first and the second part of the sentence seem not to fit together, first is on process, second is on method to measure.
370: fine?
373: smaller bubble sizes unclear in context; do you mean smaller microbubbles or nanobubbles?
379: delete minute
387: concentration of stock solution required.
388: synthetic vs real wastewater, when used in experiments unclear in results section, check and revise.
390: do you have characteristics the real wastewater used, normally, this should be presented in the SI.
394: Check Figure assignment as Fig 8 is both in line 361 and 439.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have given the detailed response and addressing almost the concerns raised. However, after carefully reviewing the revisions, I still have several concerns on the manuscript.
1. Novelty Concerns: While your study focuses on the removal of smaller-sized microplastics (MPs, 1-50 μm) using nanobubble (NB)-assisted flotation, this approach does not represent a substantial departure from existing methodologies. Previous studies have already demonstrated that nanobubbles can assist microbubbles during the flotation process (e.g., 10.1016/j.seppur.2017.06.007). Additionally, I have identified some other relevant studies (e.g., 10.1016/j.hazadv.2022.100139; doi.org/10.1016/j.jwpe.2023.104637) that have considered the removal of smaller-sized MPs using modified dissolved air flotation techniques. These studies collectively diminish the novelty of your approach. Additionally, a more comprehensive review of existing flotation studies to remove MPs should be performed. The incremental improvement in targeting smaller MPs, while important, does not sufficiently differentiate your work from these established techniques.
2. Significance of Techniques Used: The application of mathematical models to analyze bubble-particle interactions and the exploration of radical generation by nanobubbles, while useful, do not introduce advanced or groundbreaking techniques. These approaches are well-documented in the literature, and their application in your study does not provide a significant leap forward in the field. The existing methods for bubble-particle dynamics simulation are robust and have been extensively applied.

Reviewer #2

(Remarks to the Author)
This is a revised version of a previously submitted manuscript that I reviewed. Overall, the authors did a good job in addressing both reviewer comments, including explaining better the novelty, an additional literature analysis, some additional experiments and improved figures.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed well all the comments.

Reviewer #1

The issue of small-sized microplastic pollution has garnered significant attention in recent years. This manuscript explores the potential of micro-nanobubble technology in effectively removing these particles. By shedding light on both the removal efficiency of microplastics across four different sizes and potential supplementary benefits for wastewater treatment, this study highlights the potential of nanobubble-assisted flotation as a promising approach to tackle the challenges associated with eliminating small-sized microplastics. While the topic is intriguing and likely to interest the broad audience of Nature Communications, the overall quality of the manuscript falls short, and the study lacks novelty to some extent. Specific suggestions and comments for enhancement are provided below:

1. The primary concern revolves around the ***novelty of this work*** as a whole. Several studies, e.g., 10.1016/j.watres.2017.07.005 and 10.1016/j.chemosphere.2023.139011, have previously proposed DAF processes for microplastic removal. Many of these studies have reported removal efficiency exceeding 90% even without the addition of coagulants. The performance of MB-F and MNB-F in fine microplastic removal in the present study did not appear compelling compared to other existing studies utilizing conventional DAF methods. In addition, some studies have reported that NBs could assist the MBs during the flotation process (e.g., 10.1016/j.seppur.2017.06.007), and facilitate the removal of organics (e.g., 10.1016/j.seppur.2009.12.021). These prior findings may diminish the perceived novelty of the presented study. The authors should also further strengthen the discussion, explicitly highlighting what sets their work apart from existing research and emphasizing the unique advancements introduced by their study.

Response: We acknowledge and appreciate the concerns raised by the reviewer regarding the novelty of our work. The reviewer's constructive comments and the provision of relevant literature have significantly enhanced the quality of our manuscript. While it is true that there are studies on the removal of microplastics (MPs) and other pollutants using Dissolved Air Flotation (DAF), microbubbles (MBs), and nanobubbles (NBs), these primarily focus on larger-sized MPs (50 µm - 5 mm). Our research uniquely addresses the removal of smaller-sized MPs (1 µm -50 µm), filling a critical gap between scientific understanding and practical applications.

Novelty: Recent research on microplastic (MP) removal from wastewater has primarily addressed larger-sized MPs ***(50 µm - 5 mm)***. However, increasing attention is now being given to smaller-sized MPs ***(1-50 µm)*** due to their ubiquity and harmful effects. These small-sized MPs are difficult to manage because of their high mobility and large surface area allow them to carry microcontaminants, posing further risks to marine ecosystems and human health. Despite their prevalence and significant impact, there is a ***substantial knowledge gap*** in accurately quantifying, analyzing, and removing small-sized microplastics (MPs) in wastewater treatment. Our novelty lies in addressing these challenges, effectively bridging the gap between scientific understanding and practical solutions.

Practical significance: Our research introduces a practical solution for efficiently removing

small-sized microplastics (MPs) from wastewater through an innovative nanobubble (NB)-assisted flotation technique. This method outperforms traditional microbubble flotation in removing MPs in particular as small as ***1~ 10 µm*** and irregularly shaped particles from both real and synthetic effluents, while also reducing coagulant dosage, thereby enhancing eco-friendliness.

Scientific advancement: We employed ***advanced techniques*** for precise characterization and quantification of MPs, integrated mathematical models to analyze ***bubble-particle interactions at the nanoscale*** and explored ***radical generation by NBs*** for organic contaminant removal. Additionally, our ***economic analysis*** reveals that this nanobubble-assisted approach is cost-effective and scalable compared to conventional systems, promising significant advancements in wastewater treatment and marine ecosystem protection.

In response to Reviewer 1's comments, which referenced the claim that "*Many of these studies have reported removal efficiency exceeding 90% even without adding coagulants",* we have carefully examined the cited source, '10.1016/j.chemosphere.2023.139011'. This paper by Monira et al. (2023) stated that '*Few studies have investigated the performance of the DAF process for MP removal (Talvitie et al., 2017a, Talvitie et al., 2017b; Esfandiari and Mowla, 2021; Sol et al., 2021).*

For clarification, here are our findings from the specific articles mentioned:

1. **Talvitie et al., 2017 (10.1016/j.watres.2017.07.005):** This study reports on DAF removal of MPs larger than 20µm using a high concentration of polyaluminium chloride (40mg/L), a flocculant that acts similarly to coagulants. This indicates that while coagulants were not used, flocculation played a crucial role in achieving high removal efficiencies.
2. **Esfandiari and Mowla, 2021 (10.1016/j.psep.2021.05.027):** The authors explored the influence of operational parameters on the removal of a range of polyethylene MP sizes (10µm-5mm), achieving removal efficiencies between 74% to 94% under varying conditions. Importantly, they noted that the removal efficiency drops to only 25-30% when the coagulation/flocculation step is omitted.
3. **Our manuscript** reports that while removal of larger MPs (>20µm) can be highly effective (80-90% removal), the challenge remains with smaller-sized MPs which have not been adequately addressed in existing literature. By focusing on this underserved area, our study contributes novel insights into the removal mechanisms and efficiencies for smaller MPs, thereby advancing the field and providing practical solutions for wastewater treatment.

In response to the reviewer's comment, the following paragraphs and a table are added to the revised manuscript, highlighting the research gap, stressing the novelty, summarizing existing literature, and facilitating direct comparison with existing literature.

Text and table added to summarize existing literature:

Line 40-51: It was reported that in water matrices, microplastic (MP) abundance increases by 1.6 to 7.9 times with every tenfold reduction in particle size[2]. Wastewater treatment plants are considered one of the main point sources of MP discharge, particularly those in the smaller size range (< 100 µm) that are not effectively separated by conventional pretreatment and primary and secondary treatment processes and are dominant in the effluent[3]. The ecotoxicological impact of small-sized MPs is of greater concern due to their increased likelihood of ingestion by aquatic organisms and their higher surface area-to-volume ratio, which renders them efficient carriers of micropollutants and pathogens[4,5]. For instance, studies have shown that mussels can ingest MPs ranging from 3 to 10 µm, and crabs are known to accumulate spherical MPs of 8 to 10 µm in size through feeding and via their gills[6,7]. Consequently, there is a growing interest in the development of more effective treatment methods to enhance the removal of small-sized MPs from wastewater[8–10].

Line 55-64: Previous research has examined the efficacy of flotation processes for MP removal and has generally found them to be effective for larger-sized MPs, yet most studies do not cover MPs in the smaller-sized range. For instance, one study measured the removal of MPs (>20 µm) in secondary wastewater using full-scale dissolved air flotation (DAF) with a pre-treatment of 40mg/L of polyaluminium chloride[16]. They reported a removal efficiency of 95% with grab sampling and 48% with 24-hour composite sampling. Another study conducted experiments using a lab-scale DAF jar tester and compared the removal of MPs across different size ranges. They found that the removal efficiency for 2-5 µm, 5-10 µm, and 15-20 µm MPs was lower than 35%, 45%, and 85%, respectively[17]. Table S.1 comprehensively reviews published studies on flotation for MP removal.

Text added to highlight the research gap and need and significance of the study:

Line 80-95: In response to the significant challenge posed by the removal of small-sized MPs during wastewater treatment, this study introduces a novel approach that specifically targets the effective removal of small-sized MPs ranging from 1 to 50 µm by combining NBs to the existing microbubble flotation (MB-F) process and utilizing varying concentrations of AlCl3 coagulant (5-25 ppm). The efficacy of this combined micro-nanobubble flotation (MNB-F) technique was tested in both synthetic and real wastewater environments and compared with nanobubble flotation (NB-F) and traditional MB-F. Recognizing the intricate challenges of understanding particle-bubble interactions due to the small sizes of MPs and NBs, numerical flotation models were employed to elucidate the underlying mechanisms for enhanced flotation processes from a microscopic perspective. Additionally, we evaluated the reactivity of NBs in water and compared the concurrent removal of total organic carbon (TOC) from wastewater using both MB-F and MNB-F. The results derived from both the experimental setups and mathematical modeling provide compelling evidence that NBs play a meaningful role in enhancing existing flotation processes. By offering an improvement over existing technologies,

this study holds considerable practical significance for wastewater treatment facilities aiming to reduce MP pollution more effectively.

Table S1. Review of existing flotation studies to remove MPs.

No. of studies and reference	Set-up	MPs characteristic	Solution	Pretreatment type and dosage	Pretreatment condition	Flotation condition	MPs removal efficiency	Sampling method	Quantification method
1[16]	Full-scale DAF	**Polymer type:** 13 types of naturally occurring MPs; **Size:** >20 μm **Concentration:** 2-2.3 microplastic/L	Secondary wastewater	**Flocculant:** Polyaluminium Chloride **Dosage:** 40mg/L	N/A	N/A	95%	Sampling at a depth of ~1m Volume: 2L-1000L	Stereo microscopy
							48%	24-h composite sampler	
2[18]	Flotation cell	**Polymer type:** PE; **Size:** >10 μm; **Concentration:** 1.2mg/L	Synthetic greywater	**Coagulant:** $FeCl_3 \cdot 6H_2O$ Dosage: 10mg/L; 15mg/L; 20mg/L	Rapid mixing (350 rpm) 1 min; Slow mixing (100 rpm) 15 min	Recirculation ratio = 20 %, flotation speed=30 cm/min, flotation time=5 min, saturation time=25 min Pressure: 4-6 bar; pH:6-8; Flow rate: 500-1000mL/min;	48.68%-67.69%	Remaining treated water in the flotation cell	Stereo microscopy
				Coagulant: $AlCl_3 \cdot 6H_2O$ **Dosage:** 10mg/L; 15mg/L; 20mg/L)			74%-94.5%		
				N/A	N/A		25-30%		
3[17]	Flotation cell	**Polymer type:** PE; PET; PA66 **Size:** 2-106 μm **Concentration:** 100mg/L	Deionized water	**Bubble modifier:** CTAB **Dosage:** 0.6-1.4 mg/L	A dose of bubble modifier was added to the saturator prior to flotation	N/A	Optimal removal at a dosage of 1.2mg/L: PA: 43.8%; PET: 58.5%; PE: 68.9%	500mL of supernatant	Laser particle analyzer
				Bubble modifier: PDADMAC **Dosage:** 0.4-1.2mg/L			PA: 46.3% (0.8 mg/L-optimal) PET: 72.6（1.0mg/L-optimal）		

							PE: 46.3% (1.2mg/L-optimal）		
				N/A	N/A		PA: 32.7% (0.5Mpa-optimal) PET: 38.9%（0.4Mpa-optimal） PE: 48.7%（0.4Mpa-optimal）		
4[19]	Column flotation	**Polymer type:** PE, PP, PVC, PMMA **Size:**10–600 μm **Concentration:** 500mg/L	Deionized water with Tween-20	N/A	N/A	Flotation time: 2min Flow rate: 2500mL/min	PE: 83.3% PP: 79.3% PVC: 66.4% PMMA: 82.8%	Remaining Treated water in the flotation column	High-resolution camera
5[20]	Column flotation	**Polymer type:** PES, PAN, PA, PP **Size:**5–3500 μm **Concentration:** 10, 50, 500 5000mg/L	Synthetic Laundry wastewater	**Flocculant:** polyacrylamide **Dosage:** 1ppm,4ppm	N/A	Flow rate: 0.5-1.6 SLPM	Fibers & particle with size >20um and initial concentration >10mg/L can achieve almost 100% of removal	Samples from treated water	Weight difference method (assisted by Confocal Microscopy when needed)
6[21]	Froth flotation cell	**Polymer type:** PP, PE, ABS, PS, PET, PVC **Size:**100-1000 μm **Concentration:** 666.67mg/L	Deionized water with kerosene	**Flocculant:** methyl isobutyl carbinol **Dosage:** 0.02mL/L	Mixing: 900rpm	Flotation time: 1 min Flow rate: 1300mL/min	PP & PE: >96% ABS, PS, PET and PVC: 89, 93, 96, and 89%	Samples from froth and from treated water	Weight difference method

2. Another major concern pertains to the implications and practical significance of this study. For instance, the study exclusively focuses on spherical polystyrene microbeads, whereas real wastewater contains various types and shapes of microplastics. If MNB-F exerts varying effects on these diverse particles, the relevance of the study to real-world scenarios may be diminished, potentially limiting the value of the findings. In practical applications, the complex water matrix present in real wastewater may pose challenges to micro-nanobubble generation devices. While upgrading conventional DAF systems to MNB-F may offer potential benefits in terms of improved microplastic/organic removal, it is essential to carefully evaluate the associated costs and weigh them against the expected benefits to determine the feasibility and cost-effectiveness of such upgrades in practical applications.

Response: We agree with the reviewer that it is crucial to expand our research to include various types and shapes of microplastics to better understand the implications for real-world scenarios. Additionally, we recognize the importance of assessing the practical significance and economic viability of our system.

