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Biodegradation of Low-Density Polyethylene (LDPE) using Yeast Isolates from Plastic Waste Biofilms

(Biodegradasi Polietilena Ketumpatan Rendah (LDPE) menggunakan Pengasingan Yis daripada Biofilem Sisa Plastik)

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ABSTRACT

Low-density polyethylene (LDPE) is an abundant and widely commercialized petroleum-based synthetic thermoplastic. It has a high molecular weight and a very hydrophobic surface. The strong C–C bond also makes LDPE resistant to biological attacks. Biodegradation presents a promising eco-friendly solution for tackling plastic waste. This study aimed to investigate the potential of yeast from plastic waste to degrade LDPE. Yeast was isolated from various plastic-polluted areas in Surabaya and Banyuwangi, Indonesia. The screening test was performed on mineral salt medium agar (MSMA) supplemented with polyethylene powder. The biodegradation test was conducted for 4 weeks in Mineral Salt Medium Broth (MSMB) with LDPE film. The ability of the isolates to degrade LDPE was evaluated by measuring the reduction in dry weight of plastic (% degradation), yeast growth via Optical Density (OD600 nm), Scanning Electron Microscopy (SEM), and Fourier Transform Infrared Spectroscopy (FTIR) analysis. Screening on MSMA showed that 13 isolates could degrade polyethylene, as indicated by the formation of clear zones. The five best isolates were used for further biodegradation tests. The yeast isolate M.3.0.1 exhibited the highest degradation percentage of 1.1474±0.0888%. It demonstrated increased growth in the test medium, as indicated by an increase in optical density. In addition, SEM analysis showed a change in the morphology of the LDPE surface, and FTIR analysis showed a change in the transmittance value for the test plastic.

Keywords: Biodegradation; low-density polyethylene; plastic; waste; yeast

ABSTRAK

Polietilena berketumpatan rendah (LDPE) ialah termoplastik sintetik berasaskan petroleum yang banyak dan dikomersialkan secara meluas. Ia mempunyai berat molekul yang tinggi dan permukaan yang sangat hidrofobik. Ikatan C–C yang kuat juga menjadikan LDPE rintang terhadap serangan biologi. Biodegradasi memberikan penyelesaian mesra alam yang berpotensi untuk menangani sisa plastik. Penyelidikan ini bertujuan untuk mengkaji potensi yis daripada sisa plastik untuk merendahkan LDPE. Yis diasingkan dari pelbagai kawasan tercemar plastik di Surabaya dan Banyuwangi, Indonesia. Ujian saringan dilakukan pada agar medium garam mineral (MSMA) yang ditambah dengan serbuk polietilena. Ujian biodegradasi telah dijalankan selama 4 minggu dalam Mineral Salt Medium Broth (MSMB) dengan filem LDPE. Keupayaan pencilan untuk merendahkan LDPE dinilai dengan mengukur pengurangan berat kering plastik (% degradasi), pertumbuhan yis melalui Ketumpatan Optik (OD600 nm), Mikroskopi Elektron Pengimbasan (SEM) dan analisis Spektroskopi transformasi Fourier infra merah (FTIR). Saringan pada MSMA menunjukkan bahawa 13 pencilan boleh merendahkan polietilena, seperti yang ditunjukkan oleh pembentukan zon jernih. Lima pencilan terbaik telah digunakan untuk ujian biodegradasi selanjutnya. Pengasingan yis M.3.0.1 menunjukkan peratusan degradasi tertinggi iaitu 1.1474±0.0888%. Ia menunjukkan peningkatan pertumbuhan dalam medium ujian, seperti yang ditunjukkan oleh peningkatan ketumpatan optik. Di samping itu, analisis SEM mendedahkan perubahan dalam morfologi permukaan LDPE dan analisis FTIR menunjukkan perubahan dalam nilai penghantaran untuk plastik ujian.

Kata kunci: Biodegradasi; plastik; polietilena berketumpatan rendah; sisa; yis

INTRODUCTION

Global plastic production reached 370 million tons in 2019, with estimates suggesting it will rise to 1.1 billion tons by 2050 (Geyer 2020). Approximately 60% of plastic production will enter the environment as waste (Zhang et al. 2021). Indonesia is recorded to produce around 7.8 million tons of plastic waste every year, with 4.9 million tons of plastic waste not being appropriately managed (World Bank 2021). Based on the data from Meijer et al. (2021), Indonesia is among the five highest-ranking countries contributing to plastic waste worldwide.

Plastic is a synthetic polymer typically produced through a polymerization process, which makes it difficult to break down (Kumari et al. 2023). These products are essential for human needs and are extensively used daily. The popularity of plastic stems from several advantages, including lightweight, high durability, flexibility, and low production cost (Nayanathara Thathsarani Pilapitiya & Ratnayake 2024). Polyethylene (PE) plastic is a widely used type of plastic. PE is a mixture of ethylene polymers with the chemical formula $(C_2H_4)n$ (Basmage & Hashmi 2020). LDPE, a type of polyethylene, is known for its flexibility, strength, tear resistance, slight transparency, chemical resistance below 60 ºC, and water resistance (Lubis, Muis & Siregar 2020). These properties make LDPE widely used in everyday applications in packaging materials such as plastic bags, shrink wraps, and containers. Additionally, LDPE is used in various consumer products like toys, squeeze bottles, and medical devices (Burnd & Yrick 2021; Tuteja, Vyas & Sand 2024). However, the excessive use of these products and inadequate waste management lead to the accumulation of plastic waste in the environment, causing environmental pollution and disrupting ecosystems (Kumar et al. 2021).

