

# Limitations of Nanomaterials in Enhancing Plastic Biodegradation: Evidence from Two Failed Experimental Attempts

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## Abstract

Nanomaterials (NMs) have been proposed as catalysts to enhance the degradation of organic pollutants in combination with biology. However, to date, there have been no attempts to use NMs to promote the biodegradation of plastics. We conducted two separate experiments combining NMs with microorganisms and plants. The bacterium *Bacillus cereus* was used in combination with multi-walled carbon nanotubes (MWCNTs) in a minimal salt medium. A second phytoremediation experiment used a soil–water cultivation system with watercress (*Nasturtium officinale*) and MWCNTs. Neither approach yielded significant improvements in polyethylene (PE) plastic film degradation. In fact, NM addition reduced degradation rates. These results highlight the context-dependent limitations of NMs in real-world biodegradation systems and underscore the need for better-designed NMs with improved stability and bioavailability in biological systems.

## Keywords

Nanomaterials, plastic degradation, *Bacillus cereus*, MWCNTs, phytoremediation, negative results

## 1. Introduction

Nanomaterials (NMs) have been widely explored in recent years as tools to enhance the bioremediation of environmental pollutants (1, 2). Studies have shown that certain NMs can promote microbial and plant-based remediation of heavy metals and organic contaminants, such as arsenic (As) (3), lead (Pb) (4) and polycyclic aromatic hydrocarbons (PAHs) (5, 6). In some cases, NM-based immobilization platforms have even been developed to improve the stability and bioavailability of remediation agents (7). These promising results have led to growing interest in applying NMs to emerging contaminants, including plastics.

However, research on the use of NMs to enhance plastic degradation remains limited. Most studies involving plastic remediation have focused on microbial consortia or phytoremediation systems without the involvement of NMs (8, 9). For example, some plants, such as lettuce (*Lactuca sativa* L.), have been used to test the degradation of plastic (10). Yet the reported efficiencies are often extremely low, sometimes below 4 percent even after 120 days of exposure, or they are only applicable to biodegradable plastics, which do not represent the persistent polymers that dominate environmental pollution (11, 12).

Given this gap, we hypothesized that adding NMs, specifically multi walled carbon nanotubes (MWCNTs), which have been widely reported to enhance the remediation of organic pollutants (5, 13), could improve the biodegradation of polyethylene (PE) plastic film. Unfortunately, the final result of this experiment was negative. Hence, we hope these findings contribute to a more realistic understanding of how NMs behave in complex biological systems and help others avoid similar pitfalls in future research.

## **2. Materials and Methods**

All experiments were conducted at University College London, United Kingdom. Each treatment was performed in triplicate to account for biological and experimental variability. This study consisted of two parallel experiments: (a) the first investigated the combination of MWCNTs with plastic degrading bacteria, and (b) the second examined the combination of MWCNTs with watercress in a hydroponic system.

### **2.1 Materials**

The plastic films used in both experiments were a double layer black commercial grade PE film purchased from Amazon (Brand: Ramofy). This type of film is commonly used for packaging applications as shown in the product operating manual. MWCNTs with diameters ranging from 5-20nm and consisting of 4 to 8 layers (Brand: Shilpent). For the plant experiment, gardening soil was acquired from the B&Q UK (Brand: Verve Bulb Planting Compost). The seeds of Watercress (*Nasturtium officinale*) were purchased from Amazon.

### **2.2 Microorganism Degradation of PE Plastic Film with MWCNTs**

To identify a suitable bacterial strain for the degradation experiment, we first reviewed the literature on plastic degrading microorganisms. Previous studies have reported a range of bacteria and fungi capable of colonizing plastic surfaces or utilizing plastic polymers as carbon sources. For example, Jachimowicz et al. identified microplastic degraders belonging to the genera *Acidovorax*, *Gordonia*, *Pseudomonas*, *Sphingomonas*, and *Sphingopyxis* in biofilms (14). Auta et al. found that only two isolates, *Bacillus cereus* and *Bacillus gottheilii*, grew on a synthetic medium containing different microplastic polymers as the sole carbon source (15). Fungal strains such as *Aspergillus caespitosus*, *Phialophora alba*, *Paecilomyces variotii*, and others have also been investigated for their ability to produce ligninolytic enzymes including laccase, manganese peroxidase, and lignin peroxidase, which can break down low density polyethylene films (16). Based on this review, we selected *Bacillus cereus* as the model organism for our experiments. The strain is Biosafety Level 1, making it safe to handle in a standard laboratory setting without specialized containment. It is commercially available, inexpensive to culture, and grows rapidly under standard conditions.

