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UTILIZATION OF PINEAPPLE PROCESSING LIQUID WASTE IN BIODEGRADATION OF DISPOSABLE FACE MASK BY BACTERIA FROM LAMPUNG BAY

Pemanfaatan Limbah Cair Nanas dalam Biodegradasi Masker Sekali Pakai oleh Bakteri dari Teluk Lampung

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ABSTRACT

The designation of COVID-19 as a global pandemic led to an increased use of single-use face masks, which result in waste that is difficult to degrade and has the potential to release microplastic fibers into the environment. This study aims to examine the impact of adding pineapple peel liquid waste (LCN) as a growth medium for the biodegradation of single-use face masks by bacterial isolates obtained from the waters of the Lampung Bay. The study investigates how LCN affects the efficiency of mask degradation by microorganisms, as well as how the ratio of LCN mixed with other growth media, such as Nutrient Broth (NB), influences the degradation process. The biodegradation process was carried out using a biostimulation technique, where bacterial isolates were incubated in media containing LCN at a 1:1 ratio. The degradation process lasted for 15 days, with the results being analyzed using gravimetry and Fourier-Transform Infrared Spectroscopy (FTIR). Gravimetric results showed a greater weight reduction in treated masks compared to the control masks. FTIR analysis also indicated changes in the intensity of functional groups in the degraded layers of the masks, as well as the emergence of C≡C functional groups in the second and third layers. This study demonstrates that the addition of LCN can accelerate the biodegradation of single-use face masks, offering a new approach for managing mask waste.

Keywords: *Biodegradation, Pineapple processing liquid waste, Disposable face mask, Microplastics, Lampung bay*

ABSTRAK

Penetapan COVID-19 sebagai pandemi global meningkatkan penggunaan masker sekali pakai yang menghasilkan limbah yang sulit terdegradasi dan berpotensi melepaskan serat mikroplastik ke lingkungan. Tujuan dari penelitian ini adalah untuk mengkaji pengaruh penambahan limbah cair nanas (LCN) sebagai media pertumbuhan terhadap degradasi masker sekali pakai oleh isolat bakteri yang diambil dari perairan Teluk Lampung. Pada penelitian ini, dirumuskan pengaruh LCN terhadap efektivitas degradasi masker oleh mikroorganisme, serta bagaimana rasio campuran LCN dengan media pertumbuhan lain, seperti Nutrient Broth (NB), dapat mempengaruhi proses degradasi masker. Metode yang digunakan adalah biodegradasi dengan teknik biostimulasi, di mana isolat bakteri diinkubasi dalam media yang mengandung LCN dengan rasio 1:1.

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Proses degradasi berlangsung selama 15 hari, dan hasil degradasi masker dianalisis dengan gravimetri dan Fourier-Transform Infrared Spectroscopy (FTIR). Hasil gravimetri menunjukkan penurunan berat masker perlakuan lebih besar dibandingkan masker kontrol. Analisis FTIR juga menunjukkan perubahan intensitas gugus fungsi pada lapisan masker yang terdegradasi, serta kemunculan gugus fungsi C≡C pada lapisan kedua dan ketiga. Penelitian ini menunjukkan bahwa penambahan LCN dapat mempercepat proses degradasi masker sekali pakai, menawarkan pendekatan baru dalam pengelolaan limbah masker.

Kata kunci: *Biodegradasi, Limbah Cair Nanas, Masker Sekali Pakai, Mikroplastik, Teluk Lampung*

INTRODUCTION

In March 2020, the World Health Organization (WHO) declared the COVID-19 virus outbreak a global pandemic. The government has implemented various measures to contain the spread of the COVID-19 virus, including hand washing with soap and water, social distancing and wearing masks (Phelps and Cooke, 2020). The increase in COVID-19 cases in Indonesia has increased the number of people wearing disposable masks. Disposable masks can cause waste that is harmful to health and the environment.

A disposable face mask consists of three layers. The innermost layer is made of hydrophilic material that serves to prevent body fluids such as saliva from escaping from the inside of the mask (Morgana et.al., 2021). The middle layer is made of synthetic non-woven materials such as polypropylene which functions as a filter to attract aerosols and particles (which are negatively charged) by electrostatic forces (Bhattacharjee et al., 2020). And the outermost layer is made of waterproof hydrophobic materials such as polypropylene which serves as a defense against contaminants from outside the mask (Christita et al., 2018).

