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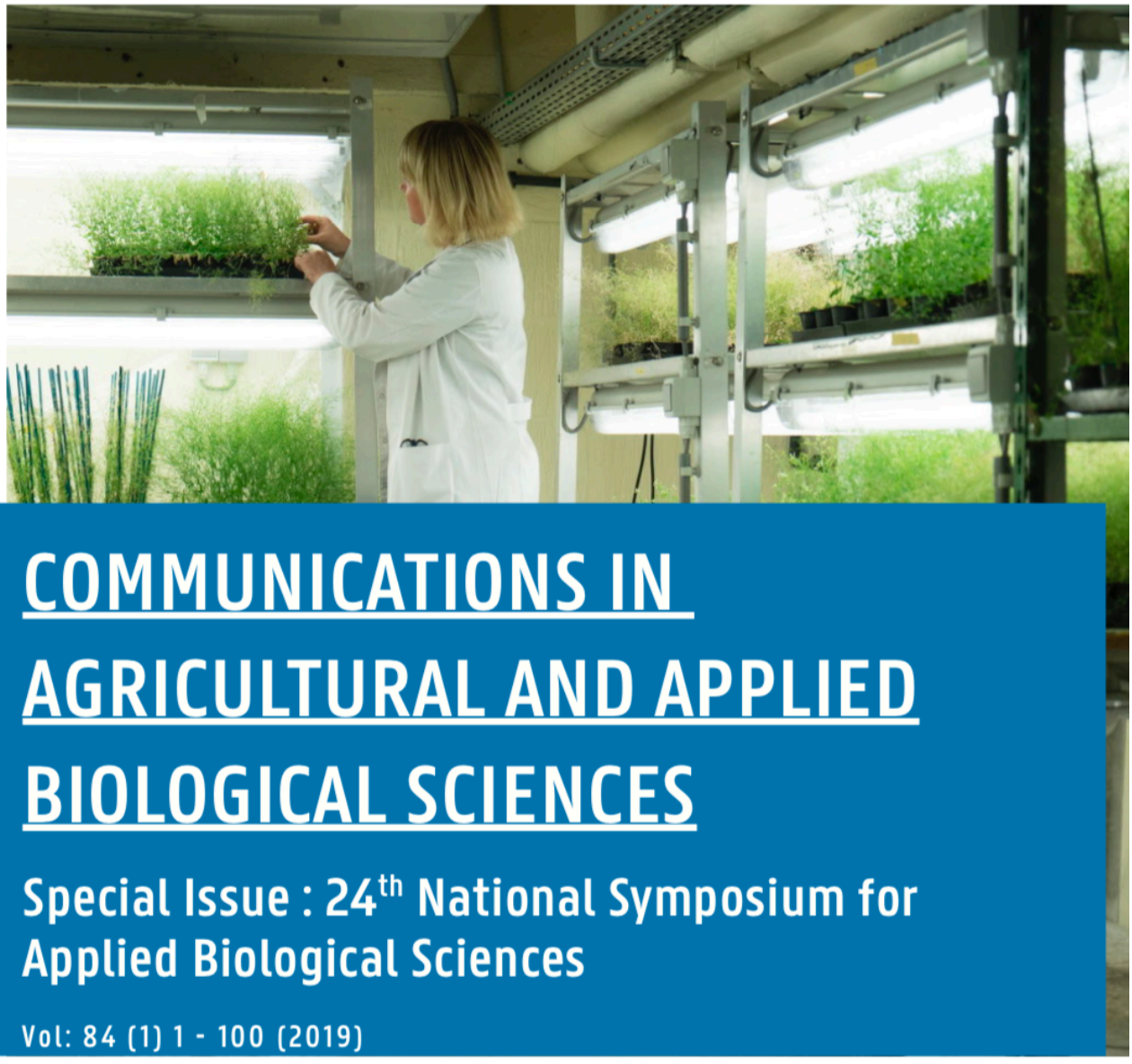

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PROCEEDINGS

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FACULTY OF BIOSCIENCE ENGINEERING
GHENT UNIVERSITY

FEBRUARY 4TH, 2019

Website
http://www.nsabs2019.ugent.be/

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The National Symposium on Applied Biological Sciences (NSABS2019) is jointly organized by the following institutes:

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The organizing committee of NSABS2019 is grateful for the financial and logistic support by the following institutes and their doctoral schools:

- UGent
- KU Leuven
- UAntwerp
- VUB
- ULB
- UCL
- ULg
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PREFACE

With great pleasure the organizing committee presents the proceedings of the 24th National Symposium on Applied Biological Sciences (NSABS 2019).

This symposium has its roots at Ghent University, where the first conference in the series was organized in 1995. In the following years, other Belgian universities joined the organizing committee and the symposium took place in different cities throughout the country. Currently, KU Leuven, UAntwerp, UGent, ULg, UCL, UMONS, ULB and VUB are involved in reviewing, programming, promoting and financially supporting the conference. The main purpose of the conference has been preserved throughout the years: to explore and to bring together the Belgian expertise in the field of Applied Biological Sciences, focusing mainly on contributions of PhD students.

The 24th edition of NSABS is organized at the Faculty of Bioscience Engineering at Ghent University. About 150 abstracts were submitted for review by researchers from the participating institutions, of which 33 were selected by the organizing committee for oral presentations and about 100 for poster presentations. In the light of the ongoing debates on climate change and sustainability and the fact that much of the research conducted at the participating institutions is either directly or indirectly related to these topics, the focus of NSABS 2019 is on 'Sustainability now'. In this way, we hope to showcase how the research conducted at these institutions supports the ongoing political and social discussions on these matters. Coinciding with this central theme, for the first time, the organizing committee has taken on the challenge of organizing a carbon neutral symposium in the sense that participants their carbon footprint is compensated and we offer a vegetarian and sustainable catering.

Special thanks go to the members of the organizing committee for their commitment and valuable input and to the sponsors of this event, being the doctoral schools of the involved universities, Air Liquide, Arcelor Mital, JEOL, LGC, Novolab, Qiagen, BaseClear, VWR and our partner for the symposium EOS.

We hope that you enjoyed NSABS 2019 and we look forward to a fruitful, interactive, informal, enjoyable, open-minded and memorable experience.

Prof. dr. ir. Jan M. Baetens

On behalf of the organizing committee

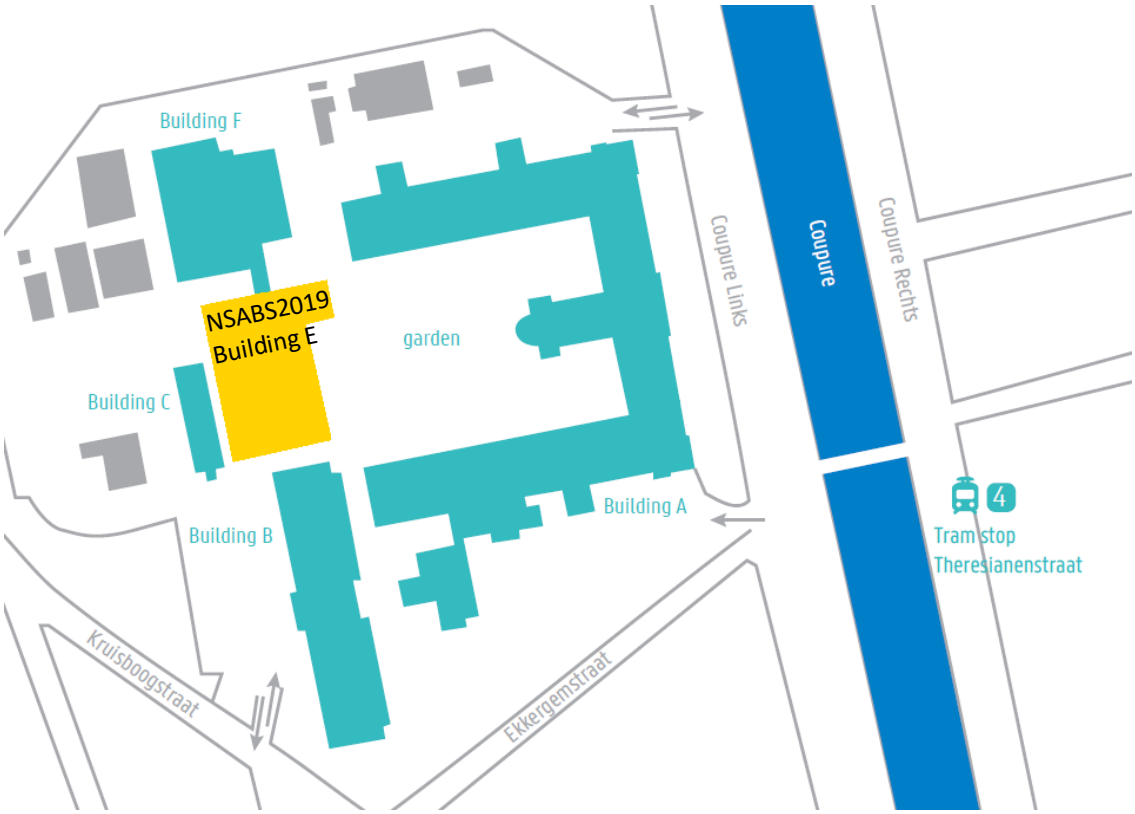
24th National Symposium on Applied Biological Sciences (NSABS2019)

4 February 2019, Ghent University, Coupure links 653, 9000 Gent

Time schedule and rooms

08:30	Installing posters & registration - **Foyer**		
09:00	Plenary session 1 - **E1** Introduction & *Arjen Hoekstra* - The environmental footprint of humanity		
10:00	Environment 1 - **E2**	Biotechnology 1 - **E3**	Animal sciences & aquatic ecology - **E4**
10:55	Poster session - **Foyer**		
11:30	Environment 2 - **E2**	Biotechnology 2 - **E3**	Green chemistry & technology - **E4**
12:15	Poster session & lunch - **Foyer**		
13:30	Plenary session 2 – **E1** *Willy Verstraete* - Science and enterprises		
14:00	Sustainability now - **E2**	Plants & crops - **E3**	Food technology, safety & health - **E4**
15:25	Poster session - **Foyer**		
15:50	Plenary session 3 - E1 Awards & *Jean-luc Doumont* - The three laws of communication		
17:00	Reception - **Foyer**		

Location



ORAL PRESENTATIONS

Room E2	Room E3	Room E4
Environment 1	**Biotechnology 1**	**Animal sciences & aquatic ecology**
O1. 10:10 - 10:25 *Yankai Xie* - Recurrent free nitrous acid exposure insufficiently suppressed nitrate formation in a mainstream partial nitritation/anammox system	**O4. 10:10 - 10:25** *Weiqiang Zhu* - Sludge storage potential for mainstream partial nitritation/anammox: Winter bioaugmentation with stored summer biomass	**O7. 10:10 - 10:25** *Pieterjan Verhelst* - Downstream migration of European eel in an anthropogenically regulated freshwater system: Implications for management
O2. 10:25 - 10:40 *Valentine Cyriaque* - Plasticity of microbial communities in metal-impacted river-like microcosms	**O5. 10:25 - 10:40** *Joris Van Lindt* - Pathological aggregation of an ALS-linked protein via liquid-liquid phase separation	**O8. 10:25 - 10:40** *An Bautil* - Dietary arabinoxylan-oligosaccharides kick start arabinoxylan degradation in the ageing broiler
O3. 10:40 – 10:55 *Benoît Destrée* - Root system responses of D. glomerata and M. sativa to water deficit in pure or mixed stands	**O6. 10:40 – 10:55** *Dimitri Boeckaerts* - Predicting phage-host specificity	**O9. 10:40 – 10:55** *Laura Hoebeke* - Automated recognition of people and identification of animal species in camera trap images
Environment 2	**Biotechnology 2**	**Green chemistry & technology**
O10. 11:30 – 11:45 *Christin Bobe* - Improving geophysical resource exploration using probabilistic inversion methods	**O13. 11:30 – 11:45** *Neha Sachdeva* - Cost efficient cultivation of *Arthrospira* sp. using alternative nitrogen sources based on biochemical and proteomic profiling of biomass	**O16. 11:30 – 11:45** *Emma Deniere* - Comparison of the ozone and ozone-activated peroxymonosulfate process for the degradation of pharmaceutical residues in water
O11. 11:45 – 12:00 *Reinhart Van Poucke* - Application of biochar to stabilize metals in contaminated soils	**O14. 11:45 – 12:00** *Peter Rubbens* - Predicting microbial biodiversity using flow cytometry and 16S rRNA gene amplicon sequencing	**O17. 11:45 – 12:00** *Benjamin Claessens* - Combining shape-selective zeolites for the efficient recovery and purification of biobutanol via adsorption
O12. 12:00 – 12:15 *Daniele Da Re* - Looking for ticks from Space: using remotely sensed spectral diversity to assess Amblyomma and Hyalomma tick abundance	**O15. 12:00 – 12:15** *Annalisa Cartabia* - A semi-hydroponic cultivation system associating Boraginaceae plants with arbuscular mycorrhizal fungi for optimal production of plant secondary metabolites	**O18. 12:00 – 12:15** *Hannelore Peeters* - Photocatalytic self-cleaning coatings with embedded plasmonic gold nanoparticles
Sustainability now	**Plants & crops**	**Food technology, safety & health**
O19. 14:10 – 14:25 *Tine Vergrote* - Model-based analysis of greenhouse gas emission reduction potential through farm-scale digestion	**O24. 14:10 – 14:25** *Simon Backx* - Identification of a new algicidal compound derived from quorum sensing signalling molecules	**O29. 14:10 – 14:25** *Ali Sedaghat Doost* - Whey protein isolate - almond gum nano-complexes as potential green and sustainable alternative for synthetic surfactants
O20. 14:25 – 14:40 *Reindert Devlamynck* - Valorisation of agricultural wastewaters into alternative proteins using duckweed	**O25. 14:25 – 14:40** *Bianca Drepper* - Frequently frozen flowers? Towards assessing future frost risks in Belgian orchards	**O30. 14:25 – 14:40** *Huong Pham* - Changes in the soluble and insoluble fractions of orange juice during storage in relation to non-enzymatic browning
O21. 14:40 – 14:55 *Janne Spanoghe* - Exploring the feasibility of photohydrogenotrophically grown	**O26. 14:40 – 14:55** *Nea Fatimata* - Variability of essential oil composition and biological activities of Lantana	**O31. 14:40 – 14:55** *Elien Alderweireldt* – Effect of processed meat related compounds on colon cells

purple bacteria as novel microbial protein source

O22. **14:55 – 15:10**
David De Pue - Safeguarding Natura 2000 habitats from nitrogen deposition by tackling ammonia emissions from livestock facilities: unequal efforts and uneven effects

O23. **15:10 – 15:25**
Laurens Van Hoecke - Chemical Hydrogen Storage as a Fuel in the Shipping Industry

rhodesiensis Moldenke from Côte d'Ivoire

O27. **14:55 – 15:10**
Sathyanarayan Rao - Relationship between electrical anisotropy of soil-root continuum and geometrical architecture of root system

O28. **15:10 – 15:25**
Ann Van Holle - Biochemical markers to evaluate the impact of terroir on the brewing value of hops

O32. **14:55 – 15:10**
Sofie Rousseau - Fundamental research on factors influencing mineral bioaccessibility in plant-based food systems: the case of pectin degree of methoxylation and concentration of co-existing minerals

O33. **15:10 – 15:25**
Magda Costa - Yeast proteomics during beer fermentation: challenges and prospects

AWARDS

There are five best presentation awards:

Best oral presentation awards

- JEOL best oral presentation award: €250
- Qiagen best oral presentation award: €250
- EOS Sustainability award: 1y free subscription to EOS + write article for EOS

Best poster presentation awards

- ArcelorMittal best poster award: €150
- Air Liquide best poster award: €150

During talks and poster sessions, reviewers from all universities and supporting sponsors evaluate the presentations. One poster award is awarded from the public. Every attendee can submit his/her three best posters at the registration desk.

SUSTAINABILITY NOW!

This year's symposium is organized around the theme of 'Sustainability now!'. With this theme, we hope to bring together researchers who contribute to sustainability in the different applied fields of biological sciences. This symposium also takes place within the wider commitment of UGent to sustainable principles. The goal of this sustainability vision is for UGent to become a leading knowledge institute for a future that is ecologically, socially and economically sustainable within a local and global context.

One concrete application of this policy relates to academic gatherings like this symposium. Academic conferences can have a huge environmental impact. The primary cause is the use of airplanes as transportation, as outlined recently by Caset et al. (2018) in their paper "Meet & fly: Sustainable transport academics and the elephant in the room". Since NSABS is a national symposium, its climate impact is limited compared to most international conferences. However, in the context of the main theme, we have committed to making the symposium climate neutral. To achieve this, we will primarily avoid emissions and in addition compensate the emitted amounts of CO_2.

