Biotechnological possibilities for waste tyre-rubber treatment

Olle Holst[1,*], Bengt Stenberg[2] & Magdalena Christiansson[1]
[1] *Dept. of Biotechnology, Centre for Chemistry and Chemical Engineering, Lund University, P. O. Box 124, S-221 00 Lund, Sweden;* [2] *Polymer Technology, Royal Institute of Technology, S-100 44 Stockholm, Sweden* (* author for correspondence)

Abstract

Every year large amounts of spent rubber material, mainly from rubber tyres, are discarded. Of the annual total global production of rubber material, which amounts to 16–17 million tonnes, approximately 65% is used for the production of tyres. About 250 millions spent car tyres are generated yearly in USA only. This huge amount of waste rubber material is an environmental problem of great concern. Various ways to remediate the problem have been proposed. Among these are road fillings and combustion in kilns.

Spent tyres, however, comprise valuable material that could be recycled if a proper technique can be developed. One way of recycling old tyres is to blend ground spent rubber with virgin material followed by vulcanization. The main obstacle to this recycling is bad adhesion between the crumb and matrix of virgin rubber material due to little formation of interfacial sulphur crosslinks. Micro-organisms able to break sulphur-sulphur and sulphur-carbon bonds can be used to devulcanize waste rubber in order to make polymer chains on the surface more flexible and facilitate increased binding upon vulcanization. Several species belonging to both Bacteria and Archaea have this ability. Mainly sulphur oxidizing species, such as different species of the genus *Thiobacillus* and thermoacidophiles of the order of *Sulfolobales*, have been studied in this context.

The present paper will give a background to the problem and an overview of the biotechnological possibilities for solutions of waste rubber as an environmental problem, focusing on microbial desulphurization.

Introduction

The white sap of the South American tree *Hevea brasiliensis* forms the basis of a large and global industry, the rubber industry. The South American Indians were familiar with the material and used it for various purposes already when the first Europeans came in the late 15th century. The Indians called the tree Ca-hu-chu (kautschuk!) or 'the crying tree'. Since then rubber technology has evolved. The vulcanization process was invented by Charles Goodyear in 1839. By adding a few percentage of elemental sulphur to a natural rubber (NR) and heating the material to 140–180 °C under pressure (40 atm) the original sticky and viscous material was converted to a non-sticky and elastic material. The ability of rubber to extend several hundred percent in length upon the application of external stress, with virtually immediate and complete recovery on release of the stress, cannot fail to fascinate both the scientist and the layman. In addition to NR many synthetic rubbers have been developed and together with sulphur several curing agents and additives are used for their manufacturing. Today, rubber materials are used in a wide variety of high tech products. The largest single rubber product is tyres. The global rubber consumption in 1996 was estimated to be 16–17 million tons, of which 65% were used for tyre production. About 250 millions tyres are generated yearly in USA and in Sweden the number is 6 millions (Godfrey 1996; Rapra 1996; White 1996).

After usage, spent car tyres, are a world wide solid waste problem. The recommended priority order for how to reduce the volume of this kind of waste material should be: reduction of consumption,

Table 1. A typical recipe of a rubbermix for tyre surfaces (phr = parts per hundred rubber)

Compound	phr	Compound	phr
Natural rubber (NR)	100	Stearic acid	2,5
Carbon black N 330	40	Retarders	0,2
Sulphur	2,3	Antioxidizers	1
Accelerator	0,5	Antiozonants	1
Zinc oxide	3	High aromatic oils (HA)	4
		Anti-sunchecking agents	0,5

reuse of the product, recycling of the material, energy recovery and, as the final option, deposition of the product. Due to the chemical crosslinking, it is not possible to simply melt and reshape rubber materials, as can be done with e.g. thermoplastics like polyolefins (e.g. polyethylene, polypropylene). In order to reuse rubber materials special operations like grinding, pyrolysis and other thermal methods come into question. During treatment by these methods the material may become degraded to a certain extent. The waste tyre market is growing fast and in 1994, 55.4% of the scrap tyres generated in USA were reused in some way. Still rubber producers are distrustful to recycled material and large quantities of spent tyres are today used as fuels or for civil engineering purposes, like road fillings (Leblanc 1996; Novelli 1995; Rouse 1995; White 1996). Many of these applications are just concealed deposition and are not a satisfactory solution to the waste problem. Other methods and processes need to be developed. Research activities on how to overcome the problem with spent car tyres are extensive and the number of reports on the topic is nearly as comprehensive (e.g., Beckman et al. 1974; Crane et al. 1978; Droscher 1995; Liaskos 1994; Smith 1978; Swor et al. 1980). The present paper will give a background to the problem and an overview of the biotechnological possibilities for solutions of waste rubber as an environmental problem, focusing on microbial desulphurization.

Rubber materials

Rubber tyres are normally made out of vulcanized natural rubber (NR), butadiene rubber (BR) and/or styrene butadiene rubber (SBR) in various mixtures and combinations. NR and SBR are relatively inexpensive and make up the largest quantities of polymers in rubber products. The pure rubber has to
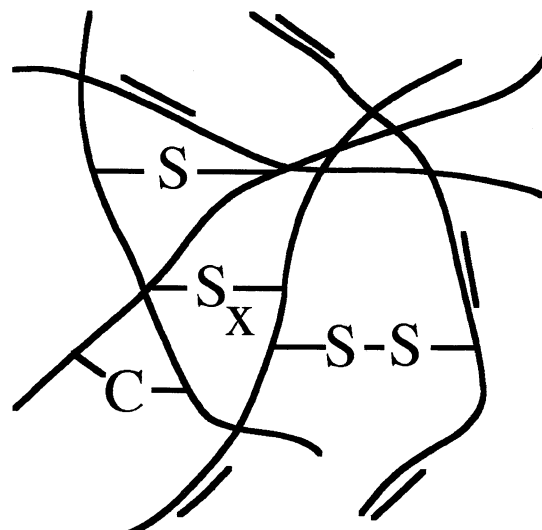

