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Manuscript

Quantification of synthetic polyesters from biodegradable mulch films in soils

Taylor F. Nelson[†], Stephanie C. Remke[‡‖], Hans-Peter E. Kohler[§], Kristopher McNeill[†], and Michael Sander[†*]

[†] Institute of Biogeochemistry and Pollutant Dynamics, ETH Zurich, 8092 Zurich, Switzerland

[‡] Drinking Water Chemistry Group, Water Resources and Drinking Water, Swiss Federal Institute of Aquatic Science and Technology (Eawag), 8600 Dübendorf, Switzerland

[‖] School of Architecture, Civil and Environmental Engineering (ENAC), Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

[§] Environmental Biochemistry Group; Environmental Microbiology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), 8600 Dübendorf, Switzerland

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*To whom correspondence should be addressed.
E-mail: michael.sander@env.ethz.ch
Phone: +41-(0)44 632 8314
Fax: +41-(0)44 633 1122

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ORCID Numbers of Authors:
Taylor F. Nelson: 0000-0003-0719-4402
Stephanie C. Remke: 0000-0001-9991-2162
Hans-Peter E. Kohler 0000-0001-7667-0762
Kristopher McNeill: 0000-0002-2981-2227
Michael Sander: 0000-0003-3383-2041

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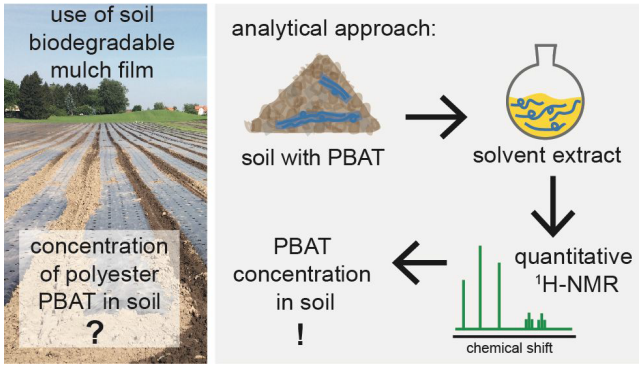


Abstract

Soil biodegradable mulch films composed of the polyester polybutylene adipate-*co*-terephthalate (PBAT) are increasingly used in agriculture. Analytical methods to quantify PBAT in field soils are needed to assess its soil occurrence and fate. Here we report an analytical method for PBAT in soils that couples Soxhlet extraction or accelerated solvent extraction (ASE) to quantitative proton nuclear magnetic resonance (q-^{1}H NMR) spectroscopy detection. ^{1}H NMR peak areas of aromatic PBAT protons increased linearly with PBAT concentrations dissolved in chloroform ($CHCl_3$), demonstrating accurate quantitation of PBAT by q-^{1}H NMR. Spike-recovery experiments involving PBAT addition to model sorbents and soils showed increased PBAT extraction efficiencies into $CHCl_3$ with methanol (MeOH) as co-solvent, consistent with MeOH competitively displacing PBAT from H-bond donating sites on mineral surfaces. Systematic variations in solvent composition and temperatures in ASE revealed quantitative PBAT extraction from soil with 90/10 volume% $CHCl_3$/MeOH at 110-120 °C. Both Soxhlet extraction and ASE resulted in complete recovery of PBAT added to a total of seven agricultural soils covering a range of physicochemical properties, independent of whether PBAT was added to soils dissolved in $CHCl_3$, as film, or as particles. Recovery was also complete for PBAT added to soil in the form of a commercial soil biodegradable mulch film with co-extractable polylactic acid. The presented analytical method enables accurate quantification and biodegradation monitoring of PBAT in agricultural field soils

Introduction

Agricultural production heavily relies on the use of plastics – a practice coined plasticulture – to increase crop yields while lowering the consumption of water for irrigation and the use of agrochemicals, such as pesticides and fertilizers.[1] The agricultural plastic market is dominated by polymeric films,[2-6] among which mulch films have the largest share (approximately 40% of the agricultural plastic films market; with an estimated 3 million tons used annually by 2021).[4-8] Mulch films have thicknesses in the µm-range and are applied directly to the soil surface to control weed growth, preserve soil moisture content, increase soil temperature, and improve soil structure.[1,2,5,6,9-12] Incomplete recovery of the thin mulch films from the soils after use, however, leads to the transfer of residual polymer films into agricultural soils. Because conventional mulch films are composed of persistent polyethylene (PE),[13] these remnant film pieces accumulate in soils over time and thereby threaten soil health and productivity.[1,2,9-12] A promising approach to overcome the accumulation of such plastic films in soils is to replace conventional mulch films with soil biodegradable polymeric mulch films, which are designed to be completely degraded *in situ* by soil microorganisms after ploughing the used films into soils. Biodegradation in soils is considered a viable end-of-life treatment because used mulch films are typically heavily contaminated by soil and plant debris, rendering the films difficult to recycle and leaving incineration or landfilling as the more economical, but less sustainable, end-of-life treatment options.[2,12,13]

Commercialized soil biodegradable mulch films are commonly composed of bio-based and fossil-based aliphatic and aliphatic-aromatic (co-)polyesters.[13,14] Biodegradation of these films relies on soil microorganisms utilizing the polyesters for energy production —under conversion of polyester carbon to CO_2 in oxic soils (referred to as "mineralization")— and for forming new microbial biomass.[15,16] Film biodegradation is commonly assessed through laboratory soil incubations coupled to respirometric analyses of formed CO_2 as the ultimate

biodegradation end product.[17,18] Such laboratory respirometric analyses are required by biodegradation standards which typically stipulate extensive polymer mineralization over a defined time span (e.g., 90% mineralization – either as an absolute amount or relative to the mineralization of an accepted biodegradable reference material – in soils over two years for soil biodegradable mulch films in the EU soil biodegradable mulch film standard EN 17033-2018).

Compared to respirometric analyses of mulch film biodegradation in laboratory soil incubations, such analyses are difficult (if not impossible) to conduct under less-controlled conditions in the field. As a consequence, determining mulch film biodegradation *in situ* in agricultural field soils has been challenging. Methods used so far, such as visual inspection of the disappearance of films, measuring mass loss over time, and capturing changes in the physicochemical and mechanical properties of remnant polymer film pieces, cannot distinguish mere physical disintegration of films from true biodegradation.[18,19] Accurate quantification of total residual polymer would serve as a more robust proxy for polymer biodegradation, but so far has been challenging to achieve.[20] However, it would be of vital importance to have available such analytical methods that could be used as viable alternatives to respirometric analyses for the assessment of mulch film biodegradation in the field.

