Chemosphere 368 (2024) 143739

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Assessing the aquatic biodegradation potential of polymeric excipients for pharmaceutical formulation

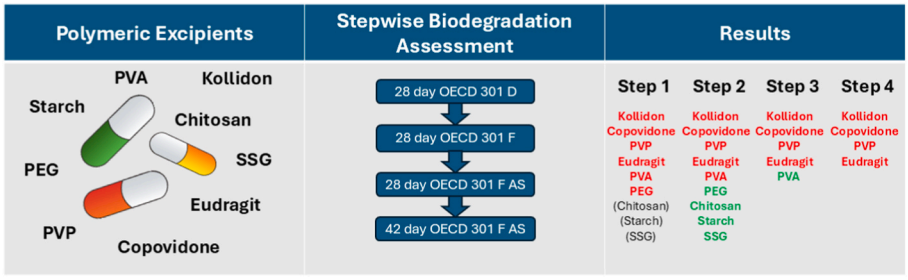
Mila Bading *, Oliver Olsson, Klaus Kümmerer **

*Institute of Sustainable Chemistry, Leuphana University Lüneburg, C13.205, 21335, Germany*

## HIGHLIGHTS

- Biodegradation assessment of ten polymeric excipients using OECD 301 standard tests.
- Variations in biodegradation extents of PEG and PVA under different test conditions.
- Environmental persistence identified in five out of the ten polymeric excipients.
- Prolonged tests for 'non-biodegradable' compounds did not increase biodegradation.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

Handling Editor: Alvine C.Mehinto

*Keywords:*
Water-soluble polymers
Biodegradability
OECD 301
Wastewater
Nitrification

## ABSTRACT

Polymeric excipients (PEx) are essential in drug formulation but raise environmental concerns upon wastewater release post-administration due to their potential detrimental effects to life-histories of freshwater vertebrates and invertebrates. Ten pharmaceutical polymeric compounds were assessed in a stepwise environmental biodegradation assessment according to standard OECD 301 guidelines to thoroughly evaluate biodegradability of these compounds. Polyvinyl alcohol (PVA), polyethylene glycol (PEG), chitosan, maize starch, and sodium starch glycolate (SSG) were found to be 'readily biodegradable,' although PVA and PEG showed variation across employed test systems. PEG and PVA did not degrade in OECD 301D tests having low microbial density and diversity. In contrast, in the OECD 301F tests i.e., higher microbial density and diversity, PEG exhibited 73.0 ± 3.3 % biodegradation, while PVA showed 91.2 ± 8.0 % biodegradation with secondary effluent and activated sludge, respectively. Polyvinyl pyrrolidone (PVP), Copovidone, Kollidon CL, and Eudragit derivatives EPO and L100-55 were categorized as 'non-biodegradable' (< 10 % biodegradation). No increase in degradation was observed after 42 days. This indicates their environmental persistence. This study lays the groundwork for a comprehensive understanding of the biodegradation potential of pharmaceutical polymers. It considers the influence of test conditions, inoculum sources, and compound characteristics. The environmental persistence of certain PEx underlines the urgent need to use more environmentally biodegradable alternatives in drug formulation.

* Corresponding author.
** Corresponding author.
*E-mail addresses:* mila.bading@leuphana.de (M. Bading), klaus.kuemmerer@leuphana.de (K. Kümmerer).

https://doi.org/10.1016/j.chemosphere.2024.143739
Received 3 June 2024; Received in revised form 5 November 2024; Accepted 11 November 2024
Available online 29 November 2024

## 1. Introduction

Polymers are key formulation excipients for pharmaceutical drug products. Pharmaceutical excipients are ingredients that are considered pharmacologically inert and play crucial roles in drug formulations. Their functionalities range from their use as binders, disintegrants, and diluents to coatings, among others (Debotton and Dahan, 2017). The environmental risks associated with water-soluble polymers (WSPs) have recently gained attention due to concerns of their persistence and toxicity in freshwater ecosystems, however their environmental impact is still largely unexplored. (Arp and Knutsen, 2020; Huppertsberg et al., 2020; Albright and Chai, 2021; Robison-Smith et al., 2024). The WSPs polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), polyacrylic acid (PAA) and polyethylene glycol (PEG) are extensively utilized as excipients by pharmaceutical industry (Debotton and Dahan, 2017). Apart from their use in the pharmaceutical industry, these polymers find applications in various sectors such as personal care products, cosmetics, agrochemicals, household cleaning products, and water treatment (Nigro et al., 2023). The production volume of WSPs in Europe can be estimated by examining the annual production of the respective monomers, as these monomer products, mainly used in polymer synthesis, are registered under REACH (Huppertsberg et al., 2020). In Europe, the estimated production volumes exceed $10^3$ tons per year (PVP) (ECHA (I)), between $10^5$-$10^6$ tons per year (PVA and PAA) (ECHA (II); ECHA (III)) and more than $10^6$ tons per year (PEG) (ECHA (IV)). Despite extensive industrial applications of polymeric excipients (PEx), quantitative data on their environmental concentrations are currently scarce (Huppertsberg et al., 2020). Available studies revealed PVP concentrations ranging between 0.9 mg $L^{-1}$ and 7 mg $L^{-1}$ in effluents from wastewater treatment plants, as well as concentrations around 0.1 mg $L^{-1}$ in river water (Antić et al., 2011). In addition, polyethylene oxide (PEO) concentrations in effluent and surface water samples have been recently reported to reach up to 20 μg $L^{-1}$ in effluent and concentrations exceeding 1 μg $L^{-1}$ in surface water samples (Pauelsen et al., 2023). The ubiquitous presence of water-soluble polymers in the environment raises concerns about their potential ecotoxicological effects (Nigro et al., 2023; Robison-Smith et al., 2024). A recent study reported reproductive impairment in the aquatic organism *Daphnia magna* when exposed to PVP, PVA, PEG or to PAA at concentrations ranging from 5 to 10 mg $L^{-1}$ (Mondellini et al., 2022). In addition, Nigro et al. observed ecotoxicological effects of PVP, PVA, and PAA on zebrafish embryos at three concentrations (0.001, 0.5, and 1 mg $L^{-1}$), calculated as expected environmental concentrations, by monitoring alterations in swimming behavior. Therefore, PEx that reach the aquatic environment pose potential risks to aquatic systems (Nigro et al., 2023).

Under the umbrella of the 'European Green Deal', the EU has implemented the 'Zero Pollution Action Plan for Air, Water and Soil' (EC, 2019; EC, 2021). As part of this action plan, the 'Chemicals Strategy for Sustainability' has been adopted, which catalyzed the current REACH revision to include the registration of polymers (EC, 2020). Further, the EU Taxonomy, a classification system identifying environmentally sustainable economic activities, is guiding companies towards aligning with green standards and fostering environmental responsibility. The pharmaceutical industry has recently come under the scope of this regulation, and in this context, the EU Taxonomy emphasizes the preference for the use of more biodegradable ingredients in drug products (EC, 2023). Against this background of the EU's policy at transitioning towards a toxic-free environment, it is necessary to provide standardized data on the biodegradation of PEx.

We recently carried out a comprehensive analysis in accordance with OECD 301 guidelines of cellulose-based polymers used in the pharmaceutical industry and developed a graded classification score of their biodegradability to lay the groundwork for aligning with the 'benign-by-design' concept (Bading et al., 2024). Building upon this work, our current study, aimed for a thorough biodegradation assessment focusing on non-cellulose-based polymers, which represent the most frequently used polymers in drug product development. We analyzed 10 different PEx including the vinyl polymers PVP, Copovidone, Kollidon, and PVA, along with Macrogol 6000 (PEG), the natural polymers chitosan, maize starch, its derivative sodium starch glycolate (SSG), and the polymethyl methacrylate polymers (PMMA) Eudragit EPO and Eudragit L100-55.