Figure 1. Vulcanized rubber materials containing mono-, di- and polysulphidic crosslinks as well as carbon- carbon bonds. The average distance beween crosslinks is 10 μm.

be crosslinked, i.e. vulcanized, to become strong and elastic over a wide temperature range. Unsaturations in the polymer provide sites for vulcanization. Crosslinking and cure are used synonymously to the word vulcanization. A typical tyre formula is presented in Table 1. Depending on the formulation of the rubber recipe and the vulcanization parameters applied either polysulphidic, disulphidic or monosulphidic crosslinks are formed (Figure 1). Also carbon-carbon bonds can be formed. Different rubber materials are used in different types of tyres and also in different parts of the tyres. NR, SBR and BR swell in contact with oil and their mechanical properties are lost. NR also shows limited thermo-oxidative resistance and cannot be used for long times at temperatures above 70–80 °C. Every monomer unit in NR contains an unsaturated bond, which is susceptible to thermo-oxidative degradation, attack by ozone or degradation by UV-light. Synthetic alternative rubber materials have been developed which show good resistance to swelling in oil and exposure to elevated temperatures. Synthetic rubber materials which can withstand elevated temperatures for long times in aggressive environments have been developed. Spent rubber is thus a mixture of materials of a wide variety of origins. For detailed information about rubber materials and rubber technology, the reader is refered to Blow & Hepburn (1982), Brydson (1988) and Mark et al. (1994).

Techniques for grinding spent rubber material

The scrap tyres are normally first shredded into larger pieces (avarege size 20 × 20 mm) and then ground to less than 1 mm. Spikes, cords and textiles are subsequently removed. Several grinding methods for rubber have been developed (Burgyon et al. 1976; Khait 1997; Klingensmith 1991). The conventional method of grinding is ambient milling, either dry or wet. In spite of its name, ambient milling generates heat, especially in compounds with high modulus and this can result in some degradation of the rubber surface. A relative large surface area is generated making the rubber more prone to oxidation. However, a large area can be an advantage from other aspects. Cryogenic grinding was first developed in the 1960s. In Surahammar, Sweden, a plant for cryo-grinding of spent car tyres is runned by Returdäck AB. Shredded tyres are cooled in liquid nitrogen (-196 °C). The brittle material is ground to fine particles in hammer mills, sieved and remaining cord and steel are removed. Generally cryo-ground rubber has a smooth surfaces (Phadke et al. 1984). Depending on product requirements and costs, a cryogenic or ambient milling process is selected (Klingensmith 1991; Liaskos 1994). The grinding step is of importance for the outcome in the subsequent microbial treatment (see below).

Physical properties of rubber partly made from old material

One way to reuse ground rubber is to mix and vulcanize it with new virgin material for production of various rubber goods (e.g. tyres). Today, materials for certain applications contain a few percent ground material because it increases stiffness and tear strength, improves mixing and processing by facilitating removal of enclosed air and has a positive effect on the cost. Additions over a few percent can, however, be troublesome. The mechanical and thermal properties of mixed rubber largely depend on the particle size and the amount of recycled material used (Burford & Pittolo 1983; Phadke 1984; Swor et al. 1980). Cryo-ground rubber vulcanized with natural rubber showed an increase in mooney viscosity, modulus and stiffness, while tensile strength, elongation at break and rebound resilience decrease. Tear properties were not affected by addition of cryoground rubber material (Phadke et al. 1984). Törnsten (1994) mixed cryo-ground material with sizes less than 0.2 mm and 0.4 mm with NR, SBR and EPDM (rubber of ethylene-propylene-diene monomer). Increasing amounts of untreated cryo-milled rubber mixed with natural rubber gave increased physical relaxation and lowered the scorch time, tensile strength, elongation and nozzle swelling. In the SBR mixture, increasing amounts of old rubber gave an increase in physical relaxation and shear stiffness, while the scorch time, reometer moment and tensile strength decreased. Scanning electron microscopy (SEM) analyses of fractures showed aggregation of old particles and bad adhesion. From SEM analyses it is clear that process failure is associated with de-wetting of the crumb particles, with the ultimate failure occuring by growth of the voids formed (Burford & Pittolo 1983). These studies highlight the necessity of developing methods for modification of the recycle rubber to enhance the formation of crosslinks between crumb and virgin material upon revulcanization. If this is achieved, an increased amount of recycled material in remixes could be used.

Modification methods

In an earlier review, Warner (1994) summarizes several chemical and physical methods of modification of the rubber material to be recycled. New methods are constantly suggested. The mechanism behind chemical methods is either to add a compound that modifies the surface (e.g., Bauman 1995; Dierks 1996) or totally reverses the crosslinking (e.g., Kohler & O'Neill 1997; Myhre & MacKillop 1996). Some of the methods are commercially available as is the modified rubber thus produced.

Chemical modification techniques sometimes require usage of hazardous chemicals. Physical methods are not impaired with this but might on the other hand require a lot of energy. Isayev has developed an ultrasonication technique for devulcanization (Isayev et al. 1995; Isayev et al. 1997; Johnston et al. 1997; Levin et al. 1996). Shear extraction is another alternative (Khait 1997). Bioprocessing could become an inexpensive and non-toxic alternative.

Microbial deterioration of rubber

One possible way of getting rid of spent rubber could be to degrade it using microorganisms. Spent rubber could be used as substrate for microorganisms provided the structure can be efficiently degraded (Kawai

