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Application of Microorganism to Pulping and Bleaching Processes[*1]

Koki Sakai[*2]

펄프 및 漂白工程에서의 微生物應用[*1]

坂井 克己[*2]

ABSTRACT

The application of white-rot fungi to pulping and bleaching processes has been studied at the Wood Chemistry Laboratory in Kyushu University, cooperatively with the Biotechnology Laboratory of Kobe Steel, Ltd. Some successful results of the studies for a biomechanical pulping process, biobleaching of hardwood and softwood kraft pulp, as well as chlorine free biobleaching of oxygen-prebleached hardwood kraft pulp are dealt with. Biological treatment of the pulp bleaching effluent is also described.

1. INTRODUCTION

The pulp and paper industry in the world is now faced with pressure from environmental lobbies and concern for saving energy and wood resources, even though the industry has continually tried to improve its processes and products. Recent developments in biotechnology[1~3] suggest that the application of microorganisms to the pulp and paper industry may give rise to new aspects of the technologies for overcoming these pressures and concerns.

Fungi, rather than bacteria, are the main decomposers of wood lignin which mainly joins fiber cells together in the wood tissues. Thus, some ligninolytic fungi may be used to soften or loosen the wood structure so that electric energy to produce acceptable pulp can be saved to a certain extent in a mechanical pulping process. Another potential utilization of lignin degrading fungi may be the biobleaching of KP since the brown color of UKP is associated with the residual lignin in the pulp.

It is also expected that fungi treatment can reduce the color of the effluent from pulp bleaching processes. Fungi with superior and selective ligninolytic activity are required for all these purposes. In this connection, Nishida *et al*[1] recently isolated a fungus, named IZU−154, which was very active and highly

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[*2] 日本九州大學 農學部 Department of Forest Products, Faculty of Agriculture, Kyushu University, Japan

selective in lignin degradation.

Based on the above-mentioned prospect, we have studied the biomechanical pulping[5], biobleaching[6-8] and biological treatment of a bleach effluent[9]. Some successful results are given below.

2. BIOMECHANICAL PULPING

Biomechanical pulping processes using white-rot fungi were investigated by many researchers[10-14] to reduce energy consumption during production of mechanical pulp since one of major disadvantages of the mechanical pulping is that large amounts of electrical energy are required to produce acceptable pulp. According to the recent papers of Leatham *et al.*[13, 14] supplemental nutrients and long incubation time, at least four weeks, were necessary for the fungal treatment of aspen chips to save refining energy in the biomechanical pulping and to improve the pulp strength properties.

Therefore, we investigated how much the supplemental nutrients and treatment time can be minimized since these are very important variables in fungal treatments if the practical feasibility of the biomechanical pulping is concerned.

2.1 Lignin biodegradation with white-rot fungi

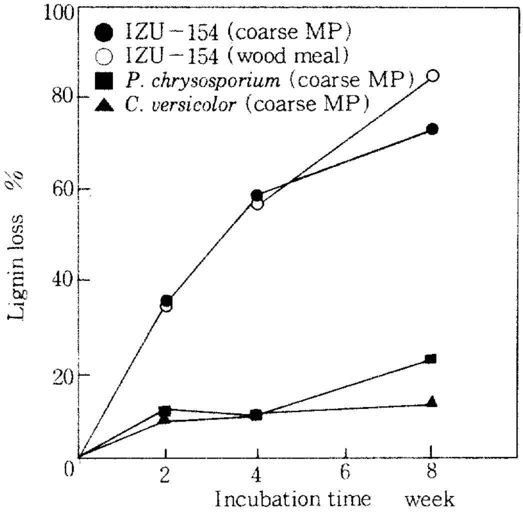

Fig. 1. Delignification of beech wood meal and mechanical pulp (MP) with some typical white-rot fungi.

