

# Development of a Method for Anodic Degradation of Lignin for the Analysis of Paleo-Vegetation Proxies in Speleothems

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Here we present an electrochemical method for the anodic oxidation and subsequent degradation of lignin in speleothems to utilize the resulting lignin oxidation products (LOPs) as paleo-vegetation markers. LOPs were analyzed using an ultra-high performance liquid chromatography (UHPLC) system coupled to a high-resolution mass spectrometer (HRMS). The

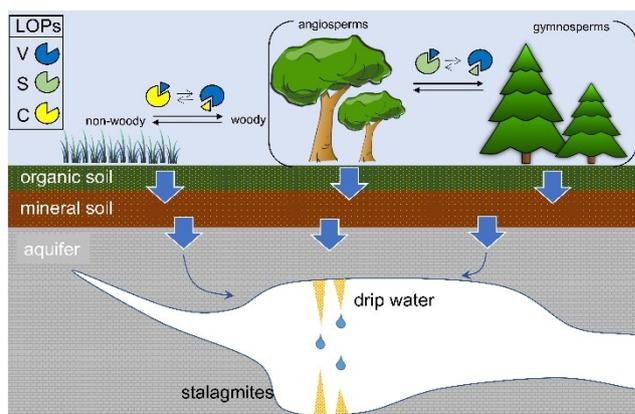
method presented here achieved comparable or even higher LOP concentrations than established CuO and CuSO<sub>4</sub> oxidation methods. The method represents a new tool for the analysis and reconstruction of paleo-vegetation and has the potential to be applied to other climate archives.

## Introduction

Speleothems are well established climate archives because they can grow continuously over thousands of years and can be dated with unprecedented precision using the <sup>230</sup>Th/U method.<sup>[1]</sup> Due to the stable conditions in caves, speleothems undergo only minor physical and chemical changes over time.<sup>[2]</sup> Traditionally, speleothem research mainly used inorganic climate proxies, such as  $\delta^{13}\text{C}$  or  $\delta^{18}\text{O}$  values or trace elements, while organic proxies played only a minor role.<sup>[3]</sup> However, with the improvement of techniques for organic trace analysis, more and more organic compounds have been investigated as potential proxies.<sup>[4]</sup>

One of them is lignin, a biopolymer found exclusively in vascular plants. The main constituents are sinapyl-, coniferyl-, and coumaryl alcohol units linked by stable ether and C–C bonds.<sup>[5]</sup> To analyze the lignin, it is broken down into monomeric units by oxidative digestion. The resulting lignin oxidation products (LOPs) can be divided into three groups according to their phenolic: the guajacyl moiety (V), the syringyl group (S) and the cinnamyl group (C).<sup>[6]</sup> The composition of the polymer with respect to these groups allows conclusions to be drawn about the different types of vegetation from which the lignin originates. According to a study by Hedges and Mann,<sup>[7]</sup> it

is possible to distinguish between angiosperms (flowering plants, enclosed seed-bearing plants) and gymnosperms ("naked seed"-bearing plants) as well as woody and non-woody lignin by comparing the two parameters C/V and S/V. Here, C is the sum of all lignin oxidation products in the C-group, etc. This is possible because only non-woody plant parts produce substantial amounts of cinnamyl units and only angiosperms produce substantial amounts of syringyl units. Guajacyl moieties are ubiquitous. The origin of lignin and the pathway of the polymer into the speleothem is shown in Figure 1. Hedges and Mann visualized their results with scatter plots and define limits for each result (Figure 3). However, their data were limited to a small number of samples and are further constrained by the method used for digestion and analysis. Therefore, their results may rather be considered as a guideline and only applied to the variations between individual samples in a consecutive set of samples. In addition, the parameter  $\Sigma 8$ , representing the sum of the concentration of all 8 analyzed LOPs, is used as a general measure for the amount of lignin in the sample.