1994). Studies on the topic are abundant. One must keep in mind that these studies are in most cases related to the prevention of microbial attack and subsequent degradation of the rubber. Rubber should withstand all kinds of harsh environments such as extreme heat or cold, ozone, radiation of ultraviolet light and also microbial attack. In many applications rubbers are used in environments where microorganisms are abundant such as sewage pipes, soil, water etc (Blake et al. 1955; Cadle & William 1980; Rook 1955). In these applications the resistance towards microbial attack is of utmost importance. In fact rubber recipes have been formulated to increase the stability and prevent microbial attack. Many studies have been made on microbial degradation of rubber materials aiming to either prevent or enhance mineralisation (Cundell & MacKillop 1973; Cundell et al. 1973; Keursten & Groenevelt 1996; Kwiatkowska et al. 1978; Williams 1986; Zyska et al. 1971; Zyska 1981). Most studies are dealing with micro-organisms belonging to the *Actinomycetes* (e.g., Tsuchii 1995; Heisley & Papadatos 1995; Low et al. 1992). Jendrossek et al. (1997) found 46 rubber degrading strains among 1220 tested all belonging to the *Actinomycetes* group. Tsuchii et al. (1996) have used species of *Nocardia* for rubber degradation. Steinbuchel has reported solubilisation of natural rubber of both pure rubber and vulcanized latex by using species of *Gordona* (Linos & Steinbuchel 1996; Steinbuchel & Linos 1997). Biosurfactants were believed to facilitate degradation of the rubber during adhesive growth. Vulcanized rubber in the form of latex is rather easily degraded while rubbers from spent truck tyres are more resistant (Tsuchii et al. 1997). Microbial adaptation to growth on polymer material and adhesion of co-substrate increases degradation. The mechanisms behind microbial rubber degradation are often oxidation and chain scission of the polymer backbone (Tsuchii et al. 1990), which does not result in materials suitable for reuse.

The effect of compounding chemicals on the microbial breakdown of vulcanized natural rubber by a *Nocardia* sp. has been investigated (Tsuchii & Takeda 1990). It was found that increased amount of additives such as carbon black (a filler) and cyclohexylbenzothiazole sulphenamide (CBS, an accelerator) as well as elemental sulphur increased the resistance towards microbial degradation. The addition of these compounds is most certainly causing a decreased access for the microorganisms to the rubber matrix.

An interesting approach would be to combine partial microbial degradation with physico-chemical methods to obtain a rubber material better suitable for recycling. To our knowledge no such experiments have been done. The possibility to use micro-organisms for detoxification prior to further bioprocessing requires further investigation.

Microorganisms for desulphurization of solid material

Several mesophilic and thermophilic microorganisms are known to oxidize sulphidic minerals like pyrite, copper sulphide and arseno pyrites (Brombacher et al. 1997; Johnsson et al. 1993; Sand et al. 1995). These microorganisms are chemolithotrophes and derive energy from the oxidation of various sulphur compounds. Carbon for cell growth is obtained from CO_2. These microorganisms have successfully been used for desulphurization of coal, mineral oil and clays as well as for the leaching of metals from ores (Karavaiko & Lobyreva 1994; Larsson et al. 1994; Maka & Cork 1990; Ryu et al. 1995; Shennan 1996). The genus *Thiobacillus* is among the most studied sulphur oxidizers. *T. ferrooxidans* can utilise metal sulphides such as pyrite. Other species are *T. thiooxidans, T. caldarius* and *T. thioparus.* Several sulphur oxidizing species have been isolated from acidic hot springs all over the world. They belong to the domain Archaea and the order Sulfolobales. They have optimal growth temperatures of 65–85 °C and many are also acidophilic, preferably growing around pH 1.4 −3 (Segerer & Stetter 1992). *Sulfolobus acidocaldarius* and *S. solfataricus* have been tested in coal desulphurization experiments (Kargi & Robinson 1982; Larsson et al. 1995). *Acidianus brierleyi* has been reported to remove both inorganic (Olsson et al. 1993) and organic sulphur in coal (Khalid et al. 1990). Two other species of sulphur oxidizing and ore leaching microorganisms are *S. metallicus* (Huber & Stetter 1991) and *Methallosphaera sedula* (Huber et al. 1989). *M. sedula* has been used for desulphurization of pyrite and coal (Clark et al. 1993).

The desulphurization of organically bound sulphur is of great interest since a large part of the sulphur in oil and coal are bound to organic molecules. Structures similar to dibenzothiophene (DBT) are very common in oil and thus DBT has been used as a model molecule for desulphurization of organically bound sulphur in several studies (Afferden et al. 1993; Krawiec 1990). Strains of the genera *Pseudomonas, Rhodococcus, Paenibacillus* and *Bacil-*

lus are reported to have the ability to oxidize DBT (Krawiec 1990; Kilbane II & Jackowski 1992; Kilbane II 1991; Konishi et al., 1997) and DBT is completely mineralised by *Brevibacterium* sp. DO (Afferden et al. 1993). The active enzyme in *Brevibacterium* sp. DO is, however, thought to be intracellular, which makes it less useful for desulphurization of complex materials like rubber. *R. rhodochrous* IGTS8 is able to use DBT as a sole sulphur source but not as a carbon source (Kilbane II & Jackowski 1992). *R. rhodochrous* is proposed to degrade DBT according to the '4S pathway', which means that DBT is converted to sulphoxide/sulphone/sulphonate and sulphate ions. The carbon frame is only slightly oxidized with the end products biphenyl, monohydroxybiphenyl and 2,2′-dihydroxybiphenyl (Krawiec 1990; Warhurst & Fewson 1994).

An interesting approach is the anaerobic reduction of sulphur from polysulphides by the hyperthermophilic archeaon *Pyrococcus furious* (Tilstra et al. 1992). Tilstra and co-workers found that *P. furiosus* liberated hydrogen sulphide when grown in the presence of various sulphides, e.g., dinaphtyl sulphides. Although only model compounds have been tested so far it would be of great interest to try anaerobic sulphur reducers in biodesulphurization processes.

Desulphurization of rubber materials

Microorganisms and incubation conditions

Already decades ago, it was reported that sulphur oxidizing microorganisms might be capable of oxidizing sulphur inclusions in rubber (Thaysen 1945). In 1945 problems with fire hoses were noted in England. Sulphuric acid was found in water remaining in the hoses after use. Eventually micro-organisms were identified as the cause of the acid formation. Sulphur oxidizing micro-organisms had oxidized the sulphur present in the rubber to sulphate, i.e. sulphuric acid. This was later confirmed by Torma and Raghavan (1990), who studied sulphur utilisation from newly made pulverised rubber materials by *T. ferrooxidans* and *T. thiooxidans* aiming to facilitate recycling of spent rubber. Eight synthetic rubbers with unknown composition, but with sulphur contents from 1.2 to 15.5%, were tested. A mixed culture of *T. ferrooxidans* and *T. thiooxidans* added to the rubber material containing 15.5% sulphur showed the highest formation of sulphate. In a shake-flask experiment with a slurry concentration of 40 g/L, pH 2, performed during six days 350 ppm sulphate was formed in comparison with 33 ppm for a sterile control. This was confirmed by respiration studies in Warburg respirometers where the increase in sulphate concentration was proportional to the initial pulp density of rubber material up to about 8%. Torma and Raghavan (1990) suggest that there might be an effect of toxic compounds when exceeding a critical concentration.

