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Studies on the Influence of Mixed Culture from Buried Soil Sample for Biodegradation of Sago Starch Filled Natural Rubber Latex Gloves

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Abstract. The addition of sago starch into natural rubber (NR) latex compounds was aimed to accelerate the degradation process of the end products including gloves as an alternative to reduce solid waste disposal problem. In this research work, the degradation of sago starch filled natural rubber (SS/NR) latex gloves by a mixed culture from buried soil sample was investigated. The Gram staining test of mixed culture showed the Gram-negative bacteria are the dominant species as an effective bacterium to degrade the SS/NR latex gloves. The starch hydrolysis test was performed to confirm the existence of starch-degrading bacteria in mixed culture that consume sago starch from the gloves. Based on the biodegradation rate results obtained, the existence of starch-degrading bacteria together with rubber-degrading bacteria were observed, which accelerate the biodegradation of SS/NR latex gloves by 53.68%. Meanwhile, the biodegradation rate for sago starch unfilled natural rubber (control) latex gloves were only 50.31%. Thus, the addition of sago starch has shown an increased in the biodegradation rate, which can be used as alternative method to overcome the solid waste disposal problem.

1. Introduction

Latex gloves have been designed for single use, which makes their disposal becomes the worldwide solid waste problem. The rubber waste can be eliminated by either burned or used as landfills, but these processes caused serious pollution [1]. One of the alternative ways to overcome this environmental problem is by addition of natural biodegradable fillers such as starch base materials into the gloves formulation [2]. Although natural rubber (NR) latex is able to biodegrade, the incorporation of starch can accelerate the microorganism activities, thus able to decompose the composites effectively. It is reported that the bacteria as well as *fungi* have the ability to degrade the rubber but the process is slow [3].

Sago starch commonly used as filler or matrix to produce biodegradable composites due to the incorporation of the starch not only assists in the disintegration of polymer composites but also inexpensive and naturally abundant [4]. Polysaccharide chain of amylose and amylopectin from the starch is consumed by microbes in the soil to produce enzymes such as amylases and glucosidases that break the glucosidic linkages in starch [5]. Furthermore, it was suggested that microbes favored to consume simpler glucosidic linkages in starch during biodegradation periods compared to polyisoprene molecules of NR latex alone [3].

In biodegradation process, the rubber-degrading bacteria consume NR as the sole carbon and energy sources for their growth. The rubber has to be cleaved extracellularly into low-molecular-weight compounds; so that it can be transported across the cell membrane and used for metabolism [6]. The structure of NR, which has one functional group of carbon-carbon double bond per isoprene unit, is the primary target of NR-cleaving enzymes called rubber oxygenases

[7]. The secretion of rubber oxygenases by rubber-degrading bacteria is used to initiate the break-down of the polymer and to consume the generated cleavage products as a carbon source.

To date, the previous studies have shown most of microorganism only degrade latex samples without fillers [8-9] while the studies about the effect of the integration of sago starch into latex compounding on microbial degradation are still limited. Thus, this paper aims to investigate the influence of mixed culture from the buried soil sample on the biodegradation of sago starch filled natural rubber (SS/NR) latex to confirm the existence of starch-degrading bacteria that consumed the sago starch from the gloves which accelerate the biodegradation process.

2. Materials and Methods

2.1. Materials

NR latex with the initial properties of dry rubber content (DRC) of 60.08wt%, total solid content (TSC) of 61.00wt%, mechanical stability time (MST) of 1140 s, and volatile fatty acid number (VFA no.) not exceeding 0.2 was purchased from Zarm Scientific and Supplies Sdn. Bhd. (Penang, Malaysia). Meanwhile, sago starch dispersion was produced in-house and other compounding ingredients such as diethyldithiocarbamate (ZDEC), zinc oxide (ZnO), potassium hydroxide (KOH), 2,2'-Methylene-bis (4-methyl-6-tert-butylphenol) and sulfur were obtained from Farben (Malaysia) Sdn. Bhd. In addition, the Gram staining kit such as crystal violet, Gram's iodine, ethyl alcohol and safranin was purchased from Pro-Lab Diagnostics Inc. (United Kingdom).

2.2. Preparation of NR latex compound and gloves

The NR latex compound was prepared with 31.00 ± 0.20wt% of TSC. The preparation of NR latex compound was carried out in accordance with a previous study [3]. The compound was pre-vulcanized at a temperature of 70°C and the process was stopped until the degree of vulcanization has achieved chloroform number 2. Then, the compound was left overnight under room temperature for the latex maturation process. The formers with pre-layered of calcium nitrate were dipped into the maturated compound and cured in the oven for 25 minutes at 110°C. The gloves were stripped with the assistance of talc powder to avoid the gloves from sticking.

2.3. Biodegradation procedure and weight loss analysis

The gloves were buried in the soil for 5 months for biodegradation process. Upon collection, the films were rinsed and dried at room temperature to a constant weight and the weight was recorded. Weight loss of the samples with time was used to determine the degradation rate of the samples by following Equation 1:

$$\text{Weight loss (\%)} = [(W_i - W_d)/W_i] \times 100 \quad (1)$$

Where W_i is the initial dry weight of the sample and W_d is the dry weight of the sample after biodegradation process.

