EGG-M--90382

DE91 006122

JAN 1 5 1991

BIODESULFURIZATION OF RUBBER MATERIALS

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ABSTRACT

One of the most challenging problems in municipal waste treatment is the recycling of polymeric waste materials. The present study has demonstrated the applicability of biotechnological principles in the desulfurization of rubber using shake flask and Warburg respirometric techniques. In terms of oxygen uptake and specific rate of oxygen uptake, it was found that the mixed culture of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* was more efficient in this process than the individual pure cultures of these bacteria. Furthermore, the mixed cultures resulted in ten times higher sulfur removals from rubber relative to those of sterile controls. Additional studies are needed to elucidate the mechanisms of biodesulfurization of rubber. It is expected that the development of this process may provide a solution to recycling of car tire materials.

INTRODUCTION

The rapid depletion of the available landfill sites for the disposal of urban wastes has created a need for the development of new alternative management methods (Paul, 1978; Anonymous, 1989). Within the proposed alternatives, recycling methods are especially gaining acceptance, since these are leading to a direct reduction of the disposable waste volume. With respect to the polymeric waste materials, the effective disposal of rubber is of particular concern motivated by the fact that yearly millions of car tires are discarded in the United States. These represent about two thirds of the total rubbery wastes (Paul, 1985). Current disposal methods involve entombing of rubbery wastes with dirt. However, under these conditions, rubber remains unaltered for an unforeseeable period of time (Raghavan and Torma, 1989). Recently, a number of processes have been suggested for reclaiming or recycling the waste rubber. One of these is the blending of waste rubber with a commercial polymer, i.e., polyurethane (Anonymous, 1989). The processing of this blended material leads to a new polymer product, in which, scrap rubber acts as a filler. However, such an approach cannot effectively reprocess the entire amount of rubber waste. Other investigators (Tsuchii and Takeda, 1990) suggested that biodegradation processes may be important in the recycling of rubbery materials. In 1945 it was reported that *Thiobacillus ferrooxidans* may be capable of oxidizing the sulfur inclusions in rubber (Thaysen et al., 1945). For the next twenty years no related activity was done. Then, Turner (1965) expressed the opinion that such bacterial action may lead to deterioration in the mechanical properties of rubber. Next, it was Rodriguez (1971) who proposed that sulfur in rubber may be used as a source of energy by certain microorganisms. The biodesulfurization of rubber is a developing technology, which has to reach application maturity. Still, although the data are preliminary in nature, the ultimate application of biotechnological principles in the remediation of rubber wastes may provide a potential solution to the problem. The eventual development of a biodesulfurization process for waste rubber, will create new opportunities and challenges for the research community and polymeric waste recycling industry.

RUBBER MATERIALS

Rubber is a high-molecular-weight polymer of cis-1,4 isoprene (Billmeyer, 1984), which is also called a "thermoplastic elastomer" that can be stretched to at least twice its original length, and upon releasing the force, it will retract to its original dimension (Brydson, 1978; Rosen, 1982). The double bounds contained in the natural and synthetic (butadiene-styrene, BS) rubbers provide sites for vulcanization with sulfur to improve their physical properties. The largest volume item is the car tires containing typically less than 60% polymer (Allen, 1972; Rosen, 1982), as shown in Table 1. In addition to those tire components given in Table 1, rubber tires may contain varying amounts of inorganic (minerals, metal wires) and/or organic (cords, fibers) fillers and pigment materials.

TABLE 1. COMPOSITION OF A TYPICAL CARE TIRE	
Components	Parts by Weight
Butadiene/styrene(75/25)	100
Carbon black	50
ZnO	5
Stearic acid	3
Sulfur	2
Extending oil	10
Accelerators	0.95

Because of the very complex nature of the car tires, this biodesulfurization study was carried out with special rubber samples containing 2 to 16% of sulfur.

MICROORGANISMS

A number of mesophilic and thermophilic microorganisms exists that can oxidize sulfide minerals and reduced valence sulfur compounds (Landesman at al., 1966; Karavaiko, 1985; Ehrlich, 1981). These microorganisms are chemolithoautotrophs (Smith and Hoare, 1977) that derive energy from the oxidation of the above substrates and their carbon for cell material production from CO_2. The most frequently studied mesophilic bacteria are *Thiobacillus ferrooxidans* (Torma, 1977 and 1987; Ingledev, 1986), *T. thiooxidans* (Buchanan and Gibbons, 1975), Leptospirillum ferrooxidans (Balashova et al., 1974), *T. organoparus* (Karavaiko, 1985), *T. thioparus* (Karavaiko, 1985). The thermophilic species include *Sulfobacillus acidocaldarius* (Brock, 1978; Brierley and Brierley, 1973), sulfur reducing *Sulfobacillus thermosulfidooxidans* (Golovacheva and Karavaiko, 1978). The above bacteria have been shown to be effective in the desulfurization of coal (Hutchins et al., 1986; Dugan, 1986; Torma and Gundiler, 1987; Klein et al., 1988), a problem similar in many respect to that of rubber. In both cases, coal and rubber, sulfur content is composed of inorganic and organic portions.

