Solving Pitch Problems in Pulp and Paper Processes by the Use of Enzymes or Fungi

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Pitch problems in pulp mills are often caused by the resinous materials (pitch) in wood which comprise approximately 2–8% of total composition depending upon the species and time of year. Traditional methods to control pitch problems include natural seasoning of wood before pulping and/or adsorption and dispersion of the pitch particles with chemicals in the pulping and papermaking processes, accomplished by adding fine talc, dispersants and other kinds of chemicals. Within the last five years, two new and different methods of combatting pitch, both of which are biotechnological in basis, have been developed independently and are now used industrially. Hata and colleagues of Nippon Paper Industries developed a pitch control method using the enzyme lipase, which catalyzes the hydrolysis of triglycerides. Farrell and colleagues of Sandoz Chemicals Biotech (now known as Clariant) developed a method of pitch control and biocontrol using a fungus developed in the laboratory from the same type of organisms which cause natural aging, the Ascomycetes. This fungus is non-colored and prevents the staining and decrease of brightness normally associated with aged wood.

Advances in Biochemical Engineering/
Biotechnology, Vol. 57
Managing Editor: T. Scheper

1 Introduction

In today's pulp and paper industry, pitch trouble is often caused by the resinous materials (pitch) in wood. These are some of the materials ("extractives") that are extracted from wood during the pulping process, and comprise about 2–8% of its total composition, depending upon the species and time of year. Not all of the extractives are troublesome, most problems occurring in pulping and papermaking when there are shifts in pH and/or temperature. During the pulping process, these resinous materials are released from wood and later stick to the tile and metal parts including the rolls and wires of the papermaking machines. The pitch also stains the felts and canvas, and eventually reaches the dryer section. This pitch accumulation can cause paper spotting and web breaks on the machine, which are severe problems in production. Pitch content and severity of problems from pitch vary with wood species. The pitch of pines, including loblolly, slash, and red pines is known to cause serious problems. Hardwood pitch, particularly from tropical hardwood species, and eucalyptus can also be detrimental.

Traditional methods of controlling pitch problems include seasoning of wood before pulping. Seasoning requires raw wood logs (roundwood) to be left outdoors for several months or chips to be piled and left for weeks. It is the most commonly used method around the world because wood extractives are decomposed during the seasoning process. However, accompanying seasoning are potential losses due to biological deterioration, such as decreased pulp brightness and pulp yield. Moreover, seasoning increases working capital costs due to high wood inventory and land use. Thus, this method is often unacceptable, especially in areas where space is limited. Another method used to reduce the accumulation of pitch is the adsorption and dispersion of the pitch particles with chemicals in pulping and papermaking processes. This is accomplished by adding fine talc, dispersants, and other kinds of chemicals.

In Japan, red pine is the most important wood for groundwood pulp. Red pine groundwood pulp has high opacity and printability. Therefore, it is an indispensable pulp for the manufacture of newsprint and light weight paper. However, the red pine groundwood pulp contains a large amount of pitch. Hata et al. conducted fundamental research and determined that pitch trouble was caused by triglycerides within the resinous materials in wood [1]. These triglycerides form a nucleus upon which other resinous materials tend to accumulate, causing pitch troubles. Hata et al. developed a new pitch control method using the enzyme lipase [1–3]. This method was put into practice in a large scale papermaking process as a routine operation in the early 1990s, and was the first case in the world in which enzyme was successfully applied in the actual papermaking process.

In the USA, Farrell and coworkers independently and concurrently were also studying biotechnological solutions to pitch problems [4, 5]. These studies were initiated with the goal of solving pitch problems in loblolly pine by the application of a fungus developed in the laboratory from the same Class of organism which causes natural aging, the Ascomycetes [6]. This organism, *Ophiostoma piliferum*, belongs to the sap-staining type of organisms, but was bred in the laboratory to be colorless and non-staining. Thus, the positive benefits of aging were achieved with increases in pulp brightness. Also, by directly applying the fungus to the wood chips or logs, the effect of aging was accelerated. This product, marketed as Cartapip, was the first case in the world where an organism was commercially successfully applied prior to pulping to achieve beneficial effect [7, 8].

