ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

Biodegradation of Hydrogels from Oxyethylated Lignins in Model Soils

Lars Passauer,*[,†] Till Hallas,[‡] Ernst Bäucker,[§] Gisela Ciesielski,[∥] Sascha Lebioda,[⊥] and Ute Hamer[#]

[†]Dresden Institute of Wood Technology, Institut fuer Holztechnologie Dresden gemeinnuetzige GmbH, Zellescher Weg 24, 01217 Dresden, Germany

[‡]Institute of Plant and Wood Chemistry, Technische Universitaet Dresden, Pienner Straße 19, 01737 Tharandt, Germany

[§]Institute of Forest Utilization and Forest Technology, Technische Universitaet Dresden, Pienner Straße 19, 01737 Tharandt, Germany

[∥]Institute of Soil Science and Plant Nutrition, Technische Universitaet Dresden, Pienner Straße 19, 01737 Tharandt, Germany

[⊥]Institute of Wood Research, Federal Research Institute for Rural Areas, Forestry and Fisheries, Leuschnerstraße 91, 21031 Hamburg-Bergedorf, Germany

[#]Institute of Landscape Ecology, University of Muenster, Heisenbergstraße 2, 48149 Muenster, Germany

ABSTRACT: Since cross-linked hydrogels from oxyethylated lignins (OELs) are progressively more regarded as water-retaining soil improvements based on sustainable and biorenewable resources, an effort is made here to gain some insight into the biodegradation behavior of these materials. For this purpose, model soils with defined sand/lignin and sand/OEL ratios were incubated in a closed system under laboratory conditions, and carbon dioxide evolved by microbial lignin and OEL decomposition was determined. OELs with different oxyethylation/cross-linking degrees and water absorption capacities were included into the experiments and compared with respective types of parent technical soft wood and hard wood lignins. The results suggest a medium- to long-term biodegradability of OELs ensuring a long-term functionality of the hydrogel materials on the one hand and a subsequent integration of respective degradation products into the natural carbon cycles on the other hand. It was found that the short-term carbon mineralization rates of OELs are markedly lower compared to that of parent lignins and strongly dependent on (1) lignin type, (2) cross-linking/oxyethylation degrees of OEL, (3) corresponding swelling properties, and (4) OEL concentrations in model soils.

KEYWORDS: *Biorenewable materials, Lignin, Cross-linking, Hydrogel, Soil rehabilitation, Carbon mineralization*

INTRODUCTION

Hydrogels (HGs) and superabsorbents (SAs) are highly hydrated polymer gels with macromolecular three-dimensional networks that swell but do not dissolve in water.[1] Depending on their particular properties HGs are used for a large number of applications such as implants, wound dressings and tissue engineering,[2] drug delivery systems,[3] electrode and ultrasonic gels,[4] food packaging,[5] in a multitude of sanitary and cosmetic products,[1] and as stimuli−responsive smart materials in microsystems technology.[6] Less known but highly required is their application as soil amendments to increase the water retention particularly of sandy soils in arid and semiarid regions.[7,8] In such soils, HGs increase the water holding capacity and decrease drought stress for plants,[9] diminish leaching of pesticides, fertilizers, and plant nutrients,[10] improve the survival rate of saplings, and increase biomass production in general.[11] Nevertheless, regulatory commitments in various countries aim at the biodegradability of soil conditioners in general, in order to protect soil and ground or fresh water resources from accumulating contaminants or toxic loads and to include degradation products into the natural materials cycles.[12] On the other side, an adequate stability of HGs in soils is desired providing for a mid-term or long-term functionality of the materials at most over more than one growing period. HGs currently applied as soil amendments are from the poly(acrylic acid)/polyacrylamide types.[8,12] From an ecological point of view, those materials did not become widely accepted in this field of application because they are nonrenewable, contain potentially toxic monomers (e.g., acrylamide), and are biodegradable only to a limited extent. In common agricultural and garden soils, degradation rates of polyacrylate-based SAs varied between 0.2% and 0.82% within 11 to 14 weeks depending on the cross-linking degree of the polymer, soil type, and temperature ranges.[12,13] Other studies have shown that

Received: February 23, 2015
Revised: August 2, 2015
Published: August 6, 2015

DOI: 10.1021/acssuschemeng.5b00139
ACS Sustainable Chem. Eng. 2015, 3, 1955−1964

polyacrylate-based SAs are generally degradable by some fungal species, but mineralization rates during incubation experiments turned out to be low with 0.8−3.2% within 11−14 weeks.[14,15] HGs based on chemically modified and cross-linked polysaccharides such as starch,[16] cellulose,[17] chitosan,[18] pectin,[19] or alginates[20] represent environmentally friendly alternatives to conventional acrylate/acrylamide-based HGs. Applied in soils and composts, those materials were found to be quickly degraded with degradation rates between 25−95% within 4−14 weeks depending on the type of polysaccharide, cross-linking degrees, incubation temperatures, and soil/compost types.[19,21,22] The fast decomposition hinders their nonrecurrent application as water-storing materials in soils for periods longer than few weeks or months. For that, more stable HGs are needed, which should be, nevertheless, biodegradable in principle. A less known but promising approach for such purposes is using lignin as a raw material for making HGs[23,24] because lignin is known as a recalcitrant but generally biodegradable biopolymer.[25] As byproducts of pulp and paper manufacturing, technical lignins are increasingly considered as renewable sources for the production of fine chemicals and innovative materials.[26,27] In that context, lignin HGs represent a novel class of lignin-based functional materials and can be obtained, inter alia, by simultaneous cross-linking and oxyethylation of technical and oxidatively modified lignins with poly/oligo(oxyethylene)-α,ω-diglycidyl ethers PEGDGE/OEGDGE.[24,28] HGs from oxyethylated lignin (OEL) were found to improve soil physical and soil hydraulic properties considerably.[28,29] Because lignin as a plant residue plays a key role for humification processes ins soils,[30,31] OELs not only represent a water-storing soil amendment but also are additionally considered as precursors for the formation of soil humic substances, rendering them attractive materials for soil preservation and rehabilitation.[29]

To gain some insight into the biodegradation behavior and persistence of OELs in soils, in the current study soil respiration experiments were performed with models soils under laboratory conditions. For this purpose, defined sand/lignin and sand/OEL model soils were incubated in a closed system, and carbon dioxide evolved as a final reaction product of lignin and OEL biomineralization was determined. Corresponding biomineralization rates were related to structural and morphological aspects of technical lignins as well as swelling properties and oxyethylation degrees of respective OEL variants.

MATERIALS AND METHODS

Technical Lignins. For the experiments, pine kraft lignin (IND; Indulin AT; MeadWestvaco, U.S.A.), spruce organosolv lignin (S-OSL; organocell lignin; further Organocell GmbH, Germany), and beech organosolv lignin (B-OSL; vTI Institute of Wood Research, Germany) were used. The latter was used for the first time to obtain an oxyethylated beech organosolv lignin. Functional group contents of technical lignins used are specified in Table 1 and were determined as follows: OCH_3 groups according to Zeisel−Viehböck−Schwappach,[32] COOH groups by potassium iodide/iodate method,[33] CO groups by oximation,[34] total OH groups by deacetylation of peracetylated samples and quantification of the released acetic acid,[35] and phenolic OH groups by selective aminolysis of acetylated lignin with pyrrolidine and quantification of the resulting 1-acetylpyrrolidine by GC.[35] Differing from this, OH and COOH group analysis of B-OSL was accomplished using ^{31}P NMR spectroscopy as described elsewhere.[36,37] NMR analysis of phosphitylated lignin was performed in a VARIAN Mercury 400 MHz spectrometer. All chemicals used were from analytical grade.

Table 1. Functional Group Contents of Lignins Used for OEL Preparation (S-OSL, Spruce Organosolv Lignin; IND, Indulin AT; B-OSL, Beech Organosolv Lignin)

	S-OSL[a]	IND[a]	B-OSL[b]
OCH_3	12.84	12.80	21.20
OH_{phen}	4.64	6.29	5.50
OH_{aliph}	5.64	6.50	3.76
CO	–	0.11	–
COOH	0.49	0,54	0.63

[a]Values obtained from wet chemical analysis. [b]Values obtained from ^{31}P NMR spectroscopy.

