Polymer Degradation and Stability 107 (2014) 91–97

Contents lists available at ScienceDirect

Polymer Degradation and Stability

journal homepage: www.elsevier.com/locate/polydegstab

The influence of different Tween surfactants on biodesulfurization of ground tire rubber by *Sphingomonas* sp.

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ARTICLE INFO

Article history:
Received 9 January 2014
Received in revised form
21 April 2014
Accepted 26 April 2014
Available online 6 May 2014

Keywords:
Sphingomonas sp.
Ground tire rubber
Tween surfactants
Biodesulfurization

ABSTRACT

In present work, three non-ionic surfactants (Tween 20, Tween 60 and Tween 80) were used to improve the affinity between the lipophilic ground tire rubber (GTR) and hydrophilic microbes. The growth characteristic of the *Sphingomonas* in the co-culture process was studied. The effects of different Tween surfactants on the biodesulfurization of GTR were investigated. Tween 20, among these three surfactants, showed best effect on enhancement of biodesulfurization. Results of SEM-EDS showed that the amount of sulfur in rubber surface layer of 0–4 μm was significantly decreased by 67%. XPS analysis data showed that the area of S–S bonds and S–C bonds were decreased while the S–O bonds were obviously increased. The mechanical properties of desulfurized-GTR/styrene butadiene rubber composite were improved. Also, the mechanism of the surfactants in improving the affinity between the GTR and *Sphingomonas* was proposed.

1. Introduction

With rapid development of world industry, annual demand of rubber products increases gradually. However, spent rubber cannot be degraded through nature because cross-linked rubber products have a stable three-dimensional network structure. Therefore, recycling of rubber material has been an issue of environmental concern since 20th century. Many researchers utilize microwave [1], ultrasonic [2,3] and chemical agents [4,5] to recycle rubbers. By breaking the cross-linked bonds in rubbers, their chains become reflow and easily processable. The desulfurized-rubber is then revulcanized, either as it is, or mixed with virgin material to make rubber products with common performance.

In recent years, biodesulfurization has become a potentially attractive way to recycle rubber. This method is an effective and selective process. Microorganisms, exhibiting ability to produce desulfurized enzymes, can selectively break crosslinked sulfur bonds on rubber surface while remain the main chains intact [6]. In 1945, it was firstly reported that sulfur oxidizing microorganisms might be capable of oxidizing sulfur in rubber. Thaysen et al. [7] noted that sulfuric acid was found in water remaining in the fire hoses after use. Eventually microbes were identified as the cause of the acid formation. Romine, R. A. and Romine, M. F. [8] used three bacterial strains (*Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Sulfolobus acidocaldarius*) to desulfurize dibenzothiophene and studied the mechanism of biodesulfurization. They proposed a “4S” metabolic pathway through which the sulfur crosslinks were metabolized by *S. acidocaldarius* into sulfoxide/sulfone/sulfonate/sulfate. Meanwhile, researchers indicated that treated ground tire rubber (GTR) would achieve good chemistry reactive if bioprocess was stayed at the first three steps. Thus the modified GTR could add into virgin rubber with high loading amounts and perform with good mechanical properties. Fliermans [9] screened a thermophilic bacterium from a hot spring, which was used to modify GTR surface. After microbial treatment, GTR also exhibited excellent surface chemistry reactive. The additives in rubber had some adverse effect on bacterial growth [10]. Ethanol was used to remove these toxic additives. Some fungi were also applied to rubber detoxification [11]. Li et al. reported that *T. ferrooxidans* [12], *Thiobacillus* [13] and *Sphingomonas* [14] were used respectively to recycle GTR, and *Sphingomonas* showed the strongest biodesulfurized activity among these three bacteria.

However, rubber is lipophilic thus it can’t dissolve in aqueous phase, which allows microbes to stay on the rubber surface for a short time. In other words, biodesulfurization effect still needs to improve. Therefore, it is important to increase the affinity between rubber and microorganism, raising the chance of microbial

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http://dx.doi.org/10.1016/j.polymdegradstab.2014.04.025
0141-3910/

contacting with rubber. Surfactant exhibits a unique ability to increase two-phase affinity. Thus it has a wide range of applications in biodegradation field. Kim et al. [15] evaluated the effect of several surfactants (Brij-30, Tween 80 and Triton X-100) on the biodegradation of polycyclic aromatic hydrocarbons (PAHs). The results showed that the PAHs solubility was linearly proportional to the surfactant concentration when above the critical micelle concentration. Thus the formation of micelle was the key to PAHs degradation. Bautista et al. [16] investigated the effect of *Enterobacter* sp., *Pseudomonas* sp. and *Stenotrophomonas* sp. on degradation of PAHs. Three non-ion surfactants, i.e. Tween 80, Triton X-100 and Tergitol NP-10, were respectively added into culture medium to compare their influence on biodegradation process. Results showed that Tween 80 had some toxicity for bacterium growth at early times but after the first 24 h bacterium growth rapidly and exhibited good biodegradation effects for PAHs. On the other hand, Triton X-100 and Tergitol NP-10 had severe toxicity for bacterium growth and biodegradation effects for PAHs didn't exhibit. Yao et al. [6] mixed Tween 80 with waste latex, and then the mixture was added into *Alicyclobacillus* culture medium. This technique avoided surfactant toxicity on microbial growth.

However, to the extent of our knowledge, there are few reports on how to select proper surfactants to enhance biodesulfurization effect. The specific mechanism and their relation to surfactants and rubber surface properties still remain unclear.

