# Novel Bio-Degradable Lignin Reinforced NBR Composites

K. AGARWAL[*1], M. PRASAD[1], R. B. SHARMA[2] & D. K. SETUA[1]

[1] Defence Materials and Stores Research and Development Establishment
DMSRDE (Post Office), G. T. Road, Kanpur-208013, India

[2] Defence Institute of Advanced Technology, Girinagar, Pune 411025, India

***ABSTRACT*-Lignin is evolved from wood having feature of good biodegradation properties. The samples were exposed to standard culture media for fixed intervals of time and evaluated for changes in their physical properties, thermal stability and morphology. Rubber vulcanizates were analyzed for physico-mechanical properties and thermal stability, and compared with fillers like phenolics resin and carbon black and lignin. Fillers are used to reinforce polymers to improve properties of matrices for biological activity. Lignin reinforced rubber biocomposite has been found to produce superior elongation properties compared to phenolic resin but inferior to carbon black. The development of commercially viable "green products" based on natural resources for both matrices and reinforcements for a wide range of applications is on the rise. This effort includes new pathways to produce natural polymers with better mechanical properties and thermal stability using nanotechnology and use of natural polymers to make biodegradable plastics and their composites with lignin. The present study was initiated to isolate and characterize a number of NBR-degrading bacteria from various ecosystems in India. It also suggests that rubber-degrading bacteria might be useful for the disposal of discarded rubber products (waste management). This paper presents the effect of different bacteria through in vitro antimicrobial activity in NBR, NBR with phenolic resin, NBR with carbon black and Lignin reinforced Rubber composites. These NBR composites were tested against five pathogenic bacteria Bacillus subtilis, Escherichia coli, Streptococcus mutans, Staphylococcus aureus and Pseudomonas aeruginosa isolated from clinical samples mainly for SEM studies.**

***Keywords: Bio-Degradable; Lignin; NBR; SEM***

- To whom correspondences to be addressed

Corresponding Author Phone (O): +91 512 2402360, +91 512 2451759-78
Ext. 467
Fax: +91 512 2450404
e-mail: kavita.agarwal2005@gmail.com

## I. INTRODUCTION

Disposal of waste rubber is a worldwide problem. Research efforts on the development of biodegradable polymers by chemical degradation route have been provoked by the action of microorganisms such as bacteria, fungi and algae [1]. The manifestation of biodegradable polymers lies in their conversion into a degradable polymer by primary degradation mechanism through the action of metabolism by microorganisms. Natural polymers (e.g., proteins, polysaccharides, nucleic acids, etc.) are degraded in biological systems by oxidation and hydrolysis [2]. In case of synthetic polymers, the microbial utilization of their carbon backbone as a carbon source is, however, essential [3]. Biodegradable materials have proven capability to decompose through natural biological processes in the most common environment, within a year into non-toxic carbonaceous soil, water or carbon dioxide [4]. The chemical structure responsible for functional group stability, reactivity, hydrophylicity and swelling behaviour are important factors affecting biodegradability of polymeric materials. Other important factors are inter alia, physical and physico-mechanical properties, such as molecular weight, porosity, elasticity and morphology (crystalline, amorphous) [5,6].

Lignin is an abundant renewable resource material next to cellulose and is generated in huge quantity in the papermaking and cellulosic ethanol industries. It is anticipated that upcoming ethanol bio-refineries and producers of bio-diesel will generate large quantity of lignin with better chemical properties than paper pulp-lignin. In general, lignin is a light-weight (half the density of talc or calcium carbonate), stiff and brittle bio-polymer but rarely used as a bio-filler in plastic compounds. About 2% of the lignin obtained from paper or bio-refineries is used in value added applications such as isolation of chemicals, as phenol replacement in phenol-formaldehyde formulations, applications in adhesive and asphalts, lignin based polyurethane and other polymer like polyethylene oxide (PEO), polypropylene(PP), polyvinyl alcohol (PVA), polyethylene chloride (PVC) and their blends [7]. However, very limited studies have been conducted on the application of lignin in rubber blends. Use of lignin in natural rubber blends has been reported to decrease both the scorch time as well as optimum cure torque of the blends with increasing

concentration of lignin between 0 to 40 phr [8]. Setua et al. [9] reported a detailed study of lignin reinforced nitrile rubber (NBR) composites. Varied proportions of lignin up to 50 phr plus hexamine have been incorporated in medium acrylonitrile (37%) containing NBR to study their processing characteristics and physico-mechanical properties, such as reinforcement, oil and fuel resistances, ageing and thermal stability, etc. Efficacy of lignin has been found to be superior to phenolic resin-hexamine or carbon black filled compounds.

Structural feature of lignin has β-O-4 linkage (Fig. 1) between the phenylpropane units as one of the most dominant linkages. The various functional groups on these units could give lignin a unique but very complex structure with profound impact on its reactivity [10, 11]. Lignin mostly contains methoxyl, phenolic hydroxyl, and few terminal aldehyde groups, but only a small proportion of the phenolic hydroxyl groups are free since most of them are occupied in linkages to neighboring phenylpropane units. Carbonyl and alcoholic hydroxyl groups are incorporated into lignin during enzymatic dehydrogenation [11, 12].

The strategy of bacteria to degrade synthetic polymers is to first adhere to polymer surface and then to secrete enzymes that can initiate degradation by reducing the polymer chain lengths and eventually produce molecules (precursors for its bio synthetic reactions) so that they can grow. *In vitro* antibacterial activity on rubber composites containing different filler has been reported [13-15]. *Streptococcus mutans* is a facultative anaerobic, gram-positive coccus-shaped bacterium commonly found in human oral cavity. *S. mutans* is known to be a major causative organism for plaque. Green tea extract, a customary drink after every meal in Japan, is known to contain several polyphenols that inhibit the growth of *S. mutans*. *Bacillus subtilis* is a ubiquitous naturally occurring saprophytic bacterium that is commonly recovered from soil, water, air and decomposing plant material. *Pseudomonas aeruginosa* is also a common bacterium found in soil, water, skin flora, and most man-made environments throughout the world.

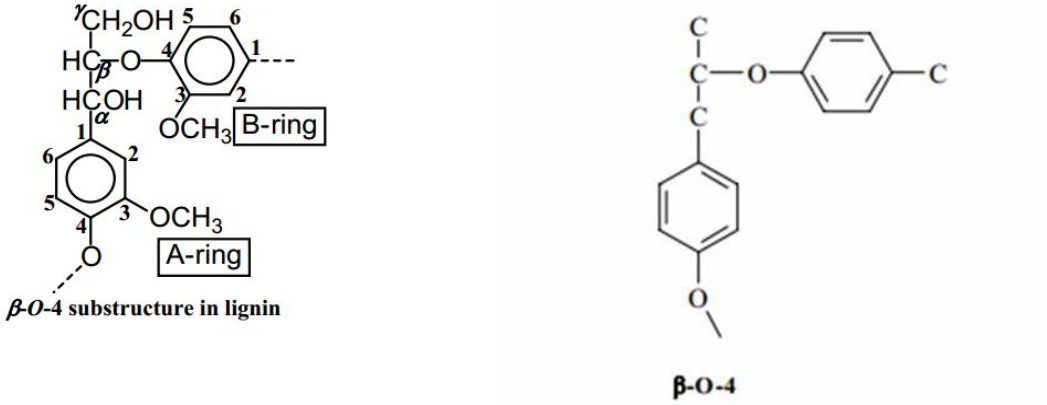


Fig. 1A β-O-4 linkage of lignin.

