BACTERIAL DEGRADATION OF NATURAL RUBBER LATEX FILM BLENDED WITH METROXYLAN SAGO PITH FORM

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ABSTRACT

The demand of rubber products is increasing time to time. Due to the high consumable and disposable of rubber latex products, hence remain inert to degradation and leading to their accumulation in the environment. An attempt to combine Natural Rubber (NR) latex system with other degrading materials have been made to facilitate biodegradation process. This paper shows the degradation of natural rubber (NR) and NR latex film blended with metroxylan sago pith waste (NR/TSPW latex film) by isolated Bacillus cereus ATCC 14579. B. cereus is proved able to utilise rubber as sole source of carbon and energy. The biodegradation studies were analyzed by growth profile, weight loss, protein content and tensile strength test. Fourier Transform Infrared Spectroscopy (FTIR) analysis was also used to confirm the biodegradation process. In shake cultures, OD of culture increased by 19.2% from the initial inoculum after 14th cultivation days with NR/TSPW latex film. An increase in protein content up to 0.037 mg/g with 12.377 % weight loss of film were obtained after biodegradation. Tensile test result shows that tensile strength and elongation break are decreased by 10.203%. Bacillus cereus ATCC 14579 could provide a biotechnological solution to the waste rubber disposal problem.

Keywords: Biodegradation; metroxylan sago pith waste; rubber latex.

INTRODUCTION

Rubber products takes a long time to degrade naturally and causes serious environmental problems in the municipal landfills. The methods of re-using and recycling rubber products are inefficient, not environmental friendly and required sophisticated equipment to deal with the environmental problems addressed [1]. Thus, some attempts have been made to exploiting starch-containing waste as filler to form biopolymers [2]. Previous research works [3-5] has proven that the use of sago starch can enhance the biodegradation properties of latex products. In biodegradation process, gram-positive microbial will produce latex-clearing protein (Lcp) and starch hydrolysis enzyme to hydrolyze polyisoprene and starch, respectively [6]. In previous studies, most of gram-positive NR degrading bacteria belongs to the group of actinomycetes [7-11]. However, there is only one report about degradation of NR with Bacillus genus, which is *Bacillus sp.* SBS25 [12]. However, food crisis issue raised and treated *Metroxylan sagu* pith waste (TSPW) is used to replace sago starch in the NR compounding system. TSPW is a fibrous residue rich in (60–80 wt%) sago starch which is formed from the sago starch extraction process. The sago starch is extracted from the rasped pith of the sago palm [13,14]. Therefore, NR latex is blended with TSPW to accelerate the biodegradation process of films. This study focus on identification of Bacillus genus NR degrading bacteria and its biodegradation process on NR latex and NR/TSPW latex film.

METHODOLOGY

Natural Rubber (NR) Latex Film Samples

The NR films samples were prepared. Each sample was buried separately in compost soil for 12th week of biodegradation period. In the meantime, TSPW/NR latex films were prepared for the further studies.

Isolation of Rubber-degrading Bacteria

Approximately 1 g of each soil buried NR latex films was added to 50 mL of mineral salts medium in a 250 mL flask. The flask was shaken on 150 rpm at 30°C for 3 days and it was serially diluted. Subsequently, 100 µL of each dilution samples were spread on latex overlay agar. All plates were incubated at 30°C for 7 days. Bacterial colonies with different morphology were streaked on a fresh nutrient agar (Sigma-Aldrich).

According to Braaz and colleagues [15], latex overlay agar was prepared by the overlay technique. The composition of mineral salts medium used in latex overlay agar was followed the ingredients described by Heisey and Papadatos [16]: 2.0 g/L $NaNO_3$, 0.5 g/L $MgSO_4$, 0.5 g/L KCl, 0.01 g/L $Fe_2(SO_4)_3.H_2O$, 0.14 g/L KH_2PO_4, 1.2 g/L K_2HPO4 and supplemented with 0.02 g/L of yeast extract. Meanwhile, 15 g/L of bacteriological agar powder was mixed into medium for agar preparation. During the agar preparation, 0.6% of purified Natural Rubber (NR) latex was added to mineral salts medium. The starch agar (5 g/L peptone, 3 g/L meat extract, 2 g/L soluble starch and 2 g/L yeast extract) were prepared for further studies.