Biodegradation is a method for handling environmental pollution using microbes (Bahl et al. 2020). Many studies have focused on the formation of biofilms on plastic surfaces, giving rise to the hypothesis that within the microbial community of a plastisphere, there will be microbes capable of degrading plastic (Zadjelovic et al. 2022). Extensive research has focused on microorganisms like bacteria and molds for degradation; however, there is a notable gap in the exploration of yeast for plastic degradation. This study investigated the potential of yeast isolates from plastic waste in plastic-polluted areas to degrade LDPE.

MATERIALS AND METHODS

PLASTIC MATERIAL

The plastic used for the screening test is Polyethylene powder (medium density, CAS Number 9002-88-4) produced by Sigma-Aldrich Chemical Co. (USA). For the biodegradation test, a PAS-in multipurpose plastic clip bag (thickness 40 µm), with the LDPE logo on the packaging was obtained from Surabaya, Indonesia. It was cut into 1 × 1 cm squares for the biodegradation assay and sterilized using 70% alcohol and UV light for 30 min (Hussein et al. 2015).

ISOLATION OF YEAST FROM PLASTIC WASTE

Yeast was isolated from plastic waste biofilms in various plastic-polluted areas in Surabaya and Banyuwangi, Indonesia (Figure 1). During the sampling stage, plastic pieces were collected using tweezers, cut to a size of 1 cm × 1 cm using scissors, and then placed into sterile 50 mL falcon tubes. Each Falcon tube containing one piece of plastic debris was supplemented with 2 mL of sterile distilled water. The mixture was then homogenized using a vortex for 10 s to separate the yeast from the plastic sample. Next, 0.1 mL of suspension from each Falcon tube was transferred to a Petri dish containing Yeast Malt Extract Agar (YMEA) medium, consisting of 20 g/L agar, 10 g/L dextrose, 3 g/L malt extract, 5 g/L pepton, 3 g/L yeast extract, and supplemented with 200 mg/L chloramphenicol.

SCREENING ASSAY

The screening test was conducted on Mineral Salt Medium Agar (MSMA) media supplemented with powdered polyethylene. The media composition included 3 g/L NH_4NO_3, 5 g/L K_2HPO_4, 1 g/L NaCl, 0.2 g/L $MgSO4.7H_2O$, 0.25 ml/L Tween-20, 15 g/L agar. For the test, 10 g of powdered polyethylene, previously sterilized by soaking in alcohol for 1-2 h and exposed to UV light for 30 min, was added to the MSMA media. Subsequently, 19 yeast isolates were inoculated onto the media and incubated for approximately 7-14 days (Brunner et al. 2018). Positive screening results for polyethylene-degrading yeast isolates were indicated by the formation of a clear zone around the yeast colony after staining with 0.1% Coomassie Blue (CB). The CB solution was applied to the screening media, spread evenly, and left for 20 min. This was followed by adding a destaining solution, which was left for 25 min, after which the solution in the media was discarded to observe the clear zone in the screening media (Rana & Rana 2020).

BIODEGRADATION ASSAY

The biodegradation test consisted of five treatment groups, each supplemented with a different yeast isolate. The five yeast isolates used in the biodegradation test were those that exhibited the largest clear zone diameter. In addition, a control group was included, which contained no yeast supplementation. Each treatment group, including the control, consisted of 20 LDPE sheets measuring 1 × 1 cm squares, placed in 250 mL Erlenmeyer flasks, to which 90 mL of Mineral Salt Medium Broth (MSMB) media had previously been added. The MSMB composition, based on previous research (Gilan, Hadar & Sivan 2004; Sekar et al. 2011) with modifications, consisted of 1.0 g/L NH_4NO_3, 0.1 g/L $MgSO4.7H_2O$, 1.73 g/L K_2HPO_4, 0.68 g/L KH_2PO_4,

0.02 g/L $CaCl_2.2H_2O$, 0.1 g/L yeast extract; 0.03 g/L $FeSO_4.7H_2O$, and 4 g/L NaCl. Then, each treatment group was supplemented with 10 mL of yeast isolate suspension, while the control group was replaced with sterile distilled water. Several loops of yeast isolates were transferred into sterile distilled water to prepare the yeast suspension until the Optical Density (OD) value reached 0.5 at λ600 nm by measurement using a spectrophotometer. The experiment was conducted with two replications per treatment and control group. The LDPE biodegradation process was carried out for four weeks. Weekly, two Erlenmeyer flasks from each group were randomly selected for biodegradation analysis and pH measurement.

ASSESSMENT OF BIODEGRADATION

MEASUREMENT OF OD VALUES OF PLASTIC BIOFILM

The OD value of the plastic biofilm was measured by placing the plastic into MSMB using sterile tweezers. The plastic was then immersed in 9 mL of sterile 0.90% physiological saline and vortexed for 10 min. The resulting suspension was measured for its OD value using a spectrophotometer at λ600 nm.

