

Development of lignin-based biodegradable polymer from Agrowaste

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ABSTRACT

Biosourced and biodegradable polymers have garnered substantial attention for their role as sustainable and environmentally friendly alternatives to traditional synthetic polymers, finding diverse applications across various fields. The escalating global demand for food production and a surge in agricultural activities have resulted in a significant increase in agrowaste generation. In India, an agrowaste annual output ranging between 85-100 million tonnes is observed, with wheat straws contributing substantially to approximately one-third of the total agro residue [6]. Addressing the challenge of agrowaste utilisation, we present an innovative methodology for converting wheat straw into a Biodegradable polymer (acetyl ferulic acid polymer). The process involves the extraction of lignin through an acid-base neutralisation reaction, with the qualitative confirmation of lignin presence achieved through Safranin dye testing. The extracted lignin undergoes oxidation via the Nitrobenzene Oxidation (NBO) method, facilitating the conversion into Vanillin through sidechain oxidation of the complex lignin structure. The subsequent acetylation of Vanillin is carried out to obtain acetyl ferulic acid, which is then polymerised utilising zinc acetate as a catalyst. Glycerol is employed as a plasticiser, and the biodegradability of the resulting polymer is assessed within a controlled ambient decomposition environment in the laboratory. Decomposition data is subjected to modelling using Wolfram Mathematica, yielding the decomposition time. Our findings assert that the developed biodegradable polymer requires approximately 105 days to decompose, achieving a degradation rate of approximately 99.98%. This newly synthesised biopolymer is posited to exhibit compatibility with existing biodegradable polymers, thus contributing to the advancement of sustainable polymer materials.

INTRODUCTION

Synthetic plastics are one of the almighty innovations paved for massive development yet provided with their bane for non-biodegradability, leading to several environmental and ecological issues. According to the Central Pollution Control Board of India [1], the estimated plastic waste generation during 2019-20 was approximately 34,69,780 Tons Per Annum (TPA) [1]. Only 9% of plastic waste is recycled while another 19% is incinerated, 50% ends up in landfill, and 22% goes into uncontrolled dumpsites, is burned in open pits or ends up in terrestrial or aquatic environments, especially in poorer countries [2]. As these hazardous plastics continuously pollute the natural environment, the development and production of environmentally conserved biodegradable plastics are rapidly expanding to trim down our reliance on synthetic plastics.

Straw is a softwood produced as a byproduct of crop production at harvest. Straw is removed from the grains during harvest and ends up being piled or spread out in the field depending on whether it was harvested manually or using machines. According to the International Rice Research Institute, globally, roughly 800 to 1,000 million tons of rice straw per year are produced in the Philippines, with about 600 to 800 million tons per year being produced in Asia. This continues to rapidly increase due to the shorter turnaround time required for intensified rice cropping [7]. As far as wheat straw is concerned, India produces 100 million tons yearly[3].

Wheat and Rice straw contain 17.5 – 30 % and 15.6 – 25 % dry weight of Lignin, respectively. Lignin is a heavily branched and complex molecule that can be degraded using the Nitrobenzene Oxidation (NBO) Method, eventually giving rise to Vanillin [Figure 1]. Vanillin is the key component responsible for forming Biodegradable plastics, comparable to polyethylene terephthalate (PET). Vanillin can be acetylated using Sodium Acetate and Acetic Anhydride to form acetyl ferulic acid, which can be polymerised using Zinc Acetate $Zn(OAc)_2$ and processed further to form Bioplastics.

