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Isolation of Natural Rubber Latex Degrading Bacterial Consortium from Rubber Plantation Area

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Abstract

The huge quantity of waste rubber materials can cause environmental problems that are the accumulation of recalcitrant organic compounds in the environment which need great concern, sufficiently long (persistence) that they have undesirable effects. Most commonly-used fibers and natural polymers are natural rubber, especially starches, gelatin, wood and cotton are inherently biodegradable under favourable conditions although the intensity of degradation decreases with the increasing molecular weights of the polymers. Natural rubber (NR) is both a photo synthetically-renewable resource and an environmentally degradable material. It is an unsaturated high molecular weight polymer, and in nature, is expected to degrade very slowly in comparison with other natural polymers. Various physical, chemical and biological methods have been proposed to solve this problem. NR-degrading bacteria are widely distributed in soil, water and sewage and interestingly, with some exceptions, belong to the actinomycetes. As a group, the bacteria and actinomycetes are characterized by an ability to digest complex materials and to use unusual molecules and are thus significant decomposers in soils. Present review focuses on the degradation of natural rubber by different microbes involved in degradation, analysis of degradation products and biodegradation pathway for technically important polymers. This review may provide an opportunity for further studies about the applications of biotechnological processes used as a tool for rubber waste management.

Keywords: Actinomycetes; Bacteria; Biodegradation; Natural rubber

Introduction

Latex is a naturally occurring polymer of isoprene often known as cis-1,4-polyisoprene with a molecular weight of 100,000 to 1,000,000. Some natural rubber sources like guayule are composed of trans-1,4-polyisoprene, a structural isomer that has similar, but not identical, properties.

Polyisoprene can also be created synthetically, producing what is called synthetic natural rubber, while the natural and synthetic routes are completely different. Natural Rubber Latex (NRL) is a specific type of rubber derived from milky sap of the *Hevea brasiliensis* tree that is refined and treated to form the raw liquid latex used to make gloves. It is also used to manufacture numerous diverse products namely tyres, clothing, medical products and toys. Currently the rubber is harvested mainly in the form of the latex from some trees. This natural rubber latex is a sticky, milky colloid drawn off by making incisions into the bark and collecting the fluid in specific vessels. This process is known as "tapping". The latex is then refined to rubber ready for commercial processing and a solution of ammonia can be used to prevent the coagulation of raw latex while it is being transported from its collection site [1].

Natural Rubber (NR) contains a minimum of 90% rubber hydrocarbon together with small amounts of proteins, resins, fatty acids, sugars, and minerals [2]. The main constituent of natural rubber is cis-polyisoprene. Rubber particles are found to be surrounded by a single membrane of phospholipid with hydrophobic tails pointed inward.

Once the rubber is vulcanized, it will be turned into a thermoset and most of the rubber in everyday use is vulcanized to a point where it shares properties of both; i.e., if it is heated and cooled, it is degraded but not destroyed. This natural rubber latex is also a natural, biodegradable product, containing no petroleum by-products or dioxins that enables disposal by either land fill or incineration without environmental damage. Organic impurities present in the rubber can support microbial growth as well [3,4]. There are several evidences that these natural rubbers that can be degraded by the number of fungi and bacteria present in the soil [5-11].

Since accumulation of rubber waste in the environment is becoming a worldwide problem [12], it is worth trying to develop a microbial remedy for the disposal of natural rubber waste [13]. The main constituent of natural rubber is a compound called cis, 1-4 polyisoprene which is relatively resistant to microbial decomposition when compared with many other natural polymers. At the same time, there were many reports published before implying the application of

microorganisms in the treatment of the effluents of natural rubber latex [14]. The extent, vigor and rate of microbial degradation are influenced by the factors like the rubber formulation, the microorganism present and their interaction with its surrounding environment. Rubber wastage and the disposal of waste tires have become two major challenges of rubber industry that leads to much environmental pollution. Thus, numerous remedies of physical, chemical and biological methods have been proposed to solve this problem.

Bacteria can use and degrade natural rubber latex as the sole source of carbon and energy were isolated from different ecosystems. About 42 rubber-degrading bacteria were isolated and all rubber-degrading isolates were identified as a member of Actinobacteria which is a Gram-positive bacteria characteristic of forming large group of mycelia. Interestingly no Gram-negative bacteria could be isolated. The growing problem of environmental pollution caused by synthetic plastics has led to the search for alternative materials like biodegradable and renewable plastics. Of the biopolymers presently under development, starch or natural rubber is one of the promising alternatives. This problem has extensively triggered research into finding and producing alternative synthetic materials and recent researches widely search for multipurpose, versatile, biodegradable plastics [15,16].