PERCENTAGE OF DEGRADATION

The biodegradation value was determined by measuring the reduction in the dry weight of the plastic. Twenty pieces of LDPE plastic from the biodegradation media were soaked with 2% SDS for 4 h, then rinsed with distilled water and dried using an oven at 60 ºC (Gilan, Hadar & Sivan 2004). The plastic weight loss percentage formula refers to previous research (Kuswytasari et al. 2023).

$$\text{Degradation Percentage} = \left[\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}}\right] \times 100\%$$

SCANNING ELECTRON MICROSCOPE (SEM) ANALYSIS

Morphological changes resulting from the polymer biodegradation test were observed using a SEM (Thermo Fisher Scientific, Netherlands). LDPE plastic from the biodegradation media were soaked with 2% SDS for 4 h, then, rinsed with distilled water to remove the attached cells and dried using an oven at 60 ºC. The films were sputter-coated with gold using Luxor Au/Pt SEM Coater (IB-FT GmbH, Germany), followed by visualization under SEM at a magnification of up to 20,000. SEM analysis was conducted at the beginning and end of the incubation period, focusing on the LDPE plastic sample with the highest dry weight reduction percentage.

FOURIER TRANSFORMED INFRARED SPECTROSCOPY (FTIR) ANALYSIS

FTIR analysis was employed to observe changes in the characteristic pattern of absorption bands, indicating alterations in the material composition of LDPE plastic. This research utilized the Thermo Scientific Nicolet iS10 FTIR spectrometer equipped with the Attenuated Total Reflection (ATR) technique. LDPE from the biodegradation media

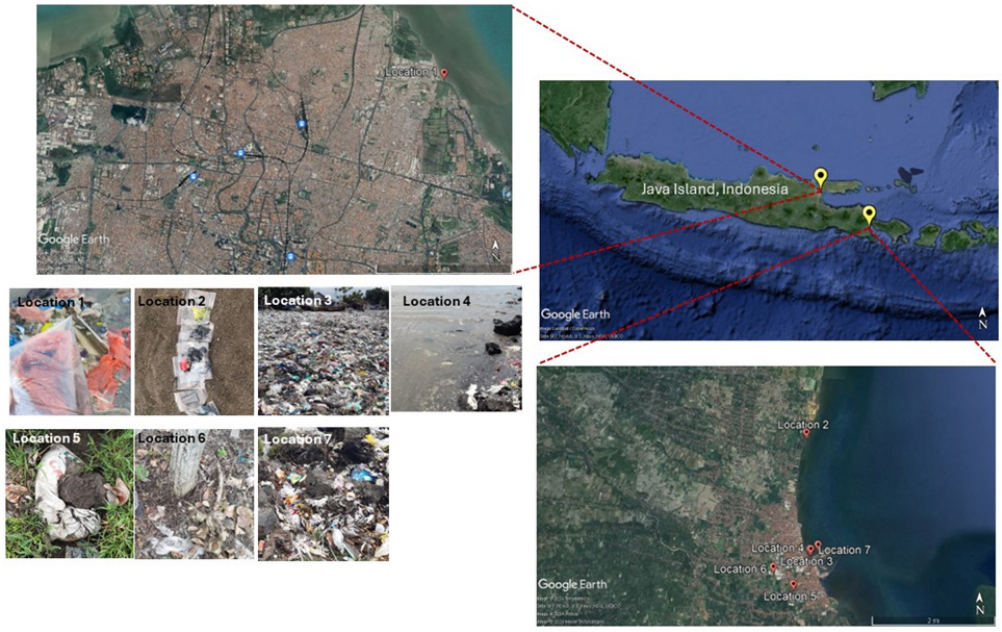

FIGURE 1. Sampling location in various plastic-polluted areas in Surabaya and Banyuwangi, Indonesia (Source: Google Earth Pro, 2024)

were soaked with 2% SDS for 4 h, then rinsed with distilled water to remove the attached cells and dried using an oven at 60 ºC. FTIR analysis was conducted at the beginning and end of the incubation period, specifically focusing on the LDPE plastic sample from the biodegradation test with the highest dry weight reduction percentage. Absorbance was measured in the IR-medium region at wave numbers 400-4000 cm^{-1} (Kuswytasari et al. 2023).

RESULTS AND DISCUSSION

SCREENING OF POLYETHYLENE-DEGRADING YEAST

The isolation of yeast from plastic waste biofilm resulted in 19 yeast isolates. Screening tests for polyethylene-degrading yeasts were performed on all isolates. Yeast isolates capable of utilizing polyethylene formed a clear zone around the yeast colonies (Figure 2) after staining with 0.1% CB on the 14th day of observation. The results showed that 12 yeast isolates could form clear zones and had the potential to degrade polyethylene. The diameter of the clear zones formed by each yeast isolate is shown in Table 1.

As shown in Table 1, yeast isolates capable of degrading polyethylene can form clear zones with varying diameters. The formation of a clear zone indicates that the isolate can degrade polyethylene. This activity results from enzymatic degradation by the yeast (Brunner et al. 2018). Additionally, the clear zone around the yeast can also form due to its ability to utilize polyethylene as the primary carbon source (Rachmawati et al. 2021). A larger diameter of the clear zone suggests increased enzyme secretion by the yeast, potentially enhancing its ability to degrade polyethylene (Asmi, Baharuddin & Febryanti 2022).