2 Extractive Degradation during "Natural" Storage

Living cells are contained in the bark, foliage, and sapwood when the tree is cut. These cells remain viable for periods of up to six months when the wood is stored. The living cells in the wood rays (ray parenchyma) respire and release heat. Bacteria and fungi are provided with good growth conditions during this heat generation, and the starches and simple sugars of the rays and subsequently by the extractives of the wood can be metabolized as a source of carbon and energy. This metabolism results in an overall decrease of pitch with storage of wood.

The outside storage of pulpwood was introduced in the 1920s as whole logs (roundwood), and in the early 1950s as chips [9]. This method was the direct result of the need to stockpile wood as inventory to mills, to handle intermittent flow of chips to the mill, and to season wood, which resulted in decreased resin deposition. The reduction in pulp brightness and yield during storage was shown to be of the same order whether the wood had been stored as chips or as roundwood [10]. Conditions which affect the wood were shown to be the following: species of wood, time of cutting, removal of bark, presence of insects, methods of piling, length of storage time, general housekeeping conditions in the woodyard, and climatic conditions, especially temperature and moisture. Temperature appeared to be the single most important factor affecting distribution and prevalence of microorganisms in various sections of the chip pile in one study on microbiological effects of seasoning on hardwoods [11]. Outside storage of white spruce (*Picea glauca*) and lodgepole pine (*Pinus contorta*) showed decreased wood substances by 3.8% and 4.5% respectively after 6 months. Most of this decrease was attributed to pitch components [12]. This study also showed that pine kraft pulp yield increased based on seasoned chips, though spruce kraft pulp yield decreased slightly with time of storage.

Seasoning has been recommended for pine unless the recovery of maximum tall oil and turpentine yields was desired [13]. Eighty percent losses in tall oil and turpentine yields resulted after 30 weeks storage. Pulp strength in this and other studies was shown not to be affected by seasoning, with the possible exception of tear strength. Although seasoning chips reduces pitch troubles, the negative effects of seasoning on various wood species were also thoroughly studied in North America and Europe [14, 15]. The single most detrimental effect was loss of brightness of mechanical and sulphite pulps after storage, particularly with softwoods [10].

3 Degradation of Wood Extractives by Fungi

A variety of wood-inhabiting fungi including molds, sap-stain, brown-rots, and white-rots are capable of degrading wood extractives.

3.1 Molds

Although molds are capable of degrading wood extractives, the contribution of molds to extractive degradation is expected to be minimal because molds grow less prolifically in wood than do other wood-inhabiting fungi. Molds grow best on wood that is very wet or that has been exposed to very high humidity for a long time. On softwoods, molds grow mainly on wood surfaces. On hardwoods, molds can enter the wood at exposed parenchyma, vessels, and ruptured cells and can move throughout the wood by rupturing pit membranes [16]. Nilsson and Asserson have shown that the following molds degrade wood waxes in liquid culture: *Penicillium roqueforti*, *Penicillium funicolosum*, *Rhizopus arrhizus*, and *Trichoderma lignorum* [17]. In addition, they showed that the following molds could reduce the ethanol/benzene (1:2) extractive content of wood chips: *R. arrhizus*, *Gliocladium viride*, *Penicillium rubrium*, *T. lignorum*, and *Aspergillus fumigatus*. The wood chips were

Table 1. DCM extractive content of nonsterile southern yellow pine treated with various molds

Fungal Species	Extractives (%) Control[1]	Extractives (%) Treatment	Reduction %
Phlebia roqueforti	3.34	2.16	35
Leptographium terrebrantis	2.27	1.92	15
Verticicladiella truncata	2.27	1.96	14
Diplodia pinea	2.27	2.01	11
Codinaea sp.	2.27	2.07	9
Aureobasidium pullulans	3.34	3.26	2

[1] The control was chips that had been frozen at − 20 °C since the start of the experiment

stored at 35°C and sampled at 30 days. The type of wood chips tested was not given.

Iverson et al. screened various molds for their ability to degrade wood extractives [18]. Nonsterile southern yellow pine chips were inoculated with 10^4 to 10^8 colony-forming units/g wet weight wood and incubated at room temperature for 2 weeks. As shown in Table 1, the best fungus tested was *P. roqueforti*, which reduced the dichloromethane (DCM) extractive content by 35%.

