

The Potential of Marine Yeast and Its Interaction with Mold in Degrading High-Density Polyethylene (HDPE)

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Abstract

Indonesia is one of the countries that contributes significant amounts of plastic waste to coastal environments. High-density polyethylene (HDPE) is a petroleum-based synthetic thermoplastic and the primary source of environmental plastic pollution. The stable aliphatic chains with strong C–C bonds in HDPE render it recalcitrant to microbial degradation. Bioremediation through biodegradation by microorganisms can be an effective and environmentally friendly solution for eliminating HDPE from ecosystems. Several studies have suggested that microbial consortia may enhance plastic biodegradation. However, the effectiveness of marine yeast-mold interactions in HDPE degradation remains poorly understood. This study aimed to evaluate the potential of marine yeast to degrade HDPE and assess its effectiveness when combined with molds for HDPE degradation. The biodegradation experiment was conducted over 30 days using Mineral Salt Medium Broth (MSMB) and plastic sheets. The parameters assessed during biodegradation included degradation effectiveness (%), total count of heterotrophic microorganisms (CFU/mL), and Scanning Electron Microscope (SEM) analysis. The results show that marine yeast has the potential to be used alone or in combination with molds. The highest value was achieved by the P2 treatment group in week 4, which consisted of a consortium of marine yeast and mold, with a value of 4.855%. However, this was not significantly different from P1, which consisted of a consortium of marine yeast with a value of 4.17%. The SEM test results indicated biofilm formation in all treatments, suggesting plastic biodegradation activity.

Keywords

Biodegradation, High-Density Polyethylene, Marine Yeast, Mold, Waste

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1. INTRODUCTION

Indonesia, the United States, and China contribute the most significant amounts of plastic waste to coastal environments (Law et al., 2020; Ministry of Environment and Forestry, 2020). Studies conducted from 2015 to 2019 revealed that the volume of marine waste in Indonesia is estimated to be between 0.27 and 1.29 million tons per year. This amount represents up to 10.1% of the global marine plastic waste (Wang et al., 2022). Jambeck et al. (2015) mentioned that Indonesia is the second-largest producer of marine plastic waste in the world, generating 3.22 million tons of unmanaged plastic waste annually, of which 0.48 to 1.29 million tons comes from illegal dumping, plastic waste production in coastal areas, and fishing and industrial activities (Li et al., 2016).

High-Density Polyethylene (HDPE) is a polyethylene thermoplastic derived from petroleum (Kumar et al., 2011).

This class of plastic is widely produced and commonly used in various products and packaging (Balasubramanian et al., 2010). HDPE is degraded more slowly than LDPE because HDPE has a less branched structure, resulting in a denser composition and slightly higher chemical resistance than Low-Density Polyethylene (LDPE) (Mbuge et al., 2011). With fewer tertiary carbons and a higher molar mass, HDPE challenges microorganisms to access the polymer chains (Sudhakar et al., 2008; Fontanella et al., 2010). The extensive use of HDPE poses a significant environmental threat to terrestrial and marine ecosystems because it degrades slowly and poses challenges for its volumetric disposal after use (Balasubramanian et al., 2010).

Bioremediation, involving the biodegradation of HDPE by microorganisms, can be an effective and environmentally friendly solution for eliminating plastic from ecosystems (Zeenat et al., 2021; Miri et al., 2022). Marine yeast has been reported to be a versatile biodegradation agent (Kutty

and Philip, 2008). Unlike terrestrial yeast, marine yeast possesses unique characteristics, including high osmotic tolerance, higher productivity of specific enzymes, and efficient and economical production facilitated by shorter generation times (Kutty and Philip, 2008; Zaky et al., 2014). In addition to yeast, molds have been studied for their ability to degrade plastic. Molds can survive stressful conditions and exhibit saprotrophic properties, producing various plastic-degrading enzymes (Khatua et al., 2024).

Previous research has isolated several yeasts with codes BI.4.1.1, K.5.0.2, K.3.1.2, and BI.1.0.2 from plastic marine debris in the coastal area of Surabaya and Banyuwangi, East Java, Indonesia (Alami et al., 2025a). Molecular identification showed that isolates BI.4.1.1 and K.3.1.2 were identified as *Rhodotorula mucilaginosa* (Alami et al., 2025b), whereas isolate K.5.0.2 was identified as *Candida parapsilosis* based on ITS sequence analysis (Alami et al., 2023). Isolate BI.1.0.2 was previously characterized using the Integral System YEASTS Plus biochemical identification kit, with the results indicating *Candida famata* (Sari, 2021). Additionally, Kurniawati and Shovitri (2018) reported that the mold of *Aspergillus terreus* (LM 1021) demonstrated the ability to degrade LDPE by 12.2% in 30 days of incubation. *A. terreus* secretes lipase and alkane hydroxylase to facilitate plastic degradation.