OEL Preparation. This was performed by chemical cross-linking/oxyethylation of lignin in 3.3 M aq. NaOH with poly(ethylene) glycol diglycidyl ether ($M_w \sim 526$ g mol^{-1}; Sigma-Aldrich, Germany) as described elsewhere.[24,28] Cross-linking degrees were selectively adjusted by varying the PEGDGE/lignin ratio (0.65, 0.75, and 1.0 mmol PEGDGE g_{Lig}^{-1}). Essential parameters for the preparation of gels used for incubation experiments are summarized in Table 2. For analytical purposes, OELs were dialyzed in deionized water using cellulose regenerate membranes (Carl Roth GmbH, Germany). Corresponding xerogels were obtained by air-drying of purified and neutralized OEL at 40 °C.

Table 2. Selected Parameters for the Preparation of OEL Used for Biomineralization Experiments and Respective Gel Sample Codes

raw material	lignin/aq. NaOH ratio (w/v)	PEGDGE/lignin ratio (mmol g_{Lig}^{-1})	sample code
S-OSL[a]	1:1.47	0.65	$S\text{-}OEL_{0.65}$
S-OSL[a]	1:1.47	0.75	$S\text{-}OEL_{0.75}$
S-OSL[a]	1:1.47	1.00	$S\text{-}OEL_{1.0}$
B-OSL[b]	1:2.01	0.75	$B\text{-}OEL_{0.75}$
IND[c]	1:2.18	0.75	$I\text{-}OEL_{0.75}$

[a]Spruce organosolv lignin. [b]Beech organosolv lignin. [c]Indulin AT.

Free Swelling Capacity. Free swelling capacity (FSC) of OEL, which is the amount of deionized water a certain amount of a dehydrated xerogel (XG) counterpart can absorb and retain, was determined gravimetrically as described earlier.[28] The FSC values are given in g_{H2O} g_{XG}^{-1}.

CN Elemental Analysis. This was conducted in duplicate using a CN analyzer VARIO EL III (Elementar Analysensysteme, Germany). CN contents are given in % (w/w) of absolute dry material.

Size-Exclusion Chromatography. Molecular weight distributions and average molecular weights of technical lignins and soluble fractions of OEL were determined according to Ringena et al.[38] For the measurements, 0.4% lignin/OEL solutions were prepared and filtered through 0.45 μm regenerated cellulose membranes. SEC was performed using the following system: degasser DG-4400 (Phenomenex, U.S.A.), autosampler AT 100 (TSP), pump Smartline 1000 (Knauer, Germany), column oven Croco Cil (ERC, Switzerland), UV-detector LC 1200 UV−vis (Polymer Laboratories, Germany, detection wavelength: λ = 280 nm). DMSO:H_2O

DOI: 10.1021/acssuschemeng.5b00139
ACS Sustainable Chem. Eng. 2015, 3, 1955−1964

(9:1 v/v) added with 0.1% w/v LiBr was used as eluent, and a PL PolarGelM column set was applied (Varian, U.S.A.). Flow rate was 0.5 mL min^{-1} at 60 °C at a pressure of 0.76 MPa. Calibration was performed using polyethylene glycol and poly(ethylene oxide) standards (190−82,000 g mol^{-1}; Polymer Laboratories, Germany).

Scanning Electron Microscopy. SEM images of lignin and OEL particles were obtained using a JEOL T330A microscope (JEOL, Ltd., Japan) operating at 15 kV acceleration voltage. For SEM analyses, dried samples were double coated (vaporizing unit EMITECH K950 carbon coater, Emitech, Ltd., U.K.) and finally sputter-coated (ion sputter JEOL JFC 1100E) as described earlier.[28]

Sand/Hydrogel Model Systems and Biomineralization Experiments. The biodegradation of organic matter finally yields carbon dioxide and water and can be indirectly estimated in terms of carbon dioxide release.[39] In the present study, CO_2 release of incubated sand/lignin and sand/OEL model soils (with lignin and OEL concentrations of 0.0 [control], 0.5, 1.0, and 2.0% w/w, respectively) was determined according to ISO 16072[40] with some modifications. Purified (acid-washed, calcined) sea sand (Merck, Germany) was used as soil matrix for the model systems (1) because HGs for soil improvement are mainly applied in sandy soils with naturally low water holding capacities, (2) to prevent CO_2 adsorption on clay minerals/soil humic substances/clay humus complexes, which would cause an underestimation of effective lignin/OEL biomineralization rates, and (3) to eliminate the potential biodegradation of soil organic matter, which would distort real lignin/OEL biomineralization rates.

For the experiments, 20 mg of pure sand (control experiment), homogenized sand/lignin, and sand/OEL mixtures (each with n_i = 5 replicates) were transferred into perforated centrifuge glass tubes, which were placed into airtight incubation bottles (250 mL wide mouth bottles, Schott-Duran, Germany; Figure 1) filled with 20 mL of 0.05 M NaOH.

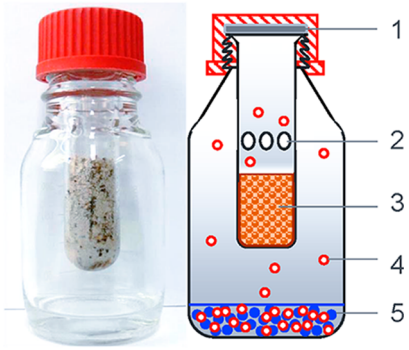


Figure 1. Image and schematic representation of an incubation bottle with a model soil: (1) screw cap with seal, (2) centrifuge tube with perforations, (3) model soil: sand/OEL mixture, (4) carbon dioxide, and (5) aq. sodium hydroxide solution with adsorbed carbon dioxide.

The model soils were wetted with ultrapure water to 50−60% of the previously determined water holding capacity WHC.[41] Furthermore, 1 mL aqueous nutrient solution (12.3 g L^{-1} KH_2PO_4 + 9.4 g L^{-1} K_2HPO_4 + 11.7 g L^{-1} NH_4NO_3) and 1 mL inoculum was added to the model soil as described elsewhere.[38] The inoculum was obtained from the Oe- and Oa-horizons of a Podzolic Cambisol at Tharandter Wald, Germany) using a method described by Hamer and Marschner.[42] The model soils were incubated in darkness for 14 days at 20 °C. The CO_2 release was determined after 1, 3, 7, and 14 days by adding 5 mL 0.5 N $BaCl_2$ to the aq. NaOH solution in each case. Thus, CO_2, which was absorbed by aq. NaOH, was precipitated as $BaCO_3$ under consumption of an equivalent of NaOH. Unspent NaOH was determined by titration with 0.05 M HCl. The CO_2 evolution [mg_{CO2} g_{soil}^{-1}] of each incubation step i (= 1, .., 4 after 1, 3, 7, and 14 days, respectively) was determined as

$$ {}^{i}R_{CO_2} = \frac{(\bar{V}_b - \bar{V}_s) \times 1.1 \times f_{NaOH}}{m_s \times \omega_s} \quad (1) $$

where $\bar{V}_b$ is the HCl consumption of the control [ml], $\bar{V}_s$ is the HCl consumption of the sample [mL], 1.1 is the conversion factor [−] (1 mL 0.05 M NaOH corresponds to 1.1 mL CO_2), f is the normality factor [−], m_s is the sample weight at 60% WHC/FSC (wet soil plus lignin/OEL) [mg], and ω_s is the relative dry solids content of the soil sample.[40]

Summation of the CO_2 release of the incubation steps i yielded the total CO_2 release after 14 days of incubation $\bar{R}_{CO_2}$ [mg_{CO2} g_{soil}^{-1} 14 d^{-1}].[40]

The carbon mineralization for the incubation steps i ${}^{i}R_C$ [mg_C g_{soil}^{-1}] and the total carbon mineralization (during 14 d of incubation) $\bar{R}_C$[mg_C g_{soil}^{-1}] is yielded using ${}^{i}R_C = {}^{i}R_{CO_2} \times 12.01/44.01$, and $\bar{R}_C = R_{CO_2} \times 12.01/44.01$, respectively, with 12.01 the molar mass of carbon and 44.01 the molar mass of carbon dioxide.[40]

The percentage of carbon mineralization C_{min_i} [% of $C_{Lig/OEL}$] of the incubation step i was calculated by

$$ C_{min_i} = \frac{{}^{i}R_C \times m_{ds}}{c_{CLig/OEL} \times m_{Lig/OEL}} \times 100 \quad (2) $$

with m_{ds} is the dry substance of soil sample [g], $c_{CLig/OEL}$ is the carbon content of lignin/OEL [%] and $m_{Lig/OEL}$ is the dry mass of lignin/OEL in the soil sample [mg].[40]

Summation of C_{min_i} for each incubation step i yielded the percentage of carbon mineralization $\sum C_{min}$ after 14 days of incubation [% of $C_{Lig}/_{OEL}$ 14 d^{-1}].

