

Microbial Desulfurization for NR Ground Rubber by *Thiobacillus ferrooxidans*

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ABSTRACT: This study concentrated on microbial desulfurization for NR ground rubber by *Thiobacillus ferrooxidans* with sulfur oxidizing capacity. NR ground rubber was desulfurized in the modified 9K medium during the cultivation of *T. ferrooxidans*. FTIR-ATR and XPS spectra and the increase of SO_4^{2-} in the medium indicated that the main chains of the polymer were not broken by *T. ferrooxidans*, and S-S linkages on the surface of ground rubber were partly oxidized to sulfoxide and sulfone, and at last partly oxidized to SO_4^{2-} . Cumulative sulfur convention of ground rubber was 16% (w/w), which means 16% of sulfur has been fully oxidized to SO_4^{2-} after 20 days' incubation. A sulfur oxidative scheme was proposed to explain the microbial desulfurization by *T. ferrooxidans*. Physical

properties were determined on carbon black enforced SBR vulcanizates compounded with desulfurized ground rubber of 40 phr loading. Preferable tensile strength and elongation at break were obtained for SBR vulcanizates filled with desulfurized ground rubber if compared with that one obtained using ground rubber without modification. Scanning electron microscope photographs and DMA results suggested good interface coherence between desulfurized ground rubber and SBR matrix. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 116: 2768–2774, 2010

Key words: *Thiobacillus ferrooxidans*; microbial desulfurization; ground rubber; mechanical properties; dynamic mechanical properties

INTRODUCTION

Millions of tons of waste rubber have been discarded by human beings every year. Methods like burning and burying, outdoors deposit, have wasted precious rubber resources, caused severe pollutions and wasted land resources because of the stockage of tyres in wasteyards. Waste rubber can be reclaimed by chemical and mechanical processes.^{1–3} Chemical reclaiming methods might cause poisonous gas releasing, while mechanical reclaiming methods results in immense energy consumption.^{4,5} Grinding waste rubber to ground rubber is more environmental concerned and less energy consuming than ultrasound and microwave reclaiming methods. However ground rubber is not compatible enough to be used as fillers in applications that requires mechanical demanding. Ground rubber surfaces need to be modified or desulfurized to make it more compatible to the matrix.

Inorganic or covalent sulfur utilizing through microbial metabolism is a new method of desulfurization. *Thiobacillus ferrooxidans* was widely used in many applications, such as metallurgy,^{6,7} biorefining of diesel fuel,^{8,9} and rubber desulfurization. In 1945 Thaysen¹⁰ reported that sulfur in rubber hoses was oxidized by *T. ferrooxidans*. Desulfurization was proved by both the release of SO_4^{2-} in medium and surface chemical group analysis of rubber. The sulfur content of rubber decreased 7.8% after desulfurization for 30 days and oxidation state of sulfur was detected on the surface of rubber.¹¹ The content of sulfone group on NR surface increased during desulfurization.¹² Virgin rubber filled with desulfurized ground rubber maintained high mechanical properties, compared with unfilled rubber.^{13,14} Oxygen uptaken experiment in Warburg respirometers revealed mixing culture of *T. ferrooxidans* and *T. thiooxidans* was more effective than any pure cultivation of them.¹⁵ The elasticity modulus of vulcanizates filled with desulfurized GTR (ground tire rubber) was lower than that of untreated vulcanizates filled with GTR.¹⁶

In this article, *T. ferrooxidans* with high desulfurization activity was screening. The Fe^{2+} concentration of culture medium was chosen according to the growth of *T. ferrooxidans* and the amount of pissophane precipitation. NR ground rubber was

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desulfurized in the medium of *T. ferrooxidans*. To propose the mechanism of microbial desulfurization, the chemical groups on the surface of desulfurized NR were analyzed by FTIR-ATR and XPS, and SO_4^{2-} concentration of medium was monitored by turbidimetric analysis. Desulfurized ground rubber was then incorporated in SBR matrix. The fracture surface morphology, the mechanical and dynamical mechanical properties of SBR vulcanizates filled with ground rubber and desulfurized ground rubber were evaluated to study their effects in the final properties of SBR compounds.

EXPERIMENTAL

Materials

NR was provided by Xishuang Banna Eastwind Farmland, China. SBR 1502 was supplied by China Petrochemical Co., Qilu Branch. Carbon Black N330 was obtained from Tianjin Dolphin Carbon Black Development Co. Other compounding ingredients were brought from chemical reagent shops of Beijing.

