

Full Length Research Paper

Synthesis of copolymer from lactic acid-polyethylene terephthalate (LA-PET) copolymerization

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Bio-plastic has been a need of the hour for the past few decades and the usage of lactic acid (LA) in the production of bio plastic opens a new window to the field. Polyethylene terephthalate (PET) thermoplastic polyester with excellent tensile and impact strength, chemical resistance, clarity, process ability, and transparency has become a bane to the society. The co polymerization increases the mechanical strength of LA. In this study, the production and optimization of the copolymer of LA –PET was done. The blend of sugarcane bagasse and corn stover was used for the production of LA by incubating with *Lactobacillus* sp. for 24 h. *Lactobacillus* sp. is a potent producer of lactic acid and crude curd was used as the source. The optimization in lignin breakdown by 10% NH₄OH and 2% NaOH and acid hydrolysis by 10% H₂SO₄ of the blend was done. The process parameters in the copolymerization LA-PET using p-toluene sulfonic acid, ethylene glycol and stannous chloride were also analyzed. The products formed were confirmed by the FTIR analysis at various transmittance peaks at 1681, 3008 and 3610 cm⁻¹. The process of production of the copolymer proves to be a green technology by decreasing the PET contamination of the environment and also helping in the production of bio-degradable plastics in the run.

Key words: Lactic acid, copolymer, bioplastic, polyethylene terephthalate.

INTRODUCTION

Lactic acid is one of the most important organic acid today, due to its recently gained attention of its biodegradable nature and of its potential polymer application. Conventionally, it is produced by the fermentation of the cellulosic materials with the help of microbes (Sharon and Sharon, 2003; Ahmed and Discher, 2004) and extraction of primary metabolite lactic acid. Previous reports have indicated the sole usage of corn stover or bagasse as a substrate for lactic acid production but the results

indicated lower yield and extraction difficulties. Polyethylene terephthalate (PET) is a thermoplastic resin of the polyester family and is used in synthetic fibers. PET is produced by the polymerization of ethylene glycol and terephthalic acid (Chetna and Madhuri, 2003). Ethylene glycol and terephthalic acid, when heated together under the influence of chemical catalysts, produces PET in the form of a molten, viscous mass that can be spun directly to fibers or solidified for later processing as a plastic

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(Abdelaal et al., 2008). It is synthesized by the tranesterification reaction between ethylene glycol and dimethyl terephthalate (Zulfiqar et al., 2006). The presence of a large aromatic ring in the PET repeating units, gives the polymer, notable stiffness and strength, especially when the polymer chains are aligned with one another in an orderly arrangement (Fariyal et al., 2004). The substance is not classified as dangerous according to Directives 67/548/EEC and 1999/45/EC.

Bio-plastic has been the need of the hour since plastics started showing their true nature. Bioplastics have proven advantageous over their relative plastics, by their easy degradation in the environment without reverse effects. There have been various approaches in producing these plastics, but only few found success. Natural polymers from plants such as cellulose and starch, have been functionally modified and crafted to thermoplastic or synthetic polymers which in few cases, have added monomers like lactic acid (Achani et al., 2011). The material produced represents a high-molecular polymer which is practically insoluble in water (Magdy et al., 2008).

In this study lactic acid was produced from bagasse and corn stover blend which was delignified using 2% NaOH and 10% NH₄OH (Dennis et al., 2004) and sequentially it was acid hydrolyzed using 10% H₂SO₄ resulting in cellulose breakdown to glucose. This serves as the carbon source for the microbe to act on and produce lactic acid (Zulfiqar et al., 2006). The study also employs the usage of copolymerization technique to produce bioplastic consisting of LA and PET. The copolymerization is carried out, after the glycolysis of PET to BHET using ethylene glycol and further grafting to lactic acid (Chetna and Madhuri, 2003) to finally yield the product polymer.

MATERIALS AND METHODS

Raw material

Bagasse obtained from Bannari Amman Sugars Private Ltd., were dried at 70°C for 15 h or until dry weight of the bagasse is measured as constant. Then, it was milled and homogenized to obtain small particles of approximately 5 mm in size and stored at cool temperature. Corn stover which was purchased locally from Sathyamangalam market was also dried at 80°C for 4 h and ground and homogenized to smaller particles of size 2 mm and stored at cool environment. All the chemicals used in the study were purchased from Himedia Laboratories, Mumbai, India and of analytical grade.