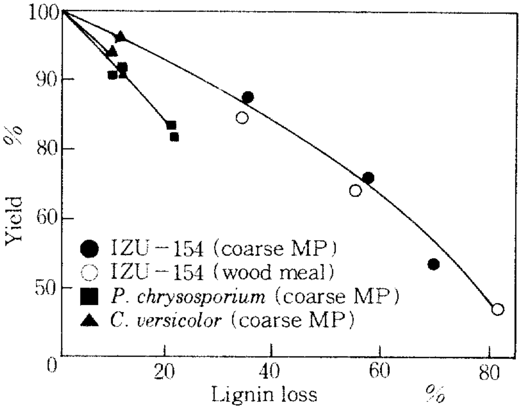

Fig. 2. Relation between yield and lignin loss during incubation with fungi.

Beech coarse mechanical pulp and wood meal were incubated with the mycelia of the fungus IZU−154, *Coriolus versicolor*, and *Phanerochaete chrysosporium* at 30℃ for 2−8 weeks. It should be noted that no supplemental nutrients are added to the incubation system. The rates of delignification by these fungi are shown in Fig. 1. The fungus IZU−154 extensively degraded lignin of both the coarse mechanical pulp and the wood meal to give rise to lignin loss of 35, 60 and 70−80% after two, four and eight weeks of incubation, respectively. This rate was much faster than those brought about by the other two fungi.

As judged from the relationship between yield and lignin loss in Fig. 2, the fungus IZU−154 preferentially degraded the lignin portion compared with the carbohydrate portion. At the same lignin loss, yields after the treatment with IZU−154 were much higher than those with the other fungi. In other words, this fungus has high selectivity of lignin degradation. No clear difference was observed in the selectivity between the coarse mechanical pulp and the wood meal. Therefore, incubation of the coarse mechanical pulp with the fungus IZU−154 seems to constitue an effective biological treatment in the biomechanical pulping processes.

2.2 Biomechanical pulping without supplemental nutrients

It was reported that fungal treatment without adding nutrient in medium deteriorated the pulp strength properties and did not contribute to the refining energy saving, for both hardwood[10)] and softwood[11)] mechanical pulping processes. Thus, the fungal treatments were carried out with some supplemental nutrients in many studies previously reported on biomechanical pulping.[10−14)] In the present study, however, coarse mechanical pulps were treated with the fungus IZU−154 without adding any supplemental nutrients since the fungus extensively and selectively degraded lignin as mentioned above.

At first, beech wood coarse mechanical pulp was inoculated with only the mycelia of the fungus and incubated for seven days at 30℃. The fungal treated and untreated pulps were bleached with hydrogen peroxide (H_2O_2 2%, NaOH 2%, Na_2SiO_3 3% on pulp) at 70℃ for 2h, and sequentially refined in a PFI mill without washing out bleaching agents. As measured by the number of revolutions in the PFI mill to develop a given freeness, energy requirement for refining the fungal treated pulp were decreased by a half after the H_2O_2 beaching or by two-thirds without bleaching, compared with those of the untreated pulp (Table 1). The fungal treatment increased the pulp strength valuses to approximately twice.

For pine biomechanical pulping, electric energy required to produce a 200ml CSF pulp by the disk refining was measured before and after the fungal treatment for 14 days. The energy consumption was about 2,100 kWh/t with the untreated spruce coarse mechanical pulp, while that with the fungus-treated one was about 1,400 kWh/t. Thus, energy saving brought about 34%. The fungal treatment is supposed to degrade the lignin portion in the coarse mechanical pulp and/or to soften the pulp so that the individual wood fibers can be separated more easily. As shown in Table 2, the fungal pretreatment improved tensile index by about 36%, but did not considerably affect other strength properties. This may be caused by decreased damages of the individual

Table 1. Strength and optical properties of beech biomechanical pulps

H_2O_2 bleach	Incubation time, days	Freeness ml CSF	Tensile index, Nm/g	Burst index kPa · m²/g	Tear index mN · m²/g	Brightness %
Before	0	130	8.7	0.35	0.86	40.8
	7	160	9.6	0.74	1.65	37.6
	14	135	14.6	1.12	1.99	38.1
After	0	150	9.6	0.52	1.21	64.7
	7	150	19.8	1.05	2.48	65.7
	14	140	23.7	1.36	2.71	67.4

