Environmental Technology

Newsletter | December 2023

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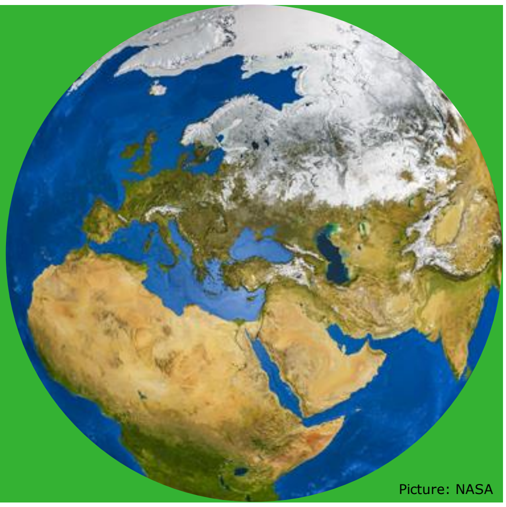


Picture: NASA

News

Original new format for EU-ISMET conference

During the sixth European Meeting of the International Society for Microbial Electrochemistry and Technology (ISMET), held in Wageningen last September, the participants were in for a surprise: the organizing committee had chosen for a totally different format for the conference, that should stimulate contacts between scientists.

The program showed shorter presentations and longer breaks allowing scientists to talk and interact. ETE's new Chair, Annemiek ter Heijne, initiated this new approach. She argued that the essence of a conference is meeting and exchanging information between scientists, and that a more relaxed and informal atmosphere would contribute. The invitation for the conference meeting previewed its unique design:

"The meeting will allow you to meet and interact with leading experts in the field and present your research in a unique setting featuring lively keynote presentations, interactive sessions, poster pitches, oral presentations, and social activities."

Interactive Kick-off

Redesign the conference set-up

ETE scientist Sanne de Smit, part of the organizing committee explains: 'Together with David Strik, Tom Sleutels, Philip Kuntke, Shih-Hsuan Lin and several PhD students, we tried to redesign the conference set-up to encourage more informal contact between scientists. Therefore, the traditional in-depth presentations held by the participants, were replaced by short, five-minute pitches, focusing on the essence of the research, followed by long, interactive breaks, where the details of the topic could be discussed in person.

Easy going interactions

Poster sessions were also replaced by a 'One Minute Madness', where researchers pitched their poster in just one minute, followed by informal discussions. The possibility for scientists to present their research in an alternative, more creative format, like a short movie, a play, or even a cartoon, added to easy-going interactions. 'To stimulate the contacts, the poster presenters gave colored stickers to the participants of the poster session', de Smit says. 'The person receiving most stickers was awarded the 'most interactive participant' prize.

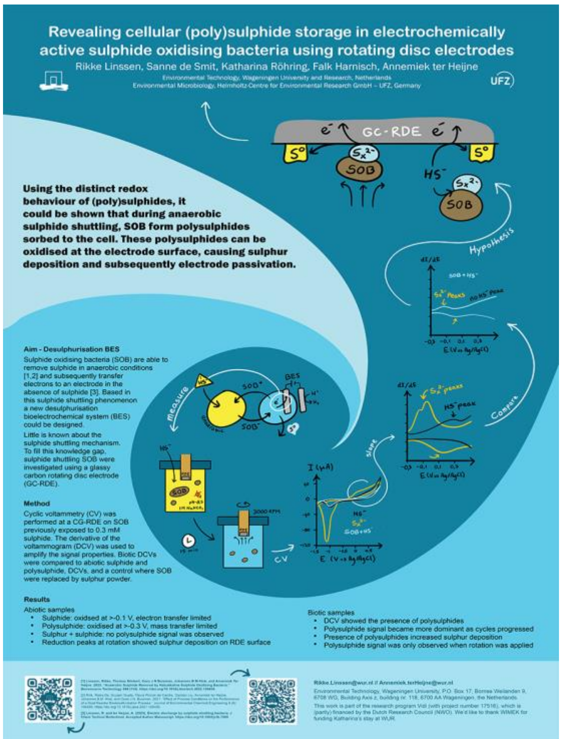
Innovation award for Xiaofang Yan

In-depth conversations

The new, creative conference format was received very well by the participants and interactions were indeed more effective. ‘Quite a few people said they were a bit skeptical at first, but really liked the new format, since it was more focused on having real in-depth scientific conversations’, de Smit says. ‘Due to the longer breaks, there was more conversation time and due to informal activities, like sport and pub quizzes, it was easier to connect to people.’

