

Biodegradation of Alkali-O₂ Oxidized Lignins Used as Dispersants

Minna Vikman,* Olesya Fearon, and Anna Kalliola

Large quantities of lignin are produced as by-streams via chemical pulping and emerging biorefinery processes. These lignins are typically water-insoluble; however, they can be converted into a water-soluble form by chemical modifications. A novel LigniOx technology solubilizes lignin using alkali-O₂ oxidation. The product can be used for bio-based dispersants. This study evaluated the biodegradability of alkali-O₂ oxidized kraft, organosolv, and hydrolysis lignin. The oxidized lignins exhibited higher biodegradation in soil and in aquatic environments in comparison to a commercial kraft lignin and a commercial lignosulfonate. In soil, the biodegradabilities of oxidized lignins were 19 to 44%, whereas the reference lignins exhibited only 5 to 12% conversion to CO₂. Biodegradation of the oxidized lignins and references in the aquatic environment increased in a similar order as in the soil environment, although the degradation in each sample was slightly smaller than in the soil. The improved biodegradability of the oxidized lignins was due to the altered chemical structure of lignin. Compared to the untreated lignin, the oxidized lignin contained structures formed in aromatic ring opening reactions, making the lignin more accessible to microbial degradation. In addition, the oxidized lignin contained carbon originating from small organic compounds, which are easily biodegradable.

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INTRODUCTION

Lignin is the second most abundant biopolymer on earth after cellulose. In plants, the aromatic polymer acts as a crosslink between cellulose and hemicellulose, giving structure to the cell wall. The hydrophobic nature of lignin enables water transport, and it also protects plants against parasites and disease. Large quantities of lignin are produced as by-streams from chemical pulping and emerging biorefineries by applying *e.g.*, organosolv pulping or enzymatic methods to fractionate lignocellulose material. The lignin by-streams are currently used for energy, although some lignin-based products have been commercialized. Lignin represents the largest source of bio-based aromatic compounds to substitute the synthetic counterparts on the market (Fatehi and Chenin 2016). For example, kraft lignin is used to replace phenols in phenol formaldehyde resins for plywood and laminate production. Furthermore, similar to lignosulfonates, sulfonated kraft lignin is used as a dispersant, although the market for sulfonated lignin is currently significantly smaller than for lignosulfonates (Aro and Fatehi 2017; Li and Takkellapati 2018). Lignosulfonates are originally water soluble lignins that have anionic charge due to the presence of sulfonic acid groups. Lignosulfonates are also applied in minor volumes as emulsifiers, crystal

growth modifiers, and complexing agents. In some cases, lignosulfonates are further modified to improve their applicability.

A novel LigniOx oxidation process uses O₂ under alkaline conditions to solubilize water-insoluble lignins (Kalliola *et al.* 2015; 2022). The process has been demonstrated to work for lignins originating from soda, kraft, or organosolv pulping processes, as well as hydrolysis residue lignin from bioethanol production. The alkali-O₂ oxidized lignins have an increased anionic charge that stems mainly from carboxylic acids introduced to the lignin structure. The oxidized lignins have been demonstrated to work as a concrete plasticizer and a versatile dispersant for inorganic pigments and special carbon black (Kalliola *et al.* 2015; 2017; 2018; Fearon *et al.* 2021). Dispersing agents prevent the aggregation of particles, thus enhancing the processibility and quality of the product. Anionic dispersants from fossil origin cover most of the dispersant market, but lignosulfonates are also used to some extent. The oxidized lignins have been shown to outperform lignosulfonate products and even some of the synthetic products based on naphthalene sulfonates, polycarboxylate ethers, and polyacrylic acids. Recently, a concept of LigniOx process for producing lignin dispersants in a feasible manner at a kraft pulp mill has been introduced (Kalliola *et al.* 2022).

Lignin has utilization potential in the agrochemical market. Lignin could potentially be used as a fertilizer, pesticide, soil improver and plant growth regulator (Ahmad *et al.* 2021). In addition, utilization of lignin for antiscaling and chelation agent has been reported (Wikander *et al.* 2006; Gonçalves and Soto-Oviedo 2002). In some of the applications, alkali-O₂ oxidized lignin derivatives could be considered as a substitute for oil-based polyacrylic acids. The oil-based polyacrylic acids are reported to have limited biodegradability resulting in the accumulation of the product in the environment (Gaytán *et al.* 2020).

