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Universities of Applied Sciences Projects at ILMAC and SCS Booth

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For the second time after 2013 the SCS is partnering with the four Departments/Institutes of Chemistry at Universities of Applied Sciences (UAS) to present successful and innovative projects with a direct link to industrial application. The projects show that scientific research at UAS has become an essential pillar in the Swiss academic landscape and provides important contributions not only to the education of highly qualified employees but also to innovation in companies of all sizes. UAS became an integral part of a successful and innovative industrial location and help to keep Switzerland an attractive place for existing and new companies.

ILMAC as the trade fair for Laboratory and Process Technology, specializes strongly on innovation and application in industry and provides a perfect platform to present UAS initiatives and projects to a broader public.

A total of eleven projects from all four UAS chemistry/life science departments in Switzerland were accepted by the evaluation committee. The assessment process looked at the innovative potential and the practical application. The projects will be presented as posters and, if possible, also with concrete prototypes or samples at the joint booth of the SCS and the UAS. You can find a preview of all projects here.

A selection of initiatives are presented as short lectures in the ILMAC Forum as follows:

Wed, September 21, 2016, 10.15–10.45
Analytical sciences: development of new analytical methods and applications.

10.15 ***Semi-Quantitative Analysis of Transglutaminase in Food Samples Using a Lateral-Flow Immunoassay***
Sebastian Tschirren, FHNW

10.30 ***Fully Automatized Newborn Screening for Acylcarnitines, Amino Acids and Steroids by LC-MS***
Christian Berchtold, FHNW

Thu, September 22, 2016, 10.00–10.45
Biotechnology and bioanalytics: innovations in the fields of biotechnology, diagnostics and medicine.

10.00 ***A Novel Approach to Assess the Bioactive and Cytotoxic Potential of Novel Biomaterials: the Agar Diffusion Scratch Assay***
Manfred Zinn, HES-SO Valais-Wallis

10.15 ***Standardized Expansion of Human Mesenchymal Stem Cells (hMSCs) in Wave-mixed Single-use Bioreactors***
Dieter Eibl, ZHAW

10.30 ***Phosphate Recovery Using Scale-up Microbial Electrolysis Cell***
Fabian Fischer, HES-SO Valais-Wallis

Fri, September 23, 2016, 10.00–10.45
Process technology and engineering: efficient and sustainable solutions for industrial processes.

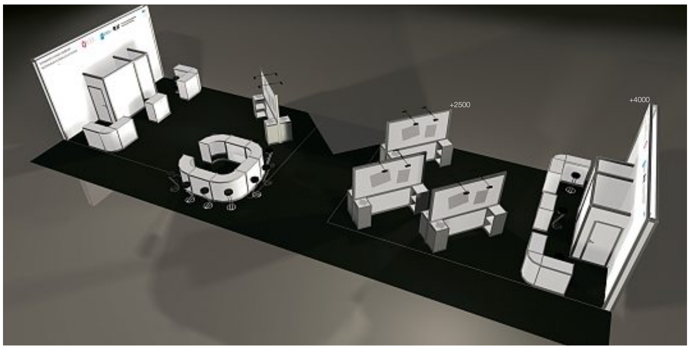
10.00 ***Design eines neuartigen Rohrreaktors für homogene und heterogene Mehrphasenreaktionen***
Wolfgang Riedl, FHNW

10.15 ***Production of Poly(3-hydroxyalkanoates) Biopolymers from Syngas Using Rhodospirillum rubrum: Turning Waste into Treasure***
Manfred Zinn, HES-SO Valais-Wallis

10.30 ***Development of a New Generation Coffee Roaster – The 'InfinityRoast' A Success Story of Collaboration between the ZHAW and Bühler AG***
Chahan Yeretzian, ZHAW

We would be very happy to welcome you as a guest at our booth or as a visitor in one of our project presentation session.

Booth of the SCS, the UAS and its Partner Organizations



SCS Booth: Hall 1.1, C110, right next to the ILMAC Forum

SCS offers you at its booth/bar coffee and refreshments and a comfortable place to relax during your ILMAC visit.

Booth Partners of the Swiss Chemical Society are

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Semi-Quantitative Analysis of Transglutaminase in Food Samples Using a Lateral-Flow Immunoassay

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Commercially available transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) is an enzyme which is able to crosslink proteins and peptides covalently. Thus, this specific protein is used in industrial food processing to modify product properties. For meat products, transglutaminase may enhance texture, elasticity and taste. Moreover, it is used to glue pieces of chopped meat together to form meatballs or hamburgers.[1,2] Transglutaminase is permitted for use in meat in Switzerland, but declaration is required.[3] The Cantonal Laboratories are responsible for checking declarations. This is currently done with a time-consuming ELISA (enzyme-linked immunosorbent assay) requiring sophisticated laboratory equipment (*e.g.* a plate reader and multi-channel pipettes) and numerous buffers. For the development of a faster and less complex alternative, we considered a lateral-flow assay. The lateral-flow assay is an immunochromatographic system comprising a cassette, a nitrocellulose strip, three types of antibodies and a latex-particle to visualize the binding of the detection antibody to transglutaminase and/or control antibody. This type of assay does not require any additional instrumentation, aside from a pipette and it is much faster. However, it is not quantitative, only positive and negative decisions can be made. The ELISA is a sandwich-immunoassay and provides high specificity and sensitivity. The detailed setup is depicted in Fig. 1.

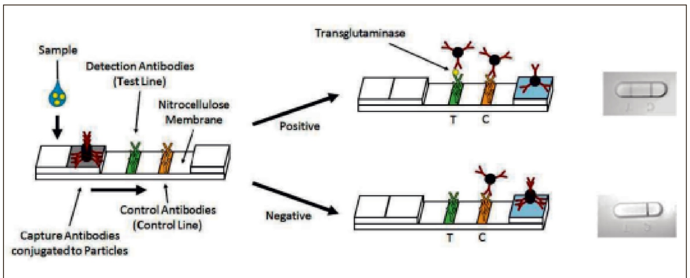

Fig. 1. Left: Schematic setup of the lateral-flow assay. Transglutaminase (yellow), capture antibodies conjugated to latex-particle (red), detecting antibodies (green), control antibodies (orange). Right: Positive and negative result using meat specimens.

A meat specimen is cut into small pieces and buffer is added. After shaking for ten minutes at room temperature, a sample of the supernatant is taken (no centrifugation is required). After application of the sample, the test is evaluated by eye after 15 minutes. If both the control and test line become black, the test is positive. If only the control line appears, the test is negative. The test is invalid if no line is observed. This test was successfully pre-validated in comparison to the original ELISA using ten meat samples (pork, beef, salmon and poultry), resulting in no false-positive or false-negative results. The detection limit lies below 20 ng/mL transglutaminase in salmon extract.

[1] Y. Zhu, A. Rinzema, J. Tramper, J. Bol, *Appl. Microbiol. Biotechnol.* **1995**, *44*, 277.
[2] M. Motoki, K. Seguro, *Trends Food Sci. Technol.* **1998**, *9*, 204.
[3] 'Verordnung des EDI über Lebensmittel tierischer Herkunft', **2014**.
[4] MTG-Nachweis in Lebensmitteln *via* ELISA: 3rd Version, A. Thommen, **2015**.

