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Biodegradation of Natural Rubber by Actinomycete No. 4 in a Continuous Culture System

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Abstract: The degradation of natural rubber in a continuous culture system using actinomycete has been investigated at three exposure times (30, 60 and 90 days) employing diagnostic matching as well as the evaluation of mechanical and chemical properties. The biodegradation was carried out in a packed bioreactor varying three operating parameters; temperature (20°C, 30°C, 40°C and 50°C) pH (5,6,7 and 8) and aeration rate (0, 0.3, 0.5 and 1 vvm). The actinomycete growth characteristics were also investigated and found to be the best in a minimal media with a concentration of 0.1 g/l glucose. The actinomycete was able to grow in both glucose and non-glucose environments. In diagnostic matching, the morphology of degradation was matched with actual conditions using scanning electron microscopy. The morphologies observed were; striations, asperities, voids and bulk degradation. The intensity of biodegradation runs parallel with morphology in the order thus; striations, asperities, voids and bulk degradation. Asperities were observed at 60 days and temperatures of 20-40°C, striations at 40°C and an exposure time of 60 days. The morphologies with change in rate of aeration were voids and bulk disintegration. Voids were observed for all samples at aeration rates of 0.3-1 vvm with onset at 30 days exposure. The morphology of degradation for the change in pH was voids with onset at 30 days exposure time. The voids then grew with increase in pH as well as exposure times, but did not manifest as bulk degradation at the end of the exposure, at the higher temperatures. The morphology of degradation was matched to the mechanism, enabling a mapping of the process to be made.

Key words: Biodegradation, natural rubber, actinomycete No. 4

Introduction

Concerning the relatively slow degradation of polymers in the environment, there has been spurred research into the biodegradation of natural and synthetic rubbers (Tsuchii *et al.*, 1985). Being a natural rubber producing country, much interest in Malaysia (Ikram *et al.*, 1995) as well as in other countries (Jendrossek *et al.*, 1997) has been shown in the biodeterioration of natural rubber (NR). The earliest reported work on the microbial degradation of natural rubber was made by Spence and Van Niel (1936), that measured biodegradation phenomena as the weight loss of the hydrocarbon. Researchers then could not ascertain whether the microbial attack on the rubber hydrocarbon was due to the carbon source in the polymer backbone or due to the other constituents in the rubber such as the compounding ingredients (Linos *et al.*, 2000). Experiments were invariably carried out on highly purified natural rubber.

In this work, NR samples were investigated for biodegradation in the presence of actinomycetes following the work of Borel *et al.* (1982). Electron microscopy was used to study the mechanism of biodegradation by morphological presentation of the degraded NR samples. This is called diagnostic matching, a technique often used in materials science and engineering.

Materials and Methods

The source of natural rubber: Natural rubber (SMR-L), referred to as NR was obtained from the Rubber Research Institute of Malaysia and used in the as-received condition.

Culture stock preparation: Actinomycete No. 4 culture stock was obtained from the Rubber Research Institute of Malaysia. The culture was subcultured on 5 petri dishes in minimal rubber media, then incubated at 30°C for 5 days till spore formation. The spores were harvested by adding 15 ml sterilized distilled water and scrapped out using wire. It was left for 30 minutes. The media was then filtered with sterile muslin.

Bioreactor design and experimental methods: The packed bioreactor consists of several main components, that is a packed reactor, air and media inlets, air and media outlets, a temperature controller and water jacket (Fig. 1). The reactor packing was

stainless steel wire (Amway scrub buds). The biodegradation process was effected at temperatures of 20- 50°C (T20-T50) exposure time of 0 to 3 months (C1-C3) and pH of 5 to 8 (pH 5-8).

