Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioresource Technology 97 (2006) 171–177
BIORESOURCE TECHNOLOGY

Productivity of laccase in solid substrate fermentation of selected agro-residues by *Pycnoporus sanguineus*

S. Vikineswary [a,*], Noorlidah Abdullah [a], M. Renuvathani [b], M. Sekaran [c], A. Pandey [d], E.B.G. Jones [e]

[a] *Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[b] *Institute of Postgraduate Studies, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[c] *Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[d] *Biotechnology Division, Regional Research Laboratory, CSIR, Trivandrum 695 019, India*
[e] *National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Phaholyothin Rd, Klong 1, Klong Luang, Pathumthani 12120, Thailand*

Received 31 January 2005; received in revised form 31 January 2005; accepted 2 February 2005
Available online 20 June 2005

Abstract

A comparative study on solid substrate fermentation (SSF) of sago 'hampas', oil palm frond parenchyma tissue (OPFPt) and rubberwood sawdust with *Pycnoporus sanguineus* for laccase production was carried out. Optimal mycelial growth of *Pyc. sanguineus* was observed on all the substrates studied over a 21 days time-course fermentation. Laccase productivity was highest during degradation of sago 'hampas' and OPFPt and a range from 7.5 to 7.6 U/g substrate on the 11th day of fermentation compared to degradation of rubberwood sawdust with a maximum laccase productivity of 5.7 U/g substrate on day 11 of SSF. Further optimization of laccase production was done by varying the inoculum age, density and nitrogen supplementation. SSF of OPFPt by *Pyc. sanguineus* gave maximum productivity of laccase of 46.5 U/g substrate on day 6 of fermentation with a 30% (w/w) of 4 weeks old inoculum and 0.92% nitrogen in the form of urea supplemented in the substrate.

The extraction of laccase was also optimized in this study. Recovery of laccase was fourfold higher at 30.6 U/g substrate on day 10 of SSF using unadjusted tap water at pH 8.0 as extraction medium at 25 ± 2 °C compared to laccase recovery of 7.46 U/g substrate using sodium acetate buffer at pH 4.8 at 4 °C. Further optimization showed that laccase recovery was increased by 50% with a value of 46.5 U/g substrate on day 10 of SSF when the extraction medium was tap water adjusted to pH 5.0 at 25 ± 2 °C.

Keywords: Laccase; *Pycnoporus sanguineus*; Oil palm frond parenchyma tissue; Solid substrate fermentation

1. Introduction

In Malaysia, lignocellulosic agroindustrial residues are abundant and readily available (Vikineswary et al., 1997). The palm oil processing industry, sago starch processing industries as well as industries that produce rubber wood products are producing large amounts of residues. Currently, these agroresidues are either allowed to decay naturally in the fields or burnt. These residues are, however, potential substrates for microbial conversion via solid substrate fermentation into value-added products such as enzymes (Pandey et al., 2000). In an oil palm plantation approximately 11 tonnes of pruned fronds are generated per hectare of mature oil palm per year. The felling of old palms yields about 115.4 kg dry frond matter per palm, too. About 35% of the oil palm frond is oil palm parenchyma tissue (OPFPt) and it has been reported to be suitable for

* Corresponding author. Fax: +60 3 79674178.
E-mail address: viki@um.edu.my (S. Vikineswary).

0960-8524/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.biortech.2005.02.015

production of laccase via SSF using *Pleurotus sajor-caju* (Ling, 1994). Sago 'hampas' is a starchy lignocellulosic by-product generated from pith of *Metroxylon sagu* (sago palm) after starch extraction. In Sarawak, in the Sibu Division alone about 50–110 tonnes of sago hampas are produced daily. 'Hampas' contains approximately 66% starch and 14% fibre on a dry weight basis of which about 25% is made up of lignin (Chew and Shim, 1993). Kumaran et al. (1997) had reported that sago 'hampas' can be used as substrate for the production of laccase via SSF using *Ple. sajor-caju*. In Malaysia, rubberwood sawdust is used to cultivate *Ple. sajor-caju* on a commercial scale and the spent compost is an optimal source of lignin modifying enzymes (Avneesh et al., 2003).

