

# A protective biofilm for wood preservation

*By Mr. Michael Sailer*

*pilot realised with Regge Hout B.V./Xylotrade B.V*

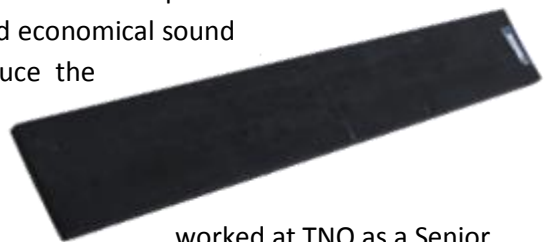
## Introduction

Partners grow2build – introduction of companies and persons involved

Regge Hout (earlier Reef Hout) is a wood processing company with its own manufacturing facilities in Goor (Overijssel, The Netherlands). The company has long experience in outdoor wood applications and durability aspects, and possesses extensive knowledge and network in the timber industry. Regge Hout has gained experience in research and development projects which ensures an efficient and quick market introduction of the microbial coating concept. Due to a consequent policy in e.g. the use of FSC certified tropical hard wood Regge Hout achieved a good reputation as a wood supplier for building and construction purposes. The annual turnover of Regge Hout in 2013 was approximately 11 million euros, which was achieved by 30 employees. Regge Hout uses an expert-based, R&D oriented approach in the development and application of timber solutions. In recent years Regge Hout recognized that a more efficient use of wood resources with a higher added value is required to remain competitive. The research project Biofilm was initiated with the research institute TNO and is actually on the way to be implemented into the market. The protection of wood with a microbial coating will be realized together with IMEnz Bioengineering.

Xylotrade B.V. is a company dedicated to the commercialization of the biofilm. Frans van Rooijen, CEO of Regge Hout, and M. Sailer, senior researcher of Saxion, are involved in the founding this company.

IMEnz Bioengineering is a biotech company with ample experience with the optimization and upscaling of microbial fermentation processes. Preferably, abundant agricultural waste streams of low value and by-products are exploited as nutrients. This contributes to the development of sustainable production processes. For the current project efficient and economical sound fermentation processes are essential in order to produce the micro-organisms for the microbial coating at sufficient qualities and quantities for validation studies.



Main researcher of the project, M. Sailer, has previously worked at TNO as a Senior Research Scientist for more than 10 years in the assessment of interactions of lignocellulose materials, water and micro-organisms. The interaction of these partners in this project will create synergistic scientific effects and increase the application perspective in building practice.

Biofilms on substrates are one of the most stable biological systems on earth. Forming biofilms on materials is being examined exhaustively. An overview over the most recent developments can be found in the proceedings of the first European congress on microbial biofilms (2009). From this overview it is clearly stressed that functional application of biofilms for protection of materials in



combination with vegetable oils is an unexplored area. In general, research on biofilms focuses on prevention of biofilm formation, in widely different application domains.

In addition to the enhanced ecological protection of wood, the EAWP Biofilm makes it possible to upgrade the technical performance specifications of European wood to the level of tropical hardwood. This opens up new opportunities for the European wood sector.

### Technological Readiness Level

A major breakthrough in the functional use of biofilms was recently achieved (Sailer *et al*, 2010;). On wood treated with vegetable oils, usually establishes a biofilm consisting for more than 90% of the yeast like fungus *Aureobasidium pullulans*. It turns out this biofilm protects the wood. This was demonstrated with wood of low durability, which was covered with a biofilm of *A. pullulans*. The samples were not degraded after a period of more than 10 years of outdoor exposure.

The biofilm is a self-repairing system consisting of a new innovative protection principle using vegetable oils like e.g. linseed oil and a living biofilm created by *Aureobasidium pullulans*. It is completely based on natural substances and the fungus used is frequently occurring in different variants all over the world (ubiquitous fungus). Due to its complete biological origin the wood-oil-biofilm combination is completely recyclable and environmentally friendly and not harmful to humans.