Table 2. Refining energy and strength of softwood biomechanical pulps

Wood species	Treatment	Time days	Refining energy kWh/t	Tensile index, Nm/g	Burst index kPa · m^2/g	Tear index mN · m^2/g
Pine	Untreated	0	2073	17.3	1.26	7.67
	Fungus(IZU−154)	14	1367	23.5	1.55	7.32
	Fungus+glucose	7	1401	20.9	1.43	8.40
Spruce	Untreated	0	2627	33.3	2.77	8.54
	Fungus(IZU−154)	10	2700	38.3	2.76	8.22

wood fibers during the refining of softened and partially delignified coarse mechanical pulp and/or increased interfiber bonding during the sheet formation.

For spruce wood, the energy consumption on the disk refining was about 2,700 kWh/t for untreated pine coarse mechanical pulp, while that after the fungal treatment for 10 days was about 1,700 kWh/t at 200ml CSF. As shown in Table 2, energy saving obtained with fungal treatment was by about 35%. Tensile index was improved by 17% after fungal treatment.

2.3 Attempts to reduce incubation time

In order to minimize the incubation time in our biomechanical pulping process, glucose was added as a supplemental nutrient to the pine coarse mechanical pulp at the start of the treatment with IZU−154. After seven days of the treatment, energy saving obtained was by about 32%(Fig. 3 and Table 2). Both tensile and burst indexes were improved by the fungal treatment. These data indicate that addition of glucose can reduce the incubation period to one-half that without supplemental nutrients for exhibiting the same effects on energy saving and improving pulp strength.

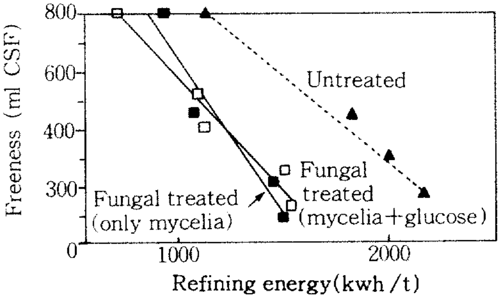

Fig. 3. Freeness of pine pulp as a function of refining energy consumed by disk refining.

In conclusion, an application of the fungal treatment to both hardwood and softwood mechanical pulping processes resulted in substantial reduction of refining energy, and in improving the pulp strength properties. It is notworthy that these results were obtained by only 1-week fungal treatment. To our knowledge, no other study has succeeded in saving energy and in improving pulp strength such a short incubation period.

3. BIOBLEACHING OF KRAFT PULP USING A WHITE-ROT FUNGUS

The effluent from the pulp bleaching processes which use chlorine-based chemicals is of growing environmental concern because it contains numerous chlorinated organic substances, including mutagenic chlorinated phenols and dioxins.[15,16] There is a great interest, therefore, in eliminating or at least reducing the use of chlorine-based chemicals in bleaching by means of white rot fungi because they are the most attractive candidates for biological removal of residual lignin from kraft pulp. Kirk and Yang were the first to recognize that white-rot fungi could partly delignify unbleached kraft pulp[17]. Tran and Chambers observed delignification of kraft pulp in treatment with *Phanerochaete chrysosporium*[18]. More recently, hardwood kraft pulp treated with *Coriolus versicolor* showed an increase in brightness and a corresponding decrease in residual lignin.[19, 20]

In our study four white-rot fungi (IZU−154, *C. versicolor, P. chrysosporium,* and *C. hirsutus*) were tested for their ability to directly bleach unbleached kraft pulps.