Disposable masks are made of polypropylene fibers, high-density polyethylene and may contain other polymeric materials such as polyester, polyurethane, polystyrene, and polyacrylonitrile. This type of fiber is difficult to degrade because it has resistance to chemicals, air, sunlight, and heat (Prata et al., 2021). One method that can be applied is bioremediation, which is known to be more environmentally friendly than other methods such as incineration and pyrolysis. In that method, microorganisms are used with the addition of organic compounds such as nitrogen and phosphorus which are widely found in food industry waste (Sheldon and Long, 2023).

Information on the biodegradation of disposable masks is still limited. This is because the constituent material of disposable masks contains polypropylene which is difficult to decompose. Based on several journals including research conducted by Auta et al. in 2018, it was explained that polypropylene microplastics can be degraded using microorganisms such as Bacillus sp. and Rhodococcus sp. This is evidenced by a weight loss of 6.4% by Rhodococcus sp and 4% by Bacillus sp after passing the incubation stage for 40 days (Auta et al., 2018). Disposable masks have also been confirmed through research conducted by Zhou et al. to be a place for bacteria to grow. This is evidenced by the results of ARGs (Antibiotic Resistance Genes) analysis after incubating in the estuary for 30 days the number of bacteria increased by about 1.07×10^{12} for surgical masks (S. Zhou et al., 2022).

In this study, the degradation process was carried out biologically using a selected bacterial isolate from Lampung Bay waters, incubated with pineapple processing liquid waste (*limbah cair nanas*/LCN) through the biostimulation method. This method involves adding organic compounds, such as nitrogen and phosphorus from food industry waste, which is LCN, to enhance microbial activity, a strategy that has been less explored in biodegradation studies. LCN is a cost-effective alternative to commercial media such as Nutrient Broth, and its use in the biodegradation of disposable masks adds novelty to the field. While other studies focus

on biodegradation of plastics, such as polypropylene, in marine environments (Castañeda et al., 2024), few explore the use of organic, locally sourced media like LCN. This makes the current study innovative by applying biostimulation with LCN, offering both environmental and economic benefits. LCN, with a pH of 6.5 and high organic content, provides an environment for bacteria to thrive, and if improperly disposed of, it can contribute to environmental contamination (Miljaković et al, 2020).

MATERIALS AND METHODS

Place and Time of Research

This research was carried out in October 2023 – March 2024, located at the Microbiology and Chemical Engineering Laboratory, Sumatra Institute of Technology, South Lampung, Lampung.

Materials

Disposable Face Masks Sample

The 3-ply dispossable face mask used in this study were obtained from the marketplace. First, the straps and support were separated from the mask, and the mask was cut into uniform pieces measuring 0.5 x 0.5 cm. The cut pieces were then sterilized by autoclaving at 121^{o}C for 15 minutes. After sterilization, the samples were stored in a closed container to avoid contamination before use in the degradation process.

Pineapple Processing Liquid Waste (LCN) & *Nutrient Broth* (NB)

The growth media used for the bacterial isolates were a mixture of Nutrient Broth (NB) and Pineapple Processing Liquid Waste (LCN) in a 1:1 ratio. The NB medium was prepared by dissolving 8 grams of NB in 1000 mL of distilled water, while the LCN medium was made by dissolving 200 mL of LCN in 1000 mL of distilled water. The media were mixed using a magnetic stirrer to ensure homogeneity, followed by sterilization in an autoclave at 121^{o}C for 15 minutes. The pH of the media was chosen to ensure optimal bacterial growth conditions, with the NB medium having a neutral pH of 7 (Merck Millipore, 2022), and the LCN medium having a slightly acidic pH of 6.5. According to Kim et al. (2018), the pH range suitable for most bacterial species is between pH 3 and 9.

Bacterial isolate

The bacterial isolate used in this study was obtained from Lampung Bay, as described by Deviany et al. (2023). The bacterial isolate was morphologically and biochemically characterized and identified as a Gram-positive bacterium. The adaptation process was carried out in stages using the biostimulation method, where additional media such as LCN were provided as nutrients.