CO_2-COMPENSATION

Several initiatives exist to compensate CO_2 emissions. Some compensate the emitted CO_2 by investing in green energy (e.g. carbonaltdelete.eu) or in energy-efficient household equipment such as cooking stoves (e.g. fairclimatefund.nl) in developing countries. Other initiatives compensate by protecting forests (e.g. co2logic.com) or by planting trees (e.g. treecological.be).

For this symposium, we opted for the Belgian initiative Treecological. This initiative of BOS+ and Ecolife compensates CO_2 by reforestation in Ecuador. These tropical forests sequester 15 tons of CO_2 per hectare per year. Treecological charges 17 euros per ton CO_2. At this price, they can plant, conserve and protect an amount of trees that will sequester 1 ton of CO_2 over 20 years. Researchers at Ghent University monitor the CO_2-sequestering of the planted forests, to guarantee that the expected quantity of CO_2 is captured. Local organisations and inhabitants are involved to ensure that the planted forest corresponds to their needs. A biodiverse, healthy forest, often enriched with agroforestry, guarantees an income and added value for the local residents, which in turn enables them to better protect the forest.

MINIMISING OUR ECOLOGICAL FOOTPRINT

Transportation

Participants are strongly encouraged to travel to the symposium by public transport or under their own power. Participants who come by foot or by bike save 172 g CO_2 per travelled km compared to travelling by car, while those coming by train will save 130 g CO_2 per travelled km.

The amount of CO_2 emitted for each vehicle is given below, using the assumptions adopted by De Lijn. "Tank to wheel" refers to the direct emissions during combustion. "Well to tank" refers to the indirect emissions of the fuel, emitted during exploitation, refinement, transportation and distribution. In the final CO_2-calculations by Treecological, the indirect emissions for production and maintenance of the vehicle and infrastructure are also accounted for. For the example of a car, these emissions increase its total pollution by 40%. (Source: treecological.be).

	CO_2-emission tank to wheel (kg/km)	**Emission including well to tank (TTW * 1.3) (kg/km)**	**Assumptions**
Average car	0,13	0,17	Diesel, 6,5 l/100 km (= 172 g CO_2/km, 1,35 passengers)
Average bus	0,08	0,10	Diesel, 40 l/100km, 14 passengers
Average tram De Lijn	0,00	0,00	De Lijn bus 100 % green (hydropower) energy (20 g CO_2/kWh)
Train NMBS	0,03	0,04	
Bike/Foot	0,00	0,00	

Catering

The coffee breaks, lunch and reception will be provided by Roof Food. These young entrepreneurs cultivate vegetables and spices on the roof of their business in Ghent. With the ingredients directly from the garden, they provide catering that is not only tasty and original, but also organic, seasonal, vegetarian and short-chain. All food and drinks will be transported by bike from their location to the symposium venue. The emissions of the catering (mainly cooking) are estimated at 509 kg CO_2(estimated by Treecological, based on information from Ecolife.be).

Other sources of pollution

To limit paper use, the conference proceedings will only be provided online. Other sources of CO_2 such as the remaining printing (conference program and posters, estimated at 25 kg CO_2), as well as the heating and electricity of the buildings (estimated at 44 kg CO_2), will be compensated via Treecological.

PLENARY TALKS

PROF. DR. IR. ARJEN Y. HOEKSTRA

THE ENVIRONMENTAL FOOTPRINT OF HUMANITY: A REFLECTION ON CONSUMPTION PATTERNS, TRADE AND PLANETARY BOUNDARIES

After so many years of research we still lack a coherent picture of what sustainability means. Hoekstra will put a few pieces together by reflecting on sustainability from a production, consumption as well as a trade perspective, while moving from the local to the global discussion and back. He will discuss tensions and inevitable trade-offs between efficient resource use, fair sharing of resources, and environmental sustainability, and between strategies of control and resilience. Human's environmental footprint will be introduced and put in the context of planetary boundaries. Having done most research in the field of water for food and energy, Hoekstra will go into more depth by reviewing humanity's water footprint and addressing the question of sustainable water use.

Biography

Arjen Hoekstra is professor in Water Management at the University of Twente, visiting prof at the Lee Kuan Yew School of Public Policy, National University of Singapore, and Editor-in-Chief of Water. He specialises in policy analysis and sustainability science, with a focus on water in relation to food and energy. Hoekstra is father of the new interdisciplinary research field of water footprint assessment, which addresses the relations between water management, consumption and trade He is founder of the internationally operating Water Footprint Network and founding member of the Planetary Accounting Network. His books were translated into several languages and include The Water Footprint of Modern Consumer Society (Routledge, 2013), The Water Footprint Assessment Manual (Earthscan, 2011) and Globalization of Water (Wiley-Blackwell, 2008).

PROF. DR. IR. WILLY VERSTRAETE

SCIENCE AND ENTERPRISE: THE TRENDS TO CONSIDER

We start by some philosophical considerations. According to Gude (2015), science is not a necessary pillar of society. Yet Spinoza (1677) has pointed out that it is our nature that drives us to explore for more and better. We then overview the 'place of science' in universities and in industry, as it evolved over the last decades. We conclude with a few considerations about 'becoming a professional'.

Biography

Willy Verstraete was born on April 25, 1946 in Beernem (Belgium). He graduated in 1968 from the Gent University as bio-engineer. In 1971, he obtained a PhD degree in the field of microbiology at the Cornell University, Ithaca (USA). Since 1971, he worked at the Ghent University, first as assistant and since 1979 as professor and head of the Laboratory of Microbial Ecology and Technology (LabMET - Faculty of Bioscience Engineering). Since October 2011, he has become emeritus professor. Prof. Verstraete has been instrumental in the creation of several successful spin-offs, both in Belgium and abroad in the field of applied microbial ecology (environmental technology; health; food and feed). He currently is active member of the board of three of these companies. He has received countless awards to distinguish his work, and even has a new archaeal phylum name after him: the Verstraetearchaeota. Since 2014, he ranks in the list of Highly Cited Researchers, due to the fact that several of his papers belong to the top 1% most cited papers of his field. The whole of his scientific work totals at a h-index of 100. The sum of his cited papers is about 45 000. Since April 2016, Willy Verstraete is elected president of the board of the Science Foundation FWO of Flanders, Belgium.

DR. JEAN-LUC DOUMONT

THE THREE LAWS OF COMMUNICATION

Useful as each of them can be, a large body of tips and tricks is impossible to remember, at least in a practical, usable way, unless it is structured into a balanced, meaningful hierarchy. This talk proposes and illustrates three simple yet solid ideas that lead to more effective communication and that underpin every other guideline: easy to remember, readily applicable, and always relevant—in short, valuable for the rest of your life.

Biography

An engineer (Louvain) and PhD in applied physics (Stanford), Jean-luc Doumont is acclaimed worldwide for his no-nonsense approach, his highly applicable, often life-changing recommendations on a wide range of topics, and Trees, maps, and theorems, his book about “effective communication for rational minds.” For additional information, visit www.principiae.be.

Lecture session

ENVIRONMENT

RECURRENT FREE NITROUS ACID EXPOSURE INSUFFICIENTLY SUPPRESSED NITRATE FORMATION IN A MAINSTREAM PARTIAL NITRITATION/ANAMMOX SYSTEM

Yankai Xie[1], Michiel Van Tendeloo[1], Weiqiang Zhu[1], Lai Peng[2], Siegfried E. Vlaeminck[1]

[1]University of Antwerp, Belgium.
[2]Wuhan University of Technology, China.

Carbon- and energy-lean nitrogen removal in sewage treatment plants can be achieved by switching from conventional nitrification/denitrification to partial nitritation/anammox (PN/A) process, which requires 100% less organic carbon and 60% less aeration [1]. PN/A is already a mature technology for treatment of sludge reject water, but its implementation under mainstream conditions is still challenging as the low temperature and nitrogen levels do not easily facilitate the required suppression of nitrite oxidizing bacteria (NOB) and retention of anoxic ammonium-oxidizing bacteria [2].

The objective of this study was to achieve mainstream PN/A (no COD) using a rotation biological contactor (RBC). An existing PN/A reactor was converted from sidestream to mainstream conditions by lowering temperature (30 to 19°C) and gradually decreasing influent concentration (from 1144 to 47 mg NH^{4+}-N L^{-1}). To avoid overloading, the loading rate was lowered from 497 to 178 mg N L^{-1} d^{-1}. In a later phase, a weekly free nitrous acid (FNA) treatment was conducted to selectively suppress NOB (24h; 3.7±0.2 mg FNA-N/L).

After the conversion, the total nitrogen (TN) removal decreased from 80±5 to 39±10%, and the relative NO^{3-} production increased from 18 to 64%, highlighting the increased activity of NOB. Although the FNA treatments did not promote the TN removal, a further NOB boom was avoided, indicated the insufficient NOB suppression. Interestingly, batch tests showed changes in maximum NOB-activity: 35 and 13 mg N g^{-1} VSS day^{-1}, before and after FNA treatment respectively. NOB suppression in this study was not straightforward, potentially linked to the biofilm structure of the biomass, and dissolved oxygen levels experienced during rotation.

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[1] Mulder, A (2003) The quest for sustainable nitrogen removal technologies. Water Sci Technol 48:67–75.
[2] Verstraete, W., Vlaeminck, S.E. (2011). ZeroWasteWater: short-cycling of wastewater resources for sustainable cities of the future. Int. J. Sustain. Dev. World Ecol. 18: 253-264.

PLASTICITY OF MICROBIAL COMMUNITIES IN METAL-IMPACTED RIVER-LIKE MICROCOSMS

Valentine Cyriaque[1], Augustin Geron[1,2], David C. Gillan[1], Ruddy Wattiez[1]

[1]University of Mons, Belgium.
[2]University of Stirling, Scotland.

Metal contamination of soil and sediment is a serious environmental issue because of biotoxicity and bioaccumulation. Despite elevated metal concentrations, the river sedimentary microbial communities sampled nearby the MetalEurop foundry (Northern France) display an unexpected higher diversity in comparison with upstream unpolluted control site (Férin). Recent methodological improvement of 16S rRNA profiling has enhanced analytical accuracy and revealed heavy metals as drivers of the microbial community structure. In the present study, a comparative follow-up of the evolution of sediment microbial communities from Férin was performed in static microcosms with fresh river water turn-over and supplemented or not in metal solution (Cd: 38.1 mg/kg, Cu: 100.0 mg/kg, Pb: 913.8 mg/kg and Zn: 3218.5 mg/kg). Taxonomical profile evolution was studied by 16S rRNA gene Illumina sequencing. Functional insights on metal resistance adaptation were obtained using quantitative PCR on czcA and pbrA genes coding for metal efflux pumps. Proportion of incP-plasmid evolution was followed by quantitative PCR approach. Taxonomical analyses shown a higher specific richness and equitability in metal-added microcosms. This increase can be explained by metals acting as community-manager together with community coalescence. Quantitative PCR analysis coupled with taxonomic evolution suggest a step by step adaptation through the selection of different metal-resistant bacteria acting as “Public Good” providers.

ROOT SYSTEM RESPONSES OF D. GLOMERATA AND M. SATIVA TO WATER DEFICIT IN PURE OR MIXED STANDS

Benoît Destrée[1], Mathieu Javaux[1], Xavier Draye[1]

[1]Université Catholique de Louvain, Belgium.

Climatic projections indicate that Belgium might face a significant change in rainfall regime with up to 25% less precipitations in Summer by the end of the 21st century. This would lead to drought stresses for plants. Grasslands that make for a large part of Belgian agricultural system would be amongst the most affected by these drier conditions and would be likely to see their yield decrease. To tackle the issue, UCLouvain launched the ForDrought project in 2012, whose overal goal is to improve the performance of forage crops to cope with climate change.

The specific objectives of this study were to observe the responses of root systems of grassland plants when facing a water deficit and to assess the effect of plant species association. The species studied were Dactylis glomerata and Medicago sativa.

Large pits were dug in front of experimental agricutural parcels to carry out different measurements on the root system. Root numbers in each pit were counted, soil samples containing roots were taken and the roots were analyzed genetically to determine their species. In addition, yield was determined four times over the course of one year.

The results showed that a water deficit had an impact on the number of roots of both species. In addition, the yield was negatively impacted by water deficit while no clear effect was visible for the association of species. It must be noticed that, quite surprisingly, the genetic analyses were unable to detect Dactylis when it was alongside alfalfa leaving room for further improvement.

IMPROVING GEOPHYSICAL RESOURCE EXPLORATION USING PROBABILISTIC INVERSION METHODS

Christin Bobe[1], Ellen Van De Vijver[1], Marc Van Meirvenne[1]

[1]Department of Environment, Ghent University, Belgium.

In exploration geophysics, physical methods are used to characterize physical properties of the subsurface. Anomalies in these measurements can often be associated with deposits, such as mineral or hydrocarbon resources, groundwater reservoirs, or waste and contamination plumes.

The search for the cause of an anomaly in geophysical measurements is a kind of inverse problem. For example, gravity measurements can be inverted to gain knowledge about the subsurface density distribution. Inverse problems are typically ill-posed, as can be seen from the fact that many subsurface density profiles satisfy a given set of surface data. While classical inversion methods apply mostly unphysical regularization to derive a single subsurface model, in Bayesian techniques probability density functions (PDFs) are used to characterize the solution to the inverse problem. A main advantage is that these PDFs provide an uncertainty measure for the inversion result. The full Bayesian solution often requires an extensive search of the model parameter space. Thus, the computational expense is high compared to classical inversion methods. Yet, knowledge of uncertainties can be crucial when inversion results are used for decision making. For example, in environmental risk assessments, uncertainties of inversion results translate to uncertainties of economic implications of different land and resource management strategies.

We introduce the Kalman ensemble generator (KEG) as a solver for inverse problems. Using the KEG, PDFs are approximated as Gaussian, avoiding an exhaustive search of the model space. Instead, an ensemble of random samples from the PDF is used to approximate the mean and standard deviation of the PDF. We use the KEG for the characterization of subsurface electrical conductivity and magnetic susceptibility inverted from surface frequency-domain electromagnetic measurements.

APPLICATION OF BIOCHAR TO STABILIZE METALS IN CONTAMINATED SOILS

Reinhart Van Poucke[1], Caleb E. Egene[1], Yong S. Ok[2], Frederik Ronsse[1],
Erik Meers[1] and Filip M.G. Tack[1]

[1]Department of Green Chemistry, Ghent University, Belgium.
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The European Environment Agency reported in 2007 that approximately 250.000 contaminated sites in Europe require a clean-up. Most commonly, these sites are contaminated with metals. The potential of biochar to sorb metals and reduce their concentrations in soil solution was investigated as a function of feedstock and pyrolysis conditions.

Different types of feedstock were selected: shredded pine wood (a lignin rich feedstock), grass (a hemicellulose rich feedstock), cow manure (a digested and nutrient rich feedstock) and egg shell (an inorganic feedstock). The feedstocks were pyrolyzed at 2 different temperatures (350°C and 550°C) with 2 different residence times (10 and 60 min). The produced chars were then tested for their capacity to sorb metals. The biochar produced from cow manure and pyrolyzed at 550°C for 10 min showed the best adsorption whereas biochars produced from wood performed the weakest.

The manure-based chars, and to a lesser extent grass-based chars, were characterized by high concentrations of phosphates and carbonates. These anions play an important role in metal sorption because they bind cations and can form insoluble compounds with the metals.

When mixed with contaminated soil from the Campine region, the application of 4% cow manure biochar resulted in a 90% and 95% reduction in Cd and Zn, respectively, compared to the control. After one month of incubation in a technosol from a former silver-lead mine site located in France, the same cow manure biochar was able to retain 99.79% of Pb compared to the blank. The promising result of this experiments emphasize the potential of biochar to immobilize heavy metals in soil and water systems, thus reducing the phytotoxicity.

LOOKING FOR TICKS FROM SPACE: USING REMOTELY SENSED SPECTRAL DIVERSITY TO ASSESS AMBLYOMMA AND HYALOMMA TICK ABUNDANCE

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Tick distribution and populations dynamics models commonly use Satellite Remote Sensing products (SRS) as proxy of environmental covariates [1]. However, epidemiological studies do not exploit the whole potential of SRS products, often considering only a) the portion of the electromagnetic spectrum useful for vegetation indexes and b) the assemblage of habitats' resources experienced by vectors and hosts at single-pixel values rather than spanning across pixels. Spectral diversity computed across neighboring pixels is an indicator of habitat heterogeneity [2] which, relying on the Resource Based Habitat Concept [3], could be linked to tick resources availability. In fact, several studies have shown how tick abundance is higher in heterogeneous areas, presumably reflecting a greater abundance of hosts, feeding and reproduction sites [4]. In this study, we assessed the capacity of SRS spectral-based landscape diversity indexes to inform on the habitat of two tick genera, Amblyomma and Hyalomma, in Benin (Western Africa). Specifically, we tested for i) association between spectral diversity indices and heterogeneous landscapes and, ii) whether their inclusion brings added ecological meaning for predicting tick abundance. Higher spectral diversity was correlated to mosaicked landscapes and positively related to Hyalomma abundances only. Though taxa dependent, our approach showed a goodness of fit between predicted and observed value between 13-20% using one-variable models, which is a promising outcome considering the sampling and scale limitations. Spectral diversity indexes coupled to the classical SRS methods revealed to be a complementary approach bringing interesting ecological considerations into disease biogeography field.