Fully Automatized Newborn Screening for Acylcarnitines, Amino Acids and Steroids by LC-MS

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Newborn screening is a public health service provided by most countries around the world aimed at screening newborns for a list of serious genetic and metabolic disorders. Early diagnosis of those conditions can help prevent their further development which, when untreated, often results in brain damage, organ damage or even death. A routine neonatal screening procedure requires that a health professional takes a few drops of blood from the baby's heel, applies them onto a filter paper and sends the samples to a laboratory for a number of analytical tests. This dried blood spot (DBS) analysis combines the ease of sampling with early diagnosis. This is well established in newborn screening around the world. In Switzerland, 80'000 newborns are screened for inborn defects every year. However, traditional methods in this field are laborious as they are based on punching and offline extraction. Even more critical, sometimes sample confusion is caused due to electrostatic effects, which directs punched samples into the wrong tube or well.

Newly available automated extraction systems, *e.g.* the CAMAG DBS-MS 500, allow a fully automated analysis workflow, which includes quality control of the spots during the entire process. Especially the issue of accidently confused samples (or punched discs) is solved, since the cards remain intact. This revolution in newborn screening and the subsequent development step in dried blood spot analysis has been the focus of a CTI (Commission for Technology and Innovation) funded project. Project partners are CAMAG AG, the University Children's Hospital Zurich and the School of Life Sciences of the University of Applied Sciences and Arts Northwestern Switzerland FHNW. One of the major goals of this project was to establish a fully automated and fast screening method for acylcarnitines, amino acids and steroids by LC-MS.

The important step in this project was to integrate a panel of steroids into a screening method and to introduce a fast and reliable chromatographic dimension. This is challenging since acylcarnitines, amino acids and steroids have very different physico-chemical properties according to solubility, polarity and ionization potential, which require careful selection and optimization of extraction, chromatographic and MS-ionization parameters. In addition, blood as a complex and inhomogeneous matrix adds an additional challenge into this composite method development procedure. Finally, a validated method according to ICH standards was developed and implemented at the Swiss children's hospital in Zurich.

It has been shown that the new direct elution method has significant advantages compared to the traditional method: It covers more analyte classes, and it is fully automated. Thus, after the sample card has been placed in the DBS-500 no further manual step is needed. As soon as the analysis is started all DBS-cards are scanned by the camera and all samples are automatically extracted and measured sequentially. Finally, most of the analytes have been detected in higher sensitivity compared to the manual routine method. The combination of these 25 biomarkers allows the screening for several diseases in less than 2.5 minutes per sample. Our approach significantly improves not only newborn screening programs, but also opens new avenues for dried blood spot analysis in other fields such as screening for doping, therapeutic drug monitoring, or point of care analytics.

Phosphate Recovery Using Scale-up Microbial Electrolysis Cell

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Partners: BAFU, LONZA, STEP de Sion, ARA Worblental, STEP de Martigny, SATOM, fenaco, BFE, Commune de Bagnes, ERZ Zürich, Saia Burgess.

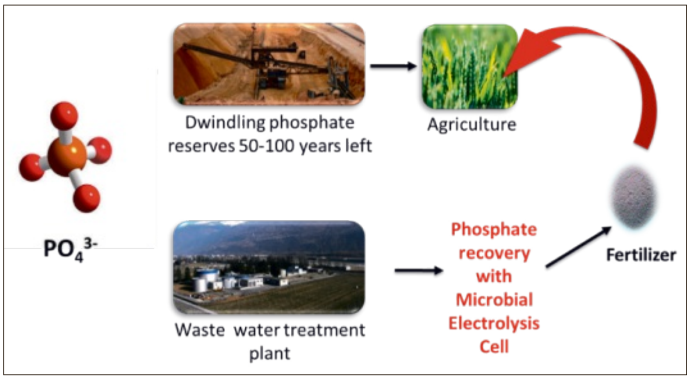

Fig. 1. Today's and the future phosphorous mining to produce phosphate fertilizers and industrial phosphorous.

Introduction

Phosphate fertilizer was produced in a most sustainable manner using a bioelectrical systems reactor. It is designed as a self-propelled process using energy contained in waste water for phosphate extraction, which is equally found in waste water.[1] In addition the microbial fuel cell produced electricity while extracting phosphate. Process modeling and kinetic analyses were performed and a scale-up reactor constructed.

Phosphate Availability

Phosphate daylight mining is possible in a small number of countries such as Morocco, USA and China (Fig. 1). Phosphate reserves will last only for the next 50 to 80 years.[2] The largest reserves are found in Morocco, while other producers possess far less of this essential resource. The quality of traded phosphate will decline and the fertilizer industry will need to invest heavily in the purification of low quality phosphate rock. Due to the reduced availability of phosphate it is already subject to export taxation. Beside this, the growing world population and the reduced availability of arable land per capita also increases fertilizer demand. All in all, phosphorus will become expensive and unaffordable for many with the grim consequence of a decreasing world population if the problem is not resolved. Based on this a new phosphate recycling business is predicted to emerge fueled by rising phosphorus prices.

Bioelectric Phosphate Recovery Processing

The principally investigated process was phosphate remobilization from iron phosphates contained in digested sewage sludge with a microbial fuel cell or microbial electrolysis cell (Fig. 2). Chemical reaction engineering was the tool to obtain kinetic data and derive models to plan pilot plant operation.

Kinetic data to optimize the phosphate recovery process were generated with a semi-artificial system using a well-defined 50 mL setup. The analyzed conditions comprised temperature,

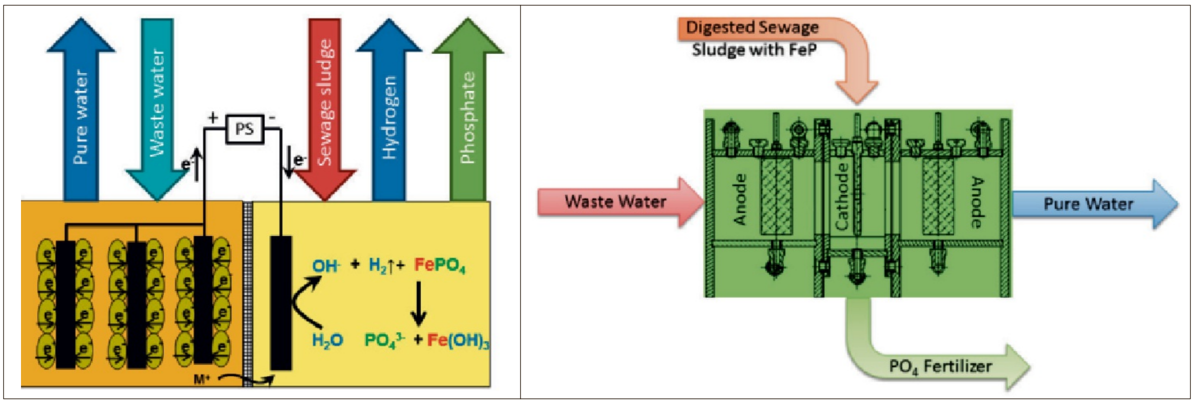

Fig. 2. Left: Principal phosphate recovery reactions in a microbial electrolysis cell. Right: Cut of the 3 liter scale-up microbial fuel/electrolysis cell used in the project.