**Figure 1.** Schematic representation of the origin of lignin and the pathway of the polymer into the speleothem.

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**Table 1.** Concentrations of the V-, S-, and C-group LOPs, the sum of all eight LOPs ( $\Sigma 8$ ), and the ratios C/V and S/V of the speleothem sample determined using different oxidation methods.

Sample	$\Sigma S [\mu\text{g}\cdot\text{g}^{-1}]$	$\Sigma V [\mu\text{g}\cdot\text{g}^{-1}]$	$\Sigma C [\mu\text{g}\cdot\text{g}^{-1}]$	$\Sigma 8 [\mu\text{g}\cdot\text{g}^{-1}]$	C/V	S/V
30 °C, 0.10 C/g	194.8 ± 9.6	242.7 ± 21.7	88.4 ± 6.0	526.0 ± 37.3	0.45 ± 0.05	1.25 ± 0.17
30 °C, 0.15 C/g	334.6 ± 30.2	121.4 ± 10.6	56.8 ± 2.1	512.8 ± 42.9	0.17 ± 0.02	0.36 ± 0.06
30 °C, 0.20 C/g	154.6 ± 11.4	203.4 ± 14.9	67.3 ± 1.2	425.3 ± 27.6	0.44 ± 0.04	1.32 ± 0.19
50 °C, 0.10 C/g	407.1 ± 28.0	241.8 ± 23.0	83.8 ± 2.0	732.8 ± 53.0	0.21 ± 0.02	0.59 ± 0.10
50 °C, 0.15 C/g	385.9 ± 34.8	253.2 ± 18.0	88.7 ± 1.8	727.7 ± 54.6	0.23 ± 0.03	0.66 ± 0.11
50 °C, 0.20 C/g	475.4 ± 33.8	191.5 ± 20.2	86.8 ± 3.0	753.7 ± 57.1	0.18 ± 0.02	0.40 ± 0.07
Set 1	69.6 ± 10.1	86.0 ± 9.0	13.3 ± 0.6	168.9 ± 19.7	0.19 ± 0.04	1.24 ± 0.31
Set 2	63.2 ± 2.7	12.0 ± 0.9	4.5 ± 0.2	83.6 ± 4.7	0.07 ± 0.01	0.20 ± 0.04
CuO	135.5 ± 1.7	195.1 ± 5.4	21.9 ± 0.9	349.5 ± 8.0	0.17 ± 0.01	1.47 ± 0.06
CuSO <sub>4</sub>	341.7 ± 24.1	79.7 ± 28.9	46.0 ± 0.5	467.4 ± 53.5	0.13 ± 0.01	0.23 ± 0.10

Traditionally, CuO,<sup>[8]</sup> nitrobenzene,<sup>[9]</sup> or tetramethylammonium hydroxide (TMAH)<sup>[10]</sup> were used for the oxidative/thermal degradation. However, large sample volumes, impurities of the chemicals used, or a variety of possible reaction products make it difficult to adapt to analytical purposes.<sup>[11,12]</sup> More recently, Yan and Kaiser introduced a method based on alkaline CuSO<sub>4</sub> oxidation, which is particularly suitable for small sample volumes.<sup>[13]</sup>

However, all established methods result in toxic waste, are costly and time consuming, and can cause relatively high blank values. In addition, the composition of the reaction mixture and thus the resulting quantity of LOPs depends on several factors, such as the organic carbon content and the presence of oxygen. A new approach is highly desired to simplify the procedure and obtain more reproducible results.