In contrast to many conventional polymers that are insoluble in organic solvents, aliphatic and aliphatic-aromatic co-polyesters used in soil biodegradable mulch films readily dissolve in chlorinated solvents, such as chloroform. Solubility in organic solvents is a prerequisite for applying established extraction techniques for recovering these polyesters from soils, such as Soxhlet extraction and accelerated solvent extraction (ASE).[21] Past work demonstrated the principle feasibility of using such extraction techniques for extracting biodegradable polyesters from soils.[22-24] Yet, reliable determination of polyester extractability and recovery from different soils remained missing in these studies because adequate methods

for quantification of extracted polyesters were not available. Methods depending on gravimetric determinations of the extracted polyester mass after solvent removal are ill-suited for reliable quantification as they are susceptible to artifacts arising from any co-extracted soil matrix components (e.g., natural organic matter).[22,25] Also methods that depend on hydrolytically converting the extracted polyester into mono- and short oligomers, followed by their quantitative analysis by gas- or liquid-chromatography coupled to mass spectrometry (GC-MS and LC-MS, respectively)[24,26] are not well suited for routine and fast quantification of extracted polyesters. Such methods are labor and time consuming, often require derivatization of the formed oligomers and monomers and standard compounds for quantification are not readily commercially available.

The goal of this work was to develop analytical methods to quantify polyesters used in biodegradable mulch films in soils. We chose the aliphatic-aromatic co-polyester polybutylene adipate-*co*-terephthalate (PBAT) for method development because PBAT is used extensively in commercial soil biodegradable mulch films and serves as model for the larger class of synthetic biodegradable polyesters. In the first part of this work, we present a method for accurate and precise quantification of PBAT dissolved in $CDCl_3$ using internal standard quantification and quantitative proton NMR spectroscopy (q-^{1}H NMR). Analysis by ^{1}H-NMR can be conducted directly on the soil extract after its re-constitution in $CDCl_3$. Therefore, neither extensive purification of the extract, nor any chromatographic separation and chemical hydrolysis and/or derivatization of the PBAT are required prior to the analysis. In the second part, we present a methodology for systematically assessing Soxhlet and AS extraction of PBAT both from model sorbents (including both common soil minerals and synthetic particles) and from natural soils with a range of physicochemical properties.

Materials and Methods

Chemicals. Chloroform ($CHCl_3$) and methanol (MeOH) (both HPLC grade) were from Fischer and deuterated chloroform ($CDCl_3$) (99.8 atom% D) was from Amar. We stored $CHCl_3$ and $CDCl_3$ with 4 Å molecular sieves (Sigma-Aldrich) to remove water. 1,4-dimethoxybenzene (DMB) (> 99% purity) was from TCI. We used all solvents and chemicals as received. Polybutylene adipate-*co*-terephthalate (PBAT) was provided by BASF SE. Most of the experiments were conducted with PBAT that was synthesized according to a previously published protocol,[27] and had a number-averaged and weight-averaged molecular weights of M_n= 18'300 Da and M_w= 56'100 Da, respectively (i.e., a polydispersity index M_w/M_n= 3.1), a glass-transition temperature of T_g= -31 °C and a melting temperature of T_m= 127 °C. Few selected experiments were conducted with extrusion blown PBAT films (circular cut pieces with 1 cm diameter and thickness of 25 μm), or we cryo-milled the synthesized PBAT material and collected the fraction of particles with a diameter < 300 μm.

Commercial film. We used a biodegradable mulch film from Sansonnens (Estavayer, Switzerland; certified as "OK biodegradable soil" (TÜV Austria)) which was composed mainly of PBAT and polylactic acid (PLA).

Model sorbents and natural soils. Silica (SiO_2) particles with different sizes (i.e., glass beads with diameters 150–212 μm and ≤ 106 μm and specific surface areas of SSA= 0.012 and 0.029 $m^2\ g^{-1}$, respectively; glass spheres with diameters 9–13 μm and SSA= 0.60 $m^2\ g^{-}1$; and silicon dioxide with diameters 0.5–10 μm and SSA= 6.18 $m^2\ g^{-1}$) were from Sigma-Aldrich. Polytetrafluoroethylene (PTFE) particles with two sizes (i.e., diameters of 35 and 1 μm with SSA= 0.55 and 9.18 $m^2\ g^{-1}$, respectively) were from Sigma-Aldrich and a PTFE foil was taken from a laboratory stock. Goethite (SSA = 13.04 $m^2\ g^{-1}$) was from Bayer (Bayferrox 910, Germany), gibbsite ($Al(OH)_3$, ≥ 99.63%, SSA = 0.78 $m^2\ g^{-1}$) from Merck (Germany), and montmorillonite and kaolinite (SWy and KGa with SSAs of 4.91 and 8.29 $m^2\ g^{-1}$) were from

the Source Clay Minerals Repository (USA). For method development, we primarily used LiHof soil, which was collected from an agricultural research center (Limburgerhof, Germany). To demonstrate efficient extraction of PBAT from different soils, we included Pahokee Peat II (PP) soil with a high organic carbon content (International Humic Substances Society (IHSS); USA) (SSA = 0.26 m^2 g^{-1}; organic carbon content = 40.9%, organic nitrogen content = 3.40% (published by IHSS)[28]) and six standard agricultural soils of varying soil types and physicochemical properties from LUFA Speyer (Germany). **Table 1** lists selected physicochemical properties of the seven main agricultural soils used for extractions. We determined the specific surface area of all model sorbents and soils using a Nova 3200e (Quantachrome, USA) and the N_2-BET method. All remaining soils parameters for LiHof soil were determined according to reference methods of the Swiss Federal Research Stations[29] and for LUFA soils were provided by LUFA Speyer and determined according to good laboratory practices (GLP).

Table 1. Selected physicochemical properties of agricultural soils used for extractions.

soil	texture [a]	sand [b]	silt [c]	clay [d]	SSA [e]	organic-C	total-N	pH	CEC [f]
		——— mass % ———			m^2 g^{-1}	——— mass % ——			meq 100 g^{-1}
LiHof	sandy clay loam	63	16	20	1.18	0.84	0.10	7.0	7.68
LUFA 2.1	sand	86	11	3	0.88	0.71	0.06	4.9	4.2
LUFA 2.2	sandy loam	76	16	8	1.58	1.59	0.17	5.4	9.7
LUFA 2.3	sandy loam	60	34	6	4.11	0.67	0.08	5.7	7.5
LUFA 2.4	loam	33	41	26	24.68	2.03	0.22	7.3	33.0
LUFA 5M	sandy loam	58	31	11	5.48	1.02	0.13	7.3	17.4
LUFA 6S	clay	25	34	41	34.58	1.77	0.18	7.2	26.5

[a]texture classified according to USDA soil classification system; [b]sand refers to soil particles with diameter 0.05 – 2.0 mm; [c]silt refers to soil particles with diameter 0.002 – 0.05 mm; [d]clay refers to soil particles with diameter < 0.002 mm; [e]SSA = Specific Surface Area, as determined by N_2-BET; [f]CEC = Cation Exchange Capacity

General extraction procedure.

