Tailored Lignocellulose-Based Biodegradable Matrices with Effective Cargo Delivery for Crop Protection

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**ABSTRACT:** Controlled release and targeted delivery of agrochemicals are crucial for achieving effective crop protection with minimal damage to the environment. This work presents an innovative and cost-effective approach to fabricate lignocellulose-based biodegradable porous matrices capable of slow and sustained release of the loaded molecules for effective crop protection. The matrix exhibits tunable physicochemical properties which, when coupled with our unique “wrap-and-plant” concept, help to utilize it as a defense against soil-borne pests while providing controlled release of crop protection moieties. The tailored matrix is produced by mechanical treatment of the lignocellulosic fibers obtained from banana plants. The effect of different extents of mechanical treatments of the lignocellulosic fibers on the protective properties of the developed matrices is systematically investigated. While variation in mechanical treatment affects the morphology, strength, and porosity of the matrices, the specific composition and structure of the fibers are also capable of influencing their release profile. To corroborate this hypothesis, the effect of morphology and lignin content changes on the release of rhodamine B and abamectin as model cargos is investigated. These results, compared with those of the matrices developed from non-banana fibrous sources, reveal a unique release profile of the matrices developed from banana fibers, thereby making them strong candidates for crop protection applications.  

**KEYWORDS:** Banana paper, Sustainable agriculture, Pulp refining, Abamectin, Wrap and plant  

**INTRODUCTION**  
Worldwide awareness for the conservation of natural resources is leading to the use of various non-wood plant fibers as alternatives to wood pulp in the manufacture of paper and paperboard.1−4 Materials derived from non-wood plants (also known as lignocellulosic materials) primarily consist of three important components: cellulose (35−50%), hemicellulose (20−35%), and lignin (10−25%).5 Cellulose is responsible for imparting mechanical strength to the plant; hemicellulose is capable of developing interfiber bonds; while lignin is the major hydrophobic component of plant fibers, consisting of phenyl propane units, and is associated with the natural decay resistance of the plants.6 Among various non-woody plants, fiber removed from the banana plant (*Musa spp.*) is found to consist of a comparatively higher content of cellulose (44−54%) with a low amount of lignin (6−13%).7,8 Bananas are also the world’s most exported fresh fruit (US$ 10 billion/year), with global exports (excluding plantains) reaching a record total of 18.1 million tons in 2018.9 It is estimated that wastes produced by a single banana plant is 80% of its mass, which is mostly used as animal feed or fuel.10,11 Generation of abundant wastes from banana harvest, taken together with comparatively low content of lignin and higher amount of cellulose, makes the wastes of banana harvests attractive candidates for paper production.8 Previously, considerable research had been conducted on sorption and release properties of products and/or wastes produced by plants, such as gums, rice husk, sawdust, jute, and banana fiber.12−15 Other than exploring the utility of banana fibers in composites and sorbent materials,10,11,13,15,16,17 recently some research groups have reported production of paper from banana fiber for various applications, including feminine hygiene products and wrapping paper.18−20 However, to the best of our knowledge, there has been no detailed research on understanding of the process–structure–property relationship of matrices produced from banana paper and its effect on the cargo release profile.  

In this study, we undertake an in-depth examination of the impact of variation in physical characteristics of banana paper in order to engineer banana fiber-based biodegradable matrices capable of controlled release of loaded cargo molecules (such
as pesticides) for better crop protection management in nematode-infested soils. Pesticides have been an essential component of pest management schemes for many years, but concerns about environmental contamination and non-target impacts have led to increasing restrictions on broad-scale application. Generally, only ~10% of the pesticide is available to the crops, while the remaining 90% becomes part of the surrounding environment, i.e., soil, water, and air, resulting in damage to the environment as well as human health.31,32 A controlled release of pesticide, or any other active ingredient, would be ideal to maintain its effective concentration over a stipulated period while applying a lower initial volume. Recently, several studies have focused on the utilization of either biopesticides or biodegradable release media for controlled release of pesticides.21−26 In addition, different bio-based materials such as lignin, chitosan, alginates, and plant virus nanoparticles are being considered for encapsulation of agrochemicals.27−30 However, the cost of the process involving complicated steps is a major barrier to applying these approaches on a large scale. The effects of the use of nanomaterials on the environment and human health are also limiting factors for implementation of the technology. Our group has recently examined a promising approach for controlled release and targeted delivery of fluopyram and abamectin using biodegradable cellulose diacetate-based nanofibers electrospun directly onto soybean seeds.25 The scaling up of the electrospinning process, however, remains a challenge.

