From waste to resource for a greener, biobased world

NEWSLETTER - JANUARY/FEBRUARY 2019

Welcome to the second FALCON newsletter and thank you for joining us!
For more information on the FALCON project, visit the FALCON website www.falcon-biorefinery.eu.

A reminder - What is the EU FALCON project?

FALCON is a project funded by the EU that aims at turning the lignin-rich industrial waste stream from second generation biofuel plants into higher value products, such as marine fuels, fuel additives and chemical building blocks. FALCON stands for "**F**uel **a**nd chemicals from **l**ignin through enzymatic and chemical **con**version"

Biofuels are a promising alternative to fossil fuels, one of the contributors to global warming. However, the use of crops to generate biofuels is under strong economic pressure. In addition, large amounts of waste are generated in biofuel production. But what if we could use this waste to make biofuel production more profitable and sustainable?

The main component of this waste is lignin, the glue that holds plants and wood together. It is estimated that by 2050 biofuels will generate approximately 200 million tons of lignin. This offers tremendous opportunities for using it as a starting material for many products. However, at present most of this waste is burned to produce green energy (heat or electricity) for the biofuel plant. One challenge is to develop new technology to convert lignin into other valuable products.

FALCON is a project funded by the European Union's Horizon 2020 Program to tackle this challenge. It aims to develop an environmentally friendly, low-energy demanding process to turn lignin waste into value-added bioproducts. Falcon will make use of enzymes from fungi and bacteria, which are excellent at breaking down plant fibers. Combining the power of enzymes with other innovative technologies, lignin waste will be turned into an oil that can be readily converted into:

- Marine fuel, a green alternative to conventional fuels currently used.
- Fuel additives to make road transportation more sustainable and more efficient.
- Chemical building blocks to produce bioplastics and other biobased materials.

These new, sustainable products could replace fossil fuels and conventional plastics, as well as help bio refineries transition towards a "zero waste" production. Overall, the project will place lignin transformation at the heart of the economic and ecological viability of biofuels.

3rd FALCON Meeting Lyon

The third FALCON Governing Board meeting took place in Lyon in France from February 6-8, 2018 at the TOTAL Facilities. The meeting started with a visit to the Total compound. The rest of the first day and part of the second day was reserved for the separate Work Package summary meetings, whereas the Work Package meetings took place on the second and third day. The coordinator was pleased to notice that all Work Packages were on schedule.

Results from Work Package 2

During the second year of FALCON project, the work in **WP2 – Laccase mining, production and characterization** has focused on the identification and characterization of novel and existing laccases from MetGen, KNAW and UH, aiming to discover the best candidates to be applied in further research and development related to the end-user applications. The laccase development work in WP2 has proceeded smoothly and all the planned milestones for WP2 have been reached as scheduled.

MetGen has selected the five most promising bacterial laccase variants to be used for the lignin depolymerization tests. All these laccases variants showed promising activity against phenolic substrates. Results showed that stable operation conditions of the selected laccases candidates are between temperature of 60-85°C and pH of 8-11, which makes them good candidates for the operations in alkaline conditions, where lignin expresses highest solubility and reactivity towards oxidation and depolymerization. In addition, MetGen's laccases showed high solvent tolerance in alcohol solvents. They were also subjected to impact tests with the studied lignins. In neutral and

alkaline conditions, these laccases had an impact on the molecular size distribution of the studied lignin, indicating that laccases can oxidize and radicalize lignin in the tested conditions. These characteristics merit their selection for the further lignin depolymerization studies and a larger scale production of lignin-oil. All laccase variants showed good expression and production yields in MetGen's *E. coli* production platform and different candidates were produced in a pilot-scale (400 l) and provided to FALCON partners for further testing.

KNAW has characterized four new ascomycete fungal laccases. Two of them are active under mild alkaline conditions towards model substrates and two are acidic laccases. They all showed optimal activity and stability at 25-30°C, which corresponds with the growth conditions of the fungi they originate from. The enzymes also tolerate low concentrations of solvent. These enzymes were tested for their ability to depolymerize lignin under alkaline conditions, which has an advantage for lignin solubility, as well as mild acidic conditions. Based on the lignin depolymerization studies, the ascomycete enzymes work better under mild acidic conditions and are susceptible to high alkaline conditions. One ascomycete laccase shows promising lignin depolymerization with the assistance of a mediator, and is selected for further improvement by rational mutagenesis.

UH has tested seven basidiomycete fungal laccases for their suitability in lignin depolymerization. Basidiomycete laccases were recombinantly produced in the yeast *Pichia pastoris*, which is a commonly used host organism for eukaryotic protein production. Laccase activity was detected on phenolic substrates at pH range 3-8 and due to the lignin extraction method, the laccases that were active on neutral conditions were selected for detailed studies. The optimal conditions for the two selected laccases were at 60-70°C and pH 7-8. All the selected laccases tolerated alcohol solvents and dioxane, which were used as co-solvents in lignin depolymerization experiments. The best laccase candidate showed lignin depolymerization both with and without small molecular weight mediators. The depolymerization rate in terms of MW distribution was ΔM_N -15-55% depending on the reaction conditions. This laccase was selected for up-scaled production.

Results from Work Package 3

The activities and obtained results in **WP3 – Process design and pilot for low-sulphur heavy fuel oil production** during the second year of FALCON are developing positively towards the set objectives and milestones of the project.

