

Comparative studies on poly- β -hydroxybutyrate (PHB) with gelatin and PHB with starch as a finished fabric

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ABSTRACT

The aim of the study was to isolate the PHB producing strains from soil samples, quantitative screening, extraction and estimation of PHB. The preliminary step involves the preparation of PHB, gelatin, starch, PHB with gelatin and PHB with starch as a polymer solution. The production of PHB with gelatin and starch coated textiles laminates by pad dry cure method. The determination of the physical, chemical and functional properties of the developed textile laminates. Comparative studies of invitro degradation of developed textile laminate (PHB with gelatin / PHB with starch) for biomedical application.

Keywords: PHB, strains, soil samples, gelatin.

1. Introduction

Synthetic Polymers obtained from petrol causes air pollution because of its slow degradability in soil. For this reason, a microbial biopolymer has gained importance because of its easily degradable nature. Environmental pressures are forcing on polymer manufactures to consider biodegradable polymers as an alternative polymeric material. Poly- β -hydroxy butyrate (PHB) and poly- β - hydroxyalkanoic acids, biodegradable thermoplastics can be produced from a wide range of substrates using bacteria (Khanafari *et al.*, 2006). To combat challenges due to environmental pollution, Poly- β -hydroxy butyrate (PHB) has been found as eco-friendly and best alternative biopolymers (Schlegel *et al.*, 1970). PHB is a biodegradable, biocompatible, partially crystalline thermoplastic produced by various microorganism (Barnard and Sanders, 1989). Bio textile is defined as a structure composed of textile fibers and designed for its performance and interaction with cells and biological environment. Textile industry continues to look for eco-friendly, biodegradable polymer that substitute for toxic non-biodegradable textile fabrics e.g., fabrics that generate tissue/cells and self cleaning fabrics. Fabrics transparent coatings on fine fabrics were equal to fragile but strong, durable fabric. The fabric coated with Poly- β -hydroxybutyrate (PHB) has increased surface porosity, decreased crystallinity, better tensile and mechanical properties (Fredrik *et al.*, 1994). Poly- β -hydroxybutyrate (PHB) has been found as eco-friendly substitute and ideal candidate for making non-toxic biodegradable polymer (Miller and Williams, 1987).

2. Materials and method

2.1. Isolation of PHB producing organisms from soil samples (Yilmaz *et al.*, 2005)

The soil samples were collected in and around Coimbatore district. One gram of soil sample was suspended in 100 ml of sterile distilled water and shaken vigorously for 2 min. The diluted soil samples were heated at 60°C for 30 min in water bath. Then the liquid was serially diluted and plated on nutrient agar medium. The plates were incubated at 37°C for 24-48 hours. The isolated colonies were selected and subcultured on minimal agar medium for further studies (Cappuccino, 1992) and the production of PHB was determined as a preliminary screening method, the sudan black staining technique.

2.2. Quantitative screening for PHB Producing Isolates

The selected strains were grown on minimal broth (pH 7) under standard conditions and incubated at 37°C. During incubation, samples were retrieved after every 24 hours for 4 days (24-72 hours) to quantify the production of PHB (µg/ml) by chloroform extraction method.

2.3. Extraction of Poly-β-hydroxybutyrate (Mekala *et al.*, 2011)

PHB produced from the selected and standard isolates were extracted by the following procedure. About 10 ml of the bacterial cultures (24-96 hours) grown in minimal broth was retrieved at an interval of 24 hours and centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and the pellet was suspended with 2.5 ml of 4% sodium hypochlorite solution and 2.5 ml of chloroform. The pellet suspension was incubated at 30°C for 1 hour. After incubation, the suspension was centrifuged at 1,500 rpm for 10 min. After centrifugation, three phases were obtained. The upper phase consisted of hypochlorite solution which was removed and the middle phase (chloroform containing undisturbed cells) was separated by filtration from the bottom phase (chloroform with PHB). The extracted chloroform phase was used to quantify the production of PHB by measuring the absorbance at 230 nm.

