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Degradation of the rubber in truck tires by a strain of *Nocardia*

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Abstract

A strip of tread compound cut from a truck tire was degraded only slightly when it was used as the sole growth substrate for a strain of *Nocardia*. On the contrary, its degradation was markedly enhanced by addition of a strip cut from a latex glove which the organism readily utilized as a growth substrate. When a glove strip was added, the biomass concentration in the experimental flask became more than 10-fold higher than the control without a glove strip and the colonization of the tire strip was significantly enhanced.

After 8 weeks' cultivation, about 28% of the tire strip was disintegrated into very small black particles (mostly less than 30 μm in diameter) and the weight of the remaining unchanged portion of the strip was about 49% of the initial weight.

Four kinds of truck tire treads were attacked in differing degrees by the organism under the same conditions. The treads containing more than 70 phr (parts per hundred of rubber) of natural rubber were considerably attacked, while those with a natural rubber content of less than 55 phr were attacked only slightly. The microbial activity against the rubber in the side wall of a truck tire was relatively high, but the inner liner was hardly attacked and the bead rubber not at all.

Introduction

Waste rubber, like waste plastic, is becoming a worldwide waste disposal problem. One particular concern is used tire disposal because of the huge number of tires produced and discarded annually and of the potential environmental hazard should a tire stock pile catch on fire (Smith & Klingensmith 1991). Consequently, it is very important and worth trying to develop a microbial process for waste tire disposal (Nickerson & Faber 1975).

Although passenger tire treads are made principally from synthetic elastomers, natural rubber (NR) is primarily used for heavy-duty truck tires. Since the recent development of the radial tire, NR usage has further increased in truck tire components (Bernard et al. 1984).

There have been many reports about microbial degradation of vulcanized NR in soils and water flows (Tsuchii 1995). The rate of degradation, however, was generally very slow. Biological and chemical oxidation of tire wear particles were also reported (Cadle & Williams 1980, Dannis 1974). However, direct and quantitative evidence of microbial degradation of rubber polymers in the particles has not been demonstrated yet.

We recently found that the rubber in truck tire treads made principally of NR was degraded rather rapidly under some special conditions by a rubber-degrading strain of *Nocardia* and here report the conditions and results of the degradation tests.

Materials and methods

Tires and rubber products

Four kinds of heavy-duty truck tires were gifts received from Muraoka Rubber Industries Co., Ltd. in 1987. Bias tire A was made by Bridgestone Tire Co., Ltd.

and bias tire B by Dunlop Co., Ltd. Radial tire C was a product of Yokohama Rubber Co., Ltd. and radial tire D of Dunlop Co., Ltd. Rubber strips with a length of 120 mm and a diameter of 0.3 to 0.5 mm were taken from tread parts of the tires. Tread cap (TC) samples were cut from near the surface (within 5 mm) and base tread (BT) samples were cut from near the carcass or belt. The strips were extracted with a mixed solvent of acetone and chloroform (30:70) in order to remove chemicals with microbiocidal activities, and weighed after the complete removal of the solvent *in vacuo* before use. In the case of tire D-TC, the content of low molecular weight organic compounds (chloroform or acetone soluble) was about 6% and the content of carbon black and inorganic compounds (boiling *ortho*-dichlorobenzene insoluble) was about 30%.

A latex glove (Phoenix brand, 0.1 mm thickness) was obtained from Sunrex Japan Co., Ltd. Film-like strips with a width of 5 mm and a length of 120 mm were cut from the glove and used as a co-substrate for the degradation test of tire rubbers. The glove contained about 3% of chloroform soluble components and 3% of hot toluene insoluble constituents.

Microorganism and preculture

An actinomycete, *Nocardia* sp. strain 835A, the strongest decomposer of solid rubber in our culture collection, was used throughout this study (Tsuchii et al. 1985). A preculture of the organism grown on glove rubber for 19 days was used as the inoculum for the degradation test. A test flask was inoculated with 10 ml of the preculture containing 2.0 x 10^7 viable cells per ml of the medium. The viable cells in the preculture were counted by plating 0.1 ml aliquots of the appropriate dilution onto yeast extract-malt extract agar (Pridham et al. 1956).