3.2 Basidiomycetes

Basidiomycetes, including white-rot and brown-rot fungi, extensively colonize wood. Brown-rot fungi preferentially degrade wood polysaccharides including cellulose and cause rapid decreases in its degree of polymerization. Brown-rotted wood usually shows virtually no decrease in total lignin content. Rather than degrading lignin, brown-rot fungi modify it by oxidation and demethylation of methoxy groups. White-rot fungi are the predominant degraders of lignin in nature. Some species of white-rot fungi preferentially degrade lignin to wood polysaccharides, and other species degrade all wood components simultaneously. The Basidiomycetes have been shown to degrade pitch extractives.

Several fungi observed to have "biopulping" activity have also been shown to degrade wood extractives. Biopulping involves the use of fungally treated chips to obtain pulping benefits such as reduced energy used during mechanical pulping or improved chemical pulping efficiency. Lim and Cho have shown that the ethanol/benzene (1:2) extractive content of oak treated with *Phanerochaete chrysosporium* decreased by 46% after twelve weeks [19]. Treatment of sterile southern yellow pine wood chips with *P. chrysosporium* for 2 weeks resulted in a 21% reduction in dichloromethane (DCM) extractives [20]. Fischer et al. have shown that the DCM extractive content of sterile loblolly pine chips treated with *C. subvermispora* decreased after four weeks by 32% [21]. In addition, they showed that both *C. subvermispora* and *P. chrysosporium* decrease the DCM extractive content of sterile spruce chips [21].

Various Basidiomycetes were screened for their ability to degrade wood extractives [18]. Sterile southern yellow pine was inoculated with 10^4 to 10^8 colony-forming units/100 g wet weight wood and incubated for 2 weeks at room temperature. As shown in Table 2, *P. chrysosporium* performed best, reducing DCM extractives by 51%. *Hyphodontia setulosa, Perenniporia subacida, P. gigantea*, and *Phlebia tremellosa* also performed well, reducing extractives by about 40%. Poller and Schultze-Dewitz investigated the effect of two brown-rot fungi, *Coniophora puteana* and *Gloeophyllum saepiarium*, and one white-rot fungus, *Phellinus igniarius*, on the extractive content of pine [22]. All three fungal treatments resulted in large reductions in extractive content.

Table 2. Extractive content of sterile southern yellow pine treated with various Basidiomycetes

Fungal species	Control Extractives (%)	Treatment Extractives (%)	Reduction (%)
Phanerochaete chrysosporium	2.19	1.30	41
P. subacida	3.34	2.01	40
P. gigantea	3.34	2.03	39
P. tremellosa	1.98	1.21	39
H. setulosa	1.98	1.20	39
Coriolus versicolor	1.98	1.28	36
Inonotus rheades	3.34	2.18	34
Trichaptum abietinium	4.70	3.13	33
C. subvermispora	3.34	2.18	29
Trichaptum biforme	4.70	3.13	24
Schizophylum commune	2.50	2.03	17
Sistotrerma brinkmanii	2.44	2.17	11
Pleurotus ostreatus	2.44	2.30	6
Alurodiscus sp.	2.44	3.70	0
Ganoderma collosum	2.29	1.75	0
Phellinus igniarius	2.44	2.48	0

3.3 Sap-Stain Fungi

Sap-stain fungi rapidly colonize the sapwood of logs and wood chips. These fungi grow mainly in ray parenchyma cells and are capable of deeply penetrating logs and wood chips. In addition, these fungi can grow within resin canals, tracheids, and fiber cells, and penetrate simple and bordered pits, occasionally forming boreholes through wood cell walls. Sap-stain fungi are not capable of degrading the major components of the wood cell wall: cellulose and lignin. Hemicellulose is degraded to a very slight degree. Extractives and simple sugars found in the parenchymal cells are the major nutrient source for these fungi. Sap-stain fungi cause a characteristic staining of sapwood, resulting in a blue, black, grey, or brown discoloration of the wood. Sap-stain causes major economic losses in the lumber and mechanical pulping industries. Problems with sap-stain are most prevalent in warm, humid climates and when wood with a high sapwood content is used.

Common species of sap-stain on softwoods include: *Ophiostoma ips*, *O. piliferum*, *O. piceae*, *Aureobasidium pullulans*, *Leptographium lundbergii*, *Alternaria alternata*, *Cephaloascus fragrans*, *Cladosporium* spp., *Lasiodiplodia theobromae*, and *Phiolophora* spp. [23]. Common species of hardwood sap-stain include: *Ophiostoma pluriannulatum*, *Ceratocystis moniliformis*, *L. theobromae*, *Ceratocystis rigidum* [23]. Many of these species are capable of degrading wood extractives. Extractive degradation by *Ophiostoma* spp., particularly *O. piliferum* and *O. piceae*, has been most widely studied.