*Bacillus cereus* was cultured following established protocols (17, 18). Luria Broth (LB) liquid medium was prepared by dissolving 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 1 L of distilled water. The solution was autoclaved at 121 °C for 20 minutes. After cooling, the medium was inoculated with *Bacillus cereus* and incubated in a shaking incubator at 37 °C for 24 hours (19). The culture was then centrifuged at 5000×g for 4 minutes. The supernatant was discarded, and the cell pellets were washed twice with sterile distilled water. The washed pellets were lyophilized and suspended

in phosphate buffered saline at pH 7.4, prepared by mixing 1.8 mmol/L  $\text{KH}_2\text{PO}_4$ , 10.1 mmol/L  $\text{NaH}_2\text{PO}_4$ , 2.7 mmol/L  $\text{KCl}$ , and 137 mmol/L  $\text{NaCl}$  in distilled water and autoclaving under the same conditions (20).

All experiments were carried out in dark glass bottles. Minimal Salt Medium (MSM) was used as the medium, which is the mixture solution of 1 L distilled water or deionized water contains:  $\text{K}_2\text{HPO}_4$  6.0 g;  $\text{KH}_2\text{PO}_4$  5.5 g;  $\text{Na}_2\text{SO}_4$  2.0 g;  $\text{KCl}$  2.0g;  $\text{NH}_4\text{NO}_3$  2.0 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.2 g; Trace metal salt solution 1.0 mL. Trace metal salt solution:  $\text{EDTA} \cdot 2\text{Na}$  500 mg per liter of water,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  10 mg,  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  5 mg,  $\text{H}_3\text{BO}_3$  30 mg,  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$  24 mg,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  5 mg,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  5 mg,  $\text{Ca}(\text{OH})_2$  50 mg, pH 7.2.

The bottles were placed on an orbital shaker at 150 rpm. Groups include:

- (a) Bacillus + Plastic films (200 mg/L) + MWCNTs (100 mg/L);
- (b) Bacillus + Plastic films (200 mg/L) + MWCNTs (200 mg/L);
- (c) Plastic films + MWCNTs (100 mg/L);
- (d) Plastic films + MWCNTs (200 mg/L);
- (e) Plastic films only;
- (f) Bacillus only.

Plastic films were weighed before and after 7 days of incubation. After removal, films were rinsed with distilled water and dried for 24 h before weighing. The recovery rate of PE plastic film is  $100\% \pm 0.01\%$ .

### **2.3 phytoremediation of PE plastic film with NMs**

The phytoremediation experiment was conducted in sterile glass petri dishes (90 mm diameter) to avoid any plastic contamination. Each dish was filled with Milli-Q water just below the rim, creating a shallow liquid layer. A pre-weighed piece of PE film ( $0.07 \pm 0.005$  g) was placed flat on the bottom of each dish, and 0.05 g of nutrient soil was added to provide a substrate for plant growth and root anchoring (21). Watercress seeds were germinated on moist filter paper for 48 hours. Ten germinated seeds with visible radicles were transferred to each dish.

The dishes were then placed in a greenhouse under controlled conditions: temperature  $25 \pm 1$  °C, 16 h light / 8 h dark photoperiod, with light provided by plant growth lamps. The device was shown in Figure 2(b) in Section 3. Three experimental groups were set up:

- (a) CK (MWCNTs only);
- (b) Watercress only;
- (c) Watercress + MWCNTs.

100 mg/L MWCNTs were added as a suspension directly into the water at the start of the experiment. The water level was checked every 2 days and replenished with sterile Milli-Q water to maintain the initial volume. On the 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> days, plastic films were collected. They were rinsed gently with deionized water to remove soil particles and adhered biomass, then dried in a fume hood for 24-36 hours until no change in weight. Degradation rate was calculated as the percentage of weight loss relative to the initial weight based on reported research (11). The plant growth indicators, such as root length, plant height, and biomass, are measured after the plants have been washed.