Methods of the degradation tests

To examine the microbial degradation of tire strips, growth experiments in which a glove strip was added as a co-substrate for the growth were carried out. A tire strip (20 to 30 mg) and a glove strip (60 to 70 mg) were added to 100 ml of mineral salt medium in a 300 ml Erlenmeyer flask. The flask was aerated by stirring with a magnetic stirrer at 600 rpm and 30 °C. The composition of the mineral salt medium was reported previously (Tsuchii et al. 1996). We prepared a set of replicate flasks, and analyzed them in pairs at regular time intervals. After 8 weeks of incubation, the tire strip was recovered from the flask and weighed after being dried *in vacuo*. In order to digest and remove microbial cells tightly attached to the strip, the strip was then boiled for 10 minutes with 1N NaOH and thoroughly washed with water and weighed again after being dried *in vacuo*. The weight (%) of the strip recovered after the alkali treatment was calculated according to the following equation: 100 x Residual strip after alkali extraction/ Initial weight. The weight losses of the strips incubated without the organism were less than 1% even after alkali treatments.

Estimation of small black particles

When microbial cells tightly attached to the strip of TC of tire D were digested with alkali, a considerable amount of small black particles was released from the strip. They were collected by centrifugation, washed with water, and weighed after being dried in vacuo (Table 1, 2.0 mg).

The cells and small black particles suspended in the culture medium were also collected by centrifugation, washed with water, and weighed after being dried *in vacuo*. The mixture of cells and particles (Table 1, 30.1 mg) after 8 weeks of cultivation was then treated by boiling with 1N NaOH. Most of the cells (about 90% by weight) were digested by the alkali and the remaining particles (5.3 mg) were collected according to the procedures described above.

Determination of the polymer composition

0.5 mg of the tire compound was pyrolyzed at 590 °C for 3 sec using a JHP-22 Curie-point pyrolyzer (Japan Analytical Industry Co., Ltd.) and the pyrolysis products were analyzed with a Shimadzu GC-14B gas chromatograph equipped with a flame ionization detector. A stainless steel column (3 m) packed with 25% silicone DC 200 on 60-80 mesh shimalite AW-DMCS was used at an oven temperature of 140 °C. N_2 gas was used as the carrier at a flow rate of 35 ml/min.

The polymer composition was calculated on the assumption that three basic types of rubber were used as elastomeric materials in compounding the tire treads: polyisoprene (NR), polybutadiene (BR), and styrene-butadiene rubber (SBR). The analysis of these materials was based on an estimation of the dimers (dipentene and vinylcyclohexene) and the monomeric styrene (Kreshen & Tucker 1974; Sugiki & Yamamoto 1972).

Table 1. Microbial growth and suspended solid in culture

Rubber strip and Incubation period	Residual weight of strip[a] (mg) before alkali treatment (A)	after alkali treatment (B)	Small particles[b] released by alkali treatment (C) (mg)	C/(A-B)	Suspended solid[c] (mg) before alkali treatment (D)	after alkali treatment (E)	E/D
[d]Tire strip (25mg)[e] +glove strip (65mg) 56 days	17.5 ± 0.6[f]	12.2 ± 0.5	2.0 ± 1.0	0.38	30.1 ± 0.7	5.3 ± 0.6	0.17
[g]glove strip (65mg) 14 days	38.3 ± 8.2	3.2 ± 0.2	3.2 ± 0.3	0.09	10.4 ± 1.2	0.8	0.08
[g]glove strip (65mg) 19 days	11.5 ± 8.1	0	1.3	0.11	16.3 ± 1.3	1.4	0.09

[a]Weight of the remaining unchanged portion of the strip.
[b]Released from strip.
[c]Suspended solid in culture medium.
[d]Tire D-TC (tread cap).
[e]Initial weight of strip.
[f]Mean ± deviations.
[g]Glove strip alone.

