sion of cellulose to ethanol has many advantages over the two-step process consisting of a yeast fermentation after the cellulose is hydrolyzed by chemical treatments or by fungal cellulase preparations. Much more work has to be done, including studies with actual waste under technical conditions, before a final judgement can be made. But the direct process seems one of the most promising alternatives for ethanol production from cellulosic waste.

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Degradation of cellulose

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Microorganisms degrading cellulosic materials

One of nature's most important biological processes is the degradation of lignocellulosic materials into carbon dioxide, water and humic substances. Different kinds of microorganisms are involved in the process of degrading woody materials, but it is mainly a task for fungi. Bacteria are considered to have only a limited capability of wood degradation. The strong wood-degrading effect that fungi have has to do, in part, with the organization of their hyphae which gives the organisms a capacity for penetration.

Different types of fungi give rise to different types of wood rot. One normally distinguishes between soft-rot, brown-rot and white-rot fungi[1]. Fungi from the first 2 groups mainly attack the polysaccharides of wood and other lignocellulosic materials while the white-rotters also are capable of a substantial attack on the lignin. The degradation of the different compounds in lignocellulosic materials is catalyzed by enzymes produced by the respective microorganisms. Knowledge of these reactions may be of importance for the conversion of biomass into chemicals and fuels.

The enzyme mechanisms for cellulose degradation by fungi are known in great detail and will be summarized below. The corresponding enzyme mechanisms for lignin degradation are less known and will not be subject to description in this article.

Enzyme mechanisms involved in cellulose degradation

The enzyme mechanisms involved in cellulose degradation have been particularly well studied in 2 fungi,

namely the white-rot fungus *Sporotrichum pulverulentum*[2] and the mold *Trichoderma reesei*[3] (the fungus *T. viride* QM6a and strains derived from it are now referred to as *T. reesei*).

The fungus *S. pulverulentum* produces 3 different types of hydrolytic enzymes, namely a) 5 different endo-1,4-β-glucanases which attack at random the 1,4-β-linkages along the cellulose chain; b) 1 exo-1,4-β-glucanase which splits off cellobiose or glucose units from the non-reducing end of the cellulose; c) 2 1,4-β-glucosidases which hydrolyze cellobiose and water-soluble cellodextrins to glucose and cellobionic acid to glucose and gluconolactone[2].

It has been generally accepted that essentially the same picture is also true for cellulose hydrolyses by *T. reesei*[4]. However, a few differences have been recognized such as the number of the various hydrolytic enzymes, the degree to which the β-glucosidase activity is bound to the fungal cell wall, etc. The action of the exo-glucanase in *S. pulverulentum* differs from the action of the corresponding enzymes in *T. reesei* in that the exo-glucanase from *S. pulverulentum* splits off both glucose and cellobiose, while the exo-glucanases from *T. reesei* only split off cellobiose[3]. However, to degrade crystalline cellulose a synergistic action between endo-1,4-β- and exo-1,4-β-glucanases seems necessary for both fungi. Crystalline cellulose is not attacked by one of these types of enzymes alone. Amorphous cellulose is, however, degraded by both types of enzymes separately[2,3]. In a recent paper by Gritzali and Brown[5] a much simpler enzymic pattern of the fungus *T. reesei* QM9414 has been suggested compared with that previously found[4]. Using a different cultivation technique, only 1 endo-glucanase and 2 exo-glucanases were obtained[5]. Instead of being grown on cellulose, glucose was used as a carbon source. After washing, the glucose grown cells were transferred to a buffer where the hydrolytic cellulose degrading enzymes were induced by the addition of sophorose. Polyacrylamide gel electrophoresis of the concentrated culture solution revealed that the separation pattern of cellulases induced by sophorose is much simpler than the separation pattern of enzymes obtained after several days of cultivation on cellulose. It seems therefore likely that the apparent multiplicity of cellulases in cellulose culture is due to protein modification by proteases. Indeed, Nakayama et al.[6] reported that partial proteolysis of an endo-glucanase from *T. viride* yielded enzymes with a changed substrate specificity and protein structure. In *S. pulverulentum* the 5 endo-glucanases are very similar in molecular weight, amino acid composition, etc. However, they differ somewhat in function[7]. Recent investigations of culture solutions after growth of *S. pulverulentum* on cellulose have demonstrated the existence of 2 different proteases, 1 of carboxy-peptidase and the other of chymotrypsin type. These enzymes seem to influence the release of endo-1,4-β-glucanases from the fungal cell wall and also appear to modify the fungal cell wall[8,9]. Whether or not these enzymes are responsible for the multiplicity of endo-glucanases in *S. pulverulentum* is not known. However, the recent finding of Gritzali and Brown[4] concerning the very simple enzyme picture in *T. reesei* QM 9414 when the cellulases are induced by sophorose in a short term culture points to this possibility. An investigation into the effect of similar cultivation conditions on the endo-glucanase pattern in *S. pulverulentum* will be undertaken.