Data Analysis and Statistical Evaluation. Total mineralization rates $\sum C_{min}$ (% of C_{OEL} 14 d^{-1}) of model soils with 1% w/w of different OEL variants were statistically evaluated using RStudio 0.98.1017 (open source integrated development environment for R).[43] One-way ANOVA was performed to test, if there is an effect of the factors (1) lignin type (with k = 3 factor levels: S-OSL, B-OSL, and IND; n_i = 5 replicates per level and $n = k \times n_i$ = 15 samples) and (2) lignin/PEGDGE ratio (k = 3: 0.65, 0.75, and 1.0 mmol g_{Lig}^{-1}; n_i = 5, n = 15) on the biomineralization of the respective HGs in the model soils. Multiple comparisons of means was performed using the Tukey test to specify which groups (factor levels) within factor 1 and 2 are significantly different from each other. Homogeneity of variance and normal distribution of data assumed for ANOVA were verified using the Fligner−Killeen, Bartlett, and Shapiro−Wilk test, as described elsewhere.[44]

RESULTS AND DISCUSSION

FSC and CN Contents of OELs. Oxyethylation and cross-linking of lignins with PEGDGE yielded HGs with FSC values in the range from 8.59−49.50 g_{H2O} g_{XG}^{-1} (Table 3). Generally, sorption and swelling properties of OELs were found to be

DOI: 10.1021/acssuschemeng.5b00139
ACS Sustainable Chem. Eng. 2015, 3, 1955−1964

Table 3. CN Contents and C/N Ratios of Parent Lignins and Corresponding OEL Variants, and FSC Values of Respective OELs Used for Incubation Experiments

sample	$C_{Lig/OEL}$ [%]	$N_{Lig/OEL}$ [%]	C/N [−]	FSC [g_{H2O} g_{XG}^{-1}]
Lignin				
S-OSL[a]	60.97 (0.00)	0.13 (0.11)	469	−
B-OSL[b]	63.07 (0.45)	0.25 (0.04)	252	−
IND[c]	64.37 (0.11)	0.41 (0.04)	157	−
OEL[d]				
$S\text{-}OEL_{0.65}$	59.73 (0.15)	0.08 (0.00)	747	49.50
$S\text{-}OEL_{0.75}$	59.45 (0.14)	0.07 (0.01)	849	34.56
$S\text{-}OEL_{1.0}$	58.63 (0.19)	0.07 (0.01)	838	13.56
$B\text{-}OEL_{0.75}$	60.39 (0.20)	0.14 (0.01)	431	8.59
$I\text{-}OEL_{0.75}$	60.95 (0.16)	0.24 (0.04)	254	9.55

[a]Spruce organosolv lignin. [b]Beech organosolv lignin. [c]Indulin AT. [d]Oxyethylated lignin; values in brackets correspond to standard deviations.

strongly dependent on the oxyethylation/cross-linking degrees and the resulting pore volume of the gels.[28,45] Upper cross-linking densities increase polymer−polymer interactions and counteract osmotically driven polymer expansion and are therefore associated with swelling inhibition. This is true for S-OEL variants oxyethylated/cross-linked with 0.65, 0.75, or 1.0 mmol PEGDGE per g lignin, featuring declining FSC values with 49.50, 34.56, and 13.56 g_{H2O} g_{XG}^{-1}, respectively. B-OEL and I-OEL each cross-linked with 0.75 mmol PEGDGE possessed significantly lower FSC (8.59 and 9.55 g_{H2O} g_{XG}^{-1}, respectively) related to $S\text{-}OEL_{0.75}$ (34.56 g_{H2O} g_{XG}^{-1}) indicating that the swelling properties of the gels are strongly influenced by the lignin type used for gel preparation.[24,28,29] Gel materials and a respective unmodified organosolv lignin used for biodegradation experiments are exemplarily depicted in Figure 2.

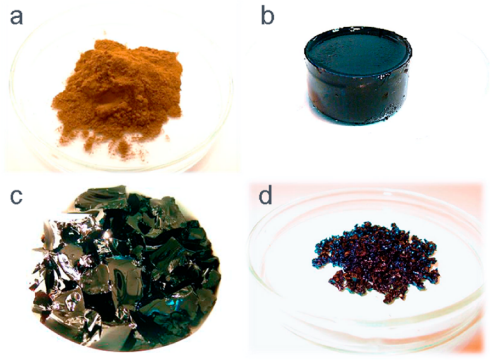


Figure 2. Images of (a) S-OSL lignin powder, (b) PEGDGE cross-linked S-OEL gel bloc, (c) swollen and neutralized S-OEL hydrogel, and (d) air-dried S-OEL xerogel granulate.

Carbon contents were found to be slightly decreased after lignin oxytehylation from 63.1 (B-OSL) and 64.4% (IND) to 60.4 ($B\text{-}OEL_{0.75}$) and 61.0% ($I\text{-}OEL_{0.75}$) (Table 3). This is due to the introduction of ether and hydroxyl group containing oligo(oxyethylene)/oligo(oxyethylene glycol) (OOE/OOEG) substituents into the lignin macromolecule as it was described earlier.[45] For S-OEL variants, C contents decreased from 60.97% for unmodified lignin to 59.73%, 59.45%, and 58.63%, respectively, depending on the lignin/PEGDGE ratio and the respective oxyethylation degree which varied between 54−72% for OELs from spruce organosolv lignins cross-linked with 0.5−1.0 mmol g_{Lig}^{-1}.[40] N contents ranged between 0.13 and 0.41% for parent lignins corresponding well with literature data[46,47] and decreased by 50% after oxyethylation (0.08−0.24%). Nitrogen present in lignin has been attributed to proteinaceous structures bound to the lignin polymer in immature plant tissues,[48] proteins in parenchyma cells of wood, cambium and inner bark, or to impurities from lignin isolation.[48] Resulting C/N ratios, which were found to be strongly affecting the microbial activity, biodegradation, and humification of organic substrates in soils[31] were in the range between 157−469 for parent lignins and markedly higher (254−849) for respective OEL variants (Table 3).

Carbon Mineralization: Lignin vs OEL. In Figure 3a and b, the biomineralization of model soils with 1% w/w lignin or 1% w/w of the corresponding OEL variants cross-linked with 0.75 mmol PEGDGE during 14 days of soil incubation is shown.

For parent lignins, carbon mineralization—mainly connected with decarboxylation of methoxy groups and carbon loss from the lignin side chain[31,49]—was found to increase exponentially with low initial rates in the range from 0.1% to 0.3% at 1 or 3 days of incubation, respectively (Figure 3a). Total carbon mineralization rates at the end point of the experiment ($\sum C_{min}$) were 0.9% (B-OSL), 1.8% (IND), and 3.2% (S-OSL) and are in good accordance with mineralization rates of isolated lignin in silty and loamy agricultural and forest soils over comparable time periods (0.5−3.89% after 2−6 weeks).[50,51] Generally low biodegradation rates of lignin are due to a low microbial assimilation of lignin C explained by the fact that soil microorganisms do not use lignin as sole C source.[52,53] Rather, lignin C utilization was reported to take place when a more easily utilizable C source (e.g., polysaccharides) is present.[25] Nonetheless, some microorganisms, particularly fungi, have developed the necessary enzyme systems to break lignin apart and are, thus, able to degrade larger amounts of lignin relative to carbohydrates from plant materials.[25,52] The initial reactions are mediated by extracellular lignin and manganese peroxidases, primarily produced by white-rot fungi. Actinomycetes can also decompose lignin but typically degrade less than 20% of total lignin present.[54,55] Lignin degradation is primarily an aerobic process, and under anaerobic conditions, it can persist for long periods.[56] Ultimately, a substantial part of lignin was reported to remain in the form of fairly modified lignin polymers in soils[50] and are successively transformed over long-term periods (years, decades) into soil humic substances, namely, humic and fulvic acids and humin. Lignin humification includes mechanisms involving both (1) lignin modification (lignin theory) and (2) lignin fragmentation to phenolic substructures, subsequent oxidation, and repolymerization of resulting quinones (polyphenol theory).[30,31]