It thrives not only in normal atmospheres, but also in hypoxic atmospheres. It uses a wide range of organic materials for food; in animals, the versatility enables the organism to infect damaged tissues or those with reduced immunity. *Escherichia coli* is facultatively anaerobic with a type of metabolism that is both fermentative and respiratory. They are either nonmotile or motile by peritrichous flagella. Most *E. coli* strains are harmless and these harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin $K_2$ [16] and by preventing the establishment of pathogenic bacteria within the intestine [17,18]. *Staphylococcus aureus,* a bacterium of the genus *Staphylococcus,* is gram-positive facultative anaerobic cocci that are microscopically observed as individual organisms, in pairs, and in irregular grape like clusters. The ability to clot plasma continues to be the most widely used and generally accepted criterion for the identification of *S. aureus.*

It is therefore apparent that there is still a need to develop a low-cost biodegradable material from polymer and lignin. The present investigation aimed towards generating a low-cost biopolymeric material comprised of oil/fuel-resistant nitrile rubber (NBR), which is primarily used in a variety of components as seals, gaskets, diaphragms, etc. in Defence and Aerospace equipment. The overall goal of this work is to examine the mechanism of bio-degradability of lignin filled NBR and the effect of addition of lignin on mechanical and thermo-degradation properties of these rubber composites. Comparison was also made to see efficacy of lignin vis-à-vis conventional carbon black filler and phenolic resin used in NBR as adhesion promoter as well as a crosslinking agent.

## II. EXPERIMENTAL DETAILS

### *A. Materials*

NBR (grade Perbunan 3307) obtained from Bayer A.G., Germany. Kraft Lignin was obtained from Pulp and Paper industries of Kanpur. Rubber reinforcing phenolic resin (grade PR 202) was obtained from M/S Hardcastle Wand Ltd., Mumbai. SRF carbon black (grade N 765) was obtained from Philips Carbon black Ltd., Durgapur. Hexamethylene tetramine was obtained from BDH, Germany. Commercial grades of MBTS, TMT and sulphur were obtained from M/S Aliga Rubber Works, Kanpur.

### *B. Procedure*

The formulations of the mixes are given in Table 1. Mixing was carried out in a conventional laboratory open mill (150 mm x 330 mm) at 30-40$^{o}$C according to ASTM method D 15-70. Different ingredients were added as per the sequence mentioned in Table 1. The mixes were vulcanized for 30 min at 150$^{o}$C and under the pressure of 4.5 MPa in a hydraulic press having electrically heated platens. Tensile testing was done as per ASTM method D 412-51T using dumb-bell specimens.

TABLE 1 FORMULATION OF MIXES

| **Mix nos. / content of mix (parts by weight)** | | | | |
|---|---|---|---|---|
| **Ingredient** | A | B | C | D |
| **Nitrile Rubber** | 100 | 100 | 100 | 100 |
| **Zinc Oxide** | 15 | 15 | 15 | 15 |
| **Stearic acid** | 1.5 | 1.5 | 1.5 | 1.5 |
| **Lignin** | - | - | - | 50 |
| **Phenolic Resin** | - | 50 | - | - |
| **Carbon Black** | - | - | 50 | - |
| **Hexamine** | - | 5 | - | 5 |
| **Sulfur** | 0.3 | 0.3 | 0.3 | 0.3 |
| **MBTS** | 1 | 1 | 1 | 1 |
| **TMT** | 3 | 3 | 3 | 3 |

Tear strength of the vulcanizates was determined using un-notched 90$^{o}$ angled tear test piece (die C) as per ASTM method D 624-48. TGA module 2950 with TA controller 3100 was used for thermo-gravimetric analysis of the vulcanizates at a heating rate of 20$^{o}$C per min in the thermal range between RT to 700 $^{o}$C under constant nitrogen gas flow rate of 60 ml per min. The fracture surfaces of the tensile test specimens and the culture test specimens were carefully cut without touching the surface. The surfaces were subsequently sputter coated with gold and studied under a Carl Zeiss EVO50 LVSEM for failure mechanism. It has already been reported earlier by Setua et al. (19-22) that the failed surfaces under various modes of physical testing, wherever available, offer a good area of research on the studies of failure mechanism for rubbers and rubber based composites. Dissipation of stress during failure generates some permanent features on the fracture surface that persist even after the failure and help to correlate the topography with observed physico-mechanical properties.

### *C. Antimicrobial screening*

The antimicrobial activity of the Lignin reinforced Rubber composites and other NBR composites were tested against five pathogenic bacteria, *B. subtilis, E. coli, S. mutans, S. aureus* and *P. aeruginosa* isolated from clinical samples.

### *D. Preparation of inoculum*

Stock cultures were maintained at 4 °C on slopes of nutrient agar. Fresh plates were raised from stock cultures by transferring a loopful of cells from the stock cultures to new agar plates and incubated at 37°C. Active cultures for experiments were prepared by transferring a loopful of cells from the fresh plates to test tubes of Luria Bertani (LB) broth and were incubated with agitation for 18 hrs at 37 °C. The cultures were diluted with fresh LB broth to achieve optical densities corresponding to $2.0x10^{6}$ colony forming units (CFU/ml).

### *E. Antimicrobial susceptibility test*

*In vitro* antimicrobial activity was screened by using LB agar media obtained from M/S Himedia Co. Ltd., (Mumbai). The LB plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 5 min and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. A, B, C and D loaded disks (≈6mm) were placed on the surface of medium and the compound was allowed to diffuse and the plates were kept for incubation at 37 °C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter [23-26]. In the same process, lignin filled rubber composites also possessed *in vitro* biological activity with several pathogens.

### *F. Effect of inoculums size*

The effect of changes to the rate of degradation of the polymer composites was identified using five different pathogenic bacteria, *B. subtilis, E. coli, S. mutans, S. aureus* and *P. aeruginosa* isolated from clinical samples. The strategy of bacteria to degrade polymers is to first adhere to polymer surface, then secrete enzymes that can initiate degradation by reducing the polymer chain lengths and eventually produce molecules that can be assimilated and converted by their metabolic process to provide energy and precursors for their bio-synthetic reactions so that they can grow. *In vitro* antibacterial activity on rubber composites embedded different filler loaded disks were placed on the surface of the medium and the compound was allowed to diffuse and the plates were kept for incubation at 37 $^0$C for 24 hrs. In this process filler reinforced rubber composites also possessed *in vitro* biological activity with several pathogens and showed very good antibacterial results.

## III. RESULTS AND DISCUSSION

The physico-mechanical properties can be explained on the basis of the mechanisms of different filler actions in NBR composites as enumerated in Table 2. The solution theory can explain the mechanical properties of the phenolic resin and hexamine composition (mix B). Phenolic Resin is a semisolid in the ground state, but when added to NBR it behaves like a solute. When NBR absorbs resin, it lowers its melting point (i.e. 40-50°C) to below room temperature. The resin filled composition, therefore, should be more flexible in contrary to the list of physical properties shown in Table. 2. In this case, the excess resin at 50 phr likely to exceed the solubility limit of NBR, lower flexibility as separate phase to and thereby, of the matrix. Further, the moisture in air released ammonia and HCHO from hexamine, which, in turn, underwent crosslinking of the resin, resulting in its intermolecular cross-linking. This is responsible for higher tensile strength, tear strength, hardness and modulus values as compared to mix A. It also resulted in drop in elongation at break. As expected, the carbon black reinforced composite (mix C) is superior to others, and all observation is agreeable with the chain slippage mechanism of strengthening or bound rubber theories.