2.4. Standardisation of binder concentration for PHB coated cloth with gelatin and starch powder

As a preliminary step, the selection of textile binder and the concentration of the binder were standardised by dissolving about 0.7 g of PHB powder in 10 ml of binder solution (isopropanol) and about 0.3 g of binder (gelatin/starch) was dissolved in 100 ml of distilled water, both the mixtures were mixed properly such that the binder and the PHB powder dissolves well. The binder solution was made for two concentrations of gelatin/starch and PHB (7:3, 5:5). De-starched 40's count cloth (10×10 cm) was dipped in the PHB with binder solution for half an hour. The cloth was allowed to dry in oven (50°C for 30 min) or dried under sunlight.

2.5. Pad dry cure method (Rajendran *et al.*, 2006)

The coating of PHB with gelatin and PHB with starch powder on 40's count cloth was passed through a padding mangle (R.B. Electronic and Engineering, Mumbai), running at a speed of 15 m/min with a pressure of 1kgf/cm² to remove excess solution. The cloth was then kept in oven at 50°C for half an hr for drying and curing process was done under 110° C for 1hour.

2.6. Characterisation of developed textile laminates

2.6.1. Physical characterisation of developed textile laminates-SEM

Scanning electron microscopy (SEM) was used to examine the morphology of the coated fabrics (PHB with gelatin and PHB with starch) and the control fabric. The samples were sonicated at 20 KHZ for 3 cycles of 5 min each. Fracture surfaces of the samples were made by breaking of extruded tapes in liquid nitrogen. The fragments with fracture surfaces were glued on metal holder and covered by platinum layer in normal atmosphere. After sample preparation, the photographs of the sample were taken by Scanning Electron Microscope (Model-JEOL-6390 under the magnification 150X to 300,000X, accelerating at the voltage 0.5 to 30 kV).

2.6.2. Chemical characterisation of developed textile laminates - FTIR

FTIR Spectroscopy was a form of vibrational spectroscopy, the sample was irradiated with infrared radiation and absorption of the radiation stimulates vibrational motions by depositing quanta of energy into vibrational model (Diem and White, 1999 and Wenning *et al.*, 2002). The developed fabric, PHB with gelatin and PHB with starch was subjected to FTIR spectroscopic analysis. Spectra were recorded in 4000 cm^{-1} to 400 cm^{-1} range.

2.7. Physical and functional analysis of developed textile laminates

2.7.1. Water repellency test of developed textile laminates (spray test method 1941 by AATCC RA63)

Water repellency test was to measure the resistance of developed fabric to wetting process by water and helps to find out the water repellent efficacy of PHB with gelatin and PHB with starch powders applied onto the developed and control fabric materials. The water was allowed to spray on the mounted fabric (spray time – 25 to 30 sec). The distilled water at $27 \pm 1^\circ\text{C}$ ($80 \pm 2^\circ\text{F}$) was poured into the funnel and allowed to sprayed on developed (PHB with gelatin and PHB with starch) and control fabric. The standard chart for water repellency test was observed from table 1.

Table 1: Standard chart for water repellency test

Values	Spray test rating
Value 100	No sticking or wetting of specimen face.
Value 90	Slight random sticking or wetting of specimen face.
Value 80	Wetting of the specimen face at spray points.
Value 70	Partial wetting of the entire specimen face beyond the spray points.
Value 50	Complete wetting of the entire specimen face beyond the spray points.
Value 0	Complete wetting of the entire face of the specimen.

2.7.2. Absorbency of developed textile laminates – (wicking, wetting, warping 1954 by Aatcc ra 34)

Absorbency test was performed in order to measure the propensity of a material to take in or retain a liquid, in the pores and interstices of the material. The completeness and the uniformity of the PHB with gelatin and PHB with starch developed fabrics depend upon the absorbency. The time required was calculated using stop watch up to 60 sec maximum for the surface of the liquid to loss its specular reflectance. The watch was stopped and the elapsed time was recorded. The standard chart of absorbency test was observed from table 2.