Lignin extraction

10% NH₄OH and 2% NaOH was used in order to separate the lignin from bagasse and stover. The experiment was carried out at 37°C for 24 h. Total amount of sample used was 1 g and the composition used in the subsequent trials of the experiments were: a) 1 g of bagasse, b) 0.5 g of bagasse and 0.5 g of corn stover and c) 1 g of corn stover. In the second case, the concentrations of

NaOH and NH₄OH were maintained constant along with the amount of sample. Here the compositions used were: a) 0.2 g of bagasse and 0.8 g of corn, b) 0.4 g of bagasse and 0.6 g of corn, c) 0.6 g of bagasse and 0.4 g of corn, and d) 0.8 g of bagasse and 0.2 g of corn. After a period of 24 h, the samples were filtered cautiously using Whatmann No.1 filter paper. The residues collected in the filter paper were dried in a hot air oven at 190°C and filtrates were taken for analysis. The lignin content present in the soluble fraction was measured in visible spectrophotometer at an absorbance of 549 nm (Zulfiqar et al., 2006).

Acid hydrolysis

After lignin extraction, the dried residue was subjected to treatment with 10% HCl/H₂SO₄ for hydrolysis (Wathana et al., 2012). The entire process was carried out in a controlled environment of temperature of 120°C and for a time period of 60 min (Pattana et al., 2009; Ziyu et al., 2009).

Lactic acid production

Lactobacillus sp. was isolated from the butter milk by centrifuging 15 mL of the sample at 5000 rpm for 5 min at 4°C after which the supernatant was discarded (Bridget et al., 2011). The debris or the pellet left, was thoroughly mixed with PBS and made up to a volume of 10 mL. The cultured strains in PBS were then, added to the acid hydrolyzed samples of bagasse and corn stover. The setup with mixture was placed in an orbital shaker for a time period of 24 h and at 100 rpm and 37°C. After 24 h, the samples were taken and again subjected to centrifugation at 8000 rpm for 3 min and following which the supernatant was extracted for the estimation process (Bertrand et al., 2011; Woranart et al., 2012).

Lactic acid estimation

For the evaluation and estimation of lactic acid, the lactic acid produced has to be extracted from the supernatant by using n-butanol as a solvent for its extraction (Kanungnit and Panarat, 2011). So for, the extraction purpose, n-butanol and the samples were taken at a ratio of 4:1 in a separating funnel. Both n-butanol and the supernatant were taken in the separating funnel and mixed thoroughly and allowed to stand for about 1 h resulting in complete phase separation. Here, the aqueous phase consists of the lactic acid and thereby requires simple removal of bottom phase. The extracted lactic acid was then taken for titration process, where NaOH was taken as the titrant and phenolphthalein as the indicator for the titration purpose. The presence was estimated based on the color produced. Its presence can be confirmed by using p-phenyl phenol reagent, where 0.7 mL of supernatant was added with 3 mL of 96% sulphuric acid, followed by heating for 10 min in boiling water bath and then cooling it to room temperature for about 30 min. The cold solution was added with 50 µl 4% copper sulphate and 100 µl p- phenyl phenol (prepared by dissolving 1.5% of the reagent in 95% ethyl alcohol) which provided a chromogenic complex. The absorbance for lactic acid is measured in UV-VIS double beam spectrophotometer at 570 nm (Ghosh and Ghosh, 2012). This method is otherwise called Kimberly Taylor method.

Glycolysis of PET

40 g of PET was taken and cut into very thin films. The cut pieces were taken in a 500 mL two necked round bottom flask with a reflux condenser attached to it. To it, 70.5 mL of ethylene glycol was added which was dried before (that is completely free from moisture) followed by the maintenance of a constant temperature of

Table 1. Weight of lignin produced by the samples using two reagents NaOH & NH₄OH.