In contrast, several studies have reported that the dual application of microbes from bacterial or fungal groups can enhance the effectiveness of plastic degradation (Satlewal et al., 2008; Sowmya et al., 2015; Skariyachan et al., 2022). To the best of our knowledge, this is the first report on the use of a marine yeast–mold consortium for HDPE biodegradation. Marine yeast isolated from marine plastic debris may possess ecological adaptations that differ from those of terrestrial microorganisms, making them well-suited for co-culturing with molds. In this study, HDPE biodegradation by a marine yeast–mold consortium was evaluated and compared with that by marine yeast alone.

2. EXPERIMENTAL SECTION

2.1 Materials

High-density polyethylene (HDPE) sheets were used as substrates for biodegradation experiments. HDPE were prepared from commercially available disposable plastic gloves (Bagus®, Indonesia). According to the product label, the gloves were manufactured from 100% food-grade HDPE, had a nominal thickness of 10 µm, and were BPA-free. The gloves were obtained from a local supermarket in Surabaya, Indonesia, and cut into 1 × 1 cm pieces before use in the biodegradation assay. Detailed information on polymer characteristics, including molecular weight, density, and degree of crystallinity, was not specified by the manufacturer. Four marine yeast isolates (K.5.0.2, BI.1.0.2, BI.4.1.1, and K.3.1.2), previously selected based on their biodegradation potential, and *Aspergillus terreus* (LM 1021) were obtained from the Microbiology Laboratory, Department of Biology,

Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia. Seawater and distilled water were used to prepare the medium. The culture medium components included dextrose, peptone, malt extract, yeast extract, agar, K₂HPO₄, KH₂PO₄, MgSO₄·7H₂O, NaCl, FeSO₄·H₂O, NH₄NO₃, and CaCl₂·2H₂O. All chemicals used were of analytical grade and obtained from the Department of Biology, Institut Teknologi Sepuluh Nopember.

2.2 Methods

2.2.1 Biodegradation Test by a Single Isolate of Marine Yeast in Degrading HDPE

The marine yeast isolates BI.4.1.1, K.5.0.2, K.3.1.2, and BI.1.0.2 were subcultured on Yeast Malt Extract Agar (YMEA) medium, which comprised 10 g dextrose, 5 g peptone, 3 g malt extract, 3 g yeast extract, and 20 g agar in 1000 ml seawater. After incubation for 2 days (Kutty and Philip, 2008), the yeast culture was suspended in sterile physiological water (NaCl 0.85%) until it reached an optical density (OD) of 0.5 at λ 600 nm. Subsequently, approximately 10% of the yeast suspension was added to a fresh Yeast Malt Extract Broth (YMEB) medium. YMEB medium is YMEA medium but without agar. The yeast suspension was then incubated in an incubator shaker at a speed of 120 rpm for 3 days (Alami et al., 2017).

The biodegradation test was conducted in 90 ml of Mineral Salt Medium Broth (MSMB) media, composed of 0.85 g K₂HPO₄, 0.34 g KH₂PO₄, 0.05 g MgSO₄·7H₂O, 2 g NaCl, 0.015 g FeSO₄·H₂O, 0.5 g NH₄NO₃, and 0.01 g CaCl₂·2H₂O in 100 ml of seawater supplemented with 10 ml of yeast culture. A mineral salt medium was employed to create carbon-limited conditions for the biodegradation assay. The medium supplied only essential inorganic nutrients and did not contain any easily metabolized organic carbon. Consequently, microbial growth depended on the utilization of HDPE as the sole carbon substrate during the incubation period. HDPE was previously cut into 1 cm × 1 cm squares and sterilized using 70% ethanol and UV radiation for 45 minutes (Li et al., 2012) to eliminate surface-associated microorganisms. Three pieces of sterile HDPE were aseptically placed into a bottle containing the medium and incubated for 4 weeks at room temperature under continuous rotary shaking at 120 rpm.