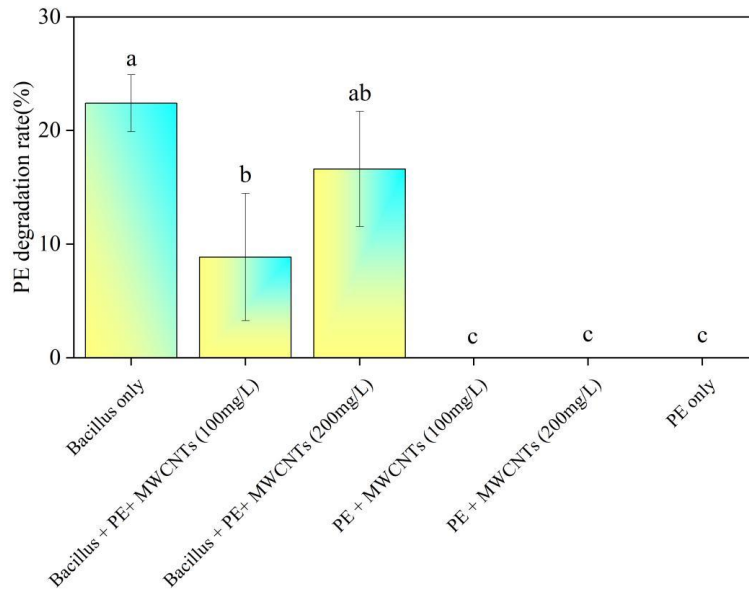
## 2.4 Data treatment and statistical significance testing

All experimental data were sorted in Microsoft Excel. Subsequent data visualization were performed using OriginPro 2021 and R 4.5.1. All errors are expressed as standard deviations (SD). Statistical significance was assessed using analysis of variance (ANOVA) in R.

## 3. Results and Discussion

### 3.1 Ineffective Attempt to Enhance Microbial Plastic Degradation Using MWCNTs

After 7 days of incubation, the plastic films were retrieved and weighed to calculate degradation rates. As shown in Figure 1, the results showed considerable variation across the experimental groups. The highest degradation rate was observed in the group containing *Bacillus cereus* alone, which reached  $22.4 \pm 2.51\%$ . It should be noted that the apparent weight loss may partially reflect surface erosion or biofilm removal rather than complete polymer mineralization (22). For example, physical erosion of the plastic surface may release micro particles too small to recover during washing. The addition of MWCNTs at 100 mg/L reduced the degradation rate to 8.9%, significantly lower than *Bacillus cereus* only group, despite the presence of both bacteria and MWCNTs. When the NMs concentration was increased to 200 mg/L in the presence of bacteria, the degradation rate recovered to 16.6%, though it remained lower than the bacteria only group and the difference was not statistically significant. No measurable degradation was observed in any of the abiotic control groups. The plastic films incubated with MWCNTs alone, whether at 100 mg/L or 200 mg/L, showed zero weight loss, as did the plastic only control. This confirms that the PE plastic films were stable under the experimental conditions and that any weight loss observed in the biotic groups can be attributed to microbial activity.



**Figure 1. Mean degradation rates of PE films after 30 days incubation with *Bacillus cereus* and MWCNTs.**

These results suggest that adding MWCNTs did not enhance PE degradation and may have interfered with bacterial activity. Nanotube aggregation likely reduced the available surface area for catalysis, limiting any potential benefit (23). Aggregation of MWCNTs was also observed in our samples, which was one of the reasons an orbital shaker was used during incubation. The porous structure of individual materials can also reduce bioavailability due to strong adsorption and pore entrapment under actual background due to soil or dust (5, 24). Therefore, this is not an isolated case, and it has also been confirmed in the remediation of other organic pollutants, such as PAHs (25) and polybrominated biphenyl ethers (PBDEs) (24). It is also possible that the MWCNTs exerted mild toxicity on *Bacillus cereus*, inhibiting its metabolic activity over the 30 day incubation period, as shown in our previous study (26).