A recent multi-laboratory study (McDonough et al., 2023) demonstrated high reproducibility of OECD 301B and 301F test protocols for assessing polymer biodegradation, endorsing their suitability for polymeric substances. Among others, PEG and PVA were found to be readily biodegradable. While available data suggest that PVP may not biodegrade due to the lack of hydrolyzable or oxidizable functional groups in its carbon backbone, these data do not align with OECD 301 guidelines (Trimpin et al., 2001; Swift, 1994). However, the extent of PVP degradation may be influenced by factors such as the origin of activated sludge and the presence of other substrates (Julinová et al., 2012), rendering its biodegradability unclear. We included these polymers (PVA, PEG and PVP) to generate directly comparable biodegradation results, thereby establishing a consistent biodegradation dataset.

For this research study, we employed a stepwise method with sequentially conducted screening biodegradation tests, each incorporating increasing levels of microbial density and diversity. This approach aims not for authorization but provides in-depth insight into biodegradation performance and allows for the comparison of results obtained under different test conditions. By varying the size and source of the inoculum, we will gain insight into possible mechanisms influencing probability and extent of biodegradation (Bading et al., 2024).

This comprehensive systematic analysis of biodegradability according to different OECD 301 protocols supports the selection of environmentally benign excipients and promotes the development of pharmaceutical products with enhanced biodegradability, aligning with the 'benign-by-design' concept.

## 2. Materials and methods

### 2.1. Chemicals

Polyvinyl pyrrolidone, Kollidon CL (crosslinked polyvinyl pyrrolidone) and Kollidon VA 64 (Copovidone, polyvinyl pyrrolidone–vinyl acetate copolymer) were provided by BASF (Ludwigshafen, Germany). Macrogol 6000 (PEG) was purchased from Ter Hell & Co. GmbH (Hamburg, Germany). Polyvinyl alcohol 28–99 (degree of hydrolysis 99 %, MW ~ 130,000) was obtained from Merck Chemicals GmbH. Starch 1500 was provided by Colorcon Ltd. (Dartford, UK). Sodium starch glycolatewas purchased from Roquette (Lestrem, France). Eudragit EPO and Eudragit L100-55 were obtained from Evonik Operations GmbH (Essen, Germany). Sodium azide, sodium acetate and chitosan were purchased from Sigma Aldrich (Steinheim, Germany).

### 2.2. Biodegradability in OECD 301D and OECD 301F tests

The assessment of biodegradation involved two OECD 301 screening tests, i.e., the optode-based (Fibox 3, PreSens, Regensburg, Germany) closed bottle test (CBT, OECD 301D) (OECD, 1992; Friedrich et al., 2012) and the manometric respirometry test (MRT, OECD 301F) using the OxiTop® system (OC110 system, WTW GmbH, Weilheim, Germany) (OECD, 1992). The latter was performed in two different variants using different inoculum sources. The analytical endpoint was oxygen consumption for both CBT and MRT. In addition, for soluble test compounds, the dissolved organic carbon (DOC) elimination (ASI-V autosampler, TOC-VCPN analyzer, Shimadzu, Germany) was monitored within the MRT test set-up after 28 days to confirm oxygen uptake results. As illustrated in Fig. 1, the experimental approach followed a stepwise procedure to increase the probability of biodegradation as follows. First, the test compounds were subjected to a CBT with secondary effluent and hence low bacteria density (step 1). It is important to note that the CBT is applicable to water-soluble test substances under

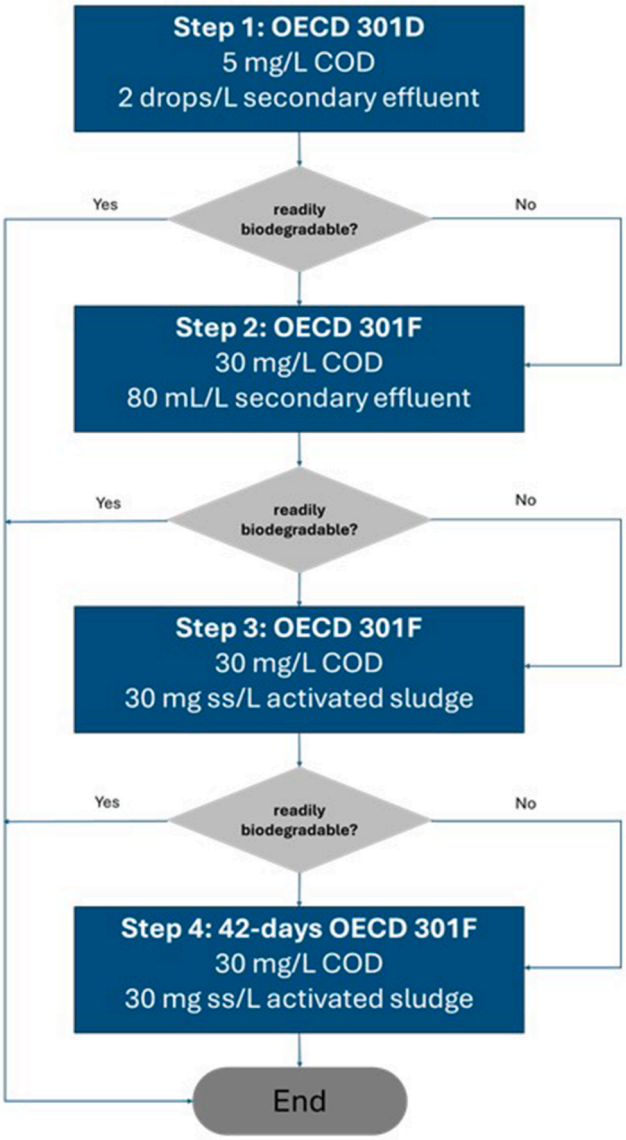



**Fig. 1.** Workflow of the biodegradability testing.

our test conditions. Substances that are not water-soluble were directly submitted to step 2. Substances that failed the CBT (step 1) were submitted to a MRT with secondary effluent and higher bacterial density (step 2). Substances neither biodegrading in the CBT nor in the MRT with lower bacterial mass were tested in a MRT with higher bacteria mass and diversity using activated sludge (MRT-AS, step 3). In addition, substances not meeting the ready biodegradability criteria in the MRT-AS over 28 days, were tested in an extended 42-day MRT (step 4). The CBT and MRT concentrations of the PEx were set at 5 mg $L^{-1}$ chemical oxygen demand (COD) and 30 mg $L^{-1}$ of COD, respectively (Fig. 1). These concentrations were determined through Merck Spectroquant® photometric COD cell tests in the range of 5–80 mg $L^{-1}$ or 300–3500 mg $L^{-1}$ according to DIN ISO 15705. Table S1 presents the determined COD values for the assessed PEx. The monomeric compounds used in this study included sodium acetate and N-methyl-2--pyrrolidone (NMP). Sodium acetate served as a reference compound, while NMP was included due to its structural similarity to the PVP monomer with respect to the presence of a lactam ring. For the monomeric compounds in this study, the initial concentrations were based on their theoretical oxygen demand (ThOD) and were set at 5 mg $L^{-1}$ and 30 mg $L^{-1}$ of ThOD for the CBT and MRT, respectively. The CBT and MRT were inoculated with 2 drops $L^{-1}$ (~200 μL $L^{-1}$) and 80 mL $L^{-1}$ of secondary effluent from a municipal sewage treatment plant (AGL Abwasser, Grün & Lüneburg Services GmbH, Lüneburg, Germany, 325000 eq. inhabitants), respectively (Fig. 1). The secondary effluent was filtered through a filter paper before use. For the 28-day MRT-AS and 42-day MRT-AS 30 mg suspended solids $L^{-1}$ of AS from the same treatment plant (see above) was utilized (Fig. 1). The sludge was washed three times with tap water before use to reduce the organic matter. The mineral media were prepared according to the corresponding OECD guidelines 301D and 301F (OECD, 1992). All tests consisted of 'blank', 'quality control' (sodium acetate), 'test series' and 'toxicity control'. The MRT also contained a 'sterile control' containing 320 mg $L^{-1}$ $NaN_3$ to monitor abiotic degradation. CBT test runs were performed in parallel with two test bottles each for the blank value, quality control, test series and toxicity control. For MRT, each test compound was run in two test bottles, along with one toxicity control, one sterile control, and three blanks and three quality control bottles. Replicates of CBTs and MRTs ($n \geq 2$, as indicated in Table 1) were performed for each test compound. To obtain the 42-day MRT-AS, the duration of the test for two MRT-AS replicates was extended to 42 days to monitor prolonged biodegradation.