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Lecture session

BIOTECHNOLOGY

SLUDGE STORAGE POTENTIAL FOR MAINSTREAM PARTIAL NITRITATION/ANAMMOX: WINTER BIOAUGMENTATION WITH STORED SUMMER BIOMASS

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Partial nitritation/anammox (PN/A) is an energy-efficient, carbon-lean alternative to conventional nitrification/denitrification for nitrogen removal from wastewater since it requires 60% less aeration, 100% less organics and produces 75% less waste sludge1. It relies on two types of microorganisms: aerobic and anoxic ammonium-oxidizing bacteria (AerAOB and AnAOB), and on the suppression of nitrite-oxidizing bacteria (NOB). Its application for sewage treatment, so-called mainstream PN/A, is challenging, partly due the slow growth rate of AnAOB2, especially at lower temperatures. This problem may be tackled by storing excess PN/A sludge, mainly harvest over the summer, and re-inoculate this in winter, but only if the microbial activity can be preserved. For now, storage of mainstream biomass has not been studied. Therefore, the objective of this study was to explore several preservation methods.

PN/A sludge from a 7m³ mainstream pilot-scale installation was stored for two months in three 1m³ plastic containers at ambient temperature (7-15°C) and four 10L containers at 4°C. Three storage conditions were tested: continuous presence of $NaNO_2$, $NaNO_3$ or neither of them.

Results showed that the presence of $NaNO_3$ had the best results with AnAOB activity lost of 18% and 44% at 4°C and ambient temperature, respectively, compared to 35% and 65% in absence of $NaNO_2$ and $NaNO_3$. Moreover, the presence of $NaNO_3$ at 4°C showed the smallest AnAOB decay rate of $0.0266d^{-1}$ when it was recalculated to the reference temperature of 20°C with the Arrhenius equation.

This study demonstrated that the continuous presence of $NaNO_3$ resulted in the highest preservation of AnAOB activity, thus enabling the long-term storage of PN/A sludge. The $NaNO_3$ was also cost-effective for application. The presence of $NaNO_2$ can lose more NOB activity than AOB, but they lose much anammox activity. In the ongoing experiments, different biomass types and microbial community analyses will be tested in the longer-term.

PATHOLOGICAL AGGREGATION OF AN ALS-LINKED PROTEIN VIA LIQUID-LIQUID PHASE SEPARATION

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease leading to paralysis and death. The disease is characterized by inclusions in the affected neurons. Recently it was discovered that liquid-liquid phase separation (LLPS) is responsible for the formation of membraneless organelles. Interestingly, many of the proteins involved in ALS also undergo LLPS. Because proteins in the high density droplets of LLPS are highly concentrated, they are at high risk for aggregation. Consequently the misregulation of LLPS granules is thought to lead to ALS.

To study the liquid-to-solid transition of phase separated proteins, we used the disordered domain of hnRNPA2. We developed a protocol to quickly induce phase separation. The resulting formation and growth of high density droplets was studied by dynamic light scattering and fluorescence microscopy. The analysis of the laser scattering pattern of the droplets and the detection of the fusion of droplets, confirmed their liquid like behavior. We also observed the aggregation of the droplets. The aggregates did not appear to be fibrous, nor did they stain thioflavin-T positive – a typical characteristic of ordered fibrils. This lead us to hypothesize the ALS-linked aggregates are not of fibrous nature (as observed in Alzheimer's disease), but rather arise by amorphous aggregation.

Our results also suggest a direct link between LLPS and aggregation. Further studies in this aggregation process will provide new insights in the importance of LLPS in neurodegenerative pathology.

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PREDICTING PHAGE-HOST SPECIFICITY

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Pathogenic bacteria increasingly show resistance to antibiotics. The need for alternative, more specific approaches keeps growing year by year. A promising alternative approach to this problem is to fight bacteria with their natural enemies, bacteriophages. These bacterial viruses are highly specific to only one or a few bacterial species. Phages also represent a sustainable solution, as they continuously coevolve with their bacterial hosts, allowing them to regain the upper-hand after resistance formation. Phages employ specific proteins to recognize a suitable bacterial host. Often, these proteins are located on tail fibers and tail spikes. By studying bacteria-phage interactions and the proteins involved, important proteins can be identified and synthesized in the lab using recombinant DNA technology. These proteins can be used to create synthetic viruses with modified host specificity. In my research, I have developed machine learning models that learn patterns from tail fiber and tail spike protein data. A variety of machine learning models attempted to classify the proteins in three different categories. These categories corresponded to the bacterial hosts that these proteins are linked to. Every model scored above 89 percent across several performance metrics, indicating that tail protein data can be used appropriately to predict bacterial hosts. Ultimately, this research can aid in the development of alternative ways to combat bacterial pathogens by creating synthetic viruses that are much more specific.

COST EFFICIENT CULTIVATION OF *ARTHROSPIRA* SP. USING ALTERNATIVE NITROGEN SOURCES BASED ON BIOCHEMICAL AND PROTEOMIC PROFILING OF BIOMASS

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The genetic robustness of *Arthrospira* sp. (Spirulina) and its ability to adapt to variable cultivation conditions (pH, temperature, nitrogen sources etc.) has increased the commercial viability of this oxygenic cyanobacteria. Due to its wide range of applications in therapeutic, food, nutraceutical, biofuels and various other commercial sectors, the global Spirulina market is estimated to be valued at USD 1.8 million by 2026. Furthermore, the versatility of *Arthrospira* sp. to assimilate waste nitrogen sources (urea, ammonium, nitrite etc.) makes it a viable candidate in the field of waste water management. In order to harness the potential of *Arthrospira* sp. for waste water management, it is first required to study the metabolic response of this photosynthetic cyanobacteria to the varying concentrations of the waste nitrogen sources. With this objective in mind, the present study combined the proteomic analysis with various bioengineering tools (light use efficiency, reactor configuration, modelling etc.) to study the adaptation of *Arthrospira* sp. to variable concentrations (upto 120 mM) of urea, ammonium, nitrate and nitrite, under both batch and continuous conditions of a photobioreactor. The study also evaluated the effect of individual and combined nitrogen sources on the proteomic profile, photosystems and energy parameters (NADH, NAD, NADPH, NADP etc.) of the cyanobacterial cells. This comprehensive meta-analysis of biomass at cellular level, thus opens new avenues towards solving the issues of waste management using photosynthetic cells. Furthermore, the proposed cultivation techniques could also be harnessed for the development of bio-refinery concept using photosynthetic cells for commercially viable production of high value co-products by using alternative and cost efficient nitrogen sources.

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PREDICTING MICROBIAL BIODIVERSITY USING FLOW CYTOMETRY AND 16S RRNA GENE AMPLICON SEQUENCING

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Microorganisms are vital components in various ecoystems on Earth [1]. In order to investigate the microbial biodiversity, researchers have relied on the analysis of 16S rRNA gene sequences from environmental DNA for over a decade [2]. However, this technique is often expensive and most importantly time-consuming. Flow cytometry has been proposed as an alternative technique to characterize microbial diversity. It is an optical technique, able to characterize a number of phenotypic properties of individual cells very fast. It has been shown that the so-called cytometric diversity correlates well with the genotypic diversity, as determined by 16S rRNA gene amplicon sequencing [3,4]. When samples have been analyzed by both flow cytometry and 16S rRNA gene amplicon sequencings, machine learning models can be used to used to estimate the biodiversity for samples for which no sequencing data is available. In this work, we propose an improved fingerprinting strategy which can be incorporated in predictive models. We applied our approach on two different types of datasets, the first containing synthetic microbial communities, the second containing freshwater microbial communities. Our results show that biodiversity can be inferred effecively in both types of ecosystems. This highlights the potential of flow cytometry to study microbial community dynamics in a fast and efficient way.

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A SEMI-HYDROPONIC CULTIVATION SYSTEM ASSOCIATING BORAGINACEAE PLANTS WITH ARBUSCULAR MYCORRHIZAL FUNGI FOR OPTIMAL PRODUCTION OF PLANT SECONDARY METABOLITES

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Anchusa officinalis, from the Boraginaceae family, is known for its therapeutic properties including anti-inflammatory, anti-viral and anti-bacterial [1, 2]. Such activities are attributed to the production of different bioactive metabolites (e.g. naphthoquinones, flavonoids and phenols). These plants interact with various microorganisms including arbuscular mycorrhizal fungi (AMF). The soil fungi are obligate biotrophs that establish symbiotic associations with plants, providing minerals to their hosts in exchange for photosynthates. They also increase the resistance/tolerance of plants to abiotic stresses [3] and are reported to stimulate the production of secondary metabolites (SMs) [4].

To enhance the production of SMs and to detect the role of the AMF, a semi-hydroponic cultivation system was set up consisting of pots containing A. officinalis plants mycorrhized (M) or non-mycorrhized (NM) with the AMF Rhizophagus irregularis (MUCL 41833) [5]. The pots were connected via silicon tubes to glass bottles containing a nutrient solution which circulated for 42h using peristaltic pumps. The solution was sampled at eight different times and, at the end of the circulation, the plants were harvested and roots and shoots were lyophilized separately. The extraction and semi-quantification of the SMs was assessed in the solutions, shoots and roots using HPLC-PDA-ELSD, HPTLC and UHPLC-HRMS analysis. Different statistical software and databases have been used for the identification of SMs.

Interestingly an up-regulation of specific SMs (such as 3,4-(Methylenedioxy)cinnamoyl alkannin) and a down-regulation of other SMs (an unknown compound with MW: 526,2262) were observed in the shoot extracts of the M plants as compared to the NM plants. Conversely, no differences were found in the SMs present in the solutions.

This experiment suggested that AMF may impact (e.g. stimulate or inhibit) the SMs content of A. officinalis. Further trials are planned to identify SMs and to assess the optimal conditions for their production in A. officinalis colonized also by other AMF strains.

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Lecture session

ANIMAL SCIENCES

&

AQUATIC ECOLOGY

DOWNSTREAM MIGRATION OF EUROPEAN EEL (*ANGUILLA ANGUILLA* L.) IN AN ANTHROPOGENICALLY REGULATED FRESHWATER SYSTEM: IMPLICATIONS FOR MANAGEMENT

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Connectivity between freshwater habitats and marine areas is heavily obstructed by migration barriers, leading to a high pressure on diadromous eel populations. Migration barriers attribute to the 98% decline of the European eel (*Anguilla anguilla L.*) population. A better understanding of eel behaviour regarding these barriers is needed for water managers to take proper mitigation actions. We tracked 50 eels by means of acoustic telemetry between July 2012 and January 2015 and analysed their migration behaviour in a Belgian polder area. On their way to the Schelde Estuary, eels face several migration barriers such as a pumping station, a weir and tidal barriers. The telemetry study did reveal significant delays and searching behaviour near those barriers. Depending on nothing but their accumulated fat, delays can have a serious impact on the fitness of the eel by wasting precious energy resources needed for a successful trans-Atlantic migration. In addition, delays and searching behaviour can also increase predation risk. The obtained knowledge can contribute to efficient management such as improved fish passage and guidance solutions.

DIETARY ARABINOXYLAN-OLIGOSACCHARIDES (AXOS) KICK START ARABINOXYLAN DEGRADATION IN THE AGEING BROILER

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Endoxylanases are commonly used in broiler feeds to improve the nutritional quality of the feed. One of the proposed mechanism of action of these enzymes is the generation of arabinoxylan-oligosaccharides (AXOS). It is however hypothesized that the age-related development of the microbiota in broilers influences the efficacy of these enzymes. The objective of this study was to measure age-related changes in and impact of AXOS addition on arabinoxylan (AX) degradation in the GI tract of broilers. A feeding trial was performed on 480 chicks(Ross 308) receiving a wheat-based diet supplemented with or without 0.5% AXOS. Digesta and faecal samples were collected at six different ages and examined for AX contents and digestibility, intestinal viscosity and microbial endoxylanase activities. In the first two weeks, microbiota were already able to solubilize part of the wheat water-unextractable arabinoxylan (WU-AX), thereby increasing intestinal viscosity and water-extractable arabinoxylan (WE-AX) content in the GI tract. Establishment of an adult microbial community at three weeks resulted in a further increase in the solubilisation of WU-AX and WE-AX fermentation. Remarkably, AXOS supplemented birds younger than 10 d showed higher wheat AX solubilisation and fermentation compared to control birds. Overall, this study shows that broiler age is an important factor in determining AX digestion. Moreover, AXOS will kick start AX degradation, speeding up the development of a fibre fermenting microbiome in young broilers.

AUTOMATED RECOGNITION OF PEOPLE AND IDENTIFICATION OF ANIMAL SPECIES IN CAMERA TRAP IMAGES

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Camera traps are increasingly being used in wildlife monitoring. The great advantage of camera traps in comparison with other sampling methods is that accurate data can be collected without the animal being collared or tagged, nor the researcher being present. However, such camera trapping frameworks produce high volumes of images which often need to be reviewed and annotated manually. Convolutional neural networks can be used to automate this labour intensive process.

In our work, we use manually labelled images from a camera trap study conducted by the Research Institute for Nature and Forest in collaboration with Hasselt University to train a convolutional neural network for identifying animal species. Images can be automatically labelled or the network can be incorporated into annotation applications to provide a suggestion to the users and as such speed up the annotation process. Given the limited number of manually labelled images, we incorporated hierarchical classification into the neural network. In this way, the user can adapt the level of detail of the classification, depending on the reliability of the predictions.

In addition to conveying the presence of species, the images may contain other useful information, for example animal attributes and behaviour. Therefore, getting help from wildlife enthusiasts via citizen science may be desirable to annotate the large amounts of data. However, since cameras are mounted in public nature reserves, passers-by may have triggered the camera traps. For privacy reasons, images showing people cannot be made public. These images need to be removed before the data can be made available to volunteers. This can be automated by training the network to recognise people in addition to identifying animal species.

Lecture session

GREEN CHEMISTRY

&

TECHNOLOGY

COMPARISON OF THE OZONE AND OZONE-ACTIVATED PEROXYMONOSULFATE PROCESS FOR THE DEGRADATION OF PHARMACEUTICAL RESIDUES IN WATER

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INTRODUCTION

To increase water quality and re-use, advanced oxidation processes (AOPs) are of key importance to degrade emerging organic micropollutants. A large number of studies have proven the efficiency of ozone-based AOPs in synthetic (clean) water and in secondary effluent from wastewater treatment plants (Benitez et al., 2009; Chys et al., 2017). These studies show, however, that the performance of AOPs reduces if the matrix becomes more complex. Rapid scavenging of both ozone and hydroxyl radicals by bulk organic matter reduces the amount of radicals that remain available for the micropollutant removal (Choi et al., 2014; Souza et al., 2014). Recently, AOPs based on the generation of sulfate radicals have gained attention (Waclawek et al., 2017; Wang et al., 2018). These radicals are characterized by a higher selectivity, exemplified by lower reaction rate constants with organic matter than hydroxyl radicals while maintaining high reactivity towards a large set of micropollutants. Sulfate radicals can be generated by activation of peroxymonosulfate (PMS), e.g. through reaction with ozone (Yang et al., 2015; Cong et al., 2015), but this technique has been scarcely investigated so far and fundamentals need to be explored yet. In this study, mechanisms of the O_3/PMS process are studied through the degradation of a set of selected pharmaceuticals in synthetic clean water.

MATERIAL AND METHODS

Chemicals

The target pharmaceuticals were chosen based on two criteria: 1) relevance in the environment, and 2) ozone reaction rate constants. Ketoprofen and metronidazole are compounds with a low ozone reaction rate constant (< 250 $M^{-1}s^{-1}$) while metoprolol, venlafaxine, carbamazepine and moxifloxacin have high ozone reaction rate constants (> 1000 $M^{-1}s^{-1}$). Atrazine and pCBA were also included in the degradation experiments as reference compounds, and have low ozone reaction rate constants (< 250 $M^{-1}s^{-1}$).

