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Environmental Chemistry

Assessing the Biodegradability of Tire Tread Particles and Influencing Factors

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Abstract: Abrasion of tire tread, caused by friction between vehicle tires and road surfaces, causes release of tire wear particles (TWP) into various environmental compartments. These TWPs contribute to chemical, microplastic, and particulate matter pollution. Their fate remains largely unknown, especially regarding the extent and form in which they persist in the environment. The present study investigated (1) the biodegradability of tread particles (TPs) in the form of ground tire tread, (2) how accelerated ultraviolet (UV) weathering affects their biodegradability, and (3) which TP constituents are likely contributors to TP biodegradability based on their individual biodegradability. A series of closed-bottle tests, with aerobic aqueous medium inoculated with activated sludge, were carried out for pristine TPs, UV-weathered TPs, and selected TP constituents; natural rubber (NR), isoprene rubber (IR), butadiene rubber (BR), and treated distillate aromatic extracts (TDAE). Biodegradation was monitored by manometric respirometry, quantifying biological oxygen consumption over 28 days. Pristine TP biodegradability was found to be 4.5%; UV-weathered TPs showed higher biodegradability of 6.7% and 8.0% with similar and increased inoculum concentrations, respectively. The observed TP biodegradation was mainly attributed to biodegradation of NR and TDAE, with individual biodegradability of 35.4% and 8.0%, respectively; IR and BR showed negligible biodegradability. These findings indicate that biodegradability of individual constituents is decreased by a factor of 2 to 5 when compounded into TPs. Through scanning electron microscopy analysis, biodegradation was found to cause surface erosion. Processes of TP biodegradation are expected to change throughout their lifetime as new constituents are incorporated from the road and others degrade and/or leach out. Tire emissions likely persist as particles with an increased fraction of synthetic rubbers and carbon black. *Environ Toxicol Chem* 2024;43:31–41. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Aerobic biodegradation; Environmental fate; Microplastics; Runoff; Tire wear

INTRODUCTION

Abrasion of tire tread presents an environmental concern because it introduces a continuous release of tire wear particles (TWPs) into natural and urban environments (Boucher et al., 2020; Knight et al., 2020). Emissions of TWPs contribute to particulate matter air pollution (Avagyan et al., 2014; Kole et al., 2017), chemical pollution by leaching of additives

(Abernethy et al., 1996; Gualtieri et al., 2005; Wik & Dave, 2006), and microplastic pollution due to their microscopic size and polymeric composition (Boucher & Friot, 2017; Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2015; Kole et al., 2017). It has been estimated that TWPs are one of the biggest contributors to microplastic pollution (Boucher et al., 2020; Knight et al., 2020), which has raised concerns regarding their persistency and potential adverse effects in the receiving natural environments (Wagner et al., 2018).

The chemical composition of tire tread includes polymers, fillers, oil, vulcanizers, and additives used for tire preservation (Table 1). The specific combination of the polymeric blend and the rest of the compounds depends on the tire use and manufacturer (Eisentraut et al., 2018; Wagner et al., 2018). Tire wear particles are generated by two types of wear mechanisms,

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TABLE 1: Typical composition of tire tread and tire particles expressed in weight percentage (w/w)

Category	Typical compounds	Content (% w/w)
Rubber polymers	Natural rubber, isoprene rubber, butadiene rubber, styrene–butadiene rubber	40–60
Fillers	Carbon black, silica, silane coupling agents	19–35
Oils and plasticizers	Mineral oils, mild extract solvates, treated distillate aromatic extracts	12–20
Vulcanization agents	Sulfur, zinc oxide, stearic acid, zinc stearate	2–5
Additives	Antioxidants, antiozonants, preservatives, desiccants, waxes, processing aids	5–10

Tire particle constituents are divided into rubber polymers, fillers, oil and plasticizers, chemicals of the vulcanization system, and additives (ChemRisk, 2008; Kreider et al., 2010; Null, 1999; Rodgers & Waddell, 2013; Scandinavian Tire & Rim Organization, 2004; Wagner et al., 2018; Wik & Dave, 2009).

both of which are the result of friction between the tire tread and road surface: (1) mechanical shear force, which creates coarser TWP by abrasion (Fauser et al., 2002; Kreider et al., 2010; Nguyen et al., 2018; Sommer et al., 2018; Thorpe & Harrison, 2008), and (2) thermal wear, which creates finer particles when local heat spots cause evaporation of volatile compounds that later precipitate (ChemRisk, 2008; Fauser et al., 2002; Kole et al., 2017; Park et al., 2018). Combined, this generates TWPs ranging from 5.6 nm to 350 µm (Foitzik et al., 2018; Kreider et al., 2010). They are predominately within the size range of 20 to 220 µm (Kreider et al., 2010; Sommer et al., 2018) with an elongated and cylindrical shape (Kreider et al., 2010; Sommer et al., 2018; Wagner et al., 2018). Their specific size and shape, however, depend on the characteristics of the vehicle, tire, road, and driving style (Alexandrova et al., 2007; Dannis, 1974; Thorpe & Harrison, 2008). In the course of generation, TWPs are combined with other car emissions (e.g., brake wear particles) and road material, forming tire and road wear particles (TRWPs; Kreider et al., 2010; Panko et al., 2013; Sommer et al., 2018). Subsequently, road dust may adhere to the surface of TRWPs as they roll across the road, coating them with a layer of mainly minerals and metals (Sommer et al., 2018).