Biodegradation Test

The washed films were cut into small pieces and the exact initial weight was recorded. Approximately 1 g of films were dissolved in 100 mL of acetone for 12 hours and allowed to dry, respectively. The treated films were sterilized by autoclaving prior added into culture medium. Thereafter 10 % of starter culture (*Bacillus cereus* ATCC 14579) was transferred into 150 mL of mineral salts medium in a 500 mL flask. Approximately, 100 mg of treated films was added into the culture. The culture was cultivated on 150 rpm for 14 days at 30°C. The experiment was carried out with three replicates for each NR and TSPW/NR latex films. Control set was prepared with minerals salts medium added with treated films, respectively without inoculums. The optical density (OD) of culture were measured at 600nm. The films were removed from culture before centrifugation, washed and dried in oven until constant weight were achieved. The final weight was recorded. Control was harvested at 14th days of cultivation.

Molecular Identification

Molecular analysis was carried out by Centre for Chemical Biology (CCB), Penang, Malaysia. GF-1 Bacteria DNA Extraction Kit was used to obtain pure DNA sample. Further, the purity of DNA was determined by Nanodrop 2000 Spectrophotometer. The 16s rRNA gene was amplified by PCR using universal primers 16S-27F and 16S-1492R. PCR was carried out under the following conditions: 94°C (3 min), 30 cycles of 94°C (30 s), 55°C (30 s) and 72°C (1.4 min) and a cycle of final extension at 72°C (5min). The obtained sequences were analysed using BLAST analysis provided by NCBI (National Centre for Biotechnology Information, https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Protein Determination

Protein contents of *B. cereus* grown at different NR latex film were determined according to the method of Bradford [20]. The harvested cultivation media to be tested were prepared after filtering the culture via syringe filters 0.45µm pore size, respectively. The 10 µL of sample was mixed with 200 µL Bradford reagent (5x dilution, Sigma-Aldrich) in a 96 well microtiter plate. The solution was incubated at room temperature in dark condition for 10 minutes. The absorbance was then measured at 595 nm by using microplate reader (Halo MPR-96, Dynamica Ltd). Four samples were prepared and the average values were calculated.

Weight Loss

Upon collection after 14th days cultivation, the films were rinsed and dried at 40 °C to a constant weight and the weight 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 ids the dry weight of sample after biodegadation,

Mechanical Properties Test

Tensile strength test was conducted using Instron® 3366 Machine (Norwood, MA, US) according to ASTM D 412-06 at room temperature. The control NR and TSPW/NR latex films were cut into dumbbell shapes. The thickness of each sample was recorded. The crosshead speed was set at 500mm/min. Four samples were prepared and the average values were calculated.

Fourier Transform Infrared (FTIR) Analysis

A Fourier Transform Infrared (FTIR) spectrometer (Perkin-Elmer Model Series 2) was used to study the changes in chemical functionality of latex samples. The equipment was operated with a resolution of 4 cm^{-1} and peak height was used to represent the IR intensity, which is expressed as absorbance. IR spectra were collected by using attenuated reflectance. The FTIR spectra were recorded in the scaning range of 4000 – 400 cm^{-1}.

RESULTS AND DISCUSSION

Isolation of Rubber-degrading Bacteria

There are various bacteria can hydrolyse starch, however, an ability to hydrolysed rubber is restricted to a few genera [11]. Therefore, the research was conducted first to select the rubber-degrading bacteria rather than to test their ability to degrade modified NR. The modified NR used is TSPW/NR latex films.

The isolate *B. cereus* was selected because of its most rapid growth on latex overlay agar. Although it non-clear zone former, it showed positive reaction to iodine on starch agar plates by producing clearing zones on it. *B. cereus* is Gram-positive bacteria [Figure 1] with 2-7 mm in diameter. These non-pathogenic bacteria were found to have irregular, with undulate, crenate and fimbriate edges, and matt or granular textures as described in the [17]. However, the drawback of latex overlay agar is not all rubber-degrading bacteria can form clear zone area on such plates. It's because only little amount of isoprene available on the plate that are not compatible with some bacteria growth system [11].