The effect of three non-ionic surfactants (Tween 20, Tween 60 and Tween 80) respectively on the biodesulfurization of ground tire rubber (GTR) by *Sphingomonas* sp. Strain was studied. The proper adding technique of surfactant was confirmed. The desulfurized effect was evaluated through measuring desulfurized depth, swelling value and sulfur content of desulfurized ground tire rubber (DGTR) sheet. The physical properties of desulfurized ground tire rubber/styrene butadiene rubber (DGTR/SBR) composite were measured. The mechanism of surfactants effect in co-culture-desulfurization process was discussed based on analysis of lipophilic chains length of the Tween molecule.

2. Materials and methods

2.1. Materials

The ground tire rubber (GTR) used in this study was supplied by Puyang Rubber Factory (Henan, China). Styrene-Butadiene rubber (SBR 1502) was supplied by Qilu Branch of China Petrochemical Co. (Shandong, China). Carbon Black N330 was provided by Dolphin Carbon Black Development Co. (Tianjin, China). Three non-ion surfactants (Tween 20, Tween 60 and Tween 80) were provided by Yili Fine Chemical Co. (Tianjin, China). Relevant chemical names and molecular formulas of these surfactants are shown in Table 1. Other chemicals were bought locally. All chemicals used for this work were analytical grade.

2.2. Microorganism and culture medium

Sphingomonas sp. was isolated from coal mine soil in Sichuan Province, China. It was cultured in a medium that contained KH_2PO_4 4.0 g/L, $K_2HPO_4•3H_2O$ 4.0 g/L, $MgSO_4•7H_2O$ 0.8 g/L, NH_4Cl 0.4 g/L, $CaCl_2$ 0.01 g/L, $Na_2S_2O_3•5H_2O$ 10.0 g/L, glucose 2 g/L, peptone 1.0 g/L and yeast extract powder 0.1 g/L. The cultured temperature was 30 °C and pH was 6.5.

Table 1
Chemical names and molecular formulas of Tween surfactants.

Surfactants	Chemical name	Molecular formula
Tween 20	Polyoxyethylene (20) sorbitan monolaurate	$C_{58}H_{114}O_{26}$
Tween 60	Polyoxyethylene (20) sorbitan monostearate	$C_{64}H_{128}O_{26}$
Tween 80	Polyoxyethylene (20) sorbitan monooleate	$C_{64}H_{124}O_{26}$

2.3. Biodesulfurization process

Sphingomonas sp. was cultured in 250 mL flask in shaker incubators. Each flask was filled with 100 mL medium. The culture medium of *Sphingomonas* sp. had been autoclaved at 115 °C. Microbe was incubated into the medium after cooling down to room temperature. The inoculum of *Sphingomonas* sp. strain was 10% (v/v). And the cultivation temperature was 30 °C. Stirring speed was 200 rpm.

Before desulfurization, GTR and GTR sheets were immersed in 75% ethanol (v/v) for 24 h in order to kill the microbes attached to it and remove harmful additives. Detoxicated GTR was filtered out and dried in a sterile cabinet. And Tween surfactants were mixed with GTR and GTR sheets. After 48 h incubation, the mixture (surfactants, GTR and GTR sheets) was added into the culture medium. The amount of GTR and surfactants were 2.5% and 0.1% (w/v), respectively. And the control group did not add surfactants.

After desulfurization for 20 days, DGTR samples were filtered out and washed by distilled water for 1 h, dried at room temperature.

GTR represents the ground tire rubber was immersed for 20 days under the same culture conditions without inoculation. $DGTR_{ctr}$, $DGTR_{20}$, $DGTR_{60}$ and $DGTR_{80}$ represent the ground tire rubber desulfurized by *Sphingomonas* sp. without Tween, with Tween 20, Tween 60 and Tween 80, respectively.

2.4. Preparation of GTR or DGTR sheets and SBR composites

The GTR without virgin rubber stock was molded into a rubber sheet at 15 MPa and 150 °C, after being passed through a two-roll mill with a roller spacing of 0.5 mm for 20 min. The cured rubber sample was cut into small squares (10*5*1.2 mm, 0.043 ± 0.001 g).

Raw SBR was masticated on the two-roll mill, blended with process additives and 20 phr GTR or various DGTR for 10 min. The basic formulation included SBR-1502 100 phr, ZnO 4.0 phr, Stearic 2.0 phr, carbon black N330 30 phr, Accelerator D 0.6 phr, Accelerator DM 1.2 phr, sulfur 2.0 phr.

About 35 g of the GTR/SBR or DGTR/SBR compounded rubber stock after 24 h of storage were placed in a mold and pressed by the platens press (Shanghai, Model XLB-DQ). The each samples was cured at 150 °C, 15 MPa for the optimum cure times ($t = t_{90}$). The t_{90} was obtained from an Oscillating Disk Rheometer (Beijing, Model P3555B).

2.5. Characterizations and measurements

2.5.1. GTR size distribution and morphology observation

The size distribution of GTR was obtained by the OMEC LS-Pop3 laser particle size distribution analyzer, China. The morphology of GTR particles was observed by the Hitachi S-4800 SEM, Japan. The samples were vacuum plated with platinum for electrical conduction.

2.5.2. Microorganism biomass

Microbe growth development was monitored during incubation in this study. The method was as followed: taken 5 mL liquid from a culture flask, then diluted it to a certain concentration by distilled water, lastly its optical density at 600 nm (OD600) was measured on a Spectrophotometer (Chongqing, China, Model XSZ-H3). OD600 of the sample represented biomass.