The global annual emission of TWPs is estimated at approximately 3.4 to 5.9 million tonnes per year, with emissions per capita ranging from 0.2 to 5.5 kg/cap/year (Baensch-Baltrusch et al., 2020; Kole et al., 2017). The TWP emission factor of a vehicle ranges from 3.45 to 1200 mg/km, depending on the number and type of tires, as well as the type of vehicle, road, and driving style (Baensch-Baltrusch et al., 2020; Wagner et al., 2018). Mainly, TWPs are generated on roads and in urban areas (Kole et al., 2017). Approximately 0.1% to 10% are estimated to become airborne (Grigoratos & Martini, 2015; Panko et al., 2013; Park et al., 2018; Wik & Dave, 2009) and will later deposit in both aquatic and terrestrial environments (Kole et al., 2017). The nonairborne fraction is transported off the road by runoff water into either nearby soils, surface waters, or a sewer system connected directly or indirectly to wastewater-treatment plants (WWTPs; Kole et al., 2017). Three-quarters of particles in surface waters are estimated to be retained in sediments, with the remaining quarter reaching ocean waters (Kole et al., 2017). The majority of particles in WWTPs are retained in sludge and end up in agricultural soil when sludge is used as fertilizer (Nizzetto et al., 2016). Overall, it is estimated that 25% to 67% of TWPs end up in soils (Fauser et al., 2002; Kole et al., 2017) and that 48% reach the oceans (Boucher & Friot, 2017). Today,

microrubber originating from tires has been found in air, soil, and sediment and deposited on plants (Fauser et al., 2002; Panko et al., 2012; Salma & Maenhaut, 2006; Wik et al., 2008) and is estimated to account for 30% of the volume of microplastics in rivers, lakes, and oceans (Sommer et al., 2018).

Despite the availability of information on their generation, transportation, and sinks, the environmental fate of TWPs is currently an understudied topic, with fundamental questions that remain unanswered. This includes how long and in which form they persist in the environment. Specifically, there is little quantitative knowledge on (1) the overall degradability of TWPs (Wagner et al., 2022), (2) which TWP constituents degrade or persist, and (3) how degradability of constituents changes when combined into tread, when released as TWPs, and finally when they persist in the environment as TRWPs.

The aim of the present study is to contribute to filling these knowledge gaps by studying the biodegradability of TWPs and their individual components. Ground vulcanized rubber (defined as crumb rubber within 0.1–1 mm per Halle et al., 2020) created from the grinding of tire tread was used as a proxy for TWPs, henceforth referred to as “tread particles” (TPs). The main objectives of the present study were to (1) assess the extent to which TPs biodegrade under standardized test conditions and how accelerated ultraviolet (UV) weathering affects this, (2) assess which TP constituents are likely contributors to the observed TP biodegradability based on their individual biodegradability, and (3) evaluate how the biodegradability of constituents is affected by their occurrence as a mixture in TPs. These objectives were addressed through a series of closed-bottle tests with an aerobic aqueous medium inoculated with activated sludge. Biodegradation was monitored by manometric respirometry, quantifying the biological oxygen consumption during the incubation period.

MATERIALS AND METHODS

Test substrates

Experimental tests were carried out with TPs, UV-weathered TPs, and the following TP constituents: natural rubber (NR), isoprene rubber (IR), butadiene rubber (BR), and treated distillate aromatic extracts (TDAE; Vivatex 200; Klaus Dahleke). We selected NR and TDAE because of their common and high fractional occurrence in TPs (Table 1) and because of their suspected susceptibility to biodegradation. In the present study they comprised 38% to 42% (Supporting Information, Table S1) and 12% to 20% of the tested TPs (Table 1),

respectively. Synthetic rubbers were assumed to be non-biodegradable, and BR was tested to confirm this; IR is a synthetic rubber based on isoprene like NR. It was included in the test because it was developed as a potential replacement for NR for its purer isoprene content (Rose & Steinbüchel, 2005; Wagner et al., 2018) and because NR is a raw material with a limited supply (Salehi et al., 2021). All tested materials were obtained from Genan A/S (Viborg, Denmark), a company specialized in end-of-life tire recycling. The TPs (Genan Fine rubber powder) were received as a granulate, created by cryogenic grinding of different types of used tires (mainly passenger car tires [~80%] but also truck tires [20%]), with added 0.5% SiO₂ as flow agent. Particle properties are listed in Supporting Information, Table S1. Degradation experiments were carried out with a subfraction of the TP granulate with a particle size of $\leq 125 \mu\text{m}$, which was achieved by sieving. This size fraction was chosen to better align with the predominant size range for TWPs of 20 to 220 μm (Kreider et al., 2010; Sommer et al., 2018). The resulting size distribution of the TPs was estimated to be approximately 20% 0 to 50 μm , 58% 50 to 100 μm , and 22% 100 to 125 μm , based on a sieve analysis from Genan. Unvulcanized NR, BR, and IR (*cis*-1,4-isoprene content $\leq 97\%$) were received as block samples. Cryogenic grinding and sieving were used to obtain particle sizes $\leq 125 \mu\text{m}$. Properties of the TDAE oil are listed in Supporting Information, Table S2. Accelerated weathering of TPs $\leq 125 \mu\text{m}$ was achieved by UV photodegradation by UVA-340 lamps, with an irradiance strength of 9 W/m^2 , in an 8:4-h light:dark cycle, for 6 weeks, at a temperature of $20 \pm 2^\circ\text{C}$, and relative humidity of $36 \pm 5\%$.

Biodegradation tests

Tests for ready biodegradability were performed in accordance with the Organisation for Economic Co-operation and Development (OECD) 301F test guideline (OECD, 1992). The principles of this method coincide with the newer International Organization for Standardization (ISO) 14851:2019 (ISO, 2019), with minor variations related to, for example, suspended solid concentration of the inoculum ($<30 \text{ mg/L}$ vs. $>30 \text{ mg/L}$, for OECD 301F and ISO 14851, respectively). Respirometric tests were carried out using OxiTop[®] instrumentation (WTW), employing manometric monitoring of microbial respiration (biological oxygen demand [BOD]) as a measure of substrate biodegradation. Table 2 summarizes information for the performed biodegradation tests. Substrate concentrations corresponded to a theoretical oxygen demand (ThOD) of approximately $75 \text{ mg O}_2/\text{L}$, if all added substrate was to be completely mineralized. The ThODs of TPs and TDAE were estimated based on chemical oxygen demand (COD) measurements, performed according to standard methods using K₂Cr₂O₇ as oxidizing agent (Clesceri et al., 1998). The ThODs of NR/IR and BR were calculated assuming a pure content of polyisoprene and polybutadiene, respectively. All biodegradability tests were carried out in 510-ml amber glass bottles to which 359 ml of OECD mineral medium was added together with seven drops of allylthiourea and 0.6 ml inoculum.

