APPLICATION OF FUNGI AND FUNGAL PRODUCTS IN BIOPULPING
PART I: BIOPULPING OF WOOD

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Biopulping is a solid-substrate fermentation (SSF) process where lignocellulosic materials are treated with fungi prior to pulping to improve pulping. Research has focussed on the utilization of lignin degrading fungi for biopulping, but the only commercial process currently available, utilizes *Ophiostoma piliferum*, a sap staining and not a lignin-degrading fungus. Filamentous fungi are well adapted for biopulping because of their ability to penetrate and transfer enzymes into the woody substrate, but because biopulping is a SSF process, it requires control of temperature, moisture and aeration. Biopulping has been evaluated for improvement of mechanical and chemical pulping as well as for depitching and the control of degradation of the wood. The diversity of these pulping processes requires that different SSF processes should be developed to achieve unique benefits. The aim of these reviews, is to consider the work done on different pulping methods and to elucidate the benefits and economic implications of biopulping.

INTRODUCTION

Biopulping has been defined as the treatment of lignocellulosic materials with lignin-degrading fungi prior to pulping[1]. This definition stresses the use of lignin degrading fungi. However, it has been demonstrated that a biopulping effect can also be achieved with *Ophiostoma piliferum*, a fungus that does not degrade lignin[2,3]. Improvement of biopulping by *O. piliferum* has been achieved through the reduction of wood extractives to improve penetration of pulping chemicals. Wood chips have also been treated successfully with fungal enzymes to improve the penetration of pulping liquor[4,5]. The process that utilizes lignin degrading fungi as well as the process based on *O. piliferum* have been developed to a stage where they can be applied on a mill scale[6,7]. These procedures are aimed at the treatment of wood chips in a solid-substrate fermentation (SSF) process. Filamentous fungi are ideally suited for biopulping because of their ability to penetrate and transfer enzymes into the woody substrate[8,9].

Despite the unique ability of fungi to successfully modify wood and to improve the pulping process, biopulping is hampered by several obstacles that require engineering and management solutions[1,10,11]. One of the most important problems is the control of competing microorganisms. Fungi such as *Trichoderma* spp. and *Aspergillus* spp. are important in this respect[8,12], but pre-sterilization of wood chips is regarded as uneconomical[3]. Freshly cut wood also contains inhibitory compounds such as monoterpenes, to which white-rot fungi are especially susceptible[12,13,14]. A certain measure of asepsis of wood chips as well as reduction of the inhibitory compounds in wood can be achieved with a brief steam treatment[11,15]. The SSF process occurs outdoors on chip piles during the normal storage, but the fungi that are recommended for utilization, require a relatively controlled environment for growth. Special measures to control temperature, moisture and aeration must, therefore, be taken[1,11]. The engineering problems that have to be considered in the development of SFF processes have been discussed in detail elsewhere[10] and they will not be repeated here.

The special requirements of SSF apply to all of the biopulping methods. However, the specific processes of biomechanical pulping, biochemical pulping and pitch control all have unique requirements to obtain different benefits. Different types of fungi are used to achieve the specific aims with each method[3,6,11,16,17,18,19] and process parameters need to be changed accordingly.

BIOMECHANICAL PULPING

Mechanical processes are responsible for 25 % of the worldwide production of pulp[1]. Mechanical pulps are characterized by their high yield, that is obtained at the cost of high energy inputs[20]. These pulps also have a reduced strength compared to chemical pulp. The aim of biomechanical pulping is, therefore, to reduce the energy consumption during pulping and to improve pulp strength[6]. Development of such a biopulping procedure has mainly been focussed on thermomechanical pulping where wood chips (which are easier to treat than roundwood) are used. The benefit of biomechanical pulp is that it has similar properties to that of chemithermomechanical pulp[21]. It could, therefore, compete with this type of pulp for a share of the world market.

The biopulping consortia of industry and universities at the Forest Products Laboratory of the USDA Forest Service developed a method of biomechanical pulping over a period of eight years [15]. *Ceriporiopsis subvermispora* was identified as the most efficient fungus for biopulping of soft and hardwood. A United States patent was issued for this method[22]. Application of *C. subvermispora* on *Pinus taeda* resulted in a 42 % saving in energy, 32 kN/g improvement of burst index and 67 mN m²/g improvement of tear index[15]. A reduction of pulp brightness was experienced during initial trials, but this problem was solved by bleaching with alkaline hydrogen peroxide or sodium hydrosulphite. Brightness stability was lower than refiner mechanical pulp, but higher than chemithermomechanical pulp.