MetGen has designed, optimized and implemented a streamlined and state-of-the art progress-flow for scale-up and optimization of process and product development. MetGen has successfully implemented Standard Operation Protocol (SOP) to produce its newly developed laccases at pilot

scale and industrial scale. MetGen has already up-scaled six laccase variants developed during the FALCON project to a larger scale, with the two most prominent candidates produced at pilot scale (400 l). A full technology transfer to an industrial scale (50 m^3) is planned for the most prominent laccase candidate in the next period. MetGen continues to provide FALCON partners novel extremophilic laccases for lignin valorization. In addition, MetGen's has offered the FALCON consortium the access to its proprietary lignin valorization technology. BBEPP will utilizes this technology in WP5 for the production of lignin oil.

UH has developed two separate purification method for the lignin biomass prior to enzymatic oxidation to enhance the efficiency of the lignin-oil formation. One method includes the removal of salts by washing and mild acidification to remove carbohydrates, followed by alkaline extraction and re-precipitation. The other method includes formic acid treatment combined with filtration and re-precipitation. The materials obtained by depolymerization in WP3 have been further tested in WP5 for chemical depolymerization of lignin crude oils to non-oxygenated mono-aromatics and oxygenated mono-aromatics

BBEPP further optimized the method for lignin purification taking into consideration the quality and economics of the process. This also resulted in reducing the number of pre-treatment steps required before lignin depolymerization. BBEPP developed and optimized a continuous enzymatic lignin depolymerization system without mediator addition. The continuous system supports the separation of the low molecular weight monomers due to enzymatic depolymerization. A solvent extraction protocol is currently under development for the recovery of the lignin oil.

Results from Work Package 4

During the first two years of the FALCON project, the groundwork laying tasks and activities regarding **WP4 – Marine fuel and fuel additive applications of lignin crude oil** have been commenced and progressed towards the envisioned objectives and milestones.

In close cooperation with the entire FALCON project consortium, the project partners of WP4, **Total**, **WinGD** and **PI**, determined the desired Lignin Derived Fuel Oil (LDFO) properties and published these in a report. The herein listed LDFO specifications concern the most critical properties with respect to practical fuel applications.

Similar to the aforementioned (LDFO) requirements, the desired lignin-based fuel additive properties have been determined and published in a report. Based on pre-screening, this report recommends various candidates.
For both reports, Total and WinGD contributed by collecting the specifications from international standards, internal and external reports, as well as from their colleagues working in the related industry.
Additionally, the foundations have been laid for effectively collecting all relevant lignin- and LDFO sample properties, next to performing the first exploratory Compression Ignition (CI) engine tests with baseline fuels and exploratory Spark Ignition (SI) engine tests with model component based fuel additives.

Results from Workpackage 5

The activities in **WP5 – Upgrading lignin crude oil to fuel additives (value chain 2) and chemical building blocks (value chain 3)** have concentrated on generation of pools of aromatic compounds from lignin-derived oil and production of microbial aromatic metabolic enzymes in the second year of the FALCON project. The progress is well in line with the objectives and milestones of FALCON.

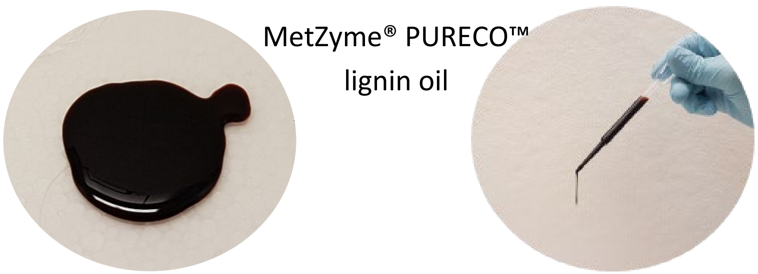
UH has subjected different types of lignin model compounds and enzyme-treated purified lignin to chemical treatment to evaluate suitable reaction conditions for lignin cleavage and modification in order to convert the low-sulphur lignin-derived heavy fuel oil to fuel additives and mono-aromatic building blocks. The cooking conditions were adjusted according to the optimization results. Promising results with respect to lignin depolymerization have been obtained.

The work on fungal and bacterial aromatic metabolic enzymes for bioconversion of the aromatic compounds from the lignin-derived oil has been continued at UH and KNAW. UH has focused on basidiomycete fungal aromatic metabolic enzymes and cloned 10 candidate enzyme encoding genes to fungal and bacterial production hosts. Optimization of production conditions in bacterial host has been performed.

KNAW has produced 22 enzymes involved in the conversion of aromatic compounds. Eleven enzymes originate from bacteria, three from yeast and eight from filamentous fungi. Seven of the eight enzymes originating from filamentous fungi have not been described before. The activity of 13 enzymes was determined and the other nine will soon be analyzed. The enzymatic activity was observed on monomeric aromatic compounds that are released from lignin during microbial degradation. These enzymes can be used to produce aromatic compounds and chemical building

blocks of great industrial interest, such as the flavour agent vanillin and the plastic precursors styrene and *cis,cis*-muconic acid.

MetGen has utilized its proprietary lignin fractionation technology combining MetGen's novel MetZyme® PURECO™ enzymatic technology for lignin depolymerisation in alkaline conditions together with robust UF and NF membrane operations in a continuous mode to produce and separate small oligomeric, dimeric and monomeric lignin units. These lignin units were formulated as lignin oil like substance by solvating them to organic solvent mixtures with a high lignin loading.



Results from Work Package 6

So far the work **in WP6 – Economic and life cycle assessment** focused on industrial state-of-the-art base case scenarios for two value chains, namely marine fuels and gasoline fuel octane booster. The results of the investigations, using the same tools and level of detail as later for the FALCON processes and products, will be used for benchmarking the final FALCON results. This will allow FALCON to quantify the achievements of the project in terms of economic and environmental sustainability.

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Agenda

Next FALCON Governing Board meeting in Gent January 28-29, 2018

FALCON Workshop “Lignin Valorization towards fuels, chemicals & materials”, Gent, January 30

The Consortium

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