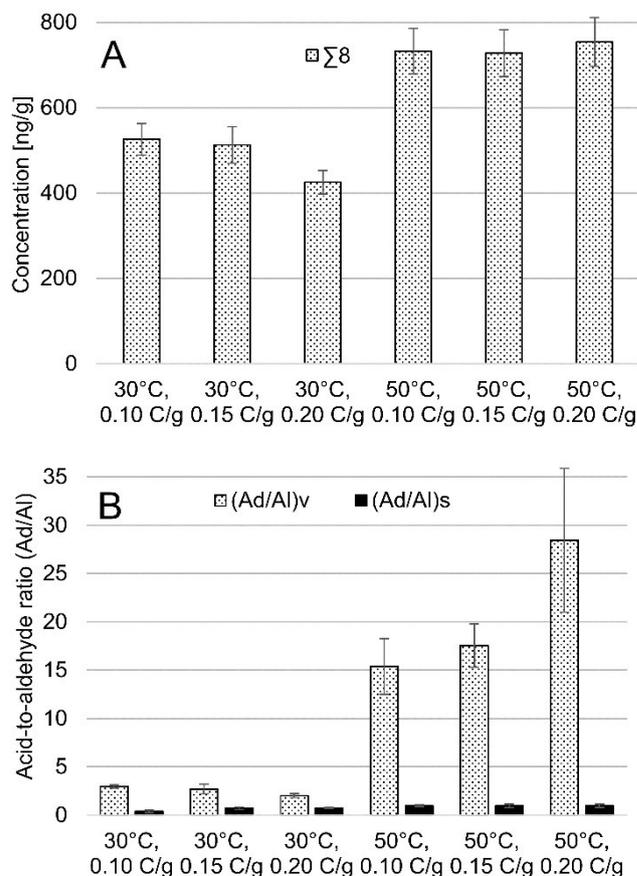
Electrosynthesis seems to be a promising alternative for the conversion of lignocellulose biomass.<sup>[14–16]</sup> Since only electricity serves as oxidizing and/or reducing agent, the generation of reagent waste is avoided, and the atomic efficiency increased. In addition, electric current is an inherently safe, renewable, and cost-efficient reagent.<sup>[15,16,17]</sup> Thus, it meets the criteria of “green chemistry”.<sup>[18]</sup> In recent decades, electrochemical degradation of lignin has been investigated to produce low molecular fine chemicals in a sustainable manner.<sup>[14]</sup> Several applications for the electrochemical conversion of liginosulfonate,<sup>[19,20]</sup> Kraft lignin,<sup>[21,22–29]</sup> organosolv lignin,<sup>[30,31]</sup> and native(-like) lignin<sup>[32]</sup> have been published here. High temperature electrolysis (> 100 °C) has been established for efficient electrochemical depolymerization of technical lignins, such as liginosulfonate, Kraft and organosolv lignin, into valuable aromatic fine chemicals (mainly vanillin).<sup>[20,29,31]</sup> In general, nickel-based anodes were commonly used for the electrochemical production of monoaromatics from lignin.<sup>[20,22–29,31]</sup> For example, the Waldvogel laboratory reported the anodic degradation of lignin to produce vanillin using Ni electrodes in a simple undivided set-up.<sup>[22–24,29,31]</sup> Detailed mechanistic studies of the electrocatalytic anodic conversion of lignin utilizing nickel anodes were provided by Utley *et al.* Here, the formation of low molecular weight products, such as vanillin, was associated with the oxidation of phenolic structures in the lignin *via* oxygenated Ni(III) species at the anode surface.<sup>[20,33]</sup>

In this study, we developed a new electrochemical degradation method using nickel anodes for the analysis of lignin in

speleothems and present the results in comparison with the established CuO and CuSO<sub>4</sub> degradation methods.

## Results and Discussion

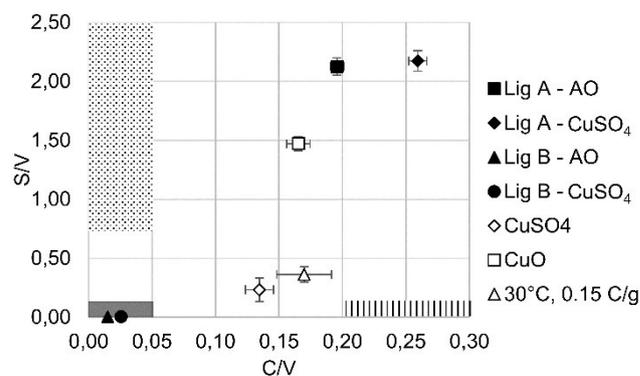
Two different temperatures and three different amounts of electric charge applied were tested. The results are summarized in Table 1 and illustrated in Figure 2A. All conditions tested lead to a noticeable formation of LOPs with  $\Sigma 8$  values ranging from



**Figure 2.** A: Comparison of LOP concentration obtained for different anodic oxidation conditions. B: Comparison of the acid-to-aldehyde ratios (Ad/Al) for the V- and S-group obtained for different anodic oxidation conditions.