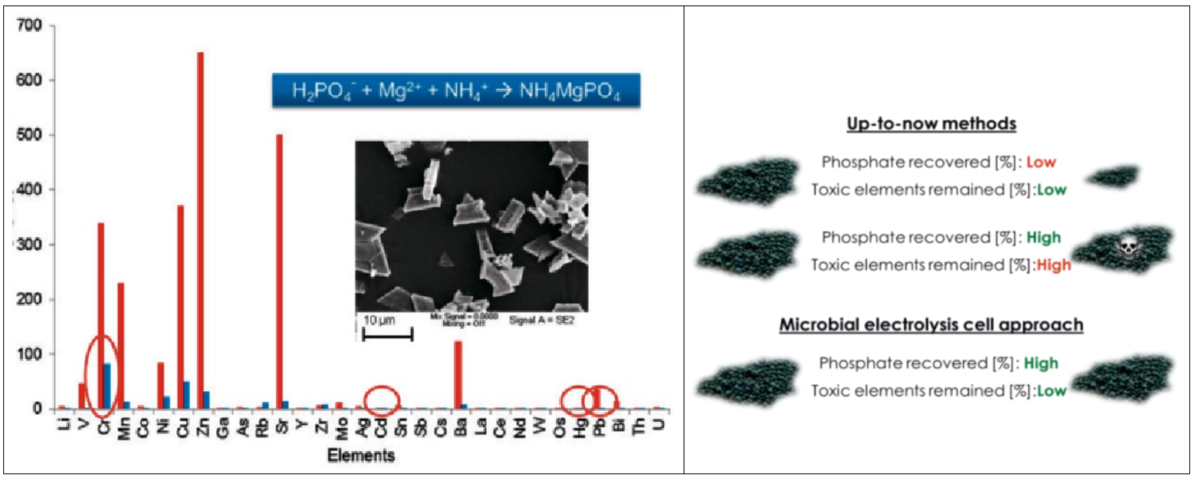

Fig. 3. Left: Heavy metal contaminates in digested sewage sludge (red) and in elemental impurities in struvite fertilizer after remobilization (blue). Right: Comparison between the novel wet sludge MFC/MEC phosphate extraction process and the widely reported ash extraction methodologies.

pH, stirring velocity, sludge concentration and particle size. The kinetic data allowed rate constants, reaction orders and the activation energy to be determined. These data were then analyzed with the help of the fluid particle shrinking-core kinetic model. It showed that the most important reaction resistance was the diffusion of the *in situ* generated renewable base (OH^-) in the sludge particle. The same modeling approach also allowed examination of the electron reduction mechanism. The most influential reaction engineering parameters were found to be pH > temperature > stirring rate.

Phosphate was recovered in up to very high yields of 95% using optimized process parameters.[3] Initially, the scale-up process took about 3 months and with adapted processing the remobilization was completed within a day's time; and this at the scale-up level. The ortho-phosphate containing supernatant catholyte was decanted and phosphorous precipitated as struvite (Fig. 3). This fertilizer was purer than the stringent Swiss legislation requires.[4] The novel bioelectric process generates beside this also a chemical base if needed and is proposed as an alternative for the less sustainable chlorination process used in industry. Another side product is phosphate-free digested sewage sludge useful as biofuel in cement production. A further side product is biohydrogen, and finally potassium is accumulated in the cathode.

Conclusions

The microbial electrolysis cell enables fast and quantitative phosphate recovery for iron phosphate contained in wet digested sewage sludge. It is possible to produce bioelectricity while generating the fertilizer using microbial fuel cell conditions. Scale-up showed that microbial electrolysis cell conditions enable fast processing. The product is not only a fertilizer but also reagent grade phosphate, chemical base, hydrogen and phosphate free sludge as biofuel can be obtained. The most significant result was that the fertilizer contained very low quantities of lead, mercury, cadmium, chromium and other toxic metals fulfilling stringent legislative requirements for recycling fertilizers.

[1] F. Fischer, C. Bastian, M. Happe, E. Mabillard, N. Schmidt, *Biores. Technol.*, **2011**, *102*, 5824.
[2] D. Cordell., J.-O. Drangert, S. White, *Global Environ. Change*, **2009**, *19*, 292.
[3] F. Fischer, G. Zufferey, M. Sugnaux, M. Happe, *Environ. Sci.: Processes Impacts* **2015**, *17*, 90; Also published in the '*Themed collection': 2016 Environmental Science: Processes & Impacts 2015 Most Downloaded Articles*.
[4] M. Happe, M. Sugnaux, C.P. Cachelin, M. Stauffer, G. Zufferey, T. Kahoun, P.-A. Salamin, T. Egli, C. Comninellis, A.-F. Grogg, F. Fischer, *Biores. Technol.* **2016**, *200*, 435.

A Novel Approach to Assess the Bioactive and Cytotoxic Potential of Novel Biomaterials: The Agar Diffusion Scratch Assay

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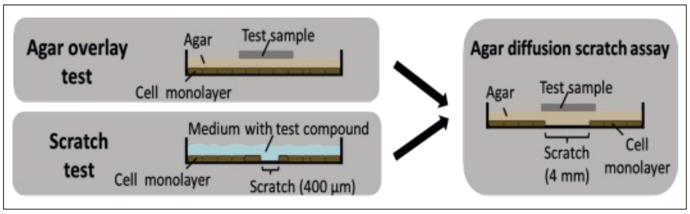

Fig. 1. The recombination of the agar overlay test (ISO10993-5) and the wound healing assay (scratch test) gives new information whether a biomaterial or a chemical solution embedded in an agar test sample is promoting or reducing cell activity (cell migration).

Many physiological processes, such as tissue repair and regeneration, as well as immune system responses rely on cell migration. *In vitro*, cell migration can be affected by numerous different alterations in cell physiology, *e.g.* gene expression, signalling, and/or a modified interaction with the extracellular matrix. Therefore, using cell migration as a bioactivity indicator allows cell performance to be evaluated in a quantitative, qualitative, and time-dependent manner.

One of the most prominent tests is the scratch assay, also known as the wound healing assay. Shortly, a gap, called scratch or artificial wound, is generated by removing a lane of cells within a confluent cell monolayer. Cells on the edge of this gap will migrate into the cell-free space until cell–cell contacts limit further migration. Depending on the compound concentration in the culture medium, the rate of migration might differ, which is evaluated by comparing microscopic pictures. Two key limitations of this test are known: Firstly, its inability to establish a chemical gradient of the test compound within the cell culture dish (impossible to detect a concentration-effect relationship). Secondly, the disperse migration pattern of the cells makes it rather difficult to quantify accurately the migrated distance.

In contrast, the agar overlay test, a method part of the guideline ISO 10993-5 allows cytotoxicity to be measured by indirect contact and gives a dose response. In this assay a sub-confluent cell culture, *e.g.* mouse fibroblasts, is overlaid by a thin layer of agar. Thereafter the test material is placed centrally on top of the agar and as a result, cells are exposed to a concentration gradient of the released compound due to the radial diffusion in the agar. However, this test allows only a qualitative assessment of cytotoxicity, which is done by grading the size of the zone of dead cells around the sample by means of cell morphology and/or by a selective staining of living and/or dead cells. The limits of this test are the following: Firstly, only acute cytotoxic effects of released compounds can be detected. Secondly, released compounds which affect cell functionality without being cytotoxic cannot be identified.