Any dispersants used in an agricultural product or in water treatment can end-up in the soil, fresh water, or the marine environment. Therefore, to ensure environmental safety, biodegradability is an important property. Biodegradability is not dependent on the origin of material (biobased or synthetic); rather, it is a function of the physicochemical properties (Sapea 2021). Lignin in plants is insoluble, has a complex structure, and lacks hydrolysable linkages (Ayeronfe *et al.* 2018). In nature, white-rot fungi are identified as the most effective degraders of lignin. In addition, many bacteria are shown to be capable of degrading and solubilizing polymeric lignin and lignin-related compounds (reviewed by Datta *et al.* 2017 and Tuomela *et al.* 2000). However, as demonstrated earlier (Tuomela *et al.* 2001; Vikman *et al.* 2005; Li *et al.* 2020), lignin is not completely mineralized to CO₂ during microbial degradation and generally gives poor results in biodegradability tests. This study evaluates the influence of alkali-O₂ oxidation on biodegradability of lignin in soil and in aquatic environment compared to a commercial softwood kraft lignin and lignosulfonate product.

EXPERIMENTAL

Materials

Alkali-O₂ oxidized lignins were produced from different sources using molecular oxygen as the oxidizing agent (Kalliola *et al.* 2022). The lignins used for oxidation were unwashed softwood kraft lignin (from Metsä Fibre Rauma mill), softwood hydrolysis lignin (from St1 Kajaani demo plant), and wheat straw organosolv lignin (provided by

Fortum). After oxidation, the solutions of the oxidized lignins were post-treated by membrane filtration to concentrate and purify the lignin product with a method similar to that described by Kalliola *et al.* (2022). The post-treated samples are referred to as LigniOx-KL, LigniOx-HL, and LigniOx-OSL, respectively. In addition, a solution of oxidized organosolv lignin without the post-treatment was used and is referred to as Crude LigniOx-OSL. All the alkali-O₂ oxidized solutions were alkaline with a pH close to 10.

Commercial softwood kraft lignin (BioPiva™, Domtar) and partly desulfonated lignosulfonate (Vanillex®, Nippon Paper group) were used as references, and are referred to as KL-Ref and LS-Ref, respectively.

Characterization of Samples

The organic and inorganic carbon content of the samples were analyzed according to SFS-EN 13137 (2001) with Skalar Primacs 100- analyzer. The total carbon, hydrogen, nitrogen, oxygen and sulfur content of the solid samples (KL-ref and LS-ref) were analyzed by elemental analysis Flash 2000 EA CHNS-O (Thermo Fisher Scientific). Lignin content of the oxidized lignins was measured by UV absorbance at 280 nm and the molar mass of the lignins was analyzed by alkali size exclusion chromatography. The methods are described in detail in Jääskeläinen *et al.* (2017). The amount of small aliphatic acids (formic and acetic) in the oxidized lignin were determined by capillary electrophoresis (Keskinen *et al.* 2017).

Non-volatile carboxylic acids were analysed as their trimethylsilyl derivatives by gas chromatography mass spectrometry (GC-MS). For the analysis, samples were dissolved in water, acidified with 3 M hydrochloric acid, and extracted twice with ethyl acetate. The resulting extracts were evaporated dry and trimethylsilylated in pyridine with N,O-bis(trimethylsilyl)trifluoroacetamide, containing 10% of trimethylchlorosilane. Citric acid was added as the internal standard before the extractions. The GC-MS runs were performed with an Agilent 7890 A GC system, equipped with an Agilent 5975 C Inert MSD detector and a DB-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The applied oven temperature program was 1 min at 80 °C, 10 °C/min to 300 °C, and 10 min at 300 °C. The MS identifications were based on the use of an in-house MS library available at VTT, the commercial Wiley database, and existing literature collections. The peak areas were used for the indicative concentration calculations, without any corrections.

Elemental composition (Ca, Fe, K, Mg, Na, S, Mn) of lignin samples were analyzed by inductively coupled plasma optical emission spectroscopy after solubilization with HCl/HF/HNO₃-treatment.

Anionic charge in the oxidized lignin products was determined at pH 6 by applying polyelectrolyte titration (SCAN-W 12:04) combined with streaming current detection (Mütek™ PCD-05 and titrator T3, BTG Instruments GmbH, Germany). Here, a solution of about 1 wt% in terms of oxidized lignin was prepared using 0.01 M sodium citrate buffer pH 6. The solution was titrated with a cationic polymer, polyDADMAC. Lignin charge (mmol/g) was computed based on the amount of polyDADMAC consumed at the point where the measured potential passes zero.

Methods to Test Biodegradability

Biodegradation in soil environment

The biodegradability of the lignin samples in soil was evaluated using the standard method ISO 17556 (2012) based on CO₂ evolution. Instead of passing CO₂-free air over the soil and then determining the carbon dioxide content of the air as described in the standard, CO₂ concentration was analyzed from the gas phase by IR analysis. Nitrogen and carbon content of the soil were evaluated using elemental analyzer Flash 2000 EA CHNS-O (Thermo Fisher Scientific).

Soil was collected from an agricultural field at the University of Helsinki, Helsinki, Finland. The pH of the soil was 6.4, organic matter content 9% (of the dry matter, dm) and C/N ratio of 19. Soil was sieved to a 2 mm particle size, and the final soil moisture was adjusted to 24% (circa 30% of the water holding capacity). Samples were mixed with 75 g (dm) of soil in a 1000 ml glass bottle sealed with a septum and screw cap with a hole. The final concentration of the lignin sample in the test bottles ranged from 330 to 2000 mg per bottle corresponding *ca.* 25 to 250 mg of organic carbon. Three replicates were prepared for the lignin samples, for the blank and for microcrystalline cellulose, which was used as a reference compound.

