Biodegradability of Cellulose Fibers, Films, and Particles: A Review

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Cellulose fibers are an abundant material that is well known for its biodegradability. Various forms of cellulose, such as cotton, paper pulp fibers, and microcrystalline cellulose can be regarded as benchmarks for biodegradability, when comparing other materials. However, as revealed by the literature, broad ranges of time and extent of biodegradation have been reported for cellulose. These large ranges can be attributed not only to environmental factors but also to the presence of lignin, the degree and perfection of crystallinity, the size and density of the physical specimens, and chemical modifications to the cellulose, if any. Studies also have shown differences in biodegradability associated with the selection of test methods. Although cellulose is subject to well-known enzyme-promoted mechanisms of biodegradation, the evolution of plant materials has favored development of some resistance to decay, *i.e.* recalcitrance. Cellulosic materials are clearly less biodegradable than starch. However, they are more biodegradable than various synthetic or bio-based plastics, as well as some cellulose derivatives, which persist in ocean water or soils for very long periods. This review indicates that cellulose biodegradability, while generally rapid and natural, has a rate and extent that depends on a complex and sometimes subtle set of environmental and chemical factors.

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INTRODUCTION

This article reviews published findings related to the relative biodegradability of cellulose fibers, including factors affecting their accessibility to enzymatic attack. Interest in this topic has been spurred by reports of fibers and related materials remaining either after conventional wastewater treatment (López Alvarez *et al.* 2009; Ghasimi *et al.* 2016; Libardi *et al.* 2022), or when cellulosic matter is discharged to other environments as rinsewater after laundering (Ladewig *et al.* 2015; Zambrano *et al.* 2019). In addition, there have been concerns about slow biodegradation of cellulosic fibers that reach ocean environments (Zambrano *et al.* 2020; Nagamine *et al.* 2022; Royer *et al.* 2023). Issues to be reviewed include the rates and extent of cellulose fiber biodegradation under a variety of conditions, including fresh water, aerobic and anaerobic wastewater treatment environments, seawater, soil burial, and composting. Literature is examined to shed light on mechanisms of biodegradation, as well as factors that can promote or inhibit those natural processes. Common examples of cellulose fibers include sanitary tissue fibers, cotton and rayon textile fibers, and pulp fibers present in packaging, as well as in printing and writing papers.

Biodegradability of cellulose fibers can be viewed as a continuum, in which specific materials exposed to defined environmental conditions can be compared to reference materials. Such a perspective is illustrated in Fig. 1. In this article the term environmental will be used broadly, including both natural conditions and those in wastewater treatment facilities, unless specified.

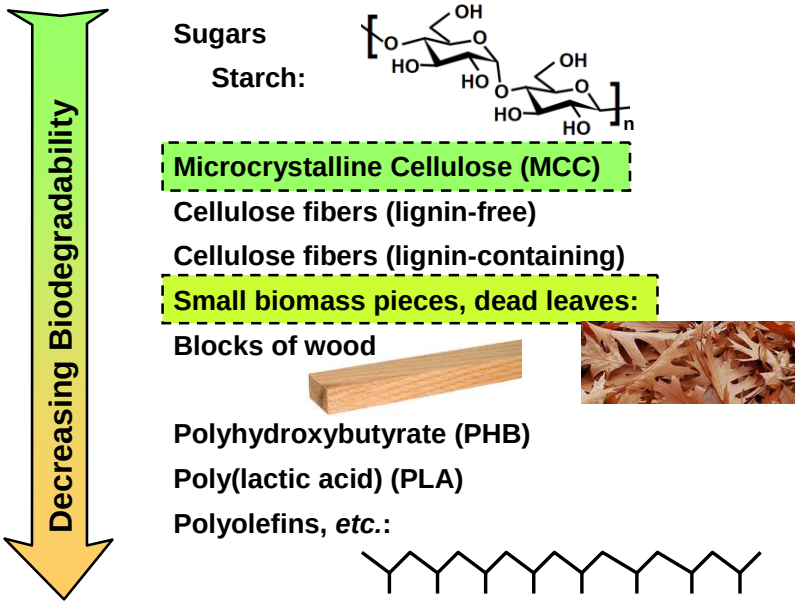

Fig. 1. Relative biodegradability of various other polymers compared with that of some different forms of cellulose and wood

As an example of a reference material, every year in temperate climates one can expect masses of leaves to fall from deciduous trees, such that they not only blanket the ground, but many of them pass into streams and eventually into oceans. The aquatic biodegradation of leaf litter has been studied (Sakamaki and Richardson 2008; Raposeiro *et al.* 2014). It was reported that half a year was sufficient for biological breakdown of

leaves placed in tidal flat environments (Sakamaki and Richardson 2008). Raposeiro *et al.* (2014) reported between about 15% and 95% mass loss of leaves with no added nutrients or bacteria in 28 days in the North Atlantic island of São Miguel in the Azores, depending on the tree species in different fresh-water streams. In addition to such natural reference points, microcrystalline cellulose (MCC) is often used by researchers as a benchmark from which to judge the relative biodegradability of other cellulosic materials in selected environments.

The fact that bioplastics, despite their plant-based origins, are not equally biodegradable was highlighted in recent work by Kwon *et al.* (2024), who compared biodegradation in aerobic water conditions. Pure poly(lactic acid) (PLA) was not degradable under the studied conditions, whereas poly(β-hydroxybutyrate) (PHB) readily degraded. Moreover, incorporation of just 25% of PLA into a mix of the two polymers, followed by melt-bending and extrusion, yielded fibers that showed only 11% degradation. The differences in biodegradability among bioplastics have been shown to be attributable to differences in crystallinity, hydrophobicity, and chemistry (Kwon *et al.* 2023b).

Table 1. Highlights from Studies Considering the Biodegradability of Microcrystalline Cellulose (MCC)

Highlights from Cited Studies	**% Degraded/ test period**	**Citation**
MCC biodegradation was compared in shaken enzyme solutions with that of Douglas fir wood, with optional steam-explosion pretreatment.	70% / 2 days	Esteghlalian *et al.* 2002
MCC biodegradation was compared to that of sugarcane bagasse in enzyme solutions.	53% / 2 days	Wada *et al.* 2010
Factors affecting the biodegradation rate of MCC were studied in enzyme solutions, with discussion that the data can be used as a reference for other cellulosic materials.	40-80% / 2 days	Yu *et al.* 2012b
MCC biodegradation in enzyme solutions was compared with that of bleached softwood fibers and bacterial cellulose.	63% / 2 days	Kafle *et al.* 2015
MCC was found to be more biodegradable than toilet paper and various other cellulosic fiber types under aqueous mesophilic and thermophilic anaerobic conditions of wastewater treatment.	86-91% / (tests stopped when no more methane was generated)	Ghasimi *et al.* 2016
MCC biodegradation in aerated river water was used as a reference for cotton, rayon, and polyester fibers.	84% / 245 days	Zambrano *et al.* 2019
MCC was used as a reference for studying biodegradation of fibers released during the laundering of cellulose and polyester-based textiles.	80% / 35 days (lakewater) 100% / 35 days (activ. sludge) 70% / 35 days (seawater)	Zambrano *et al.* 2020
MCC was used as a biodegradability reference point when comparing three commercial plastics under aerobic composting conditions.	86% / 60 days	Rossetti *et al.* 2021
MCC biodegradation was compared to that of various plastic and cellulosic fibers, including Lyocell.	100% / 30 days (marine) 50% / 30 days (fresh water)	Royer *et al.* 2021

Further work showed that mixtures of the more degradable PHB with less degradable polymers such as PLA and polypropylene gave rise to micro- or nanoplastic particles, which tend to build up in the tissues of marine organisms (Kwon *et al.* 2023a, 2024).

Table 1 provides highlights from studies that have considered the biodegradation of MCC, often in comparison with cellulosic fibers of various types. Other studies have considered various less-biodegradable classes of material, such as bioplastics (Bhagwat *et al.* 2020; Royer *et al.* 2023) and synthetic plastic items, including fibers and fabrics (Cooke 1990; Li *et al.* 2010; Zambrano *et al.* 2020; Kwon *et al.* 2021; Royer *et al.* 2021, 2023).

Another aspect to be considered in this article is the different environments in which biodegradation is important. As shown in Fig. 2, a rough division of categories can be drawn based on relatively dry to water-saturated environments (*e.g.* soils, composting, and landfilling) *vs.* aqueous environments (*e.g.* fresh water, seawater, wastewater treatment). Note that although the figure illustrates the possibility of collecting methane that forms within landfills, such collection is often incomplete or may be absent. Further information about composting (Hubbe *et al.* 2010; Reyes-Torres *et al.* 2018; Ruggero *et al.* 2019; Wu *et al.* 2022) and wastewater treatment technologies (Hubbe *et al.* 2016; Srivastava *et al.* 2022; Wang *et al.* 2022a) has been published.

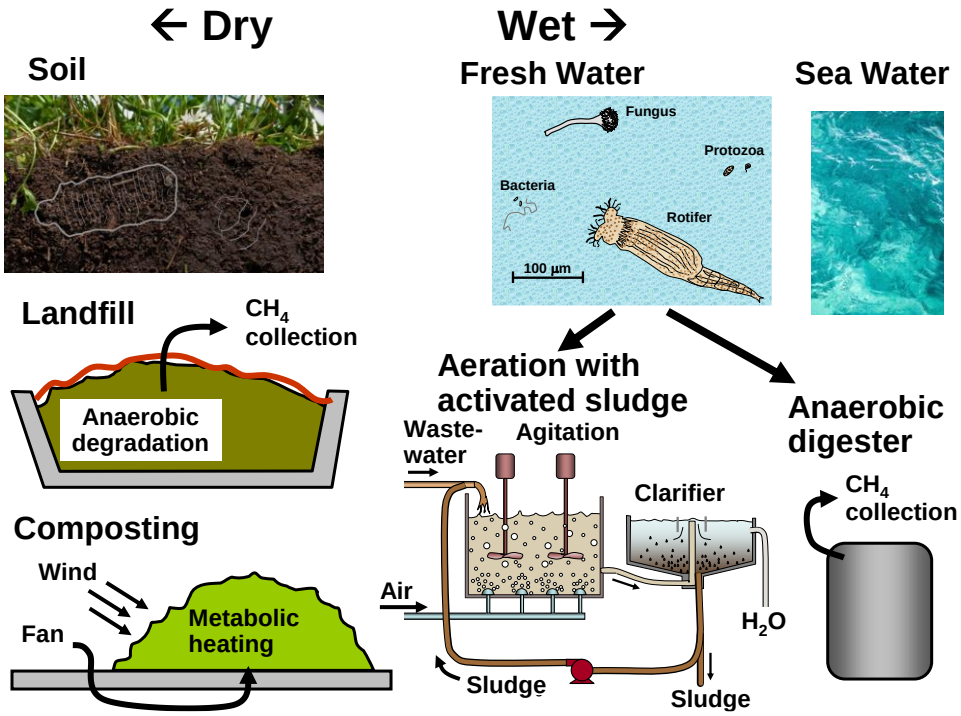

Fig. 2. Seven contrasting environments in which biodegradation of cellulose fibers can be expected to have different rates and controlling factors. The figure is arranged such that the gentler environments, with correspondingly slower biodegradation, are towards the top, whereas more biodegradative environments, often with higher temperatures, appear towards the bottom.

Though broader ranges of cellulosic material are considered in this review, the primary focus will be on lignin-free fiber-based products, such as cotton and bleached kraft fibers, the latter of which is the main component of most flushable sanitary paper products. The distinction can be important, since, as will be described in more detail later, lignin can substantially slow down biodegradation (Reyes-Torres *et al.* 2018; Wu *et al.* 2022).

Synthetic plastic materials generally were not considered in the literature covered in this article. A general finding is that many such plastics show much lower rates of biodegradation, or even no measurable biodegradation under conditions that lead to substantial degradation of MCC and other cellulosic materials. For instance, Zambrano *et al.* (2019) reported about 4% biodegradation of polyester after about 245 days in an aqueous aerated system. Under the matched conditions, the biodegradation of cotton was 76% and MCC was 83%. Royer *et al.* (2021) compared seawater biodegradation based on measurements of fiber diameter. Polyester fibers showed a 5% decrease after 7 months of exposure, whereas lyocell regenerated cellulose fibers showed a 20% decrease after about one month. Li *et al.* (2010) reported about 13% biodegradation of polyester fabric under conditions giving 23% biodegradation of cotton under large-scale compositing conditions (ASTM D 5988-03). Royer *et al.* (2023) reported essentially undetectable levels of marine biodegradation of polylactic acid (PLA), polyethylene terephthalate (PET), and polypropylene (PP), under various marine conditions and times that gave substantial biodegradation of cotton, rayon, lyocell, and modal cellulosic fibers (*e.g.* about 50% to 70% degradation in 7 days and about 80% in 28 days). In a review article, Cooke (1990) draws a distinction between so-called biodegradable synthetic plastics, such as aliphatic polyesters, polyurethanes, some polyamides, polyvinyl alcohol, polyvinyl acetate, polyacrylates, *vs.* non-biodegradable ones, including polyolefins, polystyrene, and aromatic polyesters. Even if a plastic material is prepared from plant materials, as in the case of PLA, one should not automatically assume that it is biodegradable (Royer *et al.* 2023). Thus, Bhaghwat *et al.* (2020) urge testing of each material under the environments of interest, following available standards when possible.

Various plant-based materials, such as starch, chitin, proteins, hemicelluloses, lignin, lipids, and natural rubber, generally fall outside of the primary focus of this article. Attention has been paid to these substances in other reviews, some of which are listed in Table 2. A general rule, which is supported by entries in this table, is that specific enzymes are needed, often in combination, to achieve effective biodegradation of each unique natural polymer.

Table 2. Natural Polymers and Enzymes Associated with their Biological Degradation

Polymer Type - Natural	Associated Enzymes	Selected Citations
Starch	Amylases	De Souza & Magalhaes 2010; Farooq *et al.* 2021
Chitin	Chitinases, proteases, chitin deacetylase; chitosanases, lytic polysaccharide monooxygenases	Younes & Rinaudo 2015; Kaczmarek *et al.* 2019
Proteins	Proteases	Tavano 2013; Kim *et al.* 2014
Cellulose	Endoglucanase, cellobiohydrolase, β-glucosidase	Teeri 1997; Horn *et al.* 2012; Wang *et al.* 2013a
Hemicelluloses	Xylanases (endo & exo), β-xylosidase, α-arabinofuranosidase, esterases	Saha 2003; Girio *et al.* 2010; Houfani *et al.* 2020
Lignin	Laccase, peroxidases	Datta *et al.* 2017; Chio *et al.* 2019; Khan & Ahring 2019
Lipids	Lipases, lipoxygenase	Shah 2005; Reis *et al.* 2009
Natural rubber	Oygenase, lipoxygenase, peroxidase	Rose & Steinbüchel 2005

Scope of the Problem

The term "cellulose fibers," which defines the focus of this article, generally will follow the literal meaning of the words, thus excluding cellulose derivatives such as cellulose acetate. Cellulose derivatives will be considered only briefly, to show how they contrast with either natural cellulosic fibers or regenerated cellulose fibers, such as rayon. A reason to focus on biodegradability of cellulose fibers at this time is that large amounts are routinely discharged to natural environments.

As illustrated in Fig. 3, two of the most prominent sources are from the flushing of toilets and the discharge or rinse water from laundering. An average US citizen, in 2018, used 12.7 kg of toilet paper, whereas lesser *per capita* amounts were used elsewhere (Armstrong 2018). Table 3 summarizes reported information of the total amounts of cellulosic fibers discharged to wastewater treatment systems, with emphasis on toilet paper as a major source. As reported by Gupta *et al.* (2018), the relative amounts of cellulose in various wastewater and sludge specimens can be determined with high accuracy.

Table 3. Reports of Cellulosic Fibers, Mainly Toilet Paper, Routinely Discharged to Wastewater Treatment Facilities or the Natural Waterways

Highlights from Cited Studies	Citation
To test the biodegradability of "very large quantities" of cellulose fibers discharged to wastewater, the authors used a bag method, allowing for long periods of degradation. Cotton strings required over 70 days to fully biodegrade in water.	Edberg & Hofsten 1975
Amounts of cellulose present in municipal wastewater, mainly attributable to toilet paper, was estimated to be about 400 thousand tons per year in Japan. Sedimentation was suggested as a way to separate and recover the fibers.	Honda *et al.* 2000, 2002
Between 12% and 27% of incoming solids to a wastewater treatment plant consisted of cellulose.	López Alvarez *et al.* 2009
Between 38% and 43% of incoming cellulose fiber material in a wastewater treatment plant still remained after the typical aerobic biodegradation process, depending on the thermal conditions.	Ghasimi *et al.* 2016
Toilet paper was found to be the main source of chemical oxygen demand in sewage.	Chen *et al.* 2017
Cellulose constituted about 31% and 33% of the total suspended solids in wastewater treatment facilities in the Netherlands and Canada.	Ahmed *et al.* 2019
Cellulose represents about 25% of the chemical oxygen demand of a typical municipal wastewater treatment system.	Khan *et al.* 2022
The content of cellulosic material in municipal wastewater was about 21 to 28% by mass, depending on the measurement method.	Libardi *et al.* 2022

Likewise, Table 4 highlights studies that have helped to quantify amounts of textile cellulose fibers discharged to wastewater. Two factors that tend to promote detachment of fine particles, including some of the fibers, in the course of laundering are the agitation and the usage of detergents (Zambrano *et al.* 2019). As described in a recent review article (Hubbe *et al.* 2022), laundry detergents are designed to promote separation between fibers such as cotton and other attached solids.