425.3 ng·g<sup>-1</sup> to 753.7 ng·g<sup>-1</sup>. The  $\Sigma 8$  stays at the same level with the increase of the applied charge from 0.1 C to 0.15 C per g speleothem. When the amount of charge is further increased to 0.2 C per g speleothem, the  $\Sigma 8$  value drops significantly at 30 °C, while it remains at the same level at 50 °C. The decreasing concentration can be explained by over-oxidation of the polymer leading to the formation of units, which were not investigated in this study. This implies that if such highly oxidized compounds are a desired product, the application of higher charge amounts might be advisable. At 50 °C, the  $\Sigma 8$  value is significantly higher, even at 0.1 C·g<sup>-1</sup>. This is due to the fact that as the electrolysis temperature increases, the (oxidative) degradation of the polymer is also enhanced.<sup>[29]</sup> Therefore, a larger amount of low molecular weight compounds is generated. However, these monoaromatic compounds are often more prone to anodic conversion, resulting in highly oxidized species. This is evident from our data, as the main contribution to the  $\Sigma 8$  value comes from the V-group, more precisely from vanillic acid (Figure 2B). Moreover, an increasing ratio between vanillic acid and vanillin ((Ad/Al)<sub>v</sub>) is observed, applying higher amounts of charge, indicating the over-oxidation of the polymer and/or LOPs. Although the  $\Sigma 8$  values for oxidation at 50 °C are all significantly increased compared to the reaction at 30 °C, these conditions are considered unsuitable for analytical purposes because monomeric units of the V-group appear to be formed preferentially over all other LOPs. This would distort the S/V and C/V ratios, making the analytical technique unsuitable to provide reliable results. This behavior be due to the partial degradation and over-oxidation of the polymer and LOPs at the higher electrolysis temperature, where the corresponding vanillyl units (V) are partially more stable to over-oxidation and/or more easily anodically depolymerized. Therefore, the electrolysis temperature of 30 °C and an applied charge amount of 0.15 C·g<sup>-1</sup> were chosen for all further experiments and comparisons since these conditions gave the highest  $\Sigma 8$  value at relatively low (Ad/Al) values.

To demonstrate the effectiveness of the anodic oxidation, a set of samples was prepared that was only thermally degraded (Set 1). In addition, a set of samples was prepared that was subjected to anodic conversion only, but not to subsequent thermal degradation (Set 2). The results are shown in Table 1. The  $\Sigma 8$  value for Set 1 is at 168.9 ng·g<sup>-1</sup> and the  $\Sigma 8$  value for Set 2 is at 83.6 ng·g<sup>-1</sup>. Thus, both values are significantly lower than the  $\Sigma 8$  value of the anodic oxidation and subsequent thermal degradation of 512.8 ng·g<sup>-1</sup>. The results illustrate that both steps are necessary for the successful formation of LOPs from lignin. The anodic oxidation converts the strong ether and



**Figure 3.** A: S/V versus C/V values of the speleothem sample, and lignin samples determined using anodic oxidation (AO), CuSO<sub>4</sub> oxidation, or CuO mediated oxidation.