Preparation

Preparation of ground rubber

Vulcanized NR was pulverized into ground rubber on a two-roll mixing mill for 10 min. And it is of broad distribution with irregular shape. The size was less than 0.4 mm. The components of ground rubber was NR: 100, zinc oxide: 4, stearic acid: 2, accelerator DPG (Diphenylguanidine): 0.6, accelerator MBTS (Dibenzothiazyl disulfide): 1.2, sulfur: 2 phr (per hundred rubber).

Preparation of SBR compounds

When SBR became fluidic on mixing mill, ground rubber was added into SBR. Other agents were mixed to get SBR compounds. Curing and physical properties of filled SBR vulcanizates were tested to evaluate the effect of desulfurization of ground rubber. The formulation used was shown in Table I.

T. ferrooxidans cultivation

T. ferrooxidans was selected from soil of an iron mine in Xinlong, Hebei Province, China. Strain of *T. ferrooxidans* was separated and purified between 9K medium¹⁷ and International Streptomyces Project (ISP) medium¹⁸ at 30°C repeatedly, 9K medium contains: $(\text{NH}_4)_2\text{SO}_4$: 3.00, KCl: 0.10, K_2HPO_4 : 0.50, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.50, $\text{Ca}(\text{NO}_3)_2$: 0.01, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 44.3 (g/L) and it is adjusted to pH 2.5 with

TABLE I
Formulation of SBR Compounds Filled with Ground Rubber and Desulfurized Ground Rubber

Material/phr	A1	A2
SBR 1502	100	100
Ground rubber	40	0
Desulfurized ground rubber	0	40
Zinc oxide	4	4
Stearic acid	2	2
Carbon Black N330	30	30
DPG	0.6	0.6
MBTS	1.2	1.2
Sulfur	2	2

H_2SO_4 . Sterilization of the medium was accomplished by filtering and autoclaving.

B1–B4 medium were used to test the dependence of *T. ferrooxidans* growth on Fe^{2+} concentration. Fe^{2+} concentration in B1–B4 medium is 9/2, 9/4, 9/8, and 9/16 g/L, respectively. The other ingredients of B1–B4 medium are the same as 9K medium.

Desulfurization process

T. ferrooxidans is sensitive towards aromatic compounds in the ground rubber. Before desulfurization, ground rubber was leaching by 75% (v/v) ethanol for 24 h to remove harmful additives.

T. ferrooxidans was cultured at 30°C in a shake flask, vigorous shaking at 170 rpm. After 1 day, ground rubber, about 2% (w/v) of culture medium, was added in. After 20 days, ground rubber was filtered out and washed by tapping water.

Characterization

Fe^{2+} and SO_4^{2-} analysis

1, 10-phenanthroline method was used for determination of Fe^{2+} in trace quantities, according to ASTM E 394-2000. Turbidimetric test method by a spectrophotometer was used to determine SO_4^{2-} concentration, according to ASTM D 516-2002.

Strain morphology

The morphology of *T. ferrooxidans* was observed by an FEI-XL30 ESEM (environmental scanning electron microscope).

Fracture surface morphology study

The morphology of the fracture surface of the vulcanizates was observed on a scanning electron microscope, Hitachi S-4700 SEM. The fracture ends of tensile specimen were vacuum-plated with gold for electrical conduction.

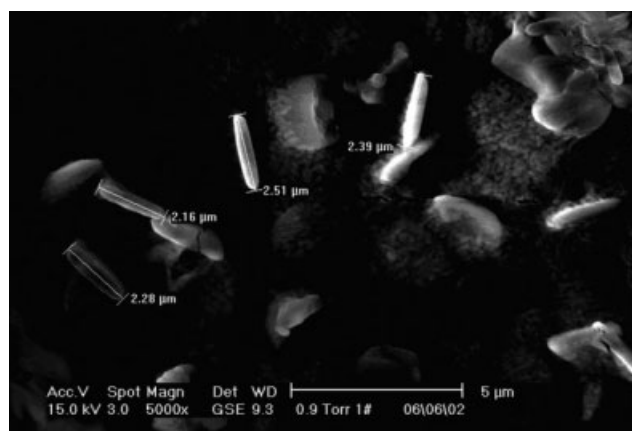


Figure 1 SEM photograph of *T. ferrooxidans* on ISP medium.