2.7.3. Measurement of abrasion resistance (ASTM D 3885-07)

Abrasion resistance test was performed to measure the abrasion resistance of developed (PHB with gelatin and PHB with starch) and control fabrics. The fabrics of about 3 x 4-1/2 inches (7.5 x 11 cm) was required for each test (ie) one for the machine directions and the other for the cross machine direction test (Flex Tester).

Table 2: Standard chart of absorbency test

Time	Result
Shorter the average time	More absorbency
5 sec or less time	Adequate absorbency

2.7.4. Measurement of air permeability (ASTM-D-737-2004)

The air permeability of developed (PHB with gelatin and PHB with starch) and control fabrics was analyzed by Kawabata Evaluation System (KES-F8-API) automatic air permeability tester. The air permeability of a fabric was the volume of air measured in cubic centimetres passed / sec through 1 cm² area of the fabric at pressure of 1 cm of water. The air resistance of a fabric was measured by the time in sec for 1 cm³ of air to pass through 1 cm² area of a fabric under a pressure head of 1 cm of water. The porosity of the fabric was the ratio of air space to the total volume of the fabric expressed as a percentage.

2.7.5. Tensile strength (IS 1969/85)

A tensile testing machine was to determine the breaking strength and elongation of developed fabrics (PHB with gelatin and PHB with starch) and control fabrics. The test was performed either wet or dry and samples were cut in both the warp and weft directions. The specimen was mounted in the clamp of the testing machine.

2.7.6. Tear strength (Elmendorf method ASTM D 1424-07)

The tearing strength of the developed (PHB with gelatin and PHB with starch) fabrics and control fabric were measured by the tongue (slip rip) procedure using constant-rate-of-extension tensile testing machine recording.

2.8. Comparative studies of invitro biodegradation of developed laminates

The developed laminates were cut into rectangle (5x5x0.05mm) and incubated in phosphate buffer saline (PBS) at 37°C in the presence of trypsin (0.1mg/ml). After the incubation for requisite time (12 hours), the samples were washed with distilled water. The degradation effects of treated sample were studied under scanning electron microscopy.

3. Results and discussion

3.1. Isolation of PHB Producing Organisms from Soil Samples

All the ten isolates from soil samples were observed under the direct dilution and plating on minimal agar supplemented with 2% glucose. All the ten isolates were grown on the minimal agar (production media for PHB). The isolates (B1-B5) were observed with positive for the presence of lipophilic PHB granules as a dark gray colour on minimal media due to the absorption of Sudan black stain. The isolates were selected based on the maximum adsorption

pattern for further studies. Burdon *et al.*, (1942) confirmed the greater value of dye and modified the procedure for demonstrating intracellular fatty material present in bacteria by preparing microscopic slides of bacteria stained with alcoholic Sudan black B solution.

3.2. Extraction of Poly- β -hydroxybutyrate (PHB) (chloroform extraction method) (Mekala *et al.*, 2011)

PHB produced from the selected and standard strains were extracted by chloroform extraction method. Three phases were obtained, the upper phase with of hypochlorite solution which were removed and the middle phase (chloroform containing undisturbed cells) were separated by filtration from the bottom phase (chloroform with PHB).

3.3. Estimation of poly- β -hydroxybutyrate by Spectrophotometric methods (Mekala *et al.*, 2011)

The standard curve was plotted by preparation of PHB standard solution at different concentration (100-1000 μ g/ml). About 2ml of concentrated sulphuric acid were added to all tubes and kept in boiling water bath for 10 min for the conversion of PHB into crotonic acid. After cooling, the absorbance was measured at 230 nm using UV spectrophotometer (Systronics 180) and standard graph was plotted and observed from figure 1. About 2ml of concentrated sulphuric acid were used as blank.