Our analytical method requires dry (water-free) samples for PBAT quantification. We therefore first dried all sorbents (i.e., minerals and soils) by freezing them overnight, followed by freeze-drying under vacuum at 0.01 mbar for at least 24 hours (Alpha 2-4 LD plus, Christ, Germany). In a second step, we loaded the sorbents into the extraction chambers (Soxhlet), extraction cells (ASE), or amber vials (ultrasonication) before adding PBAT. For most spike-recovery experiments, we added PBAT by transferring aliquots from a PBAT stock solution in $CHCl_3$ (note that the transferred amount of PBAT was experiment dependent), followed by letting the $CHCl_3$ evaporate. At the same time, we transferred the same volume of the PBAT chloroform solution also directly either into empty 1.5 mL amber vials (whenever samples were prepared in another laboratory and needed to be transported back to our laboratory) or directly into NMR tubes, added $CDCl_3$ with a known amount of internal standard, and finally quantified the transferred PBAT using q-^{1}H NMR. These extraction-free samples served as controls for recovery calculations of PBAT. For selected spike-recovery experiments, we additionally transferred PBAT to soils both in the form of powder or foil (i.e., powder was mixed into the soil with a small spatula and PBAT foil was completely covered by soil prior to starting the extraction). In these cases, we prepared extraction-free controls by weighing PBAT powder aliquots or foil pieces into amber vials, added $CDCl_3$ with a known amount of internal standard, and quantified the transferred PBAT using q-^{1}H NMR.

Soxhlet extractions. We added the dried sorbents into the extraction chamber of micro-Soxhlet extractors (ChemGlass, USA; extractor volume 6 mL) onto glass wool that we placed at the bottom of the chambers to provide support for the sorbent. We subsequently added PBAT onto the surface of the sorbent, and placed a second layer of glass wool on top of the sorbent. The round bottom flask at the bottom of the extractor contained 20 mL of extraction solvent (either pure $CDCl_3$, pure $CHCl_3$, or $CHCl_3$/MeOH mixtures) and a PTFE stir bar. We heated the

assembled extraction apparatus under reflux and stirring for 8 hours, followed by letting the apparatus cool back to room temperature. For experiments in which we extracted into $CHCl_3$ or $CHCl_3$/MeOH solvent mixtures, we subsequently completely removed these solvents under a continuous stream of compressed air, followed by reconstituting the dried extracts in 2 mL of $CDCl_3$ containing a known amount of the internal quantification standard DMB. For experiments in which we extracted into $CDCl_3$, we spiked a known amount of DMB directly into the extraction solvent. An aliquot of each $CDCl_3$ extract solution was then transferred to an NMR tube for q-^{1}H NMR analysis.

Accelerated solvent extractions. ASE was conducted with a Dionex ASE 350 instrument. We first fitted glass fiber filters (type D28 ASE 350, Dionex) into the stainless-steel ASE extraction cells (10 mL volume, Thermo) before adding a known mass of dried sorbent into the cell, followed by adding PBAT (see above). We subsequently loaded the extraction cells into the ASE autosampler tray for automated extraction. We used either pure $CHCl_3$ or $CHCl_3$/MeOH mixtures as extraction solvents. Unless otherwise noted, extractions were run at 120 °C and 105 bar. Each sample was extracted in three sequential extraction cycles and collectively collected into one extraction vial (total volume of 40–50 mL collected into 60 mL clear glass vials). One extraction cycle consisted of the following steps: (1) heating of the extraction cell to the set temperature, (2) filling of the cell with the solvent and pressurizing the cell to set extraction pressure; (3) static extraction conditions for 10 minutes; (4) rinsing the cell with 10 mL (corresponding to the total cell volume) of solvent; (4) N_2-purging of the cell for 90 seconds. After extraction, we quantitatively transferred each extract into a glass sample tube (Büchi, Switerland) which was placed in a parallel sample evaporator (Syncore Analyst; Büchi, Switerland) in which the solvent was removed under heating (50 °C), horizontal shaking (90 min^{-1}) and vacuum (ramped from 1’000 to 200 mbar over 30 minutes, then held at 200 mbar for 2 hours). We subsequently re-dissolved the dried extract in 2 mL $CDCl_3$ containing a known

amount of the internal standard DMB. An aliquot of this solution was then transferred to an NMR tube for q-^{1}H NMR analysis.

Ultrasonication extractions. We extracted PBAT (2.5 mg) either from silica (1.5 g) or LiHof soil (2.5 g) in 20 mL glass amber vials into 10 mL of either pure $CHCl_3$ or a 90/10 vol% $CHCl_3$/MeOH mixture. We also added the same amount of PBAT into empty 20 mL glass amber vials which served as sorbent-free controls for recovery calculations of PBAT. Following addition of the solvent (mixture), we shook the vials on a horizontal shaker (300 rpm) for 5 minutes, followed by sonication of the vials in a sonication water bath (USC 600 D, VWR; 10 minutes at a power of 130 W) cooled with ice. After sonication, we stored the samples for 45 minutes on the lab benchtop. We repeated the above steps two more times but after the third sonication, we directly centrifuged the vials (2500 rpm for 5 min), followed by transferring 1 mL of the supernatant liquid into 2 mL glass amber vials. We then evaporated the solvent and reconstituted the dried extract in 1 mL of $CDCl_3$ containing a known amount of DMB, then transferred this solution to an NMR tube for q-^{1}H NMR analysis.

Quantification of PBAT by q-^{1}H NMR.

We quantified PBAT in $CDCl_3$ by q-^{1}H NMR (Bruker Avance III 400 MHz NMR; Bruker 5 mm BBFO 400 MHz Z-Gradient probe). We added DMB as internal quantification standard because of the similar ^{1}H chemical shifts of the aryl protons in PBAT and DMB and of the alpha carbon protons in butanediol in PBAT and methoxy carbon protons in DMB. The peaks corresponding to these protons were well-resolved (see **Figure 1a,b** for chemical structures of PBAT and DMB, and the corresponding NMR spectra with annotated peaks, respectively). For each sample, we acquired 16 dummy scans and 128 measurement scans with a pulse width of 8.3 µs, and set a delay time between scans of 15 seconds. The number of scans was selected to increase the signal-to-noise ratio of the spectra, while minimizing the overall analysis time (~45 minutes per sample). The pulse width and delay time were selected to optimize accuracy,

as detailed in section S1 of the Supporting Information. NMR spectra were analyzed using the software MNova (Mestrelab, Spain). We first referenced chemical shifts to the ^{1}H peak of residual $CHCl_3$ (δ = 7.26 ppm). We subsequently performed a manual phase correction to the spectra to smoothen the baseline and set it to approximately zero intensity values. We manually integrated ^{1}H peaks at δ= 8.09, 4.40, and 4.12 ppm for PBAT and δ= 6.84 and 3.77 ppm for DMB (**Figure 1b**). We used well-resolved peak areas (A_i) of the aryl protons of PBAT (δ= 8.09 ppm) and DMB (δ= 6.84 ppm) for quantification. We used the area ratio of peaks at 4.40 ppm (alpha protons in a butanediol monomer adjacent to a terephthalate monomer; $A_{\text{B–T }^1\text{H}}$) and 4.12 ppm (alpha protons in a butanediol monomer adjacent to an adipate monomer; $A_{\text{B–A }^1\text{H}}$) to calculate the ratio of terephthalate (T) to adipate (A) in the extracted PBAT (i.e., $\text{ratio}_{\text{T:A}}$) according to Eq. 1:

$$\text{ratio}_{\text{T:A}} = 2 \cdot \frac{A_{\text{B–T }^1\text{H}}}{(A_{\text{B–T }^1\text{H}} + A_{\text{B–A }^1\text{H}})} \quad \text{Eq. 1}$$