Laccases are able to depolymerize lignin (Kawai et al., 1999) and delignify wood pulps (Bourbonnais et al., 1997) when they are combined with various low molecular weight electron transfer agents. Hence, laccase is potentially applicable for pulp bleaching (Srebotnik and Hammel, 2000) and detoxification of phenolic pollutants (Collins et al., 1996; Johannes et al., 1996; Hublik and Schinner, 2000). *Pycnoporus cinnabarinus* and *Pyc. sanguineus* have been reported to produce laccase as the sole lignolytic enzyme (Eggert et al., 1996; Sigoillot et al., 1999; Pointing, 2001) and this is beneficial for industrial applications, as purification steps are both costly and time consuming.

The aims of this study were (a) to assess the potential of selected agroresidues for laccase production via SSF using *Pyc. sanguineus*, (b) to optimize extraction and recovery of enzymes from SSF and (c) to optimize the inoculum age and size and nitrogen supplementation of the substrate for laccase production.

2. Methods

2.1. Fungal source

The stock cultures of *Pyc. sanguineus* CY788 were maintained on potato dextrose agar (PDA) slants at 4 °C. The fungus was transferred to PDA plates and incubated for seven days at 27 °C before koji development using autoclaved precooked whole wheat grains.

2.2. Selection of agro-residues as substrate for laccase productivity

The three substrates selected for this study were sago 'hampas', rubberwood sawdust, and OPFPt. Sago 'hampas' was collected from Hup Guan Sago Factory in Johor, OPFPt was collected from United Plantations Sdn. Bhd. in Teluk Intan, Perak, and rubberwood sawdust was collected from a mushroom farm in Semenyih, Malaysia.

Solid substrate fermentation of sago 'hampas' and rubberwood sawdust were followed according to Kumaran et al. (1997). Ten grams of sterilized sago 'hampas' and rubberwood sawdust were supplemented with nutrient solution containing (w/v): 0.2% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$ and 0.38% nitrogen (N) in the form of filter sterilized urea. Solid substrate fermentation of OPFPt was according to Ling (1994). Ten grams of sterilized OPFPt was supplemented with 1% $CaCO_3$ and 0.46% N in the form of filter sterilized urea. The moisture content for each substrate was adjusted within the range of 75–85% (v/w). The contents of the flasks were inoculated with 10% (approximately 2.35 ± 0.05 g wet weight) of *Pyc. sanguineus* whole wheat grain inoculum and incubated at 25 ± 2 °C in static condition. The fermentation was carried out for 21 days and at suitable time intervals, three culture flasks of each substrates were randomly sampled for laccase assay.

The contents of each flask were extracted with 100 ml of cold 50 mM sodium citrate buffer (pH 4.8). A spatula was used to break the solid culture into smaller particles. The flasks were subsequently transferred to an incubator shaker at 4 °C and 200 rpm for approximately 18 h (Kumaran et al., 1997). After shaking, the contents were centrifuged at 9000 rpm for 20 min. The crude culture filtrate containing fungal enzymes were stored at −20 °C for 24 h prior to laccase assays.

2.3. Laccase assay

Laccase activity was determined by the increase in the absorbance due to the formation of tetramethoxy-azobis methylenequinone from the reaction of laccase with syringaldazine (Harkin and Obst, 1973; Leonowicz and Grzywnowicz, 1981). One unit (U) was defined as the amount of enzyme producing one unit change in absorbance/min at λ = 525 nm.

2.4. Optimization of extraction and recovery of laccase

OPFPt was chosen for further studies as laccase productivity was the highest among the substrates investigated. Solid substrate fermentation of OPFPt was carried out as described in the previous section and at suitable time intervals, the optimum method of laccase extraction was determined. The above extraction method was modified by changing the incubation temperature to 4 °C and 25 °C and using sodium acetate buffer at pH 4.8 or tap water having pH 8.0 as the extraction medium.