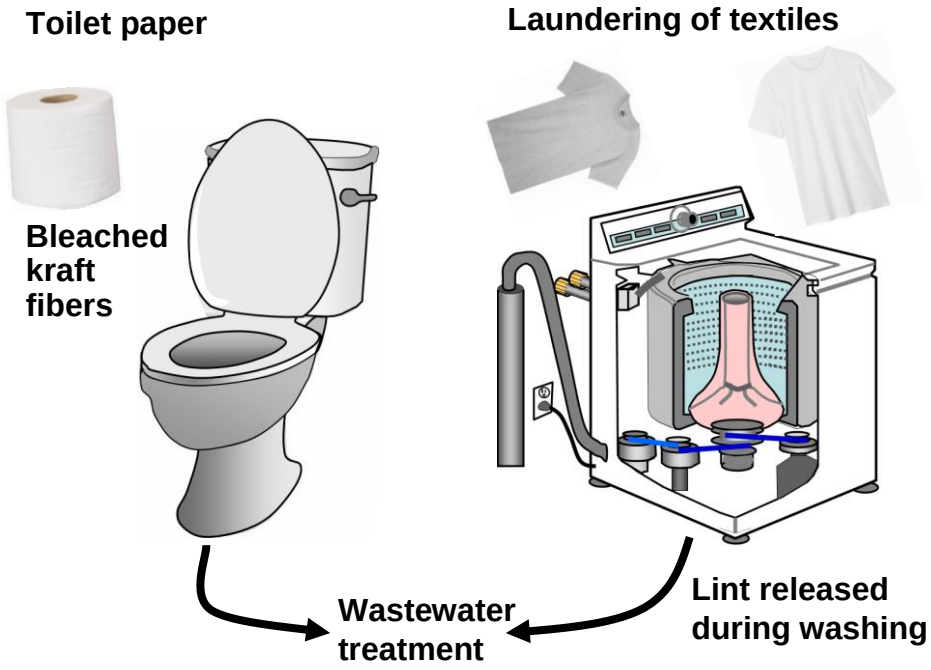

Fig. 3. Illustrate of two major sources of cellulosic fibers in municipal wastewaters. Part of figure (washing machine) previously published as an original drawing by the author (Hubbe *et al.* 2022)

Table 4. Reports of Cellulosic Fibers Discharged to Wastewater Due to the Laundering of Textile Items

Highlights from Cited Studies	Citation
Reported that washing of a single garment can release over 1900 fibers.	Ladewig *et al.* 2015
An average of 0.3% of the mass of garments was discharged in rinsewater from laundering.	Hartline *et al.* 2016
A cumulative amount of *ca.* 0.33% of the mass of cotton garments was released during five sequential washing cycles. The amount released per cycle decreased and then stabilized at less than half the amount of the first cycle.	Sillanpää & Sainio 2017
Cotton clothing released 0.02 to 0.44% of the mass of the garment during accelerated washing.	Zambrano *et al.* 2019
>80% of the microfibers released from the washing of 50/50 polyester/cotton blended fabrics are cotton.	Haap *et al.* 2019; De Falco *et al.* 2020
The greatest proportion of shed cotton fibers were those below 0.2 mm in length.	Frost *et al.* 2020
Commercial nonwoven wipes and meltblown textiles were found to release 1 to 65 mg of microfiber material per gram tested in a standardized laundering test. They released about 4 mg/g microfiber material in a dry shaking test.	Kwon *et al.* 2022
Textile microfibers (*i.e.* fibers small enough to be released during laundering) have been detected in waterways. Published findings are summarized.	Smith *et al.* 2024

Recent research indicates the pervasive presence of both synthetic and natural microfibers in natural environments. Suaria *et al.* (2020) discovered fibers in 99.7% of samples collected across six oceanic basins, with the majority being dyed. Polymers

identified were typical of textiles, including 8.2% synthetic, 79.5% cellulosic, and 12.3% of animal origin. Athey *et al.* (2020) reported that microfibers constitute 87 to 90% of anthropogenic particles in lake sediments, with 41% identified as modified cellulose. Indigo-dyed cotton, common in denim jeans, accounted for 12 to 23% of microfibers analyzed. Similarly, Miller *et al.* (2017) estimated that 50% of fibers collected from the Hudson River, USA, were non-plastic, with cotton fibers identified *via* FTIR. Challenges associated with these studies include the fact that the amounts of various fibers initially entering the water were not known.

As will be discussed in this review, there are several feasible paths by which cellulosic fibers may reach natural environments, including waterways and eventually the sea, though the relative amounts are difficult to quantify. Wastewater treatment operations can be expected to partly biodegrade cellulosic fibers, and most of the rest will end up in sludge, which is often landfilled, composted, or applied to land as a soil amendment, as will be discussed later. However, one can expect a wide variation in the operational efficiency of wastewater treatment, or even its absence, in different regions and situations, considering different parts of the world, seagoing ships, and overflow of combined wastewater and stormwater systems during high volume events. In addition, some cellulosic fibers may come from careless littering, from wind-blown lint, and illegal dumping, all of which will be hard to quantify.

In addition, uncertainties persist regarding the origin of these cellulosic fibers. Current high throughput spectroscopic techniques face challenges in distinguishing between wood-based, cotton, and regenerated cellulose fibers. It is worth noting that these studies often lack morphological evaluations of the cellulosic fibers to confirm their origin, highlighting the need for comprehensive analyses to better understand their sources and pathways into natural environments. Issues related to contamination can interfere with many kinds of identification methods. For instance, some investigators may be assuming that any dyed fiber must be cotton; however, dyes and fluorescent whitening agents may be present in wood pulp fibers.

Circular Economy Issues

While the main focus of this review article is on biodegradability, it is important not to lose sight of an emerging imperative for industrial processes to move in the direction of circularity (Corona *et al.* 2019). In other words, rather than seeking the best ways to "throw away" materials after their single or multiple uses, society needs to find ways to employ such materials as building blocks for making other valued items. For instance, there is increased interest in the reuse of textile fibers at the end of a garment's or bedsheet's useful life (Jia *et al.* 2020). There also has been consideration of recovering used fibers from toilet paper and their beneficial use for other purposes (Honda *et al.* 2002; Ruiken *et al.* 2013; Ghasimi *et al.* 2016; Ahmed *et al.* 2019; Cipolletta *et al.* 2019; Li *et al.* 2019, 2020; Liu *et al.* 2022). Such issues will be considered further later in this article.

CELLULOSE BIODEGRADATION BASICS

In a broad sense, biodegradation is the natural process by which cellulosic materials, including cotton and paper pulp fibers, are decomposed in either natural environments or during wastewater treatment and composting. At the very end of biodegradation, the main product will be carbon dioxide. However, as discussed in this

section, other final products such as methane can result if the materials are subjected to anaerobic conditions. Fiber materials may accumulate in various environments in cases where biodegradation is sufficiently slow. As noted earlier, the fibers may enter various environments by such means as industrial discharges (*e.g.* insufficient wastewater treatment), flushing (*e.g.* insufficient treatment of municipal wastewater), and careless discharges, including littering.

Enzymes, which are complex protein structures, are the main catalysts by which cellulosic materials break down in natural environments and in wastewater treatment plants. These catalysts are synthesized by various organisms, mainly bacteria and fungi (Bhat and Bhat 1997; Esteghlalian *et al.* 2002; dos Santos *et al.* 2023). For degradation of cellulose fibers, the most important category is the cellulases, which will be considered next. Other enzymes can be important when the fibers contain hemicellulose, lignin, or possibly additional functional groups as a result of some kind of chemical derivatization or sizing treatment of the fibers. In addition, many of the wood-derived fibers present in municipal solid waste will be highly lignified and rich in hemicellulose.

In nature, and in most wastewater treatment operations, the enzymes are supplied directly by the bacteria and fungi that happen to be present. Because cellulosic material can be regarded as food for such organisms, the populations of bacteria and fungi may proliferate, depending on the amounts of such resources that are present. For some industrial purposes, and also as a means of studying the underlying mechanisms, it is also possible to isolate and use specific enzymes, such as the cellulase enzymes that catalyze the breakdown of cellulose.

Teamwork is an important theme to bear in mind when aiming for highly effective biodegradation, either in a wastewater treatment plant or in a natural environment. As will be shown, different classes of cellulases work together in a cooperative manner to bring about suitably rapid and substantial biodegradation of the cellulose (Wang *et al.* 2013a). Likewise, biodegradation of the hemicellulose and lignin each require multiple enzyme types working together to achieve efficient and effective results. In many cases, an optimized community of bacterial and possibly fungal organisms will have become acclimated to the cellulosic material present, wherein different microbial members of the community are specialized in the production of different enzymes (Peng *et al.* 2016; Lillington *et al.* 2020). Libardi *et al.* (2022) proposed the optimization of a cellulase mixture, using municipal wastewater as a medium, for effective biodegradation. Though such a strategy is bound to be effective, the usual practice is just to let nature take its course in the competition and proliferation of microorganisms to achieve a suitable overall effect with less direct involvement of technologists or treatment plant operators.

In very general terms, it is well known that the presence of lignin tends to block access of cellulase enzymes to the fibers surfaces and that enzymatic degradation can be accelerated by increasing the accessible surface area of the cellulosic materials. However, as noted by Esteghlalian *et al.* (2002), it is still not possible to make predictions of enzymatic hydrolysis rates based on information about lignin levels and the capacity of the surfaces to adsorb enzymes.

Cellulases

Endo attack

Figure 4 provides an overall scheme of cellulose biodegradation, consistent with current literature (Teeri 1997; Horn *et al.* 2012). In this figure, sets of parallel, straight black lines represent crystalline cellulose domains, whereas non-parallel parts with curves

in the lines represent non-crystalline nano-scale regions. Little circles with light blue filling represent non-reducing ends of cellulose chains, whereas back-filled circles represent reducing ends. Short, barbell-like features with a glycosidic bond connecting a non-reducing end and a reducing end symbol represent cellobiose. As shown, the endoglucanases (shown as bright green features) have the job of random cleavage of cellulose chains in the non-crystalline zones (Wang *et al.* 2013b). Such cleavage involves hydrolysis, which entails the addition of a water molecule. Splitting of the glycosidic bond with a water molecule results in formation of an -OH group on each side of the broken bond. Thus, they are especially effective at reducing the chain lengths within cellulosic materials. However, since typical cellulosic materials such as cotton and wood pulps have quite high levels of crystallinity, *e.g.* 45 to 87% (Salem *et al.* 2023), one can expect there to be a plateau molecular mass remaining, even after extensive endoglucanase action (Chang *et al.* 2021). That is because the portions of cellulose chains that lie within the crystalline domains generally will not be susceptible to attack by endogluconase, which is represented by the bright green features in Fig. 4.

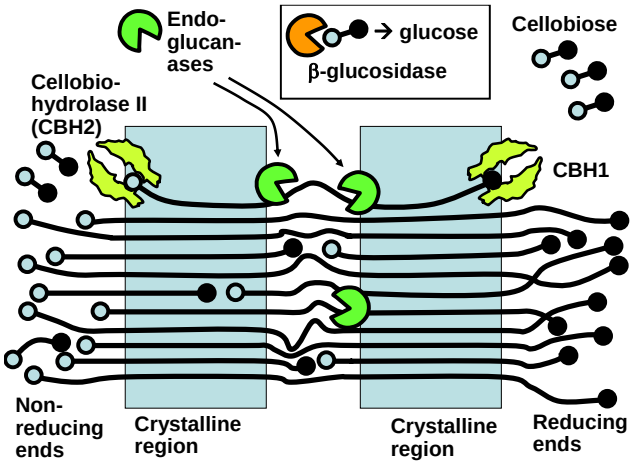

Fig. 4. Schematic diagram showing the roles of three classes of cellulase enzyme relative to the biodegradation of cellulose (adapted from Teeri 1997)

Exo attack

The exoglucanases (shown as CBH2 and CBH1 in Fig. 4) have the function of un-zipping and cleaving off small pieces, mainly cellobiose, starting from the cellulose chain ends, mainly at the surfaces of crystals. They can be advancing from either reducing or non-reducing ends of the chains; thus, two different subclasses of such enzyme are needed (Teeri 1997; Horn *et al.* 2012; Wang *et al.* 2013b). Evidence to support the pictorial view shown in Fig. 4 was obtained by Lee *et al.* (2000). They showed atomic force images of "tracks" left by cellobiohydrolase enzymes in the course of traversing the surfaces of cellulose fibers. By contrast, endoglucanase had the effect of smoothing the surfaces and peeling away the loose debris.

Oxidative cleavage-type cellulases

In addition to the well-known endo- and exoglucanase categories, as just described, a specialized type of cellulase has been reported to have the ability to hydrolyze intermediate points within the exposed crystalline regions of cellulose, thus creating new reducing and non-reducing ends of chains that can then be attacked by exoglucanases (Horn

et al. 2012; Wang *et al.* 2013b). A key difference is that these oxidative endoglucanases do not need to extract the cellulose chains from their associated crystal phases to do their work. The cited work does not give evidence as to whether or not such enzymes play a role when biodegradation occurs in natural environments.

Solution-phase

Cellulase enzyme systems appear to have evolved in such a way as to be inhibited by the presence of their reaction byproducts in solution, *e.g.* cellobiose (Zhao *et al.* 2004). Such a strategy makes sense in terms of the survival of specific cellulase-producing microorganisms. The produced cellobiose presumably can be used as food for the microorganism itself, after the final hydrolysis to glucose. Thus, the species may benefit by a mechanism that slows down the usage of the remnant of solid cellulose in the mixture. The breakdown of the small fragments, such as cellobiose, is catalyzed by another main category of cellulases, the β-glucosidases (Teeri 1997), which are represented in Fig. 4 as a bright orange item. The tunnel-like features in these enzymes require the cellobiose or other small cellulose oligomers to diffuse into the tunnel, whereby the glycosidic bonds can be cleaved. Because this class of enzymes does not break cellulose chains, it has been found useful in some modifications of textile fibers (Teeri 1997). This issue merits further research attention, since textile cellulose fibers that had been treated in this way might be more susceptible to biodegradation by other cellulase enzymes when they enter the environment.

Fragmentation effects

In addition to the hydrolytic effects of cellulases, it has been proposed that certain of them play a role in fragmenting the cellulosic material (Saqib and Whitney 2006). This type of effect is illustrated schematically in Fig. 5. The large dark green arrow, pointing in the leftward direction of the figure, represents this type of enzymatic action, which does not need to involve cleavage of bonds. The cited authors proposed that such ability can explain some of the differences in biodegradative ability of different cellulases, especially when attacking different kinds of cellulosic materials. Such observations are consistent with the findings of Wang *et al.* (2012), who observed general swelling of cellulose material in response to cellulase action. Presumably, an enzyme acting in the manner depicted in Fig. 5 could, at least for a time, increase the thickness of a cellulose particle in the course of bringing about fragmentation.

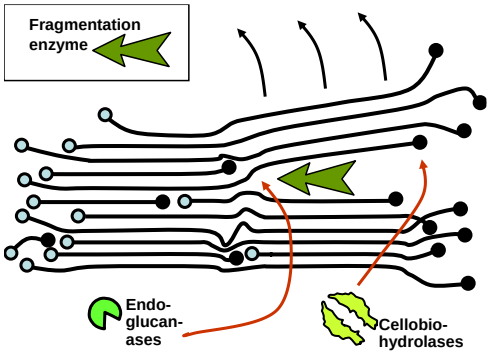

Fig. 5. Schematic diagram suggesting a proposed fragmentation-inducing effect of certain enzymes, which would be expected to help open up lignocellulosic structures at the nano-scale, thus promoting hydrolysis reaction by the same of other enzymes

Biodegradation of different parts of the cellulose

Having considered the different cellulase components separately, it next makes sense to envision how the different types of enzymes tend to focus their activity on different parts of a cellulose particle or fiber. General attack on the amorphous regions (not within crystalline zones) of cellulose in the fibers is brought about by endoglucanases (Hosseini and Shah 2011), which break glycosidic bonds at random intermediate points in the chains. The next category is the exoglucanases (*i.e.* the cellobiohydrolases), which begin their degradative work at the ends of cellulose macromolecular chains, mainly focusing on the crystalline zones (Wang *et al.* 2013b). It appears that such work is direction-dependent; some of the exoglucanases have the ability to start at the reducing ends of cellulose chains, whereas others start at the non-reducing ends. As these enzymes do their work, they break off small groups, usually as cellobiose (Teeri 1997; Medve *et al.* 1998; Homma *et al.* 2013b) and likely also some cellotriose (Medve *et al.* 1998).

Depending on the goals of the treatment, different recipes of cellulase enzyme can be selected. Esteghlalian *et al.* (2002) proposed that when one's purpose is to modify the fiber surfaces for specialized applications, then treatment with single types of cellulase, *e.g.* endoglucanase, might be advantageous. However, for purposes of general and efficient biodegradation of the whole material, a complete "multicomponent" cellulase combination is preferred. In addition, higher enzyme concentrations and times of treatment are recommended to achieve complete breakdown. For polishing (depilling or aging) of textile fabrics, a multicomponent cellulase is also recommended, but with a relatively low dosage and an optimized exposure time (Esteghlalian *et al.* 2002).

Enzyme immobilization

It has been widely reported that the progress of a cellulase macromolecule, relative to its work of breaking down cellulose, can be greatly slowed down if it becomes immobilized at surfaces (Huang *et al.* 2022a; Zhao *et al.* 2023). The phenomenon is widely referred to as nonproductive binding. In particular, it has been shown that such binding onto lignin surfaces can impede the action of those enzymes (Berlin *et al.* 2005). The phenomenon is illustrated schematically in Fig. 6.

The black feature in the figure represents lignin, and the several cellulase items resting on the lignin are envisioned as being attached with enough energy such that they spend much of their time immobilized on those surfaces. In such cases, the binding has been attributed to non-specific adsorption that is favored by the more hydrophobic nature of lignin (Huang *et al.* 2022b). Evidence in support of the binding mechanism is provided by studies in which increased activity of cellulase was achieved by the addition of surfactant (Lin *et al.* 2015). Presumably, the surfactant can enable the release of cellulase from lignin and thereby free it to do its work on nearby cellulose structures.

A further possible strategy to promote more effective cellulose breakdown has been to select cellulase strains having lower affinity for lignin surfaces (Berlin *et al.* 2005). Yet another approach has been to apply a sulfonate treatment of the fiber material, which is akin to mild sulfite pulping (Lou *et al.* 2013; Wang *et al.* 2013c; Huang *et al.* 2022b). Such treatment tends to render the lignin surface more hydrophilic and thus less prone to bind cellulase.