The Degradation Effectiveness (DE) was measured at weeks 0, 2, and 4. The percentage of DE was calculated based on the dry weight loss of HDPE before and after incubation period. The HDPE was removed from the degradation medium, rinsed with distilled water (Gilan et al., 2004), and dried overnight in an oven at 60°C before being weighed. The percentage of plastic weight reduction or degradation effectiveness was determined using the following formula (Kyaw et al., 2012):

$$\text{Degradation Effectiveness}(\%) = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100\%$$

Meanwhile, after weeks 0, 2, and 4, microbial growth was counted following the standard Total Plate Count (TPC) method.

2.2.2 Compatibility Assay

The experiment involved interactions among marine yeast isolates (K.5.0.2, BI.1.0.2, BI.4.1.1, and K3.1.2) and between marine yeast and mold within a Petri dish containing Potato Dextrose Agar (PDA) media following established protocols (Alexopoulos et al., 1996; Dalal and Kulkarni, 2014; Sangdee et al., 2016). The interaction between marine yeast and mold was achieved by streaking one marine yeast onto a Petri dish and simultaneously transferring cut hyphae of *A. terreus* nearby. The Petri dish was then incubated at room temperature for 24 h. The absence of an inhibition zone suggests that the selected microorganisms were compatible for co-cultivation, as per the criteria outlined by Asri and Zulaika (2016).

2.2.3 Consortium Preparation

Approximately 10% of the yeast suspension with an optical density (OD) of 1 at λ 600 nm was inoculated into a 90 mL YMEB medium and incubated in an incubator shaker at 120 rpm for three days (Alami et al., 2017). *A. terreus* was cultured in a fresh PDA and incubated for five days. Subsequently, the culture was homogenized with approximately 5 mL sterile distilled water and transferred into a culture bottle as a concentrated mold suspension. One milliliter of the mold suspension was transferred into 9 mL of sterile distilled water, followed by the next transfer for a dilution series until a mold suspension of 10^6 conidia/mL was reached (Syamsi et al., 2019).

2.2.4 Biodegradation Test by Consortium

The treatments were designed into four types: control (K), treatment 1 with the addition of a consortium of four marine yeast isolates (P1), Treatment 2 (P2) with the addition of a consortium of four marine yeast isolates and *Aspergillus terreus* (LM 1021), and Treatment 3 (P3) with the addition of *Aspergillus terreus* (LM 1021) alone. The details of each treatment are performed in Table 1. HDPE was cut into 2 cm $\times$ 2 cm pieces and sterilized using 70% ethanol and UV radiation for 45 minutes (Li et al., 2012). Three pieces of each plastic type were placed inside a bottle containing 90 mL of Mineral Salt Medium Broth (MSMB) and 10 mL of microbial culture. The culture was then incubated at room temperature under continuous rotary shaking (120 rpm). The Degradation Effectiveness (DE) and microbial growth were measured after weeks 0, 1, 2, 3, and 4.

2.2.5 SEM Analysis

Morphological changes resulting from the plastic degradation test were observed using a Scanning Electron Microscope (SEM) (Carl Zeiss (Evo Ma 10), England) with a magnification of up to 20,000 $\times$. SEM analysis was conducted only at

the end of the incubation period for each treatment group, without repetition.

2.2.6 Data Analyses

Data on biodegradation by single yeast isolates were analyzed descriptively. Data from the consortium experiment were analyzed using IBM SPSS Statistics version 25. The normality of the data was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Because the degradation effectiveness data did not meet the assumption of normality ($p < 0.05$), differences among treatments were analyzed using the Kruskal–Wallis test. Microbial growth (TPC) data were analyzed using the Brown–Forsythe test followed by the Games–Howell post hoc test. Statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

The results of the biodegradation test revealed that the four marine yeast isolates with codes K.5.0.2, BI.1.0.2, BI.4.1.1, and K3.1.2 exhibited potential for degrading HDPE plastic. This is supported by the degradation effectiveness (%) and yeast cell concentration (log CFU/mL), as shown in figure 1. No measurable weight loss was detected at Week 0 following the sterilization procedure, suggesting that ethanol treatment and UV exposure under the conditions applied did not produce detectable degradation prior to microbial incubation. A four-week incubation period was selected to evaluate the initial biodegradation potential of the tested microorganisms. During this period, the microorganisms were expected to attach to the HDPE surface, form biofilms, which represents the early stage of polyethylene biodegradation (Restrepo-Flórez et al., 2014).

