Development of the *Penicillium verruculosum*-cellulase complex for the SSF-process of the bio-ethanol production up to pilot scale in a master plan for the utilization both the carbohydrate content and the lignin for the production of valuable products from wheat straw

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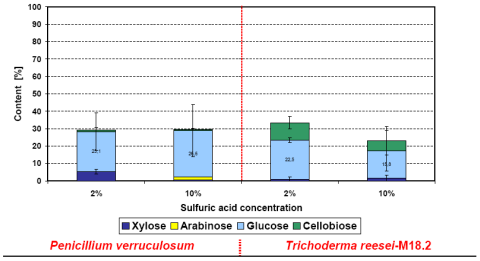
The state-of-the-art

In the past sixty years, various procedures both for the production and the application of cellulolytic enzymes have been developed. The state-of-the-art is characterised by a lack of sufficiently suitable enzyme complexes, which allow the enzymatic breaking up of lignocellulosic biomass for the economic utilization of the carbohydrate content for the fermentation to bioethanol. This is mainly caused by a non-optimal composition of the enzyme complexes as well as by its low specific activity, especially in case of cellulase.

Scale-up of the pre-treatment process of the lignocellulosic substrate for the release of the carbohydrate content could not be implemented economically due to the polymeric structure of the lignin.

The aim of the project

is first, to develop an *P. verruculosum*-enzyme complex for the efficient saccharification of the carbohydrate content in lignocellulosic biomass, particularly wheat straw, in a process of simultaneous saccharification and fermentation (SSF) to bioethanol. Secondly, we plan a pre-treatment-process, which makes it possible to use the lignin content for material application. Thirdly, we plan to expand our knowledge about the mechanism of the saccharification of lignocellulosic biomass in the SSF-process for the bioethanol production. Using wheat and maize straw as lignocellulosic substrate, the developed enzyme complexes are investigated in SSF for bioethanol production up to pilot scale.



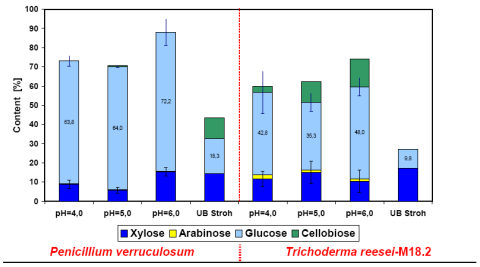


Fig. 3: Comparison of *P. verruculosum* with *T. reesei*-M18.2:
Comparison of the pre-treatment with formic acid (NP) and sulfuric acid
Comparison in composition of the wheat straw hydrolysates
(UB = wheat straw not pre-treated)

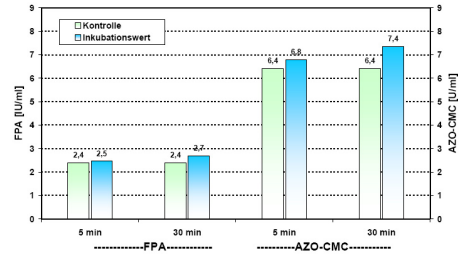


Fig. 4: Influence of lignin on the activity of the *P. verruculosum*-cellulase
(incubation with 3% kraftlignin INDULIN-AT, 30°C; control without lignin)

References:

[1] Letzel, Anne-Catrin. Herstellung von Bioethanol mittels simultaner Verzuckerung und Fermentierung (SSF) von Lignocellulose. 2009, Diplomarbeit, TU Bergakademie Freiberg

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[3] Bönsch, K. Molekularbiologische Untersuchungen zur C-Katabolitrepression von extrazellulären Enzymen an *Penicillium verruculosum*. Diss. 2003, Universität Leipzig

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Present results

In previous investigations we have developed a *P. verruculosum*-cellulase complex which is favoured for the SSF-process using pre-treated wheat straw for the ethanol production. This enzyme complex exhibits significant advantages in comparison with the worldwide used *T. reesei*-cellulase complex. This is demonstrated both in higher resistance towards ethanol and in an advantageous composition of the cellulose-hydrolysate (Fig. 1-3). Furthermore, the activity of the *P. verruculosum*-cellulase is not influenced by lignin (Fig. 4).

The *P. verruculosum*–mutant strain forms an enzyme complex with a higher specific activity towards MCC in comparison with the *T. reesei*-production strains. This is mainly caused by increased ß-glucosidase content in the *P.verruculosum*-enzyme complex (e.g. [3], [4]). The genetically fixed excretion rate of about 15 mg enzyme protein per hour per gram mycelium is in the same range like at high yielding production strains, however, in contrast to *T.reesei*, this high excretion rate can not yet realized during the whole fermentation process. Two *P.verruculosum*-mutant strains are as safe deposit in the DSMZ.

