The Impact of Lignin Content on the Biodegradation of Virgin Paper Pulps in Soil and Marine Environment

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Paper pulp is a lignocellulosic fibrous material used in the industrial production of paper and board products. In addition to cellulose and hemicellulose, paper pulp contains 1 to 20% lignin, depending on the raw materials and pulping process used. Lignin is a heterogenous aromatic polymer that is hydrophobic and more resistant to microbial degradation compared to the easily biodegradable cellulose and hemicellulose. In this study, the biodegradation of paper pulps containing varying amounts of lignin was examined in soil and marine environments using ISO testing methods. Lignin significantly reduced the mineralization of paper pulps to CO_2 in both environmental conditions, and a strong inverse correlation between lignin content and the mineralization to CO_2 was observed. A similar impact was observed with natural materials containing lignin, such as birch sawdust. Since the calculation of biodegradability in most ISO and EN standards is based solely on the concept of mineralization to CO_2, materials containing lignin can receive poor values in these tests. The implications of this for standardized requirements of biodegradability and possible options to overcome testing deficiencies are discussed.

DOI: 10.15376/biores.19.2.2452-2465

Keywords: Biodegradation; Mineralization; Lignin; Paper pulp

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INTRODUCTION

Paper pulp is the primary raw material used in the industrial production of paper and board products (Alén 2007). Pulp is a lignocellulosic fibrous material prepared by chemically or mechanically separating cellulose fibers from virgin wood or other sources, such as cotton, bamboo, or recycled paper products (Suhr *et al.* 2015). The composition of paper pulp varies according to the raw materials and the pulping and bleaching processes, with the major constituents being cellulose, hemicellulose, and lignin, while the remainder comprises other organic and inorganic materials (Echa 2023). The final lignin content of the paper pulp ranges from 1 to 20%, as shown in Table 1.

Wood can be converted to pulp through mechanical, chemical, or a combination of both methods (Alén 2007). In mechanical pulping, wood is disintegrated by refining or grinding without the use of chemicals, and the majority of the lignin remains in the pulp, albeit with the dissolution of some organics. The most common chemical pulping process is kraft pulping (*i.e.*, sulphate process), which accounts for approximately 80% of the world's pulp production (Suhr *et al.* 2015). In this process the cooking chemicals such as NaOH and Na_2S are used to dissolve lignin and a portion of the hemicellulose from the wood chips.

Table 1. Average Lignin Contents of Wood and Various Pulp Types (Alén 2000; Hakkila and Verkasalo 2009; Lahtinen *et al.* 2014)

Wood / Pulp type	Lignin- % (dry weight)	Remarks
Wood	Softwood (coniferous) 25-30% Hardwood (non-coniferous) 20-25%	Cellulose 40-45% Hemicellulose 25-35% Pectin, starch, proteins extractives
Mechanical pulp	Lignin ~20c%	Mechanical process, majority of the original lignin not removed, composition similar to the used raw material
Unbleached chemical pulp	Residual lignin 2 to 5%	Cooking chemicals used to dissolve lignin
Bleached chemical pulp	Residual lignin 1%	Bleaching used to remove remaining lignin
Chemithermo-mechanical pulp (CTMP)	Residual lignin ~20%	Combination of chemical, thermal, and mechanical processes are used to produce pulp
Recycled pulp	Depends on the composition of the raw material, 0 to 20%	Composition difficult to define, can contain residues of chemical additives

After the dissolution stage, paper pulp is washed, and 96 to 99% of the used chemicals are recovered (Suhr *et al.* 2015; Bajpai 2018). The brightness of mechanical or chemical pulp can be enhanced through bleaching (Alén 2007), which may involve the use of chlorine dioxide, oxygen, ozone, and peroxide (Suhr *et al.* 2015). Since paper pulp contains only a small quantity of chemical residues, various chemicals and additives are required when pulp is used to manufacture paper, cardboard, or packaging materials. These functional chemicals and process aids are employed to optimise the specific properties of the product and enhance the runnability of the process (Krogerus 2007).