2.4. *Starch hydrolysis test*

1 g of each soil buried control and SS/NR latex gloves were mixed with 50 ml of MSM in a 250 ml flask. The flasks were agitated at 150 rpm for 3 days at 30°C. Subsequently, 100 µL of each sample was spread on latex overlay agar. The plates were then incubated at 30°C for 3 days. To detect starch-degrading bacteria, the most abundant dissimilar morphology of the mixed culture from the incubated plates was transferred to a fresh starch agar for the iodine test. The iodine test was conducted by flooding the plates with Gram's iodine for 10 minutes [10].

The latex overlay agar used in the starch hydrolysis test was prepared by the overlay technique according to Braaz and co-workers [11]. The composition of mineral salts medium (MSM) used in latex overlay agar was based on the ingredients described by Heisey and Papadatos [12] and supplemented with 0.02 g/L of yeast extract. In the meantime, 15 g/L of bacteriological agar powder was mixed into the culture medium for agar preparation. During the agar preparation, 0.6% of purified NR latex was added to MSM. Meanwhile, 3 g/L meat extract, 5 g/L peptone, 2 g/L soluble starch and 15 g/L bacteriological agar under pH 7.2 was used to prepare the starch agar.

2.5. *Gram staining test*

A loopful of the culture has been placed on the slide and it was fixed with the heat in order to kill the bacteria in the smear, adhere the smear to the slide and allow the sample to more readily take up the stain. The staining was done by flooding staining reagents by the order: crystal violet, Gram's iodine, ethyl alcohol and safranin. The results were observed under oil immersion (100x) using a light microscope.

3. Results and Discussion

There are various bacteria can hydrolyze the starch, which called starch-degrading bacteria. The existence of this type of bacteria is the key factor to determine the effectiveness of incorporation of sago starch into latex compounding for biodegradation purposes which was done by measuring the biodegradation rate of the buried gloves.

Figure 1 showed the positive reaction towards iodine on starch agar plates which containing bacteria from SS/NR latex gloves. The formation of the clear zone around the bacteria indicates the starch was hydrolyzed which means the bacteria were capable of breaking down the starch into maltose through the activity of the extra-cellular α-amylase enzyme. Based on the results obtained, it is proven that the mixed culture from buried sample contains the starch-degrading bacteria which are very beneficial for the application of SS/NR latex gloves.

As sago starch being introduced into the NR latex system, the starch employed the void within NR latex colloidal particles and remains as cementing substances for the films [13-14]. The cementing mechanism among starch and NR latex particles depends on the interaction of random coiled amylose and lipids in rubber particles [13]. As the hydrolysis process occurs in amylose, the linkages are broken and reduced the effective interaction with rubber particles and hence decreased the physical integrity of SS/NR latex gloves. Thus, it increased the feasibility of the SS/NR latex gloves to biodegrade.

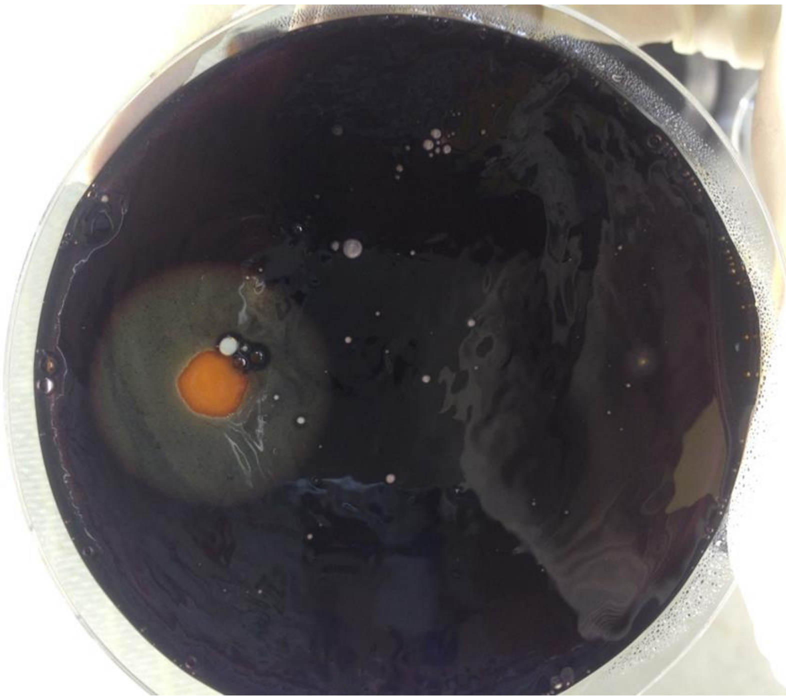

Fig. 1. Clear zone formation on the starch agar plate

Figure 2 showed the biodegradation rate of the control and SS/NR latex gloves throughout the 5 months of cultivation in the soil. Although both types of gloves showed a sign of degradation, the biodegradation of SS/NR latex gloves gave the higher rate. It is proven that the existence of starch-degrading bacteria to consume the starch can accelerate the biodegradation of SS/NR latex gloves by 53.68%. Meanwhile, the absence of starch-degrading bacteria in control latex gloves gave lesser biodegradation rate i.e. 50.31%.