The sulfur removal from rubber by microbial action can be given by the following reactions:

Inorganic portion:

$$S^{\circ} + 1.5\ O_2 + H_2O \xrightarrow{\text{bacteria}} H_2SO_4 \qquad (1)$$

Organic portion:

$$R\text{-}S_x\text{-}R + Y\ 1.5\ O_2 + Y\ H_2O \xrightarrow{\text{bacteria}} Y\ H_2SO_4 + R\text{-}S_{x-y}\text{-}R \qquad (2)$$

or

$$R\text{-}S_x\text{-}R + X\ 1.5\ O_2 + X\ H_2O \xrightarrow{\text{bacteria}} X\ H_2SO_4 + R\text{-}R \qquad (3)$$

where R represents the organic component of rubber.

In this study, individual pure cultures of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* as well as their mixture were used.

MATERIALS AND METHODS

Rubber Samples

Eight pulverized synthetic rubber samples used in this study were provided by Goodyear Tire and Rubber Company, Akron, Ohio. Their identification numbers and sulfur content are shown in Table 2. The first four vulcanized rubbers are styrene-butadiene and the composition of the next four are not available. Their surface characteristics and sulfur distribution are compared in Figure 1, using a Hitachi HHS-2R scanning electron microscope equipped with energy dispersive X-ray (EDX) capability. For SEM examination, the rubber samples were first molded into epoxy resins, then polished with 200, 400 and 600 grit papers and finally with diamond paste and silk cloth (Clough and Quintana, 1985). The specimens were individually wrapped with aluminum tape, then colloidal carbon was applied to one of their ends. To avoid accumulation of electrical charge on the rubber surface, the specimens were coated with a thin layer of carbon.

TABLE 2. GOODYEAR RUBBER SAMPLES		
Sample No.	Designation	Sulfur Contents (%)
1	SBR # 709	1.2
2	SBR # 710	3.4
3	SBR # 711	7.2
4	SBR # 712	15.5
5	SMR # 713	1.2
6	SMR # 714	3.4
7	SMR # 715	7.2
8	SMR # 716	15.5

Bacteria

The cultures of *T. ferrooxidans* and *T. thiooxidans*, used in this study were routinely grown on an adapted Silverman and Lundgren (1959) nutrient medium, in which, the energy source, ferrous iron, was replaced by elemental sulfur. The mixed culture of these organisms was prepared with equal amounts of *T. ferrooxidans* and *T. thiooxidans* present in growth medium. When growth reached the late logarithmic phase, an aliquot of the homogenized suspension was transferred into a new medium to maintain the stock culture, or it was used as an experimental inoculum.

For the Warburg manometric studies the pure or mixed cultures of *T. ferrooxidans* and *T. thiooxidans* were produced in 30 dm^3 batch reactors, which were charged with 18.5 dm^3 basal salt nutrient medium (Silverman and Lundgren, 1959), 1.5 dm^3 bacterial inoculum and 10 g elemental sulfur and incubated at 35°C and 150 rpm. Periodically, samples were removed from the reactor and analyzed for sulfuric acid contents, and when growth reached the late logarithmic phase, the process was stopped. The bacterial cells free of substrate were collected from the decanted culture solution by centrifugation (Torma, 1978). The solutions were first centri-fugated at 4080 * g gravitational force for 5 minutes. The super-natant fluid containing the bacteria was carefully removed from the flasks so as not to disturb the colloidal and solid particles. Then, this fluid was subjected to centrifugation at 39100 * g