BIODEGRADATION OF LDPE

The percentage reduction in the dry weight of plastic is a parameter used to assess the plastic biodegradation process. Figure 3 displays the percentage reduction in the dry weight of the LDPE plastic over the 4-week incubation period. Based on Figure 3, in the first week of incubation, there was no decrease in dry weight for any of the isolates, resulting in a 0% reduction. It also shows that the percentage reduction in dry weight continues to increase until the fourth week of incubation. In this study, isolate M.3.0.1 exhibited the highest percentage reduction in the dry weight of LDPE in the fourth week of incubation, reaching 1.1474±0.0888%.

The degradation value reaching a percentage of 1.1474 ± 0.0888% within 4 weeks is considered quite good, considering that this study still used a single isolate and the type of plastic used was PE which has characteristics that are difficult to degrade because it is composed of a stable aliphatic chain with ethylene monomer (C_2H_4) n so that it is recalcitrant (Shah et al. 2008; Tokiwa et al. 2009; Zadjelovic et al. 2022). A study by Tao et al. (2023) found that Rhodococcus strain A34 bacteria from plastic waste could also reduce plastic weight by 1% over 30 days. Likewise, a study conducted by Yang et al. (2024) using mixed fungi consisting of *Alternaria* sp. and *Trametes* sp. showed that the rate of weight loss of LDPE film reached 0.66 ± 0.06% within a 30-day incubation period. Several other studies have been reported by Kopecká et al. (2022) showed that the degradation value using bacteria on 3 × 3 mm HDPE film was 0.5613-1.7808 during an incubation period of 30 days. Meanwhile, another study reported by Elsamahy et al. (2023) stated that there was degradation activity using a yeast consortium isolated from termites, consisting of *Sterigmatomyces halophilus*, *Meyerozyma guilliermondii*, and *Meyerozyma caribbica* yeast on 2.5 × 2.5 cm^2 plastic sheets, which could achieve a degradation percentage of up to 19.2% -43.6% (during 45 days). However, the type of plastic used was LDPE in sheets that had been treated with UV for 4 h, and added with 0.3 mL of Tween 80 to increase yeast colonization on the LDPE surface. The description of the results of PE degradation, as reported by various previous studies, shows that PE degradation with its recalcitrant characteristics requires pretreatment both physically and chemically, which can change the structure of the plastic so that the plastic is more susceptible to biodegradation and uses microbes in the form of a consortium (Zhang, Ding & Yuan 2022).

Additionally, the OD value of the biofilm increased across all treatments during the 4 weeks of incubation. In the initial week, the OD values of the biofilm for all isolates were notably low, with some isolates like M.2.0.1 and M.4.0.4 having OD values almost similar to the control. However, by the 2nd and 3rd weeks of incubation, there was a substantial increase in the OD.

Based on Figure 3, the 0% reduction in dry weight in the first week suggests that the yeast isolate did not exhibit biodegradation activity during this period. This is likely due to the isolate still adapting to the transfer to MSMB media, which initially provides minimal nutrition (Wahyuningsih & Zulaika 2018). The reduction in the dry weight of LDPE plastic by the M.3.0.1 yeast isolate exhibited a slow increase during the 2nd and 3rd weeks of incubation, followed by a rapid increase in the fourth week. This pattern suggests that M.3.0.1 yeast underwent a favorable growth phase, leading to the formation of a thicker biofilm during the 2nd and 3rd weeks of incubation. Subsequently, as the biofilm matured, M.3.0.1 yeast optimized its utilization of LDPE plastic. Consequently, during the fourth week of incubation, there was a significant increase in the percentage of dry weight reduction by M.3.0.1 yeast isolate. These findings are consistent with research by Cheng et al. (2021), which indicates that microbes are more effective in performing the biodegradation process when they reach the mature biofilm phase.

Biofilms adhered to plastic surfaces are known to alter the physicochemical properties of plastics and can lead to damage on the plastic surface, triggering the plastic

biodegradation process and resulting in an increase in the percentage reduction in dry weight (Afianti et al. 2022). The observed increase in the percentage reduction in the dry weight of LDPE by the M.3.0.1 isolate may also be attributed to the isolate's capability to produce extracellular enzymes with high activity in degrading LDPE plastic.

The increase in OD value indicates that yeast cells can grow in an environment with minimal nutrition by utilizing a carbon source in the form of LDPE plastic (Damayanti, Sulaiman & Ibrahim 2020). The low OD value of the biofilm was caused by yeast isolates that were still adapting to the MSMB incubation medium and had not yet formed a biofilm (Martins et al. 2016). The high increase in the OD value of the biofilm in the M.3.0.1 and M.4.0.1 isolates suggests that these isolates have good ability to form biofilms, as indicated by the thicker biofilm on the LDPE test plastic during the 2nd and 3rd weeks of incubation. In the fourth week of incubation, the increase in the OD value of the biofilm was relatively low. This is likely because, in the 4th week of incubation, the yeast isolate had begun to enter the mature phase, leading to a dispersal mechanism or spread in the test medium. This is consistent with research

