

Full Length Research Paper

A microbiological technique for the separation of *Hibiscus sabdariffa* L. fibers

Pilane Vaithanomsat^{1*}, Poom Phusanakom², Waraporn Apiwatanapiwat¹ and Molnapat Songpim¹

¹Enzyme Technology and Waste Management Research Unit, Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Kasetsart University, 50, Chatuchak, Bangkok 10900, Thailand.

²Toyota Motor Asia Pacific Engineering and Manufacturing Company Limited, 99 Moo 5, Ban Ragad, Amphur Bang Bo, Samutprakan 10560, Thailand.

Accepted 29 June 2009

This study is related to a process for the separation of *Hibiscus sabdariffa* L. fibers using living microorganisms. The selected fungal strains, *Datronia* sp. and *Oligoporous* sp., were isolated from natural sources in Thailand and were previously shown to be capable of degrading lignin in pulp and paper industrial wastewater. The process described here is comprising the fungal-treated fibrous of *H. sabdariffa* L. barks in certain conditions. The fungal inoculum size, retting time and retting temperature were important in the experiments and the retting efficiency was based on the visual characteristics of resulting fibers. In addition, the pectinolytic enzymes released from the fungi in retting conditions were shown to correspond to the separation of *Hibiscus sabdariffa* L. fibers.

Key words: *Hibiscus sabdariffa* L., fibers, separation, microbiological process.

INTRODUCTION

Hibiscus sabdariffa L., an ecological plant became a topic of the world due to its characteristics with growth speed quicker than normal plants, powerful absorption of carbon dioxide more than normal plants and strong water purification power. The advantage of kenaf has been known for a long time but its production has gradually decreased because of the development of less expensive items made by industrialized synthetic fibers. Due to its coarseness and stiffness, the traditional products of kenaf are package bag, cordage and ropes, which are low-grade products. The combination with plastic fibers has drawn attention as an alternative in a variety of industries. However, retting or a treatment degrading the pectin-rich middle lamella connecting adjacent fiber cells to release bast fibers is the predominant problem in preparation (Yu and Yu, 2007). Soaking kenaf stem or bark in water is a common way of kenaf fiber extraction. During retting, microbial activity causes a partial degradation of the components that bind tissues together, thereby separating the

cellulosic fibers from non-fiber tissues (Akin et al., 2007). This technique is extremely dependent on the weather and water source, and therefore soaking time varies and is very difficult to control. As a result, a better natural fiber retting method has been sought for some time (Sharma and Faughey, 1999; Akin et al., 2001). Normally, stored premature wood is rapidly attacked by microorganisms, mainly fungi (Lopez et al., 2007). White-rot fungi are fungi belonging to the class Basidiomycetes capable of degrading these wood polymers (Lopez et al., 2007). The use of fungi not only aims at making production more effective but also at an environmentally friendly production (Li et al., 2009). As a result, by a suitably controlled fungal treatment it is possible to apply such a technique for plant fiber separation. WO/2003/080812 on novel white rot fungus and use thereof in wood pretreatment disclosed a novel white rot fungus, *Physisporinus rivulosus* strain T241i, and the biopulping process associated with paper making, utilizing said fungus. The fungus strongly reduced the amount of wood extractives. The first article about application of the white-rot fungi, *Datronia* sp. and *Oligoporous* sp., for specific purposes was in 2006 when Apiwatanapiwat and coworkers (Apiwatanapiwat et al., 2006) isolated *Datronia* sp. and *Oligoporous* sp. from natural

*Corresponding author. E-mail: aappln@ku.ac.th. Tel.: 66-29428599. Fax: 66-25620338