In *S. pulverulentum* an oxidative enzyme of importance for cellulose degradation has been discovered in addition to the hydrolytic enzymes described above[10]. The enzyme has been purified and characterized and found to be a cellobiose oxidase, which oxidizes cellobiose and higher dextrins to their corresponding onic acids thereby using molecular oxygen. The enzyme is a hemoprotein and also contains a FAD group. It is not yet known whether this enzyme also oxidizes the reducing end group formed when 1,4-β-glucosidic bonds are split through the action of endo-glucanases. It was recently reported by Vaheri[11] that cultures of *T. reesei* grown on cellulose also contained gluconolactone, cellobionolactone and cellobionic acid. These findings indicate that *T. reesei* also produces an oxidative enzyme involved in cellulose degradation. However, further confirmation is necessary in this case.

The fungus *S. pulverulentum* has 3 means of converting cellobiose. The first is hydrolysis by the 1,4-β-glucosidases. The second is through the already described enzyme cellobiose oxidase and the third is through the enzyme cellobiose: quinone oxidoreductase[12,13]. This enzyme is of importance for the degradation of both cellulose and lignin. Although the enzyme seems to be involved in both lignin and cellulose degradation, the highest yields of the enzyme were obtained when cellulose powder was used as a carbon source. In *S. pulverulentum* development of cellobiose: quinone oxidoreductase activity and cellulolytic activity occurred simultaneously. The enzyme is relatively specific for its disaccharide substrate, while the requirements on the quinone structure are less specific and the enzyme is able to reduce both ortho- and para-quinones. A reaction scheme for the enzyme is presented by Eriksson[2] where also a total reaction scheme for cellulose degradation in *S. pulverulentum* is given.

Regulation of endo-1,4-β-glucanases in the white-rot fungus *S. pulverulentum* has recently been investigated using a newly developed sensitive method[14]. The method is based upon the viscosity lowering effect of endo-1,4-β-glucanases on solutions of carboxy-methyl cellulose (CMC). The effect of inducers and repressors can be determined with the method as well as whether

the enzymes are localized on cell wall surfaces or actively released into the surrounding medium. The results show that cellobiose causes induction of endo-1,4-β-glucanases at concentrations as low as 1 mg/l. It was also shown that glucose causes catabolite repression of enzyme formation at concentrations as low as 50 mg/l. Mixtures of inducer and repressor give rise to a delayed enzyme production compared with solutions of inducer only.

Studies of the mold *T. reesei* QM6a using the same technique show that cellobiose under our conditions was not an efficient inducer. However, sophorose causes induction of endo-1,4-β-glucanases at a concentration of 1 mg/l. The comparison between the regulation of endo-1,4-β-glucanase production in the 2 fungi also demonstrates several other important differences. Thus, a solution of CMC alone induces enzyme formation in *S. pulverulentum* but not in the *T. reesei* strain. Under our experimental conditions no endo-1,4-β-glucanases were actively excreted into the solution by *T. reesei.* The enzymes were bound to the cell wall. However, *S. pulverulentum* released the enzymes into the medium although they first appeared bound to the cell wall. It was recently shown by Gritzali and Brown[5] that sophorose gives rise to active excretion of endo-1,4-β-glucanases into the culture solution of *T. reesei* QM 9414. The differences in the results of the two studies must be due either to the differences in the fungal strains or in cultivation conditions.

Production of fuels and chemicals from cellulosic materials

The increasing pressure upon the fossil fuel resources has given rise to a world-wide interest in the production of liquid fuels and chemicals from renewable resources. An area which at present attracts very special attention is the production of ethanol by fermentation of sugar from lignocellulosic materials. Cellulose can be hydrolyzed to soluble sugars by acids or enzymes. Drawbacks of the acid processes include low yields due to degradation of the products, production of inhibitors affecting the ethanol formation by the yeast, corrosion of the equipment and high capital costs. It therefore seems as if, in the future, an enzymic hydrolysis process would be preferred. If this will be the case, it is an absolute requirement that the lignocellulosic material first be delignified. Different methods exists to do so and one example is the so called IOTECH process[15].

A prerequisite for enzymatic saccharification of cellulosic materials is that cellulose and hemicellulose degrading enzymes be cheaply produced. Production can be achieved by cultivation of fungi as well as of bacteria. Enzyme productivity can be enhanced by several means. Primarily, it can be brought about by selection. Genetic manipulation can give rise to strains of microorganisms hyperproducing with respect to one or several of the 3 different hydrolytic enzymes involved in cellulose degradation. It also seems reasonable to assume that DNA-hybridization techniques will be used in the future for large-scale production of these saccharification enzymes.