First, to better address real-world scenarios, we conducted additional experiments to include the removal of irregular-shaped MPs: three different polymers commonly found in the natural environment: ***1) polystyrene, 2) polyethylene, and 3) polypropylene terephthalate (PET).*** Furthermore, we recognized that the low level of MP concentration used in the initial tests (1 mg/L) may not accurately reflect practical conditions for detection and analysis. Hence, we increased the MP concentration to 4 mg/L in the subsequent experiments. This modification was necessary because irregular-shaped MPs, which are commonly white or transparent, can be challenging to detect accurately at low concentrations. A separate section on MPs removal is discussed in the revised manuscript as follows:

Line 328-355: Building on this demonstration of NBs' positive effect on the removal of spherical PS MPs, we expanded the investigation to include irregular-shaped MPs comprised of three different polymer types, PS, polyethylene (PE) and polyethylene terephthalate (PET) on their removal efficiency through MB-F and MNB-F processes. The results indicated that the removal efficiencies for irregular-shaped PS and PE were comparable, whereas those for PET were notably lower. In the flotation process, density is the primary determinant of removal efficiency among different polymer types. Particles with higher densities tend to settle more readily, posing challenges for removal via flotation[57]. Consequently, the removal efficiencies for PS and PE, whose respective densities are 0.88–0.96 g/cm³ and 0.96–1.05 g/cm³, were similar and higher compared to PET, which has a density of 1.38 g/cm³ (**Fig. S7**).
Furthermore, irregular-shaped MPs generally exhibited higher removal efficiencies than spherical MPs. The edges and corners of irregular particles could facilitate the thinning and rupture of the liquid film between the bubble and particle, thus reducing the critical induction time for particle capture and enhancing the collision efficiency with the bubbles[58,59]. A previous study comparing the removal of spherical and non-spherical PE MPs sized 50-60 µm via MB-F reported removal efficiencies of approximately 83-90% for irregularly shaped MPs, compared to about 52-53% for spherical MPs[57]. In our study, spherical PS and irregular PS did not show a significant variation in their removal efficiency, which may be attributed to the presence of a large number of irregular particles smaller than 1 µm. Additionally, since the

irregular-shaped MPs were only available in white/transparent colors, a higher MP concentration (4mg/L) was utilized compared to the tests for spherical particles (1mg/L). This adjustment, which potentially influenced the comparative results, was necessary to ensure that the turbidity meter accurately reflected the concentration. Future studies should further explore these aspects to elucidate the dynamics of MP removal across different shapes and polymer types. However, regardless of MP's polymer type and their shapes, we found a clear enhancement in removal efficiency with the use of MNBs. The presence of smaller bubbles can increase collision and adhesion efficiency, and thereby improve removal efficiency across all polymer types.

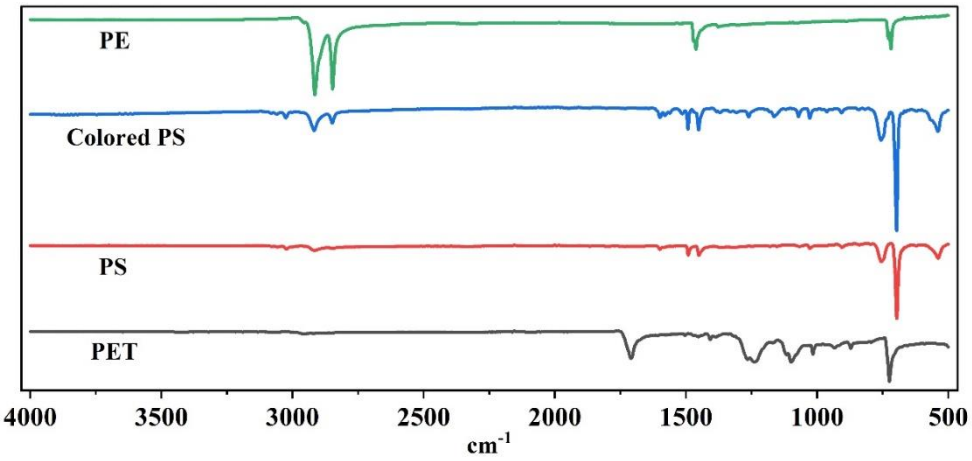


Figure S2. FT-IR spectrum of the purchased commercial microplastics.

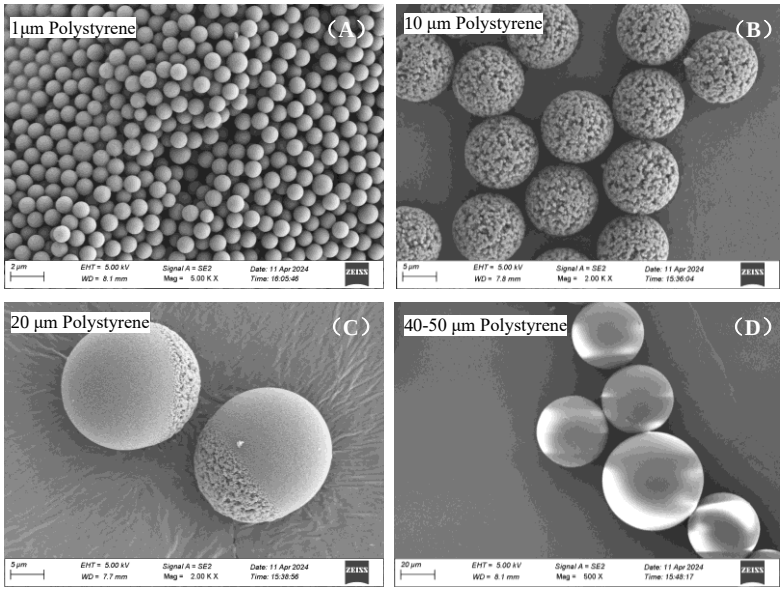


Figure S3. FE-SEM image of the purchased commercial spherical colored PS MPs.

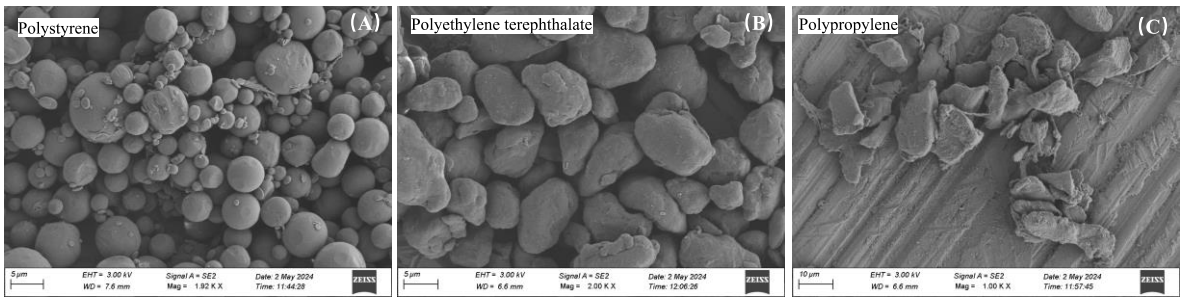


Figure S4. FE-SEM image of the purchased commercial irregular PS, PE, and PET MPs.

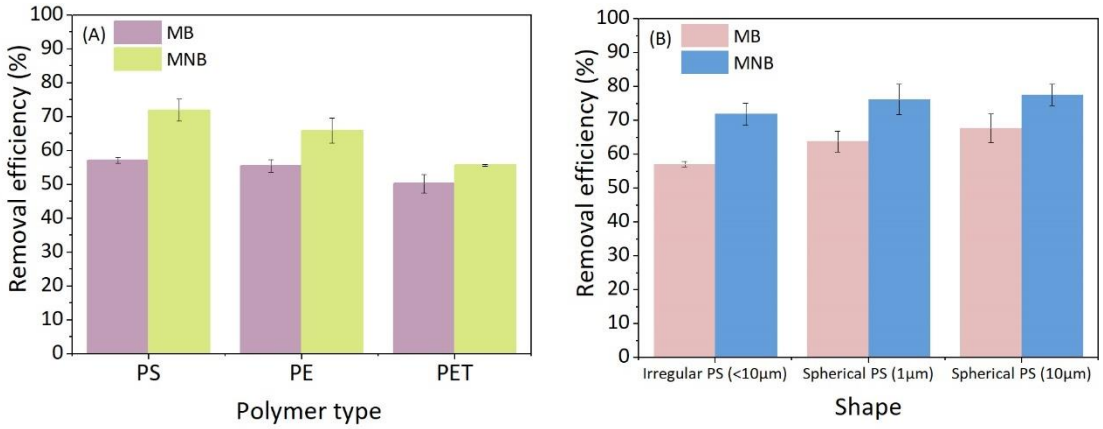


Figure S6. The effect of different polymer types and polymer shapes on removal efficiency. (A) Comparison of irregular PS, PE, and PET with size <10μm MPs removal by MB-F and MNB-F at $AlCl_3$ dosage of 10ppm. (B) Comparison of irregular PS (<10μm) at $AlCl_3$ dosage of 10ppm and initial concentration of 4mg/L with spherical PS of size 1μm and 10μm at $AlCl_3$ dosage of 10ppm and initial concentration of 1mg/L.

Second, in response to the challenges to micro-nanobubble generation devices with complex water matrix and the economic viability of NB-assisted DAF, we direct attention to our recently published review paper "***Nanobubbles in water and wastewater treatment systems: Small bubbles making a big difference***" (doi.org/10.1016/j.watres.2023.120613). Our review includes case studies and real-world applications demonstrating successful integration of NB generators in diverse wastewater treatment settings, including industries like dairy, oil, and gas [22]**.** These cases confirm the robustness of NB technology in handling complex water matrices effectively.

Lastly, Economic Viability, Our review also assesses the economic aspects, highlighting that although initial costs for NB-assisted DAF systems are higher than conventional systems, they offer significant savings in energy and maintenance costs. Long-term savings are estimated at up to 40% over ten years, making NB technology a cost-effective option for large-scale wastewater treatment[22]**.** This provides an initial estimation of the cost-effectiveness of NB-assisted DAF and its practicability in large-scale wastewater treatment applications.

While this study primarily focused on the efficacy of nanobubble (NB) technology in small

sized microplastic (MP) removal, it did not include a detailed cost-effectiveness analysis. Recognizing the importance and relevance of economic evaluations, we have acknowledged this limitation within our research in the Revised Manuscript as follows:

Line 434-447: The findings of this study underscore the critical role of NBs in conjunction with MBs in enhancing the removal efficiency of small-size MPs. This enhancement offers promising implications for the broader application of this technique in eliminating other fine suspended particles commonly found in wastewater.
While the practical implementation of NBs presents a viable path forward, one concern would be the feasibility of replacing existing techniques with NB technology, our preliminary economic assessment comparing the estimated costs of MB-F and MNB-F, published in a previous study, revealed that while the initial investment costs for MNB-F are higher than those for MB-F due to higher capital costs, the operational costs are 20% lower, as MNB-F reduces electricity consumption and chemical requirements[18]. Hence, it was projected that after one year of operation, the overall costs of MNB-F will become lower than those of MB-F. However, more studies are necessary to provide a more comprehensive economic assessment of NB technology implementation.

[*REDACTED*]

Figure S9. [*REDACTED*]

3. On the other hand, the injection of MBs- or MNBs-enriched deionized water into the flotation jar testers at a flow rate of 240-300 mL/min raises concerns regarding the adequacy

of this flow rate to transport microbeads to the top space of the tester due to their small mass. This scenario could inadvertently lead to overestimating the effectiveness of MBs/MNBs-F due to flow-induced movement rather than the action of bubbles alone. Without comparative tests against a standard method like conventional DAF, it becomes challenging to accurately gauge the efficacy of MNB-F and MB-F. The addition of MBs- or MNBs-enriched deionized water may further dilute the concentrations of microplastics (also TOC), potentially affecting the accuracy of the results.

Response: We appreciate the opportunity to clarify the specifics of the flow rates used in our experiments for microplastic (MP) removal, and to address the concerns regarding potential flow-induced movement effects. 1) The flow rate of 240-300 mL/min used in our jar tester is consistent with standard practices in flotation processes. As detailed in Table S1 of our supplementary materials, comparative studies in larger flotation columns and tanks often employ flow rates ranging from 500 to 2500 mL/min. This benchmarking helps to establish that our chosen flow rates are well within the typical range for such experiments. 2) In flotation systems, both at full-scale and lab-scale, air-saturated water is typically introduced horizontally into the tank rather than being directly purged from the bottom (as highlighted in Figure X). This method promotes effective bubble-particle interactions whereby the bubbles, once formed, transport the particle aggregates towards the water surface. 3) The potential for flow-induced movement was also tested by the removal of irregular PS MPs using an $AlCl_3$ dosage of 10 ppm by purging DI water at the same speed, compared with the MB-F and MNB-F treatments. As depicted in Figure X, compared to MB-F and MNB-F, the removal efficiency by purging DI water at the same speed is significantly lower, with less than 30% removal.

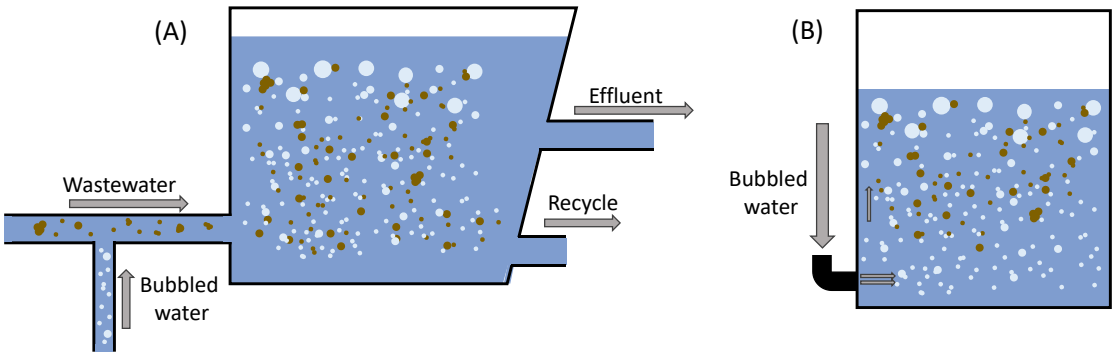


Figure R1. The comparison of industrial scale and bench scale flotation processes.

Besides, the statement by reviewer on '*The addition of MBs- or MNBs-enriched deionized water may further dilute the concentrations of MPs (also TOC), potentially affecting the accuracy of the results.*' was already considered in our study. As shown in the method section 'MPs removal quantification', we have excluded the effect of dilution due to the purging of saturated air on MPs removal in our calculation by multiplying a dilution factor($\frac{\text{Initial water volume in the flotation cell}}{\text{Final water volume in the flotation cell}}$) when calculating the initial MP concentration. The equation provided in the material and method is as below, and we have added a sentence to clarify the concern further:

Line 540-548: Finally, the overall removal efficiency was calculated based on the difference in the area coverage or turbidity of the MPs on the filter before and after flotation using the following equation. To account for the effect of dilution on removal effects, a dilution factor was introduced when calculating the initial MP concentration.

$$\text{Initial MPs concentration} = \frac{\text{The sum of MPs area coverage or turbidity before sample} \times \text{Initial water volume}}{\text{Final water volume}}$$

The overall removal efficiency was calculated as follows:

$$\text{Removal Rate (\%)} = \frac{\text{Intial MPs concentration} - \text{Final MPs concentration}}{\text{Initial MPs concentration}} \times 100\%$$

On the other hand, we did not include a dilution factor for the TOC concentration results. This decision was made because we included results for TOC removal at a coagulant dosage of 0 ppm, which were conducted to determine whether radicals produced by NBs degraded organics. Compared with the TOC concentration before flotation, we found that at 0 ppm, the observed removal was solely due to dilution. When accounting for a dilution factor, the removal results would be zero or negative.

4. L55, why is the average removal stated to reach 72% ±61%?

Response: The figure of 72% ± 61% was cited from the study by Leslie et al. (doi.org/10.1016/j.envint.2017.01.018). This study analyzed influent and effluent data collected on the same day from seven different wastewater treatment plants (WWTPs) in the Netherlands, resulting in the calculated average MP removal rate with a high standard deviation of 61%. To enhance clarity and avoid potential confusion among readers, we have decided to exclude this specific reference from our manuscript. Instead, we have expanded our literature review to include additional studies that provide a broader overview of MP removal efficiencies across various WWTPs, as illustrated in the updated Figure X.