The concentration of CO₂ in the gas phase of the bottles was measured at regular intervals by immersing a needle through septum of the bottles and directly measuring the CO₂ concentration by a Servoflex MiniFoodPack 5200 infrared analyzer (Servomex, UK). The bottles were aerated to remove excess CO₂, closed with a septum and screw cap, and the CO₂ concentrations were measured for a second time to ensure that CO₂ was removed from the bottles. The net CO₂ production evolved from the test materials was calculated by subtracting the average amount of CO₂ produced in the blank soils from the amount of CO₂ produced in the test material jars. The biodegradation percentages were calculated as ratio of the net CO₂ production and the theoretical CO₂ production calculated on the basis organic carbon content of the tested material (except for LS-ref, in which case total carbon content was used to calculate theoretical CO₂ production).

Biodegradation in aquatic environment

Biodegradability of the samples (Table 1) in aerobic aquatic conditions was studied using the EN ISO 14593 (1999) standard method. Microcrystalline cellulose (20 µm, Aldrich, USA) and water-soluble sodium benzoate (Merck, Germany) were used as positive references.

The test compound, as the sole source of carbon and energy, was added to a mineral salt medium inoculated with a mixed population of microbes from a wastewater treatment plant (Suomenoja, Espoo, Finland). Solid samples were weighed directly to the bottles, and liquid samples were added by preparing stock suspension before addition to the bottles. The final concentration of sample in the bottle was 6 to 60 mg of organic carbon/L. Bottles were incubated in sealed vessels in aerobic conditions at +20°C in the dark. Three replicate bottles were analyzed after 7, 13, 25, 61, 91 and 153 days of incubation. Concentrated orthophosphoric acid (> 85 % mass per volume) was injected to each test vessel to lower the pH of the medium to below 3, bottles were stirred for 1 to 3 h, and the temperature was allowed to equilibrate to room temperature. The concentration of CO₂ in the air phase of the headspace bottles was measured by immersing a needle through the rubber cap of the headspace flask and directly measuring the carbon dioxide concentration by a Servoflex MiniFoodPack 5200 infrared analyzer (Servomex, UK). The biodegradation percentages of samples were calculated similarly as described for soil biodegradation test.

RESULTS AND DISCUSSION

The potential end-uses of the LigniOx lignins include application as an ingredient in agricultural products or as an antiscaling agent in water treatment or processes. Understanding the biodegradability of the oxidized lignins is especially important with these applications as the LigniOx lignin will be directly applied to soil or an aquatic environment and can potentially accumulate to the environment. Therefore, the biodegradability of the alkali-O₂ oxidized lignins was examined both in agricultural soil and in aquatic conditions. Untreated kraft lignin and a commercial lignosulfonate-based dispersant were analyzed as references.

Composition of Oxidized Lignin Products

The organic and inorganic carbon content as well as the lignin content in the samples are shown in Table 1. The lignin content in the oxidized lignin products ranged from 59.2 to 77.1% of dry matter (Table 2; Fig. 1). In addition to lignin, the oxidized lignins contained considerable amount of sodium, carbonates, and sulfates (Table 2; Fig. 1).

All products, except LigniOx-HL, contained 1.5 to 10.8% (dm) of small organic compounds, which are degradation products of the lignin and formed during the oxidation process. The membrane post-treatment of Crude LigniOx-OSL solution reduced the amount of small organic compounds from 10.8 to 4.7 % of the dry matter in the solution (Fig. 1).

Molar mass (M_w) of oxidized lignins varied from 3750 to 25890 Da and the anionic charge at pH 6 from 2.1 to 3.5. The anionic charge can be attributed to carboxylic acids and hydroxyl-*p*-quinones introduced in lignin during oxidation and more over turning lignin into a biobased dispersant. While the oxidized lignins are soluble under the conditions of pH 6, the charge could be determined unlike for kraft lignin (KL-Ref), which is only soluble under highly alkaline conditions (Kalliola, 2015). Chemical characteristics of the references *i.e.* kraft lignin (KL-Ref) and lignosulfonate (LS-Ref) are shown in Table 3.

