Lignin-Based Pesticide Delivery System

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ABSTRACT: The potential of lignosulfonates as widely underutilized byproducts of the pulp and paper industry for the synthesis of a biodegradable pesticide carrier system was assessed in this study. Design of experiment software MODDE Pro was for the first time applied to optimize lignosulfonate granule production using Myceliophthora thermophila laccase as a biocatalyst. Enzymatic cross-linking was monitored using size exclusion chromatography coupled online to multiangle laser light scattering, viscosity measurement, and enzyme activity. The determined optimal and experimentally confirmed incubation conditions were: 33 °C, 30 cm³/min O₂ supply, and 190 min reaction time. The granules were thereafter loaded with 2 g/kg 3,6-dichloro-2-methoxybenzoic acid (Dicamba), a broad-spectrum herbicide. According to the HPLC analysis, complete release of Dicamba was achieved after 48 h of release. This study showed the green production of a 100% lignosulfonate-based biodegradable solid carrier with potential application in agriculture.

INTRODUCTION

Lignin is a major byproduct of the pulp and paper industry, produced at a quantity of 50–60 million tons per year. It is an extremely valuable biopolymer and a renewable resource, but today only 2% of the total lignin extracted is exploited for value-added products, while the rest of it is mostly burned for low-cost energy production.¹ Therefore, there is a need for lignin utilization in the production of value-added materials to make the biorefinery process more profitable. Thanks to advances in valorization of each renewable component of the lignocellulosic biomass, new marketable products are being developed.¹² Using enzymes to modify lignin-based structures avoids the use of toxic chemicals, thus providing a valid biotechnological approach.¹ Laccases belong to a very broad and diverse superfamily of multicopper oxidases (MCOs): they generally contain a cluster of four copper atoms, which constitute their active site. Thanks to their natural origin, nontoxicity, mild operating conditions in which they are active, and the very broad range of oxidized substrates, fungal enzymes like laccases are very valuable tools for industrial applications.¹³ Laccases can be used to valorize lignosulfonates by utilizing their ability to generate reactive radicals that cross-link their side chains and form long-chain polymers.⁵⁻⁷ No addition of mediators to the reaction mixture is needed and only oxygen has to be supplied; it is possible, therefore, to control laccase reactivity by just increasing or diminishing the oxygen supply.⁸ This approach is useful to alter lignin properties like molecular weight and water solubility and make the polymer more suitable for applications as binders, plasticizers, and adhesives in agriculture for the production of novel agrochemical carriers, as well as in storage and release systems.⁹

The massive use of pesticides poses serious public concerns since very large amounts of substances applied to ensure a good harvest can be dangerous for human health and natural ecosystems. Dispersion of pesticide residues due to over-application or misuse often results in groundwater pollution and consequently in eutrophication, toxicity, and air pollution, as well as in a decrease of soil quality.¹⁰ Approximately 95% of herbicides are released into the environment.¹¹ Therefore, there is a need to optimize the use of pesticides and an urgency to introduce ecological strategies for their application and effectiveness in the field, to avoid massive environmental pollution. Innovative solutions are needed to allow a better control of the use of pesticides as valuable tools for agriculture in the future.¹²¹³ Agrochemical carriers and delivery systems are increasingly being recognized as important systems to deliver agrochemicals and minimize leaching, thereby preventing environmental pollution and ecosystem damage while reducing adverse human health effects.¹⁴ As summarized by Campos et al.,¹⁵ currently used products are generally made from synthetic materials such as petroleum-based polymers, for...
example, polysulphone, polyacrylonitrile, polyurethane, and polystyrene. Using low-cost, bio-based polymers like lignosulfonate, recovered from process side streams, is an innovative and promising solution that also promotes circular bioeconomy and environmentally friendly processes. A study by Huang et al. shows the production of an environmentally friendly polymer structure for the encapsulation of photosensitive pesticides. Also Liu et al. developed a pH-responsive controlled-release nanoparticle from sustainable resources. Lignin-based products generally decompose to form humic acid, a natural soil fertility-enhancing material. Dicamba is resistant to oxidation and acid, a natural soil fertility-enhancing material.19–21 For this study, Dicamba was chosen as the model pesticide because of its avid use in agriculture. Dicamba is resistant to oxidation and hydrolysis and stable in weak acid and alkaline solutions; it is highly soluble in water (6.5 g/L at 25 °C), which can be a problem in case of runoff events because it is highly mobile in soil (it does not bind to soil particles) and therefore can easily reach and solubilize in groundwater and be dispersed in the environment. It is rather persistent in soil, with a half-life ranging between 1 and 4 weeks depending on soil type. It is a very good representative of many products that are currently used and its properties are similar to those of other products.22,23

The overall objective of the present study is to investigate the possibility to produce a stable biodegradable bio-based carrier system for pesticides by utilizing enzymatic cross-linking of lignosulfonates to produce granules.