3.1 Effect of fungal treatment on pulp brightness

Samples of UKP were inoculated with each of four different fungi and incubated at 30℃ for 3−15 days. No nutrients were added at the start of the incubation. Fig. 4 shows that treatment with IZU−154 produced a sharp increase in pulp brightness, while brightness increased only slightly with the other three fungi. Pulp kappa number decreased with increasing brightness, as shown in Fig. 5, indicating that fungal treatment removed residual lignin in the pulp. Furthermore, fungus IZU−154 preferentially degraded the lignin portion compared with the carbohydrate portion of the pulp, as the pulp yield was much higher with fungus IZU−154 than with the other fungi at the same brightness and kappa number (data not shown). Therefore, it can be concluded that the superior brightness observed for fungus IZU−154 can be ascribed to its superior and selective ligninolytic activity.

Paice *et al.* reported that *C. versicolor* could directly bleach hardwood kraft pulp under conditions of low pulp consistency and supplemental nutrients.[19, 20] However, more extensive increases of pulp brightness were obtained using IZU−154 under conditions of high pulp consistency and no supplemental nutrients as mentioned above. This was done to

- Maintain the concentration of lignin-degrading enzyme at a high level.
- Facilitate the transfer of oxygen to the reaction sites of UKP.
- Minimize the size of the bioreactor for practical operation at a mill.

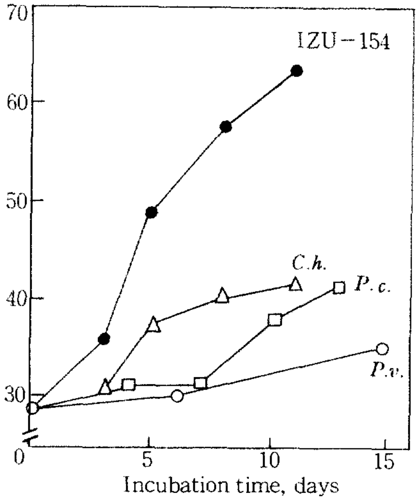

Fig. 4. Pulp brightness as a function of incubation time.

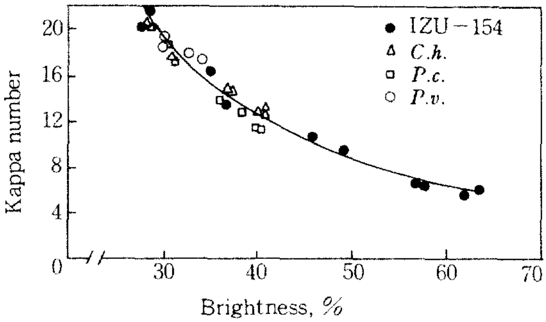

Fig. 5. Pulp kappa number as a function of brightness for kraft pulps treated with whit-rot fungi.

Table 3. Bleachng conditions and optical properties for conventional 5-stage bleaching and biobleaching processes

Bleaching sequence	Dosage, % on pulp						Brightness, %	
	C	E_1	D_1	E_2	D_2	As effective chlorine	Before aging	After aging*
CEDED (Conventional process)	5.0	3.6	0.8	0.2	0.3	7.89	88.8	84.2
FCED (Biobleaching process)	1.4	0.8	0.3	…	…	2.19	88.1	85.3

*at 105℃ for 1h.

3.2 Partial replacement of chemical bleaching by biobleaching

One objective for fungal bleaching would be to reduce the dosage of the chlorine-based chemicals used to bleach kraft pulp. Table 3 shows that a combined fungal treatment (F) for five days and chemical bleaching process (CED sequence) gave a pulp of 88% brightness at 94% yield, almost the same brightness as that obtained using a conventional CEDED bleaching sequence. The FCED bleaching process could decrease the chemical dosage in the C, E, and D stages by 72%, 79% and 63%, respectively, compared with the conventional bleaching process.

Further reduction of the chemical dosage can be achieved by a prolonged fungal treatment in an FED or FEDED sequence. However, a slight loss in pulp yield is unavoidable for this strategy.

