Nordic Forest Research
Co-operation Committee (SNS)

ANNUAL STATUS REPORT for PROJECT **YEAR 2014**

Please notice that the size of text sections in the form can be adjusted if needed.
The length of the report should not exceed 3 pages. Supplementary information can be attached. Deadline: March 1st 2015

1. Project title	Exploring novel oxidative biocatalysts for tailored wood fibre modification
2. Project leader (name, address, telephone, e-mail)	Professor Vincent Eijsink; Norwegian University of Life Sciences (NMBU), P.O. Box 5003, N-1432 Ås, Norway; Phone: +47 67232463, vincent.eijsink@nmbu.no
3. Duration	2013-2015
4. Project status	Does the project develop according to the plans? Yes Please describe: Important results have been obtained, especially in the area of discovering new LPMOs (oxidative enzymes) and in our studies on how to exploit these enzymes in the best possible way. At the same time, alternative methods for controlled oxidation by other enzymes are being explored in Helsinki. The network is very strong, in part as a result of an important researcher exchange in 2013. The three partners actively work together and are in contact on a monthly basis.
5. Activities during the reporting year	Project meetings: There have been no dedicated project meetings because there simply was no need for this after the strong coordination effort that was done in 2013. The partners have met several times at conferences and the like and there have been ad hoc E-mail and telephone contacts. Scientific meetings: None. Scientific research with joint focus: See below. There has been lots of such research. Sample exchange: Enzymes have been exchanged between Copenhagen and NMBU (Ås). Samples of oxidized sugars generated at NMBU have been transferred to Helsinki spring 2014. Fiber samples before and after pretreatment and/or enzymatic treatment have been exchanged between partners. Researcher mobility: After his long stay in 2013, Ås post-doc Bjørge Westereng has been working in the Copenhagen labs for shorter periods of time in 2014. Joint project applications between the partners: Copenhagen and NMBU are joint partners on a Horizon 2020 application, which was initiated late 2014. Furthermore, Copenhagen and NMBU are partners in a large national Norwegian project on oxidative enzyme systems in wood modification (“BioMim”) that was granted late 2014.
6. Results achieved during the reporting year	University of Helsinki: Work with galactose oxidase was continued focusing on novel aerogels from galactomannan (GM) and –xyloglucan (XG). Specific enzymatic oxidation of GM

	and XG was used to prepare hydrogels which were further dried to aerogels (Mikkonen et al. 2014). In the next step, XG and GM aerogels were reinforced with nanofibrillated cellulose (NFC) (Ghafar et al. submitted). Adding NFC before enzymatic oxidation entrapped the NFC in the three-dimensional structure of the GMox/XGox hydrogel matrices. A correlation study showed that the properties of the aerogels and the reinforcement by NFC were greatly dependent on the type of biopolymer (GM and XG) and their interaction with NFC. The pores in aerogels were approximately 125–250 µm in diameter. Such macroporous structures are suitable in tissue engineering applications. The benefits of the enzyme mediated gelation technique include the good preservation of the native properties of NFC. This can be used in the future for surface modification of NFC, for various applications, before or after entrapping within the gel matrix. The NFC-reinforced polysaccharide-based aerogels can find applications such as water absorbents, providing mechanical support for food packaging, biocompatible delivery systems, tissue-engineering scaffolds, and encapsulation of active components, such as antioxidants. The structures of obtained aerogels are being studied in detail using synchrotron x-ray microtomography at Paul Scherrer Institut Villigen, Switzerland (Ghafar, manuscript in preparation). An other enzyme-mediated method for introducing oxidized functionality to XG, GM and NFC is the TEMPO – laccase system, which is selective to primary hydroxyl groups in polysaccharides. This method for XG, GM and NFC activation and functiona-lization is studied in more detail during the last project year. <u>University of Copenhagen:</u> The work has focused on 2 paths: 1. Developing an assay for detection of LPMO enzyme activity on solid surfaces (e.g. cellulose fibers) 2. Long range electron transport between cellulose and lignin through low molecular weight electron shuttles 1. The fluorescent assay based on labeling of carboxyl groups with a diazo compound (FDAM) has been applied to native i.e. non-modified lignocellulose with some success, the work is ongoing and will be finalized. This work opens a road to new fiber modification tools. 2. The recent discovered cellulose oxidizing LPMO enzymes require both electron donors and acceptors and on pure cellulosic substrates under laboratory conditions ascorbic acid has been found highly efficient. In collaboration with NMBU we have found that lignin is an important part of the LPMO redox cycle under *in vivo* conditions. This cycle is built upon both the lignin macromolecule and low molecular weight lignin, where the latter functions as an electron shuttle providing a cyclic long range electron transport mechanism. We believe that this is a new fundamental insight and the results are now in process for publication. These findings couple the modifications of two fiber types, cellulose and lignin. The research on the mechanisms and role of electron donors is continuing in other projects.