Funny and accessible

The appreciation for an ‘out of the box’ approach was also illustrated by the poster prize, that was awarded to ETE PhD researcher Rikke Linssen. ‘Her research deals with the bacterial conversion of hydrogen sulfide (H_2S) into solid sulfur’, de Smit says. ‘Linssen illustrated her complicated research in a funny and accessible cartoon that was obviously highly appreciated by the participants.’



Best Poster Award for the creative poster of Rikke Linssen

More appreciation

But there was more appreciation for the ETE scientists. Xiaofang Yan was awarded the prize for Best Oral Presentation as well as ISMET Innovation Award for the Best Technological Development for her research on ammonium (NH_4^+) oxidation. And also, organizing ETE scientist Sanne de Smit herself scored with the Discovery Award for Best Scientific Paper for her research on the role of metals in the bacterial conversion of CO_2 into fatty acids.

Awarded publications:

Xiaofang Yan

Xiaofang Yan, Dandan Liu, Johannes B. M. Klok, Sanne M. De Smit, Cees J. N. Buisman, and Annemiek ter Heijne: Enhancement of ammonium oxidation at microoxic bioanodes, 2023 *Environmental Science & Technology.*
*D*OI: https://doi.org/10.1021/acs.est.3c02227

Sanne M. De Smit

Sanne M. De Smit, Thomas D. van Mameren, Yiduo Xie, David P. B. T. B. Strik, Johannes H. Bitter: Trace metals from microbial growth media form *in situ* electro-catalysts, 2023 *Electrochimica Acta.*
DOI: https://doi.org/10.1016/j.electacta.2023.142722

New tenure tracker for ETE

Since March 2023, Gabriel Sigmund has joined ETE in a tenure track position focusing on sorption technologies to reduce contaminants in soil and water. Sigmund has a solid background in soil and water remediation and will now focus on the mobility of toxic substances.

‘One of my main interests is the behavior of so-called Persistent Mobile and Toxic substances, PMTs. Their mobility and persistence determine their fate in the environment as well as in engineered systems such as (waste)water treatment plants’, Sigmund explains. ‘I mainly focus on PMTs like pharmaceuticals, pesticides and also PFAS.’ Many of these compounds are highly mobile, and don’t stick easily to stuff. Often, they can form negatively charged ions, which are repelled by many surfaces, including negatively charged soil minerals and organic matter. Sigmund: ‘This results in a complex environmental behavior and generally a higher mobility.' Consequently, traditional removal methods like adsorption to activated carbon or sand filtration, can fall short. Therefore, new removal methods in (waste)water treatment are crucial to avoid that these compounds enter the environment.

Complex behavior

One of the focal points of Sigmund's research is to develop key expertise on the complex behavior of mobile, ionizable contaminants and study their sorption behavior. 'One of my research questions a is how strongly these PMT compounds will bind to different materials and how mobile they are', Sigmund explains. 'I will also develop screening methods to assess this mobility as well as the persistence of PMTs. My research also has a technological component, where I develop sorbents specifically designed to remove PMTs from a range of polluted waters.'

Great environmental costs

Currently, activated carbon is one the most widely used substances in water purification technologies. Generally, this material originates from coal or coconut shells. However, the sourcing and transportation of these materials has great environmental costs. Therefore, Sigmund aims to develop more sustainable carbon-based absorption materials, for example from the wood or cork fraction in waste streams. Sigmund: 'Using such alternative materials is an important step towards reducing the environmental footprint of water purification technologies.'

