Nordic Forest Research
Co-operation Committee (SNS)

ANNUAL STATUS REPORT for PROJECT **YEAR 2013**

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 2014

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, P.O. Box 5003, N-1432 Ås, Norway Phone: +47 6496 5892, 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, networking activities have been strong, and we have had an important researcher exchange catalysing intensified collaboration (see below).
5. Activities during the reporting year	Project meetings: March (Ås), September (Copenhagen). In addition, there have been (many) ad hoc E-mail and telephone contacts. Scientific meetings: Due to synergy with the PolyRefNorth network (meeting in Copenhagen, September 2013), no separate meeting was organized in 2013. Scientific research with joint focus: See below Sample exchange: Enzymes have been exchanged between Copenhagen and NMBU (Ås). Samples of oxidized sugars have been generated at NMBU and will be transferred to Helsinki Spring 2014. Researcher mobility: Ås post-doc Bjørge Westereng has been working in the Copenhagen labs for most of 2013; financed from other sources. Joint project applications between the partners: Not yet. However, operational collaboration between Copenhagen and NMBU has been intensified beyond what the SNS grant finances Education (Norway and Finland): 28 doctoral students and supervisors from the Finnish BIOREGS graduate programme (http://blogs.helsinki.fi/bioregs-gs) visited the Norwegian University of Life Sciences (NMBU) in Ås, Norway on September 4, 2013. A joint seminar was arranged with 8 talks.
6. Results achieved during the reporting year	University of Helsinki: A MS-based method based on the reactivity of carbonyl groups was developed for analysis of oxidized structures, i.e. carbonyl groups, in oligosaccharides. The methods is based on that a hemiacetal compound is formed with methanol, by which carbonyl groups can be differentiated from carboxylic acid groups. In the normal situation, carbonyl groups react with water to form hydrates, which have the same *m/z* as carboxylic acid. This method, a major step forward for the study of oxidative processes in biomass, will now be used for analysis of various project samples, including samples provided by the other partners. Work on galactose oxidase has focused on novel aerogels from

	galactomannan (GM) and –xyloglucan (XG) and the first article has been published (Mikkonen et al. 2014). Specific enzymatic oxidation of GM and XG) was used to prepare hydrogels. The compressive moduli of the aerogels were dependent on the oxidation, polysaccharide type, freezing method, and ambient moisture. The oxidized GM and XG aerogels were no longer water-soluble, in contrast to their starting materials, but absorbed liquid water 40 and 20 x their initial weight, respectively. Focused ion beam SEM showed that the inner structure of oriented aerogels from oxidized GM consisted of honeycomb architecture with a pore diameter of some tens of μm. On the other hand, corresponding aerogels from oxidized XG seemed to contain longer capillars oriented in the freezing direction. *The enzymatic hydro- and aerogel preparation method is considered a green way to obtain novel, functional products from polysaccharides.* The demonstrated concept can be used to prepare various types of functional materials, with reinforcements or active components mixed with the polysaccharides before gel formation, entrapping them in the matrix. The next step in the project is to reinforce the aerogels with nano-sized cellulose. The obtained aerogels are biobased and biodegradable, and prepared without hazardous chemicals in an aqueous environment. Notably, both GM and XG are acceptable for food and packaging use. <u>University of Copenhagen</u> The work has focused on 2 paths: -Developing an assay for detection of LPMO enzyme activity on solid surfaces (e.g. cellulose fibers) -Long range electron transport between cellulose and lignin through low molecular weight electron shuttles A challenge for oxidative fiber modification is to measure the level of oxidative actions. For the cellulose oxidizing LPMO enzymes there is a strong interaction with hydrolytic enzymes. But detection of the sole enzymatic oxidation of solid cellulose is a challenge. For this we are developing a fluorescent assay based on labeling of carboxyl groups with a diazo compound (FDAM). The LPMO enzyme is supplied by NMBU (the Norway partner). The work is ongoing and we use TEMPO oxidized cellulose as a reference to quantify the level of oxidation. By this work we will pursue the possibilities for oxidative modification of cellulose and oxidative nanofibers to be delivered to the other partners will be prepared. The recent discovery of cellulose oxidizing LPMO enzymes has focused much upon the action on cellulose. However, the reaction requires 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 may hold many implications for our understanding and exploitation of the oxidative reactions on both carbohydrates and lignin. <u>NMBU</u> NMBU has been working on characterizing novel LPMOs, which are one of the main classes of enzymes with potential for oxidative fiber modification. We have discovered novel LPMO activities that can be used to generate

	new types of oxidations (to be reported in more detail later) and we have developed a wide range of (challenging) analytical methods for in-depth LPMO characterization. Importantly, one outcome of this work is the generation of (quite unique) standard samples of a wide variety of oxidized oligosaccharides, which the other partners can use in their work. NMBU has been collaborating intensely with Copenhagen, as reported above.
7. Publishing and communication during the reporting year International scientific peer reviewed journals, other scientific publications, short communications, web etc.)	Publications: **Mikkonen, K.S., Ghafar, A., Parikka, K. and Tenkanen, M.**Prospects of polysaccharide aerogels as modern advanced food materials, Trends Food Sci. Technol. 34 (2013) 124-136. **Mikkonen, K.S., Parikka, K., Suuronen, J.-P., Ghafar, A., Serimaa, R., and Tenkanen, M.** Enzymatic oxidation as a potential new route to produce polysaccharide aerogels, RSC Advances, 2014, in press, doi:10.1039/C3RA47440B. **Isaksen T., Westereng B., Aachmann F.L., Agger J.W., Kracher D., Kittl R., Ludwig R., Haltrich D., Eijsink V.G.H., Horn S.J.** A C4-oxidizing lytic polysaccharide monooxygenase cleaving both cellulose and cello-oligosaccharides J Biol Chem. 2013 Dec 9. [Epub ahead of print] Presentations: **Tenkanen, M**. Galactose oxidase opens novel ways for tailored natural polysaccharides, 10th Carbohydrate Bioengineering Meeting, April 21-24, 2013, Praque (talk). **Canella D., Westereng B., Jørgensen H., Eijsink V.G.H., Felby, C.** Mechanistic studies of LPMOs kinetics acting on lignocellulosic substrate: implications for industrial applications. Presentation at the Gordon Research seminar on Cellulosomes, Cellulases & Other Carbohydrate Modifying Enzymes in August 3-4 2013 (poster & short talk). **Westereng B.** Structure and function of copper-dependent Lytic Polysaccharide MonoOxygenases; Lecture at "Enzymatic hydrolysis of soluble carbohydrates" Søminestationen, Denmark, 26-27.09.2013 (talk).
10. Short economic report (overview) of the reporting year	Costs University of Helsinki: Personnel costs (salaries with social security costs): 5 808.81 €, Consumables: 673.32 €, Traveling: 289.72 €, Other: 171.00 € Overhead: 1 422.03 €; Total: 8 364.88 € (remaining funds to be transferred to 2014 & 2015) University of Copenhagen: Direct costs were 600 Euro. Most of the activities in 2013 have been covered by self-financing and remaining SNS funds will be transferred to 2014 and 2015 (where they will be needed to keep project activities afloat). Norwegian University of Life Sciences (NMBU): Personnel costs (salaries with social security costs): €97,83, Consumables: €0 Traveling: €186,36, Other, incl exchange loss: €2389,51, Overhead: €15,38; Total: € Most of the activities in 2013 have been covered by self-financing and remaining SNS funds will be transferred to 2014 and 2015 (where they will
11. Date and signature	Date: March 1, 2014 Signature of project leader: