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## A biobased, bioactive, low CO<sub>2</sub> impact coating for soil improvers†

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Lignosulfonate-based bioactive coatings as soil improvers for lawns were developed using laccase as a biocatalyst. Incorporation of glycerol, xylitol and sorbitol as plasticizers considerably reduced the brittleness of the synthesized coatings of marine carbonate granules while thermal enzyme inactivation at 100 °C enabled the production of stable coatings. Heat inactivation produced stable coatings with a molecular weight of 2000 kDa and a viscosity of  $4.5 \times 10^{-3}$  Pas. The desired plasticity for the spray coating of soil improver granules was achieved by the addition of 2.7% of xylitol. Agriculture beneficial microorganisms (four different *Bacillus* species) were integrated into the coatings. The stable coatings protected the marine calcium carbonate granules, maintained the viability of the microorganisms and showed no toxic effects on the germination and growth of model plants including corn, wheat, salad, and tomato despite a slight delay in germination. Moreover, the coatings reduced the dust formation of soil improvers by 70%. CO<sub>2</sub> emission analysis showed potential for the reduction of up to 3.4 kg CO<sub>2</sub>-eq. kg<sup>-1</sup> product, making it a viable alternative to fossil-based coatings.

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### Introduction

Lawns have become a key part of landscape designs in urban centres occupying parks; golf courses; botanical, zoological and home gardens; institutional green spaces; playground/school grounds; street spaces, *etc.* Lawn management requires frequent mowing, irrigation and application of fertilizers for optimal performance and aesthetic quality, which are attributed to many negative environmental impacts.<sup>1</sup> European homeowners spend billions of euros and typically apply 10 times the amount of pesticides and fertilizers per hectare on their lawns than farmers do on crops.<sup>2,3</sup> Most of these agro-

chemicals end up polluting the environment, for example, fertilizers emit greenhouse gases and also cause eutrophication of local streams, lakes, and coastal zones.<sup>1</sup>

The negative effects caused by the release of fertilizers into the environment are putting pressure on agrochemical producers to improve the efficiency of their products. Ideal fertilisers should have a higher nutrient use efficiency, as a consequence of which detrimental effects on soil, water, and atmospheric environments are reduced while at the same time enabling a single fertiliser application during a growing season.<sup>4</sup> However, currently, the nutrient use efficiency (NUE), defined as the percentage of a nutrient that contributes to biomass production, for nitrogen based fertilizers ranges from 30 to 50%.<sup>5</sup> To compensate for these losses, agrochemicals are applied in excess amounts,<sup>5</sup> meaning at least 70% of the fertilizer is lost either through leaching thereby causing eutrophication and also increasing the release of nitrous oxide,<sup>6</sup> a powerful greenhouse gas emitted into the atmosphere that contributes to climate change. Intensely managed lawns have 70% higher global warming potential from direct emissions compared to lawns that are not fertilized or irrigated.<sup>7</sup>

To reduce the loss and negative effects of agrochemicals on the environment, coating materials for fertilisers have been developed. However, many coatings are fossil based and/or non-biodegradable such as those based on polyethylene, polysulfone, polyvinyl chloride, polystyrene, polyacrylic acid latex, polyvinyl alcohol, polyethylene, polydopamine, cellulose

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acetate, polyacrylonitrile, copolymers of butylene-succinate-*co*-dilinoleate and polyethylene-succinate-*co*-terephthalate copolymers of polyvinyl alcohol (PVA) and polyvinylpyrrolidone.<sup>4,5,8,9</sup> Some of these polymer-coatings decompose extremely slowly or not at all in soil, leading to undesirable accumulation of microplastics potentially posing a threat to terrestrial wildlife and food security.<sup>10,11</sup> Apart from accumulating in the soil, microplastics can be transported to water bodies, contributing to marine plastic pollution and putting marine organisms at risk.<sup>12</sup> Furthermore, several conventional fertilizer coatings can even be toxic to plants or change the soil's pH, which is undesirable.<sup>4</sup> The European Commission, in the frame of the Circular Economy Action Plan, has put forward a proposal to restrict some intentionally added microplastics in products, including polymers used in fertilizers, by 2021.<sup>13</sup> By 2026 only polymers that meet defined biodegradability standards will be allowed on the market.<sup>14</sup>

Therefore, there is growing interest in developing bio-based coating materials obtained from renewable sources. Among the bio-based polymers, lignosulfonate is emerging as a highly promising versatile material. Unlike many other bio-based materials, lignosulfonates have an inherent structure containing abundant reactive functional groups (aliphatic and phenolic hydroxyl groups and carbonyl groups) and unshared electron pairs on the oxygen atoms in these groups, making them natural chelators of many important agricultural metal ions such as potassium, calcium, magnesium, manganese, *etc.*<sup>15</sup> Furthermore, the abundant phenolic functionalities of lignosulfonates stabilize the soil's organic nitrogen and thereby inhibit two detrimental events occurring in soils: nitrification and the unwanted volatilization due to soil urease activity.<sup>15</sup> Currently only 2% of the 100 million tonnes of lignin side streams produced yearly by the global pulp and paper industry is utilised,<sup>16</sup> and the remaining 98% is burned in the processes releasing 1.63 kg CO<sub>2</sub> kg<sup>-1</sup> lignin.<sup>17</sup> Contrary to this, applying lignosulfonates to the soil can enable the capture of CO<sub>2</sub> and improve the soil properties. Lignosulfonates biodegrade in the soil and transform it into humic acids which improve the soil structure, nutrients and water retention and are hence beneficial for crop growth.<sup>18</sup> Lignosulfonates are approved for use in organic crop production in the USA as published in the identification of petitioned substances report of the U.S. Department of Agriculture.<sup>19</sup> Therefore their broad application in agriculture as fertilizer components<sup>20</sup> and even attempts to develop gels formed by chemical synthesis using formaldehyde as an agent<sup>21</sup> have been reported. Although the suitability of lignosulfonates for agriculture is well-known, as fertilizer carriers, binders, and dispersants are in commercial use, to date no suitable technologies have been developed to exploit their remarkable properties for fertilizer coatings.<sup>22,23</sup> This would both allow the replacement of currently used petrol-based coatings and exploit the beneficial effect of lignins on the soil. Several strategies including the use of simple physical coatings without modification of technical lignins (kraft, soda and lignosulfonates), chemically modified coatings through ammoxidation, acetylation,<sup>24</sup> Mannich reaction, esterification, and hydroxymethyl-

ation combined with blending and even attempts to use glycerol as a plasticizer resulted in defective coatings.<sup>18,25</sup> Furthermore, chemical processes such as acetylation,<sup>26</sup> hydroxymethylation, and esterification of lignin use toxic chemicals like formaldehyde.<sup>18,27,28</sup> Likewise other applications such as the application as dispersing agents or stabilizers have even been patented, but they often involve the necessity of harsh reaction conditions and toxic or harmful chemicals.<sup>29,30</sup> Often lignosulfonates are used as is without any modification merely as fillers or in rather low amounts.<sup>31,32</sup>