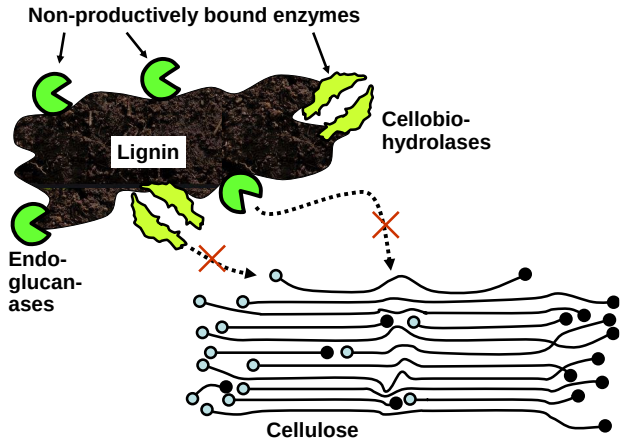

Fig. 6. Schematic illustration of the non-productive binding of cellulase to the more hydrophobic surface of lignin, which has the potential to arrest its interactions with cellulose

Enzyme denaturing

All enzymes are subject to gradual loss of function, *i.e.* denaturation, over the course of time, depending on such conditions as increasing temperature. Efforts have been underway to identify or develop thermo-stable cellulases, with the goal of speeding up the transformation of underutilized cellulose to sugar, which can be fermented to produce ethanol and other valuable biofuels or monomers to be used in organic synthesis (Patel *et al.* 2019). Certain enzymes, called extremophiles, are able to perform well outside of the typical thermal ranges of enzymes, though even those enzymes have their limits of heating, beyond which they break down rapidly (Daniel *et al.* 2008). Tamo *et al.* (2020) showed that such decline could be reduced by encapsulation of cellulase in alginate, thus immobilizing it. In addition, the hydrolytic effect was enhanced when the cellulase was encapsulated.

Hemicellulose Biodegradation

Cellulose fibers derived from wood, as is common in toilet paper for instance, will contain substantial amounts of hemicellulose, often in the range 10 to 25% in the delignified material (Duchesne *et al.* 2001; Neto *et al.* 2004). Though cotton is mostly cellulose, it can contain about 2% of hemicellulose (Kim and Ralph 2014). Compared to cellulose, the hemicellulose is low in molecular mass, having a molecular mass in the range of 10,000 to 40,000 g/mole (Dahlman *et al.* 2003; Bai *et al.* 2012). Considering that anhydroglucose, a common repeat unit in some hemicellulose, has a mass of 162 g/mol, these values correspond to degrees of polymerization (DP) in a range from about 62 to 247. By contrast, the DP of cellulose has been reported to be in the range 300 to 1700 in wood pulps and in the range 800 to 10,000 in cotton and some other plants (Klemm *et al.* 2005). The amorphous nature of hemicelluloses and the presence of carboxylic acid functional groups cause them to swell in water, leading to hydrogel behavior (Gabrielii *et al.* 2000). The hemicellulose can be expected to be accessible to enzymes, which require water-filled passages in the size range of one or more nanometers, depending on the specific case (Mäki-Arvela *et al.* 2011). Whereas cellulose requires just three classes of enzymes, complete hemicellulose degradation can require more, due to a somewhat more complex chemical composition (Houfani *et al.* 2020). The complexity of hemicelluloses, leading to several different points of potential enzymatic attack, is illustrated in Fig. 7. The catalyzed

chemical reaction, in those cases where a pointer is shown acting on an oxygen atom, generally can be described as the addition of water, *i.e.* hydrolysis.

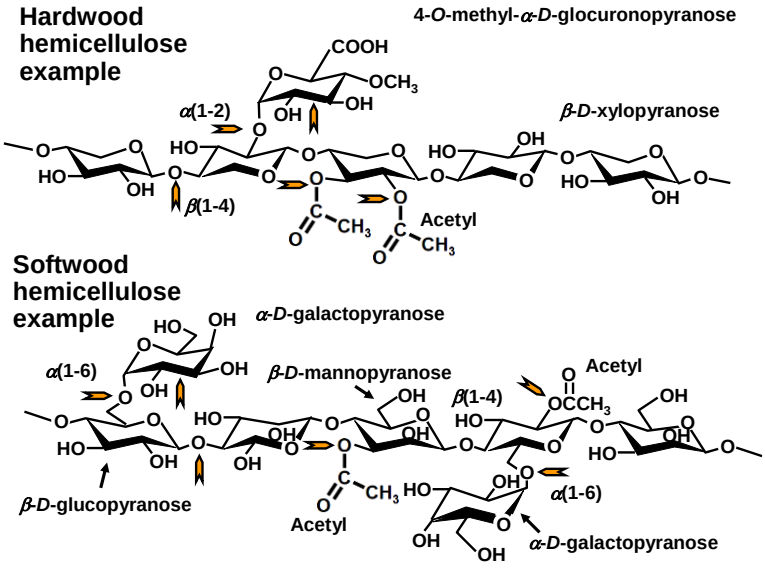

Fig. 7. Sketch of reported points of enzyme attack onto hemicellulose structures. The orange pointer suggests some main points of enzymatic attack, including various glycosidic bond types and deacetylation. The text labels in the figure refer to different sugar monomer units or functional groups present in natural hemicellulose structures.

Aquatic biodegradation was carried out by Kwon *et al.* (2021), comparing three types of hemicellulose with two types of lignin and with MCC as the point of comparison. Results are provided in Fig. 8.

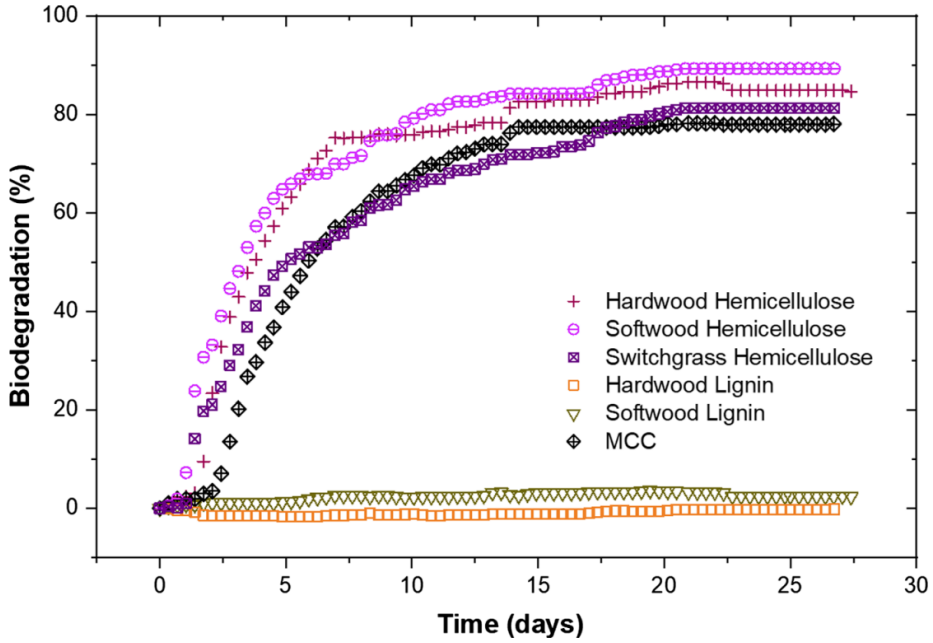

Fig. 8. Aquatic biodegradation compared for three hemicellulose specimens, two lignin specimens, and MCC (Kwon *et al.* 2021). Permission to reuse figure was from the copyright owner, Springer. The inoculum was biosolids from a wastewater treatment plant.

As shown, all three of the hemicellulose specimens achieved somewhat higher biodegradation after 27 days (80 to 90%) in comparison to MCC (78%). These findings are consistent with the relatively hydrophilic and non-crystalline nature of hemicellulose, as described above. By contrast, conventional MCC has been reported to have a degree of crystallinity in the range of 48 to 96% (Ardizzone *et al.* 1999; Eichhorn and Young 2001; Ibrahim *et al.* 2013).

Lignin Biodegradation

As was shown in Fig. 8, there was a large contrast between the lignin specimens and both MCC and the hemicellulose specimens, relative to the observed extents of biodegradation in an aquatic environment. The two lignin specimens showed about zero to 4% biodegradation, in comparison to over 78% for the other specimens under the conditions of testing in that case. These differences can be tentatively attributed to the relatively dense and hydrophobic nature of lignin. It should be noted that there is no lignin at all in cotton, and the level is close to zero in common tissue paper products, for which the fibers are obtained from kraft pulping and bleaching to remove the lignin. However, there can be roughly 20 to 30% of lignin content in various species of wood and various non-wood biomass sources.

Fungi are the main source of lignin-biodegrading enzymes in natural environments (Carvalho *et al.* 2009). When wood-based papermaking pulp is prepared by mechanical refining, the cellulose fibrils are often protected by a layer of lignin. This is especially the case for thermomechanical pulps, for which in ingoing wood chips are treated with steam under pressure ahead of the mechanical refining process. At the temperatures employed, above the ambient boiling point of water, the lignin becomes deformable, such that it becomes the point of separation when the fibers are mechanically pulled apart from each other (Irvine 1985; Mattsson *et al.* 2021). Such a manner of separation leaves the resulting fibers surrounded by a layer of lignin. In theory, treatment with lignin-degrading enzymes, such as manganese peroxidases and laccases (Bugg *et al.* 2011), can enable subsequent action by cellulases (Sun and Cheng 2002; Huang *et al.* 2022b). However, the hydrophobic nature and complex, random structure of lignin means that the enzymatic breakdown of lignin tends to be relatively slow (Datta *et al.* 2017; Janusz *et al.* 2017).

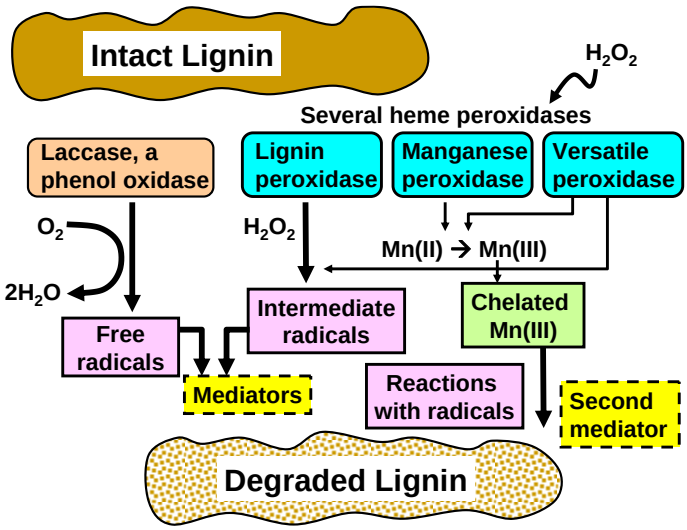

Fig. 9. Representation of some major types of lignin-degrading enzymes

Figure 9 illustrates the way in which communities of different enzymes can participate in the biodegradation of lignin (Javaid *et al.* 2019). Of the major classes of ligninolytic enzymes, laccase serves the function of generating oxidative free radicals by the conversion of oxygen to water. Meanwhile, parts of lignin's complex structure are subjected to three main groups of heme-type peroxidases, which employ hydrogen peroxide to oxidize Mn(II) to Mn(III), which is temporarily held in chelated form. The accumulated radical species undergo further reactions, with the help of mediators, leading to breakdown of connecting linkages within the lignin structure.

In addition to blocking the access of cellulase enzymes to cellulose, the presence of lignin also can lead to non-productive binding of the cellulase, as noted already (Li *et al.* 2022). When a cellulase enzyme is essentially immobilized onto the relatively hydrophobic surface of lignin, it cannot contribute effectively to cellulose decomposition. Products that do not have lignin, such as cotton, will not have this issue affecting their biodegradation.

Recalcitrance in General

Natural wood-based fibers generally show a resistance to biodegradation, *i.e.* a recalcitrant nature, that goes beyond what can be attributed to each of its main components individually. Factors that can help explain this recalcitrance, in addition to the crystalline nature of the cellulose, will be considered in more detail later in this article. In general terms, the factors can be listed as hydrophobicity (mainly due to the lignin), density of the material (resulting from the details of biosynthesis), and the presence of covalent bonds between lignin and the polysaccharides, especially the hemicellulose (Tarasov *et al.* 2018). It should be noted that both cotton and bleached kraft pulp fibers are generally free of the concerns just mentioned, which is consistent with their only moderate resistance to biodegradation. By contrast, the complex, multicomponent nature of natural woody materials has been proposed as a main contribution to difficulty or slowness in biodegradation (Carvalho *et al.* 2009).

Wood extractives, which include lipids, waxes, fats, phenolic compounds, and alkaloids, are quantified by extracting wood – often in the form of wood chips – by means of nonpolar solvents (N'Guessan *et al.* 2023). The levels of wood extractives in the wood chip types commonly used for production of papermaking pulps are generally rather low, *e.g.* about 1 to 4% for trees grown in temperate climates (Shebani *et al.* 2008). However, due to their hydrophobic nature, it might be expected that they could tend to either non-productively bind cellulase enzymes or to cover the fiber surface, thereby blocking the action of those enzymes. It has been argued, however, that by the time the wood has been chemically pulped and bleached, almost all of the extractives have been removed (Liu *et al.* 2012). In fact, it has been suggested that removal of the extractives may play a significant role in enabling the work of cellulase enzymes (Auxenfans *et al.* 2014). Likewise, Wang *et al.* (2018) concluded that structural features of the biomass are more important than factors such as extractives content in affecting rates of enzymatic hydrolysis. The waxes that are present on the outside of some cotton items, especially before scouring (Chung *et al.* 2004), are likewise hydrophobic, thus raising an expectation that they might tend to bind cellulase enzymes or block their access to the cellulose.

ENVIRONMENTS

Rates and extents of biodegradation of cellulose fibers depend on many factors, and these can be grouped together using the labels environments, fiber-related factors, and conditions that can be adjusted by technologists. These will be considered in this and the following two main sections. Many studies have been conducted regarding the biodegradability of cellulosic fibers in wastewater, especially during processes of wastewater treatment. Other environments of concern include seawater, soil, landfill facilities, and composting systems. The form of cellulose input to each system will vary, with a high variety of lignocellulosic materials disposed of in landfills (chemical and mechanical pulps, wood) and treated in composting systems (*i.e.*, yard waste).

Wastewater and its Treatment Facilities

Wastewater

When cellulosic fibers are flushed or when they are discharged during the rinse cycle of a washing machine, they are essentially in a fresh-water environment. But as the suspended matter travels through a drainage system, the conditions may not be optimized to favor their biodegradation. Nagamine *et al.* (2022) considered the analogous environment of river water. The cited authors reported that such an environment was conducive to initial fragmentation of the cellulose, but complete biodegradation was not necessarily achieved. The degree of biodegradation in river water increased with the passage of time, though the rate of biodegradation gradually decreased over the course of 30 days, which was the study period. Based on the results of biological oxygen demand tests, the river water environment was much more conducive to biodegradation than either brackish water or seawater, in decreasing order. When the results were analyzed based on weight loss, there was less difference. The study compared ramie, mercerized ramie, and fully regenerated cellulose fibers.

Wastewater treatment in general

The first step in a typical wastewater treatment operation involves settling by gravity, *i.e.* primary clarification. It can be expected that a majority of the cellulosic fibers present in the incoming effluent will have been removed from the water already in such an operation prior to any aerobic or anaerobic stage of treatment. Thus, Ahmed *et al.* (2019) found that about 80% of cellulose present in the influent to a typical aerobic wastewater treatment plant was physically removed by a primary clarifier operation, upstream of the activated sludge operation. The initial separation from the water phase is settling, by gravity. This usually happens within a wide, circular tank of wastewater (the clarifier), in which the incoming flow enters at the center, and the clarified water is collected as it overflows at the outside edges of the tank. The primary sludge, after it has settled to the floor of the clarifier, is thickened first by raking and then by pressing. The common fate of such primary sludge is landfilling, though land application is also used (Chynoweth *et al.* 1992; Wang *et al.* 2008). For example, it has been reported that about half of the wastewater sludge residuals from kraft pulping are placed in landfills, with land application making up much of the remainder (Meyer 2022). Because primary clarification operations depend on such fiber attributes as density and size, one can expect roughly equal efficiency of clarification for various cellulose fibers originating from sanitary tissues, textiles, or other sources.

Relative to the potential biodegradation of cellulose fibers, an important aspect of a wastewater treatment facility is whether the biological treatment stage employs an aerobic or an anaerobic treatment stage (Hubbe *et al.* 2016). Each of these options can allow for at least some cellulose biodegradation, but the operations are quite different, as described next.

Aerobic activated sludge wastewater treatment

For both municipal and industrial wastewaters in the US, activated sludge systems, using aeration, are commonly used to reduce the biological demand (BOD) before discharge of the treated water (Hubbe *et al.* 2016; Liu *et al.* 2022). Since cellulosic matter in wastewater contributes to the BOD, there has been an expectation that such treatment ought to biodegrade cellulosic materials. A standard BOD test measures the consumption of oxygen during the microbial metabolism of an aqueous sample (sometimes with suspended plant materials) over a period of five days. As shown by the examples highlighted in Table 5, it has been found that such biodegradation is usually only partial in the case of cellulose fibers. Thus, results of standardized BOD tests generally cannot be assumed to include more than a fraction of the cellulose that is present in a specimen. As noted in 1975 by Edberg and Hofsten, full decomposition of cellulose fibers may require several weeks of retention time before the remaining secondary sludge from the biodegradation process is settled, thickened, and sent to a landfill (Hubbe *et al.* 2016). In practice, wastewater treatment facilities are run in such a way as to emphasize the quality of outgoing water rather than minimizing the quantity of sludge, which would include cellulose. Thus, the undegraded portion of cellulose, following a cycle of aerobic wastewater treatment, is likely to be included within thickened sludge that is hauled to a landfill or land applied. Based on most of the data in Table 5, activated sludge treatment can be expected to decompose and remove 60 to 90% of the cellulose remaining after the primary (settling) stage.