Scanning electron microscope observation

Tire strips after microbial degradation were fixed in 2% glutaraldehyde, dehydrated with a graded ethanol series, and coated with gold before examination. They were observed under a JEOL scanning electron microscope, model JSM-5310, with 10 to 20-kV acceleration.

Results

The effect of glove rubber on microbial degradation of tire rubber

Degradation of the tread rubber was markedly accelerated by the addition of soft type NR products or unvulcanized raw rubber, which was readily utilized as growth substrate by the organism. When the organism grew on a strip cut from the tread cap (TC) of tire D as the sole growth substrate, a small number of colonies of varying size were observed on the surface after 8 weeks (Figure 1a) and the weight of the portion of the strip remaining unchanged after alkali treatment was 87% of the initial weight. When the inoculum size was increased from 5 ml to 20 ml, the weight of the tire strip after alkali treatment decreased from 92% to 84%. On the contrary, when incubated with a glove strip, the surface of the tire strip was covered with many large colonies (Figure 1b) and the weight of the strip recovered after alkali treatment was only 49%. In the rest of this study, a glove strip was always added to the medium of the degradation test.

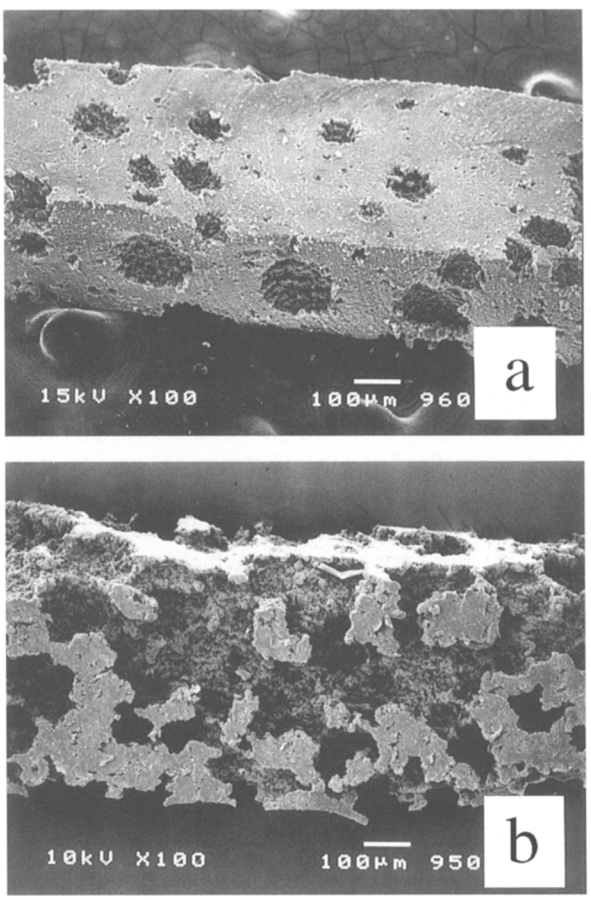


Figure 1. The effect of glove strip on degrdation of tire strip. Scanning electron micrograph (SEM) of the strip of tire D-TC after alkali treatment. (*a*) 8 weeks' incubation without glove strip. (*b*) 8 weeks, with a glove strip.