FTIR-ATR

Fourier transform infrared analysis was performed on a TENSOR 27 Fourier transform infrared spectrometry (BRUKER OPTIK GMBH, Germany), equipped with a Ge-ATR crystal. The operating conditions were 4 cm^{-1} resolution with 64 scans being averaged.

X-ray Photoelectron spectroscopy

XPS was tested to analyze the surface elements and bonding states on surface exposed to liquid medium. XPS measurement was carried on Escalab I 250 XPS, Thermo Electron.

Sample testing

The compounds were vulcanized at a platen press, with 15 MPa pressure at 150°C , according to ASTM D 2084-07. t_{s1} is scorch time, and t_{90} is optimum cure time determined from an oscillating disk rheometer. Physical properties were measured according to ASTM D 2240 (hardness), ASTM D 412 (tensile strength), and ASTM D 624 (tear strength), respectively.

Crosslink density test

The test specimens, about 0.05 gram, were immersed into toluene at 30°C for 72 h. They were then removed from the solvent, wiped with tissue paper to remove the excess solvent from the surface, and weighed. Crosslink density was calculated by Flory-Rehner equation.^{19–21} For styrene butadiene rubber, the interaction parameter χ is 0.31 in toluene.

Dynamic mechanical analysis

The dynamic mechanical analysis of SBR vulcanizates samples was measured using a dynamic mechanical thermal analyzer (VA30000 1dBDM, FRANCE VISCOANALYSEUR). The test specimens

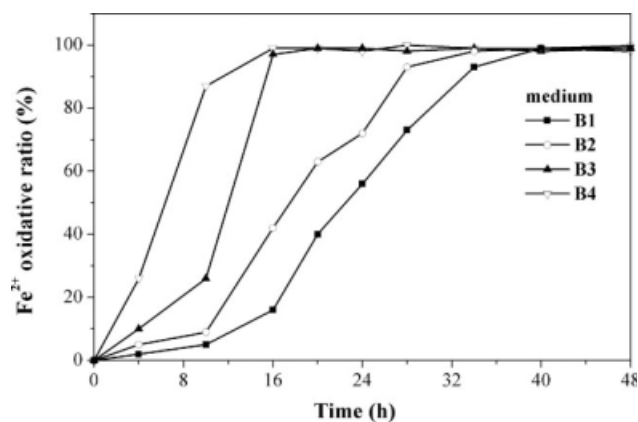


Figure 2 Fe^{2+} oxidative ratio in B1–B4 medium by *T. ferrooxidans*.

for DMA were cut from molded sheets, approximately 2.0 mm thick. The run conditions were tension mode at a fixed frequency of 10 Hz with strain amplitude of 0.1%. Temperature range was from -100 to 100°C , at a heating rate of $3^\circ\text{C}/\text{min}$.

RESULTS AND DISCUSSION

Growth curves of strain

T. ferrooxidans formed bacterial colonies of 0.5–1 mm in diameter on ISP medium. The morphology of *T. ferrooxidans* was shown in Figure 1. It could be seen from Figure 1 that *T. ferrooxidans* is rod-shaped, with about $2\ \mu\text{m}$ in length and $0.5\ \mu\text{m}$ in diameter. Basic Local Alignment Search Tool (BLAST) proved *T. ferrooxidans* in this work has 100% sequence identity to DXH strain in National Center of Biotechnology Information (NCBI).

The growth curves of *T. ferrooxidans* were expressed by Fe^{2+} oxidative ratio in medium, which are illustrated in Figure 2. Fe^{2+} oxidative ratio increased at the beginning of incubation, and then flatted early or later in different medium. The time

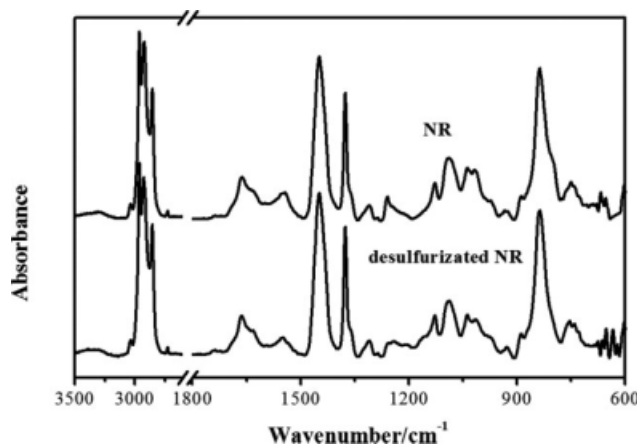


Figure 3 FTIR absorbance spectra of NR and desulfurized NR vulcanizates sheets.