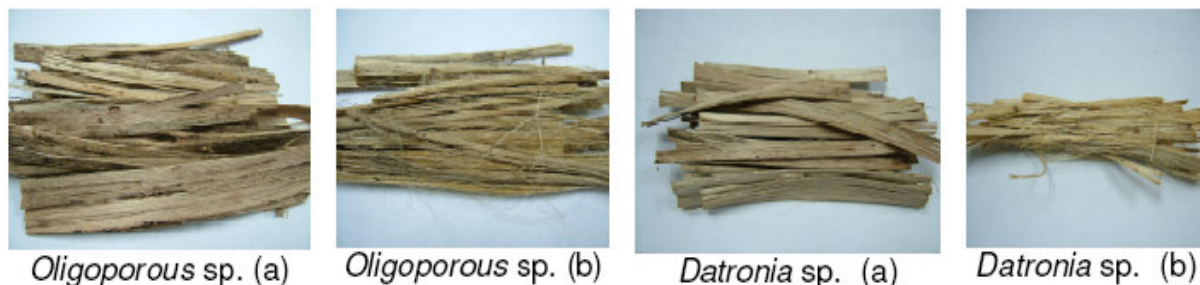


Figure 1. The characteristics of *Hibiscus sabdariffa* L. fibers after being retted by *Oligoporus* sp. and *Datronia* sp. in liquid (a) and solid (b) treatments for 4 weeks.

sources in Thailand. Its ability in decolorization of waste water from pulp and paper mill by decomposing or degrading extractives such as lignin compound was investigated. Li et al. (2008) applied the low cost method, namely bag retting with white-rot fungi, to hemp fiber. The resulting fibers showed an increase in tensile strength. The aim of this study is to evaluate the feasibility of white-rot fungi, *Datronia* sp. and *Oligoporus* sp., in environmentally-friendly, efficient retting processing of *H. sabdariffa* L.

Experimental

Raw material and microbial culture

H. sabdariffa L., which was collected from the Khonkhan province in Thailand, was used in this study. After harvesting, the non-retted green outer barks were separated by hand and dried under sunlight. Before subjecting to the experiments, the barks were sterilized (121°C, 15 min) to get rid of all the contaminants. The cultures of white-rot fungi, *Datronia* sp. and *Oligoporus* sp., were kindly supplied by Apiwattanapiwat et al. (2006), to be used in this study. The fungi were subcultured and maintained in Potato Dextrose Agar (PDA) slants and stored at $5 \pm 1^\circ\text{C}$ until use. To prepare the inoculum, the fungi were transferred onto a fresh PDA plate and incubated at 30°C for 7 days. After that, they were inoculated in Erlenmeyer flasks containing sterilized basal media (g/L: glucose 10.0; K_2HPO_4 1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5; KCl 0.5; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.01; NH_4NO_3 1.75) and incubated at 30°C for 7 days and kept for further experiments.

Fiber separation by liquid-fungal treatment

Sterilized dried non-retted barks were inoculated with the fungal suspension of either *Datronia* sp. or *Oligoporus* sp., and incubated at 30°C in plastic bags to promote the growth for 4 weeks. The amount of fungal inoculum was varied between 5 - 25% on a dry weight basis of barks, different retting period i.e. 1, 2, 3 and 4 weeks under different temperature that is, 30, 40 and 50°C were studied. Soaking wastewater was sampled during the testing period to determine the activity of pectinolytic enzyme. Barks were subjected to sterilization to get rid of all remaining fungi. The fungal-retted *H. sabdariffa* L. fibers were washed with clean water and dried. Evaluation of the retting was based on the visual separation characteristics of the obtained fibers.

Fiber separation by solid-fungal treatment

White-rot fungal inoculums, as mentioned above, were filtered

through Whatman filter paper No.1 to obtain fungal cells. Sterilized dried non-retted barks were inoculated with either *Datronia* sp. or *Oligoporus* sp. cell pellets and incubated at 30°C in plastic bags to promote the growth for 4 weeks. The amount of fungal inoculum varied between 5 - 25% on a dry-weight basis of bark. The retting period (1, 2, 3 and 4 weeks) and temperature (30, 40 and 50°C) were also studied. The appropriate buffer was added into the reaction in order to extract substances produced by the fungi. The retted *H. sabdariffa* L. barks were subjected to sterilization to get rid of all remaining fungi. Activity of pectinolytic enzyme which was released by the fungal strains in the soaking wastewater was quantitatively determined. The resulting *H. sabdariffa* L. fibers were washed with clean water and dried. The evaluation, of the retting degree, based on the visual fiber separation characteristics was determined.