We plan to use a unique wrap-and-plant (W&P) approach to explore the efficacy of banana fiber-based matrices as controlled release media, wherein we “wrap” seed/seed pieces with active ingredient impregnated banana paper and “plant” them in the soil, ensuring no impediment to seed germination. Without relying on high-technology processing, our W&P approach is focused on facilitating sustainable crop protection for smallholder farmers across the developing world, without damaging the soil chemistry because of biodegradable nature of the seed wraps. Initial studies from our group introduced this W&P methodology, demonstrating promising results shown by the banana paper as compared to abaca, softwood, and hardwood paper.41 However, an in-depth analysis of the variation in processing conditions, their effects on the properties of the matrices, and the role of the chemical components (e.g., lignin) of banana fiber is needed to better understand its potential utilization as an effective matrix for the tunable release of various types of cargo. Our study involves (1) additive-free fabrication of various matrices from banana fibers via a basic papermaking process and (2) testing of the developed matrices for their strength and release profile so that they can serve as effective seed wraps without any compromise on the integrity of the wrap as well as the seed germination process. In contrast to a typical papermaking processes, our focus is to prepare the matrices from fibers produced from banana plants through mechanical pulping (refining) and to study the effect of variations in refining on its strength, structure, and release profile. Mechanical pulping (refining) involves mechanical treatment of a specific concentration of fiber slurry in water through the application of compression and shear force on the fibers, which results in several changes in their structure and properties, depending on the extent of refining.32,33 We use two different types of cargos, i.e., rhodamine B (Rhb) as a model molecule and abamectin (Abm) as a model pesticide, to study the release profile of paper. RhB is a water-soluble basic dye used in various biotechnology applications and also as a water tracer.15 Abm is a macrocyclic lactone that exhibits strong activity against a variety of nematodes by paralyzing them by binding with their nerve and muscle cells.34,35 One major drawback of using Abm for crop protection is its poor mobility due to low water solubility and high binding capacity to soil, which makes it unavailable to the nematodes. Although it has been previously suggested that the phenylpropionate units in lignin molecules are mainly responsible for the binding and release mechanism of the lignocellulose matrices for any cargo molecule,31,33 these studies have not examined the effect of lignin content or other factors that may come into play. We believe that the structure and composition of the fiber also play a significant role in determining its cargo release profile. Since we are developing paper-like matrices via additive-free processing of banana fiber, these matrices will be referred to as paper or banana paper hereafter in this work. For a better understanding of the process, we have compared the release profiles of banana fiber-based matrices (banana paper) to those of two control non-banana papers with a noticeable difference in morphology and lignin content.

### EXPERIMENTAL SECTION

#### Materials

Banana fiber was procured from the agricultural unit of Earth University, Costa Rica, where it was obtained by processing the wastes of banana harvest. Typically, chopped parts of banana plant are biologically fermented with the inoculum of Bacillus and actinomycetes for 5 days. Later, the fermented banana fibers are mixed with 95−98% water in agitation tanks and then cast as sheets which are hydraulically pressed to remove moisture. The sheets are finally dried in the sun to reduce the moisture content to 7% before shipping to North Carolina State University. Rhodamine B (Rhb, ≥95%) dye was purchased from Millipore Sigma and used without further purification. For lignin content measurement, sodium thioulate solution (0.2 N), potassium iodide solution (1 N), sulfuric acid (4 N), potassium permanganate solution (0.1 N), and starch indicator were provided by Fisher Scientific. Abamectin (Abm) (97%) was supplied by Alfa Aesar. Liner (P1) and copy (PS) papers that were used as controls were provided by the Forest Materials Department at NC State University. The recipe for nematode growth media (NGM) and M9 buffer was adapted from Wormbook.36 Caenorhabditis elegans (C. elegans) strain N2 (wild type) were obtained from the Caenorhabditis Genetics Center (CGC). Reagent grade acetone (99.5%) and HPLC grade acetonitrile (99.8%) were purchased from Millipore Sigma. Deionized water (pH: 5.77 ± 0.13) was used throughout the experiments, except while making paper handsheets.