Here we present a novel assay termed agar diffusion scratch assay, which combines the advantages of the scratch assay and the agar diffusion test, and additionally rules out the above-mentioned key limitations (Fig. 1).[1] In addition, it remains a simple, low-cost assay and may be used for a broad range of applications like biocompatibility and cytotoxicity testing as well for more specific fields like cancer and angiogenesis research.

[1] M. Pusnik, M. Imeri, G. Deppierraz, A. Bruinink, M. Zinn, *Sci. Rep.* **2016**, *6*, 20854.

Acknowledgements
The authors would like to thank the HES-SO Valais-Wallis for their financial support under the Socle R&D grant.

Standardized Expansion of Human Mesenchymal Stem Cells (hMSCs) in Wave-mixed Single-use Bioreactors

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The primary aim of our research project was the characterization of fluid flow and prediction of shear stress levels in wave-mixed single-use bioreactors with one-dimensional motion which are operated with microcarriers. Furthermore, the suitability of the suspension criteria described by Schirmaier *et al.*[1] was investigated in respect of the expansion of human adipose tissue-derived stromal/stem cells (hASCs).

The results of the biochemical engineering and cell cultivation studies (Fig. 1) demonstrate the general suitability of the BIOSTAT RM 20|50 for the expansion of hASCs. As predicted, transient fluid flow states were found. The NS_{1W} and NS_{1WU} are strongly dependent on the microcarrier-medium combination, the microcarrier concentration and the bag working volume and were defined as the suspension criteria derived from stirred bioreactors. Interestingly, the differences between the lower and upper suspension criteria were smaller in the wave-mixed (2.5–8%) than in the stirred single-use bioreactors (20–40%). The fluid flow velocities ranged between 0.03 m/s and 0.33 m/s, and decreased significantly with increasing working volume. In addition, the fluid flow velocities were more strongly influenced by the bioreactor rocking angle than the rocking rate. This was also the case for the specific power input values (P/V), which were between 34.4 W/m^3 and 156 W/m^3. Specific power inputs and local shear stresses ran in parallel. The mean shear stresses (2.79×10^{-3} Pa – 18.3×10^{-3} Pa) were in an uncritical range for the expansion of hASCs and comparable to values reported for stirred single-use bioreactors.[2] Only the maximum volume-weighted shear stresses were higher in the wave-mixed bioreactor. However, these values occurred only in a low proportion of the entire bag volume, and therefore had little impact on cell growth.

The first proof-of-concept cultivation of the hASCs, which was performed at the rocking angle/rate combination of 4°/31 rpm (NS_{1WU}), was successful. The approach enabled 2.85×10^8 hASCs to be harvested after nine days of cultivation while maintaining stem cell quality. Moreover, the established regression model facilitates the rapid prediction of optimum boundary conditions for suspension studies and subsequent cell cultivations. The model can also be used for further adherent cells such as those in vaccine productions.

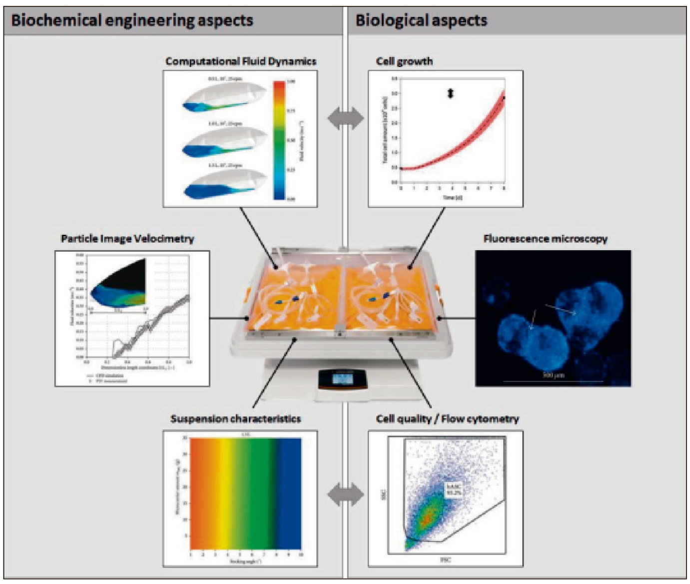

Fig. 1. Combined biochemical engineering and biological aspects of the research project, whose first results were published in ref. [2].

[1] C. Schirmaier, V. Jossen, S. C. Kaiser, F. Jüngerkes, S. Brill, A. Safavi-Nab, A. Siehoff, C. van den Bos, D. Eibl, R. Eibl, *Engin. Life Sci.* **2014**, 14, DOI:10.1002/elsc.201300134.
[2] V. Jossen, C. Schirmer, D. S. Mostafa, R. Eibl, M. Kraume, R. Pörtner, D. Eibl, *Stem Cells Int.* **2016**, Article ID 4760414, DOI: 10.1155/2016/4760414.

Design eines neuartigen Rohrreaktors für homogene und heterogene Mehrphasenreaktionen

Tobias Leonhardt[a], Andreas Zogg[b], Pascal Müller[b], Cédric Hutter[b], Julius Jeisy[c], und Wolfgang Riedl[a]*

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Zahlreiche potentiell gefährliche, chemische Reaktionen wie Nitrierungen oder Oxidationen mit reinem Sauerstoff können in den derzeit üblichen Mehrzweck-Rührkesselanlagen der pharmazeutischen Industrie nicht durchgeführt werden. Daher gehört es zur notwendigen operationellen Tätigkeit im Bereich der Verfahrensentwicklung, diese Syntheserouten *a priori* für die technische Produktion in bestehenden Anlagen nicht in Betracht zu ziehen.

Um diesen Umstand zu ändern, soll im Rahmen dieser Arbeit eine Laboranlage konzipiert werden, welche es erlaubt, derartige Reaktionen inhärent sicher durchzuführen.

Motiviert und initiiert durch das derzeitige Vorgehen innerhalb der chemischen Verfahrensentwicklung in der pharmazeutischen Industrie wird ein Designvorschlag für eine neuartige Reaktionsapparatur für inhärent sichere, stark exotherme, mehrphasige Reaktionen erarbeitet. Die primäre Entwicklungsaufgabe ist die Realisierung einer semi- und vollkontinuierlichen Anlage, die eine inhärent sichere Betriebsweise innerhalb des Scale-ups in bestehende Produktionsanlagen zulässt. Darüber hinaus wird aufgezeigt, dass es der neuartige Mehrphasen-Rohrreaktor vermag, eine hohe Produktivität zu erreichen, die mit den üblichen Pharma-Produktionsanlagen zu vergleichen ist.