Physico-mechanical properties can also explain the mechanism of lignin action in NBR vulcanizates as enumerated in Table 2. Addition of lignin increases the structural complexity due to inefficient employment of sulfur in cross-link formation. Extra sulfur, however, causes main chain modification as pendent groups terminated with accelerator residue and higher zinc sulfide formation. Presence of lignin (mix D), therefore, causes improper vulcanization, and generates lower values of tensile strength and higher elongation at break compared to pure NBR. Improvement in hardness and modulus values are primarily due to reduction in volume fraction of rubber for the 50 phr filled lignin composition rather than any reinforcement.

Thermo Mechanical analysis (TMA) is an ideal thermal technique for estimation of the dimensional stability of sample over a temperature range or continuous maximum working temperature of polymer for functional properties. The failure of a sample can also be assigned to sample or component when it can no longer fulfill the functional requirement expected out of it. Fig. 1B shows the dimensional change vs. temperature for the control as well as that of the exposed samples. It can be seen from the figure that dimensional change is reduced or dimensional stability of the bio-exposed sample is increased in the range between general service temperatures up to 175 $^0$C. This may be attributed to the action of bio-agents and change in microstructure of sample during bio-exposure. The zig-zag pattern of change in slope of dimensional change vs. temperature plot of control sample and the extent to which it is reduced for the exposed sample in comparison in the temperature range of RT to 175$^0$C indicate strong action of microbes during bio-exposure of the sample. However, dimensional change rate of the exposed sample is increased beyond the temperature 175 $^0$C. This may adversely affect the change in properties of bio-exposed for maximum service temperature in actual application.

Addition of lignin as such in NBR (mix D) impairs the tensile strength and likewise the elongation at break compared to unfilled vulcanizate (mix A), implying a non-reinforcing nature of this filler (Table 2). Addition of lignin increases the structural complexity due to inefficient utilization of sulfur in cross-link formation. Extra sulfur, however, causes main chain modification as pendent groups terminated with accelerator residue and higher zinc sulfide formation. Presence of lignin, therefore, causes improper vulcanization and also generates lower values of tensile strength.

TABLE 2 PHYSICAL PROPERTIES OF THE COMPOSITES

| | **Composites*** | | | |
|---|---|---|---|---|
| **Property** | **A** | **B** | **C** | **D** |
| **Tensile strength, Kg/cm$^2$** | 23.27 | 50.0 | 151.2 | 14.8 |
| **Modulus at 100% elongation, Kg/cm$^2$** | 0.92 | 51.0 | 18.5 | 10.9 |
| **Elongation at break, %** | 290 | 127 | 150 | 175 |
| **Tear strength, Kg/cm** | 11.97 | 40.3 | 56.4 | 12.1 |
| **Hardness, Shore A** | 40 | 85 | 55 | 55 |

Composites (A) NBR, (B) phenolic resin lignin reinforced NBR, (C) carbon black (D) lignin reinforced NBR, (d) modified lignin reinforced NBR

**Parentheses in column E indicate values after soil burial test for 200 days.

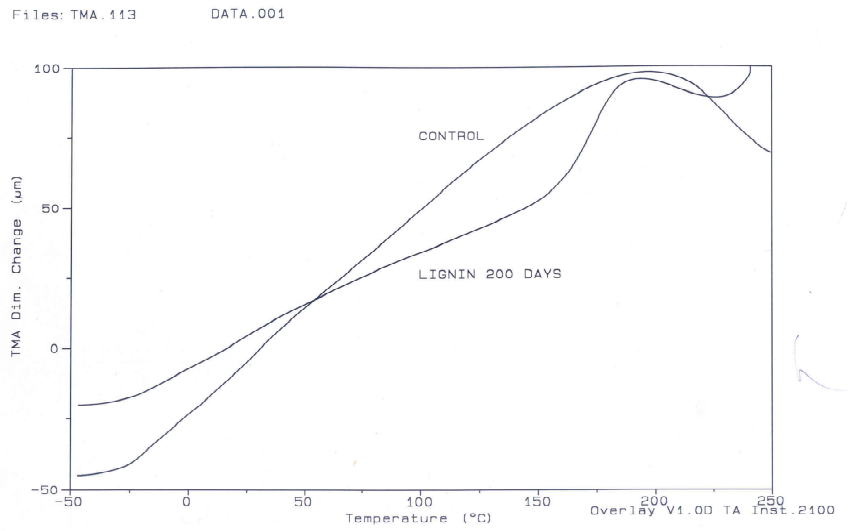


Fig. 1B TMA of the control as well as that of the exposed Lignin reinforced NBR composite

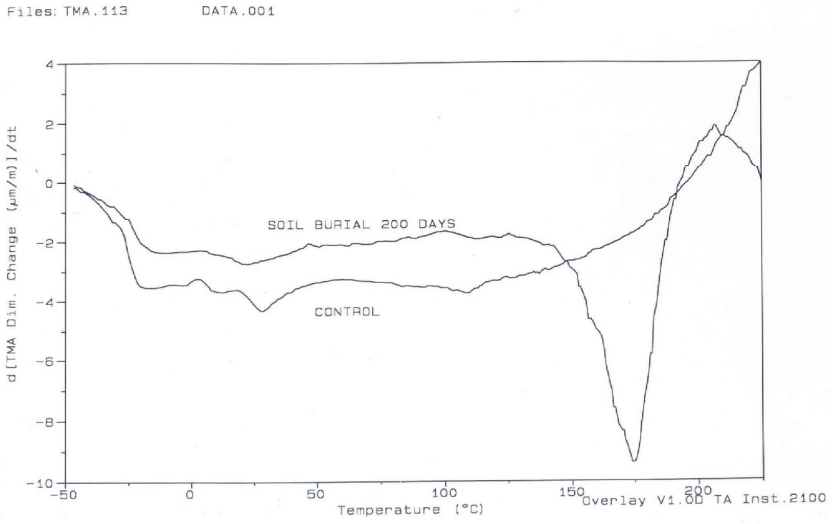


Fig. 1C Derivative TMA of the control as well as that of the exposed Lignin reinforced NBR composite

Improvement in modulus and hardness of mix D compared to mix A was primarily due to the addition of non-reinforcing filler in a large quantity, which caused a marked reduction in the elongation properties. Similar observation in case of short-fibre rubber composites has been reported earlier by Setua [26, 9]. A very long elongation (5%) modulus (e.g. secant modulus) ($E_s^5$) parameter was evaluated to derive 100% modulus ($E_s^5$ x 20) values as a real measure of reinforcement for non-reinforcing filler present in a larger volume fraction in rubber composites. Improvement in tear strength and modulus occurred as a result of enhanced reinforcement by lignin in crude version. The unexposed lignin reinforced NBR plot of TGA is shown in Fig. 2A. The TGA graphs of lignin reinforced NBR with S. mutans (Fig. 2B) and lignin reinforced NBR composite with *S aureus* (Fig. 2C) show different stages of decomposition after *in vitro* antibacterial activity. Table 3 and Table 4 give the TGA findings of five different bacteria on NBR and lignin reinforced NBR, respectively.