We used the T:A ratio to quantify the amount of extracted PBAT, $n_{\text{PBAT,measured}}$ (mg), based on the ratio of the area of the PBAT-aryl proton peak ($A_{\text{aryl PBAT }^1\text{H}}$) to the area of the DMB-aryl proton peak ($A_{\text{aryl DMB }^1\text{H}}$) as Eq 2:

$$n_{\text{PBAT,measured}} = \frac{A_{\text{aryl PBAT }^1\text{H}}}{A_{\text{aryl DMB }^1\text{H}}} \cdot \frac{n_{\text{DMB}}}{M_{\text{DMB}}} \cdot M_{\text{PBAT}} \cdot \frac{1}{\text{ratio}_{\text{T:A}}} \quad \text{Eq. 2}$$

where n_{DMB} is the known mass of DMB added to the extract, and M_{DMB} and M_{PBAT} are the molecular weights of DMB (= 138.17 g mol^{-1}) and of the repeat unit B-A-B-T in PBAT (= 420.45 g mol^{-1}), respectively. We report extraction efficiencies as a percentage (%) of the amount of PBAT added, $n_{\text{PBAT,added}}$ (mg) (Eq. 3):

$$\text{extraction efficiency} = \frac{n_{\text{PBAT,measured}}}{n_{\text{PBAT,added}}} \cdot 100 \quad \text{Eq. 3}$$

Results and Discussion

Quantification of PBAT by q-^{1}H NMR

We assessed the accuracy and precision of q-^{1}H NMR to quantify PBAT by analyzing a set of ten calibration standards of varying PBAT concentrations (from 0.01 – 10 mg PBAT mL^{-1}; single analysis of three replicates for each PBAT concentration) spiked with a constant amount of the internal standard DMB and prepared in $CDCl_3$. **Figure 1a** shows the chemical structures of PBAT and DMB. The symbols mark the protons that we chose for PBAT quantification, with the corresponding peaks highlighted in a representative ^{1}H NMR spectrum (standard with 1 mg PBAT mL^{-1}) in **Figure 1b**. PBAT concentrations were calculated according to Eq. 2. As can be seen in **Figure 1c**, there was a linear relationship with a slope of one between the measured and the nominal PBAT concentrations. This finding and the small variability between triplicates (standard deviations of measured PBAT concentrations between triplicate standards were on average 2% relative to the mean value of the triplicates) demonstrated that PBAT quantification by q-^{1}H NMR was both accurate and precise. The ^{1}H NMR-signal peaks of the protons that are not marked in **Figure 1a** showed partial overlap and were not suitable for quantification.

Based on peak heights, we determined the limit of detection (LOD) and that of quantification (LOQ) for PBAT in $CDCl_3$ as 1.3 µg mL^{-1} and 4.4 µg mL^{-1}, respectively (see section S2 of the Supporting Information for the procedure and calculation of LOD and LOQ). We judge this LOQ to be sufficiently low for using q-^{1}H NMR to follow PBAT biodegradation in field soils. This judgment is based on estimated environmental concentrations, as detailed in section S3 of the Supporting Information.

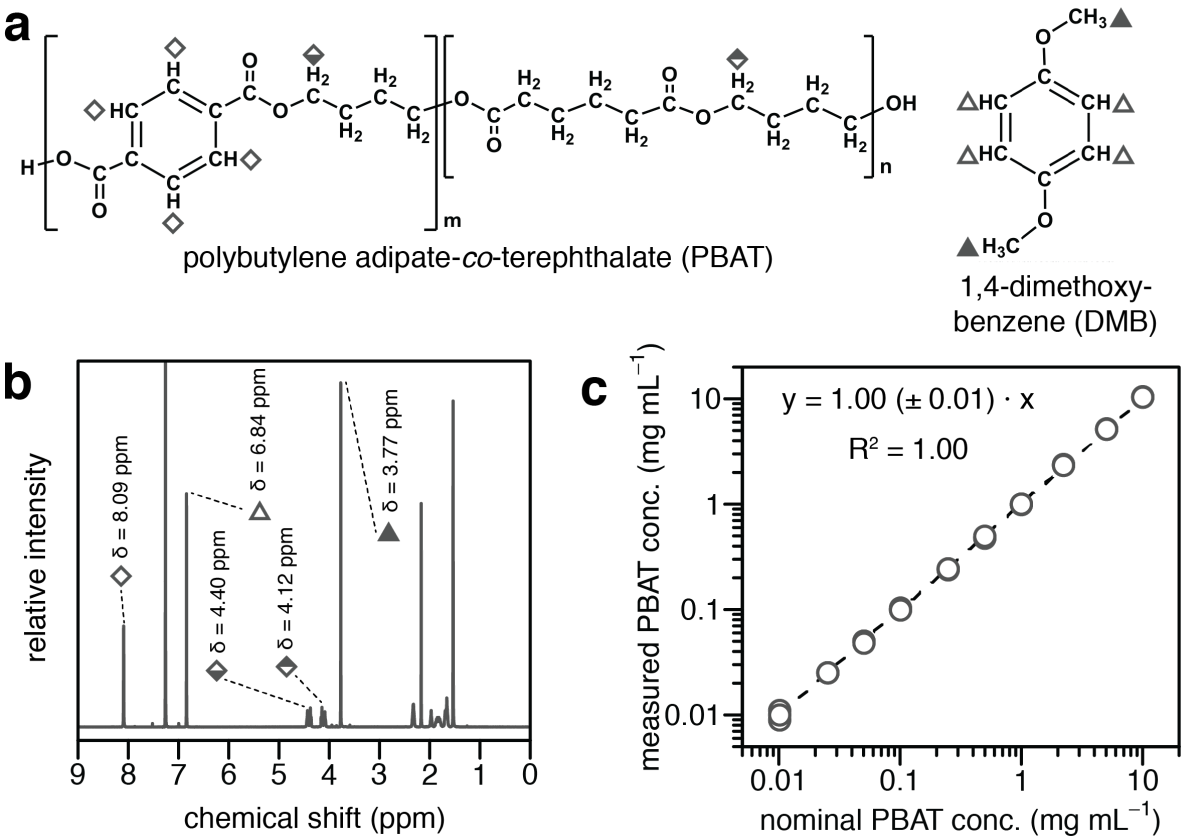


Figure 1. a) Chemical structures of polybutylene adipate-*co*-terephthalate (PBAT) and the internal quantification standard 1,4-dimethoxybenzene (DMB), with symbols indicating protons used for PBAT quantification. **b)** A representative ^{1}H NMR spectrum of PBAT and DMB in $CDCl_3$ (concentration standard with 1 mg PBAT mL^{-1}). The symbols marking peaks correspond to marked protons in panel a. **c)** Linear increase in the measured PBAT concentration using quantitative ^{1}H NMR vs. the nominal PBAT concentration in the concentration standards prepared in $CDCl_3$. Single analysis was performed on three standards prepared at each PBAT concentration. The dashed line is a linear least squared fit of the measured versus the nominal PBAT concentration (slope of unity (1.00 ± 0.01)).