Materials and Methods

Collection of latex material

The liquid latex sample was collected from Kerala rubber plantation area (Kottayam). Ammonia solution was used to prevent the coagulation of raw latex while it is being transported from its collection site.

Sample collection

The soil sample was collected from Kerala rubber plantation area. They were collected from the rooting part of the rubber trees.

Media used

A mineral salts medium was used for the acclimatization of the bacterial consortium (Table 1) [17].

Table 1: Media composition.

Media composition	g/L
di-Potassium hydrogen phosphate (K_2HPO_4)	1
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.5
Potassium Nitrate (KNO_3)	1
Distilled water	1 L
pH	7

Acclimatization

Prepared 100 ml mineral salts medium each in two different conical flasks. Then to one of the conical flasks 1 ml of preserved latex solution was added and to the other conical flask 0.5 ml of the same latex solution with 0.10% of yeast extract as nitrogen source. Then they were sterilized in the autoclave. After sterilization once the media was cooled to each of them 5 grams of the collected soil sample was inoculated. Then it was allowed to grow or kept for incubation for 2 days.

After the incubation the microbial growth was observed under microscope. So, it was found that the growth of microbes in the medium that was inoculated with 1 ml of latex solution is higher. Then both the cultures containing 0.5 ml latex as well as 1 ml latex were pelletized at 10,000 rpm for 10 minutes. The supernatant was discarded, and the pellets were dissolved in a sterilized freshly prepared 100 ml of mineral salts medium with 0.10% yeast extract as a nitrogen source to enhance bacterial growth. Figure 1 shows the pelletized bacterial culture solution.

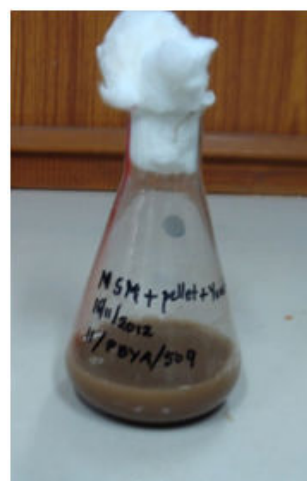


Figure 1: Pelletized bacterial culture solution.

Isolation of different types of bacterial isolates from the mixed consortium

Using the pelletized culture solution serial dilution was done was spread plated on sterile nutrient agar plates from the dilutions 10^{-5} , 10^{-6} and 10^{-7} . Then the different types of individual isolated bacterial colonies were taken based on their morphological characters and streaked on other freshly prepared nutrient agar slants in sterile screw cap tubes. These bacterial slants were preserved for further experiments.

Determination of the molecular weight of the NR-latex solution

For more accurate evaluation of the average Molecular Weight (MW) of the collected NR-latex solution a capillary Ubbelohde viscometer was used. The collected NR-latex solution was diluted with distilled water at different

concentrations like 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.10. Then according to the time taken by each of those samples to reach their specific end point in the viscometer (**Table 2**), the specific viscosity was calculated for each sample (**Table 3**). To find out specific viscosity the given equation was used, where t_0 - the time taken by water and t_s - time taken by the different diluted latex sample.

Table 2: Time taken by each sample.

Sample conc.	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1	Dist. water
Time taken (sec.)	110	100	90	80	78	75	83	87	90	92	72

Table 3: Specific viscosity of each sample.

Sample	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09	0.1
Specific viscosity	0.527	0.388	0.25	0.11	0.083	0.042	0.152	0.21	0.25	0.28

$$\text{Specific viscosity, } \eta_{sp} = \frac{t_s - t_0}{t_0}$$

From these specific viscosities of the individual samples a graph was plotted, and the intrinsic viscosity was calculated (**Figure 2**).