Table 5. Highlights from Studies on the Extent of Cellulose Fiber Biodegradation in Aerated (Activated Sludge) Wastewater Treatment Facilities

Highlights from the Study	Percent Degraded	Citation
96 h at ~ 54 degrees F in aerobic sludge: 96 h at ~ 74 degrees F in aerobic sludge:	19.9% 87.7%	Hurwitz *et al.* 1961
Aerobic treatment for 70 days or more	100%	Edberg & Hofsten 1975
Activated sludge treatment for 4 to 5 weeks	60%	Verachtert *et al.* 1982
90 days, for cotton, ramie, and rayon fibers	30 to 60%	Niu *et al.* 2012
Conventional 6 to 7 day activated sludge treatment	70 to 90%	Ahmed *et al.* 2019
Cotton & rayon yarns, evaluated after 15 to 30 days	>70%	Zambrano *et al.* 2020
Various kinds of cellulosic fibers, 27 days	46 to 90%	Kwon *et al.* 2021
Aerobic wastewater treatment for 3 or 15 days	78 to 90%	Kim *et al.* 2022
Cotton microfibers, tissue, flushable wipes, MCC	60 to 86%	Smith *et al.* 2024

Smith *et al.* (2024) compared the aerobic environments associated with wastewater treatment plants, lakewater, and seawater. Their results corresponding to the freshwater environments (wastewater treatment and freshwater conditions) are given in Figs. 10 and 11, respectively. As shown, the cotton microfibers (those likely to be released from textiles) achieved the highest levels of biodegradation under each of the conditions of testing, reaching about 85% and about 96%, respectively. Under the aerobic wastewater treatment

conditions, the MCC relatively quickly (over about 20 days) reached about 60% biodegradation, and then it remained at that level until the end of testing (105 days). By contrast, the tissue paper and the flushable (cellulose) wipes exhibited an initially slower but more persistent biodegradation, eventually surpassing that of the MCC under the wastewater treatment conditions. A likely explanation for the different rate behavior of the MCC is that though it has a smaller particle size, which can favor its biodegradation, it also typically has a higher degree of crystallinity, which will tend to slow down the process. Under lakewater conditions, the tissue paper, flushable wipes, and MCC had generally similar trends. The apparent negative values for biodegradation of the non-flushable wipes under lakewater conditions may be tentatively attributed to algal growth. Such growth might account for an increase, rather than a decrease in mass during exposure to lakewater.

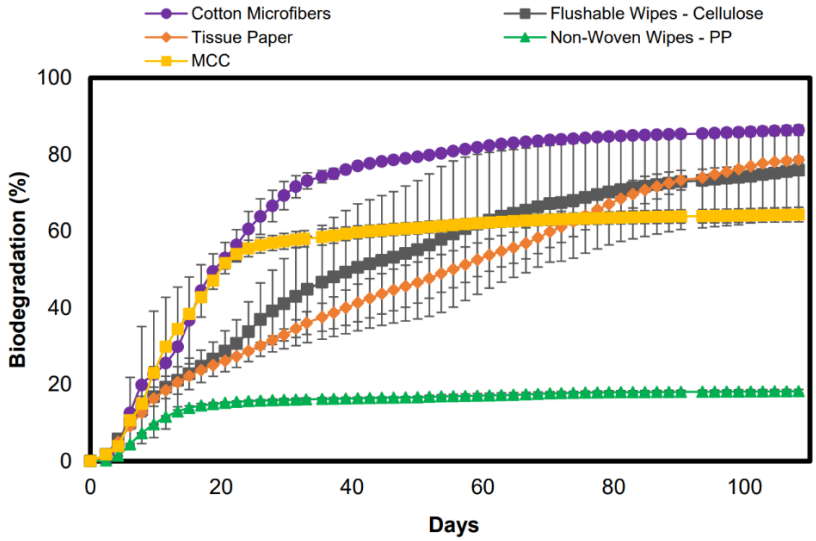

Fig. 10. Biodegradation results for various commonly flushed items under conditions of wastewater treatment (Smith *et al.* 2024). Permission to reuse figure obtained from R. A. Venditti, copyright holder. The PP wipes were mainly comprised of polypropylene but also contained 28% of cellulosic fibers.

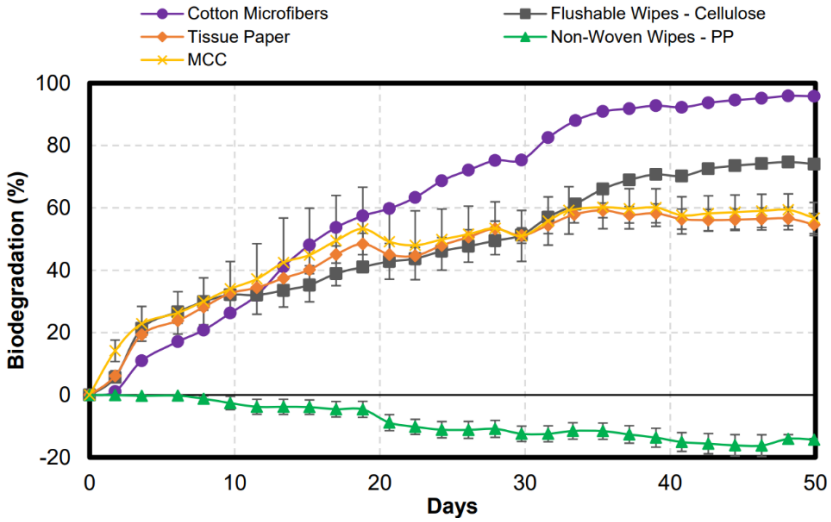

Fig. 11. Biodegradation results for various commonly flushed items under lakewater conditions (Smith *et al.* 2024). Permission to reuse figure obtained from R. A. Venditti, copyright holder. The PP wipes were mainly comprised of polypropylene but also contained 28% of cellulosic fibers.

Anaerobic wastewater treatment

Though it requires a higher capital investment, there is interest in anaerobic wastewater treatment systems due to their tendency to yield less sludge that needs to be hauled away (Chan *et al.* 2009), and also the fact that such systems produce methane or hydrogen (Shen *et al.* 2015), which can be captured and burned as fuel. The mechanisms involved in anaerobic biodegradation of cellulose and other components of biomass have been reported (Li *et al.* 2018; Peces *et al.* 2018). Peces *et al.* (2018) showed that regardless of the composition of inoculants at the start of anaerobic digestion, over time the microbial community within bioreactors tended to converge in a predictable, repeatable way. In other words, the evolution of community composition can be viewed as deterministic rather than resulting from differences in starting inoculum conditions. On the other hand, anaerobic biodegradation of cellulose has been shown for specific populations of microbes, such as those in the rumens of cattle (Zhang *et al.* 2017). Li *et al.* (2018) showed that the relative order of biodegradation, under anaerobic conditions, was hemicellulose > cellulose > lignin, except that cellulose was the most favorable source when the goal is to produce methane. A study by Yu *et al.* (2012a) showed that the anaerobic biodegradation of microcrystalline cellulose (MCC) proceeds by development of channels in the size range of 400 to 500 nm within the MCC.

Table 6 summarizes some findings from such systems. Although some of the numbers shown in Table 6 may appear favorable relative to aerobic systems, Edberg and Hofsten (1975) pointed out that long treatment times are more commonly employed in anaerobic treatment systems. Thus, the size of the equipment may have to be suitably scaled up relative to aerobic treatment to accommodate given amounts of flows and amounts of incoming solids to be treated. However, operating costs may be lower than in aerobic systems, since it is not necessary to pump air or to use fountains or sprays.

Toilet paper typically exhibits a lower biochemical methane potential compared to mixed food waste (Cho *et al.* 1995; Naroznova *et al.* 2016), which can be attributed to its high cellulose content. Despite this, toilet paper can be effectively co-digested alongside food waste and human feces without diminishing the overall methane yield. Such methane is regarded as beneficial, since it can be fully captured and used as fuel or in other applications. This approach has been shown to be feasible for valorizing the primary organic wastes generated in households (Kim *et al.* 2019).

Table 6. Highlights from Studies on the Extent of Cellulose Fiber Biodegradation in Anaerobic Treatment Facilities

Highlights from the Study	Percent Degraded	Citation
Sulfite pulps in various anaerobic sludge systems for 50 to 125 days	70 to 95%	Edberg & Hofsten 1975
Cotton anaerobically digested as sludge for up to 35 days	55 to 75%	Verachtert *et al.* 1982
Different materials from a pulp & paper mill compared from various literature sources and time periods	30 to 90%	Meyer & Edwards 2014
Various papermaking pulps, based on CH_4 production within about 400 days	90 to 95%	Wang *et al.* 2015
Toilet paper ~ 7.5 days in a bioreactor making CH_4	100%	Chen *et al.* 2017

Seawater Biodegradation of Cellulose

Although the world's seas represent a huge area, and a huge volume, there are some common features, such as salinity and pH, which remain relatively constant. On the other hand, the sea contains varying temperatures. In particular, the cold temperature and absence of light deep in the sea can be expected to slow down biological processes. Table 7 lists highlights from studies dealing with cellulose biodegradation in real or simulated seawater. As shown in the table, wide ranges of biodegradability have been reported for cellulose fibers in seawater. Studies involving matched tests in both fresh water and seawater have indicated substantially lower biodegradation in the seawater (Zambrano *et al.* 2020; Nagamine *et al.* 2020; Miyaji *et al.* 2023). Possible reasons for the difference include possible inhibition of enzymatic actions due to salinity (Miyaji *et al.* 2023), or maybe a lower availability and concentration of cellulase enzymes in ocean environments.

Table 7. Highlights from Studies on Cellulose Fiber Biodegradation in Seawater

Highlights from the Study	Percent Degraded	Citation
Cotton, rayon, & MCC for 35 days	46 to 71%	Zambrano *et al.* 2020
MCC in seawater, 30 days	85%	Royer *et al.* 2021
Cellulose "readily degradable" in seawater	No report	Wang *et al.* 2021
Ramie, mercerized ramie, and rayon 30 days, BOD	3 to 14%	Nagamine *et al.* 2022
Emphasis on comparing lab *vs.* field data	30 to 100%	5 Gyres Inst. 2023
Cellulose powder (presumably MCC) for 30 days	12%	Miyaji *et al.* 2023
Bioreactor for 28 days; cotton & regenerated cellulose	76 to 82%	Royer *et al.* 2023
Cotton microfibers, tissue, flushable wipes, MCC	70 to 78%	Smith *et al.* 2024

Tests by the 5 Gyres Institute (2023) showed essentially complete biodegradation of paper straws after about 16 weeks in a marine environment. However, tests of bamboo utensils (forks) showed only about 30% biodegradation in 64 weeks. It is not reported, but it is possible that the commercial tableware listed above may have contained substances in addition to cellulose or bamboo. It follows that these cited results will need confirmation.

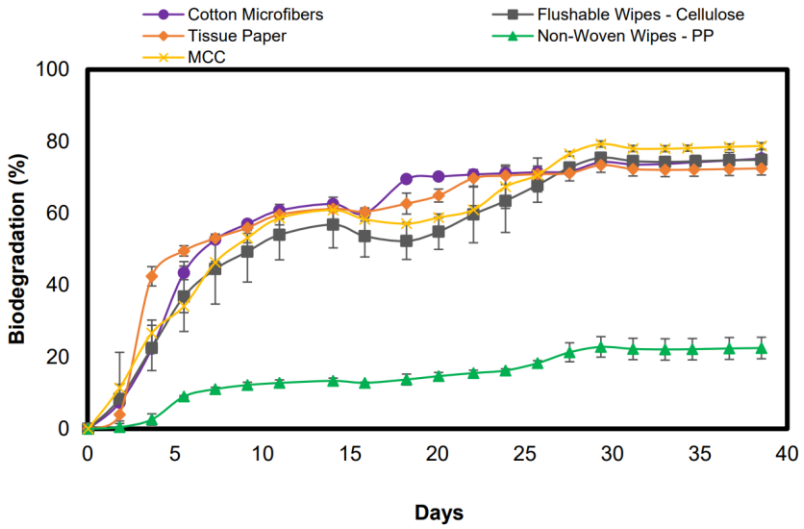

Fig. 12. Biodegradation results for various commonly flushed items under seawater conditions (Smith *et al.* 2024). Permission to reuse figure obtained from R. A. Venditti, copyright holder. The polypropylene (PP) non-woven wipes had a main component of polypropylene but also contained 28% cellulose.

Figure 12, which features biodegradation results from seawater exposure of various materials, shows a strong contrast between those products that were cellulose based, in comparison to the non-woven wipes, for which the main component was polypropylene fibers (Smith *et al.* 2024). The cellulosic products, which included cotton microfibers, tissue paper, microcrystalline cellulose, and flushable wipes made from cellulose fibers, all were between 30 and 50% degraded after five days, followed by more gradual degradation up to about 30 days, and then a stagnation in the degradation process.

Soil Biodegradation of Cellulose

Due to the possibilities of littering and airborne transportation of dryer lint, *etc.*, substantial quantities of cellulose can become subject to degradation in natural soils. Standard evaluation protocols are available (Tyagi *et al.* 2022). Table 8 features highlights from studies dealing with biodegradation in soil.

Table 8. Highlights from Studies on the Extent of Cellulose Fiber Biodegradation in Soil

Specimens and Key Aspects of the Study	Percent Degraded	Citation
Films of regenerated cellulose; weight loss 30 days; kraft paper	70% 55%	Zhang *et al.* 1996a
Cotton and rayon textiles evaluated after 14 days soil burial, based on tensile strength	~ 100%	Salerno-Kochan *et al.* 2001
Cotton evaluated as a control relative to Ag-treated cotton specimens; 12 days soil burial; loss of DP	10%	Klemencic *et al.* 2010
Textile fabrics, 90 days soil burial	23 to 28%	Li *et al.* 2010
Cotton evaluated after 12 days soil burial based on DP loss	20%	Simoncic *et al.* 2010a
Softwood mechanical pulp, 14 days; Other wood pulp	20% 57%	Dobrin *et al.* 2012
TEMPO-oxidized NFC (an experimental material) with different counter-ions for 12 days	2 to 100%	Homma 2013a
All-cellulose composites formed from rayon after 70 days of soil burial	38 to 73%	Kalka *et al.* 2014
Analytical grade cellulose after 29 days in soil	84 to 88%	Mistriotis *et al.* 2019
Cellulose film regenerated in ionic liquid with most of degradation within three weeks	~100%	Zhao *et al.* 2019
Cellulose film regenerated from an ionic liquid completely biodegraded in soil. Evaluations done after 7 and 14 days	100%	Ai *et al.* 2020
Bacterial cellulose film after 3 and 7 days of burial	50 to 100%	Zahan *et al.* 2020
Numerous natural cellulosic fibers, with cotton showing the highest soil biodegradation in 32 days	60 to 100%	Margariti 2021
Cellulose hydrogel nanocomposites were judged to be fully biodegradable in soil.	~ 100%	Das *et al.* 2023

Tests by the 5 Gyres Institute (2023) showed that test conditions affect the results of biodegradability testing. The study considered six types of natural environment, to which the specimens were exposed. The test locations were Maine, Florida, and California, and in each case Coastal marine environments were compared with terrestrial environments relative to their effects on biodegradation. Weight loss, after exposure, gentle cleaning, and drying, was used as the measure of biodegradation. Fragmentation of the specimens was

judged visually. Key factors were found to be soil type, temperature, and moisture content. The synthetic plastics polystyrene and polyethylene showed essentially no biodegradation over 32 weeks, whereas paper straws were in a similar category to biodegradable bioplastics.

Landfill

Substantial quantities of cellulosic materials are sent to landfills. Milbrandt *et al.* (2024) estimated that in 2019, 110 million tons of used paper and cardboard were managed domestically in the US, and that approximately 56% was landfilled, 38% was recycled, and 6% was combusted. The authors noted that the landfilled waste represents a loss of value, and that recycling and energy production (by combustion) can be much better options. Johnson *et al.* (2022) estimated that 11.6 million metric tons of cotton waste are generated annually during production activities in the cotton mill. The authors advocated non-traditional end uses, including biofuels and composites, as alternatives to landfilling.

Problems inherent in landfill operations include a slow rate of biodegradation (De La Cruz and Barlaz 2010). Biodegradation rates for all materials including cotton textiles increase with moisture content. Decay rate constants for municipal solid waste were estimated to be 0.02, 0.04, 0.08, and 0.12 yr^{-1} (proportional amount per year) for dry, average, wet, and bioreactor conditions, as defined in De La Cruz and Barlaz (2010). In addition, different portions of the cellulose-based content of a landfill may remain undecomposed, depending on the material (Barlaz 1998). Undecomposed fractions of cellulosic materials, based on kg C per dry kg of waste, rose in the order of office paper (0.05), food waste (0.08), old corrugated containers (0.26), coated paper (0.34), and old newspapers (0.42), among others. Though methane is evolved from landfills, the process occurs over years to decades, and not all of the generated methane will be collected. Methane collection efficiencies, over a 100-year period, have been estimated to range between about 64% to 88%, when assuming various scenarios corresponding to practices in the US (Barlaz 2009). Collection efficiency may be lower, depending on the age of the facility, its location in the world, and local regulations.

Compost

Composting can be a practical way to stabilize various biodegradable wastes to a form that can be added to soil with beneficial effects, such as moisture retention (Briassoulis *et al.* 2010; Hubbe *et al.* 2010). Composting can be defined as the aerobic biodegradation of organic materials under engineered conditions. Heat generated by metabolism during the aerobic degradation typically results in increased temperature within the pile. Though such heating often accelerates the process, temperatures above about 60 °C can lead to self-sterilization (MacGregor *et al.* 1981). A distinction can be made between industrial composting conditions, in which the interior of the pile rises in temperature to about 60 °C, compared to home composting, in which such temperature rises may be lower or in doubt. Table 9 lists highlights from studies in which cellulose fibers and related materials were subjected to industrial composting. With the exception of a few studies showing very high degradation levels of nanocellulose films and certain regenerated cellulose films, the extents of cellulose degradation generally fell within a range of about 30 to 80%. Within such ranges, some of the highest levels were obtained in systems that had been inoculated with bacteria, and sometimes also with fungal treatment. In general, the rates of degradation have been reported to decline with the passage of time.