Several parameters for efficient SSF with *C. subvermispora* were also optimized[15]. Mycelial suspensions of the fungus as well as pre-colonized chips were effective biopulping inocula and aeration was required, but at a low flow rate. One obstacle was the inability of *C. subvermispora* to colonize unsterilized wood chips[11], notwithstanding earlier statements that this fungus could be applied to unsterilized chips[23]. *Phanerochaete chrysosporium*, on the other hand, was able to colonize unsterilized chips at its optimal growth temperature of 39 °C[15].

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Phanerochaete chrysosporium was, however, less efficient than *C. subvermispora* to improve pulping. Steaming wood chips at atmospheric pressure resulted in a sufficient degree of asepsis, to allow *C. subvermispora* to colonize chips. Economic evaluation of a biomechanical pulping process showed that treatment of chips in a packed bed reactor yielded a return on investment of 21 % before tax. However, when a chip pile based system was used, the return on investment was at least 106 %. These calculations were based on an industrial chip pile that had been modified to regulate moisture and temperature. The packed bed bioreactor consisted of a bed of chips that had been designed to control process conditions, but required larger capital investment[15].

A major variable cost factor in the economic evaluation of biopulping is the cost of inoculum[23,24]. Initial studies showed that a very high inoculum dosage of 3 kg/ton wood (dry weight) was required for efficient biopulping. It was then discovered that corn steep liquor (CSL) could be added as an inexpensive nutrient source to improve colonization[25]. By adding CSL, the inoculum requirement was reduced to 0,25 g/ton wood[15].

Analysis of the waste water from the first pass of treated aspen chips through the refiner indicated that biopulping had reduced the environmental impact[15]. The toxicity was substantially reduced but chemical oxygen demand (COD) values were higher. The increase in COD values was ascribed to the release of products resulting from lignin degradation by the fungi.

In more recent developments, *Phlebiopsis gigantea* has been identified as a fungus that can potentially be applied for biopulping[18,26]. This fungus is able to grow on a variety of hard and softwoods and, because it is a primary colonizer of fresh wood, it can compete effectively with contaminating microbes. The fungus can grow well at temperatures as high as 37 °C. *Phlebiopsis gigantea* offers protection against blue stain and is also able to reduce the extractives content of wood by up to 69%. Studies have shown that this fungus can be applied to logs directly after felling, thereby allowing biopulping to start in the forest[26].

Treatment of *Pinus taeda* and *P. resinosa* wood with *Phlebiopsis gigantea* resulted in reduced energy consumption during refining (up to 27 % on *P. resinosa*). Burst strength (17 %), tear strength (20 %) and tensile strength (13 %) were improved, but pulp brightness was reduced[26]. This technique to treat logs instead of chips could lead to significant savings, because chip sterilization and chip pile aeration is *not* required.

BIOCHEMICAL PULPING

The application of biopulping in chemical pulping has not been researched to the same extent as biomechanical pulping[21]. However, the effect of fungal treatment on wood has been investigated for the two most important chemical pulping methods namely kraft and sulphite pulping[20] as well as organosolv pulping[27].

Biosulphite pulping with white-rot fungi

The use of sulphite pulping has declined in recent years. It is a process that takes place in an acidic pulping liquor that contains a high percentage of free sulphur dioxide[28]. The sulphite base may be calcium, sodium, magnesium or ammonia. Two research groups, one based in Austria[29] and the other in the U.S.A.[30], have focussed on the biological treatment of wood prior to sulphite pulping. Scott *et al.*[30] have evaluated the effect of fungal treatment on *P. taeda* chips for different sulphite processes. Chips were treated for two weeks with two strains of *C. subvermispora* and pulped in a semi alkaline sodium based sulphite as well as an acidic calcium sulphite process. The first process resulted in pulp with a kappa number that was reduced by 27 % compared to the untreated control or, alternatively, a 30 min shorter pulping time to reach the same kappa number. A significant reduction of yield (3,5 %) was also observed. Similar results were obtained with both fungal strains, but strain CZ-3 resulted in the greatest improvement when the calcium based method was used. The kappa number of calcium sulphite pulp was reduced by 49 %, while the yield remained similar to untreated wood. An alternative benefit was pulping time that was reduced by 30min. The improvement by the fungal pretreatment was ascribed to degradation of lignin or its modification for easier removal. In these trials, no change in chemical consumption was observed. However, the authors commented that *C. subvermispora* was selected for application in biomechanical processes and that it might not be the most suitable organism for biosulphite pulping[30].