Table 1. Organic and Inorganic Carbon, and Lignin Content in the Samples

Sample	Origin of the lignin	Form	Organic carbon (g/kg)	Inorganic carbon (g/kg)	Lignin content (g/kg)
LigniOx-KL	Softwood kraft lignin	liquid	124.2	1.1	175
LigniOx-OSL	Wheat straw organosolv lignin	liquid	125.3	1.4	189
LigniOx-HL	Softwood hydrolysis lignin	liquid	71.0	3.7	110
Crude LigniOx-OSL	Wheat straw organosolv lignin, crude solution	liquid	97.8	5.0	139
KL-Ref	Softwood kraft lignin, acid washed	powder	462	<0.2	619
LS-Ref	Desulfonated sodium lignosulfonate	powder	nd	1.1	766

nd = not determined

Table 2. Characteristics of Oxidized Lignins

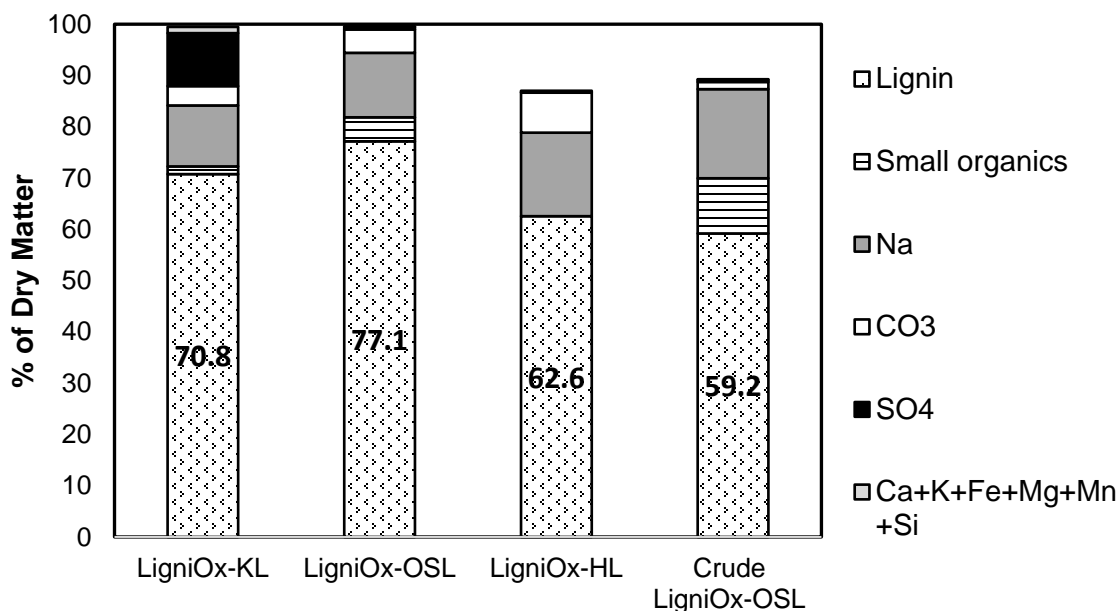
Oxidized Lignin Sample	Dry Matter	Lignin	Small Organics	Na	CO ₃	SO ₄	Other inorg.*	Mw	PD	Charge at pH 6
	%	% of dm						Da	-	mmol/g
LigniOx-KL	24.8	70.8	1.5	11.9	3.8	10.3	1.2	4750	2.9	-3.5
LigniOx-OSL	24.5	77.1	4.7	12.6	4.6	0.5	0.1	3960	2.8	-2.4
LigniOx-HL	17.5	62.6	n.d.	16.3	7.8	0.1	0.2	25890	12.8	-3.7
Crude LigniOx-OSL	23.4	59.2	10.8	17.4	1.4	0.4	0.1	3570	2.8	-2.1

*Ca+K+Fe+Mg+Mn+Si

Table 3. Chemical Characteristics of the Reference Materials Kraft Lignin KL-Ref and Lignosulfonate LS-Ref

Characteristic	KL-Ref	LS-Ref
Dry matter (%)	64.2	93.4
Mw (Da)	3700	5830
Polydispersity	2.9	3.3
Carbon (% of dm)	68.9	49.0
Hydrogen (% of dm)	5.6	5.2
Nitrogen (% of dm)	0.1	0.04
Oxygen (% of dm)	27.2	26.3
Sulphur (% of dm)	2.1	1.3
Ash (% of dm) at 550°C	1.9	22
Sugars (% of dm)	1.5	8.0
Lignin (% of dm)	96.4	82.0
Soluble in water at 20°C	pH >11	pH >7.5

Elemental composition, molar mass (M_w) and lignin content of both samples analyzed as described in this manuscript. Other properties of KL-Ref characterized by Borrega *et al.* (2020) and properties of LS-Ref by Borenien (2019).

