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# Effect of Microbial Degradation (*Streptomyces* Spp) on Molecular Weight of Natural Rubber

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## Abstract

**Degradation of natural rubber by *Streptomyces* spp using dilute solution viscosity measurement technique was studied. Natural rubber latex was dissolved in xylene and exposed to *Streptomyces* spp for 1, 2 and 3 weeks. The degradation by these species was monitored by measuring the intrinsic viscosity for the various time of exposure. In the absence of the degrading, the intrinsic viscosity for rubber solution is 0.58 g/dl, while when subjected to 1, 2 and 3 weeks exposure in organism (*Streptomyces*), the intrinsic viscosity for the rubber solution are 0.54 g/dl, 0.52 g/dl and 0.48 g/dl respectively. The result shows that the intrinsic viscosity decreased with an increase in time of exposure of rubber solution to the degradant. This is expected since longer time of exposure to the degradant will provoke chain scission and subsequent reduction in molecular weight which is a measure of the rate of degradant of the polymer.**

***Keywords:* *Degradation; Latex; Streptomyces spp; Viscosity; Xylene***

## Introduction

Natural rubber degradation by micro-organisms has been under investigation for several years [1]. It is obvious that bacteria as well as fungi are capable of degrading rubber, although rubber bio-degradation is a slow process [2,3]. The introduction of the latex agar plates which consist of a bottom agar layer of mineral salt medium and a layer of latex agar on top for isolation and cultivation of rubber degrading micro-organisms was an important achievement [4,5]. Micro-organisms growing on such plates form a clear zone around their colonies and when about 1, 220 different bacteria were investigated for their ability to degrade rubber using the latex overlay agar plate technique, 50 clear zones forming actinomycetes were identified [6]. However, growth of some of the strains on natural rubber led to significant weight loss (10% to 30%) of the material used as well as the average molecular weight of the rubber. The main constituent of natural rubber is the poly (cis-1, 4-isoprene) with an average molecular mass of about $10^6$ Da. Since natural rubber contains a small amount proteins, resins, fatty acids, sugars and minerals together with a minimum of 90% rubber hydrocarbon [3,7], it is still produced in large amounts ($10^7$ tons/yr) from the rubber tree known as (Hevea brasiliensis) [7]. Thus, bacterial involved in the microbial degradation of rubber have

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been characterized, identified and have their corresponding genes cloned. More so in the past decade, studies on microbial degradation were made and only little is known about the occurrence of natural rubber degrading bacteria [8]. The present study were initiated to isolate and characterized a number of natural rubber degrading bacterial from various ecosystems and its usefulness for the disposal of discarded rubber products [9]. Therefore, identification of rubber metabolizing microorganisms potentially could provide a biotechnological solution to this problem.

## Experimental

### Materials used for culturing organism

- Exposed later coagulum (Rubber Research Institute of Nigeria, Iyanomo, Benin, Edo State)
- Gallenkamp incubator
- Distilled water
- Wire lobe
- Autoclave
- Flame burner
- Microscope
- Petri dishes
- Glass hockey
- Agar plate

### Equipment's used for viscometric analysis

- Ubbelohde viscometer (Size 3)
- Stop watch
- Test tubes
- Micro wave oven
- Volumetric flask
- Solvent (Xylene)
- Stirrer rod
- Measuring cylinder/beaker
- Electronic weighing balance
- Constant temperature water bath
- Suction pump
- Latex/latex crumb

### Preparation of culture medium

20 g of agar was dissolved in 750 ml of distilled water by steaming 5 g of Nacl, 2 g of $K_2HPO_4$, 1g of $MgSO_4$. $7H_2O$, 0.04 g of $CaCl_2$, 0.02 g of $FeSO_4$, $7H_2O$ and 0.01 g of $ZnSO_4$ were used. $7H_2O$ were dissolved and steamed in 250 ml of distilled water. The agar solution was added to the solution of the salts and mixed thoroughly. The solution was sterilized at 115°C for 20 min in an autoclave and was allowed to cool at room temperature.

**Procedure for culturing organism:** Long exposed latex was soaked for about 168 h in water and filtered. About 10 ml to 15 ml of the filtrate were poured in an agar plate and incubated for about 48 h to activate the growth of the organisms present in the filtrate. 0.1 g inoculum sp. was collected from the plate for several dilutions. A sub inoculum of 0.1 ml and 1.0 ml were separately cultured using the spread and pour plate techniques. For the spread plate, a glass hockey was used to spread the 0.1 ml evenly over the surface of the plate incubated and then monitored for growth. For the pour plate, 1.0 ml was carefully dropped on an empty sterile Petri dish and 10 ml of the culture medium were added to mix with the inoculum. The plates were incubated at 25° to 35°C. The colonies were observed for distant color, biochemical reaction, carbohydrate utilization and gram staining for microscopic view. On confirmation of the organism, pure sub-cultures were made using the wire loop and were incubated.