Science: Closing the plastic cycle: bioplastic conversion into chemical building blocks

Due to a push towards the production of more nature-based materials, biobased plastics will slowly replace traditional oil-based plastics. But effective recycling, closing cycles, is crucial to make these new plastics sustainable. To be future proof, ETE scientists David Strik heads a project where he and his team are developing effective bioplastic recycling. 'We have shown that microorganisms can effectively transform bioplastics into valuable chemical building blocks that can be reused.'

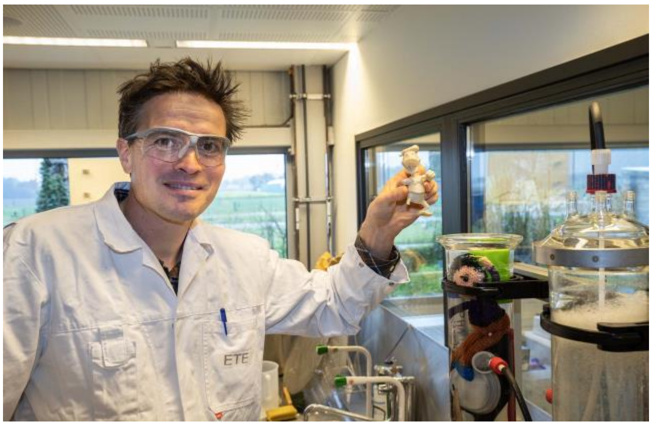
The lab at ETE sometimes holds some surprising features, but this is one of the most peculiar ever: a glass reactor column of about 50 centimeters high and 10 centimeters in diameter is stuffed with, what looks like, a pile of plastic waste: plastic toys, a spoon, a lid of a coffee cup and other undefined plastic garbage. A second column is filled with water, constantly aerated with stream of bubbles. Both columns are connected with plastic tubes, to allow a constant water exchange. 'This is our demonstration reactor, where we illustrate our new project about plastic recycling', ETE scientists David Strik says with a smile. He opens the reactor top and takes out a whitish plastic toy: 'This is Donald Duck. He is made from bioplastic. During several weeks, microorganisms in the reactor will completely break him down. Over time, all these plastic items will be completely degraded.' Although Donald's future looks quite grim, he plays an important role in the experiments of Strik, that may change the world of plastic recycling.

Solve the plastic issue

Plastics are an indispensable part of our society with a huge variety of applications. However, if not managed properly, they are also a substantial source of pollution for the environment like soils, water systems, oceans, our food system and even our own body. The increasing use of single-use, disposable plastics accelerates this problem. Dealing with disposed plastics still is a problem. Plastics are partly mechanically downcycled, where it loses part of its original value, for example, by turning various types of plastic foils into plastic granulates that are used to make plastic buckets. In addition, significant amounts of remaining plastic waste materials are incinerated leading to pollution. Therefore, a transition towards biobased plastics, those made from biomass, could solve the plastic issue, especially if effective recycling technologies can be developed. 'While plastics are very versatile and essential for society, the growing production of nature-based plastics with nature-based additives is eminent and needed', Strik states. In his project he aims to develop technologies that will effectively reuse the future big volumes of biobased plastics, closing cycles and minimizing waste. And the first successful results are already published.

Versatile bioplastics

Strik's project focuses on the most used biodegradable plastics. They all consist of polymers: long, chain-like molecules that consist of repeating, similar units, the so-called monomers. Polylactate (PLA)-based bioplastics are manufactured from lactic acid, originating from all kinds of plant-based streams, like sugars or other agri-food residues. The second type of biobased plastics are the poly-hydroxyl alkanoates (PHA), a very versatile family of bioplastics, made by microorganisms, that use it as energy storage. Finally, Strik is investigating, the so-called thermoplastic starch-based bioplastics, TPS used for bags or agricultural mulch films. Strik: 'We are focusing our research on the efficient reuse and recycling of biodegradable biobased PLA, PHA and TPS mixtures.'



