Desulfurization of Vulcanized Rubber Particles Using Biological and Chemical Methods

Cristian Valdés

Universidad Catolica del Maule

Camila Hernández

Universidad Catolica del Maule

Rodrigo Morales-Vera

Universidad Catolica del Maule

Rodrigo Andler (✉ randler@ucm.cl)

Escuela de Ingeniería en Biotecnología, Universidad Católica del Maule, Talca, Chile

<https://orcid.org/0000-0001-6627-2701>

Research

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Abstract

Currently, recycling or degradation treatments for tires are an enormous challenge. Despite efforts to dispose of or recycle it, rubber waste is increasing year by year worldwide. To create a rubber-recycling system, several researchers have proposed tire desulfurization. In this study, we compare two methods: one biological, using *Acidobacillus ferrooxidans* in shake 250 mL flask experiments, and one chemical using, for the first time, microwaves and an aqueous solution. The results of these methods were analyzed through sulfate quantification, cross-linking differences, Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy with energy disperse spectroscopy (SEM-EDS). We observed that the amount of sulfates generated by the chemical system was 56 mg / L, which was 10-times higher than the biological system, which generated 5.3 mg / L. Similarly, after cross-linking studies, a 36% higher decrease after the chemical treatment was observed. When using FTIR analysis, the disappearance of characteristic bands corresponding to functional groups containing sulfur bonds was observed by treating the sample with both desulfurization mechanisms. Morphological changes on the rubber surface structure was also demonstrated by SEM-EDS analysis with the appearance of holes, cracks and changes in the porosity of the material. This work analyzed two different non-aggressive desulfurization mechanisms that might be used as sustainable methods for rubber recycling processes.

1. Introduction

Millions of tires are discarded every year, representing up to 12% of the total accumulated solid waste. This type of waste increases annually by around 17 million tons. Recent studies project that, by 2030, about 1200 million tons of tires will be discarded every year [1]. Discarded tires generate problems as accumulation of mosquitos, and cause of unwanted fires generating toxic vapors [2]. The main constituent of tires is rubber, which can be obtained from fossil sources (synthetic rubber) or from the tree *Hevea brasiliensis* (natural rubber; [3]. When analyzing the dry weight of natural rubber, it consists of 90% poly(*cis*-1,4-isoprene) and less than 10% of other constituents such as proteins, carbohydrates, lipids, resins, and inorganic salts [4]. For commercial purposes, rubber is subjected to a vulcanization process where the poly (*cis*-1,4-isoprene) chains are cross-linked between them by heat in the presence of sulfide [5]. The process is based on adding a percentage of elemental sulfide to rubber, which is subsequently subjected to heat at 140 °C and 4 atm of pressure. As a result, the original material, which is sticky and non-elastic, becomes a non-sticky and elastic material. Due to cross-linking, it is not possible to deform the material using heat, as it is done with thermoplastics such as polyolefins (polyethylene or polypropylene) [6]. In addition, to improve abrasion resistance, carbon black is added to the rubber during vulcanization [7]. Carbon black together with silica are the second major components of a tire, after the polymer of rubber [8].

Since procedures such as tire burning, tire burying, and depositing tires on landfills are harmful, several methods and mechanisms for rubber degradation have been studied [9, 10]. Rubber waste can be treated by chemical, biological, and mechanical processes. However, as a result of the high degradation resistance of rubber materials, no clean and efficient processes have been established to date. One of the

reasons that vulcanized rubber is difficult to degrade is due to the presence of disulfide bridges, which link the rubber molecules together in a strong bond [11]. Consequently, a first desulfurization step is essential for improving the overall degradation process.

Biological desulfurization methods as rubber degradation of ground tires has been reported. [12], observed using Fourier Transform Infrared Spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) analysis, how *Thiobacillus ferrooxidans* was able to breakdown sulfur crosslinks. In another work using the same bacteria, the bioconversion from sulfide to sulfate was studied. After a 20-day cultivation, an 8% conversion was reached with changes in the physical properties of the material [13].

