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Analysis of environmental biodegradability of cellulose-based pharmaceutical excipients in aqueous media

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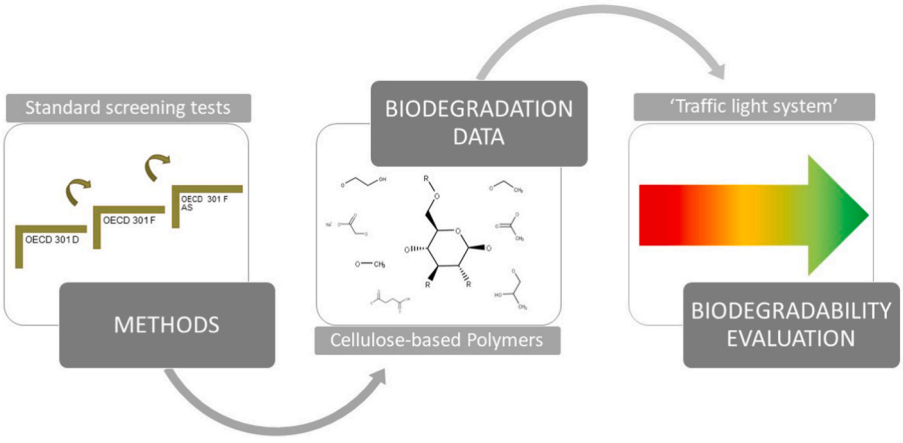
HIGHLIGHTS

- First systematic study on biodegradation of 14 cellulose-based pharmaceutical excipients using OECD 301 standard methods.
- None of the tested cellulose derivatives met the criteria for 'readily biodegradable' classification.
- Identification of potential inhibitory effects on inoculum respiration for 10 compounds.
- Development of a 'traffic light system' for grouping the substances, highlighting structure-biodegradability relationships.

GRAPHICAL ABSTRACT



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ABSTRACT

Pharmaceutical cellulosic polymers will inevitably reach natural water systems if they are not removed after entering wastewater. Biodegradation of organic chemicals in sewage or in the aquatic environment is an important removal mechanism. In this study, we investigated the environmental biodegradation of 14 cellulose derivatives commonly utilized as pharmaceutical excipients using three different test systems that are based on the closed bottle test (OECD 301D) and the manometric respirometry test (OECD 301F). For the different cellulose derivatives tested, we observed varying degrees of biodegradation ranging from 0 to 20.4 % chemical oxygen demand (COD). However, none met the criteria for classification as 'readily biodegradable'. In addition, 10 out of 14 cellulose derivatives and/or their possible transformation products formed during the experiments, may exhibit possible toxic inhibitory effects on the inoculum. This includes one or several derivatives of hydroxy propyl methyl cellulose, hydroxy propyl cellulose, methyl cellulose, ethyl cellulose, and hydroxy ethyl cellulose. Based on the results obtained, we have developed a graded classification score ('traffic light system') for excipient biodegradation. This could help streamline the assessment and classification of cellulose derivatives concerning risk of persistence and potential adverse environmental effects, thereby assisting in the prioritization

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of more favorable compounds. In the long term, however, excipients should be designed from the very beginning to be biodegradable and mineralizable in the environment ('benign by design').

1. Introduction

Polymers, both water-soluble and water-insoluble, represent a major group of formulation excipients in solid oral dosage forms, referred to as 'polymeric excipients (PEs)'. Their functionality ranges from their use as binders and disintegrants to film coatings, to modifiers of release kinetics of the active pharmaceutical ingredient (API) (Debotton and Dahan, 2017). Absorption of PEs by the gastrointestinal track is generally considered negligible. PEs are regarded as pharmacologically inert, non-metabolizable, and excreted primarily in feces as part of the unabsorbed material (Bauer and Lehman, 1951). Consequently, their potential high release into the aquatic environment after excretion could result in considerable environmental concentrations of PEs if they are not removed within sewage treatment or the natural environment. Concerning the overall environmental exposure to PEs, the recent restriction proposal on microplastics by the European Chemicals Agency (ECHA) has estimated the annual usage volume of a single, not further specified PE to range between 500 and 2700 tons, specifically within diffusion-controlled systems (i.e., controlled release dosage form) in Europe (ECHA Annex XV Restriction Report, 2019). However, this estimation does not include water-soluble polymers and polymers used in immediate-release forms, suggesting that the total environmental pollution from PEs could be substantially higher than this estimated range.

