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Biodegradability of NR gloves in soil

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The environmental fate of NR gloves buried in soils was studied with several synthetic alternatives for 12 months in clayey Munchong series and sandy Holyrood series soil. Residual weight recovery and loss of tensile properties, supplemented by measurements of microbial populations and loss of film thickness were used to compare the extent of biodegradation of the glove materials. Residual weight measurements indicated a substantial degradation of the NR glove pieces but neoprene and nitrile were not degraded under such conditions. The weight loss of NR glove pieces in Munchong series soil was significantly higher than in the Holyrood series soil. Degrading NR glove pieces showed a significant loss of tensile strength and elongation at break after four weeks in both soils, with a significant increase in hardness. Vinyl showed some losses in weight that were probably confined to plasticiser and other additive losses. Significant losses of thickness were shown by NR (54%) and vinyl (37%) glove pieces but not for neoprene and nitrile. When averaged over soils and sampling times, the population densities of microorganisms on the various glove materials were of the order: bacteria; NR, vinyl > neoprene, nitrile; fungi; NR > vinyl > neoprene, nitrile; and actinomycetes; NR > neoprene > vinyl > nitrile. The biodegradation of used NR gloves in municipal landfills is discussed.

Rubber is a natural product with organisms for its biodegradation widely present in the natural environment. The rate and extent of microbial degradation will be influenced by the rubber formulations, the microorganisms present and their interactions with the environment. Early efforts made to study the biodeterioration of rubbers goods manufactured from NR have been reviewed by Cundell and Mulcock[1] and Zyska[2,3] with varying results under different test conditions. Thus, Shaposnikov *et al.*[4,5] showed that pure strains of some bacteria caused up to a 55% loss in weight of thin rubber sheets in 70 days, and pure cultures of actinomycetes a 43% loss in 45 days. The presence of fungi increased the weight loss due to actinomycetes to 52%. Low *et al.*[6] had used fungi and actinomycetes to degrade diluted latex for six weeks and found reductions of 17% and 24% in molecular weights due to a fungus and an actinomycete, respectively. Exciting evidence began to emerge when Tsuchii *et al.*[7] used the actinomycete *Nocardia* sp. strain 835A to degrade thin latex glove films where weight losses reached 75% after a two-week cultivation period to produce two fractions of degraded low molecular mass isoprene. Kajikawa *et al.*[8] completely degraded latex glove films in batch cultures using laboratory fermenters with the same strain after 45 days. Heisey and Papadatos[9] isolated 10 actinomycetes from soil that reduced the

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weight of vulcanised rubber from latex gloves by 10%–18% in six weeks

In pure culture, rubber-degrading enzymes from the bacteria *Xanthomonas* sp produce random scissions of NR to isoprene oligomers (acetonyl polyprenyl acetoaldehyde) with average molecular weight of 10^4 that is further degraded to form acetonyl diprenyl acetoaldehyde as the degradation product[10] This work by Tsuchii and Takeda[10] was the first evidence for the existence of an extracellular polyisoprenoid oxygenase in a Gram negative bacteria

The emergence of various elastomeric materials for production of gloves has posed an environmental problem for years, and their growing use is expected to raise concerns about their environmental impact as a result of their disposal by incineration and landfilling The present study examines the environmental degradation of NR gloves in the complex physicochemical and biochemical environment of natural soils

MATERIALS AND METHODS

Gloves

Normal, powdered NR examination gloves (Run 24/97) were from the Rubber Research Institute of Malaysia (RRIM) Glove Plant, polyvinyl chloride (vinyl) gloves were commercial sample, Neoprene® (polychloroprene) gloves were a gift from Ansell Malaysia Sdn Bhd, and Nitrile (RS NS 100) from Riverstone Resources Sdn Bhd, Selangor

The glove materials were cut into large (15×9 cm^2) and small (2×4 cm^2) pieces and buried in a horizontal position in the shallow soil layers

Soils and Experimental Units

The unamended soils used were the well-characterised heavy-clay Munchong series soil (Tropeptic Haplorthox) and the light-textured sandy Holyrood series soils (Typic Quartzipsamment) collected from the RRIM field station Their physical and chemical properties have been described elsewhere[11]

The soils (0–15 cm depth) were air-dried and sieved (<5mm) and filled into $52 \times 38 \times 15$ cm ($L \times B \times H$) plastic containers (17 2 kg/container, Munchong series, 19 8 kg soil/container, Holyrood series) to within 2 5 cm from the top The containers were kept in a room at 25°C and watered weekly to maintain a 40% field capacity by weight throughout the experiment

