

## Isotopes 2019

The Cross-Disciplinary Conference on Stable Isotope Sciences Raitenhaslach, Germany – July 7 – 12, 2019





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## **Scientific Program**

## Sunday, July 7

- until 16:00 Arrival of shuttle buses from Munich and Salzburg train station/ airport
- until 18:00 Registration
- 17:00 18:00 Welcoming reception at Klostergasthof
- 18:00 19:30 Dinner
- 19:30 19:45 Welcoming from organizers
- 19:45 20:30 Evening session Topic: Forensics

THOMAS PIPER (German Sport University Cologne, Germany): Isotope ratio mass spectrometry in sports drug testing

21:00 – 22:00 Shuttle buses to hotel

## Monday, July 8

until 08:30	Breakfast
08:30	Shuttle buses from hotel to Raitenhaslach

#### 09:00 - 10:45 Morning Session 1 - Topic 1: Advances in Analytics I

- 09:00 09:45 MATTHIAS GEHRE (Helmholtz Center for Environmental Research UFZ, Germany): Methodical advances in multi-element compound-specific stable isotope analysis using elemental analyzer- and gas chromatography-techniques
- 09:45 10:05 SIMON LEITNER (BOKU, Austria): UAV-based gas monitoring systems for the underpinning of urban, agricultural and industrial emission roadmaps – a methodological approach
- 10:05 10:25 JOACHIM MOHN (EMPA, Switzerland): A user guideline for N<sub>2</sub>O isotopocule analysis by laser spectroscopy: how to retrieve accurate results using QCLAS, CRDS or OA-ICOS analysis?
- 10:25 10:45 JULIAN RENPENNING (Thermo Fisher Scientific, Germany): Coupling of GC-IRMS with high-resolution mass spectrometry (GC Orbitrap) for final confirmation in drugs and metabolites research
- 10:45 11:15 Coffee break

#### 11:15 - 12:40 Morning Session 2 - Topic 3: Isotope Effects in Chemistry

- 11:15 12:00 MATHEW VETTICATT (Binghamton University, USA): *Kinetic isotope effects in organocatalysis and transition-metal catalysis*
- 12:00 12:20 JOHN GLANCY (University of Bath, UK): Computational simulation of mechanism and isotope effects on acetal heterolysis as a model for glycoside hydrolysis
- 12:20 12:40 DANIEL O'LEARY (Pomona College, USA): Isotopic perturbation of hydrogen bond equilibria
- 13:00 14:00 Lunch
- 14:15 16:15 Shuttle buses between hotel and Raitenhaslach
- 16:30 18:00 Poster session
- 18:00 19:30 Dinner

#### 19:30 – 20:35 Evening Session – Topic 4: Isotope Effects in Biology and Enzymology I

- 19:30 20:15 STEVEN SCHWARTZ (University of Arizona, USA): Heavy enzymes, artificial and natural enzymes and what they both teach us about enzyme design
- 20:15 20:35 ANDREW BENNET (Simon Fraser University, Canada): Kinetic isotope effect measurement for carbasugar analogue covalent inhibitors
- 21:00–22:00 Shuttle buses to hotel

### Tuesday, July 9

until 08:30Breakfast08:30Shuttle buses from hotel to Raitenhaslach

#### 09:00 - 10:45 Morning session 1 - Topic 2: Computations & Theory

- 09:00 09:45 IAN WILLIAMS (University of Bath, UK): Hessian-based QM and QM/MM calculations of isotope effects
- 09:45 10:05 MASANORI TACHIKAWA (Yokohama City University, Japan): Path integral simulation for accurate calculation of hyperfine coupling constants of hydrogenated and muoniated molecules
- 10:05 10:25 AGNIESZKA DYBALA (Lodz University of Technology, Poland): Vapor pressure isotope effects on evaporation from pure organic and aqueous phases – a theoretical study
- 10:25 10:45 VICENT MOLINER (Universitat Jaume I, Spain): Theoretical predictions of enzymatic rate constants and kinetic isotope effects: from origins to applications
- 10:45 11:15 Coffee break

#### 11:15 - 12:45 Morning session 2 - Topic 4: Isotope Effects in Biology and Enzymology II

- 11:15 12:00 SAM HAY (University of Manchester, UK): What are the signatures of tunnelling in enzyme-catalysed reactions?
- 12:00 12:45 ADAM OFFENBACHER (East Carolina Unversity, USA): Temperature dependent kinetic isotope effects resolve the origins of proficient enzymatic C-H activation
- 13:00 14:00 Lunch
- 14:15 16:15 Shuttle buses between hotel and Raitenhaslach
- 16:30 18:00 Poster session
- 18:00 19:30 Dinner
- 19:30 20:55 Evening session Topic 4: Isotope Effects in Biology and Enzymology III
- 19:30 20:15 SAMANTHA HARDMAN (University of Manchester, UK): The use of stable isotopes in ultafast spectroscopy of enzymes and photoreceptors
- 20:35 20:55 MARCO FARREN-DAI (Simon Fraser University, Canada): Mechanistic evaluation of glycoside hydrolases covalent inhibitors: enzymatic kinetic isotope effect and computational studies
- 21:00–22:00 Shuttle buses to hotel

### Wednesday, July 10

until 08:30 Breakfast08:30 Shuttle buses from hotel to Raitenhaslach

#### 09:00 - 10:45 Morning session 1 - Topic 7: Environmental and Water Chemistry / Microbiology I

- 09:00 09:45 THOMAS HOFSTETTER (Eawag & ETH Zürich, Switzerland): Effect of metabolic constraints on the observable isotope fractionation during aerobic biodegradation of hexachlorocyclohexanes
- 09:45 10:05 JOHANNES BÜSING (University of Tübingen, Germany): Using dual element isotope analysis ( $\delta^{13}C$ ,  $\delta^{37}Cl$ ) to gain mechanistic insights into enzymatic tetrachloroethene degradation of three different reductive dehalogenases
- 10:05 10:25 IVONNE NIJENHUIS (Helmholtz Centre for Environmental Research UFZ, Germany): Mechanistic and enzymatic insights in reductive dehalogenation by Dehalococcoides mccartyi strain BTF08
- 10:25 10:45 Томаяz Кирек (University of Oklahoma, USA): Biological and abiotic natural attenuation of 1,2-Dibromoethane (EDB) at Kirtland AFB, USA
- 10:45 11:15 Coffee break

#### 11:15 - 12:40 Morning session 2 - Topic 7: Environmental and Water Chemistry / Microbiology II

- 11:15 12:00 ANAT BERNSTEIN (Midreshet Ben-Gurion, Israel):
   Applying stable isotope analysis on increasing scales –microcosm, field, and multi-field-sites studies
- 12:00 12:20 MICHAELA LÖFFLER (Helmholtz Centre for Environmental Research UFZ,Germany): *Tracking hydrogenase activity with hydrogen stable isotopes*
- 12:20 12:40 CHARLOTTE BOPP (Eawag, Switzerland): Impact of oxygen activation on substrate isotope effects of enzymatic oxygenations
- 13:00 14:00 Lunch

#### 14:00 – 22:00 Excursion to Salzburg and conference dinner at panorama restaurant Hohensalzburg Fortress

The journey will allow us to visit the old city of Salzburg and explore some of its highlights for two hours before the conference dinner. At dinner, we will enjoy at the restaurant location high above the rooftops of Salzburg a nice view over the city. Travelling back to medieval times, we will be eating like the archbishops and be entertained by funny and exciting games from the time of the archbishops. Master of ceremonies will guide us through the evening and introduce us to medieval music, customs and traditions of the archbishops.

#### Scientific Program | Wednesday, July 10

14:00 Departure of shuttle buses from Raitenhaslach
15:30 - 17:30 Free time to explore Salzburg
17:45 Departure by cable-car (Mönchsberg 34, S020 Salzburg) up to the restaurant
18:00 Beginning of dinner/program
22:00 Departure of shuttle buses back to Burghausen/Raitenhaslach

### Thursday, July 11

until 08:30 Breakfast08:30 Shuttle buses from hotel to Raitenhaslach

#### 09:00 - 10:45 Morning session 1 - Topic 5: Isotopologues

- 09:00 09:45 SHUHEI ONO (Massachusetts Institute of Technology, USA): Measurements of doubly substituted methane isotopologues (<sup>13</sup>CH<sub>3</sub>D and <sup>12</sup>CH<sub>2</sub>D<sub>2</sub>) using mid-infrared spectroscopy
- 09:45 10:05 STEFANO BERNASCONI (ETH Zürich, Switzerland): Measuring the temperature of geological processes with carbonate clumped isotopes
- 10:05 10:25 KRISTÝNA KANTNEROVÁ (Empa / ETH Zürich, Switzerland): New reference frame for clumped N<sub>2</sub>O isotopic analysis
- 10:25 10:45 MIN ZHU (Helmholtz Centre for Environmental Research UFZ, Germany): 3D (<sup>13</sup>C, <sup>2</sup>H, <sup>37</sup>Cl) Isotope analysis for HCHs transformation by cobalamin
- 10:45 11:15 Coffee break

#### 11:15 – 12:40 Morning session 2 – Topic 5/7: Isotopologues/ Environmental and Water Chemistry/ Microbiology II

- 11:15 12:00 GERALD REMAUD (University of Nantes, France):
   <sup>2</sup>H, <sup>13</sup>C and <sup>15</sup>N isotopomers titration and profiling: the use of Nuclear Magnetic Resonance spectrometry (NMR) for Position-Specific Isotope Analysis (PSIA)
- 12:00 12:20 NATALIA MALINA (Helmholtz Centre for Environmental Research UFZ, Germany): Analysis of phenolics by gas-chromatography – isotope ratio mass-spectrometry (GC-IRMS): method development and application
- 12:20 12:40 JING WEI (Empa, Switzerland): Quantifying N<sub>2</sub>O production pathways of waste water treatment plants using <sup>15</sup>N tracer
- 13:00 14:00 Lunch
- 14:15 16:15 Shuttle buses between hotel and Raitenhaslach

#### 17:00 – 17:40 Afternoon Session – Topic 7: Environmental and Water Chemistry/ Microbiology III

- 17:00 17:20 ТЕТУАNA GILEVSKA (University of Toronto, Canada): In situ biodegradation rates in contaminated sediments via a high resolution multielement isotopic approach
- 17:20 17:40 ANN OJEDA (University of Toronto, Canada):
   Useful statistical methods for compound-specific isotope analysis in contaminant hydrogeology
- 18:00 19:30 Dinner

#### 19:30 - 20:35 Evening session - Topic 6: Earth/Planetary Sciences, Biogeochemistry and Ecology I

- 19:30 20:15 КАТНЕRINE FREEMAN (The Pennsylvania State University, USA): Tracking life's fingerprints with molecules and isotopes
- 20:15 20:35 JÜRGEN SCHLEUCHER (Umeå University, Sweden): New ecophysiological information from intramolecular isotope variation: methods, analysis and implications for biogeochemistry
- 21:00–22:00 Shuttle buses to hotel

## Friday, July 12

until 08:30	Breakfast
08:30	Shuttle buses from hotel to Raitenhaslach

#### 09:00 - 10:50 Morning session 1 - Topic 6: Earth / Planetary Sciences, Biogeochemistry and Ecology II

- 09:00 09:45 DEB JAISI (University of Delaware, USA): Linking phosphate oxygen isotope effects to phosphorus cycling, from molecular to ecosystem scales
- 09:45 10:05 ANDREA WATZINGER (University of Natural Resources and Life Sciences (BOKU), Austria): Future precipitation patterns in agroecosystems affected carbon and nitrogen turnover – a green manure stable isotope labelling study
- 10:05 10:50 MARTIN JISKRA (University of Basel, Switzerland): Mercury stable isotopes as an eye-opener on the role of vegetation in global mercury cycling
- 10:50 11:15 Coffee break

#### 11:15 - 12:20 Morning session 2 - Topic 1: Advances in Analytics II

- 11:15 12:00 DANILO SCIARRONE (University of Messina, Italy): Simultaneous detection by isotope ratio and quadrupole mass spectrometry coupled to multidimensional gas chromatography for the analysis of complex samples
- 12:00 12:20 NATALIA IVLEVA (Technical University of Munich, Germany): Stable Isotope Raman Microspectroscopy for Nondestructive Analysis of Microorganisms on the Single-Cell Level
- 12:20 12:40 Prizes & closing
- 13:00 14:00 Lunch
- 14:00 Departure of shuttle buses to Munich and Salzburg train station/airport

Abstracts: Oral Presentations

### Isotope ratio mass spectrometry in sports drug testing

Thomas Piper<sup>1</sup>, Mario Thevis<sup>1,2</sup>

<sup>1</sup> German Sport University Cologne, Institute of Biochemistry, Center for Preventive Doping Research, Am Sportpart Müngersdorf 6, 50933 Köln, Germany

<sup>2</sup> European Monitoring Center for Emerging Doping Agents (EuMoCEDA), Cologne/Bonn, Germany

The application of isotope ratio mass spectrometry (IRMS) to doping control analysis is a growing scientific field encompassing different approaches to improve the detection of cheating athletes.

Since more than 15 years the determination of carbon isotope ratios (CIR) enables to confirm abnormal steroid profiles found in athletes. The steroid profile is based on the measurement of six or more different urinary steroid concentrations such as those of testosterone (T) and epitestosterone (E) and corresponding concentration ratios. Elevated ratios of T/E can be due to either testosterone doping or confounding factors like bacterial contamination or ethanol intake. Only by IRMS measurements of urinary T or T-metabolites it is possible to unambiguously confirm the anti-doping rule violation. This approach has recently been improved by the introduction of a so-called long-term metabolite (epiandrosterone), which enables to prolong the detection of T-doping considerably.

A comparable approach enables to identify the source of so called pseudo-endogenous steroids such as boldenone, 19-norandrosterone, and formestane that represent doping substances but, in rare cases, can also be produced endogenously.

While most of the IRMS techniques in doping controls focus on steroids as target analytes, first investigations into other classes of compounds have been successfully accomplished and, as in case of AICAR, already implemented into routine sports drug testing programs. AICAR is produced naturally in humans and beneficially modulates insulin action and glucose uptake which consequently led to its inclusion into the so-called Prohibited List of the World Anti-Doping Agency (WADA).

In rare cases, CIR might fail to identify the source of urinary T-metabolites. Here, hydrogen isotope ratios (HIR) have been implemented in doping controls. Unfortunately, a considerably large overlap in isotope ratios between endogenously produced steroids and pharmaceutical preparations has been substantiated, complicating the straightforward application of this method.

But HIR proved to be extremely valuable in the detection of formerly unknown metabolites of synthetic steroids like metandienone, methylstenbolone, 2-androstenone, or the selective androgen receptor modulator YK11. Excretion studies employing the deuterated analogs of the compound under investigation in combination with HIR and high resolution/high accuracy mass spectrometry allows for unambiguous identification of (nearly) all urinary metabolites of the administered compound.

In this presentation, an overview of the exciting and challenging field of the application of IRMS in doping control analysis will be provided. Using authentic case reports, the added value of IRMS to sports drug testing is demonstrated that significantly contributes to fair decision-making processes.

Oral Presentations: Monday, July 8

## Methodical advances in multi-element compound-specific stable isotope analysis using elemental analyzer- and gas chromatography-techniques

#### Matthias Gehre

Helmholtz Centre for Environmental Research - UFZ, Permoserstr. 15, 04318 Leipzig, Germany

During the last two decades several new on-line techniques for the analysis of hydrogen carbon, nitrogen, chlorine and sulfur stable isotope ratios were introduced which paved the way towards multi-element compound-specific stable isotope analysis (ME-CSIA). Through the isotope measurements of several elements of a compound, new possibilities of a more detailed process elucidation and understanding were made possible or greatly improved.

The presentation will focus on gas chromatography (GC) and elemental analyzer (EA) applications coupled with gas isotope ratio mass spectrometer (IRMS) or multi-collector inductively coupled plasma mass spectrometer (MC-ICPMS) as detector. Practical methods for high and low flow systems will be presented. Moreover, major and irreconcilable isotopic discrepancies as a result of incomplete conversion of the sample to the respective analyses gas will be discussed. A central bottleneck for ME-CSIA is the missing of adequate isotopic reference materials which can be used to normalize the isotope results via two-point calibration to international scales. Additionally, the feasibility and the value of ME-CSIA for forensic inquiries and environmental questions will be addressed.

## UAV-based gas monitoring systems for the underpinning of urban, agricultural and industrial emission roadmaps – a methodological approach

<u>Simon Leitner</u><sup>1</sup>, Wendelin Feichtinger<sup>2</sup>, Stefan Mayer<sup>2</sup>, Florian Mayer<sup>2</sup>, Rebecca Hood-Nowotny<sup>1</sup> & Andrea Watzinger<sup>1</sup>

<sup>1</sup>University of Natural Resources and Life Sciences Vienna, Institute of Soil Research, Konrad-Lorenz-Straße 24, 3430 Tulln, Austria

<sup>2</sup> Combinnotec Ges.m.b.H, Heiligenkreuzer Str. 466, 2534 Alland, Austria

Currently sampling of the atmosphere for gas emission measurements involves building towers or hiring airplanes – capital-intensive methods. Easy access to unmanned aerial vehicles (UAV) has opened-up new opportunities for remote gas sampling. The project Iso-2-Drone aims to develop and produce a modular UAV-based gas monitoring system for emission measurements to substitute current technologies. A key feature of the UAV-attached gas sampler design was the ready-to-use nature of the system. This meant that the system was designed to mesh with commonly available equipment, using collection vials which can be easily and immediately measured by common continuous flow - isotope ratio mass spectrometer (CF-IRMS) instrumentation. The target compounds comprise the three major natural greenhouse gases  $CH_4$ ,  $CO_2$  and  $N_2O$  to be measured at natural abundance and ambient levels.

Sample preparation of the glass vials (20 mL headspace vials) is achieved by repeatedly evacuating and helium refilling cycles to prevent ambient air contamination. On the UAV-attached sampler atmospheric air is sampled passively by pressure compensation of the vacuum. Both a prototype device and a UAV-attached sampler have been designed, built and are currently tested.



Figure 1: Chromatographic separation of CH<sub>4</sub>, CO<sub>2</sub> and N<sub>2</sub>0.

The measurement setup in the lab comprises of two autosamplers, a purge & trap system (VSP 4000, IMT Innovative Maschinentechnik GmbH) and headspace sampler (CTC CombiPal, Chromtech GmbH)) in order to switch from ppb range necessary for  $CH_4$  and  $N_2O$  to a ppm range for  $CO_2$ . Each autosampler transfers sampled air to a Restek Micropacked Column (Shin Carbon ST 100/120,

2m x 1mm ID and 1/16" OD) within a Thermo Scientific Trace GC Ultra heated up from 40°C to 175°C by 12°C per minute. CH<sub>4</sub> eluted from the column is oxidized to CO<sub>2</sub> in a combustion reactor held at 1000°C. CO<sub>2</sub> and N<sub>2</sub>O pass through a high temperature conversion reactor held at 200°C, which was shown not to alter the isotopic composition of carbon and oxygen in CO<sub>2</sub> and nitrogen in the N<sub>2</sub>O. The IRMS (Thermo Scientific DeltaV Advantage) continuously scans the intensity of the mass-to-charge ratios of mass 44, 45 and 46 (Figure 1). While  $\delta^{13}$ C and  $\delta^{18}$ O are referenced against a CO<sub>2</sub> working gas, the  $\delta^{15}$ N value is referenced against the  $\delta^{15}$ N<sub>AIR</sub> of a N<sub>2</sub>O working gas. We are currently determining the precision of the method which will be presented at the conference.

## A user guideline for N<sub>2</sub>O isotopocule analysis by laser spectroscopy: how to retrieve accurate results using QCLAS, CRDS or OA-ICOS analysis?

Stephen J. Harris<sup>1,2</sup>, Jesper Liisberg<sup>3</sup>, Longlong Xia<sup>4</sup>, Jing Wei<sup>5</sup>, Kerstin Zeyer<sup>5</sup>, Longfei Yu<sup>5</sup>, Matti Barthel<sup>6</sup>, Benjamin Wolf<sup>4</sup>, Bryce F.J. Kelly<sup>1</sup>, Dioni I. Cendón<sup>2</sup>, Thomas Blunier<sup>3</sup> & Joachim Mohn<sup>5,\*</sup>

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<sup>3</sup> University of Copenhagen, Centre for Ice and Climate, Niels Bohr Institute, Copenhagen, Denmark

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<sup>5</sup> Empa, Laboratory for Air Pollution/Environmental Technology, Dübendorf, Switzerland

<sup>6</sup> ETH Zürich, Department of Environmental Systems Science, Zürich, Switzerland;

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Over the last two decades, research involving N<sub>2</sub>O site specific isotopic analysis has been stimulated by continuing analytical progress in isotope-ratio mass-spectrometry (IRMS) and more recently mid-infrared laser spectroscopy. This development has been triggered by the invention and availability of quantum cascade lasers (QCL), which offer high optical power in continuous wave operation at room temperature. QCL light sources have been combined with different detection schemes such as direct absorption (QCLAS), cavity ring down (CRDS) and off-axis integrated cavity output (OA-ICOS) to realize compact, field-deployable analyzers.

The availability of temporal resolved  $N_2O$  isotopic information in real-time will deepen our process-level understanding of the nitrogen cycle. It will also open up entirely new research areas that will attract an increasing number of application-oriented scientists. Provided that the novel laser spectrometers produce compatible and thus accurate results (i.e. traceable to the international isotope ratio scales), the implementation of these instruments will lead to a further dissemination of  $N_2O$  isotopic research.

We will present results of an inter-comparison study on the three most common commercial N<sub>2</sub>O isotope analyzers, including Aerodyne Research (dual QCLAS, with/without TREX), Picarro (G5131-i) and Los Gatos Research (Model 914-0027). Most importantly, gas matrix effects were investigated by determining the dependence of N<sub>2</sub>O isotope deltas on the analysis in an "ambient" N<sub>2</sub>/O<sub>2</sub>/Ar/CO<sub>2</sub>/ CH<sub>4</sub>/CO versus a simplified N<sub>2</sub>/O<sub>2</sub>/Ar or N<sub>2</sub>/O<sub>2</sub> matrix. In addition, spectral interferences of enhanced trace gas concentrations (CO<sub>2</sub>, CH<sub>4</sub>, CO, H<sub>2</sub>O) were characterized and strategies for removal tested. Repeatability, drift and dependence of isotope deltas on N<sub>2</sub>O concentrations were also quantified and compared among instruments. Based on these results a calibration strategy was established and the accuracy of individual analyzers assessed combining the studied uncertainty contributions.

Our study will guide the selection of instruments for specific applications (e.g. ambient air versus incubation studies), and foster the development of  $N_2O$  isotope reference gases optimized for laser spectrometers currently ongoing within the EMPIR project "Metrology for Stable Isotope Reference Standards (SIRS)".

## Coupling of GC-IRMS with high-resolution mass spectrometry (GC Orbitrap) for final confirmation in drugs and metabolites research

Julian Renpenning, Andreas Hilkert, Dieter Juchelka & Mario Tuthorn

#### Thermo Fisher Scientific , Germany

Compound-specific isotope analysis (CSIA) via gas chromatography and subsequent isotope ratio mass spectrometry (GC-IRMS) is a well-established analytical approach to a variety of samples from routine and research areas, such as environmental forensics, food authentication, energy exploration, paleo-climate and metabolism research.

Concomitant data are critical to qualify the true identity of a compound. However, this has become mandatory in confirmation of so-called adverse analytical findings. By coupling GC-IRMS with the Q Exactive GC system, the isotopic compositions and the comprehensive qualitative and quantitative sample information is now simultaneously accessible from a single injection with high levels of selectivity, sensitivity, and confidence.

In the last years, the online coupling of GC-IRMS with an organic MS system has been established for confirmatory analysis in doping control samples. In analysis of banned drugs in sports, GC-IRMS allows distinguishing between endogenous steroids from their synthetic analogs in urine by the determination of <sup>13</sup>C/<sup>12</sup>C isotope ratios for doping control. However, it is also necessary to know metabolic fate of drugs because knowing metabolites accelerates the drug discovery and method development process. Coupling of GC-IRMS with high-resolution mass spectrometry allows tracking down drug metabolism by the IRMS system, while the Q Exactive GC provides simultaneously the structural information of all monitored metabolites.

Here we present case studies on how isotope fingerprints have been effectively investigated to fight emerging threats in drug abuse.