Sample	reagent	Composition	weight of lignin
a	NH ₄ OH (24 h)	Bagasse	0.018
b		Corn	0.03
c		Blend	0.082
a	NH ₄ OH (48 h)	Bagasse	0.036
b		Corn	0.031
c		Blend	0.13
a	NaOH	Bagasse	0.029
b		Corn	0.04
c		Blend	0.142

180°C in an oil bath for about 5 h (Abdelaal et al., 2008). In this, zinc acetate was used as the catalyst and the amount taken is 0.2% by weight of PET used. The reaction, occurring in the round bottom flask was basically glycolysis which will finally result in the production of BHET.

PET-LA copolymerization

35 g of BHET was made to react with lactic acid in the presence of stannous chloride (SnCl₂.2H₂O) which was taken to be around 0.5 to 0.8 weight % along with p-toluene sulfonic acid (0.91 mol/mol of SnCl₂) (Achanai et al., 2011). This reaction mixture was again taken in a 500 mL round bottom flask which was to be immersed in an oil bath at a temperature of 180 to 210°C kept for 6 to 8 h. As the process continues, water and glycol was removed from the reaction simultaneously and the remaining mixture was dissolved in chloroform, which was later precipitated in methanol and filtered and dried at 90°C (Jin et al., 2001).

RESULTS AND DISCUSSION

Weight of lignin

Weight of lignin extracted was calculated after evaluating the dry weight (Table 1) of the residue containing the delignified mixture of bagasse, corn and their blend. The process included two reagents for delignifying, that is NaOH and NH₄OH. After evaluation, it was found that the amount of lignin separated by using NaOH was high compared to NH₄OH (Table 1, Figure 1).

Efficiency of lignin removal

To check the efficiency of lignin separation performed on different substrates like bagasse, corn stover and also the blend of both bagasse and corn, percentage efficiency was calculated (Table 2). By comparing the observations, it was found that the amount of lignin removed was high when bagasse and corn stover were

taken up as a blend rather than individual delignification (Figure 2).

Blend composition

From the previous trials, the higher lignin separation in blend was further optimized by measuring various compositions of blend of bagasse and corn (Table 3). The process of delignification with 10% NaOH was repeated and it was found that blend with a composition of bagasse taken at 0.8 g and corn at 0.2 g showed high lignin content removal. The results were conformed and evaluated using visible spectrometer at 549 nm (Zulfiqar et al., 2006) (Figure 3). This blend provided the best composition for producing lactic acid and further for the production of bioplastic.

Lactic acid production and extraction

The production of lactic acid involved *Lactobacillus* sp., and its production during the bacterial growth curve was tracked for the efficient removal of lactic acid and extraction. It was determined that the lactic acid production reached a peak at the end of 3 h. This also in turn decreased the pH of the media to the acidic level of 4.5 (Ghosh and Ghosh, 2012). The graphical representation of the same is given below (Figure 4).

Extraction of lactic acid

The separation of lactic acid from the media was a strenuous task and the extraction was done using n-butanol. The extraction process was carried out in a 4:1 ratio of butanol with respect to sample taken (Kanungnit and Panarat, 2011). The extraction showed high efficiency (Figure 5a) and this was attributed to the direct solvation between n butanol and lactic acid. The further increase of the organic phase was not performed since n -butanol is partially miscible in water. It was also noted that the extraction process when varied with respect to pH, showed maximum degree of extraction at pH 1 (Figure 5b). Thus, the decrease in the pH of the media due to lactic acid production contributed significantly to its extraction process using n-butanol. The mechanism behind this being solvating extraction method of n-butanol. The higher distribution co-efficient at low pH is mainly due to the availability of unassociated lactic acid form, which at low pH presents itself in a large manner.

Bis(2-hydroxyethyl) terephthalate (BHET) formation

BHET was formed by the glycolysis process, where PET was reduced to BHET by distilling it with proportions of ethylene glycol (Sharon and Sharon, 2003). The