3.4. Development of coated textile as laminates

The complete coating of PHB with gelatin and PHB with starch (5:5) were observed from figure 2a and figure 2b. Kissa (1984) reported that the relative humidity of the air and the volume of air passing over fabric depend on the choice of material, air space of the material and size of the material.

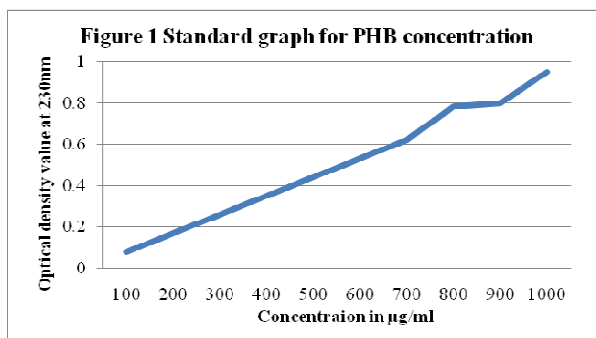


Figure 1: PHB Concentration



Figure 2a: Coating of PHB with gelatin starch on textile fabric



Figure 2b: Coating of PHB with starch on textile fabric

3.5. Pad dry cure method (Rajendran *et al.*, 2010)

The coating of PHB with gelatin and PHB with starch on 40's count cloth was performed by pad dry cure method. The physical property changes in fabrics as smooth drying proper ties are developed in cotton by pad-dry-cure process. The maximum relative humidity being required in the wet-fix and the minimum in the poly-set cottons.

3.6. Characterization of developed textile laminates

3.6.1. Physical characterization of developed textile laminates-SEM

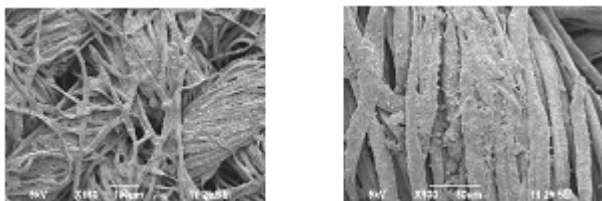


Figure 3a: SEM image of PHB with gelatin developed textile laminate

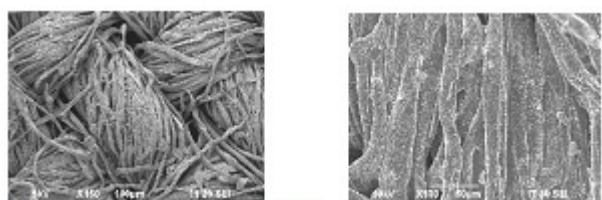


Figure 3b: SEM image of PHB with starch developed textile laminate

The morphology of developed and control textile laminates were studied under scanning electron microscope (SEM) as a large spherulite (a semi crystalline polymer) material on 150X and 500X magnification were represented in figure 3a, 3b and 3c which revealed the property as crystallinity and more brittle as a fine spherulite. Hahn *et al.*, (1995) reported that the PHB granules are released from the cell content exposing the granule morphology in the FE-SEM photography. The PHB granules were found as electron dense granules of spherical to oblong shaped, while the bacterial cells were long and rod shaped.

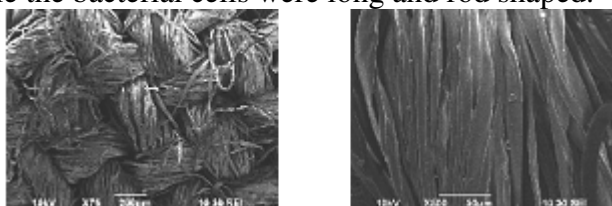


Figure 3c: SEM image of control textile laminate

3.6.2. Chemical characterization of developed textile laminates– FTIR

The FTIR spectrum of coated fabrics (PHB with gelatin, PHB with starch) and control fabric were observed. On comparison of FTIR spectrum of coated fabrics, the vibrational stretch observed at $\sim 3297.6\text{ cm}^{-1}$ and $\sim 3333.3\text{ cm}^{-1}$ of peak 1 (figure 4a and figure 4b) showed the presence of $-\text{OH}$ and $-\text{CH}_3$ group.