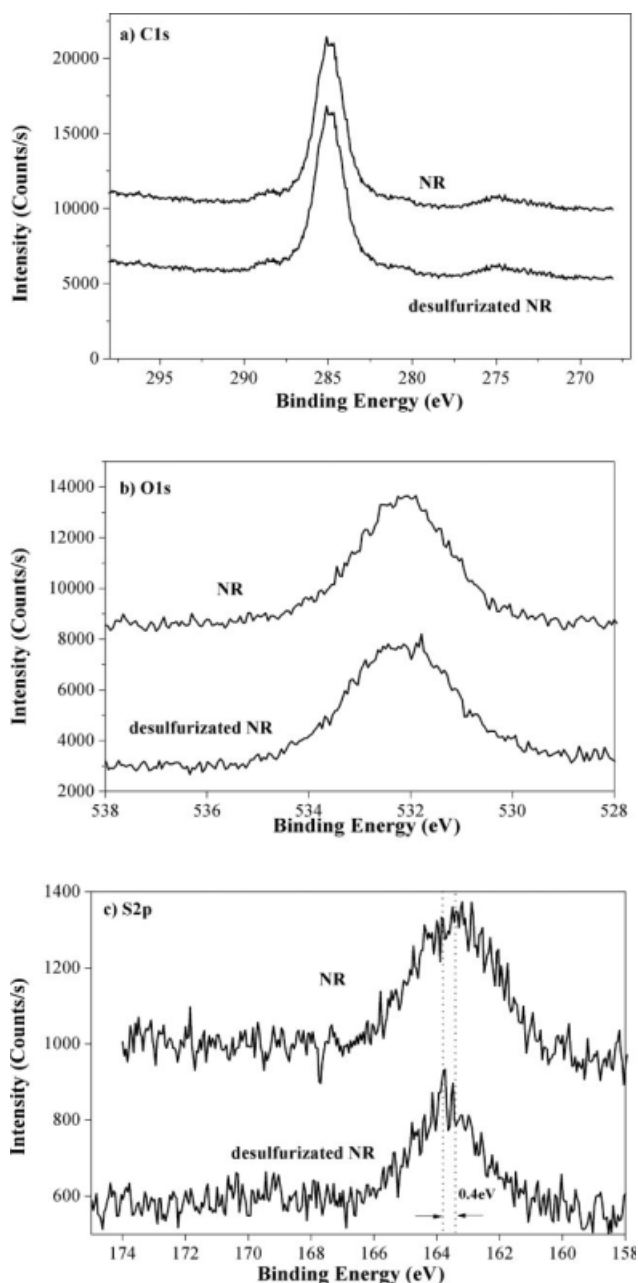


Figure 4 XPS spectra of NR and desulfurized NR vulcanizates sheets, a-C1s; b-O1s; c-S2p.

of completely oxidizing Fe^{2+} to Fe^{3+} increased with the initial Fe^{2+} concentration. Therefore, the highest stable phase biomass existed in B1 medium. But large amounts of precipitation were observed in B1 and B2 medium, while none appeared in B3 and B4 medium. Therefore B3 medium was chosen for desulfurization.

Effect of desulfurization for vulcanized NR

FTIR-ATR spectrum

The absorbance spectra of NR vulcanizates and desulfurized NR are shown in Figure 3. The spec-

trum of desulfurized NR corresponded well with the spectrum of vulcanized NR. Amide bands at 1652 and 1545 cm^{-1} and polysaccharide bands in the region from 900 to 1200 cm^{-1} were not shown on surface of desulfurized NR, which proved the removal of bacteria biofilm. The decreases of $\nu(\text{C}=\text{C})$ band at 1640 cm^{-1} and $\delta(=\text{CH}_2)$ band at 835 cm^{-1} were not obvious, and no $\nu(\text{C}=\text{O})$ band at 1700 cm^{-1} appeared for the desulfurized NR. These indicated that *T. ferrooxidans* did not oxidize C—C and C=C backbones. In the region of 1160 – 1030 cm^{-1} , there was no new peak for $\nu(\text{S}=\text{O})$. It is possible that the content of oxidized sulfur is too low to be detected by FTIR-ATR.