TABLE 3 TGA RESULTS OF NBR WITH DIFFERENT BACTERIA

| Sample | I weight loss | II weight loss | Main Step of degradation | | | | Residue at 600 °C |
|---|---|---|---|---|---|---|---|
| | | | Onset Temp. | Mid point Temp | Main weight loss | End temp. | |
| **NBR** | | 3.304 | 416.42 | 446.41 | 79.37 | 465.43 | 17.03 |
| **NBR+ Streptococcus mutans** | 2.003 | 3.224 | 433.91 | 447.91 | 71.70 | 479.47 | 22.62 |
| **NBR+ Bacillus Subtilis** | .8526 | 3.505 | 430.30 | 442.79 | 80.94 | 475.42 | 13.87 |
| **NBR+ Pseudomonas aeruginosa** | 1.083 | 2.86 | 428.36 | 460.73 | 80.13 | 479.69 | 15.52 |
| **NBR+ Escherichia coli** | 0.5789 | 2.841 | 425.76 | 455.74 | 79.50 | 474.80 | 17.17 |
| **NBR+ Staphylococcus aureus** | 1.103 | 2.675 | 424.92 | 437.24 | 81.68 | 470.18 | 13.90 |

### *A. Study of Bacterial actions on NBR*

TGA results in Table 3 clearly exhibit that all the bacteria show very little effect on NBR in case of I weight loss and II weight loss. In the main step of degradation, bacterial actions of all the bacteria are such that they start the process of degradation at higher temperature as compared to that of unexposed NBR. Maximum enhancement of onset temperature is found in case of A1 bacteria and also results in decrease of main weight loss, however all other bacteria have negligible effect on main weight loss as compared to unexposed NBR, as also supported by residual data.

TABLE 4 TGA RESULTS OF LIGNIN REINFORCED NBR COMPOSITES WITH DIFFERENT BACTERIA

| Sample | I weight loss | II weight loss | Main Step of degradation | | | | Residue at 600 °C |
|---|---|---|---|---|---|---|---|
| | | | Onset Temp. | Mid point Temp | Main weight loss | End temp. | |
| **NBR+ Lignin** | 3.758 | 14.92 | 420.21 | 433.38 | 50.29 | 459.53 | 28.09 |
| **NBR+ Lignin+Streptococcus mutans** | 3.613 | 5.198 | 403.23 | 425.35 | 65.41 | 463.33 | 22.19 |
| **NBR+ Lignin+Bacillus Subtilis** | 4.922 | 11.51 | 424.27 | 449.36 | 57.55 | 466.79 | 18.71 |
| **NBR+ Lignin+Pseudomonas aeruginosa** | 4.592 | 9.940 | 429.57 | 453.32 | 57.88 | 471.96 | 24.30 |
| **NBR+ Lignin+Escherichia coli** | 5.384 | 12.88 | 429.70 | 451.04 | 55.08 | 471.53 | 22.55 |
| **NBR+ Lgnin+Staphylococcus aureus** | 5.406 | 12.42 | 426.72 | 438.90 | 50.44 | 469.60 | 17.44 |

### *B. Study of Bacterial actions on Lignin reinforced NBR*

In case of I weight loss, results in Table 4 clearly show that Streptococcus mutans bacterium exhibits almost negligible effect on the degradation process, whereas all other bacteria show reaction with the samples and the maximum action was found in case of Staphylococcus aureus . In case of II weight loss, all the bacteria restrict the degradation process. Maximum restriction was found for Streptococcus mutans and minimum for Escherichia coli. Now in case of main step of degradation, Streptococcus mutans shows the maximum action on the sample by lowering the onset temperature whereas all other bacteria show less reactiveness as compared to that of Streptococcus mutans, as strongly supported by mid point temperature data as well as main weight loss data. Lastly, TGA results clearly show that all the bacteria reacted with the sample effectively and lowered the residual weight at last.

### *C. Effect of addition of lignin on NBR: A thermo-analytical study*

By comparing the corresponding values in Table 3 and Table 4, it is found that the addition of lignin in NBR enhances the initial degradation processes, as clearly evidented by I weight loss and II weight loss data, and bacteria Escherichia coli and Staphylococcus aureus are found to be more effective than the other bacteria. In the main step of degradation, the addition of lignin in NBR exhibited negligible effect on the onset temperature except in the case of Streptococcus mutans treated sample, as also supported by the mid point temperature data. A decrease in main weight loss was observed when lignin is added in NBR.

Minimum decrease is found in the case when the sample was treated with Streptococcus mutans bacteria. Consequently, residual weight data also strongly supports this observation.

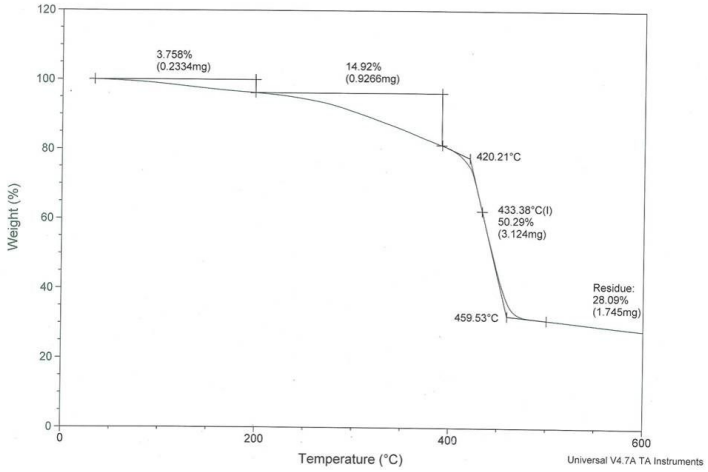


Fig. 2A TGA plots of unexposed lignin reinforced NBR

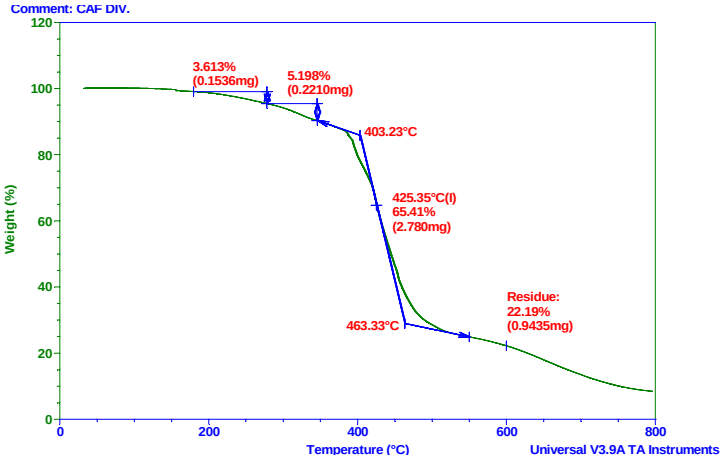


Fig. 2B TGA plots of lignin reinforced NBR after *in vitro* antibacterial activity with S. mutans