In all experiments, the pH values on days 0, 28, and 42 were measured to ensure they were within the range of 6.0–8.5, as required by the OECD guidelines (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 the testing duration (OECD, 1992). Biodegradation extent was expressed as the ratio of biological oxygen demand (BOD) to COD. Biodegradation of less than 5 % at test end was defined as 'no biodegradation'. The lag phase was defined as time from start of a test until degree of biodegradation reached 10 %.

### 2.3. Classification of biodegradability

According to the comprehensive data gathered in this study, the PEx were categorized based on their degradation levels into the following subgroups as previously outlined: 'readily' in line with the OECD test guidelines (≥ 60 %), 'moderately' (20–59 %), 'slightly' (5–19 %), and 'non-biodegradable' (< 5 %) (Bading et al., 2024).

## 3. Results and discussion

### 3.1. Biodegradability according to OECD 301D and F

In our study, we investigated the environmental aquatic biodegradability of 10 pharmaceutical grade PEx. For all tests, the validity criteria were met according to the OECD test guidelines (OECD, 1992). None of the tested compounds was toxic to the inoculum according to the guidelines, biodegradation of the toxicity test vessels containing both the reference compound, and the test substance exceeded 25 % within 14 days (OECD, 1992). The obtained biodegradation results are summarized in Table 1. In the OECD 301D (closed bottle test, CBT) with secondary effluent as the inoculum source, we found no biodegradation (< 5 %) for any of the PEx tested (Table 1). Maize starch, chitosan, and SSG were not tested in the CBT due to their insolubility in water. The OECD 301F results revealed that Macrogol 6000 (PEG), PVA, maize starch, SSG, and chitosan classified as 'readily biodegradable' according to the OECD 301 test guidelines (OECD, 1992). By day 28 of testing, these compounds displayed oxygen consumption values > 60 % of COD. Importantly, the 10-day window criterion does not apply to polymers, as they are known to degrade sequentially (OECD, 2006). All other investigated PEx were classified as 'not readily biodegradable' (Table 1).

We observed a notable difference in the classification for 'readily biodegradability' of PVA and PEG between the OECD 301D and OECD 301F tests (Table 1). This suggests that under stringent CBT test conditions ($10^4$ - $10^6$ approx. cells $L^{-1}$), a sufficiently high biomass of competent degraders may not have been reached. This could have

**Table 1**
Biodegradability of polymeric excipients in OECD 301D (secondary effluent), OECD 301F (secondary effluent) and OECD 301F AS (activated sludge). Presented as an average of replicates($n \geq 2$) with ± showing standard deviation.

| | OECD 301D | OECD 301F | OECD 301F AS | 42 d OECD 301F AS | Results according to OECD |
|---|---|---|---|---|---|
| | Biodegradation level [% COD] | | | | |
| PVP | −0.2 ± 2.1 [b] | −4.0 ± 1.4 [a] | 0.0 ± 1.4 [b] | 1.8 ± 5.6 [a] | not readily biodegradable |
| Kollidon CL | n.a. | −4.4 ± 2.8 [a] | −6.7 ± 4.6 [c] | −0.8 ± 4.0 [a] | not readily biodegradable |
| Copovidone | −3.0 ± 2.6 [b] | −7.6 ± 4.7 [a] | −0.1 ± 3.9 [b] | 0.8 ± 4.0 [a] | not readily biodegradable |
| Macrogol 6000 (PEG) | 1.6 ± 1.4 [a] | 73.0 ± 3.3 [a] | | | readily biodegradable |
| PVA | −2.9 ± 2.2 [b] | 5.7 ± 3.3 [a] | 91.2 ± 8.0 [a] | | readily biodegradable |
| Maize starch | n.a. | 69.2 ± 7.3 [a] | | | readily biodegradable |
| Sodium starch glykolate | n.a. | 60.3 ± 10.6 [a] | | | readily biodegradable |
| Chitosan | n.a. | 125.8 ± 0.0 [a] | | | readily biodegradable |
| Eudragit EPO | −2.1 ± 1.9 [a] | 6.9 ± 5.2 [a] | −5.0 ± 6.3 [c] | −1.0 ± 9.5 [a] | not readily biodegradable |
| Eudragit L100–55 | −1.5 ± 3.0 [a] | −3.7 ± 3.5 [c] | 6.0 ± 4.7 [b] | 6.5 ± 4.0 [a] | not readily biodegradable |

n.a.=not applicable (water-insoluble test compounds).
[a] n=2.
[b] n=4.
[c] n=6.

prevented PVA and PEG biodegradation within the 28-day period. On the other hand, the OECD 301F tests contain higher levels of biomass ($10^7$ - $10^8$ approx. cells $L^{-1}$) 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 (OECD, 1992).

In the following sections, we will provide more details on the biodegradation potential of the different excipients using the different test set-ups (Fig. 1).