Low initial carbon mineralization rates (0.1−0.3%) and further progression of lignin mineralization found in our experiments agree well with previous findings from Erikson et al.[25] The low onset degradation rates of lignin were reported to be caused by the low lignolytic enzyme activity during the primary growth phase of white-rotting fungi and actinomycetes, involved in lignin degradation.[25] For some typical white-rotting fungal species, which have been observed in a wide range of forest ecosystems in Europe and North America[57] and which were most likely present in the inoculum used for the current experiments, lignin degradation was found to be reduced if C/

DOI: 10.1021/acssuschemeng.5b00139
ACS Sustainable Chem. Eng. 2015, 3, 1955−1964

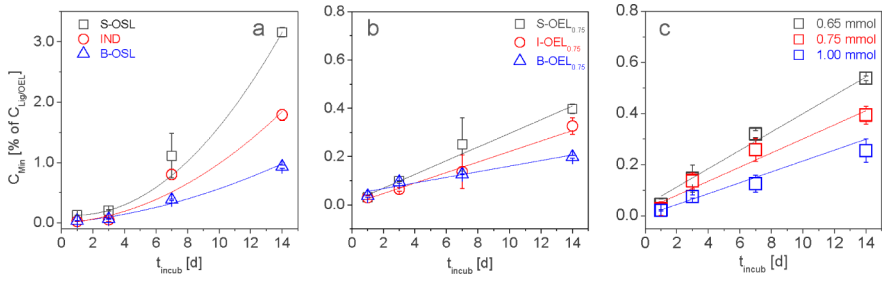


Figure 3. Development of carbon mineralization in model soils with (a) 1% w/w lignin (IND, Indulin AT; S-OSL, spruce organosolv lignin; B-OSL, beech organosolv lignin), (b) 1% w/w of the hydrogel variants I-OEL$_{0.75}$, S-OEL$_{0.75}$, and B-OEL$_{0.75}$, and (c) 1% w/w of the hydrogel variants S-OEL$_{0.65}$, S-OEL$_{0.75}$, and S-OEL$_{1.0}$. Bars represent standard deviations, n_i = five replicates per factor level.

N ratios decrease or can be triggered by carbohydrate limitation or N starvation.[25,52] Otherwise, soft rot fungi decompose lignin more rapidly when N is abundant,[58] even after adding small amounts of N (<0.2%) to lignocellulosic materials incubated with white-rot fungal cultures.[59] The former effect could be a reason for the higher degradation rates of S-OSL, which possessed much lower N contents (0.13%) and significant higher C/N ratios (469) related to B-OSL (N = 0.25%; C/N = 252) and IND (N = 0.43%; C/N = 154; Table 2). Different mineralization rates of unmodified lignins used in this study may also be caused (1) most likely by differences in their average molecular weights (S-OSL, 5,400 g mol^{-1}; IND, 7,400 g mol^{-1}; B-OSL, 3,400 g mol^{-1}) and molecular weight distributions (Figure 4a), (2) particle sizes (S-OSL, 2.5−50 μm; IND, 50−150 μm), (3) particle morphology (Figure 5a and b), and (4) by the botanical origin and corresponding structural differences of the materials (e.g., different proportions of guaiacyl/syringyl units), reflected by marked differences in methoxyl group contents of soft and hardwood lignins (Table 1). For the softwood lignins (IND, S-OSL), higher molecular weights and bigger particles are connected with lower degradation rates and vice versa, which is probably due to smaller specific surface areas of bigger particles (decreased microbial accessibility), lower proportions of phenolic OH groups in high-MW fractions of lignin,[60] and higher contents of recalcitrant carbon−carbon linked substructures (e.g., biphenyls, methylene linked phenols) in kraft lignins (IND).[61] Despite of lower average molecular weights (M_w = 3,400 g mol^{-1}), higher contents of low-M_W fractions (Figure 4a), and smaller particles (particle size, 1−30 μm; Figure 5c) B-OSL was found to be the most recalcitrant lignin variant under the selected experimental conditions. These results are probably due to physiological conditions and expressed enzyme systems of the dominating microbial species in the inoculum, most likely specialized in the decomposition of plant litter and lignin from softwoods such as Norway spruce (*Picea abies* Karst.) and Scots pine (*Pinus sylvestris* L.), which are the dominating tree species on the forest site from which the inoculum was obtained.

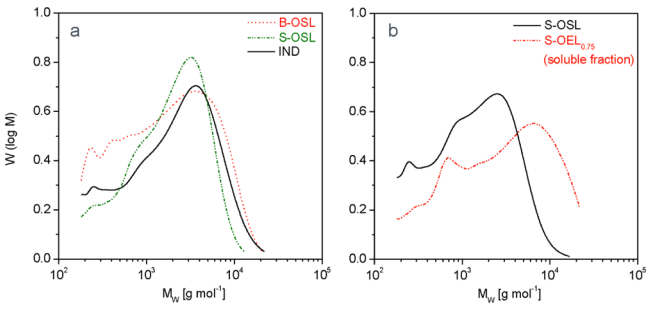


Figure 4. (a) Molecular weight distributions (MWD) of spruce organosolv lignin (S-OSL), Indulin AT (IND), and beech organosolv lignin (B-OSL). (b) MWD of S-OSL and the soluble fraction of the respective OEL type S-OEL$_{0.75}$.

Corresponding OEL variants cross-linked with 0.75 mmol PEGDGE featured only a slight linear increase of C_{min} within the incubation period starting at about 0.05% and 0.1% after 1 and 3 days of incubation and reaching values of 0.2%, 0.3%, and 0.4%, at the endpoint of the experiment (Figure 3b). Those

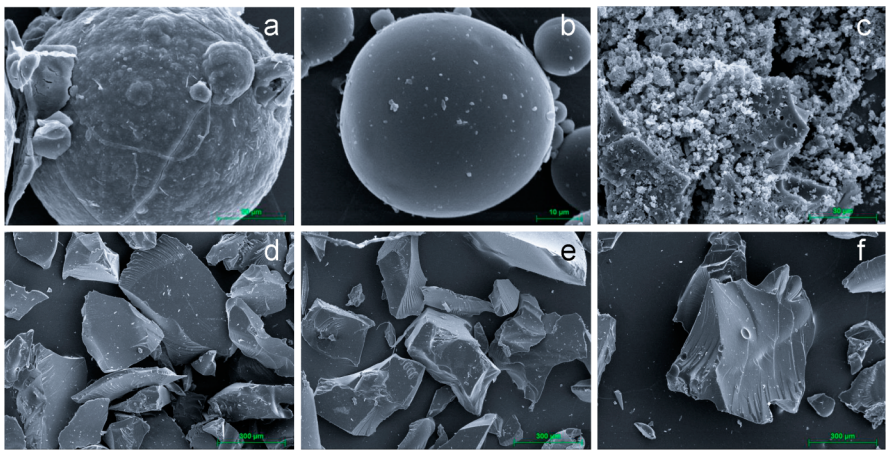


Figure 5. SEM micrographs of (a) pine kraft lignin Indulin AT, (b) spruce organosolv lignin, and (c) beech organosolv lignin and corresponding air-dried OEL particles (d-f).