**Fig. 1.** Composition of dry matter in oxidized lignins (according to Table 2)

Biodegradation of Oxidized Lignin

The oxidized lignins LigniOx-KL, LigniOx-OSL, LigniOx-HL, and Crude LigniOx-OSL showed clearly higher biodegradability in soil compared to softwood kraft lignin KL-Ref and the commercial lignosulfonate LS-Ref (Fig. 2). Biodegradation was in the range 19 to 44% for the oxidized lignins, whereas the reference lignins gained only 5 to 12% biodegradation. The highest biodegradability was obtained with the oxidized lignin produced from wheat straw organosolv lignin Crude LigniOx-OSL with 45% of the sample converted to CO₂ in the 6 months incubation time in soil. Biodegradability of the commercial lignosulfonate LS-Ref was 12% after 6 months of incubation. This was higher compared to the kraft lignin KL-ref, which biodegraded 5% in the same incubation time. The enhanced biodegradability of LS-Ref over KL-Ref could be due to the modifications, *e.g.* partial desulfonation, of the lignosulfonate product (Vanillex N) during the manufacturing process (Nippon Paper 2022). LS-Ref also contained a higher amount of sugars than the KL-Ref (Table 3), which may have increased its biodegradability.

Biodegradation of microcrystalline cellulose was 71% after 6 months, indicating that the test was valid. According to the ISO 17556 MC should exhibit over 60% biodegradability after the plateau is reached or at the end of the test. The test period is typically six months but can be extended if the plateau phase has not been reached.

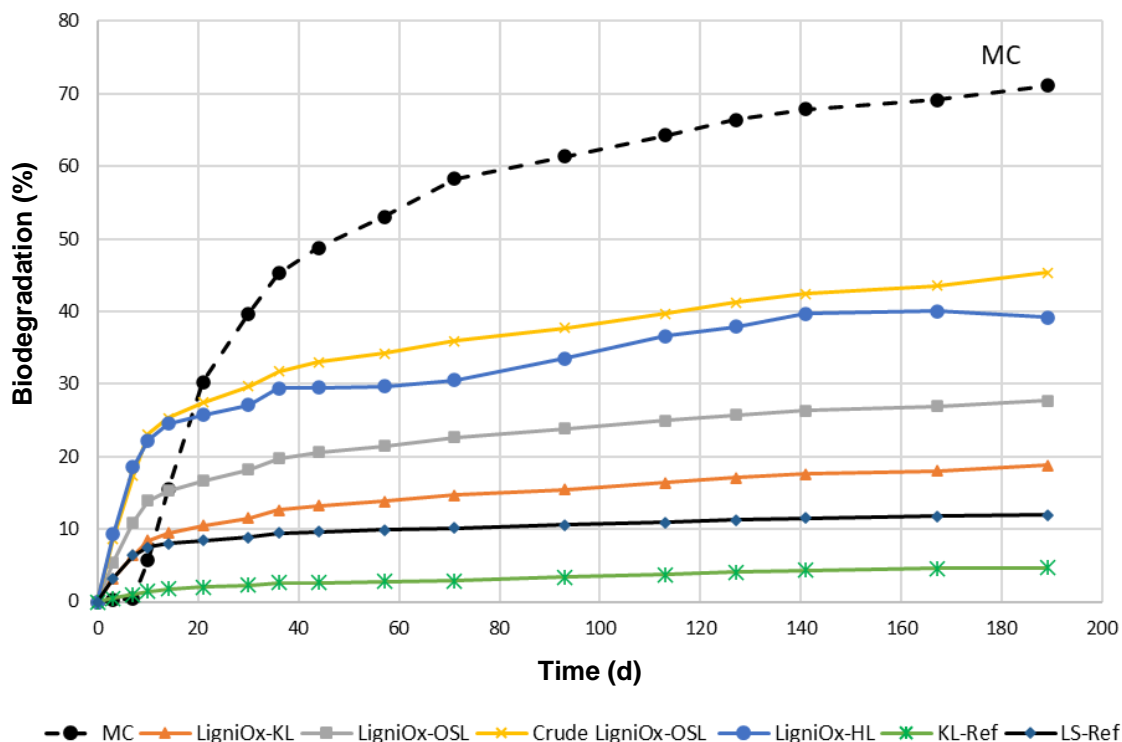


Fig. 2. Biodegradation of oxidized lignins and lignin references in soil environment. MC = microcrystalline cellulose

Biodegradation of the oxidized lignins and references in the aquatic environment was similar, though slightly smaller, than in the soil environment (Fig. 3). Although the inoculum from the wastewater treatment plant and the agricultural soil both contain a diverse microbial community, the soil was a more optimal environment for degradation of

lignin-containing materials. The maximum biodegradation of the oxidized lignins in the aquatic environment ranged between 11 and 30%, whereas in the soil, the maximum biodegradability was between 19 and 45%. Similarly, as in the soil environment, Crude LigniOx-OSL achieved the highest conversion to CO₂ (30%) of all the oxidized lignin concentrates tested in the aqueous environment. Biodegradabilities of the reference lignins KL-Ref and LS-Ref were 8 and 11%, respectively. The biodegradability of kraft lignin KL-Ref was higher than what was been reported by Kwon *et al.* (2021). They noted that softwood and hardwood kraft lignin showed no biodegradation in the aquatic ISO 14851 (2019) biodegradation test.