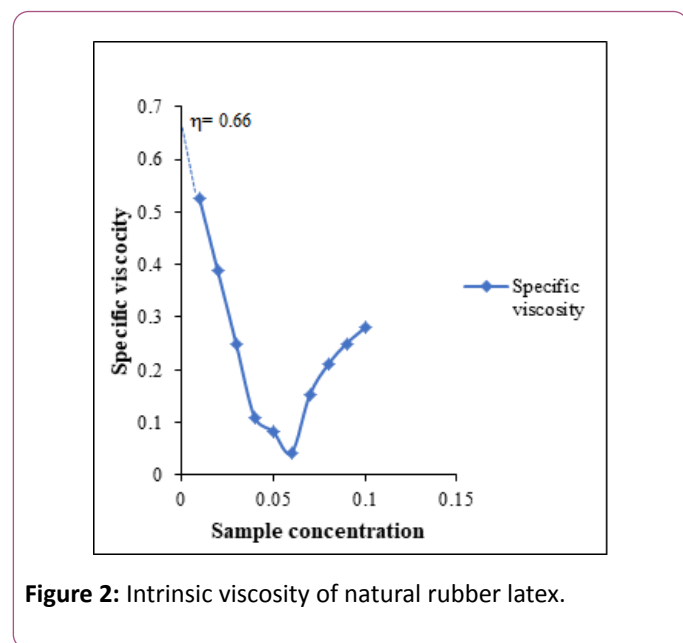


Figure 2: Intrinsic viscosity of natural rubber latex.

Using this intrinsic viscosity value, the molecular weight of the latex was calculated with help of an equation given below that includes Mark-Houwink constants K and α . According to Robert O Ebewele [18],

$$\text{Intrinsic viscosity, } \eta = KM\alpha$$

Determining the mineralization rate of NR-latex by the mixed microbial consortium

The percentage mineralization rate taken place at each concentration on each day was determined with the help of titration method and growth OD. Biodegradation of the poly

(cis-1,4-isoprene) and poly (trans-1,4-isoprene) hydrocarbon chains to carbon dioxide can be obtained by determining the respiratory CO_2 released during cultivation of cells in presence of polyisoprene as sole carbon source [19]. Determination was carried out in tightly closed glass bottles and boiling tubes by using the property of $\text{Ba}(\text{OH})_2$ to precipitate CO_2 as BaCO_3 . The bottles, containing 50 ml of mineral salts medium, 0.10%-0.50% of milled poly (cis- 1,4-isoprene) or poly (trans-1,4-isoprene), and the boiling tubes containing 15 ml of 0.2 M $\text{Ba}(\text{OH})_2$ solution inoculated with 1 ml of the pelletized culture grown for 48 h. Consumption of HCl for precipitation of CO_3^{2-} as BaCO_3 was determined for each period by titrating with 0.1 M HCl according to the following equation, yielding the percentage mineralization.

Mineralization (% CO_2)

$$= \frac{\text{Required amount of HCl (ml)} \times 0.1}{\text{C content of amount of poly (1,4-isoprene) applied (mmol)} \times 2}$$

As indicator, phenolphthalein was added, and the end point of titration was determined by alteration of the colour from magenta to uncoloured. The growth OD value for each of the concentrations on each day was also taken. This growth OD value was measured with the help of UV-spectroscopy. It was done by diluting each sample with distilled water at 1:10 ratio.

Determining the % mineralization rate at different concentration

In sterilized boiling tubes 15 ml of 0.2 M $\text{Ba}(\text{OH})_2$ was poured. Then sterilized bottles were taken and freshly prepared mineral salts medium of 50 ml was added to each of them. To each of the 5 set of bottles containing mineral salts medium add 0.05 μl , 0.1 μl , 0.15 μl , 0.2 μl and 0.25 μl of NR-latex solution indicating 0.10%, 0.20%, 0.30%, 0.40% and 0.50% respectively. These bottles containing medium along with latex was sterilized. Followed by which 1 ml of the pelletized culture solution was added to each of those bottles. These bottles were connected to each boiling tube containing the $\text{Ba}(\text{OH})_2$ solution with sterilized plastic tubes and they were properly

sealed. Then these boiling tubes containing $\text{Ba}(\text{OH})_2$ solution was also properly sealed. Then they were kept for overnight incubation. Since for each concentration there were 5 culture bottles, each culture was assorted for determining each day's mineralization rate from day 1 to day 5 continuously. After incubation their mineralization rate was determined with the help of titration method. Thus, it was possible to find out at which concentration maximum mineralization takes place (Figure 3).



Figure 3: Experimental setup for mineralization of latex.