Unlike these previous studies, this study investigates a whole green chemistry process, that employs laccase enzymes to polymerize and tailor the properties of lignosulfonate coatings. Laccases are oxidoreductase enzymes that are widely distributed in nature, able to react with a variety of aromatic substrates including lignosulfonates, and generate reactive species with the concomitant reduction of molecular oxygen to water. The most attractive feature of laccase lies in its ability to generate reactive species that are able to cross-react among themselves or with other (functional) molecules. This characteristic enables the tailoring of lignosulfonate properties to synthesize materials with certain property profiles. Despite earlier promising studies on lignin modification using laccases, the need for electron mediators, the high enzyme costs, and the lack of mechanistic understanding had considerably limited the viable use of laccases to process lignin. Recently we developed a versatile laccase-based technology that allows tailoring the lignosulfonate polymerization process and enables us to transform low molecular weight water-soluble lignosulfonates into high molecular weight water-insoluble polymers with potential applications for making many different functional materials<sup>33,34</sup> including agrochemical delivery systems<sup>35</sup> without the need for mediators. Based on this success, this study involved the enzyme-based synthesis of novel multifunctional biobased, bioactive non-toxic lignosulfonate coatings that reduce fertilizer leaching and hence its negative environmental impact. This involved specifically controlled enzymatic polymerization combined with enzyme activation and the addition of precise amounts of plasticizer to achieve certain properties suitable for coating synthesis (such as the use in coating machines and/or for spray coating) as well as improved coating performance (stability, caking, *etc.*). This study also, for the first time, shows the possibility of incorporating plant growth promoting microorganisms *i.e.* *Bacillus* species into the formulation. The enzymatically polymerized lignin coatings incorporating plant growth promoting *Bacillus* species are used for promoting the growth of turf grass. It is expected that incorporating *Bacillus* spp, widely known as plant growth promoters, which under stress conditions solubilize nutrients needed by the host plant,<sup>36</sup> will help promote plant growth. Their ability to support the nutrient uptake of the plant in soils by interacting with the root system has been described<sup>37,38</sup> and a number of products have been commercialized such as Kodiak (*B. subtilis* GB03), Quantum-400 (*B. subtilis* GB03), Rhizovital42 (*B. amyloliquefaciens* FZB42)<sup>39</sup> and Rhizovital C5 (*B. atrophaeus*, included in this study).



## Materials and methods

### Laccase activity assay

The activity of laccase from *Myceliophthora thermophila* was determined by photometrically monitoring the oxidation of ABTS (azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) at 420 nm as previously described by Weiss *et al.* 2020.<sup>35</sup> ABTS is oxidised by laccases and the concentration of the resulting green-blue coloured cation radical can be correlated with the enzyme activity.<sup>40</sup> For the enzyme activity assay, 50 mM sodium phosphate buffer pH 7 and 10 mM ABTS solution were prepared. The reaction was performed in a 96-microwell plate. For the blank, 170  $\mu\text{L}$  of phosphate buffer were added to 50  $\mu\text{L}$  of the ABTS solution. For the enzymatic reaction, 170  $\mu\text{L}$  of the enzyme solution diluted at a ratio of 1 : 30 000 in sodium phosphate buffer were prepared in triplicate and added to 50  $\mu\text{L}$  of ABTS solution. The laccase activity was determined as follows:

$$\text{Enzymatic Activity} \left[ \frac{\text{U}}{\text{mL}} \right] = (\text{slope}_{\text{test}} - \text{slope}_{\text{blank}}) \times \frac{1}{\epsilon} \times \frac{1}{d} \\ \times \frac{V_{\text{total}}}{V_{\text{enzyme}}} \times \text{DF}$$

The enzyme activity refers to the amount of enzyme necessary to catalyse the conversion of 1  $\mu\text{mol}$  of substrate per minute under specific assay conditions.

### Enzymatic synthesis of lignosulfonate coatings

Liquid magnesium lignosulfonates supplied by SAPPI were used as a raw material for all polymerisation experiments (Specifications on the composition are provided in the ESI†). The liquid lignosulfonates were dried in large ceramic pans for 3–7 days in a 70 °C oven until it was bone dry (*i.e.* 100% dry matter). The dried lignosulfonates were then weighed and dissolved in MilliQ water to obtain the desired lignosulfonate concentration (7% w/v, 8% w/v, 9% w/v) and pH adjusted to 7 using 5 M sodium hydroxide solution.

The level of oxygen saturation in the solution was monitored by using oxygen sensors positioned on optical spots. The reaction was started by introducing 10 Units per mL of laccase and continuously aerating at 30  $\text{cm}^3 \text{min}^{-1}$  at room temperature. A laccase with an average activity of 1273  $\text{U mL}^{-1}$  from *Myceliophthora thermophila* (MtL) purchased from Novozymes (Novozym 51003) was used for lignosulfonate cross-linking.

To reduce the brittleness of pure lignosulfonate polymers, various plasticizers (sorbitol, xylitol and glycerol of reagent grade and purchased from Sigma-Aldrich GmbH) at different concentrations (1 : 2, 1 : 3, 1 : 4 ratios of plasticizer: dry matter content of lignosulfonates) were separately added to the lignosulfonates during polymerization.

### Size exclusion chromatography

The changes in the molecular weight of lignosulfonates before and during polymerization as well as during the inactivation process were measured using a high performance liquid chromatography (HPLC) system equipped with a quaternary/binary pump, a 1260 series autosampler from Agilent

Technologies (Palo Alto, CA), a DAD (diode array detector) and a refractive index (RI) detector system (Agilent Technologies 1260 Infinity), as well as a MALS HELEOS DAWN II detector from Wyatt Technologies (Dernbach, Germany) was used. The column system consisted of a PL aquagel-OH MIXED Guard precolumn (PL1149-1840, 8  $\mu\text{m}$ , 7.5  $\times$  50  $\text{mm}^2$ , Agilent, Palo Alto, CA) and a PL aquagel-OH MIXED H separation column (PL1549-5800, 4.6  $\times$  250  $\text{mm}^2$ , 8  $\mu\text{m}$ , Agilent, Palo Alto, CA) with a mass range of 6–10 000 kDa. Lignin samples were diluted with the eluent to a concentration of 1  $\text{mg mL}^{-1}$ . The injection volume was 100  $\mu\text{L}$ . The mobile phase consisted of 50 mM  $\text{NaNO}_3$ /3 mM  $\text{NaN}_3$  and had a total runtime of 92 min. The Agilent Software Openlab Chemstation CDS and ASTRA 7 software from Wyatt Technologies were used for data acquisition and data analysis.

BSA was used for the normalization, band broadening, and peak alignment of the MALS detector.