T. thioparus was reported to utilise more sulphur from powder accumulated from recapping of truck tyres than *T. ferrooxidans* and *T. thiooxidans*, both in pure and mixed cultures: i.e 4.70% for *T. thioparus* and 3.96% for the mixed culture (Löffler et al. 1993). They used another cultivation technique. A sulphurous substrate was added with the inoculum which had to be consumed before the sulphur content in the rubber was used. Desulphurization of the rubber started first around day 10 and levelled off around day 30. *T. thioparus* grows at neutral pH which is close to the pH of suspended rubber particles and might, therefore, be advantageous. Particle size had a strong effect on the amount of sulphur accessible for microorganisms; the degree of desulphurization decreased with increasing particle size. Calculated on a simple sphere model, the finest fraction, i.e., 0.1–0.2 mm was desulphurized in a surface layer of 1–2 μm. Effects on crosslinking density were studied by swelling particles in gaseous toluene but evidently the desulphurized fraction was too small to show differences between microbially treated and untreated material (Löffler et al. 1995). Also Löffler and co workers (1995) have studied the effect of pulp concentration and found a linear behaviour up to 20%. At higher concentrations the slurry became viscous and shear forces are believed to influence the microorganisms. Romine and co-workers (1995) used ground tyre rubber (74 μm) to screen six cultures of microorganisms for desulphurization activity. After seven days of treatment with *S. acidocaldarius* 13.4% (w/w) of the sulphur was removed which exceeded the amount extracted by a mixed culture of *T. ferrooxidans* and *T. thiooxidans*. No release of sulphate could be detected for *R. rhodochrous* or the mixed culture ATCC 39327. The compostion of this culture is not known. The formation of sulphone and sulphoxide groups were followed with Fourier Transformed Infrared Spectroscopy (FTIR) and X-ray analysis for near edge surfaces (XANES). The FTIR absorbance was strongest after 2–3 days and then slowly disappeared. After seven days the ground rubber was completely stripped of surface sulphur.

Table 2. Microorganisms tested for desulphurization of rubber

Microorganism	Type of rubber	Reference
Thiobacillus ferrooxidans	SBR, NR, GTR	Torma & Raghavan 1990, Löffler et al. 1993, Romine et al. 1995, Christiansson et al. 1997
Thiobacillus thiooxidans	SBR, GTR	Torma & Raghavan 1990, Löffler et al. 1993, Romine et al. 1995
Mixed culture *T. ferrooxidans* and *T. ferrooxidans*	SBR, GTR	Torma & Raghavan 1990, Löffler et al. 1993, Romine et al. 1995
Thiobacillus thioparus	GTR	Löffler et al. 1993, Löffler et al. 1995
Rhodococcus rhodochrous IGTS8[1]	GTR	Romine et al. 1995
ATCC 39327 (mixed culture)	GTR	Romine et al. 1995
Acidianus brierleyi[1]	GTR	Christiansson et al. 1998
Sulfolobus acidocaldarius	GTR	Romine et al. 1995
TH2Lund[2]	GTR	Christiansson et al. 1998

GTR ground tyre rubber of unknown composition; NR fresh natural rubber; SBR fresh styrene-butadiene rubber
[1] tested but unsuccessful; [2] an archaeal isolate.

The result was evaluated by tensile and elongation testing. Ground tyre rubber desulphurized for 7 days was blended with virgin rubber material in amounts up to 20% and vulcanized. No positive effect of the desulphurization was detected. On the contrary good properties were only obtained with ground tyre rubber desulphurized with *S. acidocaldarius* for 72 hours. On 15% (w/w) loading of treated rubber the elastic modulus was 15% higher than the untreated control. Romine et al. (1995) explain the difference in results with formation of sulphone and sulphoxide functionalities. These are easily reduced in the presence of elementary sulphur and are thus believed to be important during revulcanization. According to this theory the process had to be optimised to yield as many sulphones and sulphoxides as possible. However, sulphur bridges in rubber material are quite disperse with approximately 100 monomers between each sulphur functionality. Every unsaturation in the hydrocarbon backbone can, of course, be accessible during a second revulcanization, provided that appropriate mixing between virgin and old polymer chains is achieved. By obtaining a more loose surface, the flexibility of chains increases and a higher degree of mixing can be expected. This is probably the most important advantage with techniques for splitting crosslinks in vulcanized rubber materials. Rubber material treated with a mixed *Thiobacillus* culture had a lower elastic modulus than the control, but the reasons were not further identified. A summary of the strains used for desulphurization is given in Table 2. Romine et al. (1998) have patented a method for enzymatic devulcanization of waste rubber. Enzymes from sulphur oxidizing microbes are used for selectively breaking the S crosslinks in vulcanized rubber in the surface layer. Also this process is halted at the sulphoxide or sulphone step so that the devulcanized layer is reactive with virgin rubber.

Sulphur oxidizers are known for their ability to withstand harsh environment such as low pH ($<$3) and high temperature ($>$65 °C). Species were chosen in accordance with experiences from previous studies concerning coal desulphurization (Clark et al. 1993; Olsson et al. 1993; Olsson et al. 1995) with thermophilic microorganisms, and from reports in literature (Kilbane II & Jackowski 1992; Löffler et al. 1993; Löffler et al. 1995; Raghavan et al. 1990; Romine et al. 1995; Torma & Raghavan 1990). The strains used are: *T. ferrooxidans, T. thioparus, R. rhodochrous, A. brierleyi* and TH2 Lund. TH2 Lund is an unidentified archaean isolate and grows at 65 °C on Allens medium supplemented with yeast extract, elemental sulphur or pyrite.