3.3 Biobleaching of softwood kraft pulp by white-rot fungi

The white-rot fungi were applied to delignification and brightening of unbleached softwood kraft pulp in order to establish a biobleaching process for SWKP. When treated with the fungus IZU−154, brightness of mill-made SWKP reached 27 and 52% from 23% after 5 and 12 days of incubation, respectively as shown in Fig. 6. At the same time, kappa number decreased from 40.0 to 29.1 and 6.9, respectively.

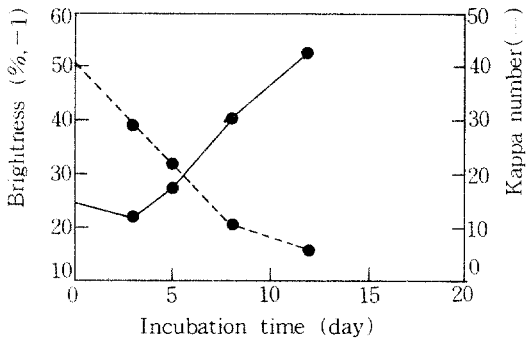

Fig. 6. Brightening and delignification of SWKP with the fungus IZU−154

Reid *et al.* reported that brightness of SWKP was decreased in the earlier stage of incubation with the white-rot fungus *Trametes (Coriolus) versicolor* in agiatated cultures and did not regain its initial level until the passage of 14days[21]. By the treatment with IZU−154, however, brightness went up to the initial level in a few days and continually increased during the incubation, though a slight and temporary decrease of brightness was observed in an early phase of the IZU−154 was much smaller than those with other fungi as compared at the same kappa number,

Table 4. Bleaching conditions and optical properties for mill-made SWKP.

Bleaching sequece	Dosages, % on pulp						Brightness, %		PC number	Yield %
	C	E_1	D_1	E_2	D_2	As effective chlorine	Before aging**	After aging**		
CEDED (conventional Bleaching)	9.0	6.4	1.0	0.5	0.5	12.95	84.2	82.4	0.38	91.4
F*CED (Biobleaching)	2.4	1.7	0.4	…	…	3.45 (△73%)	84.6	83.4	0.25	91.4

* Softwood(mill-made) UKP was treated for 6 days and kappa number was decreased from 40.0 to 14.9(△kappa=63%)

** at 105℃ for 60 min.

indicating that this fungus selectively degraded residual lignin, even if SWKP was used as a substrate but no nutrients such as glucose were added.

A combined fungal and chemical bleaching process was investigated in order to obtain a fully brightened SWKP. At the first stage mill-made SWKP was treated with IZU−154 for six days (F stage) to decrease kappa number form 40.0 to 14.9. Subsequently, this fungus-treated SWKP was bleached with the CED sequence. Chemical dosages were controlled to give approximately the equal brightness values for the pulps bleached with FCED and CEDED sequences. Consequently, a six-day treatment with IZU−154 could reduce the chemical dosages by 73%, 75% and 73% in C, E and D stages, respectively, to get the same brightness.

No obvious differences were observed between the strength properties of FCED and CEDED bleached pulps (data not shown). It should be especially noted that tear index was not decreased by FCED process, whereas Reid *et al.* reported that tear index of the fungal-treated pulps was deteriorated significantly.

In conclusion, FCED bleaching can produce bleached kraft pulp with very small chlorine charge. This bleached pulp has sufficient optical and strength properties. It is considered that active and selective lignin-degrading ability of IZU−154 leads to these successful results.

4. CHLORINE-FREE BLEACHING OF KRAFT PULP

The effluent from multi-stage bleaching of unbleached kraft pulp (UKP) contains many kinds of low molecular weight chlorinated aromatics, such as chlorinated phenols and dioxins that are acutely toxic and aliphatic low molecular wight unstable aldehydes that are recognized as being mutagenic.[15, 22-24] Therefore, the environmental concerns have let us to seek alternative ways to eliminate the use of chlorine-based chemicals in bleaching.