Untreated straw (A), pre-treated straw according the NP-process (B), wheat straw-lignin resulting from the NP-process (C)

The objectives of the project are

shown with the master plan in Fig. 5.

Optimization of the *P. verruculosum*-strain

Investigations to switch-off the c-catabolite repression in *P. verruculosum* using methods of molecular biology and genome analysis, including comparison with the genome of T.reesei-production strains which was published in 2007

Optimization of the fermentation process

Investigations on inducing the cellulase- and hemicellulase components in *P.verruculosum*, including stillage as substrate [cf. 5], to get knowledge for the optimization both the medium and the feedback-controlled fed-batch-techniques

Pre-treatment of the lignocellulosic substrate

Scale-up of the pre-treatment process for the lignocellulosic substrate; e.g. optimization of the NP-procedure

Optimization of the SSF-process

Saccharification of the carbohydrates by the *P.verruculosum* enzyme complex, yeast strain optimization

Scale-up of the SSF from lab scale to pilot plant

Scale-up of SSF for bioethanol production on basis of wheat- or maize-straw up to pilot scale using the optimized *P. verruculosum*-enzyme complex; Economic evaluation

The pre-treatment of wheat straw was operated with formic acid according to the so called Natural-Pulping-Process [2]. This procedure proved to be useful for the following SSF-process. The lignin could be separated nearly completely in a workable quality (cf. photo). We have already R&D activities concerning the production of biopolymers on the basis of lignin (cf. www.era-ib-lignin.eu).

The ethanol yield in SSF-experiments with the yeast *Kluyveromyces marxianus* was about 65% in lab-scale. An interaction of the yeast with the *P. verruculosum* cellulase, e.g. proteolysis, could be eliminated by the addition of stillage or other protein sources in the fermentation broth.

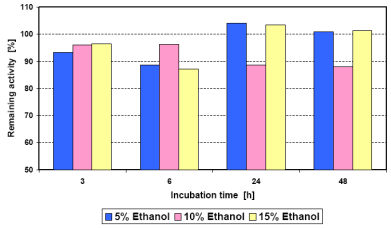

Fig. 1: Activity of the *P. verruculosum*-cellulase complex dependent on the ethanol concentration at 30°C and MCC as substrate
(remaining activity up to 48h in comparison with the control)

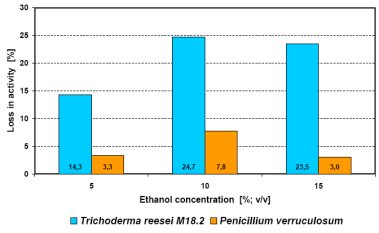

Fig 2: Comparison of the ethanol resistance between *T. reesei*- and *P. verruculosum*-cellulase at different ethanol concentrations, saccharification of microcrystalline cellulose (MCC)

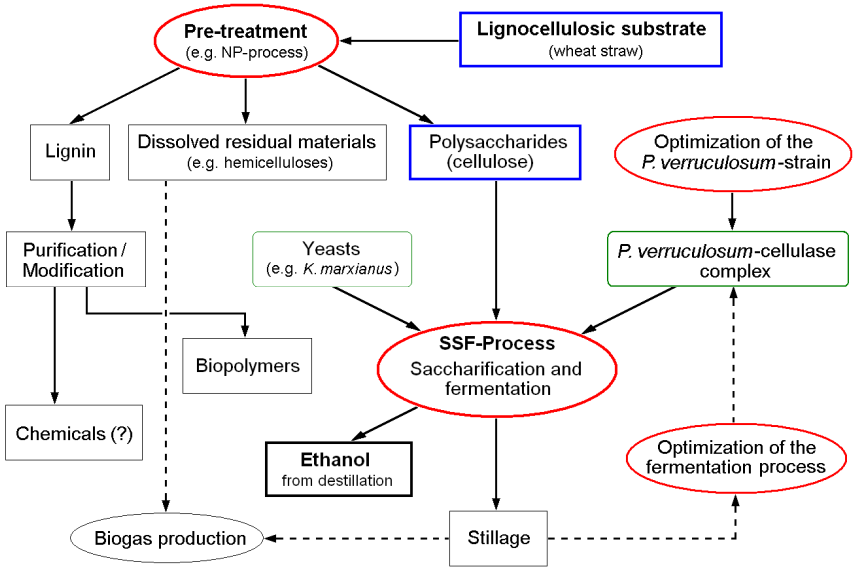

Fig. 5: Master plan