## Kinetic isotope effects in organocatalysis and transition-metal catalysis

#### Mathew J. Vetticatt

Department of Chemistry, Binghamton University, 25 Murray Hill Road, Vestal, NY 13850 USA

Our research program utilizes experimental and theoretical <sup>13</sup>C kinetic isotope effects (KIEs) to investigate mechanisms of catalytic reactions. In the area of asymmetric organocatalysis, this approach is utilized to identify new mechanistic pathways and uncover fine details of complex catalytic cycles. These studies provide the basis for the rational design of novel concepts in asymmetric catalysis. Our more recent efforts address challenging mechanistic questions in the area of transition-metal catalysis.

This seminar will provide an overview of our research program and present unpublished results from (a) a mechanistic study of an organocatalytic Mannich reaction, (b) an investigation of the transmetalation step of the Suzuki-Miyaura reaction, and (c) the development of a novel mechanistic probe for the rapid determination of <sup>13</sup>C KIEs using 'designed' starting materials.

#### References

[1] Ashley, M. A.; Hirschi, J. S.; Izzo, J. A.; Vetticatt, M. J. J. Am. Chem. Soc. 2016, 138, 1756-1759.

- [2] (a) Izzo, J. A.; Poulsen, P. H.; Intrator, J. A.; Jørgensen, K. A.; Vetticatt, M. J. Isotope Effects Reveal an Alternative Mechanism for "Iminium-Ion" Catalysis. *J. Am. Chem. Soc.* 2018, *140*, 8396-8400. (b) Bandar J. S.; Sauer, G. S.; Wulff, W. D.; Lambert, T. H.; Vetticatt, M. J. *J. Am. Chem. Soc.* 2014, *136*, 10700-10707.
- [3] Macharia J.; Victor, W.; Hong, Y.; Harris, L.; Hirschi, J. S.; Evans, G. B.; Vetticatt, M. J. Angew. Chem. Int. Ed. 2017, 56, 8756-8760.

## Computational simulation of mechanism and isotope effects on acetal heterolysis as a model for glycoside hydrolysis

#### John Glancy<sup>1</sup> & Ian Williams<sup>2</sup>

<sup>1</sup> Centre for Sustainable Chemical Technologies, Department of Chemistry, University of Bath, Bath BA2 7AY, UK <sup>2</sup> Department of Chemistry, University of Bath, Bath BA2 7AY, UK

KIEs offer powerful probes for mechanism and TS structure in enzyme-catalysed reactions, as long as their experimentally determined values and variations can be correctly interpreted.<sup>[1,2]</sup> It is common for analyses of TS structure, based upon KIEs for multiple isotopic substitutions, to consider force-constant changes only as functions of molecular geometry.<sup>[3]</sup> However, work in our group suggests that consideration of the electrostatic environment is also necessary, as force-constant changes can depend on the relative permittivity of the medium. If so, there would be important implications for the interpretation of experimental KIEs in mechanistic enzymology.

This work outlines our efforts to accurately calculate the isotope effects, including the effects of the electrostatic environment on the reactant and transition states, for the hydrolysis of 2-(*p*-nitrophenoxy)tetrahydropyran **1**. Both QM and QM/MM molecular dynamics techniques have been used.

DFT calculations for the equilibrium isotope effect for deuterium substitution at the anomeric centre  $C_{\alpha}$  in **1**, with continuum solvation, show significant variation in the range of relative permittivity  $2 \le \epsilon \le 10$ . One-dimensional scans of potential and free energy for cleavage of the bond between  $C_{\alpha}$  and the nucleofuge do not show a transition state. A two-dimensional free-energy surface that considers also the distance between  $C_{\alpha}$  and a nucleophilic water indicates a pre-association  $D_N^*A_{Nint}^{**}$  ( $S_N^2$  intermediate) mechanism with a transition state involving nucleophilic attack upon an ion-pair intermediate, and this is supported by good agreement between the mean values of the calculated and experimental  $\alpha$ -D KIEs.<sup>[4]</sup>



This work sheds new light on an established mechanism and supports the hypothesis that interpretation of KIE data requires accurate modelling of both the geometry and the electrostatic environment of the transition state.

#### References

- [1] P. F. Cook, Enzyme Mechanism from Isotope Effects, CRC Press, 1991.
- [2] W. W. Cleland, J. Biol. Chem. 2003, 278, 51975-84.
- [3] P. J. Berti, K. S. E. Tanaka, Adv. Phys. Org. Chem. 2002, 37, 239-314.
- [4] I. A. Ahmad, S. L. Birkby, C. A. Bullen, P. D. Groves, T. Lankau, W. H. Lee, H. Maskill, P. C. Miatt, I. D. Menneer, K. Shaw, J. Phys. Org. Chem. 2004, 17, 560–566.

#### Isotopic perturbation of hydrogen bond equilibria

#### Daniel J. O'Leary

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Our group is interested in developing NMR methods for detecting intramolecular OH/OH hydrogen bonds in solution. Our approach has employed the synthesis of model compounds containing structurally well-defined diol, triol, and tetrol units. These compounds have shed light on two NMR methods for hydrogen bond detection, namely: (1) the method of isotopic perturbation of equilibria involving partially deuterated hydroxyl groups, and (2) measurement of hydrogen bond-mediated scalar *J* couplings between hydroxyl groups.<sup>1-3</sup>

We are also pursuing theoretical investigations of equlibrium isotope effects (EIEs) in these systems. For example, the preference of deut-erium for the bridging hydrogen D bond in mono-deuterated 2,6-dihydroxybenzaldehyde (1b) is due to antag-onistic enthalpic and entropic contributions to the overall isotope effect. These calculations use harmonic vibrational frequencies and directly compare enthalpy-entropy partitioning with the more traditional Bigeleisen-Mayer approach.<sup>4,5</sup>

Another area of investigation seeks to understand the titration behavior of NMR-determined EIEs in hydrogen-bonded systems (a hydrogen bond-induced isotope shift is observed in <sup>1</sup>H hydroxyl spectra of OH/OH and OH/OD isotopologues). The negative EIE for diol 2 dissolved in CD<sub>2</sub>Cl<sub>2</sub> (-57 ppb) is found to reach a limiting and reduced value (-20 ppb) when methanol is titrated into the sample. Conversely, the positive EIE for diol 3 dissolved in CD<sub>2</sub>Cl<sub>2</sub> reaches a maximum value (+17 ppb) in the presence of a small amount of methanol and then goes to zero at high additive concentrations. We ascribe these different responses to strong (diol 2) vs. weak (diol 3) intramolecular hydrogen bonds and we are using computational chemistry to further understand these phenomena. Our presentation will summarize our investigations of diols 1-3 and related systems.

#### References

[1] D.J. O'Leary, D.D. Hickstein, B.K. Hansen, P.E. Hansen, J. Org. Chem. 75 (2010) 1331-1342.

- [2] C.E. Anderson, A.J. Pickrell, S.L. Sperry, T.E. Vasquez, T.G. Custer, M.B. Fierman, D.C. Lazar, Z.W. Brown, W.S. Iskenderian, D.D. Hickstein, D.J. O'Leary, Heterocycles 72 (2007) 469-495.
- [3] N.M. Loening, C.E. Anderson, W.S. Iskenderian, C.D. Anderson, S.D. Rychnovsky, M. Barfield, D.J. O'Leary, Org Lett 8 (2006) 5321-5323.
- [4] O.M. Ogba, J.D. Thoburn, D.J. O'Leary, Spreadsheet-Based Computational Predictions of Isotope Effects, in: D.J. Tantillo (Ed.), Applied theoretical organic chemistry, World Scientific, New Jersey, 2018, pp. 403-450.
- [5] A.C. Brueckner, S.L. Cevallos, O.M. Ogba, D.M. Walden, M.P. Meyer, D.J. O'Leary, P.H.-Y. Cheong, Onyx, version 1.0; Oregon State University: Corvallis, OR, USA & Pomona College: Claremont, CA, USA, 2016.



## Heavy, artificial, and natural enzymes and what they both teach us about enzyme design

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This talk will focus on new results we have obtained studying heavy enzymes, series of more proficient laboratory evolved artificial enzymes, and some systems on which there has been controversy as to mechanism. This work is aimed at addressing the seeming never ending dispute of dynamics vs. electrostatics, and if dynamics slow or fast or both. As an example, there has been hot debate as to the importance of these issues in Catechol-O-methyl transferase. We address these questions for all the systems studied using unbiased simulation techniques.

## Kinetic isotope effect measurement for carbasugar analogue covalent inhibitors

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The enzyme-catalyzed transfer of carbohydrates from a donor to an acceptor are critical reactions for all life. As a result, the mechanisms for these substitutions at the anomeric carbon of sugars have been studied extensively. Generally, these enzyme-catalyzed reactions occur by either two inversions

of configuration (retaining) or one (inverting). The enzyme that hydrolysis glycosides are the glycoside hydrolases (GH), which have been grouped into >150 families based on sequence and structure (Henrissat, B. 1991; Lombard, V., Ramulu, H. G. et al. 2014).

We have recently shown that carbocyclic structural analogues of carbohydrates are mechanism-based covalent inhibitors of glycoside hydrolases (Shamsi Kazem Abadi, S., Tran, M. et al. 2017; Ren, W., Pengelly, R. et al. 2018). In order to understand how these covalent inhibitors react within the active site of glycoside hydrolases we have undertaken detailed mechanistic investigations of the reaction of these types of covalent inhibitors both in aqueous solution (intrinsic reactivity) and within the active site of several glycoside hydrolases. Our studies are a combination of the experimental determination of kinetic isotope effects and hybrid quantum mechanical/molecular mechanics (QM/MM) theoretical calculations.



Structures for two carbasugar glucose mimics that are covalent inhibitors of  $\alpha\mbox{-glucosidases}.$ 

Recent results and their implication for the modes of action of these mechanism-based covalent inhibitors will be discussed.

#### References

- Henrissat, B. (1991). A classification of glycosyl hydrolases based on amino acid sequence similarities, *Biochem. J.* 280: 309-316.
- [2] Lombard, V., Ramulu, H.G., et al. (2014). The carbohydrate-active enzymes database (CAZy) in 2013, *Nucleic Acids Res.* **42**(D1): D490-D495.
- [3] Ren, W., Pengelly, R., et al. (2018). Revealing the mechanism for covalent inhibition of glycoside hydrolases by carbasugars at an atomic level, *Nat. Commun.* **9**: 3243.
- [4] Shamsi Kazem Abadi, S., Tran, M., et al. (2017). New class of glycoside hydrolase mechanism-based covalent inhibitors: Glycosylation transition state conformations, *J. Am. Chem. Soc.* **139**: 10625–10628.
- Inhibition of Glycoside Hydrolases by Carbasugars: Design, Synthesis, and Structural and Computational Studies" Nat. Comm., 9:3243 (2018)

Oral Presentations: Tuesday, July 9

### Hessian-based QM and QM/MM calculations of isotope effects

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Kinetic isotope effects (KIEs) are important experimental probes of mechanism for chemical reactions, the vast majority of which occur in condensed phases. Traditional methods for their calculation are molecular in nature, which is fine for gas-phase reactions but generally inadequate for systems in which coupling between a substrate and its environment is significant. Reactions occurring in polar solutions or in enzyme active sites are important examples of those which should be considered as having 'supramolecular' character. The unifying feature of both old and new versions of the most common approach to isotope-effect calculations is the use of a Hessian matrix of force constants within the harmonic approximation.[1] Atomic subsets of supramolecular systems, whose isotopically sensitive vibrational frequencies are explicitly considered, may be (surprisingly) small without significant error for KIEs provided that several conditions are satisfied. (a) All degrees of freedom of the subset must be considered. (b) The Hessian matrix of second derivatives of energy with respect to the subset degrees of freedom must be evaluated within an adequately large environment whose electrostatic properties are accurately described. (c) The subset should be large enough to include all atoms necessary for an adequate description of the reaction-coordinate vibrational mode. (d) KIEs should be evaluated as simple quotients of isotopic partition function ratios determined independently for the reactant state and the transition state averaged over thermally accessible configurations.

Examples from recent computational studies of (i) EIEs for transfer of carbenium and oxacarbenium cations from water to a non-polar solvent and (ii) KIEs on enzyme-catalyzed methyl transfer are discussed.[4]

#### Reference

 I. H. Williams, P. B. Wilson, in Simulating Enzyme Reactivity: Computational Methods in Enzyme Catalysis, eds. I. Tuñón, V. Moliner, Royal Society of Chemistry, Cambridge, ISBN: 978-1-78262-429-5, 2017.

## Path integral simulation for accurate calculation of hyperfine coupling constants of hydrogenated and muoniated molecules

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A considerable amount of knowledge for muonium (Mu; complex of positive muon and electron) chemistry has been accumulated for over 30 years [1]. Compared with a proton, positive muon ( $\mu$ +) has a smaller mass and larger magnetic moment. Because of these unique features, Mu is used as the muon spin resonance/rotation/relaxation ( $\mu$ SR), where hyperfine coupling constant (HFCC) is a good index for the magnetic interaction between electron and muon spins.

For instance, the HFCC value of muoniated acetone radical (Mu-ACE, Figure 1) is measured by Per-

cival et al [2] as 10.27 MHz at 300 K (reduced using the proton magnetic moment). However, the reduced HFCC value for Mu-ACE is calculated as -5.8 MHz with the conventional DFT calculation [3], where the quantum effect of nuclei and thermal effect are excluded.

In this study, thus, we performed on-the-fly ab initio path integral molecular dynamics (PIMD) simulation [4, 5], which can include these effects, to Mu-ACE and hydrogenated acetone radical (H-ACE). Our HFCC values for Mu-ACE and H-ACE are calculated as 32.1 and 3.97 MHz, respectively, which are in reasonable agreement with the corresponding

pendence on HFCC values is due to the large quantum effect



experimental values of 10.3 and 1.51 MHz. Such mass-de- Figure 1: Structure of Mu-ACE.

We will also show other results for other muoniated and hydrogenated molecular species.

#### References

of muon.

[1] P. W. Percival, Radiochemica Acta, 26, 1 (1979).

- [2] P. W. Percival, J.-C. Brodovitch, K. Ghandi, B. M. McCollum and I. McKenzie, J. Am. Chem. Soc. 127, 13715 (2005).
- [3] R. M. Macraea and I. Carmichael, Physica B, 326, 81 (2003).
- [4] K. Yamada, Y. Kawashima, and M. Tachikawa, J. Chem. Theory Comput., 10, 2005 (2014).
- [5] Y. Oba, T. Kawatsu, and M. Tachikawa, J. Chem. Phys., 145, 064301 (2016).

## Vapor pressure isotope effects on evaporation from pure organic and aqueous phases – A theoretical study

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Evaporation is one of the important pathways of volatile organic compounds (VOCs) attenuation driven by the vapor pressure that these compound possess. To quantify this process one can study the isotopic composition of the compound phases. Depending on the isotopic composition of a compound phase (liquid vs vapor) such analysis can lead to either normal (liquid phase enriched in the heavier isotopologue) or inverse (vapor phase enriched in the heavier isotopologue) vapor pressure isotope effects (VPIEs). According to isotope effect theory, VPIEs are mainly governed by intermolecular forces of a different kind. However, till the date, evaluation of isotope fractionation during evaporation process is still a developing experimental field, for example, the little experimental data available mainly refers to pure organic phase evaporation while more realistic environment like aqueous solutions is unexplored but more important. Different experimental setups frequently result in an inconsistent and/or misleading interpretation of the results. Hence, it is necessary to seek independent tools that allow not only for interpreting experimental results, but also offering additional information that could not be achieved through the experiments.

The aim of the presented study is threefold. First, we wanted to test some of the available computational approaches to predict VPIEs and compare them with the experimentally determined values. For this purpose, we selected dibromomethane (DBM) and bromobenzene (BB) as representatives of brominated VOCs, clear examples of aliphatic and aromatic compound, respectively, ethanol (ETH), benzene (B), chloroform (TCM), and triethylamine (TEA). Second, with the use of carbon and bromine isotopic analysis, we explored possible differences in the direction of measured and predicted isotope effects. Finally, with insight coming from a computational study we made an attempt to investigate possible origins of observed differences. To this end, the path integral formalism of quantum chemistry, as well as ONIOM scheme and QM cluster calculations, were used.[1] We also performed an energy decomposition analysis within the symmetry-adapted perturbation theory (SAPT) to comprehend the nature of the intermolecular forces and check whether there is any correlation between the prevailing type of interaction and the VPIE magnitude.

[1] Vasquez, L.; Rostkowski, M.; Gelman, F.; Dybala-Defratyka, A.: Can PIMD Make a Good Approximation for Vapor Pressure Isotope Effects Prediction for Organic Solvents? A Comparison to ONIOM QM/MM and QM Cluster Calculation, J. Phys. Chem. B, 122 (2018) 7353, DOI: <u>10.1021/acs.jpcb.8b0344</u>

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## Theoretical predictions of enzymatic rate constants and kinetic isotope effects: from origins to applications

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Computational Chemistry techniques based on the combination of Quantum Chemistry and classical Molecular Mechanics (QM/MM) have been extensively applied to the study of enzyme catalysis. Merging these techniques with experimental methods has allowed to acquire a deep knowledge of the reaction mechanisms of these complex but highly efficient biocatalysts at molecular level.

We will focus in this communication on aspects such as the controversial debate on whether protein dynamics are linked to the chemical reaction step,<sup>1</sup> the role of the quantum tunelling and the electrostatic effects contributions to catalysis,<sup>2</sup> or the relevance of compression effects in enzymatic methyl transfer reactions.<sup>3</sup>

The magnitudes acquired from computational studies, such as rate constants and kinetic isotope effects (KIEs), can be compared with experiments. The results obtained for chemical reactions catalyzed by enzymes, considered as robust when experiments and theory match each other, can be exploited by industry since these gigant molecules are able to perform difficult synthetic reactions without the need of extreme temperatures, high pressures or toxic chemicals.<sup>4</sup> In addition, the detailed knowledge of enzymatic processes at molecular level can be the bedrock in the design of new drugs.<sup>5</sup>

Recent results obtained in our laboratory in these lines of research will be summarized in this communication.

#### References

- Luk, L.Y.P.; Ruiz-Pernía, J. J.; Dawson, W. M.; Roca, M.; Loveridge, E. J.; Glowacki, D. R.; Harvey, J. N.; Mulholland, A. J.; Tuñón, I.; Moliner, V.; Allemann R. K. Proc. Nat. Acad. Sci. USA. 2013, 110, 16344–16349.
- [2] Krzemińska, A.; Moliner, V.; Świderek, K. J. Am. Chem. Soc. 2016, 138, 16283–16298.
- [3] Świderek, K.; Tuñón, I.; Williams, I.H.; Moliner, V. J. Am. Chem. Soc. 2018, 140, 4327-4334.
- [4] (a) Świderek, K.; Tuñón, I.; Moliner, V.; Bertran, J. Arch. Biochem. Biophys. 2015, 582, 68–79. (b)
  Nödling, A.R.; Świderek, K.; Castillo, R.; Hall, J.W.; Angelastro, A.; Morrill, L.C.; Jin, Y.; Tsai, Y.; Moliner, V.; Luk L.Y.P. Angew. Chem. Int. Ed. 2018, 57, 12478–12482. (c) De Raffele, D.; Martí, S.; Moliner, V. ACS Catal. 2019, 9, 2482–2492.
- [5] (a) Kholodar, S. A.; Ghosha, A. K.; Świderek, K.; Moliner, V.; Kohen, A. Proc. Natl. Acad. Sci. USA. 2018, 115, 10311–10314. (b) Ren, R.; et al. Nature Commun. 2018, 9, Article number: 3243. (c) Serrano-Aparicio, N.; Świderek, K.; Moliner, V. Eur. J. Med. Chem. 2019, 164, 399–407.

### What are the signatures of tunnelling in enzyme-catalysed reactions?

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While it is well established that thermally-activated quantum mechanical tunnelling of light particles (electrons and light atoms, typically hydrogen) plays a role in many enzyme-catalysed reactions (1), there are few definitive experimental signatures of atomic (e.g. hydrogen) tunnelling and no clear method of estimating the relative tunnelling contribution from typical experimental data. As most enzyme reactions involve the binding/capture of freely diffusing substrate(s), reactions are typically initiated by mixing and experimental conditions must then be compatible with liquid water (the solvent). This precludes the classic test of tunnelling: the observation of temperature-independent rate constants at cryogenic temperature. Instead, H-tunnelling is usually inferred from kinetic isotope effects (KIEs) that are larger than the semiclassical limit. Often the temperature dependence of the reaction is also measured over the experimentally accessible range (~ 278 - 313 K for mesophilic enzymes) and these data analysed using variations of Arrhenius, Eyring or Marcus theory. The apparent Arrhenius and Eyring activation parameters allow some quantitative comparison of different reactions, but do not directly provide any information about tunnelling, while the validity of parameters derived from non-adiabatic models such as Marcus theory are questionable due to the partially adiabatic nature of these reactions (2). Here, we use the correlation found between apparent activation enthalpy and entropy terms observed across series of enzyme variants (e.g. 3,4) and tunnelling contributions determined using computational chemistry (e.g. 5) to begin to define new signatures of hydrogen tunnelling, which can be used to interpret typical experimental kinetic data measured for enzyme-catalysed reactions.

#### References

- (1) Hay S, Scrutton NS. (2012) Good vibrations in enzyme-catalysed reactions. Nat Chem. 4, 161-8
- (2) Hay S, Johannissen LO, Sutcliffe MJ, Scrutton NS. (2010) Barrier compression and its contribution to both classical and quantum mechanical aspects of enzyme catalysis. Biophys J. 98, 121-8.
- (3) Longbotham JE, Hardman SJ, Görlich S, Scrutton NS, Hay S. (2016) Untangling Heavy Protein and Cofactor Isotope Effects on Enzyme-Catalyzed Hydride Transfer. J. Am. Chem. Soc., 138, 13693–9
- (4) Geddes A, Paul CE, Hay S, Hollmann F, Scrutton NS. (2016) Donor-Acceptor Distance Sampling Enhances the Performance of «Better than Nature» Nicotinamide Coenzyme Biomimetics. J Am Chem Soc. 138, 11089-92
- (5) Delgado M, Görlich S, Longbotham JE, Scrutton NS, Hay S, Moliner V, Tuñón I (2017) Convergence of Theory and Experiment on the Role of Preorganization, Quantum Tunneling, and Enzyme Motions into Flavoenzyme-Catalyzed Hydride Transfer. ACS Catal., 7, 3190–8

## Temperature dependent kinetic isotope effects resolve the origins of proficient enzymatic C-H activation

#### Adam R. Offenbacher

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Enzyme catalyzed C-H activation is one of the most prevalent and fundamental reactions to occur in biological catalysis and their mechanistic understanding is sought to aid in bioinspired catalyst design. Native enzymes catalyzing such reactions are often observed with temperature independent kinetic isotope effects ( $\Delta E_a = E_a(D) - E_a(H) \cong 0$ ). The magnitude of this kinetic parameter provides a critical descriptor for the precision of the active site along the reaction coordinate. Introduction of perturbants, including (local or remote) site-directed mutations, pressure, and surface modifications, is often accompanied by increases in the magnitude of  $\Delta E_a$  (i.e. > 0) even in cases where the rate constants remain unaltered. Decades of accumulated experimental data could be rationalized from the emergence of a multi-dimensional, nonadiabatic quantum tunneling model, in which the  $\Delta E_a$  serves as a robust kinetic 'ruler' for an enzyme's effectiveness to achieve hydrogenic wavefunction overlap at the tunneling ready state.<sup>1</sup> In this talk, an overview of the theoretical model will be presented with references to semi-classic experimental data, mostly drawn from the kinetic and spectroscopic studies on the quintessential tunneling enzyme, soybean lipoxygenase. A more recent study using the  $\Delta E_a$  to provide kinetic resolution for the impact of surface glycosylations on tunneling efficiency for a lipoxygenase orthologue will be discussed.

#### Reference

[1] Klinman, J. P.; Offenbacher, A. R.: Understanding biological hydrogen transfer through the lens of temperature dependent kinetic isotope effects. *Acc. Chem. Res.* 2018, 1966-1974.

## The use of stable isotopes in ultrafast spectroscopy of enzymes and photoreceptors

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Stable isotopes can be used in many ways to probe biological reactions on ultrafast timescales, two such methods will be discussed here.