Ground spent car tyres was the target material for rubber desulphurization. Initial experiments were made on cryo-ground tyres with a particle sizes less than 0.4 mm or 0.2 mm. The particle size is of vital importance for good mechanical properties of a material containing a mixture of fresh- and spent rubber material (Burford & Pittolo 1983; Phadke et al. 1984). As a general rule a better material is obtained with smaller particles. A smaller particle size is also advantageous for microbial rubber desulphurization because a larger surface area is exposed to the microorganisms. However, costs for grinding increase almost exponential with decreasing particle size. Grinding techniques
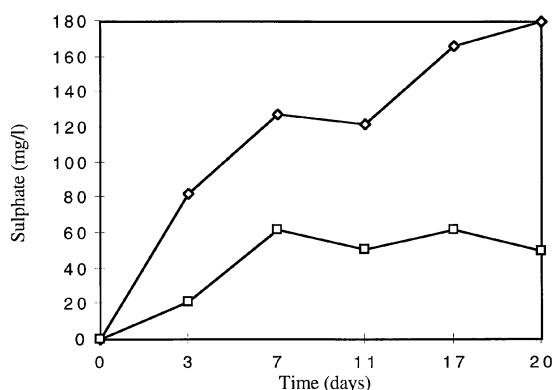
Figure 2. Formation of sulphate during treatment of cryo-ground spent rubber material with *Thiobacillus ferrooxidans.* Treatment with *T. ferrooxidans* ($\Diamond$) and sterile control ($\square$).

might be of importance for results of revulcanization, as different methods result in different surface shapes.

The easiest method to follow sulphur oxidation is to measure the sulphate release and the pH drop of the growth medium. Initial experiments resulted in an increase of the concentration of sulphate corresponding to a desulphurization of approximately 8 wt% when *T. ferrooxidans* was growing on cryo ground spent car tyre rubber during 20 days compared to 3 wt% for the control (Figure 2). TH2 Lund reduced the sulphur content in rubber with 1.5% compared to 0.6% for the control (Christiansson et al., 1997). Neither *A. brierleyi* nor *T. thioparus* exhibited growth in the presence of cryo ground spent rubber material.

Working with old rubber has some drawbacks. It is a heterogeneous material that can contain a lot of sand and soil. More important, the composition of the polymer-backbone is unknown, because tyres are made of many different rubber types with various properties. This makes physical testing of rubber blends containing reused material difficult to interpret. We decided to conduct desulphurization experiments with freshly made ground rubber in the subsequent experiments. Tyres are mainly made of natural rubber (NR), styrene butadiene rubber (SBR) and/or butadiene rubber (BR) so test pieces of tyre rubber materials based on these polymers were made, ground and tested for desulphurization with *T. ferrooxidans* and TH2 Lund. Both species were completely inhibited by the fresh made materials. Leaching of the rubber materials in water at appropriate temperatures corresponding to growth temperatures for the different microorganisms before cultivation did not have any effect. Nevertheless, the solute from a second leaching did not inhibit

the growth of *T. ferrooxidans* or TH2 Lund, indicating that both water soluble and more hydrophobic substances are responsible for toxicity. Leaching of compounds inhibiting Archaeas was also observed during coal desulphurization (Olsson et al. 1989). However, the problem was alleviated by subsequent leaching in water (Olsson et al. 1989).

Toxicity

Rubber materials contain several chemicals, which are added to enhance vulcanization, prevent degradation, make the material more durable, softer, or less expensive. Several reports are written on bacterial and fungicidal effects of rubber chemicals aiming to prevent degradation of rubber materials (Cundell & Mulcock 1973; Cundell et al. 1973; Williams 1986; Zyska 1981). Twenty-one rubber chemicals of rubber grade were tested for toxicity towards four species of microorganisms. No quantitative studies were made. The chemicals tested were applied in concentrations expected operating at a rubber slurry concentration of 10%w/v. *T. ferrooxidans,* TH2 Lund, *A. brierleyi* and *R. rhodochrous* was tested. *T. ferrooxidans* tolerated most of the investigated chemicals. On the other hand, the archaeas, *A. brierleyi* and TH2 Lund turned out to be sensitive to several of the tested chemicals. At a higher cultivation temperature (>65 °C) leaching will be more extensive, but differences in cell walls and cell membranes between Bacteria and Archaea are also reflected. Tetramethyl thiurame disulphides (TMTD) and tetramethyl thiurame monosulphides (TMTM) are accelerators and vulcanizing agents, and inhibited growth of both *T. ferrooxidans* and the archaeas (Christiansson et al., 1997). Other tested accelerators showed little or no inhibition of growth, i.e. dibenzothiazyl-disulphide (MBTS), cyclohexylbenzothiazole sulphenamide (CBS) and N-oxydiethen-2-benzothiazole-sulfenamide (MBS). Growth was not obtained in the presence of dimethylbutyl-p-phenylenediamine (6-PPD) by any of the tested species. Interestingly, also pure SBR-polymer (50 g/l) affected growth of all tested strains except *R. rhodochrous.* Leaching of toxic compounds is a less serious problem when working with ground rubber material that is older, has been exposed to weather and wind, and can therefore be expected to contain lower amounts of water soluble and volatile inhibitory compounds. However, our experience is that the tolerance of the sulphur oxidizing microorganisms is very much dependent on the batch of spent ground material.

Table 3. Average concentration of sulphur (arbitrary units) on surface and interior of NR- rubber obtained with scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy in samples before treatment (1), after cultivation of *Thiobacillus ferrooxidans* (2) and in a sterile control sample (3)

Sample	surface (left)	centre	surface (right)
1: untreated	77.1	69.8	123.5
2: *T. ferrooxidans*	58.1	80.5	64
3: Sterile control	62.3	64.3	61.6

Detoxification can be achieved by extracting the rubber with organic solvents such as acetone or ethanol before bio-processing (Christiansson et al. unpubl.).