Despite extensive industrial applications of polymers, which extends far beyond their use in pharmaceutical industry, quantitative analytical methods to detect polymers in the environment, especially for water-soluble ones, are currently lacking (Huppertsberg et al., 2020). Therefore, quantitative data on their occurrence in the aquatic environment are scarce. Recently, for the first time, environmental data on the concentration of polyethylene oxide (PEO), a commonly used water-soluble polymer within pharmaceutical formulations, in wastewater and surface water samples have been reported (Pauelsen et al., 2023). The authors found that even the readily biodegradable polymer, PEO reached concentrations in the range of μg L^{-1} and raised concerns about the potential environmental impact of non-biodegradable polymers (Pauelsen et al., 2023). Biodegradation represents a key factor determining environmental persistence of chemicals. However, currently only a few studies have evaluated environmental biodegradation of PEs. Their focus was mainly on petroleum-based polymers such as PEO, polyvinyl alcohol and polyvinyl pyrrolidone (Menzies et al., 2023; McDonough et al., 2023; Julinová et al., 2012; Julinová et al., 2018; Vaňharová et al., 2017; Trimpin et al., 2001). However, polymers based on natural occurring substances ('bio-based') constitute the majority of pharmaceutical excipients. This includes in particular cellulose derivatives, such as Hydroxypropyl Methyl Cellulose (HPMC) and Carboxy Methyl Cellulose (CMC). They represent one of the broadest classes of 'bio-based' PEs within pharmaceutical dosage forms (Klein, 2009). Importantly, however, 'bio-based' does not necessarily imply environmental biodegradability (Kwon et al., 2023). Despite their large production volumes in the hundred thousand tons range (Thielking and Schmidt, 2006) and widespread use in various industrial sectors, including the pharmaceutical industry, environmental (bio)degradation remains largely unexplored. In addition, available data on cellulose derivatives with pharmaceutical application are mainly limited to Microcrystalline Cellulose (MCC) Cellulose Acetate (CA) and CMC, which have demonstrated environmental biodegradability (Menzies et al., 2023; Komarek et al., 1993; Van Ginkel and Gayton, 1996). It is well-documented that the biodegradation of the latter two cellulose derivatives declines as their derivatization, i.e., degree of substitution (DS), increases (Erdal and Hakkarainen, 2022). Yet, a systematic study to assess biodegradation of cellulose derivatives is missing. In this study, we used standardized conditions in line with the OECD 301 guidelines to determine environmental biodegradation of 14 pharmaceutical grade cellulose derivatives (Table 1). We employed a modified version of the closed bottle test (CBT, OECD 301D) (OECD, 1992; Friedrich et al., 2012) and the manometric respiratory test (MRT, OECD 301F) (OECD, 1992). This provided novel insight into biodegradation potential and behavior of modified celluloses under different 301 test conditions and revealed structure-biodegradability relationships.

2. Materials and methods

2.1. Chemicals

Table 1 lists the cellulose derivatives used along with their chemical structure, chemical name, trade names, substituent content(s) in percent, degree of substitution (DS), molar substitution (MS) and chemical formulas. Viscosity data and molecular weights of the analyzed cellulose derivatives can be found in the supplementary material (Table S1). Five different viscosity grades of Hydroxypropyl Methyl Cellulose (HPMC) and one viscosity grade of Methyl Cellulose (MC) were obtained from Colorcon Ltd. (Dartford, UK). Hydroxypropyl Cellulose (HPC) in the grades L, SL and M were provided by Nisso Nippon Soda Co., Ltd. (Tokyo, Japan). The Hydroxyethyl Cellulose (HEC) grade Natrosol 250 HX pharm was obtained from Ashland Industries Nederland B.V. (Zwijndrecht, Netherlands). Ethyl Cellulose (EC) Ethocel Standard 10 FP Premium was purchased from Nutrition&Biosciences Ltd. (Beaminster, UK). Hydroxypropyl Methylcellulose Acetate Succinate (HPMCAS) was obtained from Shin-Etsu Chemical Co. Ltd. (Tokyo, Japan). CarboxymethylCellulose (CMC) cross-linked and Microcrystalline Cellulose type 101 (MCC) were obtained from JRS pharma (Rosenberg, Germany). CMC linear was purchased from Ashland. Sodium azide, sodium acetate and N-allylthiourea were purchased from Sigma Aldrich (Steinheim, Germany).

2.2. Evaluation of ready biodegradability

The environmental biodegradability was investigated employing the CBT (OECD, 1992) and the MRT (OECD, 1992), the latter in two different variants using different inoculum sources. The inoculum used for the CBT derived from secondary effluent of a municipal sewage treatment plant (AGL Abwasser, Grün & Lüneburg Services GmbH, Lüneburg, Germany, 325,000 eq. inhabitants). The MRT, on the other hand, was performed with both activated sludge and secondary effluent. Substances that did not pass the CBT test were submitted to MRT using secondary effluent. Substances neither biodegrading in the CBT nor the first MRT were tested in the MRT using activated sludge (MRT-AS). Our experimental design pursues a gradual increase in the probability of biodegradation from the CBT to the MRT-AS, firstly by increasing the inoculum volume and secondly, the microbial diversity with AS (Table S2). The importance of microbial diversity and density for biodegradability was demonstrated in a previous study, where a reduced total cell number accompanied by reduced detectable diversity led to a decrease in 4-nitrophenol biodegradation (Goodhead et al., 2013). Due to the polydisperse nature and heterogenous substitution pattern of the cellulose derivatives assessed, calculation of the theoretical oxygen demand (ThOD) was not possible. Therefore, their biodegradation rate was calculated as the ratio of biological oxygen demand (BOD) to the chemical oxygen demand (COD). The COD values were measured by a reaction kit, i.e., Merck Spectroquant® photometric COD cell tests in the range of 5–80 mg L^{-1} according to DIN ISO 15705. For water-insoluble

cellulose derivatives, we directly transferred the test substance in the COD cells with a concentration range of 300–3500 mg L^{-1}. Table S3 presents a comparison between experimentally determined COD values and ThOD calculations for the cellulose derivatives. The ThOD values were calculated approximately by assuming an endless chain and using the single building block (monomer) as the basis, as previously described (Kümmerer et al., 2011).