Cellulose and hemicellulose are easily biodegradable polymers, whereas lignin's hydrophobic nature, branched three-dimensional structure, phenolic units, and carbon-carbon bonds are among the factors that make it resistant to microbial degradation. Lignin is an amorphous and heterogeneous polymer that acts as a crosslink between cellulose and hemicellulose in the plant cell wall. It is composed primarily of p-coumaryl, coniferyl, and sinapyl alcohol units (Datta *et al.* 2017). White-rot fungi (classified in Basidomycota phylum) are identified as the most effective degraders of lignin and are typically found in decaying wood, soil in forest, and agricultural fields (Tuomela *et al.* 2000). It has been suggested (Varadachari and Ghosh 1984; Fustec *et al.* 1989; Inbar *et al.* 1989) that in addition to polysaccharides and nitrogenous compounds, lignin contributes to the formation of humus. Although the fate of lignin in soil and the humification process is still not well understood, humus is known to have an important role in carbon and nutrient cycling, and it improves the physical properties of soil (Thevenot *et al.* 2010; Datta *et al.* 2017). It has been speculated that in a marine environment bacteria play an important role in lignin degradation (Ley *et al.* 2023; Lu *et al.* 2020), but their contribution to the carbon cycle remains unclear.

Most standardized biodegradability testing methods in aerobic conditions are based on the concept of mineralization, by which organic carbon is transformed to CO_2 by microbial action. Mineralization of polymeric substances to CO_2 and H_2O contain several phases, including depolymerization, assimilation of the formed monomers and oligomers inside microbial cells, and mineralization (reviewed by Innocenti and Breton 2020). In reality, some of the organic carbon is also transformed to microbial biomass, but if biodegradability testing is continued long enough, most of the microbial biomass carbon is eventually mineralized to CO_2. Therefore (and partly because of the analytical challenges) microbial biomass carbon is not considered by researchers, and the level of biodegradation is calculated by comparing the amount of CO_2 evolved in the test with the theoretical amount and expressed in per cent.

In the case of the lignin-containing materials, this established way to calculate biodegradability has some drawbacks. Under industrial composting conditions, only a portion of the lignin is mineralized to CO_2, and a reduced mineralization has been observed for lignin-containing paper pulps and paper products (Vikman *et al.* 2002; Venelampi *et al.* 2003). According to Tuomela *et al.* (2001), this reduced mineralization occurs because a substantial fraction of lignin was incorporated into humic compounds during composting, with only a minor part being converted to CO_2. A similar behaviour has also been suggested for the soil environment using ^{14}C-labeled model compounds representing lignin (Martin and Haider 1979; Martin *et al.* 1980). Furthermore, in an aquatic environment with microbes from a wastewater treatment plant, a clear correlation between lignin content and biodegradability was demonstrated (Kwon *et al.* 2022). This study focused on soil and marine environments, and a wide variety of lignin-containing paper pulps and products were included. The implications of lignin content and reduced mineralization to CO_2 for standardized requirements of biodegradability are also discussed.

EXPERIMENTAL

Samples

Various pulps, one paper sample, and microfibrillated cellulose with different lignin contents were selected for this study (Table 2). Two types of unbleached chemical pulps were evaluated: pulp samples were taken before drying (dry weight 12.2%) and after drying (dry weight 91.5%). Microcrystalline cellulose MC (Sigma Aldrich, CAS 9004-24-6) and kraft lignin (Sigma Aldrich, CAS 8068-05-1), which are commercial by-products from the kraft pulping process, were used as reference compounds. The dry weight was measured by drying the sample at 105 °C overnight. The volatile solids or organic matter were determined by heating the dried sample at 550 °C for at least 4 h. Carbon content of the materials and nitrogen and carbon content of the soil were evaluated using an elemental analyzer Flash 2000 EA CHNS-O (Thermo Fisher Scientific). Lignin contents in the samples were determined after a two-stage acid hydrolysis as described by Yue *et al.* (2020). Klason lignin content, *i.e.*, the insoluble residue from the acid hydrolysis, was determined gravimetrically. Acid soluble lignin was determined from the hydrolysate based on UV at 215 and 280 nm using equation described by Goldschmid (1971). Total lignin content shown in Table 2 is the sum of gravimetric and acid-soluble lignin.

Paper pulps and bleached paper were milled cryogenically with liquid nitrogen and sieved for a particle size below 500 µm. Unbleached chemical pulp with a dry weight of

12.2% and softwood sawdust were dried at 60 °C before milling. Microfibrillated cellulose was added directly to the testing bottles without any pretreatment.

