The effect of pH-temperature on plastic allowance for Low-Density Polyethylene (LDPE) by *Thiobacillus* sp. and *Clostridium* sp.

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Abstract. LDPE are plastic polymers, widely used because they are affordable, waterproof, and flexible, hence difficult to degrade naturally. This study aim therefore is to degrade LDPE plastic using a biotechnology approach with a mixture of *Thiobacillus* sp. and *Clostridium* sp. at a variety of pH and temperatures. Mixed bacterial cultures were grown on Stone Mineral Salt Solution (SMSs) with a batch system, thereafter, gravimetric method was used to determine the difference in weight and Fourier Transformer Infrared Spectroscopy (FTIR) was used to determine changes in functional groups. To obtain the optimum condition that produces the highest degradation, researchers conducted the experiment at temperature varied from 25, 30, 35o C and pH 5, 7, 9. The highest removal of LDPE occurred at 30oC with pH 7. Gravimetrically, mixed cultures of *Thiobacillus* sp. and *Clostridium* sp. was capable of degrading LDPE plastic with an allowance of 2-7% for 30 days. Considering that this research produces a relatively small allowance, further research is needed to exploit the potential of using both bacteria in degrading LDPE plastic.

1. Introduction

Plastics are classified as one of the complex polymers with a very long degradation lifespan. This is because its structure posses a long, repetitive chain molecule, which takes a lot of time to break into short chain molecules [1]. Every year, more than 140 million tons of synthetic polymers are produced worldwide and their stable polymer characteristics causes them to have a limited natural degradation cycle. Furthermore, the pollution by water-soluble plastics and its waste poses a major environmental problem [2], its accelerated accumulation causes changes [3] like clogging of water ways and soil pollution when leaching occurs. Many animals die when they eat plastic waste and when they are trapped [4].

Plastics can be degraded by chemical, thermal, photoxidation and biodegradation mechanisms, which take a long time (depending on the molecular weight of the polymer) and some types of plastic require up to 1000 years to be degraded [5]. Furthermore, they are physically or chemically deteriorated by environmental factors, such as light, heat, humidity, chemistry, or biological activity [6].

The most commonly used thermoplastic group is Polyethylene. This is a polymer made from a long chain of ethylene monomers, they occur in different forms, which include HDPE (High-Density Polyethylene), LDPE (Low-Density Polyethylene) and LLDPE (Linear Low-Density Polyethylene). They are very hydrophobic compounds which do not react chemically and is not capable of being fully
decomposed by microbes [7], this is due to the recalcitrant nature of the biodegradation process, which further results in its rapid accumulation in nature. Some microbes capable of degrading polyethylene have so far been isolated from soil, seawater, compost, and activated sludge [8].

The government has made various efforts to solve the problem of plastic waste, using physical, chemical, and biological methods (natural microbes) to degrade polymers. Currently, enzymatic biodegradation is the most widely used treatment for plastic waste, which involves changing the material through microorganism activity [9]. Generally, plastics decompose aerobically, producing carbon dioxide and water in the process and anaerobically, producing carbon dioxide, water, and methane [10]. These methods do increase the rate of degradation without causing environmental damage. [11].

Bacteria used to degrade LDPE include Bacillus megaterium, Pseudomonas sp., Azotobacter, Ralstoniaeutropha, Halomonas sp. [12]. In addition, Brevibacillus sp. and Bacillus sp. have protease enzymes that influence the biodegradation process of polyethylene [13].

The factors that influence this process are temperature, pH, contact time, type and number of microbes, nutrient availability, microbial consortium, pollutant characteristics and salinity [14]. Biodegradation of LDPE plastic by Pseudomonas putida occurred at pH 6.5, temperature of 37°C, and shaker incubator speed of 180 rpm for 7 days [15]. Further research is however needed to process plastic waste with a biological approach in order for the amount of waste to be minimized. This laboratory scale study is aimed determining the ability of mixed cultures of Thiobacillus sp. and Clostridium sp. bacteria as biodegradation agents on LDPE plastic. The study is focused on identifying the effect of temperature and pH on the degradation process of LDPE plastic.

2. Research methods

2.1. Mechanical LDPE preparation
The plastic used in this study is Low-Density Polyethylene (LDPE) prepared mechanically by cutting it into 1x1 cm microplastic which was further sterilized using 70% alcohol for 15 minutes in a Laminar Flow Air Cabinet, rinsed with distilled water and then dried in an oven at 50°C for 24 hours [16].