## RESULTS AND DISCUSSION

### Synthesizing the Binder for Granule Production.

The lignosulfonate-based binder was produced through laccase-mediated cross-linking of LS, resulting in water-insoluble, high-molecular-weight, and high-viscosity structures. By utilizing the knowledge gained from preliminary experimental data, a two-leveled full factorial design was chosen. As shown in Table 1, the MODDE Pro design of experiment proposed 11 cross-linking reactions that were performed and respective responses (molecular weights and viscosities) were recorded. In general, the molecular weight and viscosity increased with increasing temperature, reaction time, and oxygen supply. However, the viscosity did not always increase at the same level as the molecular weight, indicating that the oxygen supply and temperature influence the chain formation in the cross-linked structure.24

The conditions provided by the MODDE Pro design of experiment were very useful for the optimization process as they lead to the production of cross-linked lignosulfonates with suitable properties for making granules. Through probability analysis, the optimal incubation conditions were determined as 33 °C, 30 cm³/min O₂ supply, and 190 min reaction time, as shown in Figure 1. The resulting binder showed very good properties in terms of sprayability and granule formation as well as very good reproducibility. Previously, the design of experiment software MODDE Pro was also proven useful in determining the optimal conditions to obtain lignin nanoparticles25 and in revealing the key factors and their influences in cocoa bean fermentation.26 In fact, statistical modeling using design of experiments (DoE) and response surface methodology (RSM), as shown in Figure 1, are useful in simultaneously dealing with multiple factors in experimental design, which can otherwise be extremely challenging and cost-ineffective. DoE has been used extensively in food technology for process optimization, microbiology, various sensory analyses,27 and as demonstrated in this study can be used for determining the optimal conditions for synthesizing cross-linked materials. This shows the ability of the program to show the interaction of responses (temperature, binder concentration, and pH). The cross-linking process during the enzymatic reaction is not entirely clear yet, but the statistic model was proven to be useful anyhow. By providing the molecular weight through size exclusion chromatography coupled to multangle laser light scattering, also the cross-linking structure could be incorporated and therefore helped strengthen the statistic model applied.

The resulting binder was then analyzed for its molecular weight, viscosity, and chemical properties. Remarkably, the molecular weight exceeded the predicted molecular weight by far being on average 4485 kDa with radii of around 70 nm, whereas the viscosity was still low at 1.47 × 10⁻¹³ Pa s, which lead to a binder that was still sprayable but with a tighter cross-linked structure advantageous for granule production. This exceeded the target, as 2000 kDa was the initial aim to have a suitable material for coating. Indeed, the MODDE Pro design of experiment was very useful in establishing the optimal conditions for producing the binder that produced stable granules. The granules were further analyzed by FTIR spectroscopy including all DoE products. Figure 2 shows the spectra obtained from native lignosulfonates, experiment N1 with a low degree of cross-linking, and experiment N8 which...

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**Table 1. Resulting Molecular Weights and Viscosities after Performing the Design of Experiment Reactions**

| experiment | temperature °C | oxygen flow cm³/min | time min | molecular weight kDa | Viscosity Pa s |
|------------|----------------|---------------------|----------|----------------------|--------------|
| N1         | 20             | 10                  | 60       | 673                  | 0.004        |
| N2         | 50             | 10                  | 60       | 903                  | 0.004        |
| N3         | 20             | 60                  | 60       | 825                  | 0.004        |
| N4         | 50             | 60                  | 60       | 2967                 | 0.040        |
| N5         | 20             | 10                  | 360      | 3510                 | 0.380        |
| N6         | 50             | 10                  | 360      | 1861                 | 0.420        |
| N7         | 20             | 60                  | 360      | 2162                 | 0.380        |
| N8         | 50             | 60                  | 360      | 6241                 | 0.300        |
| N9         | 35             | 35                  | 210      | 2690                 | 0.290        |
| N10        | 35             | 35                  | 210      | 2949                 | 0.330        |
| N11        | 35             | 35                  | 210      | 2475                 | 0.330        |

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**Figure 1.** Response contour plot of the optimized conditions for lignosulfonate cross-linking.
formed the insoluble material and the optimized binder. N1 and N8 were two of the reactions performed for obtaining the optimized binder. By comparing these spectra, the cross-linking behavior can be best illustrated. In unison with the molecular-weight responses from size exclusion chromatography, they show the difference in bond formation depending on the reaction conditions.