The first stage of adaptation involved incubating the bacterial isolate in a mixed medium of NB and LCN at a 9:1 ratio for 5x24 hours at room temperature (±25^{o}C) on a shaker. Following this, the bacterial isolate was re-adapted using varying ratios of NB and LCN (8:2 and 7:3) under the same conditions.

Bacterial Growth Curve Analysis

The bacterial growth was monitored using a spectrophotometer. Colonies from solid media were inoculated into 100 mL of NB (7:3) medium and incubated at room temperature on an orbital shaker. For every 15 minutes, 2 mL of bacterial culture was sampled to measure the turbidity at 600 nm. Absorbance readings were recorded over time to determine the bacterial growth phases necessary for the degradation process.

Methods

Biodegradation of Disposable Face Mask

The degradation process was carried out with variations in the ratio of LCN: NB media (1:1) to examine the effects of different media compositions on the degradation process. The culture from the final log phase (1 ml) was then transferred to an Erlenmeyer flask containing 40 mL of one of the following media variations: LCN: NB (1: 1), LCN, and NB. Several pieces of disposable masks were added into the culture and incubated at room temperature for 15 days. Initial weight of mask samples were measured using analytical balance. LCN and NB media without bacteria inoculation was used as negative control.

Gravimetric Analysis

After 15 days of degradation, the degraded mask samples were filtered and washed with 70% alcohol for 10 minutes, and then dried using an oven at 60°C for ±3 days to ensure complete drying before measuring weight loss. The weight of the degraded masks was measured using an analytical balance. The percentage of weight loss of the mask samples was calculated using the following formula:

$$\%weight\ loss = \left(\frac{W_0 - W}{W_0}\right) \times 100$$

Where W_0 is the initial weight of the sample and W is the weight after degradation (Mohan et al., 2016).

Fourier transform infrared (FTIR) Analysis

FTIR analysis was conducted to investigate the structural changes and identify potential chemical modifications in the mask samples before and after degradation, with a frequency range of 4000-600 cm^{-1}.

RESULTS AND DISCUSSION

Adaptation of Bacterial Isolate in LCN-NB Medium

The bacterial isolate selected for the degradation process was first adapted to the pineapple liquid waste (LCN) media. This adaptation was necessary to prepare the bacterial isolate for the degradation media containing LCN. Generally, bacteria require inorganic substances, such as salts containing Na, K, Ca, Mg, Cl, S, and P. Additionally, bacteria need nutrients containing C, H, O, and N, which are essential for synthesizing protoplasm (Sutanto and Suarsini, 2016). The nitrogen, phosphorus, potassium, and organic C content in LCN media are crucial for providing essential nutrients to the degrading bacteria (W. Zhou et al., 2022). To prepare the LCN medium, a dilution process was initially carried out with a ratio of aquadest: LCN (5:1), and the LCN media composition was analyzed (see Table 1). The high organic C content in LCN can serve as an energy source for metabolism and bacterial growth (Wang et al., 2019).

Table 1. Pineapple liquid waste composition

No	Parameter	Unit	*Result*
1.	Nitrogen	%	0,05
2.	P-total	%	<0,02
3.	Kalium	%	0,18
4.	C-Organik	%	43,13

The adaptation process occurred in several stages, with mixed media consisting of Nutrient Broth (NB) and Pineapple Processing Liquid Waste (LCN) in varying ratios: 9:1 (NB: LCN), 8:2 (NB: LCN), and 7:3 (NB: LCN). The bacterial isolate demonstrated successful growth after adapting to the 7:3 ratio. This indicates that the bacterial isolate can thrive with a higher concentration of LCN, which could be attributed to the favorable nutrient composition of the media, including the organic carbon that facilitates microbial growth. This adaptation is consistent with findings from Wu et al. (2020), where bacterial growth was enhanced by media containing organic carbon sources.