Firstly the isotope (e.g. <sup>2</sup>H, <sup>15</sup>N, <sup>13</sup>C) labeling of protein and co-factor structures has been used extensively as a tool to assign spectral features in the mid infra-red region of the spectrum, by exchanging elements for their heavier isotopes the frequency of their bond vibrations are subtly changed. The photoreactions of many photoreceptors have been investigated this way, using time-resolved infra-red (TRIR) spectroscopy.<sup>1,2</sup> Studies of the reductive half reaction of a model flavo-enzyme using various combinations of isotope labels have shown that both the flavin and the protein affect the reaction chemistry, but in different ways. In addition to the "heavy" flavin and protein slowing the reaction it is suggested that there is a mass-dependent vibronic coupling of protein and flavin motions to flavin electronically excited state(s).<sup>3</sup> In order to investigate the interactions between the flavin excited state and the surrounding protein environment TRIR measurements were performed, and due to the isotope-labeling of the protein and co-factor it was possible to observe the vibrational coupling between co-factor and protein.

Secondly, it is a well acknowledged fact that solvent (water) interactions often play a large part in the reactions of proteins, either by stabilizing reaction intermediates, or more directly as proton donor or acceptors. Phytochromes are bilin-containing photoreceptors that are typically sensitive to the red/far-red region of the visible spectrum. By performing time-resolved spectroscopy in the visible region spanning the complete time range of the photoreaction (ps-ms) in  $H_2O$  and  $D_2O$  buffers we were able to identify kinetic isotope effects associated with each of the individual steps of the photocycle.<sup>4</sup>

#### References

- Brust, R. *et al.* Proteins in Action: Femtosecond to Millisecond Structural Dynamics of a Photoactive Flavoprotein. J. Am. Chem. Soc. 2013, 135 (43), 16168–16174.
- [2] Iuliano, J. N. *et al.*. Variation in LOV Photoreceptor Activation Dynamics Probed by Time-Resolved Infrared Spectroscopy. Biochemistry 2018, 57 (5), 620–630.
- [3] Longbotham, J. E. *et al.*. Untangling Heavy Protein and Cofactor Isotope Effects on Enzyme-Catalyzed Hydride Transfer. J. Am. Chem. Soc. 2016, 138 (41), 13693–13699.
- [4] Choudry, U.; Heyes, D. J.; Hardman, S. J. O *et al.*, Photochemical Mechanism of an Atypical Algal Phytochrome. ChemBioChem 2018, 19 (10), 1036–1043.
## Mechanistic evaluation of glycoside hydrolases covalent inhibitors: enzymatic kinetic isotope effect and computational studies

Marco Farren-Dai<sup>1</sup>, Weiwu Ren<sup>1</sup>, Natalia Sannikova<sup>1</sup>, Oluwafemi Akintola<sup>1</sup>, Katarzyna Świderek<sup>2</sup>, Vicent Moliner<sup>2</sup>, Robert Britton<sup>1</sup> & Andrew Bennet<sup>1</sup>

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The study of glycoside hydrolase's (GH's) mechanism and reactivity has produced clinically relevant drugs and viable leads for the treatment of viral disease, lysosomal storage disorders and cancer. In this work, we computationally investigated the mechanism of covalent labelling by recently reported glycosidase covalent inhibitors (CIs) in order to hone the design of these compounds so as to target biologically important glycosidases.



**Fig. 1: a.** Double bond participation promotes leaving group departure **b**.Carbocation formed is stabilized by delocalization **c**. Nucleophilic capture by an active site residue **d**. Hydrolysis of enzyme-inactivator complex

The Bennet lab has recently developed allylic CIs that utilize the catalytic machinery to alkylate an active site residue, thus inactivating the enzyme. (1,2) These CIs alkylate GHs by stabilizing formation of the allylic carbocationic TSs formed by leaving group (LG) departure. Thus, mimicking the natural substrate's pyranosylium ion-like transition state (TS). The enzyme-inactivator covalent bond is eventually hydrolyzed, restoring activity (**Fig.1**).

This mechanism was investigated with an enzymatic kinetic isotope effect (KIE) experiment using <sup>13</sup>C, <sup>2</sup>H, and <sup>18</sup>O isotopologues of the covalent inhibitor, which were then used to confirm the findings of a computational analysis. Molecular dynamics simulations based on hybrid quantum mechanics/ molecular mechanics (QM/MM) potentials were utilized to thoroughly analyze the catalytic itinerary of the covalent inhibitor (**Fig. 1**). The free energy surfaces associated to every chemical step depicted in **Fig.1** allowed description of the structures of the different states (reactants, intermediates, transition states and products) involved in the inactivation of the enzyme the CIs. Ground state and transition state structure analysis allowed generation of theoretical KIEs that were compared to the experimental KIEs for validation. This study allows for determination of the structures and energy barriers along the catalytic iteinerary of the CI, providing insights which will aid in the design of more selective GH covalent inhibitors.

- Shamsi Kazem Abadi, S.; Tran, M.; Yadav, A. K.; Adabala, P. J. P.; Chakladar, S.; Bennet, A. J. "New Class of Glycoside Hydrolase Mechanism-Based Covalent Inhibitors: Glycosylation Transition State Conformations" J. Am. Chem. Soc. 139, 10625–10628 (2017).
- [2] Ren, W.; Pengelly, R.; Farren-Dai, M.; Shamsi Kazem Abadi, S.; Oehler, V.; Akintola, O.; Draper, J.; Meanwell, M.; Chakladar, S.; Świderek, K.; Moliner, V.; Britton, R.; Gloster, T. M.,; Bennet, A. J."Mechanistic Insight into Covalent Inhibition of Glycoside Hydrolases by Carbasugars: Design, Synthesis, and Structural and Computational Studies" Nat. Comm., 9:3243 (2018)

Oral Presentations: Wednesday, July 10

## Effect of metabolic constraints on the observable isotope fractionation during aerobic biodegradation of hexachlorocyclohexanes

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Compound-specific isotope analysis (CSIA) has become an important approach to study mechanisms of enzymatic transformations of organic pollutants as well as to assess both extents and pathways of biodegradation processes in contaminated environments. To obtain insights into the kinetics and isotope effects of enzymatic pollutant transformation, investigations typically focus on the initial reaction steps leading to organic products that may be channeled into common metabolic pathways. However, this approach precludes the identification of potentially confounding contributions of co-metabolic transformations of the substrate by enzymes expressed for the downstream metabolism of pollutant-degrading bacteria.

We studied the effect of such metabolic constraints on the observable substrate isotope fractionation for the aerobic biodegradation of hexachlorocyclohexane (HCH). Biodegradation HCH is initiated through two successive dehydrochlorination reactions catalyzed by variants of the lindane dehydrochlorinase (LinA). Subsequently, the haloalkane dehalogenase LinB is responsible for further hydrolytic dechlorinations of the tetrachlorocyclohexadiene intermediate to less chlorinated hexenols through nucleophilic substitution reactions. To that end, both enzymes are expressed constitutively in HCH-metabolizing bacteria.

While we have recently explored the magnitude of the apparent <sup>13</sup>C- and <sup>2</sup>H-kinetic isotope effects for the LinA and LinB-catalyzed transformations of  $(+)-\alpha$ -,  $(-)\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -HCH isomers, the impact of simultaneous and potentially competitive cometabolic transformation of HCH isomers is unexplored. Using  $\delta$ -HCH as a model pollutant, we developed enzyme assays for the study of the simultaneous reactions of HCH isomers with activity-based mixtures of LinA2 and LinB from *Sphingobium indicum* B90A at different LinA2:LinB mixtures. Our results illustrate that the simultaneous presence of the two enzymes indeed impacts the observable C and H isotope fractionation. These observations imply that the observable isotope fractionation associated with aerobic biodegradation of HCH could, in principle, reflect simultaneous dehydrochlorination and hydrolytic dechlorination and thus potentially limit the diagnostic power of CSIA.



# Using dual element isotope analysis ( $\delta^{13}$ C, $\delta^{37}$ Cl) to gain mechanistic insights into enzymatic tetrachloroethene degradation of three different reductive dehalogenases

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Reliable quantification of tetrachloroethene (PCE) biodegradation using compound-specific isotope analysis (CSIA) is hampered due to highly variable carbon isotope enrichment factors ( $\varepsilon_c$ : -0.4 ‰ to -19.0 ‰) (Nijenhuis et al., 2005, Cichocka et al., 2008, Cretnik et al., 2014). Variations of observable isotope fractionation (i.e.  $\varepsilon$ ) during a (bio)chemical reaction can result from differences in the underlying reaction mechanisms and/or masking effects by rate-limiting steps (Elsner, 2010). Analysis of dual element isotope fractionation allows to discern between both factors. While distinct reaction mechanisms are typically associated with different dual isotope slopes, masking effects show no element specific isotope fractionation and consequently do not affect dual isotope slopes (Elsner et al., 2005, Elsner, 2010).

To elucidate whether different reaction mechanisms of microbial PCE degrading enzymes (PCE-Rd-hAs) cause the reported variability of  $\varepsilon$ , we determined carbon and chlorine isotope fractionation of PCE of several *Desulfitobacterium* strains. Systematic selection of strains harboring a PCE-RdhA enzyme degrading PCE either to trichloroethene (RdhA<sub>TCE</sub>; n=2), 1,2-*cis*-dichloroethene (RdhA<sub>cDCE</sub>; n=2) or co-metabolically (RdhA<sub>co</sub>; n=1) allowed us to compare isotope enrichment factors and dual isotope slopes of three different PCE-RdhAs.

RdhA<sub>TCE</sub> exhibited pronounced isotope fractionation of -19.4 ‰ (avg.) for carbon and -5.7 ‰ (avg.) for chlorine, whereas isotope fractionation of RdhA<sub>cDCE</sub> was significantly less pronounced with -5.4 ‰ (avg.) and -2.1 ‰ (avg.) for carbon and chlorine, respectively. Co-metabolic degradation showed intermediate isotope fractionation ( $\varepsilon_{\rm C}$ : -9.8 ‰;  $\varepsilon_{\rm C}$ : -3.4 ‰) compared to the range given by Rd-hA<sub>TCE</sub> and RdhA<sub>cDCE</sub>. Dual isotope slopes were similar for all tested strains and ranged between 2.4 ± 0.3 to 3.7 ± 0.2 suggesting that PCE degradation proceeds via the same reaction mechanism for the tested PCE-RdhA enzymes. Consequently, observed variations of  $\varepsilon$  presumably arise from masking effects. As cell envelope properties (cell wall



structure, localization of RdhA) differ also for tested strains with identical RdhAs, we exclude distinct magnitudes of masking by PCE uptake limitations through cell walls and cytoplasmic membranes to cause the determined variability of  $\varepsilon$  as previously reported (Renpenning et al., 2015). Therefore, observed variations of  $\varepsilon$  for the distinct types of RdhA arise from differences in rate-limiting steps within the enzymatic multistep reactions, such as diffusion in the enzymatic substrate channel or enzyme-PCE association. Thus, our study provides evidence for an identical reaction mechanism, but differences in enzymatic reaction kinetics that cause varying magnitudes of  $\varepsilon$  during PCE degradation.

#### References

Nijenhuis I., Andert J., Beck K., Kästner M., Diekert G., Richnow H.H., 2005. Stable IsotopeFractionation ofTetrachloroethene during Reductive Dechlorination by Sulfurospirillummultivorans and Desulfitobacteriumsp. Strain PCE-S and Abiotic Reactions withCyanocobalamin. Appl. Environ. Microbiol. 71(7), 3413-3419.

- Cichocka D., Imfeld G., Richnow H.H., Nijenhuis I., 2008. Variability in microbial carbon isotope fractionation of tetra- and trichloroethene upon reductive dechlorination. Chemosphere , 71(4), 639-648.
- Cretnik S., Bernstein A., Shouakar-Stash O., Löffler F., Elsner M., 2014. Chlorine Isotope Effects from Isotope Ratio Mass Spectrometry Suggest Intramolecular C-Cl Bond Competition in Trichloroethene (TCE) Reductive Dehalogenation. Molecules 19, 6450-6473.
- Elsner M., 2010. Stable isotope fractionation to investigate natural transformation mechanisms of organic contaminants: principles, prospects and limitations. J. Environ. Monit. 12(11), 2005-2031.
- Elsner M., Zwank L., Hunkeler D., Schwarzenbach R.P., 2005. A New Concept Linking Observable Stable Isotope Fractionation to Transformation Pathways of Organic Pollutants. Environ. Sci. Technol. 39(18), 6896-6916.
- Renpenning J., Rapp I., Nijenhuis I., 2015. Substrate Hydrophobicity and Cell Composition Influence the Extent of Rate Limitation and Masking of Isotope Fractionation during Microbial Reductive Dehalogenation of Chlorinated Ethenes. Environ. Sci. Technol. 49(7), 4293-4301.

## Mechanistic and enzymatic insights in reductive dehalogenation by Dehalococcoides mccartyi strain BTF08

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*Dehalococcoides mccartyi* strain BTF08 has the unique property to couple dechlorination of tetrachloroethene and vicinally halogenated alkanes to ethene with growth by using them as terminal electron acceptors. The genome of strain BTF08 encodes 20 genes for reductive dehalogenases (RdhA) including those described to dehalogenate tetrachloroethene (PceA), trichloroethene (TceA) and vinyl chloride (VcrA)<sup>1</sup>. However, thus far it is unknown which RdhAs are expressed *in vivo*, their substrate specificity and the reaction mechanisms catalyzed by these corrinoid-dependent enzymes. Previously, competing reaction mechanisms were observed for trichloroethene during dechlorination with vitamin  $B_{12}^{-2}$ . Additionally, computational modeling predicted distinct reaction mechanisms for higher vs. lower chlorinated ethenes<sup>3</sup>. As the reaction mechanism may depend both on enzyme structure and chlorinated substrate, we investigated the dual-element (C/Cl) compound-specific stable isotope fractionation patterns during dechlorination by cells with defined RdhA inventories. We determined the expression pattern of RdhAs during cultivation with different electron acceptors and the enzymatic activity of specifically induced cells to correlate expressed proteins with enzymatic activity and inferred reaction mechanism.

During dechlorination of chlorinated ethenes a novel PCE-RdhA, different from the formerly identified PceA and VcrA was detected, whereas TceA of *D. mccartyi* strain BTF08 was expressed only when cultivated on 1,2-dichloroethane. Independent of the enzyme inventory, carbon and chlorine compound-specific stable isotope analysis suggested two distinct reaction mechanisms for the dechlorination of (i) *cis*-dichloroethene and vinyl-chloride and (ii) tetrachloroethene. Furthermore, two patterns could be observed for 1,2-dichloroethane, during dehaloelimination by cells cultivated with different electron acceptors and distinct RdhA inventory. In summary, we could link specifically expressed RdhA proteins with a biochemical function and provide an insight into the apparent enzyme and substrate-specific reaction mechanisms by *D. mccartyi* strain BTF08.

- 1. Pöritz, M. et al. Genome sequences of two dehalogenation specialists *Dehalococcoides mccartyi* strains BTF08 and DCMB5 enriched from the highly polluted Bitterfeld region. *FEMS Microbiol Lett* **2013**, *343*, (2), 101-104.
- 2. Heckel et al., Chlorinated Ethene Reactivity with Vitamin B-12 Is Governed by Cobalamin Chloroethylcarbanions as Crossroads of Competing Pathways. *Acs Catal* **2018**, *8*, (4), 3054-3066.
- Ji, L. et al, Mechanism of Cobalamin-Mediated Reductive Dehalogenation of Chloroethylenes. *Acs Catal* 2017, *7*, (8), 5294-5307.

### Biological and abiotic natural attenuation of 1,2-Dibromoethane (EDB) at Kirtland AFB, USA

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This presentation describes the results from an application of carbon compound-specific isotope analysis (CSIA) to evaluate 1,2-dibromoethane (EDB) attenuation at Kirtland AFB, New Mexico, USA. Historical accidental fuel releases at the site included aviation gasoline that contained EDB, resulting in a large plume of over 1.5 km length, ~150 m below ground surface (Figure 1) exceeding a USEPA regulatory concentration of 0.05  $\mu$ g/l. The site has undergone extensive monitoring and remediation efforts. For this project, samples of groundwater were collected and analyzed for C isotope ratios in

EDB. The analysis posed significant challenges due to low µg/l concentrations of EDB combined with high mg/l concentrations of total VOCs at several locations. CSIA required EDB extraction from large volumes of water (up to 1 liter) to recover enough mass of EDB for analysis (achieved by using a custom closedloop purge and trap apparatus developed at the University of Oklahoma) and chromatographic separation of EDB from the high quantities of non-target VOCs by orthogonal two-dimensional GC. The results from field samples were evaluated to determine baseline conditions prior to the initiation of engineered remediation. Large enrichments of <sup>13</sup>C for EDB in groundwater samples were observed throughout the site, rela-



tive to the typical <sup>13</sup>C-depleted values of manufactured EDB (Figure 1) providing clear evidence of past EDB degradation. To identify the mechanisms of degradation, a simple model was developed, to predict the extent of <sup>13</sup>C enrichment for various processes, with their attendant enrichment factors. The presence of abiotic hydrolysis of EDB can be always taken for granted and the rates of the process can be estimated, based on groundwater temperature and pH (Koster van Groos et al. 2018). The isotope ratios of EDB measured in the samples collected from the distal half of the plume are consistent with a hydrolysis, suggesting that this mechanism is the dominant process controlling EDB attenuation in this area, where EDB concentrations are low and the groundwater is aerobic. On the other hand, a shift in observed isotope fractionation closer to the source area was inconsistent with that predicted by hydrolysis of EDB alone. The observed results near the source area matched a degradation process with enrichment factor of approximately -8 per mil, a value consistent with laboratory results for biological anaerobic dihaloelimination by three different cultures analyzed previously (Koster van Groos et al. 2018). The results from CSIA are generally consistent with the extent of biodegradation predicted by the concentrations of putative degradation products (bromine, ethene). In summary, CSIA permitted identification of the area where biodegradation (as opposed to abiotic hy-

drolysis) appears to be the dominant process of EDB attenuation. A follow up active bioremediation/ biostimulation effort is underway at the same area, with CSIA monitoring the efficacy of the process.

#### References

[1] Koster van Groos. P. G.; Hatzinger, P.B.; Streger, S.H.; Vainberg, S.; Philp, R.P.; Kuder, T.: Carbon Isotope Fractionation of 1,2-Dibromoethane by Biological and Abiotic Processes. Environmental Science & Technology. 2018, 52, 3440-3448.

## Applying stable isotope analysis on increasing scales – microcosm, field, and multi-field-sites studies

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In the last two decades, Compound-Specific Isotope Analysis (CSIA) became an accepted tool for studying degradation processes of organic pollutants in affected environments. Research in varying scales provide broad knowledge on degradation processes, from revealing the degradation mechanism at the enzymatic scale, up to characterizing natural attenuation processes at the field scale. Extending research to multiple sites at a regional scale may provide additional (and possibly more universal) knowledge on environmental processes, yet is practically more complicated and not as commonly performed.

My talk will start with reviewing some of my recent work on the microcosm and field scales. **On the microcosm scale**, I will show isotope fractionation results determined for microbial oxidation of halogenated phenols, as well as for the degradation of the brominated herbicide bromoxynil. **On the field scale**, I will present isotope results for different halogenated groundwater contaminants in a site which is characterized by a large number of brominated and chlorinated organic pollutants (the Neot Hovav site).

The second part of my talk will concentrate on a recent multiple-sites field study of trichloroethylene (TCE). The aim of this study was to evaluate the importance of aerobic co-metabolic degradation of TCE in contaminated groundwater. Microbial degradation of TCE may follow two pathways, where the most commonly detected is anaerobic reductive dechlorination. This first process leads to observable indicative intermediates, providing clear evidence for the occurrence of the process. Aerobic degradation of TCE is a second process to be considered. This process is promoted co-metabolically as a result of auxiliary substrates oxidation (such as oxidation of methane, propane, ammonium, etc.). As this process does not result in the accumulation of easily detectable metabolites, our knowledge on its significance in contaminated sites often remains vague. To evaluate its importance, we first show that isotope analysis of  $\delta^{13}$ C vs.  $\delta^{37}$ Cl in TCE may potentially (yet not always conclusively) assist in pinpointing the occurrence of this process. Based on that, the research question was tackled by a combination of microcosm, molecular, and isotope ( $\delta^{13}C/\delta^{37}Cl$ ) data collected from  $\approx 100$  contaminated wells along the Israeli Coastal Aquifer. The research identified a wide microbial potential for co-metabolic degradation of TCE throughout the aquifer, as indicated by significant degradation in groundwater microcosms, as well as by functional gene abundance in groundwater. Nevertheless, no unambiguous isotope proof was obtained for the actual occurance of co-metabolic TCE oxidation in groundwater.

### Tracking hydrogenase activity with hydrogen stable isotopes

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A hydrogen-based renewable power system requires storage of hydrogen  $(H_2)$  in underground reservoirs. The potential oxidation of  $H_2$  in such reservoirs by lithoautotrophic microorganisms is an important but less studied aspect. Our long-term goal is to develop a stable isotope tool based on changes in the  $\delta^2$ H signature of  $H_2$  during oxidation by hydrogenases to detect and monitor potential biological losses during underground storage.

Most microorganisms are equipped with at least one hydrogenase. Hydrogenases are a diverse class of enzymes catalyzing the reversible cleavage of molecular  $H_2$  into two electrons and two protons. During  $H_2$ -consumption, a subsequent superimposing isotope exchange with water takes place quasi-simultaneously, resulting in an inverse isotope effect (Vignais and Billoud 2007).

We questioned whether the exchange reaction can occur independently from the  $H_2$  cleavage reaction. For this,  $H_2$  oxidation of cell suspensions of the sulphate-reducing model organism *Desulfovibrio vulgaris* Miyazaki was inhibited by the addition of sodium molybdate, circumventing electron flow to the final electron acceptor sulphate (Peck 1959; Wolin and Miller 1980; Biswas et al. 2009). Although  $H_2$  was not consumed,  $\delta^2 H$  values changed to isotope equilibrium with water, demonstrating that the exchange reaction still proceeds in the absence of the  $H_2$  cleavage reaction.

Our study shows that the isotope exchange is directly linked to the enzyme's activity and can be tracked with a GC-IRMS setup with a precision of  $\delta^2 H = 0.7 \pm 0.4$  ‰.

- [1] Vignais, P. M., and B. Billoud. 2007. ,Occurrence, classification, and biological function of hydrogenases: an overview', *Chemical Reviews*, 107: 4206-72.
- [2] Peck, Harry D. 1959. ,The ATP-dependent reduction of sulfate with hydrogen in extracts of Desulfovibrio desulfuricans', *Proceedings of the National Academy of Sciences*, 45: 701-08.
- [3] Wolin, MJ, and Terry L Miller. 1980. ,Molybdate and sulfide inhibit H2 and increase formate production from glucose by Ruminococcus albus', *Archives of Microbiology*, 124: 137-42.
- [4] Biswas, Keka C., Nicole A. Woodards, Huifang Xu, and Larry L. Barton. 2009. ,Reduction of molybdate by sulfate-reducing bacteria', *BioMetals*, 22: 131-39.

## Impact of oxygen activation on substrate isotope effects of enzymatic oxygenations

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Enzymatic oxygenations are among the most important reactions that initiate biodegradation and detoxification of organic pollutants in contaminated environments. With compound-specific isotope analysis (CSIA), the extent of transformation can be monitored based on a thorough understanding of the kinetic isotope effects (KIEs) for this type of reaction. However, the overall rate of oxygenation is often governed by the enzymatic activation of molecular  $O_2$ . Unfortunately, little is known about the contribution of enzymatic  $O_2$  activation to the rate of oxidative pollutant removal and its implications for the application of CSIA. Recent results showed that oxygenations by flavin-dependent monooxygenases only lead to measurable substrate isotope fractionation in enzymes activating  $O_2$  prior to substrate binding, whereas  $O_2$  activation upon substrate binding masked the isotope effect of subsequent hydroxylations [1]. Although Rieske non-heme iron dioxygenases (RDOs) belong to the latter group, distinct substrate-dependent variations in carbon isotope fractionation were observed. This variability for the same type of reaction suggests substrate-specific alterations in enzyme kinetics and may compromise the application of CSIA for monitoring oxygenation reactions.