Determining the best carbon and nitrogen (C and N) source for the bacterial consortium

To the sterilized glass bottles, 0.10% of latex was added along with 50 ml mineral salts medium in each bottle. To each of those bottles 500 mg of C and N sources like glucose, lactose, sucrose, (C-sources) beef extract, yeast extract, ammonium sulphate and ammonium nitrate (N-source) were added respectively. These bottles were sterilized and 1 ml of pelletized bacterial culture was added to each of the bottles. They were connected to the sterilized boiling tubes containing 15 ml of 0.2 M $\text{Ba}(\text{OH})_2$ solution with sterile plastic tubes and were properly sealed. Then it was kept for overnight incubation. After the 24 h of incubation the % mineralization rate for each of the C and N source were calculated by the same titration method. Then the growth OD of each of those cultures was also measured with the help of a UV-spectroscopy. Thus, it was possible to determine which carbon and nitrogen source helps in the maximum mineralization.

Determining the % of mineralization rate of NR by individual bacterial isolates

About 50 ml of Mineral Salts Medium (MSM) was poured to sterilized bottles. The 0.10% of the NR-latex solution was added to each those bottles and autoclaved. Then 1ml of the individual bacterial isolates was inoculated to each of the 5 set of bottles. Each bottle was connected to boiling tubes containing 15 ml $\text{Ba}(\text{OH})_2$ solution and kept for overnight incubation. The mineralization rate by each of those bacteria was determined by the titration method. Growth OD value was also measured on each day with the help of the UV spectroscopy. Thus, by titration method it was possible to

determine which bacterial culture gives the maximum mineralization or degradation of the latex compound.

Biochemical characterization for morphological identification of bacterial isolates

All the isolated bacterial strains were studied through initial tests like motility test and gram staining. The morphological and biochemical characterization of the bacterial isolates were done with the help of tests like IMViC test, oxidase test, carbohydrate fermentation test (glucose, sucrose, raffinose), triple sugar iron agar test, catalase test, lipid hydrolysis, phenylalanine deaminase test and urease test. Antibiotic sensitivity of bacterial species was tested by Kirby Bauer's procedure to find out the resistant pattern to various antibiotics like ampicillin, ciprofloxacin, tetracycline, erythromycin, nalidixic acid and norfloxacin. The sensitivity and resistance of each of the bacterial strains were determined.

Results

Screening and isolation of bacterial consortium from soil sample

In the mineral salts medium, which was inoculated with 5 g of the collected soil sample and the liquid NR-latex, microbial growth was observed when visualized under light microscope. This bacterial consortium was pelletized and after plating it on nutrient agar individual bacterial isolates were obtained as Figure 4 shows and they were preserved on nutrient agar slants.

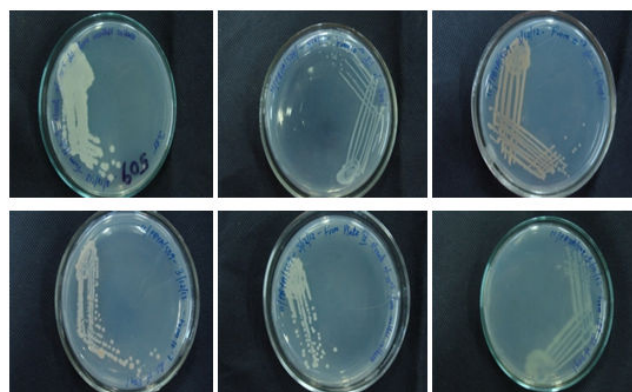


Figure 4: Growth of individual bacterial strains on nutrient agar.

Average molecular weight of latex solution

The latex solution was diluted with distilled water at different concentrations and their specific viscosity was calculated. With the calculated specific viscosity of each sample a graph was plotted. From that graph, intrinsic viscosity was found out by extending the straight line to the y-axis in the graph. Thus, the intrinsic viscosity (η) was equal to 0.66.

By substituting the value of intrinsic viscosity in the equation given below, the average molecular weight of the liquid latex was found.

Intrinsic viscosity, $\eta = KM\alpha$

Thus, the average molecular weight of the natural rubber latex was 45,184.08.