After the final adaptation stage (7:3), the bacterial isolate was used in the degradation process. The bacterial morphology at each stage was confirmed through Gram staining. Previous studies have suggested that the isolate was Gram-positive (Deviany et al., 2023), which is consistent with the results in this study. Gram-positive bacteria possess a thick peptidoglycan layer in their cell wall, giving them a high affinity for crystal violet during Gram staining (Purwaningsih and Wulandari, 2021). As shown in Figure 1(b), the adapted isolate from the 7:3 (NB:LCN) media produced a purple color after Gram staining, confirming its Gram-positive nature. The peptidoglycan structure in Gram-positive bacteria allows enzymes to interact with and break down polymers, facilitating the degradation process (Walter and Mayer, 2019).

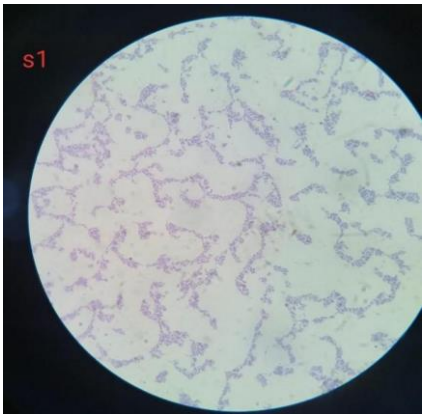


Figure 1. Gram staining test on bacterial isolates, (a) gram staining of bacterial isolates with NB media, (b) gram staining of bacterial isolates with NB:LCN media ratio (7:3).

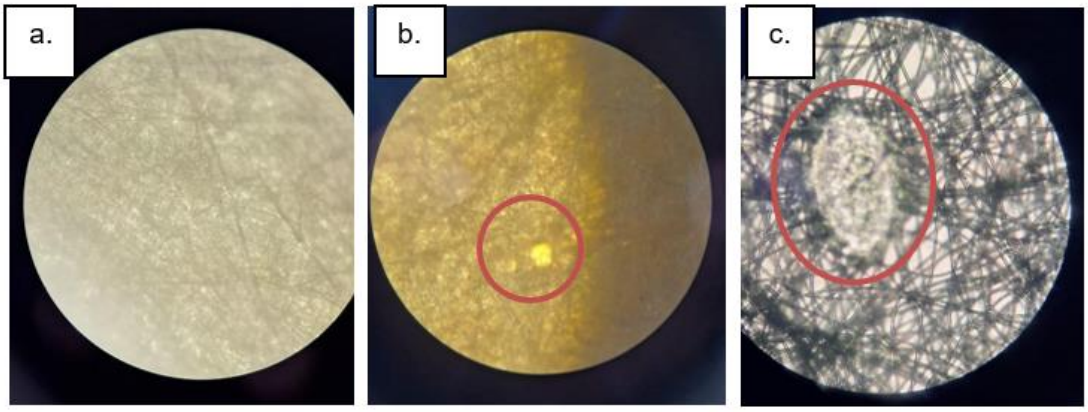


Figure 2. Microscope observation of mask samples after degradation test in (a) Control (0:1) dan (b.) second layer in LCN:NB (1:1); (c.) first layer in LCN:NB (1:1)

Preparation of microbial inoculation and testing for degradation of disposable masks

Bacterial growth phase is typically divided into four phases: the lag phase, the exponential (log) phase, the stationary phase, and the death phase. In the lag phase, bacteria exhibit metabolic activity but has not been followed by bacterial cell division. The exponential phase follows, where the growth rate is constant, with cells reproducing through binary fission. In the log phase, cellulose enzymes will be produced which can help to break down cellulose and release reducing sugars as the final product. Finally, the bacterial population will enter the stationary phase where the bacterial growth rate is the same as the death rate, resulting in decreased cell growth. Finally, the death phase where the number of living cells decreases and population growth decreases rapidly (Kochan et al., 2020).

In the present study, the 105th minute of growth was selected for degradation testing. This time point was chosen because, after the log phase, bacterial growth stabilizes and the enzymes that degrade polymers have had sufficient time to act on the pollutants. Additionally, in the stationary phase, bacterial cells will not experience growth but have an active metabolism that can still produce secondary products (Shuler and Kargi, 2022).