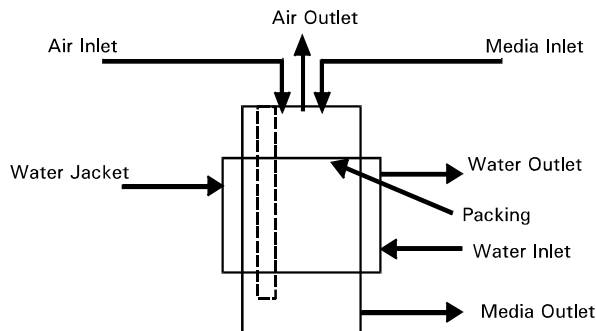


Fig. 1: Schematic figure of packed bioreactor

Sample preparation for scanning electron microscopy (SEM): NR samples were sputter-coated with gold using a sputter-coater (Bio-Rad SC 500) and the morphology was observed using electron microscopy in the scanning mode (Phillips XL-30) at a magnification of 500x.

Process runs: The biodegradation process was carried out in a packed bioreactor varying three operating parameters; temperature (20°C, 30°C, 40°C and 50°C), pH (5,6,7 and 8) and aeration rate (0, 0.3, 0.5 and 1 vvm).

Results and Discussion

The effect of temperature on morphology: The NR sample for this particular investigation was observed at a pH 7 and 1 vvm aeration rate. Three morphologies were observed on all samples viz. surface asperities, striations and bulk degradation. The control NR morphology (SMR-L 0) is shown in Fig. 2.

Surface asperities: Surface asperities were observed on the second month at 20°C till 40°C (Fig. 3). Surface asperities may be the evidence of sporulation.

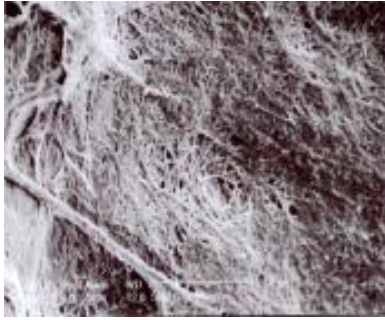


Fig. 2: SMR-L – 0

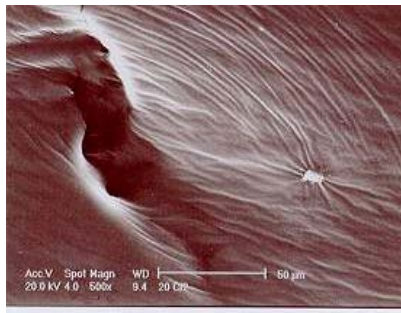


Fig. 3: Temperature 20°C-one month (C1)



Fig. 4: Temperature 20°C-3 months (C3)

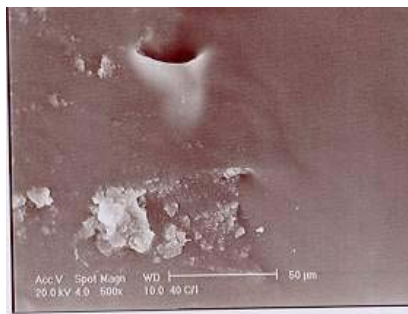


Fig. 5: Temperature 40°C-1 month (C1)

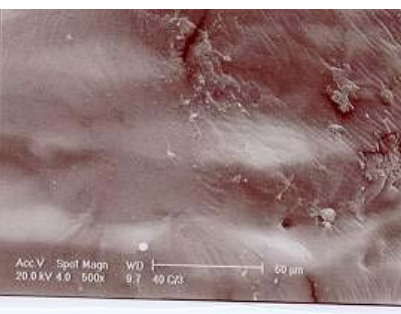


Fig. 6: Temperature 40°C-3 months (C3)



Fig. 7: Temperature 50°C-3 months (C3)

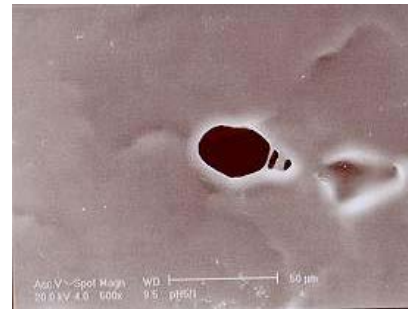


Fig. 8: pH 5- 1 month (C1)

