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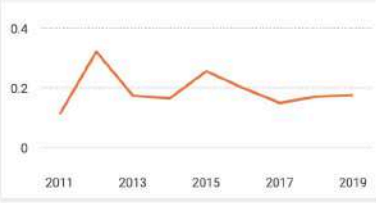
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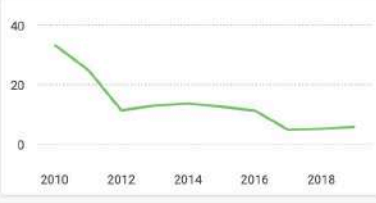
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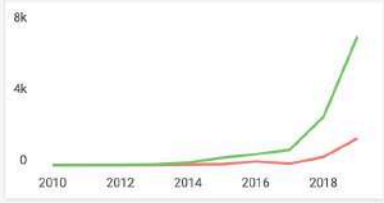
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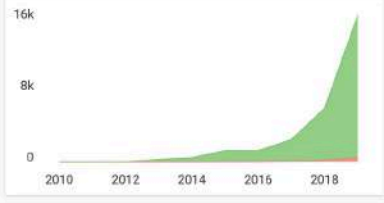
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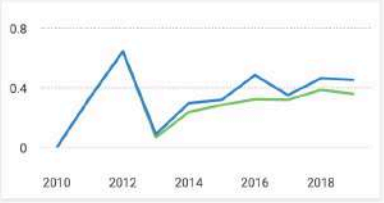
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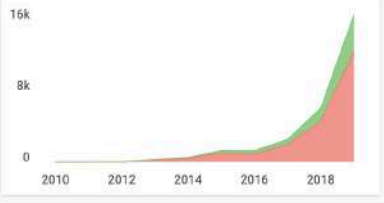
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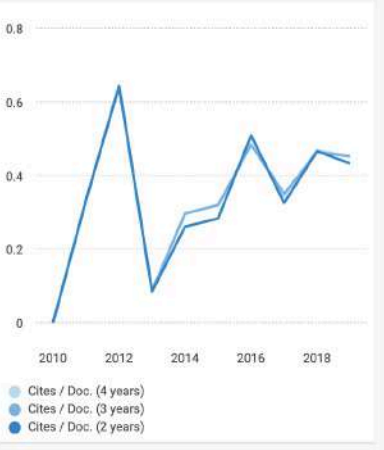
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Biodegradation of LLDPE plastic by mixed bacteria culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp.

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Abstract. Plastics take more than 400 years to naturally degrade. This study therefore aims to degrade Linear Low Density Polyethylene (LLDPE) plastic using a mixture of culture bacteria *Pseudomonas aeruginosa* and *Brevibacterium* sp. Pieces of LLDPE plastic measuring 1 x 1 cm², weighing 10 grams are put into containers containing Nutrient Broth (NB) growth media. The biodegradation process is carried out for 30 days with the test parameters being temperature (°C) and acidity (pH). The Gravimetric method was used to determine weight, while Fourier Transform Infrared (FTIR) analysis was used to actuate the morphology, changes in structure and surface of polyethylene. The results showed that mixed bacterial culture has the potential to degrade LLDPE plastic with a removal percentage of 2-7%, which is based on gravimetric analysis at 25°C, acidity value (pH) 7 for 30 days. Therefore, this study proves that mixed bacterial cultures degrades LLDPE plastic by using it as a carbon source.

1. Introduction

Presently, one of the common environmental problems not properly handled is plastic waste. Over the past few years, there has been an increase in the amount of waste generated by plastic due to its increasing amount of usage [1]. Plastics are polymers that are resistant or resistant to microbial activity so that their existence can last for years in nature. The most commonly used thermoplastic group is Polyethylene (PE) plastic [2]. Polyethylene (PE) is a polymer consisting of a long chain ethylene monomer (C₂H₄). HDPE, LLDPE and LDPE are types of polyethylene [3]. Polyethylene (PE) plastics have resistant properties that cause problems that are very alarming in nature. In most countries, pollution due to plastic is caused by improper recycling and waste management systems [4].