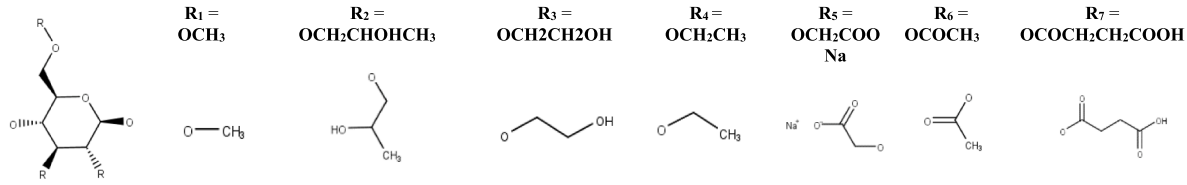
The pass levels to be classified as readily biodegradable are at least 60 % of the measured chemical or calculated theoretical oxygen demand meeting the required 10-day window (OECD, 1992). In addition, the biodegradation of the toxicity test containing both the reference compound and the test substance must exceed 25 % within 14 days (OECD, 1992).

According to the OECD guidelines, the ready biodegradability of the test compound was investigated in closed flasks at a constant temperature (20 ± 1 °C) in the dark within 28 days (OECD, 1992). In Table S2 the different test conditions are summarized (Table S2). Biodegradation of less than 5 % within 28 days was defined as 'no biodegradation'.

2.2.1. Closed bottle test (OECD 301D)

A solution of the PE corresponding to a chemical oxygen demand (COD) of 5 mg L^{-1} prepared in a mineral medium according to OECD guidelines was inoculated with secondary effluent (OECD, 1992). The inoculum concentration of 200 μL L^{-1} was selected to ensure that the oxygen consumption in the blank flasks after 28 days remains below 1.5 mg dissolved oxygen L^{-1}according to the OECD guidelines (OECD, 1992). The test system consisted of "blank", "quality control" using sodium acetate as a readily biodegradable substance, the "test series" and the "toxicity controls". An optode-based technique (Fibox 3, PreSens, Regensburg, Germany) was used to monitor the degree of aerobic biodegradation by measuring oxygen concentration at each day (modified CBT) (Friedrich et al., 2012).

Table 1
Structures of cellulose derivatives including the amount of derivatization in [%] expressed in terms of DS (degree of substitution) and MS (molar substitution).



Chemical name	Trade name	Content [%] *	DS [a]	MS [b]	Chemical formula
Hydoxypropyl methyl cellulose	Methocel K3 LV	R_1 22.9 R_2 7.7	1.3	0.2	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z]_n$
Hydoxypropyl methyl cellulose	Methocel K100 LV	R_1 22.9 R_2 8.7	1.3	0.3	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z]_n$
Hydoxypropyl methyl cellulose	Methocel K4M	R_1 22.5 R_2 7.6	1.3	0.2	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z]_n$
Hydoxypropyl methyl cellulose	Methocel K100M	R_1 21.8 R_2 9.9	1.3	0.3	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z]_n$
Hydoxypropyl methyl cellulose	Methocel E5 LV	R_1 29.5 R_2 9	1.7	0.3	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z]_n$
Hydroxypropyl cellulose	Nisso HPC SL	$R_2$70.2		6.6	$[C_6H_7O_2(OH)_x(R_2)_y]_n$
Hydroxypropyl cellulose	Nisso HPC L	R_2 72.5		7.4	$[C_6H_7O_2(OH)_x(R_2)_y]_n$
Hydroxypropyl cellulose	Nisso HPC M	R_2 73.5		7.8	$[C_6H_7O_2(OH)_x(R_2)_y]_n$
Hydroxyethyl cellulose	Natrosol 250 HX pharm	R_3 46.8		3.2	$[C_6H_7O_2(OH)_x(R_3)_y]_n$
Ethyl cellulose	Ethocel 10 FP	R_4 48.4	2.5		$[C_6H_7O_2(OH)_x(R_4)_y]_n$
Hydroxypropyl methyl cellulose acetate succinate	Hypromellose acetate succinate	R_1 22.2 R_2 6.2 R_6 7.9 R_7 14.7	1.3 0.2	0.2 0.2	$[C_6H_7O_2(OH)_x(R_1)_y(R_2)_z(R_6)_m(R_7)_i]_n$
Carboxymethyl cellulose (linear)	Carmellose	not specified	0.8		$[C_6H_7O_2(OH)_x(R_5)_y]_n$
Carboxymethyl cellulose (cross-linked)	Croscarmellose	not specified	0.7		$[C_6H_7O_2(OH)_x(R_5)_y]_n$
Methyl cellulose	Methocel A4C	R_1 29.8	1.7		$[C_6H_7O_2(OH)_x(R_1)_y]_n$