Mineralization of different concentration of latex by the bacterial consortium

Based on the mineralization percentage and growth of bacterial consortium a graph was plotted (Figure 5). In the present study, effect on latex solution at different concentrations (0.10%, 0.20%, 0.30%, 0.40%, and 0.50%) by the bacterial consortium was examined. From the graph, it was found that maximum mineralization of latex happens at 0.10% concentration. The maximum mineralization took place on day 5 in the culture that contained 0.10% concentration of latex giving maximum value of 5.98×10^{-8} . The maximum growth of the bacterial consortium was seen on the third day in 0.10% concentration of latex culture solution and the lowest growth was seen in the 0.40% concentration on fourth day giving values of about 0.997 and 0.050 respectively. While the growths of bacterial culture at other latex concentrations like 0.20%, 0.30%, 0.50% was giving different growth values. From the graph, on day 3 the growth of bacterial consortium was maximum. When the growth OD graph was compared with the mineralization graph it was found that the mineralization rate has a sudden increase in all the culture solution having different concentration of latex solution.

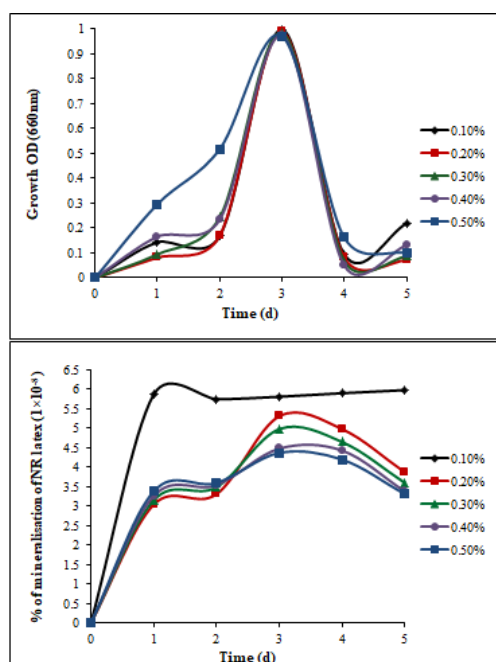


Figure 5: Mineralization of different concentration of latex by the bacterial consortium.

Likewise, the carbon and nitrogen source which can enhance the mineralization rate was thus determined by the same method.

Mineralization of 0.10% latex with different nitrogen sources

Different nitrogen sources were added to the mineral salts medium for the enhancement of bacterial growth. From the experiment it was found that the maximum mineralization was taken place on day 5 in the culture solution that was treated with yeast extract giving a value 6.14×10^{-8} . While the growth OD value was giving maximum value in the culture solution treated with beef extract on day 3 giving a value about 0.297. These growth OD values were observed with the help of UV-spectroscopy at 660 nm. Figure 6 shows that each of the cultures having different nitrogen sources like beef extract, yeast extract, ammonium sulphate and ammonium nitrate as their nitrogen source have an initial bacterial growth. Their growth later decreased but gradually starts increasing again. The culture having beef extract showed its maximum growth on day 3. Yeast extract also showed increased growth of the bacterial consortium of about 0.288 on fifth day. Ammonium nitrate showed the lowest growth of bacterial consortium on day 5 giving a value about 0.092. In the same way the carbon source which can enhance the mineralization was also determined.

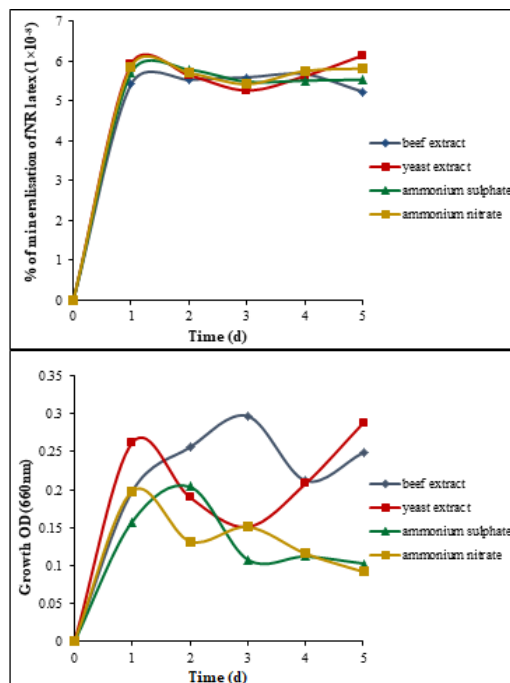


Figure 6: Mineralization of 0.10% latex with different nitrogen sources by the bacterial consortium.