#### Handsheets Production

Banana fibers were soaked in water overnight and were subsequently diluted to a 3% consistency (wt% of fiber in the mixture) with tap water. The fibers were beaten for different intervals ranging from 1 to 30 min in a laboratory valley beater as per the TAPPi T200 standard method. To monitor changes in the fiber structure and drainage behavior, freeness of each sample of pulp was measured using Canadian Standard Freeness (CSF) tester as per the TAPPi T227 method. A standard laboratory British handsheet mold was used to prepare at least 10 circular handsheets (6.25 in. diameter with a grammage of 70 g/m²) from the pulp following TAPPi T205 standard method. For ease of understanding, different handsheets are named according to preparation conditions, as shown in Table 1. After production, handsheets were conditioned at a temperature of 23 °C and a relative humidity of 50% according to TAPPi 402 (all the steps involved in paper production are shown in Figure S1). Multiple samples of the handsheets were regularly tested for characterizing the release profile using RhB and Abm and the associated mortality of the model nematode C. elegans. Detailed
information about the setup for the bioavailability assays is mentioned in the characterization section on pesticide bioavailability.

**Characterization.** The lignin content of different types of fibers was measured by a thiourea oxidation process following TAPPi T236 test protocol. The burst strength of hand sheets was tested with an L&W tester according to the TAPPI T414 test method. The tensile strength was measured using an L&W tensile tester according to the T 494 test method. Air resistance of the hand sheets was tested with an L&W tester according to the TAPPI T 414 test method. The column temperature was held at 40 °C whereas the mobile phase was a mixture of 80% acetonitrile and 20% deionized water with a flow rate of 1.0 mL/min. An optimal absorbance wavelength of 245 nm was applied to the detector, while the injection volume was 10 μL in each case. % Abm released was determined through the following formula:

\[
\% \text{Abm released} = \frac{(Q_0 - Q_f)}{Q_0} \times 100
\]

where \( Q_0 \) is the amount of Abm released (μg) and \( Q_f \) is the dye sorbed on each type of paper.

**2. Pesticide Release Studies. Abm Release Profile.** Following the already established protocol by our laboratories, \(^25,27,31\) we prepared a dilution of Abm from a 2 mg/mL stock solution in acetone, in order to achieve a final concentration of 32.5 μg of Abm/m² on each sample after spraying. Using circular disks of 7 mm diameter from different samples of paper, we soaked the disks in 3 mL of DI water in closed vials and left on a VWR rocking platform at a rate of 6 rpm to ensure homogeneous mixing of the active ingredient in the release medium. All experiments were conducted in triplicate. The vials were removed from the rocking platform after regular intervals ranging from 30 min to 2 weeks and aliquots were removed from each vial for the quantification of Abm through high-pressure liquid chromatography (HPLC). A Shimadzu HPLC equipped with an autosampler and a diode array UV−vis detector was used to determine the concentration of Abm, for which a Phenomenex Kinetix C18 column (150 × 4.6 mm², 2.6 μm particle size) was suitable. The column temperature was held at 40 °C whereas the mobile phase was a mixture of 80% acetonitrile and 20% deionized water with a flow rate of 1.0 mL/min. An optimal absorbance wavelength of 245 nm was applied to the detector, while the injection volume was 10 μL in each case. % Abm released was determined through the following formula:

\[
\% \text{Abm released} = \frac{(Q_0 - Q_f)}{Q_0} \times 100
\]

Table 1. Comparative Properties of Handsheets Prepared from Banana Fiber Unrefined Pulp (BP-0) and Pulp Refined for 1 min (BP-1), 2 min (BP-2), 3 min (BP-3), 5 min (BP-5), 10 min (BP-10), 20 min (BP-20), and 30 min (BP-30)

| Appearance | BP-0 | BP-1 | BP-2 | BP-3 | BP-5 | BP-10 | BP-20 | BP-30 |
|------------|------|------|------|------|------|-------|-------|-------|
| Brightness | 4.0  | 3.5  | 3.2  | 3.0  | 2.8  | 2.5   | 2.2   | 2.0   |
| Proportion | 554.5 mm² | 10   | 8    | 6    | 4    | 3     | 2     | 1     |
| Weight     | 0.15 g | 0.12 | 0.10 | 0.09 | 0.08 | 0.07  | 0.06  | 0.05  |
| Root Penetration (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | none |

*Root penetration test was not conducted for BP-1, BP-3, and BP-20.*
each case was determined from the number of immobile (rigid movement and linear shape) nematodes as compared to total number of nematodes in the well, using the following relation:

\[
\% \text{ mortality} = \frac{100 \times \text{number of immobilized } C. \text{ elegans}}{\text{total number of } C. \text{ elegans added}}
\]

All experiments including the controls (PBS with paper without Abm and PBS only without paper or Abm) were conducted in triplicate. A detailed schematic explaining the experimental setup for the bioavailability studies is shown in a subsequent section (Figure 4B).