XPS analysis

As is shown in Figure 4, the contents and bonding states of C, O, and S elements for vulcanized NR and desulfurized NR were detected by XPS. C peak of desulfurized NR was almost the same as vulcanized NR, which indicated main chains of polymer were not oxidized by *T. ferrooxidans*. The change of O peak of desulfurized NR was not noticeable, while S peak of desulfurized NR decreased comparatively. In addition, S peak shifted about 0.4 eV to a high binding energy. Peak energy at 163.7 eV represents C—S and S—S bonds, and peak at 164.1 eV represents sulfoxide and sulfone. It showed that C—S and S—S bonds were decreased and more oxidized sulfur was produced on the surface of desulfurized NR. This result could be explained by that crosslinking sulfur was partly oxidized to sulfoxide and sulfone. Peak of the final oxidation products (SO_4^{2-}) at 169 eV was not existed, because it was broken away from the rubber surface.

Sulfur conversion

No substances containing sulfur were added into medium after ground rubber was added in. SO_4^{2-}

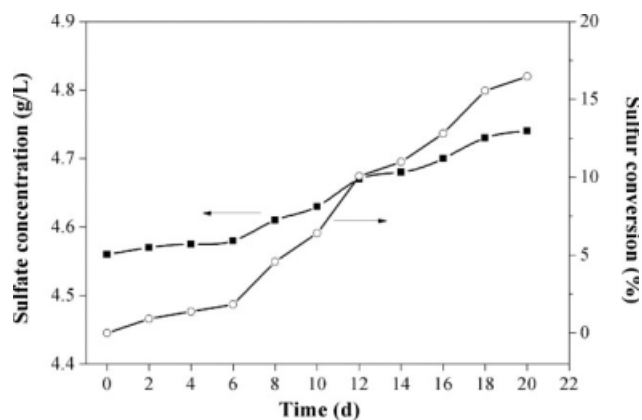


Figure 5 SO_4^{2-} concentration in the medium and sulfur conversion percentage of ground rubber.

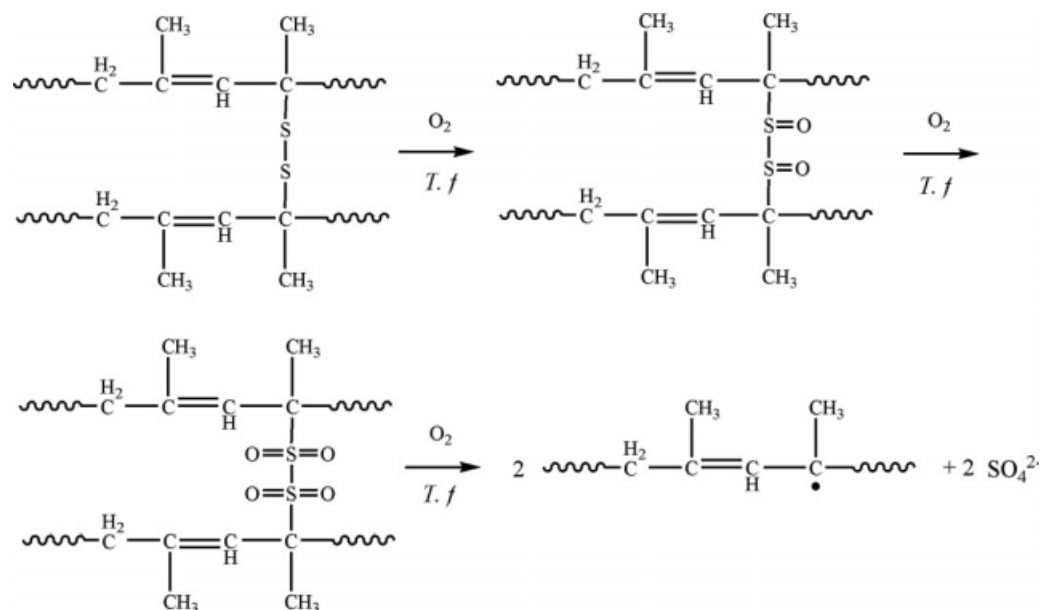


Figure 6 Proposed scheme for the desulfurization of sulfur linkages by *T. ferrooxidans*.

concentration in cultivation and corresponding sulfur conversion of rubber were shown in Figure 5. SO_4^{2-} concentration increased with cultivation days, from initial 4.56 to 4.74 g/L at 20 days. According to the law of conservation of matter, sulfur conversion in ground rubber is calculated to be 16%. The increase of SO_4^{2-} proved that part of the crosslinking sulfur on the surface of ground rubber was fully oxidized to SO_4^{2-} by *T. ferrooxidans*.