Measurement of pectinolytic enzyme and single fiber tensile testing

Pectinase enzyme was determined as pectate lyase (PAL) activity using the method of Silva *et al.* (1993). The single *H. sabdariffa* L. fibers resulting from each treatment were tensile strength tested in accordance with the ASTM Standard Test Method and Young's Modulus for High-Modulus Single Filament Materials (ASTM D3379-75, 1989) and smell tested according to the instruction of Toyota Motor Asia Pacific Engineering and Manufacturing Company Limited.

RESULTS AND DISCUSSION

The effect of retting parameters on fiber separation by fungal treatment

Figure 1 demonstrated the *Hibiscus sabdariffa* L. fibers obtained from the 4-week incubation with selected fungal strains. It showed that the fibers resulted from the solid treatment were softer and better separated when compared with those from the liquid treatment. This might be due to the solid treatment providing a more preferable growing environment for the fungi (Lopez et al., 2007). It could also be explained that fungal secreted essential for fiber separation were released in sufficient quantity (Yu and Yu, 2007). Figure 2 illustrated the effects of retting time period (1, 2, 3 and 4 weeks) by the selected fungi in the solid treatment on the characteristics of fibers. The results indicated that more fiber separation was obtained when the retting period was longer as also suggested by Yu and Yu (2007). Figure 3 showed the effects of retting



Figure 2. The effects of retting time period (1, 2, 3 and 4 weeks) by *Datronia* sp. and *Oligoporous* sp. at 30°C in the solid treatment on the characteristics of *Hibiscus sabdariffa* L. fibers.

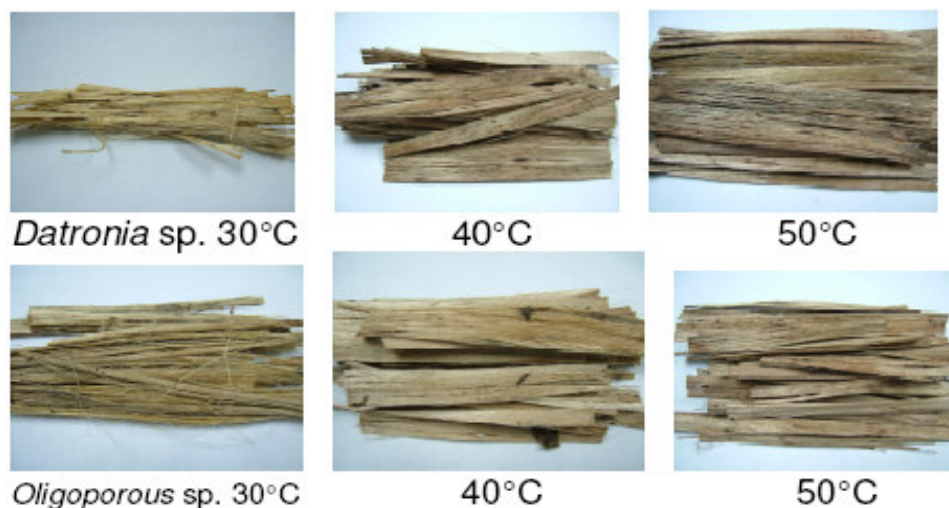


Figure 3. The effects of retting temperature (30, 40 and 50°C) by *Datronia* sp. and *Oligoporous* sp. in the solid treatment for 4 weeks on the characteristics of *Hibiscus sabdariffa* L. fibers.

temperature (30, 40 and 50°C) by the selected fungi in the solid treatment for 4 weeks on the characteristics of fibers. Unsurprisingly, the best fiber separation by both fungi was obtained under the temperature at 30°C. This was also supported by the study of Yu and Yu (2007) in that 30 - 32°C was an appropriate temperature for the retting of kenaf fibers by an unknown isolated fungus. Temperature was one of the most important factors affecting microbe growth. When temperature was too low, the metabolism speed was low or microbes were dormant (Yu and Yu, 2007). While the temperature raised, microbes grew faster. However, if temperature was too high, the growth of microbes would be limited (Yu and Yu, 2007).