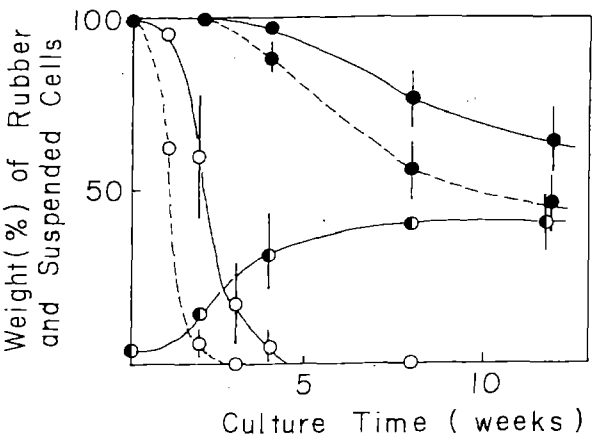


Figure 2. Time course of microbial degradation of a tire strip (tire D-TC) in the presence of a glove strip.
Symbols: —●— , weight(%) of tire strip before alkali treatment; - - ● - - , weight(%) of tire strip after alkali treatment; —○— , weight(%) of glove strip before alkali treatment; - - ○ - - , weight(%) of glove strip after alkali treatment; —◐—, cells and small black particles suspended in medium expressed as weight% of the initial weight of glove strip. The vertical lines in the figure represent the deviations in at least triplicate experiments.

Time course of the degradation of a tread rubber

A typical time course of the degradation of a strip of tire D-TC is shown in Figure 2.

The glove strip was rapidly degraded and utilized as growth substrate by the organism. Mycelial growth was observed on the surface of the glove strip on day 3. Many colonies with diameters of 20 to 100 μm were observed on day 5 and the whole surface was covered with thick mycelial growth on day 7. The mycelial cells fragmented into very small particles (less than 1 μm in diameter) and were released into the culture medium on day 14. At the same time the strip was torn into small fragments and the weight loss of the glove strip reached more than 90% after alkali treatment. By day 21, the glove was almost completely degraded and the culture medium exhibited a tinge of gray.

On the contrary, the tire strip was very slowly degraded after a lag time. Many small, hemispherical colonies with diameters of 10 to 20 μm were observed on the surface of the tire strip on day 7 and many mycelia and cells were also observed (Figure 3a). The diameter of the colonies reached 20 to 40 μm on day 14. Then cracks were observed on some of the colonies and pits filled with very small particles were observed in some other colonies (Figure 3b). Each colony had a small number of short mycelia and cocoid cells at the peripheral part, but the center part was filled with very small particles and included almost no cells of normal size. Pits filled with small particles can been seen beneath the colonies and the depth of the pits was about 20 μm (Figure 4a). The small particles contained in the pits were released by alkali treatment of the strip. However, the weight loss of the tire strip was negligible at this point even after the alkali treatment.

Large round pits filled with very small particles could be seen on the surface of the tread on day 28 (Figure 3c, up to 100 μm in diameter) and cells of the ordinary size were hardly observed. The weight loss of the strip after the alkali treatment was then 10 to 15%.

With the progress of the degradation, very small black particles were released from the strip of tread and the culture medium became dark gray or black. After 8 weeks' cultivation, the weight of the particles in the medium, recovered after alkali treatment of the mixture of cells and the particles, was 5.3 mg (20±2% of the initial weight of the strip, Table 1,E). The diameters of most of these particles were less than 30 μm.

Almost all the surface of the strip was covered with a number of large pits after 8 weeks' cultivation. Some of the pits were filled with small particles and some were empty. Upon alkali treatment of the strip, very small black particles (Table 1, C, 2.0 mg, 8±4% of the initial weight of the strip) were again released from the strip and many large, deep cavities appeared on the surface of the strip. Although the depth of the cavities reached more than 70 μm, plain smooth surface areas remained between the cavities (Figure 4b). The weight of the portion of the tire strip remaining unchanged after the treatment was 12.2 mg (49±4%).

The effect of polymer compositions

Microbial degradation of strips cut from the treads of four kinds of truck tires was examined in relation to their polymer compositions (Table 2). The content of NR was higher in radial tires than in bias tires, and the microbial degradation of the rubber in radial tires was more extensive than that of bias tires. BT of tire A, which had the highest content of NR among the bias tires, was considerably degraded and the weight of the portion of the strip remaining unchanged was about 70%. TC of radial tire C, which had a small amount of NR, was hardly degraded. BT of tire C and TC of tire D had high NR contents and the recovered weights of the strips were less than 50%. BT of tire D, however, was not so readily degraded in spite of its high NR content.