### 3.2. PVA and PEG mineralization: potential co-metabolic processes

No degradation was observed for either compound in the OECD

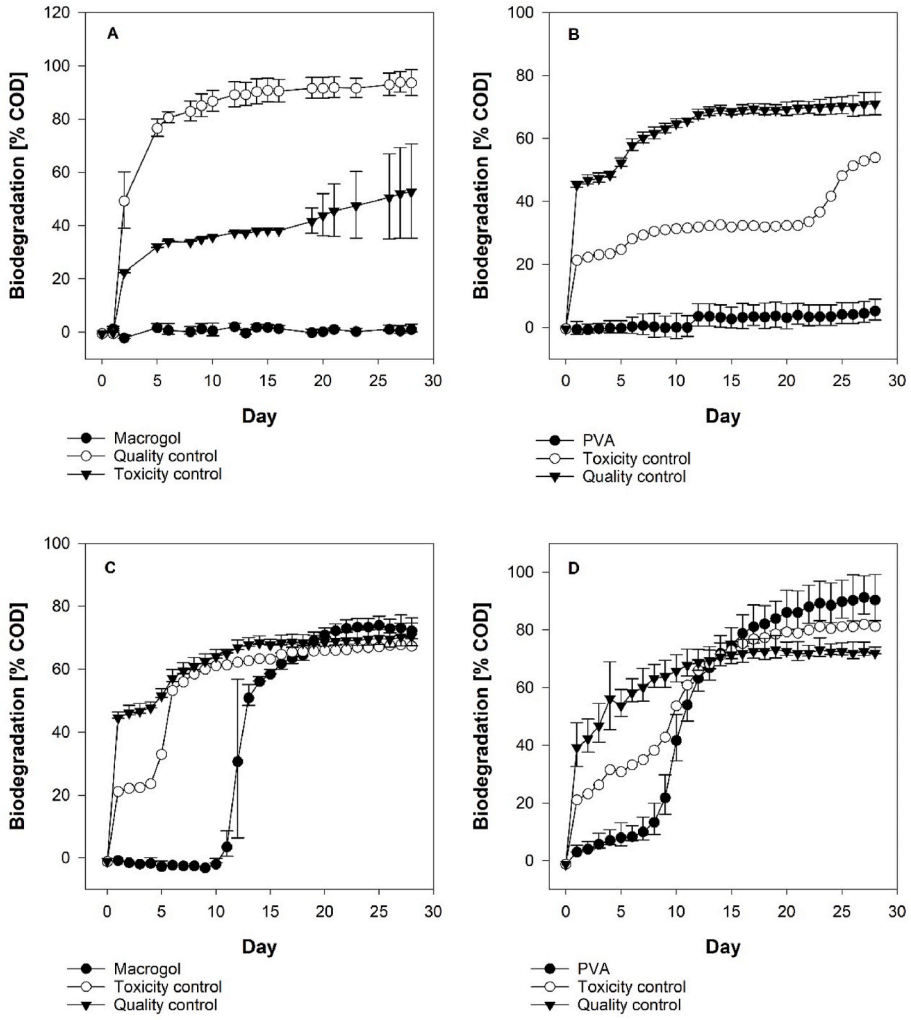



**Fig. 2.** Biodegradation of **A)** Macrogol (PEG) in an OECD 301D test **B)** PVA in an OECD 301F test **C)** Macrogol (PEG) in an OECD 301F test and **D)** PVA in an OECD 301F AS test. Presented as an average of replicates($n = 2$) with error bars showing standard deviation.

301D (CBT) (Table 1). Within the CBT toxicity vessels, however, PEG showed an increase in biodegradation from day 16 onwards (Fig. 2A). This observed increase in biodegradation in the toxicity flasks could be explained by two potential mechanisms, or a combination of both. Firstly, the observed increase could be linked to co-metabolic biodegradation by ammonia-oxidizing microorganisms (AOM). Several studies have observed co-metabolism of organic micropollutants along with ammonium oxidation (Kennes-Veiga et al., 2022). In our test set-up, nitrification processes (nitrification phase) typically occur between days 12 and 22 and are initiated by the oxidation of ammonium in the mineral salt medium (Bading et al., 2024). The presence of sodium acetate (reference compound) might have enhanced growth of nitrifying bacteria, thereby favoring co-metabolic biodegradation of PEG from day 16 onward with the onset of nitrification processes. Secondly, the availability of sodium acetate as an easily metabolizable substrate increased overall microbial growth, leading to the critical mass of competent strains required for biodegradation measurement in the CBT flasks. It is possible that both co-metabolic activities (ammonium oxidation) and enhanced microbial growth due to the presence of sodium acetate contributed to the observed increase in PEG biodegradation in the toxicity flasks. In this experiment, we noticed a high variability in the toxicity controls towards the end of the assessment period, as indicated by the high error bars showing standard deviation (Fig. 2A). One possible explanation for the apparent variability in the degree of biodegradation may arise from different onsets of the delayed nitrification processes. Nitrifying bacteria, which are known for their slow growth, typically begin nitrification towards the end of the testing period under our conditions (Bading et al., 2024; Chhetri et al., 2022). The precise starting point of this process within the 28-day test duration may vary and thus the length of this test period may not have been sufficient to fully capture these processes. This could account for the differing levels of PEG metabolism and co-metabolism observed.

Similar observations were noted for PVA in MRT with secondary effluent, where biodegradation increased from day 22 onwards in the toxicity flasks (Fig. 2B). The DOC removal of the toxicity control of 91.3 % (Fig. S1) confirmed full mineralization of PVA.

The subsequent OECD 301F tests (MRT and MRT-AS), i.e., increased microbial density and diversity, showed substantial biodegradation for PEG and PVA reaching 73.0 ± 3.3 % (96.2 % DOC removal, Fig. S2) and 91.2 ± 8.0 % (87.8 % DOC removal, Fig. S3) with secondary effluent and AS, respectively (Table 1). In the case of PEG within the MRT using secondary effluent, biodegradation increased rapidly after a 12-day lag phase. In addition, in the MRT toxicity flasks, the lag phase for PEG was reduced from 10 to 5 days (Fig. 2C). This reduction in lag phase could be attributed to the readily available sodium acetate facilitating the growth and establishment of a critical mass of PEG degraders. In summary, the lag phase duration decreased in the following order: CBT-toxicity vessel (19 days) > MRT-test vessel (12 days) > MRT-toxicity vessel (5 days).

PVA, on the other hand, achieved ultimate biodegradation (mineralization) in MRT with AS by day 28 after a lag phase of 7 days (Fig. 2D). In contrast, PVA biodegradation was neither observed in the CBT toxicity bottles nor in the MRT test bottles with secondary effluent as the inoculum due to lower microbial biodiversity and density compared to activated sludge. Consequently, the use of the CBT and MRT with secondary effluent as inoculum may be limited in assessing the full extent of biodegradation for PVA within the standard 28-day timeframe. The MRT-AS test system renders the test for ready biodegradability more potent and indicative of the biodegradation potential of PVA. The findings align well with a recent study evaluating different PEG and PVA materials biodegradation in an OECD 301B test setup with activated sludge. Low molecular weight PEG materials (MW 4000, 25,000, and 35,000) rapidly biodegraded, exceeding 70 % $ThCO_2$ in 28 days (Menzies et al., 2023). The lag time of the different PEG derivatives varied between 4 and 9 days and increased with higher MW (Menzies et al., 2023). However, the lag phase of 4 days determined in the study by Menzies et al. (2023) for the low MW of 4000 Da contrasts with our results from the MRT, which showed alag phase of 12 days for a 6000 Da PEG derivative. This observation could be due to the fact that secondary effluent was used as inoculum in our study, as opposed to AS, i.e. higher microbial density and diversity, which was used in the previous study.

The MRT-AS results on the biodegradation of PVA, with a degree of hydrolysis (DH) of 99 % and a molecular mass of ~130,000 Da, show that high DH and MW values do not significantly hinder biodegradation under these test conditions. Consistent with this result, other studies reported that PVA materials with a MW of 10,000 to 130,000 Da and a DH of 79 % and 88 % achieved ThCO2 degradation of more than 75 % by day 28, indicating no significant effect of MW and DH on the mineralization rate (Menzies et al., 2023).

The observed biodegradation of both PEG and PVA can be explained by established oxidative biodegradation pathways in literature. For PEG, the biodegradation process involves a series of sequential oxidation steps targeting the terminal hydroxyl group. This group is progressively oxidized to form carboxylic acid moieties. Subsequently, the terminal ether bond undergoes oxidative cleavage, resulting in the depolymerization of PEG by the removal of one glycol unit at a time (Eubeler et al., 2010). PVA, with its repeating 1,3 diol structure, follows a two-step biodegradation process. Initially, diketone structures are generated, which are subsequently broken down through hydrolase or aldolase reactions, leading to the production of acetic acid, which is further metabolized within the TCA cycle (Wilkes and Aristilde, 2017).