5. There are many small mistakes scattered throughout. For instance, Figure 1 (A) and (B) are incorrectly labeled. Additionally, the current Figure 1(A) appears blurry. Furthermore, the unit of nanobubble concentration in Figure 1(C) is also incorrect. The caption for Figure 3(D) is missing.

Response: Thank you for highlighting the mistakes in our manuscript. All the mistakes were corrected, and the entire manuscript was carefully checked, enhanced, and proofread again before the submission.

6. L135-136: it is noted that there was no significant difference between MB-F and MNB-F regarding the removal efficiency of both sizes of microplastics. This observation raises questions about the necessity of subsequent work.

Response: As the reviewer correctly noted, our test showed no significant differences in removal efficiency between MB-F and MNB-F for MPs sized 40-50μm and 20μm. However,

the key insight from these findings was the limitation of MB-F in effectively removing smaller MPs, which are more abundant and pose higher ecotoxicological risks.

Our subsequent studies focused on removing smaller-sized MPs (10μm and 1μm), where we observed clear improvements with MNB-F compared to MB-F. The inclusion of nanobubbles appears to enhance the interaction with smaller MPs, improving removal efficiencies and addressing a critical gap in MP remediation which necessities the subsequent work.

7. L292-293: the standard curve for the calibration of the APF methods may not be necessary, as indicated by Figure 6(B), given that it is a well-established determination method.

Response: We agree with the reviewer that the calibration curve for the APF method is not necessary in the main text due to its established nature. We have moved this detail to the supplementary information to maintain the conciseness of the manuscript. The updated figure is as follows:

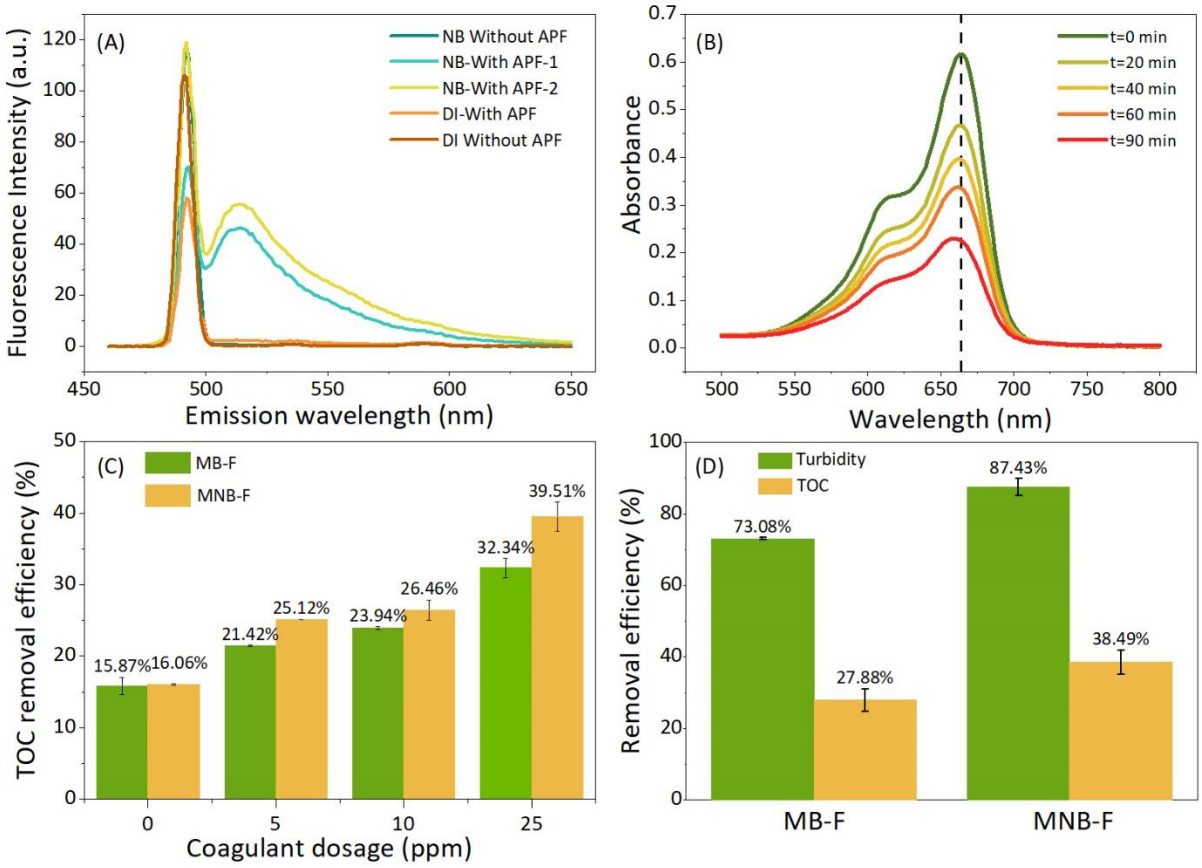


Figure 7. Assessing NBs' reactivity in water: **(A)** hydroxyl radical identification using APF method; **(B)** in-situ methyl blue degradation during NB generation; **(C)** comparison of TOC removal by MB-F and MNB-F at dosages of 5, 10, 25 ppm (independent t-test, $p<0.05$). **(D)** The removal of turbidity and TOC from treated primary wastewater by the two flotation processes at AlCl3 dosage of 10 ppm.

8. L317-318: In fact, it is possible to determine the concentration of naturally occurring microplastics in wastewater. However, the background concentrations in the real wastewater were not provided in this study.

Response: As suggested by the reviewer, ***we have added a section 'preliminary analysis of MPs in effluent from chemically enhanced primary treatment.*** The detailed results and method are as follows:

Line 97-116: Characterizing MPs, particularly those of smaller sizes, is challenging and often neglected, leading to a scarcity of data on their distribution in water bodies[29]. The few studies conducted on the size distribution of MPs in the influents and effluents of wastewater have reported that large MPs are more easily removed, leaving mostly small-sized MPs in the effluent (**Fig. 1(A)**). The observed limited efficiency in removing small-sized MPs suggests that a significant quantity of these particles continues to be released into natural water bodies. We performed a preliminary analysis of MP concentrations and sizes was performed to determine the size distribution of MPs in the effluent after chemically enhanced primary treatment (an additional step of coagulation and sedimentation after primary treatment). A Raman microscope was used to identify MPs larger than 5 µm, and an Optical Photothermal Infrared (O-PTIR) spectroscopy system was used to quantify MP particles between 1-5 µm. Selected spectra from the O-PTIR and Raman analyses are summarized in **Fig. S1**. After quantifying three sub-samples, we obtained an average of 25.67 ± 8.33 particles per 100 mL of the sample for MPs larger than 5 µm and an average of 204.53 ± 73.06 particles per 100 mL of the sample for MPs in the range of 1-5 µm. Similar to previous studies, which noted a pattern where the abundance of MPs increases with the decrease in particle size in various aquatic environments, this study also found a higher number of MPs in the smaller size range (**Fig. 1(B)**)[30–38]. The characterization results emphasize the urgent need to enhance the removal of small-sized MPs during water treatment processes.

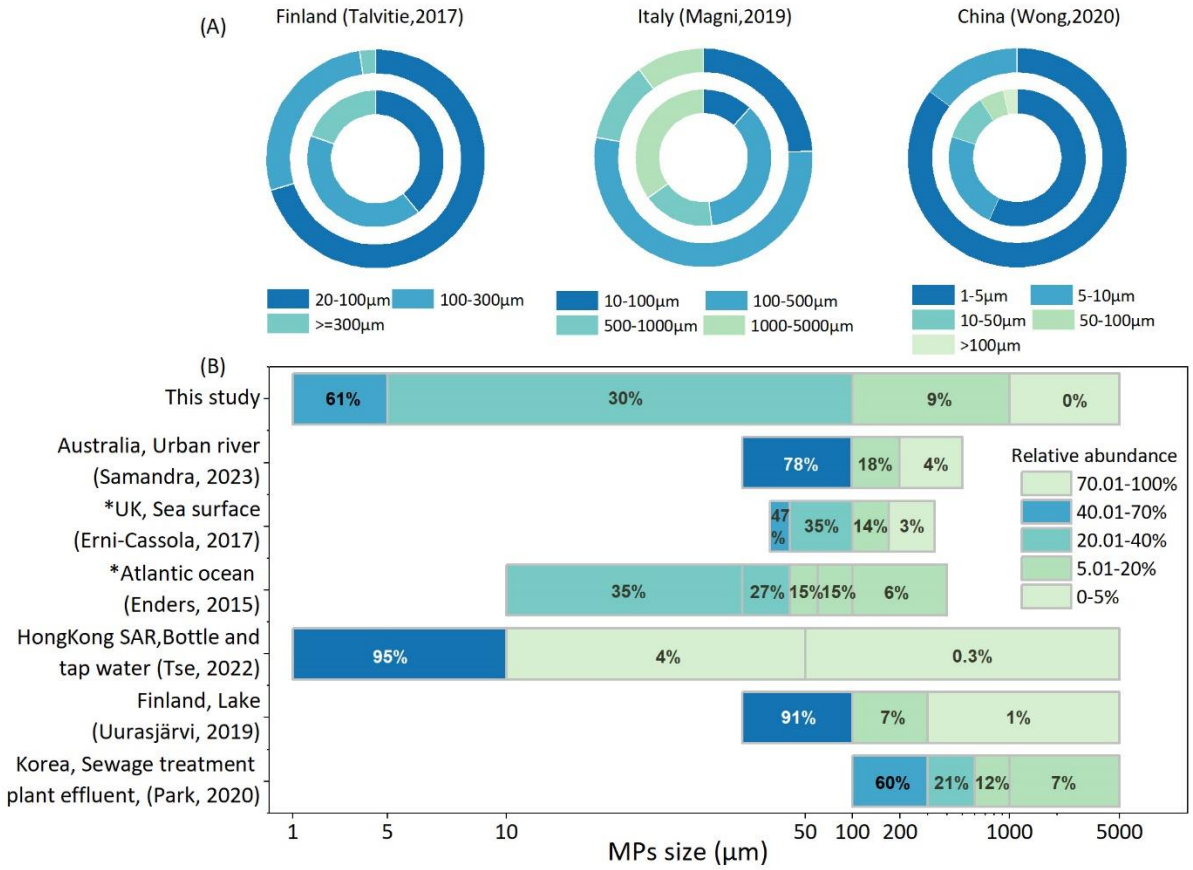


Figure 1. MP size distributions in various water matrices around the globe. **(A)** Percentage composition of MPs in the influent (inner circle) and effluent (outer circle) of wastewater treatment facilities. **(B)** Size distribution of MPs in surface water locations. *Data extracted from figures in referenced studies using WebPlotDigitizer.

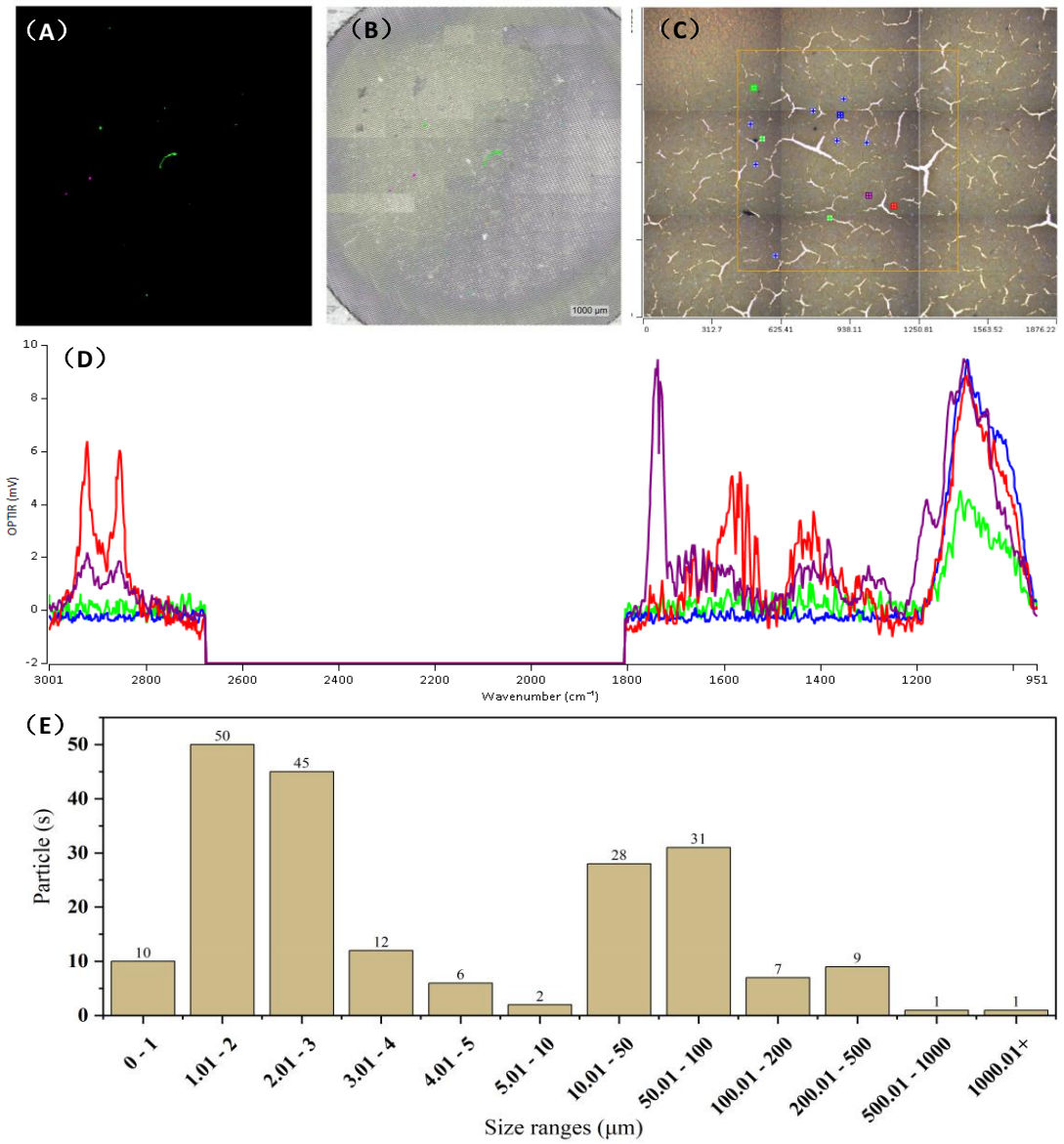


Figure S1. MPs Characterization in Treated Wastewater. (A-B): A selected Raman scanned area identified on a 5µm stainless steel filter. (C-D): O-PTIR scanned area and polymer spectrum identified within a 1000 µm² area on an 800 nm pore size, gold-coated polycarbonate filter. The four spectra (top panel) represent silicone polymer (blue), polyvinyl fluoride (red), methyl-cellulose (lime), and cellophane (purple), corresponding to the scanned area shown in the bottom panel. (E) Size distribution of MPs identified in wastewater samples.