In the general information area of the spectra, a very broad band between 3650 and 3100 cm\(^{-1}\) is visible, corresponding to the presence of \(-\text{OH}\) stretches. The presence of an additional band in this region for experiments N8 and N1, specifically between 3450 and 3300 cm\(^{-1}\), can be due to the presence of water in solid samples that can generate a spurious band. Between 3000 and 2840 cm\(^{-1}\), there is a band that appears pointier and sharper in the native LS and in N1, indicating the asymmetric stretching of C–H and fully saturated carbons (–CH\(_3\) and –CH\(_2\)-alkanes), while for N8 and the optimized binder, the conformation seems broader, indicating the presence of –OH stretching and hydrogen-bonded carboxylic acid dimers.\(^{28}\) This can be a possible marker of the oxidation of lignosulfonate and of the new bonds that are formed. The triplet present at 1506, 1453, and 1422 cm\(^{-1}\) is attributable to the stretching of C=C, CH\(_2\), CH\(_3\), aldehydes, and methoxy (–OCH\(_3\)) groups. The doublet at 1370–1330 cm\(^{-1}\) is moreover characteristic of CO\(_2\) symmetric stretches.\(^{29}\) The peaks between 1300 and 1000 cm\(^{-1}\) depend on various vibration bands such as C=O, C–H, and C–O. For example, the medium peak at 1260 cm\(^{-1}\) corresponds to C=O stretching and carboxylic acid dimers; the band at 1216 cm\(^{-1}\) as well as the highest one at 1020 cm\(^{-1}\) correspond to para-substituted benzenes.\(^{30,31}\)

Figure 3 shows a smaller part of the spectrum, only the fingerprint region between 1700 and 680 cm\(^{-1}\). To ease the view, spectra were shifted vertically. At 1390 cm\(^{-1}\), in the highly cross-linked experiments (N8 and optimized binder), a small peak appeared, together with another small but broader one at 1375 cm\(^{-1}\); these two bands can indicate the presence of more aldehydes, CO\(_2\) stretches, and carboxylic acid salts. At 1160 cm\(^{-1}\), a small band is present in all of the curves except the untreated LS; this medium absorption band corresponds to C=O and C=O stretching and mono-substituted benzenes. Some different conformations of the bands are also visible between 1100 and 1060 cm\(^{-1}\), the area that corresponds to the asymmetric stretch of aliphatic ethers and to aliphatic primary and secondary alcohols: the behavior of the N8 and optimized binder curves in this part of the spectrum is similar, while it looks much different for the native LS and N1. As is clarified in the zoom of Figure 3, in fact, a small peak around 1065 cm\(^{-1}\) is present for N8 and

Figure 2. FTIR spectra of native lignosulfonates and enzymatically cross-linked lignosulfonates at different conditions in terms of oxygen supply, reaction time, and temperature.

Figure 3. FTIR spectra of the fingerprint region for the enzymatic cross-linking of LS highlighting the main differences caused by the cross-linking reaction.
optimized binder, and there is also a shift in the peaks from 1090 cm\(^{-1}\) (for the native LS and N1) to 1080 cm\(^{-1}\) (for the N8 and optimized binder), highlighted in a black arrow. Other differences like these can be seen at wavelengths 950, 920, and 880 cm\(^{-1}\), all signifying ring vibrations, C–H bending, meta-substituted benzenes, as well as vinyl esters. Finally, at 830 cm\(^{-1}\), a small peak, present in all spectra except the native LS, can be observed. At this wavelength, also isopropyl groups and functional groups like RR\(^1\)-C═CHR\(^2\) absorb, suggesting the formation of new bonds between different LS residues.\(^{30-34}\)

**Synthesis of Pure Lignin-Based Granules and Dicamba Release.** The granules produced using the lab granulation device had a size range of 2–4 mm diameter and were visually characterized by electron microscopy (Figure 4).