2.2. Bacterial cultivation in growth media
Mix culture of living Thiobacillus sp. and Clostridium sp. were used in this experiment, obtained from the Laboratory of Environmental Biology/Microbiology, Environmental Engineering Department, Trisakti University, Jakarta, Indonesia. The bacterial was multiplied to meet the needs of this research, thereafter, the mixed culture was inoculated on SMSs (Stone Mineral Salt Solution) for 7-14 days, placed in the incubator at room temperature and pH 7. Growth media was further made from a solution of 0.5 g calcium carbonate (CaCO3), 2.5 g of ammonium nitrate (NH4NO3), 1 g of sodium hydrogen phosphate (Na2HPO4.7H2O), 0.5 g of monopotassium phosphates (KH2PO4), 0.5 g magnesium sulfate (MgSO4.7H2O) and 0.2 g of magnesium chlorides (MnCl2.7H2O). All of which were dissolved in one liter of distilled water.

2.3. The effect of temperature and pH
To determine the effect of temperature and pH, as much as 2% LDPE (w / v) and 10% (v / v) mixed cultures of bacteria were put into SMSs, pH 7, incubated in shaker incubators at a speed of 180 rpm and varying temperatures (25, 30 and 35 °C) for 30 days each.

Having obtained the temperature that gave the highest allowance results, this study advanced to determine the optimum pH by entering 2% LDPE (w / v) and 10% (v / v) mixed bacterial cultures into SMSs media, incubated in the shaker incubator at a rotatory speed of 180 rpm, at different pH levels (5, 7, 9) for 30 days each.

Determination of the excised microplastic LDPE at various temperatures and pH was carried out gravimetrically [17] and Fourier Transform Infra-Red (FTIR) analysis was also conducted to determine the changes that occurred in the functional group.

The percentage decrease in LDPE plastic weight is determined using the formula:
% weight loss = \frac{a - b}{a} \times 100\%

a = Microplastic weight of LDPE before being degraded
b = Microplastic weight of LDPE after being degraded

3. Results and discussion

3.1. The effect of temperature and pH

The results showed that bacterial culture was able to grow on growth media containing the toxic LDPE and there was a minor reduction in microplastic weight in all treatments. Figure 1 shows the highest allowance for LDPE using the gravimetric method was obtained at a temperature of 30°C at 5.3% in 30 days, this decrease in microplastic weight indicates that the LDPE biodegradation process has occurred though it was very slow.

![Figure 1](image1.png)

**Figure 1.** Removal percentage of LDPE plastics by temperature’s effect.

Figure 2 shows the highest allowance for LDPE using the gravimetric method at a pH of 7 which is 6.4% on 30 days, this decrease in weight is an indicator that the LDPE biodegradation process has occurred. Variations in temperature and pH provide a relatively small allowance (less than 10%) within 30 days and this process ran slowly because LDPE plastic is a xenobiotic material. This is a term applied to many recalcitrant organic chemicals such as synthetic chemicals which are not found in nature, hence difficult to degrade [18].

![Figure 2](image2.png)

**Figure 2.** Removal percentage of LDPE plastics by pH’s effect.
Polyethylene is a stable polymer consisting of long chains of ethylene monomers that cannot be easily degraded by microorganisms. However, some lower molecular weight PE oligomers (MW = 600-800) can be degraded by Actinobacter sp., while high molecular weight PE is difficult to degrade [19]. The mixed bacterial culture utilizes LDPE plastic as a source of carbon for its growth [20].

3.2. FTIR analysis
This was carried out to confirm the biodegradation process, characterized by changes in functional groups.

![Figure 3. Spectrum LDPE plastics biodegradation by FTIR before degradation process.](image1)

![Figure 4. Spectrum LDPE plastics biodegradation by FTIR after degradation process.](image2)
Figure 3 and Figure 4 show the LDPE spectrum before and after treatment. In the range of wave numbers 2500-3000 cm\(^{-1}\) which is a typical peak of PE compounds, the initial intensity was observed at 0.052 (Figure 3), which further decreased to 0.029 (Figure 4).