* Certificates of analysis

[a] DS = Degree of substitution

[b] MS = Molar substitution

2.2.2. Manometric respiratory test (OECD 301F)

The test is performed with higher inoculum density and test concentration compared to the CBT (Table S2). For each test compound two test bottles, one toxicity and one sterile control and three blank and three quality controls were run. The sterile control contained sodium azide (320 mg L^{-1} NaN_3) to monitor abiotic degradation. The concentration of each test substance corresponded to a COD of 30 mg L^{-1}. All test bottles were inoculated with an aliquot of effluent derived from a municipal sewage treatment plant (AGL Abwasser, Grün & Lüneburg Services GmbH, Lüneburg, Germany, 325,000 eq. inhabitants). To 1 L of medium 80 mL of inoculum were added resulting in a higher bacterial density in each test bottle compared to the CBT (Table S2). The OxiTop® (OC110-system, WTW GmbH, Weilheim, Germany) was used as measuring system. Biodegradation was determined by measuring the negative pressure in the closed bottle system that occurs when oxygen is taken up by the microbial population (biological oxygen demand, BOD) to transform organic carbon into CO_2 as described elsewhere in more detail (Trautwein and Kümmerer, 2011).

2.2.3. MRT with activated sludge (MRT-AS)

An additional MRT was performed utilizing 30 mg suspended solids L^{-1} of activated sludge from the same sewage treatment plant as mentioned above. The sludge was washed three times to lower the content or organic matter.

3. Results and discussion

3.1. Biodegradability

In this study, we monitored the ready biodegradability of 14 pharmaceutical grade cellulose derivatives and MCC. All biodegradation curves are available in the supplementary material (Fig. S1, Fig. S2, Fig. S3). For all tests, the validity criteria were met according to the OECD guidelines (OECD, 1992). In all studies, the positive control, sodium acetate, reached > 60 % mineralization by day 14 indicating inoculum activity and meeting test validity criteria (OECD, 1992). The biodegradation extents of all toxicity controls exceeded 25 % indicating no general toxicity to the inoculum of the test compounds (OECD, 1992). In all OECD 301D studies the oxygen uptake values of the blank flasks were below 1.5 mg L^{-1} following OECD requirements (OECD, 1992). The inoculum blanks of all 301F tests reached < 60 mg L^{-1} oxygen uptake at day 28 and over the duration of the study aligning with the OECD criteria (OECD, 1992). For all OECD 301 studies, the replicate variability of biodegradation extent at day 28 was < 20 % meeting the OECD 301 validity criteria (OECD, 1992). The obtained biodegradation results are summarized in Table 2. We found that MCC, tested in the MRT only due to its insolubility in water, biodegraded rapidly reaching 92.3 ± 3.3 % with secondary effluent and 88.1 ± 4.0 % with AS at day 28 (Fig. 1, Table 2). All cellulose derivatives investigated were classified as 'not readily biodegradable' according to the guidelines (Table 2) (OECD, 1992). Virtually no biodegradation (< 5 %) was observed for all cellulose derivatives tested in the CBT with secondary effluent. Similarly, in the MRT with secondary effluent, i.e., increased microbial density and thus diversity (Table S2), most modified celluloses showed no biodegradation (Table 2). Exceptions to this were HPMCAS (17.2 ± 2.6 %), CMC cross-linked (18.4 ± 3.3 %) and CMC linear (14.3 ± 2.6 %) (Fig. 2A, Table 2). For the Methocel K group (HPMC) only, the MRT-AS yielded a readily detectable increase in biodegradation (Fig. S3A, Table 2). The low viscosity grades, K3 and K100 LV (Table S1) yielded the highest biodegradation values of 21.4 ± 6.6 % and 15.8 ± 8.0 %, respectively. Methocel K100 M (highest viscosity grade) (Table S1) showed the lowest biodegradation rate of 11.2 ± 2.7 % within this group (Table 2). The molecular weight corresponds to the viscosity of each derivative within the Methocel K group (Table S1). The results obtained for the Methocel K group showed decreasing biodegradation with increasing molecular weight (reflected in increasing viscosity) suggesting that higher molecular weight compounds in this group hinder biological degradation.

The lack of biodegradation (< 5 %) observed for all cellulose derivatives in the CBT can most likely be attributed to the low microbial density and reduced diversity. The significant impact of inoculum source on biodegradation is supported by a previous study in which 25 %

Table 2
Biodegradability of cellulose derivatives in OECD 301D (secondary effluent), OECD 301F (secondary effluent) and OECD 301F AS (activated sludge). Presented as an average of replicate systems (n ≥ 2) with ± showing standard deviation.