Closing the cycle

Strik's aim is to develop a robust method to degrade the bioplastics into the so-called carboxylates. These chemicals are the building blocks of long-chained fatty acids, like caproate, acetate and butyrate. The industry can use these as raw materials to synthesize all kinds of chemicals, from lubricants, paints and also bioplastics, closing the cycle. The first step of Strik's method is to breakdown the mechanically grinded bioplastics using hydrolysis, where the long, molecules are broken down into monomers, like crotonate and 3-hydroxy-butyrate (Fig. 1). 'We aim to optimize reactor conditions, by tuning reactor temperature and pH for the best performance of a mixed microbial culture', Strik explains. 'This way we aim to achieve efficient chemical and biological hydrolysis of the microplastic mix.' The scientists also want to find out which processes and conversions take place at different pH and temperatures. Strik: 'This can be a real puzzle.'

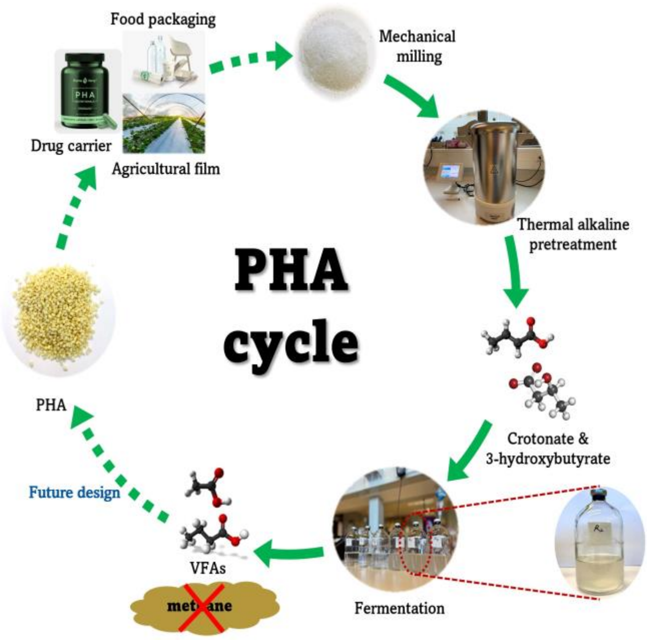


Fig. 1. Schematic representation of the degradation of bioplastics. After thermal and alkaline pretreatment (hydrolysis), mixed culture, anaerobic fermentation results in volatile fatty acids, that can subsequently be used for new bioplastic, or many other applications.

Important building blocks

After hydrolysis, the second step involves anaerobic fermentation of the hydrolyzed bioplastic (Fig. 1). 'In an open, mixed culture of microorganisms, obtained from a food waste fermentation process, the bioplastic monomers are fermented into a spectrum of carboxylates, important building blocks for many industrial products', Strik explains. 'One of the important conversions include the microbial transformation of lactate into acetate and propionate. These longer molecules also have more applications for the industry.'
Although the bioplastic waste streams are currently still too small to make large scale recycling a viable option, the first promising results from Strik's research show the proof of principle. 'We have convincingly shown that our methods works', he says. 'By using real bioplastic waste, consisting of a mixture of different bioplastics, we managed to eventually

synthesize carboxylates by using hydrolysis combined with microbial fermentation.' Bad news for plastic Donald Duck, whose fate looks grim, but he may be get a second life as another Disney figure or maybe a plastic spoon. And Donald's rebirth is great news for bioplastic recycling and a more efficient use of precious commodities in our society.

Selected publications:

Microbial Recycling of Polylactic Acid Food Packaging Waste into Carboxylates via Hydrolysis and Mixed-Culture Fermentation. Strik, P.B.T.B. and Heusschen B. 2023. *Microorganisms 11*(8), 2103. https://www.mdpi.com/2076-2607/11/8/2103

Microbial Recycling of Bioplastics via Mixed-Culture Fermentation of Hydrolyzed Polyhydroxyalkanoates into Carboxylates. Jin Y., de Leeuw K., and Strik, P.B.T.B. 2023. *Materials, 16*(7), 2693. https://www.mdpi.com/1996-1944/16/7/2693

Science: Cleaning oil-contaminated soil using bacteria and a bit of oxygen

Using microorganisms and a smart design, PhD researcher Dilan Aydin, developed a method to effectively clean soil, contaminated with oil-derived hydrocarbons, like benzene, toluene, ethylbenzene and xylene (BTEX). Aydin: 'We have shown that by creating a so-called micro-aerobic environment, where a little bit of oxygen is present, microorganisms can effectively clean hydrocarbon-contaminated soil.'