Figure 4 showed the effects of fungal inoculum size (5, 10, 15, 20 and 25%) by the selected fungi at 30°C in the solid treatment for 4 weeks on the characteristics of fibers. The results demonstrated that better fiber separation was achieved when the inoculum size increased. This also corresponded to the study by Yu and Yu (2007). However, when too many microbes existed in the retting condition, nutrients would be limited (Yu and Yu, 2007). Apart from the visual characteristics of resulting fibers, the smell of the fibers was also considered for retting efficiency. It demonstrated that the best condition to obtain least smell fibers was achieved by *Datronia* sp. under the solid treatment. In conclusion, the most appro-



Figure 4. The effects of fungal inoculum size (5, 10, 15, 20 and 25%) by *Datronia* sp. and *Oligoporosus* sp. at 30°C in the solid treatment for 4 weeks on the characteristics of *Hibiscus sabdariffa* L. fibers.

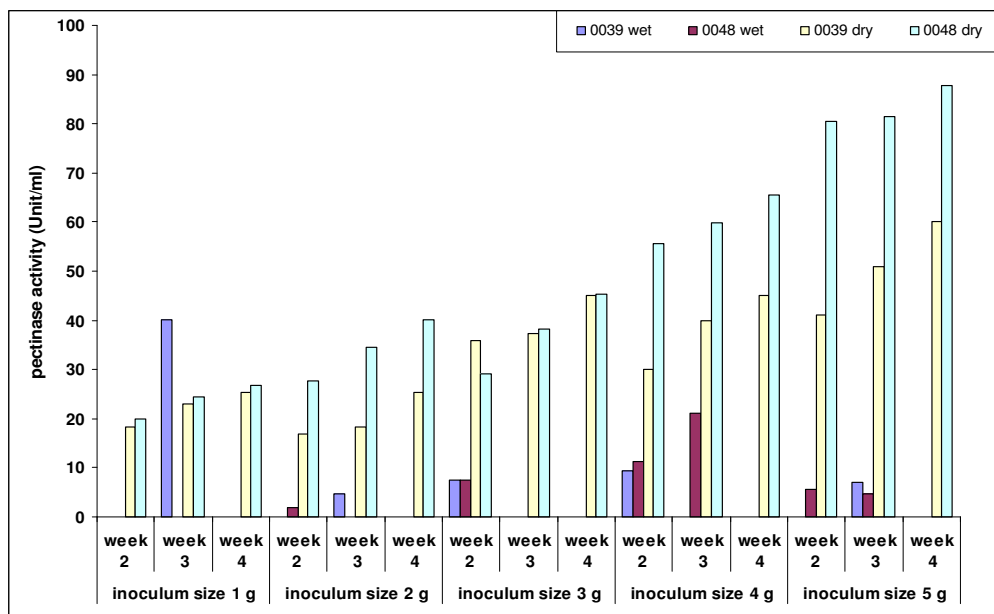


Figure 5. The effects of inoculum size and retting time on released pectinolytic enzymes of *Oligoporosus* sp. and *Datronia* sp. after solid and liquid retting for 4 weeks.

appropriate condition for *H. sabdariffa* L. fiber separation was incubation at 20% *Datronia* sp. at 30°C under the solid treatment for 4 weeks.

Furthermore, the activity of pectinolytic enzymes sampled from the soaking wastewater of each fungal treatment also corresponded to the incubation period and fungal inoculum size (Figure 5). It showed that the activity of pectinolytic enzymes was higher as the fungal inoculum size increased. Likewise, the increase of the incubation period of *H. sabdariffa* L. barks with selected fungi positively resulted in higher pectinolytic enzymes activity. As a result, the increase in pectinolytic enzyme activity was

related to the better efficiency of fiber separation. Thus, the released pectinolytic enzymes from the fungi might play an important role in the separation of *H. sabdariffa* L. fibers. However, the mechanism of these white-rot fungi for fiber separation seemed to be appropriate in only solid treatment which was the natural environment for the fungal growth.