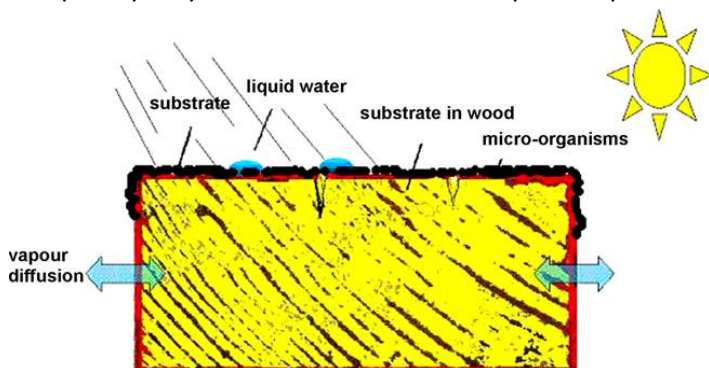


Figure 1

### Current IP and freedom to use

The fundamental principles of the production and application of this organism to protect wood has been laid down in patent EP1704028 (B1) and US7951363 (B2) by TNO). Regge Hout has obtained the exclusive rights to use these patents in the development, production and marketing of the biofilm concept.

## Purpose

### description

Common surface treatments, products and techniques consist of dyeing, thermal treatment and acetylation. The applied preservatives mostly consist of chemical additives considered biocides. The use

of these toxic substances is increasingly restricted by legal regulations. In some countries for example, the use of CCA (copper chromium arsenic) impregnated wood is no longer allowed. Also some metal-free biocides, such as creosote, are subject of discussion. Due to these developments, the use of indigenous renewable raw materials, such as timber, becomes increasingly limited despite the governments' aim to encourage the use of sustainable produced and processed materials. Furthermore, sustainable initiatives such as the actual certification processes in the building industry require the use of sustainable products throughout the entire building process. In addition, the sustainable and cost-efficient use of available resources and raw materials poses an increasing European societal challenge and calls for strategic and practical solutions which can be adopted by a wide variety of companies active in the wood industry.

The goal of the project is to enhance the use of indigenous raw materials and introducing a sustainable and ecological wood preservation solution, thereby increasing the competitiveness of the European timber industry.

production of advanced prototype

The project contributes to the practical development of the treatment concept by producing the following results:

- A biofilm on wood fit for industrial use and market replication
- Upscaling of the total biofilm production process chain
- Demonstration of biofilm properties
- Preparation of market introduction with industrial partners

## Results

technical performances

Resistance against micro-organisms:

The technical performances related to resistance of oil-biofilm treated wood against micro-organisms have been initially assessed in standard tests according to an adapted EN 113 (laboratory) test and EN 252 (outdoor in ground) test. According to the initial EN 113 test the resistance is depending on two



Figure 2: In ground test according to EN 252

factors, the oil uptake and the quality (performance) of the biofilm. Both of course are influenced by the wood specie used. In the indicative tests pine (*Pinus sylvestris*) sapwood was tested. In this combination the biofilm improved durability e.g. from class II to durability class I. With an uptake of approximately round 400 kg/m<sup>3</sup> linseed oil and the biofilm, durability class I is achieved. Due to the high oil content the risk of excessive oil secretion has to be accepted, as well as the higher costs due to the additional linseed oil used. With significantly less oil (100 kg/m<sup>3</sup>) durability class II/III is more realistic, resulting in less/no risk of oil secretion and significantly lower costs. For most out of ground timber applications durability classes II or III will be sufficient.

Similar results have been achieved in the EN 252 in ground test (fig. 2) which was started in 2009. After 5 years in ground exposure durability class II was achieved with the combination of biofilm and linseed oil impregnation.

Adhesion and esthetical appearance of the biofilm is very much depending on factors like fermentation conditions and formulation of the biofilm. Compared to a normal coating the biofilm is a bit softer but more water repellent. This improves the dimensional stability of the wood and reduces crack forming on the surface. This is an important factor which is contributing to a longer service life of the wood.

Figure 3: Impregnation vessel with linseed oil treated timber



Figure 4 : Comparison of different treatments after 3 years exterior exposure

:

## Fermentation

Besides the technical performance related to service life other aspects like the economic feasibility of the fermentation are crucial. A huge number of optimization tests have been carried out to optimize the technical and economic feasibility successfully together with IMEnz. Nevertheless, improving the efficiency and costs of the fermentation processes will remain crucial elements in the development to achieve a sound business model.