The agglomerate structure seen in Figure 4 was a result of the production process. The granules were formed from the powder and glued together by the optimized lignin binder synthesized by enzymatic cross-linking. The granules had pores and cavities where, ideally, the added Dicamba solution could penetrate and be entrapped in the cross-linked lignosulfonate structure in type 1 granules. Once added to the buffer solutions, the herbicide should then be released. In type 2 granules, Dicamba was added during the cross-linking process, and therefore a tighter entrapment was expected, since the herbicide could be built into the grafted structure. Figure 5a,b shows the release profile of Dicamba in different buffer solutions. Also pH had a minimum effect on the release of the herbicide showing no significant differences after 6 h, where all of the Dicamba was released in Milli-Q water and in phosphate buffer of pH 7. Whereas at pH 9 and pH 3, Dicamba was only completely released after 48 h, showing that at these pH values Dicamba is better bound to the granules. In a study on pesticide mobility, Dicamba was found to be relatively mobile. pH had a strong influence on Dicamba mobility; at pH less than 4.2, Dicamba was not readily leached from soils. In a pH range of 5.0–6.7, Dicamba mobility increased with increasing pH, but at pH above 6.7, there was no increase in mobility.\(^{38}\) For weak aromatic acids such as Dicamba and 2,4-D, phytotoxicity increases as soil pH increases and reaches a maximum at pH 6.5.\(^{39}\) Anyhow the major amount of Dicamba was released after 3 h for all experiments. Although different entrapment methods were used, both granule types show the same release properties. It is very likely that the hypothesis that the addition during the grafting process would lead to a better entrapment was wrong due to the fact that Dicamba rarely binds to soil particles. The produced granules, anyhow, are designed biodegradable, and therefore behave similarly to soil.\(^{22}\) Therefore, the desired bonding of Dicamba and the granule material was not reached. The shown burst release is unfavorable for the desired application. Roy et al. comprehensively summarized that matrix release systems are not very well developed yet, but hold the possibility to revolutionize the field. This first prototype of a carrier system will be further developed. The lack of binding capability of the pure lignosulfonates matrix for Dicamba has to be further investigated. Compared to other delivery systems such as nano-encapsulation, a controlled release has yet to be developed.\(^{17,37}\) By further tuning the enzymatic cross-linking process and possible addition of other materials to the reaction, the incorporation of Dicamba could be improved in the future.
Although only minor differences in terms of Dicamba release were detected, the two granule types displayed quite different behavior in terms of stability. In Figure 6, pictures of the granules before and after the release studies are shown. Although both granule types look the same prior to the release experiment, after 1 week, major differences can be observed depending on the pH values of the experiments as well as the granule type. In Figure 6a, the type 1 granules have a more compact structure especially at pH 7, whereas at pH 9, their structure is very much degraded. This behavior is due to the tendency of lignosulfonate to ionize in the alkaline region. Overall, the structural integrity of type 1 granules at pH 3 and pH 7 as well as in Milli-Q water is very good. Type 2 granules (Figure 6b) on the other hand were less stable than type 1 granules under all conditions. Granule sizes significantly decreased after the release studies, showing the faster degradation and less stability of type 2 granules. In addition to the good performance concerning the stability of type 1 granules, very good water retention of the granules was observed. As can be seen in Figure 6 also, the granules were hydrophilic, thereby absorbed water. This was also observed by Legras-Lecarpentier et al. when enzymatically cross-linked lignosulfonates were used to produce alginate–lignosulfonate fertilizer granules.

**CONCLUSIONS**

The technique used for producing type 1 granules resulted in stable granules even after 1 week of release in buffer solutions at pH 3 and pH 7 as well as in Milli-Q water. The granule structure facilitated impregnation with Dicamba and consecutively its release. These results show the successful design of a fully biodegradable carrier system using the MODDE Pro.
design of experiment suitable for pesticide release. In addition to acting as a carrier system, the cross-linked lignosulfonates can also serve as soil conditioners since they decompose into humic acid. Therefore, this study showed the green production of a 100% lignosulfonate-based biodegradable solid carrier with potential application in agriculture.