C–C bonds of lignin into weaker oxidized bonds, which undergo cleavage by the subsequent thermal degradation.<sup>[34]</sup>

To compare the anodic oxidation method with established methods, both the CuSO<sub>4</sub> and CuO oxidation method were performed. The results are shown in Table 1 and Figure 3. The  $\Sigma 8$  values obtained (CuSO<sub>4</sub>: 467.4 ng·g<sup>-1</sup>; CuO: 349.5 ng·g<sup>-1</sup>) are comparable to those obtained by the anodic oxidation method (512.8 ng·g<sup>-1</sup>). The S/V and C/V ratios are also similar, and all lie approximately in the same region of the scatter plot (see Figure 3). Minor differences in the results are to be expected and result from the different mechanisms involved in the formation of the LOPs and the general large variability in the composition of natural lignin. Overall, these results indicate that the newly developed anodic oxidation method provides robust LOP values that allow for sound interpretation of the S/V and C/V ratios.

The method was applied to two different types of commercial lignin powder to verify that the C/V and S/V ratios obtained agreed with the literature, and again to verify that the results were comparable to those obtained by the CuSO<sub>4</sub> oxidation method. The results are listed in Table 2 and illustrated in Figure 3. Application of the anodic oxidation method to both wheat straw and mixed woods lignin (Lignin A) and conifer wood lignin (Lignin B) results in  $\Sigma 8$  values of about 1600 μg·g<sup>-1</sup>, corresponding to a conversion factor of 0.16% (w/w). The  $\Sigma 8$  values resulting from the CuSO<sub>4</sub> oxidation method are only 1176 μg·g<sup>-1</sup> for Lignin A and 588 μg·g<sup>-1</sup> for Lignin B, respectively. These values correspond to conversion factors of about 0.12% and 0.06% (w/w). This shows that the anodic oxidation method can degrade various lignins more effectively

**Table 2.** Concentrations of the V-, S-, and C-group LOPs, the sum of all eight LOPs ( $\Sigma 8$ ), and the ratios C/V and S/V of the lignin samples determined using different anodic oxidation (AO) or CuSO<sub>4</sub> oxidation.

Sample		$\Sigma S$ [μg·g <sup>-1</sup> ]	$\Sigma V$ [μg·g <sup>-1</sup> ]	$\Sigma C$ [μg·g <sup>-1</sup> ]	$\Sigma 8$ [μg·g <sup>-1</sup> ]	C/V	S/V
Lignin A	AO	194.8 ± 9.6	492.8 ± 8.9	1047.3 ± 17.4	96.4 ± 0.9	1636.5 ± 27.2	0.20 ± 0.01
	CuSO <sub>4</sub>	334.6 ± 30.2	342.4 ± 5.6	744.5 ± 17.6	88.8 ± 1.0	1175.7 ± 24.2	0.26 ± 0.01
Lignin B	AO	154.6 ± 11.4	1573.9 ± 22.6	4.4 ± 0.2	23.7 ± 0.1	1602.0 ± 22.8	0.02 ± 0.00
	CuSO <sub>4</sub>	407.1 ± 28.0	570.6 ± 6.8	2.8 ± 0.3	14.6 ± 0.2	588.0 ± 7.2	0.03 ± 0.00

than the established  $\text{CuSO}_4$  oxidation method. Again, the  $C/V$  and  $S/V$  ratios of the two different methods are very similar, and the ratios for each of the two different lignin species fall within the regions of the scatter plot expected according to the regions defined by Hedges and Mann.<sup>[7]</sup> This last test also confirmed the suitability of anodic oxidation for the analytical degradation of lignin.

## Conclusion

We developed an electrochemical method for the anodic oxidation and subsequent degradation of lignin in speleothems to analyze the resulting LOPs as paleo-vegetation markers. The new method achieved comparable or even higher LOP concentrations than the established  $\text{CuO}$  and  $\text{CuSO}_4$  oxidation methods. The method also speeds up sample preparation and reduces the risk of contamination input from the chemicals used. In addition, the use of electricity as the terminal oxidant avoids the use of stoichiometric amounts of heavy metals. Furthermore, the oxidation step can be easily adopted to the particular sample matrix, e.g. organic carbon content, and thus could be useful for the analysis of other environmental samples such as soils or sediments. However, the method has so far only been applied to a homogenized speleothem sample and should therefore be further tested with a variety of different speleothem samples to confirm the presented results.