TABLE 2: Experimental overview of the biodegradation tests: inoculum concentration (expressed as total suspended solids, used test substrates, general substrate theoretical oxygen demand [ThOD]), and substrate test concentration [expressed as both ThOD and mass]

	Inoculum (mgTSS/L)	Substrate ThOD (g O ₂ /g)	Test ThOD (mg O ₂ /L)	Test mass (mg)
TPs pristine	15.0	1.67	75.0	11.2
TPs weathered	15.0	1.67	75.0	11.2
	37.4	1.67	75.0	11.2
NR	20.6	3.25	74.0	8.3
IR ^a	20.6	3.26	74.1	8.3
BR ^a	20.6	3.25	73.8	8.3
TDAE	20.6	2.55	75.0	11.0

^aIR and BR had one replicate.

For tire particles and treated distillate aromatic extracts, ThOD was estimated based on chemical oxygen demand measurements. For natural rubber (and isoprene rubber) and butadiene rubber, ThOD was calculated assuming a pure content of isoprene and butadiene, respectively.

TSS = total suspended solids; TPs = tire particles; NR = natural rubber; IR = isoprene rubber; BR = butadiene rubber; TDAE = treated distillate aromatic extracts.

The addition of microbial inoculum corresponded to a total suspended solids (TSS) concentration of 15 to 21 mg TSS/L (Table 2). The inoculum was activated sludge taken from an aerated tank at Mølleåværket WWTP (Lundtofte, Denmark) on the day of the experiment and kept aerated in the laboratory. For weathered TPs, an additional test with higher inoculum concentration (37 mg TSS/L) was performed, in an effort to maximize TP biodegradation. Test bottles were sealed with rubber tops containing two NaOH pellets (for trapping of microbially generated CO₂) and OxiTop lids. Bottles were incubated for 28 days at $20 \pm 2^\circ\text{C}$ and continuously stirred on magnetic stirrers at 180 to 450 rpm. The BOD was measured as a pressure decrease every 1.87 h throughout the incubation period.

Replicates, inoculum blanks, positive controls, and inhibition tests

In accordance with the OECD 301F protocol, two bottles containing test substances and inoculum were prepared for TPs (pristine and weathered), NR, and TDAE. Because of test capacity limitations, BR was included as a single replicate, with the primary aim of confirming its presumed nonbiodegradability, while emphasizing that additional tests would be necessary to properly validate findings. Similarly, IR was tested in a single replicate because its current use in tire tread production is very low, whereby its degradability has little influence on overall TP degradability at present. Therefore, the present study can be viewed as an initial exploration of IR degradation, which requires further investigation if there is a significant shift toward the use of IR in tires in the future. Following the OECD 301F protocol, two bottles of inoculum blanks and one positive control bottle were prepared for each round of biodegradation tests. Inoculum blanks were prepared with inoculum in medium without added substrate, yielding inoculum BODs ranging from 1.1 to 13 mg/L over the 28 days. Positive controls were prepared with sodium

acetate (NaOAc)—a readily biodegradable substrate—to confirm that microbial degradation occurred with the inoculum used. For these experiments, no lag phases were observed for the NaOAc biodegradation, and >60% of the ThOD was reached within 10 days, except for one test run reaching 60% after 16 days (Supporting Information, Figure S1). The positive controls showed that lack of biodegradation was unlikely to be caused by poor microbial fitness. Furthermore, tests for inhibition of microbial activity due to added TP were prepared, containing both TPs (pristine or weathered) and NaOAc as substrate. It was found that TPs did not inhibit microbial activity because NaOAc was mineralized readily and with no lag phase in the presence of TPs (Supporting Information, Table S2), illustrating that lack of biodegradation was unlikely to be caused by toxicity.

Data treatment

The average BOD of the duplicate inoculum blank bottles was subtracted from all BOD values obtained in test bottles to calculate the BOD related to substrate biodegradation. Biodegradability was calculated as BOD/ThOD. Final biodegradation (expressed as a percentage of ThOD) was calculated based on the final three time points for the BOD measurements, to account for fluctuations in measurements. In some cases, a pressure drop occurred immediately on incubation because of the temperature of the medium being higher than the incubation room temperature. In such cases, BOD measurements were initiated after 12 h, to ensure acclimatization. Negative values were set to zero.

Scanning electron microscopy analysis

A visual analysis with an electron microscope was completed to detect possible changes in TP surface structure. Scanning electron microscopy (SEM) analysis was performed on pristine and weathered TPs before and after biodegradation, using an Inspect S50 microscope (FEI). Samples were prepared for SEM by filtration and rinsing with ethanol, after which they were dried in Petri dishes and finally transferred onto aluminum tape and placed into the sample holder. A 10 to 30- μ m scale was used, and the Everhart-Thornley detector (ETD) was used to obtain the pictures.

RESULTS

Biodegradation of pristine and weathered TPs

The extent of TP biodegradability under standardized test conditions was investigated, as well as how this was affected by accelerated UV weathering. Figure 1A shows the progress in biodegradation of pristine and weathered TPs over a 28-day period (inoculum concentration 15 mg TSS/L), as well as biodegradation of weathered TPs at augmented inoculum concentration (37 mg TSS/L), expressed as a percentage of the theoretically obtainable amount, that is, ThOD. The values for final degree of biodegradation at day 28, listed in Figure 1A,