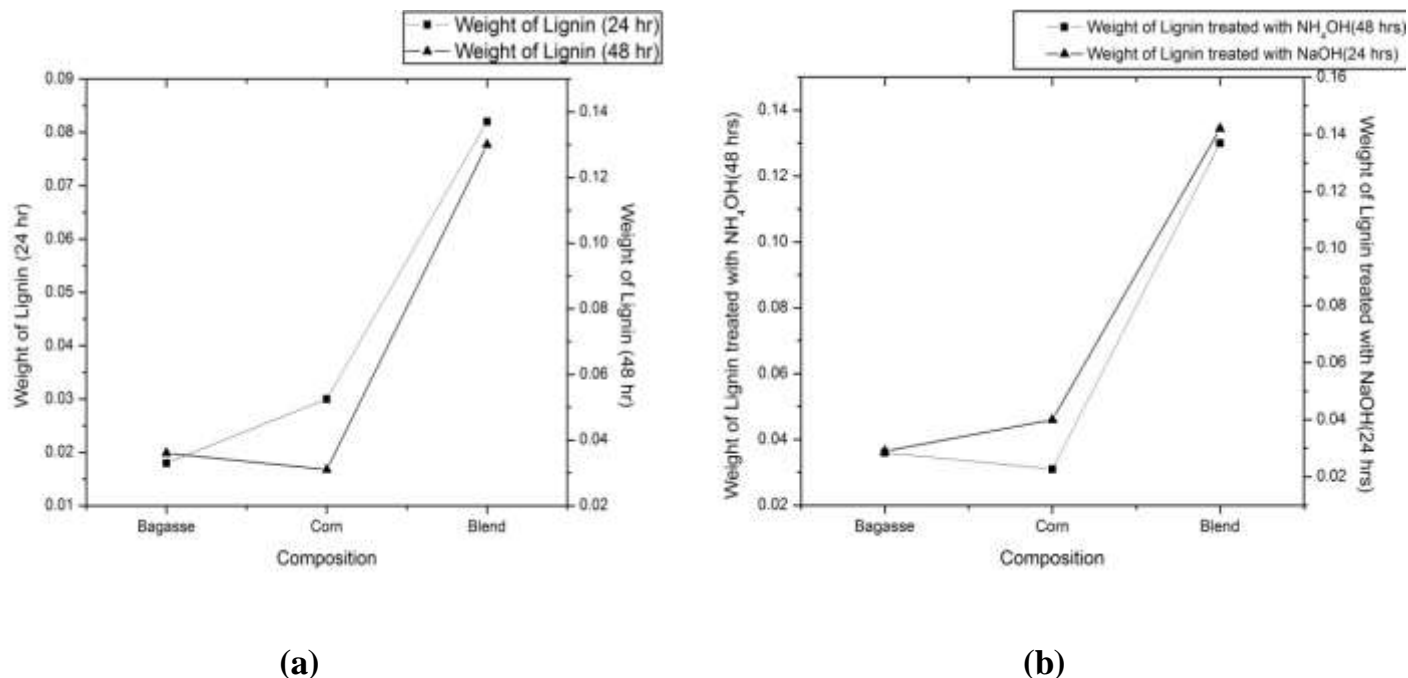


Figure 1. a) Amount of lignin removed on treatment with 10% NH₄OH at 24 and 48 h. The amount of lignin removed was determined spectrophotometrically at 549 nm. **b)** This graph depicts the amount of lignin removed from bagasse, corn and the blend on treatment with NH₄OH for 48 h and NaOH for 24 h.

Table 2. The efficiency of lignin removal by the two reagents NaOH & NH₄OH.

Sample	Reagent	Composition	Percentage efficiency of lignin removal	OD (549 nm)
1	NH ₄ OH (24 h)	Bagasse	18	0.012
2		Corn	28.30	0.022
3		Blend	40.19	0.079
4	NH ₄ OH (48 h)	Bagasse	33.96	0.052
5		Corn	30.097	0.029
6		Blend	63.41	0.091
7	NaOH	Bagasse	29	0.023
8		Corn	38.83	0.068
9		Blend	69.26	0.1

formation of a needle like white crystals on the inner walls of round bottom flask at 180°C during the glycolysis process of PET was visually confirmed and they were separated manually

Copolymerization

During the copolymerization process, the temperature was maintained at 180 to 200°C throughout the process. After completion of the copolymerization, both chloroform

and methanol were added resulting in the formation of two layers with a white precipitate at the bottom part and in the upper part, all the non-copolymerized waste residues were obtained. After filtering and drying of the precipitate, a white powder was obtained which had a melting point of 170°C confirming the formation of copolymer and on melting it at the characteristic melting point, thin films were obtained. The thin films were rigid and tensile in nature even after 2 weeks and the characteristic reaction taking place is depicted in Figure 6. The thin films formed were sent for FTIR analysis and the results