## EXPERIMENTAL SECTION

**Materials.** The lignosulfonate used in this work originated from spent liquor of the sulfite wood pulping process and was supplied by a company partner. A laccase with an average activity of 1273 U/mL from *Mycelophtora thermophila* (MtL) purchased from Novozymes (Novozym 51003) was used for lignosulfonate (LS) cross-linking. The herbicide 3,6-dichloro-2-methoxybenzoic acid (Dicamba) was purchased from Sigma-Aldrich GmbH (Germany). HCA was used for the normalization, band broadening, and application.40,41

**Cross-Linking of Lignosulfonates.** Dried lignosulfonate was used to prepare 8% (w/w) lignosulfonate solutions for the binder, and 9% (w/w) lignosulfonate solutions were used for the production of cross-linked LS powder for making the granules. The pH was adjusted to 7 using 5 M NaOH. When the conditions of temperature and oxygen content were reached, the reaction started by injecting 166.7 nkat/mL MtL. The level of oxygen saturation in the solution was monitored with oxygen sensors positioned on optical spots previously immobilized in the reaction vessels (FireSting-O2, PyroScience, Germany).

**Design of Experiment for Optimal Binder.** The design of experiment software MODDE Pro (Sartorious, Germany) was used to optimize the production of a suitable LS binder for granule production. The optimal reaction condition combinations were determined by assessing the interaction between temperature, oxygen flow, and reaction time, as shown in Table 2. Molecular weight and viscosity were chosen as responses. This method is readily applied in many fields for similar applications.40,41

**Size Exclusion Chromatography.** The molecular weight of cross-linked lignosulfonates was monitored using a liquid chromatography system equipped with a quaternary/binary pump, an autosampler 1260 series from Agilent Technologies (Palo Alto, CA), a DAD (diode array detector) and an refractive index (RI) detector system (Agilent Technologies 1260 Infinity), as well as a MALLS HELEOS DAWN II detector from Wyatt Technologies (Dernbach, Germany). The SEC column system consisted of a precolumn PL aquagel-OH MIXED Guard (PL1149-1840, 8 μm, 7.5 × 50 mm², Agilent, Palo Alto, CA) and a separation column PL aquagel-OH MIXED H (PL1549-5800, 4.6 × 250 mm², 8μm, Agilent, Palo Alto, CA) with a mass range of 6–10,000 kDa. The lignin samples were diluted with the mobile phase to a concentration of 1 mg/mL. The injection volume was 100 μL. The system was operated with 50 mM NaNO₃/3 mM NaN₃ and had a total runtime of 90 min. The Agilent Software Openlab Chemstation CDS as well the ASTRA 7 software from Wyatt Technologies were used for data acquisition and data analysis. BSA was used for the normalization, band broadening, and alignment of the MALLS detector.

**Viscosity Measurement.** The rheological properties of the samples were measured with a rotational rheometer CVO 50 (Bohlin Instruments, U.K.). Cross-linked lignosulfonate (1 mL) was placed on the plate, and the measurement was carried performed in a 96-microwell plate. For the blank, 170 μL of phosphate buffer was added to 50 μL of the ABTS solution. For the enzyme reaction, 170 μL of enzyme solution was prepared in triplicates and added to 50 μL of the ABTS solution. Measurements at the UV/vis spectrophotometer started immediately, at 420 nm.

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\text{enzymatic activity} \left[\text{U/mL}\right] = \left(\frac{\text{slope}_{\text{test}} - \text{slope}_{\text{blank}}}{\varepsilon} \times \frac{1}{d} \times \frac{V_{\text{total}}}{V_{\text{enzyme}}} \times DF\right)
\]

where \(\varepsilon\) is the extinction coefficient at 420 nm [mM⁻¹ cm⁻¹]; \(d\) is the path length [cm]; \(V_{\text{total}}\) is the total reaction volume [mL]; \(V_{\text{enzyme}}\) is the enzymatic solution volume [mL]; and DF is the dilution factor.

The laccase activity refers to the amount of enzyme necessary to catalyze the conversion of 1 nmol of substrate per second in specific assay conditions and was given as nKat.

**Table 2. Experimental Conditions Used to Obtain the Optimal LS Binder Properties**

| experiment | temperature [°C] | oxygen flow [cm³/min] | time [min] |
|------------|------------------|------------------------|------------|
| N1         | 20               | 10                     | 60         |
| N2         | 50               | 10                     | 60         |
| N3         | 20               | 60                     | 60         |
| N4         | 50               | 60                     | 60         |
| N5         | 20               | 10                     | 360        |
| N6         | 50               | 10                     | 360        |
| N7         | 20               | 60                     | 360        |
| N8         | 50               | 60                     | 360        |
| N9         | 35               | 35                     | 210        |
| N10        | 35               | 35                     | 210        |
| N11        | 35               | 35                     | 210        |