Localization of desulphurization activity

Measuring dissolved sulphate in the medium has the drawback that it does not show the effect of partly oxidized sulphur bridges. Thus nothing is known about the sulphur left on the surface of the rubber material. The crosslink is broken, but sulphate is not formed. We have used two analytical surface methods to detect decreases in sulphur content, i.e., scanning electron microscopy (SEM) in which the elemental constituents were analysed by an energy-dispersive X-ray spectrometer (EDS) and Electron Spectroscopy for Chemical Analysis (ESCA). EDS measures the relative amount of an element on a scanned surface (Heinrich & Newbury 1991). Results from toxicity studies were used to produce a suitable material for desulphurization. It was based on natural rubber, preservative chemicals were excluded and titanium oxide was added as internal standard. Sheets of the material were cut in flakes, desulphurized with *T. ferrooxidans* and analysed with SEM/EDS. The flakes were scanned over areas of 10 μm and the sulphur content was measured as arbitrary units based on the titanium content of the two surfaces. The sulphur was not depleted but the amount had decreased on the surface of rubber flakes treated with *T. ferrooxidans* (Table 3). The same rubber material was also analysed with ESCA. This technique is based on measurements of differences in bond energies between elements and it is thereby possible to distinguish oxidized sulphur from elemental sulphur or sulphides. A higher content of oxidized sulphur species was detected on the surface for the microbial treated sample than in controls and interior of the sample (Christiansson et al. 1998). However, the total sulphur content was low,

i.e. less than 2%, and thus on the detection limit, so quantitative measurements could not be made. Nevertheless, ESCA is a good technique to follow changes in sulphur species upon desulphurisation. More has to be done on analyses of the product after rubber desulphurization on a molecular level. For optimal recycling, most of the sulphur will react upon vulcanization and form cross links, yet traces of dissolved unreacted elemental sulphur will remain in the rubber after vulcanzation. Some sulphur is adsorbed to the surface of carbon black and will not contribute to the vulcanization. Sometimes, vicinal cross links are formed that act as single links but demand the double amount of sulphur. None of the published studies concerning microbial desulphurisation have distinguished between elemental sulphur present and sulphur actually contributing to crosslinks (Löffler et al., 1993; Löffler et al. 1995; Raghavan et al. 1990; Torma and Raghavan 1990; Romine et al. 1995).

Conclusions

Studies have shown that sulphur can be removed, as revealed by sulphate formation, from spent rubber material by microbial action. As seen by advanced analytical techniques (SEM-EDS, ESCA) the removal is only a surface phenomenon. Sulphur on the surface is partly removed and partly oxidized. Toxicity of rubber additives may be a problem and has to be taken into account.

So far only few species have been tested for their sulphur removal ability and new strains should thus be screened for. Anaerobic treatment is an interesting possibility in need of investigation. The concept of using rubber degrading micro-organisms in combination with sulphur oxidizers has, to our knowledge, not been explored and may prove useful.

Pilot scale studies are necessary to investigate technical and economical feasibility.

Extensive physical testings are needed for evaluation of the properties of the new products. New knowledge generated through the research may be used in production of rubber possible to desulphurize for a more efficient recycling.

References

Afferden M v, Tappe D, Beyer M, Truper HG & Klein J (1993) Biochemical mechanisms for the desulfurization of coal-relevant organic sulfur compounds. Fuel 72: 1635–1643

Bauman DB (1995) High-value engineering materials from scrap rubber. Rubber World 207(5): 30–33

Beckman JA, Crane G, Kay EL & Laman JR (1974) Scrap tire disposal. Rubber Chem. Technol. 47: 597–625

Blake JT, Kitchin DW & Pratt OS (1955) A microbial process discussion: the microbiological deterioration of rubber insulation. Appl. Microbiol. 3: 35–39

Blow CM & Hepburn C (1982) Rubber technology and manufacture. Butterworths, London

Brombacher C, Bachofen R & Brandl H (1997) Biohydrometallurgical processing of solids: a patent review. Appl. Microbiol. Biotechnol. 48: 577-587

Brydson JA (1988) Rubber materials and their compounds. Elsevier Science Publishers Ltd, London

Burford RP & Pittolo M (1983) Fracture morphology of rubber compounds containing recycled rubber powder. J. Mat. Sci. Lett. 2: 422–424

Burgyon MO, Leaker GR & Krekic Z (1976) Rubber Chem. Technol. 44: 404

Cadle SH & Williams RL (1980) Environmental degradation of tire particles. Rubber Chem. Technol. 53: 903–914

Christiansson M, Stenberg B & Holst O (1997) Influences of rubber chemicals on microbial desulfurization of waste tire rubber In: Verachtert H & Verstraete W (Eds) Int. Symp. Environ. Biotechnol. Vol. 2 (pp 331–334). Oostende, Belgium

Christiansson M, Stenberg B, Wallenberg LR & Holst O (1998) Reduction of surface sulphur upon microbial devulcanization of rubber materials. Biotechnol. Lett. 20: 637–642

Clark TR, Baldi F & Olson GJ (1993) Coal depyritization by the thermophilic Archaeon Metallosphaera sedula. Appl. Environ. Microbiol. 59: 2375–2379

Crane G, Kay E & Laman JR (1978) Scrap tire disposal procedures. Rubber Chem. Technol. 51: 577–599

Cundell AM & Mulcock AP (1973) The effect of curing agent concentration on the microbiological deterioration of vulcanized natural rubber. Int. Biodeter. Bull. 9(4): 91–94

Cundell AM, Mulcock AP & Hills DA (1973) The influence of antioxidants and sulphur level on the microbiological deterioration of vulcanized rubber. Rubber J. 155: 22–35

Dierks IW (1996) Solutions to the rubber waste problem incorporating the use of recycled rubber. Rubber World 214(5): 25–31

Droscher M (1995) Economic and ecological aspects of feedstock recycling. Kautsch. Gummi Kunstst. 48: 146–149