4. Conclusion

Based on the research conducted, it can be deduced that the ability of *Thiobacillus* sp. and *Clostridium* sp. to degrade LDPE plastic is influenced by temperature and acidity (pH) which was marked by the percentage decrease in the weight of LDPE plastic by 2-7% at a temperature of 30\(^{\circ}\)C and pH 7, further indicating a biodegradation process. The mixed culture of bacteria is however able to grow in the Stone Mineral Salt Solution (SMSs) media to which 10 grams of LDPE was added using it as a carbon source for its growth.

References

[1] Bhardwaj H 2012 Microbial Population *Associated With Plastics Degradation*
[2] Joshi P A and Jaysawal S R 2010 Isolation and characterization of poly-\(\beta\) hydroxyalkanoate producing bacteria from sewage sample *J of Cell and Tissue Research* **10** 2165-2168
[3] O’Brine T and Thompson R C 2010 Degradation of plastic carrier bags in the marine environment *Marine Pollution Bulletin* **60** (2010) 22792283
[4] Usha R, Sangeetha T and Palaniswamy M 2011 Screening of Polyethylene degrading Microorganisms from Garbage Soil *Libyan Agric. Res. Cen. J. Intl.* **2** 2165-2168
[5] Pramila R and Ramesh K V 2011 Biodegradation of Low Density Polyethylene (LDPE) by fungi isolated from municipal landfill area *J. Microbiol Biotechnol* **1** 131-136
[6] Tokiwa Y, Calabia B P, Ugwu C U and Aiba S 2010 Biodegradability of Plastics *Int J Mol Sci* **10** 3722-3742
[7] Kavitha R, Mohanan A K and Bhuvaneswari V 2014 Biodegradation of low density polyethylene by bacteria isolated from oil contaminated soil *International Journal of Plant, Animal and Environmental Sciences* **4**(3) 601- 610
[8] Yoon M G, Jeon H J and Kim M N 2012 Biodegradation of Polyethylene by a Soil Bacterium and AlkB Cloned Recombinant Cell *J Bioremed Biodegrad* **3** (4) 1-8
[9] Kaseem M, Hamad K, and Deri F 2012 Thermoplastic starch blends; A review of recent works *Polymer Science Series A* **54**(2012) 165-176
[10] Alshehri F 2017 Biodegradation of Synthetic and Natural Plastics by Microorganisms *Journal of Applied & Environmental Microbiology* **5**(1) 8-19
[11] Asmita K, Tanwar S and Shanthag T 2015 Isolation of Plastic Degrading Micro-organisms from Soil Samples Collected at Various Locations in Mumbai, India *International Research Journal of Environment Sciences* **4**(3) 77 – 85
[12] Chee J Y, Yoga S S, Lau N S, Ling S C, Abed R M M and Sudek K L 2010 Bacterially Produced Polyhydroxyalkanoate (PHA); Converting Renewable Resources into Bioplastics *Appl Microbiol & Microbiol Biotech, a Mendez Vilas (Ed) Current research, technology and education topics in Applied Microbiology and Microbial Biotechnology* **2** 1395-1404
[13] Sivan A 2011 New Perspectives in Plastics Biodegradation *Curr Opin Biotechnol* **22** 422-426
[14] Sihag S 2014 Factors Affecting the Rate of Biodegradation of Polyaromatic Hydrocarbons *International Journal of Pure & Applied Bioscience* **2**(4) 185-202
[15] Al-Jailawi, Majid H, Rasha S, Ameen and Ali A Al-Saraf 2015 Polyethylene degradation by *Pseudomonas putida* S3A 90-97
[16] Talkad M S, Maria S, Raj A and Javed A 2014 Microbial Degradation of Plastic (LDPE) & domestic waste by induced mutations in Pseudomonas putida *International Journal of Ethics in Engineering & Management Education* **1**(5) 2348-4748
[17] Mahdiyah D and Mukti B H 2013 Isolation of Polyethylene Plastic Degrading-Bacteria *Biosci. Inter.* **2**(3) 29-32
[18] Evans T J 2013 Reproductive Toxicology of Male and Female Companion Animals In Small Animal Toxicology (WB Saunders) 167-202
[19] Ghosh S K, Pal S and Ray S 2013 Study of microbes having potentiality for biodegradation of plastics Environ Sci Pollut Res 20 4339-4355
[20] Agustien A, Mifthahul J and Akmal D 2016 Screening Polyethylene Synthetic Plastic Degrading-Bacteria from Soil Der Pharmacia Lettre 8(7) 183 – 187