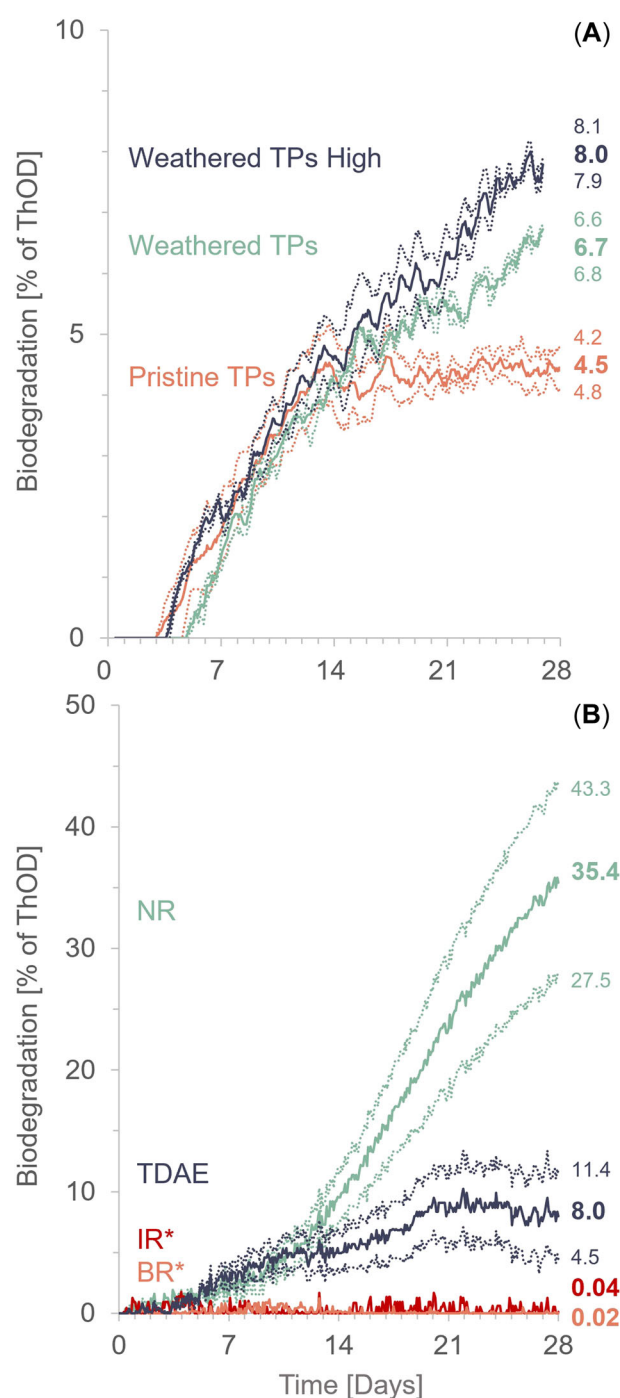


FIGURE 1: The 28-day biodegradation (expressed as a percentage of theoretical oxygen demand) of (A) tire particles—pristine (total suspended solids [TSS] of 15 mg/L), weathered (TSS of 15 mg/L), and weathered along with a high inoculum concentration (TSS of 37.4 mg/L)—and (B) natural rubber, isoprene rubber (IR), butadiene rubber (BR), and treated distillate aromatic extracts (TSS of 20.6 mg/L). The lines represent a moving average of seven data points, equivalent of 15 h. The bold line is the average of two replicates, which are presented as dotted lines. In (B) *IR and BR each had one replicate. The 28-day biodegradation, listed on the right side, is based on the final three biological oxygen demand measurements. The y-axis limit is set to (A) 10% and (B) 50% because all measurements were within this range. ThOD = theoretical oxygen demand; TPs = tire particles; NR = natural rubber; TDAE = treated distillate aromatic extracts.

show <10% of the theoretically obtainable amount. This level of biodegradation would traditionally be considered a part of the lag phase in a standard biodegradability test of a readily biodegradable compound. Nonetheless, the curves in Figure 1A indicate that biodegradation was initiated during the 28 days of incubation. Biodegradation of pristine TPs occurred mainly within the first half of the 4-week experiment, reaching 4.0% (3.3%–4.7%) of ThOD after 14 days, after which it decelerated, reaching 4.5% (4.2%–4.8%) of ThOD after 28 days. Similar to pristine TPs, the biodegradation of TPs subjected to accelerated UV weathering reached 4.3% (4.0%–4.5%) of ThOD and 4.7% (4.3%–5.2%) of ThOD after 14 days for the low and high inoculum concentrations, respectively. Unlike pristine TPs, in the final 2 weeks, the weathered TPs did not level off but continued to degrade throughout the experiment, surpassing the pristine TPs. At the end of the 28-day period, the weathered TPs with increased inoculum concentration showed the highest biodegradation, reaching 8.0% ThOD (7.8%–8.1%). Efforts to increase TP weathering by intensified UV exposure (6 weeks of constant 9 W/m² irradiance instead of an 8:4-h light:dark cycle) did not result in a higher TP biodegradation after 28 days (data not shown).

Biodegradation of individual constituents

Figure 1B summarizes the degree of total biodegradation obtained after 28 days for NR, IR, BR, and TDAE and the respective biodegradation curves. It is evident from Figure 1B that BR, as expected, exhibited no biodegradation; but, more surprisingly, neither did IR. Low biodegradation was observed for TDAE (8.0% [4.5%–11.4%] of ThOD), which appeared to level off after approximately 2 weeks of incubation. The highest degree of biodegradation was observed for NR, for which the lag phase ended at day 13 (i.e., 10% of ThOD was reached), and 35.4% (27.5%–43.3%) of ThOD was reached after 28 days. Through visual analysis and Excel curve fitting (Supporting Information, Figure S3), NR appeared to follow exponential growth for approximately 2 weeks (from day 2 to 16), with a degradation rate of 0.21 day^{−1} ($R^2 = 0.986$). For the remaining days, biodegradation followed a more linear biodegradation path (Supporting Information, Figure S3). Though NR is clearly being biodegraded, it cannot be classified as a readily biodegradable compound.