The Griftpark in Utrecht city is a popular recreation place, but 150 years ago, the green oasis looked completely different. For almost a century, a huge factory extracted gas from coal, that was used for lighting and heating. This process releases a cocktail of chemicals, including hydrocarbons, like BTEX. These waste chemicals were discharged into the soil or water As a result, the soil under the Griftpark still is contaminated with these compounds. During the 1950's, the plant was closed and in the 1990s, a first step to clean the contaminated soil was taken: the area was sealed by applying 30-meter-deep slurry walls around the site and the top soil was cleaned using microorganisms. Currently, contaminated ground water is continuously pumped to the wastewater treatment plant, four kilometers away. A yearly cost of 300.000 euro. 'The cleaning of Griftpark was one of the largest and most expensive cleaning operations in The Netherlands', Aydin says. 'And despite the efforts, the deeper soils are still highly contaminated.' This poses a serious risk to the deeper layer of ground water, since the contaminants are quite mobile and may easily seep from the first, contaminated aquifer through the porous clay layer and reach the deeper second aquifer, that is used for drinking water (fig. 1).

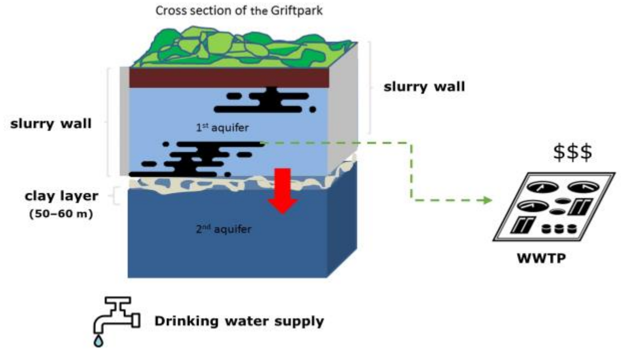


Fig. 1. Schematic representation of a cross section of Griftpark.

Utrecht municipality was looking for a more permanent solution and decided to fund a large cleaning operation, where different institutions, like Deltares and the University of Utrecht collaborate in project BestParc. ETE contributes with developing methods to enhance the microbial degradation of the contamination.

Make bacteria happy

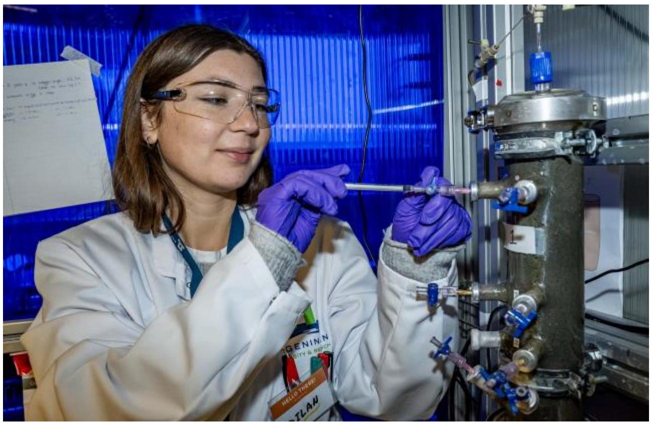
'The soil and groundwater pollution in Griftpark is slowly degraded by the microorganisms present', Aydin says. Especially in the top layers, oxygen is present and this helps the microorganisms to metabolize and breakdown the chemicals quicker.' But in the deeper soil layers, conditions are virtually anaerobic: no oxygen is present and the microorganisms use other compounds, like nitrate (NO_3^-), sulfate (SO_4^{2-}) and iron (Fe^{3+}) to replace oxygen. But these are less efficient to help in the bacteria's metabolism, resulting in a very slow breakdown. Therefore, a substantial risk that these chemicals spread to deeper layers exists. 'To stimulate and speed-up contaminant breakdown, my research focuses on how to make the bacteria happy, so they work more', Aydin says with a smile. 'So, we designed several lab experiments to find out how we could achieve that. The goal was not only to find a solution for Griftpark, but also to develop a proof of principle that optimized soil and groundwater contaminant degradation.'