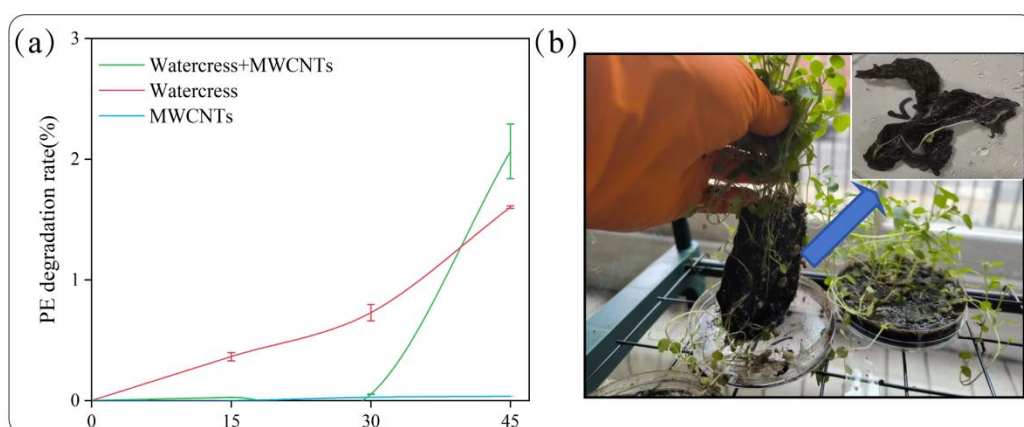
The partial recovery of degradation at the higher MWCNTs concentration may reflect changes in aggregation behavior or surface chemistry at higher particle densities, which is different from others. Previous introduced studies on other organic pollutants such as PAHs and PBDEs have shown that lower concentrations of MWCNTs tend to have weaker inhibitory effects (24, 25). For example, 10 mg/kg was more effective than 100 mg/kg, and 100 mg/kg was more effective than 1000 mg/kg (25). This may suggest that in our experiment, inhibition and promotion occurred simultaneously. On one hand, MWCNTs may have exerted inhibitory effects through adsorption and toxicity. On the other hand, their adsorption and fixation properties may have reduced the direct exposure of microorganisms to PE, thereby alleviating stress (26). However, it is clear that under the current conditions, these opposing effects did not reach a stable and mutually reinforcing balance that would result in a net positive outcome.

### **3.2 Ineffective Attempt to Enhance Plastic Degradation in a Plant System Using MWCNTs**

After 15, 30 and 45 days of incubation, the degradation rates of PE films remained

extremely low across all groups, and no meaningful enhancement was observed with the addition of MWCNTs as shown in Figure 2.

At 15<sup>th</sup> d, the Watercress only group showed a degradation rate of 0.36%, while the Watercress + MWCNTs group showed only 0.03%. The MWCNTs only control showed no measurable degradation at this time point. By 30<sup>th</sup> d, the Watercress only group had reached 0.73%, while the Watercress+MWCNTs group remained at 0.05%. The MWCNTs only control showed 0.03% degradation. At the final time point of 45<sup>th</sup> d, the Watercress + MWCNTs group reached 2.07 ±0.23%, while the Watercress only group reached 1.60±0.01%. The MWCNTs only control remained near zero at 0.04% degradation. At the same time, we did not detect any differences in plant growth. Figure 2(b) shows that the plant roots are wrapped around the PE plastic film. This might be a way for the plants to extend their roots to obtain nutrients, but the relationship with degradation remains uncertain.



**Figure 2. (a) Mean degradation rates of PE films over 45 days in a watercress hydroponic system with and without MWCNTs; (b) Experimental device diagram and the entanglement phenomenon of Watercress roots and plastic film.**

These values are far below any practical relevance for plastic remediation. After 45<sup>th</sup> d, the highest degradation rate observed was just over 2%. The presence of watercress did contribute to some degradation, as both groups containing plants showed measurable weight loss by the end of the experiment, while the MWCNTs alone group did not. However, the addition of MWCNTs did not enhance this effect. In fact, the Watercress + MWCNTs group showed consistently lower degradation than the Watercress only group at 15<sup>th</sup> d and 30<sup>th</sup> d, and only slightly exceeded it at 45<sup>th</sup> d by less than 0.5%.