The potential of fungal pretreatment of wood chips for magnesium based sulphite pulping has been demonstrated in collaboration with Leykam-Mürztaler (now Sappi Gratkorn)[29]. It was found that several fungi were able to reduce the kappa number of birch pulp and also increase brightness of pulp, but with a loss of strength. It was also observed that the beneficial effect for biochemical pulping was obtained by a different mechanism from biomechanical pulping. The effect of fungal treatment on mechanical pulping was ascribed to the reduction of the binding capacity of fibres. In chemical pulping, the beneficial effect is caused by an increase in lignin solubility[29].

Biokraft pulping with white-rot fungi

Kraft pulping is an alkaline process with cooking liquor that contains sodium hydroxide and sodium sulphide[28]. Kraft pulping accounts for 80 % of the world chemical pulp production[20]. Biokraft pulping has, however, been restricted to studies using blue-stain fungi[2], studies utilizing white-rot fungi on hardwood[31,32,33], and one study on softwood[12]. Valuable information does, however, exist on the kraft pulping properties of softwood that has been decayed by white-rot fungi under natural conditions[34,35]. These studies focussed on the effect of wood from decadent stands on kraft pulping parameters. The degradation of wood occurred under uncontrolled conditions and results can, therefore, only be applied to biopulping to a limited extent. The most obvious benefit of fungal pre-treatment, is the reduction of lignin content[32,33] or alternatively reduction of the pulping time[30,33]. These improvements also seem to be associated with reduction in pulp yield[32,34] and increased chemical consumption[35,36].

In one study, aspen chips were compressed into bales after application of *Phanerochaete chrysosporium* inoculum and covered with foil[31]. Sufficient fungal growth was obtained with this method without the addition of nutrients, sterilization of chips or stringent control of incubation conditions. However, the cost of strapping and foil wrapping was not specified. Some loss of wood mass occurred during incubation, but changes in pulp yield was not determined. The strength properties of pulp from treated wood were improved and freeness as well as brightness was reduced[31].

Our own studies on the kraft pulping of softwood treated with *Stereum hirsutum* showed a substantial reduction of the lignin content of pulp[12]. Pine chips were treated for three weeks and pulping conditions varied to determine the optimal pulping conditions for fungal treated wood. Under optimal conditions, a 30 % reduction in kappa number was observed. Pulping time could also be reduced to obtain pulp with the same kappa number as the control which could be translated to increased pulp production. However, due to non-selective delignification that occurred, the pulp yield was reduced. Most of this reduction in yield occurred as loss of wood mass before pulping, but some of the reduction also occurred during pulping. The degree of polymerization was not negatively influenced by fungal action, but was found to be a factor of kappa number. The most important disadvantage of this process was an increased alkali consumption[12]. It is, however, possible to reduce the use of chemicals during the bleaching stages when pulp with a lower kappa number is used[37].

Organosolv pulping

One of the more recent developments to reduce the environmental impact of the pulping industry has been the application of organosolv pulping. Organic solvents such as ethanol are utilized to eliminate toxic sulphur-containing wastes[27]. Fungal treatment of small samples (15 g) of aspen wood can be combined with aqueous-ethanol pulping to reduce kappa number (Table 1). An increase in yield was obtained with this method, because an apparent increase in cellulose yield occurred during pulping. However, wood mass was reduced by all three of the fungal strains used and when yield was calculated on a mass balance basis, cellulose yield was reduced (Table 1). Selective delignification was, nonetheless, still improved during biopulping and yield increased by two percentage points when wood treated with *Trametes villosus* was pulped to the same kappa number as untreated wood[27]. This biopulping process has, to our knowledge, not been scaled up.

BIOPULPING WITH CARTAPIP®

Ophiostoma piliferum is a primary colonizer of softwood and is also involved in sap staining[38]. A melanin deficient strain of this fungus[39] has been sold commercially since 1990[40] and is currently the only fungal product that is available for commercial biopulping[7]. The inoculum consists of lyophilized mycelium and conidia and is sold under the trade name Cartapip®97. Cartapip® is suspended in fresh water and sprayed onto wood chips before stacking[41]. The fungus is able to colonize freshly cut wood and utilize sugars and extractives in the wood, but is unable to degrade cellulose or lignin[41]. The fungus colonizes wood via ray parenchyma cells and resin canals and disrupts pit membranes[38]. It is, therefore, applied to control staining and decay of wood chips, reduce pitch in mechanical pulp and for biochemical pulping[7]. An incubation time of seven to fourteen days is required to achieve any benefit.