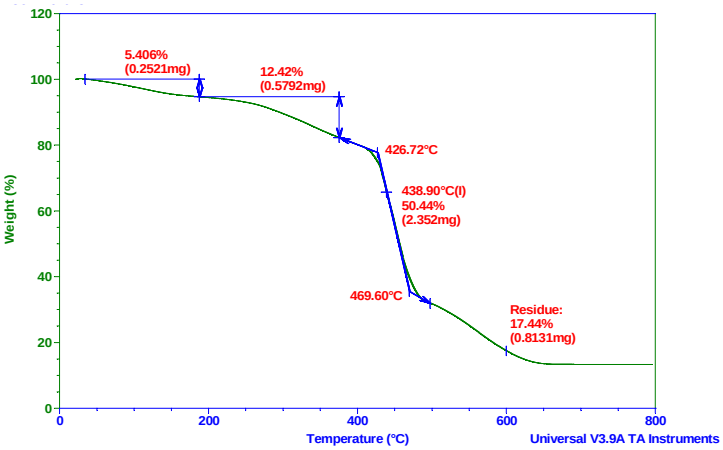


Fig. 2C TGA plots of lignin reinforced NBR composite after *in vitro* antibacterial activity with *S. aureus*

In vitro antibacterial activity of Streptococcus mutans with different rubber composites A. NBR, B. NBR with phenolic resin, C. NBR with carbon black, and D. NBR with lignin. composites

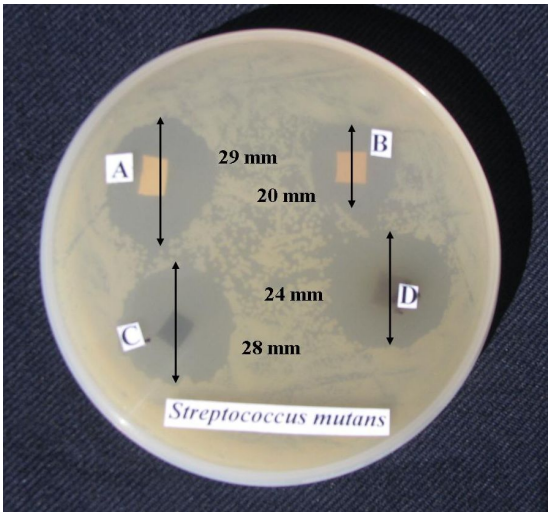


Fig. 3 Inhibition zone sizes of different NBR composites by *S. mutans*

*In* vitro studies shows the inoculums size of 2 $\times 10^6$ CFU/ml at 37 $^0$C and the inhibition zone sizes of different samples; NBR shows the inhibition zone in the diameter of 26 mm, NBR with phenolic resin gives the 20 mm-diameter inhibition zone, NBR with carbon black gives the inhibition zone in the diameter of 24 mm and NBR with lignin gives the larger inhibition zone in the diameter of 28 mm after 24 hrs.

Therefore, during the evaluation period of the present study, NBR did not demonstrate any antibacterial effect as demonstrated by SEM (Fig. 4A). The SEM was sufficient to detect the pits on Figs. 4B, 4C and 4D. The maximum small pits and voids are found present in Fig. 4D.

*In vitro* studies shows the the inoculums size of 2 $\times 10^6$ CFU/ml at 37 $^0$C and the inhibition zone sizes of different samples; NBR shows the inhibition zone in the diameter of 24 mm, NBR with phenolic resin gives the 14 mm-diameter inhibition zone, NBR with carbon black gives the inhibition zone in the diameter of 21 mm and NBR with lignin gives the larger inhibition zone in the diameter of 26 mm after 24 hrs.

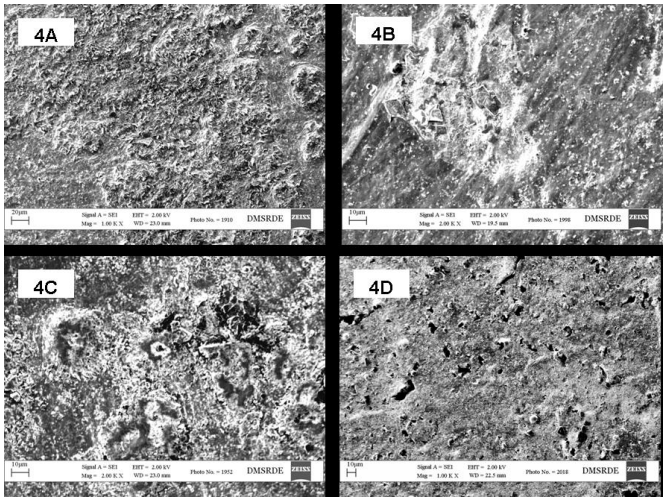


Fig. 4 SEM micrographs of *in vitro* antibactrerial activity of *S. mutans* with different rubber composites

The SEM investigation revealed that the no pits or cracks were observed on Fig. 6A. Little deep and wide depressions were commonly observed on the surface. SEM results of NBR with phenolic resin (Fig. 6B) indicate that propagules of *B. subtilis* were

dispersed randomly as individual endospores on the surface. However, small single-layered shallow colonies composed of between a few to as many as a dozen endospores were observed periodically on surface of NBR with carbon black (Fig. 6C). Individual endospores of *B. subtilis* were often observed embedded in preexisting surficial cracks and pits on the surfaces. The many deepest pits and highly cracked surfaces were observed on lignin reinforced NBR composite (Fig. 6D).

*In vitro* antibacterial activity of *Bacillus Subtilis* with different rubber composites. A. NBR, B. NBR with phenolic resin, C. NBR with carbon black and D. NBR with lignin composites

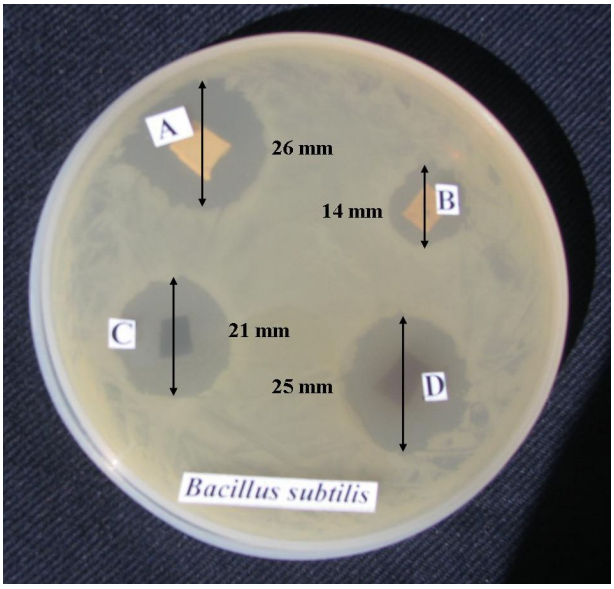


Fig. 5 Inhibition zone sizes of different NBR composites by *B. Subtilis*

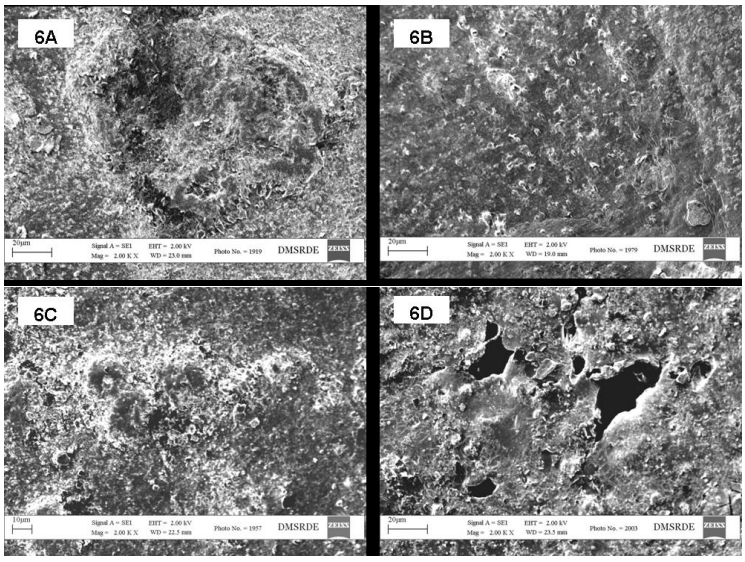


Fig. 6 SEM micrographs of *in vitro* antibactrerial activity of *B. Subtilis* with different rubber composites.