## Experimental Section

Analytical standards of acetosyringone (97%), acetovanillone ( $\geq 98\%$ ), para-coumaric acid ( $\geq 98\%$ ), ethylvanillin (99%), ferulic acid (99%), syringaldehyde (98%), and syringic acid ( $> 95\%$ ), as well as L-ascorbic acid (SigmaUltra), copper(II) oxide ( $> 99\%$ ) and ammonium iron(II) sulfate (99%) were purchased from Sigma-Aldrich. An analytical standard of vanillin (99%) was obtained from Acros Organics, and an analytical standard of vanillic acid (98%) was obtained from Alfa Aesar. Sodium hydroxide (pellets,  $\geq 99\%$ ), hydrochloric acid (HCl, suprapure, 30%), and water (LC-MS-Grade) were purchased from Merck KGaA. Mixed lignin from wheat straw and various kinds of wood (Lignin A) was purchased from Bonding Chemical. Lignin from mainly coniferous wood (Lignin B) was obtained from BASF SE. Ammonium hydroxide solution ( $\text{NH}_3$ , analytical grade, 25%) was obtained from Honeywell Fluka. Solid-phase extraction columns (Oasis HLB, 6 mL tubes, 200 mg packing material, Oasis HLB, 3 mL tubes, 60 mg packing material, and Oasis HLB, 1 mL tubes, 30 mg packing material) were purchased from Waters. Ultrapure solvents (LC/MS grade) acetonitrile (ACN) and methanol (MeOH) were obtained from VWR and Carl Roth, respectively. Ultrapure water with 18.2 M $\Omega$  resistance was produced using a Milli-Q water system from Merck Millipore (Darmstadt, Germany).

For all experiments, aliquots of powder standard prepared from a speleothem from Ifri Inouqben cave, northern Morocco, were used. The age of this stalagmite is beyond the limit of the  $^{230}\text{Th}/\text{U}$ -dating method. Therefore, it is not suited for paleoclimate reconstruction, but is ideal as a standard.<sup>[35]</sup> The speleothem was ground coarsely and then homogenized to create a standard material with uniform analytical distribution.

Aliquots of 3 g of the standardized speleothem sample were dissolved in 3 mL HCl (30%) per g speleothem and then diluted 1:1 with ultrapure water. To ensure repeatability, every experiment was conducted fourfold and one blank sample was treated the same way.

The 200 mg HLB cartridges were preconditioned with 6 mL each of MeOH and ultrapure water, acidified to pH 1–2 with HCl (30%). The speleothem solution was filtered through paper filters (Whatman, Grade 40, 8  $\mu\text{m}$  pore size) to prevent undissolved materials from clogging the cartridges and was loaded onto the cartridges using sample reservoirs. The cartridges were washed twice with 6 mL of acidified ultrapure water and then dried by drawing air through the cartridges with a vacuum pump. The lignin was eluted with 10 portions of 500  $\mu\text{L}$  MeOH. The solvent was subsequently evaporated under a gentle stream of nitrogen at 30 °C.

The residue was dissolved in 2.5 mL NaOH (1 mol·mL<sup>-1</sup>) by sonification for 10 min at 45 °C. The solution was transferred into undivided reaction cells with a capacity of 10 mL made from PTFE. (Figure 4<sup>[36]</sup>). The procedure was repeated, and the sample solutions were combined. Nickel anodes with an active surface area of 1.5 cm<sup>2</sup> were used. A magnetic stirrer ensured homogeneous mixing. Electrolysis was performed at either 30 °C or 50 °C in galvanostatic mode with a current of 17 mA, a current density of 11.3 mA·cm<sup>-2</sup>, and a terminal voltage of 1.7–1.8 V. The applied electric charge was varied between 0.10 C, 0.15 C, and 0.20 C per g speleothem.