Experimental setup & design

All batch experiments were performed in glass beakers containing a total aqueous volume of 25-30 mL. The initial micropollutant concentration was 5 µM and both single compound and mixture-experiments were performed. In all experiments, the pH was fixed at 7.5 - except for the experiments with varying pH - by the addition of 10 mM phosphate buffer. The reaction was initiated by adding a defined volume of a PMS and ozone stock solution, and the molar ratio between ozone, PMS and micropollutants was kept constant. Samples were withdrawn at regular time intervals, and

subsequently quenched by Na_2SO_3. PMS was measured at the end of every experiment (to avoid any interferences with ozone) by a spectrophotometric method using potassium iodide (Liang et al., 2008).

To identify the main radicals contribution in the O_3 and O_3/PMS process, tert-butanol (t-BuOH) and methanol (MeOH) were added to the experimental solution. It is assumed that t-BuOH only scavenges $^{\bullet}OH$ radicals while MeOH scavenges all radicals that are present in the experiments. This assumption is justified as the reaction rate constant of t-BuOH and $^{\bullet}OH$ is about 3 orders of magnitude higher than that of t-BuOH and $SO_4^{\bullet-}$, while the reaction rate between MeOH and $^{\bullet}OH$ is only 70 times higher than that of MeOH and $SO_4^{\bullet-}$.

RESULTS

Removal of micropollutants in single compound- and mixture experiments

Figure 1 shows the removal (C_t/C_0) of pCBA, KET, ATZ and METR during the single-compound experiments. During the first minute of reaction in the O_3/PMS process, more than 90% of the initial compound concentration was removed. Compared to the ozonation process, this is an enhancement of 6 to 45%, which results from the addition of PMS. Furthermore, the results of the O_3/PMS/t-BuOH process indicate that sulfate radicals are able to remove at least 57% of the compounds. This is in contrast to the O_3/t-BuOH process in which only ozone is available for the removal of ozone-recalcitrant compounds, reducing the removal efficiency to 5%, 17% and 13% for respectively pCBA, KET and ATZ. Due to the higher ozone reaction rate constant of METR, a relatively fast removal is still noticed during O_3/t-BuOH. However, the low removal of METR during O_3/PMS/MeOH shows that competition occurs between PMS and METR for ozone. This competition effect was also observed during the degradation of fast O_3 reacting compounds (data not shown). However, for these compounds, no significant difference was noticed between the O_3 and O_3/PMS processes.

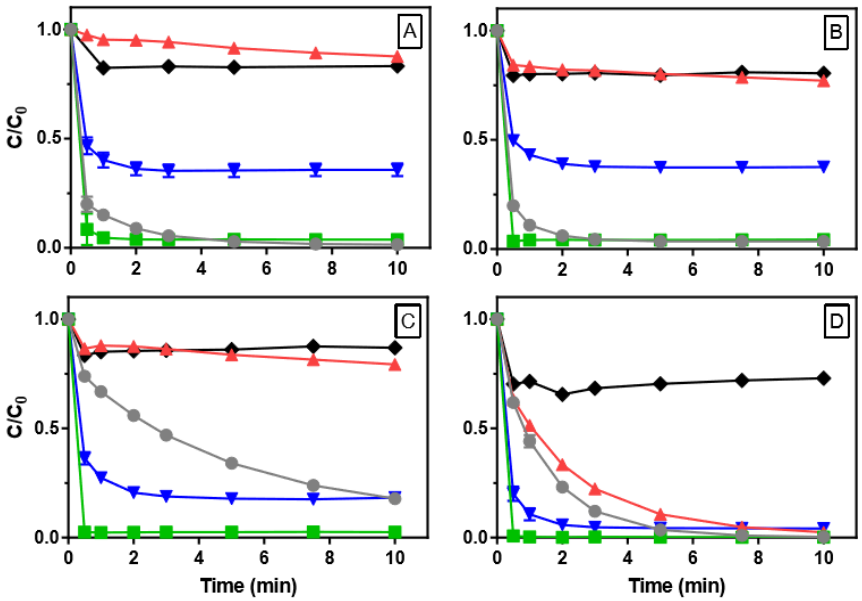

Figure 1. Removal of A) pCBA, B) KET, C) ATZ, D) METR in the (●) O_3, (■) O_3/PMS, (▲) O_3/t-BuOH, (▼) O_3/PMS/t-BuOH, and (♦) O_3/PMS/MeOH processes. Conditions: [µP] = 5 µM, [O_3] = 5 mg/L, PMS:O_3 (molar ratio) = 1:1, [t-BuOH] = 1 mM, [MeOH] = 10 mM, pH = 7.5

The relative contributions of ozone and the radicals in the O_3/PMS process are shown in Figure 2 for the single-compound and 4-compound-mixture experiments. These results show that sulfate radicals play an important role during the O_3/PMS process as their contribution is more than 50%. The high contribution of sulfate radicals in the O_3/PMS process ensures a removal that is less affected by the presence of $^{\bullet}$OH scavengers in a (waste)water matrix.

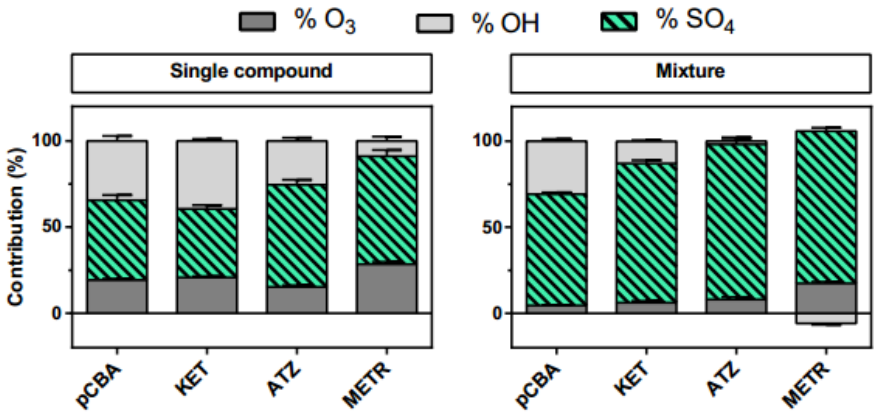

Figure 2. The contribution of O_3, $^{\bullet}$OH and $SO_4^{\bullet-}$, calculated at t = 1 min in both the single-compound and 4-compound-mixture experiments during the O_3/PMS process. Conditions: [µP] = 5 µM, Σ[µPs] = 20 µM, O_3:µPs (molar ratio) = 20:1, PMS:O_3 (molar ratio) = 1:1, [t-BuOH] = 1 mM, [MeOH] = 10 mM, pH = 7.5.

Effect of pH and PMS: O_3 ratio

The removal of the 8 selected compounds during O_3/PMS was studied at pH 3, 7, 8 and 10. At pH 3, only direct ozone reactions occur, so the removal of the ozone-recalcitrant compounds was low. At higher pH levels, the removal efficiency of these compounds increased as ozone started to decompose into radicals. Consequently, ozone and PMS were completely consumed at pH 10. However, at pH 7-8, only a partial consumption of the added PMS was noticed. In view of avoiding PMS residues in future applications, lower PMS doses were tested by decreasing the PMS:O_3 molar ratio. Results show that even at low PMS doses, the micropollutant removal is still enhanced by the addition of PMS in the O_3 process, since the pharmaceuticals removal was only lowered by 30 – 50% at a PMS:O_3 ratio of 1:10 compared with PMS:O_3 = 1:1. However, at this lower PMS dose, up to 90% of the added PMS is consumed, showing a more efficient PMS use.

CONCLUSION

This study shows that the ozone-activated peroxymonosulfate process is a promising technique for the improvement of AOPs aiming at the removal of organic micropollutants in secondary effluent. The results show that sulfate radicals are an important species during the O_3/PMS process as their contribution is more than 50% in the overall micropollutant removal. The positive effect of PMS is mostly noticeable during the removal of ozone-recalcitrant compounds, and is characterized by a high micropollutant removal during the first minute of reaction. The novel technique shows to be effective at high pH values (>7), which is ideal for

(waste)water treatment. Furthermore, the process can be optimized by reducing the amount of PMS that is added. The experiments showed that even the addition of relatively small amounts of PMS (PMS:O_3 ratio of 1:10) already shows a significant improvement of the ozonation process. However, more experiments need to be performed to test these findings in a real water matrix.

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COMBINING SHAPE-SELECTIVE ZEOLITES FOR THE EFFICIENT RECOVERY AND PURIFICATION OF BIOBUTANOL VIA ADSORPTION

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Biobutanol is an important platform chemical which can be produced from renewable resources via the acetone-butanol-ethanol (ABE) fermentation process. However, product inhibition severely limits the final butanol concentrations (< 2 wt%), making the conventional downstream processing via steam-stripping distillation very expensive. One way to relieve product inhibition is by in situ product removal, using the fermentation gasses to strip the produced solvents from the fermenter. However, subsequent energy-intensive separation steps are still needed to completely recover and purify the produced biobutanol.

In this work, a novel adsorption-desorption process is proposed to recovery biobutanol from the vapor mixture generated via gas-stripping. Two adsorbent materials, with a different selectivity, were combined in one cycle. In a first step, biobutanol is recovered from the vapor mixture using a butanol-selective zeolite (ITQ-29). During the regeneration of this material, co-adsorbed side products are captured using a second material (Si-CHA). This material is not selective for butanol, yet is able to remove the most important side products. In this way, 99% of the adsorbed 1-butanol on ITQ-29 could be recovered at a purity of 99.5 mole-%.

PHOTOCATALYTIC SELF-CLEANING COATINGS WITH EMBEDDED PLASMONIC GOLD NANOPARTICLES

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Photocatalytic self-cleaning coatings are an interesting upcoming technology to battle surface fouling and air pollution as they can be applied as thin films on various types of substrates. The problem with current TiO_2-based photocatalytic coatings is that they only respond to the UV-part of the solar light spectrum. Modifying the thin films with plasmonic nanoparticles can enhance the solar light response as these particles interact with visible light. The plasmonic effect is strongest near the surface of the nanoparticles. Hence, embedment of the particles into the TiO_2 matrix can take more advantage of this effect than loose deposition of the particles on the catalyst's surface. The organic medium of a typical TiO_2 sol-gel solution, however, is not compatible with an aqueous solution of gold nanoparticles. To circumvent this problem, pre-fabricated gold nanoparticles are first capped with an organic stabilizer and added to the mixture to obtain a one-pot Au-TiO_2 sol-gel coating suspension. The opposite strategy was applied by Sonawane et al. where the TiO_2 matrix was made compatible with the aqueous gold nanoparticles. From the sol-gel suspensions, homogeneous and highly transparent thin film coatings are achieved after spin-coating. The photocatalytic activity of TiO_2 thin films improves upon embedding gold nanoparticles as demonstrated by stearic acid degradation tests. Films with a 3 wt% gold loading have an increased stearic acid degradation efficiency of 16% and 40% under UVA and solar light illumination, respectively compared to pure TiO_2 thin films. The newly proposed protocol is also found to generate better photocatalytic activity than the previous method by Sonawane et al. with an increase in efficiency of an entire order of magnitude. This promising new strategy can be exploited in different applications and is patent pending (PCT/EP2018/079983).

Lecture session

SUSTAINABILITY NOW

MODEL-BASED ANALYSIS OF GREENHOUSE GAS EMISSION REDUCTION POTENTIAL THROUGH FARM-SCALE DIGESTION

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Biomass can be converted to biogas (a mixture of mainly CO_2 and CH_4) and digestate in the absence of oxygen, nitrite and nitrate by a wide diversity of micro-organisms (Pavlostathis et al., 1991). This process is called anaerobic digestion. Farm-scale anaerobic digestion of manure has become increasingly popular because farmers can be (partly) energy self-sufficient through valorisation of methane. Besides, it seems a promising strategy to prevent methane emissions from long-term manure storage, as storage facilities can be coupled directly to a digester (Miranda et al., 2015).

A fit-for-purpose anaerobic digestion model was set up to estimate desired methane production in the form of biogas as well as unwanted methane emissions associated with farm-scale digestion of manure. The generally accepted Anaerobic Digestion Model No. 1 was simplified assuming hydrolysis as the rate-limiting step. The carbon footprint of several types of Flemish dairy farms was assessed based on model simulations and literature data. The scenarios assessed differed in the possible presence of a digester as well as in the manure collection and storage method. Dairy farms with fresh manure collection by a manure scraper, followed by controlled digestion and digestate storage in a gas-tight tank located outside achieved the lowest carbon footprint. Controlled digestion must take place in a properly managed and correctly dimensioned reactor as high digester methane losses and low digester retention times increase the carbon footprint significantly.

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VALORISATION OF AGRICULTURAL WASTEWATERS INTO ALTERNATIVE PROTEINS USING DUCKWEED

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The aim of this research is to recover nutrients from agricultural wastewaters and convert them into high value feed protein by growing duckweed. Due to the high dependency of the European Union on imported portein [1], substitution of imported by locally produced protein would minimise the environmental impact of its livestock production [2]. Duckweed can be used as an alternative protein source due to its high protein content and high production rate [3]. In addition, this plant can grow on nutrient concentrated water like agricultural wastewater streams [4]. Therefore, two wastewaters were analysed for their potential as a nutrient source for duckweed production in an outdoor cubicontainer cascade: an aquaculture effluent from the production of Zander (Sander lucioperca), and an optimal mix from a pig manure treatment facility. We found an average productivity of 3.2 tons protein/ha/yr, indicating that duckweed can be around three times more productive than soybean. Additionally, no significant differences between the tested media were found. Nutrient recuperation of duckweed ranged between 244 and 316 mg N/m/day and 61 and 67 mg P/m/day. Modelling of the growth indicated that optimization of the productivity and quality of duckweed could be reached by reducing nitrite concentrations in the medium and managing the climatic conditions. Therefore, this study confirms the potential of duckweed in wastewater treatment and production of proteins in a low input system and indicates potential pathways for improvement.

This research is conducted in the framework of the project Blauwe Keten, financed by the European Interreg V-program “Vlaanderen-Nederland” – see https://www.biorefine.eu/projects/blue-chain-de-blauwe-keten for more information.

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CHARACTERIZATION OF HYDROGENOTROPHIC PURPLE BACTERIA: EXPLORING THE TUNABILITY OF MICROBIAL PROTEIN

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INTRODUCTION

Nitrogen and phosphorus are both essential nutrients in the food production chain. However, the biogeochemical cycles of these nutrients are severely altered and their flows towards the biosphere and oceans are exceeding the planetary boundaries [1]. Therefore, radical changes will have to be made to sustainably meet global nutritional demands now and in the future. One of these changes is the production of microbial protein (MP) which could lower global cropland area, global nitrogen losses from croplands and agricultural greenhouse gas emissions by respectively 6%, 8% and 7% [2]. Microbial protein can be produced by a variety of microorganism such as hydrogen oxidizing bacteria (HOB), methane oxidizing bacteria, aerobic heterotrophic bacteria, yeast, microalgae, purple bacteria, etc.

It is hypothesized that metabolic versatility can cause a high tunability of the nutritional quality. Purple bacteria (PB) are therefore one of the most attractive types of single cell protein as they can grow in all combinations of photo-, chemo-, auto-, and heterotrophic conditions. Moreover, their nutritional properties are outstanding, producing by-products such as protein, carotenoids, phytohormones and vitamins [3]–[6]. The composition of essential amino acids of these bacteria is found to be similar to that of soybean meal [7] and promising results were seen in feeding trials [3]. Only five studies in literature discuss the autotrophic growth of PB with hydrogen gas as electron donor (hydrogenotrophic) [8]–[12]. These papers did not include any data on the nutritional quality.

In this study, the photoautotrophic/photohydrogenotrophic growth of purple non-sulphur bacteria (PNSB) is examined. Exploring the growth mode in a biotechnological manner and analysing the nutritional quality of the PNSB.

MATERIAL AND METHODS

Strains

Rhodopseudomonas palustris (strain: LMG 18881) and *Rhodobacter sphaeroides* (strain: LMG 2827) were ordered through BCCM (Belgian Coordinated Collections of Microorganisms) and stored at -80°C. The enrichment community of *R. capsulatus* was obtained by incubating 0.1 g VSS/L of activated sludge from a sewage treatment plant, activated sludge from a dairy wastewater treatment plant and sediment from a local pond (1/1/1 VSS ratio). A dilution to extinction method was applied to obtain the most abundant isolate which was *Rhodobacter capsulatus*.

Medium

The photoautotrophic medium used is based on Madigan et al. (1979) and adapted with the use of $NaHCO_3$ carbon source based on Rey et al. (2006). Carbon and nitrogen were dosed in a concentration of 0.33 g-C/L and 0.26 g-N/L. The buffer used in the medium was a phosphate buffer with 20 mM strength (0.59 g-P/L). However, this original medium showed a high increase in pH. Therefore three buffers (phosphate buffer, HEPES and Tris) and buffer strengths 10-30 mM were tested to optimize the medium, wherein phosphorus was never limiting.