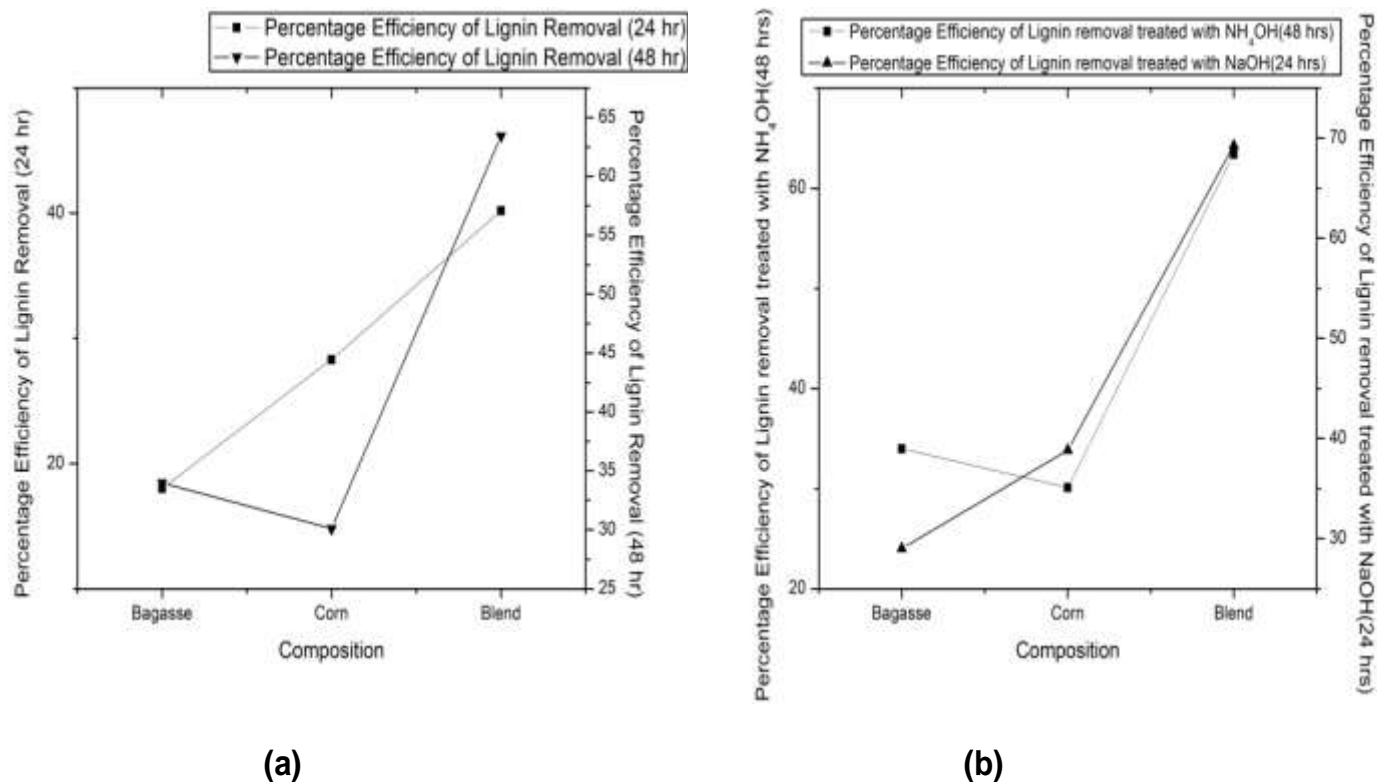


Figure 2. a) Percentage efficiency of lignin removal on treatment with NH_4OH at 24 and 48 h. The percentage efficiency of lignin removal was comparatively higher for the blend on treatment with NH_4OH for 48 h. b) Percentage efficiency of lignin removal on treatment with NaOH and NH_4OH for 24 and 48 h, respectively. Amount of lignin removed and its percentage efficiency were higher for blend on treatment with NaOH for 24 h

Table 3. The OD (549 nm) value of varying amount of bagasse and corn taken.