Biodegradation of plastic waste is one way to reduce plastic waste in nature [5]. Enzymatic biodegradation of plastic waste is one way to reduce this process in nature enzymatic. This method which utilizes enzymes possessed by microbes increases the rate at which plastics degrade without causing damage to the environment [6]. Biodegradation is a material change caused by biological activity in microorganisms such as bacteria and fungi. Plastics usually decompose both aerobically and anaerobically in landfills. The aerobic biodegradation produces carbon dioxide and water during the decomposition process, while anaerobic biodegradation produces carbon dioxide gas, water and methane [7]. Furthermore, some microbes isolated from soil, sea water, and compost degrades polyethylene [8]. Biodegradation of plastic by microbes is caused by the oxidation or hydrolysis process by enzymes from microbes which cause an aerobic or anaerobic chain chain change [9].



The biodegradation process by microbes takes place due to several factors, including temperature, soil characteristics, pH, O₂ availability, nutrients, contaminants, humidity and porosity. However, those consisting of pH, temperature, nutrients, minerals, oxygen, and humidity must match the type of microorganism utilized. Temperature and pH are the most essential factors capable of affecting the working speed of enzymes in degrading the substrate. Increased temperature rises the kinetic energy in the substrate molecules, enzymes and the degradation process. However, when the temperature exceeds its limit, it leads to denaturation, and when it is too low, it inhibits the action of the enzyme. In addition, the value of acidity (pH) is also a very important factor because only certain enzymes decompose substrates according to its activity. If the pH corresponds to the activity of the enzyme, then its ability to degrade the substrate will be more optimal [10].

During the biodegradation process, microbes form biofilms on the surface which allow them to break down high molecular weight polymers into smaller sizes or produce short chains such as oligomers and monomers through enzymatic processes [11]. Small molecules pass through bacterial and semi-permeable membranes and used as carbon as well as energy sources [12]. Enzymes produced by microbes to degrade plastic are very effective without causing damage to the environment [13].

Biodegradation of LLDPE plastic has been demonstrated in previous studies using a single culture bacterium *Serratia marcescens* which was incubated for 56 days. The result indicated that the bacteria could degrade LLDPE plastic. This is evidenced by the results of the percentage loss of plastic weight during incubation of 25% [14]. Based on the description above, this study aims to examine the culture ability of a mixture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. in degrading LLDPE type plastic.

2. Research methods

2.1. Mechanical LLDPE plastic preparation

The plastic used in this study is a Linear Low Density Polyethylene (LLDPE), with samples stripped to 1x1 cm² in size, sterilized with 70% alcohol, and dried in UV at LAF (Bio 60-M) for approximately 15 minutes. The resulting plastic pieces are rinsed with distilled water and dried in an oven at 50°C for 24 hours [15]. It is weighed using an analytical balance to determine the initial dry weight of the plastic.

2.2. Bacterial cultivation

Mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. that was collected from Biology/Environmental Microbiology Laboratory, Environmental Engineering Department, Trisakti University. It was used as a biodegrader to degrade LLDPE plastic. It was further cultivated on NA growth media for 7-14 days at room temperature and placed in an incubator for 48 hours.

2.3. Biodegradation of LLDPE plastic

LLDPE plastic removal test was carried out in 2 stages, namely optimization of temperature and acidity value (pH). The first step in determining the optimization of temperature variations was prepared Nutrient Broth (NB) growth medium in a 100 mL container added with 1 N NaOH solution to produce pH value of 7. Then, 10 grams of 1x1 cm² LLDPE plastic stripped were added, to 10% mixed culture in 80 mL of total solution volume. The solution was treated with different temperature variations, namely 25, 30, and 35 (°C). At each treatment, incubation was carried out in a shaker with a rotating speed of 180 rpm for 30 days.