As described above, the fungus IZU−154 significantly increased pulp brightness. Therefore, to establish an absolutely chlorine-free bleaching process the fungus IZU−154 was applied to delignification and brightening of an oxygen-bleached hardwood kraft pulp (HWKP). Paice *et al.* reported also the biobleaching of HWKP with *T. versicolor* which

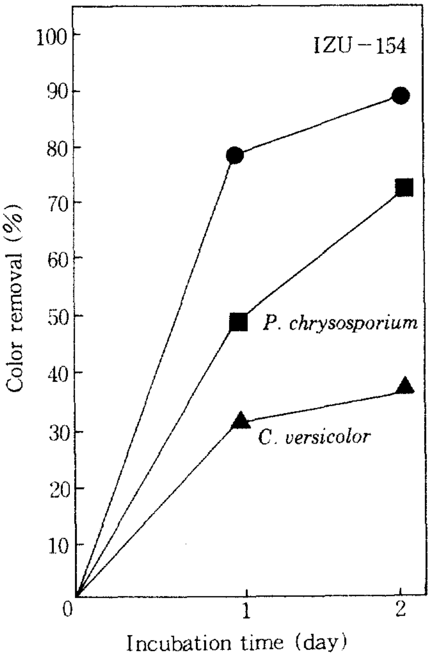

Fig. 9. Decolorization of N-E_1 effluent by white-rot fungi

IZU−154 but other additives such as ammonium tartrate as the N−source and trace elements were scarcely required.

The decolorization ability of three white-rot fungi was determined using the E_1 effluent containing 0.5% glucose. As shown in Fig. 9, for the E_1 effluent containing 10000 CU, treatment with IZU−154 resulted in 7800 CU(78%) and 8900 CU(89%) reduction of the color after one-and two-day-incubations, respectively. On the other hand, when the E_1 effluent was treated under the same conditions, only 32% and 36% of decolorization with *C. versicolor* and 49% and 72% of decolorization with *P. chrysosporium* were observed within one-and two-day-incubation, respectively. The E_1 effluent, if it contained a small amount (0.5%) of glucose, was most effectively decolorized by the fungus IZU−154. Thus, decolorization by IZU−154 was hereinafter investigated in further detail.

5.2 Effect of various additives on decolorization of N-E_1 effluent

Since adding a small amount of glucose (0.5%) to E_1 effluent was shown to be essential, various carbon sources including mannose, xylose and glycerol were tested as possible substitutes for glucose. In the presence of mannose, 86% of the color was removed by one-day-incubation, but both xylose and glycerol were noticeably less efficient than glucose which exhibited 95% decolorization.

The same concentration (28mM, that is equivalent to 0.5% glucose) of various glucose derivatives such as glucono-δ-lactone, glucuronolactone, sodium glucuronate and glucitol were added to the E_1 effluent for testing their efficiency as additives for decolorizaton. As shown in Table 4, in comparison with 53% and 78% of color reduction achieved with the E_1 effluent including glucose, addition of glucono-δlactone to E_1 effluent showed 83%

Table 6. The effect of additives on the decolorization of N-E_1 effluent by IZU−154 (initial color : 10,000 CU)

Additives	Decolorization(%) after incubation of 12h	24h
None	21	28
Glucose	53	78
Glucono-δ-lactone	83	94
Glucuronolactone	60	32
Sodium gluconate	32	36
Sodium glucuronate	17	25
Glucitol	21	29
Glyceric acid	ND	22
Glycolic acid	ND	32
Acetic acid	48	59

ND : not determined

and 94% of decolorization within 12-and 24-hour-incubation, respectively. The decolorization brought about by the addition of 0.2% (11mM) glucono-δ-lactone corresponded to that obtained by adding 0.5% (28mM) glucose. Glucuronolactone had almost the same effect on color removal as glucose did.