	OECD 301 D	OECD 301 F	OECD 301 F AS	Results according to OECD
Biodegradation level [% COD]				
MCC	n.a.	92.3 ± 3.3 [a]	88.1 ± 4.0 [a]	readily biodegradable
Methocel K3 LV	−2.7 ± 0.9 [b]	−11.9 ± 8.0 [a]	21.4 ± 6.6 [b]	not readily biodegradable
Methocel K100 LV	2.1 ± 1.3 [a]	−18.6 ± 0 [a]	15.8 ± 8.0 [a]	not readily biodegradable
Methocel K4M	−2.7 ± 1 [b]	−5.5 ± 2.1 [a]	13.8 ± 2.6 [b]	not readily biodegradable
Methocel K100 M	−2.1 ± 0.7 [b]	3.8 ± 7.3 [a]	11.2 ± 2.7 [b]	not readily biodegradable
Methocel E5 LV	−2.7 ± 2.6 [b]	−9.2 ± 0.7 [a]	7.6 ± 11.9 [b]	not readily biodegradable
HPC SL	0.5 ± 2.3 [a]	−13.7 ± 10.6 [a]	−7.4 ± 6.0 [b]	not readily biodegradable
HPC L	−5.6 ± 1.7 [b]	−10.7 ± 1.4 [a]	2.5 ± 4.0 [b]	not readily biodegradable
HPC M	1.3 ± 1.4 [a]	−17.9 ± 4.7 [a]	−1.8 ± 0.7 [b]	not readily biodegradable
Hydroxyethyl Cellulose	3.9 ± 3.9 [a]	−12.9 ± 1.4 [a]	−5.0 ± 2.6 [a]	not readily biodegradable
Methyl Cellulose	0.4 ± 0.9 [a]	−17.1 ± 2.1 [a]	−8.7 ± 5.4 [a]	not readily biodegradable
Hydroxypropyl Methyl Cellulose Acetate Succinate	n.a.	17.2 ± 2.6 [a]	6.4 ± 2.8 [a]	not readily biodegradable
Ethyl Cellulose	n.a.	−13.7 ± 2.6 [a]	2.1 ± 6.6 [a]	not readily biodegradable
CMC linear	−3.3 ± 2.9 [a]	14.3 ± 2.6 [a]	12.3 ± 9.4 [b]	not readily biodegradable
CMC cross-linked	n.a.	18.4 ± 3.3 [a]	20.4 ± 9.4 [a]	not readily biodegradable

n.a. = not applicable (water-insoluble test compounds).
[a] n = 2.
[b] n = 4.

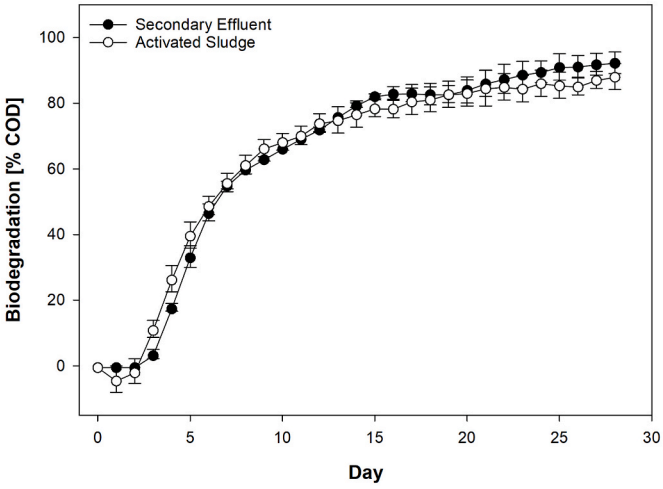


Fig. 1. Biodegradation of MCC in an OECD 301F. Presented as an average of replicate systems (n = 2) with error bars showing standard deviation.

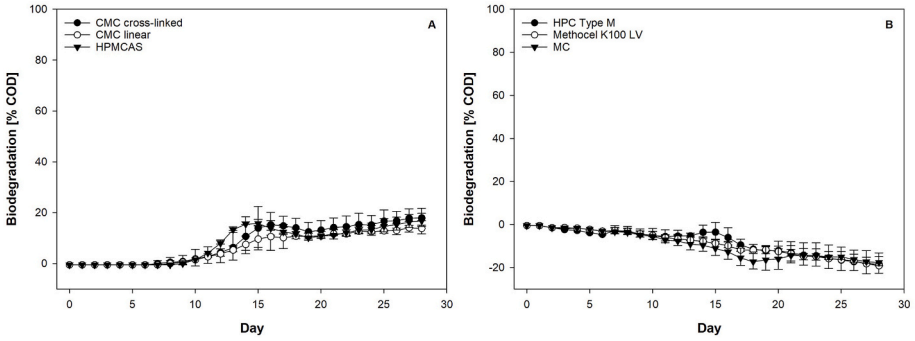


Fig. 2. Biodegradation of cellulose-based polymers in an OECD 301F using secondary effluent inoculum A) CMC cross-linked, CMC linear, HPMCAS B) HPC type M, Methocel K100 LV, MC. Presented as an average of replicate systems (n = 2) with error bars showing standard deviation.