Godfrey N (1996) Recycle or reclaim the rubber option. Materials World 10: 577–579

Heinrich KFJ & Newbury DE (1991) Electron Microprobe Quantitation. Plenum Press, New York

Heisley MR & Papadatos S (1995) Isolation of microorganisms able to metabolize purified natural rubber. Appl. Environ. Microbiol. 61: 3092–3097

Huber G, Spinnler C, Gambacorta A & Stetter KO (1989) Metallosphaera sedula gen. and sp. nov. represents a new genus of aerobic, metal-mobilizing, thermoacidophilic, archaebacteria. System. Appl. Microbiol. 12: 38–47

Huber G & Stetter KO (1991) Sulfolobus metallicus, sp. nov., a novel strictly chemolithoautotrophic thermophilic archaeal specie of metal-mobilizers. System. Appl. Microbiol. 14: 372–378

Isayev A, Chen J & Tuchachinsky A (1995) Novel ultrasonic technology for devulcanization of waste rubbers. Rubber Chem. Technol. 68: 267–280

Isayev AI, Kim SH & Levin VY (1997) Superior mechanical properties of reclaimed SBR with bimodal network. Rubber Chem. Technol. 70: 194–201

Jendrossek D, Tomasi G & Kroppenstedt RM (1997) Bacterial degradation of natural rubber: a privilege to actinomycetes? FEMS Microbiol. Lett. 150: 179–188

Johnsson DB, Ghuari MA & McGinness S (1993) Biogeochemical recycling of iron and sulphur in leaching environments. FEMS Microbiol. Rev. 11: 63–70

Johnston ST, Massay J, von Meerwall E, Kim H, Levin VY & Isayev AI (1997) Ultrasound devulcaization of SBR: Molecular mobility of gel and sol. Rubber Chem. Technol. 70: 183–193

Karavaiko GI & Lobyreva LB (1994) An overview of the bacteria and archaea involved in removal of inorganic and organic sulfur compounds from coal. Fuel Process. Technol. 40: 167–182

Kargi F & Robinson JM (1982) Removal of sulfur compounds from coal by the thermophilic microorganism Sulfolobus acidocaldarius. Appl. Environ. Microbiol. 44: 878–883

Kawai F (1994) Breakdown of plastics and polymers by microorganisms. In: Fiechter A (Ed) Adv. Biochem. Eng. Biotechnol. Vol 52 (pp 153–194). Springer, Berlin

Keursten GTG & Groenevelt PH (1996) Biodegradation of rubber particles in soil. Biodegradation 7: 329–333

Khait K (1997) New solid-state shear extrusion polymerization process for used tire rubber recovery. Rubber World 215(5): 38–39

Khalid AM, Bhattacharyya D & Aleem MIH (1990) Coal desulfurization and electron transport-linkage oxidation of sulfur compounds by Thiobacillus and Sulfolobus species. In: Pierce GE (ed) Dev. Ind. Microbiol. Vol. 31 (pp 115–126). Amsterdam

Kilbane II JJ (1991) Mutant microorganisms useful for cleavage of organic C-S bonds. US patent 5002888 19910326

Kilbane II JJ & Jackowski K (1992) Biodesulfurization of water-soluble coal derived material by Rhodococcus rhodochrous IGTS8. Biotechnol. Bioeng. 40: 1107–1114

Klingensmith B (1991) Recycling, production and use of re-processed rubbers. Rubber World 203(6): 16–21

Kohler R & O'Neill J (1997) New technology for the devulcanization of sulfur-cured scrap elastomer. Rubber World 216(2): 32, 34–36

Konishi J, Ishii Y, Onaka T, Okumura K & Suzuki M (1997) Thermophilic carbon-sulfur-bond-targeted biodesulphurization. Appl. Environ. Microbiol. 63: 3164–3169

Krawiec S (1990) Bacterial desulfurization of thiophenes: screening techniques and some speculations regarding the biochemical and genetic bases. In: Pierce GE (Ed) Dev. Ind. Microbiol. Vol 31 (pp 103–114). Amsterdam

Kwaitkowska D, Zyska BJ & Zankowics LP (1978) Microbiological deterioration of natural rubber sheet by soil microorganisms. In: Oxley TA, Allsopp D & Becker G (Eds) Biodeterioration, 4th Int. Symp. (pp 135–141). Pitman Publishing, Berlin

Larsson L, Olsson G, Karlsson HT & Holst O (1994) Microbial desulfurization of coal with emphasis on inorganic sulfur. In: G.R. Chaudhry (Ed) Biological Degradation and Bioremediation

of Toxic Chemicals (pp 493–505) Dioscorides Press, Portland, OR

Larsson L, Olsson G, Holst O & Karlsson HT (1990) Pyrite oxidation by thermophilic archaebacteria. Appl. Environ. Microbiol. 56: 697–701

Leblanc JL (1996) Plasticizing properties of heavy oils obtained from vacuum pyrolysis of used tires. Kautsch. Gummi Kunstst. 49: 194–199

Levin VY, Kim SH, Isayev AI, Massay J & von Meerwall (1996) Ultrasound devulcanization of sulphur vulcanized SBR: crosslink density and molecular mobility. Rubber Chem. Technol. 69: 104–114

Liaskos J (1994) Rubber tyre recycling. UNEP Industry and Environment 17(3): 26–29

Linos A & Steinbuchel A (1996) Investigations on the microbial breakdown of natural and synthetic rubbers. In: Kreysa G (Ed) Biodeterioration and Biodegradation Vol 133 (pp 279–286). VCH-Verlagsgesellschaft, Weinheim, Germany

Low FC, Tan AM & John CK (1992) Microbial degradation of natural rubber. J. Nat. Rubber Res. 7: 195–205

Löffler M, Straube G & Straube E (1993) Desulfurization of rubber by *Thiobacilli*. In: Torma AC, Apel ML & Brierly CL (Eds) Biohydrometallurgical Technologies, Vol 2 (pp 673–680). The Minerals, Metals & Materials Soc.