Line 484-498: Two liters of the treated wastewater sample were obtained through bulk sampling from the effluent of a chemically enhanced primary treatment plant at Siu Ho Wan Sewage Treatment Works. Three 100 mL sub-samples were taken and digested with hydrogen peroxide (H_2O_2, 30 %; Sigma Aldrich, St. Louis, MO to remove organics. Each sample was first filtered through a stainless-steel sieve (Ø = 5 µm), and MPs larger than 5 µm were characterized with a Renishaw inVia confocal Raman microscope (Wotton-under Edge, Gloucestershire, U.K.). Subsequently, the solutions were further filtered using a gold-coated polycarbonate filter (Ø = 800 nm; Sterlitech, U.S.A.) and examined with an Optical

Photothermal Infrared (O-PTIR) spectroscopy system (mIRage microspectroscope, Photothermal Spectroscopy Corp.), which combines optical photothermal infrared (O-PTIR) and Raman spectroscopy, to quantify particles between 1-5 µm in size. Specifically, following a typical procedure for MPs quantification by mIRage, four areas on the sieve were randomly selected and scanned, with each area representing 5.09% of the sieved area[67]. The average number of MPs was estimated by extrapolating the number of MPs observed in each scanned area to the total filter area.

9. L408-409, What do the authors mean by setting the recycle ratio at 20%? Did the wastewater containing microplastics flow through the micro-nanobubble generation devices? Is there a possibility that the devices intercepted a proportion of microplastics due to their small size?

Response: In our study or in general DAF-based studies, the recycle ratio refers to the proportion of air-saturated water that is reintroduced into the DAF tank/jar tester, specifically, 20% of the total volume. We would like to clarify that we used air-saturated deionized water to eliminate any influence the device might have on intercepting a portion of the MPs. We acknowledge that the term "recycle ratio" could lead to confusion due to its variable usage in the literature. To improve clarity and avoid ambiguity in our manuscript, we have revised the description of the recycle ratio as follows:

Line 513-515: The recycle ratio, which refers to the amount of air-saturated water added into the jar, was set to 20%, which corresponds to adding 400 mL of air-saturated water into 1.6 L of the initial volume in the jar.

10. Multiple methods exist for generating both microbubbles and nanobubbles. Did the choice of generation method have an impact on the removal efficiency of microplastics?

Response: As the reviewer pointed out, several established methods currently exist for generating NBs, such as hydrodynamic cavitation, acoustic cavitation, an external electrical field and solvent exchange methods. Among these, hydrodynamic cavitation remains the primary method for generating NBs in various studies and commercial applications because it consistently produces NBs with high concentrations, stability, and small sizes[37]. Given that both bubble size and concentration can influence the flotation process, it is plausible that the choice of generation method could impact the removal efficiency of MPs.

Since the goal of this study

was to test whether the presence of NBs could enhance the removal efficiency in practical terms, we used a commercial NB generator and did not further investigate the effects of different generation methods. We believe that future studies exploring how different bubble generation could affect the removal efficiency during flotation processes would be of interest. However, as long as the NB generator using any generation method is able to generate bubbles at the optimized size and concentration, we believe this will yield similar removal efficiency.

Reviewer # 2 (Remarks to authors)

In this study, Jia et al. investigate the removal of various sizes of microplastics by microbubble flotation with and without nanobubbles present. The results explain particles characterization using different techniques. The removal results are rationalized using a mathematical model. In a final section the authors also look at radical generation and TOC removal by microbubbles and explain the removal enhancing effect of nanobubbles in a schematic overview. Overall, the article is well written and structured. The results are interesting and will make a relevant contribution to this field of research. The methods description is mostly adequate, I made a few remarks. The authors struggle a bit with a consistent approach of particle and bubble determination across the different size ranges but tried their best to overcome this issue in the study. Below is a list of comments that require the author's attention.

Response: We thank the reviewer for acknowledging key findings of our research and appreciating the quality of the paper. We also appreciate reviewer's valuable and constructive comments. All reviewers' comments are carefully addressed in the revised manuscript. Please find below the point-by-point responses to your comments:

1. In addition, due to the high impact factor of the target journal I think the authors may want to elaborate stronger on the novelty and significance of this research by making a stronger literature related discussion both in the latter part of the introduction and in the results and discussion section.

Response: Thank you for your constructive feedback regarding the need to emphasize the novelty and significance of our research. We appreciate that this issue was highlighted by another reviewer and the editor as well, which underscored its importance and led us to invest significant effort in revising these sections of our manuscript.

As we have responded to editor and reviewer Q1 and also in our revised manuscript, we have included a more comprehensive review of existing studies, highlighting how our research addresses significant research gaps. We have detailed the novel contributions of our study and its practical significance, ensuring that the unique and impactful aspects of our work are clearly communicated. Through these revisions, we aim to fully convey the originality and relevance of our findings to the field.

2. 45: Meanwhile, the abundance of small-size MPs exists in high abundance, circular sentence check.

Response: Thank you for pointing out the error. The sentence has been removed during the revision process.

3. 46: A previous study investigating the particle size distribution of MPs between 10-400 μm in the Atlantic Ocean found that the abundance of MPs increased with decreasing particle size, with 10μm MPs existing in the highest abundance, which could use a more general overview reference, generally particles fragment, and their number increases.

Response: Thank you for the reviewer's constructive comments. Following these recommendations, we have included a general overview reference in which the author analyzes data from 127 literature sources, illustrating the trend of increasing MP abundance with decreasing particle size. Additionally, we have also included a new figure that incorporates several literature characterizing MPs abundance in different size categories from various study locations. This addition in the revised manuscript provides a more comprehensive overview of how MP concentration increases as particle sizes decrease as follows.

Line 97-116: Characterizing MPs, particularly those of smaller sizes, is challenging and often neglected, leading to a scarcity of data on their distribution in water bodies[29]. The few studies conducted on the size distribution of MPs in the influents and effluents of wastewater have reported that large MPs are more easily removed, leaving mostly small-sized MPs in the effluent (**Fig. 1(A)**). The observed limited efficiency in removing small-sized MPs suggests that a significant quantity of these particles continues to be released into natural water bodies. We performed a preliminary analysis of MP concentrations and sizes was performed to determine the size distribution of MPs in the effluent after chemically enhanced primary treatment (an additional step of coagulation and sedimentation after primary treatment). A Raman microscope was used to identify MPs larger than 5 µm, and an Optical Photothermal Infrared (O-PTIR) spectroscopy system was used to quantify MP particles between 1-5 µm. Selected spectra from the O-PTIR and Raman analyses are summarized in **Fig. S1**. After quantifying three sub-samples, we obtained an average of 25.67 ± 8.33 particles per 100 mL of the sample for MPs larger than 5 µm and an average of 204.53 ± 73.06 particles per 100 mL of the sample for MPs in the range of 1-5 µm. Similar to previous studies, which noted a pattern where the abundance of MPs increases with the decrease in particle size in various aquatic environments, this study also found a higher number of MPs in the smaller size range (**Fig. 1(B)**)[30–38]. The characterization results emphasize the urgent need to enhance the removal of small-sized MPs during water treatment processes.

Figure 1. MP size distributions in various water matrices around the globe. **(A)** Percentage composition of MPs in the influent (inner circle) and effluent (outer circle) of wastewater treatment facilities. **(B)** Size distribution of MPs in surface water locations. *Data extracted from figures in referenced studies using WebPlotDigitizer.

4. 60: Therefore, the development of effective treatment techniques to enhance the removal of small-sized MPs has raised great interest, requires reference(s).

Response: Thank you for pointing out the missing references. We have added three relevant references to support our statement. The sentence has been revised as follows:

Line 40-51: Consequently, there is a growing interest in the development of more effective treatment methods to enhance the removal of small-sized MPs from wastewater[8–10].

5. 64: believed?

Response: The paragraph has been changed, and the term 'believe' is removed.

6. 87: delete 'as a simple and facile'

Response: Thank you for your suggestion. The words 'as simple and facile' have been removed from the text.

7. 100-101: The consistency of the size of MPs was confirmed under a microscope, which revealed the presence of MPs with sizes of 40-50 µm, 20 µm, 10 µm and 1 µm. Change phrasing here, it needs to be clear that these are added commercial microplastics.

Response: Thank you for pointing out the need for enhanced clarity. The phrasing has been revised as follows:

Line 123-125: The sizes and polymer types of the purchased MPs were verified using a stereomicroscope, a fourier transform infrared (FT-IR) spectrometer, and a field emission scanning electron microscope (FE-SEM), (Fig. 2(A), Fig. S2, Fig. S3, Fig. S4).

8. 101: The zeta potential of the MPs at neutral pH was around -34.58 mV. Is this important, expected? Do you use Zeta potential later?

Response: Thank you for your suggestion. The zeta potential of MPs, -34.58 mV, was not used in the later section, and we have removed the sentence.

9. 103: 'The first task is', to keep the use of past/present consistent.

Response: Thank you for pointing out the inconsistency in the use of tenses. We have carefully reviewed the entire manuscript and corrected any discrepancies in the use of past and present tenses to ensure consistency throughout the document.

10. 104-107: Phrasing could be improved here. Is the size range and bubble concentrations mentioned what is also used in flotation practice, or are these parameters determined by the experimental methods used to produce such bubbles?

Response: We have further clarified the meaning of the mentioned bubble size ranges and bubble concentrations in the text:

Line 127-130: As MB-F is a well-established treatment process, extensive research has been conducted on the sizes and distribution of bubbles generated during MB-F, with the reported sizes generally ranging from 20 to 100 µm with a bubble concentration of 10^4 bubbles/mL[39–41].

11. 114: Delete 'Remarkably'.

Response: We have removed the word 'Remarkably'. The sentence is now written as: **Line 141-142:** The bubbles maintained nearly consistent sizes throughout the 21-day experiment. For example, the average size on day 7 was 168.6 nm, slightly larger than on day 1 (150.1 nm)

12. 111-121: Section requires comparison to results of others.

Response: Thank you for highlighting the need to compare the section describing the size, concentration, and stability of NBs with other studies. We have added some references and revised the paragraph accordingly:

Line 127-129: These measurements of the size, concentration, and stability of NBs align with those reported in existing literature, where generated NBs are reported to persist for over a month with sizes ranging from 100 to 200 nm and concentrations ranging from 10^6 to 10^8[19,20,43].

And

Line 147-150: Additionally, the zeta potential of the NBs measured immediately after generation using a Zetasizer was around -16 mV at neutral pH, which is similar to the typical values reported by previous studies (-50 to -20 mV under different conditions)[44]

13. Figure 1 (a) requires more explanation, what are green, red dots, and yellow areas, the size legend needs to be increased to be readable.

Response: Thank you for your suggestion to add more explanation to Figure 1(a). The different colored dots represent MPs of various sizes. We have included this explanation in both the figure and its caption. Additionally, we have increased the size of the scale bar.

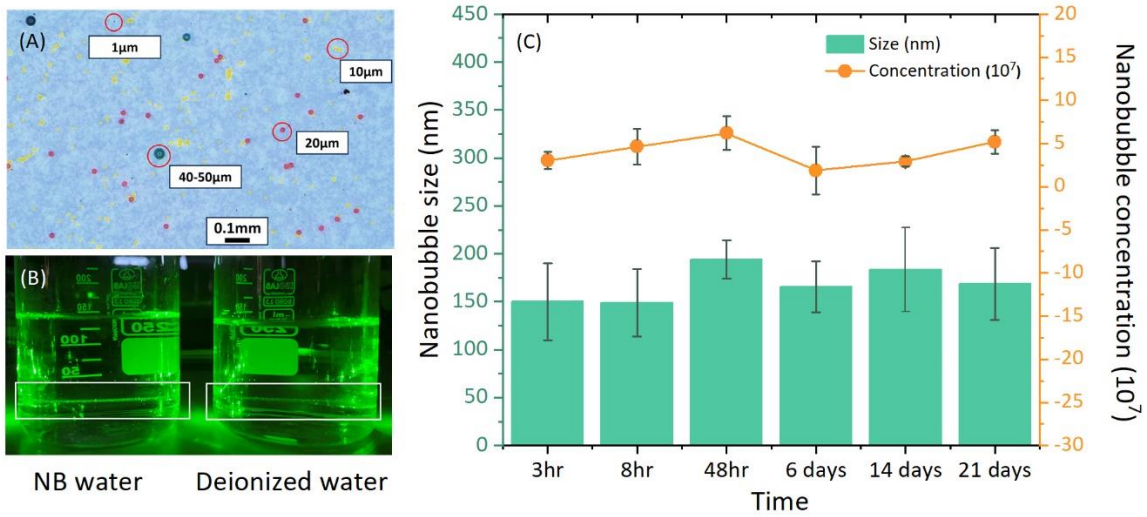


Figure 2. Visualization and characterization of NBs in DI water. **(A)** PS microplastics under microscopy (Green: 40-50µm; Orange: 20µm; yellow: 10µm; Blue: 1µm). **(B)** Differentiation of DI water and NB water by dynamic scattering using a green laser pointer. **(C)** NBs' size and concentration over 21 days.

14. 142-143: The model utilized the commonly reported bubble size distribution of NBs and MBs. This requires giving the ranges used and references.

Response: Thank you for pointing out the need to specify the ranges of commonly reported bubble sizes along with their references. We mentioned the commonly used bubble sizes in the

results section of "Characterization of Microplastics and Nanobubbles" but did not clearly indicate this in the referred part. We have modified the text as follows:

Line 174-177: We considered the commonly reported size distributions for MBs and NBs for the modeling: from 20 µm to 100 µm for MBs, with the highest intensity occurring at a size of 65 µm[139–41], and from 100 nm to 1000 nm for NBs, with the highest intensity occurring at around 300 nm[19,20].

15. 146-147: clearer visualization compared to?

Response: Thank you for pointing out the ambiguity in this sentence. We intend to explain that the modeling clearly demonstrates how bubble size influences their rising velocity. In contrast to MBs, whose rising velocity can be directly visualized and measured using a high-speed camera, the presence of NBs is difficult to visualize in situ. Therefore, they require assistance from modeling. We have modified the writing as follows:

Line 180-182: We considered the commonly reported size distributions for MBs and NBs for the modeling: from 20 µm to 100 µm for MBs, with the highest intensity occurring at a size of 65 µm[139–41], and from 100 nm to 1000 nm for NBs, with the highest intensity occurring at around 300 nm[19,20].

16. 172-174: Check this statement, this contrasts with the one in 171, and graph as well as numbers in (b) clearly shows that turbidity levels off. For this section also you need a comparison to literature in which size ranges turbidity measurements usually apply. For 1um provide linear regression analysis results. Figure 3a and b use non-linear x-axis, making it slightly difficult to see linearity, though values indicate 1um does not level off. Is the caption in (b) correct? In text 173, you indicate image analysis method used. I believe something is not correct here, have you assigned Figure in text accordingly because Figure 3d does not appear in neither caption nor text.

Response: Thank you for highlighting the issue with the calibration analysis. We have included references to studies that utilized the turbidity method for MP quantification, along with their respective size ranges for a literature comparison. Additionally, we have incorporated the linear regression analysis results (R^2) both in the text and within the figures. As you rightly pointed out, there were inaccuracies in the figure captions, which have now been corrected. The revisions are detailed below:

Line 201-223: Before conducting the removal test, two MP quantification methods were compared: an indirect method by measuring solution turbidity, and a direct method that involves filtering the MPs onto a filter paper and analyzing the MPs' area coverage on the filter paper through image analysis. **Fig. 4(A)** depicts a linear relationship between turbidity and concentration for 1 µm and 10 µm MPs (R^2=0.99 for 1µm, R^2=0.98 for 10 µm), contrasting with the less linear relationships observed for 20 µm and 40-50 µm MPs (**Fig. 4(B)**, R^2=0.95 for 20 µm, R^2=0.91 for 40-50 µm). Our results suggest that turbidity can serve as an accurate indicator for determining the concentration of 1-10 µm MPs, which aligns with a previous study that reported an R-squared value greater than 0.99 when analyzing the correlation between

turbidity and concentration for 0.1 µm, 1 µm, and 10 µm PS microplastics[47]. Another study reported a low correlation between pristine PE microplastics and turbidity, where the correlation coefficients (r) for 140 µm and 15 µm PE were 0.42 and 0.71, respectively[48]. The image analysis method, on the other hand, yielded good accuracy with linear relationships observed for 10 µm, 20 µm, and 40-50 µm MPs with R-squared all larger than 0.99 (**Fig. 4(C)**).