	NMBU, Ås, Norway: NMBU has been working on characterizing novel LPMOs, which are one of the main classes of enzymes with potential for oxidative fiber modification. Major breakthroughs were published in 2014, namely novel LPMO activities that can be used to generate new types of oxidations. We have further developed our wide range of analytical tools and we have been exchanging our knowledge in this area with the Helsinki and Copenhagen partners. We have also generated (quite unique) standard samples of a wide variety of oxidized oligosaccharides, which are used by all partners in their work. Importantly, Copenhagen and NMBU have continued their joint work on the interplay between cellulose fiber modification and lignin modification and a joint paper is expected in 2015 (see above). The work on LPMOs has yielded some C4 specific enzymes that enable completely novel ways of fiber engineering, namely through modification of the non-reducing end. These novel options are currently being explored.
7. Publishing and communication during the reporting year International scientific peer reviewed journals, other scientific publications, short communications, web etc.)	7 Scientific papers & 10 Conference presentations; see attachment #1
10. Short economic report (overview) of the reporting year	University of Helsinki: Personnel costs (salaries with social security costs): 8 022,38 €; Consumables: paid by other projects; Traveling: 571,68 €; Overhead: 1 760,93€; Total: 10 354,99 €. University of Copenhagen: Personnel costs (6 months post doc) 27322,48 €; Travel 442,93 €; Consumables 345,19 €; total 28 110,60 €. Norwegian University of Life Sciences (NMBU): Personnel costs (2 months of post-doc salary with social security costs): 13526 €; Consumables and travelling: paid by other projects; Overhead (15 % of salary costs): 2029 €.Total: 15555 €.
11. Date and signature	Date: October 15, 2015 Signature of project leader: Vincent Eijsink

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Attachment #1 - Publications 2014

Papers in scientific journals:

1. Extractability and digestibility of plant cell wall polysaccharides during hydrothermal and enzymatic degradation of wheat straw (Triticum aestivum L.); MAT Hansen, LI Ahl, HL Pedersen, B Westereng, WGT Willats, H Jorgensen, C Felby; Industrial Crops and Products, 2014, 55: 63-69.
2. A C4-oxidizing lytic polysaccharide monooxygenase cleaving both cellulose and cello-oligosaccharides; T Isaksen, B Westereng, FL Aachmann, JW Agger, D Kracher, R Kittle, R Ludwig, D Haltrich, VGH Eijsink, SJ Horn; J. Biol. Chem. 289 (2014) 2632-2642.
3. Discovery of LPMO activity on hemicelluloses shows the importance of oxidative processes in plant cell wall degradation; JW Agger, T Isaksen, A Várnai, SV Melgosa, WGT Willats, R Ludwig, SJ Horn, VGH Eijsink, B Westereng; Proc. Natl. Acad. Sci. USA111 (2014) 6287-6292.
4. Mikkonen, K.S., Parikka, K., Suuronen, J.-P., Ghafar, A., Serimaa, R., & Tenkanen, M. Enzymatic oxidation as a potential new route to produce polysaccharide aerogels, RSC Advances 4 (2014) 11884-11892.
5. Lignocellulose pretreatment technologies affect the level of enzymatic cellulose oxidation by LPMO. Rodrigues-Zuniga UF, Cannella D, Jorgensen H, Felby C. 2015. Green Chem., submitted for publication.
6. Parikka, K., Master, E.R. & Tenkanen, M., Oxidation with galactose oxidase: multifunctional enzymatic catalysis, J. Mol. Catal. B, submitted for publication.
7. Ghafar, A., Parikka, K., Sontag-Strohm, T., Österberg, M., Tenkanen, M. & Mikkonen, K.S., Strengthening effect of entrapped nanofibrillated cellulose is dependent on enzymatically oxidized polysaccharide gel matrices of wet and dry aerogels, submitted for publication.