### 3.3. PVP, Copovidone and Kollidon CL

We observed no biodegradation for PVP, Copovidone (polyvinyl pyrrolidone–vinyl acetate copolymer), and Kollidon CL (crosslinked polyvinyl pyrrolidone) in both OECD 301D/F 28-day tests and in the extended 42-day 301F test with AS (Fig. 3A, Table 1). Our findings are consistent with current research indicating resistance of vinyl polymers, except for PVA, to biodegradation (Kawai, 2010). Generally, carbon backbone polymers lacking oxidizable/hydrolysable functional groups exhibit recalcitrance to biodegradation (Kawai, 2010). In line with this assumption are practical biodegradation studies indicating that PVP is largely resistant to biological degradation in aerobic aquatic environments (Trimpin et al., 2001; Julinová et al., 2012, 2013). However, certain positive results in PVP (bio)degradation have been reported. Julinová and colleagues proposed a theoretical three-step mechanism for PVP degradation, involving the opening of the lactam ring (1), breakdown of secondary amines via aminooxidases (2), and subsequent mineralization of breakdown products (3) (Julinová et al., 2012). Their study found that approximately 8 % and 14 % of PVP was degraded when aerobic activated sludge was pre-adapted with NMP, a compound containing a lactam ring also found in PVP (Julinová et al., 2012; Julinová et al., 2012). The proposed mechanism for PVP degradation (see above) implies the release of nitrogen-containing transformation products, potentially leading to the release of ammonium and an observable nitrification phase during PVP biodegradation. However, our biodegradation curves, including the 42-day curves for the three compounds, did not show any increase in oxygen consumption related to nitrification, indicating that no nitrification phase was observed (Fig. 3A). In contrast, the monomer NMP, analyzed in a CBT, exhibited over 90 % degradation, displaying an evident nitrification phase resulting from the release of ammonium after day 22 (Fig. 3B). The lack of a nitrification phases for the PVP-based polymers indicates their resistance to biodegradation under our test conditions. The potential environmental persistence is concerning from an ecotoxicological perspective. Chronic exposure to PVP has been associated with adverse effects on aquatic organisms, including impairments of the reproductive cycles in *Daphnia magna* (Mondellini et al., 2022). Additionally, behavioral changes in *Danio rerio* and disruptions to host-parasite interactions (i.e., *Gyrodactylus turnbulli* and *Poecilia reticulata*) within freshwater ecosystems have been reported (Nigro et al., 2023; Robison-Smith et al., 2024). Increasing evidence of PVP as an emerging

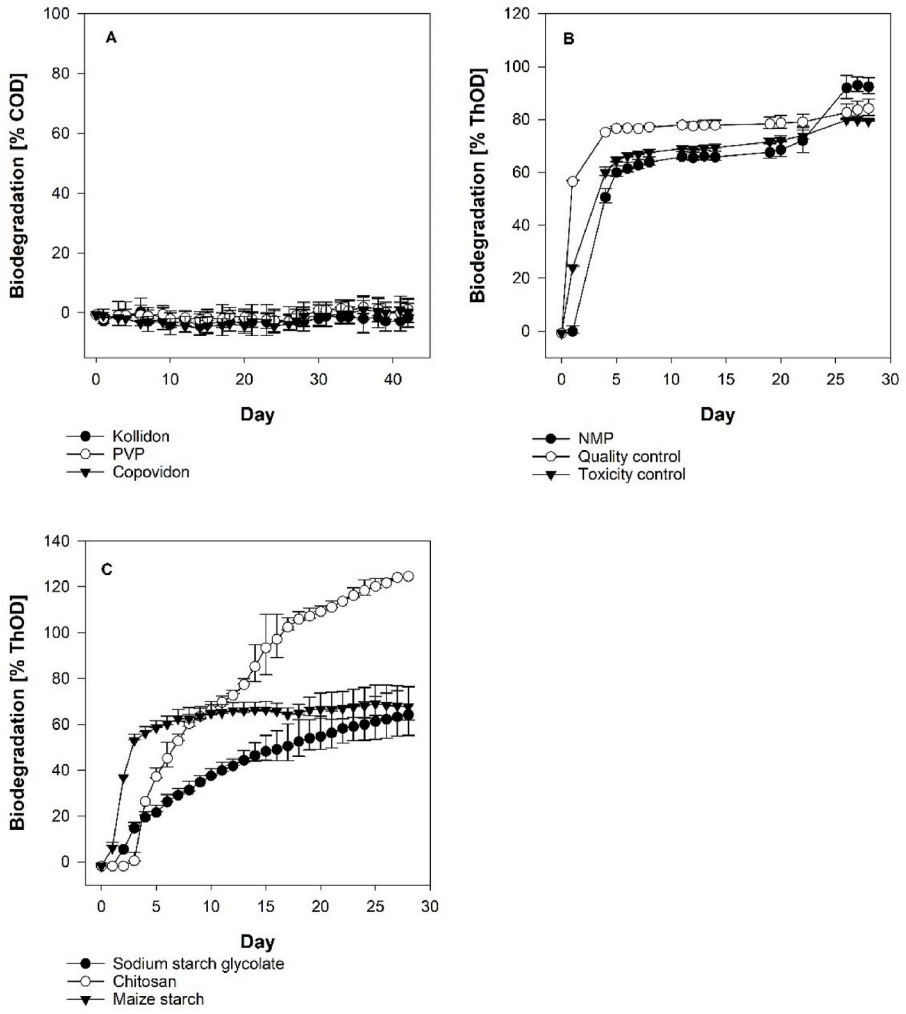



**Fig. 3.** Biodegradation of **A)** PVP, Copovidone and Kollidon CL in a 42-day OECD 301F AS test **B)** NMP degradation in an OECD 301D test and **C)** Natural and natural-based polymeric excipients in an OECD 301F test. Presented as an average of replicates(n = 2) with error bars showing standard deviation.

environmental contaminant underscores the importance of considering alternatives to vinyl pyrrolidone polymers in pharmaceutical formulations.

### 3.4. Eudragit derivatives

The assessed PMMAs, Eudragit EPO (MW 47 kDa) and Eudragit L100-55 (MW 320 kDa) showed no biodegradation in the OECD 301D (CBT). While Eudragit L100-55 did not better degrade in biodegradation within the OECD 301F (MRT) with secondary effluent, Eudragit EPO showed 6.9 ± 5.2 % biodegradation. In contrast to the results obtained with secondary effluent, Eudragit EPO showed no biodegradation in both the 28-day and prolonged 42-day MRT-AS, potentially due to its adsorption by AS solids, limiting its bioavailability to microorganisms. Hydrophobic interaction and hydrogen bond force have been found to play a major role in the adsorption of polyacrylate onto AS (Zhao et al., 2018). The 2:1:1 ratio of dimethylaminoethyl methacralyte, butyl methacrylate, and methyl methacrylate in Eudragit EPO, compared to the 1:1 ratio of free carboxyl groups to ester groups in Eudragit L100-55, might facilitate stronger hydrophobic interactions with activated sludge. For Eudragit L100-55, we observed hardly any increase in biodegradation during the MRT-AS after 42 days (Table 1). Concluding, the results show that the use of secondary effluent instead of activated sludge in the MRT may be relevant for substances prone to adsorption effects by activated sludge. Therefore, this study emphasizes the importance of considering the specific characteristics of polymers and their interactions with different test conditions when evaluating biodegradation potential through OECD 301 tests. As described for the assessed vinyl polymers in section 3.3, the limited biodegradation extents observed for the analyzed PMMAs might be attributed to the polymer's resistant carbon backbone, coupled with quaternary carbons hindering metabolism even more and their high MWs (Gaytán et al., 2021). Efficient mineralization of PAA has been reported with MWs below 1000 Da indicating the negative impact of the C–C backbone size in PAA biodegradation (Jackson et al., 2022). Several oxidative aerobic metabolic pathways have been proposed for PAA biodegradation leading to double bond formation between α- and β-carbons, analogous to the metabolic pathway of β-oxidation. However, the presence of methyl groups in the carbon backbone of the investigated Eudragit derivatives likely impedes the initial oxidation steps for double bond formation, thus inhibiting biodegradation of the carbon chain. Therefore, the action of enzymes e.g., mutases to shift the methyl group would be required, enabling PMMA biodegradation (Gaytán et al., 2021). The observed slight biodegradation (Table 1) may derive from initial sidechain removal and mineralization, aligning with the suggested two-phase degradation of PAA/PMMA involving enzymatic side group elimination (first phase) prior to backbone cleavage (second phase) (Gaytán et al., 2021). However, it is important to consider that the low biodegradation values may not definitively indicate biodegradation, as they could potentially fall within the variability and/or error range of the

