

## CHAPTER 4

### CONCLUSIONS

113 isolates from 164 basidiocarps could be isolated as pure culture. Forty-two isolates were able to show the capability to grow at 37°C and 9 isolates were capable to degrade Poly R-478 as well as *Phanerochaete chrysosporium* ATCC 34541.

Glucose showed the enhancing effect on Poly R-478 decoloration for some isolates. Lignin powder was impossible to be used as an indicator in plate assay for screening of ligninolytic fungi and could not replace of the well-known indicator, Poly R-478 by lignin powder.

The decoloration of Poly R-478 was suitable for screening of laccase producing strains.

The new isolate NP21 was identified to be *Lenzites* sp., was the most attractive candidate to be laccase producer and was selected for further studies.

The optimal conditions for laccase production from the new isolate NP21 were cultivation on rubber wood chips containing 4% (w/v) peptone at 37°C for 5 d. The enzyme activity was found up to 5.69 unit/g substrate. In the presence of 150 µM copper sulfate, the production of laccase was increased up to 9.43 unit/gram substrate.

The laccase obtained from isolate NP21 could be purified through CM-cellulofine column, DEAE-cellulose column and Sephadex G-100 gel filtration column, respectively. The purification factor was up to 893 folds and 49.6% recovery yield. The molecular weight of the purified laccase was 65 kDa.

Some properties of the purified laccase were found that pH 4.0 was optimal pH; it was inactivated at 45°C and above. The half-life of heat inactivation at 65°C was 33 min. DMP was the specific substrate for the purified enzyme from this isolate which gave the highest laccase activity. The enzyme was completely inhibited by HgCl<sub>2</sub> and NaN<sub>3</sub>. Poly R-478 and lignin powder were potentially significant inhibitors for laccase activity.

For the preliminary experiment of eucalyptus oxygen-delignifying kraft pulp (ODKP) bleaching, a mediator as HBT was possible to be an important factor for pulp biobleaching by laccase.

Although the brightness of ODKP bleaching by laccase was not so marked, these results indicated that the application of laccase in pulp and paper industry was not so far to be a promising alternative method to solve or prevent public health and environmental problems, which cause by pulp and paper mills. To date, this application of laccase is continued to improve and develop the possibility to use in paper producing industry for replacement of the traditional method like xylanases, which is expected to be the practical uses in the near future.