Table 2. Microbial degradation of tread strips

Tread rubber		Residual[a] weight(%)	Polymer composition (NR:SBR:BR)[d]
Bias tire A	-TC[b]	91 ± 2[c]	55:15:30
	-BT	70 ± 10	70:10:20
Bias tire B	-TC	97 ± 0	55:15:30
	-BT	98 ± 1	55:15:30
Radial tire C	-TC	98 ± 1	50:30:20
	-BT	22 ± 5	90: 5: 5
Radial tire D	-TC	49 ± 4	100: 0: 0
	-BT	86 ± 1	100: 0: 0

[a] Tread strip was incubated for 8 weeks with glove strip.
[b] TC, tread cap; BT, base tread.
[c] Weight(%) of the remaining unchanged portion of the strip after alkali treatment, mean ± deviations in duplicate experiments.
[d] NR, natural rubber; SBR, styrene-butadiene rubber; BR, butadiene rubber.

Table 3. Microbial degradation of various parts of tire D

Part of tire	Residual[a] weight(%)	Polymer composition (NR:SBR:BR)
Tread cap (TC)	49 ± 4[b]	100:0: 0
Base tread (BT)	86 ± 1	100:0: 0
Side wall	65 ± 2	30:5:65
Inner liner	95 ± 2	65:5:30
Bead	100 ± 1	100:0: 0

[a] Tire strip was incubated for 8 weeks with glove strip.
[b] Weight(%) of the remaining unchanged portion of strip after alkali treatment, mean ± deviations in duplicate experiments.

Comparison of tire parts

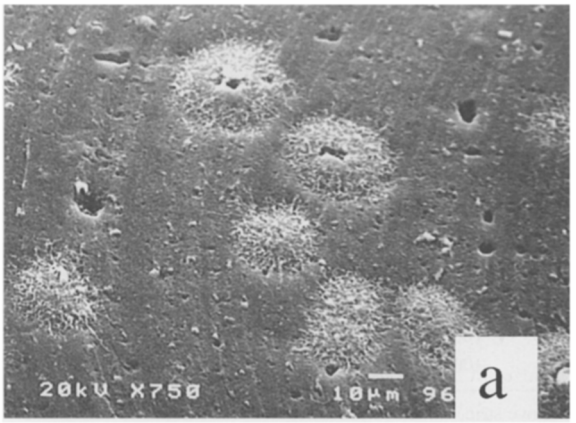
Table 3 shows a comparison of the microbial degradation of the strips from various parts of tire D. The rubber in the side wall was significantly attacked in spite of its low NR content, while bead rubber having a high NR content was not attacked at all. Although the weight loss of the strip of inner liner was very small, very small cavities were observed on the surface. No cavities could be seen on the surface of bead rubber even after the alkali treatment.

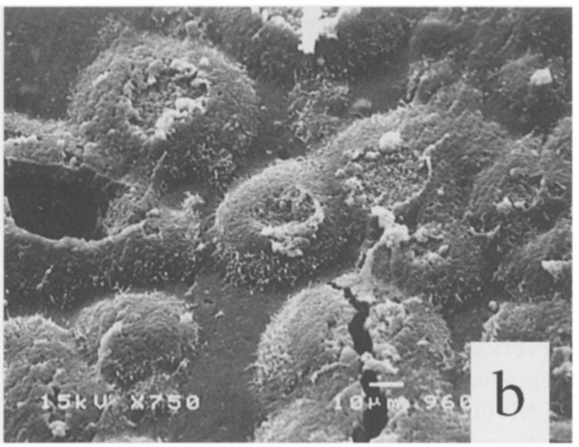
Discussions

Many reports have been made regarding microbial degradation of natural rubber (NR) products (Zyska 1988). The hydrocarbon of NR was reported to be used by strains of *Nocardia* and *Streptomyces* as a sole source of carbon and energy and colonization and penetration of latex gloves by the organisms were demonstrated by SEM (Heisey & Papadatos 1995). We previously reported that an actinomycete, *Nocardia* sp. strain 835A, was able to cause scissions of polyisoprene molecules of NR (Tsuchii et al. 1985). The strain is a very strong decomposer of solid rubber, but it does not produce an extracellular enzyme capable of degrading NR (Tsuchii 1995).