biodegradation of a CMC polymer with DS of 0.7 (in our study: 0.8) was observed in a CBT with AS after 28 days (Van Ginkel and Gayton, 1996). In contrast, our findings revealed no biodegradation in the CBT with secondary effluent (Table 2). This suggests that under the CBT test conditions used in our experiments, a sufficiently high biomass of competent degraders may not have been reached to enable CMC biodegradation within the 28-day period. In addition, given that cellulose-based polymers cannot easily be taken up into bacterial cells due to their size, a sufficiently high production of exoenzymes catalyzing depolymerization is needed prior to microbial uptake and intracellular degradation. This probably involves microbial adaptation processes to CMC, which additionally may contribute to the extended lag phase in comparison to the prior CBT study (Van Ginkel and Gayton, 1996). Consistent with this hypothesis are our MRT and MRT-AS results obtained for a subset of cellulose derivatives comprising CMC polymers (linear and cross-linked), HPMCAS, and Methocel K derivatives, which showed a considerable increase in biodegradation compared to the CBT (Table 2). In contrast to CBT, both tests have a substantially higher bacterial diversity and density in their initial phase, increasing the likelihood that suitable degraders will be present at the critical mass needed for measurable degradation within the 28-day period.

3.2. *Negative deflection in MRT*

A striking feature of the MRT biodegradation curves with secondary effluent observed for most of the cellulose derivatives tested was their negative deflection after day 12. HPC type M, MC and Methocel K100LV (HPMC) reached the highest negative biodegradation values of -17.9 ± 4.7 %, -17.1 ± 2.1 % and -18.6 ± 0 % respectively, at day 28 (Fig. 2-B–Table 2). The negative biodegradation curve is a result of subtracting the blank respiration of the inoculum ('baseline respiration') from the oxygen uptake values of the test substance. This negative deflection indicates that endogenous oxygen-consuming processes in the inoculum may be inhibited, at least in part as respiration values were below baseline respiration. Under our experimental conditions, endogenous respiration of the microorganisms (MO) increases significantly from day 12 (blank flask) suggesting that nitrification processes take place while ammonium is oxidized from the added growth medium (Fig. 3). To support this hypothesis, we conducted a MRT with secondary effluent with the nitrification inhibitor, ATU as the only test substance to measure the inhibition of oxygen uptake. We found that the oxygen uptake rates of ATU decreased significantly from day 12 onwards after subtracting the baseline respiration values (Fig. 3A). This decrease in oxygen consumption from day 12 onwards, after subtracting baseline respiration was observed for 10 out of 14 cellulose derivatives, as shown, for example, for Methocel K100LV in Fig. 3B. Thus, either nitrification processes and/or growth processes of nitrifying bacteria may be disturbed by the presence of these compounds. Nitrifying bacteria are known to be highly sensitive to nitrification inhibition by the presence of toxic compounds (Chhetri et al., 2022).

A possible explanation for the decline in metabolic activity may be the accumulation of non-metabolizable monomeric units after enzymatic cleavage in bacteria. Alpha-methyl glucoside (αMG) is an example of a non-metabolizable glucose analog (Pikis et al., 2006). Non-metabolizable sugar compounds can accumulate intracellularly after uptake by the bacterial phosphotransferase system (Pikis et al., 2006). Accumulation of sugar-phosphates, which can be taken up but not further metabolized like αMG, has been shown to be toxic to bacteria and inhibit their growth (Richards et al., 2013). However, our data do not establish whether extracellular fragmentation occurs and thus the issue remains unsolved. Extracellular cleavage is primarily influenced by the type of the substituent and the degree of substitution (DS) or molar substitution (MS), which affect the accessibility to enzymatic fragmentation (Richardson and Gorton, 2003). In general, lower DS/MS values are associated with a higher enzymatic degradation rate (Erdal and Hakkarainen, 2022). MC is the derivative with the smallest substituent group for which enzyme accessibility and thus fragmentation was expected to be high. MC can be effectively enzymatically cleaved at

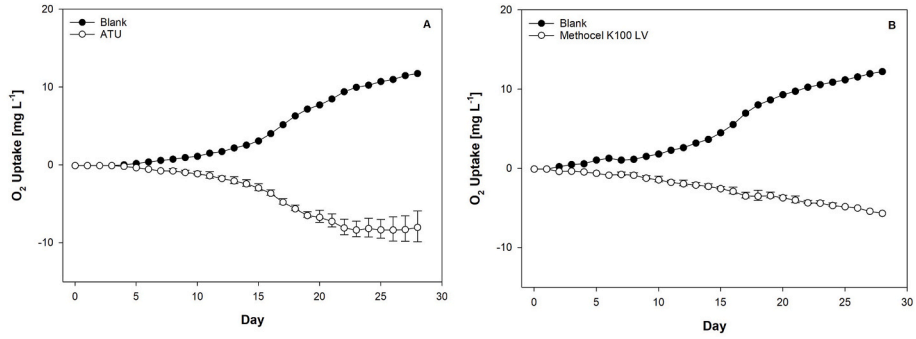


Fig. 3. O_2 Uptake [mg L^{-1}] in an OECD 301F using secondary effluent inoculum A) ATU B) Methocel K 100LV. Presented as an average of replicate systems (n = 2) with error bars showing standard deviation.