The vibrational stretch observed at $\sim 2358.52\text{ cm}^{-1}$ and $\sim 2809.4\text{ cm}^{-1}$ of peak 2 (figure 4a and figure 4b) revealed the presence of strong band of $-\text{C}=\text{O}$ and $-\text{CH}$ group. These strong bands

were absent in control fabric (figure 4c). The vibrational stretch observed at $\sim 874.56\text{ cm}^{-1}$ and $\sim 1305.5\text{ cm}^{-1}$ from peak3 (figure 4a and figure 4b) obtained from FTIR analysis of coated fabric (with gelatin and starch) revealed the presence of $=\text{C}-\text{O}-\text{C}$ and $-\text{OH}$ group.

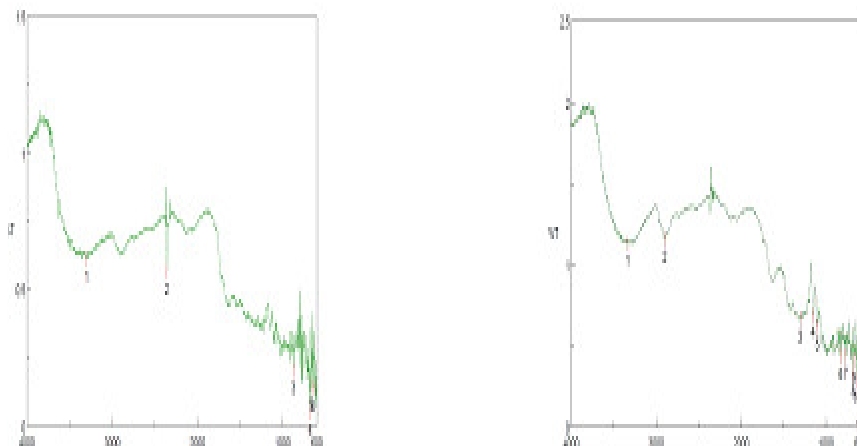


Figure 4: FTIR spectra of control textile laminates

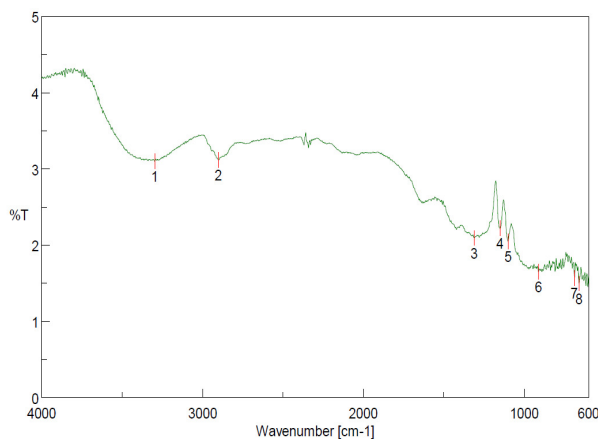


Figure 5: FTIR spectra of control textile laminates

The vibrational stretch observed at $\sim 2358.52\text{ cm}^{-1}$ and $\sim 2809.4\text{ cm}^{-1}$ of peak 2 (figure 4a and figure 4b) revealed the presence of strong band of $-\text{C}=\text{O}$ and $-\text{CH}$ group. These strong bands were absent in control fabric (figure 4c). The vibrational stretch observed at $\sim 874.56\text{ cm}^{-1}$ and $\sim 1305.5\text{ cm}^{-1}$ from peak3 (figure 4a and figure 4b) obtained from FTIR analysis of coated fabric (with gelatin and starch) revealed the presence of $=\text{C}-\text{O}-\text{C}$ and $-\text{OH}$ group. On comparison of vibrational stretch observed at $\sim 677.85\text{ cm}^{-1}$ and $\sim 1301.5\text{ cm}^{-1}$ of peak 4 (figure 4a and 4b) revealed the presence of $-\text{CH}$ and $-\text{NH}$ group. The vibrational stretch obtained from FTIR analysis of coated fabric revealed the presence of $-\text{CH}$ and $-\text{OH}$ group at $\sim 665.32\text{ cm}^{-1}$ and $\sim 1103.8\text{ cm}^{-1}$ of peak 5 (figure 4a and figure 4b). The $-\text{CH}$ and $-\text{OH}$ group were not observed in control fabric (figure 4c). The vibrational stretch observed at $\sim 874.56\text{ cm}^{-1}$ and $\sim 1305.5\text{ cm}^{-1}$ from peak 6 (figure 4a and figure 4b) obtained from FTIR analysis of coated fabric revealed the presence of $=\text{C}-\text{O}-\text{C}$ and $-\text{OH}$ group. The result of FTIR- Spectra of control fabric shows no strong band when compared with the developed fabric. The stretching bands obtained from 600 cm^{-1} to 1100 cm^{-1} were observed to be $-\text{C}-\text{O}-\text{C}$ groups and the stretching bands obtained from 2000 cm^{-1} to 4000 cm^{-1} were observed to be CH_3 groups were represented in figure 4c.