As soft type NR products, like rubber bands and gloves, are degraded rather rapidly, the microbial process has the potential for early application in practical treatment of waste products. Microbial degradation of a relatively large amount of a surgery glove made of NR was demonstrated. The semi-continuous culture of a rubber-degrading organism was continued over 150 days without sterilization of rubber and medium, and rubber films cut from the glove were efficiently degraded (Kajikawa et al. 1991).

On the contrary, hard type products with a high content of curing agent were degraded only at an extremely slow rate (Tsuchii et al. 1990) and, furthermore, the addition of carbon black as a filler made the vulcanizate more resistant to microbial attack (Kwiatkowska & Zyska 1988). The colonization of *N. asteroides* on the surface of a hard type NR vulcanizate was monitored by SEM. When the colonies were removed, the rubber beneath was found to be pitted and local penetration to depths of 220 μm was recorded, though the degradation amounted only 3% of the sample volume after an exposure of nearly two years (Hanstveit et al. 1988).





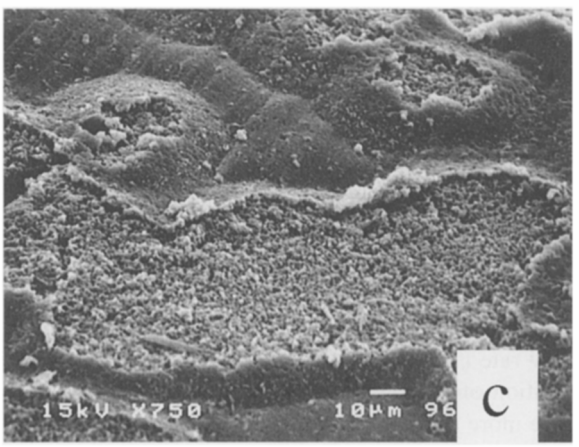
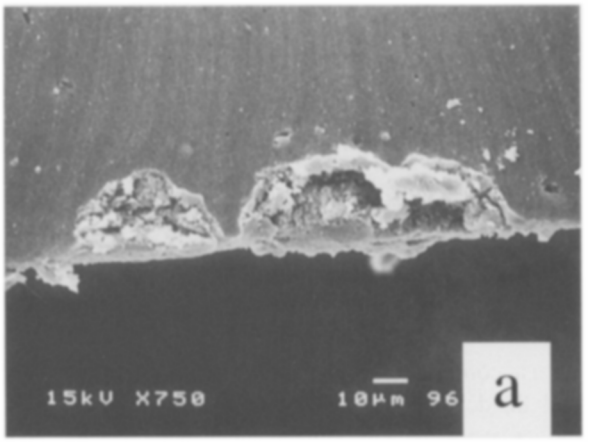


Figure 3. Microbial degradation of tire strip. SEM of the surface of tire strip (tire D-TC) incubated with glove strip. (*a*) 7 days. (*b*) 14 days. (*c*) 28 days. Bars represent 10 µm.



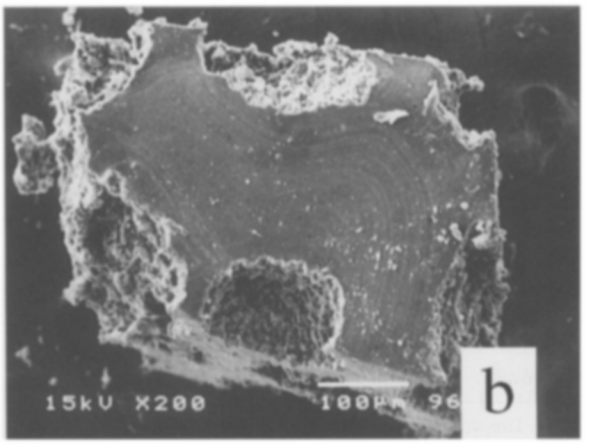


Figure 4. Cross section of tire strip. SEM of the cross section of tire strip (tire D-TC) incubated with glove strip. (*a*) 14 days, before alkali treatment. (*b*) 56 days, after alkali treatment.