Mineralization of 0.10% latex with different carbon sources

The mineralization of latex and the growth of the bacterial consortium by the different carbon sources were determined with the help of a graph. Based on the **Figure 7**, it was found that maximum mineralization was taken place on day 4 that was treated with sucrose giving a value 5.89×10^{-8} . The maximum growth of bacterial consortium was seen on day 1 giving a value 0.315 that was treated with glucose as the carbon source. In case of the growth of bacterial consortium by the different carbon sources it was found that sucrose had the maximum growth followed by lactose and then glucose. Their growth had a sudden increase initially but gradually it was decreasing.

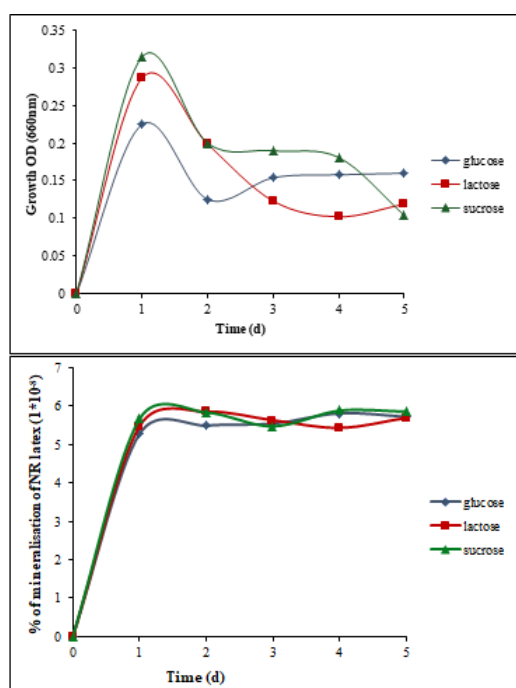


Figure 7: Mineralization of 0.10% latex with different carbon sources by the bacterial consortium.

Mineralization of natural rubber by individual bacterial strains

Based on the **Figure 8**, it was observed that the bacterial isolate that was named J-3 was able to do maximum mineralization. J-3 has done its maximum mineralization on day 5 giving a value of about 6.31×10^{-8} . In the initial stage all the bacterial isolates showed a sudden increase in mineralization of latex. But there was a gradual decrease followed by an increase again on day 5. Likewise, the growth OD value of each individual isolates on each day was also measured. The growth was highest in the bacterial isolate that was named J-4 on day 5. In case of J-1, J-3 and J-5, the growth was increasing day by day in a slow rate. But in case of J-2, J-4

and J-6 there was an increase in their growth initially followed by a decrease and a slight increase later.

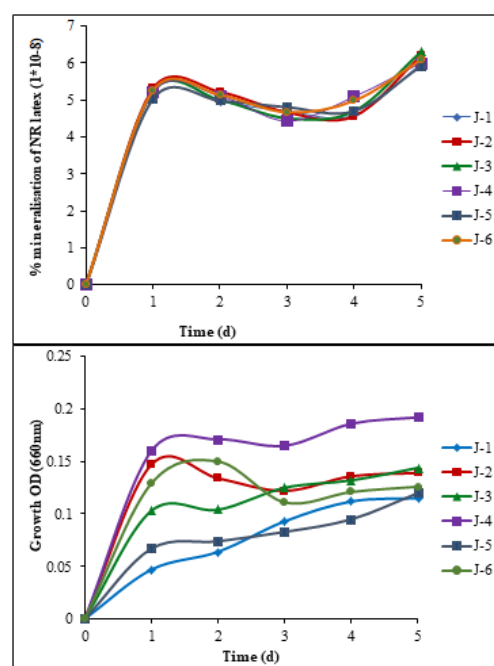


Figure 8: Mineralization of natural rubber by individual bacterial strains.

Characterization of the bacterial strains present in the consortium

The bacterial isolates were characterized according to their morphological characteristics. Further characterization of these individual bacterial isolates was done by carrying out gram staining, motility test and different biochemical tests like IMViC test, oxidase test, carbohydrate fermentation test (glucose, sucrose, raffinose), Triple Sugar Iron agar test (TSI), catalase test, phenylalanine deaminase test and urease test. The antibiotic sensitivity test was performed as well for different antibiotics like ampicillin, ciprofloxacin, tetracycline, erythromycin, nalidixic acid and norfloxacin.