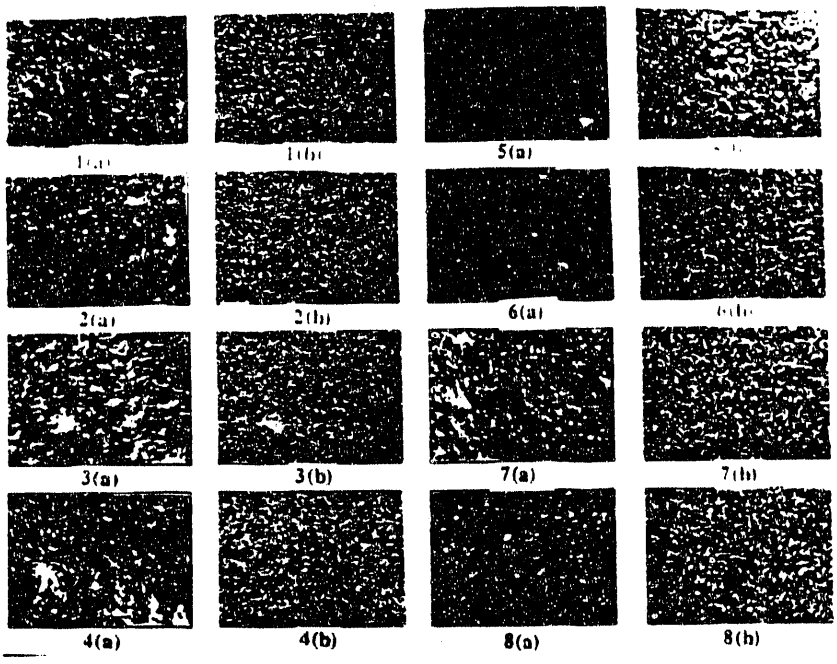

Figure 1. SEM surface characterization of rubber samples at 1000 times magnification are given in the (a) figures and their corresponding EDX-sulfur dot mapping in the (b) figures.

gravitational force for 15 minutes. The packed cells were resuspended in iron-free nutrient medium (Raghavan et al., 1990). Cellular protein was liberated by alkaline digestion (0.1 N NaOH) of the bacteria and estimated by the method of Lowry et al. (1951).

Shake Flask Experiments

Desulfurization experiments were performed in 250 cm^3 Erlenmeyer flasks containing 70 cm^3 sulfur-free nutrient medium, 0.75 to 12 g pulverized rubber and 5 cm^3 bacterial inoculum. The flasks were incubated on a Gyratory Incubator Shaker, Model G26 from the New Brunswick Scientific Co., NJ, at 35°C at 200 rpm. The weight loss associated with water evaporation was periodically compensated with distilled water. In predetermined periods of time 2 cm^3 of leach solution were withdrawn from the flasks and replaced with 9K sulfur-free nutrient medium. The leach samples were analyzed for sulfate content by ion exchange chromatography (Raghavan, 1990). The results were compared to those obtained for the sterile controls, where 5 cm^3 of a 2% solution of thymol in methanol replaced the bacterial inoculum.

Manometric Experiments

The oxygen uptake experiments were carried out in Warburg respirometers (Precision Scientific Company, Illinois, Model 15-AD-8) equipped with 16 cm^3 flasks (Umbreit et al., 1972). Each flask was charged in the main compartment with 2.0 cm^3 iron free basal salt nutrient solution (Silverman and Lundgren, 1959) and 200 mg pulverized rubber, in the center well 0.2 cm^3 of 20% KOH solution was introduced, while in the side arm 0.3 cm^3 of 10% bacterial suspension (pure or mixed cultures of *T.ferrooxidans* and *T. thiooxidans*) containing 0.3 to 0.4 mg protein were added. In the sterile controls either no bacteria were added or a 2% thymol in methanol was added to the experimental solution. The endogenous respiration experiments were done with heat-killed bacterial cells. The experiments were carried out at initial pH = 2.3, temperature 35°C, and a speed of agitation 130 strokes per minute for 2.5 hours. After 15 minutes of equilibration at the desired temperature, the reaction was started by tipping the cell suspension from the side arm into the main compartment of the flask.

RESULT AND DISCUSSION

Surface Characteristics of Rubber Samples

As indicated in Figure 1, the morphologies of rubber samples characteristically show numerous protrusions, which are uniformly distributed throughout their surfaces. Similar surface features were observed by Lin (1986) during Auger spectroscopic studies of rubber samples. All eight SBR and SMR rubber samples used in this study showed uniform distribution of their sulfur contents as depicted by the EDX dot mapping in Figure 1. The sulfur dot density increased with the increase in the sulfur concentration of the rubber samples. This tendency is an indirect reflection of the extent of sulfur participation in cross-linking of rubber (Lin, 1987).