Iverson et al. screened a variety of sap-stain fungi for the ability to degrade wood extractives [18]. Sterile southern yellow pine was inoculated with the fungi listed in Table 3 and incubated at room temperature for 2 weeks. The best

Table 3. Extractive degradation by sap-stain fungi on nonsterile southern yellow pine

Fungal Species	Control Extractives (%)	Treated Extractives (%)	Reduction (%)
C. adiposa	2.13	1.26	41
O. piliferum	3.34	2.27	32
C. adjuncti	1.98	1.44	27
C. minor	2.13	1.57	26
O. piceae	2.13	1.57	26
O. populina	2.13	1.62	24
O. abiocarpa	2.13	1.61	24
C. tremuloaurea	2.13	1.65	23
O. fraxinopennsylvanica	2.13	1.71	20
O. plurianulatum	2.13	1.73	19
E. aereum	1.98	1.61	19
C. ponderosa	2.19	1.86	18
C. penicullata	1.98	1.58	15
O. olivaceum	2.27	1.93	15
E. clavigerum	2.13	1.84	14
C. hunti	2.13	1.89	11
C. ambrosia	2.13	1.92	10
O. distortum	2.27	2.07	9
E. robustum	2.27	2.06	9
C. virescens	2.27	2.11	7
O. galeiformis	2.13	1.98	7
C. coerulescens	2.13	2.13	0
O. dryocetidis	2.24	2.27	0
O. stenorcerns	2.13	2.21	0
X. conudamae	2.44	3.16	0
X. hypoxylon	2.44	2.88	0

species for extractive reduction were *Ceratocystis adiposa*, *O. piceae*, and *O. piliferum*. Nine different isolates of *O. piliferum* were also screened on sterile southern yellow pine using the same procedure; 22% of the isolates did not reduce DCM extractives and 55% of the isolates reduced DCM extractives by 25–35%. In addition, 45 different strains of *O. piceae* were screened on sterile aspen chips. These strains can be divided into four groups based on their ability to reduce ethanol/toluene extractives: 24% of the isolates did not degrade extractives, 46% reduced extractives by 1–15%, 28% reduced extractives by 16–35%, and one isolate reduced extractives by 60%.

Chen et al. studied the effect of five sap-stain fungi on the composition of aspen and lodgepole pine extractives [24]. Four fungi were selected on the basis of high lipolytic activity from 100 fungal strains isolated from Canadian lumber mills and compared with the commercial strain, Cartapip® 97. Analysis of untreated wood showed that triglycerides were the most abundant component of both aspen and lodgepole pine sapwood extractives. The wax and steryl ester content of aspen was about 3 times that of lodgepole pine, and fatty and resin acids were the second most common component of lodgepole pine extractives but were present in very small amounts in aspen. All five fungi decreased the total acetone extractive content of aspen and lodgepole pine sapwood to

Table 4. Extractive content of sterile lodgepole pine and aspen treated with sap-stain fungi

Treatment	Aspen Extractives (%)	Lodgepole pine Extractives (%)
Control	3.09 ± 0.07	2.31 ± 0.03
Aged Control	2.88 ± 0.04	2.26 ± 0.02
Cartapip 97	2.15 ± 0.01	1.94 ± 0.03
Strain A	2.13 ± 0.02	2.08 ± 0.01
Strain B	2.22 ± 0.01	NA
Strain C	2.07 ± 0.04	1.92 ± 0.11
Strain D	2.08 ± 0.05	1.92 ± 0.01

a similar degree – 28–33% for aspen and 10–17% for lodgepole pine (Table 4). Extractive component analysis showed that all five fungi decreased triglyceride content and steryl esters/waxes content, and that four of the five fungi increased free fatty acid content.

3.4 Industrial Use of Fungi to Solve Pitch Problems

Several studies have shown that wood extractive components such as triglycerides, resin acids, and steryl esters are major components of paper machine pitch deposits [1, 2, 6]. In addition, pitch outbreaks are more common when resinous wood species are used and during seasons when wood resin content is particularly high.