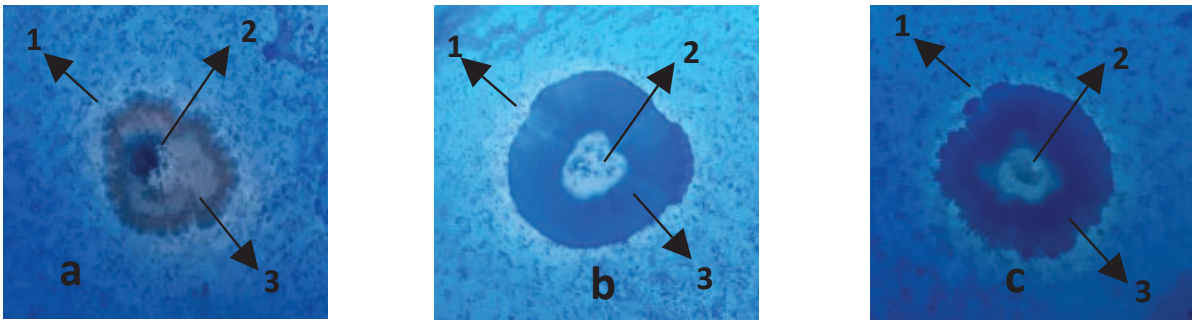

FIGURE 2. Clear zone formation by several yeast isolates (a) M.3.0.1 (b) M.3.0.2 (c) M.4.0.4. Description: 1. Clear Zone, 2. Inoculation Point, and 3. Growth Colony

TABLE 1. The diameter of the clear zone produced by yeast isolates on Mineral Salt Medium Agar (MSMA) supplemented with polyethylene powder

Isolate	Test results (+/-)	Zone diameter (mm)
K3.1.2	+	0.5
BI.4.1.1	+	0.8
M5.0.1	+	1
M5.0.3	-	0
M6.0.1	+	0.725
M6.0.2	+	0.3
M.2.0.1	+	1.1
M.2.0.2	+	0.5
M.2.0.3	-	0
M.2.0.4	+	1
M.3.0.1	+	3
M.3.0.2	+	2
M.3.0.3	-	0
M.3.1.1	-	0
M.4.0.1	+	1.4
M.4.0.2	-	0
M.4.0.3	+	0.5
M.4.0.4	+	1.8
M.4.0.5	-	0

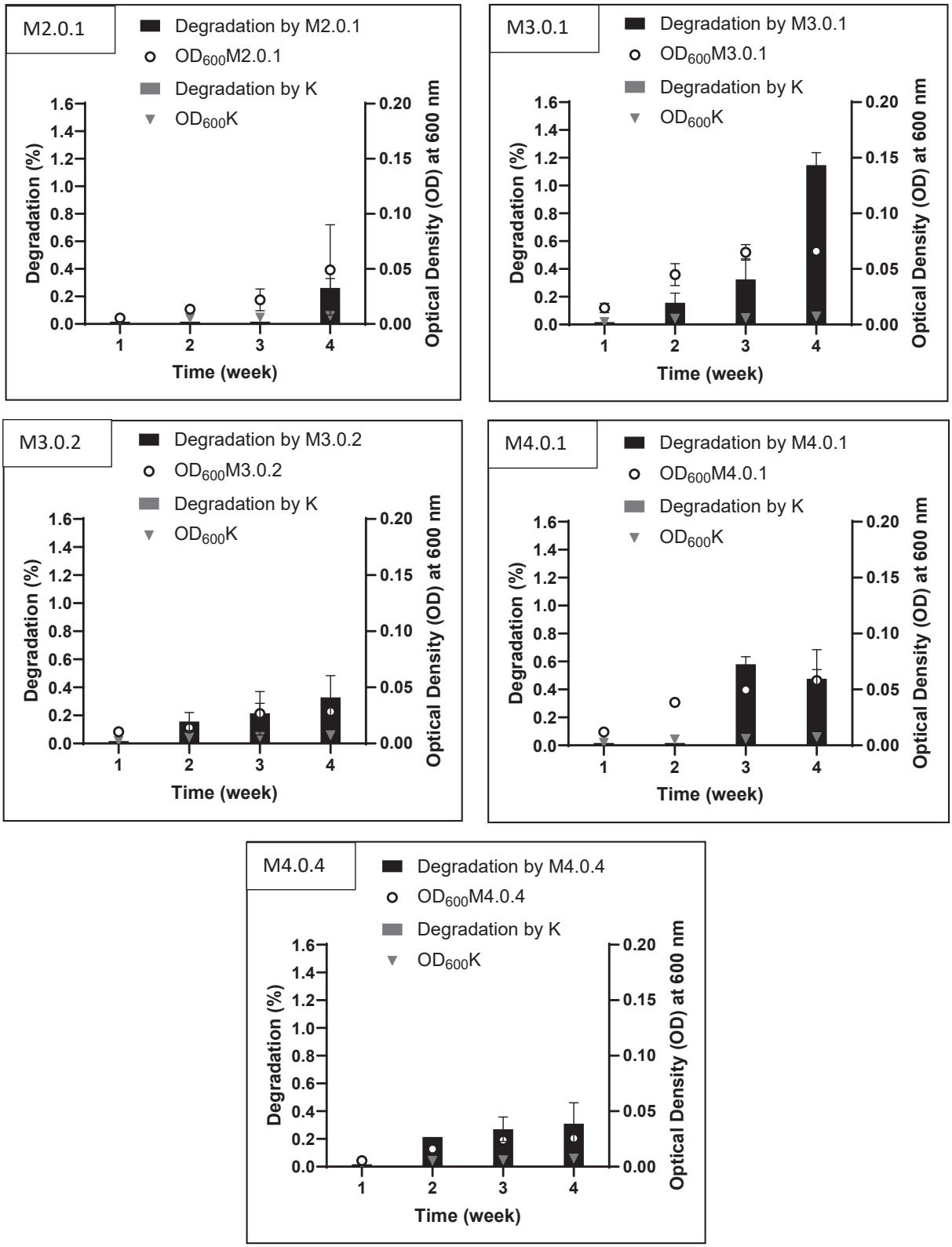

FIGURE 3. LDPE biodegradation test results by yeast isolates compared to control (K), based on the reduction in plastic dry weight (%) and yeast growth (OD600 nm) during the four-week incubation