### Viscosity measurement

The rheological properties of the samples were measured using a CVO 50 rotational rheometer (Bohlin Instruments, U.K.). This device permits the study of viscosity and elasticity of materials under different conditions. For the viscosity measurements, 1 mL of the defrosted sample was placed on a fixed plate. The shear force was applied with a 4° conical plate with a diameter of 40 mm. The measurement was carried out at a constant temperature of 20 °C and a shear rate of 200  $\text{s}^{-1}$ .

### Inactivation of laccase

The laccase was heat inactivated after polymerization to the desired size in order to produce stable coatings. To determine the optimal conditions for inactivation, 10 mL of the respective 7%, 8% and 9% coatings were treated at different temperatures (80 °C, 90 °C, 100 °C) for 5, 10, and 15 minutes in a water bath. Falcon tubes with a max volume of 15 ml were used. The stability of the polymers was then followed by monitoring the changes in viscosity during storage.

### Synthesis of bioactive soil improver coatings

Marine calcium carbonate granules (4 mm diameter) provided by Roullier were first coated with different concentrations of enzymatically polymerised lignosulfonates. Finally, the 8% coating was determined as the optimum incorporating 2.7% xylitol as a plasticizer. Six types of granules were produced, namely, (i) uncoated marine calcium carbonate granules which served as a blank, (ii) lignosulfonate coated granules which represented the negative control and (iii) granules that contained different *Bacillus* species in the lignosulfonate coating at a concentration of 2  $\times$  10<sup>6</sup> cfu  $\text{g}^{-1}$  (Table 1).

### Spore viability in coated granules

*Bacillus* species were provided by ABITEB and AIT and spore formulations were produced by ABITEP. One gram of differently coated and uncoated granules was transferred under



**Table 1** Granules applied in the experiments

Calcium carbonate	Coated	<i>Bacillus</i> species	Abbreviation	Strain collection
Yes	—	None	—	—
Yes	Yes	None	—	—
Yes	Yes	<i>B. subtilis</i>	A0 44	AIT_EKA044BA16
Yes	Yes	<i>B. licheniformis</i>	Abi 53	ABITEP Abi 53
Yes	Yes	<i>B. velezensis</i>	Abi 25	ABITEP Abi 25
Yes	Yes	<i>B. atrophaeus</i>	Abi 05	ABITEP Abi 05 Rhizovital C5

sterile conditions into 15 ml tubes filled with 10 ml of sterile 0.9% NaCl solution and 1 g of sterile glass beads (1 mm diameter). Tubes were vigorously vortexed for a couple of minutes to homogenize and suspend spores. Serial dilutions of spore suspensions were prepared by combining 100  $\mu$ l of suspension with 900  $\mu$ l of sterile 0.9% NaCl up to a dilution of  $10^{-5}$ . From each spore dilution, 25  $\mu$ l were inoculated onto each of the three Trypsin-Soy (100%) agar plates (6 cm diameter) and spread using well sterilized glass rods. Agar plates were inoculated overnight at 37% and the number of colony forming units (CFUs) were counted. In calculations, the most outliers for CFUs per plate were removed and the average of CFUs (germinated spores) per plate and dilution was calculated.

#### Attrition of granules

Attrition (the gradual destruction of dried granules in subsequent handling after production and during storage is generally a disastrous situation to be avoided, also called roll off)<sup>41</sup> of uncoated and coated granules was measured. Twenty grams of the respective granules were transferred in 50 ml Falcon tubes and stored at room temperature. Due to mechanical attrition, the generated dust was gravimetrically analysed after 1 month and 1 year.

#### Application of coatings on plants

The effects of modified lignosulfonates and/or coatings on plants were tested in plant pot trials. Lignosulfonates (3.6 w/v%) were put into 1000 ml plant pot soil volume. As plant models, corn, wheat, salad, and tomato were chosen and 16 seeds per pot were planted. Sterilized, fertile soil was used for all tests. Experiments were performed in triplicate. Germination and growth were monitored. After germination, seedlings were manually removed and only 3 plants per pot for corn, salad, and tomato, and 4 plants per pot for wheat left to grow.

#### Application of soil improvers on the golf lawn

The effect of the soil improver, when coated with different variants of the bioactive coating, was tested in greenhouse trials on the golf lawn. The grass was regularly cut and the fresh weight of the cut was gravimetrically analysed to show the biomass production over 84 days.

#### CO<sub>2</sub> emission equivalent of coating production

The system boundaries applied in the CO<sub>2</sub>-eq. assessment are cradle-to-gate, assessing the upstream and foreground processes leading to the production of a market ready coating material. The impact category assessed is the Global Warming Potential for a 100-year time horizon (GWP100a). The modelled processes are shown in Fig. 1. Information about the process parameters was obtained from SAPPI for their sulphite paper pulp factory in Austria (Gratkorn). In this installation, lignosulfonates (LS) are currently a side product of the pulping process. LS are presently used as a fuel to generate heat and power in the installation (Fig. 1: process B'). In the innovative processes investigated, LS are processed into a coating material (process train B). The processing comprises the purification of the lignosulfonate containing wastewater. The purified LS are further refined by polymerisation and addition of a plasticizer before being market ready (*i.e.* at gate). Inputs to these processes are electrical energy and heat energy, respectively (see the ESI† for assumptions). Diversion of LS from incineration implies that another energy source must be substituted for the marginal decrease in energy generation (process A). At the paper pulp plant it is envisioned to use wood residues for this purpose. Wood residues and LS are further assumed to have the same CO<sub>2</sub>-eq. emission per unit energy (*i.e.* MJ) released. Therefore, the substitution between the two materials is assumed to exhibit a net zero emission (zero burden assumption). Finally, the novel LS coating material is assumed to substitute the state-of-the-art coating products of the current industrial use (process D). Therefore, scenario analysis has been applied in this research, in which the impact of different state-of-the-art coatings (polyurethane, polyethylene) is compared to LS coatings that used different plasticizers (xylitol, glycerol).<sup>42</sup> Plasticizers were varied as they were found to cause the major share of CO<sub>2</sub>-eq. emissions.