The goal of our work was therefore to understand the contribution of O<sub>2</sub> activation to the rate of oxidative biodegradation in RDOs and to elucidate the enzyme-substrate specific roles of O<sub>2</sub> activation on contaminant isotope fractionation. To this end, we investigated the dioxygenation of a series of nitroaromatic compounds in laboratory model systems of two closely related RDOs, namely nitrobenzenedioxygenase and 2-nitrotoluenedioxygenase. We analyzed the kinetics, <sup>13</sup>C-, and <sup>2</sup>H-apparent kinetic isotope effects of organic substrates and products, quantified the stoichiometries of dissolved O<sub>2</sub> consumption, and derived <sup>18</sup>O-KIEs as described in Pati et al. [2]. We observed similar oxygen isotope fractionations suggesting that O<sub>2</sub> activation was rate-limiting regardless of substrate. However, the efficiency of oxygenation was substrate-specific and varied between 0% and 70% of the available O2, whereas the remainder was released as reactive oxygen species. The extent of this uncoupling reaction is correlated with the extent of isotope fractionation measured in the organic substrate. We hypothesize that oxygen uncoupling allows the detection of substrate isotope effects after the rate-determining O<sub>2</sub> activation by the release of the unreacted substrate and H<sub>2</sub>O<sub>2</sub> from the activated enzyme-substrate complex. Our findings provide new insights into the catalytic mechanism of aromatic dioxygenations and the application of CSIA to monitor biodegradation reactions initiated through oxygenations.

- Wijker, R. S., Pati, S. G., Zeyer, J. & Hofstetter, T. B. Enzyme Kinetics of Different Types of Flavin-Dependent Monooxygenases Determine the Observable Contaminant Stable Isotope Fractionation. *Environ. Sci. Technol. Lett.* 2, 329–334 (2015).
- [2] Pati, S. G. *et al.* Measurement of oxygen isotope ratios (180/160) of aqueous O2 in small samples by gas chromatography/isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry* **30**, 684–690 (2016).

Oral Presentations: Thursday, July 11

## Measurements of doubly substituted methane isotopologues (<sup>13</sup>CH<sub>3</sub>D and <sup>12</sup>CH<sub>2</sub>D<sub>2</sub>) using mid-infrared spectroscopy

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Methane is a significant greenhouse gas and energy resource, which generates about a quarter of electricity in the world today. It is derived from diverse sources (e.g., microbial, thermogenic or abiotic), yet differentiation between sources remains a major challenge. While, carbon ( $^{13}C/^{12}C$ ) and hydrogen (D/H) isotope ratios can tell the origin of carbon and hydrogen, abundance of doubly-isotope substituted "clumped" methane isotopologues,  $^{13}CH_3D$  and  $^{12}CH_2D_2$  may inform us the origin of C-H bonds.

We will discuss recent development of high precision analysis of doubly substituted methane isotopologues using tunable infrared laser direct absorption spectroscopy (TILDAS) instruments, as well as new insights and constraints we gained from these novel measurements. In addition to <sup>13</sup>CH<sub>3</sub>D (Ono et al., 2014), we have recently developed a TILDAS instrument to measure doubly deuterated methane ( $^{12}CH_2D_2$ ) for natural samples. We will discuss major challenges to identify spectral regions, the design of an inlet system that allows repeated measurements to improve precision, and future opportunities using the instrument.

Our research, along with others carried out by using high-resolution isotope ratio mass spectrometery instruments, has demonstrated a wealth of additional information that can be inferred from clumped isotopologues of methane (2). For example, apparent <sup>13</sup>CH<sub>3</sub>D equilibrium temperatures can clearly differentiate microbial versus thermogenic methane in marine sedimentary environments, suggesting methane formation under near-equilibrium conditions in deep subsurface environments. We also find that methane samples from sites supporting active microbial methanogenesis (such as wetlands and cow rumens) and laboratory methanogen cultures carry highly depleted <sup>13</sup>CH<sub>3</sub>D abundance that implicates the contribution of quantum mechanical tunneling (3). These unique signals can be used to trace sources of methane in a number of environmental and geologic settings as well as to investigate the mechanism of methane generation.

- [1] Ono, S., Wang, D.T., Gruen, D.S., Sherwood Lollar, B., Zahniser, M.S., McManus, B.J. and Nelson, D.D., 2014. Measurement of a doubly substituted methane isotopologue, <sup>13</sup>CH<sub>3</sub>D, by tunable infrared laser direct absorption spectroscopy. *Analytical Chemistry*, 86, 6487-6494.
- [2] Wang, D.T., Gruen, D.S., Lollar, B.S., Hinrichs, K.-U., Stewart, L.C., Holden, J.F., Hristov, A.N., Pohlman, J.W., Morrill, P.L., Könneke, M., Delwiche, K.B., Reeves, E.P., Sutcliffe, C.N., Ritter, D.J., Seewald, J.S., McIntosh, J.C., Hemond, H.F., Kubo, M.D., Cardace, D., Hoehler, T.M. and Ono, S. (2015) Nonequilibrium clumped isotope signals in microbial methane. *Science* 348, 428-431.
- [3] Gruen, D.S., Wang, D.T., Könneke, M., Topçuoğlu, B.D., Stewart, L.C., Goldhammer, T., Holden, J.F., Hinrichs, K.-U. and Ono, S. (2018) Experimental investigation on the controls of clumped isotopologue and hydrogen isotope ratios in microbial methane. *Geochimica et Cosmochimica Acta* 237, 339-356.

## Measuring the temperature of geological processes with carbonate clumped isotopes

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Carbonate clumped isotope thermometry, which is based on the measurement of the abundance of 13C-18O bonds in carbonate minerals, is a very promising tool for many applications in geosciences ranging from paleoceanography to the study of diagenesis and tectonics. The application of this tool in many fields of research, however, has been limited by the large sample sizes (15-30 mg) required by traditional measurement methods, the complex analytical methodology and contrasting published temperature calibrations which add considerable uncertainty in temperature reconstructions. With the latest improvements in small sample analysis [1] it is now possible to analyze samples of 1-1.5 mg of carbonate with errors of less than  $\pm 3^{\circ}$ C (at the 95% CI). With the introduction of a carbonate-based standardization scheme, in addition, differences in temperature calibrations across different laboratories are being resolved [2].

We will present new unpublished and published temperature calibrations showing that clumped isotopes in calcite, siderite and dolomite all have the same temperature dependence, but that individual calibrations are necessary due to the different phosphoric acid fractionation factors. These improvements in analytical methods and interlaboratory consistency is improving the applicability of this tool to many fields, such as palaeoceanography using foraminifera and reconstruction of terrestrial temperatures from paleosol carbonates. In addition, in combination with U/Pb dating clumped isotopes in carbonates can provide new insights on the burial history and tectonics in mountain belts.

- [1] Müller, I.A., Fernandez, A., Radke, J., van Dijk, J., Bowen, D., Schwieters, J., Bernasconi, S.M. Carbonate clumped isotope analyses with the long-integration dual-inlet (LIDI) workflow: scratching at the lower sample weight boundaries. Rapid Communications in Mass Spectrometry. 31, 1057-1066. 2017.
- [2] Bernasconi S.M., Müller I. A., Bergmann K.D., Breitenbach S. F. M., Fernandez, A., Hodell D. A., Jaggi M., Meckler A.N. Millan I., and Ziegler M. (2018) Reducing uncertainties in carbonate clumped isotope analysis through consistent carbonate-based standardization. Geochemistry Geophysics Geosystems 19, 2018. https://doi. org/10.1029/2017GC007385

## New reference frame for clumped N<sub>2</sub>O isotopic analysis

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The isotopic composition of nitrous oxide ( $N_2O$ ) can be used to differentiate between various biotic and abiotic sources and sinks of this greenhouse gas. Due to the high complexity of the involved processes, however, source attribution is still equivocal. Measuring doubly substituted isotopocules of nitrous oxide (so called "clumped isotopes") adds new opportunities to fingerprint and further constrain the  $N_2O$  cycle.

Our research target in this challenging topic is twofold. First, we developed an analytical technique for selective and precise analysis of clumped isotopic species <sup>14</sup>N<sup>15</sup>N<sup>18</sup>O, <sup>15</sup>N<sup>14</sup>N<sup>18</sup>O, and <sup>15</sup>N<sup>15</sup>N<sup>16</sup>O based on quantum cascade laser absorption spectroscopy. Using this approach, a precision of 0.1–0.2 ‰ for  $\delta$ 458,  $\delta$ 548 and  $\delta$ 556 with 2–3 min spectral averaging has been achieved on samples of 4 µmol of N<sub>2</sub>O in N<sub>2</sub> at 4 hPa.

Second, as no reference gases are available, we established a reference frame using a combination of two independent approaches. On one side, clumped  $N_2O$  measurements are linked to a stochastic distribution, equilibrating the N–O bond by heating  $N_2O$  over activated  $Al_2O_3$  at two different temperatures (100 and 200 °C). Alternatively, we exploit the potential of laser spectroscopy for analysis of individual isotopologue concentrations by using a set of gravimetric  $N_2O$ -in- $N_2$  gas mixtures. This latter approach is particularly valuable for clumped molecules with no known procedure for equilibration (e.g. the N–N bond in  $N_2O$  in case of  $^{15}N^{15}N^{16}O$ ).

We demonstrate that the developed QCLAS technique is a very promising complementary tool to high-resolution mass spectrometry<sup>1</sup> regarding ease-of-use, sample throughput, precision, and its inherent selectivity for the clumped isotopomers <sup>14</sup>N<sup>15</sup>N<sup>18</sup>O and <sup>15</sup>N<sup>14</sup>N<sup>18</sup>O. This technique has the potential to open the path to a broad range of prospective applications from the biogeochemical N<sub>2</sub>O cycle to stratospheric chemistry or industrial catalytic processes.

<sup>[1]</sup> Magyar, P. M., V. J. Orphan, J. M. Eiler (2016). Measurement of rare isotopologues of nitrous oxide by high-resolution multi-collector mass spectrometry. Rapid Communications in Mass Spectrometry, v. 30, p. 1923-1940.

## 3D (<sup>13</sup>C, <sup>2</sup>H, <sup>37</sup>Cl) isotope analysis for HCHs transformation by Cobalamin

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Hexachlorocyclohexanes (HCHs) were widely used until 1990s which caused heavy contamination due to the toxicity and persistent. Reductive dehalogenation, one of the most important HCHs transformation process, shows the potential for remediation of HCHs contaminated field site (Liu et al., 2017). However, the reaction mechanisms of HCHs reductive dehalogenation still need further investigation. Compound-specific stable isotope analysis (CSIA) potentially can be used for investigating the transformation mechanisms of organic compounds. In this study, experiments for the reductive dehalogenation of four HCH isomers ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) by cobalamin, the transition-metal coenzyme of several anaerobic bacteria, were conducted as a model study. The HCH isomers were chosen because of different amount of equatorial and axial chlorine substituents at the cyclohexane ring which govern the stability of HCH isomers (Beurskens et al., 1991).

The isotope composition of HCHs as well as the products (benzene and MCB) were measured by GC-IRMS (for carbon), GC-Cr/HTC-IRMS (for hydrogen) and GC-MC-ICPMS (for chlorine) (Renpenning et al., 2018). The isotope enrichment factors ( $\varepsilon_{\rm C}$ ,  $\varepsilon_{\rm H}$ ,  $\varepsilon_{\rm Cl}$ ) of HCHs for reductive dehalogenation were obtained and compared to those of hydrolysis and electron transfer reaction catalyzed by Fe(0). The reaction mechanisms were discussed based on the evaluation of AKIE<sub>C/H/Cl</sub> in different scenarios (confirmed by the position of chlorine atoms). Different electron transfer pathways were also discussed for the investigation of the reaction mechanisms. The multi-element isotope enrichment factors will provide mechanistic information of degradation of HCH isomers. Particularly the comparison of fractionation factors in abiotic reactions, such as hydrolysis, electron transfer by Fe(0) reduction and cobalamin catalyzed dehalogenation, with biodegradation reactions provided a new perspective for analyzing the fate of HCH isomers.

- [1] Beurskens, J. E.; Stams, A. J.; Zehnder, A. J.; Bachmann, A., Relative biochemical reactivity of three hexachlorocyclohexane isomers. Ecotoxicology and Environmental Safety 1991, 21, (2), 128-136.
- [2] Liu, Y., Bashir, S., Stollberg, R., Trabitzsch, R., Weiß, H., Paschke, H., Richnow, H. H. Compound specific and enantioselective stable isotope analysis as tools to monitor transformation of hexachlorocyclohexane (HCH) in a complex aquifer system. Environmental Science & Technology 2015, 51(16), 8909-8916.
- [3] Renpenning, J., Horst, A., Schmidt, M., & Gehre, M. Online isotope analysis of <sup>37</sup>Cl/<sup>35</sup>Cl universally applied for semi-volatile organic compounds using GC-MC-ICPMS. Journal of Analytical Atomic Spectrometry 2018, 33(2), 314-321.

## <sup>2</sup>H, <sup>13</sup>C and <sup>15</sup>N isotopomers titration and profiling: the use of Nuclear Magnetic Resonance spectrometry (NMR) for Position-Specific Isotope Analysis (PSIA)

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Position-Specific Isotope Analysis (PSIA) consists in the determination of the isotope content for each site of a given molecule i.e. each isotopomer of the heavy element is quantified. This is not the case when using irm-MS (isotope ratio measured by mass spectrometry, known also as IRMS), for which the molecule is converted into a single gas that will be introduced in the source of the mass

spectrometer (CO<sub>2</sub>, as example for  ${}^{13}C/{}^{12}C$ ratio). As a result, irm-MS measures the amount of heavy isotopologues versus the light one. While PSIA generates as many data as the number of isotopomers  $(d^{13}C_i)$ , for <sup>13</sup>C), irm-MS leads to one parameter only: the global (bulk) isotope composition  $(d^{13}C_{\rho})$ . The intramolecular heavy isotope fractionation should give a better picture of the process (physical, chemical, biochemical or physiological) under investigation.

Three methods are currently available for PSIA. The oldest is the chemical and/or  ${}^{13}C$  profiles in glucose obtained by NMR for C<sub>3</sub>, C<sub>4</sub> and CAM enzymatic degradation of the molecule, plants (Gilbert et al., 2012) with subsequent analysis of the resulting



fragments by irm-MS. From the  $\delta^{13}$ C of the fragments, the complete isotopic distribution can be rebuilt, assuming that all steps are quantitative or that their specific associated fractionation is known. Alternatively fragmentation is done by pyrolysis, fragmenting molecules in a ceramic oven coupled on-line to irm-MS. While this is effective for small molecules (up to 4 carbones), it is not applicable directly to larger compounds.

In contrast, isotopic quantitative NMR (irm-NMR) gives access to all spectrally-resolved sites in the compound, i.e. position-specific measurements. Historically, the measurement of position-specific <sup>2</sup>H/<sup>1</sup>H ratios (quantification of each <sup>2</sup>H isotopomer) was the first and it had been exploited for the detection of forbidden chaptalization of wine and to authenticate natural aromas (SNIF-NMR) (Jamin & Thomas, 2017). The <sup>13</sup>C equivalent methodology (irm-<sup>13</sup>C NMR) has been established only recently. The main difficulty of irm-13C NMR is meeting the requirement for a high level of precision: of the order of 1 ‰ (Jézéquel et al., 2017)! Examples on targeted molecules (mainly ethanol, vanillin) will be used to demonstrate that <sup>13</sup>C isotopic profiles, by giving access to a larger number of parameters, offer a new tool for forensic and environmental inverstigations. Furthermore, by taking advantage of recent developments in NMR, sensitivity and resolution are improved allowing the study of smaller amounts of product. In fact, a parralel between <sup>2</sup>H and <sup>13</sup>C NMR constraints will be done in terms of the amount of samples and the analysis duration.

Recent methodologies and new approaches will be also presented. For the first time intramolecular <sup>15</sup>N profiles were exploited for characterizing position-specific <sup>15</sup>N fractionation and for forensic investigations (Joubert, 2019). Furthermore, from the intrinsic properties of NMR: structure elucidation and quantification at high precision, information on both the metabolome (metabolomics) and on the isotopome (isotopomics) can be collected during the same experiment as demonstrated on olive oils (Merchak et al., 2017).

#### References

Gilbert, A.; Robins, R.J.; Remaud, G.S.; Tcherkez, G.G.B. Proc. Natl. Acad. Sci. U. S. A. 2012, 109, 18204–18209.

Jamin, E.; Thomas, F. SNIF-NMR Applications in an Economic Context: Fraud Detection in Food Products. In *Modern Magnetic Resonance*; Webb, G., Ed.; Springer, Cham, 2017.

Jézéquel, T.; Joubert, V.; Giraudeau, P.; Remaud, G.S.; Akoka, S. Magn. Reson. Chem. 2017, 55, 77-90.

Joubert V. PhD dissertation, November 30th, 2018, University of Nantes, France.

Merchak, N.; Silvestre, V.; Loquet, D.; Rizk, T.; Akoka, S.; Bejjani, J. Anal. Bioanal. Chem. 2017, 409, 307-315.

## Analysis of phenolics by gas-chromatography – isotope ratio massspectrometry (GC-IRMS): method development and application

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Phenolics are natural compounds but also widely used in industrial processes. Due to their general toxicity and high water solubility phenolics are harmful groundwater contaminants; understanding their degradation pathways is essential for a proper water risk assessment.

A recently developed technique to take insight into reaction mechanisms is compound-specific stable isotope analysis (CSIA). In previous research, carbon isotope effects were determined by liquid-chromatography isotope ratio mass-spectrometry (LC–IRMS) for major initial enzymatic reactions of aerobic and anaerobic phenol degradation pathways (monohydroxylation or carboxylation at the aromatic ring, monohydroxylation or fumarate addition to substituted methyl groups) (Wei et al., 2016). However, a drawback of the used LC-IRMS method is that it does not allow analysis of hydrogen isotopes and thus, two dimensional stable isotope analysis of phenolics transformation reactions.

In this study, a method was developed and tested for carbon and hydrogen stable isotope analysis of phenol and cresols by GC-IRMS. Two extraction techniques were applied for carbon isotope analysis of phenolic compounds in water: liquid-liquid extraction and solid phase extraction (SPE). The efficiency of SPE method reached 57, 63 and 71 % for phenol, o-cresol and p-cresol, correspondingly. The method showed the absence of isotopic shifts caused by the extraction technique for the target compounds and proved the possibility of its implementation in analysis of environmental samples. The method of hydrogen isotope fractionation determination is based on derivatization by acetylation, formation of trifluoroacetate derivatives, that don't cause the change in isotope composition due to absence of extra hydrogen addition in molecule, and measurement using chromium-based high-temperature conversion, scavenging the formation of HF (Renpenning et al., 2017). We applied the method on samples from aerobic and anaerobic phenol and cresol degrading model strains and will present first results for two dimensional stable isotope analysis of phenolics.

#### References

[1] Wei, X.; Gilevska, T.; Wetzig, F.; Dorer, C.; Richnow, H.-H.; Vogt, C. Environmental Pollution 2016, 210, 166–173

[2] Renpenning, J.; Schimmelmann, A.; Gehre, M. Rapid Communications in Mass Spectrometry 2017, 31, 1095–1102

## Quantifying N<sub>2</sub>O production pathways of waste water treatment plants using <sup>15</sup>N tracer

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Autotrophic nitrogen (N) removal by anaerobic ammonium oxidation (anammox) is an important mechanism of fixed N elimination, both in engineered and natural systems. In wastewater treatment plants, it offers the potential for operation under energy autarky and a better carbon footprint of the cleaning process. However, its process control and engineering is still under development: an optimized nitrogen removal process using nitritation-anammox must combine stable operation, high N removal efficiency and minimized  $N_2O$  emissions. The recently initiated SNF Sinergia project ISOMOL combines stable isotope analysis and molecular biological tools with process engineering approaches, to develop process designs and control parameters for application of the nitritation-anammox process in full scale municipal wastewater.

<sup>15</sup>N tracer techniques is implemented to quantify main N<sub>2</sub>O production pathways and their controls on the carbon footprint of the nitritation-anammox process. In detail, N transformation processes in aqueous phase will be traced in real-time by analysis of <sup>15</sup>N-NH<sub>4</sub><sup>+</sup>, <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> and <sup>15</sup>N-NO<sub>2</sub><sup>-</sup> using a sample preparation unit for inorganic N-species coupled with membrane inlet mass spectrometry (SPIN-MIMS) as a novel online N monitor. In addition, our existing capability at Empa for on-line analysis of <sup>15</sup>N-N<sub>2</sub>O by quantum cascade laser absorption spectroscopy (QCLAS) will be extended to the analysis of highly enriched gas samples. Temporal trends of <sup>15</sup>N-labels in aqueous phase N compounds and gas phase N<sub>2</sub>O will be interpreted using a <sup>15</sup>N tracing model to quantify the main N<sub>2</sub>O production pathways. Microbial 16S, metagenomic and metatranscriptomic analysis will support our interpretation of N transformation and N<sub>2</sub>O production in wastewater treatment plants. Beyond engineered systems our results will advance understanding of the environmental controls on anammox and thus be of high relevance to a broad scientific community, including isotope geochemistry, microbial ecology, limnology and oceanography.

## *In situ* biodegradation rates in contaminated sediments via a high resolution multielement isotopic approach

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Compound Specific Isotope Analysis (CSIA) is able to assess whether decreases in concentrations of a specific contaminant are due to *in situ* transformation or transfer processes, information that is not available solely from concentration measurements. Further, multielement isotope analysis offers detailed information on processes occuring in natural environments, especially for reactions that involve isotope fractionation of two different elements. Recent analytical developments have enabled measurements of hydrogen isotopes for halogenated contaminants, which are often targets of remediation efforts (1). In this study we have combined hydrogen and carbon CSIA with high-resolution sampling (every 3 cm) across the sediment – water interface (SWI) at a contaminated field site, to identify zones with the highest biotransformation potential. Samples were collected at four locations via passive diffusion samplers (2) from sediment contaminated with monochlorobenzene (MCB) and benzene (the dechlorination product of MCB) in a field site (3).

Concentrations of MCB and benzene decreased from the bottom to the top of the sediment column at all four locations. This decrease in concentration correlated with a progressive enrichment in  $\delta^{13}$ C (up to 5.7 ‰) and in  $\delta^2$ H (up to 24 ‰) for MCB. Benzene, meanwhile, showed the inverse trend: a significant depletion in <sup>13</sup>C by up to 7.0 ‰ and in <sup>2</sup>H by up to 123 ‰ over only 40 cm. Such large and correlated trends in multielement enrichments for MCB and depletion for benzene are consistent with isotope signatures expected for parent contaminants and resulting daughter products during *in situ* biotransformation. Importantly, in the uppermost part of the sediment, the trend for benzene reversed, with an enrichment up to 2.2 ‰ for  $\delta^{13}$ C, indicating that biodegradation of both compounds was occuring across the SWI, with benzene degrading faster than MCB (*3*). By applying the concept of representative elementary volume (REV), a critical zone of 9-15 cm, within the depth profile, with the highest biodegradation potential in the sediments was identified. These results demonstrate how the combination of concentration and multi-element isotope data at high spatial resolution can be used to quantify *in situ* rates of degradative processes and therefore evaluate whether naturally occurring processes are an effective mechanism of remediation.

- 1. J. Renpenning et al., Anal. Chem. 87, 9443–9450 (2015).
- 2. E. Passeport et al., Environ. Sci. Technol. 50, 12197-12204 (2016).
- 3. T. Gilevska et al., Water Res. 149, 632–639 (2019).

## Useful statistical methods for compound-specific isotope analysis in contaminant hydrogeology

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Historically, measurement uncertainty in compound-specific isotope analysis (CSIA) has played a key role in interpreting the extent of contaminant transformation and has constrained our ability to positively identify enrichment trends in laboratory and field data. For many traditional stable isotope measurements ( $\delta^{13}$ C,  $\delta^{2}$ H,  $\delta^{37}$ Cl), uncertainty related to measurement accuracy and precision has been discussed and reported for specific sample-introduction methods (e.g. direct injection, headspace, purge and trap) as well as for different methods of detection (e.g. IRMS, q-MS, ICP-MS). However, uncertainty introduced by mathematical manipulations of isotopic data has been largely overlooked in the literature to date. Common data analysis techniques like regression analysis of dual-isotope plots and comparisons between several sets of data can be susceptible to mathematical uncertainty that potentially biases data interpretation. A suite of statistical methods such as t-tests and z-scores can improve our ability to use CSIA measurements to interpret laboratory data and apply it to the field. Here we present the appropriate statistical methods to support mathematical manipulations and data analysis techniques for CSIA applications. Incorporating robust statistical methods into data analysis and interpretation will help validate the role CSIA plays in contaminant hydrogeology.

