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# Potential of Halophilic Bacteria from Hypersaline Waters to Reduce Polyethylene Microplastic Abundance in *Artemia* sp. Culture: A Preliminary Indication of Degradation Based on Gravimetric Weight Loss

Nanda Wahyuni Al Humaero[1*], Sadikin Amir[2], Bagus Dwi Hari Setyono[1]

[1]Study Program of Aquaculture, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram
[2]Study Program of Marine Science, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram
Jl. Majapahit No. 62, Gomong, Selaparang District, Mataram, West Nusa Tenggara 83115

**Correspondence**:
nandawahyuni177@gmail.com

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**ABSTRACT**

Polyethylene (PE) microplastic contamination in aquaculture systems can increase plastic particle exposure in zooplankton, including *Artemia* sp., while also elevating the risk of biological disturbance in cultured organisms. This study evaluated the potential of halophilic bacteria isolated from hypersaline waters to reduce PE microplastic abundance in *Artemia* sp. and to provide a preliminary indication of PE degradation based on gravimetric weight loss. A completely randomized design with six treatments and three replications was applied: P0 (without microplastics and bacteria), KN (PE 2 mg/L), KP (bacteria $10^6$ CFU/mL), P1 (PE 2 mg/L + bacteria $10^6$ CFU/mL), P2 (PE 2 mg/L + bacteria $10^7$ CFU/mL), and P3 (PE 2 mg/L + bacteria $10^8$ CFU/mL). The primary response variables were microplastic abundance in *Artemia* sp. and PE degradation efficiency, whereas growth, survival, and water quality were treated as supporting variables. The treatments significantly affected microplastic abundance in *Artemia* sp. KN produced the highest abundance (0.1100 particles/individual), whereas bacterial addition reduced the value to 0.0267 particles/individual in P1, 0.0267 in P2, and 0.0467 in P3. PE weight loss ranged from 20.0% to 31.1%, with the highest value recorded in P1. These findings suggest that a halophilic bacterial inoculum of $10^6$ CFU/mL was the most promising level for early-stage PE bioremediation in Artemia culture systems. Nevertheless, because degradation was assessed only by gravimetric loss, the result should be interpreted as a preliminary indication and still requires abiotic controls, SEM, and FTIR confirmation.

## INTRODUCTION

Microplastics have evolved from a general pollution issue into a matter of aquaculture environmental quality because these particles have now been reported in water, sediment, feed, and aquatic organisms across a wide range of production systems. Among the many polymers in circulation, polyethylene (PE) is one of the most dominant, widely used, resistant to degradation, and prone to fragmentation into micro-sized particles that persist in aquatic environments (Alimba & Faggio, 2019; Le *et al*., 2024; Saha & Chandrasekaran, 2024). In aquaculture, the relevance of PE is not limited to pollution alone; it is also important from a biosecurity perspective because plastic particles may act as carriers of additives and other contaminants and may prolong exposure in cultured organisms (Gao *et al*., 2024; Heris, 2024).

*Artemia* sp. is a highly relevant model for evaluating this risk. As a non-selective filter feeder, Artemia readily ingests suspended particles within the size range of its mouth opening. A growing body of research has shown that microplastic exposure in Artemia can reduce feeding efficiency, impair gut histology, increase oxidative stress, alter behavior, and depress biological performance under certain conditions (Varó *et al*., 2019; Wang *et al*., 2019; Kim *et al*., 2021; Kanimozhi *et al*., 2024; Kim *et al*., 2024; Vengatesh *et al*., 2024; Kim *et al*., 2025). Because Artemia is also an important live feed in aquaculture, microplastic accumulation in this organism may become an early node for particle transfer within cultured food webs (Saha & Chandrasekaran, 2024).