The development that hitherto has taken place in the area 'hyperproduction of cellulases' has been primarily in the United States[16] and also in Finland[17]. Mutations to create hyper-producing strains have been carried out mainly with the fungus *T. reesei.* DNA hybridization techniques for hyper-production of cellulases have been instituted at the Massachusetts Institute of Technology (MIT) in the United States and in the Biotechnical Laboratory at the Technical Research Center of Finland in Helsinki.

In addition to the above described enzymatic degradation of cellulose for conversion of the produced glucose to ethanol, another development is taking place. At MIT, cellulosic materials are directly, in 1 fermentation step, converted to ethanol. For this purpose, the thermophilic bacterium *Clostridium thermocellum* is used. *C. thermocellum* grows at 60 °C and degrades cellulose to glucose which, by the same organism is converted to ethanol. Another interesting bacterium, also studied at MIT, is *Clostridium thermosaccharolyticum.* As is clear from the name, also this bacterium is thermophilic but instead of using cellulose as substrate, it can convert xylan to ethanol. The ethanol concentrations obtained with these 2 bacterial strains are around 5%. Still higher concentrations are, however, expected to be reached[18].

Another interesting project concerning ethanol production from lignocellulosic materials is taking place at the University of Georgia in Athens, Georgia. Also here the work is carried out with 2 different *Clostridium* strains, namely *C. thermohydrosulphuricum* and another bacterium which is not yet named. This bacterium has been isolated from hot wells in Yellowstone National Park in USA. Both of these bacteria are extremely thermophilic and function up to temperatures of 78 °C, i.e. in the immediate vicinity of the boiling point of ethanol (78.6 °C).

C. thermohydrosulphuricum can ferment glucose, mannose, galactose, ribose, raffinose, xylose, cellobiose, sucrose, starch and pectin. However, cellulose or xylan cannot be utilized by this strain. The products formed are ethanol, lactic acid and acetic acid. The yields of ethanol are as high as 1.5 moles ethanol/mole sugar if the fermentation is carried out at 72 °C. The thermophilic bacterium which has been isolated from hot wells in Yellowstone differs from *C. thermohydrosulphuricum* by not forming spores and by producing practically the theoretical amount of ethanol, i.e. almost 2 moles/mole fermented glucose. Lactic acid, acetic acid and hydrogen are produced as by-products. By using a mixed culture of, for instance,

the bacterium *C. thermocellum* and one of the thermophilic ethanol producing bacteria, ethanol can be produced directly from cellulose. Mixed cultures have given rise to 1.4 moles ethanol/mole glucose[18].

Other biotechnical processes based on cellulosic materials

The utilization of cellulosic materials in biotechnical processes naturally depends upon the development of economically feasible processes. 2 fermentation processes for the production of fodder protein based on cellulosic waste materials from forest industries are already in use and may serve as examples. The first process is founded on the yeast *Candida utilis*, and the other on the fungus *Paecilomyces varioti*. Both of these processes have in common that the substrate is mainly monosaccharides in spent sulfite liquor. Disaccharides and higher oligosaccharides are utilized only to a very limited extent. However, on the basis of the knowledge gained in the studies of the enzymatic degradation of cellulose a fermentation process based on the white-rot fungus *S. pulverulentum*[19] has been developed. The process allows fermentation of solid as well as dissolved lignocellulosic waste and gives, as products, purified water and protein. It seems possible to use the developed technique for the total closure of the white-water system in a newsprint paper mill or in a fiberboard mill. The process has recently been studied in pilot plant scale. The results show no build-up of organic matter taking place and, thus, that all dissolved substances of lignocellulosic origin dissolved from wood in mechanical grinding can be utilized by the white-rot fungus. The fungal mycelium produced in the process can be used either as animal feed[20] or be added to the paper. Both possibilites have been tested and found feasible.

With the present escalation of oil prices it can be foreseen that renewable resources, mainly cellulosic materials, will substitute petroleum as raw material although the extent to which, cannot be surveyed at present. Knowledge of the metabolic pathways of microbial cellulose degradation will, with certainty, play an important role in this evolution.

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Biodegradation of lignin: Biochemistry and potential applications

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Introduction

An estimated 65% of our biomass is produced on land. Of that biomass, lignin is the most abundant natural polymer next to cellulose and is an important renewable source of aromatic carbon on earth. Since lignin and cellulose, together with the hemicelluloses, are the structural components of the vascular tissues of higher land plants, biodegradation of the vascular tissues is the key process in the recycling of terrestrial biosynthetic carbon.

However, lignin, which is a heterogeneous aromatic polymer containing various biologically stable carbon-to-carbon and ether linkages, is interspersed with hemicelluloses surrounding cellulose microfibrils, resulting in an organic composite material protected from the degradative enzymes of microorganisms. Therefore, elucidation of the lignin biodegradation process is essential for understanding the circumstances of the recycling of carbon on earth, for establishing technology for bioconversion of plant