## Results and discussion

#### Production of lignosulfonate coatings

Multiple parameters were monitored during the enzymatic synthesis of lignosulfonate soil improver coatings to determine optimum conditions and concentrations of lignosulfonates required. As shown in Fig. 2, aeration followed by the addition of enzyme leads to an immediate sharp decrease of the oxygen concentration to zero. The oxygen saturation is one of the most crucial drivers of enzymatic polymerization and the most influential tool to change the polymerization rate. This was utilized in previous studies for the production of oxygen scavenging films.<sup>43</sup> The online measurement of dissolved oxygen results in saturation curves giving insight into the evolution of the reaction. Previous studies<sup>44</sup> demonstrated the remarkable activity of laccase in oxidizing lignin while simultaneously reducing molecular oxygen to water. Similar to previous findings on sodium lignosulfonates the molecular weight of the coatings is highly dependent on the used enzyme as well as the substrate concentration and the initial molecular weight of



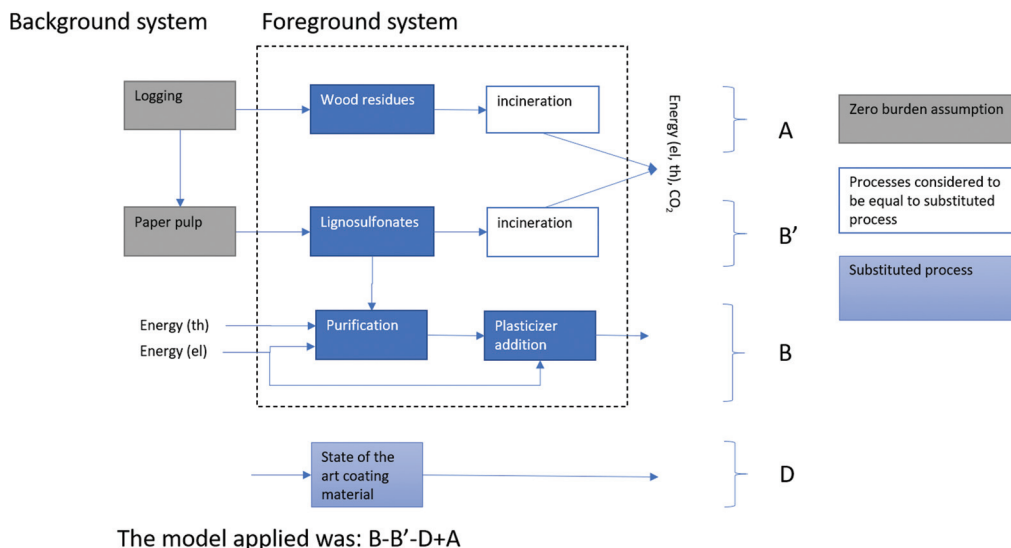


Fig. 1 Flow diagram of the processes of LS coating production as well as substituted processes.

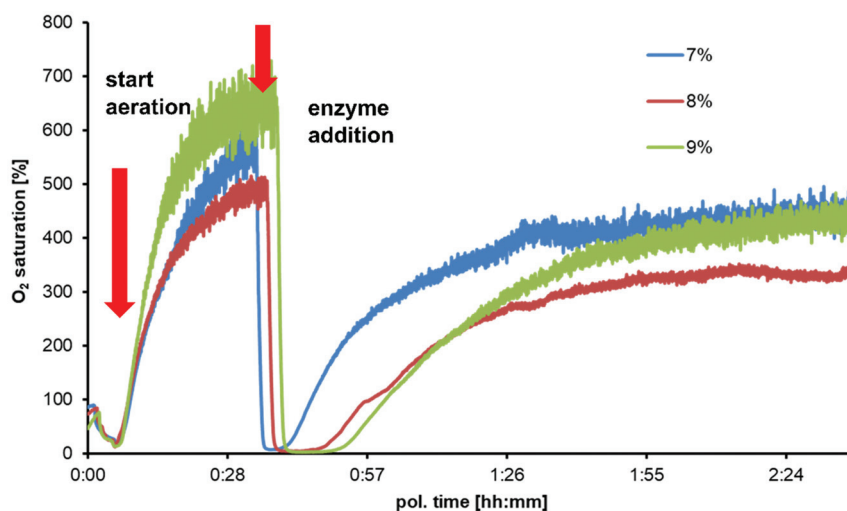


Fig. 2 Oxygen saturation during laccase catalysed polymerisation of lignosulfonates at different concentrations.

the substrate.<sup>45</sup> The results show that at constant oxygen supply significant differences can be observed depending on the lignosulfonate dry matter content. After enzyme application at 7% DM saturation levels are attained earlier compared to higher dry matter percentages (Fig. 2). The 8% DM curve remains on a lower oxygen saturation level, suggesting a higher demand for oxygen due to the better mobility of the radicals in the solution as well as the better steric availability of the substrate.<sup>46–48</sup>

Laccase polymerization of 7, 8 and 9% lignosulfonates resulted in an increase in the molecular weight from between 30 and 80 kDa to over 1500, 2000 and 2500 kDa, respectively (Fig. 4). This clearly shows that the initial concentration of lignosulfonates has a direct effect on the size of the formed polymers. As also shown in Fig. 4, the size of the formed ligno-

sulfonate polymer has also a direct effect on the viscosity of the produced coatings. These observations enable the tailoring of the properties of the produced coatings depending on the initial lignosulfonate concentration.

### Stabilization of lignosulfonate coatings

A stable and consistent coating is required for product reproducibility. Hence, the reaction must be stopped when reaching the desired degree of polymerisation to avoid ongoing laccase action during storage. To address this challenge, thermal inactivation of the laccase was investigated at 80 °C, 90 °C and 100 °C for 5, 10 and 15 minutes, respectively. Fig. 3 shows the enzyme activity in the coatings after each treatment. MtL was very stable at 80 °C retaining 40–90% of its activity in lignin samples as shown in Fig. 3. The enzyme could only be comple-

tely inactivated when incubated at 100 °C for 10 minutes. Since heat inactivation at 100 °C is a relatively harsh tool in respect of the polymer structure, the effect of the thermal inactivation on the molecular weight and viscosity of the produced polymers was investigated (Fig. 4). The structure of the lignosulfonate polymer produced with 9% dry substance was significantly stronger affected by higher temperatures and residence time compared to the other polymeric coatings. The 7% polymer was in general less viscous and had a lower average molecular weight, but was much less affected by the inactivation. Contrary to that the 8% coating showed an intermediate decrease of molecular weight and viscosity due to temperature and residence time, having similar viscosity (5 mPa s) and molecular weight (2000 kDa) values at the minimal required parameters for heat inactivation. Heat inactivation of the enzyme generally led to a decrease in both the final molecular

weight and viscosity of the coatings. However, these studies show that, considering all these factors, well defined conditions can be achieved to produce a consistent coating product.

### Screening for plasticizers and anti-dust properties

Preliminary trials with polymerized lignosulfonates produced unstable randomly disintegrating coatings that completely detached from the soil improver granules. This is in line with previous observations which demonstrated that using lignosulfonates and other industrial lignins without plasticizers or binder materials leads to brittle and shrinking materials.<sup>49,50</sup> To reduce the brittleness of the coatings, the effects of glycerol, sorbitol and xylitol as plasticizers at different concentrations were studied. The addition of these plasticizers showed no significant effect on the molecular weight of the coatings.