## RESULTS AND DISCUSSION

**Effects of Pulp Refining on Mechanical Properties of Handsheets.** Pulp refining was done through mechanical beating without the use of any chemical additives, as our focus is to explore the inherent properties of the banana fiber. Its role is evident from digital images of the surfaces of hand sheets (Figure 1A) prepared from pulp refined from 0 to 30 min, revealing a transition from a rough to a smooth surface-paper. Further details on the characteristics of the paper as affected by mechanical refining of the pulp are displayed Table 1 together with Figure 1. It is apparent from Table 1 that the freeness (drainage rate) of the pulp reduces substantially with increased refining, decreasing to one-fourth its value from 678 mL with no refining, to 185 mL with 30 min of refining. This trend is expected since fibers are shortened and become more fibrillated because of shearing and compression forces during refining thereby enhancing the available surface area for water holding and slowing drainage, i.e., have lower CSF values. Fibrillation also results in generation of microscopically hairy appearances on the fibers because of delamination of the cell wall. Rise in the number of tiny hairs on the fiber surface leads to increased tendency to develop interfiber bonds resulting in a smoother finish and compact structure of the handsheets produced from more refined pulp (Figure 1A). Figure 1B reveals that the thickness of the handsheets decreases (almost linearly) with refining as the fibers collapse under high shear and compression during refining, resulting in the dense packing of fibers and fines in the resulting paper;\(^{32}\) the paper thickness drops by half as we go from 0 to 30 min of refining time. A rise in the density of paper (Table 1) with refining is also a result of a more compact structure due to the consolidation of the fibers. One major outcome of the close packing of fibers is a smooth finish of the handsheets (Figure 1A) because of a reduction in the free space between the fibers. Close packing also increases the air resistance of the paper (Figure 1B, Table 1) by well over an order of magnitude. One should note that increased air resistance relates to lower porosity of paper, which can influence its usage as a release or sorption matrix (discussed further in the sorption/release section).

Another significant outcome of pulp refining is the variation in burst and tear indices with increased refining of the fibers. Figure 1C displays a rise in the burst index of the paper with more refining. This is understandable because increased fibrillation in more refined fibers would produce additional hydrogen bonds and a more compact structure of paper to resist any external pressure. Tear index, on the other hand, decreases with the rise in pulp refining. Since tear index is a measure of the force required to continue the tearing of paper in a specific direction, the presence of a higher number of scattered short fibers in more refined pulp can be a major reason for its decrease with refining.

![Figure 1](image_url)

**Figure 1.** Effect of pulp refining on (A) the appearance of handsheets (the refining time for each is shown on the arrow indicating increase in refining), (B) variation in thickness and air resistance with refining, and (C) strength in terms of burst and tear indices of the respective handsheets.

**Effect of Refining on Fiber Structure.** We undertook microstructural analysis of the banana fibers subjected to different conditions to better understand the physical property changes it shows with refining. As a first step, we examined SEM images of the cross section of paper produced from unrefined banana fibers (Figure 2A). It clearly shows that the fibers consist of bundles of long tubular structures or microfibrils made up of an inner hollow region (lumen) with diameter in the range of 20 μm and a thin layer of about 1 μm...
between the cell walls known as middle lamella. The long hollow lumens in banana fibers are responsible for high capillary action in the banana stem and play a major role in dictating the sorption properties of products made from banana fibers.20,39,40 During refining, the fibers undergo considerable changes in their microstructure as is evident from Figure 1, Figure 2B–D, and Figure S3. The digital image in Figure 1A and SEM micrographs of the surface sections in Figure 2B(a–d) and Figure S3A–C show that the fiber structure got compact with longer refining times leading to a smoother surface with fewer loose fibers. The compaction is more evident in cross-sectional images which show the microfibrils to collapse and the lumens to lose their morphology with refining (Figure 2B(e–h) and Figure S3(a–c)). These changes in microstructure are consistent with our results of paper getting thinner with refining time (Figure 1B). A close examination of surface sections also supports the argument developed in the previous section that the fibrillation of the banana fiber has resulted in the production of a collapsed (flat) structure with possibly stronger bonds between each other. This can result in the generation of mechanically strong handsheets produced from pulp that has been refined for a longer time.

