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Rapid screening of phytoremediation effluents by off-line tetramethylammonium hydroxide assisted thermochemolysis ☆

Juergen Poerschmann [a,*], Luciana Schultze-Nobre [b]

[a] UFZ-Helmholtz Center for Environmental Research, Department of Environmental Engineering, Permoserstr. 15, D-04318 Leipzig, Germany
[b] UFZ-Helmholtz Center for Environmental Research, Department of Environmental Biotechnology, Permoserstr. 15, D-04318 Leipzig, Germany

HIGHLIGHTS

- Thermochemolysis was applied to study the organic matter in phytoremediation systems.
- Stable intermediates such as oxidative coupling products could be detected.
- Diagnostic fatty acids pointed to terrestrial and bacterial input.
- Lignin monomers indicated the dominance of the cinnamyl and guaiacyl types.

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ABSTRACT

Tetramethylammonium hydroxide-assisted thermochemolysis performed in an off-line mode proved a useful tool in determining organic compounds in the effluent from laboratory-scale phytoremediation systems. Studies were performed with artificial wastewaters contaminated with xylenols and densely rooted *Juncus effuses* plants. Analytes in these molecular-level based studies included xylenol substrates, an array of stable intermediates such as low molecular weight carboxylic acids and oxidative coupling products (tetramethyl biphenyldiols, tetramethyl diphenylether monools), diagnostic fatty acid biomarkers, as well as lignin-, carbohydrate-, and protein-based phenols and carboxylic acids. Lignin-based breakdown products belonged to p-hydroxyphenyl- and guaiacyl-units, with lower abundance of syringyl units and the dominance of acids over phenols. Monomeric lignin-, protein- and carbohydrate-based breakdown products could not be detected in the non-treated lyophilized effluent. The formation of diketopiperazines pointed to soluble peptides and proteins. The procedure described herein can easily be applied in every modern laboratory to characterize underlying processes in phytoremediation.

1. Introduction

Phytoremediation represents an environmentally sound, cost- and energy efficient technology to treat contaminated wastewaters (Vymazal, 2011). The basic processes to remove organic contaminants, including uptake from the water column and the root zone, filtration, precipitation, (phyto)volatilization, and microbial degradation have been well studied (Seeger et al., 2013). However, molecular level characterization relating to the conversion of organic contaminants, the pattern of organic intermediates, the biomarker-based characterization of microorganisms in the phytoremediation system, etc., have not been addressed extensively thus far.

In former contributions a wide array of organic analytes arising from phytoremediation of xylenols using contaminated water-tolerant *J. effuses* was identified (Poerschmann and Schultze-Nobre, 2014) and (Poerschmann et al., 2015). Lyophilizates from reactor effluents were subjected to solvent extraction followed by derivatization to render polar molecules accessible to GC/MS analysis. Analytes under scrutiny included tricarboxylic acids generated via the ketoadipate pathway, low molecular weight carboxylic acids such as lactic and succinic acids, as well as oxidative coupling products such as tetramethyl (TeM) biphenyldiols.

The preparation scheme spanning from solvent extraction of the lyophilizates to GC/MS analysis is a multistep procedure. In addition, solvent extraction is incapable of targeting oligomeric and polymeric constituents in the effluent, as well as suspended microorganisms, which may account for a significant fraction of the dissolved/suspended organic carbon cycling in the system. Generally, this water-soluble organic matter is more biologically and chemically active than the

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* Corresponding author.
E-mail address: juergen.poerschmann@ufz.de (J. Poerschmann).

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water-insoluble pool (Said-Pullicino et al., 2007). The oligomeric and polymeric constituents originate from water to be remediated, which circulates through the rhizosphere. Water acts in this case as the solvent for water-soluble root constituents.

Another contribution to organic carbon may be provided a priori by dissolved humic organic matter, chiefly aquatic fulvic acids, which account for a widely distributed fraction in wastewaters. In the course of phytoremediation of artificial wastewaters (devoid of aquatic fulvic acids) contaminated with xylenols lasting many weeks it could be visually observed (and confirmed by UV/VIS-spectroscopy) that the circulating water turned slightly brown. Centrifugation of the circulating water resulted in a brown coloured residue. This finding, also applicable to artificial wastewaters treated with sodium azide to prevent microbial activity, suggests that low molecular weight water-soluble oligomers were extracted from the plant roots. Potential macromolecular root compartments include lignin, suberin, (hemi)cellulose, and proteins. In contrast to cellulose built up solely by glucose, lignin represents a copolymer consisting of different monomers, namely p-coumaryl-, coniferyl-, and sinapyl alcohol.

In light of the above, an analytical approach is called for that allows concomitant detection and identification of monomeric solvent extractables along with water-soluble oligomeric and polymeric organic carbon including

(i) xylenol model pollutants to allow conclusions on the removal rate
(ii) their stable intermediates such as carboxylic acids and biphenyldiols to allow conclusions on the degradation pathways of the xylenols
(iii) chemotaxonomic markers for microorganisms such as diagnostic fatty acid to allow conclusions on the activity of microorganisms
(iv) water-soluble oligomers to gather information on their capability to serve as carbon source and on their toxicological potential towards autochthonous and allochthonous microorganisms.