Sampling and Measurements

Samples were retrieved monthly after burial for up to 12 months, in 3 replications, for measurements of residual weight, changes in tensile properties and counts of viable microorganisms (bacteria, fungi and actinomycetes) and aerobic spore formers colonising the glove surfaces by a dilution plate estimation and film thickness measurements

The larger test strips were cleaned by washing in running water over a sieve and left overnight in water containing a non-ionic surfactant on a shaker to further remove adhering soil particles Samples were air-dried at room temperature for 24 h prior to weighing Subsequently the samples were used to characterise their mechanical properties, according to *ISO 37* (Type 2) standards using an Instron model 5500 tensile testing machine

The population dynamics of bacteria, fungi and actinomycetes were studied using the smaller pieces Retrieved samples were shaken

to remove excess soil and left in a bottle of sterile diluent[12] containing a non-ionic detergent (0.05%) and 1.0 mm glass beads. The bottles were shaken for 20 min (200 r.p.m.) and 1.0 ml-suspensions from 10-fold dilutions plated out on 1/10-strength tryptic soy agar (TSA) with 50 mg L^{-1} cycloheximide to estimate population densities of total bacteria and actinomycetes[13], and on peptone-dextrose agar containing a mixture of 33 mg L^{-1} final concentrations of rose bengal and 30 mg L^{-1} streptomycin for total fungi[14]. Population densities of aerobic spore formers were estimated for up to 5 months by boiling an aliquot for 10 min and plating out the suspension on TSA. Samples for initial microbial colonisation were obtained after two weeks of soil burial. The counts were expressed on the basis of retrieved material weight.

For thickness measurements, the dried glove piece was cut into smaller pieces (0.5×0.5 cm^2) before being fixed in Paraplast® tissue embedding medium (Oxford Labware, St. Louis, MO) at 56°C. Thin slices were made from the embedded subpieces and the glove widths from 100 pieces measured under the microscope at 40 × magnification.

To determine the swelling behaviour of NR glove pieces at 2, 4, 12, 16 and 20 weeks, 2×2 cm^2 pieces were cut from each dried specimen, weighed and allowed to swell in 50 ml toluene in test containers to the equilibrium state. The swollen specimens were taken out periodically to check on time taken to reach equilibrium, the excess liquid removed by blotting with tissue and the swollen weight immediately measured. The degree of swelling, Q, was calculated from the formula:

$$Q = W_2 - W_1/W_1, \quad \ldots 1$$

where W_1 and W_2 were the unswollen (dry) and swollen weights, respectively.

Statistics

Analysis of variance was carried out on all the measured variables and the treatment means compared at the $P<0.05$ probability level.

RESULTS

Weight Loss

For both soils, an almost 50% weight loss of NR pieces occurred during 16 weeks and averaged 94% after 48 weeks *(Figure 1)*. At 48 weeks, weight losses in the clayey Munchong series soil (99%) were significantly greater ($P<0.001$) than in the sandy Holyrood series soil (88%). Neoprene showed an insignificant weight loss ranging from 0%–5% in both soils over all samplings (2.3%, averaged over both soils and time). Weight losses for nitrile glove pieces were also marginal and ranged from 2%–13% (average, 6.4% over both soils and time). After 48 weeks, the weight loss due to neoprene and nitrile was not significant. Vinyl showed small weight losses ranging from 3%–22% (average, 11.6% for both soils). The mean differences due to soil were however significant (Munchong series, 13.5%; Holyrood series, 9.6%; $P<0.05$).

Averaged over time, the degradation of NR glove pieces in Munchong series soil (67%) was significantly higher than in the Holyrood series soil (59%) ($P<0.05$). The weight changes of neoprene, nitrile and vinyl in both soils fluctuated within a mean value over the time interval and were probably losses of compounding ingredients.