To analyze further the effects of refining on fiber structure, samples of pulp refined at various time intervals were collected and analyzed for what is referred to as “fiber quality”, one
measure of which is the quantity of fiber fines. Fines are the high surface area fibers generated during a typical mechanical pulping process. A high number of fiber fines in the pulp contributes toward formation of a mechanically strong and less porous paper.\textsuperscript{41,42} Fines are referred to as the fraction of pulp which passes through a 200-mesh screen of a fiber length classified according to the TAPPI test method T 261 Cm-94.\textsuperscript{32,41–43} They are further categorized into primary fines that are present during the pulping and bleaching process, and secondary fines that are generated as a result of refining.\textsuperscript{2,42,44} Generally, refining of the fiber pulp generates secondary fines, increasing overall fine content in the pulp, which is shown as the rise in fiber count with increase in refining time in Figure 2C. The change in percent length of fines, on the other hand, is inconsistent and stays almost in the same range (18–24%) when the pulp is refined from 1 to 10 min, indicating larger distribution in fiber and fine size at low refining. Such unanticipated behavior has been previously reported for pulps having a fiber percentage content of less than 10%.\textsuperscript{32,45} Considering the fact that the pulp content in our experiments is just 3%, inconsistent variation in fine content is totally understandable. However, when the pulp is refined for 20 and 30 min, we can notice a gradual decrease in the % length of the fines, which is understandable and supports the formation of more compact and less porous handsheets as displayed in Figure 1A and Table 1.

Two other interesting features of the fiber pulp which affect the properties of the paper are variation in mean kink index and fiber coarseness (Figure 2D). While there is no regular trend displayed by pulp refined for smaller intervals, there is a clear decrease in fiber coarseness of the pulp refined for 10, 20, and 30 min. A reduction in fiber coarseness can be attributed to increased fibrillation and collapsing of the fibers with refining as supported by macroscopic (Figure 1A) and microscopic (Figure 2B) structure of the handsheets. While mean kink index is indicative of an abrupt change in fiber curvature, it is apparent from Figure 2D that the kink index of the fibers is decreasing with refining time of the pulp. This result makes sense because refining results in breaking the fibers into shorter sizes which can lead to straightening of the fibers, thereby helping in stacking the fibers close together to generate stronger paper with more smooth finish.\textsuperscript{41} Reduction in the kink index is also considered an advantage to prepare mechanically strong paper since it improves the load carrying ability as well as stress distribution of the handsheets.\textsuperscript{46}

**Root Penetration Studies.** The mechanical strength of the paper plays a critical role in designing an effective seed wrap loaded with active ingredients, e.g., pesticides or soil amendments. When wrapped around the seed/seedling, a strong paper could impede growth of the root after seed germination. A weak paper, on the other hand, would tend to disintegrate in the soil, before being able to unload its cargo. Since the burst and tensile indices of banana paper (Figure 1C and Table 1) increase with refining, our challenge is to identify a threshold where the paper is strong enough to stay intact in the soil for the required time, while allowing the germinating roots to penetrate it. As displayed in Table 1 and Figure S4A, root penetration studies conducted for 2 weeks demonstrate the highest penetration (100%) in the setup that used BP-0, BP-2, and BP-5 (paper produced from unrefined, 2 min refined, and 5 min refined pulp, respectively) as the seed wrap. We observed that BP-0, when removed from the soil, had already started to disintegrate, which was undoubtedly because of its low strength—a property that clearly makes it unsuitable as a seed wrap. While the seeds wrapped with papers produced from 10 min refined pulp (BP-10) exhibit lower rates with a root penetration profile of 80%, the seed wraps prepared from BP-5 (paper produced from 5 min refined pulp) display an impressive root penetration profile (100% penetration through paper). When removed from soil, all the other papers produced from 2, 5, and 10 min refined pulp (BP-2, BP-5, and BP-10, respectively) are found almost intact while letting the germinating roots penetrate in each case. However, upon close examination of the papers, cracks were clearly visible on BP-2, and it was apparent that if kept in its present form, it would begin disintegrating within a week. BP-5, on the other hand, stayed as a single intact piece while the developing roots penetrated robustly (Figure S4C)—a tendency which suggests that the strength of BP-5 is suitable to serve as an effective seed wrap. BP-30 (produced from 30 min refined pulp), on the other hand, is too strong for roots to penetrate through and exhibit no root penetration (Table 1). Emergence shown by a few seeds wrapped with BP-30 could be attributed to developing roots growing around the stiff paper without penetrating it (Figure S4B).