For experiments with pure lignin, about 50 mg of lignin powder was weighed into the reaction cells and dissolved in 5 mL NaOH (1 mol·mL<sup>-1</sup>). Electrolysis was performed at 30 °C in galvanostatic mode with a current of 17 mA, a current density of 11.3 mA·cm<sup>-2</sup>, and a terminal voltage of 1.7–1.8 V. The applied charge was set to 30.6 C per g lignin powder.

After electrolysis, the solution was transferred into microwave digestion vessels with a capacity of 30 mL made of PTFE. The vessels were heated to 155 °C in 5 min and held at that temperature for 90 min using a 5890A Gas Chromatograph (Hewlett Packard, USA). Immediately after opening the vessels, 10  $\mu\text{L}$  of a 1  $\mu\text{g}\cdot\text{mL}^{-1}$  standard solution of ethyl vanillin in ACN was added as an internal standard, and the oxidized sample solution was acidified to pH 1–2 with HCl (30%).

The  $\text{CuSO}_4$  oxidation procedure was developed based on the method described by Yan and Kaiser (2018).<sup>[13]</sup> The residue was dissolved in 200  $\mu\text{L}$  MeOH and sonicated at 45 °C for 10 min. The solution was transferred to a PTFE microwave digestion vessel with a capacity of 500  $\mu\text{L}$ . The procedure was repeated with 100  $\mu\text{L}$  MeOH and the combined solutions were evaporated to dryness under a gentle stream of nitrogen at 30 °C. The residue was dissolved in 200  $\mu\text{L}$  NaOH (1 mol·mL<sup>-1</sup>) by sonification for 10 min

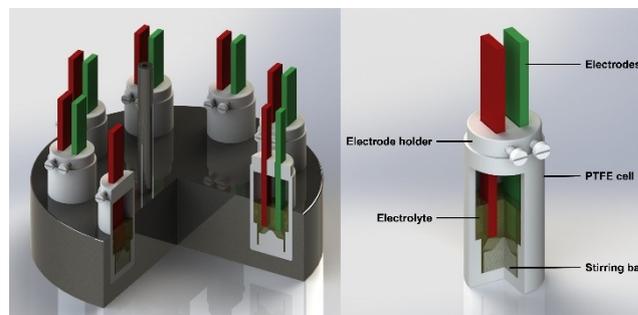


Figure 4. Schematic view of the reaction cells.<sup>[36]</sup>

at 45 °C. To each, 10  $\mu\text{L}$  of each  $\text{CuSO}_4$  solution (10  $\text{mmol}\cdot\text{L}^{-1}$  in  $\text{H}_2\text{O}$ ) and a solution of L-ascorbic acid (0.2  $\text{mol}\cdot\text{L}^{-1}$  in  $\text{H}_2\text{O}$ ) were added. The vessels were each purged with nitrogen for 20 s each and quickly sealed to ensure inert gas atmosphere. The digestion vessels were packed 5 at a time into a larger Teflon vessel and covered with 7 mL of NaOH (1  $\text{mol}\cdot\text{mL}^{-1}$ ) to ensure better heat transfer. These vessels were also purged with nitrogen for 1 min.

For experiments with pure lignin, about 50 mg of lignin powder was weighed into microwave digestion vessels with a capacity of 30 mL made of PTFE. For oxidation, 126  $\mu\text{L}$  of  $\text{CuSO}_4$  solution (10  $\text{mmol}\cdot\text{L}^{-1}$  in  $\text{H}_2\text{O}$ ), 315  $\mu\text{L}$  of L-ascorbic acid solution (0.2  $\text{mol}\cdot\text{L}^{-1}$ ), and 1060  $\mu\text{L}$  NaOH (1  $\text{mol}\cdot\text{mL}^{-1}$ ) were added. The vessels were purged with nitrogen for 1 min and quickly sealed.

The vessels were heated to 155 °C in 5 min using a gas chromatographic oven (5890 A Gas Chromatograph, Hewlett Packard, USA) and held at that temperature for 90 min. Immediately after opening the vials, 10  $\mu\text{L}$  of a 1  $\mu\text{g}\cdot\text{mL}$  standard solution of ethyl vanillin in acetonitrile was added as an internal standard and the oxidized sample solution was acidified to pH 1–2 with HCl (30%).