by Odobel et al. (2021), which states that some microbes can form biofilms and reach a mature phase within an incubation period of 15-30 days. However, in the M.2.0.1 isolate, it was discovered that the OD value of the biofilm significantly increased in the 4th week of incubation. It is assumed that the M.2.0.1 isolate had started to enter the growth phase by forming a new biofilm, as the process of biofilm formation involves repeated phases in the biofilm life cycle (Liu et al. 2023).

The similar patterns of dry weight reduction and biofilm formation in M.3.0.2 and M.4.0.4 suggest that these yeast isolates may share similar metabolic pathways. From the second to fourth week of incubation, the percentage reduction in dry weight in both increased slowly. However, the percentage reduction in dry weight of M.4.0.4 in the fourth week of incubation was significantly lower than M.3.0.2. The pattern of dry weight reduction and biofilm formation in M.3.0.2 and M.4.0.4 indicates that these yeast isolates experienced slow biofilm thickening. The slow thickening of the biofilm may be due to the limited availability of nutrients for yeast growth within the biofilm matrix, promoting utilization of the LDPE plastic as an alternative carbon source. According to research by Gupta and Devi (2020), some microbes can gradually form biofilms, leading to a partial biodegradation process.

Figure 3 also shows that M.2.0.1 and M.4.0.1 exhibit a similar trend of gradual dry weight reduction over time. M.4.0.1 appears to have a potentially faster ability to degrade plastic than M.2.0.1, achieving a reduction in dry weight by the third week, whereas M.2.0.1 achieved a reduction only in the fourth week. The low OD value of the M.2.0.1 biofilm in the first three weeks (reference for biofilm and OD connection) suggests that this isolate was still in the initial phase of biofilm formation. The rapid increase in OD observed in the fourth week likely indicates a more mature biofilm, which could explain the increased percentage reduction in dry weight of LDPE plastic observed at that time. Therefore, it can be suggested that the M.2.0.1 isolate might require a longer period for the biodegradation mechanism to become fully established.

For the M.4.0.1 isolate, it was observed that during the first to third weeks of incubation, the isolate was still in the biofilm formation and growth phase. The high OD value of the M.4.0.1 biofilm could be indicative of increased biomass or metabolic activity within the biofilm, which may be associated with biofilm growth and development. This aligns with the observed increase in the percentage reduction of LDPE plastic dry weight, suggesting that a thicker or more active biofilm might be contributing to enhanced biodegradation.

pH VALUE OF MEDIA

In this study, Figure 4 illustrates the pH values of the media over 4 weeks of incubation. It is observed that the pH of the media decreased across all treatments from an initial pH of 6.85. Notably, the pH values of M.3.0.1 and M.4.0.1 were lower compared to the other isolates. This reduction in pH is correlated with the higher OD values of the biofilm in M.3.0.1 and M.4.0.1. Measuring the pH value is one of the parameters used to determine the metabolic activity of a microorganism in the growth medium. A decrease in pH can be associated with plastic biodegradation by some microorganisms, often leading to acidic conditions (Das & Kumar 2015). In the plastic biodegradation process, carbonyl groups are formed by the action of oxidative enzymes released by microorganisms. These carbonyl group then further oxidized and produce carboxylic acids during the colonisation process to form a biofilm. The carboxylic acids can then be metabolized by microorganisms through the β-oxidation pathway and subsequently enter the tricarboxylic acid (TCA) cycle, ultimately producing carbon dioxide and water (Mohanan et al. 2020). A high OD value in the biofilm may indicate a larger population of yeast cells, potentially leading to the production of greater amounts of organic acids during plastic biodegradation. These organic acids are the result of yeast cell activity in metabolizing compounds in plastic. While the presence of organic acids can reduce the pH of the medium, optimal pH conditions for biodegradation can vary depending on the specific microorganism involved (Srikanth et al. 2022).

SEM ANALYSIS

SEM analysis was used to determine the physical morphological changes on the surface of LDPE plastic after the biodegradation process. The LDPE plastic analyzed was the test sample with the highest dry weight reduction percentage, specifically the LDPE in M.3.0.1. The results of the SEM analysis in this study are shown in Figure 5. Based on Figure 5, the LDPE plastic surface after the biodegradation process exhibits a more irregular texture with deeper grooves compared to the smoother surface before biodegradation. This suggests damage to the plastic surface after biodegradation. The observed surface damage on the LDPE plastic suggests potential weakening or increased brittleness after biodegradation, which aligns with the reported association between biodegradation and brittleness in some studies (Khruengsai, Sripahco & Pripdeevech 2021). However, the absence of clear holes or cracks in the SEM analysis could indicate that the biodegradation process in this study might not have reached an advanced stage. The relatively short incubation time (4 weeks) used in this research could be a contributing factor. Additionally, factors like nutrient limitations or the specific characteristics of the yeast isolates employed might have also influenced the biodegradation rate (Bhagwat et al. 2021). It can also be seen that the test plastic used in this research is LDPE type plastic, which has a high polymer density, between 0.91 and 0.94 g/L with crystallinity (50–60%) (Duan et al. 2021).