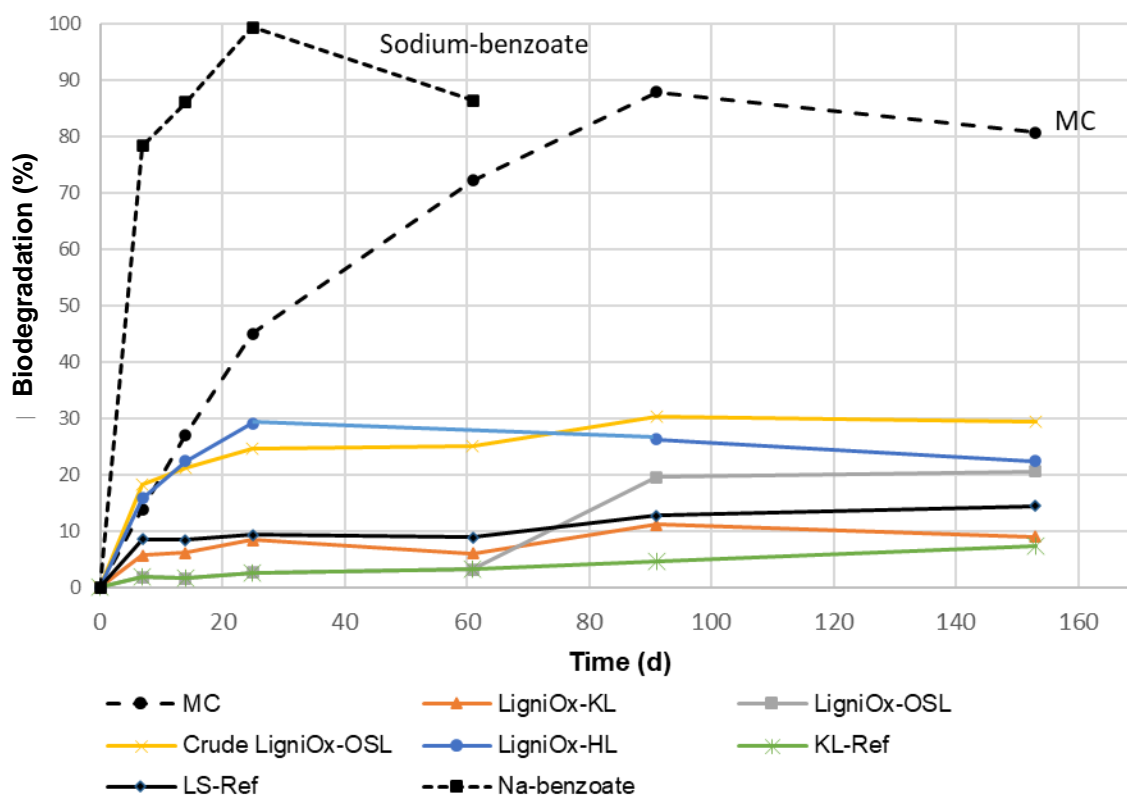


Fig. 3. Biodegradation of oxidized lignins and references in aquatic conditions. MC = microcrystalline cellulose

The Influence of Lignin Characteristics on Biodegradation

Organic carbon is used by microbes as a carbon source for growth and the biodegradation tests were dosed based on the organic carbon content (Table 1). The organic carbon in the oxidized lignin preparations originates either from the lignin structures or the small organic compounds present in the solution. The small organic molecules are degradation products of lignin released during the oxidation process (Kuitunen *et al.* 2011; Rovio *et al.* 2011). The results obtained in the biodegradation tests were based on evaluation of the complete mineralization and measurement of CO₂ evolution. It can be clearly seen that the oxidized lignins were biodegraded to a higher extent in both environments compared to the kraft lignin KL-Ref and the commercial lignosulfonate LS-Ref (Table 4). The influence of the small organics can be clearly seen by comparing the biodegradation of LigniOx-OSL before and after membrane filtration. The membrane

filtration step decreased the organic acid content from 10.8% in Crude LigniOx-OSL to 4.7% in LigniOx-OSL (Fig.1). Biodegradation of the Crude LigniOx-OSL was 45% after six months in soil environment but only 28% after membrane post treatment. The small organic compounds detected in the oxidized lignin products were mostly aliphatic carboxylic acids and vanillic acid. These compounds typically biodegrade more easily compared to compounds containing aromatic rings or compounds with a higher molecular weight (Leahy and Collwell 1990).