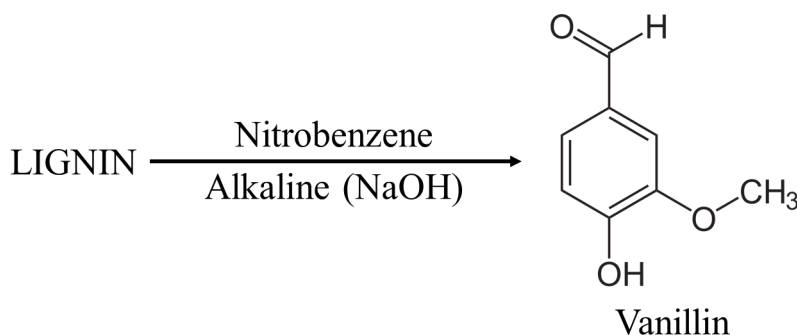


Figure 1: Reaction Scheme to form Vanillin from Lignin

EXPERIMENTS AND METHODS

Materials: Wheat straws were collected from local fields (Villages of Bakaniya and Barkheda Salem) in Madhya Pradesh, India, 25% Sodium Hydroxide, Nitrobenzene, Dichloromethane, Laboratory grade Vanillin, 30% Sodium Sulfate, Methanol, Hexane, Sulfuric Acid, Hydrochloric Acid, Zinc Acetate, EtOAc/Hexane solutions, diethyl-ether, AceticAnhydride, Glycerol, and Distilled Water

Preparation of Wheat straws for processing:

We took 10 grams of Wheat Straw, which was finely washed and ground with the grinder until the final straw size reached 1 – 2 mm. Straws were transferred into a Round Bottom Flask.

Preparation of white liquor and extraction of lignin:

We prepared the white liquor under an active alkali charge of 25% NaOH and sulphidity using 30% sodium sulfide (Na_2S) in a 3:1 ratio. A mixture of 10 grams of wheat straw and white liquor in a 6:1 ratio was refluxed at 160°C in an oil bath for 3 hours, forming a dark brown-coloured viscous solution, known as black liquor, with untreated straw residue. Vacuum filtration separated the residue, yielding black liquor as the filtrate. The black liquor exhibited an initial pH of approximately 13, subsequently neutralised with 98% pure H_2SO_4 , and gradually acidified to achieve a pH of 2-3, inducing a colour change from dark brown to peanut brown and precipitation. After overnight incubation, the solution was filtered and washed with hot water to eliminate excess H_2SO_4 , and the resulting solid lignin residue was air-dried for 24 hours at 65°C. The obtained woody brown lignin was finely ground using a mortar and pestle and quantitatively stored in vials. Qualitative testing of the lignin was conducted using Safranin dye, and the results were reported, providing insights into the efficacy of the lignin extraction process.

Synthesis of Vanillin from Extracted Lignin using the NBO method

1.5 grams of dried Lignin underwent an oxidation process facilitated by a mixture containing 52.5 ml of 2M NaOH and 3.75 ml of Nitrobenzene, acting as a mild oxidising agent under alkaline conditions to support side-chain oxidation of Lignin. The resulting dark brownish solution was obtained after refluxing the mixture in an oil bath for 3 hours at 160°C. Subsequent separation of the contents yielded two distinct layers, with the upper layer subjected to pH testing, followed by neutralisation using 98% pure HCl until the solution reached a pH of approximately 4. The neutralised solution was then vacuum-filtered through a 90mm filter paper, and the filtrate was transferred to a separating funnel. To facilitate layer separation, 20 ml of Dichloromethane (DCM) was added, and the organic layer was extracted and further purified through additional DCM extractions. The crude product obtained after rotavapor evaporation of the DCM was stored in a 5 ml vial. The final product was characterised through Thin-Layer Chromatography (TLC) against laboratory-grade Vanillin, utilising different eluents (10%, 20%, 30%, and 40% EtOAc/Hexane solutions) followed by UV irradiation and stained with 2,4 DNP, providing valuable insights into the compound's composition and purity. This comprehensive

methodology offers a systematic approach to the oxidation and purification of Lignin, contributing to the understanding of its chemical transformation under the outlined conditions.