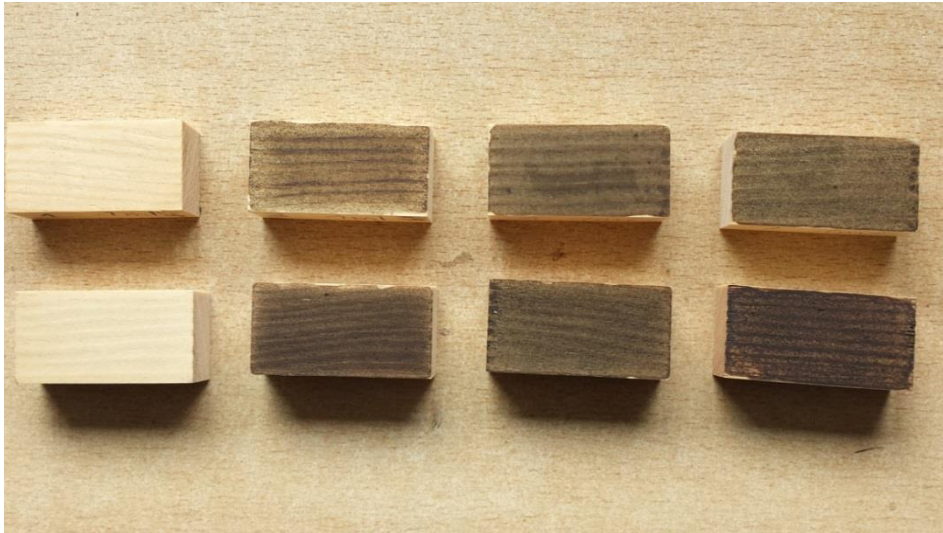


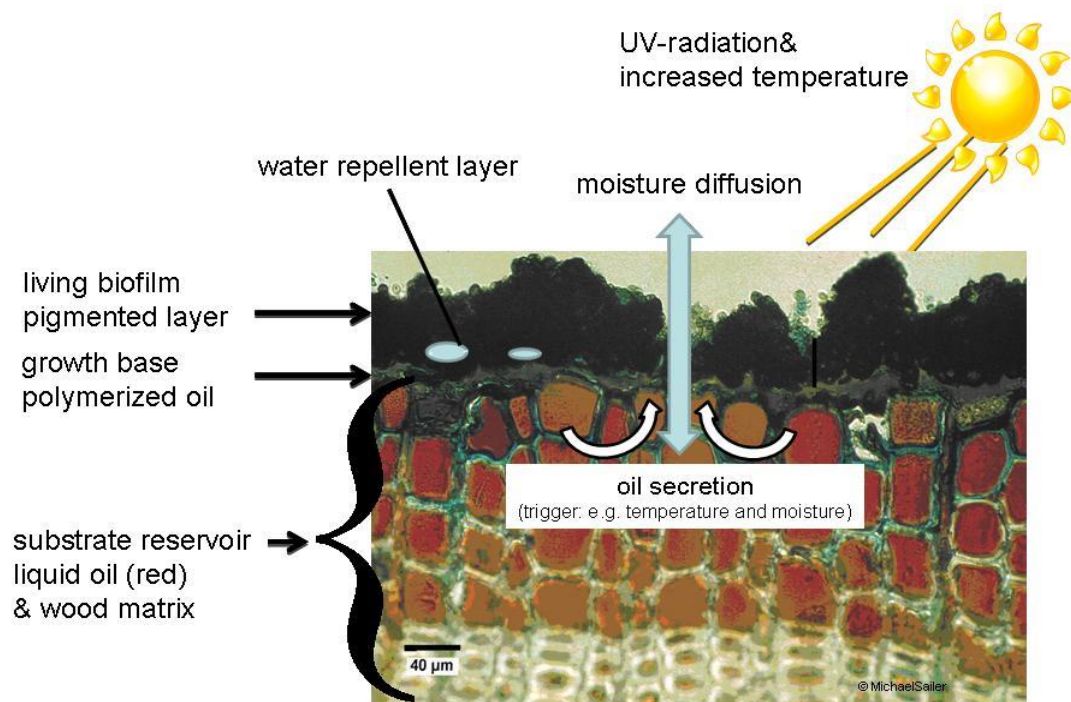
Figure 5: Quality control of different fermented fungal suspensions on wood