Table 2. Samples and their Characteristics

Sample	Sample Form	Dry Weight (%)	Organic Matter (% dw)	Total Lignin (% dw)	Carbon Content (% dw)
Microcrystalline cellulose, Sigma Aldrich	Powder, particle size 20µm	98	80.6	0.4	42.7
Kraft lignin, Sigma Aldrich	Powder	97.1	96.7	93.9	63.5
Sawdust, softwood	Particles	50.2	99.7	30.6	48.9
Unbleached chemical pulp (softwood), dry	Dry pulp	91.5	78.3	9.0	37.1
Unbleached chemical pulp (softwood), wet	Wet pulp	12.2	99.3	12.6	42.5
Bleached chemical pulp (softwood), dry	Dry pulp	92.7	99.9	1.5	42.5
Bleached paper	Paper	95.7	71.6	2.0	34.7
Bleached kraft pulp (softwood)	Dry pulp	91.7	99.8	0.8	43.0
Mixture of CTMP and chemical pulp	Dry pulp	98.1	98.7	21.1	45.8
Microfibrillated cellulose	Paste	11.0	99.9	0.9	43.4

CTMP = chemi-thermomechanical pulp; dw = dry weight

Soil Biodegradation Test

The biodegradability of the samples was evaluated using ISO 17556 (2012), based on CO_2 evolution as described by Vikman *et al.* (2022). Instead of passing carbon-dioxide-free air over the soil and then determining the CO_2 content of the air, as described in the standard, CO_2 concentration was analysed from the gas phase without passing air over the soil. Soil was collected from an agricultural field of Helsinki University situated in Helsinki, Finland. The pH of the soil was 6.4, organic matter content was 9% (dw), and the C/N ratio was 19. Soil was sieved to a 2 mm particle size, and the final soil moisture was adjusted to approximately 30% of the water holding capacity. For each replicate, approximately 500 mg of sample (dry weight) was mixed with 75 g of soil (dry weight) in a 1000 mL glass bottle sealed with a septum and screw cap with a hole. Three replicated samples were prepared for the tested materials, for blank and for microcrystalline cellulose (Aldrich, 20 µm), which was used as a reference compound. Bottles were incubated in sealed vessels in aerobic conditions at +22 °C in the dark. The duration of the test was approximately 14 months (419 days). According to ISO 17556, the test is continued until CO_2 evolution is attained and the test can be continued for up to two years.

CO_2 concentrations in the gas phase of the bottles were measured at regular intervals by immersing a needle through a septum of the bottles and directly measuring the

CO_2 concentration with a Servoflex MiniFoodPack 5200 infrared analyser (Servomex, UK). Bottles were aerated to remove excess CO_2, closed with septum and screw cap, and CO_2 concentrations were measured for a second time. The net CO_2 production evolved from the test materials was calculated by subtracting the average amount of CO_2 produced in the background samples (containing only soil, no sample) from the amount of CO_2 produced in the bottles containing the sample.

The biodegradation percentages were calculated from the ratio between the net CO_2 production and the theoretical CO_2 production calculated based on elemental carbon content of the tested material.

$$\text{Biodegradability (\%)} = CO_2 \text{ (produced)} / CO_2 \text{ (theoretical)} \times 100\% \quad (1)$$

$$\text{Relative biodegradability (\%)} = \frac{\text{Biodegradability of sample (\%)}}{\text{Biodegradability of MC (\%)}} \times 100\% \quad (2)$$

where MC is microcrystalline cellulose.

Marine Biodegradation Test

The biodegradability of the samples was evaluated using ISO 23977-1 (2021), which is based on CO_2 evolution. Sea water for the test was collected from the coast of the Baltic Sea, in Espoo in June, 2022. Collected seawater was filtered using a filter paper (Whatman 4) to remove coarse particles, 0.1 g/L of KH_2PO_4 and 0.05 g/L of NH_4Cl were added, and the seawater was preincubated at room temperature for 6 days before starting the test. The pH of the sea water was 6.9.

The test compound, as the sole source of carbon and energy, was added to 50 mL of sea water in 120 mL glass bottles sealed with rubber septum and aluminium cap. The amount of samples was 140 to 160 mg/L. Bottles were incubated in sealed bottles in aerobic conditions at 22°C in the dark. Three replicate bottles were sacrificed for analysis 7, 14, 28, 80, 120 days of incubation. Concentrated orthophosphoric acid was injected to each test vessel taken, bottles were stirred for 1 to 3 h, and the temperature was allowed to equilibrate to room temperature. CO_2 concentration 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 CO_2 concentration by a Servoflex MiniFoodPack 5200 infrared analyser (Servomex, UK). The biodegradation percentages were calculated similarly as in soil biodegradation test. The biodegradability of microfibrillated cellulose was not assessed in the marine environment.