Glove Thickness

Significant losses of thickness were shown by NR (54%) and vinyl (37%) glove pieces but

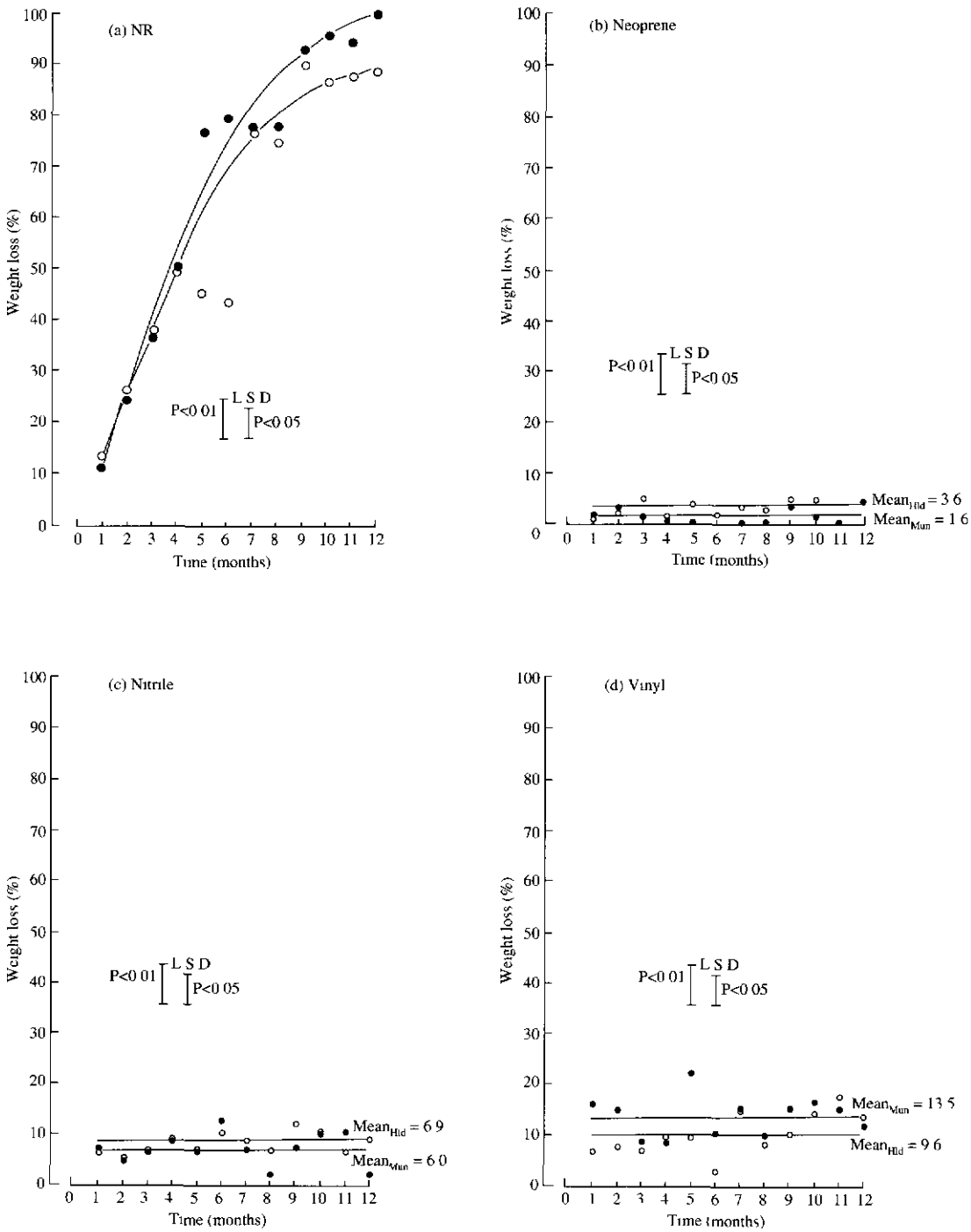

Figure 1. Weight loss of glove materials after burial in Munchong series (●) and Holyrood series (○) soil. Vertical bars represent L.S.D. values.

not for neoprene and nitrile after 48 weeks *(Table 1)*. Except for NR, the effect of soils in influencing glove thickness of neoprene, nitrile and vinyl was not significant.

Equilibrium Swelling

For a given rubber-solvent system, the greater the crosslink, the lesser is the swell. There was a slight increase in Q at two weeks followed by a decline thereafter, with significantly low values by 20 weeks *(Table 2)*. The decrease in swelling indicated an increasing crosslinking of the polymer in the biodegradation process.

Microbial Colonisation of Glove Pieces

Microbial enumerations revealed a considerable flux in the populations over the course of the year *(Table 3)*. In general, differences between the main effects of sampling times, glove material and microorganisms were highly significant ($P < 0.001$) although the differences between soils were not.