In the **Table 4** it is given that by gram staining it was found that only J-6 was gram positive rods and all other isolates were gram negative rods. By hanging drop method, it was found that all the six isolated bacteria were motile. In case of biochemical test, it was found that in indole test except J-5 all other bacterial isolates were positive. In methyl-red test except J-2 and J-5 all others were positive while in Voges-Proskauer test all the isolates gave negative results. In citrate utilization test it was found that only J-6 can utilize citrate giving a positive result. In oxidase test all the bacterial isolates gave positive results. In case of carbohydrate fermentation test, only J-6 gave negative results for sucrose and raffinose and J-4 gave negative results for glucose. For catalase test all the isolates responded positively. In phenylalanine deaminase test every isolate gave negative results while in urease test all

the isolates responded positively. In case of TSI agar test, every isolate gave acid butt and alkaline slant but only J-6 had gas production as well.

Table 4: Biochemical Tests for the six bacterial strains.

Test	J-1	J-2	J-3	J-4	J-5	J-6
Gram staining	Gram negative rods	Gram negative rods	Gram negative rods	Gram negative rods	Gram negative rods	Gram positive rods
Motility test	+	+	+	+	+	+
Indole test	+	+	+	+	-	+
Methyl-red test	+	-	-	+	-	+
Voges-proskauer test	-	-	-	-	-	-
Citrate Utilisation test	-	-	-	-	-	+
Oxidase test	+	+	+	+	+	+
Carbohydrate fermentation test	G ⁺ (+)	G (+)	G (+)	G (-)	G (+)	G (+)
	S ⁺ (+)	S (+)	S (+)	S (+)	S (+)	S (-)
	R ⁺ (+)	R (+)	R (+)	R (+)	R (+)	R (-)
Triple Sugar Iron agar test	acid butt and alkaline slant	acid butt and alkaline slant	acid butt and alkaline slant	acid butt and acid slant	acid butt and alkaline slant	acid butt and alkaline slant with gas production
Catalase test	+	+	+	+	+	+
Phenylalanine deaminase test	-	-	-	-	-	-
Urease test	+	+	+	+	+	+
In carbohydrate fermentation test: G ⁺ , S ⁺ and R ⁺ refer to glucose, sucrose and raffinose respectively						

With the help of Bergey's Manual of Systematic Bacteriology, genus level identification of the six bacterial isolates was accomplished. Thus, it was found that J-1, J-2, J-3, J-5 can be assumed to be an *Aeromonas sp.*, and J-4 found to be *Pseudomonas sp.* while J-6 can be assumed as *Bacillus sp.* Among the six different isolates, four of them (J-1, J-2, J-3, J-5) were assumed to be *Aeromonas sp.*, (J-4) was assumed to be *Pseudomonas sp.* and (J-6) was assumed to be *Bacillus sp.*

Antibiotic sensitivity test

Table 5: Antibiotic sensitivity test.

Antibiotic disc used	J-1	J-2	J-3	J-4	J-5	J-6
Ampicillin	Sensitive	Sensitive	Sensitive	Resistant	Sensitive	Resistant
Ciprofloxacin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Tetracycline	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Erythromycin	Sensitive	Sensitive	Resistant	Resistant	Sensitive	Resistant
Nalidixic acid	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Norfloracin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive

In the **Table 5** it was given that in antibiotic sensitivity test, ciprofloxacin, tetracycline, nalidixic acid and norfloracin are sensitive to all the individual bacterial strains.

Ampicillin was resistant to the bacterial strain named J-4 while sensitive to all other bacterial strains. Erythromycin was resistant to J-3 and J-4 but sensitive to the other bacterial strains.

Discussion

Rubber compounds are important group of contaminants, for which efficient treatment methods are required. Biodegradation constitutes the primary mechanism for contaminant removal. However, researches were conducted to isolate polyisoprene rubber (latex gloves) degrading microorganisms from soil. Twelve bacterial strains were isolated through enrichment technique and were screened for their capability to degrade rubber. Best growth was observed with *Bacillus sp.* S10 [20]. Likewise, in the present study, the natural rubber (latex solution) degrading microbes were isolated from soil. Six bacterial strains were isolated through enrichment technique and were screened for their capacity to degrade rubber in the liquid state. The culture assumed as *Aeromonas sp.* i.e. J-3 gave the highest mineralization % of natural rubber latex giving value about 6.31×10^{-8} within 5 days.