In Figure 1, the highest degradation percentage was achieved by isolate K5.0.2 in week 4, reaching 13.995%. This was followed by isolates BI.1.0.2, BI.4.1.1, and K3.1.2, with respective values of 12.657%, 10.025%, and 9.809%, all observed in week 4. A simple and rapid method for assessing polymer or plastic biodegradation involves measuring the dry weight loss. This loss is attributed to the formation of biofilms on the surface and within cracks of the plastic, leading to a loss of physical integrity and, consequently, a reduction in dry weight. The decrease in dry weight correlates with the surface area, as biodegradation typically begins at the polymer surface, a process referred to as biodeterioration (Das and Kumar, 2015; Cheng et al., 2021).

The four potential isolates were then tested using a microbial compatibility assay to determine whether marine yeast and molds could be co-cultivated without visible antagonistic interactions, ensuring their suitability for conducting HDPE degradation tests using the microbial consortium. Based on the results of the compatibility assay, the isolates did not form inhibition zones when crossed (Figure 2), indicating that they were compatible for co-cultivation under the experimental conditions. Although compatibility suggests that the isolates can coexist without apparent antagonism,

Table 1. Composition of Each Treatment Group

Treatment	Culture Composition
Control (K)	10 mL YMEB + 90 mL Mineral Salt Medium & Broth (MSMB) + Three HDPE Films (2 x 2 cm Each)
Treatment 1 (P1)	2.5 mL Each of Marine Yeast Isolates K5.0.2, BI1.0.2, BI4.1.1, and K3.1.2 + 90 mL MSMB + Three HDPE Films (2 × 2 cm Each)
Treatment 2 (P2)	2 mL Each of Marine Yeast Isolates K5.0.2, BI.1.0.2, BI.4.1.1, and K3.1.2, plus 2 mL of <i>Aspergillus terreus</i> LM 1021 + 90 mL MSMB + Three HDPE Films (2 × 2 cm Each)
Treatment 3 (P3)	10 mL of <i>Aspergillus terreus</i> LM 1021 + 90 mL MSMB + Three HDPE Films (2 × 2 cm Each)

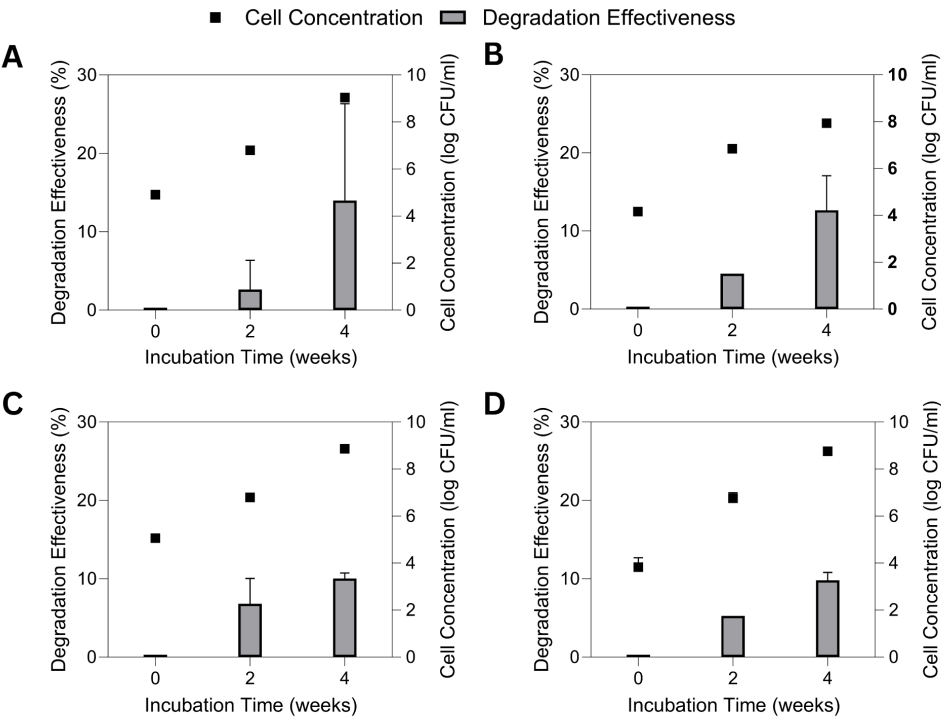


Figure 1. Biodegradation of High-Density Polyethylene (HDPE) by Four Marine Yeast Isolates. (A) K5.0.2, (B) BI.1.0.2, (C) BI.4.1.1, and (D) K3.1.2. Bars Represent Degradation Effectiveness (%), and Black Squares Represent Cell Concentration (log CFU/mL) after 0, 2, and 4 Weeks of Incubation. Error Bars Indicate the Standard Deviation (n = 2)

it does not necessarily demonstrate synergistic interactions during HDPE biodegradation. The exact mechanism of synergy within the consortium remains uncertain; however, it is assumed that when one member of the genus is unable to degrade specific organic materials, it relies on other members to provide the degradation product (Asri and Zulaika, 2016).