SEM observations specifically show a dual mechanism that can occur. A biofilm is formed that supports the attachment of microbes that can damage the LDPE surface, thus facilitating further degradation. The biofilm stage is a crucial stage in LDPE degradation. After the biofilm is formed, the biodegradation stage continues with biodeterioration, fragmentation, assimilation, and biomineralization (Yoon, Jeon & Kim 2012).

Yeast biofilms are complex and heterogeneous multicellular structures in which the cells are well protected from highly dynamic external environmental conditions. In the first step, a conditioning films (CFs) is formed, the initial layer that prepares the surface for colonization by yeast cells. Then, yeast cells attach to the surface, divide, and form microcolonies. CFs can significantly alter the surface tension, charge density, and roughness (Bhagwat et al. 2021). It comprises proteins, polysaccharides, lipids, and minerals, which can be sourced from the surrounding environment or released by microorganisms (Shineh et al. 2023). Organic molecules or polymers contained in the conditioning film (such as proteins, polysaccharides, or other molecules) can adsorb to the surface of the material and change the surface properties from hydrophobic (water-repellent) to more hydrophilic (water-attracting), thus impacting changes in the surface tension of the plastic (Shineh et al. 2023).

Then, as growth progresses, yeast cells produce extracellular matrix (ECM) and differentiate to produce elongated pseudohyphae and hyphae to thrive in low-nutrient environments. Finally, in the maturation phase, the amount of ECM increases (Mohammadi & Saris 2022). The ECM produced by some yeasts, such as Candida, consists primarily of polysaccharides, proteins, nucleic acids, and lipids, and has both positively and negatively charged sites. These components interact with each other to form a complex biofilm matrix structure. This matrix acts as an adhesive that binds the biofilm cells together, and protects the biofilm from external threats. The secretion of charged EPS components can create positively or negatively charged areas on the surface (charge density). These changes in surface charge can further influence microbial attachment (Kurniawan et al. 2015; Wall et al. 2019). The surface charge of a material affects yeast adhesion. Opposite charges can promote adhesion, while like charges can repel yeast attachment (Shineh et al. 2023).

Over time, roughness can increase with the attachment and growth of microorganisms, which results in plastic damage. Plastic damage at this early stage (biodeterioration) involves the action of several oxidative enzymes released by microorganisms and induced by several environmental physicochemical factors. This can facilitate further oxidation at the biofragmentation stage. Several yeasts have been reported to produce enzymes involved in the biofragmentation stage, such as laccase, manganese peroxidase, and alkane hydroxylase (Zhang et al. 2022). Short hydrocarbon fragments (n-alkanes) with 10–50 carbon atoms released from the biofragmentation stage are metabolized at the bioassimilation stage (Restrepo-Flórez, Bassi & Thompson 2014). These short hydrocarbon fragments can be recognized as intermediates and/or substrates for hydroxylases, monooxygenases, and oxygenases to produce alcohol compounds, which can then be oxidized to ketones by alcohol dehydrogenases, and then converted to esters by Baeyer-Villiger monooxygenases. The converted esters are then cleaved by esterases, cutinases, and lipases, which can lead to the production and β-oxidation of fatty acids; the resulting compounds can then be used as metabolites and carbon sources (as well as CO_2 and H_2O) during mineralization (Seo et al. 2023).

FTIR ANALYSIS

The biodegradation process of LDPE plastic can be confirmed by FTIR analysis, which determines changes in the chemical structure of LDPE due to the action of yeast isolates on the polymer. The LDPE plastic analyzed was the sample with the highest dry weight reduction percentage, specifically the LDPE plastic in M.3.0.1. The results of the FTIR analysis are shown in Figure 6. In Figure 6, the results of FTIR analysis of LDPE plastic before and after biodegradation show differences in the formed peaks; however, there are no additions or reductions in functional groups after the biodegradation process. Additionally, there is a change in the transmittance percentage value before and after the biodegradation process.

The changes in transmittance intensity observed in the FTIR spectra, as reported by others (Sarker et al. 2012) could be due to the activity of microorganisms degrading the LDPE and altering the chemical structure. Research by El-Sayed et al. (2024) suggests that changes in specific wavenumbers can be linked to stretching or weakening of bonds within the polymer chain, potentially indicating biodegradation processes. Compared to the control group, Figure 6 shows a significant increase in the concentration of the O-H functional group (3309 cm^{-1}). This increase likely suggests the occurrence of oxidation, hydrolysis, or the formation of carboxylate functional groups. Additionally, an elevated concentration of aliphatic C-H functional groups (2915 cm^{-1} and 2847 cm^{-1}) was detected compared to the control group, indicating structural changes in the carbon chain (e.g., branching or alkyl group formation). A slight increase in the concentration of the CH_2 functional group (722 cm^{-1}) was also observed compared to the control group, potentially suggesting structural alterations in the carbon chain (e.g., C-C bond cleavage or formation). The presence of functional groups like OH (3302-3303 cm^{-1}) and CO (1019 cm^{-1}) might be associated with additional chemical compounds introduced during LDPE plastic manufacturing (Shovitri et al. 2023).