Table 9. Highlights from Studies Dealing with the Extent of Cellulose Fiber Biodegradation under Industrial Composting Conditions

Specimens and Key Aspects of the Study	Percent Degraded	Citation
Diverse types of paper in 45 days	36 to 79%	López Alvarez *et al.* 2009
Cotton t-shirts treated with a fabric softener degraded faster than untreated cotton after 90 days of composting.	50 to 77%	Li *et al.* 2010
Filter paper, MCC, biofilms, nanocellulose, *etc.*, were evaluated over 66 days, with most of the degradation occurring during the first 30 days.	70 to 100%	Vickman *et al.* 2015
Bacterial inoculation increased the breakdown of cellulose in compost. Most biodegradation took place during the first 20 days, with slower degradation continuing to the end of the study at 36 days.	30 to 70%	Zhao *et al.* 2016
Inoculation increased cellulose breakdown during composting. Higher rates of cellulose breakdown were observed up to 18 days of composting, followed by slower rates.	30 to 63%	Zang *et al.* 2018
Cellulose films prepared from ionic liquid had been fully degraded after 2 days of composting. Cellophane was 90% hydrolyzed, and wet-strength paper was 80% hydrolyzed within the same period.	100%	Leppanen *et al.* 2020
Bacterial community enhancement promoted cellulose degradation and humus formation.	75 to 80%	Wang *et al.* 2022b
Bacteria and fungi worked synergistically; however lower biodegradation was observed for hemicellulose (50%) and lignin (21%) than cellulose.	70%	Han *et al.* 2023
Cotton fabric specimens composted for 77 days under laboratory conditions were biodegraded by 18% to 62% and showed no effect related to various indigo dyes.	18 to 62%	Alwaya *et al.* 2024
Addition of denim to the compost mixture did not have a notable effect on the rate of composting over the course of 77 days; however, those specimens containing non-cellulose fibers left contaminants at the end of composting.	Close to 100%	Schwarz *et al.* 2024

A delay in cellulose biodegradation can result when other more degradable substrates (*e.g.*, starch) are present (López Alvarez *et al.* 2009). The progress of composting can be expected to be most favorable when the C:N ratio is in the range 25:1 to 50:1 and when there is also a sufficient amount of phosphorus and moisture (Hubbe *et al.* 2010). When the mixture is too rich in cellulosic materials, the C/N ratio will be too high for optimum composting.

FIBER-RELATED FACTORS

The subsections below will consider classes of factors that have been reported to affect the rate and extent of the biodegradation of cellulose fibers under a variety of conditions. Factors related to the fibers will be considered here, namely the fiber type, the

sizes of the cellulosic entities, effects of processing of the fibers, the degree of crystallinity, and various treatments of the fiber surfaces.

Type of Cellulose Fiber or Particle

Relatively few researchers have attempted to answer questions such as "how does the biodegradability of one kind of fiber compare to that of another kind of fiber?" The work of Kwon *et al.* (2021) carried out such comparisons in aquatic environments. Results in Fig. 13 show that beached hardwood kraft achieved the highest extent of biodegradation, about 90%, which was even higher than that of MCC (78%) in the course of 27 days in fresh water. It is worth noting that not only does the chemical pulping process remove the hard-to-biodegrade lignin component, but it also renders the fibers porous in the range 2 to 50 nm (mesoporous) (Stone and Scallan 1968), thus allowing better access of enzymes to the cellulose. Next came bleached softwood fibers (74%), which also have a mesoporous nature. The slower biodegradation of the softwood fibers compared to hardwood fibers, is consistent with a larger fiber size. Unbleached fibers from linerboard took longer to start the biodegradation process, which might be attributed to the presence of considerable lignin content in the unbleached pulp used to make such products. Mechanical pulp and material from newsprint were found to have the slowest rates of biodegradation, which might be attributed to the fact that such pulps have the highest lignin content.

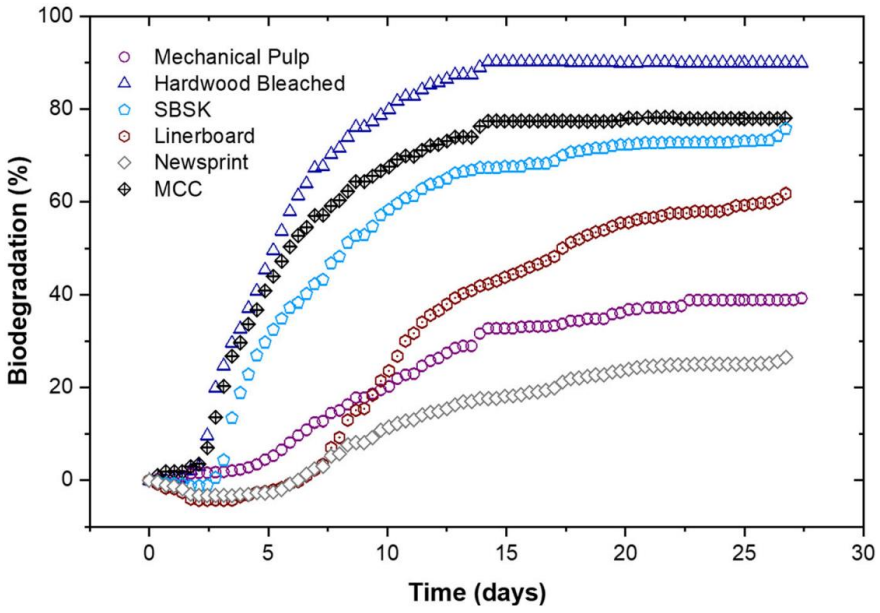

Fig. 13. Extent of biodegradation of different types of wood-derived pulp fibers (bleached kraft pulps from hardwood and softwood (SBSK = southern bleached softwood kraft), unbleached kraft pulp from linerboard, mechanical pulp and such pulp obtained from newsprint paper, all compared relative to MCC (Kwon et al. (2021). Permission to reuse figure was from the copyright owner, Springer.

Mizutani *et al.* (2002) compared the extent of enzymatic degradation of cotton fibers to that of MCC. The extent of biodegradation of the MCC was higher by a factor of 1.7 in the absence of surfactant and by a factor of 2.1 in the presence of the surfactant. The type of MCC particles used in the studies had a nominal size of 50 µm.

Smith *et al.* (2024) compared the biodegradability of cotton microfibers, tissue paper, flushable wipes, polypropylene-based wipes, and MCC under the conditions

associated with aerobic wastewater treatment, lakewater exposure, and seawater exposure. Their results for these three environments are summarized in Table 10. As shown, the cotton microfibers typically showed the greatest degradation, except that in seawater the MCC was slightly higher. High, but intermediate biodegradability was observed for the tissue paper and the flushable (cellulose) wipes, except that the flushable wipes were superior to the tissue paper and MCC in the lakewater conditions. The polypropylene-based wipes consistently achieved the lowest biodegradation, even achieving negative numbers as a result of tests done in lakewater. Positive numbers can be regarded as evidence of attachment of organic matter, such as algae.

Table 10. Comparative Extents of Biodegradation of Commonly Flushed Materials after Exposure to Conditions of a Wastewater Treatment Plant, Lakewater, and Seawater (Smith *et al.* 2024).

Samples	**Wastewater Treatment (110 days)**		**Seawater (38 days)**		**Lakewater (50 days)**	
	Mean bio-degradation	Statistical groups	Mean bio-degradation	Statistical groups	Mean bio-degradation	Statistical groups
Cotton microfibers	88.2%	b	75.8%	a, b	96.8%	a
Tissue paper	78.6%	c	72.7%	b, c	58.2%	c
Flushable wipes	75.8%	a, b, c	75.1%	c	75.3%	b
PP-based wipes	18.2%	d	22.7%	d	-14.0%	d
MCC	94.3%	a	79.0%	a	61.7%	c

Note: Materials with different letters for statistical groups were statistically different. The statistical groups were relabeled to match the order of biodegradability.

Inherent Differences in the Cellulosic Material

To begin to explain the observed differences in the extents of biodegradation of different types of cellulosic entities, as shown in the previous subsection, it can be hypothesized, first of all, that the observed differences in biodegradation are due to some inherent differences in the materials. In particular, it can be proposed that the degree of crystallinity in the cellulose can be a contributing factor. Likewise, it might be proposed that biodegradation will be affected by the proportion of lignin or the proportion of hemicellulose in the specimen.

Crystallinity

The research question to be considered here is whether the literature supports the idea that the crystalline nature or the degree of crystallinity play a governing role relative to rates or extents of biodegradation of cellulose. Strikingly high resistance to enzymatic breakdown was reported in a study of tunicate cellulose (Cheng *et al.* 2020). As reported by these authors, tunicate cellulose has an especially high degree of crystallinity (circa 94%). They observed only 7.8% hydrolysis after 96 hours of biodegradation. By contrast, 83% of high-purity cellulose obtained from kraft pulping (*i.e.* alpha-cellulose) was degraded over 72 hours under similar conditions. Amorphous cellulose exhibited even faster and more complete hydrolysis, approaching 100% hydrolysis in just 10 hours. Such findings can be rationalized based on the explanations given earlier for the functioning of

the main categories of cellulase. Thus, if it is assumed that endoglucanases are ineffective in the crystalline regions and that exoglucanases begin their work only from the ends of cellulose chains, then cellulose having a high crystallinity would be expected to reduce the extent of biodegradation.

Earlier work by Sazaki *et al.* (1979) showed that the biodegradability of cellulose could be greatly facilitated by its dissolution and regeneration, using various chemical systems. The regenerated cellulose specimens, some of which were textile fiber material, all were completely hydrolyzed within 48 hours by a fungal cellulase mixture. By contrast, cryo-milling of the native cellulose yielded only a modest increase in biodegradation (increasing the hydrolysis extent from 26% to 36%) within that time period. Though such results show clear differences, three things need to be considered. First, the final crystal form of the regenerated cellulose will have been changed from the native cellulose I to cellulose II in the regenerated cellulose specimens. In the native crystalline form, all of the chains within cellulose are facing the same direction, whereas in cellulose II, each adjacent chain faces in the opposite direction from end to end. Second, the degree of crystallinity of regenerated cellulose is often lower than it had been in the native form (Armir *et al.* 2021). Third, some decrease in degree of polymerization can be expected to result from the dissolution and regeneration processes (Liu *et al.* 2011; Dong *et al.* 2020).

Zhu *et al.* (2008) compared cellulose specimens that had been decrystallized by ball milling to different extents. Large and distinct increases in biodegradation rates were found with decreasing crystallinity. However, once again, it is to be expected that decreased crystallinity was not the only change induced in the cellulose. A ball-milling process, with its intensive damage to the cellulose crystals, can be expected to decrease the molecular mass as well (Ling *et al.* 2019). A consequence of a lower molecular mass is a higher number of ends of chains, which presumably will provide more starting points for exoglucanase.

In summary, although several studies have shown strong correlations between decreasing cellulose crystallinity and accelerated biodegradation, other factors such as molecular mass and the type of the cellulose crystals may also contribute to the increased biodegradability.

Lignification

Compared to the other main components of woody material, lignin has a more hydrophobic nature, a more complex assortment of links between the subunits, and a high proportion of aromatic groups (Li *et al.* 2016). It has been found that plant materials having higher content of lignin tend to be slower to biodegrade (Huang *et al.* 2022b). For instance, Renouard *et al.* (2017) reported that coconut coir fibers, which have a high lignin content, are less easily biodegraded in soil in comparison the flax fibers, which have a higher proportion of cellulose. In the work of Nagamine *et al.* (2022), the initial ramie fibers, which may be assumed to have lignin content, were over 8% degraded in 24 days, whereas the mercerized ramie and regenerated cellulose from ramie, which were free of lignin, were degraded by about 15% and 52%, respectively, in the same period. Kwon *et al.* (2021) compared the biodegradability of the main components of woody materials in aquatic media. Under matched conditions of 27 days of wastewater biodegradation, specimens of hardwood and softwood lignin showed less than 3% biodegradation, based on oxygen consumed, whereas specimens of cellulose and hemicellulose showed 75 to 87% degradation. The strong contrast in these numbers suggests that lignin is more difficult to

degrade. Another factor to consider is the concentrations and activities of lignin-degrading enzymes present in aqueous systems.

Some exceptions to the rule have been reported, in which lignin levels were not a good predictor of slower biodegradation. For instance, in anaerobic systems, hardwoods have been reported to degrade faster than softwoods, despite similar lignin levels (Wang *et al.* 2011). Grass is high in lignin, but it degrades faster than wood (Eleazer *et al.* 1997). The latter finding is likely explainable by the much smaller particle size, which implies greater accessibility. Here the word "size" refers especially to the thickness of a blade of grass, which will determine the approximate surface area that will be immediately accessible to enzymes, especially in the early stages of biodegradation.

Mansfield *et al.* (1999) suggested that in addition to the chemical composition, the inherent complexity of the intertwined cellulose, hemicellulose, and lignin components in plant-based materials is responsible for higher resistance to biological breakdown, in comparison to the individual parts. Thus, the biodegradation process can be expected to depend on a gradual loosening of the material's structure, eventually enabling different enzymes to access and cleave different kinds of bonds holding the material together. In principle, the overall progress could be blocked at various points due to the need for a specific enzyme capable of breaking a certain kind of linkage.

Hemicellulose

Tests by Kwon *et al.* (2020) showed that three types of hemicellulose (switchgrass, hardwood, and softwood) reached 82 to 90% biodegradation in 27 days of aquatic exposure. These values were slightly higher than the results obtained for MCC (about 77% and much higher than for lignin (3% or less). Such results are consistent with the hydrophilic and non-crystalline nature of hemicellulose.

Size of Cellulosic Entity

If one assumes that cellulolytic enzymes will mainly or initially operate at the outside surfaces of cellulosic material, then it follows that there should be a correlation between size of the cellulosic particle and the rate of biodegradation. Here, "size" refers to the external dimensions. In cases where the material is dense and non-porous, the square of the smallest main dimension would be expected to be roughly proportional to the accessible surface area, depending on such factors as roughness. However, whether or not such a relationship is observed can help to shed light on whether any pores in the material are sufficiently large to allow access to the enzymes. Kraft pulp fibers are known to be mesoporous, due at least in part to the spaces left behind when removing lignin; therefore, it makes sense to consider them separately from other kinds of cellulosic fibers. Published findings related to such questions will be considered here, starting with lignin-containing natural fibers.

As illustrated in Fig. 14, a relatively dense piece of lignocellulosic materials would be expected to begin to biodegrade at its outer surface, and progress into the interior might then depend on a gradual process of degradation of successive layers. In general, thinner fibers or fibrils will be expected to expose greater surface area. Likewise, any fragmentation of fibers that results in thin sheets or fibrils can be expected to greatly increase the accessible surface area.

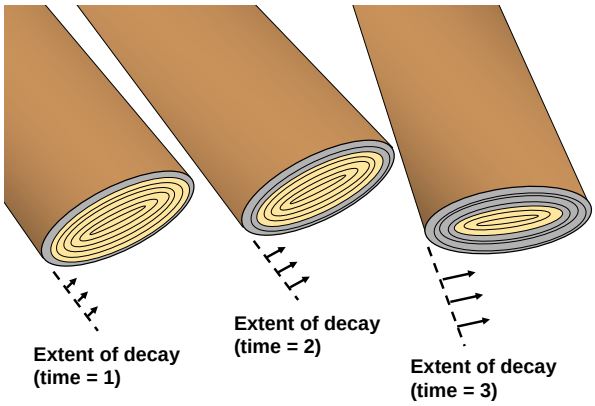

Fig. 14. Sketch of expected layer-wise degradation of dense lignocellulosic materials, depending on their dimensions

A practical way to decrease the effective size of wood-based fibers, whether they still contain lignin or not, is by mechanically refining them, *i.e.* subjecting them to repeated shearing and compression in the wet state (Gharehkhani *et al.* 2015). Chen *et al.* (2015) carried out such an enzymatic procedure with recovered newspaper fibers. Since newspapers are commonly made by mechanical separation of softwood fibers, without removing the lignin, the cell walls are expected to be relatively dense and inaccessible to enzyme access, in the natural state. Refining of the recovered fibers was found to increase the amount of sugar produced during subsequent enzymatic saccharification. Such results are consistent with the idea that refining will increase the effective surface area of the material, just as if the material had been broken into smaller pieces. Likewise, Sinitsyn *et al.* (1991) found a proportionality between the rates of enzymatic hydrolysis and the surface area accessible to enzymes following dry milling of either cellulose or lignocellulose specimens.

Steam explosion is another way that researchers have attempted to increase the areas of cellulosic materials that are accessible to enzymes (Muzamal and Rasmuson 2017). Esteghlalian *et al.* (2002) carried out such treatment on Douglas fir wood. Consistent with the goal of increasing the surface area, it was observed that the adsorption capacity of the woody material for cellulase enzymes was increased by the treatment. However, they did not observe a proportionality between enzyme adsorption and the enzymatic hydrolysis of the cellulose.

A broad range of sizes of cellulose have been evaluated for biodegradability, but in many cases the results cannot be directly compared due to chemical derivatization or other factors. For instance, Homma *et al.* (2013a,b) showed 96% degradation of TEMPO-oxidized nanofibrillated cellulose (NFC) after 12 days, but only if the counter-ion was sodium. The term "TEMPO" refers to the radical species 2,2,6,6-tetramethylpiperidin-1-yl)oxyl, which catalyzes oxidation of the C6 -OH group of cellulose to form the corresponding carboxylic acid. Since chemical derivatization often interrupts the action of enzymes (Glasser *et al.* 1994; Yadav and Hakkarainen 2021), such results may provide general support to the hypothesis that developing a small size and higher surface area of cellulosic will promote biodegradation. However, as will be argued later, those results might also be attributed mainly to a swelling effect coming from the charged nature of the material and colloidal effects.