Additional challenge

An important part of the experiments involved aerobic batch experiments, where small bottles were filled with clean sediments and ground water from Griftpark, to guarantee that bacteria that were naturally present were studied. By subsequently adding known amounts of different contaminants, the experiment was highly controlled and reproducible. 'So, we used different contamination scenario's' Aydin says. 'We investigated the breakdown of full mixtures, but we also studied the degradation of different combinations as well as single contaminants, like BTEX, but also tar oil components like indene, indane and naphthalene.' Aeration of the bottles provided an aerobic (oxygen-rich) environment, optimizing breakdown conditions. The results of this first experiment were promising and informative: all contaminants were eventually completely degraded. However, when a limited number of compounds was supplied to the microorganisms, contaminant breakdown was almost three times quicker as compared to the full mixture, as found in Griftpark. Aydin: 'The complex mixture of contaminants present in the soil of Griftpark clearly poses limitations on the breakdown. The bacteria are kind of 'overwhelmed' and can't handle the large variety of compound that well. In addition, the absence of oxygen in the deeper soil poses an additional challenge for the bacteria to quickly breakdown the contaminants present.'

Different set-ups

To further test the influence of different aerobic and anaerobic conditions on contaminant breakdown, Aydin experimented with different set-ups. Anaerobic conditions, where nitrate and/or sulfate were added to replace oxygen, revealed a much slower and incomplete degradation. When Aydin performed her degradation experiments under low-oxygen (microaerobic) conditions followed by anaerobic conditions, contaminant breakdown stopped as soon as the oxygen was depleted. 'When we reversed this experiment and started with pre-anaerobic condition,



and supplemented nitrate, there was no contaminant degradation until we added low-oxygen concentrations. This led to a more efficient breakdown than in experiment without the pre-anaerobic step.' According to Aydin, this can be explained by the breakdown of organic matter during the pre-anaerobic step, whereafter, the supplemented oxygen could be exclusively used by the microorganisms to degrade the contaminants present.

Proof of principle

The results of batch experiments were confirmed in column experiments, where a continuous flow made a more realistic, experimental environment. Aydin's studies have resulted in a proof of principle to remediate contaminated soil. 'Injecting low levels of oxygen using microbubbles in a contaminated site may help a rapid contaminant mixture breakdown', Aydin say. 'However, this should be preceded by adding nitrate and sulfate, to allow the breakdown of organic matter first, resulting a more effective use of oxygen during the breakdown of chemicals.'

Selected publication

Indene, indane and naphthalene in a mixture with BTEX affect aerobic compound biodegradation kinetics and indigenous microbial community development. Aydin, D.C., Faber, S.C., Attiani, V., Eskes, J., Aldas-Vargas, A., Grotenhuis T., and Rijnaarts H. 2023. *Chemosphere*, *340, 139761.*
https://www.sciencedirect.com/science/article/pii/S0045653523020283

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Agenda

PhD defences:

Dilan Aydin, Januari 19th, 2024, 16.00h. Biostimulation of aromatic hydrocarbon mixture degradation in a former gaswork site.

Ivonne Servin Balderas, March 5th 2024, 16.00h. The hidden pitfalls of the energy transition a study on CO2 use.

Rita Branco, April 10th 2024, 11.00h. Dissolved organic matter dosing to enhance in situ micropollutants biodegradation in drinking water aquifers.

Yue Sun, April 19th 2024, 13.30h. Bio-electrochemical system for mitigating sewer gas formation and sewer pipe corrosion.

INAUGURAL SPEECH:

Annemiek ter Heijne, February 8th, 2024, 16.00h