3.7. Physical and functional analysis for developed textile laminates

3.7.1. Water repellency test for developed textile laminates (spray test method 1941 by AATCC RA63)

On comparison of water repellency test for coated and control fabrics, observed to be increased water repellency for coated PHB fabric with gelatin (50) and PHB with starch (51) than the control fabric (0), the results were observed from table 3. Anton *et al.*, (2000) reported the fabric coated with fatty acid (2%) showed to be increased water repellency.

Table 3: The assessment of water repellency test under AATCC RA 63

Properties	Water repellency of cotton fabric		
	Control fabric	PHB with gelatin coated fabric	PHB with starch coated fabric
Water Repellency	0	50	51

3.7.2. Absorbency for developed textile laminates – (wicking, wetting, warping 1954 by Aatcc ra 34)

Table 4: The assessment of absorbency test under AATCC RA 34

Absorbency of cotton fabric			
Properties	Control fabric	PHB with gelatin coated fabric	PHB with starch coated fabric
Absorbency (seconds)	4.5 seconds	5.8 seconds	5.15 seconds

On comparison of absorbency test for coated and control fabrics, observed to be more time taken for coated PHB fabric with gelatin (5.8 seconds) and PHB with starch (5.15 seconds) than control fabric (4.5 seconds), the results were observed from table 4. Kissa (1996) reported that when the fabric is coated with gelatin, it took longer time for a drop of water to be adsorbed compared to control fabric. Schindler *et al.*, (2004) reported the fabric coated with sunflower oil ester show slight increase in water absorption

3.7.3. Measurement of abrasion resistance (ASTM D 3885-07)

Table 5: The assessment of abrasion resistance under ASTM D 3885-07

Properties	Cotton fabric – Abrasion Resistance (Flex)			
	Direction	Control fabric	PHB with gelatin coated fabric	PHB with starch coated fabric
No. of strokes to abrade the fabric	Warp	57	91	76
	Weft	67	82	71

On comparison of abrasion resistance test for coated and control fabrics, observed to be increased number of strokes (warp and weft) for coated PHB fabric with gelatin (91, 82) and PHB with starch (76, 71) than control fabric (57, 67), the results were observed from table 5. An (2001) reported the fabric is coated with gelatin, showed the increased abrasion resistance compared to control fabric.