Biodesulfurization of Rubber in Shake Flasks

The results obtained in the series of shake flask experiments are shown in Figure 2. All experiments were carried out with 3 g rubber sample (No.4) containing 15.6 g sulfur. The data points in Figure 2 represent the mean value of triplicate experiments. The efficiency of mixed culture of *T.ferrooxidans* and *T. thiooxidans* in the desulfurization of rubber can be appreciated from the difference between the inoculated and sterile runs. After six days of bioleaching, the maximum concentration of sterile experiments was 33 ppm, while in the

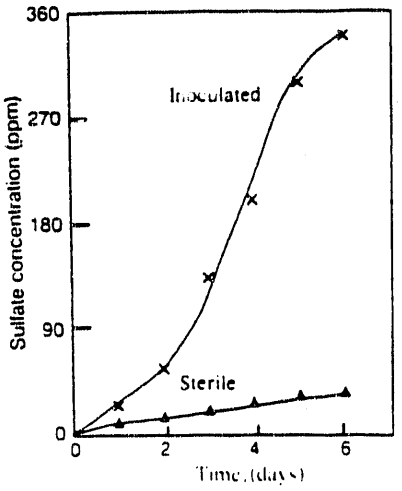

Figure 2. Biodesulfurization of rubber sample #4 by a mixed culture of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*.

inoculated runs about 350 ppm. Therefore, the bacterial desulfurization process was about 10 times faster than that of the sterile one. After six days of leaching, the sulfate concentration reached its maximum value and became independent from further increase in leaching time.

The influence of rubber concentration was studied with sample No.4, in the range of 1 to 16% pulp densities (the mass of solid rubber (g) over the total volume of leach solution multiplied by 100) using the mixed culture of *T. ferrooxidans* and *T. thiooxidans*. The results are summarized in Figure 3. The increase in the sulfate concentration was proportional with the pulp density of rubber up to about 8%, then it deviated from the straight line behavior. It is possible that at higher than 8% pulp densities, some of the rubber components were solubilized in proportions to reach high enough concentrations to be toxic to the microorganisms.

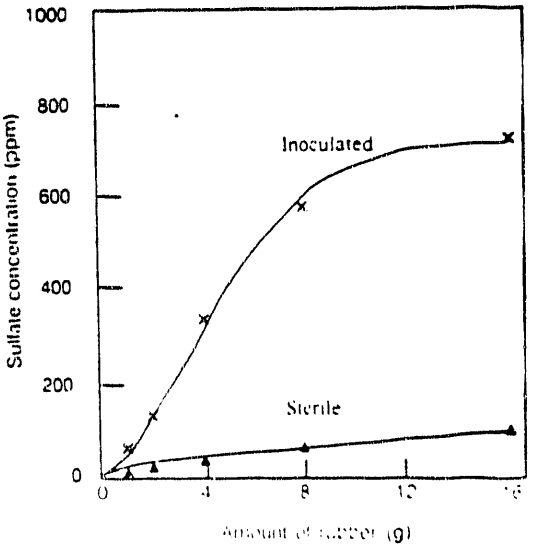

Figure 3. Effect of pulp density on the biodesulfurization of rubber sample #4 by a mixed culture of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* in 6 days of treatment.

Similar effects were observed by other investigators (Karlsson et al., 1988), who studied the effect of surfactants on biodegradation of polyethylene.

Oxygen Uptake Experiments

The metabolic activity of microorganisms during the desulfurization of rubber requires the presence of oxygen as expressed in equations 1 through 3. Consequently, the oxygen consumption can be related to the product (sulfate) formation.The kinetics of biodesulfurization of the eight rubber samples was studied in Warburg respirometers using cells free of substrates that were harvested from pure and mixed cultures of *T. ferrooxidans* and *T. thiooxidans*.

As shown in Figure 4, the best results were obtained with the mixed cells of *T. ferrooxidans* and *T. thiooxidans*, and rubber samples containing the highest amount of sulfur. A similar observation was made for the effect of sulfide concentration relative to the manometric studies of rubber sulfide oxidation (Silver and Torma, 1974). The initial rate of oxygen uptake (V; $\mu\ell O_2\ h^{-1}$) was computed from the experimental data obtained in the first 32 minutes as the slope of the straight lines shown in Figure 4. The specific rate of oxygen uptake (V_{spec}; $\mu\ell O_2\ h^{-1}$ mg protein^{-1}) was obtained by dividing V with the amount of bacterial protein present in the cells free of substrate used for experimentation. Table 3 compares the mean values of V_{sp} computed from data of triplicate runs. The large difference between the data of inoculated runs and sterile controls indicates again the effects of bacteria. Practically, there is no difference in the specific rates of oxygen uptake for the sterile controls (no bacteria present or thymol solution added), and endogenous respiration (with heat killed bacteria). The highest specific oxygen uptake rates were obtained with rubber samples containing the largest amount of sulfur (15.6%) and using substrate free cells of mixed cultures of *T. ferrooxidans* and *T. thiooxidans*. No data are available from the literature for comparison.