SEM analysis

Figure 2 presents the SEM analysis of (a) a pristine TP, (b) a UV-weathered TP, (c) a pristine TP after biodegradation, and (d) a UV-weathered TP after biodegradation with high inoculum concentration. For the pristine TP (Figure 2A), the surface of the particle appeared smooth, intact, and uniform; and the edges of the particle were sharp. On UV exposure, the weathered TP (Figure 2B) did not show clear, visible differences compared to the pristine particle (Figure 2A), yet minor irregularities in the surface could be detected. Comparing the pristine TP (Figure 2A) to the TP after

biodegradation with activated sludge inoculum (Figure 2C), slight irregularities and roughness in the particle surface were observed, and the edges of the particles were more rounded. Comparing the UV-weathered TP before biodegradation (Figure 2B) to the weathered one after biodegradation (Figure 2D), a change in the particle surface and edges could be seen. The surface of the particle was no longer uniform and had gaps. A more visible difference was observed when comparing the pristine TP (Figure 2A) to the TP subjected to both UV weathering and biodegradation with high inoculum (Figure 2D): The polymer surface after weathering and biodegradation was porous and rough, indicating that the particle surface is deteriorating and experiencing biodegradation.

DISCUSSION

Biodegradability of pristine and weathered TPs

The biodegradability of pristine TPs was found to be 4.5% of ThOD after 28 days. Despite being <10%, and thus within the lag phase, the observed biodegradation of TPs appeared to be biphasic. A potential explanation for this phenomenon is that during the first 2 weeks microbes primarily utilize readily available substrates. Because easily accessible points for microbial attack are depleted over time, this leads to a slowing down of biodegradation. Accelerated weathering seemed to increase biodegradability of TPs from 4.5% to 6.7% and 8.0% of ThOD with similar and increased inoculum concentrations, respectively (Figure 1A). Weathered TPs moreover appeared to have monophasic biodegradation kinetics over the 28-day period. The UV weathering was assumed to primarily affect the polymeric blend because photooxidation (along with thermooxidation and ozonolysis) has been found able to affect the polymeric properties by chain scission, additional crosslinking, and C-C double bond cleavage (Wagner et al., 2022). Previous literature states that abiotic processes may occur prior to or alongside biodegradation, increasing or accelerating rubber biodegradation, respectively (Cadle & Williams, 1980; Linos et al., 2000; Müller, 2005). Biodegradability of isoprene, butadiene, and styrene (also used for synthetic rubber) oligomers was also found to increase with decreasing molecular weights (Tsuchii et al., 1978, 1979, 1984), indicating that chain scission by photooxidation may enhance biodegradability. In the present study, the abiotic UV weathering of the polymer chain may thus have increased the number of sites available for microbial attack, to the point where they were not depleted over the 28-day period as hypothesized for the pristine TPs. However, biodegradation experiments with extended incubation time are needed to further investigate if the difference between pristine and weathered TPs observed in the present study is significant.

Biodegradability of individual constituents

Results of the biodegradation tests on TP constituents indicate that the 4.5% of ThOD biodegradability observed for pristine TPs may be attributed predominantly to NR and TDAE

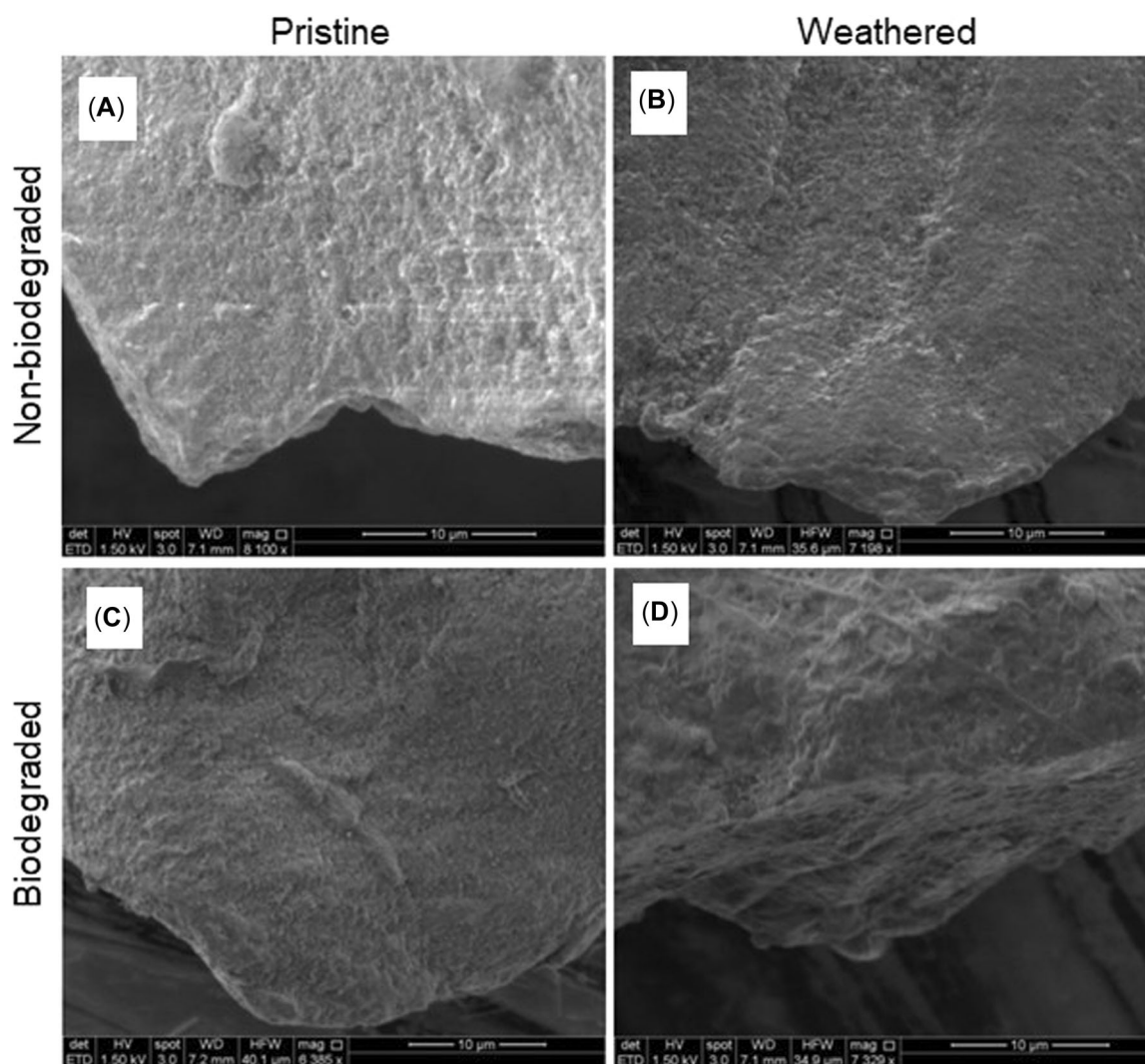


FIGURE 2: Scanning electron microscopy analysis of (A) a pristine tire particle (TP), (B) an ultraviolet (UV)–weathered TP, (C) a pristine TP after biodegradation by activated sludge, and (D) a UV-weathered TP after biodegradation with high inoculum concentration.

biodegradation because these biodegraded by 35.4% and 8.0% of ThOD, respectively, when tested individually. Both IR and BR showed negligible biodegradation over the 28-day test period, and therefore biodegradation of these polymers would not be expected to contribute significantly to the biodegradation of TPs.