RESULTS AND DISCUSSION

Biodegradation in Soil and Marine Environment

In this study, biodegradability was evaluated using ISO methods, which are based solely on the concept of mineralization to CO_2. Other factors, such as carbon transformations to microbial biomass or humic compounds, were not considered in the calculation of biodegradability. Commercial kraft lignin exhibited low mineralization to CO_2 in both soil and marine environments (Figs. 1 and 2; Table 3). After 419 days, mineralization to CO_2 was only 7 % in soil and after 120 days, and it was 13 % in marine conditions. Softwood sawdust, a potential raw material for pulp production, showed 33% mineralization in soil and 15% in a marine environment. A mixture of CTMP and chemical pulp with 21.1% lignin mineralized 52% in soil and only 28% in marine conditions.

Bleaching increased the mineralization to CO_2 of chemical paper pulps (bleached kraft pulp, bleached paper pulp), with degrees of mineralization ranging from 74% to 78% in soil and 73% to 81% in marine conditions. Without bleaching, mineralization of chemical pulp varied between 61 and 78%.

For comparison, microfibrillated cellulose was included in the test conducted in soil conditions. It biodegraded better than microcrystalline cellulose and reached 86% mineralization to CO_2 in 419 days (Figs. 1 and 2; Table 2). Similar results were obtained in industrial composting conditions, where microfibrillated cellulose manufactured from bleached birch kraft pulp by Masuko grinder biodegraded better than microcrystalline cellulose (Vikman *et al*. 2015). Microfibrillated wood cellulose have a width of 10 to several hundred nanometers, which is considerably smaller than native cellulose fibers with a width of 20 to 50 µm (Osong *et al.* 2016). This smaller diameter of fibers and larger surface area make cellulose more accessible to microbial attack.