**Rhb Release Studies.** An ideal release matrix should be capable of liberating the loaded molecules steadily, i.e., neither too fast nor too slow. Banana paper exhibits remarkable capacity as a sorbent because of the extensive network of tube-like microfibrils, facilitating transport of various materials through the plant. We can exploit similar transport properties in the paper produced from the banana fibers if special care is taken to retain its microfibrillar structure.\textsuperscript{20} Another parameter to examine for release of cargo is the lignocellulose composition of paper, particularly lignin content. The rationale behind studying release profiles of paper containing different amounts of lignin is that the presence of diverse functional groups in lignin may help to bind any exogenously applied molecule through chemical or physical bonds.\textsuperscript{31} It has also been suggested by our group in a preliminary study that hydrophobic interactions between lignin and Abm result in the controlled release of Abm.\textsuperscript{31}

Figure 3A shows the release profile of Rhb from banana papers with different lignin content along with liner and copy paper, the latter two having the highest and lowest lignin content, respectively. Table S1 shows total amount of % Rhb released by each sample after 14 days (336 h). We used Rhb as a model molecule to verify the possibility of interactions between lignin and a hydrophilic substance. Based on our observation regarding the optimum strength profile of BP-5 (previous section), we selected paper produced from 5 min refined pulp from different sources of banana fibers (P2, P3, and P4) having different lignin content and morphology as compared with two controls, i.e., non-banana papers containing high lignin (P1, liner paper) and very low lignin content (P5, copy paper). Lignin content was estimated as 19.7, 11.96, 10.25, 5.09, and 2.99 in P1 (non-banana liner), P2, P3, and P4 (banana papers), and P5 (non-banana copy paper), respectively (Figure 3B).

Figure 3A reveals that all three types of banana papers exhibit similar trends in releasing Rhb, which can be easily distinguished into two steps. The first step demonstrates fast release kinetics of the dye molecules from the paper within first 24 h of the study. The second step shows a comparatively slower release from all the banana paper. The initial fast release can be attributed to the detachment of dye molecules which
were stacked on the surface of paper, probably through weak physical bonds. The slow release in the second step likely represents movement of dye molecules, which were strongly bonded either on the surface or deeper in the bulk. Irrespective of the similar trend in release profiles of all banana papers, it is also apparent in Figure 3A that the lignin content of paper plays a key role in determining its release profile, i.e., faster release with low lignin content. Both the controls also follow similar lignin-dependent release trends, however displaying a single step release profile.

Figure 3A also shows that RhB release from the high-lignin paper (P1) is very slow as compared to the amount sorbed, while the low-lignin paper (P5) displays a fast release of the dye molecules. Based on these observations, we can surmise that the availability of multiple binding sites in lignin makes it capable of developing strong interactions with the hydrophilic dye molecules. The presence of similar functional groups in RhB and the cellulose component of banana fiber make it less likely for strong bonds to form between the cellulose and RhB. Lignin is a complex molecule, mainly hydrophobic in nature, with many functional groups such as hydroxyl, phenolic, carboxyl, carbonyl, methyl, and ether, which can easily develop strong interactions with the carboxylic group of incoming dye molecules. In fact, recent studies on lignin-based materials as sorbents for RhB contaminants have shown clear evidence of electrostatic interactions between oppositely charged functional groups of lignin and RhB. Furthermore, the strength of the bonds between the dye and the paper seems to be dependent on the total lignin content of the paper. A low amount of lignin would result in fewer interactions between the dye and lignin, therefore leading to fast release of the dye and vice versa.