Formation of acetyl ferulic acid from Vanillin and further polymerisation using Zinc Acetate as the catalyst:

A synthetic route to acetylferulic acid was undertaken through the acetylation of Vanillin. Specifically, 1.00 grams of Vanillin was combined with 1.08 grams of Sodium Acetate and 4.7 ml of Acetic Anhydride in a 25 ml round bottom flask. The resulting mixture underwent reflux in an oil bath at 140°C for 24 hours, yielding a brown-coloured solution. Following this period, the solution was poured onto 50 grams of crushed ice in a 100 ml beaker, where it was stirred for 45 minutes, forming a brown sticky substance. Subsequently, the mixture was placed in a freezer at 4°C overnight. The water was removed upon thawing, and the remaining solution was dissolved in diethyl-ether. The diethyl-ether was then evaporated, leaving a layer of acetyl ferulic acid characterised by a beige-yellow colouration. This product was subjected to a 24-hour drying period in an oven. The ensuing acetylferulic acid was polymerised using 1 mol% of Zinc Acetate as a catalyst. The reaction mixture, comprising Acetylferulic acid and Zinc Acetate, was heated to approximately 200 degrees Celsius in an oil bath for 2 hours. Following polymerisation, any residual moisture was removed by vacuuming the contents of the Round Bottom flask. This methodology presents a systematic and reproducible approach to the synthesis and polymerisation of acetylferulic acid.

Formation of Bioplastic using polymerised Acetyl ferulic acid and testing it:

The polymerised compound, upon completion of the synthesis, underwent a drying process, following which it was finely ground to yield a pale yellow-coloured powder. Subsequently, this powder was combined with 0.9 ml of glycerol, serving as a plasticiser, and approximately 1 gram of de-lignified straw fibres (obtained in the first stage). The resultant mixture underwent controlled microwave irradiation for 45 seconds, promoting effective blending of the components. The plasticised material was shaped into the desired form and subjected to further processing following microwave treatment. Specifically, the moulded material was dried in a hot air oven maintained at 45 °C for 3 days.

Biodegradability test:

A mass of approximately 0.1001 grams of the synthesised bioplastic, derived from the experimental synthesis, was utilised for investigation. Employing a 5 cm × 5 cm cotton mesh, the bioplastic was enveloped, ensuring uniform coverage. A controlled soil environment was established to simulate environmental conditions and evaluate the material's biodegradability. The soil, housed within a designated container, was deliberately moistened to foster the proliferation of indigenous soil bacteria. Subsequently, the mesh-encased bioplastic specimen was interred within the prepared soil bed and concealed beneath a layer of damp soil. During the experiment, ambient temperature conditions were rigorously regulated, maintaining a constant

range between 25 and 27°C. These controlled parameters serve to facilitate a systematic investigation into the biodegradability of the synthesised bioplastic under simulated environmental conditions.

RESULTS

Extraction of Vanillin from Wheat Straw using Nitrobenzene Oxidation Method:

Lignin extraction from wheat straws:

After refluxing the 10gms of 1-2 mm-sized straws with white liquor for 3 hours at 160°C, Black liquor(Fig 2a,b) formed. Acidification with 98% pure H₂SO₄ followed by filtration produced a cream-coloured residue (Lignin), which was collected and dried. Approximately 3.152 grams of dried Lignin is extracted(Fig 2c,d). In the Safranin test, Safranin Dye changes colour from Deep Red to Pink, indicating a positive result for the presence of lignin.