Averaged over sampling times, glove materials and soils, the mean numbers of bacteria (1.2317×10^4/mg glove piece) were significantly higher than fungi (4.4147×10^2/mg) that in turn were significantly higher than the actinomycetes (2.9702×10^2/mg). When averaged over soils and sampling times, the population densities on the various decomposing glove materials were of this order: bacteria; NR, vinyl > neoprene > nitrile; fungi; NR > vinyl > neoprene, nitrile, and actinomycetes; NR > neoprene > vinyl > nitrile ($P<0.05$).

TABLE 1. MEAN WIDTH OF NR, NEOPRENE, NITRILE AND VINYL GLOVE PIECES AFTER SOIL BURIAL*

Time (weeks)	Width (μm)			
	NR	Neoprene	Nitrile	Vinyl
0	193 a	168 b	128 c	163 a
4	170 b	170 ab	140 a–c	141 ab
8	171 b	176 a	152 ab	130 a–c
12	175 b	150 c	146 ab	133 a–c
16	147 c	166 b	149 ab	122 bc
20	152 c	169 b	152 ab	135 a–c
24	150 c	167 b	151 ab	118 bc
28	140 c	170 ab	144 a–c	126 bc
32	116 d	166 b	139 a–c	114 bc
36	114 d	165 b	151 ab	116 bc
40	102 de	169 b	153 a	128 bc
44	111 d	152 c	136 bc	132 a–c
48	90 e	167 b	130 c	103 c

*Mean of 3 replicates, averaged over two soils. For each glove material, values not followed by common letters are significantly different ($P<0.05$)

TABLE 2. WEIGH SWELLING INDEX (Q) OF NR GLOVE PIECES AFTER BURIAL IN TWO SOILS*

Soil series	Sampling stage (weeks)						
	0	0.5	1	2	3	4	5
Munchong	4.061 a–c	4.305 a	3.896 a–c	3.677 bc	3.381 c	0.758 f	n.d.
Holyrood	4.068 a–c	4.177 ab	3.789 a–c	2.387 d	1.634 de	0.880 ef	0.767 f

*Mean of 3 replicates. n.d.: not determined. Values across rows and columns not followed by common letters are significantly different ($P<0.05$)

TABLE 3. COMPARISON OF MICROBIAL COLONISATION OF GLOVE PIECES AFTER 48 WEEKS OF SOIL BURIAL*

Glove material	Nos./mg glove pieces*		
	Bacterial	Fungi	Actinomycetes
NR	12.0670 a	8.2487 c	9.3756 b
Neoprene	9.4295 b	6.2286 ef	6.3594 e
Nitrile	8.1655 c	6.2877 e	5.1914 g
Vinyl	11.8890 a	7.4878 d	5.8071 f

* *Mean of 3 replicates, as ln (X+1) data; averaged over soil and sampling times. Values across rows and columns not followed by common letters are significantly different ($P<0.05$)

Bacterial populations on NR and vinyl were consistently high throughout the samplings. Actinomycetes and fungal populations on NR only increased after 16 weeks in both soils, that caused higher overall averages relative to the other materials. The populations of actinomycetes on vinyl were generally low except for the significantly higher numbers from 24 weeks onwards obtained on pieces buried in Munchong series soil (data not shown). The populations of aerobic spore formers were low throughout the samplings, were not significantly different between the materials or soils (mean, 0.7689×10^1/mg) and declined significantly by 16 weeks and 20 weeks (data not shown).

Tensile Properties

The results are expressed as % changes from the 0-time control. For NR glove pieces, there was a significant loss of tensile strength (>90%) and elongation of break after 4 weeks in both soils *(Table 4)*, with a significant increase in modulus values *(Table 5)*. Tensile strength measurements could not be performed on NR samples beyond 12–16 weeks as part of the material had disintegrated during soil exposure.

Except for some exceptionally high values, tensile strength and elongation at break of

TABLE 4. CHANGES IN TENSILE PROPERTIES OF NR, NEOPRENE, NITRILE AND VINYL GLOVE PIECES AFTER INCUBATION IN TWO SOILS[a]