DOI: 10.1021/acssuschemeng.5b00139
ACS Sustainable Chem. Eng. 2015, 3, 1955−1964

Table 4. Test Results of One-Way ANOVA and Multiple Comparison of Means[a,b]

one-way ANOVA (biomineralization vs lignin type; $k = 3$, $n_i = 5$, $n = 15$)					
	Sum Sq.[c]	DF[d]	Mean Sq.[e]	F value	p value
Factor: Lig. type	0.099457	2	0.049728	44.35	2.863e-06 ***
Residual	0.013455	12	0.001121		
Total	0.112912	14			

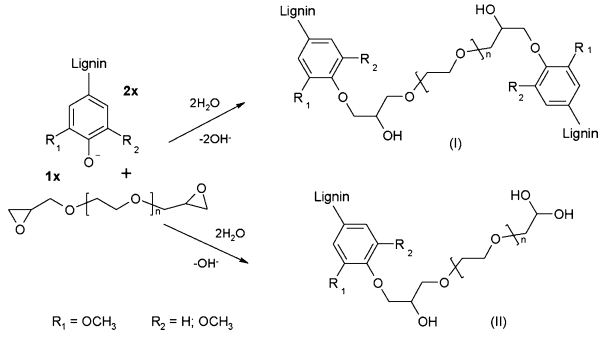
post hoc analysis (Tukey multiple comparison of means, 95% confidence level)					
	Diff[f]	lwr[g]	upr[h]	p value	α
I-OEL$_{0.75}$ − B-OEL$_{0.75}$	0.126456	0.069956	0.182956	0.000178	< 0.001***
S-OEL$_{0.75}$ − B-OEL$_{0.75}$	0.196808	0.140308	0.253308	0.000002	< 0.001***
S-OEL$_{0.75}$ − I-OEL$_{0.75}$	0.070352	0.013852	0.126852	0.015555	> 0.001***

[a]Variable, biomineralization of model soils with 1% w/w OEL; factor, lignin type; factor levels, I-OEL, S-OEL, B-OEL; each cross-linked with 0.75 mmol PEGDGE g_{Lig}^{-1}; $k = 3$, $n_i = 5$, $n = 15$. [b]Significant codes: 0, '***' 0.001, '**' 0.01, '*' 0.05, '.' 0.01, ' ' 1. [c]Sum of Squares: Sum Sq$_{factor}$ (variation between factor groups), Sum Sq$_{residual}$ (variation within factor groups), Sum Sq$_{total}$ = Sum Sq$_{factor}$ + Sum Sq$_{residual}$. [d]Degrees of freedom: DF$_{factor}$ = k − 1, DF$_{residual}$ = n − k. [e]Mean Squares: Mean Sq$_{factor}$ = Sum Sq$_{factor}$/DF$_{factor}$, Mean Sq$_{residual}$ = Sum Sq$_{residual}$/DF$_{residual}$. [f]Mean difference. [g]Lower bound. [h]Upper bound.

values are in the same order S-OEL$_{0.75}$ > I-OEL$_{0.75}$ > B-OEL$_{0.75}$ as it was found for parent lignins. One-way ANOVA of the data pictured in Figure 3b (C_{min} at 14 days) revealed that the effect of the lignin type was significant (with $F = 44.35$ and $p < \alpha$, at a significance level $\alpha = 0.001$; Table 4). A multiple comparison of means suggests that the mineralization rates of those OEL groups at 14 days are significantly different (with lwr > 0 at $p <$ 0.001) indicating the strong impact of the lignin type on the biomineralization of the respective HGs.

In comparison to parent lignins (Figure 3a), biomineralization of OEL variants were found to be markedly lower (Figure 3b), which is very probably (1) due to evidently increased molecular weights of OEL (M_w > 15,000 g mol^{-1}; Figure 4b) in relation to parent lignins (Figure 4b), (2) stronger cross-linked macromolecular networks of OELs, compared to that of unmodified lignin, characterized by a large number of molecular cross-junctions and entanglements, and (3) the compact structure of dried (Figure 5d and e) or partially swollen gel particles making their decomposition much more difficult. A key reason is (4) the etherification of phenolic substructures in OELs (Scheme 1), which prevents an oxidative attack on phenolic lignin units by phenol peroxidases typically released from lignolytic fungi (Scheme 2b).[25,52] Those fungi are able to convert phenols (I) into phenoxyl (II) and quinoid radicals (III), which can be oxidized to instable ether peroxides (IV) that undergoes spontaneous ring fission under the formation of muconic acid derivatives (V). Their carboxylic acid groups can be split off as carbon dioxide (Scheme 2a).[62]

Scheme 1. Proposed Reaction Pathway for Etherification and Cross-Linking of Phenolic Subunits of Lignin with PEGDGE in Alkaline Media



Biodegradation rates of OELs are (1) notably lower than decomposition rates of polysaccharide based HGs in sandy and garden soils (5−90% 14−28 d^{-1}),[19,21,22] clearly higher than mineralization rates of polyacrylate- and polyacrylamide-based SAs in unfertilized sandy/loamy soils (0.1−0.25% 14 d^{-1})[13] and garden soils (0.05−0.25% 14 d^{-1})[13] and (2) comparable with mineralization rates of natural soil organic matter of agricultural sandy soils in NE-Germany over comparable time periods (0.4 to 0.7% 14 d^{-1}).[63] Those results suggest that the soil microbial consortium is generally able to decompose OEL, albeit in a limited manner. Because OELs contain only small amounts of phenolic substructures, their biodegradation is probably due to lignolytic fungal species producing lignin peroxidases (LiPs), the only enzymes capable of attacking nonphenolic lignin substructures due to the formation of aromatic cation radicals.[25,52] Furthermore, it is pointed out that OELs are much more stable than environmentally friendly but quickly degradable polysaccharide-based HGs, which are, consequently, not very suitable for long-term applications to improve soil hydraulic and physical properties in a sustainable manner. In contrast, biodegradation rates of OEL comparable with mineralization rates of natural soil organic matter and as twice as high as that for less degradable polyacrylate/polyacrylamide-based SAs suggest that OELs are long-term stable HGs, which might be gradually integrated in soil organic matter similar as it was reported for isolated lignin[50] or lignocellulosic materials[30,31] by humification processes.

OEL Biomineralization: Impact of Oxyethylation Degree and Gel Concentration. Figure 3c pointed out that C_{min} of OELs decreased with increasing oxyethylation degree in the order S-OEL$_{0.65}$ > S-OEL$_{0.75}$ > S-OEL$_{1.0}$ supporting the assumption that oxyethylated substructures of OEL are more recalcitrant against microbial degradation than free phenolic lignin structures. One-way ANOVA of the respective data (C_{min} at 14 days) indicated that the effect of the PEGDGE/lignin ratio, and the oxyethylation degree of the respective HGs on their biodegradation is significant (with $F =$ 85.36 and $p < \alpha$, at a significance level $\alpha = 0.001$; Table 5). A post hoc analysis of these data (Tukey test) verified that the mineralization rates of those OEL variants are significantly different (with lwr > 0 at $p < 0.001$). Thus, a clear relation between mineralization rates of OEL variants and their cross-

DOI: 10.1021/acssuschemeng.5b00139
ACS Sustainable Chem. Eng. 2015, 3, 1955−1964

Scheme 2. (a) Hypothetical Reaction Pathway for Phenol Peroxidase-Mediated Degradation of Phenolic Lignin Substructures (M: transition metal)[57] and (b) Hindered Enzymatic Oxidation of Oxyethylated Phenols in OEL (R = OOE/OOEG substituent)

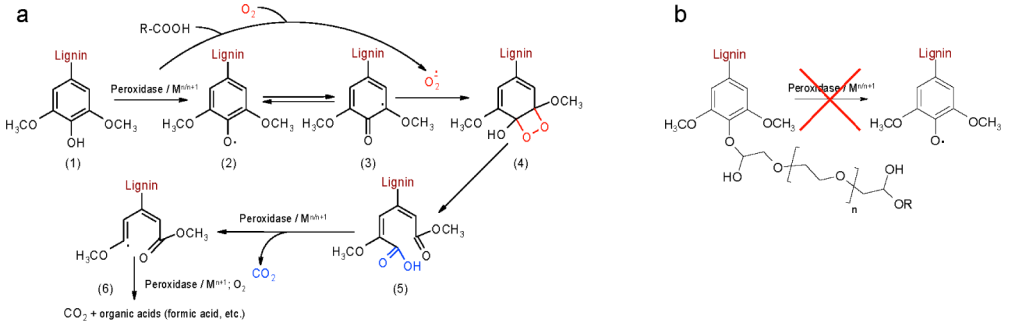


Table 5. Test Results of One-Way ANOVA and Multiple Comparison of Means[a,b]

one-way ANOVA (biomineralization vs PEGDGE/lignin ratio; k = 3, n_i = 5, n = 15)					
	Sum Sq.[c]	DF[d]	Mean Sq.[e]	F value	p value
Factor: PEGDGE/Lig	0.219327	2	0.109663	85.356	8.026e-08***
Residual	0.0005497	12	0.000039		
Total	0.02198767	14			
post hoc analysis (Tukey multiple comparison of means, 95% confidence level)					
	Diff[f]	lwr[g]	upr[h]	p value	α
0.75−1.0	0.13976	0.079281	0.200239	0.0001325	< 0.001***
0.65−1.0	0.29604	0.235561	0.356519	0.0000001	< 0.001***
0.65−0.75	0.15628	0.095801	0.216759	0.0000046	< 0.001***

[a]Variable, biomineralization of model soils with 1% w/w OEL; factor, PEGDGE/lignin ratio; factor levels: 0.65, 0.75, and 1.0 mmol PEGDGE g_{Lig}^{-1}; k = 3, n_i = 5, n = 15. [b]Significant codes: 0, '***' 0.001, '**' 0.01, '*' 0.05, '.' 0.01, ' ' 1. [c]Sum of Squares: Sum Sq_{factor} (variation between factor groups), Sum $Sq_{residual}$ (variation within factor groups), Sum Sq_{total} = Sum Sq_{factor} + Sum $Sq_{residual}$. [d]degrees of freedom: $DF_{factor} = k - 1$, $DF_{residual} = n - k$, [e]Mean Squares: Mean Sq_{factor} = Sum Sq_{factor}/DF_{factor}, Mean $Sq_{residual}$ = Sum $Sq_{residual}/DF_{residual}$. [f]Mean difference. [g]Lower bound. [h]Upper bound.