Die Ziele dieser gemeinsamen Entwicklungsarbeit werden auf dem ausgestellten Poster dargestellt. Dazu gehören Planungsunterlagen der Gesamtanlage sowie die Kernpunkte des Sicherheitskonzepts und der Risikoabschätzung, welche auf experimentell erzeugten Batch-Labordaten basieren und nach gängigen Methoden der ISSA erstellt wurden. Anhand einer ausgewählten Modellreaktion[1] wird die Methodik des Katalysatorscreenings und die Immobilisierung des Katalysators auf der Oberfläche der neuartigen Reaktionsstruktur[2] vorgestellt. Weiterhin wird das Potential des Mehrphasen-Rohrreaktors exemplarisch dargelegt.

[1] S. Ueda, H. Nagasawa, J. Am. Chem. Soc. 2009, 131, 15080.
[2] C. Hutter, 'Laser Sintered Meso Scale Reactor by Design: Characterization of Heat and Mass Transfer', Thesis ETH, **2010**.

Reproduzierbare Versuche? – Entwicklung und Charakterisierung eines innovativen Parallelreaktorsystems

Pascal Schulthess, Tobias Leonhardt, und Wolfgang Riedl*
Fachhochschule Nordwestschweiz, Hochschule für Life Sciences, Institut für Chemie und Bioanalytik, CH-4132 Muttenz, E-Mail: wolfgang.riedl@fhnw.ch

Ob Ausgangsstoff, Zwischenprodukt oder Endstufe – jedes chemische Erzeugnis erfordert eine Syntheseroute. Sticht ein Verfahren durch ressourcenschonende Eigenschaften hinsichtlich Rohstoff-, Hilfsmittel- und Energieverbrauch oder durch eine kürzere Reaktionszeit hervor, so sind damit in der Regel Verbesserungen bezüglich der Ökonomie und auch der Ökologie verbunden. Komplexbildende Katalysatoren werden aus diesem Grund seit dem Anbeginn der technischen Chemie industriell eingesetzt. Daher beruht eine Vielzahl aller im Portfolio der chemischen Industrie angebotenen Erzeugnisse auf dem Einsatz von Katalysatoren.

Auf dem Gebiet der Katalyse nimmt der Bereich der heterogenen Katalyse mit den damit verbundenen Vor- und Nachteilen einen immer grösser werdenden Stellenwert ein.[1] Die heterogene Katalyse zeichnet sich durch ein geringeres Mass an unerwünschten Nebenkomponenten aufgrund nicht vorhandener Liganden oder aufkommende Herausforderungen bei der Isolierung aus dem Reaktionsgemisch aus.

In dieser Arbeit wurde ein Parallelreaktorsystem für heterogen katalysierte, dreiphasige Reaktionen geplant, aufgebaut, in Betrieb genommen und charakterisiert. Das Ziel besteht in der kurzfristigen Bereitstellung einer kleinvolumigen Reaktionsapparatur, welche die parallele Durchführung von bis zu sechs Reaktionen gleichzeitig zulässt. Der primäre Einsatzzweck dieser Apparatur ist die Aktivitätsuntersuchung und -beurteilung unterschiedlicher Katalysatoren. Das massgebliche Beurteilungskriterium ist dabei die Erzeugung reproduzierbarer und stabiler Messergebnisse. Um dies zu erreichen, sind genaue Kenntnisse über die Stoff- und Wärmeübertragungskoeffizienten notwendig. Im Hinblick auf den Stoffübergang ist es zweckmässig, eine Unterscheidung zwischen dem Mischverhalten des eingesetzten Rührertyps und dem resultierenden Stoffübergangskoeffizienten des zudosierten Gases in die Lösung zu treffen. Mit einer ausgewählten Modellreaktion[2] wurde die Anlagencharakterisierung durchgeführt. Der Einfluss verschiedener Prozessparameter wie die Rührergeometrie, die Rührerdrehzahl, die Begasungsrate und die Blasengrösse wurden auf den kL·a - und k-Wert untersucht und mit den Versuchsergebnissen der Modellreaktion in Korrelation gesetzt.

In übersichtlicher Darstellung werden auf dem Poster neben dem realen und schematischen Aufbau der Nachweis der Reproduzierbarkeit erbracht und die Ergebnisse der Charakterisierung erläutert.

[1] G. Prieto, F. Schüth, *Angew. Chem.* **2015**, *127*, 3268.
[2] S.Ueda, H.Nagasawa, *J. Am. Chem. Soc.* **2009**, *131*, 15080.

Exhaust Air Cleaning System from Corn Stover for Reducing Ammonia Emissions from Livestock Housing

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Abstract: Ammonia emissions from animal facilities have a negative impact on environment and human health. This study develops an air cleaning system from corn stalk impregnated with phosphoric acid to recover ammonia emissions from poultry facilities and produce nitrogen fertilizer. The prototype tested in a poultry facility showed an abatement of >95% of ammonia emissions.

Keywords: Agriculture · Ammonia · Emission · Exhaust air cleaning · Reduction

Introduction

For 25 years, emissions of major air pollutants have significantly reduced except for ammonia. In Switzerland, these emissions are allocated to 90% in agricultural activities, mainly by farm livestock.[1] Ammonia emissions are due to biological decomposition of manure. Since ammonia has a negative impact on animal health, poultry and swine production facilities are ventilated to maintain indoor ammonia level below 25 parts per millions. Gaseous ammonia combines then with the acidic gas species in the atmosphere to form PM2.5 and PM10 particulate matter which can affect human health.[2] Ammonia can also be carried by wind over long distances and contributes to over-fertilization. The consequences are eutrophication and acidification of soils and biodiversity loss. Ammonia emissions from agriculture are estimated at 48,000 tons per year.[3] The soil nitrogen cycle has important losses which must be compensated by the use of industrial fertilizers. Although Switzerland has reached the 2010 targets of the Gothenburg Protocol for the reduction of ammonia emissions (–17% as compared to 1990),[3] progress remains insufficient to achieve the new objectives for 2020. For this, the Swiss Federal Council defined the goal to reduce ammonia emissions by about 40% compared to 2005.[4]

Actually the Ordinance on Air Pollution Control (OAPC) states that emissions shall be limited as far as is technically and operationally feasible and economically acceptable (*art. 4 OAPC*). Nowadays, the exhaust air cleaning systems available commercially work as a chemical scrubber. Exhaust air is treated by sprinkling and the washing water is continuously acidified with sulfuric acid to maintain a pH of about 3. This method offers a partial solution. The system allows the reduction of ammonia emissions with efficiency up to 70%, but it is expensive, cumbersome, requires a lot of maintenance and produces a large amount of sludge to be recycled.[5]

Corn Stover for Reducing Ammonia Emissions

In order to solve this problem, the company Sorba Absorber GmbH and the School of Engineering and Architecture of Fribourg, with the collaboration of the Canton of Fribourg (CleanTech Fribourg) and the Federal Office for the Environment (FOEN), developed a new air cleaning system from corn stover impregnated with phosphoric acid. Because ammonia is highly soluble in acidic medium, it is washed out and trapped chemically with phosphoric acid to give ammonium phosphate (Eqn. (1)), a fertilizer commonly used for crop production. The used filter material could be recycled as a source of nitrogen for crops thereby limiting the use of industrial fertilizers (Fig. 1).

$$H_3P0_4\ (aq) + 2\ NH_3\ (g) \rightarrow (NH_4)_2HPO_4 \qquad (1)$$

BABS™ (BioABSorber) produced by Sorba Absorber GmbH, is the spongy body obtained after removing the bark of maize stalks