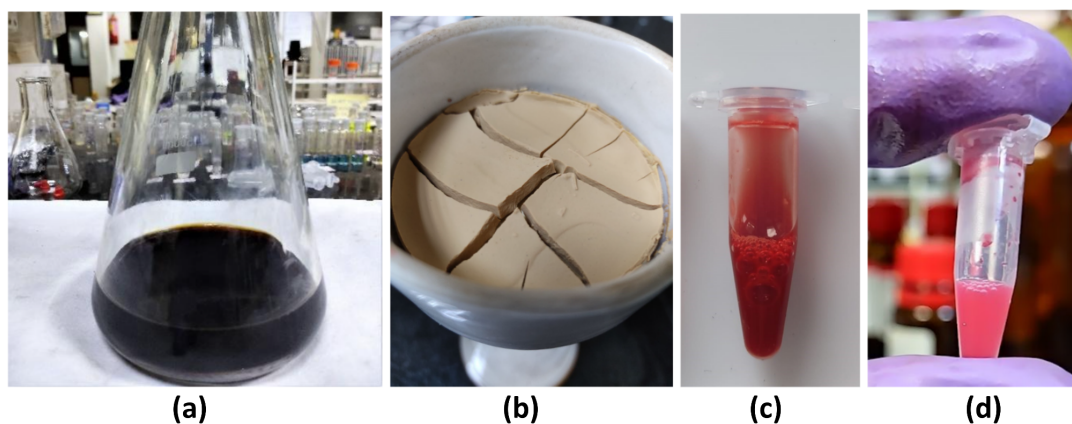


Figure 2: (a) Black Liquor formation after white liquor (basic) reflux at 160°C, (b) Acidification with 98% pure H₂SO₄ yields cream-coloured residue. (c) Safranin Control (d) Safranin dye changes colour from Deep Red to Pink, indicating the presence of lignin.

Vanillin synthesis from extracted Lignin:

1.5 grams of Lignin was refluxed with NaOH and Nitrobenzene at 160°C for 3 hours, followed by Nitrobenzene layer separation. Testing with a TLC plate against pure vanillin followed by 2,4 DNP staining showed no spots(Figure 3a), indicating no presence of vanillin in the Nitrobenzene filtrate. The water layer from reflux was acidified using 98% pure HCl and filtered using vacuum filtration. The filtrate was tested on another TLC plate against pure vanillin and showed a spot with the same R_f value as vanillin, indicating the presence of the product. The TLC plate was stained with 2,4-dinitrophenylhydrazine (2,4-DNP) to confirm the presence of a carbonyl group in the product. For the reconfirmation, the filtrate was washed with dichloromethane (DCM) 2-3 times and collected in a beaker by passing through anhydrous sodium sulfate (Na₂SO₄). The filtrate was tested

on a third TLC plate against pure vanillin and showed a spot with the same R_f value as vanillin(Figure 2b). The TLC plate was also stained with 2,4-DNP to confirm the presence of a carbonyl group in the product. The maroon band indicates the presence of vanillin. The filtrate was evaporated under reduced pressure using rota vapour to obtain the crude product as a yellow solid. The crude product was transferred to a vial(Figure 4a) and evaporated under reduced pressure to obtain the final product as a yellow solid at the end of the experiment. Vanillin is formed as the major product of this Trial. A strong vanilla-like smell appeared on smelling the Vial after 2-3 days. Many side products are formed when viewed under UV or stained with 2,4 DNP(orange-yellow solution), as shown in Figure 4(b). As side products are created, they will not interfere with further reactions as Vanillin is the major product.

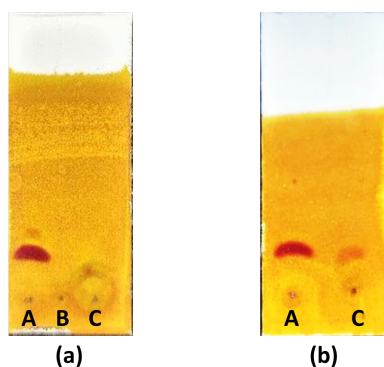


Figure 3: A - Standard laboratory grade Vanillin; B - Co-spotting of standard vanillin and extracted compound; C - Extracted compound (a) TLC Plate spotting with Nitrobenzene filtrate as extracted compound to check for the vanillin after 2,4 DNP (orange-yellow solution) staining, (b) TLC Plate spotting of acidified water filtrate to check the presence of the compound with 2,4 DNP giving faint reddish-brown band