Gravimetric analysis of mask samples after degradation process

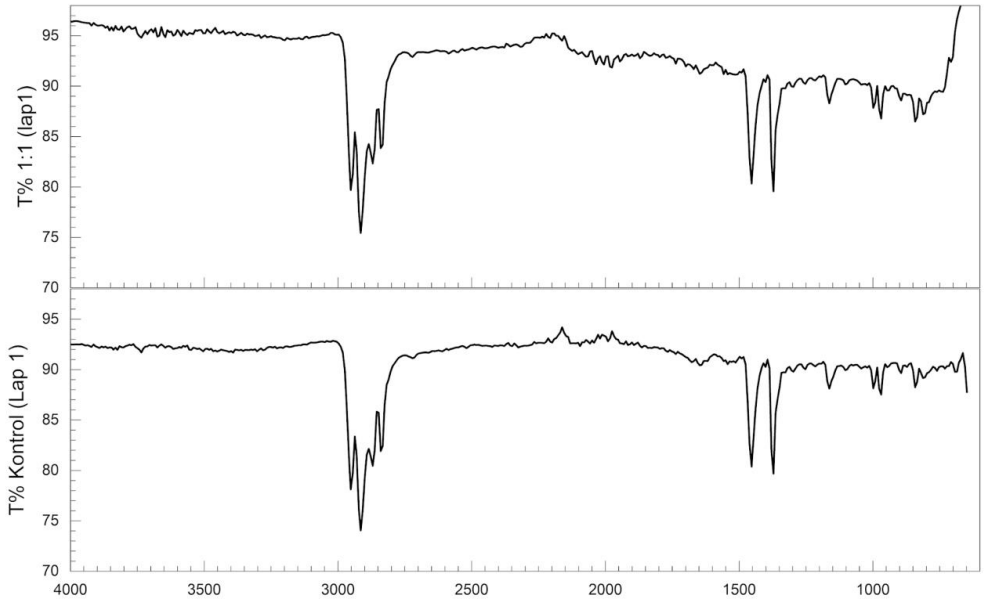
Gravimetric analysis was conducted to evaluate the weight loss of disposable masks before and after biodegradation. The analysis was performed on the mask samples with the LCN:NB (1:1) ratio and the control mask (without bacterial inoculation). The results, presented in Table 2, demonstrate a significant mass reduction in the control mask sample compared to the sample with LCN:NB (1:1) media.

Table 2. Gravimetric analysis results

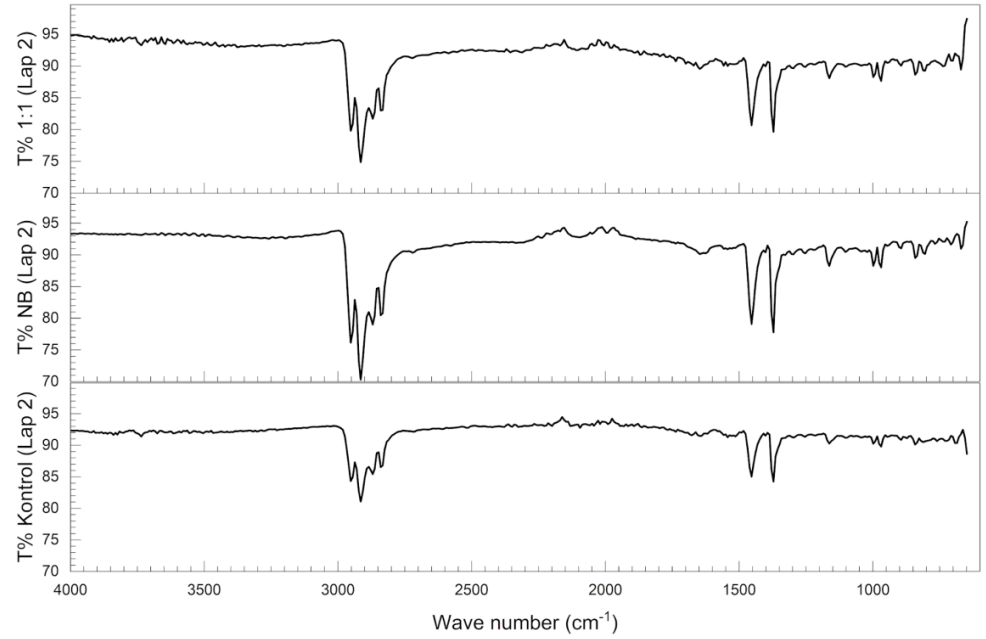
Sample	Mask Mass Before Degradation (gr)	Mask Mass After Degradation (gr)
Kontrol Masker	0,2003 ± 0,0001	0,1968 ± 0,0033
Variasi C LCN:NB (1:1)	0,2003 ± 0,0001	0,1986 ± 0,0045