Sampling stage (weeks)	NR		Neoprene		Nitrile		Vinyl	
	Munchong series	Holyrood series	Munchong series	Holyrood series	Munchong series	Holyrood series	Munchong series	Holyrood series
(a) Tensile strength (MPa)								
4	–94	–91	+ 7	+ 7	–34	– 5	+72	– 5
8	–98	–95	+18	+14	–19	+ 1	+64	0
12	–94	–95	+12	+14	–37	–20	+48	– 2
16	–	–94	+ 7	+10	–41	–28	+32	+ 7
20	–	–	– 1	+21	–53	–44	+82	– 9
24	–	–	+17	– 2	–42	–30	+55	+ 9
28	–	–	+ 4	+10	–43	–47	+69	+11
32	–	–	+10	+16	–37	–51	+60	+15
36	–	–	+ 5	+17	–50	–61	+68	+ 3
40	–	–	–14	+ 6	–47	–46	+73	+ 9
44	–	–	+11	–	–54	–74	+65	+16
48	–	–	+11	–	–57	–69	+79	+23
(b) Elongation at break (%)								
4	–73	–65	0	+ 1	+ 2	+ 1	– 9	– 8
8	–94	–91	– 5	+ 3	+ 5	– 1	– 5	– 2
12	–91	–90	+ 4	– 3	+ 2	+ 1	– 1	–13
16	–	–93	+ 3	+ 3	+ 7	+ 8	+ 2	– 2
20	–	–	– 6	+ 2	+ 9	0	–14	–23
24	–	–	+15	–13	– 1	– 3	– 3	– 7
28	–	–	+ 1	0	– 3	+ 8	–15	– 4
32	–	–	+ 7	– 4	+ 4	– 3	– 7	–12
36	–	–	– 1	+ 6	– 2	+16	–15	–17
40	–	–	–10	0	+ 3	– 1	– 5	–10
44	–	–	– 9	–	– 9	+17	0	– 6
48	–	–	– 3	–	+ 9	+ 8	–16	– 8

[a]Data expressed as % change from 0-time control; mean of 3 replicate glove pieces. For each glove material, the underlined values are significantly different from the 0-time controls ($P<0.05$). Mean tensile strengths at 0-time were 26.3, 16.5, 28.6 and 10.7 MPa for NR, neoprene, nitrile and vinyl, respectively (mean elongation: 900, 764, 562 and 404%).

TABLE 5. CHANGES IN MODULUS OF NR, NEOPRENE, NITRILE AND VINYL GLOVE PIECES AFTER INCUBATION IN TWO SOILS[a]

Sampling stage (weeks)	NR		Neoprene		Nitrile		Vinyl	
	Munchong series	Holyrood series	Munchong series	Holyrood series	Munchong series	Holyrood series	Munchong series	Holyrood series
(a) Modulus at 100%								
4	+80	+60	+18	+10	−30	−7	+ 56	0
8	–	–	+ 9	0	−33	−11	+ 69	−18
12	–	–	−18	0	−37	−11	+ 28	− 8
16	–	–	+27	+30	−22	−22	+ 33	+ 3
20	–	–	0	−30	−37	−15	+139	−38
24	–	–	−27	−20	−52	−44	+ 31	−18
28	–	–	−27	−10	−44	−52	+ 75	− 3
32	–	–	−36	−20	−52	−44	+ 44	− 3
36	–	–	−18	0	−41	−52	+ 92	− 3
40	–	–	+ 9	−40	−26	−48	+ 92	+ 3
44	–	–	−27	–	−41	−70	+ 72	+15
48	–	–	–	–	–	–	–	–
(b) Modulus at 300%								
4	–	+50	+ 6	+24	−39	− 5	+ 82	+ 1
8	–	–	+17	+6	−31	+ 4	+ 72	0
12	–	–	+ 6	+18	−37	−13	+ 37	+ 7
16	–	–	+17	+18	−37	−25	+ 29	+10
20	–	–	+11	-6	−49	−24	+106	+ 6
24	–	–	−11	+6	−44	−24	+ 51	+12
28	–	–	− 6	+6	−39	−47	+ 72	+18
32	–	–	− 6	+6	−46	−38	+ 58	+24
36	–	–	+11	+6	−39	−55	+ 82	+17
40	–	–	+17	−12	−41	−40	+ 76	+14
44	–	–	0	–	−36	−71	+ 65	+17
48	–	–	0	–	−56	−65	+ 81	+29

[a]Data expressed as % change from 0-time control; means of 3 replicate glove pieces. For each glove material, the underlined values are significantly different from the 0-time controls ($P<0.05$). The mean modulus (100%) at 0-time were 0.5, 1.1, 2.7 and 3.8 MPa for NR, neoprene, nitrile and vinyl, respectively (1.0, 1.8, 5.7 and 8.1 at 300% elongation).

neoprene in general were not significantly different from the 0-time controls after 12 months burial. For both soils, the modulus at 300% were similar to the controls.

In contrast, nitrile showed a significant decrease in tensile strength in both soils after burial although elongation at break was generally unaffected. The modulus values were also reduced with increasing sampling times.