Herein it is demonstrated that tetramethylammonium hydroxide (TMAH)-assisted thermochemolysis meets overwhelmingly the above-mentioned criteria. This approach has been shown previously to be efficient in detecting the building blocks of humic organic matter (del Rio et al., 1998), plant compartments (Mc Kinney et al., 1996), and microorganisms (Zang et al., 2001). The on-line TMAH-induced thermochemolysis has been generally carried out at 500–600 °C for 3–5 s, whereas the off-line approach, usually performed in sealed ampoules, has been characterized by sub-pyrolysis temperatures of 250 °C–300 °C as given by del Rio et al. (1998) or of 200 °C–250 °C as given by Challinor (2001), with concomitantly longer reaction times (90–180 min). The sub-pyrolysis temperature range was found to produce a suite of breakdown products which are very similar to that observed at higher (pyrolytic) temperatures (see Mason et al. (2012) and references cited therein). As an example, TMAH-assisted thermochemolysis at sub-pyrolysis temperatures was shown to allow the β-*O*-4 bond cleavage in lignin, which results in the release of lignin-based breakdown products similar to those resulting from the CuO oxidation procedure (Mc Kinney et al., 1996). As a result of thermochemolysis, ester and ether bonds in macromolecular organic matter are cleaved, resulting in the formation of monomers such as GC-amenable methyl esters of carboxylic acids and methyl ethers of alcohols. Thus, the strongly basic TMAH acts both as a chemolytic degradation agent and as a methylating agent (Challinor, 2001). Compared to conventional "dry" pyrolysis, thermochemolysis yields more diagnostic breakdown products that mirror the structural features of the macromolecular compartments listed above including lignin (Nakagawa-izumi et al., 2004), suberin (Filomena Santos Bento et al., 2001), (hemi)cellulose (Estournel-Pelardy et al., 2011), and proteins (Zang et al., 2001). In the case of lignin, chemotaxonomic distinctions (e.g. between angiosperm and gymnosperm) are possible by TMAH-induced thermochemolysis owing to the distribution of phenols and phenolic acids (Challinor, 2001). To characterize lignin-based breakdown products in more detail, an identification code was developed to distinguish surrogates of p-hydroxyphenyl-, guaiacyl-, and syringyl-units (abbreviated with P, G, S, respectively; see Poerschmann et al. (2005a) and references cited therein).

Thermally-assisted hydrolysis (more precisely: solvolysis) can be performed in both on-line and off-line modes. Only the latter was considered in this contribution owing to its simplicity; this approach can be implemented in any phytoremediation laboratory equipped with GC/MS capabilities. TMAH-assisted thermochemolysis can also act as an extraction method using (alkaline) methanol under the specific conditions of elevated temperature and pressure for analytes that do not undergo solvolysis or derivatization.

Thermochemolysis studies were conducted with lyophilizates of aerobic effluents obtained after phytoremediation of an artificial mixture of xylenols (Poerschmann and Schultze-Nobre, 2014).

2. Materials and methods

2.1. Chemicals

Xylenols, solvents, TMAH and the derivatization agents bis(trimethylsilyl)trifluoracetamide (BSTFA) and dimethyldisulfide (DMDS) were provided by Sigma-Aldrich (Munich, Germany). Isotopically labelled internal standards were purchased from Promochem (Wesel, Germany). Authentic standards including fatty acids, fatty alcohols, phenols, phenolic acids, sterols, and resin acids were available from previous projects, see Poerschmann and Schultze-Nobre (2014), and Poerschmann et al. (2005a).

2.2. Laboratory-scale constructed wetland design

The experimental design was detailed in Poerschmann and Schultze-Nobre (2014). Briefly, a glass reactor (28 cm in diameter and 30 cm in height) filled with gravel was permanently circulated to ensure conditions of complete mixing of pore water. The gravel bed was densely rooted with *Juncus effuses*, a plant widely distributed in many littoral areas and wetlands. A modified artificial wastewater was used as a medium meeting criteria for carbon, nitrogen, and sulphur concentrations as determined by German regulations (Wiessner et al., 2005). Samples were taken after 30 days of circulating flow. Experiments were performed using a mixture of 2,6-, 3,4-, and 3,5-xylenol (13.3 μg g^{-1} total influent concentration each) as the test probe.

2.3. TMAH-assisted thermochemolyis

Lyophilized effluent samples were weighted (~15 mg) and poured into glass ampoules (3 mL). Next, 2.5 mL of 25% w/w freshly prepared TMAH solution in methanol was added (~40× excess TMAH by weight). The open glass ampoule was placed in an in-house built steel autoclave and tightly closed. Thermochemolysis was allowed to take place at 240 °C for 180 min. To ensure higher yields of breakdown products, reaction temperature and holding time were both increased compared to those applied in the framework of previous experiments (220 °C, 120 min), which were focused on studying building blocks of maize plants (Poerschmann et al, 2008). After cooling to room temperature, the reaction products such as carboxylic acids and phenols were extracted from the strong alkaline mixture (solution along with the solid residue) by chloroform using the following procedure: the methanol phase was first diluted with water, then acidified to pH ~ 1.5 using 1 M HCl so as to transfer acids into their free form. The mixture was then spiked with isotopically labelled standards phenol-d_6, palmitic acid-d_2, succinic acid-d_4, and phenanthrene-d_{10}, as well as with 2,4-xylenol (the latter to quantify residual 2,6-, 3,5-, and 3,4-xylenol), then extracted twice with chloroform. This extraction scheme proved