There is a living fungus, marketed to the pulp and paper industry, which metabolizes and thus removes pitch. This fungus is a colorless strain of *O. piliferum*, an Ascomycete of the same species of that often dominates in naturally seasoned piles. Marketed as Cartapip, with different numbers denoting different strains such as 97 and 58, it is commercialized as a powder inoculum. Moreover, Cartapip use results in a biocontrol effect, i.e., the presence of Cartapip reduces growth of other, undesired organisms. One kilogram of the powder can treat about 1200 tons of wood chips. Industrial use involves dispersing the powder in mill water and spraying it onto chips as they are conveyed to a chip pile.

Selective breeding was used to obtain this isolate, which rapidly colonizes nonsterile wood chips, rapidly degrades extractives, and is colorless and nonstaining. Most *O. piliferum* strains are a bluish-black color. Growth of pigmented fungi on wood chips reduces chip brightness and increases bleach usage when these chips are used to produce TMP or sulfite pulp. Because Cartapip outcompetes indigenous microorganisms and maintains chip brightness, use of this product reduces bleach chemical usage during TMP production, in addition to reducing the extractive content of chips and pulp, and alleviating pitch problems. Use of treated chips has also been shown to increase paper strength [25]. Moreover, treatment of wood chips with Cartapip also results in improved chemical pulping efficiency [26]. Reductions in kappa number were observed during laboratory-scale kraft and sulfite pulping. Wall et al. hypothesize that the improved pulping efficiency observed experimentally is caused by more rapid and more uniform penetration of steam and cooking chemicals in the fungally treated chips [27].

Two commercially available strains of *O. piliferum*, Cartapip 28 and Cartapip 58, have been shown to degrade the extractives of both hardwoods and softwoods including aspen, southern yellow pine, red pine, and spruce [27]. Both fungi in two weeks reduced the diethyl ether extractive content of fresh nonsterile southern yellow pine wood chips by 40%, or 22% if the chips were aged but not inoculated. In addition, the white strain, Cartapip 58, maintained chip brightness. This study also showed that Cartapip 28 decreased the DCM extractive content of sterile southern yellow pine chips by 30% for a 2 week treatment. *O. piliferum* has also been shown to reduce more DCM extractives of nonsterile red pine in 31 days (48% reduction) than in 21 days (32% reduction) [18]. *O. piliferum*, strain Cartapip 97, also reduces the DCM extractives of spruce by 25% in a 2 week treatment, and the DCM extractive content of sterile loblolly pine chips by up to 35% in a 4 week treatment [21]. Cartapip 97 reduced the acetone extractive content of fresh nonsterile aspen chips by 36% after a 3 week incubation, or 13% for uninoculated aged chips [27].

Cartapip 28 and Cartapip 58 significantly decreased the fatty acid content and the unidentified compound content, which includes waxes, alcohols, and sterols, of southern yellow pine extractives [6]. In particular, Cartapip 58 decreased esterified fatty acids by 60%. Both esterified fatty acids and nonsaponifiable compounds such as waxes and steryl esters have been shown to be major components of industrial pitch deposits [6]. Both fungal treatment and natural microbial activity increased the free fatty acid content of the extractives. The increase in free fatty acid content results from initial hydrolysis of esterified fatty acids to free fatty acids. The free fatty acid content of the Cartapip-treated chips is lower than that of naturally aged chips, indicating further metabolism and removal of these components by the fungus. In addition, analysis of the individual fatty acids showed that fungal treatment significantly decreased the content of the three fatty acids found in highest concentration in the untreated southern yellow pine chips – oleic acid by 44%, linoleic acid by 64%, and palmitic acid by 45%.

The results of Cartapip treatment such as pitch removal and maintence of chip brightness and improved paper machine runnability have been documented by use in mills. In a TMP mill using southern yellow pine, a trial was performed comparing a two week period using the Cartapip product on their wood chips to a two week period without product use, and the results are shown in Table 5 [24]. Reductions in the DCM extractive content of secondary refiner pulp caused expected reductions in alum, a pitch control chemical. Because Cartapip 97 is a colorless strain that outcompetes indigenous microorganisms, its use results in brighter chips. This effect was observed as a 36.9% reduction in bleach usage along with increased paper brightness of 0.9%. In