Acidithiobacillus is a gram-negative bacterium of the chemolithotrophic type. It was initially cataloged in the genus *Thiobacillus* [14]; however, due to its high acidity tolerance, it was reclassified in the genus *Acidithiobacillus* [15]. This genus is divided into two groups: sulfur-oxidizing species and sulfur- and iron-oxidizing species. *Acidithiobacillus ferrooxidans* corresponds to the latter group [16]. This bacterium stands out for being used in mining to promote the oxidation of iron and sulfur compounds. A group of genes involved in the oxidation of reduced inorganic sulfur compounds (RISCs) has been described in *A. ferrooxidans*. Two genes homologous to doxDA encode a protein with thiosulfate/quinone oxidoreductase activity (AFE0044; AFE0048), and another 5 genes encode sulfur thiosulfate transferase proteins (AFE2558, AFE2364, AFE1502, AFE0529 and AFE0151). To date, the role they play in sulfur oxidation is not yet clear [17].

Chemical treatments are another alternative for rubber recycling purposes [18], after treating waste silicone rubber composite insulator with H₂O₂ combine with graphite in an acid medium, obtained a material with better mechanical and thermal properties [18]. Sulfuric acid has been used to treat crumb rubber in combination with ultraviolet light, as a result, the rubber's stiffness increased [19]. Surface modification of halloysite nanotubes have been reported to interact with rubber nanocomposites, using the vulcanization accelerant N-cyclohexyl-2-benzothiazole sulfenamide, improving the mechanical properties of the material [20]. Chemical methods using chemical agents and supplemented with mechanical treatments have shown positive changes of the rubber properties, 2,2'-dibenzothiazolodisulfide (DM) in combination with mechanical treatment, increases the content of oxygenated groups on the surface, increasing the swelling degree of the material 1.5 times [21].

Green chemistry approaches as ultrasound and microwaves treatments have been reported. After ultrasound treatments fine rubber particles of 100–150 µm were obtained with a with a potential devulcanized surface [22]. The energy consumption was around 2.5 times lower than a conventional mill [1]. The use of microwaves at low temperatures is also described as a devulcanization treatment. Microwave treatment allows oxidation and degradation of rubber as an environmentally friendly alternative. Even with conventional microwaves, appreciable structural rubber changes can be detected by a thermo-oxidation process [23]. Although there are methods to digest complex compounds such as rubber using microwaves, it requires a high amount of acids. Alternative methods using moderate amounts of acids are needed to fulfill the green chemistry requirements [24].

Devulcanization of rubber using microwaves is one of the most promising procedures due to its efficiency and low environmental consequences. An important advantage when compared against conventional methods is that the application of heat is homogeneous and does not depend on conduction and/or convection of heat. Studies have reported the importance of the exposure time of the samples to microwaves, affecting the cross-linking of rubber. The polymer itself, such as copolymers of isoprene-butadiene, natural rubber, and butylated rubber, are important during the desulfurization process [25]. It has been reported that the use of microwaves can decrease the toxic volatiles released in the pyrolysis of the waste crumb rubber used to generate asphalt, being a pretreatment option for this material [26].

Consequently, an efficient and environmentally friendly method is needed for the devulcanization of rubber in order to provide alternatives for recycling or degrading rubber waste. In this study, we compared two processes for the desulfurization of rubber particles. A chemical method based on microwaves at low acid concentrations, and a biological method using the bacterium *A. ferrooxidans* in shake flask experiments.

2. Materials And Methods

2.1 Microwave treatment

An Ethos Easy microwave with an SK-15 rotor was used for the chemical desulfurization treatment. A total of 250 mg of rubber particles were added to 10 ml of 1% HNO₃. The temperature program was set at 180°C for 5 minutes and 200°C for 30 min.