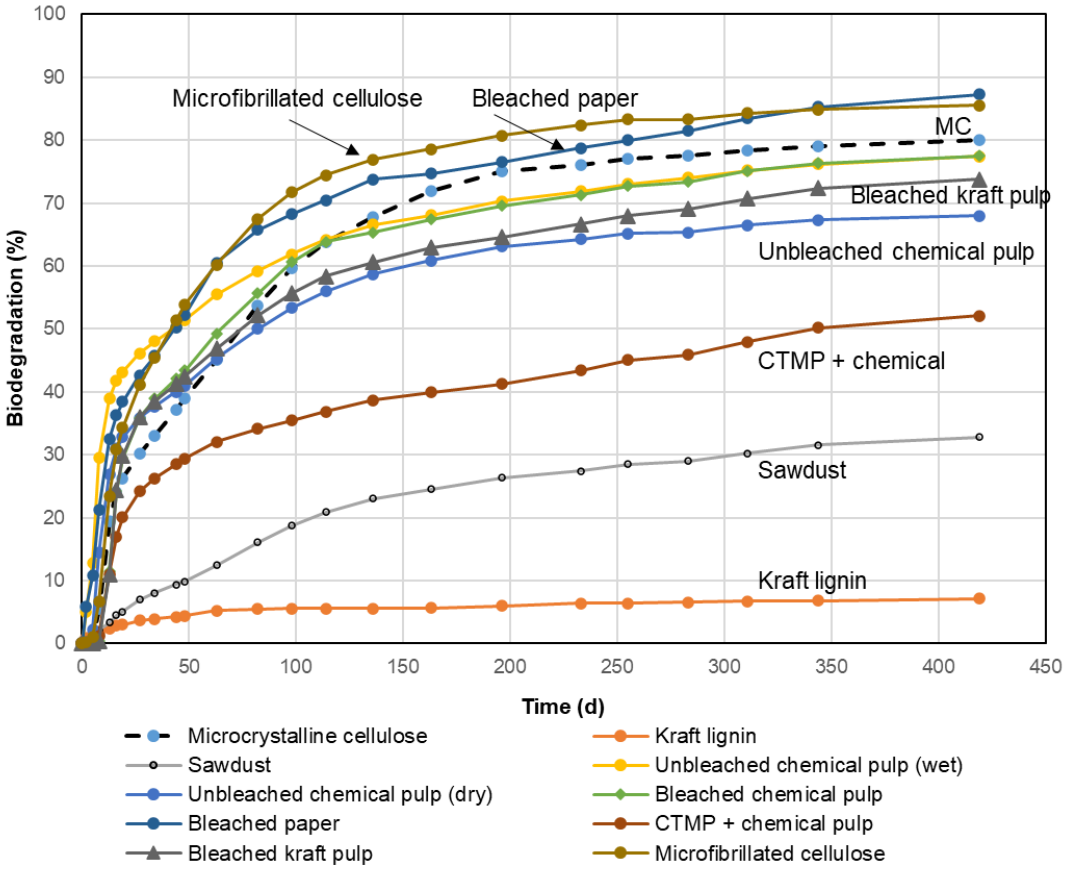

Fig. 1. Biodegradation (i.e. mineralization to CO_2) of saw dust, kraft lignin, microfibrillated cellulose and paper pulps in soil

Reference compounds are used to ensure the activity of the microbial inoculum and validate the results obtained in biodegradability tests. In both ISO 17556 and ISO 23977-1, MC can be used as biodegradable reference compound. In the soil environment, the mineralization of MC was 72% after 6 months, confirming the validity of the test.

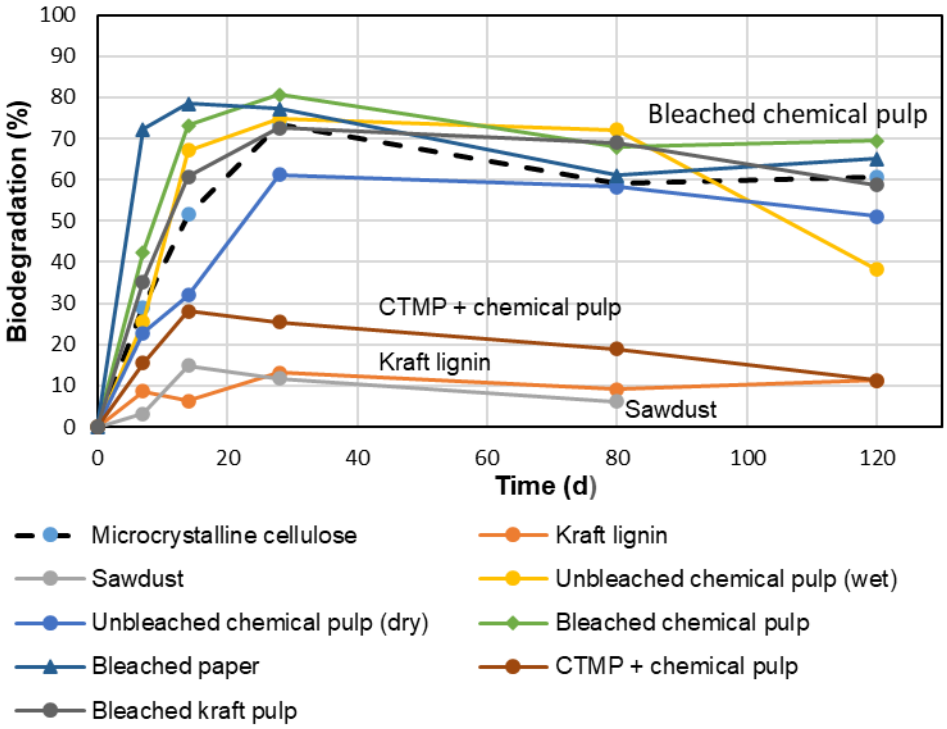

Fig. 2. Biodegradation (i.e. mineralization to CO_2) of saw dust, kraft lignin and paper pulps in marine conditions. A decreasing trend may be attributed to nutrient limitations as the test progresses.

According to the ISO 17556, MC should exhibit over 60% mineralization to CO_2 (defined as biodegradability) after the plateau is reached or at the end of the test. The test period in ISO 17556 is typically six months, but it can be extended up to two years. Additionally, marine test ISO 23977-1, which can be considered as valid as mineralization to CO_2 of MC, exceeded 60% during the test period.

Environmental conditions are specified by the ISO standards, and the selected conditions can significantly impact the biodegradability results. In the soil biodegradability test, for example, parameters such as the carbon-to-nitrogen ratio, water content, and availability of oxygen are adjusted according to the standard. Similarly, in the marine test, nutrients are added to the seawater to enhance microbial activity. It is also essential to recognize that the marine test is specifically designed for pelagic seawater from the coastal area, which could differ significantly from conditions in sea sediment. Furthermore, seawater was obtained from the Baltic Sea, where salt concentration is lower compared to the Mediterranean or the Atlantic Ocean.