Following, we used the two methods to quantify the simultaneous removal of a mixture of four different sizes of PS MPs in synthetic wastewater by MB-F and MNB-F processes and compared the results. It was found that irrespective of the quantification methods employed, MNB-F achieved a higher cumulative removal of MPs compared to MB-F, suggesting that NBs can play a role during the flotation process (**Fig. 4(D)**).

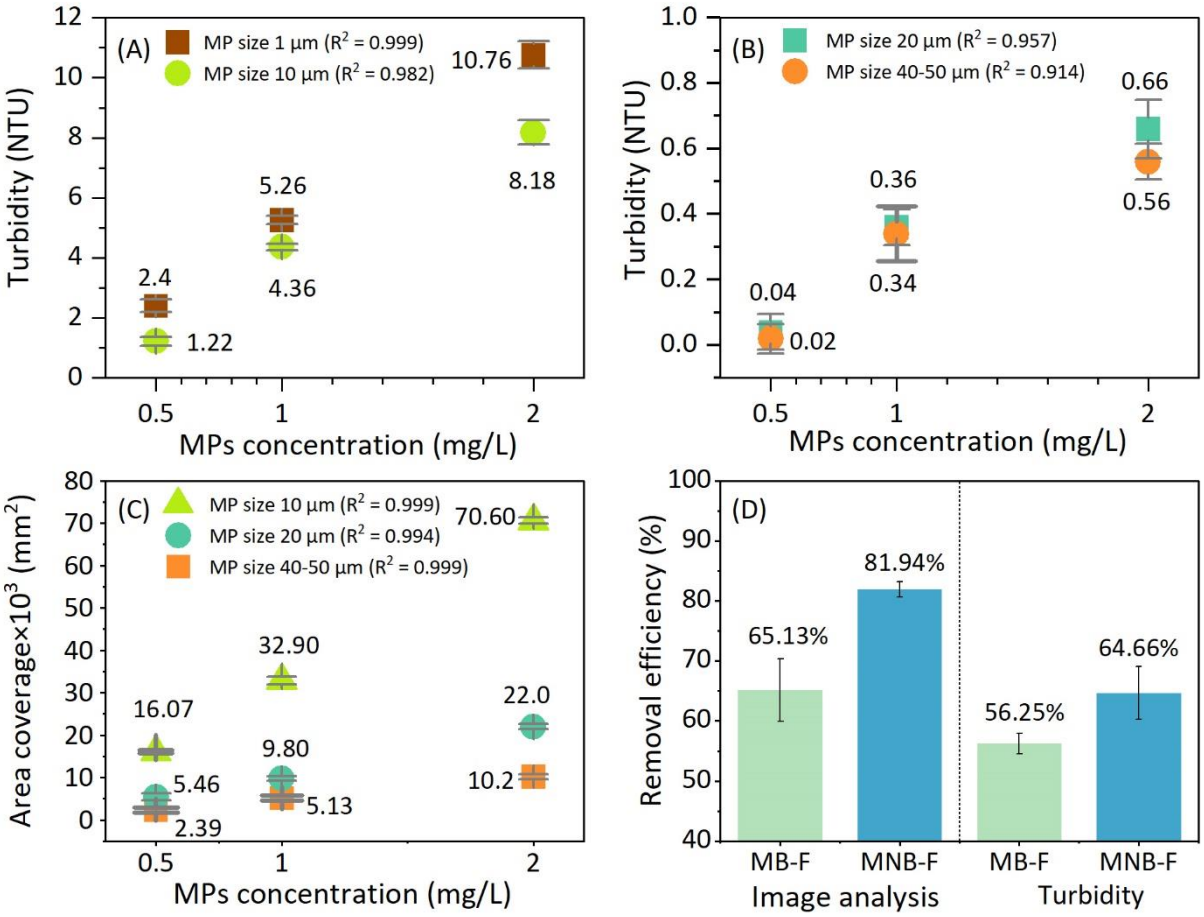


Figure 4. Comparative analysis of two MP quantification methods (turbidity calibration **(A)&(B)** and image analysis calibration **(C)**) and their simultaneous removal of a mixture of MPs of 4 sizes at $AlCl_3$ dosage of 10 ppm **(D)**.

17. Figure 3c, although methods description appears later, you need to add a remark in text or figure caption on how to understand MPs area coverage, so reader can follow.

Response: Thank you for the suggestion. We have added a remark to the text to explain the concept of MP area coverage. This addition will help readers better understand the method and

its implications. The modifications are detailed as follows:

Line 201-204: Before conducting the removal test, two MP quantification methods were compared: an indirect method by measuring solution turbidity, and a direct method that involves filtering the MPs onto a filter paper and analyzing the MPs' area coverage on the filter paper through image analysis.

18. Figure 4: caption needs to indicate that lower row in Figure is the coagulant dosage used.

Response: Thank you for your suggestion. We have revised the figure caption and the figure to express the meaning of the two rows in the x-axis more clearly.

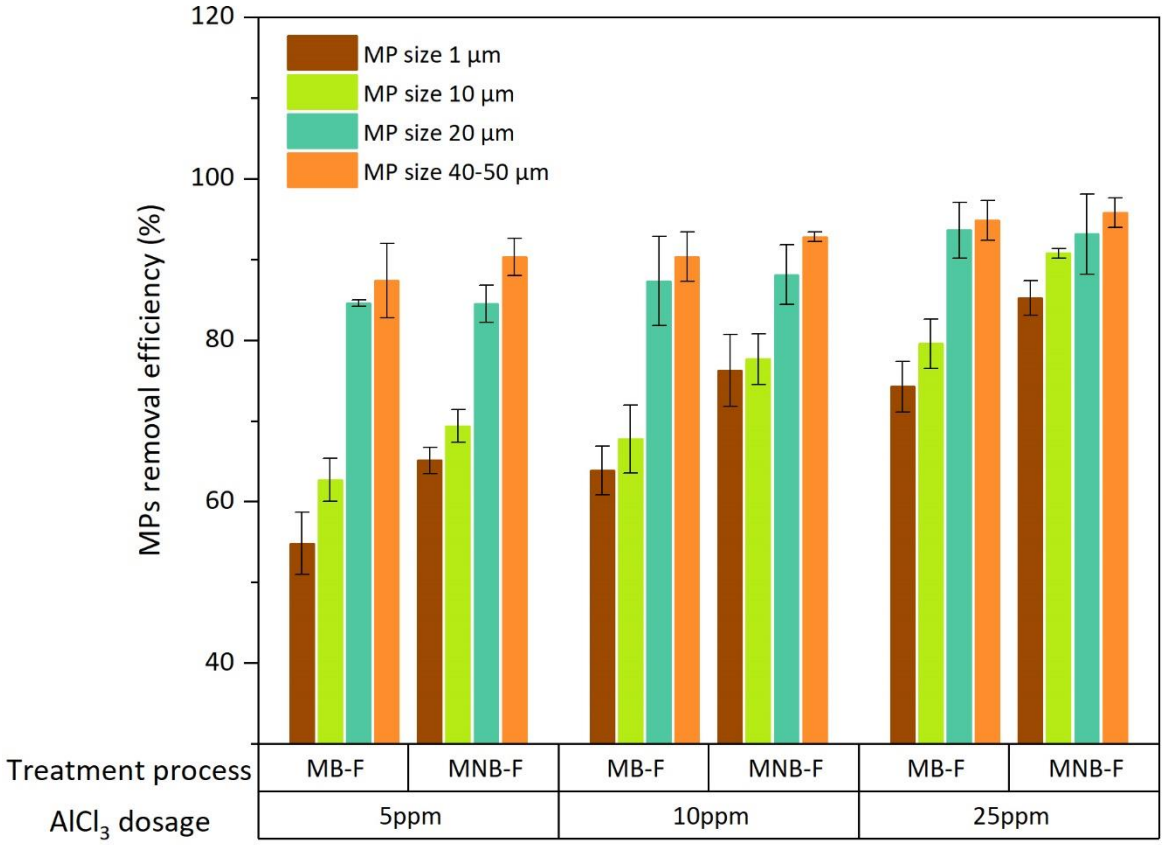


Figure 5. Comparison of removal efficiency for MPs of different sizes (1-50 µm) at different coagulant dosages by MB-F and MNB-F. Note: Removal for 1 µm MPs was measured using the turbidity method instead of image analysis, which was the method used for quantifying MPs of larger sizes.

19. 194-197 vs 227-230, watch repetitive statements.

Response: Thank you for pointing out the repetitive statements. We have removed the repetitive statements in the two paragraphs.

The original statement at Lines 194-197 has been revised to:

Line 236-239: Building upon the above analyses, a systematic investigation was undertaken to

explore the factors contributing to the improved removal of MPs. Specifically, we evaluated the impacts of MP sizes, shapes, and polymer types, and the various concentrations of $AlCl_3$ on flotation efficiency.

The original statement at Line 227-230 has been revised to:

Line 278-280: With collision and adhesion efficiencies between bubbles and particles being the key factors in determining flotation efficiency, we employed an existing flotation model to investigate how the presence of NBs affects bubble-particle interactions.

20. Figure 5: the value on the left-hand side of (d) requires explanation or should be removed.

Response: Thank you for your suggestions. The value on the left-hand side showed that the value was offset by the code. We have revised the code to remove the offset. The new figure is as below:

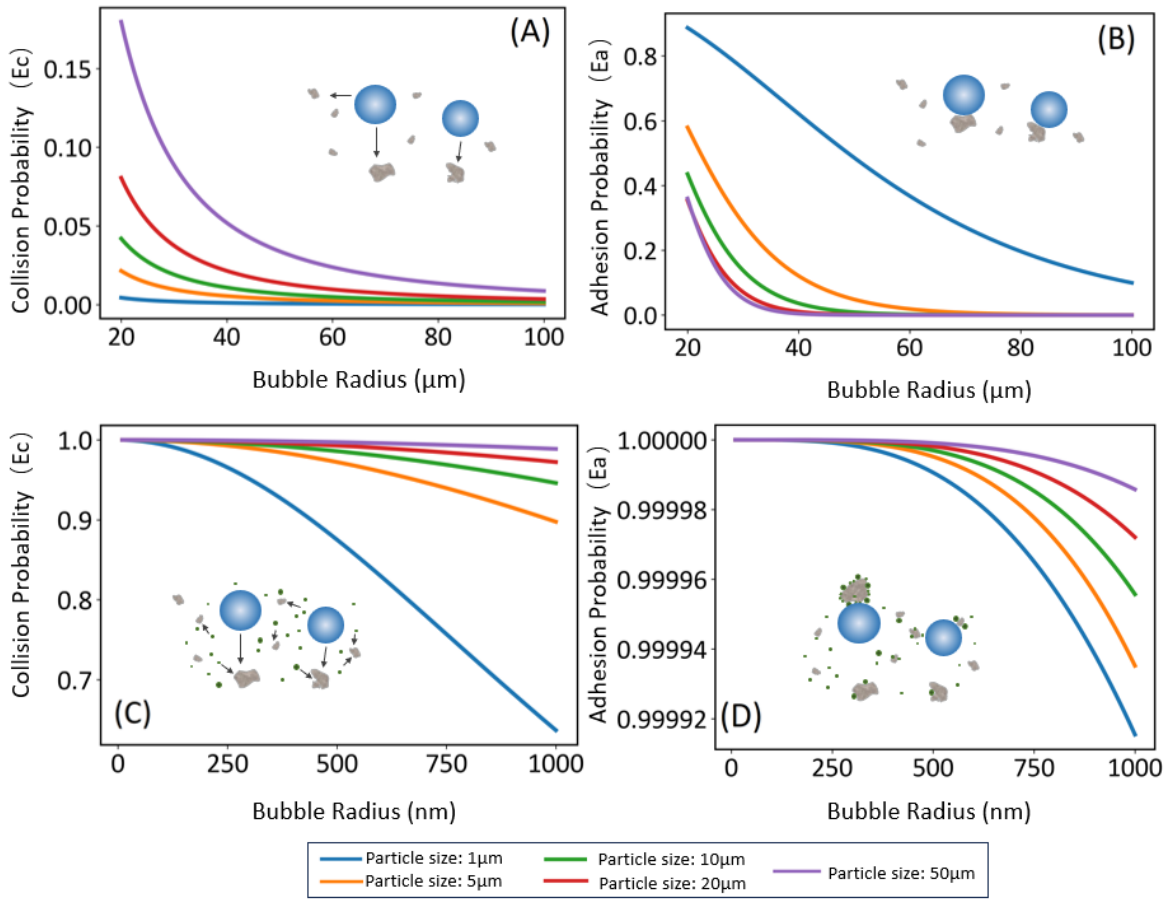


Figure 6. Simulation results on how bubble size affects collision and adhesion probabilities during the flotation process.

21. 365: replace ‘delve’ by better term. Review term ‘diminutive’.

Response: Thank you for your suggestions. We have replaced ‘delve’ and ‘diminutive’ with better terms as follows:

Line 452-455: In summary, to overcome the limitations of flotation in efficiently eliminating small-sized MPs, which pose significant environmental threats, the present investigation explored the potential of using MNB-F as a promising treatment process for the enhanced removal of these small particles. F

22. 367-369: The first and the second part of the sentence seem not to fit together, first is on process, second is on method to measure.

Response: Thank you for pointing out the problem with the sentence. We have revised the sentence as follows:

Line 459-460:. The results revealed that while MB-F was effective in eliminating particles larger than 20 µm, it was less effective at addressing MPs sized 10 µm and 1 µm.

23. 370: fine?

Response: The sentence has been revised as follows:

Line 460-463: In comparison, the application of MNB-F exhibited a substantial enhancement in the removal of a mixture of different-sized MPs, improving removal rates by a maximum of 16.8% in synthetic wastewater and 14.3% in real wastewater, respectively.

24. 373: smaller bubble sizes unclear in context; do you mean smaller microbubbles or nanobubbles?

Response: Thank you for your suggestions. We have clarified in the text that by "smaller bubbles," we were referring to nanobubbles.

Line 463-465: Flotation modeling revealed that the observed enhancement in MP removal by MNB-F can be attributed to the addition of NBs, which increased the collision and adhesion efficiencies with the particles.

25. 379: delete minute

Response: Thank you for your suggestion. The word ‘minute’ has been removed from the text.

26. 387: concentration of stock solution required.

Response: Thank you for your suggestion. We have included the concentration of the stock solution in the text:

Line 477-479: The microbeads were prepared as a stock solution (1mg/mL) and subjected to 15 minutes of ultrasonication to ensure even dispersion before use.

27. 388: synthetic vs real wastewater, when used in experiments unclear in results section, check and revise.