The residue was dissolved in 2 mL NaOH (2  $\text{mol}\cdot\text{mL}^{-1}$ ) by sonification for 10 min at 45 °C. The solution was added into a 100 mL Teflon microwave reaction vessel. The sample vial was sonicated again with 2 mL of NaOH (2  $\text{mol}\cdot\text{mL}^{-1}$ ) and the solutions were combined. The microwave-assisted CuO-mediated oxidation procedure was performed as described by Heidke et al. (2018).<sup>[12]</sup> An Ethos Plus Microwave Labstation (MLS GmbH, Germany) was used with an HPR-1000/10S high-pressure segment rotor, which can hold up to 10 reaction vessels, and an ATC-CE temperature sensor to measure the temperature inside one reaction vessel. Immediately after opening the vessels, 50  $\mu\text{L}$  of a 1  $\mu\text{g}\cdot\text{mL}$  standard solution of ethyl vanillin in acetonitrile was added as an internal standard and after clean-up, the oxidized sample solution was acidified to pH 1–2 with HCl (30%).

The 60 mg HLB cartridges were preconditioned with 3 mL each of MeOH and ultrapure water, acidified to pH 1–2 with HCl (30%). The 30 mg HLB cartridges were preconditioned with 1 mL each of MeOH and ultrapure water, which was acidified to pH 1–2 with HCl (30%), twice. The acidified oxidized sample solutions were loaded onto the 60 mg cartridges using sample reservoirs. Only the speleothem samples prepared by the  $\text{CuSO}_4$  oxidation method were loaded directly onto the 30 mg cartridges. The cartridges were washed twice with their void volume of 3 mL or 1 mL, respectively, and then dried by sucking air through the cartridges with a vacuum pump. The LOPs were eluted with 8 portions of 125  $\mu\text{L}$  acetonitrile to which 2%  $\text{NH}_3$  was added to reach a basic pH of 8–9. The eluate was evaporated under a gentle stream of nitrogen at 30 °C, and the residue was dissolved in 200  $\mu\text{L}$  acetonitrile/ $\text{H}_2\text{O}$  (1:9) by sonification at 45 °C for 10 min.

The analysis of the LOPs was carried out on a Dionex UltiMate 3000 ultrahigh-performance liquid chromatography system (UHPLC) that was coupled to a heated electrospray ionization source (ESI) and a Q Exactive Orbitrap high-resolution mass spectrometer (HRMS) (all by Thermo Fisher Scientific). To separate the LOPs, a Acquity UPLC CSH Fluoro Phenyl (PFP) column, 100 mm  $\times$  2.1 mm with 1.7  $\mu\text{m}$  particle size (Waters), was used. The injection volume was 5  $\mu\text{L}$ . A  $\text{H}_2\text{O}$ /acetonitrile gradient program was applied with a flow rate of 0.5  $\text{mL}\cdot\text{min}^{-1}$ . The gradient started with 5% eluent B (consisting of 98% acetonitrile and 2%  $\text{H}_2\text{O}$ ) and 95% eluent A (consisting of 98%  $\text{H}_2\text{O}$ , 2% acetonitrile, and 400  $\mu\text{L}\cdot\text{L}^{-1}$  formic acid). Eluent B was increased to 10% within 0.5 min and held for 4.5 min. It was then increased to 15% within 1 min and further increased to 30% in 1 min and to 50% in 0.5 min, respectively. Finally, it was increased to 99% within 0.5 min, held for 1.5 min, and then eluent

B was decreased to the initial value of 5%. The ESI source was operated in negative mode so that deprotonated molecular ions  $[\text{M}-\text{H}]^-$  were formed. ESI and MS conditions were the same as reported by Heidke et al. (2018).<sup>[12]</sup>

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## Conflict of Interest

The authors declare no conflict of interest.

**Keywords:** Anode · Electrochemistry · Lignin · Oxidation · Speleothem

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