**Degradation process by microbes:** Latex was tapped and weighed into separate beakers. The beakers were taken to the inoculation chamber for inoculation of the organism. A wire loop was used to inoculate the organism into the separate beakers containing the latex. About 10 ml to 15 ml of the culture medium was added and stirred vigorously. They were then incubated for about 120 h.

**Viscometric test:** The prepared samples for viscometer analysis were sterilized in an autoclave to terminate the activity of the organism before conducting the viscometric test. 1 g of latex was weighed and dissolved in 50 ml of xylene and was stirred to obtain a homogeneous solution. The solution was diluted in turn to obtain the following concentrations 0.2 g/dl, 0.4 g/dl, 0.6 g/dl, 0.8 g/dl and 1.0 g/dl. The water bath was equilibrated at 60°C for about 20 mins. 20 ml of xylene were poured into the viscometer held by a retort stand in the water bath and was allowed to equilibrate for about 10 mins. The flow time of the solvent was obtained. The solvent was then removed and the viscometer was rinsed with distilled water, dried at 50°C in an oven and allowed to cool to room temperature. The procedure was repeated for the different concentrations at intervals of 1 week during the degradation period.

## Results

The flow time, concentration, specific [$\eta_{sp}$] and reduced [$\eta_{red}$] viscosity values are presented in TABLES 1-4 (FIG. 1).

Flow time of xylene ($t_o$)=163 s

Temperature=60°C.

TABLE 1. **Flow time of natural rubber latex in xylene.**

| Concentration (g/dl) | Flow time (S) | ηsp (t-t0/t0) | ηred (ηsp/c) |
|---|---|---|---|
| 0.2 | 181.9 | 0.116 | 0.58 |
| 0.4 | 206.68 | 0.268 | 0.67 |
| 0.6 | 240.26 | 0.474 | 0.79 |
| 0.8 | 277.75 | 0.704 | 0.88 |
| 1 | 322.74 | 0.98 | 0.98 |

TABLE 2. **1 Week exposure.**

| Concentration (g/dl) | Flow time (S) | $\eta_{sp}$ $(t-t_0/t_0)$ | $\eta_{red}$ $(\eta_{sp}/c)$ |
|---|---|---|---|
| 0.2 | 180.6 | 0.108 | 0.54 |
| 0.4 | 205.38 | 0.26 | 0.65 |
| 0.6 | 236.35 | 0.45 | 0.75 |
| 0.8 | 273.84 | 0.68 | 0.84 |
| 1 | 319.59 | 0.961 | 0.96 |

TABLE 3. **2 Weeks exposure.**

| Concentration (g/dl) | Flow time (S) | $\eta_{sp}$ $(t-t_0/t_0)$ | $\eta_{red}$ $(\eta_{sp}/c)$ |
|---|---|---|---|
| 0.2 | 179.3 | 0.1 | 0.5 |
| 0.4 | 203.42 | 0.248 | 0.62 |
| 0.6 | 233.41 | 0.432 | 0.72 |
| 0.8 | 271.33 | 0.664 | 0.83 |
| 1 | 315.89 | 0.938 | 0.94 |

TABLE 4. **3 Weeks exposure.**

| Concentration (g/dl) | Flow time (S) | $\eta_{sp}$ $(t-t_0/t_0)$ | $\eta_{red}$ $(\eta_{sp}/c)$ |
|---|---|---|---|
| 0.2 | 178.65 | 0.096 | 0.48 |
| 0.4 | 200.16 | 0.228 | 0.57 |
| 0.6 | 228.53 | 0.402 | 0.67 |
| 0.8 | 263.41 | 0.617 | 0.77 |
| 1 | 303.28 | 0.861 | 0.86 |

