Comparative studies on crosslinked and uncrosslinked natural rubber biodegradation by *Pseudomonas* sp.

Ram Vinod Roy a, Mithu Das a, Rintu Banerjee a,*, Anil K. Bhowmick b

a Microbial Biotechnology and Downstream Processing Laboratory, Agricultural and Food Engineering Department, Indian Institute of Technology, Kharagpur 721 302, India

b Rubber Technology Center, Indian Institute of Technology, Kharagpur 721 302, India

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Abstract

A comparative study on biodegradation of di-cumyl peroxide (DCP) crosslinked and uncrosslinked natural rubber by *Pseudomonas* sp. was carried out. Decrease in organic carbon content along with the changes in tensile strength of the treated rubber, both DCP crosslinked and uncrosslinked natural rubber, indicated rubber hydrocarbon utilization by the *Pseudomonas* sp. A decrease in 60.88% MPa and 41.66% MPa was observed after five month’s old treated uncrosslinked natural rubber and DCP crosslinked rubber, respectively. Biodegradation was more pronounced in natural uncrosslinked rubber, which was further confirmed by the formation of aldehydic compounds with decrease in CH₂ stretching frequencies.

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Keywords: Biodegradation; cis-1,4-polyisoprene; Di-cumyl peroxide (DCP); Natural rubber; *Pseudomonas*; Total organic carbon

1. Introduction

Natural rubber obtained from the latex of *Hevea brasiliensis* is a high polymer of cis-1,4-polyisoprene (molecular weight \( \approx 10^6 \)), which contains more than 90% of cis-1,4-polyisoprene and less than 10% of non-rubber constituent such as proteins, carbohydrates, etc. (Subramaniam, 1995). In contrast, synthetic rubber contains little non-rubber ingredients. Natural rubber is subjected to vulcanization (crosslinking of the linear cis-1,4-polyisoprene chains) for commercial applications. Various agents used for vulcanization are sulphur, peroxides, metal oxides, radiation cure and resin cure; in general sulphur is commonly used for general purpose rubber (Bhowmick et al., 1994). Vulcanization adversely affects the biodegradation due to the presence of sulphur bridges, which reduce accessibility sites for cleavage. Moreover, addition of antioxidants prevents the microbial growth.

Several microorganisms possess rubber-degrading capacity (Heisey and Papadatos, 1995; Jendrossek et al., 1997; Steinbuchel et al., 2000; Linos et al., 2000, 2002; Tsuchii et al., 1985). Even crosslinked rubbers have been shown to be biodegradable (Tsuchii et al., 1985; Heisey and Papadatos, 1995). *Pseudomonas*, a commonly found gram negative bacterium has capacity to utilize hydrocarbons (Bhattacharya et al., 2000). *Pseudomonas aeruginosa* AL98 produced holes in a polymer film which indicated that the polymer was used as sole source of carbon and energy (Linos et al., 2000).

According to Bhowmick et al. (1994) C–C crosslinking is more resistant than C–S crosslinking and has better compression set properties. C–C crosslinking is induced by peroxides, which are basically divided into two types, peroxide with carboxylic acid group, e.g., dibenzyl peroxide and peroxide without carboxylic acid group, e.g., di-cumyl peroxide, di-tert-butyl peroxide, etc. Peroxides without carboxylic groups have lesser sensitivity to acids, higher decomposition temperatures and better compression set (Bhowmick et al., 1994).
In this work, a comparative study on uncrosslinked and di-cumyl peroxide (DCP) crosslinked natural rubber by a newly isolated *Pseudomonas* sp. was carried out. There is no other report on comparative study on biodegradation of these systems by *Pseudomonas* sp.

2. Methods

2.1. Rubber and latex

Rubber sheets of ISNR-5 (Indian Standard Natural Rubber with 0.05% dirt content) were obtained from the Rubber Board, Kottayam, Kerala, India. DCP was used as crosslinker in this study. Fresh latex was obtained from Rubber Board, Kottayam, Kerala, India.