Fiber smell testing

The results from smell test of *H. sabdariffa* L. fibers obtained from variation of the fungal inoculum sizes demon-

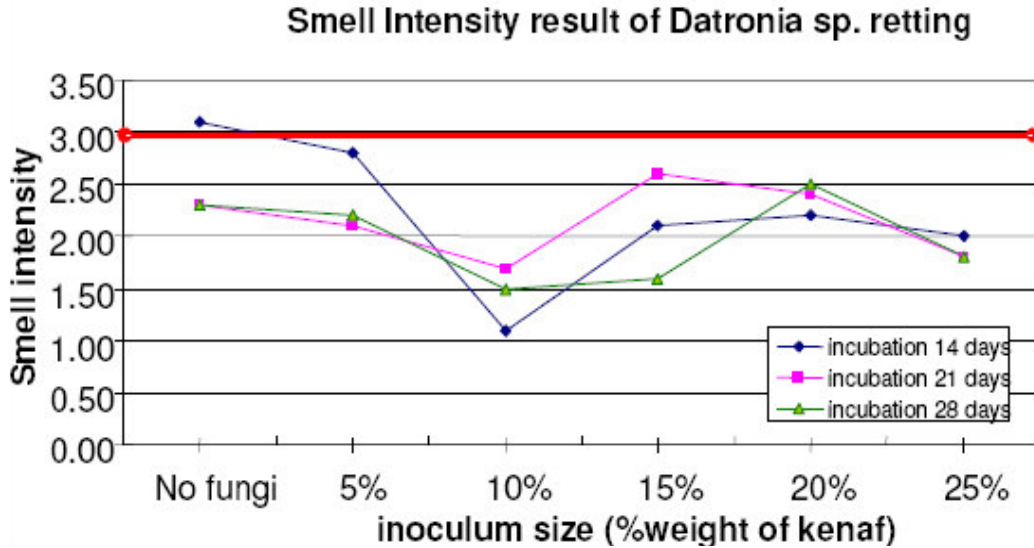


Figure 6. The effect of fungal inoculum size on the smell intensity of retted fibers by *Datronia* sp. at 30°C in solid treatment for 4 weeks.

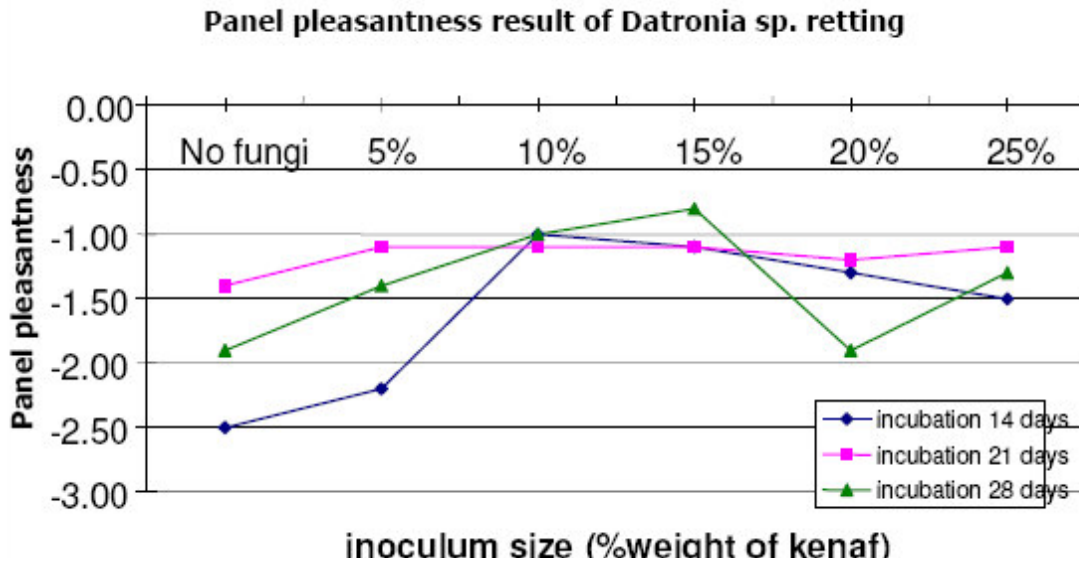


Figure 7. The effect of fungal inoculum size on pleasantness of the panels on retted fibers by *Datronia* sp. at 30°C in solid treatment for 4 weeks.