Warneke et al. [19] isolated axenic cultures capable of degrading poly (trans-1,4-isoprene) or Gutta Percha (GP) of which six bacterial strains were capable of utilizing synthetic poly (trans-1,4-isoprene) as sole carbon and energy source for their growth. In cultures with GP grains of 120 μm -250 μm , 40% of the substrate was metabolized to CO_2 within 78 days, while only 25% of the poly (trans-1,4-isoprene) granules of 1250 μm -1400 μm was mineralized in the same period by *N. takedensis* WE30. But in the present study as a carbon source natural rubber latex solution was used instead of solid latex material for the easy and faster mineralization by the bacterial strains. The bacterial consortium isolated in the present study was able to mineralize the latex up to 0.50%, in which optimum degradation was obtained in 0.10% within 5 days of incubation.

The microbial susceptibility of NR, both in the raw or in the vulcanized state, was sufficiently examined and reviewed [5,7,12,21,22]. In another study, rubber-degrading bacterial isolate was enriched from soil sample and according to biochemical and molecular characterization analysis; it was identified as *Achromobacter sp.* NRB. The obtained strain was capable of degrading and mineralizing raw natural rubber granules as well as some forms of rubber tire and was able to use it as their sole source for carbon. But in contrast to the growth in common media where carbon source is dissolved, the growth of microorganisms on natural rubber products is very slow and the effect of culture conditions on microbial growth on an insoluble solid substrate like rubber has not been well characterized [23]. In the present study, rubber-degrading bacterial consortium was enriched by different carbon and nitrogen sources like beef extract, yeast extract, ammonium sulphate, ammonium nitrate (nitrogen source), glucose, lactose and sucrose (carbon source). From the experiments it was found that beef extract served as a better nitrogen source that enhances the mineralization of latex at its maximum by the bacterial consortium. Likewise, sucrose was found to be the best carbon source that could enhance the mineralization percentage of natural rubber.

Furthermore, Na-succinate positively affected the rate of rubber degradation. Tsuchii et al. [24] reported a significant increase of rubber degradation in presence of other carbon and nitrogen sources. The presence of NH_4Cl and K_2HPO_4 were essential in degradation of rubber substrate where the first was used as N-source and the second serves as a buffer and phosphate source. In the present study, mineral salts medium used in for mineralization of natural rubber contained yeast extract as N-source which increase the bacterial growth and K_2HPO_4 used as a phosphate source helped the bacterial consortium to utilize natural rubber within 5 days.

Berekaa et al. [23] also found out that staining with Schiff's reagent indicated that the colonization efficiency was influenced with the size of rubber substrate. The colonization efficiency was increased when rubber substrate was applied in small pieces (diameter 1 cm^2) while; lower colonization efficiency was recorded in case of large size substrate (diameter 1 cm^2). A similar finding was done by Tsuchii and co-

workers [24] during the growth of *Norcardia sp.* 835A on latex gloves pieces. Thus, it was clear that the degradation of rubber was positively affected by the amount of NR granules. It was well known that increase in amount of rubber granules leads to increase in surface area of rubber substrate and hence to higher mineralization rate. While in the present study the bacterial consortium was allowed to react with liquid latex at different concentration and it was found that at the lowest concentration of latex the bacterial consortium was able to give the maximum mineralization. Purpose of the present study was to show the growth of bacterial consortium that varies from 0.10% to 0.50% of natural rubber. By performing gene manipulation and by increasing the days of incubation natural rubber present in the environment could be further mineralized by these economic friendly technologies.

Conclusion

The process of degradation of natural rubber compounds by means of microorganism is an eco-friendly manner of controlling pollution in the environment. These microbes can be obtained from anywhere like soil as well as water. Many different types of microorganisms are involved in the biodegradation of NR which promotes different methods of degradation. But it is found that it's difficult to degrade the NR products at usual environment thus further investigations are required in order to enhance the degradation rate even in usual environmental conditions. In this study it was found that soil bacteria are capable of natural rubber degradation. Even an individual bacterium can degrade natural rubber and the bacterial growth can be enhanced by providing other carbon and nitrogen sources. Thus, it is believable and gives hope that artificial rubber materials can also be degraded by the soil bacteria itself and the degradation rate can be elevated with a little incorporation of such carbon and nitrogen sources.

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