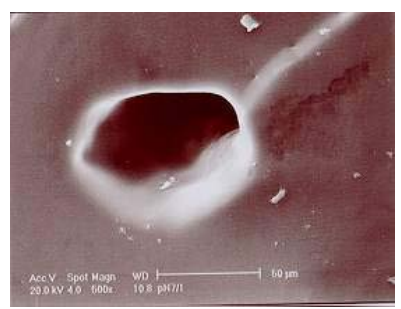


Fig. 9: pH5- 3 months (C3)

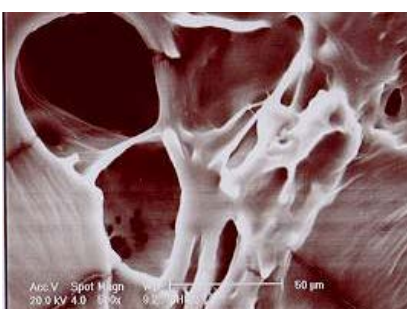


Fig 10: pH 8- 3 months (C3)

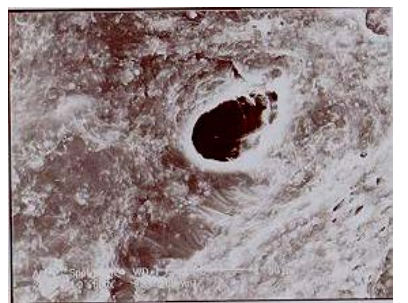


Fig. 11: 0.5vvm- 1 months (C1)

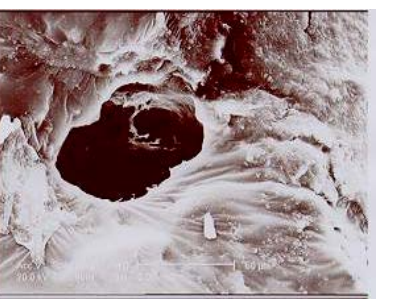


Fig. 12: 0.5vvm- 2 months (C2)

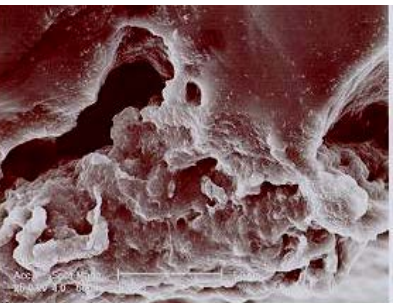


Fig. 13: 0.3 vvm- 3 months (C3)

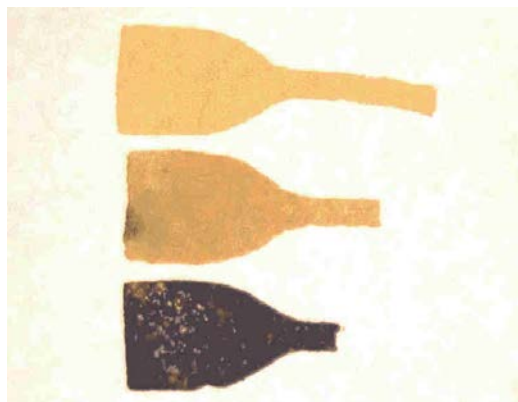


Fig. 14: Photo of NR samples showing change in physical appearance due to the degradation process.

Bulk degradation: Bulk degradation was observed on NR samples at 20°C after 3 months as well as at 30°C after the second month. Bulk degradation is represented by Fig. 4. The micrograph showed degradation, which has penetrated into the bulk structure. Process debris was also observed on the surface. The transition temperature for bulk degradation was at 30°C and transition time was 3 months for the low temperatures. If we were to assume bulk degradation is a manifestation of a degradation mechanism, then the transition points occurred at T20C3 and T30C2. The filamentous morphology was observed by other researchers such as Jendrossek *et al.* (1997) and Tsuchii *et al.* (1990), Tsuchii and Takeda (1985) and Borel *et al.* (1982). In all their reports, the morphological presentation were grooving (asperities) on the surfaces, pebbling and granular structures (asperities and striations). They also observed filamentous growth penetrating into the bulk structure as well as alteration of physical structure. These were similarly observed in our work, which we termed as bulk degradation.