*In vitro* antibacterial activity of *Pseudomonas aeruginosa* with different rubber composites. A. NBR, B. NBR with phenolic resin, C. NBR with carbon black and D. NBR with lignin. composites

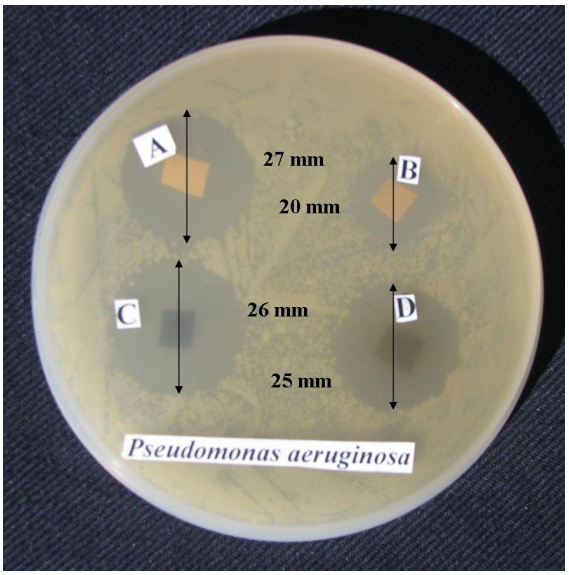


Fig. 7 Inhibition zone sizes of different NBR composites by *P. aeruginosa*

*In vitro* studies shows the inoculums size of 2 $\times 10^6$ CFU/ml at 37 $^0$C and the inhibition zone sizes of different samples; NBR shows the inhibition zone in the diameter of 26 mm, NBR with phenolic resin gives the 20 mm-diameter inhibition zone, NBR with carbon black gives the inhibition zone in the diameter of 25 mm and NBR with lignin gives the larger inhibition zone in the diameter of 27 mm after 24 hrs.

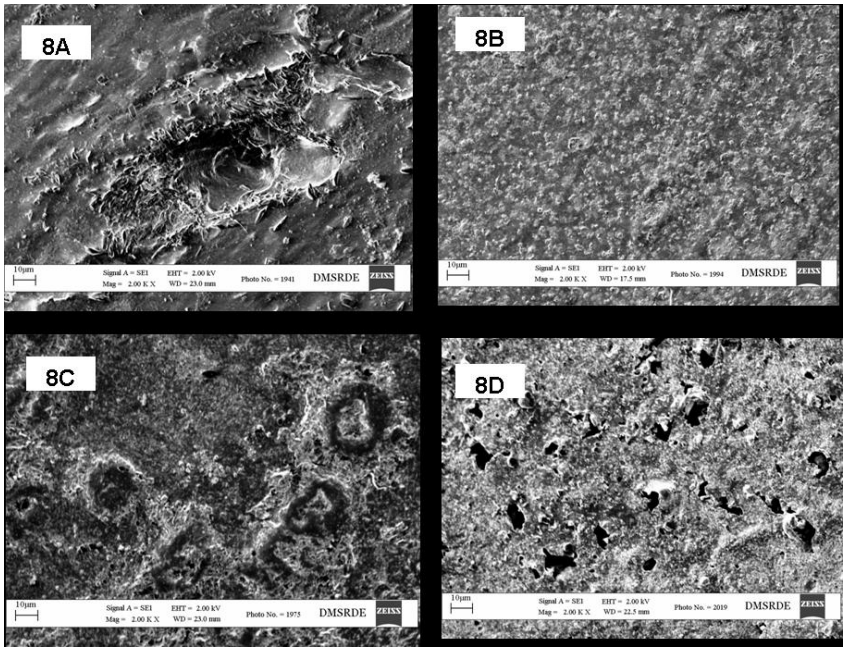


Fig. 8 SEM micrographs of *in vitro* antibactrerial activity of *P. aeruginosa* with different rubber composites

Deep and wide depressions were observed on the surface of NBR (Fig. 8A), indicating that propagules of Pseudomonas aeruginosa were dispersed randomly as individual endospores on the surface. The formation of the initial microcolonies (the stalks) in *P. aeruginosa* surface attached biofilms is due to clonal growth but no pits or cracks were observed on NBR with phenolic resin (Fig. 8B). The SEM investigation revealed that small single-layered shallow colonies were observed periodically on surface of NBR with carbon black (Fig. 8C). Many small voids and cracked surfaces were observed on NBR with lignin (Fig. 8D).

*In vitro* antibacterial activity of *Escherichia coli* with different rubber composites. A. NBR, B. NBR with phenolic resin, C. NBR with carbon black and D. NBR with lignin composites

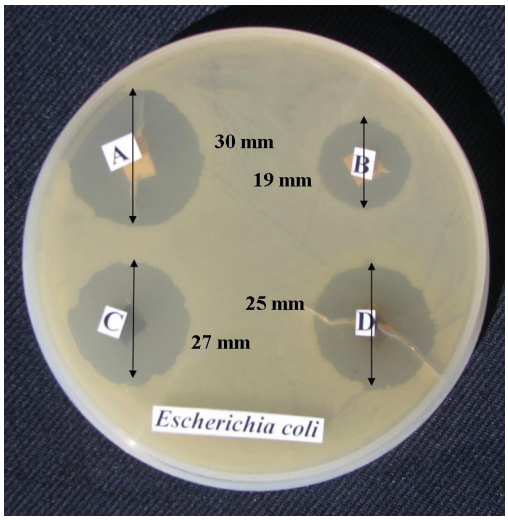


Fig. 9 Inhibition zone sizes of different NBR composites by *E. coli*

*In vitro* studies shows the inoculums size of 2 $\times 10^6$ CFU/ml at 37 $^0$C and the inhibition zone sizes of different samples; NBR shows the inhibition zone in the diameter of 27 mm, NBR with phenolic resin gives the 19 mm-diameter inhibition zone, NBR with carbon black gives the inhibition zone in the diameter of 25 mm and NBR with lignin gives the larger inhibition zone in the diameter of 30 mm after 24 hrs.

Figs. 10A – 10D show the SEM images of different substrates after *in vitro* antibacterial activity of *E. coli*. The effect of *E. coli* on the NBR surface was significantly less (Fig. 10A). Numerous distinguishable *E. coli* cells can be observed on NBR with phenolic resin as shown in Fig. 10B. In contrast, very few sparsely distributed bacteria cells could be spotted over the entire surface of NBR with carbon black (Fig. 10C). The *E. coli* was distributed not only on surface of NBR with lignin upper but also creates voids and pits on the upper surface (Fig. 10D).