One increasingly studied approach for reducing microplastic loads is microorganism-based bioremediation. Conceptually, this process begins with plastic surface colonization, followed by biofilm formation, weakening of polymer surface properties, partial depolymerization, and subsequent utilization of the resulting fragments by microorganisms. However, the efficiency of this process is strongly influenced by polymer type, environmental conditions, biofilm properties, and the composition of the microbial community involved (Wang *et al*., 2023; Gao *et al*., 2024; da Silva *et al*., 2024; Schneier *et al*., 2024). In PE, the main challenge lies in its hydrophobicity and chemically stable carbon backbone; therefore, evidence of degradation should ideally not rely solely on material weight loss but should also be supported by morphological and chemical analyses (Heris, 2024; Nguyen *et al*., 2024).

Within this context, halophilic bacteria are particularly attractive because they can function stably under high-salinity conditions, which are relevant to hypersaline waters and Artemia culture. Recent literature indicates that extremophilic microbial communities, including halophilic and halotolerant bacteria, may be associated with plastic degradation through biofilm formation and specific enzymatic activities (Atanasova *et al*., 2021; Özdemir *et al*., 2022; Pham *et al*., 2024; Krumov *et al*., 2025). Other studies have also reported that isolates from saline environments, mangroves, and aquafarm systems can reduce the mass of PE, PP, and PS with varying levels of efficiency, although their performance depends strongly on strain identity, substrate characteristics, and treatment design (Pathak & Navneet, 2023; Fang *et al*., 2024; Hossain *et al*., 2024; Yang *et al*., 2025; Afify *et al*., 2025).

Nevertheless, studies that position halophilic bacteria as agents for reducing microplastic loads within Artemia culture systems remain limited. This gap is important because aquaculture systems require two conditions to be met simultaneously: PE particles in the medium need to be suppressed, but the remediation agent applied should not worsen the biological responses of the test organism. On that basis, this study aimed to analyze the potential of halophilic bacteria from hypersaline waters to reduce PE microplastic abundance in *Artemia* sp. and to provide a preliminary indication of PE degradation in the culture

medium. To maintain interpretive caution, this article explicitly positions PE weight loss as an initial indicator rather than as a single, conclusive proof of polymer degradation.

In the local context of West Nusa Tenggara, publications shown that microplastics have already been detected in aquaculture areas of Ekas Bay and in whiteleg shrimp farming systems in North Lombok, while PVC exposure has also been reported to reduce tilapia growth and survival. These studies do not directly address PE biodegradation by halophilic bacteria, but they are highly relevant in demonstrating that microplastics have become a real pressure in aquaculture systems within the same region and therefore require applicable mitigation strategies (Sumsanto *et al*., 2024; Putrajab *et al*., 2024; Setyono *et al*., 2024).

## METHODS

### Study Period, Site, and Experimental Design

The study was conducted from August to September 2025 in the Production and Reproduction Laboratory, Hydrobiology Laboratory, and Fish Health Laboratory, Faculty of Agriculture, University of Mataram. A completely randomized design with six treatments and three replications was used, resulting in 18 experimental units. The present article focuses on two primary outcomes, namely microplastic abundance in *Artemia* sp. and the preliminary indication of polyethylene (PE) degradation based on gravimetric weight loss.

The experimental treatments were arranged as follows: P0 = without microplastics and without bacteria (system negative control); KN = PE 2 mg/L without bacteria; KP = halophilic bacteria $10^6$ CFU/mL without PE; P1 = PE 2 mg/L + halophilic bacteria $10^6$ CFU/mL; P2 = PE 2 mg/L + halophilic bacteria $10^7$ CFU/mL; and P3 = PE 2 mg/L + halophilic bacteria $10^8$ CFU/mL. The PE concentration of 2 mg/L was adapted from the microplastic exposure level used in *Artemia franciscana* by Han *et al*. (2021), who demonstrated that 2.0 mg/L produced measurable biological responses under controlled laboratory conditions. The bacterial inoculum levels were arranged as a one-log gradient ($10^6$–$10^8$ CFU/mL) to evaluate concentration-dependent effects of halophilic bacterial addition under saline conditions. This gradient was established as an experimental comparison rather than as a previously established optimum dose, while remaining consistent with the biodegradation literature emphasizing inoculum-dependent performance and the relevance of halophilic microbial systems for plastic degradation (Heris, 2024; Schneier *et al*., 2024; Krumov *et al*., 2025).