2.2. Bacterial strains and cultivation conditions

The bacterial strain used in this study was *A. ferrooxidans* ATCC 23270. For culture maintenance, the strain was grown using the medium DSMZ 70, adjusting the initial pH to 2.0. For the desulfurization cultures, a modified 9K medium was used supplemented with 1% of vulcanized rubber particles (1–2 mm diameter). Rubber particles were purchased from Trelleborg AB, Germany. The particles were previously washed using ethanol 70% v/v for 24 h. The medium composition was: (NH₄)₂SO₄, 3.00 g; K₂HPO₄, 0.50 g; MgSO₄•7H₂O, 0.50 g; KCl, 0.10 g; Ca (NO₃)₂, 0.01 g; and FeSO₄•7H₂O, 3.30 g. Two cultivation conditions were evaluated: i) with 1% w/w rubber particles and ii) without rubber particles. The pH was initially adjusted to 2 using 4M HCl and monitored during the cultivations. Cultures were performed in 250 ml shake flasks at 30°C, 150 rpm for 4 weeks. Cell growth was measured by total cell count using a Neubauer chamber. Samples were analyzed every 7 days.

2.3 Sulfate ion quantification

The sulfate ion content was measured at the beginning and at the end of the cultivation for all the biological assays, and before and after the microwave treatment. Consequently, 5 ml of supernatant (for the biological treatment) and 5 ml of the 1% HNO₃ solution (for the chemical treatment) were mixed with 100 µl of a barium chloride solution (Ba₂Cl, 20 g; acetic acid 10 N, 75 ml; glycerol 25% v/v, 25 ml). The

mixture was mixed vigorously, and the absorbance was measured using a spectrophotometer at 420 nm. Sodium sulfate was used as standard in a range of 0.02 to 1.00 mg/ μl^{-1} . Sulfate quantification data was analyzed by ANOVA using the software SPSS10. The multiple comparison test Tukey was performed for significant differences of the treatments. Sulfate analysis was performed in duplicates.

2.4 Cross-link density determination

To determine the cross-link density, the method described by Mok, Eng [9] was performed using toluene as the organic solvent. Rubber particles (0.5 g) were mixed with 1 ml of toluene and incubated for 5 days in the dark using a fume hood. After this period, the solvent was discarded, and the mass was determined (m_3). The mass of the dry polymer (m_1) was obtained by heating the particles at 70°C for 72 h. The mass of the solvent in the swollen sample (m_2) was calculated as follows:

$$m_2 = m_3 - m_1 \quad (1)$$

The volume fraction of the rubber in its swollen state (V_r) was calculated as follows:

$$V_r = \frac{(m_1 / \rho_1)}{(m_1 / \rho_1) + (m_2 / \rho_2)} \quad (2)$$

where V_r is the volume fraction of the rubber in its swollen state, and ρ_1 and ρ_2 are the density of the dry polymer sample and the density of the solvent (mol/cm^3), respectively.

The cross-link density (v) was calculated as follows [21]:

$$v = \frac{-1}{2v_s} \frac{\ln(1 - V_r) + V_r + \chi V_r^2}{V_r^{1/3} - V_r/2} \quad (3)$$

where v is the cross-link density mole per unit of volume (mol/cm^3) and χ is the Flory–Huggins polymer-solvent interaction parameter (0.35 for the rubber-toluene mixture; [27]).

2.5 FTIR analysis

The rubber particles were separated from the reaction medium by filtration using a glass filter with a porosity of 3 (nominal pore size: 16–40 μm). A 10 mg of dried rubber particles were analyzed by attenuated total reflectance (ATR)-FTIR. The infrared spectra were obtained using a FTIR spectrometer (Agilent, Cary 630) with the ATR technique. Absorbance was measured in the wavelength range of 400–

4000 cm⁻¹, whereas the resolution was 4 cm⁻¹. For each sample, 64 scans were performed, and the background was subtracted using Agilent MicroLab PC software.

2.6 Scanning Electron Microscopy (SEM)

The method was based on ASTM E1508 standard "Standard Guide for Quantitative Analysis by Energy-Dispersive Spectroscopy". The microscope used was TESCAN VEGA 3 with probe EDS BRUKER QUANTAX, samples were covered by a cathodic spray system with Palladium Gold in Hummer 6.2 equipment.