Sample	Bagasse	Corn	OD (549 nm)
1	0.2	0.8	0.047
2	0.4	0.6	0.053
3	0.5	0.5	0.063
4	0.6	0.4	0.071
5	0.8	0.2	0.086

obtained are shown in Figure 7 and Table 4. The lignin from the blend concentration (0.8 bagasse + 0.2 corn stover) using NaOH reagent and acid hydrolysis at 180°C was found to be the best optimized conditions for the production of lactic acid. The glycolysis of PET and the copolymerization process of BHET and LA were confirmed using FTIR results. The copolymerized product from lactic acid and polyethylene terephthalate has been reported before to be a biodegradable plastic. The plastic film produced after melting the powder product showed increased stability. The melting point of the powder depends on the rate to which the process of polymerization has taken place. So, based on the product

needed, the melting point of the polymer can be set approximately.

Conclusion

This material however has biodegradability, and does not render any harmful influence on the environment. They contain a large ratio of carbon from the carbon dioxide they have drawn from the air and fixed, due to the photosynthetic process. Thus, the bioplastic produced will be an opening to the near future in providing a successful sustainable environment for coming future generations.

Conflict of interests

The authors did not declare any conflict of interest.

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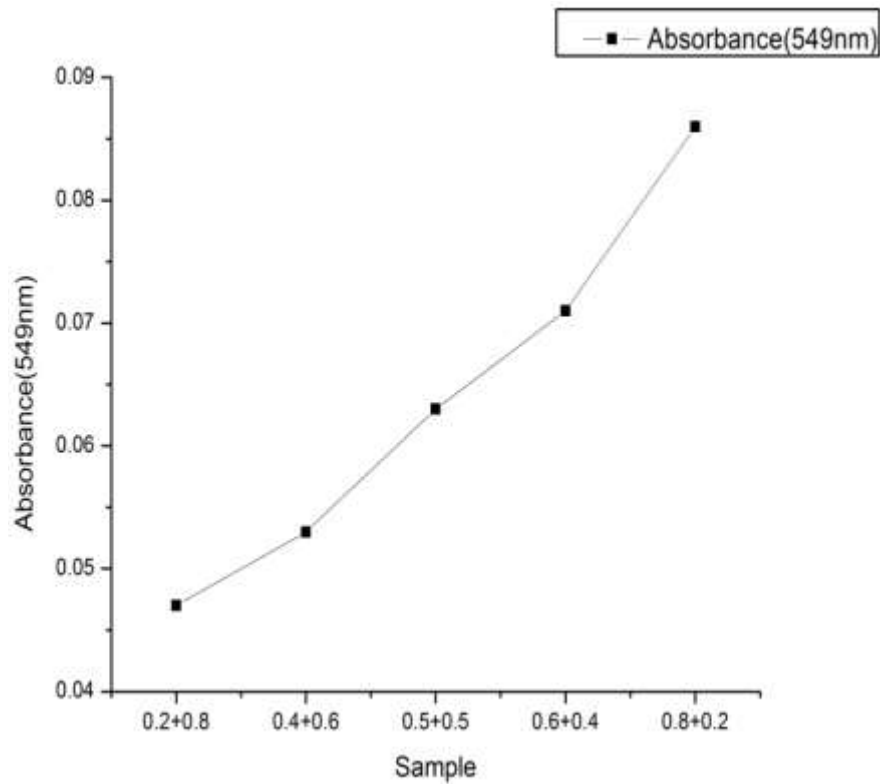


Figure 3. Blend optimization was done for various compositions of bagasse and corn and treatment with NaOH for 24 h was carried out to determine the maximum amount of lignin removal. Blend composition of 0.8 g of bagasse and 0.2 g of corn showed high absorbance at 549 nm depicting the maximum amount of lignin removal.