Table 5. Use of a depitching organism in a TMP mill

DCM extractive content of secondary refiner pulp	– 37.5%
Alum usage	– 31.7%
Bleach usage	– 36.9%
Brightness	+ 0.9%
Tensile index	+ 5.4%
Tear index	+ 3.4%
Burst index	+ 3.3%

addition, strength properties were increased, probably due to the lower extractive content of the paper. Brandal and Lindheim have shown an inverse relationship between paper strength and pitch content [28]. A two month Cartapip 97 trial at a US TMP mill using southern yellow pine showed significant reductions in the DCM extractives of wood chips and an increase in burst index [24]. A one week trial was performed at a mill in Northwestern USA using a blend of 60% lodgepole pine and spruce and 40% fir and hemlock. Only the pine/spruce mixture was treated because this mixture caused the most serious pitch problems. Cartapip 97 treatment reduced the averaged DCM extractive content of the reclaim chips by 25% [25].

4 Enzymatic Pitch Control in the Papermaking Process

4.1 Fundamental Research and Theory of Lipase Application

Initial studies to demonstrate the effect of lipases on pitch were conducted on Japanese red pine groundwood by the group of Hata and colleagues at Nippon Paper [1–3]. Subsequent studies have also been carried out on softwood sulfite pulp and birch sulfate pulp [29–32].

4.1.1 Identification of Compound Causing Pitch Trouble

A new method using an adsorption resin was established, instead of the previous solvent fractionation method, in order to fractionate red pine pitch and to determine what were the components that were sticky and causing pitch troubles [33]. Pitch compounds in red pine as well as deposited pitch were fractionated using the method and analyzed by gas chromatography. The changes in pitch compounds during the seasoning period and the contents of pitch in fresh wood were also investigated in great detail to understand the seasoning mechanism. These investigations produced the following results:

1. Pitch compounds could be fractionated into polar and nonpolar fractions [2].
2. Fresh wood contained more nonpolar compounds, especially in winter. The main component consisted of triglycerides (TG) [2].
3. 96% of the fatty acids that composed TG were oleic and linoleic acids [2].
4. TG was rapidly decomposed and reduced during seasoning [1].
5. Deposited pitch in the papermaking process always contained much TG [2].

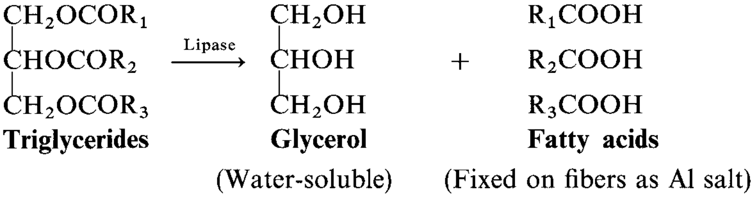
Based on these results, TG was estimated to be the key to pitch troubles. In general, nonpolar compounds such as TG may easily adhere to hydrophobic surfaces, such as rolls, by Van der Waals forces and build to become pitch deposits. It was hypothesized by Hata and his coworkers that if TG in pulp slurry could be converted to less adhesive components, pitch deposits would decrease. The conversion of TG would enable the use of fresh wood with less probability of pitch trouble.

4.1.2 *Application of Lipase*

Lipase specifically hydrolyzes TG, and thus was not expected to affect the environment or the paper quality. Three kinds of lipase, each produced by a different microorganism, were used in the original work by Hata and colleagues, and their properties are given in Table 6.

4.2 *Effect of Lipase Treatment on Prevention of Pitch Deposition*

Resinuous materials extracted from red pine wood and groundwood pulp (GP) were treated with lipase, and their adhesiveness to the hydrophobic surface was determined [1, 2]. As shown in Table 7, the pitch deposits increased when the ratio of nonpolar compounds to polar compounds increased. Thus, evidently the nonpolar compounds of the pitch materials had higher adhesiveness to hydrophobic material and seemed to play an important role in pitch deposition. TG was shown to be a key material in pitch deposition because the enzymatic hydrolysis of TG reduced pitch deposition significantly [2]. TG was hydrolyzed to glycerol and fatty acids with the lipase, and the resulting glycerol dissolved into water. Fatty acids existed in the form of aluminum salt in the presence of alum, and were dispersed into the pulp slurry and fixed on the surface of fibers.



$$\begin{array}{lcccl} CH_2OCOR_1 & & CH_2OH & & R_1COOH \\ | & & | & & \\ CHOCOR_2 & \xrightarrow{\text{Lipase}} & CHOH & + & R_2COOH \\ | & & | & & \\ CH_2OCOR_3 & & CH_2OH & & R_3COOH \end{array}$$

Triglycerides **Glycerol** (Water-soluble) **Fatty acids** (Fixed on fibers as Al salt)

4.3 Application to Papermaking Process

Since the effect of lipase on reducing pitch deposits was confirmed, the technology was applied to the actual papermaking process [2,3]. To select optimum conditions for the lipase treatment in mills, the following factors were investigated: the effects of enzyme concentration, reaction temperature, reaction time, and agitating mode on the hydrolysis of TG.

