DEVELOPMENT OF SUSTAINABLE RE-TANNING AGENTS FROM FUNGAL DEGRADATION OF LIGNOSULFONATES

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Abstract. Lignosulfonates are abundantly available by-products of the paper industry. In the vast majority of applications on leather lignosulfonates are physically blended with other chemistries to augment filling properties of the resulting products. We targeted to decrease the molecular weight of lignosulfonates using fungi to achieve increased application possibilities and to improve tanning properties. We screened various basidiomycotes for their capability to modify the molecular weight of calcium lignosulfonates and identified five species that actually polymerized the chosen lignosulfonate further. Only Irpex consors was found to depolymerize calcium lignosulfonate in surface and later in liquid cultures in our hands. We achieved a six fold reduction of the molecular weight determined by size exclusion chromatography.

1 Introduction

Lignin is the second most available bio polymer in the world only exceeded in volume by cellulose. The role that this biopolymer plays in wood is to incorporate strength into the structure. In paper factories lignin is separated from water insoluble cellulose by chemical modification producing water soluble forms of lignin. A common reaction is the sulfonation of lignin with the salt of sulfurous acids to result in lignosulfonates. These by-products are dominantly burned in the paper factories to supply heat for the energy consuming process of converting cellulose into paper. Some quantities of lignosulfonates are marketed in the construction industry, in animal nutrition, as dispersers, and as fillers, also in the leather industry. There are only few chemical modifications of lignosulfonates known. Recently, Y. Ma and P. C. Berends published the incorporation of lignin into poly-condensates using phenol sulfonic acids and several aldehydes, among them glyoxal to form water soluble tanning agents (1). Biological modification of lignin or lignosulfonates are also rare. In 2005 O. Suprano, A. Covington, and C. Evans published the degradation of lignin with the enzyme heme into monomers and condensed these with formaldehyde to obtain tanning agents in lab scale quantities (2). We targeted to decrease the molecular weight of lignosulfonates using fungi to achieve increased application possibilities. As can be seen in figure 1, the structure of lignin is a rigid one due to extensive crosslinking. Most of the phenolic OH groups required for tanning are etherified within the crosslinked structure. We targeted to reduce the molecular weight of lignin to improve penetration into the interior parts of the leather on the one hand and aimed to release more phenolic OH groups to be available for interaction with collagen and improve tanning properties. While the biological details of the degradation have been published before (3), the results for the application on leathers will be presented here.

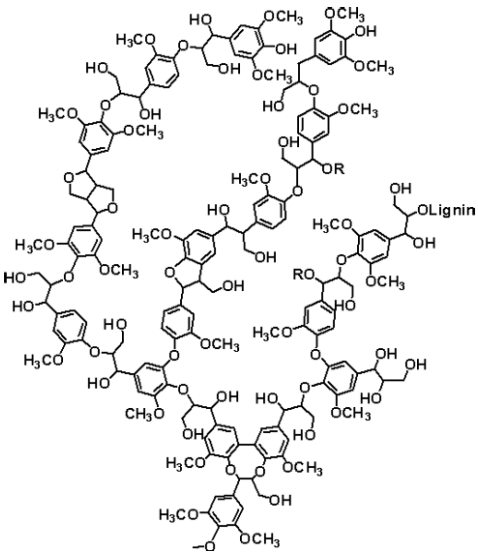

Fig. 1. Structure of lignin.

2 Materials and Methods

2.1 Screening of Fungi for the Degradation of Lignosulfonates

The biological part of the project was carried out in collaboration with the workgroup of H. Zorn at the institute of nutrition chemistry of the university of Giessen in Germany. There, various basidiomycotes were screened for their capability to modify the molecular weight of calcium lignosulfonates in two assays. The basidiomycetes used in this work were supplied from the Centraalbureau voor Schimmelcultures (CBS, Baarn, Netherlands), the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany), and the Friedrich Schiller University (FSU, Jena, Germany). Stock cultures of the fungi were maintained on standard nutrition solution agar plates containing agar, d-(+)-glucose, l-(-)-asparagine, yeast extract, and trace element solution. The fungi were grown in an incubator at 24°C under light exclusion. These stock culture could be stored at 4 °C. A piece of one square centimeter of the mycelium was transferred to an M200 plate for surface culture screening. For this first screening assay seventeen white-rot basidiomycetes were chosen assuring a wide spread of biodiversity. The calcium lignosulfonate served as the sole carbon and nitrogen source for the fungi to grow and was diluted to afford a 5% concentrated medium on agar plates in surface culture. During a cultivation period of four weeks thirteen fungi grew on M200 agar plates. Twelve fungi darkened the plates during growing, while only Irpex consors bleached the plates. In a subsequent second screening assay six fungi that had grown well in the surface culture assay were chosen and transferred to liquid cultivation including Irpex consors. Here a concentration of 0,5% of the lignosulfonate was applied and again only Irpex consors bleached the culture after a cultivation period of sixteen days. Size exclusion chromatography was employed using sulfonated polystyrene solutions ranging from 1.100 to 69.300 Dalton to determine the molecular weight distribution of the lignosulfonates. Only Irpex consors had decreased the molecular weight significantly while the other five basidiomycetes had increased the molecular weight. In a repeated trial with Irpex consors in a culture period of 56 days the molecular weight was reduced from initial 26.000 Dalton to 1.000 Dalton. Further experiments were focused on Irpex consors.