In contrast, sodium gluconate, sodium glucuronate and glucitol had practically no effect on the extent of the color removal. Addition of acetic acid effectively enhanced the decolorization of E_1 effluent, although glyceric and glycolic acids showed no effect on the color removal. The initial rate of decolorization of the N$-E_1$ effluent with 0.5% and 0.2% of acetic acid were higher than that with 0.5% of glucose within 12-hour incubation, although decolorization of the N-E_1 effluent with glucose was more effective than that obtained with acetic acid after 24-hour-incubation.

When immobilized in the MyCoPoR process, *P. chrysosporium* significantly reduce the color of E_1 effluents.[22,23] The color removal by the fungus IZU-154 corresponded to that of *P. chrysosporium* without any further biotechnological optimerization such as immobilization of the fungus and flushing with oxygen. Therefore, higher color reduction by IZU$-$154 can be expected for a possible biotechnological application.

On the other hand, We observed 59% color removal of N-E_1 effluent including 0.17% of acetic acid (28mM) within 24-hour-incubation. The addition of small amounts of acetic acid to E_1 effluents did not affect COD value of the E_1 effluents when it was analyzed by $KMnO_4$ method (data not shown). Therefore, acetic acid is expected as as possible substitute of glucose for decolorization of E_1 effluents.

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resulted in increase of pulp brightness but to the smaller extent than IZU−154 did.[19,20]

4.1 Fungal treatment of oxgen-bleached HWKP

A mill-made oxygen-bleached HWKP was inoculated with the fungus IZU−154 and incubated at 30℃ for 12 days. As shown in Fig. 7, brightness of the pulp was increased by 17 and 22 points in three and five days of the treatment with IZU−154, respectively. Kappa number was decreased from 10.1 to 6.4 by the five day treatment. Yield loss was only less than 1% for 5 days. Therefore, it can be concluded that IZU−154 degrades quite selectively even a small amount of residual lignin in oxygen-bleached HWKP.

4.2 Chlorine-free bleaching process

To establish an absolutely chlorine-free bleaching process, a fungal treatment was followed by chlorine-free chemical bleaching. A mill-made oxyen-bleached HWKP was treated with IZU−154 for three or five days to increase the brightness from 47.9% to 66.4% or 69.8%, respectively, Subsequently, these fungal treated pulps were bleached with hydrogen peroxide after alkaline extraction. Combination of oxygen-bleaching, a three-day treatment with IZU−154, an alkaline extraction and hydrogen peroxide bleaching (OF$_1$EP) gave a pulp of 85.0% and 86.3% ISO brightness with 4% and 5% charge of H_2O_2 on pulp, respectively. Moreover, when fungal treatment was prolonged to five days as shwon for

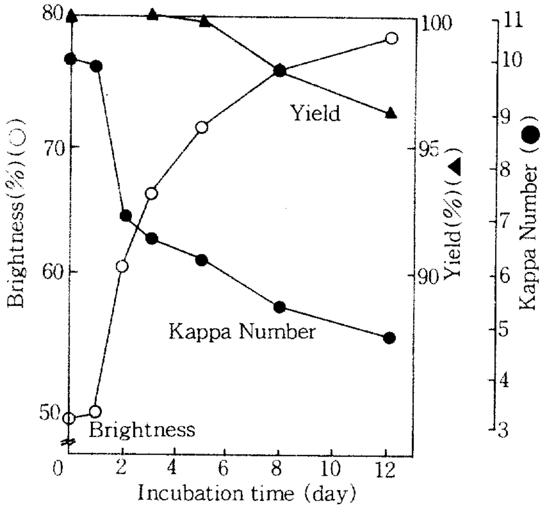

Fig. 7. Biobleaching of oxygenbl hardwood kraft pulp

Table 5. Bleaching conditions and optical properties for chlorine-free processes

Bleaching sequence	H_2O_2 %	NaOH %	Brightness(%) before aging	Brightness(%) after* aging	PC number	Yield %
O-C-E-D**			87.9	85.7	0.36	97.4
O-P$_1$-P$_2$	P$_1$: 5 P$_2$: 5	P$_1$: 2 P$_2$: 2	73.8	71.8	0.89	97.4
O-F$_1$-E-P***	4	2	85.0	83.7	0.26	97.7
O-F$_2$-E-P***	5	2	86.3	84.9	0.26	97.8
	2	2	84.3	83.5	0.17	97.1
	4	2	87.3	85.6	0.29	97.0

