

ISBN 4-9900692-4-4

Science for Sustainable Utilization of Forest Resources in the Tropics

Midterm Report of JSPS-LIPI Core University Program
in the Field of Wood Science during 1996-2000



Wood Research Institute, Kyoto University, Japan
R & D Centre for Applied Physics, LIPI, Indonesia
University Putra Malaysia, Malaysia

Production of Pulp and Paper by Using Biological Methods from Tropical Wood Resources

Period: April, 1997 - March, 1999

| | |
|---------------------|--|
| * Masaaki KUWAHARA | Wood Research Institute, Kyoto University |
| Mikio SHIMADA | Wood Research Institute, Kyoto University |
| Takashi WATANABE | Wood Research Institute, Kyoto University |
| Yoichi HONDA | Wood Research Institute, Kyoto University |
| Ryuichiro KONDO | Faculty of Agriculture, Kyushu University |
| * Bambang PRASETYA | R & D Centre for Applied Physics, LIPI |
| Triadi BASUKI | R & D Centre for Applied Chemistry, LIPI |
| Tami IDIYANTI | R & D Centre for Applied Chemistry, LIPI |
| Didiek H. GOENADI | Biotechnology Research Institute for Estate Crops |
| Yufnal AWAY | Biotechnology Research Institute for Estate Crops |
| Ridwan A. PASARIBU | Forest Products & Socio Economics Res. and Dev. Centre |
| Munir ERMAN | North Sumatra University |
| I. G. K. Tapa DARMA | Faculty of Forestry, Bogor Agricultural University |

The importance of environmental aspects in paper manufacturing processes has grown dramatically in recent years since production of pulp and paper by current chemical reactions causes global environmental problems due to production of toxic byproducts such as dioxin and chloroform. The drawback of current paper-making process has prompted us to develop environmentally-benign delignification system by use of lignin-degrading microorganisms. In this project, strong ligninolytic fungi were first screened from Indonesian forests, and the fungal isolates were applied to biopulping and biobleaching processes. As a result of extensive screening of strong ligninolytic fungi, we isolated several thermophilic fungi superior to typical white rot fungi like *Phanerochaete chrysosporium* and *Coriolus versicolor*. Application of these new isolates to biobleaching and biopulping ensured high potentials of these strains for developing the environmentally-friendly paper-making processes. Among the new isolates, a white-rot fungus from tropical oil palm plantations (K14) showed a remarkable potential for biodelignification of empty fruit bunches of oil palm trees (EFBOP). Enzymatic systems to bleach UKP by manganese peroxidase and laccase from the new isolates were also developed.

Wood Biomass is the most abundant renewable bioresource on Earth and its effective utilization in harmony with environmental safeguard is the urgent task for ensuring human activities in the twenty-first century. Wood is basically composed of three main natural polymers, cellulose, hemicelluloses and lignin. Since the separation of cellulose is hindered by the presence of lignin, biological degradation of lignin by ligninolytic microorganisms is of enormous significance in various industrial fields including production of pulp and paper from woody plants.

P. chrysosporium had no significant effects on pulp yield and kappa number reduction, although the treatment increased tear index by 16 %. Biological treatment with the strain K14 reduced 23% of energy consumption in mechanical pulping of oil palm EFB. By the pretreatment with K14, tear index of pulp sheet increased 16% and kappa number decreased 4.2% compared with control run without the fungal pretreatment.

The growing area of oil palm trees in Indonesia will become the largest in the world by the year of 2005. At that time production of the EFBOP will reach approximately 15 millions tons per year. This means that tremendous resources are available for pulp and paper, as alternatives of woody plants. The present study indicates that the EFBOP functions as a reliable substrate of white-rot basidiomycetes, and that application of biopulping of the EFBOP by using the new fungal isolates have great potentials for establishing the environmentally-friendly paper-making process from the residual biomass, EFBOP.

Biobleaching of Unleached kraft pulp

The new fungal strains isolated in Indonesia were applied to biobleaching of UKP. Among the new isolates, treatment of hardwood UKP with CPN01 and PSM01 were found to be effective for decolorizing the UKP to a greater extent than that of the standard fungal strains, *Phanerochaete chrysosporium* and *Coriolus versicolor*. Cultivation of these fungi on wood meal and liquid media demonstrated that CPN 01 can produce a high amount of manganese peroxidase (MnP) and lignin peroxidase (LiP) while PSM01 produced laccase (Lac) as its major lignin-degrading enzyme.

MnP from CPN01 was applied to biobleaching of UKP from hardwood in the presence of Mn(II) and a chelator for manganese ions. The enzymatic treatment in combination with alkali-extraction resulted in kappa number reduction and brightness gain by 13 point and 15 %, respectively. In these treatments, consistency larger than 3% was ineffective for the bleaching. In this project, biobleaching with laccase and mediators was also carried out. Treatment of UKP with laccase from PSM01 and HBT under oxygen atmosphere at 3 bar reduced kappa number by 31 %. Bleaching of the pulp by the sequence of LELEPP decreased kappa number by 90 % (Table 3, 4). Thus, significant effects on biobleaching was observed with the enzymes from CPN 01 and PSM01, ensuring the potentials of these tropical white rot fungi in environmentally-friendly paper making processes.