Table 1. Experimental Treatment Design

| Treatment | Description |
|---|---|
| P0 | Without microplastics and without bacteria (system negative control) |
| KN | PE 2 mg/L without bacteria |
| KP | Halophilic bacteria $10^6$ CFU/mL without PE |
| P1 | PE 2 mg/L + halophilic bacteria $10^6$ CFU/mL |
| P2 | PE 2 mg/L + halophilic bacteria $10^7$ CFU/mL |
| P3 | PE 2 mg/L + halophilic bacteria $10^8$ CFU/mL |

### Preparation of Bacteria, Microplastics, and Test Organisms

Halophilic bacteria were isolated aseptically from hypersaline waters/salt ponds (>30 ppt) and cultured on selective media modified with 10–20% NaCl. After 48–72 h of incubation at 30°C, morphologically distinct colonies were purified by repeated streaking. Working cultures were then prepared in high-salinity liquid media according to the inoculum level

assigned to each treatment. Because this study did not include molecular identification of the isolates, interpretation is limited to halophilic bacteria originating from hypersaline waters and is not assigned to any species.

PE microplastics were obtained from plastic bottle caps that were ground, sieved, and weighed to achieve a final concentration of 2 mg/L. The test organisms were *Artemia* sp. hatched from commercial cysts and maintained in 3-L containers at an initial density of approximately 10 individuals/mL. During the culture period, Artemia was fed *Nannochloropsis* sp. and exposed to PE and bacterial inocula according to the experimental design.

For the purposes of this article, the experiment was designed to assess the reduction of microplastic abundance in Artemia and the loss of PE weight after treatment. Accordingly, the results are relevant for evaluating the early prospects of bioremediation, but they should not yet be interpreted as conclusive evidence of polymer structural degradation without complementary analyses such as FTIR, SEM, or other polymer chemistry-based confirmation (Heris, 2024; Schneier *et al*., 2024).

**Observation of Variables and Data Analysis**

The primary variables were: (1) microplastic abundance in *Artemia* sp., calculated as the number of particles per individual after tissue destruction with $H_2O_2$, filtration, and microscopic observation; and (2) PE degradation efficiency, calculated as percentage weight loss using a gravimetric approach.

PE degradation efficiency was calculated using the following equation, following the gravimetric weight-loss method commonly applied in plastic biodegradation studies (Harrat *et al*., 2024; Krumov *et al*., 2025):

$$\mathrm{ED} = \frac{(\mathrm{W0} - \mathrm{W1})}{\mathrm{W0}} \mathrm{x}\ 100\%$$

where $W_0$ is the initial PE weight and $W_1$ is the final PE weight after treatment.

As supporting variables, the study also recorded absolute length growth, daily length growth rate, survival, and water quality. Water quality parameters consisted of temperature, dissolved oxygen (DO), pH, and salinity. Temperature was measured using a thermometer, dissolved oxygen was measured using a DO meter, pH was measured using a pH meter, and salinity was measured using a hand refractometer.

Absolute length growth was determined by measuring the total length of Artemia from the anterior tip to the posterior tip under a microscope fitted with a calibrated measuring system, following the measurement approach described by Alamsjah *et al*. (2024). Absolute length growth was calculated as:

$$\mathrm{ALG} = \mathrm{Lt} - \mathrm{L0}$$

where ALG is absolute length growth (mm), $L_t$ is the final mean length (mm), and $L_0$ is the initial mean length (mm) (Alamsjah *et al*., 2024).