Batch growth test

Growth test were performed in batch with Erlenmeyer flasks of 608 mL closed with septa, that were constantly mixed at 400 rpm. A working volume of 300 mL was taken and the headspace was flushed on designated periods for 1 minute with either a gas mixture of 10% H_2/90% N_2 or 80% H_2/20% N_2. The hydrogen gas provided the electron donor and energy was supplied by halogen lights (39.8 W/m², 400-1000 nm).

Chemical analyses and calculations

The pH was measured with edge pH (Hanna instruments). Optical density at 660 nm was measured with an UV-VIS spectrophotometer (UV-2501PC) of Shimadzu®. Maximum growth rates were calculated fitting the experimental data to the Gompertz model modified by Zwietering et al. (1990) in GraphPad Prisma 8 software. Maximum growth rates were all transformed with the Arrhenius equation $\mu_{T1}=\mu_{T2}*\theta^{(T1-T2)}$ (θ=1.07) to a temperature of 28°C [13].

The percentage of hydrogen gas in the headspace was monitored with gas chromatography (GC-2014, shimadzu, TCD detector, Shincarbon-ST 50/80 Stainless steel 2.0 m x 3.0 mm, helium as carrier gas). This percentage was then converted to a molar concentration via the ideal gas law. Protein analysis was performed with a modified lowry method [14].

RESULTS

pH buffer optimization

In a first stage the three PNSB species (*R. capsulatus, R. palustris* and *R. sphaeroides)* were used to determine the ideal buffer and buffer strength. It's important to stay below the limit of pH 8 to avoid the formation of $CaCO_3$. Overall the pH remained most stable with the use of the phosphate buffer (increase of 10%) compared to HEPES and tris (increases of 16%). Furthermore, the lower pKa of phosphate buffer (7.21) provided a lower initial pH of 6.66. While the HEPES and tris buffered samples started at 7.18 and 7.20 respectively.

Moreover, *R. palustris* displayed a better growth performance with the phosphate buffer of 30 mM with a maximum growth rate of 0.49 d^{-1} (corrected to 28°C). Maximum growth rates for the

HEPES and tris buffered objects were 0.42 and 0.43 d^{-1} (corrected to 28°C) respectively. However, all of these values are comparatively low to the 0.17-4.82 d^{-1} (28°C) found in literature [8]–[12], [15]. Overall, the phosphate buffer of 30 mM showed the best pH control and growth performance and thus was chosen in the optimized medium.

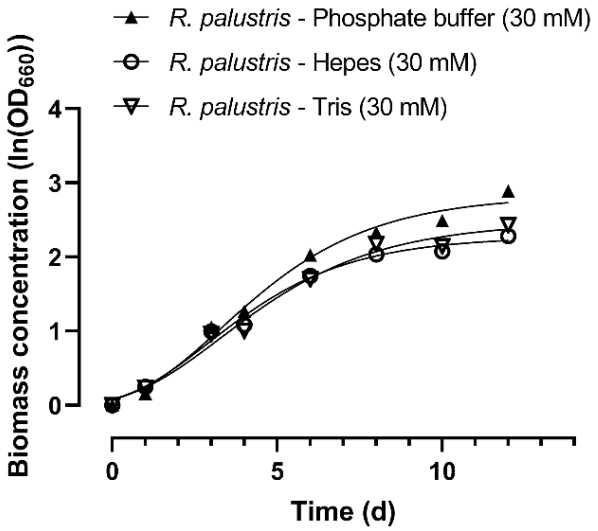

Figure 1: Photoautotrophic growth curves (10% H_2) of *R. palustris* with three pH-buffer systems at a strength of 30 mM.

Growth test with 10% H_2

Subsequently, *R. sphaeroides* was grown in the optimized medium and the effect of flushing was determined. Samples were flushed for 1 minute only once, once every 3 days or once every day. Figure 2A shows that the samples with only one flush becomes limited in growth due to a limitation in electron donor. The growth curves of the objects flushed once/3day and once/day show similar results with a μ_{max} of 0.52 and 0.48 d^{-1} respectively (corrected to 28°C). However, the pH in the daily flushed sample increased significantly more than the every 3-day flushed sample (data not shown). This is due to the flushing out of CO_2 which causes the carbon buffer system to shift, increasing the pH and losing part of the carbon available in the medium.

Hydrogen gas consumption was measured with gas chromatography resulting in hydrogen yields ranging from 8.6-9.8 g dry weight/g H_2. These are economically very promising results compared to the lower hydrogen gas yield of hydrogen oxidizing bacteria (2.3 g dry weight/g H_2 [16]). Moreover, the dependency of hydrogen gas in this photoautotrophic metabolism is shown when the cumulative hydrogen gas consumption is plotted against the growth of *R. sphaeroides* for the daily flushed sample (Figure 2B).

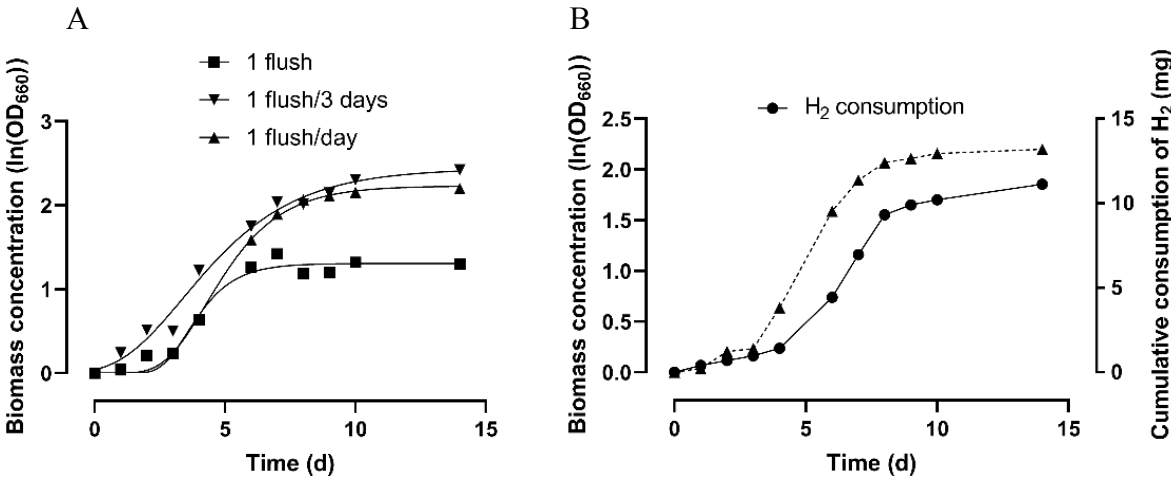

Figure 2: A) Photoautotrophic growth curves (10% H_2) of *R. sphaeroides* for different reccurences of flushing B) Photoautotrophic growth curve combined with the cumulative consumption of hydrogen gas showing the dependency between the two.

Growth test with 80% H_2

Taking into account the impact of flushing (increasing pH and decrease carbon available for growth), a new gas mixture with 80% H_2/20% N_2 was tested in order to minimize the frequency of flushing. The same PNSB species (*R. capsulatus, R. palustris* and *R. sphaeroides)* were grown in optimized conditions and maximum growth rates were respectively 3.0, 2.3 and 3.4 d^{-1} (corrected to 28°C), which are in line with values from literature (0.17-4.82 d^{-1}). Growth also proceeded faster and the stationary phase was reached within 4 days (Figure **3**), indicating the importance of sufficiently high H_2 levels to avoid kinetic limitations. Due to this more rapid consumption of H_2 an under pressure was created in the Erlenmeyer flasks. This was solved by spiking (instead of flushing) the flasks with the 80% H_2/20% N_2 gas mixture until atmospheric pressure was reached again. Overall, a carbon loss from the closed system was thus avoided.

The first nutritional analysis showed protein contents of 46, 40 and 45% of dry weight respectively for *R. capsulatus, R. palustris* and *R. sphaeroides* in the stationary phase. This corresponds to protein productivities of 0.08-0.09 g protein/L/day. As mentioned before, no nutritional data of photoautotrophically grown PNSB are available. Thus these nutritional data have to be positioned in literature by comparing with similar growth modes such as microalgae, HOB and PNSB (photoheterotrophic) as shown in Table 1.

It is seen that the protein productivities of photoautotrophic growth with H_2 are in the same order of magnitude as photoautotrophic growth of microalgae. While the photoheterotrophic PNSB and the HOB have significantly higher protein productivities. However, great optimization potentials are expected to boost the protein productivity of photoautotrophic PNSB further. The transfer of hydrogen gas to the medium has to be optimized as this was a kinetic limitation before, and might still not be optimal by switching to the 80% H_2.

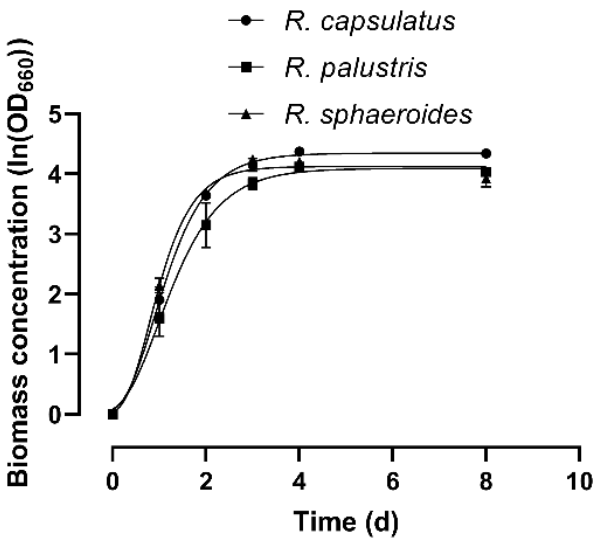

Figure 3: Photoautotrophic growth curves of *R. capsulatus*, *R. palustris* and *R. sphaeroides* grown with 80% H_2.

Table 1: Comparison between the protein productivities of different micro-organisms/growth modes. Similarities between the photoautotrophic growth mode on H_2 of the current study and the other growth modes are underlined.

	Growth mode	Protein productivity (g protein/L/day)	Source
PNSB	• Photoautotrophic with H_2 • Batch	0.08-0.09	This study
Microalgae (*Arthrospira* platensis)	• Photoautotrophic with H_2O • Batch	0.03-0.04	[17]
PNSB	• Photoheterotrophic • Semi-continuous photobioreactor	0.20-0.64	[18]
HOB	• Chemoautotrophic (H_2) • Sequencing batch reactor	1.23	[16]

DISCUSSION

Hence, the first layers of the photoautotrophic growth of PNSB on hydrogen gas for the use of microbial protein have been uncovered and show promising results. The medium was optimized with the use of a phosphate buffer of 30 mM. Initial experiments with 10% H_2 resulted in maximum growth rates of 0.42-0.52 d^{-1}, which are in the lower range found in literature (0.17-4.82 d^{-1}). Follow-up experiments with 80% H_2 gave a μ_{max} between 2.3-3.4 d^{-1}, showing the kinetic limitation of 10% H_2. Hydrogen yields were promising as they were higher than HOB, which could prove to be an economic advantage.

The protein productivities of photoautotrophic growth of PNSB is higher than the photoautotrophic growth of microalgae. However, a great optimization potential still remains to stretch this nutritional quality further. Future research will focus on changing the growth conditions (e.g. pH, light) and the subsequent effect on growth and nutritional quality. In parallel, a reactor will be designed to efficiently transfer the hydrogen gas into the medium.

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SAFEGUARDING NATURA 2000 HABITATS FROM NITROGEN DEPOSITION BY TACKLING AMMONIA EMISSIONS FROM LIVESTOCK FACILITIES: UNEQUAL EFFORTS AND UNEVEN EFFECTS

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The deposition of reactive nitrogen in Flemish habitats protected by the European Habitats Directive is currently too high to ensure the conservation objectives in these habitats, as 85% of the total surface area of Natura 2000 sites suffers from an exceedance of the critical load for nitrogen. Livestock farms are often situated in proximity of natural areas and are an important source of ammonia. Therefore, they have a high contribution to nitrogen deposition in Natura 2000 areas. To get the environmental licensing of livestock facilities in line with article 6 of the Habitats Directive, that states that projects can only be authorized after ascertaining that there's no adverse effects on protected sites, a Programmatic Approach to Nitrogen (PAN) is implemented in Flanders [1]. Next to general emission reduction targets, this PAN entails spatially-differentiated limitations on livestock farming in the vicinity of protected sites. Using an integrated modelling approach [2], we evaluated this policy on the level of individual farms and habitats. The results reveal that the PAN falls short in obtaining a significantly larger proportion of habitats without exceedance of the critical load for nitrogen. Furthermore, the policy affects some sectors more than others. Similar discrepancies are unveiled on the level of Natura 2000 areas and habitat types. Our model allows to simulate alternative policy scenarios, that balance efforts on the side of agriculture, such as technical emission abatement and a reduction in animal numbers, with habitat restoration measures.

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CHEMICAL HYDROGEN STORAGE FOR ITS USE AS A FUEL IN THE SHIPPING INDUSTRY

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To lower the emission of greenhouse gasses that are associated with burning fossil fuels, Compagnie Maritime Belge (CMB) has developed a pilot ship that uses hydrogen as fuel. On this ship, hydrogen is combusted in a controlled way in an internal combustion engine with only water as byproduct. To store the hydrogen onboard larger ships than the pilot ships, the use of classical hydrogen storage in pressurized containers is not sufficient due to the low volumetric density of these pressurized systems, and liquefaction of hydrogen is associated with a high energy loss (±40%) to cool the hydrogen below its boiling point at -253 °C. To be used more efficiently and economically on board of large ships, other methods are investigated to store the hydrogen. A promising method of storing hydrogen is to bind it chemically into molecules with a high hydrogen content and release the hydrogen on board of the ship, right before combustion. Most notable among these molecules are methanol, formic acid, ammonia and liquid organic hydrogen carriers (LOHCs). LOHCs are couples of cycloalkanes and corresponding aromatics, e.g. methyl-cyclohexane and toluene. These chemical hydrogen storage methods have already been investigated as possible carriers for hydrogen in cars, however due to the high reaction temperature (>150 °C) needed to release hydrogen from the carrier, these systems have not yet had their breakthrough. The methods seem more promising in the shipping industry as the waste heat from a ship's engine (160 – 200 °C) is much higher than in car engines, and can be used to release hydrogen from the carrier molecule.

During this oral presentation, the advantages and necessity of hydrogen as a fuel in the shipping industry will be discussed, as well as methods of fuel storage that combine safety and a higher volumetric density than conventional storage.

Lecture session

PLANTS & CROPS

IDENTIFICATION OF A NEW ALGICIDAL COMPOUND DERIVED FROM QUORUM SENSING SIGNALLING MOLECULES

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Marine diatoms are known to harbour specific bacteria in their microenvironment, many of which can engage in cell-cell communication via quorum sensing signalling molecules. One of the most abundant classes of signalling molecules in bacterial communication are N-acylated homoserine lactones (AHLs). Of these AHLs, many homologues and derivatives are known. The 3-oxo derivatives however are of particular interest as they can degrade into tetramic acids, compounds that have been shown to possess diverse bioactivities. To test if these AHLs and their derived tetramic acids can negatively influence the physiology of marine diatoms, we have synthesised N-(3-oxododecanoyl)-L-homoserine lactone (OXO12) as well as its derived tetramic acid (TA12) as representatives of this class of compounds and tested them on the model diatom, Phaeodactylum tricornutum. Additionally, we have monitored the rearrangement kinetics of OXO12 to TA12 under the growth conditions of P. tricornutum.

The effect of OXO12 on the growth of the model diatom was limited, while TA12 exhibited strong algicidal effects. These new insights in the stability and bioactivity of both the 3-oxo-AHLs and their derived tetramic acids, can shed new light on the hypothesis that these are highly important compounds for interkingdom interactions.

FREQUENTLY FROZEN FLOWERS? TOWARDS ASSESSING FUTURE FROST RISKS IN BELGIAN ORCHARDS

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The Belgian hard fruit sector (apples and pears) has been subjected to a range of meteorological and political hazards over the past decade. One of the most disastrous events was an extraordinarily severe frost night during peak flowering period of commercially grown apple cultivars, in April 2017. The phenology, including the flowering, is strongly related to air temperature during winter/early spring and can be modeled using sequential phenological models on daily mean temperatures, as described by Chmielewski *et al*. (2011). Important changes are expected to be observed in relation to warmer winters as an effect of climate change. Because both the onset of flowering and the last frost have been projected to occur earlier in the year, the challenge is to find out which trend is more dominant. Using a set of regional climate models from the CORDEX ensemble, the chance of frost to occur during flowering will be assessed for the 21st century. Thereby, differences between cultivars and between locations within Belgium are taken into consideration. Furthermore, the impact of shifting temperature regimes during the blossom period on the pollinator activity will be taken into account, knowing that the most commonly employed bee species are sensitive to colder and more variable temperatures. Ultimately, the results are supposed to support decision making when it comes to choosing adapted prevention measures on the field.