Another factor that can play a role in deciding the sorption/release profile of paper is the variation in the morphology of the fibrous network of each type of paper. Figure 3B depicts the lignin content, density, and air resistance (inversely related to porosity) of P1, P3, P4, and P5, while Figure 3C displays the difference in morphology of the samples (surface and cross-section morphology of P2 is already shown in Figure 2B). It appears that low lignin content paper (P5) consists of fibers with a compact structure and low porosity, which might have led to the inability of the dye molecules to migrate deeper into the paper and, therefore, release quickly when soaked in the release medium. This observation taken together with a lack of the two-step release profile of P5 (Figure 3A) indicates that fiber morphology of the release matrix also determines its capability to the release of the cargo. For instance, the high-lignin paper (P1) exhibits almost similar porosity and loose fibrous networks on the surface as P4 (banana paper) (Figure 3B,C(a,c)). However, the respective cross sections of P1 and P4 in Figure 3C(e,g) exhibit a combination of compressed and intact microfibrils consisting of smaller diameter lumens with

| Samples          | Lignin content (%) | Density (kg/m³) | Contact angle | Air resistance (gs) |
|------------------|--------------------|----------------|---------------|---------------------|
| Liner paper (P1) | 19.7               | 451.94         | 121°          | 6.5                 |
| Banana paper (P2)| 11.96              | 277.05         | 12°           | 45.24               |
| Banana paper (P3)| 10.25              | 316.49         | Cant be       | 10.25               |
| Banana paper (P4)| 7.2                | 329.29         | measured      | 5.5                 |
| Copy paper (P5)  | 2.99               | 702.98         | 2°            | 80.2                |
very thick walls in P1, which is in contrast to the well-organized and undamaged microfibrillar structure consisting of large sized lumens observed in P4. Interestingly, P4 (lignin content = 7.2%) displays a continuous rise in RhB release, exceeding that of P5 (lignin content of 2.99%) after 48 h. This trend can be attributed to the highly porous nature of P4 as compared to P5; i.e., low air resistance of P4 is indicative of its high porosity (Figure 3B). The presence of a larger number of pores in P4 results in a gradual release of loosely bound dye molecules even from the bulk, while the less porous P5 is unable to release all the dye molecules after 48 h of release studies. Furthermore, a very high water contact angle (WCA) of P1 (120°) represents its highly hydrophobic nature, which might have played a role in determining its release profile in an aqueous medium, as compared to the hydrophilic nature of P2 (WCA 12°), P3, P4 (WCA could not be measured for these two) and P5 (WCA 2°). Therefore, it seems that a balance between the lignin content and fiber morphology is critical for governing the release profile of each sample. Variation of the rate of release of loaded molecules from paper, depending on their lignin content, porosity, and morphology, can play a vital role in designing the seed wrap for slow or burst release of the loaded cargo, depending on the nature of the crop and edaphic environment.

**Abm Release from Various Samples.** Regardless of how the release studies of hydrophilic RhB provide a convincing picture for banana paper as compared to control papers, similar trends cannot be predicted for a hydrophobic molecule, such as Abm that can be used in real applications. To demonstrate efficacy of paper as a controlled release medium for pesticides,
we analyzed release of Abm from all the samples utilized in the dye release study. It is apparent from Figure 4A and Table S1 that there is an increase in the release of Abm with decreasing lignin content in paper; however, after a comparatively faster initial release of Abm, the low lignin content banana paper (P4) releases almost the same amount of Abm (24.33 ± 2.04%) as do the remaining banana papers (P2 and P3) at the end of a two-week study. This result indicates that the release profile of banana paper is not solely regulated by its lignin content and there are other unique features to which its sorption/release properties can be attributed. The low lignin content non-banana paper (P5), on the other hand, releases a comparatively larger amount of Abm (71.26 ± 8.98%) than RhB (37.47 ± 8.51%) within the same period, which indicates its weaker ability to bind Abm molecules. Interestingly, the high-lignin non-banana paper (P1) displays a release profile, similar to the release profiles of P2, P3, and P4. The difference in the release profile of P1 while loaded with Abm compared to RhB can be attributed to the strong hydrophobic interactions between the lignin and Abm molecules, resulting in an initial faster desorption of the surface sorbed molecules and slow release of the molecules sorbed into the bulk. However, it is notable that the rate of release of Abm from P1, P2, P3, and P4 in the first step is much slower than the release rate of RhB (Figure 3A), which can be mainly attributed to the comparatively strong intermolecular interactions between hydrophobic Abm and paper.