strated that the solid treatment by *Datronia* sp. produced fibers with most satisfactory of the panels. The comparison between liquid and solid treatments indicated that more pleasantness of the panels was obtained from the solid treatment. The results also illustrated that the fungal inoculum size did not directly affect the smell of retted fibers. Separation of the fibers, at fungal inoculum size 20% gave most fiber separation. The result from solid treatment was summarized and illustrated in Figures 6 - 9. The results showed that the smell intensity of treated fibers decreased as the fungal inoculum size decreased.

The pleasantness of treated fiber smell also varied with the fungal inoculum size. Larger inoculum size the more pleasantness of the panels. It could be concluded that the most optimum condition should be at 20% *Datronia* sp. under the solid treatment.

Conclusion

This work indicates the possibility of using white-rot fungi in *H. sabdariffa* L. fiber separation. The fungal treatment of *H. sabdariffa* L. bark with strain *Datronia* sp. and *Oligo-*

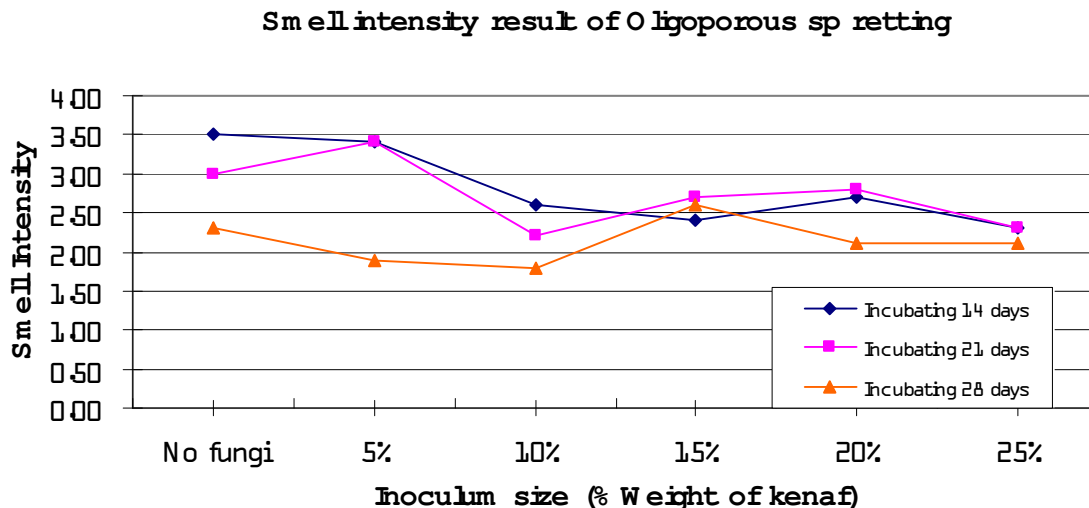


Figure 8. The effect of fungal inoculum size on the smell intensity of retted fibers by *Oligoporous* sp. at 30°C in solid treatment for 4 weeks.