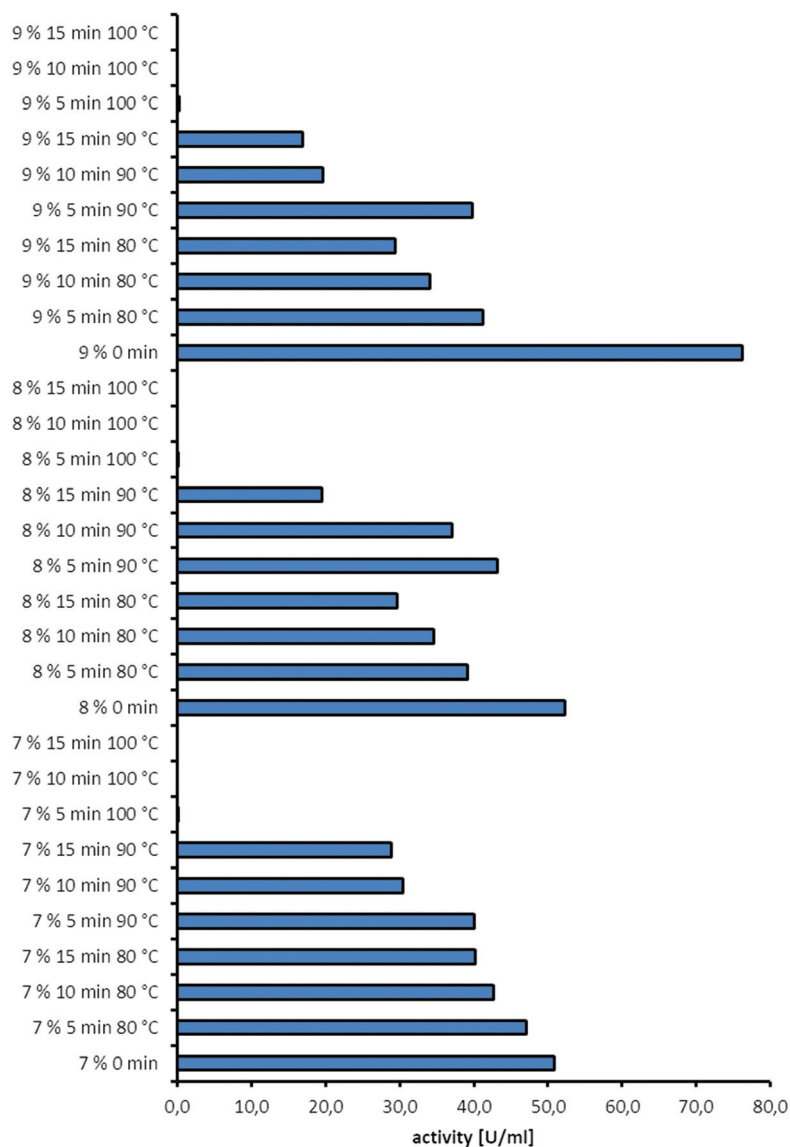


Fig. 3 Thermal inactivation of laccase. A decrease of enzyme activity after heat treatments.

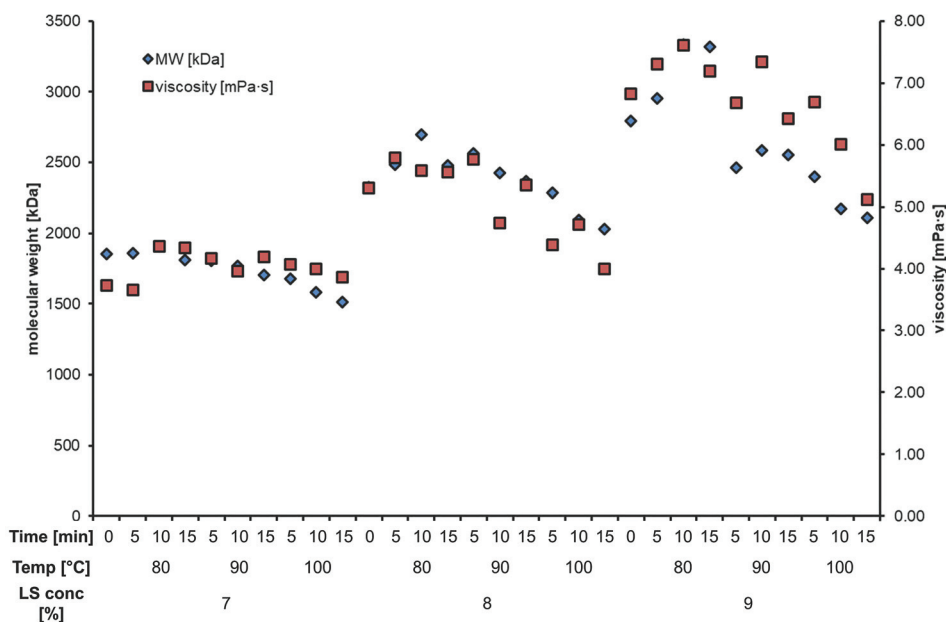


Fig. 4 Change of molecular weight and viscosity after thermal inactivation of laccase of polymerized lignosulfonates.

However, viscosity was significantly affected by the addition of plasticizers during polymerization (Fig. 5). In general, the application of plasticizers led to an increase of viscosity as previously reported by Jimenez Bartolome *et al.*<sup>34</sup> An increase in viscosity was directly proportional to the amount of added plasticizer showing the highest gain when they were added in a 1 : 2 proportion.

The performance and properties of plasticized lignosulfonate coatings were investigated on marine calcium carbonate granules. These were coated with all variants of the coatings. All the used plasticizers applied were able to reduce the brittleness of the polymerized lignosulfonates even at the lowest applied concentration. Plasticizers can be defined as low molecular weight, non-volatile compounds used as additives in order to enhance the flexibility and processability of polymers. In general, plasticizers manage to achieve this role by occupying intermolecular spaces between polymer chains, reducing

secondary forces among them, changing the three-dimensional molecular organization of polymers and at the same time thereby lowering the second order transition temperature, the glass transition temperature ( $T_g$ ), hardness, density, viscosity and electrostatic charge of the polymers.<sup>34</sup> For hydrophilic biopolymers like pullulan plasticizing effects by glycerol, sorbitol and xylitol have been reported due to the enhancement of molecular mobility by disrupting the hydrogen bonding interaction between polymer chains amongst other phenomena. The effect on various properties (mechanical, moisture content, *etc.*) depended on both the plasticizer concentration and size.<sup>51</sup> Therefore, it was imperative to investigate in this study their overall effect for this particular application in LS based coatings.