## functionalities

### Self-healing

Since the principle of the biofilm is based on living fungal cells the question arises if a self-healing principle can be used. In the case of the biofilm the wood has the function of a nutrition reservoir. Under certain circumstances (e.g. moisture and temperature changes) self-healing will be realized by extra growth of the biofilm after damages. One relevant aspect is the oil secretion to the wood surface. These functions have been demonstrated although not all principles are understood yet. Therefore in a PhD project within the Dutch National self-healing research program (IOP) more knowledge is actually generated.

The principle of the self-healing aspect is demonstrated in figure 6.



**Figure 6; Self-healing principle of the biofilm (black color) demonstrated at pine (*Pinus sylvestris*) treated with a vegetable oil (red)**

### Stability

Another advantage is the typical characteristics of the biofilm. Compared to a normal coating the surface is not closed and sealed. Under the microscope it is visible that there are a lot of fungal colonies with little space in between. The fungal colonies can therefore move together with the wood as soon as it swells or shrinks without any damage of the biofilm. An enormous advantage compared to normal coatings which frequently crack. Furthermore the biofilm will remain and grow as long as there is enough vegetable oil available. Since the fungal cells are relatively inactive they do not require much nutrients. The whole system is therefore very durable with a potential service life of at least several years.

## Production technology

An overview over the different production steps is given in figure 6.

### Production process

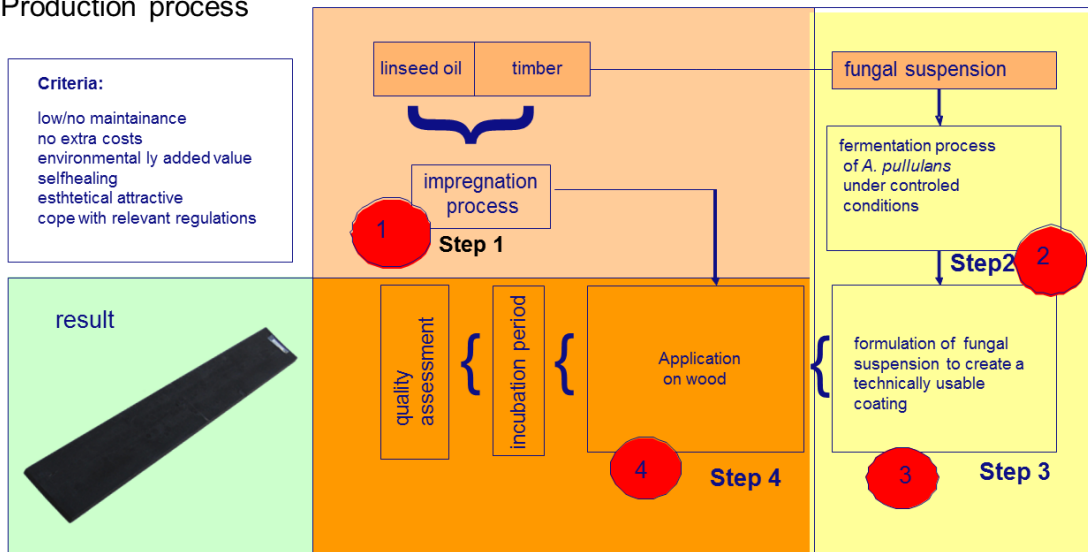


Figure 7: Overview over the different production steps

### Step 1: Impregnation of wood with linseed oil



Figure 8: Construction of the 4m<sup>3</sup> pilot impregnation plant at van der Zalm B.V.  
Foreground: the rails for loading the vessel

The pilot impregnation plant was specially constructed by van der Zalm B.V. for the impregnation of timber with vegetable oils. The pilot plant, with a length of 5m and a treatment capacity of nearly 4m<sup>3</sup> per process cycle, is about to be finished and will be delivered in august 2015. The pilot impregnation vessel is designed in such a way that it can be loaded from both sides if expansion of the impregnation capacity is needed.