2.2 Upscaling of the fungal Degradation of Lignosulfonates

The upscaling procedure was a two step sequence in submerged cultures. The culture medium consisted again of agar, d-(+)-glucose, l-(-)-asparagine, yeast extract, and trace element solution and was adjusted to a pH of 6. For the pre-culture a piece of one square centimeter of the mycelium of Irpex consors from the stock culture was cultivated with 100 mL culture medium in a 250 mL Erlenmeyer flask after Ultra Turrax homogenization. The fungal growth proceeded for seven days under light exclusion in an incubation shaker at 24°C with 150 revolutions per minute and a deflection of 25 millimeter.

For the main culture 40 mL of the preculture was treated in 400 mL solution containing culture medium and the calcium lignosulfonate in a 1 L Erlenmeyer flask for 15 days at 24°C in an incubator with 150 revolutions per minute and a deflection of 25 millimeter. As can be seen in figure 2 the molecular weight of the calcium lignosulfonate was reduced from initial 33.000 Dalton to 5.000 Dalton. This corresponds to a six fold reduction in molecular weight. The fact that in the upscaling experiment a starting molecular weight of 33.000 Dalton was observed, while during the screening the starting weight of 26.000 Dalton was measured, might be explained with the change of samples. Nevertheless, the two calcium lignosulfonates samples came from the same paper factory.

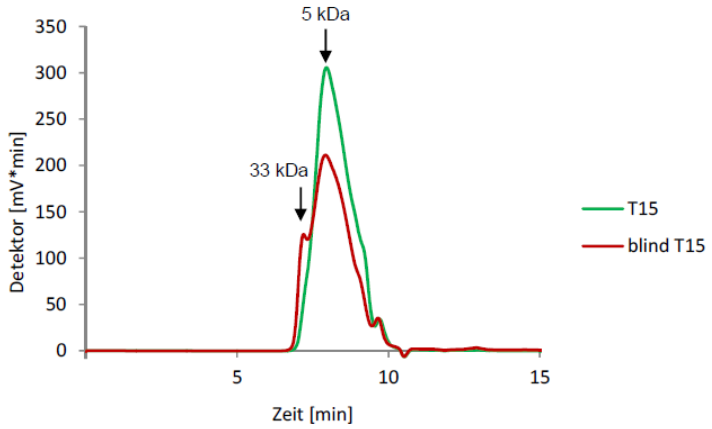

Fig. 2. Gel permeation chromatography of the degradation of calcium lignosulfonate with Irpex consors after 15 days (T15 = day 15)

2.3 Sole Tanning Method

The sole tanning was carried out with pickled bovine pelt with 150% pickle float and 25% tanning agent related to pelt weight at a pH of 3. The tanning agent was dosed in five portions over a period of one hour. After 24 hours the pH was raised with bicarbonate to afford leathers with a pH of 4 (4).

3 Results and Discussion

Vegetable tanning agents and synthetical tanning agents (syntans) on the basis of phenol both have phenolic OH groups that enable them to interact with collagen and expose a tanning effect that can be measured via shrinkage temperature of resulting leathers (4). Dispersing agents on the basis of naphthalene lack these phenolic OH groups and therefore have no tanning effect. In lignin most phenolic OH groups are etherified within the crosslinked rigid structure and are not available for tanning. Consequently, lignosulfonates have a weak tanning effect. Lignosulfonates have a rather large molecular weight of several thousand Daltons. A smaller molecular weight positively effects the penetration of tanning agent (5). Goal of treating lignosulfonates with fungi was twofold. Firstly, to degrade the molecular weight of lignosulfonates and hereby increase penetration performance of the resulting products. Secondly, to cleave some of the etherified phenolic OH group enabling formation of more free phenolic OH groups and enabling for an increased tanning effect. As can be seen in table 1 the degraded calcium lignosulfonate did not show any improved tanning effect compared to the starting material.

Table 1. Results of the sole tanning of bovine pelt with lignosulfonates and vegetable tanning agents

	Degraded Ca-Lignosulfonate (5 kDa)	**Ca-Lignosulfonate (33 kDa)**	**Pelt (no tanning agent)**	**Tara (~ 0,6 kDa)**	**Mimosa (~ 0,3 kDa)**
Shrinkage temperature	61°C	62°C	41°C	68°C	75°C
Leather quality	poor	poor	untanned	soft, round, full	softest, round, full

As a control the shrinkage temperature of pelt was determined to be 41°C. This reflects the stabilisation of collagen without any tanning agent. The two lignosulfonates increased the stabilization of collagen by about 20 degree Celsius, compared to untanned pelt. The leather quality of both pieces tanned with the lignosulfonates was insufficient. The leathers were hard and the tanning was in-homogenously throughout the thickness of the leathers, giving rise to superficial tanning effect on the flesh and grain side but not in the inner layers of the leather. The leather quality was especially insufficient compared to leathers tanned with widely used vegetable tanning agents like tara or mimosa, which were soft, round, full and evenly tanned, with mimosa achieving the softest leather. Major differences between the vegetable tanning agents and the two lignosulfonates are two fold. One difference is the molecular weight. Even the degraded lignosulfonate is still about fifteen times larger than mimosa. Another difference is the number of available OH groups which in lignosulfonates is lower than in vegetable tanning agents.

The degradation of the calcium lignosulfonate with Irpex consors did not change the number of OH groups significantly, as reflected in the basically unchanged shrinkage temperature of the degraded calcium lignosulfonate compared to the starting material. Another outcome of the work was the bleaching effect that Irpex consors exposed on the lignosulfonate. This could be a potential solution for the problem that lignosulfonates are rather dark in color and transfer this problem also to leather, giving rise to a brown shade. This could be overcome via bleaching of the lignosulfonate with Irpex consors and remains to be followed up.

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