After obtaining the optimum temperature, acidity value (pH) was determined by varying the values of 5, 7 and 9. On the 30th day, a piece of plastic sample was washed with 70% Ethanol solution to remove the mass of bacterial cells forming the remaining film in the surface layer. Plastic samples rinsed with 70% Ethanol solution are dried at 45°C for 24 hours [15] with several analytical methods used in determining the existence of allowances, including Gravimetric and Fourier Transform Infrared (FTIR) analysis methods [16].

2.4. Percentage decrease in LLDPE plastic sample weight

Plastic samples were incubated and dried, then weighed with analytical balance. This is carried out to determine the percentage of weight reduction in plastic samples gravimetrically. The following formula was used to determine it:

$$\% \text{ weight loss} = \frac{a-b}{a} \times 100\% \quad (1)$$

a = The weight of the plastic before it is degraded

b = Plastic weight after being degraded

The percentage of biodegradation was evaluated by comparing the initial dry weight of polyethylene before and after incubation. The research hypothesis is that microorganisms uses polyethylene as a carbon source other than NB media [17].

3. Result and discussion

Mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. has the ability to degrade xenobiotic polymer compounds [15]. It is an evident to reduce LLDPE plastic for 30 days with the percentage based on temperature and pH variations as shown in figures 1 and figure 2.

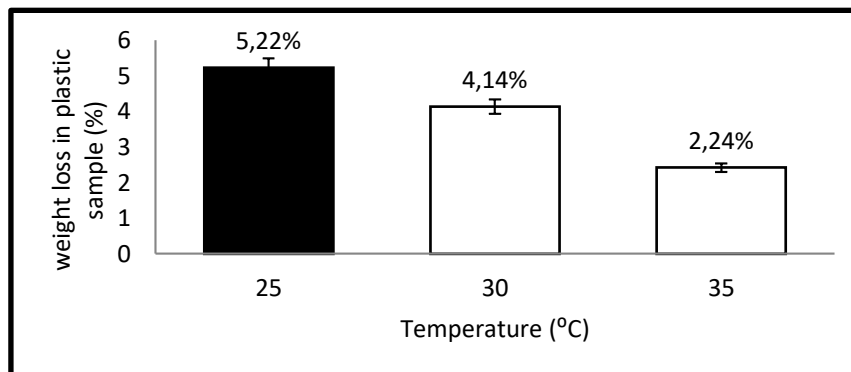


Figure 1. LLDPE Plastic Biodegradation - % of weight loss of LLDPE plastic based on temperature variations.

Mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. are able to grow well during the 30 days' incubation period in Erlenmeyer containing Nutrient Broth (NB) and 10 grams of LLDPE plastic samples, with differing temperatures, of 25, 30 and 35 (°C). The highest percentage of plastic weight allowance of 5.22% occurred at 25°C. This proves that there is a biodegradation process of LLDPE plastic by a mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp.

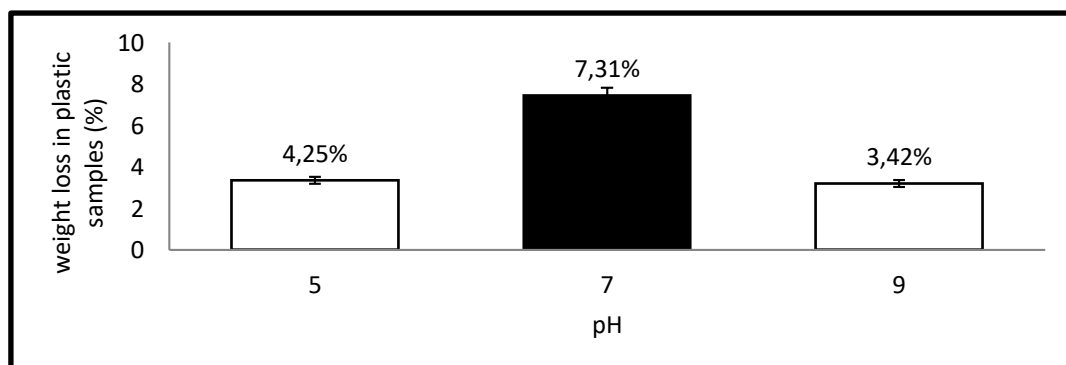


Figure 2. LLDPE Plastic Biodegradation - % of weight loss of LLDPE plastic based on pH variations.