Biodegradability of the polymeric blend. Dehydrated NR mainly contains *cis*-1,4-polyisoprene (87%–94% [w/w]) and minor amounts of proteins, carbohydrates, lipids, and minerals (Bottier, 2020; Subramaniam, 1995). It is synthesized by >2000 plant species (Rose & Steinbüchel, 2005). It is therefore plausible that microorganisms, capable of utilizing this carbon source, are present in inoculum of mixed origin, like activated sludges (Bode et al., 2001; Kanwal et al., 2015; Linos & Steinbüchel, 2005; Tsuchii, 1995). It has previously been found that unvulcanized NR was 100% biodegraded after 8 weeks by *Nocardia* sp. (Tsuchii et al., 1985) but also that any structural deviations from untreated NR resulted in highly decreased biodegradability. The latter is emphasized by the cases

of (1) vulcanized rubbers, including vulcanized NR, where biodegradability decreased significantly compared to the unvulcanized form (Cadle & Williams, 1980; Tsuchii et al., 1985, 1990); (2) IR, where biodegradability was halved when *cis*-1,4-polyisoprene content was reduced from 98% to 93% (Tsuchii et al., 1985); and (3) BR and styrene–butadiene rubber (SBR), whose structures vary significantly from NR and whose biodegradation was very limited, even when unvulcanized. Only 1% to 2% and 4% of unvulcanized BR and SBR, respectively, was biodegraded after 6 to 8 weeks of exposure to *Nocardia* sp. (Linos et al., 2000; Tsuchii et al., 1985). Likewise, biodegradability of rubber blends was found to depend on the rubber ratios. An NR:BR:SBR rubber blend of 90:5:5 was biodegraded by 38% over 8 weeks compared to only 12% biodegradation of a 55:30:15 blend (Tsuchii & Tokiwa, 1999). The TPs of the present study, showing a 4.5% 4-week biodegradation, contained a rubber blend with an approximately 40:60 ratio of NR to synthetic rubber. Thus, the studies above, connecting decreased biodegradability with structural deviation from NR, agree with the low degradation of

TP and BR observed in the present study, while the negligible biodegradability of IR was more unexpected. Contrary to our findings, a previous study measured a weight loss of 36% to 57% for TPs over 8 weeks (Tsuchii & Tokiwa, 2001), which is much higher compared to the present study. However, this may be explained by their use of a mutant strain of *Nocardia* with a strong ability to degrade tire rubber, combined with testing of TPs originating from truck tires. In general, NR content is higher in tires for heavy vehicles compared to passenger car tires. In the study by Tsuchii & Tokiwa (2001) the TPs thus had a 70% rubber content consisting purely of NR.

Biodegradability of oils. The biodegradation of TDAE was found to be approximately 8.0% of ThOD. In a study by Cadle & Williams (1980), it was also found that extender oils were degradable and that after 16 months of soil incubation, more than half of the extender oil content was oxidized enough for it to evaporate at ambient temperature. A review of mineral oils in general (covering oils made from dewaxed paraffin-based crude oils) found their biodegradability to range between 15% and 35% (Aluyor & Ori-Jesu, 2009), thus slightly above what was observed in the present study. One study found indirect evidence that biodegradation of tire polymers occurs by a cometabolic pathway, which is dependent on the presence of a metabolizable carbon source (Faber & Nickerson, 1979). In addition to being slightly biodegradable itself, the presence of oils (although their bioavailability is likely limited) may thus increase biodegradability of rubber when present in TPs, compared to rubber by itself, by facilitating polymer biodegradation through a cometabolic pathway. Elucidating this process was not a focus of the present study, and it remains unknown if and to what degree the biodegradability of rubber in TPs would decrease in the absence of TDAE oils. The possibility of such a cometabolic pathway, however, illustrates the complexity in factors influencing the biodegradability of TPs owing to its multicomponent composition.

Biodegradability of other TP constituents. Carbon black was excluded from testing in the present study because it was assumed to be nonbiodegradable, because of its structural strength (Tsuchii & Tokiwa, 2006). Cadle & Williams (1980) found that total carbon content was not reduced after 16 months of TWP degradation, which supports the choice to exclude it from the present study. Carbon black may further reduce TP biodegradation because it can protect against microbial attack (Tsuchii et al., 1990, 1996). The presence of carbon black in TPs is also likely to decrease the effect of abiotic photooxidation on the polymeric blend. With an absorption coefficient close to 1 (Razd'yakonova et al., 2013), carbon black protects TPs from UV radiation and is also thought to protect polymers from oxidative degradation because it stabilizes the free radicals that are formed during oxidation (Fock, 1968). Regarding the remaining TP constituents, the available literature suggests that polymeric silica can be biodegraded into monomers by microbes (Lauwers & Heinen, 1974); stearic acid and paraffin wax have been found

to be readily biodegradable (G. R. Williams, 1986); and there are studies showing that fungi and bacteria are capable of altering the composition of other additives (Keurstien & Groenevelt, 1996; Stevenson et al., 2008). Finally, zinc oxide, sulfur, accelerators, and antioxidants have been found to have an inhibitory effect on biodegradation (Ali Shah et al., 2013; G. R. Williams, 1986); however, the present study did not find TPs to inhibit the biodegradation of NaOAc.