Miyaji *et al.* (2023) directly compared cellulose powder, microcrystalline cellulose (MCC), and cellulose gel in three media, namely fresh water, brackish water, and sea water. Though the size distributions of each cellulose type were not specified, the degrees of biodegradation in all three media followed the order of microcrystals, gel, powder. This is notable, since the crystallinity of the MCC is expected to be higher than that of the gel, and its typical geometric width is typically larger. In addition, the gel would consist of a porous assembly of elongated cellulosic entities, whereas the MCC and powder would typically be dispersed as individual particles. Thus, the comparison among the three specimens does not provide clear support for the hypothesis about size and surface area relative to biodegradation.

In summary, there appears to be a need for studies in which particle sizes are changed, in a controlled manner, while keeping other variables constant. For example, as a means to avoid other changes in the course or reducing the particle size, one possibility would be to cool the selected cellulosic material in liquid nitrogen, followed by cryo-crushing to different extents. Steam explosion represents another option, though the high temperature can cause chemical changes, in addition to disruption of the material. In either case, parallel measurements of surface area and biodegradation outcomes are recommended. Though such tests have been carried out in the case of various lignified materials, such as wood chips (Reyes *et al.* 2016), there is a need for such work to settle theoretical questions related to the biodegradation of kraft pulps and cotton. To place such issues in context, it is well known that a typical cotton textile fiber can be 20 µm in diameter, whereas kraft pulp fibers are typically from about 20 to 30 µm in diameter. If one makes the rough estimate of about 1 nm per molecular layer and ignores the presence of a lumen space in the middle of a fiber, an enzymatic process would need to chew through 10,000 such layers to completely degrade the material.

Caution is needed in drawing conclusions about enzyme accessibility based on outer dimensions of cellulose fibers. Natural cotton is known to be porous (Mao *et al.* 2014). Especially before drying, the cited study showed that cotton fibers have a mean pore size of about 50 nm, which can be regarded as large enough to accommodate cellulase enzymes. Though the average pore size was decreased dramatically by drying, it was shown that the pores were able to open up again to approximately their original condition upon rewetting by water. On the other hand, the drying of kraft pulp fibers, as happens during the production of paper products, has been shown to permanently reduce the initially high porosity of freshly pulped kraft or sulfite fibers (Stone and Scallan 1968; Hubbe *et al.* 2007).

Effects of Processing

The term processing is used here to denote common treatments of cellulosic materials either to make them available as fibers or to modify their properties. Subsections that follow will consider delignification, mechanical refining, and chemical treatments. In principle, any process that breaks down the structure of the initial cellulosic material is of interest, since it could potentially promote further accessibility of the cellulose to enzymes. Of particular interest are chemical processes that remove lignin (*i.e.* pulping), mechanical processes that internally delaminate the cellulosic fibers (*i.e.* refining), and processes that dissolve and then reconstitute cellulose and the form of filaments of films (regeneration).

Delignification

Kwon *et al.* (2020) correlated the extent of biodegradation for a series of ten plant-based cellulosic specimens, some of which had been subjected to delignification. A strong quasi-linear trend was observed with strongly decreasing biodegradation with increasing lignin content in the range of zero to about 32%, which is the native lignin content of certain wood species. These results are consistent with the findings of Wang *et al.* (2015), who reported a higher biodegradation rate of delignified wood fibers in comparison to mechanical pulp fibers from the same wood source. Zhu *et al.* (2008) likewise reported a much higher rate of biodegradation of wood pulp fibers that had been delignified.

Such results can be rationalized by the removal of microdomains of lignin from woody material in the course of kraft pulping. Figure 15 envisions such a selective removal process involving a small section (*e.g.* 100 to 500 nm) within the cell wall of a pulped chip of wood. The cellulose is depicted in the figure as being grouped together as fibrils, which are surrounded first by a layer rich in hemicellulose, and then by lignin, which essentially surrounds the cellulose within the nanostructure of wood. As shown, full delignification of the cell wall can be expected to open up pores, thus rendering the carbohydrate portion of the material susceptible to chemical or enzymatic influences.

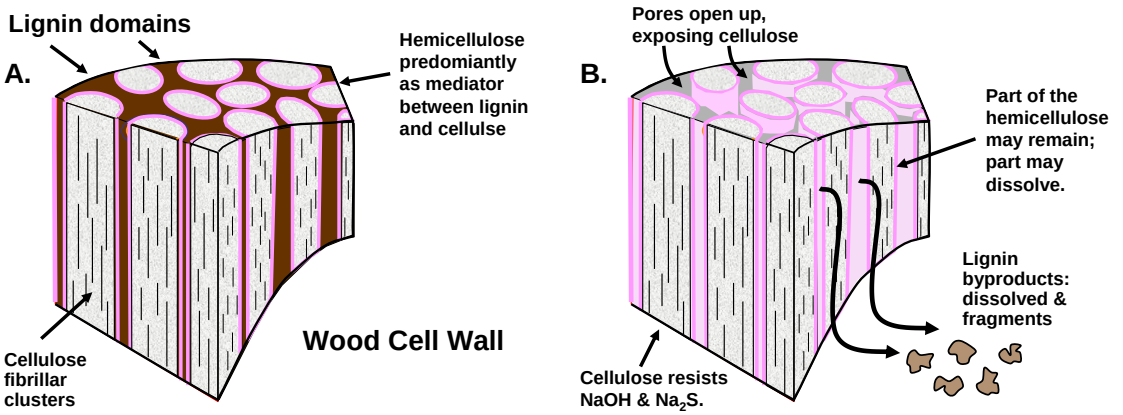

Fig. 15. Concept of removal of nanodomains of lignin, inspired by an early description by Kerr and Goring (1976); A: Small fragment of native wood, showing parts of about 10 cellulosic fibers bound together by lignin; B: Sketch of the same structure after chemical breakdown and removal of most of the lignin and some of the hemicellulose by chemical pulping, *e.g.* by the kraft process

Regeneration

The regeneration of cellulose is carried out by dissolving it and then exposing it to anti-solvent conditions. Important classes of regenerated cellulose include textile fibers such as viscose (rayon) and lyocell. As has already been mentioned in this article, regeneration changes the crystal type from cellulose I to cellulose II, so it is reasonable to expect that it might affect the material's biodegradability as well. Table 11 shows a compilation of studies that have considered the biodegradation of regenerated cellulose specimens. The seven listed studies give a consistent message: Regeneration has been found to enhance biodegradability relative to the fibers before regeneration.

Table 11. Highlights from Studies Considering Biodegradability of Regenerated Cellulose

Study Highlights	Citation
The dimethylsulfoxide-paraformaldehyde solvent system was used to prepare regenerated cellulose (lyocell type), which was much more biodegradable than the starting material.	Sasaki *et al.* 1979
Regenerated cellulose showed more rapid biodegradation in soil.	Zhang *et al.* 1996a
Mercerization of MCC with NaOH, with rinsing but not drying, resulted in the most rapid biodegradation (cellulose II hydrate), followed by the dried are rewetted cellulose II.	Wada *et al.* 2010
The biodegradation rate of viscose (rayon) fibers exceeded that of cotton and ramie.	Niu *et al.* 2012
Cellulose obtained from Whatman filter paper became much more biodegradable after regeneration from an ionic liquid solution.	Li *et al.* 2017
Rayon fibers were compared with other fibers when exposed for up to 35 days in lakewater, seawater, and activated sludge. Their biodegradation extents (about 70%, 42%, and 90% in the three listed media) were generally similar to those of cotton.	Zambrano *et al.* 2020
Fully regenerated cellulose from ramie fibers was much more biodegradable than mercerized (*i.e.* partly regenerated) ramie cellulose, and even more so than the original ramie fibers.	Nagamine *et al.* 2022
Partial dissolution of cellulose, similar to mercerization, resulted in much higher rates of soil biodegradation. Biodegradation was essentially complete in 19 days.	Tian *et al.* 2022

Chemical Treatments

For different current and potential uses, there are a broad range of chemical treatments of cellulose fibers. This subsection will consider published information related to their effects on biodegradability. Types of treatment to be considered include antimicrobial, hydrophobic, ultraviolet (UV)-blocking, and creation of cellulose derivatives.

Table 12. Highlights from Studies Considering Effects of Various Antimicrobial Treatments

Study Highlights	Citation
The effectiveness of different silver-containing treatments of cotton specimens were compared in soil biodegradation. Agglomeration of the negatively charged Al species resulted in lower effectiveness, which was attributed to less Al^+ ions released.	Klemencic *et al.* 2010
A multi-component treatment that included AgCl as a textile finish was more effective than single-component treatment relative to soil burial tests. The other components included a binder and hydrophobic silane-type monomers.	Simoncic *et al.* 2010b
Treatment of flax fibers with polyquanternium-10 at a concentration of 0.5% was sufficient to completely suppress soil biodegradation.	Renouard *et al.* 2017
Lauric acid treated bacterial cellulose was used as a coating film for antimicrobial food packaging.	Zahan *et al.* 2020

Antimicrobial treatments

Various studies involving treatment of cellulose fibers to achieve antimicrobial effects have also involved evaluation of biodegradation. Table 12 lists some highlights from such studies. Review articles on antimicrobial treatments for wood have been published (Schultz and Nicholas 2003; Freeman and McIntyre 2008; Rajput *et al.* 2023). In general, given their detrimental action towards the microbial populations, the antimicrobial treatments generally reduced the degradation rates.

Hydrophobizing treatments

It can be hypothesized that hydrophobic treatments of cellulose fibers could suppress biodegradation (Park *et al.* 2004). Studies that help to test that hypothesis are listed in Table 13. The work of Zahan *et al.* (2020) is listed again in this table because lauric acid can be used as a hydrophobic agent (Yin *et al.* 2020). The general principle of hydrophobicity contributing to resistance to biodegradation has been reviewed with respect to bioplastics and synthetic plastic materials (Hubbe *et al.* 2021). As shown in the table, the hypothesized negative effect of hydrophobic treatment on rates of biodegradation was generally supported by the experiments.

Table 13. Highlights from Studies Considering Effects of Hydrophobic Surface Treatments

Study Highlights	Citation
Slow biodegradability of cellulose acetate was attributed to its hydrophobic nature. Rayon and cotton were found to be more biodegradable in soil burial tests.	Park *et al.* 2004
A multi-component treatment that included AgCl as a textile finish was more effective in resisting biodegradation than single-component treatment relative to soil burial tests. The other components included a binder and hydrophobic silane-type monomers.	Simoncic *et al.* 2010b
Oleic acid treatment contributed to biodegradation resistance of mulch prepared by delignification of cornhusk.	Hernawan *et al.* 2020
Lauric acid treated bacterial cellulose was used as a coating film for antimicrobial food packaging.	Zahan *et al.* 2020
The onset of biodegradation was delayed by about 3 days by high hydrophobic treatment relative to unfinished cotton. Nevertheless, all cotton fabrics degraded significantly (50 to 70% by 100 days) under the conditions of this study.	Zambrano *et al*. 2021

UV-blocking treatments

Treatment with the anti-UV agent Tinuvin P helped to suppress soil biodegradation, especially during the first couple weeks of soil exposure, in comparison to oleic acid treatment alone of the delignified corn husk fiber (Hernawan *et al.* 2020). In other words, there was a lag period before substantial biodegradation.

Chemical treatments to derivatize the cellulose surface

Because microbes and their cellulolytic enzymes mainly have been evolved to deal with natural cellulosic materials, it makes sense that chemical derivatization treatments would tend to interfere with such action. Related studies are highlighted in Table 14.

Table 14. Highlights from Studies Considering Effects of Chemical Derivatization of Cellulose on Biodegradability

Study Highlights	Citation
Unmodified cotton showed about five times the biodegradation of cellulose acetate during the period of 20 to 115 days of aqueous biodegradation. However, the blend between the two showed enhanced biodegradability.	Suh *et al.* 1996
A cellulose ether phthalate was found to be slower to biodegrade than MCC in a composting test; however, a related sodium salt was more biodegradable than MCC.	Simon *et al.* 1997
Slow biodegradability of cellulose acetate was attributed to its hydrophobic nature. Rayon and cotton were found to be more biodegradable in soil burial tests.	Park *et al.* 2004
Increased acetylation decreased total sugar conversion consistently with various cellulase dosages and times.	Zhu *et al.* 2008
The presence of oxidized functional groups on TEMPO-oxidized nanocellulose inhibited their biodegradation. Removal of those groups allowed subsequent layers of cellulose chains to be biodegraded rapidly.	Homma *et al.* 2013b
A broad range of cellulosic materials were subjected to pilot-scale composting. Biodegradation was strongly repressed with an increasing degree of substitution (DS). Complete inhibition was achieved at a DS level of 1 in some cases.	Leppanen *et al.* 2020

Other treatments of fibers for textiles and papermaking

Textile fibers are often subjected to finishing treatments to aid either in their processing or in the final attributes of fabrics. Li *et al.* (2010) showed that treatment with a softener tended to enhance the biodegradation of cotton and polyester in soil, whereas treatment with a resin had the opposite effect. Likewise, Zambrano *et al.* (2021) observed the greatest degree of cotton fiber degradation in the case of fibers treated with a softener; these were about 89.6% degraded in comparison to 72.2% for the corresponding untreated cotton and 63.0% for durable press cotton fibers. Notably, cellulosic textiles can be processed with cellulase and other enzymes, aiming to achieve desired surface properties in an eco-friendly manner (Stanescu 2023). Thus, in at least some cases, the finishing treatment might already be counted as a start in the direction of full biodegradation. In aquatic environments, this phenomenon tends to be less pronounced, although the biodegradation rate can still be influenced by finishes commonly applied to cotton fabrics. Fabrics treated with crosslinking chemicals such as durable press and water repellents may exhibit initial resistance to biodegradation, particularly on the surface. However, it is noteworthy that even these treated cotton fabrics degrade noticeably under freshwater conditions, exceeding 60% within 102 days of testing (Zambrano *et al.* 2021).

Kraft fibers that are used in preparation of printing and writing papers, as well as some packaging grades, are often treated to increase the hydrophobic nature of the product (Hubbe 2007). Xerographic copy papers additionally are likely to contain fluorescent whitening agents (Hubbe *et al.* 2008). Though colorants are widely used to fine-tune the color of white papers, there are also some strongly colored paper products. Because none of these paper products is likely to be flushed, the most likely fates of printing paper, after it has been used, will be either recycling or landfill. Dyed fibers that are lost from the papermaking process in the course of recycling operations are expected to mostly end up in the thickened sludge from wastewater treatment, which most often becomes another

contribution to landfills (Hubbe *et al.* 2016). Though it is reasonable to expect that strongly dyed papermaking fibers would be more difficult to biodegrade, there is a need for studies to quantify such expected effects.

ENVIRONMENT-RELATED FACTORS

The environmental conditions present during biodegradation are often specified in standard tests. However, in many cases it may be important to know how different conditions affect cellulose fiber degradation. Conditions to be considered in this section include the microbial community or the presence of enzymes, moisture, temperature, C:N ratio, counter-ions (including salinity), and products of decomposition. Another general issue to address is the environmental relevance of various test protocols. Ideally, research can be carried out under conditions that will lead to valid predictions of what will happen either in typical wastewater treatment plants, composting systems, or natural water environments. On the other hand, future studies will also need to consider what happens at the sea floor, for instance, where the cold temperatures and lack of light would tend to slow down various routes to biodegradation of cellulose fibers that settle into such environments.

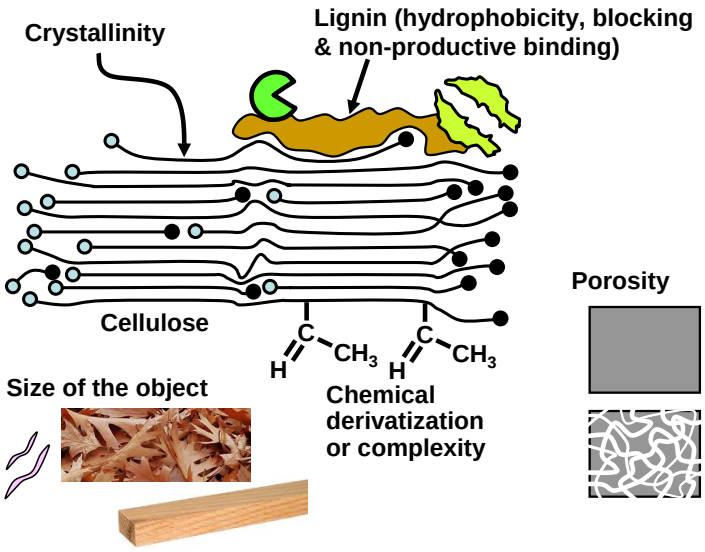

Fig. 16. Illustration of some key attributes of environments that can be expected to affect rates of biodegradation of cellulose fibers

The presence of enzymes

The essential nature of enzymes in the biodegradation of cellulose fibers and plant-based materials has been shown clearly in studies involving inoculation (Greff *et al.* 2022). Thus, Zainudin *et al.* (2022) reviewed studies in which substantial increases in the rates and extents of cellulose degradation during composting were observed after adding cellulase-producing microbes. Related work involving anaerobic conditions was reviewed by Leschine (1995). Here, emphasis was placed on the importance of microbial communities present in different environments.

As noted by Meyer and Edwards (2014), during continued or repeated exposure to certain environmental conditions, even including toxins, the microbial groups can become acclimated and thereby better able to break down the cellulose and other plant materials.

In such communities, different microbes specialize in the production of different enzymes. Rabelo *et al.* (2018) found that such optimized communities were present during anaerobic biological treatment of wastewater from pulp and papermaking. Suh *et al.* (1996) emphasized the synergistic effects of different enzymes working together in cellulose biodegradation of cotton and cellulose acetate. Zambrano *et al.* (2020) documented the microbiological communities that were present in lakewater, activated sludge, and seawater in the presence of cellulose fibers. Though the present review is mainly concerned with degradation of individualized cellulose fibers that are released by means of wastewater systems, it is worth noting that biodegradation of cellulose-containing plant materials also takes place in such environments as rice paddies and wetlands.