The most promising coatings in terms of sprayability, adhesion to the granules and their anti-dust properties were selected. In Fig. 6, the effect of the coating on roll-off pro-

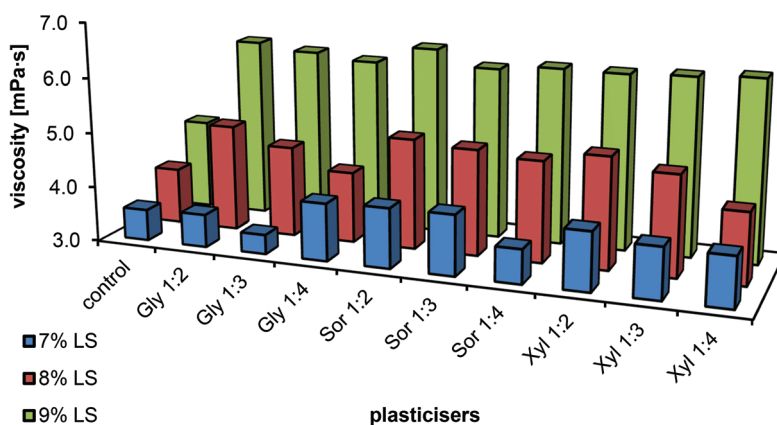


Fig. 5 Effect of different plasticisers on the viscosity of enzymatically polymerised lignosulfonates.

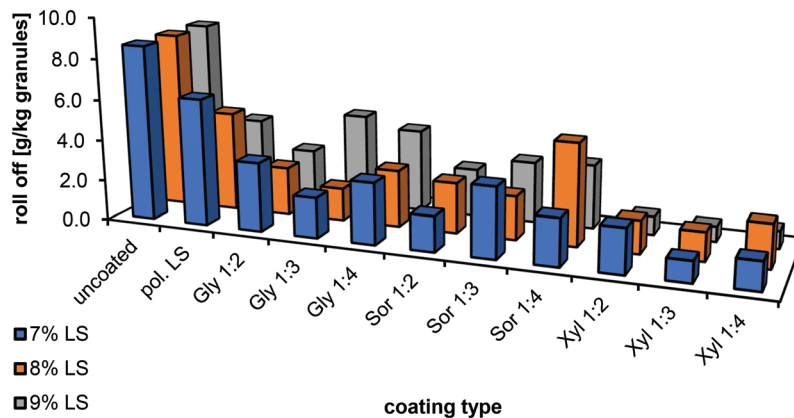


Fig. 6 Effect of different plasticisers on the roll-off (attrition) of enzymatically polymerised liginosulfonates fertiliser coatings.

duction can be seen. In polymerized lignosulfonates, without the use of plasticisers, a reduction of 28–56% of dust production could be achieved. The addition of plasticisers, however, reduced the attrition even further up to 70%, revealing xylitol to be the most suitable in the coating process.

By combining all information from these experiments, the most suitable coating was determined to be an 8% lignosulfonate containing coating with 2.7% xylitol (1:3 dry substance ratio) being added as a plasticizer, polymerized for 6 hours at room temperature and inactivated for 10 minutes at 100 °C.

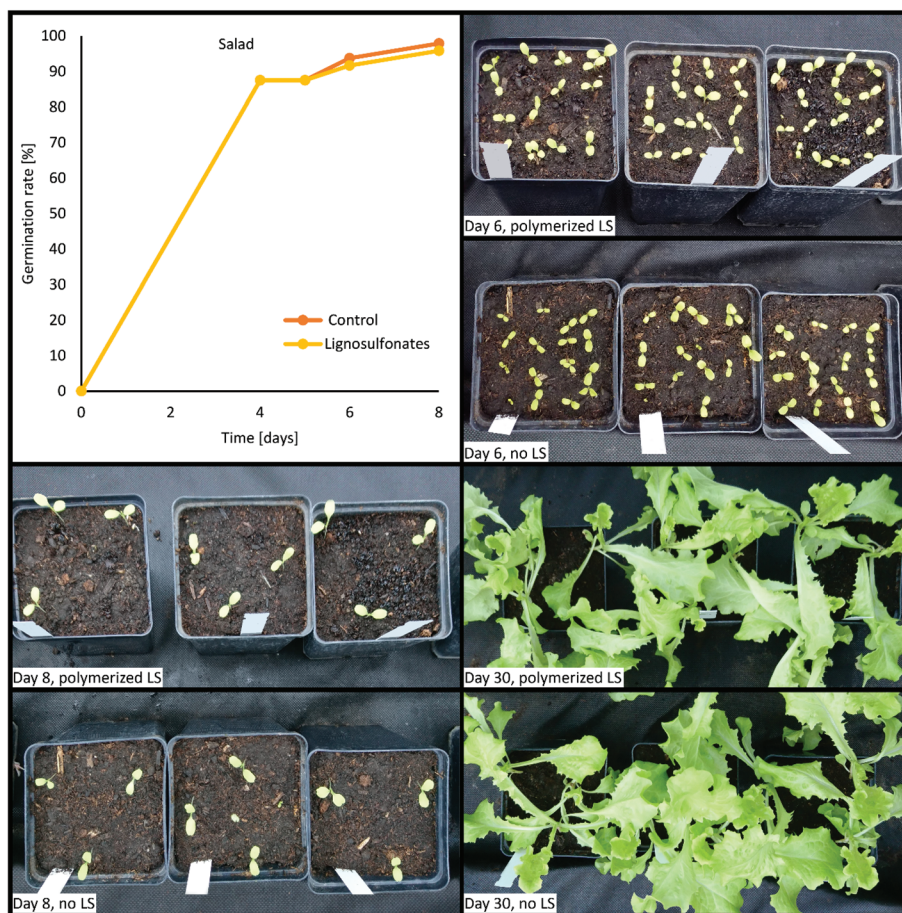


Fig. 7 Comparison of the germination rate and growth development of ice berg salad on fertile soil and fertile soil with 3.6% v/v polymerized liginosulfonates being added.



### Toxicity and safety of polymerized lignosulfonates

New materials intended for field application require extensive *in vitro* testing to ensure that they are not toxic to plants and that they support plant growth. The importance of the safety of new fertilizer products is also stated in the EU Regulation 2019/1009 of the European Parliament and of the Council.<sup>52</sup> Consequently, the effects of the synthesized coatings on the germination and growth of plants were investigated by applying 3.6% v/v of the coatings to the soil before seeding with wheat, corn, tomato, or iceberg salad under greenhouse conditions. Germination and growth were monitored for up to 31 days. Fig. 7 shows the performance of salad. No significant differences in germination or plant growth between control plants and polymerized lignosulfonate treated plants were observed.

Fig. 11–13 in the ESI† show the performances of wheat, corn, and tomato. In wheat germination rates reached their maximum after 14 days amounting to 90% in the control, and 88% in experiments containing polymerized lignosulfonates. The overall trend of the graph suggests a delay in germination between day 5 and day 14 which can be appreciated in Fig. 12 in the ESI† due to the reduced size of the seedlings. The delayed germination led to a slower growth rate at first in lignosulfonate treated plants. After decollation plant growth in control and trial pots was equivalent as little difference in the development stages were observed. In corn the germination rates reached their maximum after 8 days amounting to 98% in both the control and polymerized lignosulfonate containing experiments. The overall trend of the graph suggests a slight delay in germination between day 5 and day 8 in samples containing lignosulfonates. The delayed germination had no effect on further plant development. No significant difference in plant growth was observed. Similar to corn, tomato plants also showed a slight delay in germination between day 5 and day 11 with plants reaching their maximum germination rate of 94% in the controls and 92% in the trial plants after 11 days. No difference in plant growth and development was observed after this time point.