**Table 3. Experimental Conditions Used to Obtain the Optimal LS Binder**

| identification | composition | procedure for Dicamba addition |
|----------------|-------------|--------------------------------|
| pure lignosulfonate granules | 9% LS powder + 8% LS binder | dried LS granules soaked in Dicamba solution (40 mg in 40 mL of Milli-Q water) |
| type 1 granules | 9% LS powder + 8% LS binder | dry LS granules soaked in Dicamba solution (40 mg in 40 mL of Milli-Q water) |
| type 2 granules | 9% LS powder with ∼2 g Dicamba/kg granules + 8% LS binder | 60 mg of Dicamba, dried, milled, and used for granule production |

**Table 4. Eluent Gradient of HPLC Dicamba Analysis**

| time [min] | water [%] | acetonitrile [%] |
|------------|-----------|------------------|
| 0          | 80        | 20               |
| 1          | 80        | 20               |
| 11         | 0         | 100              |
| 14         | 0         | 100              |
| 15         | 80        | 20               |
out at 20 °C, at a shear rate of 200 s⁻¹, and a 3 s time delay. A 4° conical plate with a diameter of 40 mm was used.

**Fourier Transform Infrared Spectroscopy.** Samples of 5 mL of cross-linked lignosulfonates were stored at −80 °C in falcon tubes until lyophilization was performed at 21 °C and 0.1 mbar for 2–3 days. The resulting powder was analyzed using FTIR spectroscopy (Spectrum 100, PerkinElmer). The sample was placed on the ATR sample plate and pressed on it with a knob; the applied pressure was monitored by the software to ensure that it would be the same for all of the samples (149–150). Absorbance spectra were then obtained from 50 scans between the range of 600 and 4000 cm⁻¹ for each sample in triplicate. The average spectra from the triplicates were then identified, and the spectra from all of the samples were normalized for the entire wavelength range.

**Granule Production and Loading with Dicamba.** Two types of granules were produced differing in the mode of Dicamba loading. In general, each formulation consisted of 9% cross-linked LS powder. A lab-scale version mimicking an industrial granulation process was used for producing the granules. Essentially a small amount of the powder was put into the beaker of the granulation device and continuously sprayed with cross-linked 8% lignosulfonate as binder. The formed granules were then dried at 70 °C for 24 h. To load the granules with Dicamba, two approaches were used, as shown in Table 3. The amount of Dicamba per kg of finished granules (2 g/kg) was the same for both procedures.

**Scanning Electron Microscopy.** Scanning electron microscopy was performed on a Hitachi TM 3030 (Hitachi High-Technologies Europe GmbH, Germany) instrument to morphologically characterize the granules. To increase resolution, the samples were sputtered with platinum using a Leica EM ACE200 Vacuum Coater (Leica Microsystems GmbH, Germany).

**Release Studies.** The release of the herbicide Dicamba from the granules was investigated in Milli-Q water, 50 mM phosphate buffer pH 3 and pH 7, and 50 mM Tris-HCl buffer pH 9. The experiments were performed in falcon tubes (Fisher Scientific GmbH, Schwerte) containing 1 g of granules (with 2 mg of Dicamba) and 50 mL of solution. Experiments were conducted in triplicate at 23 °C at 80 rpm. Supernatants were regularly collected (0, 3, 6, 24, 48, 72, 120, 168 h) for HPLC analysis. The falcon tubes were refilled with 50 mL of the respective solution. The withdrawn samples were filtered with 0.45 μm syringe filters prior to HPLC analysis. The Agilent HPLC1200 series (Agilent, Santa Clara, CA) equipped with an Agilent 1200 series DAD detector was used to quantify the released Dicamba at 234 nm with measurement at 282 nm as qualifier. The injection volume was set to 25 μL at 20 °C for standards and samples on an Agilent 1200 series autosampler equipped with an autosampler thermostat from the same series. A Poroshell 120 EC-C18 (3 × 150 mm²; particle size, 2.7 μm) column purchased from Agilent (Agilent, Santa Clara, CA) was applied for separation. The column was heated to 40 °C and operated with a flow rate of 0.6 mL min⁻¹. The eluent system was composed of ultrapure water and acetonitrile (Sigma-Aldrich), each with 0.1% formic acid. The gradient profile is given in Table 4. To establish constant starting conditions, a post runtime of 5 min was included in the program. Standards were prepared in lignosulfonate solutions at the respective pH.

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