Desulfurization mechanism

A scheme of microbial desulfurization is proposed to interpret all these results. It can be described as the gradual oxidation of S–S linkages. In the process of microbial desulfurization, sulfur is oxidized by *T. ferrooxidans*; forms sulfoxide or sulfone groups; and at last leaves isoprene chains in the form of SO_4^{2-} (Fig. 6).

Properties of vulcanizates

As is seen from Table II, SBR filled with desulfurized ground rubber (A2) exhibited shorter t_{s1} , t_{90} , than A1 because of high surface reactivity of desulfurized ground rubber. Shore A hardness did not show significant change in these two vulcanizates, because the crosslink density of A2 was only 5% smaller than A1. Higher tensile strength and elongation at break of A2 may be attributed to better crosslink distribution and strong interface forces between desulfurized ground rubber and matrix. When filled in SBR matrix, more sulfur and accelerator transferred from SBR matrix to desulfurized ground rubber, and additional sulfur linkages were

formed between phases. Decrease of modulus at 100 and 300% of A2 was in coherence with the decrease of crosslink density. It showed that sulfur in ground rubber was partly removed and crosslinks were ruptured during desulfurization.

Figure 7 represents the SEM micrographs of fracture surfaces of A1 and A2. Ground rubber was peeled off from the matrix, and left lots of voids on fracture surface in A1 sample. Fracture surface of A2 showed few voids and better wetting. This indicated that desulfurized ground rubber was more compatible to the rubber matrix. There are more chemical bonds and greater interface forces between desulfurized ground rubber and rubber matrix.

Figure 8 shows the dependence of storage modulus and loss factor ($\tan\delta$) on temperature of A1 and A2. Details of the DMA data are given in Table III. In the rubbery state, the storage modulus of A2 is larger than that of A1. The increase of elasticity was obtained by the fine dispersion and strong bonding with matrix. In the glass state $\tan\delta$ is quite low, and

TABLE II
Mechanical Properties of SBR Vulcanizates Filled with Ground Rubber

	A1	A2
t_{s1}/min	2.40	2.10
t_{90}/min	7.60	7.17
Shore A hardness	58	57
Crosslink density/ $\times 10^{-5}\text{mol}/\text{cm}^3$	5.8	5.5
Tensile strength/MPa	17.0	19.1
Elongation at break/%	361	408
Modulus at 100%/MPa	1.74	1.64
Modulus at 300%/MPa	11.2	9.78
Tear strength/kN/m	28.8	28.9