*at 105℃ for 1 h.
**dosages on pulp; C : 2.1%, E : 1.2%, D : 0.7%
*** F$_1$: 3-days fungal treatment, F$_2$: 5-days fungal treatment

OF_2EP in the Table 5, a pulp of 84.3% and 87.3% brightness was obtained with 2% and 4% H_2O_2 treatment, respectively. On the other hand, the brightness of OPP pulp was only 74% in spite of its large dosage H_2O_2 and its color reversion was much higher. Therefore, the fungal treatment is very effective for establishing an absolutely chlorine-free bleaching process.

4.3 Strength properties of OF_2EP pulp

Strength properties of an OF_2EP bleached pulp were compared with those of an OCED bleached pulp as a function of sheet density in Fig. 8. The difference in strengths between these pulps was almost negligible, although the burst and tensile indexes of the OF_2EP bleached pulp were slightly higher and the tear strength of the OF_2EP bleached pulp was slightly lower than those of the OCED bleached pulp.

It can be concluded that the applying IZU−154 treatment into a bleaching process make it possible to produce fully bleached HWKP without using any chlorine-based chemicals. The bleaches pulp exhibited sufficient optical and strength properties. These results experimentally prove that it is potentially possible to establish a chlorine-free bleaching process.

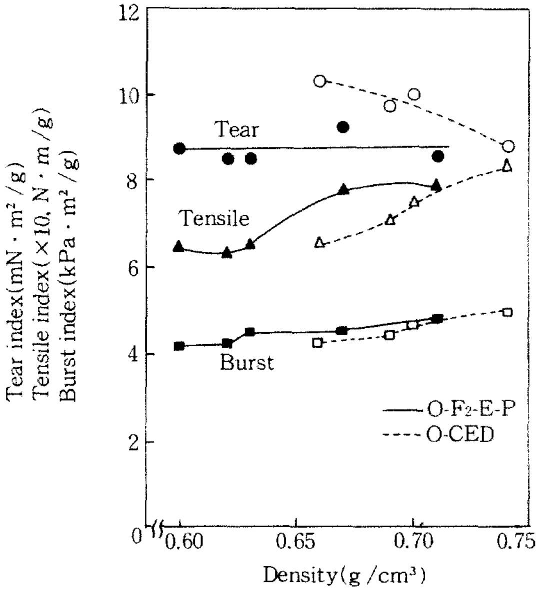

Fig. 8. Strength of OF_2EP and OCED pulp

5. TREATMENT OF KRAFT BLEACHING EFFLUENTS BY LIGNIN-DEGRADING FUNGI

In this section, the decolorization of the first alkaline extraction stage (E_1) effluents with the lignin-degrading fungi is dealt with. The kraft bleach shows dark brown color and contains numerous chlorinated organic substances.[15,16,22-24] Typical biological treatment with an aerated lagoon and/or an activated sludge systems reduces the BOD and COD of the effluent but the color of the effluent persists.[26,27] With *P. chrysosporium*, some color reduction of kraft bleaching effluents was achieved in several days in a concentrated oxygen atmosphere with or without agitation.[27] The MyCor[28] and MyCoPoR[29] processes using *P. chrysosporium* have so far been proposed as effective decolorization processes of the E_1 effluents.

5.1 Decolorization of $N-E_1$ effluent by treatment with IZU−154

Here, $N-E_1$ effluent denotes the effluent from E_1 stage in bleaching of soft-wood kraft pulp.

At first, the $N-E_1$ effluent of 7000 color unit (CU) was treated with the fungus IZU−154 under different medium conditions. The results (data not shown) indicated that a small amount (0.5%) of glucose was only necessary for decolorization of E_1 effluent by

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