3. Results

3.1 Growth of *A. ferrooxidans* in shake flasks

The *A. ferrooxidans* cultures were kept under constant agitation with a controlled temperature for 4 weeks, while cell growth and pH was monitored every 7 days. Figure 1 shows the bacterial growth of *A. ferrooxidans* and the pH evolution over time. It was possible to observe that cell growth was enhanced with the presence of rubber particles, showing maximum growth over 3 weeks, while culture without rubber in the medium showed lower growth, reaching 3.4×10^6 cells/mL. Rubber treatment with *Thiobacillus ferrooxidans* has been reported for the desulfurization of tire rubber, where a similar culture medium (modified Silverman medium) was used for a 30-day incubation period [12]. In that experiment, cell growth was negatively affected when rubber was added to the medium (after 24 h). This difference could be attributed to the fact that rubber was not treated with ethanol for partial elimination of residues present in the rubber that might prevent the growth of the bacteria. However, a similar cell density was reached in both studies [12]. Rubber treatment with the 9K medium has also been reported under similar conditions for 20 days, where the oxidative capacity of *T. ferrooxidans* towards iron was also measured [28]. In the present study, it was possible to detect a decrease in the pH in all treatments after the first week, reaching a final pH of between 1.7 and 1.8. Previous studies have shown that this pH range does not interfere with the growth performance of *A. ferrooxidans*, which can grow at pH 1 or lower [17].

The culture was subjected to analysis as sulfate quantification and rubber surface analysis by FTIR and SEM-EDS.

3.2 Sulfate ion quantification

Sulfates were quantified in order to evaluate the desulfurizing capacity of *A. ferrooxidans* and the microwave treatment. As shown in Table 1, cultures containing rubber particles and iron reached a sulfur concentration of 0.53 ± 0.08 g/L after 30 days of cultivation. An increment of sulfate was observed when rubber was present in the cultivation medium due to the low sulfate concentration reached in the absence of rubber (0.10 ± 0.03 g/L). A sulfate concentration of 0.17 g/L was reported by Li et al. [12] in cultures of *A. ferrooxidans* after 14 days. In another report using the same microorganism, 0.18 g/L of sulfate ion was obtained after 20 days of cultivation [28]. Desulfurization in latex gloves has been reported with *A.*

ferrooxidans with a release of sulphate up to 8% w/w over 20 days; this result is more efficient when comparing with *Thiobacillus thioparus* and *Acidianus brierleyi*, under the same treatment conditions [13]. It is known that *A. ferrooxidans* can couple sulfur oxidation with electron transfer with a high redox potential for CO₂ fixation, releasing sulfate ions at the end of the oxidation process [29].

For a proper comparison, the same initial concentration of rubber particles was tested in both processes. When analyzing the sulfate concentration after the chemical desulfurization with a microwave, 5.6 g/L was obtained; this is about 10 times higher than the biological treatment. No investigations were found regarding sulfate quantification by microwaves with the present technique. It is relevant to mention that the use of acids is reported as a medium of desulfurization; for example, sulfuric acid has been used for the desulfurization of fuels such as diesel and kerosene in the absence of catalysts [30]. Sulfur carbon desulfurization has also been reported in the presence of acids, and can also remove minerals [31]. Our results show the feasibility of optimizing the best balance between desulfurization and the concentration of acid to be used.

3.3 Cross-link density

The Flory–Rehner equation was employed to determine the cross-link density for the rubber particles after both the biological and the microwave treatment (Table 1). It was possible to observe differences in the cross-linking between the treated and untreated rubber particles as well as differences between the biological and chemical methods. Using the microwave method, a greater decrease in cross-linking was observed. From the untreated rubber (control), the cross-link density of the samples after the biological treatment decreased 36%, reaching a value of $6.5 \times 10^{-3} \text{ mol/cm}^3$, while a decrease of 60% was obtained after the chemical treatment with the microwave, reaching a value of $4.0 \times 10^{-3} \text{ mol/cm}^3$. In previous biological treatments of rubber where the cross-link density was studied, values of $5.8 \times 10^{-5} \text{ mol/cm}^3$ were reported [28]. Depending on the rubber content filling (phr), different cross-link densities can be obtained between 8.0×10^{-4} and $1.3 \times 10^{-3} \text{ mol/cm}^3$ [12].