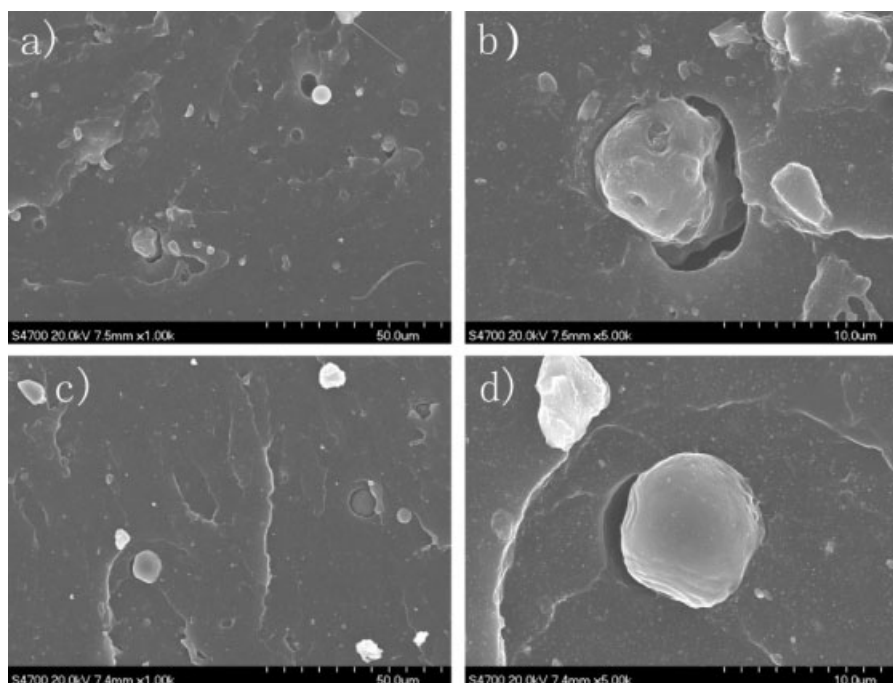


Figure 7 SEM micrographs of the fracture surface of SBR vulcanizates (a, b) A1, (c, d) A2.

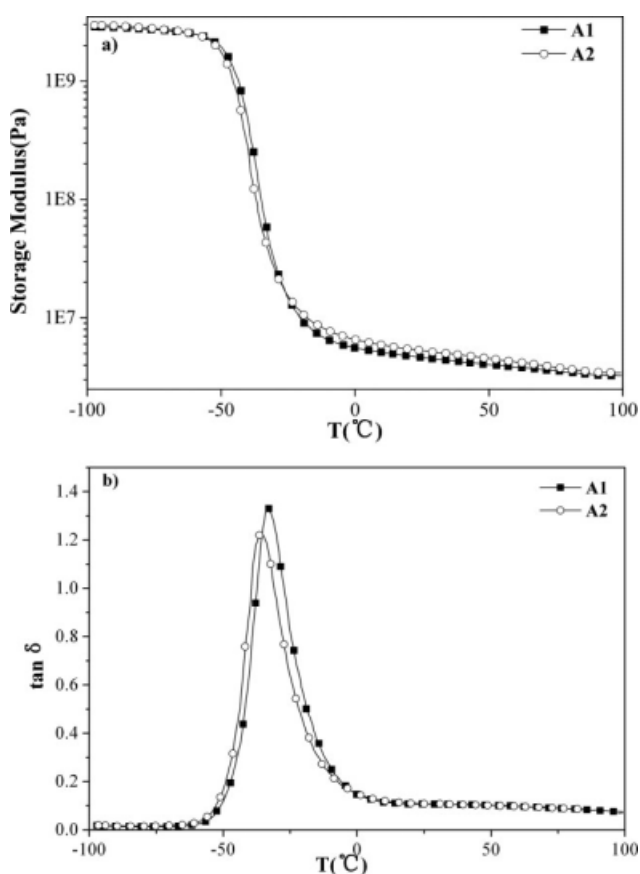


Figure 8 Storage Modulus and $\tan\delta$ vs temperature for SBR vulcanizates ground rubber filled with ground rubber and desulfurized ground rubber.

in the rubbery state, $\tan\delta$ is in the range of 0.05–0.15. Notable differences were observed in the peak position and peak amplitude. The peak position shifts to low temperature for A2. The peak amplitude decreased, while peak half-width increases. The shift of peak position was in agreement with the decrease of crosslink density. The decrease of peak amplitude suggested good interface coherence between SBR and ground rubber and widening of the half-width corroborated the better crosslink distribution.

CONCLUSIONS

T.ferrooxidans was grown better in the modified 9K medium with higher Fe^{2+} concentration. The FTIR-ATR analysis and S element peak position and area in XPS spectra of desulfurized NR indicated main chains of polymer could not oxidized to break and crosslinking sulfur was oxidized to form sufoxide and sulfone groups by *T.ferrooxidans*. The concentration of SO_4^{2-} in the medium increased, which proved

TABLE III
DMA Data of Ground Rubber SBR Vulcanizates Filled with Ground Rubber

	Peak position/°C	Peak half-width/°C	Amplitude	Area/°C
A1	-33.0	17.91	1.33	40.09
A2	-36.2	18.35	1.23	38.98

a part of sulfur was fully oxidized to SO_4^{2-} and left the macromolecular chains.

Desulfurized ground rubber was used as filler in SBR matrix. At 40 phr loading, SBR vulcanizates filled with desulfurized ground rubber showed good improvements on tensile strength and elongation at break. Furthermore, crosslink density showed a reduction for SBR filled with desulfurized ground rubber, which could be explained by microbial desulfurization and sulfur diffusion. SEM and DMA indicated good interface coherence between desulfurized ground rubber and SBR matrix.

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