DS values of up to 2.1 (Saake et al., 1998). In our study, MC with an average DS of 1.7 was investigated. Accordingly, enzyme-induced chain scission is highly likely to occur. A study conducted by Dow Chemical Co. reported 73 % $^{14}CO_2$ conversion of MC (DS ~ 1.9) with AS obtained from the Dow chemical Co. wastewater treatment plant (Blanchard et al., 1976). This finding contrasts with the 'negative degradation percentages' obtained in our study with MC (DS ~ 1.7). A possible explanation, for this apparent discrepancy is that the activated sludge used in the Dow Chemical study, was pre-adapted to the compound due to high exposure to MC and related compounds in the wastewater treatment plant. The impact of microbial adaptation on biodegradation has been described previously/is well known (Poursat et al., 2019). It is important to note that pre-adaptation of the inoculum prior to biodegradability testing will invalidate results according to OECD test guidelines (OECD, 1992). Enzymatic depolymerization has been demonstrated for highly substituted HEC (MS > 3) and HPC derivatives (Cheroni et al., 2012). Thus, taking into consideration these previous findings, partial fragmentation of the modified cellulose backbone cannot be ruled out in our experimental conditions. Consequently, this may have led to the formation of nonmetabolizable degradation products with potential adverse, i.e., toxic effects on MO (see also above).

3.3. Potential biodegradable cellulose derivatives

In contrast to the 'negative degradation percentages' of the Methocel K group obtained from the MRT with secondary effluent, they showed a significant increase in mineralization rate with activated sludge (Table 2). This indicates their potential for biodegradation in presence of activated sludge containing competent microbial degraders. Like MC (DS ~ 1.7), the analyzed Methocel K derivatives have predominantly methyl content (DS ~ 1.3), albeit to a lesser extent (Table 1). Their MS values (hydroxypropyl) of ~ 0.2–0.3 can be considered low in comparison to HPC (MS ~ 7) and HEC (MS ~ 2.5) (Table 1). The reduced DS and lower MS values compared to the mentioned cellulose derivatives explain the observed increase in biodegradation for the Methocel K group. In addition, we found that the degradation value of Methocel E5 LV, despite lower viscosity and hence molecular weight compared to Methocel K100LV, K4M and K100 M (Table S1) but with a higher degree of methyl group functionalization (DS ~ 1.7), exhibited the lowest degradation of 7.6 ± 11.9 among the HPMC derivatives in MRT-AS (Table 2). This suggests that the degree of polymerization (DP), represented by viscosity, may have a lesser influence on biodegradability compared to the extent of substitution. However, as discussed in the 'biodegradability' section for the Methocel K group, molecular weight might still have an influence on biodegradability. The fact that no plateau was reached after 28 days for the Methocel K group indicates that the degradation process was still ongoing, and the MO had not achieved complete biodegradation of HPMC derivatives within this period. This suggests that HPMC-degrading MO may need a longer adaptation time and have slower growth rate under the test conditions evaluated. Other cellulose derivatives for which partial degradation was observed and was not complete after 28 days (no plateau reached) are the CMC polymers and HPMCAS (Fig. 2A). The results of this study for linear and crosslinked CMC (DS 0.8 and 0.7) are in good agreement with the findings of a recently published study (Menzies et al., 2023). In this study, a CMC polymer with a DS of 0.6 was subjected to an 'extended' OECD 301 B test (for 148 days) and reached 20 ± 2.4 % $ThCO_2$ after 28 days and ultimately 70 ± 2.3 % (Menzies et al., 2023). This partial biodegradation is well reflected in our results from the MRT where we achieved 14.3 ± 2.6 % for linear CMC and 20.4 ± 9.4 % for cross-linked CMC (Fig. S2C, Fig. S3C, Table 2). This strongly indicates the ability of MO to utilize carboxymethylated oligomers as a carbon source after an extended adaptation phase. However, it remains unclear whether test extension would result in a plateau of incomplete degradation or of full mineralization. HPMCAS contains both ester (acetate and succinate) and ether substituents (methyl and hydroxypropyl) (Table 1). Ester linkages are commonly known to be more easily hydrolyzed than ether linkages and generally increase biodegradability (Boethling et al., 2007). The increase in biodegradation due to the interplay of esterase activity and cellulose degrading enzymes has been found in previous studies for cellulose acetate (Yadav and Hakkarainen, 2021). Hence, the increased biodegradation of HPMCAS could be due to the existence of esterase-enzymes, facilitating the initial steps of degradation, and exposing the cellulose backbone to further enzymatic degradation.