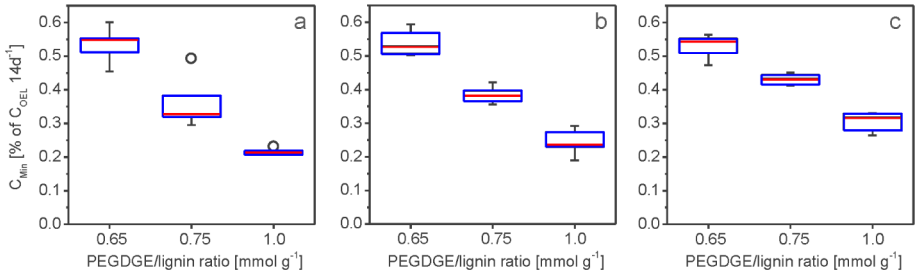


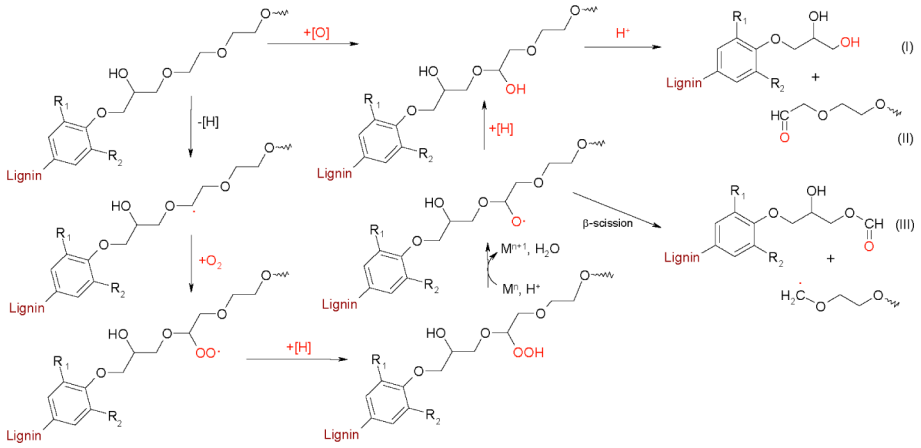
Figure 6. Impact of the PEGDGE/lignin ratio on total C mineralization values of model soils treated with (a) 0.5% w/w, (b) 1.0% w/w, and (c) 2.0% w/w of the corresponding gel variants S-$OEL_{0.65}$, S-$OEL_{0.75}$, and S-$OEL_{1.0}$, respectively. Whiskers represent standard deviations, points represent outliers, n_i = 5 replicates per factor level.

linking/oxyethylation degree is evident. Furthermore, it is indicated that differences in the biodegradation behavior of OELs with varying oxyethylation degree gradually increase over the incubation time. This phenomenon is also observable at different gel concentrations in the model soils (Figure 6). In general, C_{min} of OEL cross-linked with 0.65, 0.75, or 1.0 mmol PEGDGE g_{Lig}^{-1} ranged between (1) 0.55% and 0.22% C 14 d^{-1} at c_{OEL} = 0.5% (Figure 6a), (2) 0.53 and 0.24% C 14 d^{-1} at c_{OEL} = 1.0% (Figure 6b), and (3) 0.55% and 0.33% C 14 d^{-1} at c_{OEL} = 2.0% (Figure 6c). However, gel variants cross-linked with 0.75 and 1.0 mmol PEGDGE featured slightly increased C_{min} of 0.22%, 0.38%, and 0.42% C 14 d^{-1} for S-$OEL_{0.75}$ and 0.22%, 0.24%, and 0.33% C 14 d^{-1} for S-$OEL_{1.0}$ with increasing gel concentration. In contrast, C_{min} of S-$OEL_{0.65}$ nearly remained constant (0.55%, 0.53%, and 0.55% C 14 d^{-1}) within that gel concentration range. This is probably due to a higher content of lignin derived low molecular weight constituents and of free phenolic structures in those minor cross-linked gels which could be mineralized more easily than high molecular weight components of stronger oxyethylated/cross-linked OELs (Figure 4b).

From a structural point of view, it can be assumed that when phenolic OH groups of lignin are blocked like in OEL, formation of phenoxyl radicals, and a subsequent microbial oxidation/mineralization of those substructures by enzymes from the phenol peroxidase type[25,52] is prevented. Those effects might be reinforced by intermolecular interactions, e.g., hydrogen bonds between adjacent OOE/OOEG substituents in OEL,[45] which probably deplete the accessibility of the modified lignins to microbial attacks. Another reason for decreasing mineralization rates of OELs with increasing cross-

DOI: 10.1021/acssuschemeng.5b00139
ACS Sustainable Chem. Eng. 2015, 3, 1955−1964

Scheme 3. Hypothetical Reaction Pathway for the Biodegradation of OOE/OOEG Domains[62] in OEL (M: transition metal)



linking/oxyethylation degree is the recalcitrance of OOE/OOEG moieties in OEL itself.[64]

Despite these facts, biomineralization of OELs gels could also be due to the potential degradation of their OOE/OOEG moieties. In that context some researchers reported on the biodegradation of PEG and other alkylene oxides. Among others, it was suggested that PEG biodegradation occurs by hydrolytic cleavage of ether bonds to yield mono-, di-, and oligomers of ethylene glycol which could be used as energy and carbon source by different soil bacteria.[65] Kerem et al.[66] have been questioned a hydrolytic ether bond cleavage of alkyl ethers in soils for what physiologically unlikely strong acidic conditions are required and reported a rapid polyether cleavage by the brown-rot fungus *Gloeophyllum trabeum*. For that, a free radical fragmentation by a nonspecific extracellular Fenton system was proposed, which could be alternatively effective for OEL degradation in soils (Scheme 3). Due to that mainly primary aliphatic hydroxyls (I) and aldehydes (II, III) are formed. In experiments where PEG-etherified lignin model substrates were biomineralized either with isolated and purified fungal LiP in vitro or fungal cultures of *Phanerochaete crysosporium* in vivo, it was found that most of the oxidized substrate underwent $C_\alpha-C_\beta$ cleavage and cleavage of the $\beta-O-4$ bond of PEG-linked lignin model dimers, whereas the PEG backbone was found to be relatively stable.[67] Thus, microbial degradation of the OOE/OOEG moieties of OEL under natural conditions probably plays an underpart reflected by decreasing degradation rates with increasing oxyethylation degrees of OEL (Figures 3c and 6).