The following results were obtained from the investigation:

1. It was necessary to have a strong mixing system to keep contact between enzyme and TG for the effective reaction by enzyme.
2. Under sufficient mixing conditions, lipase 5 000 U/kgGP (300 ppm Lipase B) could hydrolyze more than 80% of TG in the surface pitch (*n*-hexane extract from GP slurry) within two hours.
3. No effect of the lipase treatment on the brightness and strength of pulp was observed.

4.4 Mill Trial I

Based on these results, the first long run mill trial was conducted using a large paper machine [2]. In this mill, the paper machine using red pine GP always had serious pitch problems because large amounts of red pine were used as raw materials for GP. Normally, 50% of unseasoned wood and 50% of wood seasoned for six months were consumed. Therefore, GP had a high content of pitch and a 30% TG content in the pitch. In order to reduce the pitch problem, it was in the past necessary to extend the seasoning period of wood to supply the mill, and the use of fine talc and dispersant was also increased. As an attempt to solve this problem, lipase was added to the groundwood pulping line just before the post refiner (Fig. 1). The operating conditions were as follows:

Paper machine:	Bel Baie II, wire width 5,080 mm
Paper product:	Yellow Telephone Directory paper (YTD) (34 g/m2) Newsprint (46 g/m2)
Pulp:	Red pine groundwood pulp (15–40%), de-inked pulp, softwood semi-bleached kraft pulp
Machine speed:	830 m/min
Production rate:	220–270 t/d
Enzyme:	Lipase A 75–125 ppm GP Lipase B 500–750 ppm GP
Reaction time:	40–60 min

An initial test was done in order to understand the proper dosage for a long run mill trial and to show the hydrolysis rate of TG by lipase in the actual papermaking process. Lipase A (125-ppm addition) on the first day of the mill trial reduced the content of TG by 74%.

For a one month lipase trial in the mill, the following parameters were compared between the usual operation and the lipase treatment operation in major products, such as newsprint and YTD: Content of the surface pitch and TG, first pass retention (FPR) of pulp and pitch, pitch deposits on the wall of the machine chest, amount of wet pitch deposits, number of defects in paper web, and dynamic friction coefficient (DFC) of paper. For 1.5–2.0% of oven-dried GP, the content of TG was 16–26% of the surface pitch [1]. Apparently the lipase hydrolyzed 70% of TG until reaching the mixing chest inlet. Furthermore, the accumulation of the pitch in the recycled white water (stock inlet, saveall) decreased to a lower level after the lipase treatment.

As shown in Fig. 2, the first pass retention (FPR) of pulp did not change with lipase addition [2]. However, the FPR of the pitch increased from 5–9% to 12–19% in YTD, and from 9–14% to 13–24% in newsprint. As the lipase hydrolyzed TG, the pitch was dispersed into the pulp slurry and distributed onto the fibers without unevenness to the surface. Lipase also prevented the accumulation of pitch in the recycled white water system. As shown in Fig. 3, pitch deposit was observed as a black piling during the usual operation. However, pitch deposits could rarely be observed after a 1-month trial with the addition of lipase. Fig. 4 clearly shows that the lipase prevented the pitch deposition on the chest wall.

In order to evaluate the pitch deposits in the wire and press sections, pitch deposit was collected from each section and measured every day. Results showed a dramatic decrease in the weight of pitch deposit with the lipase treatment compared to that of pitch in normal operation [2]. The above results strongly proved that TG in the pitch was hydrolyzed and then converted to less sticky compounds. Long term data collected by spot detectors showed that the number of defects, holes, and spots larger than 1.5 mm was reduced from 61 to 19 as a daily average by the addition of lipase. When comparing long term data between the normal and the lipase operations, it was clear that the quality of products improved with lipase use.