With the same environmental conditions, at a temperature of 25°C culture mixture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. also grow well in containers containing Nutrient Broth (NB) and 10 grams of LLDPE plastic samples with varying pH values of 5, 7 and 9. The largest percentage percentage of 7,31% occurs at pH 7. Mix Culture isolate of *Pseudomonas aeruginosa* bacteria and *Brevibacterium* sp. sticking to form a biofilm on the surface of LLDPE plastic samples which is one of the carbon sources in the Nutrient Broth (NB) media. Biofilms on plastic surfaces also grows by utilizing polymers as carbon sources [17]. In primary degradation, the main chain divides and forms oligomers or monomers owing to the presence of an enzyme secreted by bacteria to break down the main polymer chain [18-20]. Therefore, the mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. era able to degrading LLDPE plastic samples.

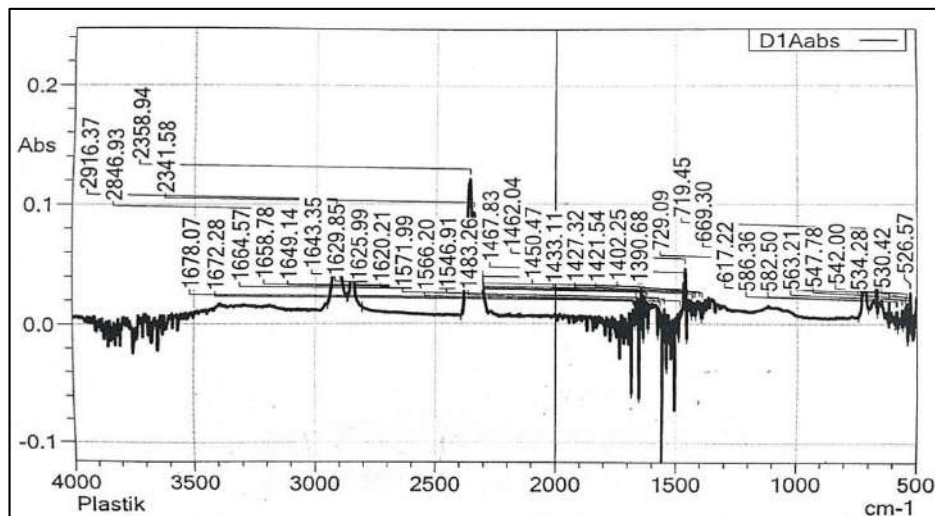


Figure 3. LLDPE Plastic Biodegradation – LLDPE plastic FTIR spectrum before treatment.