CONCLUSIONS

Three types of technical lignins and corresponding OELs with different cross-linking/oxyethylation degrees were characterized with respect to their biodegradation in model soils under laboratory conditions. OELs were obtained by cross-linking of lignin with poly/oligo(oxyethylene)-α,ω-diglycidyl ether of an average molecular weight of 526 g mol^{-1} ($n \sim 9$) in alkaline media. For parent softwood lignins, carbon mineralization rates under the selected conditions were found to be dependent on (1) average molecular weight/molecular weight distribution, (2) lignin particle characteristics, and (3) the botanical origin of lignin. Lowest degradation rates of hardwood organosolv lignin are suggested to be caused by substrate specialization of the microbial consortium of the inoculum used. Markedly lower degradation rates of OEL compared to that of unmodified lignins are attributed to (1) considerably increased molecular weights of OEL due to PEGDGE cross-linking and (2) evidently lower contents of phenolic substructures known to be directly attacked by most of the expressed enzyme systems of lignolytic fungi. This is supported by significantly decreasing C mineralization with increasing cross-linking/oxyethylation degrees of OEL. The latter is connected with decreasing swelling degrees/water contents and smaller pore volumes making stronger cross-linked gels less accessible for lignolytic fungi and actinomycetes and, consequently, more resistant against microbial attacks than slightly cross-linked and stronger swellable/hydrated OEL counterparts. Favorable properties, such as (1) general biodegradability connected with (2) moderate short-term degradation rates—(a) comparable to mineralization of stabilized soil organic matter in sandy soils, (b) markedly lower than mineralization of quickly degradable polysaccharide based HGs, and (c) notably higher than biodegradation of highly persistant acrylate/acrylamide based SAs—(3) moderate to high swelling capacities adjustable by varying lignin/PEGDGE ratio and lignin type, and 4) the potential role as biobased and renewable precursors for the formation of soil humic substances, render OEL HGs environmentally friendly and sustainable materials for versatile applications in soil improvement and soil rehabilitation.

AUTHOR INFORMATION

Corresponding Author

*Tel: +49 351 4662 369. Fax: +49 351 466 211. E-mail: Lars.Passauer@ihd-dresden.de.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Prof. B. Saake (University of Hamburg, Germany) is kindly acknowledged for providing beech organosolv lignin and data from ^{31}P NMR spectroscopy. The authors thank Dr. J. Appelt (Technische Universitaet Bergakademie Freiberg, Germany) for supporting functional group analysis, the anonymous reviewers for their valuable comments and suggestions, and Technische Universitaet Dresden for supporting this study.

REFERENCES

(1) Frank, M. Superabsorbents. In *Ullmann's Encyclopedia of Industrial Chemistry*; Wiley VCH: Weinheim, Germany, 2003.

DOI: 10.1021/acssuschemeng.5b00139
ACS Sustainable Chem. Eng. 2015, 3, 1955−1964

(2) Van Vlierberghe, A.; Dubruel, P.; Schacht, E. Biopolymer-based hydrogels as scaffolds for tissue engineering applications: a review. *Biomacromolecules* **2011**, *12*, 1387−1408.
(3) Oh, J. K.; Lee, D. I.; Park, J. M. Biopolymer-based microgels/nanogels for drug delivery applications. *Prog. Polym. Sci.* **2009**, *34*, 1261−1282.
(4) Seidel, C.; Kulicke, W.-M.; Heß, C.; Hartmann, B.; Lechner, M. D.; Lazik, W. Synthesis and characterization of cross-linked carboxymethyl potato starch ether gels. *Starch* **2004**, *56*, 157−166.
(5) Gregorova, A.; Saha, N.; Kitano, T.; Saha, P. Hydrothermal effect and mechanical stress properties of carboxymethylcellulose based hydrogel food packaging. *Carbohydr. Polym.* **2015**, *117*, 559−568.
(6) Richter, A.; Paschew, G.; Klatt, S.; Lienig, J.; Arndt, K.-F.; Adler, H.-J.-P. Review on hydrogel-based pH sensors and microsensors. *Sensors* **2008**, *8*, 561−581.
(7) Hüttermann, A.; Orikiriza, L. J. B.; Agaba, H. Application of superabsorbent polymers for improving the ecological chemistry of degraded or polluted lands. *Clean: Soil, Air, Water* **2009**, *37*, 517−526.
(8) Bhardwaj, A. K.; Shainberg, I.; Goldstein, D.; Warrington, D. N.; Levy, G. J. Water retention and hydraulic conductivity of cross-linked polyacrylamides in sandy soils. *Soil Sci. Soc. Am. J.* **2007**, *71*, 406−412.
(9) Holliman, P. J.; Clark, J. A.; Williamson, J. C.; Jones, D. L. Model and field studies of the degradation of cross-linked polyacrylamide gels used during the revegetation of slate waste. *Sci. Total Environ.* **2005**, *336*, 13−24.
(10) Ni, B.; Liu, M.; Lü, S.; Xie, L.; Wang, X. Environmentally friendly slow-release nitrogen fertilizer. *J. Agric. Food Chem.* **2011**, *59*, 10169−10175.
(11) Agaba, H.; Orikiriza, L. J. B.; Obua, J.; Kabasa, J. D.; Worbes, M.; Hüttermann, A. Hydrogel amendment to sandy soil reduces irrigation frequency and improves the biomass of *Agrostis stolonifera*. *Agric. Sci.* **2011**, *02*, 544−550.
(12) Wilske, B.; Bai, M.; Lindenstruth, B.; Bach, M.; Rezaie, Z.; Frede, H.-G.; Breuer, L. Biodegradability of polyacrylate superabsorbent in agricultural soil. *Environ. Sci. Pollut. Res.* **2014**, *21*, 9453−9460.
(13) Stahl, J. D.; Cameron, M. D.; Haselbach, J.; Aust, S. D. Biodegradation of superabsorbent polymers in soil. *Environ. Sci. Pollut. Res.* **2000**, *7*, 83−88.
(14) Mai, C.; Schormann, W.; Majcherczyk, A.; Hüttermann, A. Degradation of acrylic copolymers by white-rot fungi. *Appl. Microbiol. Biotechnol.* **2004**, *65*, 479−487.
(15) Sutherland, G. R. J.; Haselbach, J.; Aust, S. D. Biodegradation of crosslinked acrylic polymers by a white-rot fungus. *Environ. Sci. Pollut. Res.* **1997**, *4*, 16−20.
(16) Ismail, H.; Irani, M.; Ahmad, Z. Starch-based hydrogels: present status and applications. *Int. J. Polym. Mater.* **2013**, *62*, 411−420.
(17) Sannino, A.; Demitri, C.; Madaghiele, M. Biodegradable cellulose-based hydrogels: design and applications. *Materials* **2009**, *2*, 353−373.
(18) Thakur, V. K.; Thakur, M. K. Recent advances in graft copolymerization and applications of chitosan: a review. *ACS Sustainable Chem. Eng.* **2014**, *2*, 2637−2652.
(19) Chauhan, G. S.; Kumari, A.; Sharma, R. Pectin and acrylamide based hydrogels for environment management technologies: Synthesis, characterization, and metal ions sorption. *J. Appl. Polym. Sci.* **2007**, *106*, 2158−2168.
(20) Augst, A. D.; Kong, H. J.; Mooney, D. J. Alginate hydrogels as biomaterials. *Macromol. Biosci.* **2006**, *6*, 623−633.
(21) Ni, B.; Liu, M.; Lü, S.; Xie, L.; Wang, Y. Multifunctional slow-release organic-inorganic compound fertilizer. *J. Agric. Food Chem.* **2010**, *58*, 12373−12378.
(22) Nagasawa, N.; Yagi, T.; Kume, T.; Yoshii, F. Radiation crosslinking of carboxymethyl starch. *Carbohydr. Polym.* **2004**, *58*, 109−113.
(23) Thakur, V. K.; Thakur, M. K. Recent advances in green hydrogels from lignin: a review. *Int. J. Biol. Macromol.* **2015**, *72*, 834−847.
(24) Passauer, L. Highly swellable lignin hydrogels − Novel materials with interesting properties. In *Functional Materials from Renewable Sources*; Liebner, F., Rosenau, T., Eds.; ACS Symposium Series 1107, American Chemical Society: Washington DC, 2012; pp 211−228.
(25) Erikson, K. E.; Blanchette, R. A.; Ander, P. *Microbial and Enzymatic Degradation of Wood and Wood Components*; Springer: Berlin, 1990.
(26) Demirbas, A. *Biorefineries: For Biomass Upgrading Facilities*; Springer: Dordrecht, The Netherlands, 2010.
(27) Liebner, F.; Rosenau, T. *Functional Materials from Renewable Sources*; ACS Symposium Series 1107, American Chemical Society: Washington DC, 2012.
(28) Passauer, L.; Fischer, K.; Liebner, F. Preparation and physical characterization of strongly swellable oligo(oxyethylene) lignin hydrogels. *Holzforschung* **2011**, *65*, 309−317.
(29) Passauer, L., Liebner, F., Fischer, K., Katzur, J. Substrate for Soil Improvement Having Water Storing Property, Method for Producing Same and Use Thereof. Patent DE 10 2010 008 393 A1, WO 2011,098078, August 18, 2011; EP 2534188 A1, December 19, 2012; CN 000102844360A, December 26, 2012; US 20120322990 A1, June 25, 2015.
(30) Stevenson, F. J. *Humus Chemistry*; John Wiley & Sons: New York, 1994.
(31) Haider, K. From dead organic residues to humus. *J. Plant Nutr. Soil Sci.* **1999**, *162*, 363−371 (in German)..
(32) Zakis, G. E. Methoxyl Groups. In *Functional Analysis of Lignins*; Tappi Press: Atlanta, 1994; pp 3−11.
(33) Dence, C. W. Determination of Carboxyl Groups. In *Methods in Lignin Chemistry*; Dence, C. W.; Lin, S., Eds.; Springer: Berlin, 1992; pp 458−464.
(34) Zakis, G. E. Carbonyl Groups. In *Functional Analysis of Lignins*; Tappi Press: Atlanta, 1994; pp 62−75.
(35) Månson, P. Quantitative determination of phenolic and total hydroxyl groups in lignins. *Holzforschung* **1983**, *37*, 143−147.
(36) Granata, A.; Argyropoulos, D. S. 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, a reagent for the accurate determination of uncondensed and condensed phenolic moieties in lignins. *J. Agric. Food Chem.* **1995**, *43*, 1538−1544.
(37) Wörmeyer, K.; Ingram; Saake, B.; Brunner, G.; Smirnova, I. Comparison of different pretreatment methods for lignicellulosic materials. Part II: Influence of pretreatment on the properties of rye straw lignin. *Bioresour. Technol.* **2011**, *102*, 4157−4164.
(38) Ringena, O.; Lebioda, S.; Lehnen, R.; Saake, B. Size-exclusion chromatography of technical lignins in dimethyl sulfoxide/ water and dimetylacetamide. *J. Chromatogr. A* **2006**, *1102*, 154−163.
(39) Strotmann, U.; Reuschenbach, P.; Schwarz, H.; Pagga, U. Development and evaluation of an online CO_2 evolution test and a multicomponent biodegradation test system. *Appl. Environm. Microbiol.* **2004**, *70*, 4621−4628.
(40) ISO 16072: Soil Quality − Laboratory Methods for Determination of Microbial Soil Respiration; International Standardization Organization, 2002.
(41) ISO 11274: Soil Quality − Determination of the Water Retention Characteristic − Laboratory Methods; International Standardization Organization, 1998.
(42) Hamer, U.; Marschner, B. Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. *J. Plant Nutr. Soil Sci.* **2002**, *165*, 261−268.
(43) The R Project for Statistical Computing. www.r-project.org (accessed July 2015).
(44) Sachs, L.; Hedderich, J. *Applied Statistics. Collection of Methods with R.*, 14th ed.; Springer: Berlin, 2012 (in German).
(45) Passauer, L.; Struch, M.; Schuldt, S.; Schneider, Y.; Jaros, D.; Rohm, H.; Appelt, J. Dynamic moisture sorption characteristics of xerogels from water-swellable oligo(oxyethylene) lignin derivatives. *ACS Appl. Mater. Interfaces* **2012**, *4*, 5852−5862.
(46) Kühnel, I.; Podschun, J.; Saake, B.; Lehnen, R. Synthesis of lignin polyols via oxyalkylation with propylene carbonate. *Holzforschung* **2015**, in press, doi:10.1515/hf-2014-0068.