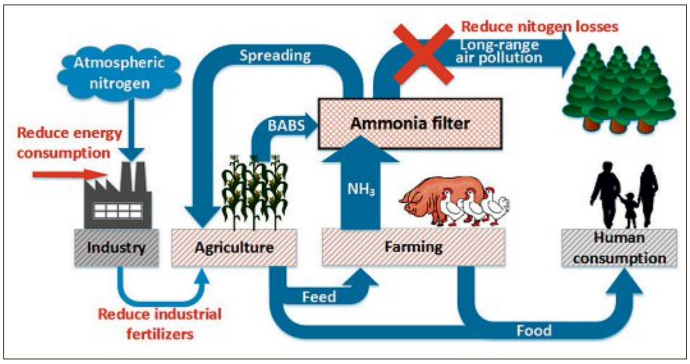

Fig. 1. Preservation of the nitrogen cycle by trapping ammonia emissions from poultry and swine facilities. Used filters can be used as fertilizer.

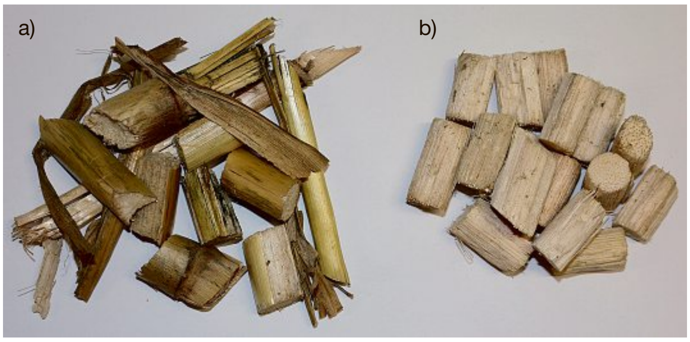

Fig. 2. Raw corn stover (a) and BABS™ after removing lives and bark (b).

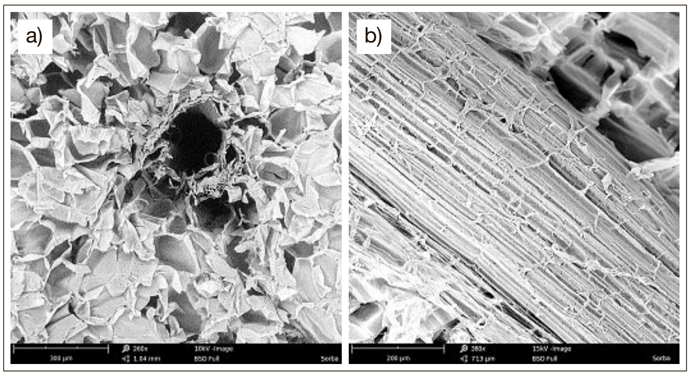

Fig. 3. Cross section (a) and longitudinal section (b) of BABS by scanning electron microscope (SEM).

(Fig. 2). It is a biosourced and biodegradable material which has a capacity of water retention up to 40 times its weight. Maize stalks are composed of large vascular bundle surrounded by a ground tissue with a large specific surface area (Fig. 3).

Ammonia Abatement in Poultry Facilities

BABS™ is impregnated with a solution of phosphoric acid and is used as a filter on the output of an air extractor of a livestock facility. Ammonia concentration before and after filter was measured simultaneously using two calibrated chemical sensor, model Libelium Smart Environment PRO waspmote Plug&Sense with temperature, humidity, pressure sensors and calibrated ammonia sensor (ref 9378-P, range 100ppm, accuracy ±0.5ppm). The filter is designed for a residence time of 0.5 second. The prototype was first tested under laboratory conditions with synthetic air of about 25 ppm of ammonia obtained by dilution of a reference gas (Carbagas BLUE 1% ammonia in air). The ammonia concentration after the filter is often below the limit of detection of 1ppm. Under these conditions, the relative ammonia absorption is >95%.

A prototype of 400 L of BABS was subsequently tested in a poultry facility during a 36-day growth cycle. Due to the low concentration of ammonia in the broiler housing, from 2 ppm to 5.5 ppm after 5 weeks, the tests were carried out only during the last two weeks. During this time a fan extractor of 1000m^3/h capacity pulsed untreated gas through the filter. The filter humidity is maintained through a timed-interval sprinkling with water. As in the laboratory tests, the concentration of ammonia at the filter output remains below 1ppm. It should be noted that after two weeks of use the amount of dust on the filter causes a significant pressure drop. There is also a partial drying of the filter media. A future study should be considered to optimize the sprinkling and add a preliminary dust filter.

Conclusion

A new exhaust air cleaning system for reducing emissions of ammonia from poultry growing unit has been developed and tested. The system consists of maize straw impregnated with phosphoric acid. The prototype tested in real condition of poultry facility showed an abatement of >95% of ammonia emissions. This strategy of ammonia emission reduction offers an economically acceptable solution for agriculture. It can be apply for poultry and swine farms and on manure storage facilities. As the substrate is fully biodegradable, spreading of used filter on crops can reduce fertilizer consumption and limit losses in the nitrogen cycle.

[1] B. Reidy, B. Rhim, H. Menzi, *Atmos. Environ.* **2008**, *42*, 3266.
[2] L. Gong, R. Lewicki, R. Griffin, *Atmos. Environ.* **2013**, *77*, 893.
[3] T. Kupper, C. Bonjour, H. Menzi, *Atmos. Environ.* **2015**, *103*, 215.
[4] 'Nitrogen-containing air pollutants affect biodiversity', *http://www.bafu.admin.ch/luft/00575/11210*, Federal Office for the Environment, **2016**, accessed June 2016.
[5] W. Gramatte, J. Johann, 'DLG Test Report 5952, MagixX-B exhaust air cleaning system', Deutsche Landwirtschafts-Gesellschaft, **2009.**

Production of Poly(3-hydroxyalkanoates) Biopolymers from Syngas Using *Rhodospirillum rubrum*: Turning Waste into Treasure

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Poly(3-hydroxyalkanoates) (PHA) are bio-based and biodegradable alternatives to conventional polymers derived from fossil fuels. In this work we assessed a novel type of production process that relies on fermentations using syngas (CO, CO_2, H_2 and N_2) as main substrate and *Rhodospirillum rubrum* as CO-metabolizing and PHA-producing strain. Syngas can be obtained from the pyrolysis of organic wastes and thus represents an inexpensive, non-food-competitive carbon source. A cutting-edge process analytical technology platform including measurements of dissolved oxygen and redox potential, gas concentrations by mass spectrometry, as well as cell concentration and PHA content by flow cytometry was set up to monitor the bioprocess and cell physiology.

Preliminary experiments revealed the difficulty of culturing

R. rubrum with syngas as sole carbon source. In particular, yeast extract and acetate addition were found to be essential for cell growth and PHA production, respectively. Moreover, cell growth was shown to be limited by CO mass transfer. In order to enhance the overall productivity, we designed a semi-continuous process with a heterotrophic, aerobic growth phase on fructose prior to the PHA production from syngas and acetate (Fig. 1). This strategy proved to be feasible but only if an anaerobic adaptation phase on fructose was included in-between. Fructose exhibited better cell growth than succinate and malate when considering both the aerobic and anaerobic phases and was therefore chosen as carbon source for the heterotrophic growth phase. We are currently further investigating the effect of the different growth conditions (aerobic, anaerobic, syngas) on the cell physiology and PHA production.