Table 3. Biobleaching of hardwood UKP by laccase-mediator system*

| Step | Condition for Laccase-Mediator system | Kappa number | Reduction (%) |
|--------------|--|--------------|---------------|
| Original UKP | – | 20.2 | – |
| L | 60 °C, 4 h Oxygen: 1 bar, (10 Unit/ g pulp) | 14.5 | 27.7 |
| L | 60 °C, 6 h Oxygen: 1 bar, (10 Unit/ g pulp) | 13.9 | 30.7 |
| L | 60 °C, 6 h Oxygen: 1 bar, (5 Unit/ g pulp) | 13.7 | 32.0 |
| L | 60 °C, 6 h Air, (10 Unit/ g pulp) | 13.4 | 33.1 |
| L | 40 °C, 2 h Oxygen: 1 bar, (10 Unit/ g pulp) | 15.0 | 25.2 |
| LEP | 60 °C, 4 h Oxygen: 1 bar, (10 Unit/ g pulp) | 9.3 | 53.5 |
| LEP | 60 °C, 6 h Oxygen: 1 bar, (10 Unit/ g pulp) | 8.0 | 60.3 |
| LEP | 60 °C, 6 h Oxygen: 1 bar, (5 Unit/ g pulp) | 6.8 | 66.3 |
| LEP | 60 °C, 6 h Air, (10 Unit/ g pulp) | 6.3 | 68.4 |
| LEP | 40 °C, 2 h Oxygen: 1 bar, (10 Unit/ g pulp) | 8.7 | 56.8 |

*L: Treated with crude laccase from PSM 01 and HBT (0.02 g/ g pulp) at 50 °C for 4 h

Table 4. Biobleaching of hardwood UKP by laccase-mediator system*

| Step | Kappa number | Reduction (%) |
|--------------|--------------|---------------|
| Original UKP | 20.2 | – |
| L | 13.9 | 31.3 |
| LE | 13.6 | 32.9 |
| LEL | 10.0 | 50.2 |
| LELE | 10.0 | 50.8 |
| LEP | 7.6 | 62.4 |
| LEPP | 2.4 | 88.1 |
| LELEP | 3.4 | 83.0 |
| LELEPP | 2.0 | 90.0 |

*L: Treated with crude laccase from PSM 01 (5 Unit/ g pulp) and HBT (0.02 g/ g pulp) at 50 °C for 4 h

List of publications

D. H. Goenadi, Y. Away, Suharyanto and T. Panji, T. Watanabe and M. Kuwahara, Pulping of empty fruit bunches of oil palm by white-rot fungi isolated from tropical plantation, *Proc. of 7th International Conference on Biotechnology in Pulp and Paper Industry*, B49-52, June 16-19th, 1998, Vancouver, Canada

B. Prasetya, Y. Srirejeki, T. Watanabe and M. Kuwahara, Acceleration of microbial lignin degradation of *Accasia mangium* wild by thermal pre-hydrolysis treatment, *Proc. of International Symposium on Emerging Technologies of Pulping and Paper Making of Fast Growing Wood*, 25-35, Nov. 23rd-25th, 1998, Guanzou, China

M. Karina, T. Watanabe and M. Kuwahara, Study on the occurrence of lignin carbohydrate bonds in kraft pulp, *Proceedings of the First International Wood Science Seminar*, 26, Dec. 6-7, 1996, Kyoto, Japan

B. Prasetya, T. Idiyanti, D. H. Goenadi, R. M. Siagian, S. Yoshida, T. Watanabe and M. Kuwahara, Production of ligninolytic enzymes of white-rot fungi from Indonesian tropical rainforest and their bleacheability on the kluft pulp of *Accasia mangium*, *Proceedings of the First International Wood Science Seminar*, 132-146, Dec. 6-7, 1996, Kyoto, Japan

M. Karina, B. Prasetya, T. Idiyanti, T. Watanabe and M. Kuwahara, Characterization of residual lignin and lignin-carbohydrate complexes from unbleached kluft pulp after fungal treatment, *Proceedings of the Second International Wood Science Seminar*, D23, Nov. 6-7, 1998, Serpong, Indonesia

B. Prasetya, T. Idiyanti, D. H. Goenadi, R. M. Siagian, T. Watanabe and M. Kuwahara, Production of manganese peroxidase from fungal isolate CPN01 and their *in-vitro* bleaching-ability on kraft pulp, *Proceedings of the Second International Wood Science Seminar*, E63-77, Nov. 6-7, 1998, Serpong, Indonesia