Overall a safe application of polymerized lignosulfonate as coatings can be deduced from the above experiments. Even if

applied in excessive amounts ( $324 \text{ g m}^{-2}$  versus  $1 \text{ g m}^{-2}$ ) no or only minor negative effects on the germination and growth rate were observed depending on plant species which was similarly reported by Stapanian *et al.* and others.<sup>53–55</sup>

In addition, to plant toxicity, the safety of coatings during transport and storage must be ensured. A detailed investigation of this was beyond the scope of this research but should be pursued in the future. Based on literature evidence it is not expected that LS coatings pose a safety hazard. The exception to this may be the coating of fertilizers containing a high concentration of ammonium nitrate. As ammonium nitrate is a strong oxidizing agent the coating with combustible organic carbon (acting as a fuel/electron donor) such as LS will likely pose a fire or explosion hazard.<sup>56</sup> According to the UN transport classification this is the case when straight ammonium nitrate with a concentration of >90% and a combustible organic carbon content of >2% are combined (classification: UN 0222 Class 1).<sup>57</sup> The coating applied in this study was 2–4% w/w of the fertilizer and does hence exceed the specified level. However, research has also shown that LS purified by dialysis can act as fire retardants in wood flour-based particleboards by increasing char formation.<sup>58</sup> Whether LS can also improve fire safety for fertilizers should be subject to further research.

### Viability of beneficial *Bacillus* strains in lignosulfonate coatings

The viability of *B. subtilis*, *B. licheniformis*, *B. velezensis* and *B. atropaueus* incorporated into the coatings was investigated. *Bacillus* spp. are known to aid plant roots in uptake of essential nutrients, such as P and N<sup>39</sup> and reduce pathogen pressure, either directly or indirectly by stimulating plant defence.<sup>59</sup> Four strains of these well-known plant growth promoters were added in the form of spore solutions to the finished coating solution before spray application. In Fig. 8 the spore viability on the surface of the granules is shown. The applied number of spores on 1 g of granules was  $2 \times 10^6$ . Despite vigorous mixing and very careful preparation of dilutions, the variation of the number of CFUs per plate of three replicates was rather high. Differences are not to be taken as of technical nature,

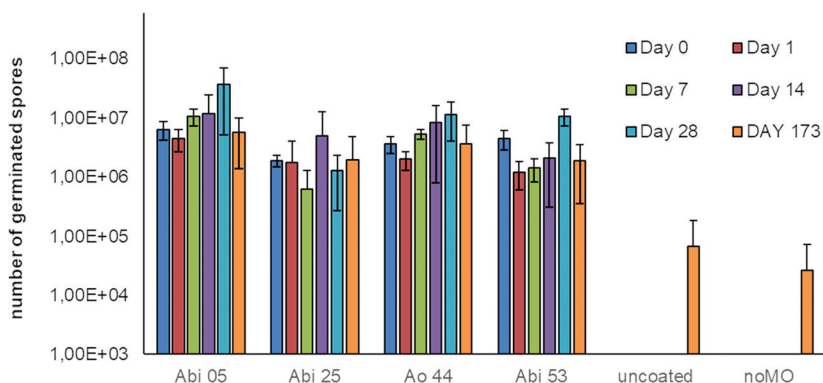


Fig. 8 Number of germinated *Bacillus* spores after 0, 1, 7, 14, 28 and 173 days of storage at room temperature.

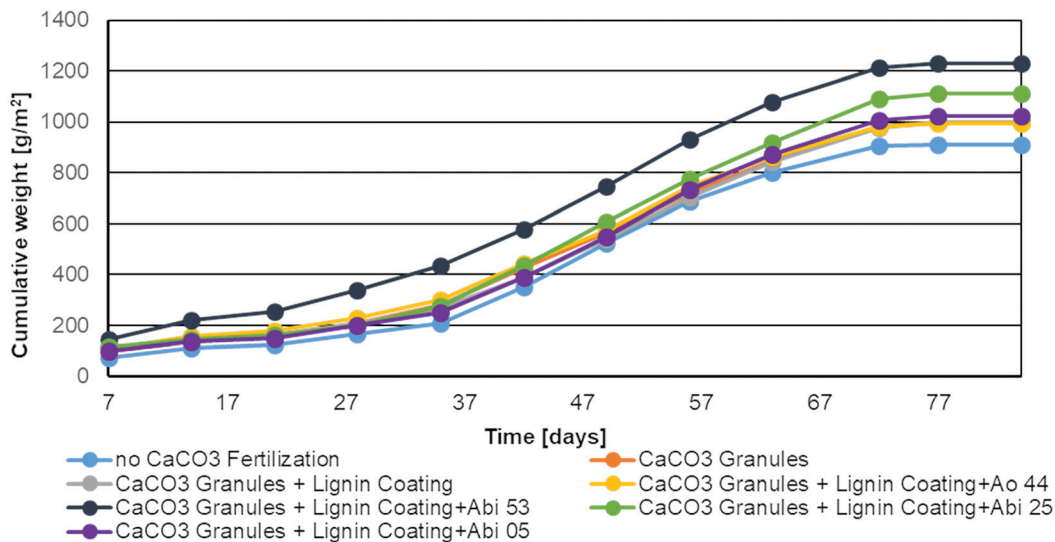


Fig. 9 Cumulative fresh weight of lawn cut when different coated soil improvers were added after germination.