Daily length growth rate was calculated as the increase in mean total length divided by the duration of the experiment, as used in aquaculture growth studies (Firdausi *et al*., 2025; Melandri *et al*., 2008):

$$\mathrm{DLGR} = \frac{(\mathrm{Lt} - \mathrm{L0})}{\mathrm{t}} \mathrm{x}\ 100$$

where DLGR is daily length growth rate (mm $day^{-1}$), $L_t$ is the final mean length (mm), $L_0$ is the initial mean length (mm), and t is the experimental duration (days).

Survival was determined from the number of live individuals remaining at the end of the experiment relative to the initial number stocked, following the standard aquaculture formula reported by Firdausi *et al*. (2025):

$$SR\ (\%) = \frac{Nt}{N0} x\ 100$$

where SR is survival rate (%), $N_t$ is the number of individuals alive at the end of the experiment, and $N_0$ is the number of individuals at the beginning of the experiment.

Data was analysed using analysis of variance (ANOVA) at a 95% confidence level, followed by Duncan's multiple range test to separate significantly different means. All interpretations in this article follow a precautionary principle: gravimetric output is treated as a preliminary indication of PE loss, whereas biological changes in Artemia are interpreted as an application context rather than as mechanistic proof of polymer degradation (Heris, 2024; Schneier *et al*., 2024).

## RESULTS

The results are presented according to the primary focus of this article, namely microplastic abundance in *Artemia* sp. and the preliminary indication of PE degradation based on gravimetric weight loss. Supporting variables, including absolute length growth (ALG), daily length growth rate (DLGR), survival rate (SR), and water quality, are presented to provide biological and environmental context for interpreting the main findings.

### Microplastic Abundance in *Artemia* sp.

Microplastic abundance in *Artemia* sp. was determined after tissue destruction, filtration, and microscopic observation, and expressed as the number of particles per individual. Differences among treatments were used to evaluate whether halophilic bacterial addition was associated with a reduction in microplastic uptake or retention by Artemia.

ANOVA from the original SPSS output showed that treatment significantly affected microplastic abundance in *Artemia* sp. ($F = 56.569$; $p < 0.001$). Duncan's test further separated KN as the highest distinct group with a mean of 0.1100 particles/individual, followed by P3 in the next group with a mean of 0.0467 particles/individual. P1 and P2 were placed in the same homogeneous group, each with a mean of 0.0267 particles/individual, whereas KP (0.0200 particles/individual) was transitional because it overlapped with both the P0 group and the P1-P2 group. P0 had the lowest mean at 0.0067. This pattern indicates that bacterial addition in the PE-exposed treatments was associated with lower microplastic abundance in *Artemia* sp., particularly in P1 and P2, relative to KN.

The pattern of mean microplastic abundance across treatments is presented in Figure 1 to clarify the position of each treatment relative to the grouping obtained from Duncan's multiple range test.

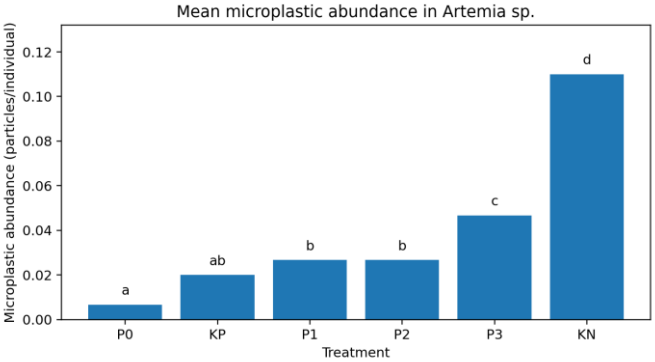


Figure 1. Mean Microplastic Abundance in *Artemia* sp. by Treatment. Different Letters Indicate Homogeneous Groups According to Duncan's Test at α = 0.05; KP Overlaps with the P0 Group as Well as the P1-P2 Group