DOI: 10.1021/acssuschemeng.5b00139
ACS Sustainable Chem. Eng. 2015, 3, 1955−1964

(47) Korntner, P.; Sumerskii, I.; Bacher, M.; Rosenau, T.; Potthast, A. Characterization of technical lignins by NMR spectroscopy: optimization of functional group analysis by ^{31}P NMR spectroscopy. *Holzforschung* **2015**, in press, DOI: 10.1515/hf-2014-0281.
(48) Whitehead, D. L.; Quicke, G. V. The nitrogen content of grass lignin. *J. Sci. Food Agric.* **1960**, *11*, 151−152.
(49) Martin, J. P.; Zunino, H.; Peirano, P.; Caiozzi, M.; Haider, K. 1982. Decomposition of ^{14}C-labeled lignins, model humic acid polymers, and fungal melanins in allophanic soils. *Soil Biol. Biochem.* **1982**, *14*, 289−293.
(50) Bahri, H.; Rasse, D. P.; Rumpel, C.; Dignac, M.-F.; Bardoux, A.; Mariotti, A. Lignin degradation during a laboratory incubation followed by ^{13}C isotope analysis. *Soil Biol. Biochem.* **2008**, *40*, 1916−1922.
(51) Donnelly, P. K.; Entry, J. A.; Crawford, D. L.; Cromack, K. Cellulose and lignin legradation in forest soils: response to moisture, temperature, and acidity. *Microb. Ecol.* **1990**, *20*, 289−295.
(52) Kirk, T. K.; Farrell, R. L. Enzymatic "combustion": The microbial degradation of lignin. *Annu. Rev. Microbiol.* **1987**, *41*, 465−505.
(53) Martin, J. P.; Haider, K.; Kassim, G. Biodegradation and stabilization after 2 years of specific crop, lignin, and polysaccharide carbons in soils. *Soil Sci. Soc. Am. J.* **1980**, *44*, 1250−1255.
(54) Basaglia, M.; Concheri, G.; Cardinali, S.; Pasti-Grigsby, M. B.; Nuti, M. P. Enhanced degradation of ammonium-pretreated wheat straw by lignocellulolytic Streptomyces spp. *Can. J. Microbiol.* **1992**, *38*, 1022−1025.
(55) Crawford, D. L. The role of actinomycetes in the decomposition of lignocellulose. *FEMS Symp.* **1986**, 715−728.
(56) Micales, J. A.; Skog, K. E. The decomposition of forest products in landfills. *Int. Biodeterior. Biodegrad.* **1997**, *39*, 145−158.
(57) Kellner, H.; Luis, P.; Pecyna, M. J.; Barbi, F.; Kapturska, D.; Krüger, D.; Zak, D. R.; Marmeisse, R.; Vandenbol, M.; Hofrichter, M. Widespread occurrence of expressed fungal secretory peroxidases in forest soils. *PLoS One* **2014**, *9*, e95557.
(58) Waldrop, M. P.; Zak, D. R.; Sinsabaugh, R. L. Microbial community response to nitrogen deposition in northern forest ecosystems. *Soil Biol. Biochem.* **2004**, *36*, 1443−1451.
(59) Yang, H. H.; Effland, M. J.; Kirk, T. K. Factors influencing fungal degradation of lignin in a representative lignocellulosic, thermomechanical pulp. *Biotechnol. Bioeng.* **1980**, *22*, 65−77.
(60) Wegener, G.; Strobel, C. Determination of phenolic hydroxyl groups in lignins and lignin fractions by means of aminolysis and FTIR spectroscopy. *Holz Roh. Werkst.* **1992**, *50*, 417−420.
(61) Dimmel, D.; Gellerstedt, G. Chemistry of Alkaline Pulping. In *Lignin and Lignans: Advances in Chemistry*; Heitner, C., Dimmel, D., Schmidt, J., Eds.; CRC Press: Boca Raton, FL, 2010; pp 349−391.
(62) Hofrichter, M. Review: lignin conversion by manganese peroxidases (MnP). *Enzyme Microb. Technol.* **2002**, *30*, 454−466.
(63) Hamer, U.; Marschner, B. Priming effects in different soil types after fructose, alanine, oxalic acid and catechol additions. *Soil Biol. Biochem.* **2005**, *37*, 445−454.
(64) Bailey, F. E., Koleske, V. *Alkylene Oxides and Their Polymers*; Marcel Dekker: New York, 1991; p 15.
(65) Haines, J. R.; Alexander, M. Microbial degradation of polyethylene glycols. *Appl. Microbiol.* **1975**, *29*, 621−625.
(66) Kerem, Z.; Bao, W.; Hammel, K. E. Rapid polyether cleavage via extracellular one-electron oxidation by a brown-rot basiodiomycete. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 10373−10377.
(67) Kawai, S.; Jensen, K. A.; Bao, W.; Hammel, K. E. New polymeric model substrates for the study of microbial ligninolysis. *Appl. Environ. Microbiol.* **1995**, *61*, 3407−3414.

DOI: 10.1021/acssuschemeng.5b00139
ACS Sustainable Chem. Eng. 2015, 3, 1955−1964