Figure 3 illustrates the results of the HDPE biodegradation test conducted on the control (K), Treatment 1 (P1) with the addition of four marine yeast isolates, Treatment 2 (P2) with the addition of four marine yeast isolates and *Aspergillus terreus* (LM 1021), and Treatment 3 (P3) with the addition of *Aspergillus terreus* (LM 1021) alone. At weeks 0 and week 1, none of the treatments showed any degradation

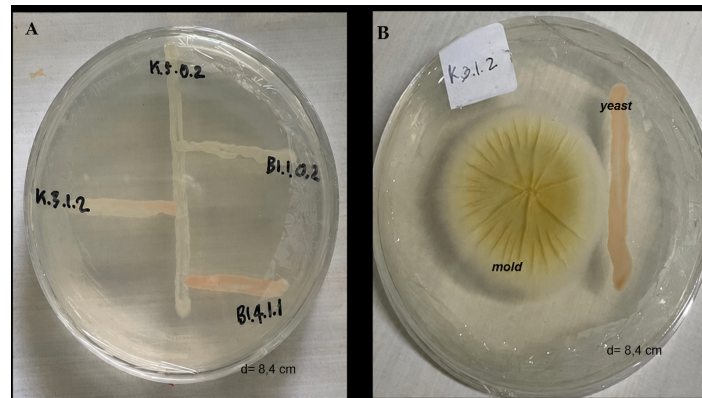


Figure 2. Compatibility Assay. (A) Compatibility among Marine Yeast Isolates. (B) Compatibility between Marine Yeast Isolate K3.1.2 (Right) and *Aspergillus terreus* LM 1021 (Left)

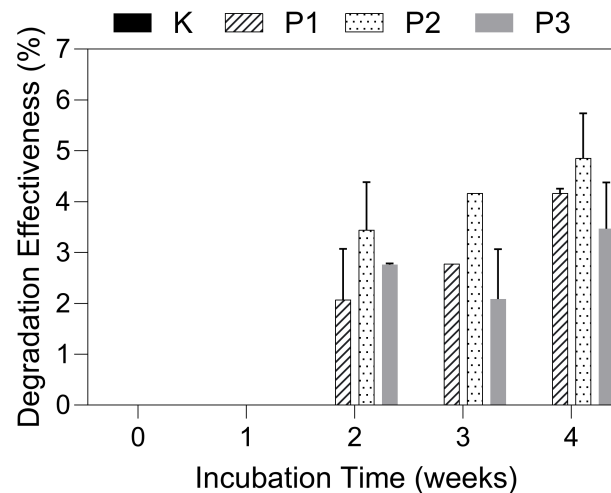


Figure 3. Degradation Effectiveness (%) of High-Density Polyethylene (HDPE) Under Different Microbial Treatments After 0, 1, 2, 3, and 4 Weeks of Incubation. K, Control (Without Microbial Addition); P1, Marine Yeast Consortium (K5.0.2, BI.1.0.2, BI.4.1.1, and K3.1.2); P2, Marine Yeast Consortium Combined With *Aspergillus terreus* LM 1021; and P3, *Aspergillus terreus* LM 1021 Alone. Error Bars Indicate the Standard Deviation (n = 2)

activity. However, from weeks 2 to 4, all treatments demonstrated HDPE plastic degradation activity. Treatment P2 consistently displayed increasing degradation effectiveness, achieving the highest degradation percentage compared to P1 and P3. Specifically, P2 degraded plastic by an average of 3.445% in the 2nd week, 4.17% in the 3rd week, and 4.855% in the 4th week. In comparison, P1, consisting of the marine yeast consortium K5.0.2, BI.1.0.2, BI.4.1.1, and K3.1.2, degraded HDPE plastic by an average of 2.075% in the 2nd week, 2.78% in the 3rd week, and 4.17% in the 4th week.