Response: Thank you for your suggestion to clarify the use of synthetic and real wastewater

in the manuscript. We utilized real wastewater for characterizing MPs to understand their size distribution in wastewater. Additionally, we tested the performance of MB-F and MNB-F in removing four sizes of MPs using real wastewater, aiming to determine whether the complex water matrix would affect the performance of the NBs. The remainder of the experiments were conducted with synthetic wastewater. We have further clarified the usage of both synthetic and real wastewater in the text as follows:

Line 162-163: The aim was to assess their efficiencies in removing MPs of two different sizes at an $AlCl_3$ dosage of 10 ppm in synthetic wastewater.

Line 219-221: Following, we used the two methods to quantify the simultaneous removal of a mixture of four different sizes of PS MPs in synthetic wastewater by MB-F and MNB-F processes and compared the results.

Line 241-243: First, we tested the removal efficiency of MPs in the sizes of 1 μm, 10 μm, 20 μm and 40-50 μm under three different coagulant dosages, i.e., 5, 10, 25 ppm in synthetic wastewater (**Fig. 5**).

28. 390: do you have characteristics the real wastewater used, normally, this should be presented in the SI.

Response: Thank you for highlighting the need to detail the characteristics of the real wastewater we used. We have added this information to the supplementary materials:

Line 483: The characteristic of real wastewater is presented in **Table S3**.

Table S3: Characteristics of the chemically enhanced primary treated wastewater collected from Siu Ho Wan Sewage Treatment Works.

Parameters	Value
pH	7.7
TDS(g/L)	11.2
Total Nitrogen (ppm)	7.7
Total organic carbon (ppm)	23.39

29. 394: Check Figure assignment as Fig 8 is both in line 361 and 439.
Response: Thank you for pointing out the issue with the figure assignment. The figure number has been corrected.

Cover Letter

Reviewer #1

The authors have given the detailed response and addressing almost the concerns raised. However, after carefully reviewing the revisions, I still have several concerns on the manuscript.

We are grateful for your dedicated time spent on our manuscript and for the valuable comments, suggestions, and concerns you raised, which have notably enhanced the quality of our work. Through careful consideration, we have effectively addressed your concerns and implemented necessary revisions in the manuscript. Our point-by-point responses are as follows:

Comment 1. Novelty Concerns: While your study focuses on the removal of smaller-sized microplastics (MPs, 1-50 μm) using nanobubble (NB)-assisted flotation, this approach does not represent a substantial departure from existing methodologies. Previous studies have already demonstrated that nanobubbles can assist microbubbles during the flotation process (e.g., 10.1016/j.seppur.2017.06.007). Additionally, I have identified some other relevant studies (e.g., 10.1016/j.hazadv.2022.100139; doi.org/10.1016/j.jwpe.2023.104637) that have considered the removal of smaller-sized MPs using modified dissolved air flotation techniques. These studies collectively diminish the novelty of your approach. Additionally, a more comprehensive review of existing flotation studies to remove MPs should be performed. The incremental improvement in targeting smaller MPs, while important, does not sufficiently differentiate your work from these established techniques.

Response: In response to your concern regarding the novelty of this work and your suggestion to provide a more comprehensive review of existing flotation studies, first, we have conducted an in-depth examination of prior studies utilizing flotation methods for microplastic removal, as in **Supplementary Table 1.** Then, we have clearly highlighted the novelty of this work and explained how it stands out from the existing literature. Additionally, we we have emphasized the originality of our work compared to the referenced studies.

Supplementary Table 1. Review of existing flotation studies to remove MPs. (*: Data extracted using WebPlotDigitizer)

Ref.	Flotation set-up	MPs characteristic	Solution medium	Pretreatment type (dosage)	Pretreatment condition	Flotation condition	MPs removal/recovery efficiency	MPs sampling and quantification method
1[4]	Full-scale DAF	**Polymer type:** 13 types of naturally occurring MPs; **Size:** >20 µm **Concentration:** 2-2.3 microplastic/L	Secondary wastewater	**Flocculant:** Polyaluminium Chloride (40mg/L)	N/A	N/A	95%	**Sampling:** sampling at a depth of ~1m with volume of 2 L-1000 L **Quantify:** count under Stereo microscopy
							48%	Sampling: 24-h composite sampler **Quantify:** count under Stereo microscopy
2[5]	Flotation cell	**Polymer type:** PE; **Size:** 10 µm- 5mm; **Concentration:** 1.2mg/L	Synthetic greywater	**Coagulant:** $FeCl_3 \cdot 6H_2O$ (10mg/L; 15mg/L; 20mg/L)	Rapid mixing (350 rpm) 1 min; Slow mixing (100 rpm) 15 min	Recirculation ratio = 20 %, flotation speed=30 cm/min, flotation time=5 min, saturation time=25 min Pressure: 4-6 bar; pH:6-8; Flow rate: 500-1000mL/min;	48.68%-67.69%	**Sampling:** Remaining treated water in the flotation cell **Quantify:** count under Stereo microscopy
				Coagulant: $AlCl_3 \cdot 6H_2O$ (10mg/L; 15mg/L; 20mg/L)			74%-94.5%	
				N/A	N/A		25-30%	
3[6]	Flotation cell	**Polymer type:** PE; PET; PA66 **Size:** 2-106 µm **Concentration:** 100mg/L	Deionized water	**Bubble modifier:** CTAB **Dosage:** 0.6-1.4 mg/L	A dose of bubble modifier was added to the saturator	N/A	Optimal removal at a dosage of 1.2mg/L: PA: 43.8%; PET: 58.5%; PE: 68.9%	**Sampling:** 500mL of supernatant **Quantify:** Laser particle analyzer

				Bubble modifier: PDADMAC (0.4-1.2mg/L)	prior to flotation		PA: 46.3% (0.8 mg/L-optimal) PET: 72.6 (1.0mg/L-optimal） PE: 46.3% (1.2mg/L-optimal)	
				N/A	N/A		PA: 32.7% (0.5Mpa-optimal) PET: 38.9% (0.4Mpa-optimal) PE: 48.7% (0.4Mpa-optimal)	
4[7]	Column flotation (Height:30 cm, Diameter:4 cm, Volume:350 ml).	**Polymer type:** PE, PP, PVC, PMMA **Size:**10–600 µm **Concentration:** 500mg/L	Deionized water with Tween-20	N/A	N/A	Flotation time: 2min Flow rate: 2500mL/min	PE: 83.3% PP: 79.3% PVC: 66.4% PMMA: 82.8%	**Sampling:** Remaining Treated water in the flotation column **Quantify:** High-resolution camera combined with image analysis by MATLAB
5[8]*	Column flotation	**Polymer type:** PES, PAN, PA, PP **Size:**5–3500 µm **Concentration:** 10,50,500,5000 mg/L	Synthetic Laundry wastewater	**Flocculant:** polyacrylamide (1ppm,4ppm)	N/A	Flow rate: 0.5-1.6 SLPM	Fibers & particles with size >20um and initial concentration >10mg/L can achieve almost 100% of removal; For a mixture of MPs at a concentration of 50mg/L at flotation time of 5, 10, 30, 60min: 372µm bubble: 89%, 93%, 100%, 100%. 451µm bubble: 78%, 87%, 91%, 91% 1578µm bubble: N.A, 79%, 94%, 95%	**Sampling:** Samples from treated water **Quantify:** Weight difference method (assisted by Confocal Microscopy when needed)
6[9] *	Froth flotation cell	**Polymer type:** PP, PE, ABS, PS, PET, PVC **Size:**100-1000 µm **Concentration:** 666.67mg/L	Deionized water with kerosene	**Frother:** methyl isobutyl carbinol (0.02mL/L)	Mixing: 900rpm	Flotation time: 1 min Flow rate: 1300mL/min	PP & PE: >96%; ABS, PS, PET, and PVC: 89, 93, 96, and 89%. At kerosene dosage of 0, 1, 2, 3mg/L: PVC: 89%, 96%, 94%, 93%. PET: 96%, 96%, 96%, 95% PS:93%, 99%, 99%, 93%. ABS: 89%,97%,98%,925. PE:96%,96%,99%, 98%. PP:97%,98%,98%,98%	**Sampling:** From both froth and treated water **Quantify:** Weight difference method

7[10]*	Electro-coagulation-flotation setup (Volume: 1L)	**Polymer type and size:** PE (150μm) and PVC (250μm) **Concentration:** 0.2 particles/mL	Deionized water with sulphuric acid, sodium hydroxide, and sodium chloride	**Electrode:** Al-Fe or Fe-Al **Electrode distance:**20 mm	**pH:** 4, 7, 10 **Current density:**10-20 A/m^2	Retention time: 10-120 min	PE: 100% in 10 min PVC: 96.8% in 10min (pH 7, 20 A/m2, Al-Fe)	**Sampling:** Sampled by pipette from the reactor at different time intervals (10, 20, 30, 40, 50, 60, 90, 120min) **Quantify:** Particle counter
8[11]	Dissolved air flotation jar tester (50L glass barrel with 35L solution volume)	**Polymer type:** Mixture of PP, PE, PA, PS, PET, PVF (for MPs with size≥50μm) **Size:** 2 to 25μm	Livestock and poultry wastewater	N/A	N/A	Settled down for 30 min	2-5: 21.50%; 5-10: 34.65%; 10-15: 41.30%; 15-20: 47.28%; 20-25: 53.80%	**Sampling:** 500mL of supernatant and sit for 10h for defoaming **Quantify:** Laser particle analyzer
9[12]	Flotation Cell; Coagulative colloidal gas aphrons (CCGAs)	**Polymer type:** PS, PMMA **Size:**5 μm **Concentration:** 30mg/L or 100mg/L	Artificial water (with spiked humic acid); Real wastewater	**Bubble modifier:** CTAB (0.5mmol/L) **Frother**: PAC (0.1mol/L)	Generation of CCFA: 6000rpm for 1.5min	Flow rate: 300mL/min Retention time:3 min Volume ratio of CCGA suspension to MP suspension: 3:5	In solution with MPs and HA and optimal PACl of 0.454mmol/L: PS: >99% and PMMA: <81%. In solution with only MPs: PS: <79% (30 mg/L initial MP concentration and 0.979 mmol/L PACl) and <69% (100 mg/L initial concentration and 0,015mmol/L); PMMA: 89%	**Sampling:** Clarified water and the floats **Quantify:** Optical microscope and turbidimeter
10[13]	Flotation column:	**Polymer type:** PET, ABS, PS **Size:**3000-5000μm **Concentration:** 1g/100mL for each type	Tap water	**Plastic modifier:** PAC (1 to 15 mg/L) **Frother:** Terpineol (23.7 mg/L)	Immersion of 1 g PET, 1 g ABS, and 1 g PS were immersed in 100 mL PAC solution	Flotation time: 3 min	PET: 100% ABS: 100% PS: 94.1%	**Sampling:** Separated waste plastics (floats) **Quantify:** Visual identification

11[14]	Dissolved air flotation jar tester (2L);	**Polymer type:** PS **Size:**10, 40-50 μm **Concentration:** 1mg/L	Synthetic seawater	**Coagulant:** AlCl3·6H2O (10mg/L)	Rapid mixing (300 rpm) 1 min; Slow mixing (30 rpm) 15 min	Recycle ratio: 20%	Microbubble: 10μm: 81.12%; 40-50μm: 85.73% Nanobubble: 10μm： 88.19%; 40-50μm: 86.34%	**Sampling:** Suspension. **Quantify:** Stereo microscopy
12[15]*	Column froth flotation:(π × 30 mm2 (radius) × 580 mm3 (height))	**Polymer type:** PET, PS **Size:**4–5 mm, 3–4 mm, 2–3 mm, 1–2 mm, 0.5–1 mm, 0.125–0.5 mm, 0.074–0.125 mm, and < 0.074 mm **Concentration:** 0.025, 0.075, 0.125, 0.175, 0.225 g/L	Synthetic marine water and industrial wastewater	**Frother:** terpineol (0–38 mg/L)	N/A	Flotation time: 4min	PS: 74μm: 86.7%; 74-125μm: 99% PET: 74μm:81.7%; 74-125μm:94.4%	**Sampling:** Floats **Quantify:** Weight difference

1) Significane of our study in targeting smaller MPs compared to literature:

After an in-depth literature review, we compared our study with existing studies on flotation for microplastic removal to provide a more direct comparison. As shown in Figure 8B, our research bridges a critical gap by focusing on the removal of smaller-sized microplastics—a less explored area in previous studies.

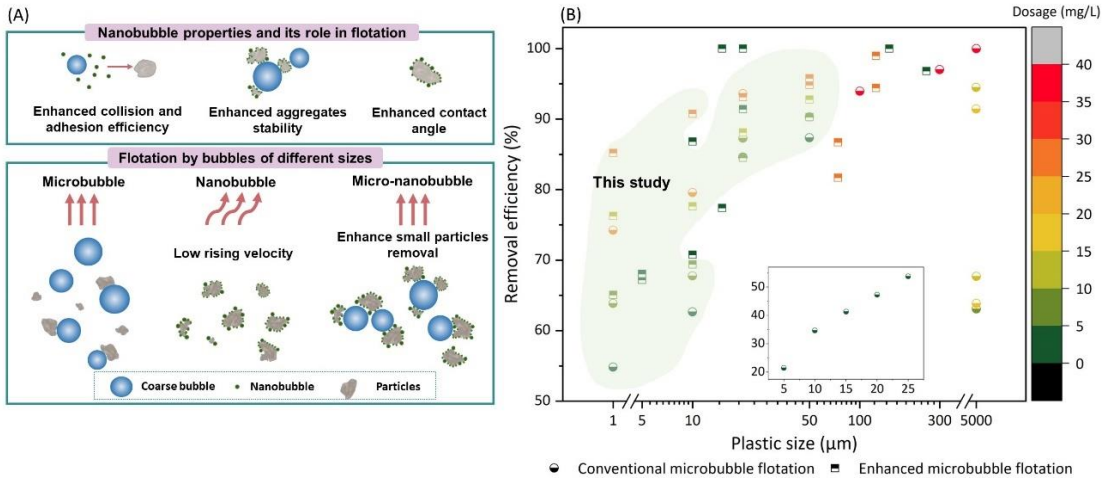


Figure 9. (A) Schematic overview of NBs' properties and the flotation mechanisms by MB-F, NB-F, and MNB-F. **(B)** Comparison between our study and existing studies using conventional and enhanced microbubble flotation processes to remove microplastics (specific data is provided in **Supplementary Note 1 and Tabe S3**).

Line 482-485:. For comparison, we summarized the removal efficiency of MPs as reported in existing studies that investigated both conventional and enhanced flotation methods alongside our own (**Fig. 9(B)**). It can be found that our research bridges a critical gap by focusing on the removal of smaller-sized MPs, an area less explored in previous studies. Furthermore, our findings offer a robust strategy that not only boosts removal efficiency but also achieves this with a relatively low use of coagulants.

Supplementary Note 1: Description of data used in comparative analysis of different studies results provided in Figure 8 (B).

The categorization of conventional microbubble flotation refers to studies that used microbubble flotation with typically applied coagulant type, while the enhanced microbubble flotation studies are those that either used unconventionally used coagulant or studies assisted with other techniques or processes. In the limited existing studies that use conventional or enhanced microbubble flotation, various parameters have been considered to assess their effects on microplastic removal. These parameters include, but are not limited to, polymer type, size, and shape; coagulant type and dosage;

flotation setup; flotation time; and flow rates. Given the diversity of variables, it is challenging to directly compare results across these studies. However, we have selected two relevant parameters for comparison with our study: polymer size and coagulant dosage. Additionally, while some studies use microplastics of specific sizes, others use a mixture encompassing a wide range of sizes. In our analysis, we have used the data for the largest size within the provided range. The specific data can be found in **Supplementary Table 3**

Supplementary Table 3. Data used for comparative analysis in Figure 8(B).