Table 2. Microplastic Abundance in *Artemia* sp. and Homogeneous Groups from Duncan's Test

| Treatment | Microplastic Abundance (particles/individual) | Homogeneous Group | Biological Meaning |
|---|---|---|---|
| P0 | 0.0067 | a | Very low background contamination |
| KP | 0.0200 | ab | No PE exposure; indicates a low-level contamination trace |
| P1 | 0.0267 | b | Exposure in Artemia was substantially reduced relative to KN |
| P2 | 0.0267 | b | Reduction effect comparable to P1 at the mean level |
| P3 | 0.0467 | c | Still lower than KN, but less effective than P1 and P2 |
| KN | 0.1100 | d | Highest accumulation in the treatment without bacteria |

**Preliminary Indication of PE Degradation based on Gravimetric Weight Loss**

The preliminary indication of PE degradation was evaluated using gravimetric weight loss and expressed as degradation efficiency (ED). In this article, ED is interpreted as a preliminary indication of PE reduction after treatment rather than definitive evidence of polymer structural degradation.

For the PE weight-loss variable, the follow-up test reported in the source manuscript indicated that P1 differed significantly from P3, but not from P2. Descriptively, PE weight loss ranged from 20.0% to 31.1%, with the highest value in P1 (31.1 ± 4.8%), followed by P2 (26.7 ± 6.2%), and the lowest in P3 (20.0 ± 4.1%). Thus, the $10^6$ CFU/mL level remained the most promising inoculum under the conditions of this study, whereas increasing bacterial density beyond that level did not improve performance proportionally.

A visualization of mean PE weight loss and standard deviation across bacterial inoculum levels is presented in Figure 2.

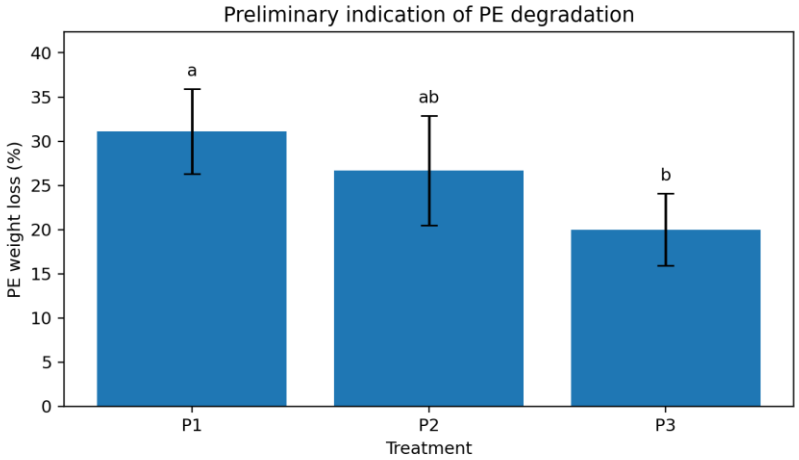


Figure 2. Preliminary Indication of PE Degradation based on Gravimetric Weight Loss in Treatments Receiving Halophilic Bacteria. Error Bars Represent Standard Deviations; Different Letters Indicate the Differences Reported in the Source Manuscript, with P1 Significantly Different from P3 and P2 Occupying an Intermediate Position

Table 3. PE Degradation Efficiency in Treatments Receiving Halophilic Bacteria

| Treatment | Bacterial Concentration (CFU/mL) | PE Degradation Efficiency (%) | Interpretation |
|---|---|---|---|
| P1 | $10^6$ | 31.1 ± 4.8 | Most promising; presumed to have the best cell-enzyme-substrate balance |
| P2 | $10^7$ | 26.7 ± 6.2 | Still effective, but lower than P1 |
| P3 | $10^8$ | 20.0 ± 4.1 | Lowest efficiency; higher density did not improve performance |

**Biological Responses of *Artemia* sp. as Supporting Variables**

Supporting biological responses were evaluated through absolute length growth (ALG), daily length growth rate (DLGR), and survival rate (SR). These variables were not used as direct proof of polymer degradation, but rather as indicators of biological performance under experimental conditions.