The larger weight loss observed in the control sample can be attributed to the absence of bacterial biomass, which led to a cleaner mask surface during weighing, thus allowing more mass to be lost. Conversely, the LCN:NB (1:1) mask sample, which was colonized by bacteria, showed less weight reduction due to the presence of bacterial biomass on the mask surface (Figure 3a and 3b). The microscope image of the LCN:NB (1:1) sample (Figure 3b) revealed holes in the mask material, suggesting microbial activity on the surface. The lower weight loss observed in this sample implies that bacterial growth and biomass attachment on the mask surface had a protective effect, reducing the overall mass loss. As shown in Figure 3c, the bacterial growth likely facilitated the metabolism of carbon sources from both the mask material and the media, contributing to microbial growth and partial degradation (Atanasova et al., 2021).

a.



b.



c.

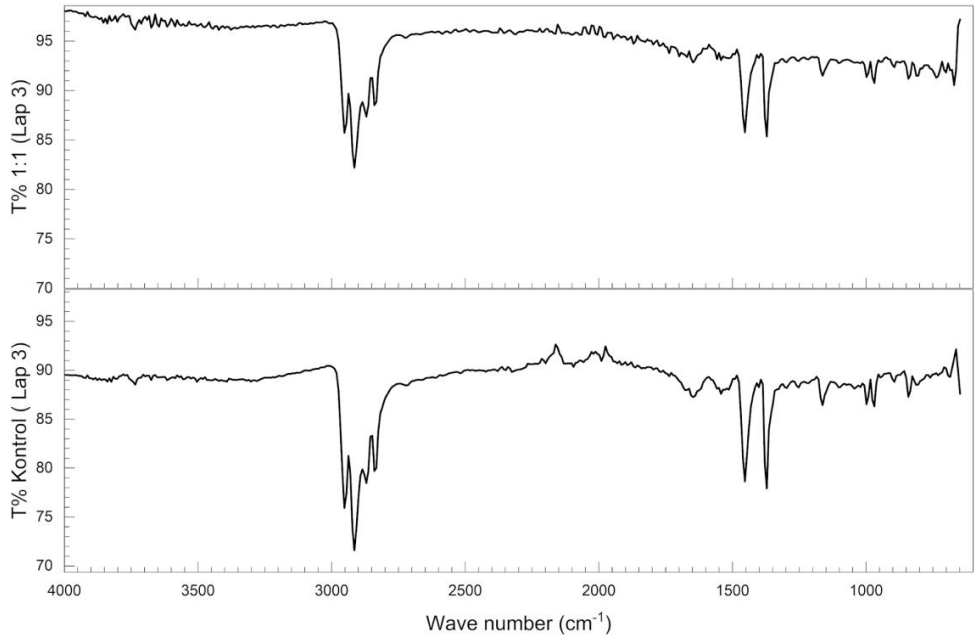


Figure 3. FTIR analysis of mask samples in LCN:NB (1:1) media (a) first layer; (b) second layer; (c) third layer

In a related study, Auta et al. (2018) examined polypropylene degradation using *Bacillus* sp. and observed a weight loss of 4% after 40 days of degradation. This suggests that the bacterial isolate used in our study is capable of secreting enzymes that break down polymer bonds, enhancing the degradation process. Initial steps in the biodegradation of polymers, such as masks, involve weakening the polymer structure and depolymerizing it into shorter chains. The bacteria likely catalyze metabolic reactions that facilitate the adsorption, desorption, and breakdown of polypropylene microplastic bonds (Auta et al., 2018). Enzymes such as laccase, manganese peroxidase, and lignin peroxidase are known to catalyze the microbial degradation of polymers (Kavitha & Bhuvaneswari, 2021).