In this study, a strip of a truck tire (tire D-TC) was found to be significantly attacked by a rubber-degrading strain of *Nocardia* when a glove strip was added to the test medium. The stimulating effect of glove rubber upon degradation of tire strip might be due to the effect of biomass produced at the expense of the glove rubber. As shown in Table 1, the preculture contained about 26 mg (dry weight) of microbial cells, of which 16.3 mg (Table 1, D) was finely dispersed in 100 ml of the medium and 11.5 mg (A-B) remained attached to the fragments of the strip. We have used only dispersed cells as inocula and 10 ml of the medium contained about 1.6 mg of cells (corresponding to 2.0×10^8 viable cells). When a glove strip was added, the biomass in the experimental flask reached as high as 45 mg on day 14 (Table 1, 10.4 mg in the medium (D) and 35.1 mg (A-B) still attached to the glove strip) and the biomass concentration was 28-fold higher than the control without a glove strip. These results strongly suggest that the rate and the extent of tire rubber degradation was enhanced by the increase in biomass. In fact, when the inoculum size was increased from

5 ml (0.8 mg dry cell weight) to 20 ml (3.2 mg), the weight loss of the tire strip (D-TC) after alkali treatment increased from 8% to 16%.

As shown in Figure 1, a small number of colonies of varying size were observed on tire strip of the control without a glove strip. On the other hand, the surface of the tire strip was covered with many large colonies when incubated with a glove strip. These results indicate that the increased amount of biomass stimulate the formation and the development of colonies. We have demonstrated that the degradation of tire strip proceeded in accordance with the progress of colonization and with the development of the colony size. Although the rubber beneath the colonies was severely disintegrated and pitted, plain smooth surface areas remained between the pitts. These observations strongly suggest that microbial growth occurred at the expense of the rubber in the tire strip and that the disintegration of the tire strip occurred only at points of direct contact with the colonies. The observations also suggest that a large amount of biomass in the medium may not directly contribute to the tire rubber degradation, but it may contribute only through the stimulation of the colonization.

Although 10 ml of the preculture contained 2.0 x 10^8 viable cells (or the aggregates of cells), only about 20/mm^2 of colonies on average was observed on the surface of tire strip incubated without a glove strip (Figure 1a), and the total number of colonies was about 5 x 10^3. These results indicate that the colonization efficiency for the tire strip was at an extremely low level. When incubated with a glove strip, the number of the colonies on the tire strip more than doubled. Assuming that the colonization efficiency was at the same level, a 28-fold increase in the biomass may be sufficient to explain the enhancement of colonization.

The adherence of microorganisms to an insoluble target substrate, like cellulose and pyrite, is considered to be a key factor in the further utilization of the insoluble substrate (Gelhaye et al. 1993; Kumar et al. 1991). Spherical holes measuring 50 to 200 mm in diameter were observed on a film of poly(hydroxybutyrate) and it was postulated that the holes were formed as the result of the colonization of a degrading bacterium (Nishida & Tokiwa 1992). We recently reported that the efficiency of colonization plays an important role in determining the degradation rate of glove rubber (Tsuchii et al. 1996). Further study will be required to improve the culture conditions for colonization and degradation of tire rubber.