Extraction of PBAT from model sorbents and soils

Extraction solvent composition

Efficient extraction of PBAT from soils requires a solvent that is both good at dissolving PBAT and is also able to disrupt potentially strong intermolecular interactions between PBAT and particle surfaces. Given that PBAT is a polyester, we expect it to be an H-bond acceptor that can form strong H-bonds with H-bond donating mineral particle surfaces in soil. Therefore, we first investigated whether an H-bond donating matrix (silica particles with H-bond donating surface hydroxyl groups) had an effect on the extraction efficiencies as compared to an apolar

323 matrix that has no H-bond donating functional groups (PTFE particles). PBAT added to the
324 two matrices was extracted into $CDCl_3$ by Soxhlet extraction and quantified by q-^{1}H NMR
325 analysis of the extract (see above).

326 **Figure 2a** shows PBAT extraction efficiencies from both silica and PTFE particles as
327 a function of particle surface area in the extractor. We systematically varied the particle surface
328 area by using differently sized particles and different total particle amounts. We obtained near
329 quantitative extraction of PBAT from both silica and PTFE particles when total particle surface
330 areas in the extractor were smaller than 0.1 m^2. These high PBAT recoveries were in the same
331 range as those we obtained when we directly added PBAT to empty extractors containing no
332 sorbent particles (i.e., recovery = 95 ± 1%; square in **Figure 2a**). We ascribe the small non-
333 extractable PBAT fraction of approximately 5% to PBAT adsorbed to glass surfaces in the
334 extractor including those of glass wool that we packed into the extractor as support for sorbent
335 particles. Extraction efficiencies of PBAT from PTFE particles remained high at larger surface
336 areas in the extractor (i.e., recovery of 85 ± 1% at a surface area of 8.96 m^2). By contrast, PBAT
337 extraction efficiencies from silica linearly decreased (R^2 = 0.87; note that **Figure 2a** is a semi-
338 logarithmic plot) with increasing surface area to only 11 ± 1% at the highest tested surface area
339 of 9.2 m^2. We ascribe the pronounced decrease in PBAT extraction efficiency with silica
340 surface area to strong H-bonding between silica silanol groups and PBAT ester groups. This
341 explanation is supported by the finding that the decrease in extraction efficiencies with surface
342 area could be well described with a simple model that assumes that the non-extractable surface-
343 adsorbed PBAT concentrations were between 0.2 to 1.0 mg PBAT m^{-2} of silica surface (shaded
344 area in **Figure 2a**). These poor extraction efficiencies of PBAT from silica into pure $CDCl_3$
345 indicated that an adequate extraction requires a H-bond accepting co-solvent that can
346 competitively displace PBAT from H-bond donating particle surfaces.
347