Fourier-transform infrared (FTIR) analysis of mask samples after degradation process

Fourier-Transform Infrared Spectroscopy (FTIR) was used to analyze changes in the functional groups of degraded mask samples. FTIR analysis provides insights into the degradation process by identifying shifts in the characteristic wave numbers corresponding to functional groups. The analysis was performed on the degradation samples, including the control mask and the mask with LCN:NB (1:1) media.

As noted previously, polypropylene (PP) is a primary component of the masks, a combination of transition metal compounds with alkyl aluminum or ZN catalyst (Zieglere Natta). PP consists of compound monomers with a structure of $(C_3H_6)_n$ which has a fairly high melting point of 165°C and a crystallization point between 130-135°C with a decrease in crystallinity of 20-25% (Gahleitner and Paulik, 2017).

Figure 4 shows a change in the peak of the wave numbers after degradation, likely due to interference from environmental factors during the degradation process. The FTIR analysis revealed a significant decrease in % transmittance for layer 2, especially in variation C. This indicates that there is absorption in the polypropylene bond due to the degradation process carried out.

Plastic biodegradation occurs due to the presence of microorganisms on the plastic surface that are able to secrete decomposing enzymes. These enzymes mainly include lipases or dehydrogenases that attack the polymer substrate after hydraulic cleavage. As a result, the polymer is degraded into smaller molecules which are eventually

converted into carbon dioxide or water through microbial metabolism (Cai et al., 2023).

Based on Figure 4, the FTIR spectra showed a peak range of 2850-2970 cm^{-1} associated with the alkyl C-H stretch, 1453.7 cm^{-1} indicating the methyl group (CH_2), and 1386.6 cm^{-1} corresponding to CH_3. The cleavage of C-H bonds affects the intensity of the CH_2 and CH_3 absorption bands. This bond cleavage can be catalyzed by enzymes such as dehydrogenase, which target the polymer substrate (Cai et al., 2023).

Additionally, hydroxyl groups (O-H) appeared in the LCN:NB (1:1) media variation at peaks 3302.4-3563.3 cm^{-1}, due to bond breakage from the oxidation process caused by bacterial biodegradation (Oliveira et al., 2022). Furthermore, C=C groups were found at peaks 1513.3-1543.1 cm^{-1} in all variations, indicative of thermos-oxidative decomposition (Andriani et al., 2022). After degradation, C≡C groups were detected at 2109.7-2161.9 cm^{-1} in the LCN:NB (1:1) layers 2 and 3, suggesting further bond breaking.

In the biodegradation of polypropylene, the main challenge is that it must first go through a change in the physical structure of the polymer to aliphatic carbon. PE and PP are plastics that have C-C chain bonds that are resistant to degradation. In addition, PP is also more resistant to damage to functional group bonds compared to PE so that it rarely decomposes by biodegradation without a pretreatment process such as exposure to high temperatures or exposure to UV light for a long time. This C-C chain can be broken by alkane dehydrogenase (Jeon et al., 2021). The appearance of a peak at 1680-1620 (alkene) which is one of the important groups in the plastic degradation process. This is where the formation of alkenes and the formation of carbonyl groups can trigger the chain cutting process which is a stage in the chemical degradation process (Khoironi et al., 2020). The process of breaking the C-C bond involves the elimination of hydrogen atoms from two adjacent carbons. In addition, bond breaking can also occur due to the oxidation process where contact occurs between the mask and the media containing H_2O compounds during the degradation process which will later produce O-H group bonds.

CONCLUSIONS

Based on the research that has been done, LCN can be used as a growth medium for bacterial isolates which will later be used for the degradation process. The degradation process resulted in a greater weight loss in the control mask compared to the variation using LCN:NB (1:1) media. This is because in the LCN:NB (1:1) variation there is biomass attachment to the surface of the mask. The results of this degradation are reinforced by FTIR analysis which states that there is a change in intensity of the functional groups C-H, C-H_2, C-H_3, and C=C in each layer of the mask sample. The second layer mask sample experienced an increase in intensity, conversely there was a decrease in intensity in the first and third layers. In addition, in the second and third layers of mask samples with NB:LCN (1:1) media, the emergence of the C≡C group was found after the degradation process.

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