From the microbiological point of view, however, the most interesting feature of tire rubber degradation may be this extremely low colonization efficiency. The low efficiency of colonization indicates the presence of a limited number of cells having an exceptionally high colonizing activity, with only one out of 40,000 viable cells (or cell aggregates) colonizing the tire strip. If we can learn the reason why some of the cells can colonize the tire strip though the majority cannot, we may be able to find a way to improve the degradation rate further. As an example, the stage of growth was reported to influence adhesion to insoluble substrate, with maximum adhesion being found during the exponential phase (Minato & Suto 1979; Gelhaye et al. 1993). An aggregate of a number of cells may have a higher inoculum potential than a single cell, or mutant cells with high colonization ability may possibly appear spontaneously. Whether one of these possibilities plays an important role in the colonization of tire strips will be a subject of further study.

By the alkali treatment, it was found that about 28% of a strip of tread rubber (tire D-TC) was microbiologicaly converted to very small black particles and that 49% remained unchanged. The amount of the particles was not corrected for undigestible materials from the cells (about 8 to 11% of cells, Table 1, C/(A-B), and E/D), and therefore represents a considerablly higher value. These results suggest that at least about 23% of the strip was truly degraded and transformed into alkali soluble fractions (soluble organic compounds and microbial cells). On the basis of the weight loss of 51% after the alkali treatment, it was concluded that about 55% was transformed to small particles and about 45% was truly degraded.

A typical formulation for truck tire tread compounds is as follows: NR, 80 (parts per hundred of rubber, phr); BR, 20; carbon black, 55; oil, 8; zinc oxide, 4; stearic acid, 2; antioxidant, 2; accelerator, 0.6; sulfur, 2.3 (Bernard et al. 1984). The content of elastomeric materials (NR and synthetic rubbers) in tread compounds is only about 60% in general and the content of carbon black is about 30%. When NR in a tread was degraded completely, the remaining carbon could be released into the medium. The size of most of the small black particles (2-30 μm) released by microbial action and alkali treatment, however, was much greater than that of carbon black (0.02–0.04 μm) and it was supposed that the particles still contained a considerable amount of NR.

All the tread rubbers examined were attacked to some degree by the organism. The weight loss of strips

after alkali treatment does not, of course, mean true mineralization. However, we could use the weight of the rubber strips recovered after alkali treatment as a measure to estimate the resistance of the products to microbial attack. The rate and the extent of the degradation of tread compounds was found to be greatly influenced by the content of NR. Rubber pieces of the tread containing more than 70 phr of NR were considerably degraded, while those with NR contents of less than 55 phr were only slightly attacked. As reported previously, strain 835A grows well on NR and synthetic isoprene rubber, but not on other types of synthetic rubber (Tsuchii et al. 1985) and it was readily expected that the NR content of tire treads would greatly influence microbial degradation. On the other hand, the effect of NR content was not clear in the degradation of various parts of a tire other than the tread part.

A tire is a product of complicated formulations with a variety of constituents. Not only the polymer composition but also the curing conditions and the filler content vary with the type of tire and with the part of the tire (Hess et al. 1984, Kaidou & Ahagon 1990). We reported previously that the resistance of the vulcanizates to microbial attack is in good correlation with the cross-link density (Tsuchii et al. 1990) and the content of curatives (sulfur and accelerators) must be the main determinant of degradability. It is also known that addition of carbon black further increases the resistance of the vulcanizate to microbial attack and that the types and the content of carbon black are probably also important parameters. The content of chemicals with microbiocidal activities may also be a factor, because many components in vulcanizates, such as accelerators and antioxidants, are known to affect microbial activities (Zyska, 1988). Properties of the rubber compounds required for the tread part are quite different from those required for other parts like the side wall or bead, and not only the polymer system of the compounds but also their carbon black reinforcement and curing system must be greatly different . This may be the reason why the NR content does not explain the differences in degradability of various parts of the same tire. On the contrary, the curing systems and the carbon black reinforcement of the tread compounds from different tires may not be so greatly different from each other, and considerable correlation between the content of NR and their susceptibilities can be expected. In any case, explanation of degradation test results on commercially available rubber products is difficult because the manufacturer is reluctant to disclose the details of his recipes and curing systems. Preparation of a series of specimens with definite formulations and examination of the effect of each component on microbial attack will be a subject of further study.

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