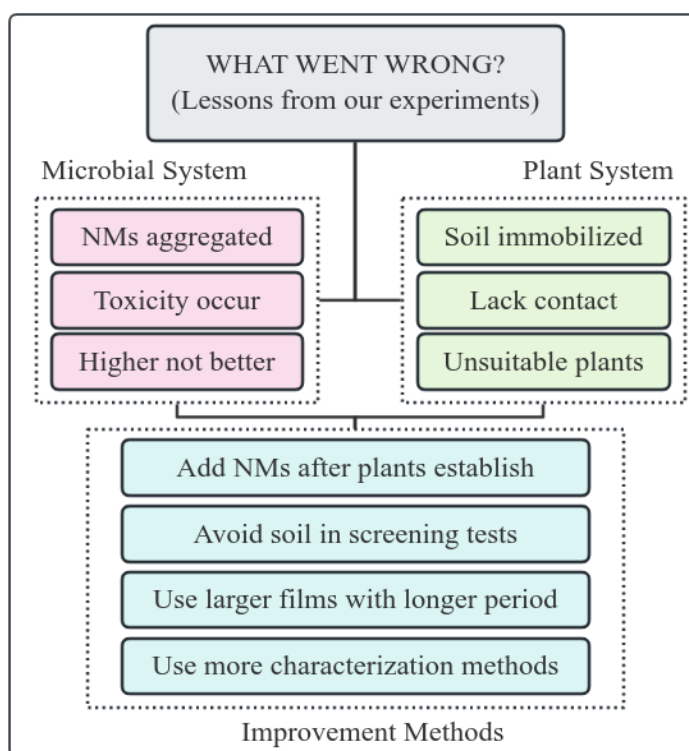
Although there is no direct evidence currently indicating that the interaction between MWCNTs and the rhizosphere environment has affected this, there are many reports on inhabited or failed phytoremediation with other NMs that can indirectly prove this. First, NMs likely became immobilized by soil particles added to the dishes, limiting their physical contact with the plastic film surfaces (27, 28). Second, the nutrient soil may have provided more accessible carbon sources for any

microorganisms present in the system, reducing any selective pressure to degrade the PE (29). Third, the plant itself may not possess or support the specific microbial communities needed to break down PE as shown in our pervious study (30).

It is also worth noting the practical challenges of measuring degradation at such low rates. With starting film weights around 0.07g, a 2% weight loss represents only 1.4mg. At this scale, small errors in film recovery, incomplete removal of soil or biomass, or slight variations in drying conditions can introduce significant uncertainty into the measurements. The accurate recovery rate data presented in Section 2 does not necessarily guarantee the overall smoothness of the process. In particular, it is necessary to take into account the possible physical properties changes of the PE film after biological treatment (31).

#### 4. Outlook

These results suggest that it is difficult to address emerging pollutants simply by adapting existing remediation systems. It is extremely difficult to achieve the goal of solving an emerging pollutant problem by simply copying the existing system (using MWCNTs) and pursuing low cost (using watercress), as these approaches cannot be implemented immediately. A large number of preliminary experiments, mechanism studies and new degradation system construction are required to address the new problems. Hence, Figure 3 provides a simple optimization experiment framework for future research.



**Figure 3. Summary of the experimental limitations and a proposed framework for improving future studies**

Four practical suggestions for researchers designing similar studies are given by Figure 3. Specific explanation as follows:

(1) Consider when and how to add nanomaterials in complex systems. In our plant experiment, soil particles immobilized the multi walled carbon nanotubes, preventing them from reaching the plastic surfaces. Adding nanomaterials after plants are already established may help maintain their mobility and availability for catalysis.

(2) Avoid using soil in initial screening tests. Soils introduce too many variables that can interfere with nanomaterial behavior. Simpler systems such as liquid media or agar should be used first to establish whether any catalytic effect exists before moving to more realistic but complex environments.

(3) Use larger plastic films and longer experimental periods. With starting film masses around 0.07 g, a 2 percent weight loss represents only 1.4 mg, well within typical measurement error. Increasing film masses to 0.5 g or more would reduce this uncertainty. Extending experiments beyond 45 days to 90 or 120 days may also allow small effects to accumulate into measurable weight loss.

(4) Include multiple measurement methods. Weight loss alone is not sensitive enough when degradation rates are low. Complementary techniques such as Fourier Transform Infrared Spectroscopy (FT-IR) can detect surface oxidation changes, while Scanning Electron Microscopy (SEM) can visualize physical erosion or microbial colonization (32, 33). These methods provide evidence of degradation even when weight changes remain minimal.

## **5. Conclusion**

Our two biodegradation experiments failed to demonstrate any enhancement of PE plastic film degradation by MWCNTs. In the microbial system, 100 and 200 mg/L MWCNTs addition did not improve and sometimes reduced degradation compared to bacteria alone. In the plant system, 100 mg/L MWCNTs did not improve phytoremediation performance. These results suggest that simply introducing NMs into biological systems is not sufficient. Future efforts must focus on ensuring physical contact between NMs and plastic surfaces, minimizing potential toxicity, and employing more sensitive analytical methods to detect early degradation signals. Importantly, publishing such negative results helps reduce publication bias in environmental nanotechnology research and provides practical lessons for designing future biodegradation experiments.

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