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VARIABILITY OF ESSENTIAL OIL COMPOSITION AND BIOLOGICAL ACTIVITIES OF *LANTANA RHODESIENSIS* MOLDENKE FROM CÔTE D'IVOIRE

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Lantana rhodesiensis Moldenke is an herb or small shrub under two meters tall which is often multi-stemmed[1]. It is used in traditional medicine for several treatments like diabetes[1], rheumatism[2], and arterial hypertension[3].

The aim of the present study was to determine the variation in the chemical composition of essential oils of L. rhodesiensis from Côte d'Ivoire. It also aimed to evaluate the in vitro antioxidant and anti-inflammatory activities of essential oils. Essential oils were obtained by hydrodistillation from leaves, stems and fruits of *L. rhodesiensis* from two localities of Northern Côte d'Ivoire. Essential oil composition was determined by GC-MS. The study of the antioxidant activity of leave essential oils was carried out by the DPPH and FRAP method; the anti-inflammatory activity was evaluated by the inhibition of protein denaturation method.

Results showed that the main constituents present in most essential oils extracted from leaves, fruits and stems were β-caryophyllene, α-copaene, δ-cadinene and α-humulene. However, thymol was the major component in three leave samples. The stem oils are also dominated by caryophyllene oxide or thymol. It may be concluded that the sesquiterpenes were the predominant molecules in all essential oil samples of *L. rhodesiensis* from Northern Côte d'Ivoire. The results of the biological activities carried out on the leave essential oil samples showed that these oils have antioxidant and anti-inflammatory activities. This study showed the interest of *L. rhodesiensis* for the treatment of rheumatism and diabetes in traditional medicine. *L. rhodesiensis* is a potential source for isolating new antioxidant and anti-inflammatory molecules.

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RELATIONSHIP BETWEEN ELECTRICAL ANISOTROPY OF SOIL-ROOT CONTINUUM AND GEOMETRICAL ARCHITECTURE OF ROOT SYSTEM

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In this work, we run finite element simulations of soil-root continuum with explicit root architecture in the finite element mesh, to understand whether a root system can be characterized using anisotropy in effective electrical conductivity. To parameterize the model, we measured complex conductivity of Maize root segments at different frequencies (IP spectra). The roots were explicitly represented with their frequency dependent electrical properties in the finite element mesh, including the properties of cortex and stele. Effective electrical anisotropy and geometrical anisotropy are examined for different synthetic root architecture generated in Crootbox software. We then relate the modeled effective electrical anisotropy to the geometrical anisotropy of root structure. To formulate geometrical anisotropy, we discuss different approaches. We also demonstrate high correlation between geometrical anisotropy of root networks and simulated electrical anisotropy of effective conductivity of soil-root continuum. The understanding of relation between geometry of root network, electrical conductivity contrast between soil-root and effective electrical property of soil-root continuum is very important to monitor rooted zone via electrical methods and our modeling results helps to give insight.

Anisotropy in electrical conductivity is a typical feature observed in geology and can be used to characterize sub-surface heterogeneity (Al-Hazaimay *et al.*, 2016). In soil-root medium, the effective electrical anisotropy factor (EAF) in electrical conductivity (EC) is due to root processes such as root water uptake, root architectural evolution, exudation and solute uptake. Under homogeneous soil conditions, (i.e. well irrigated scenario and no water uptake pattern in the soil), we hypothesize

that EAF of soil-root medium could contain information on root architecture (geometrical indices) and thus can be used for phenotyping root systems.

To support our hypothesis, we performed 2D finite-element modeling of these properties in terms of EC distribution for a series of simulated root systems, with explicit representation of the root architecture in the finite-element mesh. We then find the correlation between geometry of root network and EAF and assess under what condition will there exist high correlation and hence EAF could provide information on root architecture.

BIOCHEMICAL MARKERS TO EVALUATE THE IMPACT OF TERROIR ON THE BREWING VALUE OF HOPS

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Diverse compounds of the hops used in the brewing process are decisive for the taste and aromas of the final beer. We have recently shown that hop pellets of the Amarillo variety, grown at different locations in North-western USA (Idaho and Washington), led to divergent flavour observations in Amarillo-derived single hop beers.

In order to investigate to which extent this consideration applies to other hop varieties, cone samples were collected from 20 commercially relevant hop varieties originating from the main hop growing areas in the world and for 3 successive harvest years. For each variety, the biochemical characteristics of samples from different geographical production areas were established. The levels of hop bitter acids and hop essential oils, and especially the composition of the hop oils, have been found valuable for the differentiation of samples of the same hop variety originating from distinct growing areas. Finally, single hop beers were produced with specific hop varieties originating from different growing areas. Besides the used hop batches, the derived single hop beers were evaluated in detail from biochemically and sensorially perspectives in order to gain insights into the impact of hops and their terroirs on the final beer quality.

Taken together, it was clearly demonstrated that biochemical marker information is valuable to characterize hop samples of different geographical origins, hence, for the brewing value of hops from distinct terroirs.

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Lecture session

FOOD TECHNOLOGY

SAFETY &

HEALTH

WHEY PROTEIN ISOLATE - ALMOND GUM NANO-COMPLEXES AS POTENTIAL GREEN AND SUSTAINABLE ALTERNATIVE FOR SYNTHETIC SURFACTANTS

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There is an increasing consumer demand for more natural and environmentally friendly products known as ‘‘clean label’’ products including foods, cosmetics and pharmaceuticals. Recently, protein-polysaccharide complexes have been the subject of several studies due to their green and straightforward preparation, as well as sustainable based materials and economic costs as an alternative for synthetic surfactants with detrimental effects such as toxicity. This study investigated the interaction of whey protein isolate (WPI) with the soluble fraction of almond gum (SFAG) at varying biopolymer ratio, pH, and total polymer concentration. These negatively charged soluble complexes (-36.5 mV) with average size of around 313 nm were used to fabricate thymol Pickering stabilized emulsions. Cryo-SEM and CLSM revealed that the adsorption of the nanoparticles on the surface of the oil droplets was responsible for the stabilization of the thymol emulsions. Droplet size analyses suggested that the WPI-SFAG complexes were successfully capable to retard aggregation and/or coalescence of the droplets while native WPI showed severe creaming and coalescence after 40 days of storage. The formulated thymol oil-in-water emulsions have a potential application as an antimicrobial and antioxidant agent in different products. Our findings provide a way to design ‘‘surfactant free’’ products from sustainable natural sources for variable applications such as in food, health care, and cosmetics.

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CHANGES IN THE SOLUBLE AND INSOLUBLE FRACTIONS OF ORANGE JUICE DURING STORAGE IN RELATION TO NON-ENZYMATIC BROWNING

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Orange juice is a heterogeneous system made up of serum containing soluble compounds, and an insoluble fraction comprising cloud and pulp which have different solubility in water and/or ethanol. While the serum contributes mainly to the taste, the cloud and pulp enhance colour, flavour and mouthfeel of the juice. Colour is the most important factor in the marketing of orange juice. Therefore, the detrimental colour changes in pasteurized shelf-stable orange juice during storage, primarily caused by non-enzymatic browning reactions (NEB), reduce consumer acceptance of the juice. Although NEB has been studied intensively, a clear understanding of this problem is still missing in literature. Therefore, the aim of this work is to gain a better insight into NEB by exploring the changes during storage in the orange juice's soluble and insoluble fraction in relation to browning.

Pasteurised shelf-stable orange juice was stored at an elevated temperature (42 °C) for varying times. The non-stored and stored orange juice was fractionated into soluble and insoluble fractions using centrifugation and (stepwise-)ethanol precipitation and the changes in different fractions during storage were characterised.

The results showed that brown compounds formed during storage of the orange juice distributed over soluble and insoluble fractions. The changes in the soluble fraction, namely ascorbic acid degradation, hydrolysis of sucrose, and formation of furfural and 5-hydroxymethylfurfural had strong correlations with the browning development of the juice. In the insoluble fraction, the brown compounds were hypothesized to be linked to the protein moieties. However, the presence of brown compounds did not significantly change the monosaccharide composition, protein content as well as the molecular weight of the insoluble faction. The outcome of this study revealed that the brown compounds formed in orange juice during storage resulted from reactions involving the soluble compounds, which consecutively were linked to the insoluble compounds.

THE EFFECT OF PROCESSED MEAT RELATED COMPOUNDS ON COLON CELLS: RELEVANCE OF LONG TERM APPROACH

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INTRODUCTION

Colorectal cancer remains one of the leading causes of cancer death in developed countries, even though it is estimated that one third of cases could be avoided by a change of lifestyle factors [1, 2]. In this context, red and processed meat consumption has been correlated with colon cancer development. Red meat includes beef, lamb, veal and pork [3]. Processed meat is defined as any meat that is modified by smoking, curing, salting or the addition of chemical preservatives [4]. Based on a hazard analysis the IARC classified processed meat as 'carcinogenic' and red meat as 'probably carcinogenic' [5]. Moreover the WHO recommends an intake of less than 500 g/week [6]. Nevertheless the meat consumption worldwide keeps increasing, and processed meat products gain popularity over unprocessed meat [7, 8]. The underlying pathways are still unclear, but a number of hypotheses are currently investigated. Robust and relevant models are necessary if we want to study the mechanisms that play a role. In vitro models, if physiologically relevant, may offer an elegant alternative for human and animal studies. Colorectal cancer models are often developed with the major purpose of testing potential therapeutic compounds, but could also be of great value in the field of nutrition and health, on condition that the established models can cope with the complex food matrix. As for the colon, the epithelial cells are exposed to a mixture of metabolites resulting from the food digestion throughout the gastro-intestinal tract. Metabolites can have positive or negative effects, which can be direct or indirect (e.g. through an impact on the microbiota [9, 10]). That environment is difficult to mimic in vitro, but we believe that the best way would be to expose cell models to low doses of food metabolites for a longer period of time. Common exposure times are 24-72h thereby studying acute effects, but longer exposure would better mimic continuous presence of food metabolites in the colonic lumen as would be the case when consuming a certain food on a regular base. This approach also allows us to focus on the whole cancer development process, rather than just on cancer initiation. Cancer is a slow process, as it can take years to evolve from the first mutations to tumor formation and eventually metastasis. This is certainly true for colorectal cancer, which makes a long term setup possibly even more relevant. For this study, we hypothesized that a comparison of 72h ("short term") and 10-12 days ("long term") exposure experiments could have different outcomes, and that a long term *in vitro* study of meat metabolites and food in general could give valuable information about human health.

MATERIAL AND METHODS

Two colon cancer cell lines Caco-2 and HT-29 were exposed to metabolites that are associated with red/processed meat digestion: nitrite, hemoglobin, hemin (all (processed) meat matrix components) and sodium butyrate (a short-chain fatty acid formed by fermentation of undigested fibers in the colon. Two major setups were tested for each of the cell lines: (i) short term exposure (3 days) to a wide concentration range of the compounds, solved in serum-free cell medium (Dulbecco's modified Eagle's medium + Glutamax + 1% Penicillin/Streptomycin and 1% nonessential amino acids) and (ii) long term exposure (9-12 days) to non-toxic concentrations of each compound, in serum-containing cell medium (supplemented with 10% fetal bovine serum (FBS). Follow-up was done by high-throughput endpoint assays measuring cell viability, protein content and IncuCyte Live Cell Analysis technology.

CELL VIABILITY ASSAY

To check cell viability, an assay based on conversion of water soluble, yellow 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) compound to the insoluble, blue formazan is used. Viable cells with mitochondrial activity can complete this conversion whereas dead cells lose this ability. Consequently, the measured absorbance (590 nm) is proportional to the number of viable cells. This test was performed on 96-well plates that were seeded with 20.000 cells/well, and exposed to a solution of one of the compounds starting from 24h after seeding, until the time of measurement.

PROTEIN CONTENT MEASUREMENTS

A first protein measurement assay relies on the property of Sulforhodamine B (SRB) to bind to amino acid residues of proteins in the cells. The amount of bound SRB is then a proxy for cell number. The assay is performed on fixed cells (with TCA 50%) in 96-well plates, on which first the viability was measured. Another spectroscopic procedure used to measure the protein concentration in a solution, and thus to estimate the number of cells in a solution, is the Bradford assay. The Bradford reagent consists of Brilliant Blue G dye in phosphoric acid and methanol. The protein-dye complex causes a shift in the absorption maximum of the dye to 595 nm. In combination with the viability data, the protein content gives an idea of cell proliferation.

INCUCYTE

IncuCyte is a live-cell analysis system that automatically acquires images of 600 wells by scanning each well every two hours, providing real-time kinetic data. HT-29 cells were seeded in 96-well plates, 4000 cells per well. The incubator keeps the cells at 37°C and 5% CO_2 while the IncuCyte collects the data, making it an ideal system for long term studies. Treatments were applied 24h after seeding in six-fold. The pictures were analyzed with the IncuCyte ZOOM 2018A Software package, resulting in data for confluency degree (%) for each condition and each time point.

RESULTS

The collected data shows that long term exposure can be crucial to observe the effects of the meat metabolites. Here we discuss hemin (Figure 1) and sodium butyrate (Figure 2). 72h exposure with hemin did not result in any significant difference in cell viability or cell number (as estimated by protein content), whereas long term exposure did have an effect. For HT-29 cells treated with hemin for example, no effect on protein content was observed for the first 9 days, whereas after 12 days a clear increase of 30% is measured. For Caco-2 cells, a similar increase was observed after 9 days but not yet after 5 days of treatment. The IncuCyte data provides us with more time points and confirms that the curve of cell confluency degree (indirect measure for cell growth) for untreated cells and the curve for hemin treatment start diverging after 5 or more days.

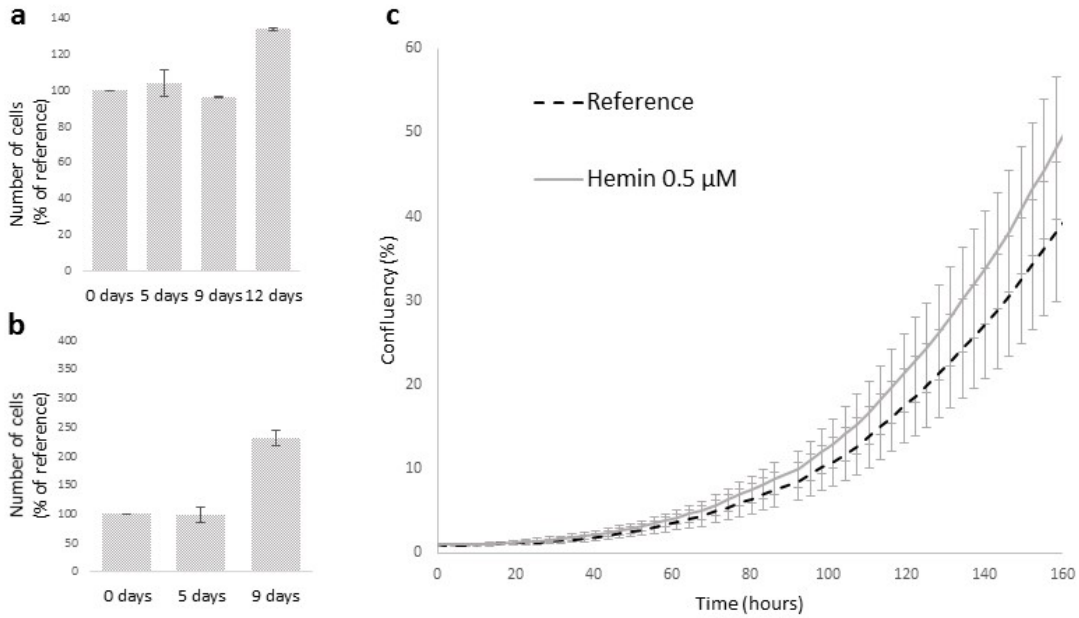

Figure 1. Estimation of cell number in function of time. (a) HT-29 exposed to hemin (0.1 µM) for 12 days, analyzed with Bradford assay at 4 time points. (b) Caco-2 exposed to hemin (0.1 µM) analyzed with Bradford assay at 3 time points. The data is expressed as relative values (%), with 100% the mean value of the untreated cells. (c) HT-29 exposed to (i) growth medium (Reference) and (ii) hemin (0.5 µM) analyzed with IncuCyte.

Moreover, in some conditions, the short and long term experiments had opposite results for cell number estimations. This was e.g. the case for Caco-2 cells treated with sodium butyrate (Figure 3). The long term treated cells display a higher cell number after 9 days for all the tested concentrations. In contrast, in the short term experiment the cell viability and cell number decreased with the butyrate concentration. This is a concentration effect known as 'the butyrate paradox' [11-13]. Again the IncuCyte curves give a good impression of the importance of the longer treatment, as the difference between the conditions is only observable in the final stage of the experiment.