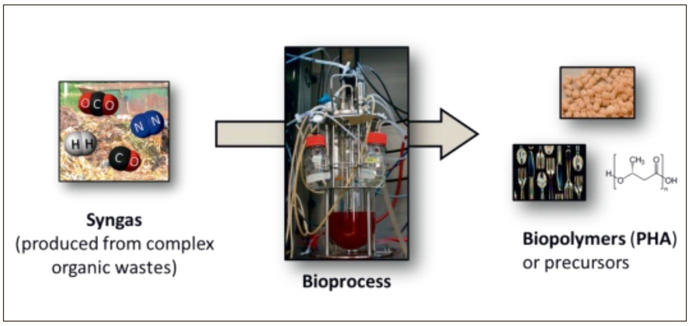

Fig. 1. PHA production from syngas and acetate.

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Development of a New Generation Coffee Roaster – The 'InfinityRoast' A Success Story of Collaboration between the ZHAW and Bühler AG

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In June 2012, a CTI project between the research group of Prof. Dr. Chahan Yeretzian at the ZHAW - Zurich University of Applied Sciences in Wädenswil and Bühler AG, headquartered in Uzwil, Switzerland was approved (No. 13897.1 PFIWIW, Duration: 30 months; Budget: CHF 1.1 Million). The project brought together two leaders in their respective fields.

The Center of Coffee Competence (CCC) at the Zurich University of Applied Sciences (ZHAW), Wädenswil: Prof. Dr. Chahan Yeretzian is the Head of the Group for Analytical Technologies and of the Center of Coffee Competence (CCC) at the ZHAW. Besides heading the CCC, he is on the Board of Directors of the Specialty Coffee Association of Europe (SCAE), the largest coffee trade association of Europe, as well as on the board of the Association for the Science and Information on Coffee (ASIC), the worldwide most important scientific association on coffee. Acknowledging that the most challenging and rewarding science is one that can be put in practice to sustainably increase the value of businesses, Prof. Yeretzian and his team engage directly with small, medium and multinational industry partners worldwide on projects along the whole value chain of coffee.

Bühler AG – Business Unit Coffee. Bühler AG is a specialist and technology partner for plant and equipment for processing basic foods and manufacturing high-grade materials. Initiated in 2010, Bühler entered the market of roaster with the stated objective to become the leading manufacturer of industrial coffee roasting equipment.

After four years of joint research and development, major progress in the understanding of the science and technology of coffee roasting was achieved. From a commercial perspective, the new *Infinity Roast*™ roaster (Fig. 1) was launched in May 2014 at the 'Interpack' in Düsseldorf, and was already sold to Swiss and international coffee roasting companies. From a scientific perspective the joined ZHAW-Bühler research led to a series of peer-reviewed publications.[1–5]

Fig. 1. The *InfinityRoast*™ represents the first generation coffee roasters, fully developed by Bühler AG. This development was supported by a CTI project, in collaboration with the Center for Coffee Competence led by Prof. Chahan Yeretzian at the ZHAW.

This project exemplified the essence of a successful collaboration between an applied university and a Swiss industry partner – supported by the CTI (Commission for Technology and Innovation).

[1] S. Smrke, S. Opitz, I. Vovk, C. Yeretzian, *Food & Function* **2013**, *4*, 1082.
[2] S. E. W. Opitz, S. Smrke, B. A. Goodman, M. Keller, S. Schenker, C. Yeretzian, *Foods* **2014**, *3*, 586.
[3] S. E. W. Opitz, S. Smrke, B. A. Goodman, C. Yeretzian, 'Methodology for the measurement of antioxidant capacity of coffee: A validated platform composed of three complementary antioxidant assays' in 'Processing and Impact on Antioxidants in Beverage', Ed. V. R. Preedy, **2014**, Chap. 26; pp 253–264, Academic Press, London; ISBN: 978-0-12-404738-9.
[4] S. E. W. Opitz, B. A. Goodman, M. Keller, S. Smrke, S. Schenker, C. Yeretzian, *Phytochem. Anal.* **2016**, submitted.
[5] B. A. Goodman, S. Opitz, S. Smrke, M. Wellinger, C. Yeretzian, 'The Non-Volatile Components of Coffee and their Properties', in 'Coffee: Chemistry, Quality and Health Implications', Ed. A. Farah, Royal Society of Chemistry, **2016**, submitted.

Life and Death of Plastics

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The European industrial demand for plastics summed up to 46.3 million tons in 2013, with the most demanding industries being packaging, construction and automobile.[1] For all these users, one of the biggest challenges is to predict how the material will withstand the test of time. Indeed, extremely diversified applications expose the base polymers to strong temperatures variations, sunlight, air, or even aggressive chemicals, all of which contribute to their degradation.[2]

Base materials suppliers provide indications of ageing based on standardized tests: for instance, they expose their compounds to a constant temperature for long periods, and then propose target temperature windows and conservative lifetimes to the application industries.[3] The problem here is that these application industries are producing final objects that will be exposed to non-constant environmental stresses (like short periods of high, out-of-specifications temperatures), and often for much longer times than advised by the base material suppliers.

In order to solve this problem, a team of chemists from the University of Applied Sciences Western Switzerland in Fribourg (HES-SO) has launched a research program in collaboration with four major industrial partners producing final polymer-based products and partially funded by the Canton of Fribourg (PST-FR).[4] These companies, namely Geberit Fabrication (composite water tubes), Jesa (plastic overmolding elements), Johnson Electric (automotive components) and Wago (electric connectors), came with their own environmental target conditions on their respective base materials, and the research team led by Prof. Dr. Pierre Brodard selected the most appropriate characterization methods.[5]

The first year of the program, called POLYAGE (2014-2015), was dedicated to chemical ageing. Two classes of plastics, based on polyethylene (PE) and polyamide (PA), were first characterized to obtain their whole thermal, structural, topographic and oxidation properties. For this purpose, we used differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), infrared and Raman spectroscopy, scanning electron microscopy (SEM) and chemiluminescence, respectively. Then, the samples were exposed to various temperature programs to force their degradation, and compared to aged products provided by our industrial partners.