3.4 Rubber particle analysis by FTIR

In order to evaluate structural variations at the surface of the particles after the desulfurization treatments. Rubber particles were recovered, dried, and analyzed using FTIR (Figure 2). It was possible to detect a variation in the polymer structure after the biological and chemical treatments corresponding to groups ($-\text{C}=\text{CH}$) at a wavelength of 874 cm^{-1} [32]. This chemical structure is characteristic of the isoprene repetitive unit in tire rubber. The band at 1360 cm^{-1} was also modified in both desulfurization approaches, which is related to ($-\text{CH}_3$) groups of the rubber structure [25]. When analyzing peaks at 750 and 1083 cm^{-1} , relating to C-S and S-O bonds respectively [25], strong variations were found. A clear modification of the group was shown after the biological treatment due to superficial exposure of the functional groups. On the other hand, those groups were lost after the microwave treatment, showing stronger variations and a release of the C-S and S-O groups from the rubber particle surface. A band close to 1452 cm^{-1} corresponding to methylene, part of the isoprene structure [32], was observed in the control

sample, and no visible in the spectra corresponding to the microwave treatment. which is also part of the isoprene structure [32]; it disappeared in the spectra corresponding to the microwave treatment. The band close to 1021 cm^{-1} was associated with rubber carbon black [32]. This band was altered after the biological treatment and was not observe after the microwave treatment.

3.5 Rubber particle analysis by SEM/EDS

SEM photographs show that the surface suffered small fractures after treating the rubber with both biological and chemical desulfurization methods (Figure 3). However, at 500x an increase in surface pores by the chemical treatment can be observed, accounting for the greater wear of the surface product of the severity of the treatment. EDS analysis shows a decrease in oxygen and sulfur content at the rubber surface after treatments, increasing the observable carbon versus the control.

4. Discussion

4.1 Biochemistry of biological and chemical desulfurization processes

The sulfur-uptake mechanism in *A. ferrooxidans* is not completely clear and some key enzymes have still not been identified. A sulfur dioxygenase (SDO) has been reported in *Acidithiobacillus*, the first enzyme reported for elemental oxidation of extracellular sulfur. SDO activity has also been detected in mitochondria, animals, and heterotrophic bacteria, where persulfide dioxygenase (ETHE1) and persulfide dioxygenase (PDO) are proposed as homologs of SDO in mitochondria and heterotrophic bacteria, respectively [33, 34]. Sulfur oxygenase reductase (SOR) is also found in *Acidithiobacillus*, which can generate molecules such as thiosulfate, sulfite, and sulfide [35]. The tetrathionate hydrolase (ThetH) protein has been reported in *A. ferrooxidans* [36]. This enzyme can also generate thiosulfate, sulphite, and sulfur from reduced sulfur species; however, ThetH's mechanism is still unclear [37, 38]. Knockout studies of this gene have been done in *A. Ferroxidans*, demonstrating the need for the ThetH to oxidize sulfur compounds present in selective media [39]. However, it has been reported that this protein could be present extracellularly (Beard et al., 2011), which suggests that this is one of the enzymes that degrades the solid rubber present outside the bacterium when treatment systems in bioreactors by heterogeneous catalysis are used.

From a biochemical point of view, treatment using microorganisms allows the action of enzymes which causes the desulfurization of the material. The ability of microorganisms to desulfurize dibenzothiophene (a sulfur compound from petroleum) through the sulfoxide-sulfone-sulfinic-sulfate (4S) pathway has been proposed, where sulfate can also be consumed as an assimilation pathway by microorganisms [40]. It has been reported in other genera of bacteria that the desulfurization performance of the dszA and dszC enzymes during the desulfurization of dibenzothiophene does not increase in the presence of metals such as Fe^{+2} , Fe^{+3} , and Cu^{+} , and no decrease in activity was detected in the presence of Ethylenediaminetetraacetic acid (EDTA), suggesting that they do not depend on

metallic cofactors [41]. Therefore, it is proposed that the presence of such enzymes would have a similar effect on the cross-linking of vulcanized rubber.

Regarding the microwave treatment, when rubber is subjected to temperatures of 200°C or more, devulcanization and shortening of the polymers is generated by rearrangement of the disulfide cross-links and the breakage of carbon–carbon bonds. The cross-links can be lost in volatile compounds in the shape of hydrogen sulfide (H₂S), carbon disulfide (CS₂), and sulfur dioxide (SO₂) [42]. This also shows that, in our microwave desulfurization tests, not all sulfur residues removed from the rubber structure would be present in the solution in forms such as sulfones, sulfates, or mercaptans.