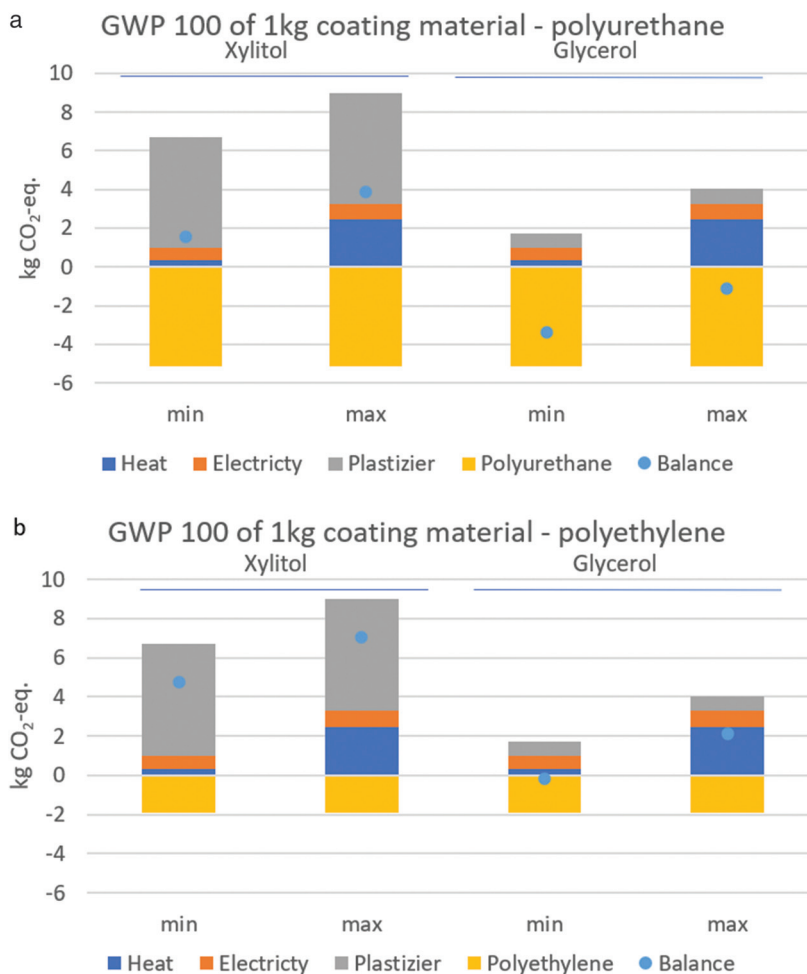


Fig. 10 Comparison of the CO<sub>2</sub>-eq. emission of LS coatings with polyurethane (a) and polyethylene (b) based coatings. Different plasticizers were modelled for each LS coating.

rather due to the nature of granule coating. Differences in the number of spores per 1 g of granules between different time points might be due to the unequal coating of granules with spores. For three species we observed a slight increase of the CFU from day 1 to day 28 potentially indicating bacterial growth. However, after 173 days of storage the CFUs were equal to or slightly lower than those at day 0. This shows that the polymerized lignosulfonate coating is a potent carrier for plant growth promoting *Bacillus* spores, which will not affect their viability. After 173 days of storage under non-sterile conditions low amounts of growth of most likely airborne Bacilli were observed in untreated and negative controls.

#### Effect of coated soil improvers on lawn cut weight

The application of the differently coated soil improvers showed significant positive effects on the biomass production of the lawns. Non-treated lawns produced significantly fewer lawn cuttings whereas samples treated with bioactive coatings had increased biomass production (Fig. 9). In addition, leaf colour and growth density were also improved. Therefore, not only the viability of the Bacilli but also their positive effects as plant growth promoters could be shown.

#### CO<sub>2</sub> emission equivalent of coating production

The results show that the impact of LS coating production with xylitol as a plasticizer exceeds the impact of coating with polyurethane by 1.6–3.9 kg CO<sub>2</sub>-eq kg<sup>-1</sup> product (Fig. 10a). However, the use of glycerol as a plasticizer leads to avoided impacts between 1.1 and 3.4 kg CO<sub>2</sub>-eq. kg<sup>-1</sup> product. The major impact of the choice of the plasticizer is evident in the fact that it accounts for about 85% of the impact in the xylitol min scenario, but for only between 19 and 43% in the glycerol scenario.

When comparing polyethylene-based coatings, the net impact of the LS coating with xylitol is further emphasized due to the lower avoided emissions of the substitution of polyethylene coatings (yellow bar – Fig. 10b). This is evident in the higher overall emissions of 4.8–7 kg CO<sub>2</sub>-eq. kg<sup>-1</sup> product in the xylitol scenario. When glycerol is used as a plasticizer, the importance of maximizing the energy efficiency is highlighted. At maximal heat energy efficiency (*i.e.* min scenario), a net CO<sub>2</sub>-eq. reduction of 0.2 kg CO<sub>2</sub>-eq. kg<sup>-1</sup> product can be realized. However, in a process with a higher heat demand (max scenario) this may lead to a net impact of 2.1 kg CO<sub>2</sub>-eq. kg<sup>-1</sup> product (Fig. 10b).

Despite the potentially higher CO<sub>2</sub>-eq. emissions, LS coatings are likely to reduce the pressure on the environment as they avoid the pollution with non-biodegradable coatings, including the pollution of the soils and oceans for example with micro-plastics. Instead, LS based coatings may increase the soil organic matter and soil organic carbon content, due to their relatively slow biodegradability and potential transformation to humic compounds.<sup>18</sup> As a result, they are likely to improve the nutrient and water retention of the soil, leading to better plant yields and a higher nutrient use efficiency.

## Conclusion

In this work a bio-based, biocompatible, anti-dust soil improver coating was investigated. The environmental non-hazardous application of polymerized lignosulfonates was demonstrated in plant trials. The model plants corn, wheat, tomato and salad showed no significant negative response to the application of the coatings. The CO<sub>2</sub> emission analysis of the coatings produced with xylitol and glycerol compared to polyurethane and polyethylene coatings showed potential CO<sub>2</sub> savings of up to 3.4 kg CO<sub>2</sub>-eq. kg<sup>-1</sup> product. The required plasticity of the coatings was best achieved by the addition of xylitol, a natural plasticizer, and the optimization of the polymerization conditions. The potential of the coating to not interfere with the combined application of the plant growth promoting microorganisms and fertilizer was further demonstrated. The coated marine calcium carbonate granules significantly improve golf lawn/sport lawn fertilization, as they provide the chance of long-term fertilization as compared to the regular liquid application of single nutrients. Future studies should investigate the safety of an LS coating in combination with other fertilizers, in particular the fire safety/explosion of combinations with ammonium nitrate.

## Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Writing – original draft: Renate Weiß, Gibson Nyanhongo, Marc Spiller, Julia Santolin, and Günter Brader. Writing – review & editing: all authors. Visualization: Renate Weiß, Sebastian Gritsch, Marc Spiller, and Julia Santolin. Supervision: Gibson Nyanhongo and Georg Gübitz. Resources: Georg Gübitz, Sylvain Pluchon, Kristin Diemel, Günter Brader, Hedda Weber, and Nikolaus Schwaiger. Funding acquisition: Georg Gübitz, Gibson Nyanhongo, and Günter Brader. Conceptualization: Renate Weiß. Data curation: Renate Weiß, Gibson Nyanhongo, Branislav Nikolic, and Günter Brader. Formal Analysis: Renate Weiß, Sebastian Gritsch, and Branislav Nikolic. Investigation: Renate Weiß, Sebastian Gritsch, and Branislav Nikolic. Methodology: Renate Weiß, Sebastian Gritsch, Branislav Nikolic, and Marc Spiller. Software: Renate Weiß and Marc Spiller.

## Conflicts of interest

No conflicts of interest are to be reported.

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