CONCLUSION

The applicability of bacteria in the desulfurization of commercially available rubber samples has been demonstrated using pure and mixed cultures of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. It was found that under the experimental conditions the bacterially-assisted desulfurization of rubber was about 10 times as efficient as the sterile (chemical) process.

The information generated in this investigation may have a far-reaching significance in the development of biodegradation methods for the remediation of polymeric wastes including rubbery waste materials.

ACKNOWLEDGEMENT

The work presented in this article was carried out at the Department of Materials and Metallurgical Engineering, New Mexico Tech, Socorro, NM.

The authors wish to express their appreciation to Dr. D. Benko, Section Head, Compound Science, Goodyear Tire and Rubber Company, Akron, OH, for providing them with the rubber samples.

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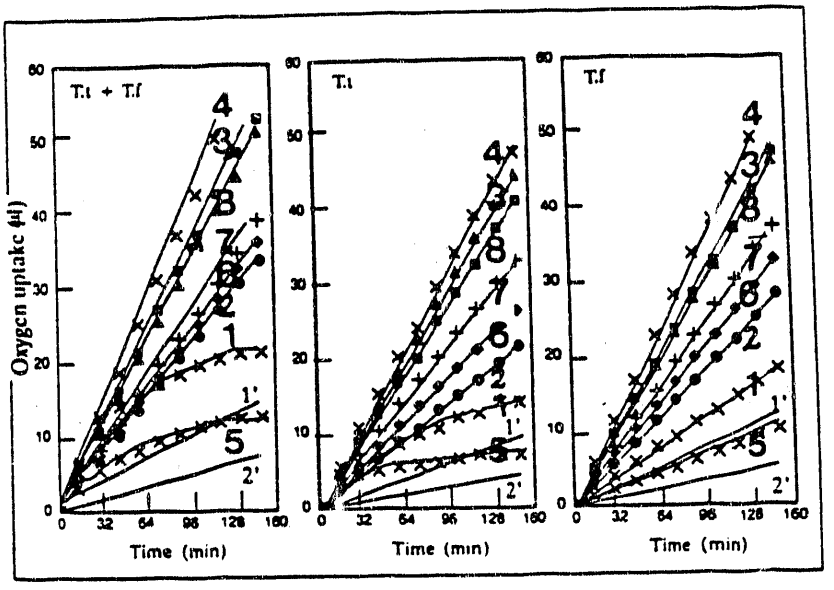

Figure 4. Oxygen uptake measurements during desulfurization of rubber samples by cells free from substrate. Where the denominations 2 through 8 of rubber samples correspond to those of Table 3 and 1′ and 2′ represent the average of sterile controls on rubber samples 1 to 4 and 5 to 8, respectively.

TABLE 3. COMPARATIVE KINETICS OF OXYGEN UPTAKE DURING BIODESULFURIZATION OF RUBBER SAMPLES BY CELLS FREE OF SUBSTRATE, HARVESTED FROM PURE AND MIXED CULTURES OF *T. thiooxidans* AND *T. ferrooxidans*.

		Specific rate of oxygen uptake $[\mu dm^3 O_2\ (mg\ protein)^{-1} h^{-1}]$							
Rubber Samples		1	2	3	4	5	6	7	8
Inoculated	A	1.13	2.23	5.58	6.69	0.96	3.43	3.97	4.60
	B	1.15	2.17	5.00	5.42	0.86	2.38	2.86	3.57
	C	1.28	2.34	6.25	7.50	0.62	4.16	4.46	4.69
Sterile (Heat-killed) Endogenous respiration	A	0.40	0.45	1.29	1.37	0.37	0.50	0.60	0.63
	B	0.39	0.40	1.25	1.33	0.37	0.42	0.45	0.48
	C	0.42	0.47	1.28	1.40	0.38	0.52	0.55	0.59
Sterile (thymole)		0.41	0.43	1.27	1.37	0.38	0.50	0.55	0.61
Sterile (no bacteria)		0.40	0.48	1.30	1.42	0.36	0.50	0.60	0.65

where A: pure culture of *Thiobacillus thiooxidans*
B: pure culture of *Thiobacillus ferrooxidans*
C: *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*

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