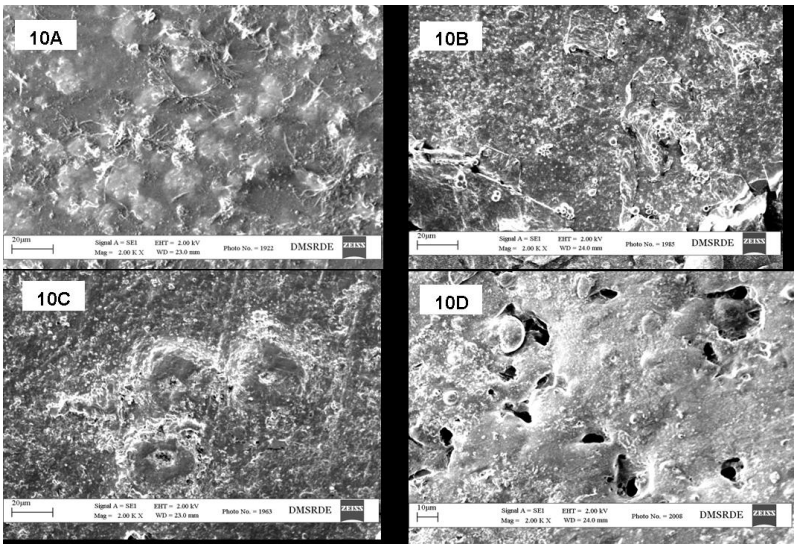


Fig. 10 SEM micrographs of *in vitro* antibactrerial activity of *E.coli* with different rubber composites

***In vitro* antibacterial activity of *Staphylococcus aureus* with different rubber composites. A. NBR, B. NBR with phenolic resin, C. NBR with carbon black and D. NBR with lignin. composites**

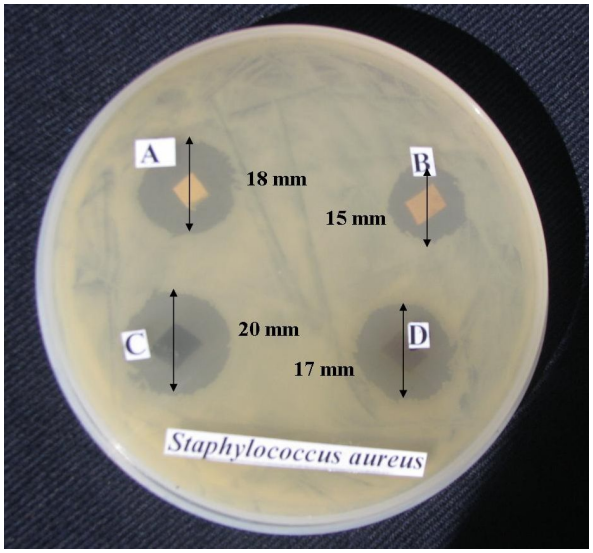


Fig 11. Inhibition zone size of different NBR composites by *S. aureus*

*In vitro* studies shows the inoculums size of 2 $\times 10^6$ CFU/ml at 37 $^0$C and the inhibition zone sizes of different samples; NBR shows the inhibition zone in the diameter of 18 mm, NBR with phenolic resin gives the 15 mm-diameter inhibition zone, NBR with carbon black gives the inhibition zone in the diameter of 17 mm and NBR with lignin gives the larger inhibition zone 20 in the diameter of mm after 24 hrs.

SEM views of *in vitro* antibactrerial activity of *S. aureus* with the different reinforced NBR composites are presented in Figs. 12A to 12D. Micrographs of NBR and NBR with phenolic resin (Fig. 12A & Fig. 12B) showed a more uniform surface than NBR with carbon black (Fig. 12C), NBR with lignin (Fig. 12D) showed bigger and more voids than NBR with carbon black (Fig. 12C), which is a positive characteristic for improving the properties of the bio-degradation of Lignin reinforced Rubber composites with antibacterial activity of *S. aureus*.

Fig. 13 shows encouraging results of FTIR spectrum of the NBR with unmodified Lignin and the spectrum was compared with the standard library and found to resemble kraft lignin [9,27]. Fig. 14 shows FTIR spectrum of lignin reinforced NBR composites and *in vitro* antibacterial activity of B. subtilis. In Table 5, structural identification of various peaks of NBR with unmodified Lignin are mentioned. Fig. 13 is the FTIR spectrum of NBR with unmodified Lignin (mix D) showing sharp peaks at 1509.2 cm-' (Aromatic ring vibration of the phenyl propane skeleton), 1436.8 cm-' (C=C stretch, aromatic rings from lignin), and 968.59 cm-' (-CH-CH- 1,2 vinyl). Appearances of peaks at 2918.7 cm-1 and 2836.7 cm-1 and 1736 cm-1 are due to Teriary –OCH3 groups, C-H stretching vibration of CH2 and CH3 group and acid or ester carbonyl groups respectively. Peaks at 1383.7 cm-1 , 1239 cm-1 and 1045.8 cm-1 show the bending vibration of OH bonds, Phenolic OH stretching and C-O stretching, respectively. Peak at 889.3 shows the 1-2-5 substituted aromaticing. Also, sharper peaks in the region between 1800 - 800 cm-' are due to enhanced filler-matrix interaction [9,26].

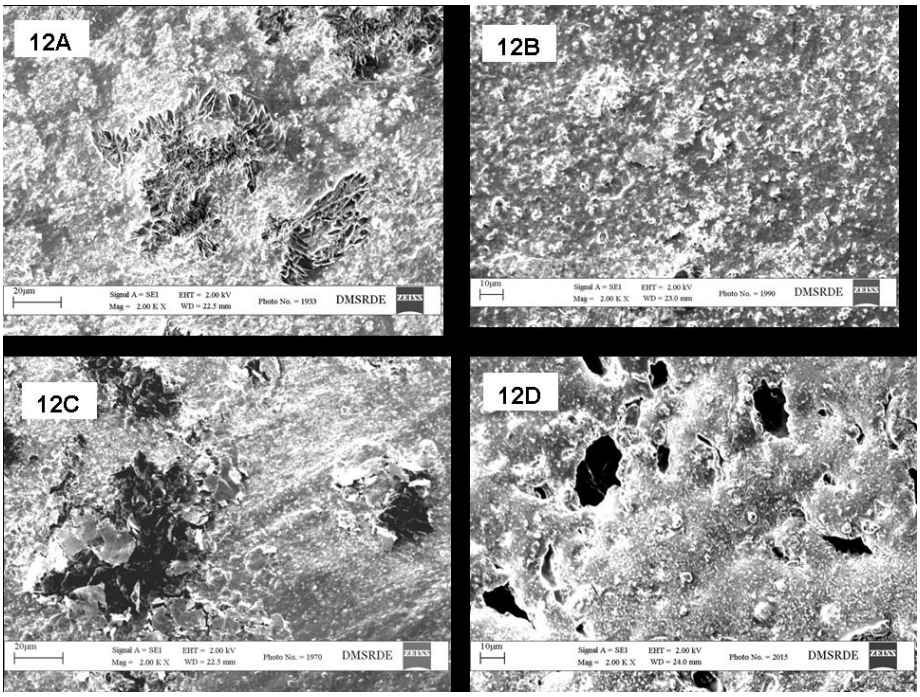


Fig. 12 SEM micrographs of *in vitro* antibacterial activity of *S. aureus* with different rubber composites