Löffler M, Neumann W, Straube E & Straube G (1995) Microbial surface desulfurization of scrap rubber crumb – a contribution towards material recycling of scrap rubber (in German). Kautsch. Gummi Kunstst. 48: 454–457

Maka A & Cork DJ (1990) Introduction to the sulfur microorganisms and their applications in the environment and industry. In: Pierce GE (Ed) Dev. Ind. Microbiol., Vol 31 (pp 99–102). Amsterdam

Mark JE, Erman B & Eirich F (1994) Science and Technology of Rubber, Academic Press

Myhre MJ & MacKillop DA (1996) Modification of crumb rubber to enhance physical properties of recycled rubber products. Rubber World 214(5): 42–46

Novelli RL (1995) Recycling tires back into tires. Scrap Process. Recycl. 52(6): 75–80

Olsson G, Larsson L, Holst O & Karlsson HT (1989) Microorganisms for desulphurization of coal: the influence of leaching compounds on their growth. Fuel 68: 1270–1274

Olsson G, Larsson L, Holst O & Karlsson HT (1993) Desulphurization of low-sulphur coal by *Acidianus brierleyi*: Effects of microbial treatment on the properties of coal. Fuel Process. Technol. 33: 83–93

Olsson G, Holst O & Karlsson HT (1995) Microbial desulphurization of different coal types. In: Pajares JA & Tascon JMD (Eds) Coal Science and Technology Vol. 24 (pp 1741–1744) Elsevier Science, B.V.

Phadke AA, Chakraborty SK & De SK (1984) Cryoground rubbernatural rubber blends. Rubber Chem. Technol. 57: 19–23

Raghavan D, Guay R & Torma AE (1990) A study of debiogradation of polyethylene and biosulfurization of rubber. Appl. Biochem. Biotechnol. 24/25: 387–39?

Rapra (1996) Rubber in the environmental age – progress in recycling. Rapra Technology Limited

Romine RA, Snowdon-Swan L (1998) Enzymatic devulcanization of vulcanized waste rubber to virgin rubber products. US 5597851A 970128

Romine RA, Romine MF & Snowdon-Swan L (1995) Microbial processing of waste tyre rubber. In: Rubber Division 148:th Fall Technical Meeting, Paper 56, Cleveland, Ohio

Rook JJ (1955) Microbial deterioration of vulcanized rubber. Appl. Microbiol. 3: 302–309

Rouse MW (1995) Application of CRM in asphaltic materials. Rubber World 5: 23–29

Ryu HW, Cho KS, Chang YK, Kim SD & Mori T (1995) Refinement of low- grade clay by microbial removal of sulfur and iron compounds using *Thiobacillus ferrooxidans*. J. Ferment. Bioeng. 80: 46–52

Sand W, Gerke T, Hallmann R & Schippers A (1995) Sulfur chemistry, biofilm and the (in)direct attack mechanism – a critical evaluation of bacterial leaching. Appl. Microbiol. Biotechnol. 43: 961–966

Segerer AH & Stetter KO (1992) The order Sulfolobales. In: Balows A et al. (Eds) The Procaryotes, Vol 1 (pp 684–701). Springer-Verlag

Shennan JL (1996) Microbial attack on sulphur-containing hydrocarbons: implications for the biodesulphurisation of oils and coals. J. Chem. Tech. Biotechnol. 67: 109–123

Smith D (1978) The rubber waste problem: Is recycling being rediscovered? Rubber World 179(3): 46–49

Steinbuchel A & Linos A (1997) Microbial degradation of natural and synthetic rubbers. In: IRC'97, pp 305–307, Nurnberg

Swor RA, Jense LW & Budzol M (1980) Ultrafine recycled rubber. Rubber Chem. Technol. 53: 1215–1225

Thaysen A, Bunker H & Adams M (1945) 'Rubber Acid' damage in fire hoses. Nature 155: 322–325

Tilstra L, Eng G, Olson GJ & Wang FW (1992) Reduction of sulphur from polysulphidic model compounds by the hyperthermophilic archaeabacterium *Pyrococcus furiosus*. Fuel 71: 779–783

Torma AE, & Raghavan D (1990) Biodesulfurization of rubber materials. In: Bioprocess Engineering Symposium, Vol 16 (pp 81–87) American Society of Mechanical Engineers, Bioengineering Division

Tsuchii A, Hayayshi K, Hironiwa T & Matsunaka H (1990) The effect of compounding ingredients on micobial degradation of vulcanized natural rubber. J. Appl. Polymer Sci. 41: 1181–1187

Tsuchii A & Takeda K (1990) Rubber-degrading enzyme from a bacterial culture. Appl. Environ. Microbiol. 56: 269–274

Tsuchii A (1995) Microbiological degradation of natural rubber. Biotransformations: Microbiological degradations of health risk compounds

Tsuchii A, Takeda K, Suzuki T & Tokiwa Y (1996) Colonization and degradation of rubber pieces by *Nocardia* sp. Biodegradation 7: 41–48

Tsuchii A, Takeda K & Tokiwa Y (1997) Degradation of the rubber in truck tires by a strain of *Nocardia*. Biodegradation 7: 405–413

Törnsten J (1994) Egenskaper hos gummimaterial som innehåller från kryomalda personbildäck. Master Thesis. Polymer Technology, KTH, Stockholm

Warner WC (1994) Methods of devulcanization. Rubber Chem. Technol. 67: 559–566

White L (1996) Pressure lower as industry acts. Eur. Rubber J. 11: 26–32

Williams GR (1986) The biodeterioration of vulcanized rubbers. Int. Biodeterioration 22(4): 307–311

Zyska BJ, Rytych BJ, Zankowicz LP & Fudalej DS (1971) Microbiological deterioration of rubber cables in deep mines and the evaluation of some fungicides in rubber. In: van der Plas H & Walters AH (Eds) 2nd Int. Biodeterioration Symp. Vol 2 (pp 256–267) Applied Science Publishers Ltd, Lunteren, the Netherlands

Zyska BJ (1981) Rubber. In: Rose AH (Ed) Microbial Deterioration, Vol 6 (pp 323–385). Academic Press, New York