Biomechanical pulping

Ophiostoma piliferum (Cartapip®) was the first fungus to be applied commercially in a biopulping process[40]. Cartapip® was develop in collaboration with Bear Island Paper Company (BIPCo), a thermomechanical pulp mill[42]. The mill has applied Cartapip® since 1991 and obtains the same benefits that were previously obtained by aging of chips[42]. However, with Cartapip® no loss of brightness due to chip staining occurred[7].

At pulp mills, the storage of wood chips is preferred to the storage of logs, because chips are more economical to handle[43]. However, during prolonged storage, contaminating fungi cause a darkening of chips that leads to decreased brightness of thermomechanical pulp[40]. One of the characteristics of *O. piliferum* is, that it is a primary colonizer that can compete strongly with other fungi on freshly chipped wood[40]. The contaminating fungi include sap-staining as well as decay fungi[44]. By reducing staining and decay, application of Cartapip® can reduce the bleaching requirement of pulp and improve pulp yield. The fungus grows in tracheids, ray parenchyma cells and resin ducts while metabolizing extractives. The disruption of parenchyma cells weakens the binding of tracheids, thereby allowing easier separation of wood fibres[38]. The benefit of loosened fibres is that energy is saved during mechanical pulping.

At BIPCo, approximately 1200 tons of freshly cut, unsterilized southern yellow pine (50 % *P. taeda* and 50 % *P. virginiana*) chips are treated per day with Cartapip® at a screw conveyor[42]. Incubation occurs on an unmodified chip pile for 14 days[7], but the chip pile is managed by turning it, to prevent overheating. Biopulping resulted in improved brightness (0,9 %), tensile strength (5,4 %), tear strength (3,4 %)[42] and burst strength (3,3 %)[7]. Pilot trials with *P. taeda* have shown that it is also possible to reduce the energy requirement for mechanical pulping with application of Cartapip®[45]. For the same energy input, pulp with a greater tensile strength was obtained. Fibre length increased and fines were reduced in lab scale trials during the same study.

Biopulping fungus		Wood mass (g)	Cellulose yield (g)*	Kappa no.
Phanerochaete chrysosporium	0 days	15,0	7,9	19,8
	15 days	13,7	7,0	12,9
	Change (%)	-8,5	-11,8	-34,8
Phanerochaete sanguinea	0 days	15,0	7,9	19,3
	15 days	14,7	7,7	14,9
	Change (%)	-1,7	-1,9	-22,8
Trametes villosus	0 days	15,0	7,9	19,7
	15 days	14,3	7,6	15,1
	Change (%)	-4,8	-4,6	-23,4

*Based on the mass balance that includes loss in wood mass.

Table 1. Effect of different fungal treatments on the wood mass, cellulose yield and kappa number of treated aspen chips (adapted from [27]).

Depitching

A large variety of compounds, found in wood, are soluble in neutral organic solvents or water. These compounds are collectively called extractives or pitch[26]. Wood extractives are important causes of production and quality problems in pulp as well as paper mills[46]. Pulp produced from wood with high pitch content has reduced strength[40] and optical properties[47]. The presence of extractives in pulp could also lead to breakage of sheets on paper machines[40].

One of the methods available to control pitch, is the application of Cartapip® to stored wood chips before pulping[40]. The fungus reduces the amount of pitch in the wood by metabolizing it[38]. Treatment of southern yellow pine chips at BIPCo resulted in reduction of triglycerides and resin acids[7]. The diminished extractives content (-37,5 %) reduced the requirement for alum (-31,7 %) to control pitch while the control of chip discolouration resulted in a reduction in the use of bleaching chemicals (-36,9 %)[42]. Application of Cartapip® reduces the time that chips have to be seasoned to reduce extractives[2]. The wood inventory can, therefore, also be reduced to save money. The effect of Cartapip® on the extractives content of wood from other soft- and hardwood species has also been determined (Table 2). The extractives content of wood from all these species was reduced by at least 11 % compared to fresh chips. This improvement was not as high when compared to aged chips, but the improvement was achieved in a shorter time (Table 2).