During the microwave treatment, microwave energy is converted into thermal energy after being absorbed by the material. The polar molecules of a medium with dielectric characteristics move in the electric's direction field where thermal movement interferes with other molecules and the obstruction of intermolecular interactions generates internal friction. This converts the energy absorbed from the electrical field into thermal energy, increasing the temperature of the system [43]. The thermal effects of microwaves can be associated with the ability to convert electromagnetic energy into thermal energy at a particular temperature and frequency; this capacity can be measured with the tangent of loss of the material ($\tan \delta$) ([44], where ϵ'' is the dielectric of material loss (which indicates the efficiency of the conversion of electrical energy into thermal energy) and ϵ' is the dielectric constant that indicates the polarization capacity [45].

Regarding the use of microwaves for treating rubber, temperature is a key factor, since it has been shown that oxidation can be generated by heat treatment of rubber and additives such as carbon black. Carbon black is an excellent microwave absorber [1, 46] inducing the heat of non-polar compounds such as isoprene. Despite, the effect of conventional microwaves on the devulcanization of rubber, in the absence of solvents, has been reported, an advantage of using acids in the treatment of rubber is the possibility of releasing toxic compounds such as metals [47]. *A. ferrooxidans* decreases its catalytic activity as an iron oxidant in the presence of metals such as zinc [48], which is a common component in rubber, used as an activator of the vulcanization process [49]. The amount of nitric acid used in this study is low, so the contaminating effect of this solvent is not significant compared to conventional rubber digestion (for elemental analysis) where pure acid is used [50]. Therefore, dilute nitric acid for microwave usage, it seems promising treatment for rubber desulfurization. The desulfurization capacity of materials such as coal in an acidic medium and in the presence of microwaves has also been reported. Up to 97% of inorganic sulfur can be removed by acid washing in combination with microwaves. In the presence of microwaves, the binding energy of C-S is weakened, facilitating its breakdown in the presence of acids; this result has been verified by FTIR analysis [43]. It is noteworthy to mention that a low acid concentration (1%) is used in the proposed chemical method, versus other described methods for treating rubber, which use high acid concentrations such as H₂SO₄ (95%). In addition, treatment with concentrated acid may require subsequent neutralization with base, which must also be in high concentrations. [19, 51].

4.2 Analysis of rubber particles after biological and microwave treatments

For the first time, the use of an aqueous phase during a microwave treatment is reported. Previously, authors have studied the effect on cross-linking using dried rubber and the microwave technique. Their results pointed out that cross-link densities after treatments were between 6.0×10^{-5} and 12.0×10^{-5} mol/cm³, using temperatures of 72°C, 115°C, 146°C, 160°C, 200°C, and 220°C [25]. Considering that the dissociation energy of the monosulfidic, disulfidic, and polysulfidic cross-links is between 268 and 285 kJ/mol; is less than the dissociation energy of a C-C bond with 351 kJ/mol [1]. Rubber desulfurization by microwave advantages adjusting parameters such as power, temperature, and incubation time, is the energy consumption, since is less energy intense than other reported treatments [52, 53].

The presence of new bands in FTIR analysis after the biological treatment indicate surface transformations possibly associated with rubber-decomposition processes. It can be seen that the chemical treatment with the microwave was more effective since the low concentration of nitric acid that was used increased the porosity of the material, facilitating the diffusion of thermal energy to break down sulfur bonds and other components that are part of the rubber surface. Tao et al., (2014) using FTIR method, observed the loss of sulfur bonds after a desulfurization treatment in an acidic and microwave medium. In the present study, the treatment of rubber using microwaves, bands corresponding to the CH₂ groups (1475–1450 cm⁻¹) and bands corresponding to the CH₃ group (1390–1372 cm⁻¹) are lost. This is a reliable indicator of the effectiveness of the microwave in combination with acid since, in the absence of acid, the persistence of these functional groups has been reported for microwave treatments [25]. Moreover, losing bands near 1096 cm⁻¹ and 1540 cm⁻¹, corresponding to ZnO and SiO₂ [32], respectively, demonstrates the capacity of this system to remove the metal oxide in the structure of the treated rubber. Analysis of the desulfurization processes by FTIR is not only useful for analyzing materials such as rubber: this technique has also been reported for monitoring different desulfurization processes as acid treatments in fuels such as kerosene, where the loss of functional groups of molecules such as mercaptans has been observed [32, 43].