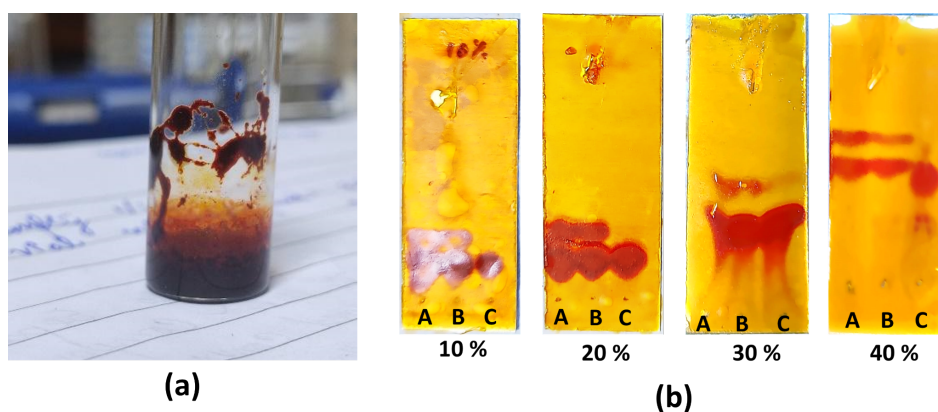


Figure 4: (a)Crude extracted vanillin with other minor products, (b) A - Standard laboratory grade Vanillin; B - Co-spotting of standard vanillin and extracted compound; C - Extracted compound. TLC staining against Laboratory–grade Vanillin using four different eluents, 10%, 20%, 30% and 40% EtOAc/Hexane solution, stained with 2,4 DNP

Formation of Acetyl ferulic acid from Vanillin and further polymerising it using Zinc Acetate:

The synthesis of poly(acetylferulic acid) was done in two steps. First, acetyl ferulic acid was obtained by acetylation of vanillin using acetic anhydride and sodium acetate as a catalyst. The reaction mixture was refluxed at 140°C for 24 hours, resulting in a dark brown liquid that solidified on cooling over ice and gave a pale yellow precipitate. The precipitate was purified by recrystallisation from diethyl ether (Figure 5a), which was then evaporated to yield acetyl ferulic acid as a thin layer (Figure 5b). Second, the polymerisation of acetyl ferulic acid was performed using zinc acetate as a catalyst under inert N₂ conditions. After polymerisation, the final product was obtained as a brown solid (Figure 5c).

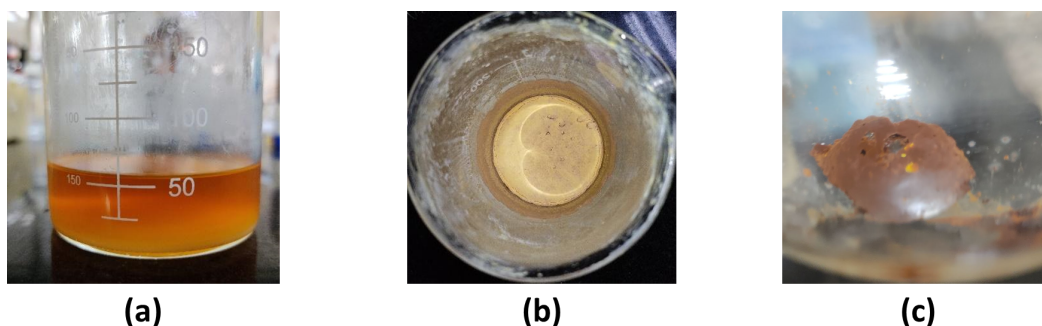


Figure 5: (a) Dissolved precipitate in diethyl-ether, (b) Acetyl ferulic acid layer formation after diethyl-ether evaporation, (c) final product obtained after polymerisation

Formation of Bioplastic using polymerised Acetyl ferulic acid and testing it:

After grinding the polymer into fine powder, a change in colour from brown to yellow (Figure 6 a,b) was observed. The polymer is then mixed with Glycerol and small stubble pieces to form a thick paste. This paste is used to mould into desired shapes and let it dry. Once dried, we obtained a 1mm thick sheet of bioplastic. The top view of the bioplastic sheet showed a smooth and uniform surface (Figure 6c).