Natural degradation of NR latex is a slow process, as crosslinks between rubber polymer chains, and the many additives and stabilizers within its structure, making it extremely resistant to biodegrade [15]. Therefore, the concept of a combination of starch into latex/rubber compounding gains much interest from other researchers. Kiing et al. [2] studied the potential of incorporating NR latex into chemically modified starch for the making biodegradable blends. They found that the modified starches that blended with 20% NR latex would still be able to biodegrade up to 50% within 12 weeks. Afiq and Azura [3] also found that sago starch is a nutritive substance to accelerate the growth of microbial colonies on the surface of NR latex films during the soil burial test. It was suggested that microorganisms favored consuming simpler glucosidic linkages in starch during biodegradation periods compared to polyisoprene molecules of NR latex films alone.

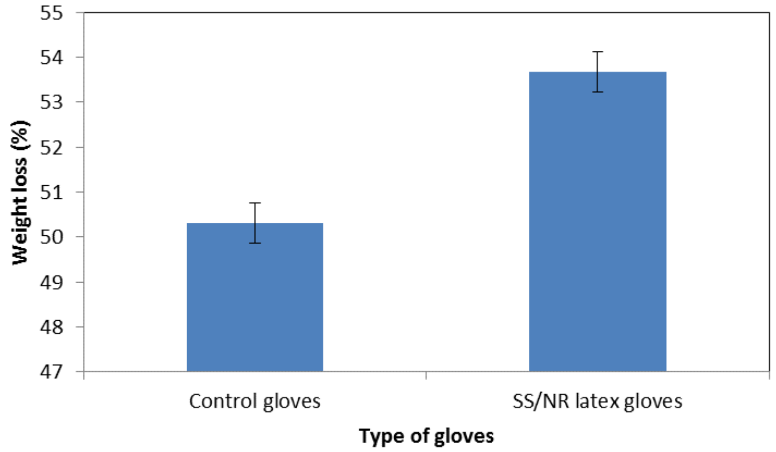

Fig. 2. Biodegradation rate of the gloves after 5 months of biodegradation periods

Further study has been done on the Gram staining to confirm the buried soil has different mixed culture. Based on Figure 3, there are lots of red stains compared to purple stains taken from SS/NR latex gloves. It indicates there have lots of residential starch- and latex-degrading bacteria that are Gram-negative.

The Gram-negative bacteria also have the capability to degrade latex/rubber composites. Linos et al. [16] found that strain AL98; a Gram-negative bacterium, was able to disintegrate NR, either in the raw state as NR latex concentrates or in the vulcanized state as NR latex glove, as well as raw synthetic *cis*-1,4-polyisoprene. Besides, Birke at al. [17] also claimed the *Xanthomonas* sp. 35Y is the only known as Gram-negative for rubber degrader.

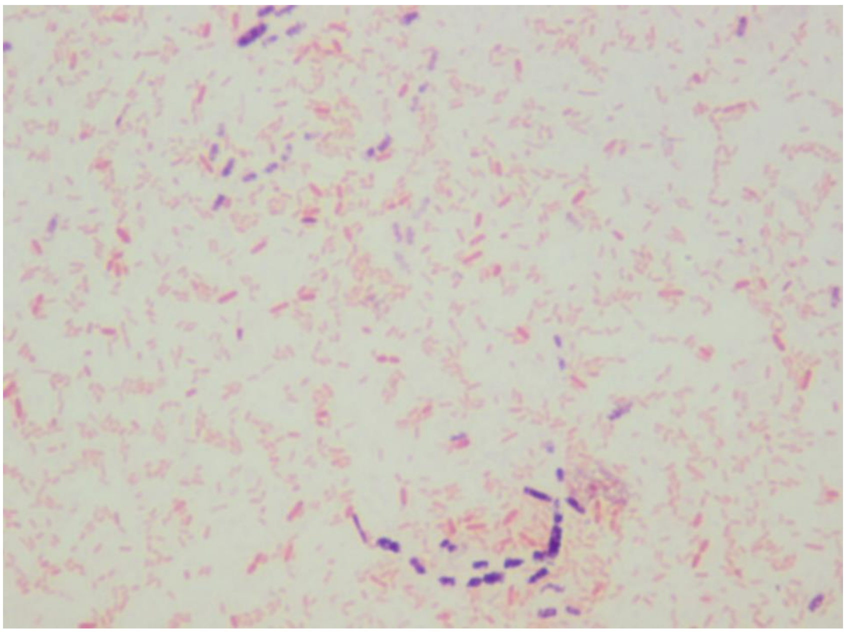

Fig. 3. Cell morphology of mixed culture under a light microscope (Magnification: 100x)

4. Conclusions

In conclusion, it was confirmed that a mixed culture from buried soil showed the potential in degrading NR latex gloves within 5 months. The existences of starch-degrading bacteria, as well as rubber-degrading bacteria, proved that it can accelerate the biodegradation of SS/NR latex gloves.

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