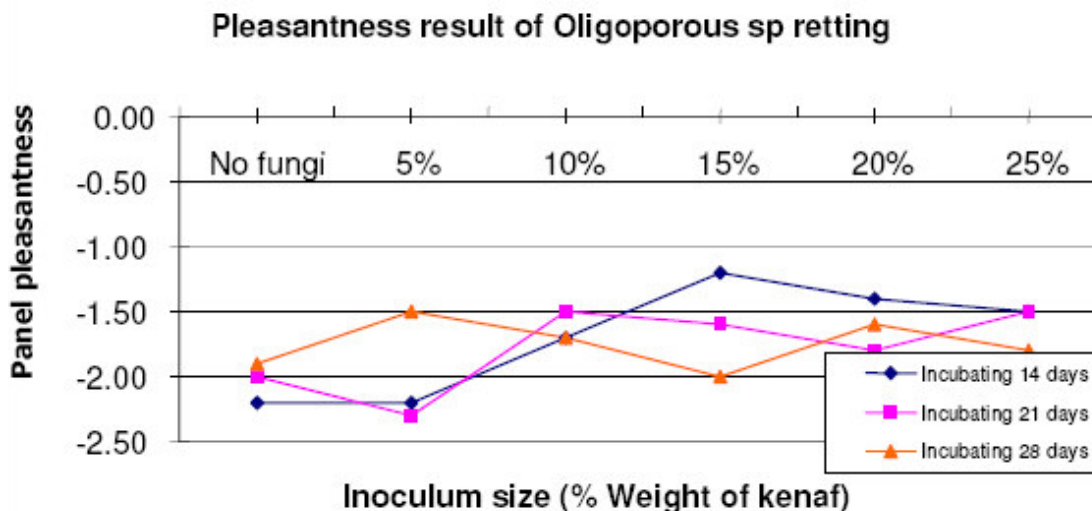


Figure 9. The effect of fungal inoculum size on pleasantness of the panels on retted fibers by *Oligoporous* sp. at 30°C in solid treatment for 4 weeks.

porous sp. caused an increase in the solubility of wood bark extractives such as pectin already after a treatment for 4 weeks as observed in the fiber separation. The increase in the solubility of pectin is likely to indicate that, as a consequence of the fungal treatment, changes occurred in the structure of pectin, promoting the separation of fibers (Akin et al., 2007). These changes are positively correlated with the production of pectinolytic enzyme as shown.

ACKNOWLEDGEMENT

The authors would like to thank Toyota Motor Asia Pacific

Engineering & Manufacturing Company Limited for financial support.

REFERENCES

Akin DE, Foulk JA, Dodd RB, McAlister III DD (2001). Enzyme retting of flax and characterization of processed fibers. *J. Biotechnol.* 89: 193-203.
 Akin DE, Condon B, Sohn M, Foulk JA, Dodd RB, Rigsby LL (2007). Optimization of enzyme-retting of flax with pectate lyase. *Ind. Crops Prod.* 25: 136-146.
 Apiwattanapiwat W, Siriacha P, Vaithanomsat P (2006). Screening of fungi for decolorization of wastewater from pulp and paper industry. *Kasetsart J. Natr. Sci.* 40(5): 215-221.
 Evans JD, Akin DE, Foulk JA (2002). Flax-retting by polygalacturonase

- containing enzyme mixtures and effects on fiber properties. *J. Biotechnol.* 97: 223-231.13
- Li Y, Pickering KL, Farrell RL (2008). Analysis of green hemp fibre reinforced composites using bag retting and white rot fungal treatments. *Ind. Crops Prod.* 29: 420-426.
- Lopez MJ, Carmen MD, Garcia V, Suarez-Estrella F, Nichols NN, Dien BS, Moreno J (2007). Lignocelulose-degrading enzymes produced by the ascomycete *Coniochaeta ligniaria* and related species: Application for alignocellulosic substrate treatment. *J. Enzyme Microbial Technol.* 40: 794-800.
- Sharma HSS, Faughey GJ (1999). Comparison of subjective and objective methods to assess flax straw cultivars and fibre quality after dew-retting. *Ann. Appl. Biol.* 135: 495-501.
- Silva DO, Attwood MM, Tempest DW (1993). Partial purification and properties of pectin lyase from *Penicillium expansum*. *World J. Microbiol. Biotechnol.* 9: 574-578.
- Yu H, Yu C (2007). Study on microbe retting of kenaf fiber. *Enzyme and Microbial Technology* 40: 1806-1809.
- Zhang J, Henriksson G, Johansson G (2000). Polygalacturonase is the key component in enzymatic retting of flax. *J. Biotechnol.* 81: 85-89.