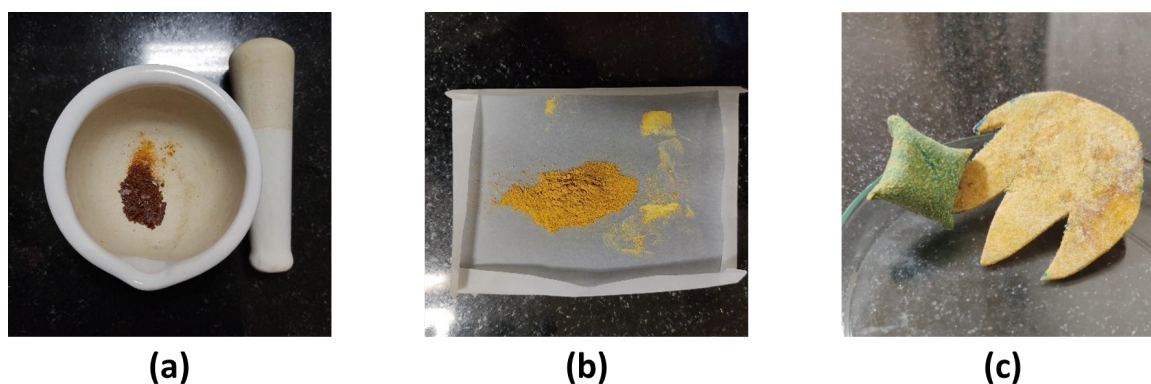


Figure 6: (a) Grinding the Biopolymer into fine particles, (b) Change in colour from brown to yellow, (c) Bioplastics made from the biodegradable polymer.

Bioplastic degradation:

We took an initial mass of 0.1001 grams of bioplastic and buried it in the soil with an ambient temperature between 25°C and 27°C. Proper moisture is provided so that the natural soil bacteria can degrade the bioplastic.

With the obtained data, we extrapolate using mathematical modelling in Wolfram Mathematica to get the total decomposition time of around 105 days (3 months)

Mass of Bioplastic (in g)	Time (in Hours)
0.1001	0
0.0904	24
0.0847	48
0.0768	72
0.0725	96

Table 1: The initial mass of 0.1001 grams of bioplastic is decomposed by the natural soil bacteria in an ambient environment. Nearly 27% of the initial mass of bioplastic is decomposed within 4 days.