Biodegradation is defined as the degradation process of organic compounds by microorganisms in the presence of oxygen producing carbon dioxide, water, mineral salts and new microbial biomass (ISO 17556). Calculations of biodegradability are typically based only on the measurements of CO₂ evolution. Other components, such as carbon incorporated in microbial biomass, are not included. It was reported earlier (Vikman *et al.* 2001) that a higher lignin content in lignocellulosic materials *i.e.*, a lower carbohydrate content, results in a lower amount of biodegradation in a compost environment. Similar correlation has been found in aquatic (Kwon *et al.* 2021) and in anaerobic conditions (Chandler *et al.* 1980; Tong *et al.* 1990). It has been demonstrated that a substantial fraction of ¹⁴C-labelled synthetic lignin is incorporated into humic substances or other insolubles during composting and only a minor part of the lignin is mineralized to CO₂ (Tuomela *et al.* 2001). The evaluation of carbon transformed to humic substances was not in the scope of this study but can be a relevant factor when carbon balance during biodegradation is considered. This process of humification is not completely understood but is considered to be an essential phenomenon in the carbon cycle and beneficial for the soil environment.

Under alkaline O₂ oxidation conditions the chemical structure of lignin is altered significantly. In addition, a minor fraction of the lignin is degraded down to the non-lignin compounds (e.g. aliphatic acids). As described by Kalliola *et al.* (2014) and drafted in Fig. 4, oxidation of lignin starts with the formation of a phenoxyl radical through electron transfer from the ionized phenolic group to O₂. Subsequent steps include reaction with the superoxide (O₂^{•-}), forming an organic hydroperoxide anion structure. Its rearrangement leads to the formation of the primary reaction products, *i.e.* muconic acid or o-kinone structure (A). These structures are vulnerable to further oxidative degradation. The hydroperoxide structure, with a pK_a value of 12 to 13, is the key intermediate in the course of oxidation of the phenolic lignin. Thus, the pH of the reaction solution determines whether the intermediate rearranges, leading to degradation (A), or induces condensation as its protonated form and decomposes back to a phenoxyl radical, which spontaneously undergoes coupling (B). For production of lignin dispersants, the oxidation conditions, especially pH, are selected to prefer the opening of the aromatic ring (A). Thereby the anionic charge in lignin is increased due to the formation of muconic acid structures and secondary oxidation products holding carboxylic acid functionalities. Compared to the untreated lignin, oxidized lignin contains less phenolic (aromatic) structures, presumably making them less hydrophobic and more accessible to microbial degradation (Fewson 1988). It has been determined that oxidation reduces the content of phenolic groups by at least 30% when compared to the starting lignin material (Tamminen *et al.* 2018). The structure of material has a direct effect on its biodegradation. Such parameters as the presence of aromatic ring, the C-C backbone length, the side groups and the molecular structure (cross-linked branched, linear) affect the rate of biodegradation (Leahy and Colwell, 1990; Sapea 2021). Biodegradability of LigniOx-OSL was found to be greater than that of LigniOx-KL. The difference could be due to the pulping process (kraft *vs.* organosolv). Softwood kraft lignin has shown to have very branched *i.e.*, condensed

structure (Crestini *et al.* 2017), while the investigations on organosolv lignin indicate that the lignin is a more linear-like polymer than kraft lignin (Cherif *et al.* 2020).

Table 4. Biodegradation and Relative Biodegradation of Oxidized Lignins in Soil and Aquatic Environments

Sample	Lignin (% of dm)	Biodegradation (%)		Biodegradation in relation to MC (%)	
		Soil 6 months	Aquatic 5 months	Soil	Aquatic
Microcrystalline cellulose, MC	-	66	81		
LigniOx-KL	70.8	19	11	28	14
LigniOx-OSL	77.1	28	21	42	26
LigniOx-HL	62.6	39	29	59	36
Crude LigniOx-OSL	59.2	45	30	68	38
KL-Ref	94.6	5	7	7	9
LS-Ref	82.0	12	14	18	13