Conference presentations:

1. 25.03.2014 Claus Felby, David Cannella, Henning Jørgensen, Vincent Eijsink and Bjørge Westereng Enzymatic Oxidation of Cellulose is linked to Lignin through Electron shuttling Mechanisms. 247th ACS meeting, Dallas TX, USA
2. 29.04.2014; Svein Jarle Horn1, Jane W. Agger1, Trine Isaksen1, Zarah Forsberg1, Roland Ludwig2, Al MacKenzie1, Gustav Vaarje-Kolstad1, Aniko Varnai1, Bjorge Westereng1 and Vincent G. H. Eijsink1; Novel structures and functions of lytic polysaccharide monooxygenases; lecture at the 36 th symposium on biotechnology for fuels and chemicals; April 28 - May 01, 2014; Clearwater Beach, FL, USA.
3. 06.2014, Mikkonen, K., Parikka, K., Ghafar, A. & Tenkanen, M., Enzymatic oxidation technology enables manufacturing of novel lightweight and stiff polysaccharide-based aerogels, 13th European Workshop on Lignocellulosic and Pulp (EWLP), Sevilla, Spain, 24-27 June 2014, 99-102. (ISBN 978-84-616-9842-4).
4. 30.06.2014, Bjørge Westereng; Novel structures and functions of lytic polysaccharide monooxygenases; Lecture at the Institute seminar at Slovak Academy of Science, Bratislava.
5. 02.07.2014; Bjørge Westereng, "Novel structures and functions of lytic polysaccharide monooxygenases". Lecture at Oxizymes, Vienna, July 1st - 4th, 2014.
6. 07.2014; NcLPMO9C, a lytic polysaccharide monooxygenase from Neurospora crassa acting on hemicellulose and soluble substrates, Trine Isaksen1, Jane W. Agger1 Aniko Varnai1, Roland Ludwig2, Svein J. Horn1, Bjørge Westereng1, Vincent G.H. Eijsink1, Poster at at Oxizymes, Vienna, July 1st - 4th, 2014.
7. 22.07.2014; The role of oxidative enzymes during enzymatic hydrolysis of lignocellulosic material at industrial conditions. Henning Jørgensen, David Cannella, Claus Felby, Vincent Eijsink and Bjørge Westereng1,3. Lecture at SIM Annual Meeting, July 20-24, 2014; Hyatt Regency St. Louis; St. Louis, MO.
8. 07.2014; H Jørgensen, D Cannella, VGH Eijsink, B Westereng, C Felby; The role of oxidative enzymes during enzymatic hydrolysis of lignocellulosic material at industrial conditions. Poster at the SIM Annula Meeting and Exhibition, July 20 – 24, St Louis, USA.
9. 31.08.2014, Vincent Eijsink, Novel tools for biomass processing – Oxidative cleavage of polysaccharides by Lytic Polysaccharide MonoOxygenases (LPMOs); Lecture at Biocat 2014, Hamburg Germany, 31.08 – 04.09.
10. 21.11.2014, Vincent Eijsink, «Structure and Function of Lytic Polysaccharide MonoOxygenases (LPMOs), novel tools for biomass processing"; invited lecture at MIE Bioforum 2014, Nemuno Sato, Ise-Shima, Japan, November 18-21, 2014.