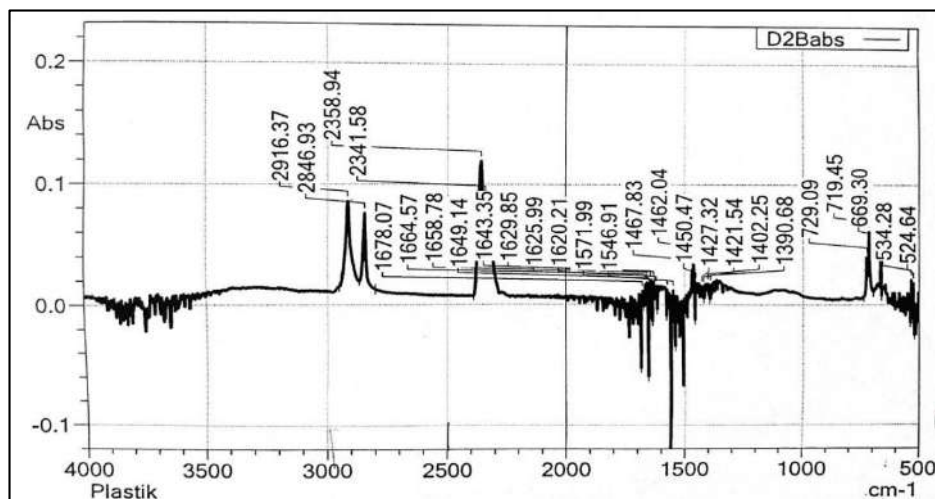


Figure 4. LLDPE Plastic Biodegradation – LLDPE plastic FTIR spectrum after treatment.

Biodegradation of LLDPE plastic was confirmed using Fourier Transform Infrared (FTIR) in the range of wave numbers 2500-3000 cm^{-1} which is a typical peak of PE compounds (Figures 3 and 4). In incubated or treated samples, there was a decrease in intensity, from an initial intensity of 0.093 at wavelengths of 2846.93 cm^{-1} and 0.121 at wavelengths of 2916.37 cm^{-1} (Figure 3) to 0.076 at wavelengths of 2846.93 cm^{-1} and 0.086 at wavelengths of 2916.37 cm^{-1} (Figure 4). Gravimetric and

FTIR analyzes that have been carried out show the ability of a mixture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. to degrade LLDPE plastic by using it as a carbon source [21].

4. Conclusion

Mixed culture of this bacterium is able to grow in the growth media of Nutrient Broth (NB) containing LLDPE plastic. This study also proved that *Pseudomonas aeruginosa* and *Brevibacterium* sp. has the potential to degrade LLDPE plastic at pH values of 5, 7, 9 and temperatures of 25, 30, 35 (°C). The highest percentage of plastic dry weight loss of 7.31% gravimetrically occurs at 25°C and pH 7. It is suspected that mixed culture of bacteria is able to utilize LLDPE plastic as a carbon source or energy source for its growth. Further studies are needed for plastic degradation by Mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. takes place faster with a higher percentage allowance.

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Biodegradation of LLDPE plastic by mixed bacteria culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp.

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The plastic used in this study is a Linear Low Density Polyethylene (LLDPE), with samples stripped to 1x1 cm² in size, sterilized with 70% alcohol, and dried in UV at LAF (Bio 60-M) for approximately 15 minutes. The resulting plastic pieces are rinsed with distilled water and dried in an oven at 50°C for 24 hours [15]. It is weighed using an analytical balance to determine the initial dry weight of the plastic.

2.2. Bacterial cultivation

Mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium sp.* that was collected from Biology/Environmental Microbiology Laboratory, Environmental Engineering Department, Trisakti University. It was used as a biodegradator to degrade LLDPE plastic. It was further cultivated on NA growth media for 7-14 days at room temperature and placed in an incubator for 48 hours.

2.3. Biodegradation of LLDPE plastic

LLDPE plastic removal test was carried out in 2 stages, namely optimization of temperature and acidity value (pH). The first step in determining the optimization of temperature variations was prepared Nutrient Broth (NB) growth medium in a 100 mL container added with 1 N NaOH solution to produce pH value of 7. Then, 10 grams of 1x1 cm² LLDPE plastic stripped were added, to 10% mixed culture in 80 mL of total solution volume. The solution was treated with different temperature variations, namely 25, 30, and 35 (°C). At each treatment, incubation was carried out in a shaker with a rotating speed of 180 rpm for 30 days.

After obtaining the optimum temperature, acidity value (pH) was determined by varying the values of 5, 7 and 9. On the 30th day, a piece of plastic sample was washed with 70% Ethanol solution to remove the mass of bacterial cells forming the remaining film in the surface layer. Plastic samples rinsed with 70% Ethanol solution are dried at 45°C for 24 hours [15] with several analytical methods used in determining the existence of allowances, including Gravimetric and Fourier Transform Infrared (FTIR) analysis methods [16].