As supporting variables, the original SPSS output also showed that treatment significantly affected absolute length growth ($F = 4{,}259.885$; $p < 0.001$), daily length growth rate ($F = 16{,}355.400$; $p < 0.001$), and survival ($F = 110.933$; $p < 0.001$). Duncan's test for all three variables yielded the same rank order of means, namely P0 > KP > P1 > P2 > P3 > KN. Descriptively, KN recorded the lowest values at 20.7533 µm, 34.9633%, and 5.3333%, respectively, whereas P1 showed partial improvement to 39.3900 µm, 46.0067%, and 14.0000%. These results support the interpretation that bacterial addition was associated with reduced PE exposure, although Artemia performance did not recover to the level of uncontaminated control.

Table 4. Supporting Biological Variables in *Artemia* sp.

| Treatment | Absolute Length Growth (µm) | Daily Length Growth Rate (%) | Survival (%) |
|---|---|---|---|
| P0 | 58.4333 | 57.2400 | 19.3333 |
| KP | 47.1733 | 50.0767 | 16.6667 |
| P1 | 39.3900 | 46.0067 | 14.0000 |
| P2 | 29.6467 | 41.4567 | 12.0000 |
| P3 | 24.2267 | 38.2700 | 10.0000 |
| KN | 20.7533 | 34.9633 | 5.3333 |

**Water Quality During the Experiment**

Water quality parameters, including temperature, dissolved oxygen, pH, and salinity, were monitored throughout the experiment to ensure that treatment responses were not primarily attributable to deterioration of rearing conditions.

Throughout the experiment, temperature, dissolved oxygen, pH, and salinity remained within ranges considered suitable for *Artemia* sp. culture. Temperature was 28.4-28.6 °C, DO was 5.3-6.4 mg/L, pH was 7.0-7.2, and salinity was 37 ppt. Accordingly, variation in responses among treatments is more reasonably attributed to PE exposure and bacterial inoculation than to extreme deterioration in water quality.

Table 5. Water Quality During the Experiment

| Parameter | Observed Range | Optimal Range | Information |
|---|---|---|---|
| Temperature (°C) | 28.4-28.6 | 25-30 | Still suitable for *Artemia* sp. culture |
| DO (mg/L) | 5.3-6.4 | 2.0-7.0 | Did not indicate severe oxygen depletion |
| pH | 7.0-7.2 | 6.0-8.4 | Within the tolerable range |
| Salinity (ppt) | 37 | 30-55 | Consistent with hypersaline/concentrated seawater conditions |

## DISCUSSION

The present study was designed to examine microplastic abundance in *Artemia* sp. and the preliminary indication of PE degradation based on gravimetric weight loss, while ALG, DLGR, SR, and water quality were included as supporting variables. Accordingly, the discussion prioritizes treatment-related patterns in microplastic abundance and ED, whereas biological responses in *Artemia* sp. are interpreted as contextual indicators of culture performance rather than as direct mechanistic proof of polymer degradation.

The high microplastic abundance in KN confirms that, in the absence of bacteria, PE particles remained available in the medium and were readily exposed to *Artemia* sp. This pattern is consistent with the non-selective filter-feeding nature of Artemia, which is known to ingest microplastic particles and to display physiological and histological responses after repeated exposure (Wang *et al*., 2019; Varó *et al*., 2019; Kim *et al*., 2021; Saha & Chandrasekaran, 2024). The fact that P0 and KP still contain particles at very low levels should also be noted, because it highlights the vulnerability of microplastic studies to background contamination. Therefore, comparisons among treatments are more informative than isolated interpretation of absolute values.