Lignin Content and Biodegradability

In addition to ensuring the activity of the microbial inoculum and the performance of the test, reference compounds can be used to calculate the relative biodegradability. Absolute and relative biodegradability of paper pulps in soil and marine environments, using MC as a reference compound, are summarized in Table 3. Relative biodegradability facilitates the comparison of results obtained from biodegradability tests in different environments, and from different test runs. Similar to absolute biodegradability, the calculation of relative biodegradability is based solely on mineralization to CO_2, and possible transformation of carbon into other components is not considered.

Table 3. Biodegradation and Relative Biodegradation of Paper Pulps in Soil and Marine Environments

Sample	Total Lignin (% of dm)	Biodegradation Measured as Mineralization to CO_2 (%)		Biodegradation in Relation to MC (%)	
		Soil 419 days	Marine 120 days	Soil	Marine
Microcrystalline cellulose, MC	-	80.1±2.5	73.4±2.8	-	-
Kraft lignin	93.9	7.1±1.5	13.1±1.1	9	18
Sawdust	30.6	32.8±5.6	14.8±1.0	41	20
Unbleached chemical pulp, wet	9.0	77.4±5.4	74.7±6.2	**97**	**102**
Unbleached chemical pulp, dry	12.6	68.0±0.9	61.2±0.4	**85**	83
Bleached chemical pulp	1.5	77.5±5.6	80.6±2.5	**97**	**110**
Bleached paper	2.0	87.3±4.4	78.5±4.3	**109**	**107**
Bleached kraft pulp	0.8	73.8±11.0	72.7±6.5	**92**	**99**
CTMP and chemical pulp	21.1	52.1±8.2	28.1±0.9	65	38
Microfibrillated cellulose	0.9	85.5±6.2	nd	**107**	nd

Values are means ±SD, nd = not determined; MC= microcrystalline cellulose. dm = dry matter.
Note: Calculations are based solely on mineralization to CO_2. In the marine test, maximum values were reported

A clear inverse correlation between the lignin content and mineralization to CO_2 of paper pulps was obtained both in soil and marine conditions (see Fig. 3). It can be observed that correlation curves had different slopes in soil compared to the marine conditions. When lignin content exceeded approximately 10% (dry weight), mineralization to CO_2 began to significantly decrease. Especially in the soil environment, low values for relative biodegradation can be explained by the incorporation of a portion of the carbon in lignin into humic compounds (Martin and Haider 1979; Martin *et al.* 1980). In addition, as demonstrated by Kwon *et al.* (2021), the lignin within fibers can reduce the biodegradability of the other components of the lignocellulosic fibers.

With the exception of commercial kraft lignin, pulps and sawdust mineralized to a higher extent to CO_2 in a soil environment. As commercial kraft lignin showed higher mineralization in marine conditions, saw dust with a lignin content of 31% and a mixture of chemical and mechanical pulp with a lignin content of 21% mineralized better in soil. Although lignin-degrading microbes have been isolated both soil and marine environment, it can be expected that agricultural soil is a more optimal environment for biodegradation of lignin-containing materials. It should be taken into account that in standardized biodegradation testing methods (as used in this study), conditions are more optimal for microorganisms than in nature. For example, in the marine test ISO 23977-1, both nitrogen and phosphorus are added to the testing system to enhance microbial activity. In the soil test ISO 17556, on the other hand, pH and moisture content are set at optimal levels at the beginning of the test.