As expected, PE-based materials are chemically decomposed by oxidation, which was easily confirmed by spectroscopy. Hence, we quantified their kinetics by chemiluminescence, a method that measures the weak light emission generated by oxidation following the Russell's mechanism.[6] By performing several isothermal measurements in a precise oxygen-containing atmosphere, we could extract the empirical Arrhenius parameters (activation energy and pre-exponential factor) of the degradation, thus allowing an exact prediction of the lifetime of the samples at any temperature.[7]

On the other hand, PA-based materials did not seem to change their chemical composition: even though their mechanical integrity was strongly fading during ageing, their infrared and Raman spectra were almost unaffected. Indeed, the fracture toughness measured by tensile tests was reduced by 40% in the hardest conditions (20 days at 200 °C), but the composition remained the same. We then found out that the structure of the material seems to change: SEM images revealed a highly inhomogeneous dispersal of the glass fibers in the reinforced PA-based samples after thermal ageing, which probably accounts for their apparent fragility (Fig. 1).[8]

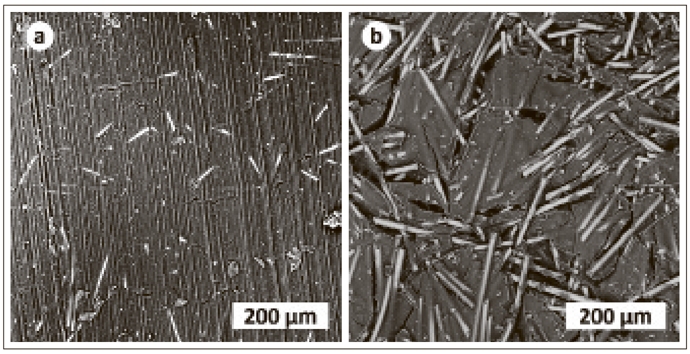

Fig. 1. SEM images of PA before (a) and after (b) thermal ageing at 200 °C for 20 days.

As a logical next step, we decided to concentrate on physical ageing in the second year of the program (POLYLIFE, 2015-2016). By physical ageing, we mean no change in the chemical composition but a modification of the structure, like the above-mentioned alteration of the glass fibers distribution in the PA

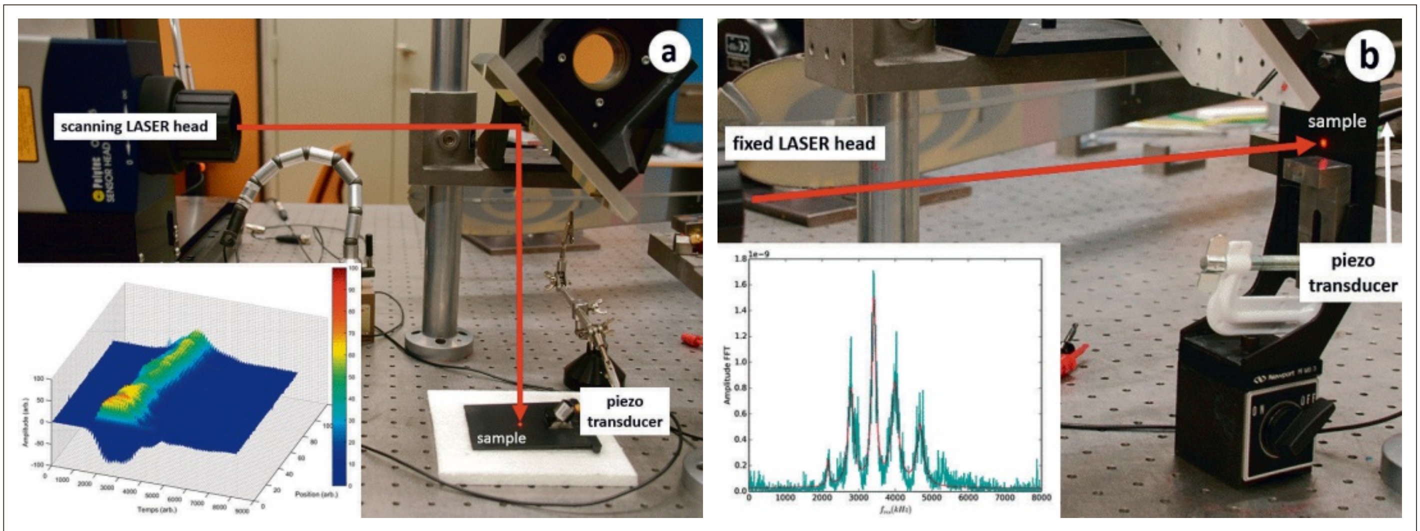

Fig. 2. Ultrasonic characterization method, with waves propagating in the plane (a) or through the sample (b).

matrix. For this purpose, and with the strong support of our mechanical engineering department, a new method based on ultrasound was developed: a piezoelectric transducer generates ultrasonic waves in the sample, and an interferometric laser head is used for detection. Two implementations have been devised: ultrasonic Lamb waves propagating in the plane of a thin flat sample and mapped by the scanning laser head, allowing determination of the elastic modulus of the material in different directions, and resonance of ultrasonic frequencies through the sample, enabling to measure more complicated, non-flat objects (Fig. 2).

These two non-destructive techniques are sensitive to any modification of the physical structure, even on final industrial products. Both approaches have already been validated, first on model aluminium samples and then on polybutylene terephthalate (PBT) objects: excellent correlation with mechanical properties obtained by macroscopic (tensile test) and microscopic (nanoindentation) methods prove their potential. We then started to measure pure and glass-fibers reinforced PBT samples having been exposed to high temperatures, as well as final manufactured objects, with interesting results in progress.

In addition, we extended the study beyond the initial PE- and PA-based samples under thermal exposition: polyethylenimine (PEI) was exposed to UV light, as well as various high-performance polyphthalamide (PPA) and polyphenylene sulfide (PPS) aged at high temperature, not to forget a high-temperature PE-based material exposed to water. For the PEI samples, we found no detectable degradation under constant UV-illumination for two months. On the contrary, for various PPA samples exposed to several temperature programs (160 °C to 270 °C, 30 h to 3000 h), tensile tests revealed a temperature-dependent exponential decay of the fracture toughness with time. Based on these results, we are now calculating the Arrhenius parameters in order to predict their lifetimes. Moreover, SEM images combined with TGA investigations of these glass fibers-reinforced PPA indicate an evaporation of the base polymer (up to 20% mass loss), whereas ageing temperatures (240 °C max) are still well below the melting point (approx. 320 °C). For the PE-based samples, first results suggest a different and faster oxidation mechanism in water than in air. Finally, the campaign of thermal ageing has just started for the PPS samples.

[1] Plastics – the Facts 2014/2015. An analysis of European plastics production, demand and waste data, PlasticsEurope, **2015**, Brussels.
[2] E. V. Bystritskaya, A. L. Pomerantsev, O. Y. Rodionova, *Chemometrics and Intelligent Laboratory System* **1999**, 175.
[3] 'Electrical Insulating Materials - Thermal Endurance Properties, Part 4-1: Ageing ovens – Single-chamber ovens', International Electrotechnical Commission, **2006**, IEC 60216-4-1.
[4] P. Morel, *KunststoffXtra* March **2015**.
[5] J. Gonthier, 'Estimer la durée de vie des polymères', *MSM Le mensuel de l'industrie* April **2015**.
[6] F. Käser, B. Roduit, *J. Thermal Anal. Calor.* **2008**, 231.
[7] Y. Mongbanziama, S. Aeby, S. Roth, P. Brodard, 'Kinetic parameters of the thermal degradation of polymers by chemiluminescence', Talk at the Annual Meeting of the Swiss Chemical Society-Photochemistry Section, Zurich, September **2015**.
[8] S. Roth, D. Parison, Projet collaboratif « PolyAge », Talk at the Journée Technologique De La Plasturgie, Swiss Plastics Cluster, Fribourg, May **2016**.