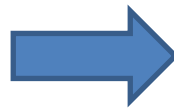
## Step 2: Fermentation of fungal suspension

The fermentation of the fungal suspension is a very important factor in the upscaling of the process.

Several methodologies can be used. It was however decided to scale up in small steps from 2 litres to 40 l and then further to 300 l batch fermentation for pilot proposes. In a last step for production 3000 l fermenters will be used. Currently, IMEnz is establishing a pilot scale production facility that will be exploited to make available larger amounts of biological coating for validation and testing and, ultimately, the market.



Figure 9: Laboratorium fementer



upscaling



Figure 10: Small industrial fermenter

## Step 3 and 4: Formulation of fungal suspension for industrial application



Figure11: Fungal suspension on a wood surface

A good coverage of the wooden surface with a pure water based fungal suspension is not feasible. Due to the lack of high viscosity most of the water based fungal suspension would flow away from the wood surface, which would not be desirable. Therefore the viscosity of the fungal suspension has to be adapted according to the requirements of the application machines figure 11. Initial tests have successfully been carried out.



Figure 11: Machine for oil applications on timber surfaces

## building requirements

Builders and architects who would like to use a new protection system based on vegetable oils and the naturally occurring fungus *Aureobasidium* are invited to have a closer look to this product. At the moment the colour dark grey/ anthracite is the typical colour which can be realised by this coating concept. This concept is designed for wooden facades and other wood exterior surfaces which have to be protected. Wood species tested up to now are pine and spruce, other wood species will be tested in future as well as the behavior of the biofilm regarding fire safety regulations.

## conclusion

### On the way to production

The first steps towards technical realisation of a unique production process to produce a biofilm based on vegetable oils and a fungal suspension from *Aureobasidium* are about to be finished. All relevant process developments steps are on time and after installation of the impregnation plant in August this year, wood up to 5m length for pilot projects will be treated with linseed oil. The basis pilot plant will be operational in the autumn of 2015. At that time, fermentation will be up scaled and at the same time the first pilots will start. Since we are still at the beginning of a new protection concept a number of research questions remain: e.g. the possibility to generate biofilms with other colours, questions about the resources for fermentation and general optimization. These points will be assessed in other projects.

## information

For detailed information:

Regge Hout B.V / Xylo Trade B.V.

Frans van Rooijen / dr. Michael Sailer

Breukersweg 9  
7471 ST Goor  
The Netherlands  
Tel +31(0)547286350  
info@reggehout.nl

## Referenties

TNO internal report (2009) EAWP biofilm development

Sailer, M.; Van Nieuwenhuijzen, E. 2008: Creation of biofilms with desired functions on surfaces. In Henk Jonkers, Jacco Booster, Leon van Paassen & Mark van Loosdrecht (Eds.), BGCE 2008. 1st international conference BioGeoCivil Engineering, 23-25 June 2008, Delft, The Netherlands, 137-142

Van Nieuwenhuijzen, E.J.; Sailer, M.F.; van Groenestijn, J.W. 2009: Quantification of growth and viability of a biofilm on wood. Eurobiofilms 2009: First European Congress on Microbial Biofilms, Rome, Italy, September 2 - 5, 2009, 147-148

Sailer, M.F.; Van Nieuwenhuijzen, E.J.; Knol, W. 2010: Forming of a functional biofilm on wood surfaces. Ecol. Eng. 36, 163–167.

Van Nieuwenhuijzen, E. J., Sailer M. F. Samson, R. A., Adan, O. C.G. (2013) Detection of *Aureobasidium* as the dominant fungus on coated wood, Proceedings: IRG/WP 13-10796, 16 pp

Elke van Nieuwenhuijzen – Poster and lecture – Oil as selective medium for the biofilm on oil treated wood - BioGeoCivil engineering conference, 14th March 2013

Sailer, M.F. 2011: EP1704028 (B1): Ecologically Protected Material

Sailer, M.F, 2011: US7951363(B2): Ecologically Protected Material

Schiele (2015) Impregmat (personal communication)