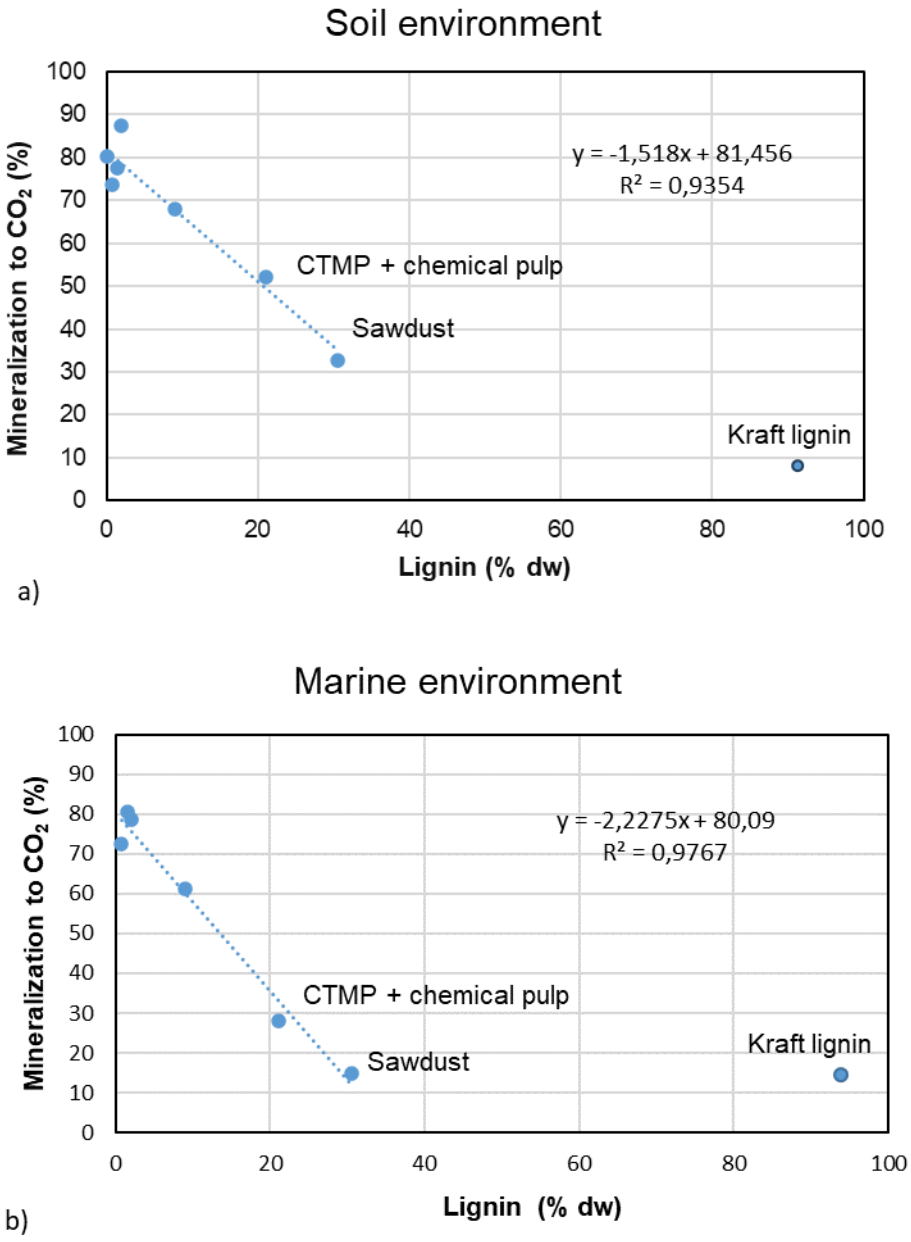

Fig. 3. Correlation between the lignin content and mineralization to CO_2 in soil (a) and marine environment (b). Commercial kraft lignin and microfibrillated cellulose were not considered in the assessment

Assessment of Biodegradability Results

There are no standardized biodegradability requirements especially targeting lignocellulosic materials in soil or marine conditions. To assess the results obtained in biodegradability tests in this study, standards that establish maximum testing times and threshold values for biodegradability in other environmental conditions and applications can be employed (Table 4). A common feature among these standards is that, in order to be considered as biodegradable, 90% of the carbon should be converted to CO_2 either as absolute value or relative to a reference material, within a specified test period and under conditions outlined in the standard. The most frequently used reference material for

calculating relative biodegradability is microcrystalline cellulose. According to the requirements set for biodegradability of mulching films in soil (EN 17033:2018), the 90 % threshold value should be achieved within a test period no longer than 24 months. In a marine environment (EN ISO 22403:2021), the threshold value for biodegradability remains the same, with the maximum testing time of 24 months. These standardized requirements are important, because they are utilized when products apply certification for biodegradability from organizations such as TÜV Austria and the German certifier DIN CERTCO.