## Tracking life's fingerprints with molecules and isotopes

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Biomarkers are organic molecular remnants of past life preserved in ancient sediments and soils. The stable isotopes carried by such fossil molecules yield insights to past organisms and their habitats and ecology. Insights provided by biomarkers and their isotopes are central to studies of climates and carbon cycle dynamics on land and in the ocean over Earth's long history. Although widely applied for these purposes, nevertheless, small samples or poor organic preservation restricts many studies due to size requirements of conventional methods for compound-specific isotope analyses (CSIA).

We recently (1) incorporated narrow-diameter capillary chromatography columns and microfluidic systems to reduce peak width, enhance separation, and minimize loss by reducing the flow diverted (or "split") from the IRMS source. This method has enabled precise carbon isotope analyses at picomolar quantities, or about 10-100 times less than required by conventional systems. Using "pico-CSIA," we have been able to measure compounds not normally included in isotope studies of past climates and environments. This presentation will highlight novel data from polycyclic aromatic hydrocarbons (PAH), and triterpenoid biomarkers from different time periods.

New analytical tools in NMR and mass spectrometry has recently led to expanding data and understanding of isotope patterns within organic compounds from different organisms and biochemistries. Extending both pico-CSIA and novel isotopologue analyses to fossil biomarkers opens new opportunities for studies of the origins of organics over Earth's history, and potentially, to organic compounds in meteorites or other planetary environments.

<sup>[1]</sup> Baczynski A. A., Polissar P.J., Juchelka D., Schwieters J., Hilkert A., Summons R.E., and Freeman K.H. (2018) Picomolar-scale compound-specific isotope analyses. *Rapid Communications in Mass Spectrometry* 32; 730-738

### New ecophysiological information from intramolecular isotope variation: methods, analysis and implications for biogeochemistry

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The IPCC considers the size of the  $CO_2$  fertilization effect as a central uncertainty in Earth system models. This is because  $CO_2$  manipulation experiments are limited in time to at most years, and impose a step increase in  $CO_2$ , instead of gradual. As a consequence, it is not clear if responses seen in these experiments hold for plants in the field, over decades, and in the presence of acclimation. We pioneer isotopomer approaches to tackle this question retrospectively using archives of plant material.

Conventional isotope applications in ecology and biogeosciences measure isotope ratios (e.g.  $d^{13}C$ ) of whole molecules. However, for primary metabolites large intramolecular isotope variation – isotopomer variation – has long been known. This variation reflects enzyme fractionations and encodes metabolic information (Schmidt 2003).

We will for present how intramolecular variation is measured by NMR (Chaintreau et al., 2013), examples for studies of intramolecular <sup>13</sup>C and D variation in trees and other plants, as well as concepts for data analysis, models to derive physiological interpretations.

First, intramolecular <sup>13</sup>C distributions of tree-ring cellulcose of several species, and of an annually-resolved *Pinus nigra* chronology (Wieloch et al., 2018). The figure displays the average intramolecular <sup>13</sup>C pattern (as ‰ deviation  $\Delta$  of carbon position C<sub>i</sub> = 1 to 6 from molecular average) of 6 angiosperm and 6 gymnosperm species. The pattern shows large intramolecular <sup>13</sup>C variation, with differences between isotopomers up to 10 ‰. Further, the pattern appears constant among all species, suggesting that it reflects fundamental preserved regulation of C metabolism. The intramolecular <sup>13</sup>C variation indicates that individual <sup>13</sup>C isotopomers of cellulose constitute distinct <sup>13</sup>C inputs into major global C pools. When glucose units with the observed



intramolecular <sup>13</sup>C pattern are broken down by different biochemical pathways, CO<sub>2</sub> with strongly differing d13C will be released; affecting isotope signals of atmosphere-biosphere C exchange fluxes. Furthermore, cluster analysis shows that tree-ring glucose exhibits several independent intramolecular <sup>13</sup>C signals, which constitute distinct ecophysiological information channels. <sup>13</sup>C fractionation by stomata/Rubisco explains only part of isotopomer variation, suggesting that whole-molecule <sup>13</sup>C analysis likely misses a large part of the isotope information stored in tree rings.

Second, in experiments on annual plants, we have found that deuterium isotopomers in photosynthetic glucose reflect the ratio of oxygenation to carboxylation at Rubisco, a central metabolic branching that is the origin of the photorespiration flux in all C<sub>3</sub> plants. Analyzing herbarium samples of several C3 species, we found that increasing atmospheric  $[CO_2]$  over the 20<sup>th</sup> century has reduced the oxygenation / carboxylation ratio in all investigated C<sub>3</sub> species, with no evidence for acclimatory reactions by the plants. Results on the moss *Spagnum fuscum* suggest a mechanism for increasing peat accumulation rates, a major global C sink (Ehlers et al., 2015). Oral presentations | Thursday, July 11 | Topic 6: Earth/Planetary Sciences, Biogeochemistry and Ecology I

Finally, results from ongoing work on deuterium isotopomers in tree rings and *Sphagnum* species. We compare data from growth chamber experiments, FACE studies and tree-ring series covering the anthropogenic  $CO_2$  since industrialization. The increase in  $CO_2$  should have led to increased photosynthesis, but this  $CO_2$  fertilization effect is poorly constrained on long time scales. A substantial part or the expected increase of photosynthesis is due to expected  $CO_2$ -driven suppression of photorespiration. Data will also be compared to herbaceous C3 species, with the goal to quantify the  $CO_2$ -driven suppression of photorespiration during recent decades.

- Chaintreau, A. et al., (2013): Site-specific C-13 content by quantitative isotopic C-13 Nuclear Magnetic Resonance spectrometry: A pilot inter-laboratory study. Analytica Chimica Acta 788, 108-113.
- Ehlers, I., Augusti, A, Betson, TR, Nilsson, MB, Marshall, J.D., Schleucher, J (2015): Detecting long-term metabolic shifts using isotopomers: CO<sub>2</sub>-driven suppression of photorespiration in C-3 plants over the 20<sup>th</sup> century. PNAS 112, 15585-15590.
- Schmidt, H. L. (2003): Fundamentals and systematics of the non-statistical distributions of isotopes in natural compounds. Naturwissenschaften 90, 537-552.
- Wieloch, T., Ehlers, I., Yu, J., Frank, D., Grabner, M., Gessler, A., Schleucher, J (2018): Intramolecular C-13 analysis of tree rings provides multiple plant ecophysiology signals covering decades. Scientific Reports 8, 5048.

Oral Presentations: Friday, July 12

### Linking phosphate oxygen isotope effects to phosphorus cycling, from molecular to ecosystem scales

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Phosphorus (P) cycling in the environment is almost entirely controlled by microbial processes. Therefore, understanding the isotope effects involved in the microbial processes is an essential step to decipher both extinct and extant ecosystem processes. Pyrophosphatase is the only known enzyme in organisms to imprint equilibrium isotope composition—which has been validated from mass spectrometry and Raman Spectroscopy methods. All other phosphatase enzymes are known to hydrolyze organic P compounds via one-way disequilibrium isotope effect, which varies both with the type of substrate and enzyme, but variation among enzymes synthesized by different organisms have also found. Still, a distinction between equilibrium and disequilibrium isotope effects can be made. On the other hand, specific phosphate pools that are not bioavailable retain source specific isotope signatures and aid in identifying sources. Together, they provide information useful to identify the role of microbial processes in the environment. Isotope mass balance has been employed to identify dominant processes in eutrophic water and has helped constrain that late stage eutrophication and dead zones are caused largely from internal cycling of P. More recently, compound-specific and position-specific oxygen isotopes, along with other stable isotopes (C, N, and H), in organic P compounds are being investigated. These developments improve resolution and thus better resolve the relative roles of anthropogenically derived sources of phosphorus on water quality.

## Future precipitation patterns in agroecosystems affected carbon and nitrogen turnover – a green manure stable isotope labelling study

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Climate change is likely to affect precipitation patterns in the future, and as such is a determining factor in agricultural systems in terms of soil organic matter mineralization, nutrient release and therefore plant production. This study investigates the impact of precipitation on two different soil types – a sandy calcaric Phaeozem and a calcic Chernozem which are representative of the Pannonian area of the Marchfeld region in Austria. A regionalized scenario RCP 6.0 derived from the 5th IPCC was used on a long-term lysimeter study in the Marchfeld, where future rainfall patterns (386 mm  $a^{-1}$ ) were compared with current precipitation (518 mm  $a^{-1}$ ) since 2011.

Bulk concentration measurements did not show clear differences in carbon and nutrient cycling between scenarios as heterogeneity between lysimeters was high. However, a more precise way of tracing carbon and nitrogen turnover in soils is using a stable isotope labelling approach. In this study, green manure (*Sinapis alba*) labelled with <sup>13</sup>C and <sup>15</sup>N stable isotopes was applied to the lysimeter soils in April 2018. Gaseous emissions, soil, plant and groundwater samples were



collected at throughout the growing season and analysed using isotope ratio mass spectrometry and a <sup>15</sup>N N<sub>2</sub>O cavity ring down isotope-laser spectrometer installed in the field.

Results showed decreased plant biomass production in the low precipitation scenario due to drought stress as indicated by increased  $\delta^{13}$ C values of the plants. Mineralization of green manure ( $^{13}$ C CO<sub>2</sub>) and label uptake into soil microorganisms ( $^{13}$ C PLFA) was shown to start within hours of application, and total CO<sub>2</sub> emissions from the calcic Chernozem was lower for the future precipitation scenario. Accordingly, isotope results showed that inorganic N (NO<sub>3</sub><sup>-</sup>) was released from the green manure more slowly under the future precipitation scenario. However, as plants grew less in summer under the future precipitation scenario, uptake of N was retarded leading to higher <sup>15</sup>N label in the soil and plants. Emissions of N<sub>2</sub>O were generally low, even after a simulated heavy rainfall event (60 mm), and initially slightly higher under the current precipitation scenario. Incorporation of <sup>15</sup>N label from the green manure into N<sub>2</sub>O was not detectable.

This study provides a nice example how isotope tools can uncover differences in soil processes which are otherwise masked due to soil heterogeneity. Lower mulch mineralisation under the future precipitation scenario came concurrent with diminished plant growth finally leading to a higher availability of inorganic N in the soils which is susceptible to leaching.

### Mercury stable isotopes as an eye-opener on the role of vegetation in global mercury cycling

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Mercury is a high priority pollutant of great concern for human and ecosystem health. Anthropogenic mercury emissions are transported through the atmosphere as gaseous elemental mercury (Hg0) before being deposited globally on land and ocean. It has been the notion over the last decades that atmospheric Hg(0) is first oxidized in the atmosphere to soluble Hg(II) before being deposited to land and ocean via rain and snowfall. The direct deposition of Hg(0) has received less attention mainly due to analytical difficulties in measuring fluxes of Hg(0). Mercury stable isotopes are a novel tool to fingerprint pathways of atmospheric mercury deposition and transformation processes. Over the last years increasing evidence from mercury isotope fingerprints suggests that 60 % to 90 % of mercury in various soils around the world originated from the direct deposition of Hg(0), rather than from Hg(II) in precipitation. During this presentation I will explain how Hg stable isotopes served as an eye-opener on the role of terrestrial vegetation as a pump for Hg(0). The uptake of atmospheric Hg(0) by vegetation appears to be a major driver of mercury storage in soils, seasonal variations in atmospheric mercury concentrations and mercury transfer to aquatic ecosystems. Finally, I will discuss the implications of the vegetation mercury pump for global mercury cycling in particular in context of climate change.

## Simultaneous detection by isotope ratio and quadrupole mass spectrometry coupled to multidimensional gas chromatography for the analysis of complex samples

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Isotope Ratio Mass Spectrometry (IRMS) is commonly recognized to be able to provide information about the geographical, chemical, and biological origins of substances. The ability to determine the source of substances stems from the relative isotopic abundances of the elements which comprise the material. By performing a separation prior to isotope ratio analysis, hyphenated techniques such as GC-C-IRMS, can provide isotopic analysis of a complex mixture, thereby providing additional information and higher discriminatory power. Since its introduction, the use of this analytical approach was not widespread due to a series of drawbacks related to chromatographic and isotopic issues. In fact, dead volumes due to the typical instrumental setup, requiring the combustion of the components followed by a drying step, often limit the separation efficiency, driving to an increased band broadening and peak asymmetry producing peak coelutions, thus falsify the measurements. Moreover, the reduced chromatographic performance increases the gas chromatographic isotope effect (or inverse isotopic effect) that generates GC peak not isotopically consistent because composed of lighter isotopes (<sup>12</sup>C, <sup>1</sup>H and <sup>16</sup>O) that elute after the isotopomers containing heavier organic compounds because of their higher volatility. The present research deals with the development of an MDGC-MS/IRMS prototype characterized by the improved resolution capability of the heart-cut mode, exploiting two different stationary phases, and the simultaneous qMS and IRMS detection of the 2D chromatographic bands. The IRMS system was optimized in terms of dead volumes enabling to overcome the extra-column band broadening effect that usually affects the commercial systems. Different applications on food [1] and flavour and fragrance samples are reported showing the enhanced performances of the prototype described.

#### Reference

 Sciarrone D. et al, Multidimensional Gas Chromatography Coupled to Combustion-Isotope Ratio Mass Spectrometry/Quadrupole MS with a Low-Bleed Ionic Liquid Secondary Column for the Authentication of Truffles and Products Containing Truffle. Anal Chem. 2018, 90, 6610-6617

## Stable isotope raman microspectroscopy for nondestructive analysis of microorganisms on the single-cell level

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Stable isotope-based analytical methods are crucial to study fluxes and to constrain processes in various scientific fields. Complementary to well-established techniques, like isotope ratio mass spectrometry and mass spectrometry-imaging methods, stable isotope Raman microspectroscopy (SIRM) opens the possibility to perform nondestructive, spatially-resolved quantitative analysis of various (in)organic and (micro)biological samples.<sup>1, 2</sup>

SIRM provides characteristic fingerprint spectra of samples with the spatial resolution of a confocal optical microscope (down to 1  $\mu$ m and even below), containing information on the chemical nature of stable isotope-labeled substances and the amount (%) of a label (based on the red-shift of bands from labeled substances). Simultaneously, these spectra deliver information on the chemical composition and structure of samples. Furthermore, this method can be performed without spectral interference of water.<sup>1</sup> Therefore, SIRM allows for *in situ* investigations of microbial communities on the single cell level. Furthermore, the sensitivity of SIRM can be significantly improved (in the range of  $10^3 - 10^6$ , up to  $10^{11}$  at so-called "hot spots") due to surface-enhanced Raman scattering (SERS), e.g. by using of Ag nanoparticles (AgNPs).

We applied SIRM and SERS for the analysis of unlabeled as well as <sup>13</sup>C-, <sup>15</sup>N- and D-labeled single bacterial cells.<sup>3-5</sup> The use of AgNPs synthesized *in situ* allowed us to perform reproducible SERS analysis. In contrast to SIRM (where *whole-organism fingerprints* for bacteria are obtained), SERS is more selective and provides information on cell surface substances. The SERS spectra are characterized by a pronounced band at around 730 cm<sup>-1</sup>, which was assigned to adenine-related compounds.<sup>4</sup> Furthermore, we studied the limitations and applicability of the SERS approach for characterization of microorganisms. Our studies suggest that the SERS signals reflect the metabolic activity of bacterial cells.<sup>5</sup>

The ability to visualize the incorporation of stable isotopes on the single cell level is not limited to planar surfaces but is extendable to three dimensions. This can provide detailed information about the 3D arrangement and metabolic activities of different microorganisms inside complex matrices, e.g. artificial biofilms.<sup>5</sup> Thus, SIRM (in combination with SERS) can open a variety of new applications, e.g. in environmental microbiology.

- [1] N. P. Ivleva, P. Kubryk and R. Niessner, Anal. Bioanal. Chem., 2017, 409, 4353-4375.
- [2] A. C. Wiesheu, R. Brejcha, C. W. Mueller, I. Koegel-Knabner, M. Elsner, R. Niessner and N. P. Ivleva, Anal. Bioanal. Chem., 2018, 410, 923-931.
- [3] P. Kubryk, J. S. Kölschbach, S. Marozava, T. Lueders, R. U. Meckenstock, R. Niessner and N. P. Ivleva, *Anal. Chem.*, 2015, 87, 6622-6630.
- [4] P. Kubryk, R. Niessner and N. P. Ivleva, Analyst, 2016, 141, 2874-2878.
- [5] R. Weiss, M. Palatinszky, M. Wagner, R. Niessner, M. Elsner, M. Seidel and N. P. Ivleva, Analyst, 2019, 144, 943-953.

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Poster presentations Topic 1: Advances in Analytics

# Graphene-modified polymer monoliths for high throughput extraction of micropollutants for compound-specific isotope analysis

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Compound-specific isotope analysis (CSIA) has been demonstrated to be highly suitable for the evaluation of sources and transformation processes of micropollutants, such as pesticides and pharmaceuticals, in laboratory experiments during the last decade. However, analytical challenges associated with low micropollutant concentrations (sub-µg/L range) in environmental water samples still limit applicability of CSIA approach to field studies due to large volumes of water necessary for extraction. Although traditional solid-phase extraction techniques are available for enrichment of micropollutants, they fail in processing large volumes in feasible timescales due to limited flow rates (i.e. few mL/min). Therefore, a high throughput concept for extraction is needed. To this end, monolith affinity filters (MAF) offer a promising solution to process large volumes of water in reasonable time (i.e. up to 1000 mL/min). MAFs are epoxy-based polymers prepared by self-polymerization of polyglycerol-3-glycidyl ether in organic solvent as porogens. A highly cross-linked structure with high porosity (79%) and large pores (i.e. from 15 to 25 µm) enables high throughput of liquid samples. In recent years, application of MAFs has been demonstrated for capturing bacteria. Nevertheless, a successful extension of the concept to organic micropollutants has not been realized. In this work, we explore the use of graphene oxide to modify the MAFs surface chemistry in order to enable pi-pi electron donor-acceptor interactions with the micropollutants.



Tailor-made MAFs were successfully synthesized using toluene and tert-butyl methyl ether as porogen with volume ratio of 3:2. Amine groups were immobilized onto the pore surface via the reaction of epoxide groups with the polyetheramine Jeffamine. Subsequently, graphene oxide (GO) was covalently attached by coupling the amine groups using dicyclohexylcarbodiimide as a coupling agent. Polymer monoliths functionalized with reduced graphene oxide (rGO) were then obtained via chemical reduction by ascorbic acid. Sorption performance of selected pesticides and metabolites, namely chloridazon, isoproturon, terbuthylazine, s-metolachlor, and 2,6-dichlorobenzamide, has been assessed on rGO-functionalized polymers, as well as on bulk GO and rGO in batch experiments. Results reveal that rGO exhibits higher sorption capacity towards chloridazon, isoproturon, terbuthylazine, s-metolachlor by factors of 5.5, 5.4, 1.8, 2.8, respectively. First results exhibit the suitability of rGO for modification of MAFs surface chemistry. Recovery efficiency of the sorbed analytes is being investigated for different eluent solvents. Optimization of operation parameters, including sample pH, volume, and flow rate will be evaluated on the base of analyte recoveries.

## References

[1] Elsner, M. and Imfeld, G., Curr. Opin. Biotech. 2016, 41, 60-72.

[2] Peskoller, C., Niessner, R., and Seidel, M., J. Chromatogr. A. 2009, 1216, 3794-3801.

## Synthesis and characterization of a calibration standard for stable chlorine isotope analysis

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For accurate stable chlorine isotope analysis, it is crucial to run isotope standards in parallel with samples. Specifically, two standards are needed which bracket the isotope values of the samples. For chlorine few international standards are available, all of them with similar isotope values near 0 ‰. The exception is USGS38, which shows an isotopic shift (-87.9 ‰), but is of limited availability. Thus, working standards for daily chlorine isotope analysis are typically characterized against only one international standard. Consequently, a second readily available standard with an isotopic shift for characterization is needed.

2,2,2-Trichloroethyl acetate was used as starting material. As depicted in panel A of the Figure, the trichloroethyl group was removed via reductive elimination by Zn under reflux conditions. After stopping the reaction by removing the zinc, silver nitrate solution was added via reductive elimination and subsequent precipitato precipitate the formed chloride as silver chloride. For tion with AgNO<sub>3</sub>. characterization via gas chromatography-isotope ration (B) Expected intramolecular isotope fractionation mass spectrometry (GC-IRMS) and gas chromatography-inductively coupled plasma-multicollector-mass spectrometry (GC-ICP-MC-MS) the international



(A) Synthesis of the silver chloride standard CT16

and the resulting strategy to recover produced chloride with pronounced changes in chlorine isotope values.

standards ISL-354 ( $\delta^{37}$ Cl = +0.05 ±0.03 ‰) and USGS38 ( $\delta^{37}$ Cl = -87.90 ±0.24 ‰) were used. Prior to characterization all substances were converted to methyl chloride.

Conversion of 2,2,2-trichloroethyl acetate produced pure silver chloride named "CT16". Characterization via GC-IRMS and GC-ICP-MC-MS gave identical values with a mean over all measurements of  $\delta^{37}$ Cl<sub>CT16</sub> = -26.82 ±0.18 ‰ (n = 16). This consensus value shows a relatively large shift when compared to most international chlorine isotope standards. This can be explained by the intramolecular chlorine isotope effect during reductive elimination according to panel B in the Figure. The large difference will be of great advantage in the future characterization of materials: Calibration against two different isotope standards will result in improved precision and trueness of daily chlorine isotope analysis.

#### References

Somsák, L.; Czifrák, K.; Veres, E. Tetrahedron Letters 2004, 45, 9095-9097.

# Instrumental Modifications of a Gas Chromatograph-Isotope Ratio Mass Spectrometer towards Sensitive and Accurate Carbon Isotope Analysis of Micropollutants

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In the recent years, reports about the detection of micropollutants in surface- and groundwater cumulated and the assessment of a compound's environmental fate using compound specific stable isotope analysis (CSIA) by gas chromatography-isotope ratio mass spectrometry (GC-IRMS) has become more and more important. The method is based on the analysis of natural isotope abundances and isotopic shifts. So far, the analysis of micropollutants is limited to the sub  $\mu$ g/L concentration range. However, as micropollutants are often detected in the ng/L range, high sensitivity, robustness and accuracy are crucial parameters for CSIA.

The aim of this study is to increase the sensitivity without introducing an isotopic discrimination. Thereto, based on the study of Baczynski et al. (1) and Tobias et al. (2) a GC-IRMS system was modified. The modification included a Rapid data acquisition (RDA) upgrade of the isotope ratio mass spectrometer and its corresponding software, a modified open-split and a new combustion reactor based on a nickel capillary with a Pt-wire. To validate the system, the frequently detected herbicides atrazine, metolachlor and acetochlor were used as model compounds.

Preliminary validation of the modified set-up resulted in accurate results with standard deviations smaller than 0.5 and a reduced peak width by the factor of three.

- 1. Baczynski AA, Polissar PJ, Juchelka D, Schwieters J, Hilkert A, Summons RE, et al. Picomolar-scale compound-specific isotope analyses. Rapid Commun Mass Spectrom. 2018;32(9):730-8.
- 2. Tobias HJ, Brenna JT. Cryofocus fast gas chromatography combustion isotope ratio mass spectrometry for rapid detection of synthetic steroid use in sport doping. Analyst. 2018;143(5):1124-32.

# HS-SPME MDGC-C-IRMS with simultaneous quadrupole MS detection: a powerful approach to discriminate between natural and synthetic truffle aroma

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Truffles are among the most expensive foods, due to their rarity and unique aroma. The white ones are available only a couple of months a year. They are more precious than the black ones, and the most valuable species is *Tuber magnatum* Pico, better known as "Alba white truffle". It is sought after for its larger size, limited quantity, and quality of taste. Given the high economical value, analytical approaches are required, for authenticity and traceability purposes. In the present research, gas chromatography coupled to combustion-isotope ratio mass spectrometry (GC-C-IRMS), has been exploited in order to discriminate between natural or synthetic truffle aroma. A powerful approach has been applied, able to evaluate the <sup>13</sup>C ratio of the key aroma compound, namely bis (methylthio) methane. A high-efficiency HS-SPME MDGC-C-IRMS with simultaneous quadrupole MS detection, has been used for the analysis of several high-value white truffles harvested in Italy, and commercial products flavored with different truffle species [1]. This application demonstrates the capability of an MDGC-C-IRMS system to overcome some of the historical problems of IRMS, associated with the combustion and measurement of impure peaks. Extra-column band broadening has been greatly reduced thanks to the optimization of the micro-combustion furnace and to the elimination of the heart-split valve, not necessary in a multidimensional configuration.