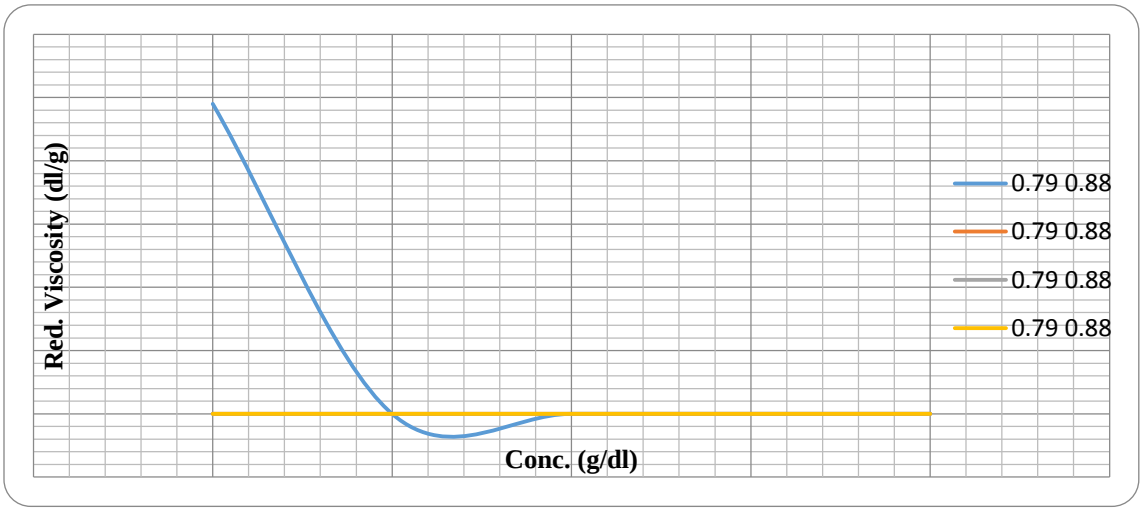


FIG. 1. **Graph of reduced viscosity ($\eta_{red}$) vs. concentration.**

## Discussion

Species of the *Streptomyces* spp has been investigated with regards to rubber degradation and the protein contents of cultures of the clear zone forming micro-organism strain were found to increase from 240 µg/ml to 620 µg/ml during incubation of

the cell with natural rubber latex after about ten weeks of incubation. However, 1% of the mutant analyzed exhibited a clear zone negative phenotype on latex outlay plates indicating a correlation between rubber and degradation due to defects in protein secretion [7,10]. Also, degradation of natural rubber by *Streptomyces* spp occur by oxidative cleavage of the micro-organism to the double bond in the backbone chain within the polymer molecular arrangement thereby feeding on the carbon contents of the rubber leading to breakdown of the man polymer chain resulting in loss of weight. Most of the degraded product detected contains aldehyde and keto groups which can be explained by oxygenases like Rox A. Thus, this degradation process occurs depending on the period of exposure of the micro-organism to the rubber to be degraded [11].

On incubation, the following observations were made as the latex coagulates:

i. Gray brown spots on coagulated latex.
ii. Odour of fermented starch.
iii. Surface irregularities of coagulated latex.
iv. Formation of rope like threads on the surface of coagulated latex on microscopic view.

The progressive decrease in intrinsic viscosity with increasing period of exposure of the microorganism leads to increase in degradation with increasing period of exposure. In other words, the gradual decrease in intrinsic viscosity leads to the decrease in molecular weight since intrinsic viscosity is empirically related to molecular weight according to Mark Houwink equation $(\eta)=KM_v^a$. Using xylene as a solvent and at a temperature 60°C, the study of degradation of natural rubber by *Streptomyces* spp using dilute solution viscosity measurement technique was determined.

## Conclusion

Based on the experiment carried out, the intrinsic viscosity of the rubber solution is 0.58 g/dl in the absence of the organism (*Streptomyces*), while when subjected to 1st, 2nd and 3rd week in the organism (*Streptomyces*) the intrinsic viscosity for the rubber solution are 0.54 g/dl, 0.52 g/dl and 0.48 g/dl respectively. This then shows that the intrinsic viscosity decreases with increase in the period of exposure of the rubber solution as well as degradation since chain scission occurred and molecular weight was reduced. However, this result confirmed that biodegradation of vulcanized rubber material is possible despite that fact that the cross linkages of the poly(cis-1, 4-isoprene) chains makes it more difficult due to reduced water absorption and gas permeability of the material. Moreover, the results also proved that when degraded, natural rubber has a remarkable lost in weight as observed in the viscometric test conducted. Therefore, dilute solution viscosity measurement techniques should be employed because it provides information about the extent to which microbes degrades rubber as well as other material characterization parameters in the study of degradation of natural rubber by *Streptomyces* species. Thus in subsequent research, the effect of varying the concentration of the degradant on natural rubber should be investigated.

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