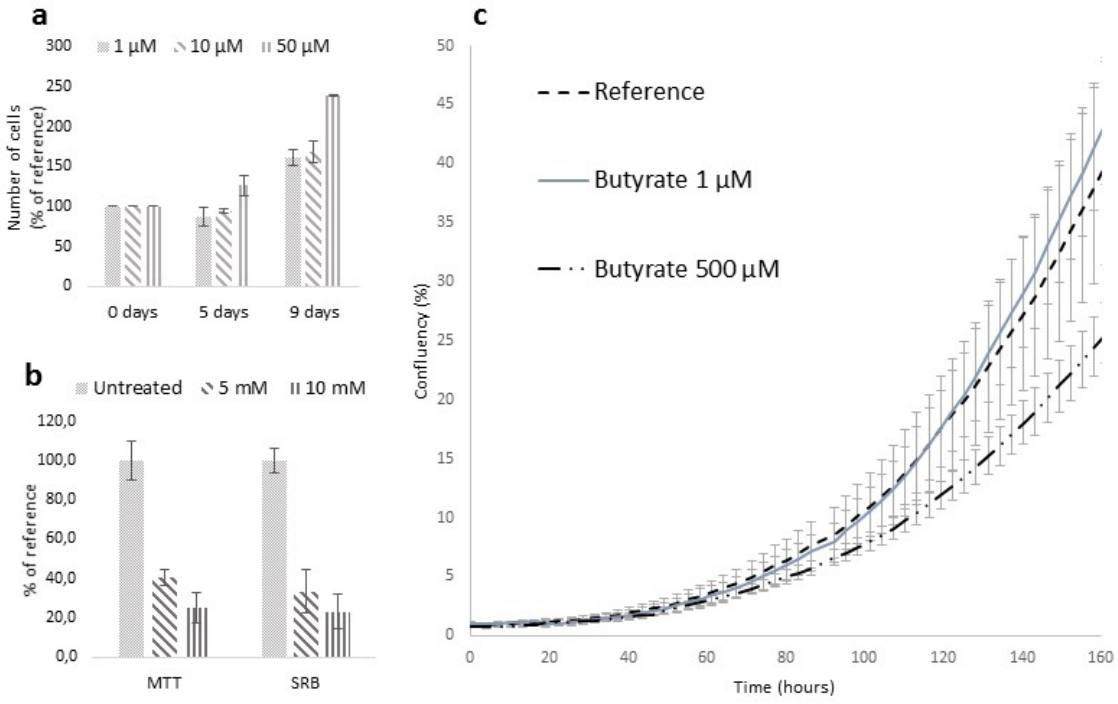

Figure 2. a) Estimation of number of Caco-2 cells exposed to three different sodium butyrate concentrations, measured with the Bradford assay at 3 time points. b) Measurement of cell viability (MTT) and protein content (SRB) after 3 days of exposure to sodium butyrate in 2 concentrations. The data is expressed as relative values (%) of the untreated cells (Reference). c) Confluency degree of HT-29 cells in 3 conditions.

From these experiments, we were able to observe differences in cellular outcome between non-treated and metabolite-treated cells and between different concentrations of the same compound, that would not have been visible in a short term study with the same concentrations. In 72h exposure experiments researchers often use higher concentration ranges because toxicity is less of a problem if the cells only have to survive for a couple of days, but as the butyrate paradox proves, the observations of short-term-high-concentration tests should be considered with care. Moreover, these high concentrations often do not occur in vivo in the lumen. Therefore we believe that the exposure of cells to metabolites should be as long as possible, in order to achieve a more relevant approach of studying the impact of the metabolites on the cells and the human health. We observed some significant effects of the meat compounds on cellular growth, which implies that meat consumption could have a direct effect on the metabolism or pathways in colon cancer cells.

CONCLUSION

We aimed to use and optimize cell-based models for food/meat research and tried to increase the relevance of established models by using a low dose-long term approach, thereby better simulating the in vivo conditions. The results indicate an effect of meat metabolites on colon cancer cells and will be further investigated in other, more complex in vitro models, still in a long term approach. The choice of setup (short term vs long term exposure) did have an impact on the results in some cases, thereby providing us with extra information about the way the cells cope with the

presence of the compounds. Therefore it is relevant to include a long term setup in the study of the impact of processed meat on colon (cancer) cells.

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FUNDAMENTAL RESEARCH ON FACTORS INFLUENCING MINERAL BIOACCESSIBILITY IN PLANT-BASED FOOD SYSTEMS: THE CASE OF PECTIN DEGREE OF METHOXYLATION AND CONCENTRATION OF CO-EXISTING MINERALS

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Plant-based foods gain more importance since they play a key role in sustainable, low-meat and healthy diets. Nevertheless, the question arises on how efficient they are to deliver minerals and if it is useful to encourage their consumption to reduce the prevalence of mineral deficiencies? Several antinutrients (e.g. pectin) may contribute to a reduced mineral bioaccessibility from plant-based foods by formation of indigestible complexes due to mineral binding. Structural characteristics of the antinutrients, as for instance the degree of methoxylation (DM) in the case of pectin, play a role in this mineral binding phenomenon which has been described before, however, only in single mineral model systems. In natural food systems, several mineral types are present together which may affect each other's bioaccessibility. Therefore, in this work, the influence of the presence of Ca^{2+} on Zn^{2+} binding capacity and bioaccessibility in mineral-pectin model systems with different DM was investigated. The results showed that increasing Ca^{2+} concentration and pectin DM reduces the Zn^{2+} binding capacity of pectin and consequently increases Zn^{2+} bioaccessibility. Moreover, an intelligible in vitro digestion procedure (only adjustment of pH, no addition of enzymes, bile salts nor digestive fluids) was found to be most appropriate to investigate the influence of pectin DM and presence of Ca^{2+} on Zn^{2+} bioaccessibility in mineral-pectin model systems.

YEAST PROTEOMICS DURING BEER FERMENTATION: CHALLENGES AND PROSPECTS

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The alcoholic fermentation of wort by yeast is a crucial step during the production of beer. The overall conditions during this process have an impact on the behaviour of the yeast cells, which is itself determined by the yeast proteome. Therefore, monitoring the yeast proteome via targeted proteomics might provide more insight into the response of the yeast cells to the fermentation conditions. Mass spectrometry proteomics has already been used to describe the dynamics of the wort untargeted proteome during nanoscale beer production but has not yet been applied to follow and quantify the yeast proteome during beer production. In this study, a framework is being developed to monitor the yeast proteome during the fermentation process of beer via liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS). Hereto, a selection of around 25 enzymes responsible for the production of desirable (e.g. esters and higher alcohols) and undesirable flavours (e.g. DMS and diacetyl), are quantified during the fermentation process, and the results are compared with the substrates assimilation profiles and the metabolites production profiles. A variety of yeast enzymes is already monitored with this technique, but the reliable quantification remains a challenge and is currently being investigated. The established framework will be used to shed new light on the reaction of different yeast strains to innovative raw materials and adjuncts, and differences in gravity and temperature. These insights may help the development of novel beer flavours and more sustainable production processes for beer.

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P4. *Mingxi Zhou* - Impact of salinity on mucilage and polysaccharides in Kosteletzkya pentacarpos with zinc stress

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P7. *Caleb Egene* - Variation in carbon and nitrogen mineralization potential of solid fractions of digestate obtained using different separation techniques

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P60. *Przemyslaw Maziarka* - Comparison between simple and detailed kinetic schemes of biomass thermal degradation – two dimensional CFD modelling of pyrolysis of anisotropic large wood particles

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POSTER CONTRIBUTIONS

Extended abstracts

PREPARATION OF BIOCHAR FROM *HYPSIZYGUS MARMOREUS* SPENT SUBSTRATE AND ADSORPTION PROPERTIES FOR METHYLENE BLUE

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INTRODUCTION

Methylene blue (MB) is one of the most commonly used substances in the textile industry. The MB compound is very easy to obtain because its cheap price and wide use in dyeing process (Ozer and Dursun, 2007). The dye molecule is very stable, thus difficult to be decomposed under natural conditions. Therefore, it is necessary to remove the dye molecule before being discharged to the environment (Yang and Qiu, 2016). Numerous technologies developed for the removal of dye contaminants from wastewater, include adsorption, membrane filtration, advanced oxidation processes, ozonation, coagulation/flocculation and biological treatment. Comparing to the conventional methods adsorption has high efficiency, cost effectiveness, and simple operation.

This study demonstrated the feasibility of biochar prepared using *Hypsizygus marmoreus* spent substrate (HMSS) for MB dye removing from aqueous solution. In this study, biochars were prepared by hydrothermal carbonization (HTC) of HMSS at temperatures at 160-240℃ with residence time of 4 hours.

MATERIAL AND METHODS

MATERIAL

Methylene blue (mass fraction> 98.5%, chemically pure) was purchased from Shanghai Zhan Yun Chemical Limited company. The experimental water is secondary distilled water, the other reagents used in the experiment are analytical pure.

PREPARATION OF BIOCHAR

The mushroom substrate used in the experiment was HMSS. The raw material was washed, dried, crushed, and sieved through a mesh sieve (0.3 mm). Biochar was prepared in 200 mL teflon tube mixed with 10.0 g biomass and 150 mL deionized water. The teflon tube was placed in a stainless hydrothermal reactor and reacted in a certain temperature (160-240℃) oven for 4 hours, then cooled in vacuum drying box to room temperature. The reaction liquid was centrifuged, the supernatant was removed, and the biochar was washed alternately with anhydrous ethanol and deionized water until a neutral solution pH was achieved. The product was then oven-dried overnight at 105℃ and grinded, the treated biochar was finally preserved in a desiccator until further use.

ADSORPTION EXPERIMENT

Adsorption experiments were conducted using 250 mL conical flask with same volume of 100 mL, and MB concentration of 180 mg/L except when studying the influence of adsorption on biochar at different MB concentration. Dosage of biochar was added to achieve liquid-solid ratios at 1.0 g/L

except when studying the influence between remove rate and dosage. The reaction temperature was held constant at 25℃. All solution pH was maintained at neutral. The samples were placed in a constant temperature shaker (Shanghai Sukun Industrial Co., Ltd.); operated at 180 rpm and for 24 h.

ANALYSIS OF ADSORPTION

After adsorption, samples were collected and centrifuged at 12000 rpm for 5 min. The supernatant was taken and the absorbance was measured by UV-2450 ultraviolet/visible spectrophotometer (Shimadzu, Japan; the wavelength was set at 665 nm). The secondary distilled water was used to zero calibration.

RESULTS

PREPARATION OF BIOCHAR

Biochars were prepared by hydrothermal carbonization of at temperatures on 160, 180, 200, 220, 240℃ with residence time of 4 hours (Figure 1). From the picture can find with the increase of temperature, the colour of biochar becomes darker and darker, which indicates that the degree of carbonization of biochar increases.

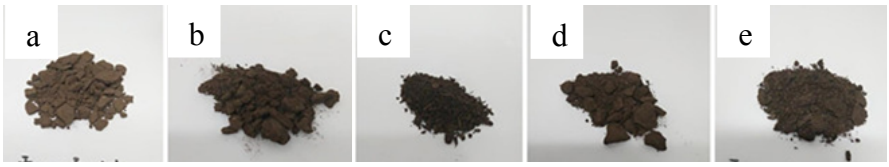

Figure 1. Biochars on a temperature of 160 (a),180 (b),200 (c),220 (d),240 (e)℃ with residence time of 4 hours.

ADSORPTION EXPERIMENT

Figure 2 shows that conical flask on the left is MB solution before adsorption and on the right is the solution after adding biochar (b) into it. From the picture we can see clearly that the colour changed from blue to colourless after adsorption, which means the biochar has strong adsorption capacity on MB.

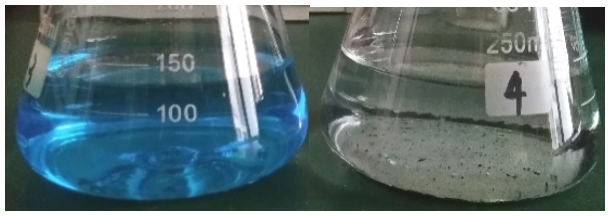

Figure 2. Example of MB solution adsorbed by biochar (Lift: before adding biochar; right: after adding biochar (b)).

ANALYSIS OF ADSORPTION

To explore the influence of biochar dosage on methylene blue removal rate, the initial dosage of biochar (b) was 0.05, 0.075, 0.10, 0.125, 0.15, 0.20, 0.25 and 0.30 mg, the volume of MB solution was 100 mL, the concentration of MB solution was 180 mg/L. After adsorption equilibrium, centrifugal sample and absorbance measurement were carried out. Figure 3 shows the influence of biochar dosage on methylene blue removal rate. From the figure 3 can find when the dosage of biochar (b) is 0.5g/L, the remove rate of MB can reach to 56.7% and the more

biochar added, the remove rate increased, we can conclude that the produced biochar has a high adsorption capacity for MB and almost 98% of the MB could be removed.

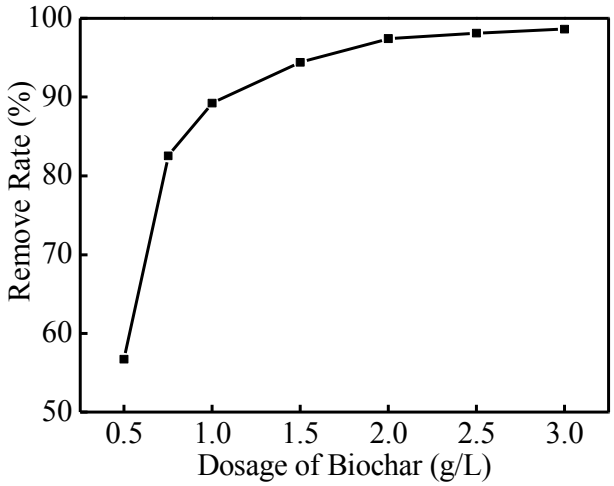

Figure 3. The influence of biochar dosage on methylene blue removal rate.

DISCUSSION

This study demonstrated the feasibility of biochar prepared using HMSS for MB dye removing from aqueous solution. The results show that the hydrothermal method is feasible and the biochar has a good adsorption capacity on MB.

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ELECTROOSMOTIC MIXING AND FOCUSING OF PS PARTICLES BY AC-ACTUATION

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SUMMARY

Application of AC electrical fields by channel-embedded electrodes or combinations of E-fields and pressure-driven flow have been developed in the past for pumping or particle focusing. [1,2] This work presents a fast and reproducible particle focusing technique by AC-actuation perpendicular to pressure-driven flow. We use a unique chip design which is completely transparent, with indium tin oxide (ITO) electrodes forming the top and bottom of the channel for AC actuation (Fig. 1). Polystyrene particles of 0.5 µm diameter were found to focus in the centreline/bottom side of the channel on a millisecond timescale by an as yet unknown focusing mechanism. Focusing speed was found to strongly depend on both flow velocity and applied voltage. Furthermore, for low axial velocities, vortexes were observed at the top side of the channel and the beads are circulating periodically.

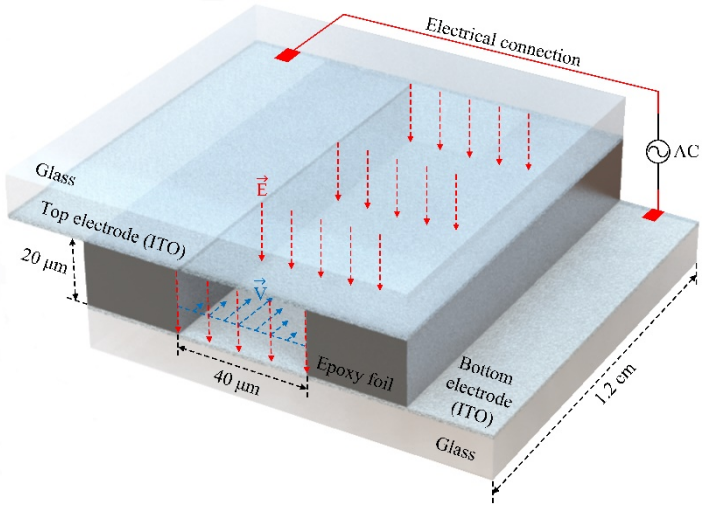

Figure 1. Schematic illustration of the setup.