3.4. Classification of biodegradability

Based on the collective data presented here, we suggest that the extent of degradation and the features of the biodegradation curves can serve as a framework to categorize the biodegradability of the studied modified celluloses into two distinct groups (Table 3). The first group (Group I) had not reached a plateau phase indicating possible ongoing biodegradation. The components of Group I can be further classified into the subgroups 'moderately' (Ia) and 'slightly/weakly' biodegradable (Ib) based on the percentage of biodegradation as previously proposed (Beiras and López-Ibáñez, 2023). In contrast, the second group (Group II) displayed a negative deflection or negligible degradation percentages (< 5 %) with an evidenced plateau phase. Thus, given lack of biodegradation in the OECD 301 screening assays, our results suggest that degradation of Group II compounds may also not occur in surface water with low bacterial density and diversity. Therefore, we recommend classifying these compounds as 'non-biodegradable' (Table 3).

Furthermore, the relationships described here between the biodegradability and factors such as DS, MS, DP, and the increase in ester linkages (see section 'potential biodegradable celluloses') allow for an extended scoring system of cellulosic excipients beyond the initial Group I/Group II categorization (Fig. 4; biodegradability is color-coded ranging from green 'readily biodegradable' to red 'non-biodegradable'). An important benefit of the traffic light scoring system is highlighted by recent data demonstrating that test extension yielded higher mineralization of polymers, indicating the inadequacy of the 28-day test duration in fully assessing biodegradation extent (McDonough et al., 2023). The scoring system allows to make predictions on which compounds are likely to undergo further biodegradation with extended test period. For example, Group I categorized compounds are likely to undergo further degradation as, indeed, has been described for CMC (Menzies et al., 2023), whereas an extension of Group II molecules may not result in further degradation. However, it is important to emphasize that one should not generally assume that additional biodegradation will occur within a longer test period. Considering the potential challenges associated with extending the testing duration, which significantly increases the time and resources required for the assessment process, the scoring system offers valuable insights in particular for both industry and regulators. Our research has demonstrated the suitability of using the '28-day OECD 301 based classification approach' (Fig. 4, Table 3) as an aid in determining whether an extended period will lead to further degradation. Moreover, it provides an initial method to prioritize PEs in pharmaceutical formulations, considering their potential environmental persistence.

4. Conclusions

To the best of our knowledge, this study is the first comparison of biodegradability data for the cellulose derivatives most used in pharmaceuticals. The data were acquired under standardized conditions and using the same source of inoculum. The outcome has laid the groundwork for a biodegradability classification system. It provides several benefits including a decision-making tool in the selection of both further investigations of biodegradability and for incorporation into pharmaceutical formulations. Moreover, the traffic light system can aid the selection of structures that may serve as potential candidates for the rational design of variants with increased biodegradability, following

Table 3
Biodegradability classification of cellulose derivatives according to OECD 301 results.

Readily biodegradable ≥ 60 %	Moderately biodegradable 20 - 59 %	Slightly biodegradable 5 - 19 %	Non biodegradable < 5 %
MCC	Methocel K3 LV CMC cross-linked	Methocel K100 LV Methocel K4M Methocel K100M Methocel E5 LV HPMCAS CMC linear	HPC SL HPC L HPC M HEC MC EC
	Group Ia	**Group Ib**	**Group II**

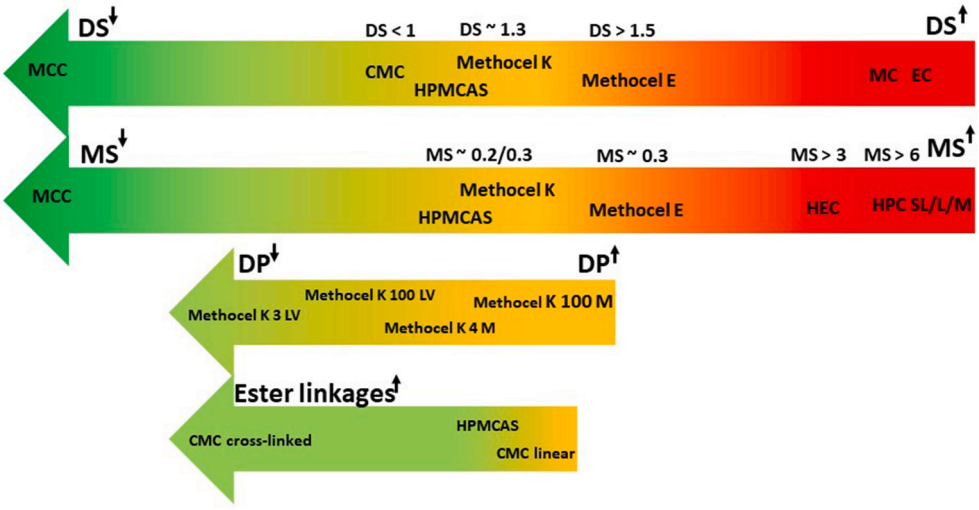


Fig. 4. Color-coded relationship between biodegradability and DS (degree of substitution), MS (molar substitution), DP (degree of polymerization), Ester linkages of cellulose derivatives.

the principle of 'benign by design'.

CRediT authorship contribution statement

Mila Bading: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Oliver Olsson:** Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. **Klaus Kümmerer:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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