Biochemical pulping

The presence of wood extractives impairs the penetration of pulping chemicals into chips, thereby increasing chemical consumption and pulping time[48]. Cartapip® can increase the porosity of wood, which allows faster penetration of pulping chemicals, by the consumption of pitch and opening of pit membranes[2]. Smaller amounts of chemicals and shorter pulping times are, therefore, required.

In one study, 10 tons of white fir (*Abies concolor*) chips were treated with Cartapip® and pulped by means of a sulphite process[2]. The K-number of pulp was reduced by 3,2 %. Pulp yield and viscosity increased by 4,2 % and 32 % respectively. These results illustrated that extractives were more important in sulphite pulping, because fatty acids are not saponified during pulping as in kraft pulping[2].

Mill-scale trials for biosulphite pulping of aspen wood have shown that K-number of unbleached pulp was reduced by 7 % while the amount of rejects was reduced by 12 % and the viscosity increased by 19 %[7]. An increase of 1 % in brightness of unbleached pulp has contributed to a 7 % to 10 % decrease in consumption of bleaching chemicals. One of the downstream benefits was the reduction in use of sizing agents that could translate to a saving of US$ 6 per ton of pulp[7].

Biokraft pulping trials with Cartapip® on hardwood have only been completed on laboratory scale[2]. Up to 20 % reduction in the active alkali requirement was obtained under these conditions or, alternatively, pulp was produced with a 29 % lower kappa number under the same pulping conditions[7]. Viscosity improved when fungal treated samples were pulped to the same kappa number as control samples[2]. Pulp yield remained unchanged, because *O. piliferum* is unable to degrade cellulose[7].

Small samples (500 g) of fresh softwood chips have been treated with Cartapip® for kraft pulping[2]. Samples that contained 70% *P. banksiana* and 30 % *Picea abies* were treated for two weeks to produce pulp with a reduced (12 %) Kappa number. Less active chlorine (9 %) was required in the D/C stage during bleaching of the pulp in an O-D/C-Eo-H-D bleaching sequence[2]. The pulp also responded better to refining.

Species	Reduction in extractives content (%)	
	Compared to fresh chips	Compared to aged chips
Southern Yellow Pine	40	22
Jack Pine (P. banksiana)	31	27
Radiata pine (P. radiata)	11	0
Red Pine (P. resinosa)	33	23
Hemlock	11	0
Aspen (Populus sp.)	40	20
Maple (Acer sp.)	26	1
Cottonwood	40	14
Birch (Betula sp.)	32	17

Table 2. Effect of treatment with Cartapip® on extractives content on wood from different species (adapted from [7]).

CONCLUSIONS

The potential of biopulping has been evaluated for most pulping methods and raw materials that are used for the production of paper pulp. It is clear that different fungi are suited to each process and that these processes must be adapted to achieve the full potential of an environmentally friendly technology. It has been demonstrated that biopulping offers some flexibility that will suit the individual requirements of mills. Pulping can, for instance, be adapted to produce chemical pulp with lower lignin content or the pulping time can be reduced to increase production[2,12].

Certain negative effects such as increased chemical consumption[12] and reduction in yield have, unfortunately, also been associated with certain biopulping processes. However, these problems could in most cases be ascribed to the utilization of fungi that were not suitable for the specific type of pulping or SSF that was not conducted under optimal conditions. Utilization of fungi that are more selective in the degradation can, for example, improve the pulp yields. It was found that incubation time plays an important role in selective delignification of wood[49] and that with long treatment times degradation became less selective. Treatment time will, therefore, play a significant role in the success of an industrial biopulping process.

The most important factors to be considered in the design of a biopulping process include the choice of organism, degree of asepsis, size and type of inoculum, control of physical conditions such as temperature and aeration, as well as the addition of nutrients[50]. Thus the most important design factors for biopulping are similar to those to be considered for all SSF procedures. The economical viability of biopulping is determined by two contributing factors. The most important capital investment, is that required for the modification of the chip pile to allow SSF[11] and the most important variable cost item, is that of the inoculum[23,24].

Environmental benefits of biopulping have been demonstrated[15], but unfortunately, environmental benefits are often difficult to quantify. Application of biopulping is, therefore, only considered on the basis of economic benefits and the modelling of pulping processes is required for the prediction of the economic benefits.

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