	Study and coagulant type	Number of data point	MPs type	MPs size	Used MPs size in figure	Dosage (mg/L)	Removal (%)	Reference
Conventional flotation process	Study 1-PAC	1	Mixture	20-100	100	40	93.96	(Talvitie, 2017)
		2		100-300	300	40	97.02	
		3		300-5000	5000	40	100	
	Study 2-Fecl3	4	PE	10-5000	5000	10	63.07	(Esfandiari, 2021)
		5		10-5000	5000	15	67.69	
		6		10-5000	5000	20	63.84	
	Study 2-AlCl3	7	PE	10-5000	4999	10	91.42	
		8		10-5000	4999	15	94.5	
		9		10-5000	4999	20	91.42	
	Study 3-No dosage	10	Mixture	2 ~ 5	5	0	21.5	(Zhu, 2022)
		11		5 ~10	10	0	34.65	
		12		10 ~15	15	0	41.3	
		13		15 ~20	20	0	47.28	
		14		20 ~25	25	0	53.8	
	this study-AlCl3	15	PS	1	1	5	54.81	This study
		16		10	10	5	62.66	
		17		20	20	5	84.585	
		18		40-50	50	5	87.35	
		19		1	1	10	63.85	
		20		10	10	10	67.74	
		21		20	20	10	87.3	
		22		40-50	50	10	90.33	
		23		1	1	25	74.23	
		24		10	10	25	79.57	
		25		20	20	25	93.6	
		26		40-50	50	25	94.82	
Enhanced flotation process	Study 1-CTAB	27	PE	2 ~ 5	1	1.2	67.2	(Wang, 2020)
		28		5 ~10	10		70.8	

		29		10 ~15	15		77.4	
		30		15 ~20	20		91.4	
	Study 1-PDAC	31	PE	2 ~ 5	1	1	68.0	
		32		5 ~10	10		86.8	
		33		10 ~15	15		100.0	
		34		15 ~20	20		100.0	
	Study 2-Terpineol	35	PS	74	74	28	86.7	(Zhang,2021)
		36		74-125	125		99.0	
		37	PET	74	74		81.7	
		38		74-125	125		94.4	
	Study 3-Electrocoagulation	39	PE	150	150	0	100.0	(Akarsu, 2021)
		40	PVC	250	250	-	96.8	
	this study-$AlCl_3$	41	PS	1	1	5	65.08	This study
		42		10	10	5	69.375	
		43		20	20	5	84.50299	
		44		40-50	50	5	90.28508	
		45		1	1	10	76.27	
		46		10	10	10	77.65	
		47		20	20	10	88.115	
		48		40-50	50	10	92.7652	
		49		1	1	25	85.19	
		50		10	10	25	90.75	
		51		20	20	25	93.135	
		52		40-50	50	25	95.7797	

2) Originality of this Work and the 3 Referenced Studies:

In response to the reviewer's concerns regarding the originality of our work, next, we would like to particularly discuss the three studies referenced by the reviewer, aiming to explain the distinctiveness of our work in comparison with these existing studies:

1. 10.1016/j.jwpe.2023.104637: **This mentioned work was conducted by our research group**, which established a foundational understanding, setting the stage for the current investigation. This study consists of two main parts: firstly, it characterized the distribution of MPs in ocean surface waters in Pui O, Hong Kong; secondly, it included a preliminary proof-of-concept for using nanobubbles in the flotation process. In this study, our initial observations indicated that MPs of 10μm and 40-50μm PS microbeads exhibited differing removal efficiencies when subjected to conventional microbubble versus nanobubble-assisted flotation. This finding prompted further exploration into the efficacy of nanobubbles in the flotation process. However, in this study, our initial experiments and analyses were limited, considering only two sizes of plastics, and the discussion on underlying mechanisms largely relied on hypothetical reasoning supported by the literature.

Building upon these preliminary findings, our current research expands the scope to more comprehensively investigate nanobubble-assisted flotation for the removal of MPs. In this manuscript, we report detailed findings from extended experiments that include: **1)** Testing a broader range of MP sizes and mixtures; **2)** Varying the dosage concentrations of flotation agents to determine the effect of flocs; **3)** Evaluating MP removal efficacy in actual wastewater samples; **4)** Conducting analyses of hydroxyl radical production and TOC removal to determine whether NBs also play a role in degradation. Following your previous suggestions, we have also included: **5)** Investigate the effect on different polymer types and shapes; **6)** Characterized the profiles of MPs in wastewater, with an emphasis on smaller sizes; **7)** Discussed the economic viability perspective of NB-assisted DAF; **8)**Provided a comprehensive review of the relevant literature to contextualize our findings within the broader field.

2. ***10.1016/j.seppur.2017.06.007*****:** The study referenced by the reviewer reported the separation of emulsified oil droplets through various flotation methods, encompassing microbubble, micro-nanobubble, and NBs techniques.

The first key distinction between this mentioned study and our research lies in the targeted pollutants: oil versus fine microplastics. While flotation techniques have long been utilized for oil separation, particularly in industrial settings, with dissolved air flotation being commercially prominent for oil removal applications, the application of

nano-sized bubbles for fine MPs remains relatively limited. Despite the existing use of NBs for removing other pollutants, employing them for the extraction of small-sized MPs (~1 micron) maintains its novelty. Furthermore, although sharing a similar concept, the referenced study falls short in providing a discussion supported by evidence of fundamental mechanistic insights. The focus of the mentioned study revolves around four flotation scenarios involving microbubbles and/or NBs, evaluating their efficiency in oil removal with varying concentrations of Dismulgan as a flocculant. However, this study overlooks the impact of oil droplet sizes on removal efficacy, lacks discussions on bubble-oil interactions at different sizes, and does not explore the potential radical generation by NBs and its effects on flotation or pollutant degradation.

In contrast, our study offers detailed mechanistic insights into how NBs enhance the collision and adhesion probabilities among microbubbles, NBs, and MP particles. We provide theoretical and experimental elucidations on how bubble and particle sizes influence flotation performance. Presented a thorough review of NB-MPs-related floatation studies. Additionally, we investigate NB-induced reactive radicals, confirming their insignificant concentration for causing substantial organic contaminant degradation in wastewater or affecting MP removal. Notably, the examination of NB-induced radicals is a focal point in NB-related research. Furthermore, we discussed the economic feasibility of NB-assisted flotation, a crucial aspect often overlooked in existing studies. With all these elements, our work presents a distinct perspective and a more comprehensive analysis, shedding light on the role of NBs and paving the way for their expanded applications in new domains.

3. *10.1016/j.hazadv.2022.100139*: This mentioned study focuses on the removal of MPs ranging from 2-5µm up to 20-25µm using conventional microbubble flotation (CDAF) as a benchmark for comparison with a modified dissolved air flotation (MDAF) system. The MDAF system incorporates chemical bubble modifiers unrelated to bubble sizes or nanobubbles. Notably, the CDAF results indicate a gradual increase in MP removal efficacy from 21.50% for the smallest MPs (2-5µm) to 53.80% for 20-25µm MPs. **Note: This is the fundamental hypothesis of our work that CDAF is not effective for small-sized MPs, and its performance increases as the MP's size increases.**

Interestingly, in the MDAF evaluation, the authors report higher removal efficiencies of 81.6% with 1.0 mg/L of CTAB and 88.3% with 0.8 mg/L of PDAC. However, specifics regarding the MP size ranges targeted in the MDAF system and removal efficiencies across different size ranges are not provided (Figure R1). The study also lacks information on the polymer types and sizes utilized in the MDAF system.

Furthermore, akin to the previous study, this work primarily showcases incremental performance enhancements rather than offering a comprehensive mechanistic overview or evidence-based discussions. Moreover, the study does not include technical aspects crucial to the topic. The primary focus of this research, as highlighted in the paper's abstract, is centered on the development of a novel hybrid system and the improved removal of antibiotics and ARGs. This sharp contrast underscores the unique perspective and depth of our study, which not only addresses these technical gaps but also delves into the intricate mechanisms underlying the NB-assisted flotation process for MPs.

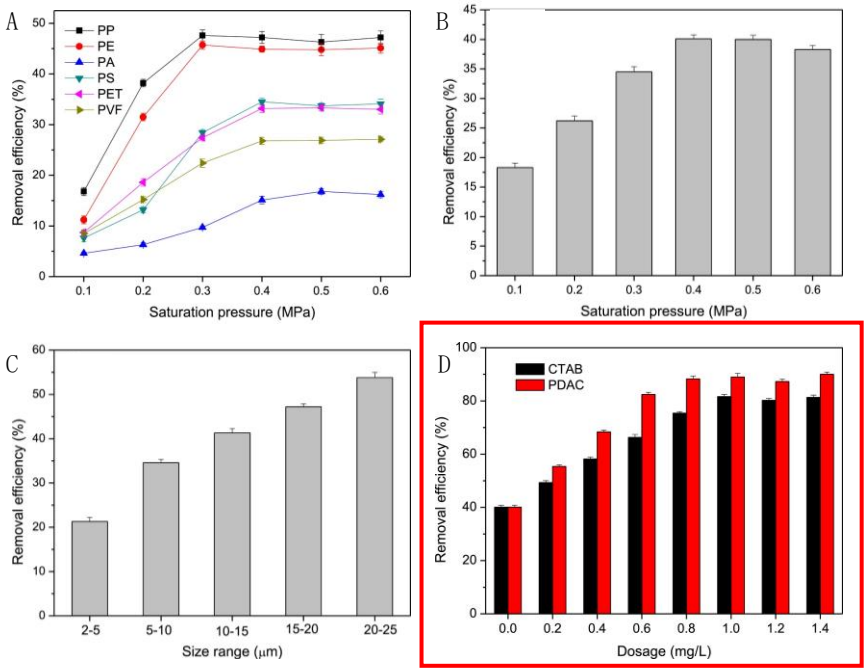


Figure R1. Removal efficiency of various types of MPs (A), total MPs (B), and various sized MPs at 0.4 MPa by CDAF. (D) Effect of the dosage of CTAB and PDAC on the removal of total MPs by MDAF at a saturation pressure of 0.4 MPa.

Moreover, as suggested by the reviewer, we have also added the following lines in the introduction to better introduce the existing studies that utilized NBs in flotation processes:

Line 82-84: Interestingly, given these properties, NBs have been reported to enhance the separation of oil droplets and amine precipitates and recover mineral particles in froth flotation[27–29].

Overall, upon the thorough review of the recommended literature and the development of detailed tables and illustrative figures for a more comprehensive literature review, we found that, whilst the basic concept of using nanobubble to aid flotation or to use

flotation for removal of MPs has indeed been present in previous literature, our study significantly expands on this by adapting and optimizing the technology for small-sized MPs removal, providing new theoretical and practical insights, and demonstrating economic and operational feasibility. We believe that these contributions present substantial novelty and address an urgent environmental need, thereby enriching our understanding of nanobubble and its application.

We would like to take this opportunity to further emphasize the distinctive contributions and novelty of our study in comparison with these existing studies.

- Compared to existing studies utilizing nanobubbles in flotation processes, our research distinguishes itself by offering: 1) A more in-depth mechanistic analysis — We delve deeper into the underlying physical and chemical interactions. This comprehensive understanding not only elucidates the fundamental principles but also paves the way for optimizing process parameters, thereby enhancing efficiency. 2) A novel application of nanobubbles — Our study introduces an innovative use of nanobubbles specifically for the removal of microplastics, a topic that has not been well-studied. This approach not only provides new insights but also opens up potential avenues for more effective environmental remediation techniques.

- Compared with studies that employ flotation for microplastic removal, our work distinguishes itself by offering: 1) Smaller Microplastic Sizes — We specifically target the removal of microplastics with smaller dimensions, which are notably more difficult to capture and have been less frequently addressed in previous research. This focus addresses a critical gap in the field, enhancing the overall impact and relevance of our findings. 2) Sustainability and Practicality — Our approach not only boosts the efficiency of microplastic removal but also prioritizes eco-friendly practices. The methodology we've developed offers real-world applicability, presenting a viable, sustainable solution that can be easily integrated into existing waste management systems for widespread use."

Comment 2. Significance of Techniques Used: The application of mathematical models to analyze bubble-particle interactions and the exploration of radical generation by nanobubbles, while useful, **do not introduce advanced or groundbreaking techniques**. These approaches are well-documented in the literature, and their application in your study does not provide a significant leap forward in the field. The

existing methods for bubble-particle dynamics simulation are robust and have been extensively applied.

Response: Thank you for your feedback regarding a specific theoretical model and experimental technique employed in our study. While the majority of existing studies on MP characterization describe particles larger than 20 µm, a limitation posed by the dominant techniques of Raman and FTIR spectroscopy, we have adpted the use of **OPTIR-Raman**, with its higher spectral sensitivity and spatial resolution allows for accurate analysis of smaller-sized MPs and, therefore, offers deeper insights into the composition of small-size MPs in wastewater effluent and highlights the problem of their insufficient removal with traditional treatment processes. Similarly, for radical generation, we employed the fluorescence-based APF method, alongside testing with Electron Spin Resonance (ESR) and High-Performance Liquid Chromatography (HPLC). Despite these latter methods failing to show clear radical peaks, as illustrated in Figures R1 and R2, which has been mentioned in the main text:

Line 407-410: 'While the EPR and HPLC methods failed to show a clear sign of radical presence in NB water, possibly due to concentrations below the detection limit 67, the APF method, validated with an H2O2 calibration test (**Supplementary Fig.9)**, displayed an •OH peak at an emission wavelength of 515 nm.'

The outcome itself provides insights into our analysis of the potential role of NBs in the flotation process.

In current NB research, understanding the dynamic behavior of NBs through direct experimental observation presents a major challenge due to their small size and Brownian motion. The implementation of modeling provides a theoretical framework to predict these interactions. Although our model might not capture all complexities, it serves as a crucial indicator to aid in understanding and explaining the behavior of NBs in our specific application.

However, in response to the reviewer's feedback and with the aim of enhancing our mechanistic analysis and providing a fresh viewpoint on the interaction between NBs and plastic particles, we have incorporated a novel **molecular dynamics simulation** analysis in this revised version. To better understand the behavior of these small-sized bubbles from a microscopic perspective, an increasing number of studies have started to use molecular dynamics (MD) simulations in NB research. MD simulations are powerful tools for understanding the physics and chemistry that govern nanoscale phenomena. In our analysis, we chose three bubble sizes and two PS particle sizes to simulate the interactions between smaller and larger bubbles and their effects on different-sized plastics. The simulation results showed that NBs can spontaneously

attach to NP by virtue of their hydrophobic nature, and the capillary bridge was formed as bubbles and particles approached each other. By far, 55 records of molecular dynamics simulation for NB in Scopus have been recorded **(Figure R2)**, and no MD simulation has been performed to understand bubble and polymer particle interactions. The use of MD simulations can provide atomistic insights into the interaction energies between bubbles and particles during attachment, assisting in elucidating how variations in bubble and particle sizes, as well as polymer types, affect attachment efficiency during the flotation process.