The SEM analysis has been used to demonstrate the changes in the rubber surface against mechanical, chemical or biological treatments [54, 55]. In this study, SEM analysis reveal morphological changes of the analyzed rubber surface after biological and chemical treatments in comparison with the control. Both treatments methods used, show a particle size of approximately 500 to 1000 µm, so the particle size is not influenced by the treatments carried out compared to a reported work [54]. The size reported in this work is compatible for the treatments of our study, facilitating its implementation in systems with lower-performance grinding. Samples observed at 500x show an increase in porosity after the chemical treatment of rubber, which is not observed in the rubber particle after the biological treatment. This result is also related to the elemental analysis of C, O and S (Table 2), where the carbon content increases in the rubber particles subjected to the treatments, especially after the application of the chemical treatment. The opposite occurs with O and S, due to the fact of the increase of sulfates detected in the reaction

mediums, probably as a result of a detachment from the rubber surface in addition to assimilation processes of these elements in the case of the biological treatment.

5. Conclusion

Rubber desulfurization was achieved through a biological process by the bacteria *A. ferrooxidans*, and a chemical process using microwaves with low nitric acid concentration. From the performed experiments it can be established that the chemical/microwave treatment was more effective than the biological treatment. This was corroborated when comparing the results regarding sulfate measurement (5.60 vs 0.53 mg/L), cross-link density (4.0×10^3 vs 6.5×10^3) for biological and chemical/microwave treatments respectively. Additionally, FTIR analysis showed clear rubber surface modification in terms of the available functional groups when both of the treatments were performed. Regarding the use of microwaves, it is worth noting the low amount of nitric acid (1%) used to promote strong structural changes in the rubber's surface. Finally, both biological and chemical methods have great potential as desulfurization processes and the combination of them might potentially increase the rubber devulcanization result, which is needed for a sustainable rubber recycling.

Declarations

Authors' contributions

CV conducted the experiments, analyzed the data and drafted the manuscript. CH conducted the experiments helped in providing information and preparation of tables and figures.. RM-V revised, corrected and commented on the manuscript. RA conceptualised, analyzed the data and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Quantification of sulfate ion and determination of cross-link densities after biological and chemical treatments. For the biological negative control, the cultivation medium did not include rubber particles. For the chemical negative control, rubber particles were suspended in 1% HNO₃ without the microwave treatment. ND: not detected. Statistical analysis showed significant differences between all the different treatments. Standard error is showed for sulfate quantification.

Treatment	Sulfate (mg/l)	Cross-link densities (mol/cm ³)
Biological	0.53 ± 0.08	6.5 x 10 ³
Biological control	0.10 ± 0.03	10.2 x 10 ³
Chemical	5.60 ± 0.19	4.0 x 10 ³
Chemical control	ND	10.2 x 10 ³

Table 2. Elemental distribution of C, O and S on rubber particles after biological and chemical desulfurization treatments.

Treatment	C (%)	O (%)	S (%)
Biological	85.6	13.1	0.4
Chemical	91.1	6.9	0.1
Control	80.6	16.1	1.5

Figures

Figure 1

Growth of *A. ferrooxidans* (●) and pH variation (▲) in shake flasks over 4 weeks. The cultivation medium was the modified 9K medium supplemented with 1% w/w rubber particles (solid line), and without rubber particles (dashed line). Measurements were done in duplicates and the standard error is presented in the figure.

Figure 2

FTIR spectra of rubber particles after biological treatment (1), after chemical treatment (3), and without treatment as a negative control (2).

Figure 3

Particle morphology and observable porosity in the analyzed samples, corresponding to control at 100x (A) and 500x (D), biological treatment at 100x (B) and 500x (E), and chemical treatment at 100x (C) and 500x (F).