analytical OxiTop® system.

### 3.5. Chitosan

Chitosan, manufactured by deacetylation of the natural polymer precursor chitin, was fully mineralized after 28 days in the OECD301F (MRT) with secondary effluent (Fig. 3C, Table 1). The degradation process of chitosan is well-established and involves enzymatic activity of chitinases, which hydrolyze glucosamine-glucosamine linkages (Qiu et al., 2022). The resulting amino sugar, glucosamine, undergoes further metabolism, generating substantial amounts of ammonia (Moye et al., 2014). Chitosan was fully mineralized after 28 days in the OECD 301F (MRT) with secondary effluent (Fig. 3C, Table 1). Following a 4-day lag phase, chitosan degradation manifested in two distinct phases, as indicated by a biphasic biodegradation curve (Fig. 3C). During the lag phase, it is likely that extracellular chitinases (hydrolases) were produced to fragment the polymer, which did not reflect in increased oxygen consumption monitoring as no oxygen is consumed during hydrolysis. The resulting amino sugar compounds are further mineralized within the microorganism's central metabolism, marking the onset of the first degradation phase. The observed increase in oxygen consumption from day 14 results from the nitrification of the ammonium produced during metabolism (nitrification phase), leading to over 100 % degradation by day 28 (Fig. 3C). Interestingly, in contrast to the findings reported here, previously published results indicated that chitosan exhibits limited mineralization under ASTM D5338 composting conditions (Gillece et al., 2024). Under the thermophilic conditions used in the study by Gillece et al. (2024), i.e., 58 ± 2 °C in a solid matrix, chitosan remained largely degradation-resistant and required acidic pretreatment to initiate biodegradation. This resulted in only approximately 40 % mineralization after 180 days (Gillece et al., 2024). These contrasting results illustrate the significant impact that environmental conditions such as temperature and medium have on the biodegradation potential of chitosan.

### 3.6. Maize starch and sodium starch glycolate

Both maize starch and SSG exhibited degradation exceeding 60 % in the OECD301F (MRT) with secondary effluent (Fig. 3C, Table 1). Maize starch displayed an immediate (day 1) increase in its biodegradation curve, reaching a distinct plateau phase after approximately 12 days (Fig. 3C). In contrast, SSG showed a 3-day lag phase and without evidenced plateau phase by day 28, indicating not yet finished but still slowly ongoing biodegradation (Fig. 3C). As it is well-established for cellulose derivatives, the extent of substitution of the glycosidic backbone significantly influences biological degradability. A previous study highlighted the critical role of the amount of derivatization expressed as degree of substitution or molar substitution in determining biological degradability (Bading et al., 2024). Further, studies confirmed inherent biodegradability of carboxymethyl cellulose (CMC) derivatives with DS values below 1 (Van Ginkel and Gayton, 1996; Menzies et al., 2023). SSG has same chemical modification as CMC, but with starch as polymer backbone. In our recent analysis, applying same test conditions to the present study, CMC linear with a DS of 0.8 exhibited 14.3 + 2.6 % degradation over 28 days (Bading et al., 2024). Extended biodegradation assessments revealed increased degradation for CMC derivative with DS < 1. For instance, CMC with a DS of 0.6 reached 20 ± 2.4 % $ThCO_2$ after 28 days and ultimately 70 % biodegradation after 148 days in an OECD 301B test (Menzies et al., 2023). In this study, we monitored biodegradability of SSG with a lower DS (0.25) compared to CMC DS (0.8). The considerably reduced level of derivatization in SSG could explain the notable increase in biodegradation observed within 28 days.

## 4. Biodegradability and applicability of OECD 301 screening tests

By applying the classification scheme established by Bading et al. (2024), the compounds, maize starch, chitosan, Macrogol 6000, PVA, and SSG used in the study can be classified as 'readily biodegradable' (Table 2). This classification scheme builds on our previous research, where we developed a framework to categorize biodegradability by considering not only the extent of degradation but also the specific characteristics of the biodegradation curves. Its aim is to predict a compound's biodegradation potential beyond the standard 28-day period. Additionally, it helps identify chemical structures that could be optimized for enhanced biodegradability (Bading et al., 2024).

In case of PVA and PEG, we observed differences in the biodegradation rates depending on the OECD 301 screening tests used (see section 3.2). The absence of biodegradation in the OECD 301D (CBT) obtained for PEG and PVA, which contrasts the readily biodegradation observed in the OECD 301F with secondary effluent and activated sludge, respectively, suggests that the OECD 301D (CBT) test conditions may not be sufficient to assess the full potential of their 'readily' biodegradation. PVA showed over 60 % biodegradation in the MRT-AS and hence classifies as 'readily biodegradable'. However, using the same test set up with secondary effluent instead of activated sludge, PVA does not undergo biodegradation (Fig. 2B) and, consequently, would not have been classified as 'readily biodegradable' according to the OECD 301 guidelines (OECD, 1992). Thus, the outcome of biodegradation assessments is context dependent, highlighting the pivotal role of both inoculum source diversity and inoculum concentration. This is in line with other studies showing that depending on the biodegradation screening

**Table 2**
Biodegradability classification according to OECD 301 results. **A)** old classification system **B)** new classification system.