Sciarrone D. et al, Multidimensional Gas Chromatography Coupled to Combustion-Isotope Ratio Mass Spectrometry/Quadrupole MS with a Low-Bleed Ionic Liquid Secondary Column for the Authentication of Truffles and Products Containing Truffle. Anal Chem. 2018, 90, 6610-6617

## Selective extraction of pesticides from surface water using crosslinked Cyclodextrin Polymers

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Compound specific isotope analysis (CSIA) is an important tool for determining the origin and fate of organic contaminants, such as pesticides, in the environment. Although analytical methods for CSIA are available for lab applications, it remains a major challenge to transfer these methods to the field-scale due to the low occurrences of pesticides in environment (i.e. ng/L range). To this

end, extraction of the pesticides from large volumes of water (i.e. 10-500 L) becomes inevitable to meet the low sensitivity of isotope-ratio mass spectrometry. Therefore, sorbents are warranted with (i) very fast kinetics to process the large volumes of water samples in reasonable times, as well as (ii) high selectivity towards the pesticides in presence of natural organic matter that is at least 104 more abundant than the pesticide.



Crosslinked Cyclodextrin Polymers (P-CDP) have recently been synthesized with high surface area that could enable their use for treatment purposes where large volumes of water are typically processed [1]. While the size of the cyclodextrin pocket is hypothesized to fit small molecules such as pesticides, natural organic matter (NOM) of larger size are presumed to be excluded. The latter assumption has not been tested and little is known about behaviour of NOM on P-CDP. In this work, crosslinked cyclodextrins with different pocket sizes, namely  $\alpha$ -P-CDP,  $\beta$ -P-CDP and  $\gamma$ -P-CDP, were synthesized and used as solid-phase extraction (SPE) sorbents to extract a selection of 16 pesticides from surface waters. Interactions between the three P-CDPs and NOM are investigated in terms of NOM size and polarity. Fractions of NOM with < 1, 1-3, 3-10, and > 10 kDa is prepared using ultracentrifugation of surface waters [2] and investigated for their behaviour on the synthesized P-CDPs in terms of sorbed amount and its aromaticity. Whereas, NOM fractions with different polarities are prepared using a set of anion and cation exchangers [3]. This contribution will systematically present recoveries of the pesticides and NOM on P-CDPs in comparison to three conventional materials and hydroxypropylated  $\beta$ -P-CDP.

### References

- Alsbaiee, A.; Smith, B. J.; Xiao, L; Ling, Y; Helbling, D. E.; Dichtel, W. R.: Rapid removal of organic micropollutants from water by a porous β-cyclodextrin polymer. Nature 2016, 529, 190-196.
- [2] Xu, H.; Guo, L.: Molecular size-dependent abundance and composition of dissolved

organic matter in river, lake and sea waters. Water Research 2017, 117, 115-126.

[3] Ratpukdi, T.; Rice, J. A.; Chilom, G.; Bezbaruah, A.; Khan, E.: Rapid fractionation of natural organic matter in water using a novel solid-phase extraction technique. Water Environment Research 2009, 81, 2299-2308.

# A novel approach for position-specific <sup>18</sup>O/<sup>16</sup>O measurement of carbohydrates from higher plants by GC-IRMS

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The <sup>18</sup>O/<sup>16</sup>O ratio at both molecular and positional levels in the carbohydrates of higher plants is a reliable proxy for the plant growth environment, and a potential indicator of the plant photosynthetic carbon assimilation mode, and its physiological, biochemical and metabolic status (Sternberg et al., 2003; 2006; Waterhouse et al., 2013). The lack of exploitable nuclear resonance in <sup>18</sup>O and <sup>16</sup>O and the extremely low <sup>17</sup>O abundance make the NMR-based PSIA (position-specific isotopic analysis) a significant challenge. In order to probe the valuable metabolic information locked into these position-specific oxygen isotopic compositions in carbohydrates, we have developed a chemical strategy for accessing the <sup>18</sup>O/<sup>16</sup>O ratios of O-3 in glucose (Ma et al., 2018). A three-step wet chemistry is employed to remove O-3 from glucose from glucose so that isotope mass balance can be applied to calculate the isotopic composition of O-3 (Scheme 1):

i) diacetonation of glucose (A); ii) chlorothionoformylation of O-3 (B); and iii) Barton-McCombie deoxyenation of O-3 with  $(n-Bu_4N)_2S_2O_8$  and HCOONa. The O-3 of glucose in a starch from a  $C_4$  plant is about 12 ‰ more enriched in 18O than the average of the 5 non-exchangeable Os in the glucose unit. This indicates that <sup>18</sup>O distribution, like that of <sup>13</sup>C and <sup>2</sup>H, in glucose (and other sugars) is also heterogeneous at natural abundance levels in glucose.



Scheme 1: The threestep wet chemistry based method for accessing the  ${}^{18}O/{}^{16}O$  of glucose O-3

- [1] Sernberg, L. S. L., Anderson, W. T., Morrison K. Separating soil and leaf water 18O isotopic signals in plant stem cellulose. Geochimica et Cosmochimica Acta, 2003, 67, 2561-2566.
- [2] Sternberg, L. S. L., Pinzon, M. C., Anderson, W. T., Jahren, A. H. Variation in oxygen isotope fractionation during cellulose synthesis: intramolecular and biosynthetic effects. Plant, Cell and Environment, 2006, 29, 1881.
- [3] Waterhouse, J. W., Cheng, S. Y., Julchelka, D., Loader, N. J., McCarroll, D. M., Switsur V.R., Gautam, L. Position-specific measurement of oxygen isotope ratios in cellulose: isotopic exchange during heterotrophic cellulose synthesis. Geochimica et Cosmochimica Acta, 2013, 112, 178-191.
- [4] Zhou, Y. P., Stuart-Williams H., Farquhar, G. D., Hocart, C. H. The use of natural abundance stable isotopic ratios to indicate the presence of oxygen-containing chemical linkages between cellulose and lignin in plant cell walls. Phytochemistry, 2010, 71, 982.
- [5] Zhou, Y. P., Yin, C. J., Yang, H. B., Su, J., Yu, H. M., Wang, Y., Zhou, S. X., Zavadlav, S. Stable isotopic evidence for the widespread presence of oxygen-containing chemical linkages between α-cellulose and lignin in Poaceae (Gramineae) grass leaves. ACS Sustainable Chemistry and Engineering, 2017, 5, 3250.
- [6] Ma, R., Zhu, Z. Y., Wang, B., Zhao, Y., Yin, X. J., Lu, F. Y., Wang, Y., SU, J., Hocart, C. H., Zhou, Y. P. Novel position-specific 18O/16O measurement of carbohydrates. I. O-3 of glucose and confirmation of 18O/16O heterogeneity at natural abundance levels in glucose from starch in a C4 Plant. Analytical Chemistry, 2018, 90, 10293.

Poster presentations Topic 2: Computations & Theory

# Development of combined plane wave and localized basis sets method toward the analysis of H/D adsorption mechanism on the metal surface

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These days, H/D isotope effects has attracted attention in computational chemistry, such as the adsorption of atoms/molecules on metal surfaces [1]. It is important to take account of the nuclear quantum effect (NQE) for such calculations. One of the powerful methods to include NQE is mul-

ticomponent density functional theory (MC\_DFT) [2]. MC\_DFT is the method based on localized (LO) basis sets, so it is used for cluster system, however, it is not suitable for the surface system. For such a surface system with delocalized electrons, plane wave (PW) basis sets in the framework of periodic boundary condition is well-known, but it excludes NQE. Therefore, we have recently developed the method with combined of PW and LO, named "combined plane wave and localized basis sets (CPLB)" method [3]. In CPLB, the system is divided into surface and cluster. Total energy with CPLB is gained by three energies: surface energy with PW (E[PW, surface]), cluster energy with PW (E[PW, cluster]), and cluster



E(PW, surface) - E(PW, cluster) + E(LO, cluster)



energy with LO (E[LO, cluster]), shown in **Fig. 1**. It is easy to calculate CPLB energy [3], however, geometry optimization program is needed for CPLB. Therefore, our purpose is implemented the geometry optimization program, and analyzing the H/D adsorbed Pd(111) surface. To treat H/D isotope effect, the MC\_DFT was used for the LO part.

**Table 1** shows the distance between nearest Pd and H/D atoms (R[Pd-H], R[Pd-D]) and geometrical change by H/D isotope effect ( $\Delta R$ ) for each method: CPLB, LO, and PW, respectively. From the comparing of H and D in CPLB, R[Pd-H] is 0.006 Å longer than R[Pd-D], which was the same trend as conventional LO method. We note that conventional PW method could not represent the H/D isotope effect. We clearly demonstrated that our developed CPLB method is a powerful tool to calculate the geometrical change by H/D isotope effect.

**Table 1** the distance between nearest Pd and H/D atoms (R[Pd-H] and R[Pd-D]) and geometrical change by H/D isotope effect ( $\Delta R = R$ [Pd-D] - R[Pd-H]) for each system: surface with CPLB, cluster with LO, and surface with PW, respectively. Unit is in Å.

System	R(Pd-H)	R(Pd-D)	$\Delta R$
Surface with CPLB	1.836	1.830	-0.006
Cluster with LO	1.779	1.773	-0.006
Surface with PW	1.818	—	_

### References

[1] T. Koitaya and J. Yoshinobu, J. Surf. Sci. Soc. Jpn., 34, 437 (2013).

[2] T. Udagawa and M. Tachikawa, J. Chem. Phys., 125, 244105 (2006).

[3] T. Ishimoto and H. Kai, Int. J. Quant. Chem., 118, e25452 (2018).

Poster presentations Topic 4: Isotope Effects in Biology and Enzymology

# Investigations on the in vivo metabolism of Methylstenbolone and detection of new long-term metabolites useful for doping control analysis

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Methylstenbolone (MSTEN; 2,17α-dimethyl-17β-hydroxy-5α-androst-1-en-3-one) is still sold as a designer steroid or nutritional supplement under brand names such as M-STEN or Nanodrol (Figure 1). Occasionally it is detected in doping control samples, predominantly tested and confirmed as the glucuronic acid conjugate of MSTEN. The absence of other meaningful metabolites reported as target analytes for its detection in sports drug testing samples can be explained with the advertized metabolic inertness of MSTEN.

In 2013, a first investigation of the human metabolism of MSTEN was published, and 2 hydroxylated metabolites were identified as potential initial testing targets for doping controls.<sup>[1]</sup> Later on, ex vivo investigations of the MSTEN metabolism revealed several additional hydroxylated metabolites found both glucuronidated and sulfated.<sup>[2]</sup> These metabolites complemented the metabolic pattern of MSTEN; yet, further research into the metabolism of MSTEN appeared warranted, especially in the light a recent publication on the in vivo metabolism of MSTEN in horses, which reported on several additional metabolites.<sup>[3]</sup>

This re-investigation was accomplished using Figure 1: Chemical structure of methystenbolone todeuterated MSTEN together with hydrogen iso- gether with the presumed structure of 2 new long-term tope ratio mass spectrometry (IRMS) in combi- metabolites nation with high accuracy/high resolution mass



spectrometry.<sup>[4]</sup> After oral administration of a single dose of 10 mg of doubly labeled MSTEN, urine samples were collected for 29 days. All samples were processed using routine doping control methods for IRMS analysis and all detected metabolites were further substantiated by mass spectrometry-based investigations.

More than 40 different metabolites still containing deuterium were detected after administration, mainly in the fraction of glucuronidated steroids but also sulfated metabolites were observed. All metabolites were investigated regarding their potential to prolong the detection time for MSTEN administrations. Besides MSTEN excreted as glucuronide conjugate, 2 additional metabolites were still detectable at the end of the study after 29 days (Figure 1). These metabolites have been further investigated regarding their chemical structure employing high accuracy/high resolution mass spectrometry.

- de Albuquerque Cavalcanti, D.; Leal, F. D.; Garrido, B. C.; Padilha, M. C.; de Aquino Neto, F. R.: Detection of designer steroid methylstenbolone in "nutritional supplement" using gas chromatography and tandem mass spectrometry: elucidation of its urinary metabolites. Steroids 2013, 78, 228–233.
- [2] Geldof, L.; Lootens, L.; Polet, M.; Eichner, D.; Campbell, T.; Nair, V.; Botrè, F.; Meuleman, P.; Leroux-Roels, G.; Deventer, K.; van Eenoo; P.: Metabolism of methylstenbolone studied with human liver microsomes and the uPA-<sup>+</sup>/<sup>+</sup>-SCID chimeric mouse model. Biomed. Chromatogr. 2014, 28, 974–985.
- [3] Choi, T. L. S.; Wong, J. K. Y.; Kwok, W. H.; Curl, P.; Mechie, S.; Wan, T. S. M.: Metabolic study of methylstenbolone in horses using liquid chromatography-high resolution mass spectrometry and gas chromatography-mass spectrometry. J. Chrom. A 2018, 1546, 106–118.
- [4] Thevis, M.; Piper, T.; Horning, S.; Juchelka, D.; Schänzer, W.: Hydrogen isotope ratio mass spectrometry and high-resolution/high-accuracy mass spectrometry in metabolite identification studies: Detecting target compounds for sports drug testing. Rapid Commun. Mass Spectrom. 2013, 27, 1904–1912.

Poster presentations Topic 5: Isotopologues

# Application of average molar weight to optimization of cascades for multicomponent isotope separation

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The distribution of the average molar weight in a cascade is an important characteristic in the separation of multicomponent isotope mixtures(Ying 1998). In this paper, the concept of average molar weight is introduced to calculate the stage number of optimal cascade to pre-optimize the multipass separating processes of cascades. This optimization method can reduce the number of optimization and shorten the running time. The results for the separation of WF6 and Xe are used as an example. Numerical calculation indicate the results obtained by this optimization method and general optimization method are consistent with the same optimization parameters and optimization target. However, the number and time of optimization were greatly shorted in the optimization process by pre-optimized of average molar weight, which is about 12% of general optimization process. Therefore, this optimization method is suitable for optimization and design of cascades for multicomponent isotope multipass separation.

## References

[1] Ying C, Zeng S. Distribution of Average Molar Weight in a Cascade for Separating Multicomponent Isotopic Mixtures[J]. Separation Science and Technology, 1998, 33(12):1861-1875.

## Isotopologue profiling for the study of metabolism and its origin

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Isotopologue profiling is a key technology for elucidating biochemical pathways and fluxes in living organisms. The observation-driven method is based on in vivo labeling experiments using simple precursors (e.g. glucose, glycerol and  $CO_2$ ) labeled with the stable <sup>13</sup>C-isotope. Experimental settings including pseudo-steady state or non-stationary state can be used for a variety of organisms such as microbes, plants, symbioses and host-pathogen situations under controlled or even natural (physiologically relevant) conditions. <sup>13</sup>C-Enrichments and positional isotope distributions in metabolic products or in selected key components are typically analyzed by high-resolution GC-MS and NMR spectroscopy. Specific isotope distributions then identify and quantify the nature of the biosynthetic pathways, the relative fluxes in the core metabolic network and even the metabolic exchange between organisms in more complex settings (i.e. symbioses or host-pathogens). With recent examples, we will illustrate the benefits and the limits of the method to elucidate pathways and fluxes in bacteria and their hosts as well as in symbiotic entities such as lichens. Moreover, it will be shown how the method contributed to recent studies of the "origin-of-life" under chemoautotrophic Fe/Ni/S-conditions.

- [1] Eisenreich, W.; Dandekar, T.; Heesemann, J.; Goebel, W.: Carbon metabolism of intracellular pathogens and possible links to virulence. Nat Rev Microbiol 8, 401-412 (2010)
- [2] Billig, S.; Schneefeld, M.; Huber, C.; Grassl, G.; Eisenreich, W. and Bange, F.-C.: Lactate oxidation facilitates growth of Mycobacterium tuberculosis in human macrophages; SciRep 7: 6484 (2017)
- [3] Mall, A.; Sobotta, J.; Huber, C.; Tschirner, C.; Kowarschik, S.; Bačnik, K.; Mergelsberg, M.; Boll, M.; Hügler, M.; Eisenreich, W.; Ivan A. Berg, I. A.: Reversibility of citrate synthase allows autotrophic growth of a thermophilic bacterium; Science 359, 563–567 (2018)
- [4] Kuhn, V., Geisberger, T., Huber, C., Beck, A., & Eisenreich, W.: A facile in vivo procedure to analyse metabolic pathways in intact lichens. New Phytologist. (2019)
- [5] Geisberger, T.; Diederich, P.; Steiner, T.; Eisenreich, W.; Schmitt-Kopplin, P. & Huber, C.: Evolutionary Steps in the Analytics of Primordial Metabolic Evolution. Life. 9(2), 50 (2019)

## Measuring all the 12 isotopologues of carbon dioxide by Raman spectroscopy

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In many applications, the identification - and quantification - of reactions in complex reaction networks can be aided by using stable isotope labels. One area of research benefitting from stable isotope labelling studies are e.g. complex gas-fluid-mineral reactions during CO<sub>2</sub> sequestration in the subsurface. During the geochemical reactions occurring simultaneously, the pool of dissolved CO<sub>2</sub> in the formation fluids is influenced by hydration of the injected CO<sub>2</sub>, the dissolution of carbonate minerals because of the concomitant pH drop, and by precipitation of secondary carbonate minerals as siderite.

For carbon dioxide, the number of isotopologues is twelve because of the numerous possible combinations between the two carbon isotopes <sup>12</sup>C or <sup>13</sup>C and three oxygen isotopes <sup>16</sup>O, <sup>17</sup>O and <sup>18</sup>O. As the isotopologues differ in the bond distances and hence strength of the carbon to oxygen bonds, all the isotopologues could be differentiated by Raman spectroscopy. As the precise quantification of the percentage of an isotope label in carbon dioxide requires the unambiguous identification of possibly overlapping signals, Raman spectra (90% <sup>18</sup>O) sealed in quartz glass capillaries. The the arrows and the isotopologue at its end. Raman spectra acquired allowed not only the



The Raman peak positions between 1180 – 1420  $\rm cm^{\text{--}1}$  for 6 of have been acquired for mixtures of  ${}^{13}C^{16}O2$ , the 12 isotopologues of carbon dioxide gas. Note that some  $^{12}C^{16}O_2$  and  $H_2^{-16}O$ ,  $H_2^{-17}O$  (90%  $^{17}O$ ) and  $H_2^{-18}O$  signals overlap, only three overlapping signals are depicted by

identification of the signals of all 12 isotopologues of carbon dioxide, but documented the isotope exchange during the equilibration of CO<sub>2</sub> and H<sub>2</sub>O. Previously, only data for 6 isotopologues have been published [e.g. 1, 2].

The analyses were carried out with a dedicated Raman system. Four optical heads, that could be positioned and moved in front of quartz glass or sapphire windows or capillaries, are connected to the Raman spectrometer by fibre optics. The signals of all four heads can be acquired at the same time. Therefore the simultaneous analysis of e.g. the gas phase, the aqueous phase, and the mineral phase in a gas-fluid-mineral system even at high pressures is possible. Data for the signals of all isotopologues of CO<sub>2</sub> with a discussion of the least overlapping signals to choose for quantification and results from a mineral dissolution study using an isotope labelling approach will be presented at the conference.

- [1] Irmer, G.; Graupner, T.: Isotopes of C and O in CO2: a Raman study using gas standards and natural fluid inclusions. Acta Universitas Carolinae - Geologica, 46: 1-2.
- [2] Windisch, C.F.; Glezakou, V.-A.; Martin, P. M.; McGrail, B. P.; Schaef, H. T.: Raman spectrum of supercritical C<sup>18</sup>O, and re-evaluation of the Fermi resonance. Phys. Chem. Chem. Phys. 14: 2560-66.

# Development of international N<sub>2</sub>O reference materials for site preference measurements

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Nitrous oxide (N<sub>2</sub>O) is one of the most important greenhouse gases in the Earth's atmosphere today, as well as a strong stratospheric ozone-depleting gas. The concentration of N<sub>2</sub>O has been rising since the Industrial Revolution due to changes in many of its sources, both natural and anthropogenic. Measurements of the four most abundant stable isotopologues of N<sub>2</sub>O ( $^{14}N^{14}N^{16}O$ ,  $^{15}N^{14}N^{16}O$ ,  $^{14}N^{15}N^{16}O$ , and  $^{14}N^{14}N^{18}O$ ) can provide a valuable constraint on source attribution of atmospheric N<sub>2</sub>O.

Recent advancements in Optical Isotope Ratio Spectroscopy (OIRS) allow for specific and high precision analysis of <sup>15</sup>N substitution in the central ( $\alpha$ ) and terminal ( $\beta$ ) nitrogen position and thus the <sup>15</sup>N site preference (SP=d<sup>15</sup>N<sup> $\alpha$ </sup> - d<sup>15</sup>N<sup> $\beta$ </sup>) in N<sub>2</sub>O. Commercial OIRS analyzers become increasingly available, complementing well-established Isotope Ratio Mass Spectrometry (IRMS), however, to date, no international standards with stated uncertainty exist that link d<sup>15</sup>N<sup> $\alpha$ </sup> and d<sup>15</sup>N<sup> $\beta$ </sup> to the AIR-N2 isotopic scale. In addition, calibration of OIRS analyzers requires N<sub>2</sub>O isotope standards in whole air at ambient amount fractions, to account for gas matrix effects and spectral interferences.

Within the framework of the EMPIR project "Metrology for Stable Isotope Reference Standards (SIRS)," we aim to develop such standards to be made available to the global community. These will be gaseous standards available as pure  $N_2O$  or  $N_2O$  diluted in whole air. To tie the  $d^{15}N^{\alpha}$  and  $d^{15}N^{\beta}$  values to the AIR-N2 scale, we are synthesizing  $NH_4NO_3$  salts with a range of  $d^{15}N-NO_3$  and  $d^{15}N-NH_4$  values, each of which will be determined precisely by an international group of laboratories. Each salt will then be thermally decomposed to achieve a quantitative conversion of  $NH_4NO_3$  to  $N_2O$ , thus providing several tie-points directly to the internationally accepted AIR-N2 isotope scale.

Here, we present the details of this aspect of the EMPIR project and specifically the methods described above.

# An examination of influences on the relationship between laser- and mass spectrometer-determined stable isotope values for carbon dioxide in plant experiments

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Lasers offer many advantages over mass spectrometers for use in plant physiological experiments. They provide continuous readings, good precision and relative ease of use and maintenance. But they also have some problems, such as being sensitive to the presence of organic molecules. The behaviour of lasers compared with mass spectrometers is also very different, requiring alternative

approaches to calibrate the laser instrument. These corrections are usually individualised depending on the experimental application. In particular we have found that concentration effects on analysis of gases are in no sense linear and that the effect on isotope analyses of mixes of gases can prove challenging to interpret: for example varying proportions of nitrogen, oxygen and water vapour will change the apparent composition of carbon dioxide mixed with them.



An example is shown in the figure where the concentration dependence of an enriched carbon dioxide is shown above an unenriched one; both curves normalised to their minimum delta. The enriched gas shows a greater delta increase with increased concentration than does the unenriched gas. The situation is complicated by the fact that the dependence is not linear: if the enrichment of a third gas was twice that of the enriched one here, the concentration dependence would not be twice as great.

We want to improve our understanding of what causes the changing responses of isotopologue concentrations to isotopic, chemical and concentration variation - even though we are able to produce highly precise and accurate data through polynomial correction curves developed from gases of known isotopic composition. In an attempt to quantify the nature of change of the laser assessed isotope values we have created maps of concentration on the X axis and delta on the Y axis. The true composition of the gas is known from the proportion of each of two gases with well tested delta values. We are then able to map the offset between the laser-reported composition of the gas and its true value as Z. The surfaces that result are not simple planes. This is perhaps not unexpected as there are no straight lines in the data: for example, the concentration dependence only appears linear over short segments but is typically described by a high-order polynomial for greater concentration spans. Errors in tracking the apparent isotopologue concentrations appear trivial until they are converted to typical stable isotopic delta values and multiplied by 1000. As an example, at an approximately ambient CO<sub>2</sub> concentration of 400 ppm, an error in 628 (<sup>16</sup>O<sup>12</sup>C<sup>18</sup>O) of 0.1 ppm produces a change of roughly 50 ‰ in d<sup>18</sup>O! These effects on the apparent concentration of an isotopologue may result from self-broadening: the gas molecules interacting and influencing their own vibrational characteristics, although a number of other factors such as slightly poor frequency fitting and baseline errors

also contribute. We also experience foreign broadening where other gases influence molecular vibrations, for example concentration dependence measurements are only valid for a gas mix where the major proportions are similar: for this reason we use the same oxygen/nitrogen mix for reference gas pulses that we use for the experiment itself. Similarly, sampled air returning from a plant sampling chamber is dried before measurement to remove any influence of water.