When a paper roll is printed on a web offset press, it is very important to prevent runnability problems such as wrinkling and uneven movement of the paper roll. This runnability performance is especially a concern in newsprint rolls. The dynamic friction of paper is thought to be related to these problems, and dynamic friction coefficient (DFC) is regarded as a quality control parameter at some Japanese paper companies. When DFC is low, the web tends to snake on the printing press. Therefore, when DFC of newsprint drops to a low level, white carbon (amorphous silica gel $SiO_2 \times H_2O$) is usually added to the paper furnish to increase DFC.

As lipase treatment was incorporated into the production process, there was an increase in the newsprint DFC and a decrease in the amount of white carbon dosage. In order to reach a certain DFC level during newsprint production, about 2% of white carbon is added in the production process. However, by incorporating the lipase treatment of GP, there was a decrease in white carbon dosage to 1%. With the lipase treatment of GP, an increase of DFC was also confirmed [3].

4.5 *Mill Trial II*

Another long term mill trial was conducted in March of 1990 [3]. Newsprint consisting of 15–35% GP was produced from red pine. This wood was normally seasoned in the mill yard for at least three months after cutting and collecting. This treatment reduced the TG content in wood and prevented severe pitch problems in the newsprint production process. However, there were still pitch deposits on the center roll of the paper machines, especially during winter, making it necessary to clean the center roll frequently. Lipase was added to make the operation smooth and to increase the unseasoned wood ratio in its furnish.

The Machine conditions during the trial were as follows:

Paper machine:	Bel Baie II, wire width 3,800 mm	
Product:	Newsprint (46 g/m2)	
Pulp:	GP (15–35%), KP, TMP, de-inked pulp	
Machine speed:	1,000 m/min	
Production rate:	200 t/d	
Enzyme:	Lipase A	80–100 ppm GP
	Lipase C	400–500 ppm GP

The long term mill trial yielded the following results:

1. The frequency of cleaning was increased during winter (from January to April). However, the addition of lipase decreased the pitch deposits on the center roll, and the frequency of cleaning decreased to the level of summer (Table 8).
2. Increase of pitch deposit was not apparent on using 50% unseasoned wood.
3. Based on the two mill trials, lipase should be added to the pulp slurry before the addition of alum.

Therefore, with the use of lipase, there was a decrease in TG content in fresh wood and reduction in the cost of seasoning and bleaching chemicals.

Lipases for hydrolysis of pitch components have subsequently been applied to other wood species and to chemical pulps with successful results. Fischer and Messner applied the commercial lipase product Resinase A 2X (Novo Nordisk A/S) to unbleached softwood sulfite pulp [29–31]. In these studies, they drew the following conclusions concerning the activity of lipase on sulfite pulps:

1. Lipase is rapidly absorbed onto pulp fiber within a minute of addition [29].
2. In a pilot mill scale treating 12 tons pulp day, with pulp at 4% consistency, 85–90% of the triglycerides were hydrolyzed, as determined by reduction of one triglyceride fraction from gas chromotography analysis [30], and the resin content of the pulp was reduced by 60% [31].

Lipases from *Candida cylindracea* have been shown to be effective in hydrolyzing triglycerides in extractives of fresh birch and birch sulfate pulp [32]. The total amount of esterified compounds in fresh birch was decreased by 34%; 50% and 65% respectively of the esterified fatty acids and saturated fatty acids were also hydrolyzed. For treatment of birch sulfate pulp, the lipases of *C. cylindracea* hydrolyzed 30% of the esterified lipids as compared to 40% hydrolyzed by Resinase A (source organism *Aspergillus* from Novo Nordisk A/S). Esters of saturated fatty acids, alcohols and sterols were hydrolyzed by both lipases, though the *C. cylindracea* lipase was incapable of degrading esters of betulaprenols and triterpenoids, whereas the *Aspergillus* enzyme hydrolyzed them to some extent.

5 Conclusions

The enzymatic pitch control method using lipase was the first successful example of the use of an enzyme as a solution to pitch problems and in the papermaking process. The fungal pitch control method, using the colorless isolant of *Ophiostoma piliferum*, was the first successful case of using a live organism as a solution to pitch problems and in the pulping process. Both technologies use biotechnology as their basis and have been successfully used in full scale industrial mills in various parts of the world.

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