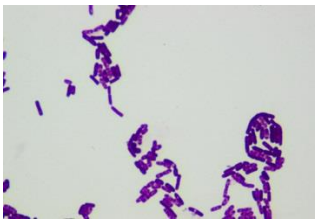

Figure 1. Cell morphology of isolate *B. cereus* under light microscope

Biodegradation Studies

The biodegradation study was conducted to further confirm the rubber-degrading ability of *B. cereus*. Figure 2(A) showed the growth of *B. cereus* in the cultivation medium. The OD of culture for TSPW/NR latex film were extremely highest. The OD of culture NR latex film increased by 10.5% from 0.227 on day 0 to 0.332 on 14th days. Meanwhile, the OD of the culture increased by 19.2 % in TSPW/NR latex films. The increment of OD from 0.204 on day 0 to 0.396 on 14th days indicated the growth of *B. cereus* faster than NR latex film.

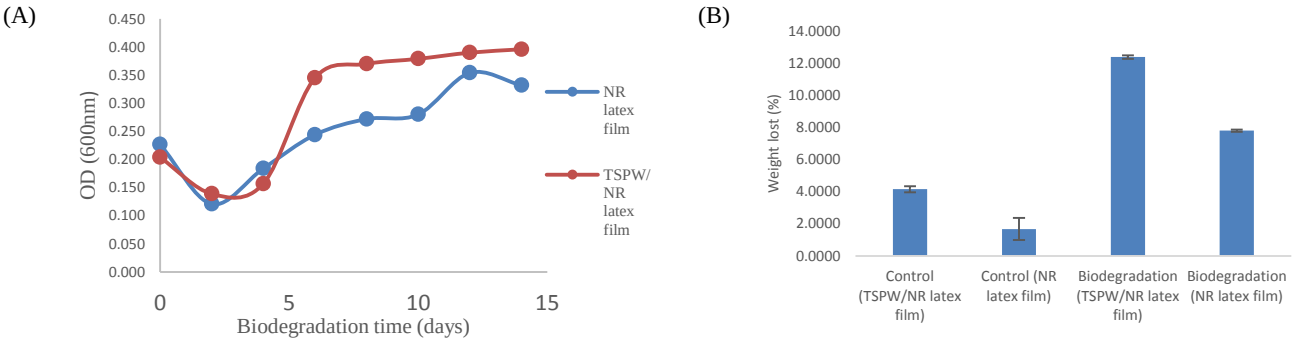

Figure 2. (A) The growth of *B. cereus* and (B) the weight loss of films after 14th days of biodegradation periods

Protein content measurement also have been used as evidences of biodegradation of NR and TSPW/NR latex films by *B. cereus*. After 14th cultivation, an increase in protein up to 0.037 mg/g after incubate TSPW/NR latex film with *B. cereus*. In contrast, an increase 0.030 mg/g in the protein content was found for NR latex film.

Table 1. Protein content after 14 days' biodegradation periods

Types of film	Protein content (mg/g)
Control (TSPW/NR latex film)	0.027
Control (NR latex film)	0.023
Biodegradation (TSPW/NR latex film)	0.037
Biodegradation (NR latex film)	0.030

Figure 2(B) showed the weight loss of the NR and TSPW/NR latex films throughout 14 days of cultivation in mineral salts by *B. cereus*. It is clearly showed *B. cereus* can accelerate the biodegradation process of films with compare to control film. Under these conditions, a weight loss of 7.799% was achieved for NR latex films and a 12.377% weight loss for TSPW/NR latex films.

Tensile strength analysis

For control films, the addition of TSPW in NR latex films decreased in tensile strength (Figure 3(A)) due to poor formation of sulphur crosslinking in the rubber particles. The TSPW/NR latex films showed the lowest tensile strength and elongation at break (Figure 3(B)) after biodegradation periods. This indicated B. cereus more favoured to hydrolyse glycosidic linkage using starch hydrolysis enzyme compared to polyisoprene chain [11]. As the breakage of these bonds, the films could not resist the stress applied hence decreased the tensile strength of the films.