| **A** | | | |
|---|---|---|---|
| **Readily biodegradable ≥ 60 %** | **Moderately biodegradable 20–59 %** | **Slightly biodegradable 5–19 %** | **Non biodegradable <5 %** |
| Maize starch<br>Chitosan<br>Sodium starch glycolate<br>PVA<br>Macrogol 6000 (PEG) | | Eudragit L100-55<br>Eudragit EPO | PVP<br>Copovidone<br>Kollidon CL |
| **B** | | | |
| **Readily biodegradable ≥ 60 %** | **Moderately biodegradable 20–59 %** | **Slightly biodegradable 10–19 %** | **Non biodegradable < 10 %** |
| Maize starch<br>Chitosan<br>Sodium starch glycolate<br>PVA<br>Macrogol 6000 (PEG) | | | PVP<br>Copovidone<br>Kollidon CL<br>Eudragit L100-55<br>Eudragit EPO |

test conditions, the outcome for a given substance can differ widely (Goodhead et al., 2013; Martin et al., 2017a). Also here, the variability is largely attributed to the inoculum and the random inclusion or exclusion of specific degraders, a phenomenon often referred to as the "biodegradation lottery" (Davenport et al., 2022). Increasing the number of microbial cells in biodegradation screening tests to environmentally representative levels can reduce this variability and enhance test reliability (Martin et al., 2017a; Martin et al., 2017b). The low levels of inoculum in the OECD 301D test, with approximately $10^1$-$10^3$ cells $mL^{-1}$, may reduce the chances of including sufficient quantities of naturally occurring competent degraders (OECD, 1992). This reduction could explain the low biodegradation potential observed in these tests and may lead to 'false negative' assignments of biodegradability within the OECD regulatory framework. For example, PEG and PVA show biodegradability in the OECD 301F test but not in the OECD 301D test. Our experimental 'step-by-step' approach revealed such differences in the requirements for biomass and diversity, which we accomplished through increasing inoculum volume and by introducing more microbial diversity and density with activated sludge to the test system (Fig. 1). To expand on these findings, further research is required to determine if these results are applicable to other readily biodegradable polymers. Additionally, gaining a deeper understanding of how the intrinsic properties of polymers affect microbial adaptation and growth mechanisms is crucial for enhancing biodegradation assessments. Further, in the toxicity controls, both PEG and PVA underwent biodegradation in the CBT and the MRT with secondary effluent, respectively. The presence of easily degradable carbon sources played a significant role in enhancing the biodegradation of these two compounds.

Eudragit EPO and Eudragit L100-55 fall into the category of 'slightly biodegradable.' PVP, Copovidone, and Kollidon CL are identified as 'non biodegradable' (Table 2A). In contrast to PEG and PVA, compounds like PVP, Copovidone, Kollidon CL, Eudragit EPO, and L100-55 lack oxidizable/hydrolysable groups within their carbon backbone structure. As a result, breaking down the carbon backbone for these compounds becomes notably challenging. This aligns with our biodegradation findings within the OECD 301 tests. In contrast to the biodegradation outcomes observed for PEG and PVA, there was minimal variability in the biodegradation results obtained across different screening tests for the PEx categorized as 'non biodegradable' and 'slightly biodegradable.' This low variability indicates a high inherent resistance to biodegradation. Importantly, none of these substances surpassed the lag phase, defined as the time to achieve 10 % degradation (OECD, 1992). The lag phase is characterized by the initial adaptation or selection and growth to reach critical mass of microorganisms and the commencement of the biodegradation process ('adaptation phase'). The absence of a lag phase for these compounds highlights their lack of susceptibility to microbial degradation. Extending the test duration for these compounds, which either did not surpass 5 % degradation or only marginally surpassed this level within the initial 28 days, to 42 days for further assessment of their biodegradation potential did not result in a readily detectable increase in degradation. Therefore, we have to consider these PEx as potentially environmentally persistent. However, higher tier simulation tests may be needed to draw definitive conclusions for regulatory persistence assessment. For these reasons, we revised the previously published classification system (Bading et al., 2024) and set 10 % degradation as the threshold to be categorize as 'slightly' biodegradable (Table 2B). By setting the threshold at 10 % degradation, the classification system accounts for the lag phase, which is a crucial indicator of the initiation of the biodegradation process.

For readily biodegradable polymers according to the OECD guidelines, more stringent test conditions within OECD 301 guidelines could be applied to gain a more thorough picture of their biodegradation potential. More research needed due to the limited mechanistic understanding of the underlying microbial processes. However, for an initial screening for potential biodegradation, it may be more suitable to first analyze the test compounds in a screening test with a higher possibility of positive outcome in biodegradation, as in this study, the OECD 301F with activated sludge.

## 5. Conclusions

This study serves two important purposes: it enhances scientific understanding and contributes to discussions on regulatory and industrial practices. With increasing pressure to adopt sustainable practices, particularly under the EU Taxonomy Regulation, there is a growing need for readily biodegradable compounds in pharmaceutical formulations. Our systematic analysis identified potentially persistent compounds such as PVP, Copovidone, Kollidon CL and the Eudragit derivatives EPO and L100-55 as well as readily biodegradable compounds such as PVA, Macrogol 6000, SSG, chitosan and maize starch. These results thus provide an initial orientation for the selection of environmentally friendly excipients and offer a solid basis for the development of pharmaceuticals with improved biodegradability, in line with the concept of "benign-by-design". In addition, our stepwise approach has shown that susceptibility to biodegradation is highly dependent on the size and diversity of the inoculum. Future research should aim at a deeper mechanistic understanding of how the physicochemical properties of polymers affect microbial degradation and adaptation processes.

## CRediT authorship contribution statement

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

## 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.

## Acknowledgments

We thank Dr. Jochen Scher for advice and insightful discussions. We are particularly grateful to Dr. Ulrich Scholz without his general support and guidance this study would not have been possible. This work was supported by funds from Boehringer Ingelheim.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143739.

## Data availability

Data will be made available on request.

## References

Albright, V.C., Chai, Y., 2021. Knowledge gaps in polymer biodegradation research. Environ. Sci. Technol. 55, 11476–11488.

Antić, V.V., Antić, M.P., Kronimus, A., Oing, K., Schwarzbauer, J., 2011. Quantitative determination of poly(vinylpyrrolidone) by continuous-flow off-line pyrolysis-GC/MS. J. Anal. Appl. Pyrolysis 90, 93–99.

Arp, H.P.H., Knutsen, H., 2020. Could we spare a moment of the spotlight for persistent, water-soluble polymers? Environ. Sci. Technol. 54, 3–5.

Bading, M., Olsson, O., Kümmerer, K., 2024. Analysis of environmental biodegradability of cellulose-based pharmaceutical excipients in aqueous media. Chemosphere 352, 141298.

Chhetri, R.K., Karvelas, S., Sanchez, D.F., Droumpali, A., Kokkoli, A., Andersen, H.R., 2022. A modified nitrification inhibition test for high-salinity wastewater. Chem. Eng. J. 429, 132460.

Davenport, R., Curtis-Jackson, P., Dalkmann, P., Davies, J., Fenner, K., Hand, L., McDonough, K., Ott, A., Ortega-Calvo, J.J., Parsons, J.R., Schäffer, A., Sweetlove, C., Trapp, S., Wang, N., Redman, A., 2022. Scientific concepts and methods for moving persistence assessments into the 21st century. Integrated Environ. Assess. Manag. 18, 1454–1487.

Debotton, N., Dahan, A., 2017. Applications of polymers as pharmaceutical excipients in solid oral dosage forms. Med. Res. Rev. 37, 52–97.

EC, 2019. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. The European Green Deal. COM, p. 640, 2019.

EC, 2020. Communication from the commission to the European parliament, the European council, the council, the European economic and social committee and the committee of the regions on chemicals Strategy for sustainability. Towards a Toxic-Free Environment COM 667, 2020.

EC, 2021. Communication from the commission to the European parliament, the council, the European economic and social committee and the committee of the regions. Pathway to a healthy planet for all EU action plan: "towards zero pollution for Air. Water and Soil" COM 400, 2021.

EC, 2023. Commission Delegated Regulation (EU) 2023/2486 of 27 June 2023 Supplementing Regulation (EU) 2020/852 of the European Parliament and of the Council by Establishing the Technical Screening Criteria for Determining the Conditions under Which an Economic Activity Qualifies as Contributing Substantially to the Sustainable Use and Protection of Water and Marine Resources, to the Transition to a Circular Economy, to Pollution Prevention and Control, or to the Protection and Restoration of Biodiversity and Ecosystems and for Determining whether that Economic Activity Causes No Significant Harm to Any of the Other Environmental Objectives and Amending Commission Delegated Regulation (EU) 2021/2178 as Regards Specific Public Disclosures for Those Economic Activities.

