The potential use of immobilized *Bacillus cereus* ATCC 14579 for biodegradation of natural rubber latex film added with *Metroxylon Sagu* pith form

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Abstract. The previous study reported that *Bacillus cereus* ATCC 14579 able to degrade natural rubber latex films added with *Metroxylon Sagu* pith waste form (MSPW/NR) and natural rubber (NR) latex films as main source of carbon and energy. Some attempts have been made to degrade them in the newly batch systems rather than disposing of them in landfill. Immobilization technique was performed to adsorb *Bacillus cereus* onto modified activated carbon. This work aims to study the potential of immobilized *Bacillus cereus* for biodegradation of MSPW/NR. The results revealed that 30% nitric acid for 2 hours impregnation time showed significant influence on *Bacillus cereus* adsorption. The highest adsorption capacity of *Bacillus cereus* was found to be 72.8% under its optimum conditions. The weight loss of MSPW/NR by 12.843 % were obtained after 14th days biodegradation period. Thus, immobilization of *Bacillus cereus* ATCC 14579 has potency to provide a biotechnological solution to the waste rubber and latex disposal problem.

1. Introduction

The increasing of various diseases outbreak across international borders are making people more concern about their healthcare awareness, such as good personal hygiene. This awareness was identified as one of the main drivers that contribute to the overall production of disposable rubber gloves around the world. However, in recent years, the waste disposal problem due to the growing consumption of latex gloves has created a demand for scarce landfill space and environmental healthcare [1,2]. Many studies have been made to encounter this problem such as the production of biodegradable gloves [3,4] and identification of rubber-degrading bacteria to accelerate biodegradation of gloves [5-11]. However, the space of landfill getting smaller by time to time.

As far as the authors are aware, there is no published analysis of NR products degradation using identified rubber-degrading bacteria. Previously, we managed to identify *Bacillus cereus* ATCC 14579 as the most effective rubber-degrading bacteria for biodegradation of natural rubber latex films added with *Metroxylon Sagu* pith waste form (MSPW/NR) [12]. It seemed of interest to create a batch system by immobilizing *B. cereus* onto activated carbon (AC) for the degradation of disposable rubber gloves. The application of this process makes bacteria able to reuse for the next biodegradation batch cycle, reduce the odor as well as site-specific targeted method.
Thus, the main objective of this study was to investigate the potential of immobilized Bacillus cereus ATCC 14579 for MSPW/NR latex films. In this study, the commercial AC were performed the chemical modification to enhance functional groups which increase the immobilization interaction of *B. cereus* onto the modified activated carbon (MAC). The immobilized *B. cereus* has tested its functionality towards biodegradation of MSPW/NR latex films as their sole carbon and energy sources.

2. Materials and Methods

*Bacillus cereus* ATCC 14579 previously isolated from buried MSPW/NR films and was used throughout this study. The culture was maintained on nutrient agar (Merck, Germany) slants and stored at 4°C. During the biodegradation process, mineral salts medium (MSM) was used in this study. The composition of MSM containing (g L\(^{-1}\)) 2.0 NaNO\(_3\), 0.5 MgSO\(_4\), 0.5 KCl, 0.01 Fe\(_2\)(SO\(_4\))\(_3\), 0.3 H\(_2\)O, 0.14 KH\(_2\)PO\(_4\), 1.2 K\(_2\)HPO\(_4\) and supplemented with 0.02 yeast extract [13]. MSPW/NR latex films were used as sole carbon and energy for culture.

Meanwhile, the modification of the coconut shell based commercial granular AC purchased from Bendosen Laboratory Chemicals, Malaysia was washed with deionized water to remove fine particles and dried at 105°C for 24 h. About 5 g of AC was impregnated with 50 mL of nitric acid (HNO\(_3\)) at 30 wt. % concentration in 250 ml conical flask with a reflux condenser, respectively. The samples were placed on a hot plate for the reflux process. The impregnation time was tested at 2 h. After cooling, MAC was separated and washed several times with deionized water until the pH of the filtrate was constant. The washed MAC were dried at 105°C in a hot air oven for 24 h. The MAC was then sieved through a 1-5 cm sieve. About 3 cm particle size MAC was used as adsorbent and isolated *B. cereus* act as adsorbate in this study.

The MAC was used *B. cereus* was cultivated in nutrient broth on 150 rpm, 30°C for 6 h. The culture concentration OD 1.5 was then centrifuged at 4000 rpm for 5 min to recover the cells. The pellet was washed three times with sterile distilled water and was suspended in 25 ml of 1% Sodium chloride (NaCl) (Merck, Germany), pH 7.0 in 100 ml flask. About 2 g of MAC was added into the flask. The flask was agitated at 45 rpm, 30°C for 24 h [14]. The MAC was separated and the final concentration of culture was monitored by turbidometry in terms of optical density (OD) using DR 2800 spectrometer at 600 nm wavelength. Scanning Electron Microscope (SEM) analysis was done to observe the attachment of *B. cereus* onto MAC. The MAC which present immobilized *B. cereus* was adhered on a sample stage and then coated with gold before subjected to U-8000, Hitachi Co. Ltd. Japan. The adsorption capacity was used to determine the adsorption of *B. cereus* onto MAC was calculated by following Equation 1,

\[
\text{Adsorption capacity (\%) = } \left[ \frac{(B_i - B_f)}{B_i} \right] \times 100\% \quad (1)
\]

where \(B_i\) is the initial concentration of *B. cereus* and \(B_f\) is the final concentration of *B. cereus* after 24 h immobilization process.