The marine yeast–mold consortium achieved 4.855% HDPE weight loss after four weeks of incubation. Under a comparable incubation period, *Cladosporium sp.* reduced LDPE weight by only $0.30 \pm 0.06\%$ after 30 days (Gong et al.,

2023), whereas a mixed fungal consortium of *Alternaria sp.* and *Trametes sp.* achieved $0.66 \pm 0.06\%$ on heat-treated LDPE (Yang et al., 2024). Differences in PE characteristics, cellular surface hydrophobicity, physical treatments, chemical additions, and environmental conditions for biodegradation can influence the differences in degradation (Kong et al., 2025). In the single-culture experiment, no comparison was made with the control because the purpose was to compare the degradation performance of the individual isolates. A control was included in the consortium experiment. Including an abiotic control in future studies would provide additional support for interpreting the weight-loss results.

Although the degradation efficiency obtained in this study was relatively low (4.855%), this result should be interpreted in the context of the highly recalcitrant nature of

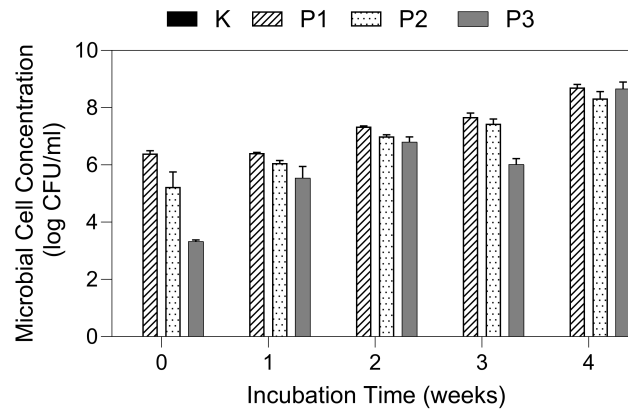


Figure 4. Microbial Cell Concentration (log CFU/mL) Under Different Microbial Treatments After 0, 1, 2, 3, and 4 Weeks of Incubation. K, Control (Without Microbial Addition); P1, Marine Yeast Consortium (K5.0.2, BI.1.0.2, BI.4.1.1, and K3.1.2); P2, Marine Yeast Consortium Combined With *Aspergillus terreus* LM 1021; and P3, *Aspergillus terreus* LM 1021 Alone. Error Bars Indicate the Standard Deviation (n = 2)

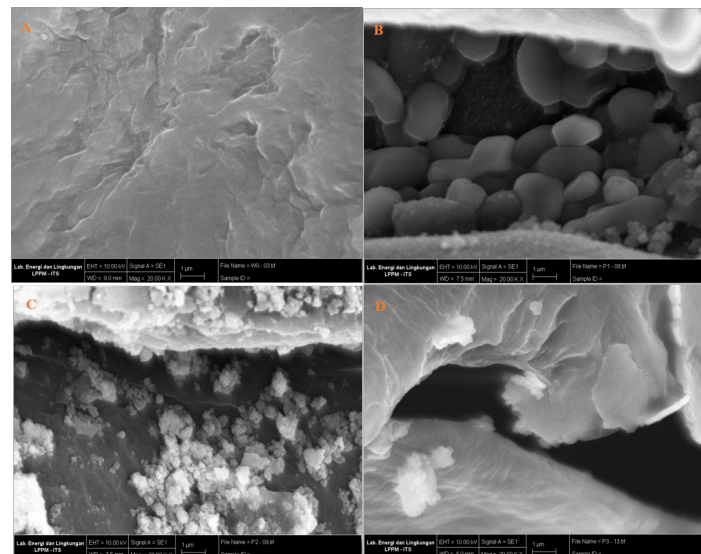


Figure 5. SEM Micrographs of the HDPE Surface after 4 Weeks of Incubation under Various Treatment Conditions. (A) K, Control (without Microbial Addition); (B) P1, Marine Yeast Consortium (K5.0.2, BI.1.0.2, BI.4.1.1, and K3.1.2); (C) P2, Marine Yeast Consortium Combined with *Aspergillus terreus* LM 1021; (D) P3, *Aspergillus terreus* LM 1021 Alone

the HDPE. The high crystallinity and chemically stable carbon-carbon backbone of HDPE make microbial degradation slow. Consequently, complete degradation is rarely achieved within a short incubation period. A recent review also highlighted that the degradation kinetics of polyethylene remain low under currently available biological processes and that complete mineralization has not yet been demonstrated (Tiago et al., 2023). Therefore, the degradation observed in this study indicates that the marine yeast-mold system can initiate HDPE biodegradation under laboratory conditions. Although the current degradation efficiency is insufficient for direct environmental application, these findings provide

a basis for further optimization of microbial interactions, culture conditions, and polymer pretreatment to improve degradation efficiency and evaluate the potential of this system as part of an integrated plastic waste management strategy.