, ECHA (I), 1-vinyl-2-pyrrolidone. EC number: 201-800-4|CAS number: 88-12-0 https://echa.europa.eu/it/substance-information/-/substanceinfo/100.001.637. .

ECHA (II), Vinyl acetate. EC number: 203-545-4 | CAS number: 108-05-4 https://echa.europa.eu/it/substance-information/-/substanceinfo/100.003.224.

ECHA (III), Acrylic acid. EC number: 201-177-9|CAS number: 79-10-7 https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15803.

ECHA (IV), Ethylene oxide. EC number: 200-849-9 | CAS number: 75-21-8 https://echa.europa.eu/substance-information/-/substanceinfo/100.000.773. .

Eubeler, J.P., Bernhard, M., Knepper, T.P., 2010. Environmental biodegradation of synthetic polymers II. Biodegradation of different polymer groups. Trends Anal. Chem. 29, 84–100.

Friedrich, J., Längin, A., Kümmerer, K., 2012. Comparison of an electrochemical and luminescence-based oxygen measuring system for use in the biodegradability testing according to closed Bottle Test (OECD 301D). Clean: Soil. Air, Water 41, 251–257.

Gaytán, I., Burelo, M., Loza-Tavera, H., 2021. Current status on the biodegradability of acrylic polymers: microorganisms, enzymes and metabolic pathways involved. Appl. Microbiol. Biotechnol. 105, 991–1006.

Gillece, T.W., Gerardi, H.K., McMullen, R.L., Thompson, W.T., Brown, D.H., 2024. Thermophilic composting as a means to evaluate the biodegradability of polymers used in cosmetic formulations. Cosmetics 11, 99.

Goodhead, A.K., Head, I.M., Snape, J.R., Davenport, R.J., 2013. Standard inocula preparations reduce the bacterial diversity and reliability of regulatory biodegradation tests. Environ. Sci. Pollut. Res. Int. 21, 9511–9525.

Huppertsberg, S., Zahn, D., Pauelsen, F., Reemtsma, T., Knepper, T.P., 2020. Making waves: water-soluble polymers in the aquatic environment: an overlooked class of synthetic polymers? Water Res. 181, 115931.

Jackson, A.W., Mothe, S.R., Ang, P., Chennamaneni, L.R., Herk, A.M.V., Thoniyot, P., 2022. Backbone degradable poly (acrylic acid) analogue via radical ring-opening copolymerization and enhanced biodegradability. Chemosphere 293, 133487.

Julinová, M., Kupec, J., Houser, J., Slavík, R., Marušincová, H., Červenáková, L., Kívar, S., 2012. Removal of Polyvinylpyrrolidone from wastewater using different methods. Water Environ. Res. 84, 2123–2132.

Julinová, M., Kupec, J., Slavík, R., Vaskova, M., 2013. Initiating biodegradation of polyvinylpyrrolidone in an aqueous aerobic environment: technical note. Ecol. Chem. Eng. 20, 199–208.

Kawai, F., 2010. The biochemistry and molecular biology of xenobiotic polymer degradation by microorganisms. Biosci. Biotechnol. Biochem. 74, 1743–1759.

Kennes-Veiga, D.M., Gónzalez-Gil, L., Carballa, M., Lema, J.M., 2022. Enzymatic cometabolic biotransformation of organic micropollutants in wastewater treatment plants: a review. Bioresour. Technol. 344, 126291.

Martin, T.J., Snape, J.R., Bartram, A., Robson, A., Acharya, K., Davenport, R.J., 2017a. Environmentally relevant inoculum concentrations improve the reliability of persistent assessments in biodegradation screening tests. Environ. Sci. Technol. 51, 3065–3073.

Martin, T.J., Goodhead, A.K., Snape, J.R., Davenport, R.J., 2017b. Improving the ecological relevance of aquatic bacterial communities in biodegradability screening assessments. Sci. Total Environ. 627, 1552–1559.

McDonough, K., Battagliarin, G., Menzies, J., Bozich, J., Bergheim, M., Hidding, B., Kastner, C., Koyuncu, B., Kreutzer, G., Leijs, H., Parulekar, Y., Raghuram, M., Vallotton, N., 2023. Multi-laboratory evaluation of the reproducibility of polymer biodegradation assessments applying standardized and modified respirometry methods. Sci. Total Environ. 901, 166339.

Menzies, J., Wilcox, A., Casteel, K., McDonough, K., 2023. Water soluble polymer biodegradation evaluation using standard and experimental methods. Sci. Total Environ. 858, 160006.

Mondellini, S., Schott, M., Löder, M.G.J., Agarwal, S., Greiner, A., Laforsch, C., 2022. Beyond microplastics: water soluble synthetic polymers exert sublethal adverse effects in the freshwater cladoceran Daphnia magna. Sci. Total Environ. 847, 157608.

Moye, Z.D., Burne, R.A., Zeng, L., 2014. Uptake and metabolism of N-acetylglucosamine and glucosamine by Streptococcus mutans. Appl. Environ. Microbiol. 80, 5053–5067.

Nigro, L., Magni, S., Ortenzi, M.A., Gazzotti, S., Signorini, S.G., Sbarberi, R., Della Torre, C., Binelli, A., 2023. Assessment of behavioural effects of three water-soluble polymers in zebrafish embryos. Sci. Total Environ. 893, 16843.

OECD, 1992. Organization for economic co-operation and development. OECD 301 Ready Biodegradability. OECD Guidel. Test. Chem. 3. Section.

OECD, 2006. Organisation for economic cooperation and development. Revised Introduction OECD Guidelines for the Testing of Chemicals. Section 3.

Pauelsen, F., Huppertsberg, S., Knepper, T.P., Zahn, D., 2023. Narrowing the analytical gap for water-soluble polymers: a novel trace-analytical method and first quantitative occurrence data for polyethylene oxide in surface and wastewater. Sci. Total Environ. 882, 163563.

Qiu, S., Zhou, S., Tan, Y., Feng, J., Bai, Y., He, J., Cao, H., Che, Q., Guo, J., Su, Z., 2022. Biodegradation and prospect of polysaccharide from Crustaceans. Mar. Drugs 20, 310.

Robison-Smith, C., Masud, N., Tarring, E.C., Ward, B.D., Cable, J.A., 2024. A class of their own? Water-soluble polymer pollution impacting a freshwater host-pathogen system. Sci. Total Environ. 907, 168086.

Swift, G., 1994. Water-soluble polymers. Polym. Degrad. Stabil. 45, 215–231.

Trimpin, S., Eichhorn, P., Räder, H.J., Müllen, K., Knepper, T.P., 2001. Recalcitrance of poly(vinylpyrrolidone): evidence through matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. J. Chromatogr. A. 938, 67–77.

Van Ginkel, C.G., Gayton, S., 1996. The biodegradability and nontoxicity of carboxymethyl cellulose (DS 0.7) and intermediates. Environ. Toxicol. Chem. 15, 270–274.

Wilkes, R.A., Aristilde, L., 2017. Degradation and metabolism of synthetic plastics and associated products by Pseudomonas sp.: capabilities and challenges. J. Appl. Microbiol. 123, 582–593.

Zhao, X., Ma, J., Ma, H., Gao, D., Sun, Y., Guo, C., 2018. Removal of polyacrylate in aqueous solution by activated sludge: characteristics and mechanisms. J. Clean. Prod. 178, 59–66.