Table 4. Standards Setting Requirements for Biodegradability *

Standard	Environment	Application	Reference Compound	Exception for Chemically Unmodified Constituents of Natural Origin
EN 13432: 2000 ISO 18606: 2013	Industrial compost, anaerobic digestion	Packaging and packaging materials	Microcrystalline cellulose	yes
EN 14995: 2006 ISO 17088: 2021	Industrial compost, anaerobic digestion	Plastic material	Microcrystalline cellulose	yes
EN 17427:2022	Home composting installations	Carrier bags of any material	Microcrystalline cellulose	yes
EN 17033:2018	Soil	Mulch film made from thermoplastic material	Well-defined biodegradable polymer (microcrystalline-cellulose powder, ashless cellulose filters or poly(3-hydroxybutyrate)).	no
ISO 22403: 2021	Marine environments	Virgin plastic materials and polymers	Microcrystalline cellulose or cellulose filter paper	yes

* All these standards require that 90 % of the carbon should be converted to CO_2 as absolute value or relative to a reference material within a test period stated in the standard.

In this study, in addition to validating the test performance, microcrystalline cellulose served as the reference compound to calculate relative biodegradability. The biodegradability of bleached chemical pulps, bleached paper, unbleached chemical pulp with a dry weight of 12%, and microfibrillated cellulose exceeded the 90% threshold for relative biodegradability in the soil and marine environment. However, the pulp containing CTMP and with a lignin content of 21% exhibited only 65% relative biodegradability in soil, and 38% in the marine environment. The relative biodegradability of kraft lignin was even lower, 9% in soil and 18% in the marine environment.

Poor values for relative biodegradability of lignin-containing materials demonstrates that microcrystalline cellulose is not the most suitable reference compound for these products. The transformation of carbon to humic compounds during

biodegradation improves soil fertility, simultaneously capturing carbon, and should be considered a positive phenomenon. Quantitatively measuring the carbon transformed into humic substances would provide a better understanding of the carbon balance during the biodegradation of lignin-containing materials. Achieving this would require the use of advanced analytical methods for the destruction of lignin structures and for the detection of formed phenol compounds (Bahri *et al.* 2006). Particularly in soil conditions, distinguishing between lignin structures originally present in the soil and those originating from the sample would be challenging. Another option to study carbon balance would involve the use of labelled compounds, such as synthetic lignin, but their similarity to materials such as sawdust and paper pulps may invite criticism.

A more practical approach for lignin-containing materials could involve the use of more suitable reference materials in standardized testing protocols or consideration of lignin content in the calculations of threshold values for biodegradability. The selection of reference material requires more research because it should be easily available and possess consistent quality properties. If an isolated lignin product is used as a reference material, it should be as representative of the original lignin in wood as possible.

Most of the standards listed in Table 4 include an exception for 'chemically unmodified constituents of natural origin'. This exception is notably absent from EN 17033, likely because the standard is aimed at thermoplastic materials (plastics) used as mulching films. Materials falling into this category are considered biodegradable without testing, but they still have to meet other requirements, such as limits for harmful compounds, disintegration, ecotoxicity listed in the standard. Currently, materials such as wood, wood fibre, cotton fibre, starch, wood pulp, or jute are mentioned as examples of this category. Based on the results obtained in this study, it is crucial to maintain this exception for paper pulps with high lignin contents within standardized requirements for biodegradability.

CONCLUSIONS

1. A noticeable inverse correlation between the mineralization of paper pulps to CO_2 and lignin content during biodegradation was detected both in soil and marine conditions. Similar impact was observed with natural materials containing lignin, such as birch sawdust.
2. The determination of biodegradability in most ISO and EN standards is based on the concept of mineralization to CO_2, and the transformation of carbon into other components is not considered. Therefore, lignin and lignin-containing materials can receive poor values for biodegradability. Possible options to overcome deficiencies in the biodegradability testing include the use of more appropriate reference materials instead of microcrystalline cellulose or consideration of lignin content in the calculations of threshold values for biodegradability.
3. Some ISO and EN standards set requirements for biodegradability, which include an exception for 'chemically unmodified constituents of natural origin'. Materials classified under this category are accepted as being biodegradable without testing. This exception is essential for paper pulps with high lignin contents and should remain in the standardized requirements for biodegradability.

ACKNOWLEDGMENTS

The authors greatly appreciate the financial support of Confederation of European Paper Industries (CEPI) for this work. Tuomo Mäkelä and Niina Torttila are thanked for skilful assistance in biodegradation testing, Jarna Teikari for lignin analysis, and Marita Ikonen for elemental analyses.

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Article submitted: December 21, 2023; Peer review completed: January 21, 2024;
Revised version received and accepted: February 13, 2024; Published: February 28, 2024.
DOI: 10.15376/biores.19.2.2452-2465