Biodegradation of lignocellulosic material by fungi – the route from organic waste to feed

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Introduction

Organic waste produced in the Netherlands consists of materials like, for example cereal straw, garden waste and prunings in municipal waste. A common characteristic of these materials is a relatively high content of lignin. Lignin in cell walls provides protection and strength to plants by forming complexes with cellulose and hemicellulose via ester and ether bonds (Buranov and Mazza, 2008). Microorganisms in an anaerobic environment, like the rumen of ruminants, are not able to degrade lignin and are therefore not able to reach the carbohydrates for fermentation (Susmel and Stefanon, 1993). To increase the nutritional value of lignocellulosic material, lignin should be degraded or modified in such a way that carbohydrates, like cellulose and hemicellulose, become available for fermentation in the rumen.
Although lignin degradation by chemical or physical treatments is described in literature, these methods do not have preference because of costs, environmental issues and the possible presence of toxic compounds. As an alternative, biological methods, like treatment of material by fungi, are described in literature. By selecting fungi which have a Generally Regarded As Safe (GRAS) status, risk of presence of toxic compounds can be reduced. Next to this, fungal treatment is lower in costs than the use of chemicals (Jalc, 2002). Most described lignin degrading fungi are white rot fungi, which produce enzymes to degrade lignin. Prior to fruiting these fungi colonize the sample with their mycelium, in this way the fungi prepare the sample for fast uptake of nutrients. Depending on the species lignin is degraded specifically without affecting nutrients.
A preliminary experiment has been done in which 11 different fungi species were tested on their ability to specifically degrade lignin in wheat straw (Tuyen et al. 2012). As measurement chemical analysis was done after fungal pre-treatment of wheat straw. In addition *in vitro* gas production was measured to see the effect of the fungal treatment on the degradability in rumen fluid. This study together with literature forms the basis of continuing experiments in the development of a technique to specifically degrade or modify lignin by fungi.

Material and methods

The 11 fungi studied (*Bjerkandera adusta*, *Ceriporiopsis subvermispora*, *Ganoderma lucidum*, *Lentinula edodes*, *Phlebia brevispora*, *Phanerochaete chrysosporium*, *Pleurotus eryngii*, *Pleurotus ostreatus*, *Schizophyllum commune*, *Trametes versicolor*, *Volvariella volvacea*) were grown on malt agar extract after which spawn was prepared on barley grains. Wheat straw was wetted (3 fold weight water:substrate), cut into pieces of ~2 to 3 cm and autoclaved twice to make sure no contaminant is present anymore. To the prepared wheat straw 5 gram of barley grains covered with mycelia was added. Fungi were grown on wheat straw at 24°C and samples were taken after 0, 7, 14, 21, 28, 35 and 49 days. Before analyses were done the material was dried and ground. Chemical analysis consisted of determination of dry matter, ash, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) according to the method described by Van Soest et al. (1973). Hemicellulose was determined by the difference between NDF and ADF and cellulose by the difference between ADF and ADL. Nitrogen content and crude protein were measured using the Kjehdahl method. To study *in vitro* degradability in rumen fluid, the *in vitro* gas production was measured by incubation of material in rumen fluid using the method described by Cone et al. (1996)..

Results and discussion

Chemical analysis on the substrate at day 49 showed that all fungi induced loss in dry matter, NDF, ADF, ADL, hemicellulose and cellulose content. Nitrogen content and consequently crude protein were increased after fungal treatment. Regarding the ratio between lignin and cellulose loss, *C. subvermispora*, *L. edodes* and *P. eryngii* were most selective for lignin degradation and left most cellulose unaffected. Material treated with these fungi also showed highest increase in *in vitro* gas production compared to non-treated material. Most efficient specific lignin degraders were found to be *C. subvermispora*, *L. edodes*, *P. eryngii*, followed by *G. lucidum* and *P. ostreatus*.

This experiment showed that fungi are capable of specific degradation of lignin and thereby increasing the *in vitro* degradability as indicated by the *in vitro* gas production assay. In literature there is already some work done on fungal degradation of lignin. However only limited combinations of fungi and substrates were studied and a well-organized study of fungal biodegradation to improve low value feeds is missing.

Based on the experiment described by Tuyen et al. (2012) the best suitable fungal species can be chosen for future research. Besides fungal species also different substrates should be considered to be investigated to develop a more general lignin degrading technique and to study in detail the changes during incubation. In a follow up experiment done by Tuyen et al. (in preparation) combinations of four different fungi (*C. subvermispora*, *L. edodes*, *P. eryngii*, *P. ostreatus*) with four different substrates (maize stover, rice straw, oil palm fronds and sugarcane bagasse) were studied. Although the rate of lignin degradation by the fungi species was different per substrate, all fungi were found to be able to improve *in vitro* degradability in rumen fluid of all substrates. The focus in this study was on chemical changes and changes in *in vitro* degradability in rumen fluid. However for the development of a fungal pre-treatment technique not only chemical but also biological changes in both substrate and fungus should be monitored to get control on the process. Chemical changes during fungal pre-treatment can be measured by methods like described by van Soest et al. (1973), which can be complemented with newer methods to detect changes in lignin content and cellulose availability. Monitoring the fungi during incubation can be done by biomass measurements, like ergosterol and chitin determinations. For visualization different microscopy techniques can be used. To obtain a full understanding of fungal delignification mechanisms of lignin degradation enzymatic assays can be used. Both enzymes produced by fungi but also the availability of compounds in the cell walls for enzymes should be taken into account. The developed method should be made suitable for large scale usage.

References

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