MATERIALS AND METHODS

Conducting and transparent ITO layers [3] (65 nm) were sputtering-deposited on fused silica wafers in 1% O2/99% Ar and an applied DC voltage with 50 W power. After sputtering, the ITO electrodes were annealed at 450oC for 30 min to increase the conductivity. A microchannel structure (40 µm width, 20 µm high) was developed on NC-epoxy foil (negative photoresist) of 20 µm thickness. Inlets and outlets were made in the glass wafers by powder-blasting. For the focusing experiments, two different types of 0.5 µm diameter polystyrene particles were used as 0.0025% w/v suspensions in unbuffered KNO3 solution (0.1 mM, pH 6.2): without surface modification (ζ-potential -47.5 mV) and with PVA-coating (ζ-potential -22 mV). Sinusoidal AC-potentials with 0.25 to 1 V peak-peak

amplitude and 1 kHz frequency were applied by a function generator. Microscopic movies of the particle movements were recorded and analyzed to characterize the focusing process and the vortexes.

RESULTS

Upon application of AC-potential, the particles migrated towards the channel centreline (Fig. 2). Figure 3 shows the focusing velocity as function of axial pressure-driven flow velocity and applied voltage amplitude. Every data point is an average velocity of 3 different particles. An increase of the bead focusing velocity with the axial pressure-driven flow velocity was observed. The bead focusing velocity also scaled with voltage V1.5. Furthermore, the focusing velocity of the two particle types was investigated for 1 V amplitude (Fig. 4). For the PVA-coated particles, the focusing velocity increased faster with flow velocity than for non-modified PS particles.

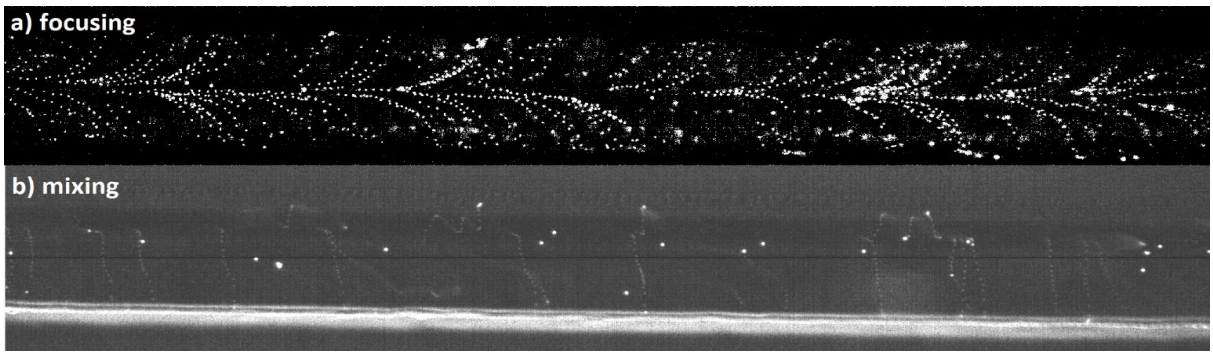

Figure 2. Polystyrene particle tracking showing series of overlaid frames during AC-actuation. a) Focusing was observed by particle movement from the sidewalls towards the centreline of the channel. Mixing was observed by particle movement starting from the middle towards the sidewalls, then circulating in vortexes. The axial pressure-driven flow for both images was low (1.56 mm/s).

The observed particle focusing is as yet unexplained. Taking into account the 1 ms oscillation actuation period, as well as the fact that the migration of the beads was unidirectional and occurring in a time span of 0.17 s (1 V) to 1.5 s (0.25 V) without axial flow and 0.07 s (1 V) to 0.09 s (0.25 V) at maximum axial flow velocity (62.5 mm/s), a force that scales quadratically with the applied electrical potential is most probable. The focusing speed and the dependence on axial flow velocity are surprising. The combination of the effects will be further investigated by a newly developed 3D-PIV imaging technique for a better understanding of this new system and for the elucidation of the focusing and the mixing mechanism. The mixing and trapping system of particles could be used for (bio) components handling in microfluidic devices for improvement of analytical techniques.

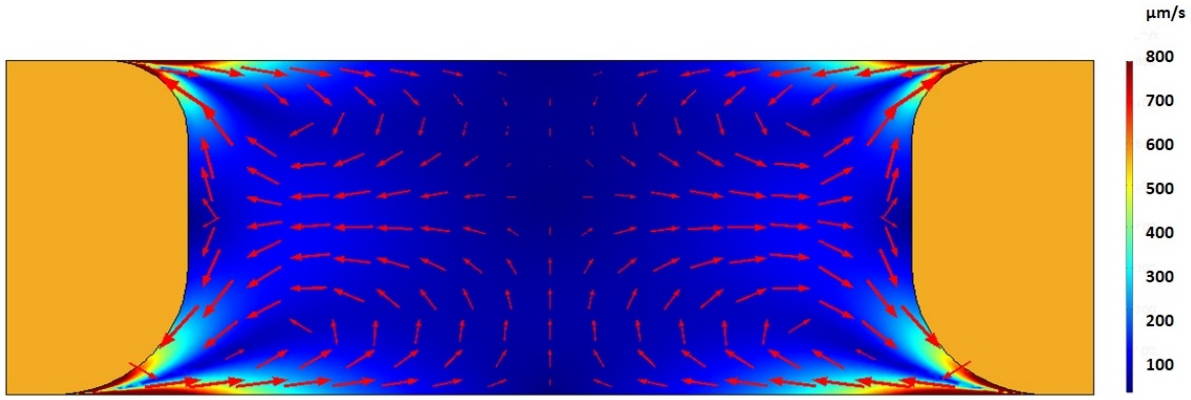

Figure 3. Average electro-osmotic flow over a full cycle, the color map shows the magnitude of the flow and the arrow field the direction.

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GROWTH AND SEPARATION OF CRYSTALS AND PROTEIN AGGREGATES IN ACOUSTOFLUIDICS

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ABSTRACT

Microfluidic devices offer a well-controlled environment for crystal nucleation and growth. Important parameters are mixing and the associated shear. Incomplete mixing results in a wide crystal size distribution and affect the morphology of the product. Bulk acoustic waves offer the possibility to quickly mix two liquid streams and to manipulate particles in the microfluidic channel. In this contribution, fast mixing of two liquid streams by acoustic streaming is demonstrated. Miconazole nitrate crystals were grown in the presence of the acoustic field, and focused on the pressure node. Growth and focusing of amyloid fibrils in the microfluidic channel has also been demonstrated.

INTRODUCTION

Mounting evidence suggests that shear flows influence the aggregation behavior of proteins [1]. Shear influences both the nucleation and crystal growth [2,3]. Acoustic streaming is a great methodology to create lateral convection in otherwise laminar flow operated channels. It offers a good strategy to reduce mass transfer resistance dramatically [4]. It could be expected that the shear patterns associated with the vortex flows of acoustic streaming will affect the crystallization/nucleation process greatly. Small particles or crystals are only affected by the Stokes drag forces and will flow along with the vortices. Larger crystals on the other hand will experience a radiation force and will rapidly focus at either the node or anti-node of the standing pressure wave in the channel [5].

In the present contribution a proof of concept has been obtained for the crystal formation and growth in presence of the acoustic field. Fast nucleation of a small pharmaceutical molecule (miconazole nitrate) was performed in the presence of acoustic streaming. The formed crystal nuclei were allowed to grow under the same conditions. Once a desired size was reached, the crystals were focused on the pressure node and separated from the main flow. Within a broader study on the influence of controlled flow on protein aggregation, we were able to visualize and focus clusters of amyloid fibrils from lysozyme. This is of great relevance since the formation of amyloid fibrils underlies a wide range of human disorders, including neurodegenerative and cardiovascular diseases like Alzheimer and cardiac amyloidosis.

EXPERIMENTAL

Rectangular microfluidic channels were etched in a silicon wafer. A width and thickness of respectively 375 µm and 310 µm were fabricated. Two inlets and outlets were provided and connected to syringe pumps by means of capillaries. A standing pressure wave was generated inside the channel with a piezo-ceramic element (PZT) having a resonance frequency at 2.0 MHz (Figure 1). Coupling of

the microfluidic chip to the PZT was done by a thin glycerol layer. An in-house built PMMA holder ensured a good connection between the chip, PZT and Nanoports. The applied voltage on the PZT was generated by a frequency generator and amplified by a RF power amplifier.

Visualization of all the experiments was done using an inverted fluorescent microscope in combination with a CCD camera. First mixing experiments were performed to quickly mix the solvent with the antisolvent. Water containing a fluorescent salt and non-fluorescent DI water were brought into contact in the microfluidic chip. Mixing was initiated by actuating the PZT at a frequency of 1.93 MHz. The recorded movies were analyzed with ImageJ software. The intensity of each pixel was related to the fluorescence intensity and eventually to the efficiency of the mixing (η). For the crystallization experiments miconazole nitrate was dissolved in dimethylsulfoxide (DMSO) (5 w/w%) and brought in the microfluidic channel at a flowrate of 2 µl/min. Water was used as an antisolvent and pumped.

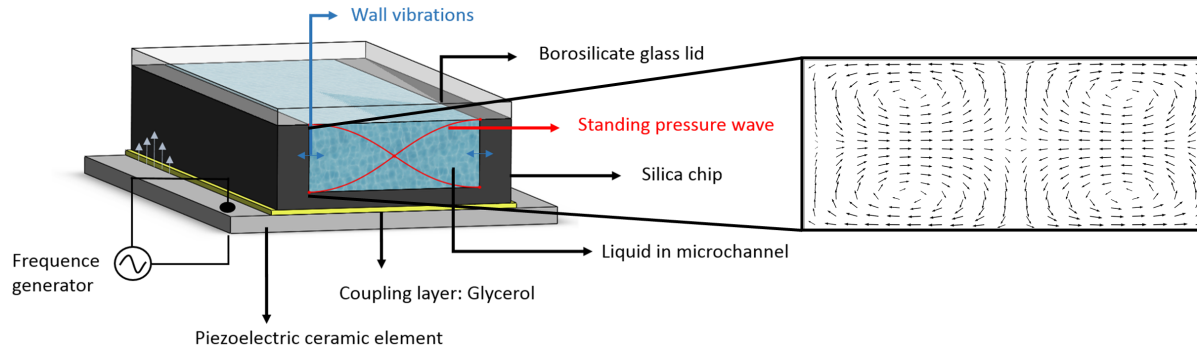

Figure 1: Schematic representation of microfluidic channel: The microfluidic channel is connected to a piezo ceramic element vibrating at 1.93 MHz. Actuation is done with a frequency generator. The vibrations are transferred to the channel wall, creating a standing pressure wave (thicknesses are not to scale). The generated vortex flow from the acoustic streaming is shown in the enlarged picture.

Through the channel at a flowrate of 8 µl/min. Crystal formation was monitored in absence and presence of the acoustic field. Crystals reaching a cut-off size were focused in the center of the microfluidic channel.

In the broader context of protein aggregates, amyloid fibrils from lysozymes were grown overnight in the microfluidic channel and without the presence of an acoustic field. Once the fibrils were formed, a flowrate of 4 µl/min was set and the acoustic field was activated.

RESULTS AND DISCUSSION

Mixing of two water streams due to the presence of an acoustic field was visualized by a camera. Figure 2a shows snapshots at different times at a fixed observation position (16 mm downstream the contact area) during the mixing process, overlaid with the concentration profile. In this case the flow rate was set to 5 mm/s and the actuation voltage to 15 Vp-p. At t = 0.60 s the channel already had a mixing efficiency of η = 0.94. The mixing efficiency has been measured for different applied voltages and flowrates (Fig. 2b). Actuation at voltages lower than 10 Vp-p does not induce any visible mixing. For a linear velocity of 5 mm/s a mixing of already η = 0.65 is reached at 10 Vp-p actuation. Complete mixing (η = 0.94) is reached at an actuation voltage of 15 Vp-p for both flowrates of 5 mm/s and 10 mm/s. To our knowledge such fast mixing in a microfluidic channel with bulk acoustic waves has not been demonstrated yet.

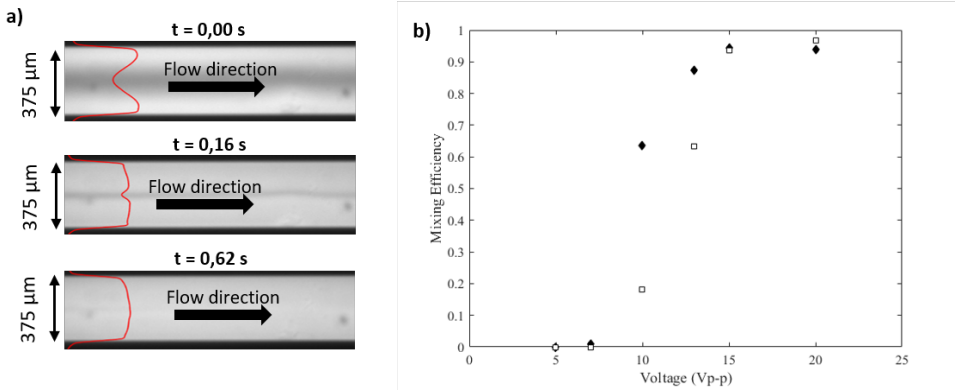

Figure 2: a) Top view of microfluidic channel. Shown is the mixing at a flow rate of 5 mm/s and an actuation of 15 Vp-p. A mixing efficiency of 94% is reached after 0.60 seconds. b) Mixing efficiency in function of applied voltage for two different flow rates, 5 mm/s (♦) and 10 mm/s (□). At lower voltages the water streams mix better for a low flow rate of 5 mm/s. The time needed for complete mixing is around 1 s and 2 s for flow rates of 5 mm/s and 10 mm/s, respectively.

Crystallization of miconazole nitrate was visualized in the microfluidic channel. In absence of the acoustic field but presence of flow, small needle like crystals appeared a short time after incubation. These crystals focused rapidly (1.28 s) upon activation of the acoustic field at a voltage of 10 $V_{p\text{-}p}$ (Fig. 3a). As a substitute for crystals, the focusing velocity was measured for 5 µm fluorescent polystyrene particles. A migration velocity that decreases as the node position is approached is observed (Fig. 3b). This is in accordance with the literature [6]. Moreover, the obtained crystal morphology is significantly different compared to crystals grown in batch. In the microfluidic reactor, small needle like crystals are observed in contrast with star shaped aggregation of needles (sea urchin morphology) obtained in batch (Fig. 3c). In the broader study of controlled shear on protein aggregates, the grown amyloid fibrils from lysozymes were focused on the pressure node upon activation of the acoustic field (Fig. 3d, 3e).

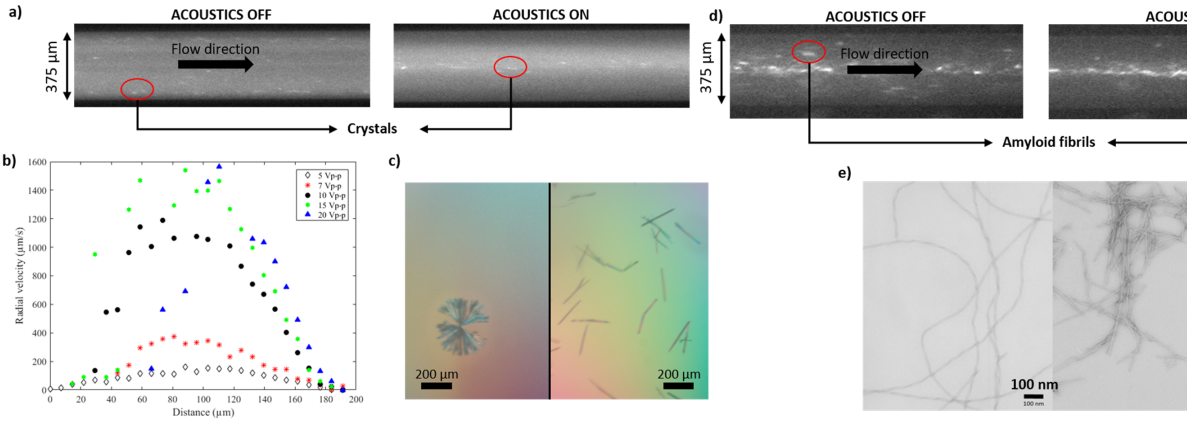

Figure 3: a) Focusing of small needle like miconazole nitrate crystals. b) Radial focus velocities of 5 µm particles as a function of five different voltages (see legend). A migration velocity that decreases as the node position is approached is observed. c) Miconazole nitrate crystals with a sea urchin morphology (left) and needle morphology (right). d) Focusing of amyloid fibrils in microfluidic channel. e) TEM micrograph of amyloid fibrils.

CONCLUSION

We have demonstrated the use of bulk acoustic wave to quickly mix two liquid streams in order to start crystal nucleation and growth. The same bulk acoustic wave have elegantly been applied to focus the formed crystals in the center of the microfluidic channel. Next to crystals, amyloid fibrils have been grown and separated in the microfluidic channel. Growth and separation by bulk acoustic wave of crystals or amyloid fibrils in microfluidic channels has to our knowledge never been achieved before.

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