The reduction in microplastic abundance in P1 and P2 indicates that halophilic bacteria added to the medium were associated with reduced PE exposure in *Artemia* sp. Mechanistically, this is consistent with literature showing that microorganisms can attach to plastic surfaces, form biofilms, modify polymer surface properties, and at certain stages accelerate the early weakening or transformation of plastic materials (Wang et al., 2023; Gao et al., 2024; Bocci et al., 2024; Schneier et al., 2024). In this article, however, that explanation is not presented as a directly proven mechanism within the experimental system, but rather as the most plausible biological interpretation of the reduced PE abundance observed in the bacteria-treated groups.

The PE weight-loss pattern shows that increasing inoculum density was not always directly proportional to improved performance. The $10^6$ CFU/mL concentration produced the highest value, whereas performance declined at $10^7$ and $10^8$ CFU/mL. Similar patterns have been reported in several PE biodegradation studies, in which success is determined not merely by cell abundance, but by the balance among surface colonization, oxygen diffusion, nutrient supply, and enzymatic synthesis efficiency (Yao *et al*., 2022; Pathak & Navneet, 2023; Hossain *et al*., 2024; Fang *et al*., 2024; Afify *et al*., 2025). At excessively high densities, biofilms may become too thick and intercellular competition may increase, thereby reducing effective enzyme-substrate contact.

Even so, this article deliberately uses the term preliminary indication of degradation rather than proof of PE biodegradation. Weight loss is a valuable indicator, but methodologically it is still insufficient to exclude contributions from fragmentation, particle release, residual moisture change, or loss during sample handling. Recent literature consistently treats gravimetry as only one component of proof, which should ideally be complemented by SEM, FTIR, crystallinity analysis, and adequate abiotic controls (Atanasova *et al*., 2021; Gao *et al*., 2024; Nguyen *et al*., 2024; Heris, 2024; Yang *et al*., 2025). Therefore, the primary strength of this study lies not in making a final mechanistic claim, but in identifying the most promising inoculum level for future testing.

From an application standpoint, P1 was the most balanced treatment because it reduced microplastic abundance to roughly one-quarter of the KN value while simultaneously producing the highest PE weight loss. However, the biological response of Artemia remained relatively low across all treatments, including the controls, especially for survival. This suggests that the rearing system was still under considerable experimental stress, and therefore the biological benefit of the bacteria should be interpreted cautiously. The Artemia literature shows that microplastics can reduce growth, damage the digestive tract, and trigger oxidative stress, but the magnitude of response depends strongly on particle shape, size, exposure duration, and media quality (Athulya *et al*., 2023; Kanimozhi *et al*., 2024; Kim *et al*., 2024; Vengatesh *et al*., 2024; Kim *et al*., 2025). Future studies should therefore integrate culture-condition optimization, molecular identification of isolates, and morphological and chemical validation of PE transformation to strengthen scientific validity and improve reviewer readiness. Its practical relevance is further reinforced by reports of microplastic contamination in aquaculture areas of Ekas Bay and whiteleg shrimp culture in North Lombok, as well as by evidence that PVC exposure can reduce the growth and survival performance of cultured fish, which supports consideration of biological mitigation approaches for coastal and hypersaline aquaculture systems in West Nusa Tenggara (Putrajab *et al*., 2024; Setyono *et al*., 2024; Sumsanto *et al*., 2024).

## CONCLUSION

Halophilic bacteria from hypersaline waters showed potential to reduce PE microplastic abundance in *Artemia* sp. and to provide a preliminary indication of PE degradation in the culture system. The best performance was obtained at an inoculum level of $10^6$ CFU/mL (P1), which yielded a microplastic abundance of 0.0267 particles/individual and PE weight loss of 31.1%. This performance did not improve at higher inoculum levels, indicating that the relationship between bacterial density and remediation performance in this system was not linear. Practically, these findings position $10^6$ CFU/mL as the most feasible initial candidate for further evaluation. However, because the degradation indicator was still based on gravimetry and was not supported by abiotic controls, SEM, FTIR, or molecular identification of the isolates, any conclusion regarding PE biodegradation must still be interpreted cautiously as a preliminary indication rather than a final confirmation.

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