Moisture Content

The presence of moisture is an essential requirement for biodegradation. Park *et al.* (2004) found a correlation between the moisture regain of dry fibers and their subsequent rate of biodegradation. Biological processes require moisture, which may differ in terms of optimum levels. Another concern is that moisture levels may be unevenly distributed, *e.g.* in an anaerobic digester or landfill. Due to the importance of this issue, especially during soil degradation and composting, there is a need for more research related to effects of limiting moisture content on cellulose biodegradation. Such studies are inherently difficult to carry out in field conditions due to uneven mixing and distribution of water.

Temperature

Though temperature is expected to have profound effects on biodegradation, it is important to bear in mind that different microbial communities and different enzymes will be favored by different temperature ranges. Thus, as noted by Ahmed *et al.* (2019), cellulose biodegradation can take place effectively over a wide temperature range, *e.g.* 14 to 25 °C in the cited work. Ghasimi *et al.* (2016) found higher rates of biodegradation under thermophilic (55 °C) conditions compared to mesophilic (35 °C) conditions. The thermophilic conditions generally shortened the time required for cellulose breakdown, though the final state after more than about 6 days of treatment was not very sensitive to temperature. These results are in agreement with those of Kim *et al.* (2022). These results also were consistent with earlier work that showed an increase in the first-order rate coefficient for anaerobic cellulose hydrolysis with increasing temperature (Ge *et al.* 2011).

Chen *et al.* (2015) found that increased temperature of an autohydrolysis pretreatment of wood material adversely affected subsequent biological degradation. The effect was attributed to closure of mesopores in the cell walls of the fibers, *i.e.* hornification.

C:N ratio

The progress of composting has been shown to be dependent on having a suitable ratio between the hydrosoluble forms of carbon and nitrogen present (Hubbe *et al.* 2010; Yu *et al.* 2020). Thus, López Alvarez *et al.* (2009) kept a range of C:N between 32:1 and 57:1 for their studies. When the goal is to degrade cellulose, the danger is likely to be too high a proportion of carbon. Though the effect of C:N ratio has been widely considered in systems that contain cellulose in some form (Greff *et al.* 2022), there has been a lack of such studies dealing with cellulose fibers as a component of compost. When considering future studies, a likely hypothesis to guide that work is that a certain range of C:N ratios may be favorable to the biodegradation of cotton or wood-derived fibers. Notably, most

standard tests to assess compostability specify C:N ratios in the range of 10 to 40, or more ideally 15 (Wyman and Salmon 2024). The level of phosphorus compared to carbon may also be considered in such future studies (Chen *et al.* 2022).

Counter-ions

Some remarkable effects related to counter-ions were shown in a series of work by Homma *et al.* (2013a,b). To begin, they showed that TEMPO-mediated oxidation (Saito and Isogai 2004) moderately decreased the biodegradability of bleached softwood kraft cellulosic fibers (Homma *et al.* 2013a). However, there were huge differences depending on the type of counter-ion associated with the carboxyl groups created by the oxidative treatment. Highest biodegradation (about 45% weight recovery after 12 days) was observed for the ammonium form and the sodium form. Essentially no biodegradation was noted with the copper counter-ion form. Only 5% loss in weight recovery was observed when the proton was the counter-ion, *i.e.* the protonated form of TEMPO-oxidized cellulose. However, tests by Homma *et al.* (2013b) showed that the protonated and deprotonated forms of TEMPO-oxidized nanofibrillated cellulose exhibited equally high rates of biodegradation in water in the presence of a crude cellulase. These results suggest that the effects reported in the first article (Homma *et al.* 2013a) were attributable to the growth and viability of the microbes and their production of enzymes in the presence of different counter-ions.

Levels of salinity can be expected to affect the types of microbes that are able to thrive in different situations. For example, it has been proposed that the brackish conditions of the Baltic Sea allowed the warship Wasa, which sank in Stockholm harbor, to remain intact for 333 years until it was recovered and preserved (Sandström *et al.* 2002). On the other hand, it has been shown that biodegradation in natural waters follows the order of fresh water > brackish water > seawater (Miyaji *et al.* 2023). In addition, the studies already cited in this work clearly show that the level of salinity present in the ocean is not a barrier to biodegradation of cellulose (Zambrano *et al.* 2020; Royer *et al.* 2021; Royer *et al.* 2023; Smith *et al.* 2024).

MECHANISTIC ISSUES

This section considers some fundamental mechanistic explanations that have been published relative to cellulose biodegradability and factors affecting it. Given the complexity of the processes considered in this article, involving biological organisms, numerous enzymes, contrasting environments, and effects of numerous controllable variables, it might be regarded as unrealistic to be able to predict rates and extents of biodegradation based on thermodynamics. Rather, one needs to look at published data to find out whether the effects predicted from thermodynamics can be regarded as rate-governing factors. Thus, after thermodynamics, the next topic to consider will be kinetics. The ways in which reaction rates and extents are affected by such variables as concentrations, temperature, and time can help support or rule out various hypotheses about mechanisms. A third category to be considered in this section is analysis of reaction byproducts. Again, such evidence can help explain some of the theoretical predictions and show whether they adequately explain what is happening in practice.

Thermodynamics and its Limitations

Inherent stability of cellulose in its crystalline form

A greater difficulty in breaking down crystalline cellulose, by various means, in comparison to non-crystalline zones, has been proposed based on thermodynamic principles (Beckham *et al.* 2011). It is possible to calculate the amount of energy that must be contributed to decrystallize different crystalline forms of cellulose. Though the cited work provides detailed analysis of cellulose crystalline states, less attention is paid to the amount of bonding energy embodied within amorphous cellulose zones. This may be a key issue to address in future work. As noted earlier, endoglucanases are expected to mainly cleave random glycosidic bonds in non-crystalline zones, whereas exoglucanases are expected to proceed from the ends of cellulose chains, proceeding along the surfaces of crystalline zones (Horn *et al.* 2012; Wang *et al.* 2013b).

Beckham *et al.* (2011) reported that the native cellulose I-beta crystalline form represents a lower energy state in comparison to other crystalline forms of cellulose, *e.g.* cellulose II, which is the common form resulting from industrially important regeneration methods, such as the viscose and lyocell processes. Based on that information, more energy is required to separate a cellulose chain from an edge of the cellulose I crystal, compared to the other crystalline forms.

Stability of cellulose associated with its contrasting crystal planes

Another reason to suspect a high degree of stability of cellulose crystalline zones is their incorporation of two contrasting bonding mechanisms in different crystal planes (Liu *et al.* 2022). Thus, relative to the plane of the anhydroglucose rings, the hydrophilic –OH groups are projected mainly equatorially, thereby contributing to hydrogen bonding within that plane (Yamane *et al.* 2006). By contrast, at 90 degrees to that plane, the chains are held together primarily by van der Waals forces. The idea is that it will be inherently difficult to break both types of bonds simultaneously. Though this concept appears to account for difficulties in dissolving cellulose by means of various solvents, it is unclear whether the same arguments can be applied to cases of enzymatic breakdown.

Inability of thermodynamic factors to account for rates of degradation

Although thermodynamic factors can explain the direction of likely changes in chemical systems, they cannot reliably predict the rates, which will be the next topic to consider. In particular, it is reasonable to expect that such modifications as coatings and chemical derivatization will slow down or block the reaction paths associated with biodegradation. For example, Zambrano *et al.* (2021) observed a small but statistically significant decrease in the rate of cotton biodegradation when comparing dyed cotton specimens to undyed cotton.

Kinetics

Important evidence to the mechanisms of rate-limiting steps in biodegradation can be obtained by examining the rates, including studies of how those rates depend on factors such as concentrations and time. Figure 17 illustrates some key factors that are likely to affect the kinetics of cellulose fiber biodegradation in different cases. As will be discussed below, this includes both the forward and reverse rates of binding of enzymes such as cellulases both to the lignin (non-productive) and to the cellulose itself (which is often productive). A mechanistic, kinetic study (Wang *et al.* 2004) showed that the biodegradation of cotton by different cellulose enzymes can be modeled based on

sequential and parallel actions of cellobiohydrolase and endoglucanase enzymes. In addition, note that when the endoglucanase (pacman item) adsorbs onto lignin, it is shown as having become immobilized. Note that the exoglucanase (shown as a claw-like shape) is shown as being possibly blocked by either lignin by itself of by the density of the cellulose structure, when approached from the polymer ends.

Other important aspects impacting the kinetics are the buildup of inhibitors (such as cellobiose) and their enzymatic breakdown, blockage of enzyme progress by lignin or other debris, such as denatured enzymes, and the status of growth, starvation, or demise of the bacterial or fungal sources of enzymes. These processes can be expected to be affected by rates of diffusion and convection, the latter of which will be affected by agitation of the mixture. There is a need for more study of kinetic aspects, especially under conditions that mimic natural environments and wastewater treatment. In addition, there is evidence that some non-enzymatic mechanisms are involved in fungal breakdown of lignocellulosic materials (Aguiar and Ferraz 2011).

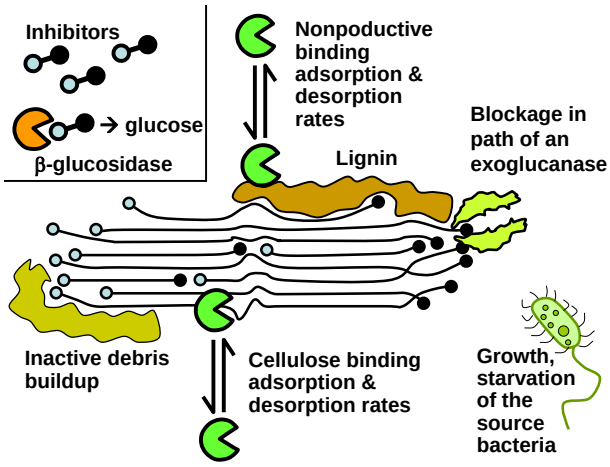

Fig. 17. Factors that can be expected to affect the kinetics of biodegradation of cellulose fibers

Results falling short of 100% degradation

One of the most striking findings, relative to the kinetics of cellulose biodegradation, has been a plateau effect in plots of cellulose hydrolysis *vs.* time. In other words, the rates of hydrolysis tend to decrease with time, and the endpoint falls well short of complete breakdown. Highlights from studies exhibiting such behavior are listed in Table 15.

Table 15. Highlights from Studies Showing Evidence Relative to the Existence of a Plateau of Incomplete Cellulose Biodegradation *vs.* Time

Study Highlights	Citation
Activated sludge degradation of cellulosic fibers was limited to about 20% in 96 hours of mesophilic treatment and 88% in the same time of thermophilic treatment.	Hurwitz *et al.* 1961
Unlike many other examples in this list, these authors showed no plateau in anaerobic digestion of cellulose, though times of greater than 100 days were required for full hydrolysis.	Edberg & Hofsten 1975

Soil burial studies over 20 to 45 days showed a maximum biodegradation of about 73% for regenerated cellulose films and perhaps lower in the case of kraft paper.	Zhang *et al.* 1996a
According to this review article, whereas theory would lead these authors to expect 100% biodegradation of cellulose, plateau levels of about 80 to 90% are typically reported.	Mansfield *et al.* 1999
Biodegradation extents in the range of about 30 to 60% were achieved in 45 days of industrial composting.	López Alvarez *et al.* 2009
Biodegradation rates of cellulose fibers and fabrics decreased with time in aerobic activated sludge treatment.	Niu *et al.* 2012
Cotton fabrics with various finishes biodegraded in soil for 154 days began a plateau in CO_2 production after 140 days but no plateau in weight loss through the end of the study.	Smith *et al.* 2012
Observations of single crystalline cellulose fibers showed marked decreases in hydrolysis rate with time.	Wang *et al.* 2012
MCC appeared to become increasingly recalcitrant to further biodegradation with the passage of time during single- and multi-stage cellulase treatment.	Yu *et al.* 2012b
All-cellulose composites prepared from rayon by means of ionic liquid treatments showed soil biodegradation levels in the range of 38 to 73%.	Kalka *et al.* 2014
Aqueous biodegradation over 96 hours showed plateau levels for MCC (70%), bacterial cellulose (about 80%), and bleached softwood kraft fibers (about 90%).	Kafle *et al.* 2015
Biodegradation levels in the range of 38 to 62% were observed for a range of cellulose types exposed to aerobic wastewater treatment under mesophilic and thermophilic conditions.	Ghasimi *et al.* 2016
Biodegradation of toilet paper fibers during wastewater treatment was generally in the range of 80 to 90% in two aerobic systems for municipal wastewater.	Ahmed *et al.* 2019
Soil biodegradation levels of about 84 and 88% were achieved after soil exposure for 209 days.	Mistriotis *et al.* 2019
A declining rate of cotton and rayon biodegradation was observed over 240 days in natural water. The plot suggests a plateau level of about 65% in the case of rayon.	Zambrano *et al.* 2019
Plots of biodegradation *vs.* time showed clear plateau features for cellulose biodegradation in fresh water, brackish water, and sea water. In lakewater, MCC showed an ultimate biodegradation of about 85%, whereas cotton showed 80%.	Zambrano *et al.* 2020
Microcrystalline cellulose (MCC) reached a plateau of 82% biodegradation within about 7 days of aqueous biodegradation and remained at that level to 27 days.	Kwon *et al.* 2021
Thin-layer chromatography-grade cellulose, presumably MCC, degraded in a quasi-linear manner to about 80% in 20 days and thereafter the rate became very slow.	Rossetti *et al.* 2021
Marine biodegradation of MCC reached about 85% in 28 days, in contrast to a theory-based curve projecting 100%.	Royer *et al.* 2021
A review of the literature estimated that about 30% of cellulose from toilet paper becomes biodegraded in typical aerobic, activated sludge wastewater treatment cycles.	Liu *et al.* 2022
Biodegradation rates in natural water were found to decline with time. The authors reported that after fragmentation, the fine fragments were resistant to further hydrolysis.	Nagamine *et al.* 2022
Marine water biodegradation levels after 28 days were generally in the range 76 to 83%.	Royer *et al.* 2023

When considering the results shown in Table 15, it is important to bear in mind some studies in which contrasting results were obtained, *i.e.* showing essentially 100% biodegradation. For instance, this is what Edberg and Hofsten (1975) observed in the case of very long anaerobic treatment cycles. Leppanen *et al.* (2020) reported essentially 100% biodegradation of many cellulose fiber types under industrial composting conditions.

Various theories have been advanced to explain the tendency of cellulose biodegradation to slow down and even to reach a plateau of stable resistance. Some of these are described below.

Production of inhibitors

Enzymatic cellulose biodegradation can be inhibited due to the buildup of decomposition products, including cellobiose (Yu *et al.* 2012b; Teugjas and Väljamäe 2013; Olsen *et al.* 2016). Thus, Van Wyk and Mohulatsi (2003) observed that cellulose biodegradation by cellulase was inhibited by the buildup of sugars. Meyer and Edwards (2014) described the buildup of inhibitors during the anaerobic treatment of wastewater from pulp and paper manufacturing. Though such effects can be expected, they do not seem to account for the full range of reported findings. In addition to the production of inhibitors to biodegradation, the hydrolysis of polysaccharides also can lead to decreases in pH (Liu *et al.* 2006), which can be expected to affect further biodegradation in various ways. However, a product inhibition effect would not account for the instances biodegradation essentially stopping at certain levels, as shown in many of the examples that were listed in Table 15. It is worth stating that the results of small batch biodegradation reactors are expected to be much more sensitive to inhibitor formation than for biodegradation in large water bodies that have a significant convective flow and thus a strong dilution and diffusion that minimizes inhibitor concentrations near the substrate.

Non-productive adsorption onto lignin and surfactant effects

Another type of inhibition of the action of cellulases can occur due to strong adsorption and apparent immobilization onto the relatively hydrophobic surface of lignin (Huang *et al.* 2022b). Such non-productive binding of the enzymes prevents them from hydrolyzing cellulose. Another way that lignin could explain some of the slow-down and plateauing of cellulose biodegradation is if some of the cellulose is encased in a contiguous layer of lignin (Huang *et al.* 2022b). However, such an explanation would not account for the many published examples in which lignin-free cellulose samples have shown plateau-type biodegradation behavior.

It has been shown in some cases that non-productive binding of cellulase to lignin can be overcome by the use of surfactants. Mizutani *et al.* (2002) showed that the nonionic surfactant polysorbate 20 enhanced the rates of cellulase-induced breakdown of microcrystalline cellulose as well as several regenerated cellulose types. The effect was attributed to overcoming a form of non-productive binding of the enzymes to the cellulose itself. Chen *et al.* (2015) found that increased levels of lignin generally inhibited cellulose biodegradation. A combination of mechanical refining and surfactant addition was found to be especially effective in facilitating cellulose hydrolysis. The refining action would be expected to render more of the cellulose accessible to the enzyme (de Assis *et al.* 2018), whereas the surfactant would be expected to facilitate reversible release of the enzyme from the lignin surfaces (Seo *et al.* 2011). Although surfactants can be expected to help to disperse enzymes from surfaces, the opposite might be expected when treating a mixture of enzymes and cellulosic fibers with a cationic copolymer of polyacrylamide, *i.e.* a

flocculant. Notably, such treatment has been shown to enhance the activity of cellulase (Reye *et al.* 2011). Presumably, the cationic polymer serves as a tether, keeping the enzyme in the neighborhood of the cellulose to be degraded without fully immobilizing it.

Starvation in confined reactors

Plateau effects observed within isolated bioreactors have been attributed to a starvation effect (Mistriotis *et al.* 2019). Thus, it was proposed that biodegradation can be expected to proceed readily during an initial period during which cellulose is abundant in the mixture. But it is reasonable to expect that microorganisms could have a feedback system that slows down the consumption of any remaining cellulose during periods of developing scarcity. The starving microorganisms were proposed to excrete inhibitory chemical agents. It is worth noting here that, whether or not the starvation hypothesis is valid, there might be other unknown contributions to plateau effects, such as depletion of essential nutrients (*e.g.* N, P) or in some cases the recalcitrant nature of lignin, if and when it is present. A search of the literature showed a need for studies addressing the question of whether the biodegradation of cellulose may be sometimes slowed down by insufficient concentrations of nutrients, including trace nutrients.