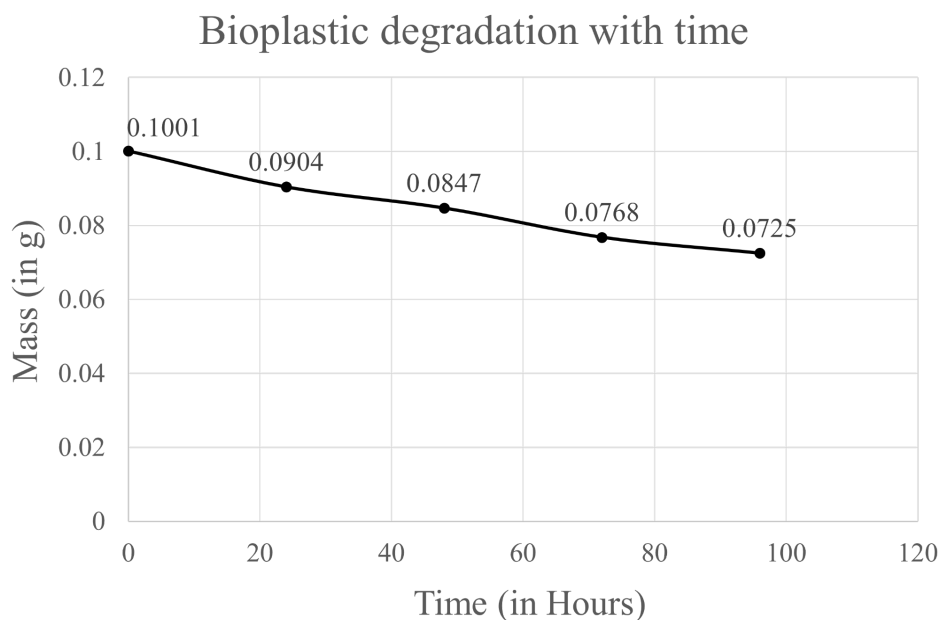


Figure 7: The graph demonstrates the decomposition of bioplastic with respect to time. The decomposition follows the exponential decay, which is fitted in Mathematica to get the total decomposition time.

CALCULATIONS

1. Mass of Lignin Extracted from 10 grams of wheat Straw: 3.152 grams (31.52 %)
 - 1.1. Safranin Dye changes colour from Deep Red to Pink
2. Determination of Vanillin (from 1.5 grams of lignin):
 - 2.1. Mass of Empty Vial = 3.300 grams
 - 2.2. Mass of Vial with Compound (Containing Side Products) = 3.401 grams
 - 2.3. Mass of Compound (Including Side Products): $3.401 - 3.300 = 0.101$ grams
3. Mass of Acetylferulic Acid (For 1 gram of Vanillin):
 - 3.1. Mass of Butter Paper = 0.249 g
 - 3.2. Mass of Paper + Compound = 0.921 g
 - 3.3. Mass of Compound = $0.921 - 0.249 = 0.672$ g
4. Mass of Polymer from 0.672g of Acetylferulic Acid:
 - 4.1. Mass of Empty Vial = 1.11 g
 - 4.2. Mass of Vial containing the polymer = 1.46 g
 - 4.3. Mass of Polymer = 0.350 g

CONCLUSION

In conclusion, we developed a lignin-based Biopolymer using Wheat straw as an agro-waste. We first extracted the lignin using wheat straw and used Nitrobenzene Oxidation of Lignin to oxidise the side chain of lignin to get the Vanillin. From 10 grams of Wheat Straw, we obtained about 3.152 grams of hard dried Lignin (31.52 %). We took 1.5 grams of lignin for the first batch and used the Nitrobenzene Oxidation method. We obtained about 0.101 grams of crude Vanillin (0.212 grams of vanillin from 3.152 grams of lignin), extracting 2.12% (w/w) of vanillin from the initial mass of wheat straw taken for the process. Vanillin is then acetylated to get the acetyl ferulic acid and polymerised using Zinc acetate as a catalyst to get the lignin-based polymer. From 1 gram of vanillin, we extracted about 0.672 grams of Acetylferulic acid, resulting in 67.2% (w/w) of extraction of Acetylferulic acid from Vanillin. We obtained about 0.350 grams of Acetylferulic acid polymer upon polymerisation of 0.672 grams of Acetylferulic acid, resulting in 52.08% (w/w) conversion of Acetylferulic acid into its polymer.

This biopolymer is mixed with the Glycerol, which acts as a plasticiser and residual straw fibres, which increases the strength of the bioplastic formed. A hard bioplastic is formed when baked in the oven for about three days. Tensile strengths and other mechanical properties need to be tested in the laboratory. Based on our simulations using Wolfram Mathematica and the initial decomposition data obtained, we found that the decomposition time of this biobased polymer takes around 105 days (3 Months) to decompose around 99.98% of the initial mass taken.

When this biopolymer is extracted at an industrial scale, it will yield enough bioplastic to be used for everyday items. The proposed process can be refined further to get a cost-effective lignin-based bioplastic with mechanical properties comparable to that of synthetic plastics such as polyethylene terephthalate (PET).

SUPPORTING INFORMATION

The supporting information is available free of charge through the ChemRxiv website. Mathematica Notebook demonstrating the simulation of Bioplastic degradation.

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