The superior degradation performance of the P2 consortium can be attributed to the presence of LM 1021, a strain of *Aspergillus terreus*. LM 1021 produces lipase and alkane hydroxylase, which are crucial for plastic degradation (Kurniawati and Shovitri, 2018). LM 1021 can penetrate and distribute hyphae throughout the polymer, making the plastic more brittle and hydrophilic, facilitating increased en-

zyme diffusion, and transformation polymer into monomers (Sáenz et al., 2019). In addition, marine yeast contributes to plastic degradation by secreting extracellular enzymes and absorbing nutrients (Chi et al., 2009; Russell et al., 2011). These enzymes play a crucial role in depolymerizing polymer chains into monomers, functionalizing hydrophobic plastics, and mineralizing monomer intermediates (Amobonye et al., 2021). Because enzyme activity was not measured in this study, the contribution of extracellular enzymes to HDPE degradation by the tested isolates remains unclear.

Treatment P3, consisting of a single isolate of LM 1021, degraded HDPE plastic by an average of 2.76% in the 2nd week, 2.085% in the 3rd week, and 3.465% in the 4th week. Notably, a decrease in the degradation process was observed in the 3rd week, possibly due to a reduction in the number of heterotrophic microbes, as indicated by the Total Plate Count (TPC) test (Figure 4). This instability is likely attributed to P3, which comprised only a single isolate. Comparing the results in Figure 3 with those in Figure 1, it is evident that HDPE degradation using the microbial consortium (Figure 3) showed a lower HDPE degradation effectiveness after four weeks of incubation. Single isolates achieved degradation percentages ranging from 9.809% to 13.995% by week 4. This difference is presumed to occur because of competition among consortium members when placed in a medium with limited nutrients.

A statistical test, specifically the Kruskal-Wallis test, was conducted to determine the effect of treatment on the percentage degradation effectiveness value. The Kruskal-Wallis test showed a significant effect of treatment on degradation effectiveness ($\chi^2 = 28.169$, $df = 14$, $p = 0.014$), indicating that degradation effectiveness differed significantly among treatments. Further analysis revealed that the degradation data between treatment groups P1, P2, and P3 at incubation times of 2, 3, and 4 weeks did not differ significantly. However, significant differences were observed when comparing the incubation times at weeks 0 and 1 with those at weeks 3 and 4. Specifically, significant differences were observed in the P2 treatment group at incubation time 0 versus P2 at incubation times 3 and 4, as well as P2 at week 1 with incubation times 3 and 4 weeks, with significance values of 0.018 and 0.007, respectively. Similar significant differences were observed for the P1 treatment group between week 0 and 4, and week 1 and week 4, with significance values of 0.020 each.

This study was designed primarily to compare the biodegradation performances of marine yeast and marine yeast-mold consortia under identical experimental conditions. Accordingly, degradation effectiveness based on weight loss was used as a comparative indicator among treatments and was interpreted together with microbial growth (total plate count (TPC)) and SEM observations. Carbon mineralization was not evaluated in the present study. Therefore, the extent of complete HDPE biodegradation could not be assessed.

The biodegradation of plastic polymers can be assessed

not only by determining the percentage of degradation effectiveness but also by monitoring the Total Plate Count (TPC). This test evaluates biodegradation activity by observing the attachment and growth of isolates on the surface of plastic polymers during a 4-week incubation period. The data on the number of heterotrophic microbes are shown in Figure 4. Based on the average Total Plate Count test results in Figure 4, the P1, P2, and P3 treatment groups showed an increasing growth trend, indicating that both yeast and mold could grow using HDPE plastic as a carbon source. However, in weeks 0 and 1 of the Total Plate Count test, all microorganisms in the test treatment experienced growth, but the degradation percentage remained at 0%. This suggests that the microorganisms had not yet utilized HDPE as a carbon source. This is likely because in week 0 and week 1, the microorganisms were still using residual nutrients from the enrichment medium (YMEB) (Nadim et al., 2016).

A statistical test, specifically the Brown-Forsythe test indicated a significant effect of treatment type and incubation time on the Total Plate Count (TPC) ($F = 87.771$, $df = 14$, 4.392 , $p < 0.001$), indicating that microbial growth differed among treatments during the incubation period. Subsequently, a post hoc Games-Howell test was performed. The results showed no significant differences between treatment groups P1, P2, and P3 at the same incubation time. However, each treatment group (P1, P2, P3) exhibited increased microbial growth over time. Further analysis revealed a significant increase in growth in the treatment group P1 at week 0, which was significantly different from P1 at week 4, as well as P3 with an incubation time of 0 weeks compared to P3 with incubation times of 2 and 4 weeks. The P2 treatment group showed an insignificant increase in growth. The consortium did not show significantly higher degradation than that of marine yeast alone. However, the present study did not investigate the interactions between these consortium members. Therefore, the reason for this difference remains unclear.