3.7.4. Measurement of air permeability (ASTM-D-737-2004)

Table 6: The assessment of air permeability (ASTM D 737-2004)

Air permeability of cotton fabric			
Properties	Control fabric	PHB with gelatin coated fabric	PHB with starch coated fabric
Air permeability (c.c/cm.sq./sec)	92.2	160	127

On comparison of air permeability for coated and control fabrics, observed to be increased air permeability for coated PHB fabric with gelatin (160 c.c/cm.sq./sec) and PHB with starch (127 c.c/cm.sq./sec) than control fabric (92.2 c.c/cm.sq./sec), the results were observed from table 6. As polyurethane polymer was confined to the surface of the yarn and fabric, the air permeability reduces through the surface effect of enhancing the resistance to the flow of air through the fabric (Anderson and Dawes, 1990).

3.7.5. Tensile strength (IS 1969/85)

On comparison of tensile strength test for coated and control fabrics, observed to be increased thickening and elongation for coated PHB fabric with gelatin (27.02, 14.52) and PHB with starch (25.88, 13.18) than control fabric (25.07, 10.99), the results were observed from table 7. Most widely used materials woven fiberglass coated with polytetrafluoroethylene (PTFE) were observed with increased strength, durability and environmental resistance. The space between the fibres has been decreased so that, the thickening of the fibres has increased after coating (Anderson and Dawes, 1990).

Table 7: The assessment of tensile strength (IS 1969/85)

Tensile strength of cotton fabric			
Direction	Control fabric	PHB with gelatin coated fabric	PHB with starch coated fabric
Warp strength (kg)	25.07	27.02	25.88
Warp Elongation (%)	10.99	14.52	13.18
Weft Strength (Kg)	20.20	25.04	23.0
Weft Elongation (%)	12.13	15.15	15.60

3.7.6. Tear strength (Elmendorf method ASTM D 1424-07)

Table 8: The assessment of tear strength (Elmendorf method ASTM D1424-07)

Tear Strength of cotton fabric			
Direction	Control fabric	PHB with gelatin coated fabric	PHB with Starch coated fabric
Warp (gf).	934.4	1094.4	1007.5
Weft (gf).	672.0	806.4	712.3

On comparison with tear strength for developed and control fabrics, the warp and weft observed to be increased for developed fabric PHB with gelatin (1094.4, 806.4) and PHB with starch (1007.5, 712.3) than control fabric (934.4, 672.0), the results were observed from table 8. The tear strength of the nylon show lesser value than the cotton fabric when the ratio $l\ c/l\ y \leq 1$ (Realff, 1991). The coated fabrics were observed with increased tear strength

which had many applications in textile industry. As the concentration increases from 20g/l - 60g/l the thickness of treated fabric also increased due to the percentage gain in weight of the fabric (Anderson and Dawes, 1990).

3.8. Comparative study of invitro degradation of developed textile laminates

3.8.1. Physical characterisation for invitro biodegradation of textile laminates

The morphological appearances of treated textile laminates (PHB with gelatin and PHB with starch) were represented in figure 5a and 5b.

The results showed that the larger parts disappeared and only chunk of degraded materials remained and little erosion was found in textile laminates. Huang *et al.*, (2007), treating of developed textile laminates and nanofibrous scaffolds with phosphate buffer saline (PBS) in the presence of depolymerase (*P.stutzeri* BM 190) in which it were observed that the nanofibrous scaffold is severely eroded when compared to textile laminates



Figure 5a: SEM image of invitro biodegradation- PHB with gelatin textile laminate

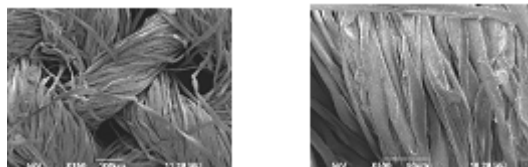


Figure 5b: SEM image of invitro biodegradation -PHB with starch textile laminate

4. Conclusion

Biodegradable polymers were developed as the matrix polymers for composite materials. The various physical characterisations done for the finished fabrics showed the quality for its application in textile industry. This work may provide a new technological route for the fabrication of environmentally friendly materials from renewable resources. Coated fabrics have gained a wide application as medical fabrics i.e. wound dressing, protective clothing etc.

5. References

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