Bioavailability of Abm-Loaded Samples. To estimate how much Abm would be biologically available to the target pests, we conducted in vitro studies using C. elegans as model nematodes following the procedure displayed in Figure 4B. While none of the banana or non-banana fiber-based matrices by themselves display any nematocidal activity, it is apparent from Figure 4C that despite variation in lignin content, all the banana fiber samples exhibit similar release profiles. Conversely, P1 (high-lignin non-banana paper) displays a slow release, in contrast to P5 (low-lignin non-banana paper), which exhibits a burst release with ∼85% of the nematodes becoming inactive during the first hour of Abm exposure. The Abm bioavailability exhibited by P1 and P5 is quite similar to their respective RhB and Abm release profiles, and taken alone seems to be consistent with our previously suggested hypothesis that lignin alone dictates release of active ingredients.31 As such, the high lignin content in P1 would cause strong binding of Abm, making it unavailable to affect the nematodes. On the other hand, weaker interactions developed between Abm and low lignin containing paper (P5) result in burst release, causing immobilization of almost all the nematodes within the first hour of exposure. However, the similar release profiles of Abm exhibited by all the banana fiber-based samples, despite difference in their lignin content, does not seem to support the previous hypotheses regarding the solitary role of lignin in regulating release profiles of the lignocellulosic materials developed from banana fibers.31 While a molecular level understanding of the binding and release of active ingredients is part of our future work, we believe that the extraordinary hierarchical microfibrillar morphology of banana fiber in the matrix is also responsible for its release characteristics. Our “wrap and plant” banana paper matrix with its “dual knob” tunable functionality thus presents itself as excellent candidate for controlled release of loaded cargos for cost-effective and enhanced crop protection in nutrient-depleted and pest-infested soils.

**CONCLUSIONS**

We have presented a sustainable wrap-and-plant approach to utilize wastes of banana harvests as tunable release medium through a cost-effective and chemical-free conversion method. Since the biodegradable nature of this matrix can be exploited in fabricating controlled release matrices for crop protection, we proposed a unique approach that can be beneficial in fine-tuning the properties of banana fibers as seed/seedling wrap. We systematically investigated the effects of pulp refining on various properties of lignocellulosic matrices produced from banana fibers. Increased refining of the pulp resulted in reduction in freeness, fiber coarseness, and mean kink index, and therefore contributed to the production of robust matrices because of fibrillation and the strong bonding of fibers. Using rhodamine B (RhB) and abamectin (Abm) as model molecules, comparative release properties of three different types of matrices developed from banana fibers were studied against two matrices developed from non-banana fibers as controls. Interestingly, all the banana fiber-based matrices exhibited similar trends in dye release, which were different from the release profiles of the controls. These studies indicate that lignin content of the matrix plays a major role in determining its release profiles because the loaded molecules are released slowly from a high lignin content matrix while a burst release of the dye is observed from a low lignin content matrix. However, the differences in the fibrillar morphology of the matrices seem to also play critical roles in tuning their respective release profiles. To further understand their effectiveness as controlled release media for the pesticides, we studied the release profile of Abm-loaded matrices via HPLC followed by in vitro bioassays. Our studies demonstrate a lignin content dependent release profile for the non-banana fiber samples. However, all the banana fiber-based matrices exhibit almost similar profiles of Abm bioavailability, regardless of variation in lignin content in individual test matrices. This finding indicates the significant role played by fiber processing and morphology in tuning the unique properties of a banana fiber-based matrix as a controlled release medium. We believe that by making use of our wrap-and-plant approach, we can design biodegradable seed wraps with tunable strength, soil integrity, and release properties.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.9b05670.

Figures S1–S7, digital images displaying various stages involved in the handsheet preparation process; showing schematics for dye sorption and release studies; SEM images of surface and cross section of paper produced from 1, 3, and 20 min refined pulp; digital images displaying results of root penetration studies using maize seed wrapped in banana paper prepared from unrefined or 2, 5, 10, and 30 min refined pulp; and selected HPLC chromatograms displaying release of Abm from P1, P2, P3, P4 and P5 after selected time intervals; and Table S1, listing average thickness, % RhB and Abm released, and % Abm bioavailability for P1–P5 (PDF)

Video S1, C. elegans in 63-well plate displaying their typical undulating movement and shape (AVI)

Video S2, C. elegans in 63-well plate showing response to Abm exposure (AVI)
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Notes
The authors declare no competing financial interest.

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