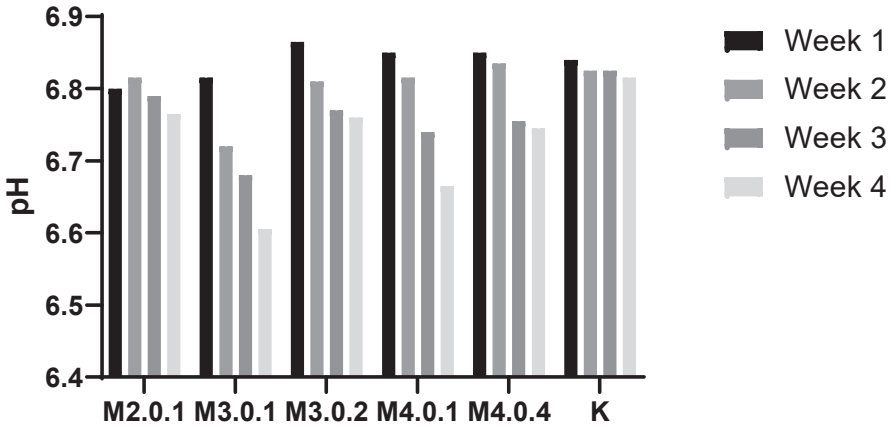

FIGURE 4. pH value of media

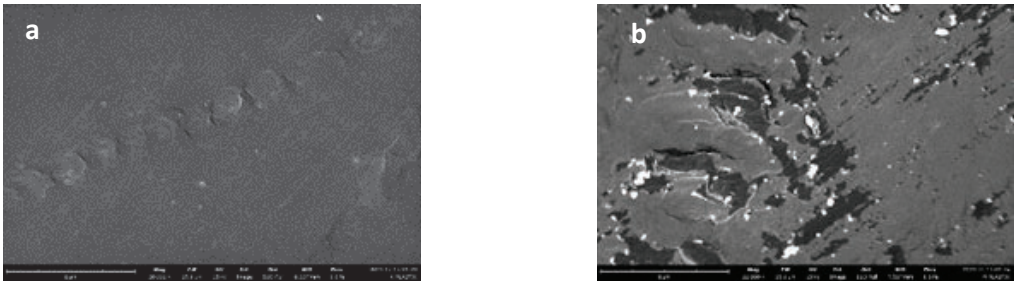

FIGURE 5. Results of SEM analysis on the surface of LDPE plastic before biodegradation (a) and after biodegradation (b) at a magnification of 20,000×

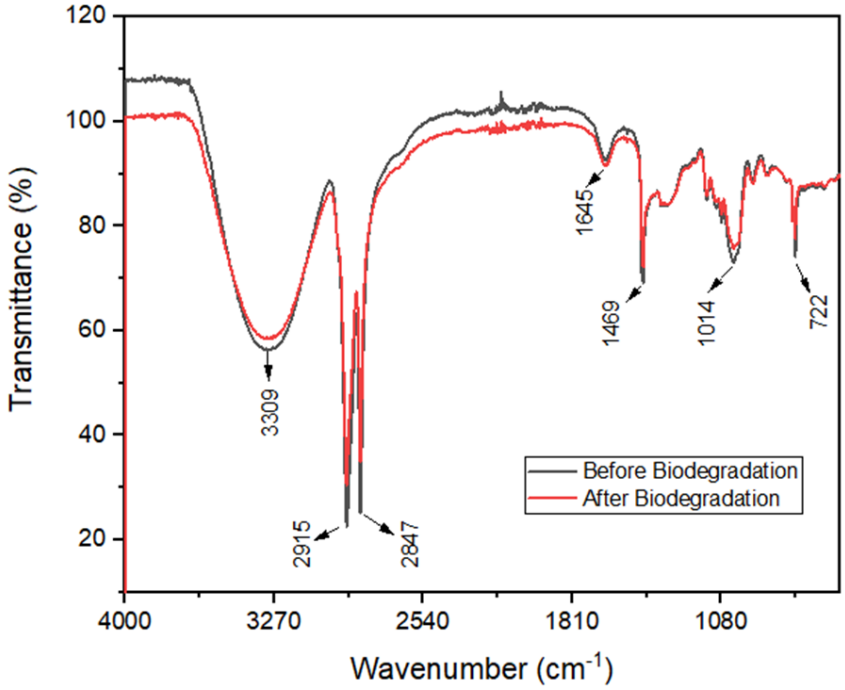

FIGURE 6. FTIR analysis results for LDPE plastic

CONCLUSION

Screening on MSMA showed that 13 isolates could degrade polyethylene, as indicated by the formation of clear zones. The five best isolates were used for further biodegradation tests. The yeast isolate M.3.0.1 exhibited the highest degradation percentage of 1.1474±0.0888%. It demonstrated increased growth in the test medium, as indicated by an increase in optical density. In addition, SEM analysis showed a change in the morphology of the LDPE surface, and FTIR analysis showed a change in the transmittance value for the test plastic. The findings suggest that these yeast isolates have the potential to contribute to eco-friendly solutions for plastic waste management.

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