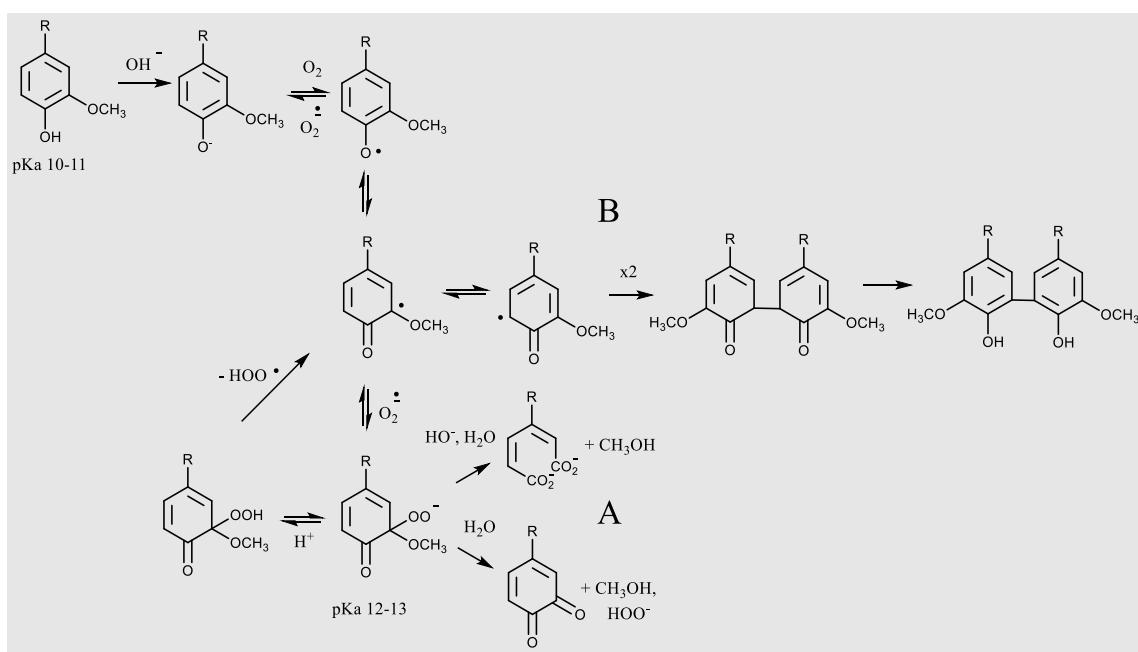


Fig. 4. Simplification of reactions during O₂ oxidation of lignin under alkaline conditions (Kalliola *et al.* 2014). Pathway A: Degradation of aromatic ring. Pathway B: Coupling of phenoxyl radicals.

Importance of Biodegradability for Lignin Applications

Limited availability and increased cost of fossil resources as well as the increased demand for more sustainable products are major factors driving the development of new bio-based products. However, all bio-based products are not automatically biodegradable. For example, although kraft lignin is produced from wood, the commercial kraft lignin is not soluble at neutral pH, has a relatively low biodegradability, a small particles size, and therefore could even be considered a microplastics in some regulations (Mitrano and Wohlleben 2020).

Biodegradation and non-toxicity are especially important for agricultural applications as the products will be in direct contact with food and feed production.

Furthermore, after application to fields, the product can remain in the soil or potentially leach to aquatic environments due to runoff or soil erosion. This study demonstrates improved biodegradability of the oxidized lignins in comparison with commercially available kraft and lignosulfonate lignins making their use potential also in agricultural applications. In addition to mineralization to CO₂, part of the oxidized lignin products is probably incorporating to humic substances during biodegradation in soil (Thevenot *et al.* 2010). The formation of humic substances is a beneficial process and should be addressed in future studies. Furthermore, the ecotoxicity of the oxidized lignins should also be investigated to ensure the environmental safety.

Oxidized lignins could substitute polyacrylic acids (PAA) and naphtha-based compounds as dispersants for inorganic pigments and special carbon black (Kalliola *et al.* 2017). The global production of PAA is around 2.0 MT/year, and PAA waste ends up in landfills, wastewaters, sediments, soil humid fractions, surface water and in rare cases to recycling (Gaytán *et al.* 2020). Clearly, the safe degradation of PAA is of importance as it accumulates to nature. For some time, PAA were considered biodegradable and therefore not harmful for human health and the environment. However, recent studies have reported negative effects from accumulation of PAA compounds in the environment and the toxicity of some of the degradation products (Gaytán *et al.* 2020). The biodegradation of sodium naphthalene-1-sulphonate has been investigated, and based on biodegradability tests, this compound is not readily biodegradable in natural environments (Registration Dossier 2022). However, based on a report available in ECHA database, the chemical is not persistent in the soil environment and the exposure risk to soil dwelling animals is moderate to low (Registration Dossier 2022). Use of a biodegradable alternative to PAA, such as the LigniOx lignins, would reduce the risk of accumulation of toxic compounds to the environment.

Recently, Rajagopalan *et al.* (2022) completed a life cycle assessment (LCA) of alkali-O₂ oxidized kraft lignin in comparison with a concrete superplasticizer product and PAA (a dispersant product). The results demonstrated that oxidized lignin performs clearly better than the commercially available oil-based products in most of the impact categories of the LCA assessment. In addition to the adequate biodegradability of the oxidized lignins presented in this study, the better performance in environmental factors in the LCA demonstrates the high potential of oxidized lignins as an environmentally friendly alternative for oil-based products.

CONCLUSIONS

1. Alkali-O₂ oxidation (LigniOx process) was found to enhance the biodegradation of lignin in soil and aquatic environments. Higher biodegradation was achieved in the soil environment compared to the aquatic environment, possibly due to the higher adaptation of the soil microbial community for lignin degradation.
2. Improved biodegradability of alkali-O₂ oxidized lignin can be attributed to changes in the chemical structure of the lignin as well as due to the lower share of lignin-based carbon of the total organic carbon content. Compared to the un-treated lignin, oxidized lignin contains fewer aromatic structures, making it more accessible to microbial degradation. In addition, oxidized lignin contains easily biodegradable small organic compounds.

3. Improved biodegradability of the lignin products will support the approach towards more sustainable processes and consumables. However, this study could be complemented by a more detailed investigation on environmental safety of the novel dispersants produced from the oxidized lignins.

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