2.2. Microorganism and culture conditions

2.2.1. Isolation of microorganism

Bacterial strain was isolated from a soil sample collected from the dumping ground of waste automobile tires. Latex solution of different concentrations such as 1%, 0.75%, 0.5%, 0.25% and 0.1% were inoculated with the diluted soil sample. The culture was isolated through spread plate method on latex-agar medium, identified from MTCC, Chandigarh (India).

2.2.2. Culture conditions

Erlenmeyer flask (250 ml) containing 100 ml of Czapek–Dox media and a natural rubber sheet (5:5:0.4 cm of length, width and thickness ratio) were sterilized at 15 psi for 15 min in autoclave and inoculated aseptically with *Pseudomonas* sp. culture suspension (1.2 × 10⁶ ml⁻¹ cells). The flasks were incubated at 30 °C on a rotary shaker with 220 rpm. Similar conditions were maintained with DCP crosslinked rubber.

2.3. Total organic carbon content

Total organic carbon content with and without microbial colonies in treated rubber samples was estimated by Walkley–Black method (Mebius, 1960). Thin film (~1 mm thickness) of treated rubber was cut weighing ~30 mg; similarly another piece was cut from the treated rubber after the microbial colonies were removed from the surface of the rubber sheet by repeated washing in water followed by 0.1% toluene, which was further confirmed through microscopic studies.

2.4. Tensile strength measurement

Tests for measuring the tensile strength were carried out according to ASTM designation D 412-98 using dumb-bell specimens. The samples were punched from natural rubber sheets with and without DCP crosslinking parallel to the grain direction using a dumb-bell dye (type C). The samples were held tight by two grips in a “Zwick” type tensile testing machine. Rate of separation of power-actuated grip was 500 mm/min, which was maintained throughout the experiment. The analysis of the treated rubber (both crosslinked and uncrosslinked rubber) and the untreated rubber was carried out at one, two, three and five months respectively.

2.5. FTIR studies

The degradation of rubber sheet was further evaluated through FTIR–ATR (Nicolet, Nexus, USA) studies. The peak characteristics of the FTIR data correspond to the presence/absence of various alkenes, vinyl and aldehyde groups, which are the products of rubber degradation (Linou et al., 2000, 2002). Studies were performed with the rubber samples taken from the treated rubber sheet after the microbial colonies were being removed from its surface.

3. Results and discussion

3.1. Total organic carbon content

Table 1

<table>
<thead>
<tr>
<th>TOC of treated natural rubber</th>
<th>Control</th>
<th>1 month treatment</th>
<th>3 months treatment</th>
<th>5 months treatment</th>
<th>6 months treatment</th>
<th>7 months treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>+DCP</td>
<td>−DCP</td>
<td>+DCP</td>
<td>−DCP</td>
<td>+DCP</td>
<td>−DCP</td>
<td>+DCP</td>
</tr>
</tbody>
</table>

- Percentage of organic carbon with microbial colonies: 80.4, 78.6, 81.7, 80, 83, 83.2, 85.3, 85.4, 86.2, 85.7, 86.6, 88.2
- Percentage increase of organic carbon with microbial colonies: −, −, 1.61, 1.78, 3.23, 5.85, 6.09, 8.65, 7.21, 9.03, 7.71, 12.21
- Percentage of organic carbon without microbial colonies: 80.4, 78.6, 78, 76.3, 77.6, 75.2, 76.8, 74, 75.2, 73.1, 74.6, 71.3
- Percentage decrease in total organic carbon without microbial colonies: −, −, 2.98, 2.92, 3.48, 4.32, 4.47, 5.85, 6.46, 6.99, 7.21, 9.28