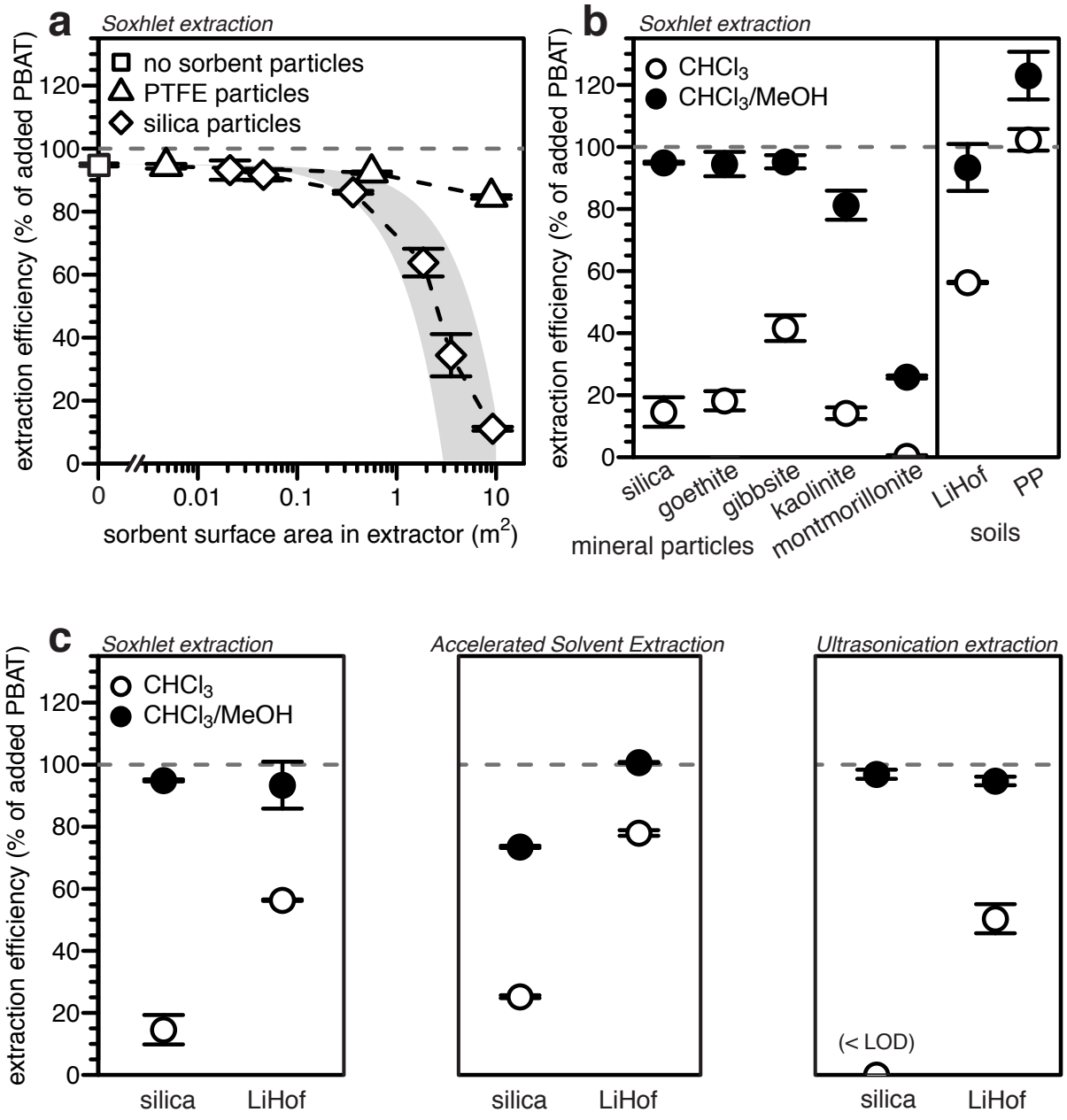


Figure 2. Extraction efficiencies of polybutylene adipate-*co*-terephthalate (PBAT) from different sorbents and soils. All extractions were performed in duplicate (or triplicate for ultrasonication), with symbols and error bars representing the mean extraction efficiency and the deviations from the mean of two extractions (or standard deviations from the mean of three extractions), respectively. The dashed gray lines help visualize 100% extraction efficiency. **a)** Extraction efficiency of PBAT from silica (diamonds) and PTFE (triangles) particles with varying total particle surface areas using Soxhlet extraction with $CDCl_3$. The extraction efficiency of PBAT added to a sorbent-free Soxhlet extractor is shown for comparison (square). The gray shaded area depicts the range of extraction efficiencies calculated assuming non-extractable PBAT concentrations on the silica particles of 0.2 (lower estimate, upper bound) to 1.0 (upper estimate, lower bound) mg PBAT m^{-2}. **b)** Extraction efficiencies of PBAT from different model mineral particles (left side) and from two soils (right side) using Soxhlet extraction. Extraction were performed with either pure $CHCl_3$ (open circles) or with a 90/10 vol% $CHCl_3$/MeOH co-solvent mixture (closed circles). **c)** Comparison of extraction efficiency

of PBAT from silica particles and LiHof soil across different extraction techniques. Extraction were performed with either pure $CHCl_3$ (open circles) or with a 90/10 vol% $CHCl_3$/MeOH co-solvent mixture (closed circles).

We considered methanol (MeOH) a potential co-solvent because it is miscible with chloroform, it can be readily removed in a solvent evaporation step after the extraction and, most importantly, because it is an H-bond acceptor that we thus expected to compete with PBAT for H-bond donating sites on silica surfaces. The fact that the co-solvent methanol can be readily removed together with the main solvent allowed us to use $CHCl_3$ (instead of the more expensive $CDCl_3$) for extractions. Only small amounts of $CDCl_3$ were then required for reconstituting the dried extract for q-^{1}H NMR analysis.

Extraction with a 90/10 vol% $CHCl_3$/MeOH co-solvent mixture instead of pure $CHCl_3$ indeed significantly increased PBAT extraction efficiencies from silica particles (**Figure 2b**). We verified enhanced extraction efficiencies in the presence of MeOH as co-solvent for other H-bond donating mineral surfaces, including the iron and aluminum (oxyhydr-)oxides goethite and gibbsite, and the clay minerals montmorillonite and kaolinite (**Figure 2b**). While extraction efficiencies of PBAT with pure $CHCl_3$ were low from all mineral particles (i.e. < 50% of added PBAT), adding 10 vol% MeOH significantly increased extraction efficiencies, for some minerals (i.e., silica, goethite, and gibbsite) to near-quantitative values.

The use of MeOH as co-solvent instead of pure $CHCl_3$ also significantly increased PBAT extraction efficiencies from two soils (**Figure 2b**). For LiHof soil, extraction efficiencies increased from 56 ± 1% to 93 ± 8% when we changed the solvent from pure $CHCl_3$ to $CHCl_3$/MeOH (90/10 vol %). We note that there was not much noise in the baseline of the ^{1}H NMR spectrum of the extract of LiHof soil, which demonstrated that there was not much interference from co-extracted soil organic matter (see **Figure S1** for a comparison of PBAT spectra in $CDCl_3$ in the presence and absence of co-extracts from LiHof and PP soils). For PP soil, a peat soil with very high organic matter content (C_{org}= 40.9%), extraction efficiencies in

390 the presence of MeOH also increased. Efficiencies over 100%, however, reflect difficulties for
391 correctly integrating proton peak areas in q-^{1}H NMR spectra due to the comparatively large
392 noise from co-extracted dissolved and fine particulate matter from the peat soil (**Figure S1**).
393 This finding implies that PBAT extraction from soils with very high organic matter may require
394 matrix-matched analytical procedures (i.e., standard addition quantification) or extract
395 purification steps. Given the exceptionally high carbon content of PP soil, we decided against
396 optimizing the extraction protocol for this specific peat soil but rather to determine extraction
397 efficiencies from a range of agricultural soils with more representative C contents (see below).

398 *Comparison of extraction techniques*

399 We chose silica and LiHof soil to compare the extraction efficiencies of PBAT using three
400 extraction techniques: Soxhlet, Accelerated Solvent and ultrasonication extraction. Consistent
401 with the results from Soxhlet extractions, ASE showed higher and near quantitative PBAT
402 extraction efficiencies from both silica and LiHof soil when we used a $CHCl_3$/MeOH co-
403 solvent mixture as compared to pure $CHCl_3$ (i.e., 93 ± 1 vs. 32 ± 1% for silica and 101 ± 1 vs.
404 78 ± 1 % for LiHof). The rather high extraction efficiencies for LiHof soil with pure $CHCl_3$ as
405 the solvent likely was due to the smaller specific surface area of that soil as compared to the
406 silica particles (i.e., 1.18 vs. 6.18 m^2 g^{-1}, respectively), as well as to the smaller fraction of
407 mineral surfaces in soils which are H-bond donating as compared to pure silica. It is noteworthy
408 that extraction efficiencies using ASE with pure $CHCl_3$ were higher than those achieved using
409 Soxhlet extraction with pure $CHCl_3$, despite the smaller number of extraction cycles in ASE
410 (programmed to 3 consecutive extractions) vs. Soxhlet (estimated minimum of 30 cycles over
411 8 hours). We ascribe the comparatively high efficiencies obtained by ASE to more efficient
412 PBAT desorption from silica and soil particle surfaces at elevated temperatures and pressures
413 used in ASE.

We additionally determined extraction efficiencies using a simple, manual ultrasonication extraction (see Materials and Methods for details). While this ultrasonication method involved only a single continuous extraction step (i.e. no solvent exchange), extraction efficiencies of PBAT from silica and LiHof into $CHCl_3$/MeOH were near-quantitative. The high efficiencies likely resulted from effective PBAT desorption from particle surfaces by additional energy provided through sonication. Given that this ultrasonication method could not be automated and was comparatively labor extensive, we carried out all subsequent extractions using Soxhlet and ASE. However, these results highlight the potential of ultrasonication extraction in laboratories that have no direct access to Soxhlet nor ASE instruments.