Finally, we are trying to determine what factors influence measurements and which of them can be corrected using a physical understanding of the interactions between the gases and the laser. Is it possible that the laser influences its own reading of the gas? If the laser energy is only absorbed by bonds vibrating at the same frequency, do we induce thermal band-broadening in the very species that we are examining? If so, does the flow rate of gas through the laser chamber affect the magnitude of that effect? Does the chamber pressure affect the measurement of the isotopologues dissimilarly?

Poster presentations Topic 6: Earth/Planetary Sciences, Biogeochemistry and Ecology

# Nitrate $(NO_3^{-})$ and ammonium $(NH_4^{+})$ supply influence carbon isotope composition of respiratory substrates and $CO_2$ emissions from tobacco plants

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The effect of inorganic nitrogen species (NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>) on the carbon isotope composition of leaf and root metabolites and respired CO<sub>2</sub> is largely unknown. Thus, we determined physiological variables, concentrations and  $\delta^{13}$ C values of tricarboxylic acid cycle intermediates and dark respired CO<sub>2</sub> ( $\delta^{13}$ C<sub>R</sub>), together with activities of key enzymes like nitrate reductase (NR), malic enzyme (ME) and phosphoenolpyruvate carboxylase (PEPC) in tobacco plants supplied with a gradient of NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup> concentration ratios.

In the leaves, as NO<sub>3</sub><sup>-</sup> supply decreased and simultaneously NH<sub>4</sub><sup>+</sup> increased, activities of NR, ME and PEPC decreased. In addition, photosynthesis and dry biomass as well as concentrations of organic acids and starch decreased along this gradient. In contrast, respiration rate, concentrations of intercellular CO<sub>2</sub>, soluble sugars and amino acids increased with increasing NH<sub>4</sub><sup>+</sup> supply. In plants supplied with higher concentrations of NO<sub>3</sub><sup>-</sup>, respired CO<sub>2</sub> was comparatively enriched in <sup>13</sup>C and decreased along a decreasing NO<sub>3</sub><sup>-</sup> gradient. And in the roots, along the N supply gradient from NO<sub>3</sub><sup>-</sup> to NH<sub>4</sub><sup>+</sup>, concentrations of malate, citrate and carbohydrates decreased together with PEPC activity, while amino acids concentrations increased.  $\delta^{13}$ C values of sugars, starch, alanine, asparagine, serine and glutamine were comparatively more positive in NO<sub>3</sub><sup>-</sup> supplied plants compared to NH<sub>4</sub><sup>+</sup> supplied plants.

The strong influence of inorganic N species on plant physiology and biochemistry conveyed a <sup>13</sup>C enrichment in leaf respired CO<sub>2</sub> of plants dominantly fed with NO<sub>3</sub><sup>-</sup>. Combining the results of concentrations and enzymatic activities together with the isotopic analysis suggest that the dominant source of respired CO<sub>2</sub> in NO<sub>3</sub><sup>-</sup> supplied plants are anaplerotically synthesized organic acids.

Despite similarities in  $\delta^{13}$ C of root respired CO<sub>2</sub> ( $\delta^{13}$ C<sub>R</sub>), the respiratory metabolism (i.e.  $\delta^{13}$ C and concentrations of metabolites and enzymatic activities) was strongly influenced by N supply. In brief, sugars and malate were the main substrates fueling respiration in all plants, however, the contribution of sugars in NO<sub>3</sub><sup>-</sup> supplied plants and malate in NH<sub>4</sub><sup>+</sup> supplied plants seemed to be higher.

# Nutrient flow in a host-parasite system by compound-specific stable isotope analysis of amino acids via LC-IRMS

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Parasitism is one of the most frequent life forms on earth. However, the complex lifecycle of many parasites makes it difficult to incorporate them into classical food web models. Recent studies high-light the importance of stable isotope analysis when investigating host-parasite systems due to its inherent ability to trace the origin and flow of organic matter. Especially compound-specific isotope analysis (CSIA) is suggested as a promising tool to investigate individual classes of nutrients and thus deliver insights into metabolic pathways [1].

Amino acids (AA), as one important class of nutrients, play a fundamental role in the metabolism of all organisms and can be either synthesized *de novo* (non-essential AA) or must be taken up through diets (essential AA). Consequently, the isotopic carbon signature of essential AA is mostly retained throughout the food web as these molecules are directly incorporated into the tissue of animals, whereas non-essential AA will undergo isotopic fractionation when being built. This relationship has been used e.g. for the reconstruction of paleodiets by analyzing human keratin and collagen samples [2], or recently to compare the  $\delta^{13}$ C isotope signatures of blow flies and their carrion food source [3], but not yet to investigate the nutrient flow in a host-parasite system.

In this study, we applied CSIA of individual AA to three-spined sticklebacks (*Gasterosteus aculeatus*) experimentally infected with the tapeworm *Schistocephalus solidus* in order to shed light on the metabolic pathways which sustain the rapid growth of the parasite inside the host's body cavity. The tapeworm takes up enough nutrients to increase its body weight several thousand times without actively feeding on host tissues, as shown by previous bulk stable isotope analysis. Host tissues (muscle, liver), the food source of the stickleback (mosquito larvae) as well as the parasites were collected during the experiment between 30 and 120 days post infection and were subsequently analyzed by an LC-IRMS system to determine the  $\delta^{13}$ C signatures of AA in compliance with already developed methods [2].

- Sabadel, A. J. M.; Stumbo, A. D.; MacLeod, C. D. (2019): Stable-isotope analysis: a neglected tool for placing parasites in food webs. Journal of Helminthology 93, 1–7
- [2] Raghaven, M.; McCullagh, J. S. O.; Lynnerup, N.; Hedges, R. E. M. (2010): Amino acid d13C analysis of hair proteins and bone collagen using liquid chromatography/isotope ratio mass spectrometry: paleodietary implications from intra-individual comparisons. Rapid Commun. Mass Spectrom. 24: 541–548
- [3] Matos, M. P. V.; Konstantynova, K. I.; Mohr, R. M.; Jackson, G. P. (2018): Analysis of the 13C isotope ratios of amino acids in the larvae, pupae and adult stages of Calliphora vicina blow flies and their carrion food sources. Anal Bioanal Chem. 410 (30), 7943-7954

Poster presentations Topic 7: Environmental and Water Chemistry / Microbiology

# Nitrate-dependent anaerobic methane oxidation coupled to anaerobic ammonium oxidation in a stratified lake

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Numerous studies have shown that the most common nitrogenous contaminant in freshwater habitats is dissolved nitrate. The greenhouse gas methane represents a more potent climate gas than  $CO_2$ and is responsible for 20% of global warming. Lakes may represent "hot-spots" of methane production. The anaerobic oxidation of ammonium (anammox) and the nitrate-dependent oxidation of methane (*n-damo*) together may account for a highly effective nitrogen loss from aquatic environments by converting  $NO_3^-$ ,  $NO_2^-$ , and  $NH_4^+$  to harmless  $N_2$  while oxidizing the potent greenhouse gas methane to  $CO_2$ . Up to now the co-occurrence of anammox and *n-damo* was a mystery in natural environments. Here we found provide evidence of the coupled process in a stratified lake and identified the microorganisms linking both processes.

# Defining lower limits of biodegradation: Isotope fractionation in chemostat reveals rate-limiting mass transfer triggering physiological adaptation

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Biodegradation of persistent micropollutants like pesticides often slows down at low concentrations (mg/L) in the environment. Mass transfer limitations or physiological adaptation are debated to be responsible. Here we explore physiological adaptation and maintenance energy requirements of an herbicide (atrazine)-degrading microorganism (*Arthrobacter aurescens* TC1) while concomitantly observing mass-transfer limitations directly by compound-specific isotope fractionation analysis (CSIA).

Isotope analysis of atrazine in chemostat experiments with whole cells revealed a drastic decrease in isotope fractionation with declining residual substrate concentration from  $\epsilon^{13}C = -5.36 \pm 0.20$  ‰ at 82 µg/L to  $\epsilon^{13}C = -2.32 \pm 0.28$  ‰ at 32 µg/L. At 82 µg/L  $\epsilon^{13}C$  represented the full isotope effect of the enzyme reaction. At lower residual concentrations smaller  $\epsilon^{13}C$  indicated that this isotope effect was masked indicating that mass transfer across the cell membrane became rate-limiting. Retentostat cultivation, finally, resulted in complete mass-transfer limitation evidenced by the disappearance of isotope fractionation ( $\epsilon^{13}C = -0.45$  ‰  $\pm 0.36$  ‰). Chemostat-based growth therefore triggered the onset of mass-transfer limitation at residual concentrations of 30 µg L<sup>-1</sup> of atrazine with a bacterial population doubling time (td) of 14 days whereas exacerbated energy limitation was induced by retentostat-based near-zero growth (td = 265 days) at 12  $\pm$  3 µg L<sup>-1</sup> residual concentration.

Retentostat cultivation also resulted in a two-fold decrease in maintenance energy requirement compared to chemostat cultivation. Proteomics revealed that retentostat and chemostat cultivation under mass-transfer limitation share low protein turn-over and expression of stress-related proteins. Mass-transfer limitation therefore effectuated slow-down of metabolism in retentostats and a transition from growth phase to maintenance phase indicating a limit of  $\approx 10 \ \mu g \ L^{-1}$  for long-term atrazine degradation. Further studies on other ecosystem-relevant microorganisms will explore the general applicability of our finding: that mass transfer limitation serves as a trigger for physiological adaptation which subsequently defines a lower limit of biodegradation.

- K. Kundu, S. Marozava, B. Ehrl, J. Merl-Pham, Ch. Griebler, M. Elsner, Defining lower limits of biodegradation atrazine degradation regulated by mass transfer and maintenance demand in Arthrobacter aurescens TC1, The ISME Journal (2019), https://doi.org/10.1038/s41396-019-0430-z
- B. Ehrl, K. Kundu, M. Gharasoo, S. Marozava, M. Elsner, Rate-Limiting Mass Transfer in Micropollutant Degradation Revealed by Isotope Fractionation in Chemostat, Environ. Sci. Technol. 53 (2019) pp. 1197–1205, DOI: 10.1021/acs. est.8b05175
- M. Gholami Gharasoo, B. Ehrl, O. Cirpka, M. Elsner, *Modeling of contaminant biodegradation and compound-specific isotope fractionation in chemostats at low dilution rates, Environ.* Sci. Technol. 53 (2019) pp. 1186–1196, DOI: 10.1021/acs.est.8b02498

## Studing co-metabolic oxidation of Trichloroethylene using $\delta^{13}C$ and $\delta^{37}Cl$ isotope analysis

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Trichloroethylene (TCE) is a carcinogenic organic chemical impacting water resource worldwide. Groundwater contamination by TCE, similarly to other contaminants, requires decision making to prevent pollution spread. In the case of TCE, biotic degradation is known to follow two distinctive

mechanisms, either by i) anaerobic reductive dechlorination, or by ii) aerobic co-metabolic degradation. While the reductive mechanism most often leads to the formation of characteristic transformation products that can be easily detected in the polluted groundwater, the aerobic co-metabolic degradation lacks indicative transformation products. Hence, aerobic co-metabolic degradation is more difficult to identify in contaminated sites. TCE breakdown by reductive vs. oxidative degradation involves different types of chemical bonds. Hence, if distinct isotope effects are reflected in dual element (carbon and chlorine) isotope values, such trends could help distinguishing



both processes in the environment. Following the knowledge gap for dual element isotope fractionation in TCE, carbon and chlorine isotopic effects for aerobic co-metabolic biodegradation were determined for pure cultures in a laboratory study. Experiments were conducted with toluene oxidizer strain *P. putida* F1 (Expressing toluene dioxygenase, TOD), and two methanotrophic strains *M*. trichosporium OB3b (expressing either soluble or particulated monoxygensase ,sMMO or pMMO), and *M. album* Bath (expressing sMMO) all known to co-metabolize TCE. Although it was hypothesized that aerobic co-metabolic degradation will not be accompanied by a chlorine isotopic effect, in analogy to abiotic permanganate oxidation, my results indicated a weak, but significant chlorine isotope effect for the methanotrophs ( $\epsilon^{37}$ Cl = 1.3±0.2 ‰, -2.9±0.5 ‰ and -2.4±0.4 ‰ for OB3b expressing sMMO, Bath expressing sMMO and OB3b expressing pMMO respectively). These findings were confirmed in an indigenous methanotrophic microbial community which was enriched from a contaminated site ( $\epsilon^{37}$ Cl = -2.9±0.9 ‰). The novel results demonstrated for the first time that the surprisingly high chlorine isotope enrichment is probably not unique for the specific strains tested, but rather may be representative for other strains as well. Dual element isotope trends were evaluated for the different strains and enzymes studied. The toluene oxidizer strain F1 ( $\delta^{13}C/\delta^{37}Cl = 38\pm 26\%$ ) presented a similar dual isotope to a formerly reported dual isotope plot for permanganate oxidation of TCE. Preliminary work using pure strain *P. mendocina* KR1 and enrichment cultures of ammonia and toluene oxidizers all shows similar trends to F1 strain. These differ significantly from reported trends for biotic and abiotic reduction processes. In contradiction to the toluene oxidizing strain F1, the methanotrophic strains (expressing either sMMO or pMMO) presented unique dual isotope enrichment with a slope of 1.2±0.4 ‰. This significant difference may stem from differences in slow mass limiting steps prior to catalysis or from mechanistic differences, and warrants further investigation. From an environmental point of view, since the isotope effect of the methanotrophs is relatively small, they may not contribute significantly to isotope shifts observed in groundwater. Thus, using dual isotope analysis can result in an under estimation of the overall co-metabolic process, if the methanotrophic contribution is excluded.

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# Carbon and nitrogen isotope fractionation along degradation of the nitrile herbicide Bromoxynil

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Bromoxynil, a brominated nitrile herbicide used mainly in field corn, wheat and barley has been classified as a possible human carcinogen and was also shown to be an endocrine disrupter impairing reproduction in birds, fish and aquatic invertebrates. In the environment, Bromoxynil is subjected to two distinct modes of degradation: abiotic degradation - mainly photodegradation, which takes place at the soil/plant surface, and biodegradation - aerobic and anaerobic - which takes place mainly in the subsurface. This study aimed to determine the isotope fractionation ( $\delta^{13}$ C,  $\delta^{15}$ N) associated with these distinct processes.

Challanged by the fact that bromoxynil is not GC-amendable, an offline purification method was first developed based on thin-layer chromatography. With the performance of the analytical method being confirmed, four sets of experiments were performed to assess the fractionation along different modes of bromoxynil degradation.

Photodegradation experiments were performed under natural conditions as well as in the laboratory under UV light (254 nm). Microbial degradation experiments were conducted under anaerobic and aerobic conditions using enrichment culures and local soil.



Bulk enrichment factors ( $\varepsilon_{bulk}$ ) calculated for photodegradation under natural condition imply insignificant carbon ( $\varepsilon_{c}$ =0.34±0.44 ‰) and normal nitrogen isotope fractionation ( $\varepsilon_{N}$ =-3.70±0.30 ‰), while under laboratory conditions enrichment factors denote a strong inverse carbon ( $\varepsilon_{c}$ =4.74±0.82 ‰) and a weak inverse nitrogen ( $\varepsilon_{N}$ =0.76±0.12 ‰) isotope fractionation. In view of these differences, and following the detected products, two different mechanisms are proposed – homolythic debromination followed by singlet-triplet converssion under laboratory conditions and free radical formation followed by homolythic debromination under natural conditions.

In the biological experiments bulk enrichment factors show negligible isotope fractionation for both carbon ( $\epsilon_{\rm C}$ =-0.11±0.52 ‰) and nitrogen ( $\epsilon_{\rm N}$ =0.18±0.15 ‰) for the anaerobic culture; the insignificant carbon isotope effect despite the fact that dehalogenation has occured may be the result of masking. The aerobic culture showed negligible carbon ( $\epsilon_{\rm C}$ =0.37±0.36 ‰) and a strong invers nitrogen ( $\epsilon_{\rm N}$ =10.56±0.36 ‰) fractionation; similar results formerly reported for an analogue pathway in dichlobenil were explained by formation of additional bond in the transition state [1].

When plotting the carbon and nitrogen stable isotope results of all four sets of experiments on a dual-isotope plot ( $\Delta^{13}$ C vs.  $\Delta^{15}$ N), a clear difference is evident between trends, enabling to distinguish between the four processes.

## References

 Reinnicke, S., Simonsen, A., Sørensen, S.R., Aamand, J., Elsner, M., 2012. C and N isotope fractionation during biodegradation of the pesticide metabolite 2, 6-dichlorobenzamide (BAM): potential for environmental assessments. Environ. Sci. Technol. 46, 1447-1454.

# Multidimensional stable isotope fingerprinting as a tool to identify the origin of the organophosphorus pesticide chlorpyrifos

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Chlorpyrifos belongs to the group of organophosphorus pesticides (OPs) which are a diverse family of insecticides in use since the 1940s. It acts on the nervous system of insects by inhibiting the acetylcholinesterase and it is one of the most widely used organophosphate pesticides in agriculture. Chlorpyrifos is considered moderately hazardous to humans by the World Health Organization (WHO 2003). The continuous and excessive use of chlorpyrifos paired with its toxicity and persistence in nature has led to significant environmental contaminations. As they have repeatedly been detected in soils, sediments, waterbodies, as well as within food and drinking water, public concerns are increasing (Solomon et al. 2014). Thus, tools are needed to understand sources, reactive transport pathways and sinks of chlorpyrifos in the environment.

Multidimensional stable isotope fingerprinting is a valuable method for the characterization of the provenance of chemicals as the isotopic profile reflects the isotopic composition of raw materials, synthetic pathways and purification processes (Gilevska, Gehre & Richnow 2015). To evaluate this approach, pure batches and commercial formulations of chlorpyrifos were collected from more than 30 manufactures in India, China and Germany. These were isotopically characterized using Elemental Analyzer – Isotope Ratio Mass Spectrometry (EA-IRMS) for analyzing the  $\delta^{13}$ C,  $\delta^{2}$ H,  $\delta^{18}$ O and  $\delta^{15}$ N isotopic composition and Gas Chromatography – Multiple Collector – Inductively Coupled Plasma Mass Spectrometry (GC-MC-ICPMS) for determining the  $\delta^{37}$ Cl isotopic signature (Horst et al. 2017).

The isotopic composition of analyzed chlorpyrifos samples varied for  $\delta^{13}$ C from -31.9 to -24.1 ‰, for  $\delta^{2}$ H from -271 to -157 ‰, for  $\delta^{18}$ O from -0.9 to 24.6 ‰, for  $\delta^{15}$ N from -3.4 to -0.1 ‰ and for  $\delta^{37}$ Cl from -1.3 to 3.1 ‰. Taking the typical uncertainties of 0.5 ‰ for  $\delta^{13}$ C, 5.0 ‰ for  $\delta^{2}$ H, 0.5 ‰ for  $\delta^{18}$ O, 0.3 ‰ for  $\delta^{15}$ N and 0.3 ‰ for  $\delta^{37}$ Cl into account the combination of different isotopic signatures gives a unique fingerprint to track sources in the environment. Thus, this study highlights the potential of multidimensional stable isotope profiling to identify sources, and provides an isotopic database of chlorpyrifos that might improve the tracing of origin, transport pathways and the environmental fate.

- [1] WHO: Chlorpyrifos in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva, World Health Organization, WHO/SDE/WSH/03.04/87, 2003.
- [2] Solomon, K.R.; Williams, W.M.; Mackay, D.; Purdy, J.; Giddings, J.M.; Giesy, J.P.: Properties and uses of chlorpyrifos in the United States. Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews), vol 231. Springer, Cham, 2014.
- [3] Gilevska, T.; Gehre, M.; Richnow, H.H.: Multidimensional isotope analysis of carbon, hydrogen and oxygen as tool for identification of the origin of ibuprofen. Journal of Pharmaceutical and Biomedical Analysis, (2015) 115, 410-417. doi: 10.1016/j.jpba.2015.07.030.
- [4] Horst, A.; Renpenning, J.; Richnow, H.H.; Gehre, M.: Compound Specific Stable Chlorine Isotopic Analysis of Volatile Aliphatic Compounds Using Gas Chromatography Hyphenated with Multiple Collector Inductively Coupled Plasma Mass Spectrometry. Analytical Chemistry, (2017) 89, 9131-9138. doi: 10.1021/acs.analchem.7b01875. Epub 2017 Aug 24.

## Mechanistic dichotomy in bacterial trichloroethene dechlorination revealed by carbon and chlorine isotope effects

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Chlorinated ethenes like tetrachloroethene (PCE) and trichloroethene (TCE) are significant groundwater contaminants. Microbial reductive dehalogenation of these substances at contaminated sites

can produce nontoxic ethene but often stops at toxic cis-1,2-dichloroethene (cis-DCE) or vinyl chloride (VC). To understand the underlying controls on the differences in reactivity and reaction (bio)chemistry, compound-specific carbon ( $\delta^{13}$ C) and chlorine ( $\delta^{37}$ Cl) isotope fractionation of TCE and *cis*-DCE can be analyzed. The magnitude of carbon relative to chlorine isotope effects (as expressed by  $\Lambda_{C/CI}$ , the slope of  $\delta^{13}$ C versus  $\delta^{37}$ Cl regressions) was recently recognized to reveal different reduction mechanisms with vitamin  $B_{12}$  as a model reactant for reductive



dehalogenase activity. Large  $\Lambda_{C/Cl}$  values for *cis*-DCE reflected cob(I)alamin addition followed by protonation, whereas smaller  $\Lambda_{C/Cl}$  values for PCE evidenced cob(I)alamin addition followed by Cl–elimination. This study addressed reductive dehalogenation in actual microorganisms and observed identical large  $\Lambda_{C/Cl}$  values for *cis*-DCE ( $\Lambda_{C/Cl} = 10.0$  to 17.8) that contrasted with identical smaller  $\Lambda_{C/}$  for TCE and PCE ( $\Lambda_{C/Cl} = 2.3$  to 3.8). For TCE, the trend of small  $\Lambda_{C/Cl}$  could even be reversed when mixed cultures were precultivated on VC or DCEs and subsequently confronted with TCE ( $\Lambda_{C/Cl} = 9.0$  to 18.2). This observation provides explicit evidence that substrate adaptation must have selected for reductive dehalogenases with different mechanistic motifs. The patterns of  $\Lambda_{C/Cl}$  are consistent with practically all studies published to date, while the difference in reaction mechanisms offers a potential answer to the long-standing question of why bioremediation frequently stalls at *cis*-DCE.

### References

Lihl, C.; Douglas, L.M.; Franke, S.; Pérez-de-Mora, A.; Meyer, A.H.; Daubmeier, M.; Edwards, E.A.; Nijenhuis, I.; Sherwood Lollar, B.; Elsner, M.: Mechanistic Dichotomy in Bacterial Trichloroethene Dechlorination Revealed by Carbon and Chlorine Isotope Effects. Environmental Science & Technology 2019 53 (8), 4245-4254.

# Differentiating sorption and degradation of phosphonate complexing agents using liquid-chromatography/ isotope-ratio mass spectrometry

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Polyphosphonic acids are strong complexing agents for di- and trivalent cations with growing commercial importance, as they are increasingly used to replace polyphosphates and aminopolycarboxylates in industrial and household applications. Although sorption is believed to be the most important process for polyphosphonate removal from the aqueous phase, only little is known about the behavior of polyphosphonates in natural and technical systems and the significance of degradation processes for their fate (Nowack, 2003; Rott et al., 2018).

A promising technique to elucidate these processes is compound specific stable isotope analysis (CSIA), which has become a versatile tool in environmental chemistry in the last two decades for differentiating sources and proving degradation of environmental contaminants (Elsner and Imfeld, 2016). Nowadays, carbon CSIA of polar, non-volatile compounds such as polyphosphonates

is possible owing to the recent development of an interface to couple liquid-chromatography and isotope-ratio mass spectrometry (LC-IRMS) (Krummen et al., 2004).

We developed a LC-IRMS method for carbon CSIA of the three polyphosphonates 1-hydroxyethane 1,1-diphosphonic acid (HEDP), amino tris(methylenephosphonic acid) (ATMP) and ethylenediamine tetra(methylenephosphonic acid) (EDTMP) and applied it to determine carbon isotope fractionation in sorption and degradation experiments.