The biodegradation studies of MSPW/NR and NR latex films were conducted using immobilized *B. cereus* onto MAC. At first, 1 g MSPW/NR and NR latex films were first dissolved in 100 ml of acetone
for pre-treatment process. The process left overnight under room temperature. About 300 mg of treated films were mixed with 2 g of immobilized B. cereus in 500 mL flask. The flask was filled with 150 ml MSM and cultivated on 45 rpm for 14 days at 30°C. Both studies were performed with three replicates and control. After 14th days of cultivation, the films were rinsed and dried at 40°C until constant weight was recorded. The weight loss of films was calculated based on equation 2,

\[
\text{Weight loss (\%)} = \left(\frac{W_i - W_f}{W_i}\right) \times 100\%
\]

where \( W_i \) is the initial dry weight of the sample and \( W_f \) is the dry weight of sample after biodegradation.

For storage stability and reusability of immobilized B. cereus, about 1 g of immobilized B. cereus was added onto 50 ml PBS solution in a 100ml flask. For storage stability study, the samples were stored at 4°C for 30 days. The biodegradation process was repeated for 5 additional cycles to observe the reusability of immobilized B. cereus. The bacteria viability of all studies was observed by DR 2800 spectrophotometer.

3. Results and Discussion

The chemical modification of activated carbon procedure is a crucial step of the immobilization of B. cereus. It was initially attempted to introduce acidic functional groups onto the surface of the activated carbon [15]. In this study, nitric acid was chosen oxidant for wet oxidation process to create oxygen functional groups on the surface of commercial AC. Previously, 30% w/v HNO3 solution was self-reflux for 2 h impregnation time with the optimum conditions were set at pH 7.0, 45 rpm, 30°C for 24 h. Thus, the highest adsorption capacity was found to be 72.8%.

Fig. 1. SEM micrograph, (a) 1500 x MAC and (b) 5000 x MAC after dominant Bacillus cereus ATCC 14579 immobilization.

The MAC and immobilized B. cereus onto MAC were examined under SEM to observe the surface characteristic of the samples. As shown in the Fig. 1(a) shows the MAC showed the presence of
numerous pores and rough surfaces where there is a possibility for the adsorption process. Fig. 1(b) shows *B. cereus* was dominant retained to the surface of MAC. Since *B. cereus* on the surface was direct contact to MSPW/NR latex films as substrates, thus it is able to degrade films as its substrate to grow and proliferate.

During the study, immobilized *B. cereus* was added into MSM media with MSPW/NR latex films in the batch system. The previous study showed *B. cereus* can utilize both starch and rubber particles as sole carbon and energy sources for their growth [12]. The conventional weight loss method is applied to prove the biodegradation of films using immobilized *B. cereus*.

The weight loss of MSPW/NR and NR latex films during incubation with immobilized *B. cereus* are shown in Fig. 2. After 14 days incubation, the weight loss in percentage of films is recorded at 12.843%. The control sample without inoculation, the weight loss of films was 1.418%. Meanwhile, the weight loss of NR latex films is 7.273% and its control is calculated at 1.673%.

![Fig. 2. The weight loss in percentage of MSPW/NR latex films with immobilized Bacillus cereus ATCC 14579 after 14th days of biodegradation periods.](image)

The results showed the immobilized *B. cereus* able to improve the biodegradation process of MSPW/NR latex films with compare to NR latex films. Few researchers agreed that the introduction of bio-filler onto latex formulation products can accelerate the biodegradation properties of the products [16-18].
Fig. 3. The weight loss and cell viability of immobilized Bacillus cereus ATCC 14579 at 4°C for 30 days.

The viability of bacteria during long-term storage and its reusability were significant for practical application and commercialization of bacterial immobilization.

Fig. 4. The reusability of immobilized Bacillus cereus ATCC 14579.

The viability of B. cereus was examined for the storage period of 30 days. As shown in Fig. 3, the weight loss percentage of films is typically decreased with decreasing cell viability of immobilized B. cereus. Initially, the weight loss percentage of films recorded at 12.843% with the cell viability concentration is at OD 0.081. After immobilized bacteria being stored for 30 days at
4°C, the bacteria concentration was decreased slightly at OD 0.078 with the weight loss of films recorded at 10.196%.

For the reusability pattern of the immobilized B. cereus, 5 cycles of reuse were tested. The results in Fig. 4 showed the weight loss of films was decreased for higher cycles. After the three cycles of regeneration, weight loss percentage of films was recorded at 10.193%. For fourth regeneration cycles and above, the weight loss percentage reduce to 10.045% and 9.611%. The reduction in the weight loss of films might be due to the detachment of the immobilized bacteria in the washing step. The results of immobilized bacteria reusability suggesting activated immobilized B. cereus can maintain their rubber-degrading activity throughout each cycle.

4. Conclusions

The results of the investigation demonstrate that applying the chemical modification of commercial activated carbon under specific conditions able to adsorb Bacillus cereus ATCC 14579 to the maximum state. The SEM micrograph results clearly showed the presence of B. cereus on the surface of modified activated carbon. It has been shown that these immobilized B. cereus able to utilize MSPW/NR latex films as the sole carbon and energy sources for their growth. The immobilized B. cereus was more stable and viable in the developed study. For commercialization, the viability of immobilized B. cereus of storage and thermal is stable and desirable. Thus, these results suggested that immobilized on modified activated carbon had a potential for practical application in the treatment of degrading rubber and latex films wastes rather than fills up the landfills.

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