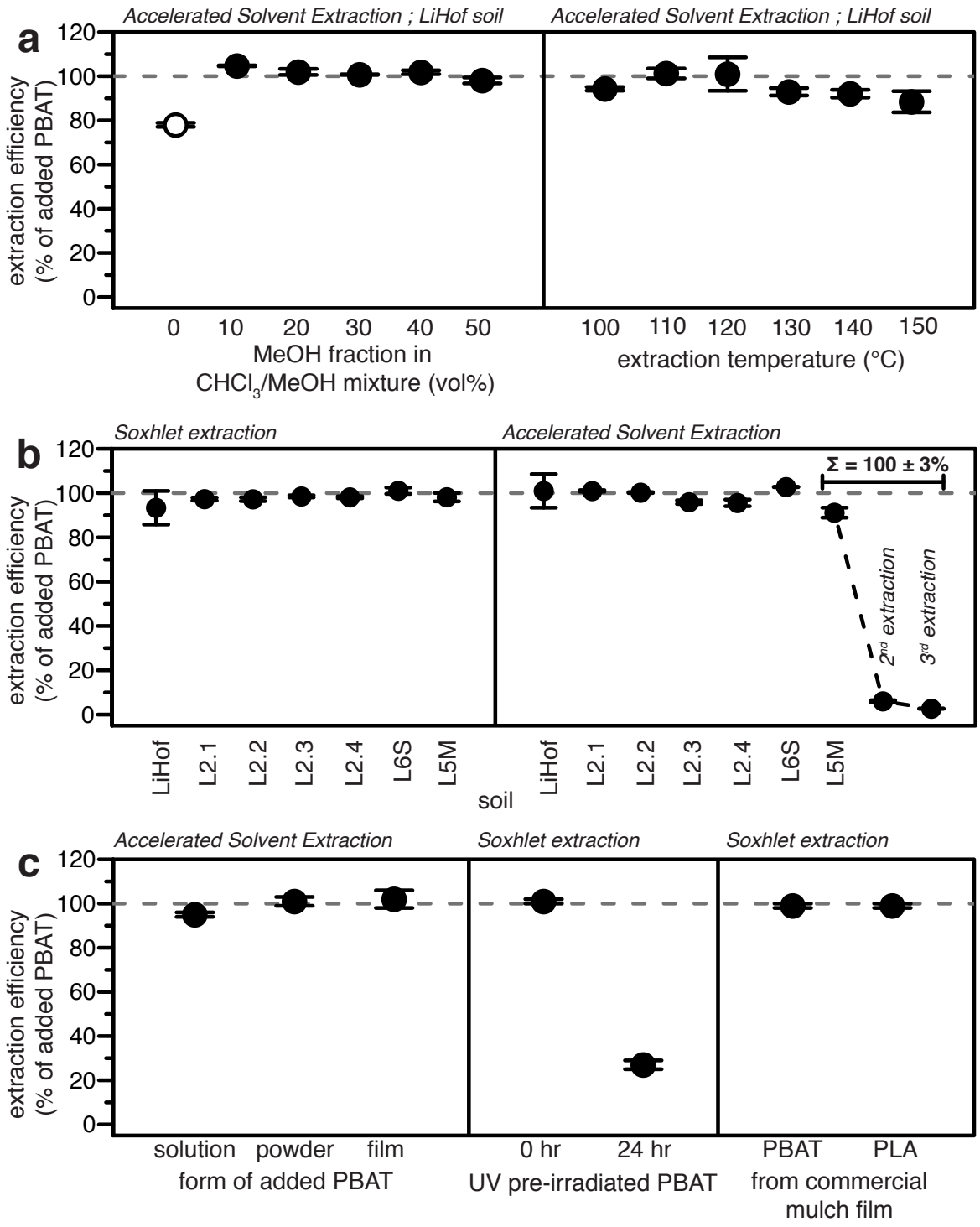
Optimization of ASE extraction parameters

In contrast to Soxhlet, ASE has the advantage of allowing for completely automated analysis and high sample throughput. For this reason, we systematically assessed how solvent composition (i.e., $CHCl_3$/MeOH volumetric ratios between 100/0 and 50/50 at a constant extraction temperature of 120 °C) and extraction temperature (between 100 and 150 °C at a constant $CHCl_3$/MeOH volumetric ratio of 90/10) affected PBAT extraction efficiencies from soils. For this assessment we focused on LiHof soil.

Consistent with the findings above, the use of only 10 vol% MeOH as co-solvent resulted in quantitative PBAT extraction from LiHof soil (i.e., 105 ± 1% vs. only 78 ± 1% in pure $CHCl_3$; **Figure 3a**). Further increases in the volume fraction of MeOH (up to 50%) did not significantly affect PBAT extraction efficiency, which remained at around 100%. For all subsequent analyses, we decided to continue to work with a volume fraction of 10% MeOH for two reasons. First, because PBAT is insoluble in pure MeOH, using only a low volume fraction of MeOH ensures high PBAT solubility in the $CHCl_3$/MeOH co-solvent system.

Second, because MeOH has a slightly lower vapor pressure than $CHCl_3$ (17.0 vs. 25.1 kPa at 25 °C),[30] a low volume fraction of MeOH facilitates solvent evaporation after extraction.

Extractions efficiencies of PBAT from LiHof were approximately 100% at extraction temperatures of 110 and 120 °C. Extraction efficiencies were slightly smaller both at lower (100 °C) and higher (130–150 °C) temperatures (**Figure 3b**). We speculate that decreased extraction efficiencies above 130 °C reflected increased hydrolysis of PBAT at elevated temperature by traces of water. ^{1}H NMR spectra of extracts collected at 150°C showed increased signal intensities over a chemical shift range from δ = 7.92–7.78 ppm. Peaks of monomeric terephthalates (δ = 7.89 ppm; in D_2O) fall into this range (**Figure S2**). Because PBAT extraction at 120 °C showed no indications for significant PBAT hydrolysis in the ^{1}H NMR spectra of the corresponding extracts, we used an ASE temperature of 120 °C in all subsequent experiments.



450

451 **Figure 3.** Extraction efficiencies of polybutylene adipate-*co*-terephthalate (PBAT) from
452 several agricultural soils. All extraction experiments were performed in duplicate, with
453 symbols and error bars representing the mean extraction efficiencies and deviations from the
454 mean of the two extractions, respectively. **a)** Assessment of changes in PBAT extraction
455 efficiency with run conditions during accelerated solvent extraction (ASE): volume fraction of
456 methanol (MeOH) as co-solvent to chloroform ($CHCl_3$) (left side; all run at extraction
457 temperature of 120 °C) and extraction temperature (right side; all using a co-solvent mixture
458 of 90/10 vol% $CHCl_3$/MeOH). **b)** Extraction efficiencies of PBAT from different agricultural
459 soils using Soxhlet extraction (left) or ASE (right). On soil L5M, we carried out three sequential

ASE steps to increase overall PBAT extraction efficiency. **c**) Extraction efficiencies of (i) PBAT that we added to LiHof soil in different forms: either dissolved in $CHCl_3$, as a powder with particles of diameter < 300 µm, or as a film with thickness of 25 µm (left panel), performed with ASE using 90/10 vol% $CHCl_3$/MeOH and 120°C; (ii) PBAT that was extrusion-blown into a film free of photostabilizers which was subsequently irradiated with UV light for 24 hours on each side prior to adding the film to the soil (middle), performed with Soxhlet using 90/10 vol% $CHCl_3$/MeOH; and (iii) PBAT and poly-lactic acid (PLA) that were added to LiHof soil in form of a commercially available biodegradable mulch film (right), performed with Soxhlet using 90/10 vol% $CHCl_3$/MeOH.