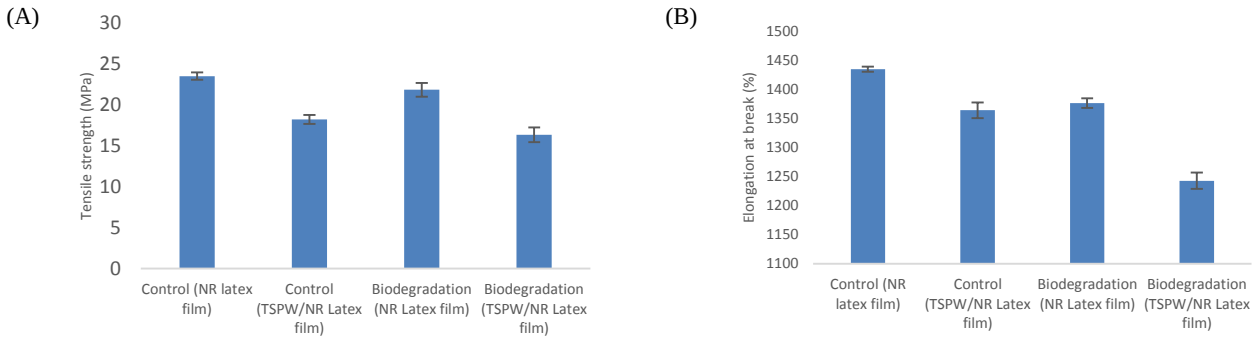

Figure 3. Effect of biodegradation process on films (A) tensile strength and (B) elongation at break

Fourier Transform Infrared Spectroscopy (FTIR) analysis

Examination of the graph [Figure 5] showed FTIR profile for different types of films. The films, which were cultivated by *B. cereus* were subjected for FTIR studies peaks were observed at the wavelength between 550-4000 cm^{-1}.

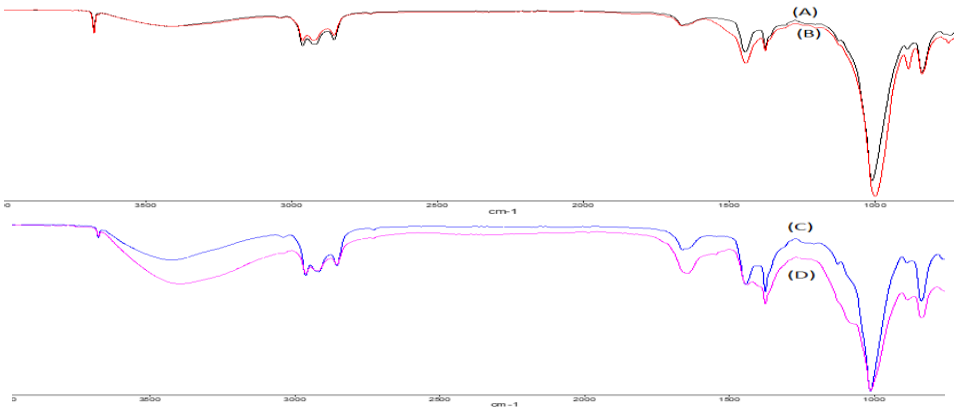

Figure 5. FTIR profile for different types of film with (A) Control (NR latex film), (B) Control (TSPW/NR latex film), (C) Biodegradation (NR latex film), (D) Biodegradation (TSPW/NR latex film)

Oxidation process cleave polyisoprene chain at doubly bonded carbon. During the bond scission, the formation of aldehyde and ketone groups can be detected as the chain is cleaved by homolysis process, with epoxide group as intermediates [8,18,19].

Table 2. Major peaks in the FTIR spectra for NR and TSPW/NR latex film

Wavenumbers, cm^{-1}	Description
3200 – 3600	OH stretching [7]
2800 – 2960	Methyl and methane group streching
1736	CO stretching vibration means the formation of aldehyde and ketones group [11]
1638	OH bending of the absorbed water
1440	Lignin of TSPW
1376	In-the plane CH bending
1010 – 1120	Unsymmetrical C-O-C and C-O bond of primary alcohol showed. Methyl and methane group vibration. The epoxide group observed.
780 – 920	Double bond vibration Methane group vibration in starch [19]

CONCLUSION

Bacillus cereus ATCC 14579 isolated from NR latex film is a non-clear zone NR degrading bacteria but showed positive result to the starch utilization. It is identified by colony morphology and 16S rRNA analysis. Most of the studies on the biodegradability have been clearly visualize by its growth profile, protein content and weight loss. Analysis of FTIR showed the decreased in double bonds and the formation of carbonyl groups (ketone and aldehyde group). The spectra proved the polyisoprene chain and sago starch were being used as carbon sources during biodegradation by B. cereus. Although the addition of TSPW decreased films tensile strength in NR latex film, it will help to promote the biodegradation process. Hence, the introduction of TSPW in rubber products can degrade by B. cereus and further, the solid waste disposal management improved.

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