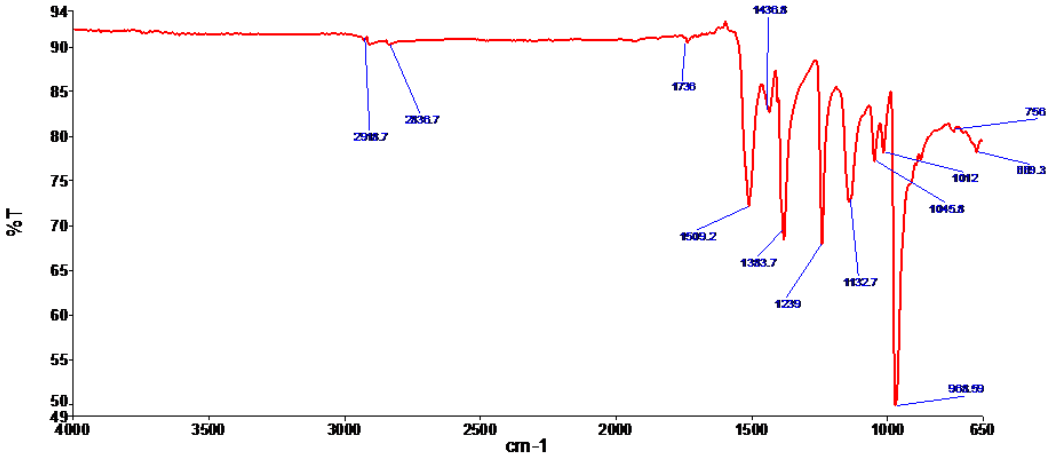


Fig 13. FTIR spectrum of unmodified Lignin reinforced NBR composite

TABLE 5 TRANSMITTANCE BAND/ WAVE NUMBER OF LIGNIN AND ITS STRUCTURAL ASSIGNMENT

| Sl. No. | Wave number ($cm^{-1}$) | Structural assignment of lignin |
|---|---|---|
| 1. | 2918.7 | Teriary $-OCH_3$ groups |
| 2. | 2836.7 | C-H stretching vibration of $CH_2$ and $CH_3$ group respectively |
| 3. | 1736.0 | Acid or Ester carbonyl groups |
| 4. | 1509.0 | Aromatic ring vibration of the phenyl propane skeleton |
| 5. | 1436.8 | C=C stretch (aromatic rings from lignin) |

| | | |
|---|---|---|
| 6. | 1383.7 | Bending vibration of OH bonds |
| 7. | 1239.0 | Phenolic OH stretching |
| 8. | 1045.8 | C-O stretching |
| 9. | 968.59 | -CH-CH- 1,2 vinyl |
| 10. | 889.30 | 1-2-5 substituted aromaticing |

Fig. 14 shows the infrared spectrum of the *in vitro* antibacterial activity of Bacillus Subtilis with lignin reinforced NBR composite. The FTIR spectrum showed absorption bands of Bacillus Subtilis at 3600-3400 cm-1 (broad peak), 1,639.6 cm-1 and 1,576.8 cm-1. These bands resulted from the stretching mode of N-H, stretching mode of the C=O bond, and the N-H stretching of proteins and peptides bonds, respectively. The bands at 2,923.6 cm-1, 1504.4 cm-1, and 1,388.5 cm-1 reflect aliphatic chains (-CH3, -CH2-) of the Bacillus Subtilis with lignin reinforced NBR composite sample. Clearly, the higher content of polysaccharide in the spectral region represent of C-O-C stretching (1150-900 cm-1) when compared with lignin reinforced NBR composite treated with the beneficial bacterium B. subtilis. Additional absorption valleys at 1441.6 indicating (C-H) aliphatic side chain may be related to predominance of hydrophobic amino acid [28-31]. These results are similar to surfactant produced by Bacillus subtilis, and indicate that there has been changes in chemical structure. FTIR absorption from lignin reinforced NBR composite produced by B. subtilis is shown in Table 6.

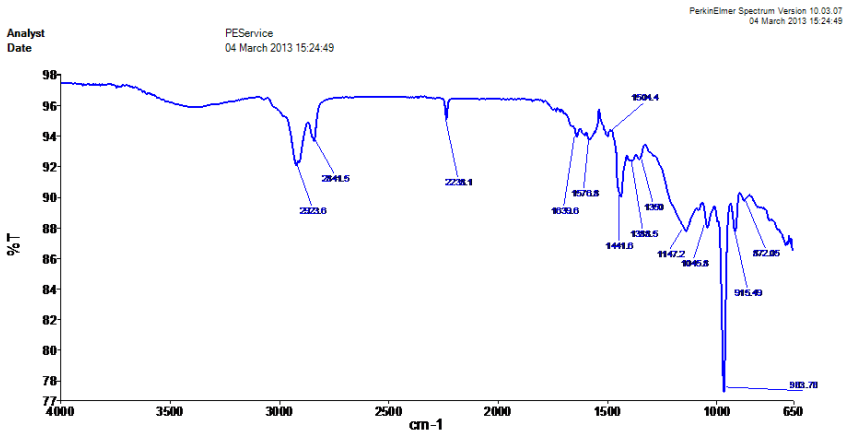


Fig. 14 FTIR spectrum of (after *in vitro* antibacterial activity of) Bacillus Subtilis with Lignin reinforced NBR composite

TABLE 6 TRANSMITTANCE BAND/ WAVE NUMBER OF LIGNIN REINFORCED NBR COMPOSITE AFTER *IN VITRO* ANTIBACTERIAL ACTIVITY WITH BACILLUS SUBTILIS

| **Sl. No.** | **Wave number (cm-1)** | **Functional group** |
|---|---|---|
| 1. | 3600-3400 | stretching mode of N-H |
| 2. | 2923.6 | Aliphatic chain (-$CH_3$, -$CH_2$-) |
| 5. | 1639.6 | stretching mode of C=O |
| 6. | 1576.8 | N-H stretching of proteins and peptides bonds |
| 7. | 1504.4 | Aliphatic chain (-$CH_3$, -$CH_2$-) |
| 8. | 1441.6 | Aliphatic chain $CH_2$ |
| 9. | 1388.5 | Aliphatic chain (-$CH_3$, -$CH_2$-) |
| 10. | 1150-900 | C-O-C stretching |

## IV. CONCLUSIONS

A great number of different applications using lignin fractions have been developed in the past. In general, the use of lignocellulosic fibers in biodegradable composites can help to generate jobs in both rural and urban areas, in addition to helping reduce waste, thus contributing to a healthier environment. In this research, phenolic resin, carbon black and Lignin were used as fillers in NBR composites. The present paper focuses on the analysis of the behaviour of antibacterial activities on NBR composites reinforced with different fillers and show good biodegradability. The consequent lignin filler content variation result proved better thermal stability as compared to phenolic resin and carbon black. Minimum thermal stability in NBR and lignin

reinforced NBR composites were obtained from E. Coli and S. aureus bacteria respectively. Lignin has the capability of replacing costlier petroleum based carbon black for applications where superior oil and fuel resistance of nitrile rubber is of primary concern. The lignin reinforced NBR composites showed slightly higher Modulus at 100% elongation, Elongation at break and Tear strength than with neat NBR. The presence of lignin in NBR composite caused improper vulcanization and also generated lower values of tensile strength. NBR composites were tested against five pathogenic bacteria. Lignin reinforced NBR composites were most affected by S. aureus and B Subtilis bacteria as proven by SEM studies. Lignin reinforced NBR composite possessed highly effective antibacterial activities and may have a wide variety of potential applications in waste management**.**

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