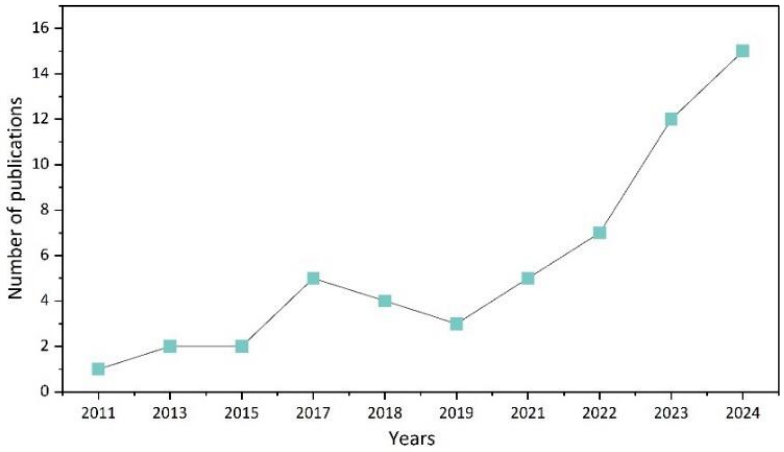


Figure R2. Overview of the number of NB MD simulation publications over the years Search in Scopus with the words 'nanobubble AND molecular AND dynamic AND simulation' in the title, abstract, and keywords on August 21, 2024.

The following discussion has been added to the result and discussion section of the manuscript:

Line 312-348: 'While conventional flotation models can provide insights into how NBs interact with particles during flotation, they are less frequently used to describe NBs due to their unique properties compared to macro or microbubbles. To better understand the behavior of these small-sized bubbles from a microscopic perspective, an increasing number of studies have started to use molecular dynamics (MD) simulations in NB research. MD simulations are powerful tools for understanding the physics and chemistry that govern nanoscale phenomena. However, due to computational limitations, most studies simulate NBs that are less than 10 nm in size, typically composed of nitrogen, argon, or oxygen gas [58–61]

In our analysis, we chose three bubble sizes (6, 15, 20 nm, denoted as B6, B15, and B20) and two PS particle sizes (6 and 12 nm) to simulate the interactions between smaller and larger bubbles and their effects on different-sized plastics. The final

equilibrium states are illustrated in Fig 7(A-H). The simulation results showed that NBs can spontaneously attach to NP by virtue of their hydrophobic nature, and the capillary bridge was formed as bubbles and particles approached each other (**Supplementary Video 1 and 2**). We further calculated the interaction energy between NP and NB to explain the adhesion probability between NP and NB. As shown in **Fig. 7(I)**, the interaction energy between NP and NB decreases with the increase of NB size for both large (PS6) and small size bubble (PS12). This trend explains the results predicted by the mathematical model well, revealing that the decrease in NB size increases the adhesion probability for both large and small NPs. To better understand the mechanisms responsible for increased interaction strength between smaller bubbles and NP. We gathered the local distributions of N_2 molecules around PS with different sizes in **Fig. 7(J-K).** We also integrated the local N2 distribution profiles to obtain the cumulative number of N2 molecules residing in the specific distance around PS. Since N_2 molecules do not interact with PS exceeding the cutoff of 1.2 nm, we only consider the distribution of N_2 molecules within 1.2 nm around PS. As shown in the figure, N_2 molecules interact strongest with PS at a distance of 3.95 Å, regardless of the bubble size. In addition, NB with a smaller size interacts with PS strongly, considering the higher peak in local number distribution profiles. As indicated in cumulative number profiles, the smaller NBs with larger curvature provide more N2 molecules to interact with PS. As a result, more N_2 molecules interact with PS at specific distances, leading to stronger interactions for PS with NBs with smaller sizes.

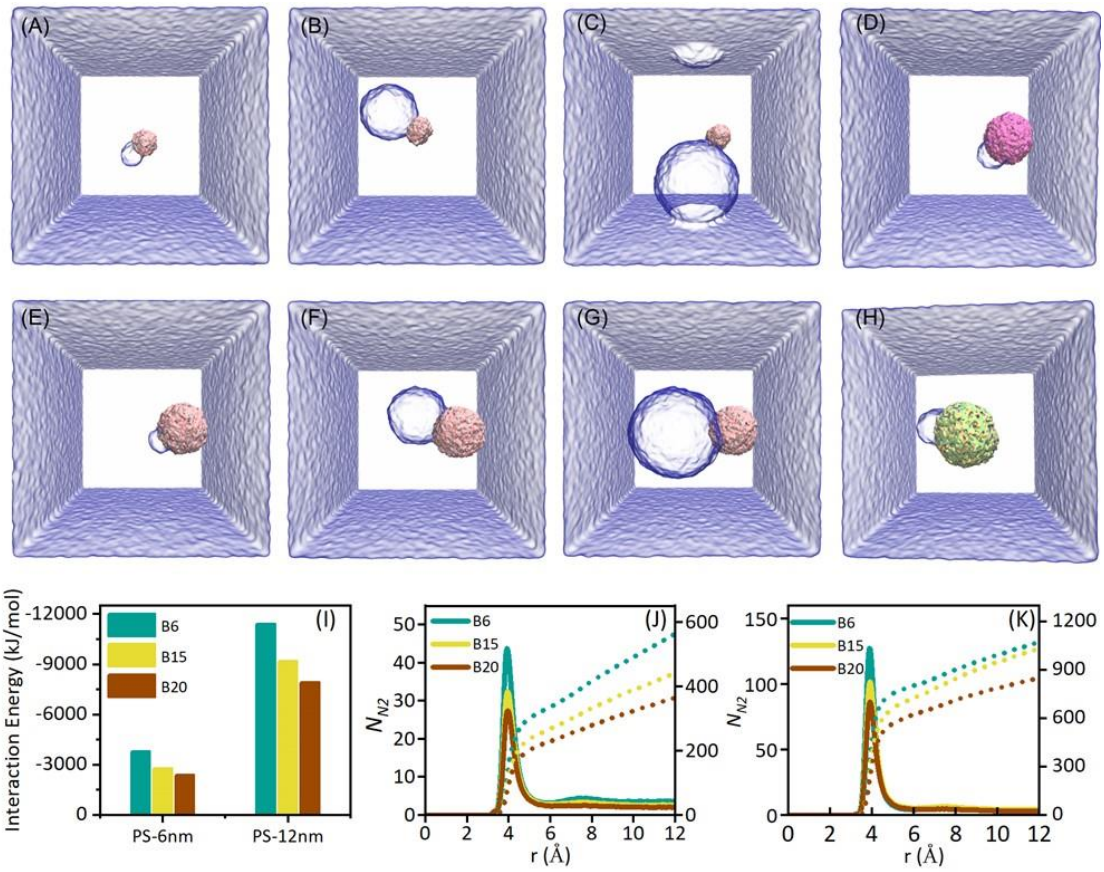


Figure 7. The final equilibrium attachment states for (A) PS6B6, (B) PS6B15, (C) PS6B20, (D) PE12B6 (E) PS12B6, (F) PS12B15, (G) PS12B20 and (H) PET12B6. The water and NP are shown as transparent and opaque surfaces, respectively, while N2 molecules are not shown for clarity. (I) Interaction energy between NB and NP with respect to different sizes and NP composition. (J)-(K) Local and cumulative distribution profiles between NP and N2 with different sizes. ’

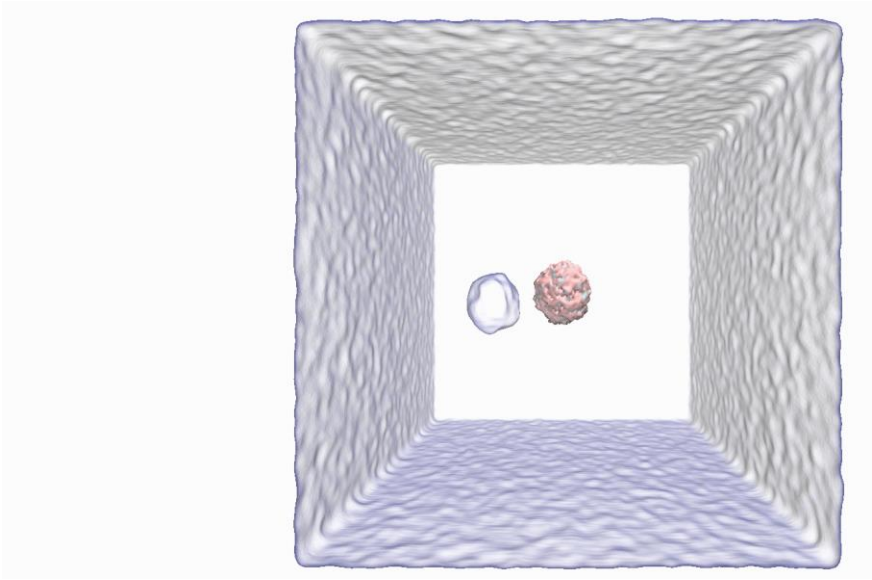
Line 376-379: ‘In addition, the interaction energy between PS and NB is comparable with that for PE and NB, which is stronger than PET and NB, also explaining the less effective removal of PET compared to PS and PE. (**Supplementary Fig.8**)’

Line 466-475: ‘In striving to understand how bubble and particle sizes would affect their interactions, two simulations were conducted using conventional flotation models and MD. The conventional flotation model indicated that nano-sized bubbles can enhance the collision and adhesion probabilities with particles. MD results showed that at the nanoscale, smaller bubbles can have greater interaction energy with particles. While these results provide insights into how NBs might behave dynamically, computational and equipment limitation make it difficult to directly observe the interactions between bubbles and particles, leaving a gap between modeling and experimentation, and highlights the need for further research.’

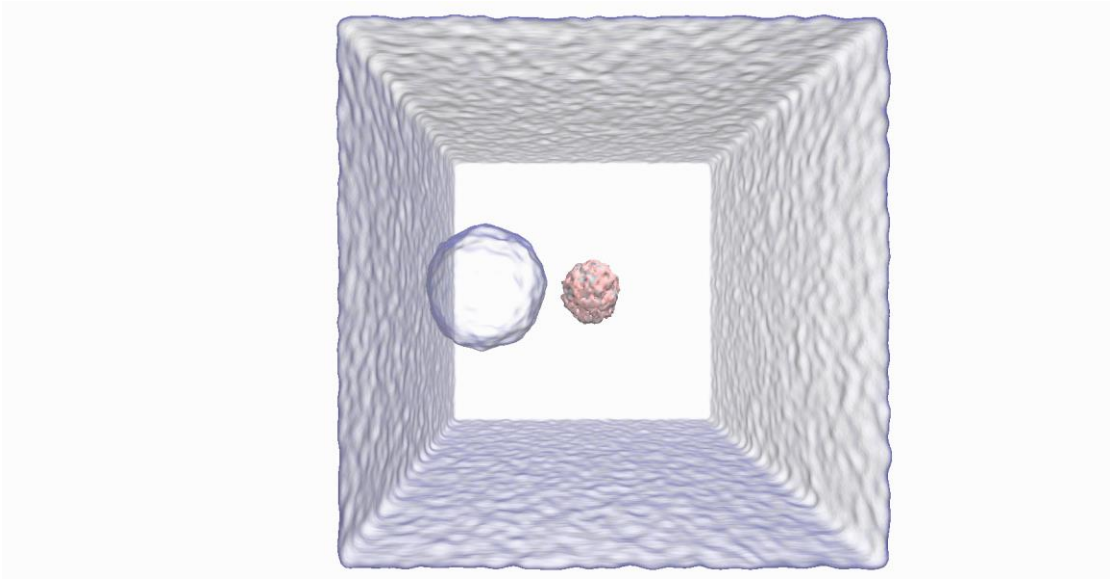
The following description has been added to the material and method section of the manuscript:

Line 605-631: 'The coarse-grain (CG) MD simulations were conducted to study the interactions between NB (6, 15, and 20 nm) and nano-sized plastics (NP, 6 and 12nm) with different polymer types (PS, PE, PET). The systems, composed of NP, N2 molecules, and water, utilized the MARTINI force field[75]. The mapping strategy of atomistic NP into CG beads and the corresponding force field parameters were taken from the previous study[76,77]. The N_2 molecules and water molecules were modeled using parameters optimized by Lin et al.[78,79] and classic MARTINI water beads, respectively. The force field parameters for different species atoms are obtained by Lorentz-Berthelot combining rules. The NP particles were generated by initially dispersing short polymer chains randomly in a large simulation box to form the initial model. The system then underwent energy minimization followed by a 20 ns relaxation at a high temperature of 600 K, and subsequently, the system was subjected to an annealing procedure from 600 K to 300 K to speed up the energy relaxation. Based on the obtained structure, a 100 ns production run was performed to obtain the equilibrium state of the NP particles. These equilibrated NP particles, along with N_2 NBs, were then placed in a $40\times40\times40$ nm^3 box and solvated with classic MARTINI water molecules. To maintain the nanobubble structure, additional diffused N2 molecules were also added to the simulation box[78,80]. Additional simulations were conducted to determine the local density of N_2 NB and diffused N_2 gas. In the composite system, NP size was controlled by the number of polymer chains, and the NB size by the N_2 molecules. The composite system model was first subjected to an energy minimization process, followed by a 20 ns relaxation process, and 300 ns production run in the NVT ensemble. The trajectories for the last 100 ns were used for analysis. The temperature in each process was maintained at 300 K using a V-rescale thermostat [81], while the pressure was kept at 1 bar using Berendsen barostat [82] for the production run. Each system was named after the sizes and components of NB and NP, e.g., PS12B6 indicates PS with a 12 nm NP and a 6 nm NB. All simulations used the GROMACS 5.1.4 package [83]. and Visual Molecular Dynamics (VMD) software rendered all snapshots [84]. '

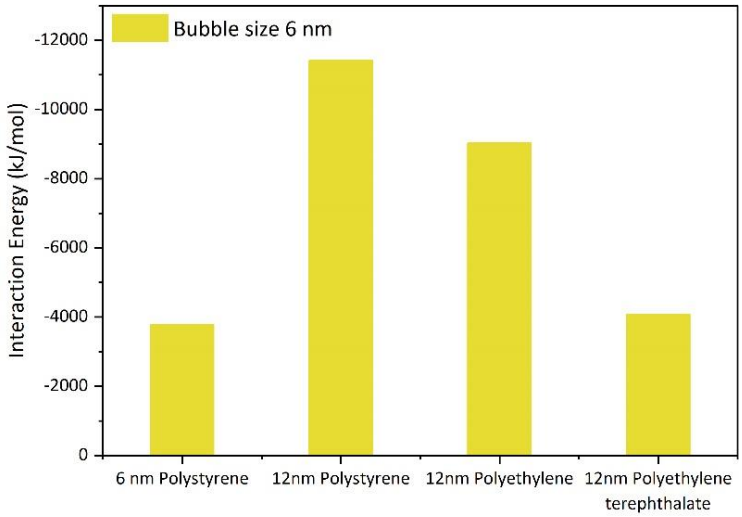
The following simultation videos have been added to the video supplementary information:



Supplementary video 1. Interaction process between 6nm PS particle and 6nm bubble.



Supplementary video 2.Interaction process between 6nm PS particle and 15 nm bubble.



Supplementary Figure 10. Comparison of interaction energy between particles with different polymer types.

In summary, although some techniques we employed are established, the context of their application—alongside the modifications and supplementary methods we incorporated—contributes significantly to advancing our understanding of NB applications in environmental remediation. We believe these efforts collectively represent a meaningful advancement in the field.

Once again, thank you very much for the time in reviewing the manuscript and all your comments and suggestions

Sincerely,

Alicia K.J An

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