There also was a significant increase in tensile strength and modulus values from the 0-time controls of vinyl samples buried in the Munchong but not Holyrood series soil although elongation at break values for both soils remained unaffected.

DISCUSSION

The residual weight loss of the NR test pieces after soil burial was consistent with the substantial reduction in tensile properties and widths, and with the highest numbers of microorganisms colonising the surfaces. The difference in the time scale of substrate utilisation as seen by the more rapid responses in the clayey Munchong series soil could probably be due to the higher nutrient contents of this soil. Strongly-weathered surface soils of the tropics are acidic and nutrient-deficient in terms of plant growth, with low *p*Hs (<4.7), are depleted in bases, and are especially low in total nitrogen (N) and available-phosphorus (P). N and P are major inorganic nutrient limitations to microorganisms in soil, and an assessment of their carbon utilisation, N and P use efficiency gives a good indication of the soil's microbial activity. In this study, microbial presence cannot be readily correlated with activity, and further experiments are planned to handle direct measures of community activity through enzymic methods to form a basis for material evaluation and process control.

Vinyl failed to mineralise in soil and the small weight losses were possibly due to plasticiser and other additive losses that also caused stimulation of microbial activity. Under such conditions, neoprene and nitrile did not degrade and both materials showed insignificant weight changes after 48 weeks that may be at the expense of the compounding ingredients worked into the product during the manufacturing process. A possible explanation for the decline in tensile strength and modulus but not % elongation of nitrile in both soils after 48 weeks is the removal and biodegradation of the additives. Similarly, the significant increase in tensile strength and modulus but not % elongation of vinyl in Munchong series soil could be due to leaching of the additives that occurred faster in the clayey soils. The changes for vinyl in Munchong series soil corresponded with its higher actinomycete populations.

Synthetic rubber compounds are more resistant to microbial attack than NR compounds[15]. Unlike natural polymers, many synthetic polymers are not biodegraded because they have not been available for a long-enough time in natural evolution for microorganisms to develop degradative enzymes that will utilise the compounds. In a previous study, Tsuchii *et al.*[7] were able to grow the rubber-degrading actinomycete strain 835A on vulcanised and unvulcanised NR as well as on synthetic isoprene rubbers but not on other types of synthetic (chloroprene, butadiene and styrene-butadiene) rubbers. The pure culture studies of Cundell and Mulcock[16] also showed that losses in weight of isobutene-isoprene (butyl), chloroprene and acrylonitrile-butadiene (nitrile) rubber strips after 18 months were very small. Polyvinyl chloride, along with other water-insoluble vinyl polymers (polystyrene, polyethylene and polypropylene) are also resistant to biodegradation[17]. Yabannavar and Bartha[18] could not degrade polyethylene and

plasticised polyvinyl chloride films in aerobic soils and found from GPC measurements, chloride release and viscosimetric tests that residual weight losses of these plastics were confined to the additives

In these tests degradation is not exclusively microbiological but include other complex physicochemical actions of the soil environment, usually the autooxidative-progressive ageing effect that can prime the material for microbial action The modulus values were higher for NR after 4 weeks incubation, indicating a hardening/ageing effect The NR test pieces develop brown spots that grew darker between two to four weeks and becoming harder and brittle by 12 weeks with an eventual breakage The route of environmental degradation of the NR glove pieces appear towards becoming stiff and brittle with time, as evidenced from the equilibrium swelling and modulus values, that subsequently break when flexed While pigmented spots develop on vinyl throughout the course of burial, neoprene and nitrile showed no morphological change

The biodegradation of used NR glove wastes in municipal landfills will be influenced by the rubber formulations and the appropriate environmental conditions Successful degradation thus cannot be left to default and control strategies include understanding the contributing ecological factors such as acceptable soil composition and properties metabolically capable microbial populations, substrate bioavailability, oxygen concentration, temperature, *p*H, moisture, the supply of inorganic nutrients, organic substrates and toxicity[19] The environmental conditions and the untreated soils used in this study may be less favourable for biodegradation since it was reported that a 50% water holding capacity and *p*H 7 5 was near-optimal for hydrocarbon biodegradation[20,21] According to Tsuchii[22], the ecological study of rubber biodegradation is still at a primitive stage, and future subjects of research will inevitably be on the waste disposal of used rubber, estimations of their degradation rates in nature, and on the biochemical and physiological studies of rubber-degrading microorganisms There is more to learn on the ecology of the process, and on the nutritional and environmental factors that could enhance rubber degradation

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