Separation of the analytes was conducted on an anion exchange column. Limits for precise iso-

Sorption of ATMP → Sorption of ATMP onto goethite

Degradation of ATMP

tope analysis were in the range of 25 mg/L to 30 mg/L (200 ng carbon on column) and thus comparable to published values for the monophosphonates glyphosate and aminomethylphosphonic acid (AMPA) (Kujawinski et al., 2013).

In preliminary experiments, we additionally determined carbon isotope fractionation for ATMP during manganese catalyzed degradation by molecular oxygen, as well as during sorption onto goethite. A kinetic isotope effect was associated with the investigated reaction pathway, whereas no detectable isotope fractionation could be observed during sorption. Thus, carbon CSIA is suitable to assign a decrease of the concentration either to sorption or degradation within the tested conditions.

The developed method allows for the first time compound specific stable carbon isotope analysis of synthetic polyphosphonate complexing agents. The results of our preliminary sorption and degradation experiments highlight the potential of CSIA using LC-IRMS to investigate the environmental fate of polyphosphonates.

#### References

- Elsner, M., Imfeld, G., 2016. Compound-specific isotope analysis (CSIA) of micropollutants in the environment current developments and future challenges. Curr. Opin. Biotechnol. 41, 60–72.
- Krummen, M., Hilkert, A.W., Juchelka, D., Duhr, A., Schlüter, H.J., Pesch, R., 2004. A new concept for isotope ratio monitoring liquid chromatography/mass spectrometry. Rapid Commun. Mass Spectrom. 18, 2260–2266.
- Kujawinski, D.M., Wolbert, J.B., Zhang, L., Jochmann, M.A., Widory, D., Baran, N., Schmidt, T.C., 2013. Carbon isotope ratio measurements of glyphosate and AMPA by liquid chromatography coupled to isotope ratio mass spectrometry. Anal. Bioanal. Chem. 405, 2869–2878.

Nowack, B., 2003. Environmental chemistry of phosphonates. Water Res. 37, 2533-2546.

Rott, E., Steinmetz, H., Metzger, J.W., 2018. Organophosphonates: A review on environmental relevance, biodegradability and removal in wastewater treatment plants. Sci. Total Environ. 615, 1176–1191.

# <sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N isotope analysis of Desphenylchloridazon to investigate its environmental fate in a systematic field study

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Desphenylchloridazon (DPC), the main metabolite of the herbicide chloridazon (CLZ), is frequently detected in water bodies at concentrations exceeding 10  $\mu$ g/L. When assessing DPC degradation in the environment, estimates can be biased if based on metabolite-to-parent-compound concentration ratios alone, in particular when the formation and transformation of the metabolite are occurring simultaneously. In this study, <sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N compound-specific stable isotope analysis (CSIA) was therefore explored for its ability to deliver complementary evidence of DPC degradation. To investigate the fate of DPC, a lysimeter study was conducted considering three scenarios deconvolving formation and transformation processes of DPC. In the first scenario, DPC was directly applied to the lysimeter surface. In this case, we found that CSIA provided evidence that there are two distinct DPC transformation processes - one process results in significant changes in the  ${}^{13}C/{}^{12}C$  isotope ratio but not in the <sup>15</sup>N/<sup>14</sup>N isotope ratio, while another one leads to changes in both <sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N isotope ratios. In the second scenario, CLZ was applied to the lysimeter surface. This case was mimicking a realistic field scenario. The results showed that during DPC formation, <sup>13</sup>C/<sup>12</sup>C isotope ratios of DPC were depleted in <sup>13</sup>C relative to CLZ, while <sup>15</sup>N/<sup>14</sup>N isotope ratios remained constant. The third scenario simulated the preferential flow. To this end, CLZ was injected below the vadose zone. Here, the influence of the topsoil on the retention of DPC was observed. In general, we found that the combination of such a systematic lysimeter study with CSIA as a complementary method to classical concentration-based methods, enabled new insights into the evaluation of DPC transformation in the field.
## Coupling of BONCAT (bioorthogonal noncanonical amino acid tagging) and SERS (surface enhanced Raman scattering) for characterization of active cells

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The stable isotope probing is known to be an efficient tool for active cells analysis, however exploring environmental samples it may disturb the studied system [1]. Most of other current techniques do not disclose who is really metabolically active (gene sequencing, electron and fluorescence microscopy), are expensive or reveal active cells in time-consuming and cost-intense mode with limitations to explore only cultured microorganisms (immunofluorescence analysis). Visualization and characterization of active cells is important for understanding of underlying processes in cell communities, e.g. biodegradation of pollutants by microorganisms, infectious disease development, immune response by lymphocytes, etc. Active cells detection is complicated because of their coexisting in communities next to dead and dormant cells.

The aim of our study is to exploit bioorthogonal noncanonical amino acid tagging (BONCAT) in order to: i) label metabolically active microorganisms in vivo with specific amino acid analogues (AAAs); ii) use click chemistry for coupling these amino acids to the metal nanoparticles (NPs); iii) take advantage of this close proximity to NPs for non-targeted visualization and characterization of active cells by surface-enhanced Raman scattering (SERS). SERS is a method based on Raman microspectroscopy that uses metal (Ag or Au) NPs to enhance the Raman signal by orders of magnitude up to 104 and higher in a rapid and non-destructive way [2].

The Incorporation of tagged AAAs by the atrazine degrader Arthrobacter aurescens TC1 was verified by fluorescent microscopy. The efficiency of the AgNPs synthesis and their distribution on the bacterial cell surface was approved using scanning electron microscopy. Raman microspectroscopy and SERS analysis with AgNPs revealed no changes in bacterial cells incorporating AAAs in media with atrazine compared to the control cells. The alkyne-modified compound 5-(1,2-dithiolan- 3-yl)-N-(prop-2-ynyl) pentamide for modifying AgNPs was synthesized according to Shi [3]. Modified AgNPs were deposited on the bacterial surface by click reaction for the SERS analysis. Obtained results indicate that incorporation of AAAs does not affect Raman characteristics of studied cells so that the tool based on BONCAT is suitable for the single-cell analysis of bacteria. Coupling BON-CAT and SERS via modified NPs can enable us to detect and characterize active cells within different microbial communities. Simultaneously this approach will be confirmed by using stable isotope Raman microspectroscopy based on detection of incorporated 13C6-D-glucose and D2O into the cell biomass [2].

- [1] Berry D., Mader E., Lee T-K, et al., Tracking heavy water (D2O) incorporation for identifying and sorting active microbial cells. PNAS. 112(2), 194-203 (2015).
- [2] Ivleva N. P., Kubryk P., Niessner R.: Raman microspectroscopy, surface-enhanced Raman scattering microspectroscopy, and stable-isotope Raman microspectroscopy for biofilm characterization. Anal Bioanal Chem. 409, 4353-4375 (2017).
- [3] Shi L., Jing C., Ma W. et al.: Plasmon resonance scattering spectroscopy at the single-nanoparticle level: real-time monitoring of a click reaction. Angew. Chem. 125(23), 6127-6130 (2013).

## Sources of uncertainty in biotransformation mechanistic interpretations and remediation studies using CSIA

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Compound specific isotope analysis (CSIA) has become an established method in contaminant hydrogeology to identify and quantify the transformation of contaminants in groundwater. In recent years, there has been an increase in the number of studies utilizing dual-isotope plots to infer transformation mechanisms of contaminants in the laboratory and the field. The slope of the linear regression of a dual isotope plot, lamda ( $\lambda$ ), is used to infer transformation mechanisms through comparison with other  $\lambda$ s. Interpreting contaminant transformation mechanisms can facilitate the optimization of these reactions in a remediation strategy for a contaminated site. While  $\lambda$  is an important and widely used parameter by practitioners applying CSIA in contaminant hydrogeology, the calculation of  $\lambda$  and its associated uncertainty can vary across different studies and laboratories. Furthermore, an investigation has not been undertaken to date to evaluate the accuracy of  $\lambda$  calculated from linear regression and the associated estimates of uncertainty. In this work, we use real and modified datasets to assess potential sources of uncertainty in estimates of  $\lambda$  by comparing three methods of regression, ordinary least squares regression (OLR), reduced major axis regression (RMA), and the York method. Results from this work demonstrate that the York method is the most robust regression method for datasets with a range of experimental conditions, the most important of which being the ratio of analytical error between the two isotope systems and the range of values for each system. These findings highlight key factors to consider that affect the reliability of interpretations using  $\lambda$  and are used to provide recommendations for standardizing, calculating and interpreting dual-isotope data.

## Bioavailability as bottleneck for biodegradation of organic micropollutants in groundwater? – Evidence from compound-specific isotope analysis in two-dimensional tank experiment

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In groundwater, organic pollutants persist at very low concentrations (sub- $\mu$ g/L), although many of them are in principle biodegradable. Bioavailability limitation is one of the factors hypothesized to constitute bottlenecks of micro-pollutant biodegradation. Compound-specific isotope analysis (CSIA) has been brought forward as a promising approach to explore the onset of bioavailability limitation in biodegradation at low-level concentrations <sup>[1]</sup>. The role of membrane permeability as a barrier for atrazine biodegradation at low concentrations has been highlighted in a recent batch study by applying CSIA <sup>[2]</sup>. However whether bioavailability limitation – i.e., rate-limiting mass transfer towards and into the microbial cell – inhibits micropollutant biodegradation in environmental systems has not yet been verified by direct observation in experiments mimicking realistic situations in groundwater.



Here we present applications of CSIA to study biodegradation of micro-pollutants in a two-dimensional sediment tank experiment as a groundwater model system. A solution of 2,6-dichlorobenzamide (BAM) at its natural isotopic abundance was continuously injected into the middle of the tank, and a gradient of high to low concentrations of BAM was established by transverse dispersion. First, abiotic experiments were conducted to study the influence of hydrodynamic dispersion on observable isotope fractionation. Subsequently *Aminobacter* MSH1 was inoculated into the tank for "biotic" experiments. We took samples at different ports at the end of the tank to draw a picture of vertical distributions of concentration, biomass, isotope values etc.

Our results show that isotopic fractionation of BAM ( ${}^{13}C/{}^{12}C$  and  ${}^{15}N/{}^{14}N$ ) induced by hydrodynamic dispersion was negligible in the abiotic experiment. In biotic experiments, the highest isotope fractionation appeared in the fringes where O<sub>2</sub> and BAM were mixed. In the area near the top and bottom of the tank, in contrast, where BAM concentrations were lowest and where BAM degradation would, therefore, be expected to be most complete, observable isotope fractionation did not further increase, but started declining. We hypothesize that these results reflect the effect of bioavailability limitation during biodegradation. When concentrations of BAM were lower than a certain threshold concentration, mass transfer of BAM towards and into the cell became rate-limiting relative to the enzyme reaction inside the cell. Consequently, observed isotope fractionation decreased. Hence, the observed decrease of isotope fractionation at low-level concentrations in the gradient of a sediment tank may provide the first direct evidence of bioavailability limitation in a groundwater model system.

- [1] Thullner, M., Kampara, M., Richnow, H.H., Harms, H., Wick., L.Y. Impact of bioavailability restrictions on microbially induced stable isotope fractionation. 1. Theoretical calculation. Environ Sci Technol., 2008.
- [2] Ehrl, B., Gharasoo, M., Elsner, M. Isotope fractionation pinpoints membrane permeability as a barrier to atrazine biodegradation in gram-negative Polaromonas sp. Nea-C. Environ Sci Technol., 2018.

#### Isotopic effect of ozonation of specific reaction sites

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The number of detected micropollutants in the water cycle has increased over the last years. One option to remove those compounds is ozonation. Ozone has a broadband action against many different classes of micropollutants but does not lead to complete mineralization. Instead transformation products are formed. [1] Which transformation products are formed depends on the reaction mechanism that in turn depends on the reaction site. The important reaction sites for ozone are C-C double bonds, C-C triple bonds, aromatic rings and amines. [2] A way to characterize the reaction site of a micropollutant is the determination of the specific isotope fractionation. In most cases, the lighter isotope (e.g. <sup>12</sup>C) reacts faster with ozone than the heavier isotope (e.g. <sup>13</sup>C). Hence, heavier isotopes are enriched in the remaining parent compound and this can be measured by compound specific stable isotope analysis (CSIA) [3]. The aim of this work is to determine the isotope fractionation of specific reaction sites, e.g. double bond (3-buten-2-ol), triple bond (3-butyl-2-ol) and aromatic ring (phenol). Different concentrations of ozone were added to the reaction solution (compound and phosphate butter (pH 7)). For the measurement with SPME-GC-IRMS 2 g/10 mL of NaCl was added to each sample.

The results show no difference in the enrichment factor between double ( $\varepsilon = -5.62 \%$ ) and triple bonds ( $\varepsilon = -5.88 \%$ ). This suggests that the primary attack of ozone is the same and the resulting reaction mechanisms are similar. For double bonds the Criegee mechanism is favored [4]. Thereby, a cleavage of C-C-bond happens. In the case of triple bonds the reaction might be similar but the C-Cbond is not completely broken. However, there is a strong difference to phenol ( $\varepsilon = -1.06 \%$ ). The enrichment effect is very small, which indicates that ozone hardly differentiates between light and heavy isotopes. The reason for this could be the attack on the whole ring and not at a specific point. This is caused by the additional electron pair from the hydroxyl group, which can be shifted to the ring and strongly activates the aromatic system [5].

- von Gunten, U., Oxidation Processes in Water Treatment: Are We on Track? Environmental Science & Technology, 2018. 52(9): p. 5062-5075.
- [2] Maier, M.P., Prasse, C., Pati, S.G., Nitsche, S., Li, Z., Radke, M., Meyer, A., Hofstetter, T.B., Ternes, T.A., and Elsner, M., *Exploring trends of C and N isotope fractionation to trace transformation reactions of diclofenac in natural and engineered systems*. Environmental Science & Technology, 2016. **50**(20): p. 10933-10942.
- [3] Elsner, M., Zwank, L., Hunkeler, D., and Schwarzenbach, R.P., A new concept linking observable stable isotope fractionation to transformation pathways of organic pollutants. Environmental Science & Technology, 2005. 39(18): p. 6896-6916.
- [4] Zhang, X.-M. and Zhu, Q., Olefinic ozonation electron transfer mechanism. The Journal of Organic Chemistry, 1997.
  62(17): p. 5934-5938.
- [5] von Sonntag, C. and von Gunten, U., Chemistry of ozone in water and wastewater treatment. 2012: IWA publishing.

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## Carbon stable isotope fractionation in the oxidation of sulfamethoxazole by ozone and chlorine dioxide

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The sulfonamide antibiotic sulfamethoxazole (SMX) is widely used in veterinary medicine (poultry-farming or aquacultures [1]) as preventive measure and is thus frequently detected in potential drinking water resources (surface or ground waters [1]). One option for removing SMX in drinking water treatment is oxidation which can be achieved for example by ozone or chlorine dioxide. However, corresponding reaction mechanisms are largely unknown, which are important to assess formation of undesired products.

Compound-specific stable isotope analysis (CSIA) can be used to investigate reactions of micropollutants such as SMX, since degradation processes may reveal specific isotopic fractionation which are related to the site of primary attack.

This study deals with the carbon stable isotope fractionation of SMX in reactions with ozone or chlorine dioxide at two different pH values (3 and 8) to investigate the oxidation of the neutral and the anionic SMX species, respectively. LC-IRMS measurements were performed as described previously by Kujawinski et al. [2].

For oxidation of the neutral SMX species at pH 3, a slight normal isotope effect is observed for both oxidative agents (Fig. 1a) [3]. In case of the anionic SMX species at pH 8 (Fig. 1b), isotopic fractionation is even slightly more pronounced for chlorine dioxide than at pH 3. Contrary, for oxidation of the anionic SMX species with ozone, the isotopic fractionation is not significant. Hence, under environmental conditions it would be possible to distinguish oxidation of SMX with ozone from oxidation with chlorine dioxide with CSIA [3]. Moreover, Dodd et al.[4] postulated that ozone either attacks at the activated aromatic ring or the nitrogen of the aniline moiety. Since hardly any carbon isotope fractionation was observed in case of the anionic SMX species, the oxidation at the nitrogen seems most likely.



Figure 1: Stable isotope fractionation of SMX in the reaction with ozone and chlorine dioxide at pH 3 and 8. Error bars represent standard deviation of triplicates. Samples were buffered with 10 mM phosphate buffer [3].

- [1] Jekel, M.; Dott, W.; Bergmann, A.; Dünnbier, U.; Gnirß, R.; Haist-Gulde, B.; Hamscher, G.; Letzel, M.; Licha, T.; Lyko, S.; Miehe, U.; Sacher, F.; Scheurer, M. Schmidt, C.K.; Reemtsma, T.; Ruhl, A.S. Selection of organic process and source indicator substances for the anthropogenically influenced water cycle. Chemosphere 2015, 125, 155–67.
- [2] Kujawinski, D.M.; Zhang, L.; Schmidt, T.C.; Jochmann, M.A., When other separation techniques fail: compound-specific carbon isotope ratio analysis of sulfonamide containing pharmaceuticals by high-temperature-liquid chromatography-isotope ratio mass spectrometry. Anal. Chem. 2012, 84 (18), 7656–63.
- [3] Willach, S.; Lutze, H. V.; Eckey, K.; Löppenberg, K.; Lüling, M.; Terhalle, J.; Wolbert, J.-B.; Jochmann, M. A.; Karst, U.; Schmidt, T. C. Degradation of sulfamethoxazole using ozone and chlorine dioxide Compound-specific stable isotope analysis, transformation product analysis and mechanistic aspects. Water Res. 2017, 122, 280–289
- [4] Dodd, M.C.; Buffle, M.-O.; von Gunten, U. Oxidation of antibacterial molecules by aqueous ozone: moiety-specific reaction kinetics and application to ozone based wastewater treatment. Environ. Sci. Technol., 2006, 40 (6), 1969– 1977

# Multi-elemental ( $\delta^{13}$ C, $\delta^{81}$ Br and $\delta^{37}$ Cl) isotope effects in biotic and abiotic degradation of 1-bromo-2-chloro-ethane

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A vast diversity of halogenated organic compounds produced in large quantities and applied in a variety of consumer and industrial products. Since many of halogenated compounds are resistant to degradation, they tend to accumulate in the environment and living organisms [1,2].

Hydrolytic dehalogenation and biodegradation are among the most possible environmental processes leading to the elimination of chlorinated and brominated aliphatic contaminants from the environment. Since C-Br bond is weaker than C-Cl bond, brominated aliphatic compounds are usually degraded easier. During the recent decades, degradation of 1,2-dichloroethane and 1,2-dibromorthane, extensively used in the past as additives in gasoline and as agriculture fumigants, has been investigated by several research groups [3, 4]. However, there is still a limited knowledge on the investigation of environmental fate of aliphatic compounds containing both chlorine and bromine atoms in the same molecule. The object of the present study was 1-bromo-2-chloro-ethane, one of the significant groundwater contaminants in Neot Hovav industrial area in the southern Israel.

The aim of the research was to investigate abiotic and biotic degradation processes of 1-bromo-2-chloroethane and evaluate isotope effects associated with these processes.

In the present work several degradation scenarios were investigated: abiotic hydrolytic degradation under alkaline conditions (pH 8), chemical oxidation by Fenton-like reagent, enzymatic dehalogenation by haloalkane dehalogenase (EC 3.8.1.5) and biotic reductive dehalogenation by culture of *Sulfurospirillum multivorans*.

Multi-elemental ( $\delta^{13}$ C,  $\delta^{81}$ Br and  $\delta^{37}$ Cl) Compound-Specific Isotope Analysis (CSIA) has been applied for tracing the degradation processes.

Abiotic degradation under alkaline conditions and enzymatic degradation by haloalkane dehalogenase followed hydrolytic debromination pathway. Both processes resulted in significant carbon isotope effects ( $\varepsilon_{C}$ , %= -27.2± 0.5 and  $\varepsilon_{C}$ , %= -29.9± 0.5, for abiotic and enzymatic process, respectively), small but detectable bromine isotope effect ( $\varepsilon_{Br}$ , %= -1.03± 0.03 and  $\varepsilon_{Br}$ , %= -1.3± 0.3 for abiotic and enzymatic process, respectively), and undetectable chlorine isotope effect.

For Fenton-like oxidation, only carbon isotope effect was observed ( $\epsilon_{c}$ , %= -3.9± 0.3). The lack of any measurable Br and Cl isotope fractionation possibly indicates that C-Br/C-Cl bond cleavage is not a rate-limiting step of the processes.

Biodegradation under anaerobic conditions resulted in significant carbon and bromine isotope effect ( $\varepsilon_{c}$  ‰= -15.6± 0.3;  $\varepsilon_{g,2}$  ‰= -1.5± 0.3). Analysis of chlorine isotope effect is still in progress.

Dual carbon-bromine isotope plot resulted in significantly different slopes:  $\Lambda_{C/Br} = 8$  for anaerobic biodegradation by *Sulfurospirillum multivorans*;  $\Lambda_{C/Br} = 21$  for abiotic hydrolysis;  $\Lambda_{C/Br} = 23$  for enzymatic hydrolytic dehalogenation and  $\Lambda_{C/Br} = \infty$  for Fenton –like degradation allowing to distinguish between degradation pathways. The results of the present study indicate that dual C-Br isotope plot can be a tool for tracking 1-bromo-2-chloro-ethane degradation in the environment.

- 1. Weisburger, E. (1977). Carcinogenicity Studies on Halogenated Hydrocarbons. Environmental Health Perspectives, 21, p.7.
- 2. De Wit, C. (2002). An overview of brominated flame retardants in the environment. Chemosphere, 46(5), pp.583-624.
- 3. Kuntze, K., Kozell, A., Richnow, H. H., Halicz, L., Nijenhuis, I., & Gelman, F. (2016). Dual Carbon–Bromine Stable Isotope Analysis Allows Distinguishing Transformation Pathways of Ethylene Dibromide. *Environmental Science & Technology*, *50*(18), 9855-9863.
- Palau, J., Cretnik, S., Shouakar-Stash, O., Höche, M., Elsner, M. and Hunkeler, D. (2019). C and Cl Isotope Fractionation of 1,2-Dichloroethane Displays Unique δ13C/δ37Cl Patterns for Pathway Identification and Reveals Surprising C–Cl Bond Involvement in Microbial Oxidation. *Environ. Sci. Technol.*, 2014, 48 (16), pp 9430–9437

### Application of multi-element compound-specific isotope analysis to complex contaminant mixtures in sediment samples

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Compound-specific isotope analysis (CSIA) is a powerful approach to demonstrate contaminant degradation in the subsurface and is often applied to volatile organic compounds (VOC) in ground-water samples. In sediments however, it can be challenging to recover sufficient amounts of VOC to perform CSIA, especially from strongly sorbing lower permeability units, which can form significant contaminant reservoirs. A prior extraction step is necessary to make mass spectrometric analysis possible. Different extraction agents, e.g. organic solvents are available for this task. A suitable solvent should be able to extract a wide range of contaminants and be water soluble to facilitate sample preparation. At the same time, using a less volatile solvent is beneficial as it is unlikely to interfere with the gas chromatographic (GC) separation and makes additional concentration steps easier.

We present results from the application of multi-element CSIA for demonstrating chlorinated and non-chlorinated hydrocarbon degradation in lower permeability layers. The study was carried out at a site where a complex mixture of chlorinated and petroleum hydrocarbons was disposed below ground decades ago, forming a downgradient plume in the heterogeneous sandy aquifer with diffusion occurring into a thin underlying aquitard. The DNAPL source was isolated from the active groundwater flow system by soil mixing with bentonite and zero-valent iron in 2008, and a study was initiated to evaluate in detail the plume response to this source treatment. As part of this study, the performance of different extraction agents to recover VOC from the aquitard sediments was evaluated. A new analytical method was developed relying on two-dimensional gas chromatography to resolve the complex mixture in the soil extracts sufficiently for precise and accurate carbon and chlorine isotope analysis. The method was applied to numerous depth-discrete sediment samples from the aquitard layer and also multi-level groundwater samples from the overlying aquifer. Multi-element CSIA makes it possible to identify the relative importance of different degradation pathways that lead to the same intermediate compound. Furthermore, it is possible to evaluate the contribution of reactive processes in the aquifer versus the aquitard to contaminant attenuation. Thus, multi-element CSIA helps to constrain reactive processes that control the long-term fate of chlorinated hydrocarbons at the site.

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