Deposition of non-hydrolysable substances

Another hypothesis is that non-hydrolysable substances may increasingly coat the surfaces of a remnant of cellulose remaining in a mixture (Kafle *et al.* 2015). These substances might even include denatured or otherwise immobilized cellulase (Yu *et al.* 2012b; Kafle *et al.* 2015). The effect has been called "enzyme blockage" (Yu *et al.* 2012b). Since there have been relatively few definitive studies related to these issues, future research is recommended. For example, tests could be carried out using optional treatments with isolated cellulose binding domains and/or denatured cellulases. The research question is whether or not such pretreatments would inhibit subsequent hydrolysis by active cellulase treatment.

Table 16. Studies that Analyzed the Presence and Concentration of Byproducts of Cellulose Biodegradation

Study Highlights	Citation
Biodegradation products of regenerated cellulose films included oligosaccharides, various sugars, organic acids, glycerol, ethanol, and formaldehyde.	Zhang *et al.* 1996b
Headspace analysis of atmosphere associated with stored books provides clues to mechanisms contributing to their degradation.	Lattuati-Derieux *et al.* 2006
Certain degradation products associated with the aging of books appeared to be decomposition products from lignin.	Dupont *et al.* 2007
Furfural and the breakdown products of fatty acids were associated with the degradation of books during storage.	Clark *et al.* 2011
Headspace analysis was used to detect breakdown products of cellulose decomposition in electrical transformers.	Bruzzoniti *et al.* 2014
Wood decay fungi, during their breakdown of alfalfa stems, produced a range of byproducts, including furfural.	Girometta *et al.* 2017
Products from anaerobic decomposition of pulp and paper mill wastewater included butyric acid along with hydrogen, methane, acetic acid, and hydrogen sulfide.	Rabelo *et al.* 2018

Reaction Product Evidence

Another approach to verifying hypotheses related to the mechanisms of biodegradation is to examine the byproducts that accumulate during cellulose biodegradation. Evidence of this type is highlighted in Table 16. In principle, the reaction products that are found in such studies can be compared to those that would be expected based on specific hypothetical reaction paths.

LIFE CYCLE ISSUES

Raised Cellulose Levels in Environments

The biodegradation of cellulose fibers can be regarded as an essential link in a potentially sustainable cycle. Human-contributed effects can be judged relative to whether they significantly affect natural cycles. Based on literature reviewed in this work, it is not so much the biodegradability, but rather the amounts of cellulose fibers introduced to wastewater that are likely to have adverse environmental effects. Published information of the amounts of such cellulose released to wastewater or released to the environment were summarized earlier in Tables 3 and 4. As has been reviewed, the rates of biodegradability of human-discharged cellulose fibers to the environment is in an intermediate range, often requiring weeks to a few months to be mostly assimilated back into the natural cycle. However, the amounts of cellulose fibers, especially the contribution of sanitary tissue, has a demonstratable effect on the capacity and energy consumption of municipal wastewater treatment plants (Wang *et al.* 2023). It is worth noting that the contribution of cellulose fibers from laundering will be a small fraction of the amount that a typical person will discharge to wastewater as sanitary tissue.

The combination of relatively large amounts of discharged fibers and moderate rates of biodegradation have some practical consequences relative to future priorities:

- To the degree that some of the discharged cellulose fibers pass through or around wastewater treatment operations, one needs to be concerned about their effects on eutrophication (Razza *et al.* 2015). In other words, they can be expected to use up available oxygen in the water as they biodegrade. This concern supports policies to promote efficient wastewater treatment technologies. Ideally, the goal should be to completely remove cellulose before discharge of treated water to waterways.
- Conventional wastewater treatment operations that are optimized for the production of purified water, rather than the full digestion of organic sludge, can be expected to result in 10 to 60% of the incoming cellulose becoming part of the sludge that requires management. The most widely used disposal alternatives for biosolids are landfills and land application. Cellulose that is buried in a landfill, when sufficient moisture is present, is expected to degrade anaerobically, with the generation of methane. The global warming potential (GWP) of CH_4 has been estimated to be about 37 times that of CO_2 (Derwent 2020). It has been estimated that 20 to 30% of the methane fails to be collected, even in modern landfill facilities (work of Levis and Barlaz 2014, listed in the Environmental Protection Agency website, EPA 2023; and Barlaz, EPA 2014). Here there appear to be opportunities for optimized composting. In ideal cases, the resulting compost might have sufficient value as a soil amendment to be sold. A concern is whether the sludge contains excessive levels of contaminants, including organic compounds, that might harm crops. In

addition, any petroleum-based fibers and microplastics present in sludge may adversely affect natural processes due to their tendency to accumulate.

Filtering to Selectively Remove Cellulose from Wastewater

As mentioned earlier in this article, the limited rate of degradation of cellulose fibers, especially toilet tissue fibers, has been found to limit the capacity and completeness of municipal wastewater treatment operations. Several authors have proposed efforts to achieve beneficial usage of such fibers by filtering the incoming water to such facilities. Highlights from related articles are listed in Table 17. The general finding from studies of this type is that the separation of cellulosic fibers from municipal wastewater is feasible.

Table 17. Studies Considering the Filtering, Recovery, and Various Alternative Beneficial Uses of Cellulose Fibers Entering Municipal Wastewater Treatment Facilities

Study highlights	Citation
Recovery of cellulose fibers from incoming municipal wastewater to a treatment plant was demonstrated.	Honda *et al.* 2002
Screens of 0.35 mm mesh size were effective in recovery of cellulose fibers from untreated municipal wastewater.	Ruiken *et al.* 2013
Fine screening was shown to be effective for the collection of cellulose fibers before municipal wastewater treatment. Those materials were shown to be 57 to 62% biodegradable under anaerobic conditions.	Ghasimi *et al.* 2016
Both gravity settling and microsieving were able to remove cellulose fibers from incoming municipal wastewater at >80% efficiency, but the microsieving was able to do so selectively.	Ahmed *et al.* 2019
The cellulose fibers recovered by filtering the intake from municipal wastewater treatment can be used in materials for construction.	Cipolletta *et al.* 2019
These authors studied the biodegradation of cellulose fibers that had not been removed from municipal wastewater, but their article discusses such filtering as an option.	Li *et al.* 2019
Screening, specialized sieving, and settling were compared for the recovery of cellulose fibers from untreated municipal wastewater. Sieves with 0.1 to 1 mm openings were found to be the most effective, allowing up to 94.5% recovery.	Li *et al.* 2020
Cellulose fiber recovery options are reviewed, with an emphasis on rotating filter belt dewatering. About 30% of the recovered cellulose in the filter sludge can be degraded biologically during reported conventional processing under aerobic or anaerobic conditions.	Liu *et al.* 2022
A life cycle assessment (LCA) showed that recovery of cellulose fibers by filtering before municipal wastewater treatment can save 8.6% of the energy needed to run the operations.	Wang *et al.* 2023

Djordjevicová and co-authors (2023) carried out work to determine whether or not the presence of cellulosic fibers would affect the operation of a typical septic tank system, which can be regarded as a type of anaerobic treatment. A matched pair of reactors was set up to mimic septic tank conditions. The process that included cellulose fibers showed similar behavior or a cellulose-free process with respect to most of the measured variables, including temperature, pH, electrical conductivity, turbidity, color, chemical oxygen

demand, and ammonia nitrogen content. Only the level of nitrate nitrogen content was higher for the system to which cellulose fibers had been added. The cited authors concluded that the presence of cellulose fibers had no important effects on the operation of the septic tank processing. The study did not include any analysis of the extent of biodegradation of the cellulose with respect to time.

Though it is clear from the studies listed in Table 17 that cellulose fibers can be effectively collected by filtering untreated wastewater at the intake of a municipal wastewater treatment plant, there has been much less attention regarding ways to achieve value from such wet and contaminated fibers. Options for the recovered fibers can include composting (with production of a stable soil component), anaerobic digestion (with the production of methane or hydrogen fuels), and possible recovery and sanitation to allow use in paper products. Regarding this last option, developmental engineers can rise to the challenge, perhaps by application of bleaching strategies (Dence and Reeve 1996; Bajpai 2012), novel cleaning and sanitation technologies (Henriksson *et al.* 2017), and other unit operations of paper recycling (Doshi and Dyer 1997), to render those fibers free of any smells or perceived contamination issues. In addition, potential uses of such fibers in construction (Cipolletta *et al.* 2019) may avoid the kind of consumer attitude problems of some other potential applications.

Henriksson *et al.* (2017) pioneered a method for recovering paper pulp, along with recyclable plastics and metals, from municipal solid waste. This process could be applied to cellulose-rich fractions obtained from wastewater processes, such as screened materials and waste sludge. By subjecting the waste material, including wastepaper, to a combination of heat and mechanical energy, it undergoes efficient repulping and unintentional sterilization. The effectiveness of repulping fibrous materials within the waste mixture crucially depends on the addition of dilution water and the application of rotational energy. Notably, this patented process, now operational at Toledo, Oregon, is owned by Georgia Pacific. The recovered and sterilized paper fiber, known as Juno™ Fiber, is transformed into new paper products.

Another option that avoids problems of consumer attitude is to convert the recovered cellulose fibers to biofuels. In principle, lignin-free cellulose fibers recovered from wastewater treatment plants have some inherent advantages. The absence of lignin makes such fibers more suitable for saccharification and fermentation to produce bioethanol and related liquid fuel products (Wang *et al.* 2013c; Taha *et al.* 2016). The downside is that such technology has yet to demonstrate large-scale economic viability (Devi *et al.* 2022). Even if economic success is achieved in the future with cellulose-to-bioethanol factories, an individual wastewater treatment plant will likely have too little recovered cellulose fiber to achieve the needed economies of scale. The wet and contaminated nature of the recovered fibers may discourage their transportation to the site of an efficient cellulose-to-ethanol facility.

Wastewater Sludge Options

Landfilling of wastewater sludge

The sludge produced during conventional treatment of municipal wastewater, with its expected content of cellulose fibers, is often landfilled (Barlaz 2006). As was noted earlier, although modern landfill facilities are set up to collect methane that is generated by anaerobic breakdown of cellulose and other biomaterials in the mixture (Kumar *et al.* 2004; Barlaz 2006; Pearse *et al.* 2018), the recovery of methane is not likely to be complete (Huber-Humer *et al.* 2008; Mønster *et al.* 2019; Huang *et al.* 2021), leading to an

environmental impact. Methane has a greenhouse gas equivalency about 37 times greater than that of carbon dioxide (Derwent 2020), noting that its ultimate impact is reduced somewhat due to a much shorter presence in the environment relative to carbon dioxide (Smith *et al.* 2012).

Other processing of wastewater sludge

More environmentally and economically advantageous options can be sought. Some possible options are illustrated in Fig. 18. Due to its content of biodegradable organic materials and moisture, the latter of which can be adjusted by use of a belt press or other thickening technology (Hubbe *et al.* 2010), municipal sludge is a good candidate for composting. The substantial carbon content due to the presence of the cellulosic fibers will be at least partly balanced by the nitrogen content from solid human waste and food waste in the mixture. As noted earlier, a delay of the composting process has been observed, depending on the addition of cellulose fibers in a compost mixture (López Alvarez *et al.* 2009). Such delays are likely attributable to the time required for completion of multiple steps in enzymatic breakage of glycosidic bonds, in combination with the relatively dense, layered structure of cellulose fibers.

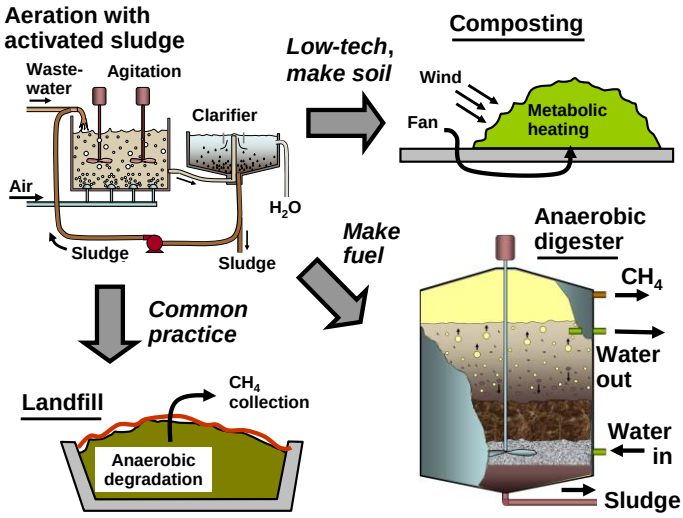

Fig. 18. Possible alternatives to the landfilling of cellulose-rich sludge from wastewater treatment

Life cycle assessment has shown that composting reduces greenhouse gas emissions in comparison to landfilling (Lou and Nair 2009). However, such predictions will depend on details, such as whether it is food waste, yard waste, a windrow composting system, or an engineered composting system (Hodge *et al.* 2016). When a mixture of biomaterials to be composted contains more readily decomposable sugar sources, such as starch, those will tend to be utilized first by microorganisms in preference to cellulose (López Alvarez *et al.* 2009). The progress of composting can be expected to be most favorable when the C:N elemental ratio is about 25:1 to 50:1 (Hubbe *et al.* 2010). When one focuses on treating waste cellulose only, there is a danger of having too high a C:N ratio. Fortunately, the composition of such compost mixtures can be optimized by adding such components as manure (Hubbe *et al.* 2010). By that means, the relative amount of available nitrogen can be adjusted to a favorable range. In addition, industrial composting can achieve sufficiently high temperatures to effectively break down the cellulose

(Leppanen *et al.* 2020). The goal in composting often is to achieve a mature compost, meaning that its organic content has been largely converted into humus-like compounds. These are mainly byproducts of lignin decomposition (Hubbe *et al.* 2010). Their slow rate of further biodegradation allows them to contribute to soil quality by holding water and binding various metal ions (Dinu and Shkinev 2020).

Biofuel production

When wastewater is treated using anaerobic conditions, valuable products can be collected, including methane and/or hydrogen (Hubbe *et al.* 2016; Nguyen *et al.* 2021), which can be used as fuels. The technology is relatively mature. However, it is important to keep one's sense of proportions. The amounts of methane or hydrogen that can be produced in a wastewater treatment operation are often about in the same order of magnitude as the amounts of heat energy and mechanical energy to run the treatment plant itself (Nguyen *et al.* 2021). Most of the methane or hydrogen collected at US wastewater treatment plants is merely flamed off (Shen *et al.* 2015). From a life cycle perspective, the bigger benefits include a net reduction in greenhouse gas emissions and a relatively low discharge of final sludge needing to be landfilled (Bagi *et al.* 2017; Zhen *et al.* 2017).

In addition to methane production, extensive research has been devoted to the conversion of cellulosic resources to liquid fuels, such as ethanol. This can be done either through enzymatic saccharification (Ceaser *et al.* 2024; Joyia *et al.* 2024) or by thermal processing (Hoang *et al.* 2021; Qiu *et al.* 2022). Of these two main approaches, the enzymatic route is generally better suited for cellulosic materials recovered from wastewater, since it does not require evaporation of the water, which has a high demand for energy. However, as noted in the review articles cited above, though both enzymatic and thermal approaches have been shown to be technically feasible, there is a challenge to compete with the current costs of fossil fuels.

Seawater, Microplastics, and Cellulose Fibers

As has been noted, the laundering of textile articles can be a source of small fibrous material, including microplastics (Ladewig *et al.* 2015; Hartline *et al.* 2016; Sillanpää and Sainio 2017; Zambrano *et al.* 2019). These will enter municipal wastewater, and presumably some of it may remain in the treated water, thereby passing into streams leading to the ocean (Ladewig *et al.* 2015). Studies have shown that it is possible to use fine filters to intercept such fibers at the household washing machine or laundromat (De Falco *et al.* 2018, 2019; McIlwraith *et al.* 2019; Erdle *et al.* 2021). However, the noted success reported in such studies suggests that filtering also could take place during wastewater treatment. Two options can be considered. As described above, there already has been work related to collecting fibrous content from incoming wastewater by filtration (see Table 17). A third option, which is likely to be the simplest and cheapest, would be to install and operate suitably fine filters at the discharge point of the wastewater treatment plant. This is where the water would be the cleanest, and thus the demands placed on such filters would be the lowest. As another version of the filtering option, cellulosic fibers and other fibers also would be fully retained on membrane filters, *e.g.* nanofiltration or ultrafiltration (Mohammad *et al.* 2015; Hubbe *et al.* 2016; Al Aani *et al.* 2020), which are sometimes employed as a polishing step when needed at the end of a wastewater treatment operation.

CONCLUDING STATEMENTS

This review has considered the biodegradability of cellulose fibers. Published studies were considered to shed light not only on the rates and extents of biodegradation, but also to understand various factors that affect biodegradation. Important factors include the presence and activity of enzymes, the type of cellulose fibers and their lignin content, and environmental conditions, which can include moisture content, temperature, C:N ratio, and the presence of counter-ions. In general, cellulose fibers, including cotton and wood-based fibers, can be regarded as a natural part of the environment, with potential to fit in well with natural cycles of biodegradation and regrowth of plants. Rates of cellulose fiber biodegradability are generally fast enough to alleviate concerns about long-term buildup of cellulose fibers in waterways. As shown in Table 5, for example, cotton typically biodegraded in the range 30% to 90% over the course of 15 to 90 days. Reported rates of cellulose biodegradation are slower than highly degradable natural polymers, such as starch, but much faster than hard-to-biodegrade synthetic polymers such as polyesters and polyolefins. Though the rates and extents of cellulose fiber biodegradation are different for soils, fresh water, seawater, and various sludge treatment operations, such as anaerobic digestion, most cellulosic material will degrade within several weeks, according to most of the studies cited in this work. An exception to this is the high solids/low liquids environment of a landfill, where biodegradation occurs over decades.

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