Previous work on marine yeasts isolated from plastic debris showed that two isolates, BI.4.1.1 and K3.1.2, were able to form biofilms on polyethylene surfaces (Alami et al., 2025a). Biofilm formation is recognized as an important early stage of plastic biodegradation, as it enables microorganisms to attach to the polymer surface and facilitates the secretion of extracellular enzymes involved in polymer degradation. The presence of a biofilm may also promote the attachment of other microorganisms, including filamentous fungi such as *Aspergillus terreus*, which could contribute to polymer degradation in mixed microbial communities. Similar observations have been reported for fungal-mediated plastic biodegradation (Khatua et al., 2024). However, the interactions between the marine yeast consortium and *A. terreus* were not evaluated in the present study. Therefore, the contribution of biofilm formation to their interaction remains uncertain. Despite this limitation, the marine yeast

consortium has the potential to be used alone or in combination with other fungi. Previous studies have suggested that interactions among consortium members may influence biodegradation efficiency under nutrient-limited conditions (Doerffer, 1992).

SEM (Scanning Electron Microscope) was used to observe microbial attachment and biofilm formation on the HDPE surface (Figure 5A-5D). After 4 weeks of incubation, microbial attachment was observed on the plastic surfaces of the P1, P2, and P3 treatments. On the surface of P1 plastic, composed of a consortium of marine yeast, numerous colonies were visible, consistent with the quantified growth of microorganisms, as indicated by the TPC value (Figure 5B). In contrast, on the surface of P2 plastic, consisting of a consortium of marine yeast and mold LM 1021, dense biofilms were observed, spreading extensively on the film surface (Figure 5C). No visible hyphae from the LM 1021 mold were observed on P2. This finding may indicate differences in microbial colonization or biofilm architecture; however, the relative abundance of yeast and mold was not quantified in the present study. This observation aligns with previous findings by Kurniawati and Shovitri (2018), who noted that LM 1021 mold isolate on a plastic surface led to biofilm formation without visible hyphae.

Biofilm formation marks the initial step in the biodegradation process (Shovitri et al., 2023), increasing the utilization of plastic polymers as a carbon supply (Fesseha and Abebe, 2019). On the surface of P3 plastic, consisting of a single isolate LM 1021, a smaller amount of microbial biofilm was observed. Overall, the SEM test results demonstrate that the consortium of microorganisms (P1 and P2) formed a stronger microbial bond on the HDPE surface than a single isolate (P3). The symbiotic growth of the microbial consortium increases biofilm formation and reduced the hydrophobicity of the plastic polymer (Mahalakshmi and Siddiq, 2015). Microbial colonization through biofilm formation aims to decrease the resistance and durability of plastic materials (Amobonye et al., 2021), facilitating HDPE contact with enzymes produced by microbes (Ren et al., 2019). The smooth surface morphology of the control plastic K (Figure 5A) indicates the absence of a microbial biofilm.

One limitation of the present study is that environmental variables, including the exact incubation temperature, pH, dissolved oxygen, and salinity, were not monitored during the biodegradation experiment. Because these factors can affect microbial growth and degradation efficiency, future studies should include their measurement to provide a more comprehensive evaluation of biodegradation.

This study provides preliminary evidence that marine yeast-mold consortia could be developed for biological treatment of plastic waste. Because the isolates were obtained from marine environments, they may be suitable for saline conditions. An ex-situ bioreactor system may offer a practical option for treating collected marine plastic waste while

maintaining conditions that favor microbial activity (Alami et al., 2025a). Further work at the pilot scale is needed to evaluate the stability and degradation performance of the consortium before larger-scale application.

4. CONCLUSIONS

Marine yeast has the potential to be used alone or in combination with molds. The highest value was achieved by the P2 treatment group in week 4, which consisted of a consortium of marine yeast and mold, with a value of 4.855%. However, this was not significantly different from P1, which consisted of a consortium of marine yeast, with a value of 4.17%. SEM test results indicated biofilm formation in all treatments, suggesting plastic biodegradation activity.

5. ACKNOWLEDGEMENT

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