Striations: Striations occurred on samples at T40C3 and continued at T50C1-C3. The transition points for time is C3 while for temperature is T50 (Fig. 6 and 7). The points which striation appeared may be seen as a transition point for process mechanism. Voids were not pervasive although seen at T40C1 (Fig. 5).

The effect of pH on morphology: The observation of NR samples for the effect of pH on morphology was carried out at the temperature of 30°C and 1 vvm. The morphology exhibited were mesovoids and filamentous degradation. It is obvious that the difference in morphology to that of the effect of temperature signaled a different biodegradation mechanism.

Mesovoids: Mesovoids ($d = 30 \mu\text{m}$) started at pH 5 C1 and were observed on all samples at C1 (Fig. 8). The mesovoids grew with exposure time: pH 5 C3 ($d = 100 \mu\text{m}$), pH 8 C3 ($d = 150 \mu\text{m}$) (Fig. 9 and 10). The growth is filamentous (Fig. 10). It is quite possible with further work to correlate quantitatively the microorganism growth rate to rate of increase in the diameter of the voids.

The effect of aeration rate on morphology: The effect of aeration rate on NR samples were made at pH 7 and a temperature of 30°C.

Mesovoids: Mesovoids were observed at 30 days for all aeration rates (Fig. 11). The rate of growth of mesovoids increased with aeration rates, e.g. at C1, 1 vvm ($d = 29.9 \mu\text{m}$) at 0.5 vvm C1 ($d = 22.10 \mu\text{m}$) at 0.3 vvm C1 ($d = 20.3 \mu\text{m}$). With increase in

exposure mesovoids increased in size until bulk degradation was observed: at 0.5 vvm C2 ($d = 39.85 \mu\text{m}$) and at 0.3 vvm C3 ($d = 56 \mu\text{m}$) (Fig. 11 and 12). At 0 aeration rate the NR sample did not manifest any of the degradation mechanism as observed in the other samples even at the maximum exposure time.

Physical appearance of degraded natural rubber: The appearance of the NR samples subjected to the biodegradation process can be observed from Fig. 14. This appearance was true for all conditions except for the sample at 0 aeration rate.

The evidence of action of actinomycete No. 4 on the natural rubber may be conclusively drawn from the change in morphologies exhibited after different exposure times with bulk degradation, most certainly evident after 3 months exposure. Previous work has only utilized electron microscopy to show the effects of degradation after confirmation with other techniques but not used in the context in this work. All the work invariably showed the final presentation and did not follow, i.e. map the mechanism of biodegradation. The technique enabled the researchers to point out that there are different mechanisms due to different parameters, but at this juncture not to be able to point the actual mechanisms. More diagnostic matching work would have to be carried out. This would involve detailed morphological study of the specimens to provide the statistical analysis. But from the study, we know that the mechanism due to the change in temperature is most complex, whilst that of the other two are similar.

From the morphology exhibited, the biodegradation mechanism of NR is heavily influenced by the operating parameters. The degradation could only commence in the presence of oxygen. The mechanism due to effect of the temperature changes were more complex compared to the change in pH as well as aeration rate. It would seem that only one mechanism was observed for the change in both pH and aeration rate. The rate of degradation was qualitatively related to the increase in mesovoids size. The main mechanisms seemed to involve the formation of filaments and attack on the bulk structure by the advent of mesovoids formation. For the change in temperature, three mechanisms existed, evident from the three morphological presentations, surface asperities, striations and bulk degradation. The transition points in the process mechanism were evident from the change in morphological presentation, which were at T20C3 (a temperature of 20°C and 3 months exposure time) and T30C2 (a temperature of 30°C and exposure time of 2 months).

Acknowledgments

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