2.4. Percentage decrease in LLDPE plastic sample weight

Plastic samples were incubated and dried, then weighed with analytical balance. This is carried out to determine the percentage of weight reduction in plastic samples gravimetrically. The following formula was used to determine it:

$$\% \text{ weight loss} = \frac{a-b}{a} \times 100\% \quad (1)$$

a = The weight of the plastic before it is degraded

b = Plastic weight after being degraded

The percentage of biodegradation was evaluated by comparing the initial dry weight of polyethylene before and after incubation. The research hypothesis is that microorganisms use polyethylene as a carbon source other than NB media [17].

3. Result and discussion

Mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. has the ability to degrade xenobiotic polymer compounds [15]. It is evident to reduce LLDPE plastic for 30 days with the percentage based on temperature and pH variations as shown in figures 1 and figure 2.

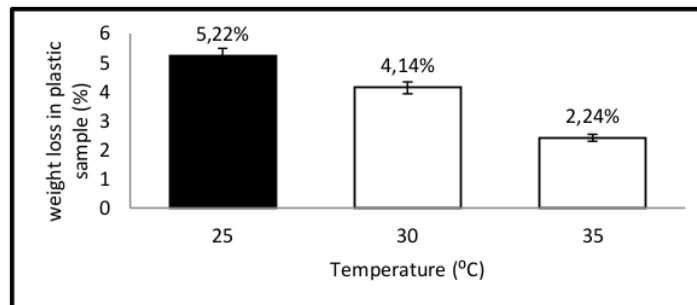


Figure 1. LLDPE Plastic Biodegradation - % of weight loss of LLDPE plastic based on temperature variations.

Mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. are able to grow well during the 30 days' incubation period in Erlenmeyer containing Nutrient Broth (NB) and 10 grams of LLDPE plastic samples, with differing temperatures, of 25, 30 and 35 (°C). The highest percentage of plastic weight allowance of 5.22% occurred at 25°C. This proves that there is a biodegradation process of LLDPE plastic by a mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp.

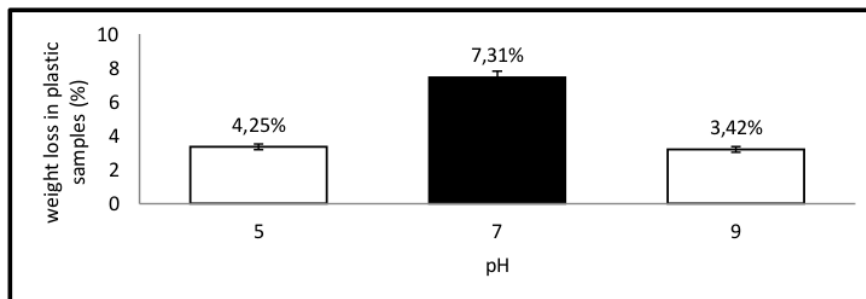


Figure 2. LLDPE Plastic Biodegradation - % of weight loss of LLDPE plastic based on temperature variations.

With the same environmental conditions, at a temperature of 25°C culture mixture of *Pseudomonas aeruginosa* and *Brevibacterium sp.* also grow well in containers containing Nutrient Broth (NB) and 10 grams of LLDPE plastic samples with varying pH values of 5, 7 and 9. The largest percentage percentage of 7.31% occurs at pH 7. Mix Culture isolate of *Pseudomonas aeruginosa* bacteria and *Brevibacterium sp.* sticking to form a biofilm on the surface of LLDPE plastic samples which is one of the carbon sources in the Nutrient Broth (NB) media. Biofilms on plastic surfaces also grows by utilizing polymers as carbon sources [17]. In primary degradation, the main chain divides and forms oligomers or monomers owing to the presence of an enzyme secreted by bacteria to break down the main polymer chain [18-20]. Therefore, the mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium sp.* era able to degrading LLDPE plastic samples.