Extraction of PBAT from different soils

To demonstrate that PBAT could also be extracted with high efficiency from other soils, we extended our spike-recovery experiments to six additional agricultural soils that covered a range of physicochemical properties, including texture and organic carbon content (**Table 1**), using both Soxhlet and AS extractions. For each experiment, we added 2.5 mg of PBAT to 2.5 g of soil (Soxhlet) or 5 mg of PBAT to 5 g of soil (ASE). With the only exception of PBAT extraction from soil 5M with ASE, PBAT extraction efficiencies by both Soxhlet extraction and ASE were within 6% of the amount added for all soils (**Figure 3b**; data for LiHof soil replotted from **Figure 2c**). While only about 91% of PBAT was extracted from soil 5M by ASE in a first extraction step, the remaining PBAT was extracted from soil 5M when we added two additional ASE extraction steps (note that we separately collected and analyzed the solvent extracts of the three steps). The combined extracted amount of PBAT over all three steps added to 100 ± 3% of the PBAT amount added. This finding suggests that efficient PBAT extraction from some soils by ASE may require a high number of extraction steps. We note that the ^{1}H NMR spectra of the LUFA soil extracts showed slightly increased baseline intensities, presumably resulting from soil organic matter co-extracted with PBAT (**Figure S3**). Yet, the complete extraction of added PBAT demonstrates that the soil co-extracts did not interfere with accurate quantification of extracted PBAT.

For all extractions discussed above, we had added PBAT as chloroform solutions to the model sorbents and soils, followed by removal of the chloroform "carrier" through evaporation

prior to starting the extractions. We decided to work with chloroform solutions of PBAT because this approach allowed for a reproducible transfer of small PBAT amounts to the sorbents as well as spreading the added PBAT across the sorbent and soil particle surfaces (thereby maximizing the possibility for PBAT adsorption to particles). Yet, field soils treated with soil biodegradable mulch films are expected to contain PBAT either in the form of thin film pieces or as film-derived particles. We therefore additionally determined PBAT extraction efficiencies using ASE from LiHof soil to which we had added PBAT as small particles or as a blown film (see Materials and Methods for details). We also achieved full recovery of PBAT added as a film and as particles (i.e., extraction efficiencies of 102 ± 4 and 101 ± 2%, respectively (**Figure 3c**, left panel). These findings demonstrate high extraction efficiencies of PBAT from soils irrespective of the form in which PBAT is present.

In this work, we demonstrated that 'pristine' PBAT could be completely extracted from soils employing Soxhlet and AS extraction. However, we recently showed that 'weathering' of non-photostabilized PBAT films by photoirradiation creates, besides other reaction products, photo-induced crosslinks between PBAT polymer chains, lowering the $CHCl_3$ solubility and the enzymatic hydrolyzability of PBAT.[31] We expected lower extraction efficiencies for such photo-crosslinked films. We therefore assessed the extractability of an extensively photo-crosslinked PBAT film from that study from LiHof soil using Soxhlet extraction. The irradiated film had a gel content (i.e., a $CHCl_3$-insoluble mass fraction) of 84 %.[31] Consistent with the decreased $CHCl_3$ solubility of the irradiated film, we found low extraction efficiencies with PBAT films when they were extensively photo-irradiated (i.e., extraction efficiency of 27 ± 2%; **Figure 3c**, middle panel) as compared to complete extraction of pristine PBAT control films. This finding suggests that preventing cross-link formation by sufficient photostabilization of PBAT-based soil biodegradable mulch films is beneficial not only for enzymatic hydrolyzability of PBAT, a key requirement for soil biodegradability, but also

ensures PBAT solubility in chloroform, a key requirement for being able to extract PBAT from soils for quantification. Future work ought to assess whether field-weathered soil biodegradable mulch films are still extractable from soils.

Implications

Here, we present an analytical methodology to quantify mulch-film derived PBAT in agricultural soils. The methodology is based on first extracting PBAT from soils by means of either Soxhlet or AS extraction followed by quantifying PBAT in the extract by means of q-^{1}H NMR.

Quantification of PBAT by q-^{1}H NMR was found to be uncomplicated and straightforward. This method is directly applicable to the extracted polyesters without further sample treatment (other than re-constituting the extract into a deuterated chlorinated solvent) such as derivatization or chromatographic separation of the extracted polyester from the co-extracted soil matrix components.[32-34] Using ^{1}H NMR spectroscopy – one of the most powerful methods for structure identification – as the detection method means that identity of the extracted polymer is highly certain. While NMR has comparatively low sensitivity compared to other detection techniques, the low sensitivity is counteracted by the fact that polyesters are composed of a very small number of distinct monomer units (typically between one and three) that consequently are present in high concentrations in the films.

Our analytical methodology opens the possibility in future work to follow PBAT biodegradation in soils through quantifying resulting decreases in the PBAT concentration. Monitoring PBAT concentrations in soils can be used to complement respirometric measurements of PBAT biodegradation in laboratory soil incubation experiments. We anticipate that the analytical methodology presented herein will be particularly useful for monitoring of PBAT biodegradation in soils directly in the field where respirometric analyses

of PBAT biodegradation cannot be implemented. We note, however, that our methodology needs to be complemented by field sampling protocols that ensure that the concentrations of residual PBAT in the collected soil samples are representative of the soil concentrations at the field scale.

We further expect that the analytical methodology presented here is broadly applicable to a large set of commercially relevant polyesters used in biodegradable mulch films, given that other polyesters are also chloroform soluble. These polyesters include polylactic acid (PLA), polybutylene succinate (PBS), polybutylene succinate-*co*-adipate (PBSA), polycaprolactones (PCL) and polyhydroxyalkanoates (PHA). For proof of concept, we used Soxhlet extraction to demonstrate quantitative extraction of PBAT and PLA (i.e., both showed extraction efficiencies of 99 ± 1%) that we added to LiHof soil in form of a commercially available soil biodegradable mulch film (**Figure 3c**, right panel). This commercial mulch film contained 70.3 ± 0.1 and 3.99 ± 0.03 mass% of PBAT and PLA, respectively, based on a q-^{1}H NMR analysis of the mulch film dissolved in $CDCl_3$ (see **Figure S4** for ^{1}H NMR spectra of the film and of PBAT and PLA concentration standards; we expect that chloroform- and methanol-insoluble fillers (e.g. starch or inorganic fillers) made up the major part of the remaining 26 mass% of the film). Furthermore, we expect that the analytical methodology is not limited to quantifying biodegradable polyesters in soil but can be extended to other natural and engineered systems, including sediments and compost material. Monitoring concentration dynamics of biodegradable polymers in these systems using the analytical methodology based on that developed in this work will help to advance an improved understanding of polymer- and environment-specific factors governing polyester biodegradability in different receiving environments. This understanding is needed for a sustainable use of biodegradable polymers in the environment, including agricultural applications.

Associated content

Supporting Information: addition information on q-^{1}H NMR acquisition parameters and LOD/LOQ calculations, mulch film application calculations, and representative ^{1}H NMR spectra of various extraction samples. This material is available free of charge via the Internet at http://pubs.acs.org.

Author information

Corresponding author: * Email: Michael.sander@env.ethz.ch

Notes

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