Changes in biodegradability from individual constituents to particles in the environment

Biodegradability of particles originating from tread abrasion varies depending on whether it is assessed based on isolated tests of their individual constituents or as the compounded particle. Particle composition and physical conditions, and therefore biodegradability, further vary throughout the lifetime of a particle, from individual constituents to TPs to TWPs and finally to TRWPs. The following sections address this and are summarized in Figure 3.

From individual constituents to TPs. The results of the present study are in general agreement with the existing literature that NR and TDAE are likely the major contributors to TP biodegradability because of their individual biodegradability and high content of TPs. However, the NR content of the studied TPs was 38% to 42% (Supporting Information, Table S1). If NR biodegraded in TPs as it did on its own (35.4% [27.5%–43.3%] of ThOD), this should result in a TP biodegradability of 10.45% to 18.19% of ThOD, attributed solely to NR. Similarly, the oil content of TPs was likely 12% to 20% (Table 1). If this biodegraded in TPs to the same extent as on its own (8.0% [4.5%–11.4%] of ThOD), TDAE biodegradation alone should result in a TP degradability of 0.5% to 2.3% of ThOD. Assuming that NR and TDAE are the only contributors to TP biodegradation, the potential TP biodegradability would be 11.0% to 20.5% of ThOD. Therefore, the observed biodegradation for pristine TPs of only 4.5% (4.2%–4.8%) of ThOD indicates that the biodegradability of individual constituents is decreased by a factor of 2 to 5 when compounded into tire tread. The cause of this may be (1) a change in NR structure caused by its vulcanization in tire tread, (2) a protection against microbial attack by the addition of carbon black, (3) an inhibition of microbial activity by toxic additives, or (4) simply the fact that in TPs the biodegradable constituents are compounded with nonbiodegradable compounds, physically hindering access for a microbial attack, especially over short periods. Results of the SEM analysis appear to validate that TP degradation, both biotic and abiotic, can serve as an attack on the particle surface, potentially leaving the core unaffected by degradation. It is noteworthy that if oils do indeed facilitate increased biodegradation of the TPs through a cometabolic pathway, then they may have partly counteracted factors decreasing biodegradability, listed above. Further research is needed to quantify the influence of these opposing processes on TP biodegradation.

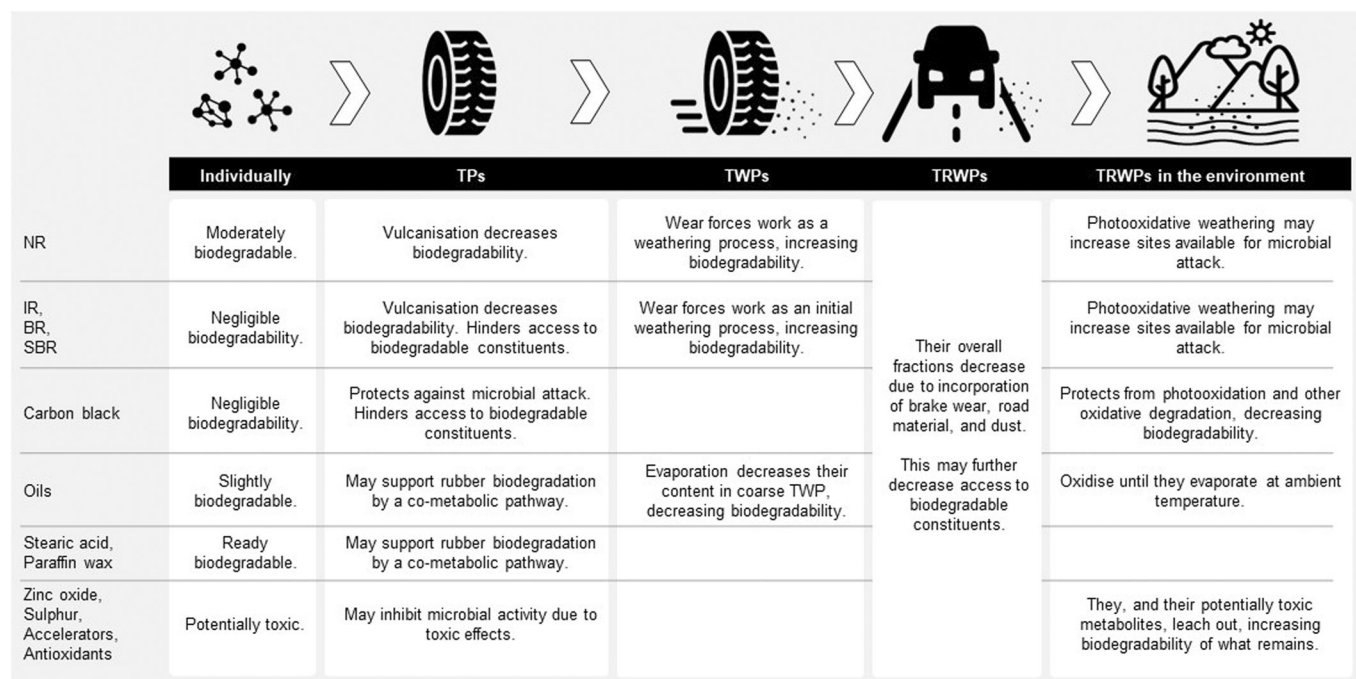


FIGURE 3: A schematic overview of how each group of tire tread constituents contributes to and affects the degradability of the main particle (the polymeric blend) and which processes they are subjected to, through the different stages of the tread mixture—from individual constituents to tire and road wear particles persisting in the environment. Based on findings of the present study and those presented in *Discussion*. TPs = tire particles; TWPs = tire wear particles; TRWPs = tire and road wear particles; NR = natural rubber; IR = isoprene rubber; BR = butadiene rubber; SBR = styrene–butadiene rubber.