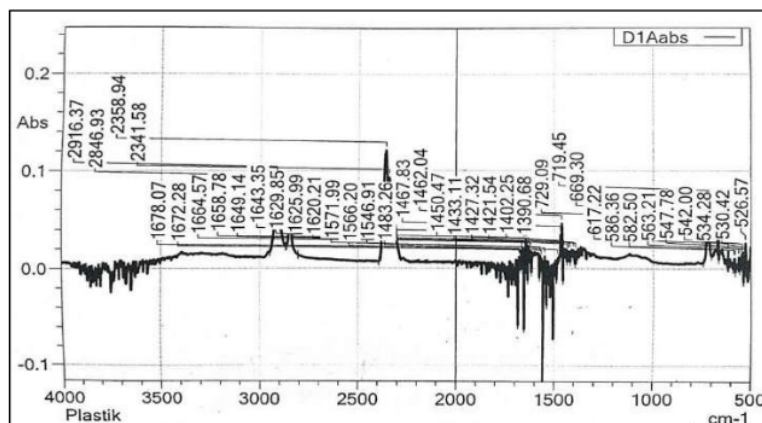


Figure 3. LLDPE Plastic Biodegradation – LLDPE plastic FTIR spectrum before treatment.

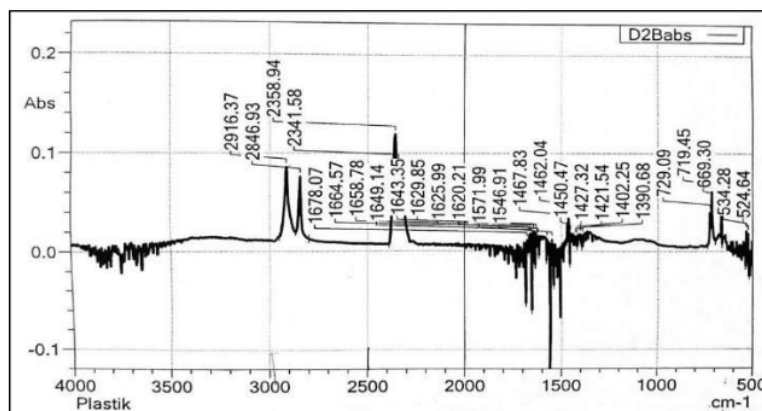


Figure 4. LLDPE Plastic Biodegradation – LLDPE plastic FTIR spectrum after treatment.

Biodegradation of LLDPE plastic was confirmed using Fourier Transform Infrared (FTIR) in the range of wave numbers 2500-3000 cm^{-1} which is a typical peak of PE compounds (Figures 3 and 4). In incubated or treated samples, there was a decrease in intensity, from an initial intensity of 0.093 at wavelengths of 2846.93 cm^{-1} and 0.121 at wavelengths of 2916.37 cm^{-1} (Figure 3) to 0.076 at wavelengths of 2846.93 cm^{-1} and 0.086 at wavelengths of 2916.37 cm^{-1} (Figure 4). Gravimetric and

FTIR analyzes that have been carried out show the ability of a mixture of *Pseudomonas aeruginosa* and *Brevibacterium sp.* to degrade LLDPE plastic by using it as a carbon source [21].

4. Conclusion

Mixed culture of this bacterium is able to grow in the growth media of Nutrient Broth (NB) containing LLDPE plastic. This study also proved that *Pseudomonas aeruginosa* and *Brevibacterium sp.* has the potential to degrade LLDPE plastic at pH values of 5, 7, 9 and temperatures of 25, 30, 35 (°C). The highest percentage of plastic dry weight loss of 31% gravimetrically occurs at 25°C and pH 7. It is suspected that mixed culture of bacteria is able to utilize LLDPE plastic as a carbon source or energy source for its growth. Further studies are needed for plastic degradation by Mixed culture of *Pseudomonas aeruginosa* and *Brevibacterium sp.* takes place faster with a higher percentage allowance.

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