+DCP: Natural rubber with DCP crosslinking.
−DCP: Natural rubber without DCP crosslinking.
tral lipids 1.0, proteins 1.6, phospholipids 0.6, inositols, carbohydrates 1.5, salts (mainly K, P and Mg) 0.5, water 58.5 and pH 10.65 (Blackley, 1997). The treated rubber samples (both crosslinked and uncrosslinked natural rubber) with microbial colony showed percentage increase in total organic carbon level which could be due to increase in microbial biomass, while treated rubber samples without microbial colony as discussed in Section 2.3, showed percentage decrease in total organic carbon level which was higher in uncrosslinked natural rubber (Table 1). Reduced level of carbon content in the treated sample indicated the utilization of rubber as carbon source by the active participation of the microorganisms, thereby leading to its degradation. The active participation of the microorganism in degradation was more in uncrosslinked natural rubber than in the crosslinked rubber.

3.2. Tensile strength

In two months treated uncrosslinked natural rubber, a decrease of 43.11% MPa was observed whereas a decrease of 60.88% MPa was noticed in third month which was stable up to fifth month. In DCP crosslinked rubber a decrease of 10.83% MPa was recorded in the second month of treatment. While the percent degradation increased gradually to 32.29 and 41.66 in third and fifth month consecutively as shown in Fig. 1. It was noticed that uncrosslinked natural rubber treated for two months showed a sharp decrease of 43.11% MPa in comparison to 10.83% of DCP crosslinked rubber. When the experiment was conducted for five months, a decrease in 60.88% MPa, indicating active participation of bacteria in the degradation of uncrosslinked natural rubber was obtained. In crosslinked rubber, a decrease of 10.83% MPa in two months treatment and a decrease of 41.66% MPa in fifth month indicated less accessible sites of hydrocarbons of crosslinked rubber to be utilized. Decrease in the percent degradation (MPa) of control natural rubber was higher than that of crosslinked rubber, probably as the double bonds were utilized and the segments of natural rubber were immobilized during crosslinking. These changes were evident from Fig. 2, which was mainly due to the degradation of the main polymer chain. There are reports on biodeterioration of susceptible and resistant NR vulcanizates with actinomycetes treatment. After two years treatment, severe pitting and penetration of the organism was detected for which the rubber sample lost 3% of its volume and lost its entire tensile strength (Linos and Steinbuchel, 2004).

3.3. FTIR spectroscopic studies

When FTIR–ATR spectra of natural rubber with and without DCP crosslinking were compared, it was found that peaks at 2961 cm\(^{-1}\) which corresponded to CH₂
stretching, showed decrease in intensities with the formation of 1735 cm\(^{-1}\) peak, which corresponded to aldehyde formation. Further decrease in CH\(_2\) and formation of aldehyde was higher in uncrosslinked natural rubber than DCP crosslinked rubber. This degradation led to the formation of aldehyde groups along with decrease of CH\(_2\) intensities, which was evident from the FTIR spectra. As crosslinked rubber was more immobilized, decrease in CH\(_2\) and aldehyde group generation was lower than natural rubber (results not shown). There are reports on application of FTIR–ATR spectroscopy revealing the insights of biodegradation mechanism of NR latex gloves by *Gordonia* sp. (Linos et al., 2002). Spectra of the treated sample demonstrated the presence of keto groups. Considering further literature data on positive detection of aldehyde groups by Schiff’s reagent test, it was assumed that the scission of the \textit{cis}-1,4-polyisoprene chain by oxygen was common among all \textit{cis}-1,4-polyisoprene degrading bacteria, irrespective of their colonization strategy.

4. Conclusion

Comparative studies of rubber degradation by *Pseudomonas* sp. on DCP crosslinked and uncrosslinked natural rubber are reported for the first time. Decrease in the total organic carbon content and tensile strength of the treated rubber and formation of aldehyde peaks in FTIR spectra indicated rubber biodegradation by the *Pseudomonas* sp. Uncrosslinked natural rubber was degraded more efficiently than the DCP crosslinked rubber.

References