From TPs to TWPs. In the present study, TPs were tested. However, the actual particles generated by wear of tire tread are TWPs. When tires are in contact with the road surface, the wear forces are strong enough to break sulfur bridges and carbon bonds. The polymeric blend of TWPs is therefore often less vulcanized than that of TPs (Russel, 1969; R. L. Williams & Cadle, 1978) and has polymers of lower molecular weight (Cadle & Williams, 1980). Because unvulcanized rubber was found to be more biodegradable than vulcanized (Tsuchii et al., 1985, 1990), wear forces may work as an initial weathering process, making TWPs more susceptible to subsequent biodegradation than their TP precursor. This is supported by Cadle & Williams (1980), who found the rubber decrease of TWPs to be 52% after 16 months in soil compared to only 4% for TPs of the same tire. The TP biodegradability found in the present study may thus be substantially lower than actual TWP biodegradability. On the other hand, oils are susceptible to evaporation during the wear processes, altering the oil content in the coarser particles formed by abrasion (Cadle & Williams, 1978; Dannis, 1974), which in turn may decrease the overall biodegradability of the coarser particles.

From TWPs to TRWPs. On integration with the road surface and environment, TWPs change further by incorporating other car emissions, pavement material, and road dust and turn into TRWPs. Therefore, TRWPs contain fractionally less polymers and more minerals and metals than TPs and TWPs (Wagner et al., 2022). Polymer and mineral content of TRWPs were found to be 13% and 53%, respectively, compared to 46% and 16% for TPs (Kreider et al., 2010). As a result, the density of

TRWPs was reported to be 1.3 to 2.2 g/cm³ (Klöckner et al., 2019; Kreider et al., 2010; Sommer et al., 2018), compared to a density of 1.1 to 1.2 g/cm³ for TPs (Kayhanian et al., 2012; Rhodes et al., 2012; Wagner et al., 2018). Because metals and minerals are not biodegradable, the percentage biodegradability of TRWPs may be lower than that of TWPs as a result. In addition, their incorporation may further decrease accessibility of microorganisms to the biodegradable contents.

TRWPs in the environment. In the environment, TRWPs are subjected to conditions different from those of standardized laboratory test conditions. The effect of weathering, observed in the present study, suggests that abiotic processes in the environment can increase TP biodegradation compared to laboratory conditions. In the environment, particles are furthermore subjected to leaching. Zinc, chromium, copper, lead, aluminum, 1-indanone, and benzothiazole have been found to leach from TPs (Halle et al., 2021; Simon et al., 2021; Wagner et al., 2022), with leaching of heavy metals increasing on UV weathering (Simon et al., 2021). Potentially toxic metabolites, formed by transformation of additives, may also leach out (Tian et al., 2021). Leachates from TPs were found to cause inhibition of algal growth (Simon et al., 2021), and removal of toxic constituents by leaching has been found to improve the susceptibility of particles to subsequent biodegradation (Fan et al., 2021; Klöckner et al., 2019; Stevenson et al., 2008). The inhibition tests of this specific study, however, showed no signs of TPs inhibiting microbial activity. The opposite process (sorption) may also occur; however, how this affects biodegradation needs further research (Wagner et al., 2022). Overall, leaching, oxidation, and

biodegradation are mentioned as the most relevant processes influencing the environmental persistence of TRWPs (Baensch-Baltruschat et al., 2020; Wagner et al., 2022). The relative importance and rate of each process are determined by which environmental compartment the particles reside in. Information on degradation of TRWPs under environmentally relevant conditions is therefore of great importance. For example, Cadle & Williams (1980) found the polymer degradation of TWP (19.5% oil, 38.9% polymers, 36.0% carbon black) to be 4% after 16 months in roadside soil. The biodegradation results of the present study, on the other hand, do not represent environmentally realistic conditions. Rather, our results provide insights into whether biodegradation can be initiated under standardized conditions, allowing for controlled experiments of contributing factors to TP biodegradation. Future testing efforts should ideally include more replicates than those required by the OECD 301 protocol, which is designed for regulatory purposes. This would increase the statistical power, thereby increasing the sensitivity and precision of test results, and ultimately allow for more robust conclusions. They should preferably test substrates that are more relevant for real-life TRWPs to elucidate the effects of devulcanization and changed polymer and mineral ratios. Experimental conditions should allow for both leaching and photooxidation to occur prior to or alongside biodegradation to investigate environmentally realistic degradation of tire wear emissions.

CONCLUSION

Particles from tire abrasion are generally considered persistent in the environment, which was confirmed by the present study finding pristine TP biodegradability to be 4.5% of ThOD. Furthermore, accelerated weathering by UV irradiation was found to increase final biodegradation by a factor of 1.5 and 1.7 with and without increased inoculum concentration, respectively, indicating potential accelerated TP degradation in the environment compared to laboratory settings. Based on the degradation patterns, biodegradation would likely have increased further for weathered materials if the test period had been extended. The present study also found NR and oils to likely be the major biodegradable constituents, reaching 35.4% and 8.0% of ThOD, respectively, after a 28-day period. The biodegradability of the compounded TPs was thus found to be less than half of what could be estimated, based on the relative proportion and isolated biodegradability of the individual components. Both IR and BR displayed very minor biodegradation over the 28-day period (0.04% and 0.02% of ThOD, respectively). In conclusion, the findings of the present study suggest that emissions of tire wear persist as particles consisting mainly of synthetic rubber polymers and carbon black. Over time, the TP content of oils and NR will be reduced as a result of biodegradation, and additives will leach out. It is likely these residual particles which contribute to long-term environmental microplastic pollution from tread abrasion. Limitations of the present study relate to the lack of insight into specific degradation kinetics and intermediates and the environmental relevance of the conditions and substrate. Thus, a

better understanding of interlinked and opposing processes governing the degradation of these complex materials in different environmental compartments is a subject of further studies, where soil degradation is particularly interesting, being the final sink for a large fraction of TWP emissions. Further studies should also consider the influence on biodegradation of the incorporation of pavement material and road dust, constituting environmentally relevant TRWPs.

Supporting Information—The Supporting Information is available on the Wiley Online Library at <https://doi.org/10.1002/etc.5757>.

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Data Availability Statement—Additional data can be found in the Supporting Information or are available on request by contacting the corresponding author, Nanna B. Hartmann (nibh@dtu.dk).

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