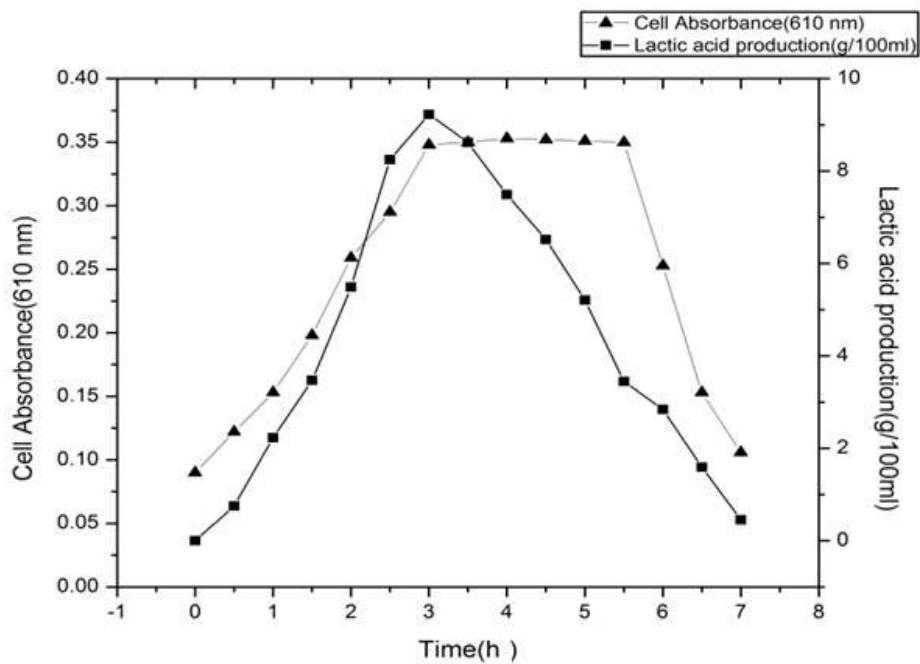


Figure 4. Comparison of Bacterial growth curve of *Lactobacillus sp.* and lactic acid production (g/100 mL) by bacteria.

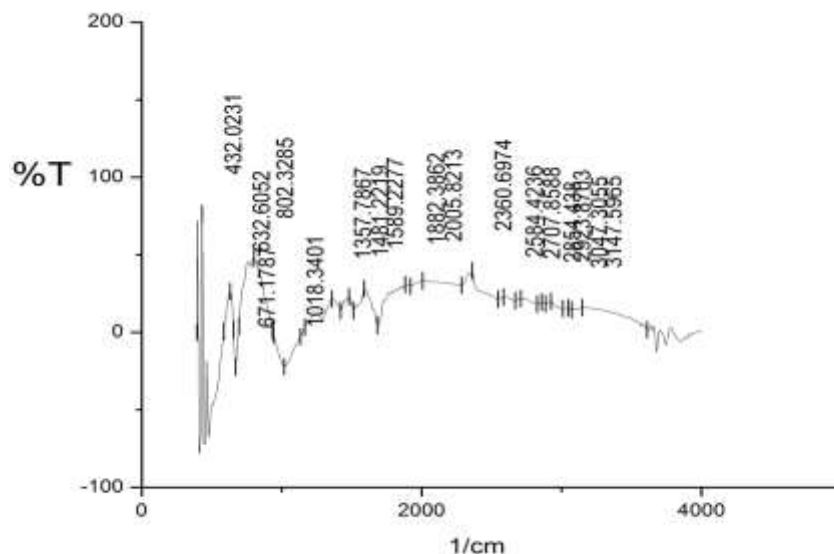


Figure 7. FTIR studies were performed to study the extent of copolymerization of lactate monomers with BHET. Absorption peaks were absorbed at the wave numbers of 1681.804 and 1134.0605 cm^{-1} representing the stretches of C=O and C-O bonds which confirms the presence of an ester group between the BHET and Lactate monomers.

Table 4. Different wave numbers showing the bond between different molecules [FTIR study].

Absorption (cm^{-1})	%T	Functional group
586.31	0.56	
655.75	1.86	Ring plane bending
694.32	3.84	C-C in plane bending
933.48	1.67	C-H in plane bending phenyl ring
1134.06	-2.83	C-O-C
1419.50	14.20	COO-
1512.08	14.01	C=C symmetric stretch
1681.80	4.40	Ar-C=O
1920.96	29.70	C-H out of plane bending
2283.55	30.34	H bonding
2545.85	21.40	C-O stretch in carboxylic group
2669.28	20.40	C-O stretch in carboxylic group
2823.58	18.17	C-C stretch
2885.30	18.44	C-H stretch
3008.73	15.30	CH aromatic
3070.45	14.60	CH ₂ symmetric stretch
3610.48	1.60	OH

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