Laccase extraction of OPFPt fermented for 10 days using *Pyc. sanguineus* was further optimized by varying the pH of the best extraction medium and using the best incubation temperature obtained in the previous section. The pH levels investigated were 4, 5, 6, 7 and 8.

2.5. Optimization of laccase productivity by SSF of OPFPt by varying inoculum age and density and urea levels

Solid substrate fermentation of OPFPt was carried out as described in the previous section but with varying inoculum ages of 2 weeks, 4 weeks and 6 weeks. The SSF was then repeated using the optimum inoculum age at a density of 10%, 20% and 30%. Laccase was then extracted using the optimum extraction medium at optimum pH and using the optimum incubation temperature determined previously.

Further studies of SSF was done using the optimum inoculum age and density with varying levels of nitrogen supplementation in the substrate. The level of urea tested were 0.46%, 0.69%, 0.92% and 1.15% N. Laccase was extracted using the optimum extraction medium at optimum pH and temperature determined previously.

2.6. Statistical analysis

In all the experiments described in this study triplicates were set up for each parameter tested. The design was completely randomized and sampling was random. The means of three replicate values for all data in the experiments obtained were tested in a one-way analysis of variance using the Statgraf programme.

3. Results and discussion

3.1. Selection of solid substrate for laccase production

All three agro-residues tested supported good growth and laccase productivity. The first signs of growth was seen two to three days after inoculation. As the culture grew older, the colour of the mycelia changed from white to reddish orange and complete colonization of the fungus was seen within 11 days of fermentation.

The profile of laccase productivity during degradation of various agro-residues by *Pyc. sanguineus* is given in Fig. 1. There was rapid increase in laccase productivity during SSF in both 'hampas' and OPFPt during the first 11 days of fermentation with maximum laccase productivity of 7.6 U/g 'hampas' and 7.5 U/g OPFPt on day 11. However, a rapid decline in laccase productivity was observed during SSF of 'hampas' which reduced to 3.1 U/g substrate at the end of 21 days of fermentation. There was, however, sustained laccase productivity during SSF of OPFPt after day 11 till day 15. After day 15, laccase productivity reduced but at a lower rate which gave 5.4 U of laccase per gram of substrate on day 21. Degradation of rubberwood sawdust produced maximum laccase of only 5.7 U/g substrate on day 11.

The highest laccase productivity during SSF of hampas using *Pyc. sanguineus* was lower compared to the

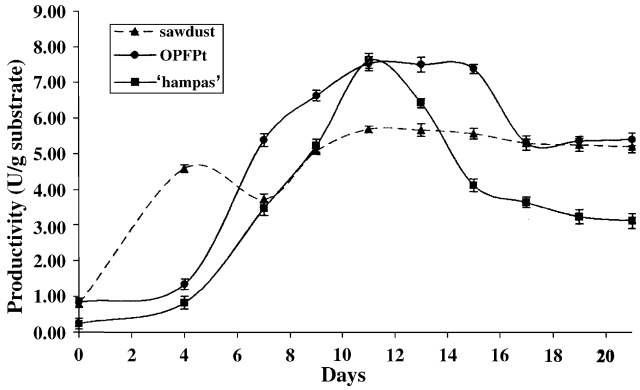

Fig. 1. Laccase productivity during SSF of various substrates using *Pyc. sanguineus*. SSF conditions: 18-days old inoculum, 10% inoculum density, 25 ± 2 °C incubation.

reported value of 10.6 U/g hampas using *Ple. sajor-caju* (Kumaran et al., 1997). Maximum laccase productivity using *Pyc. sanguineus* was also lower compared to degradation of OPFPt using *Ple. sajor-caju* which yielded maximum of 16.9 U/g substrate on day 10 as reported by Ling (1994).

However, previous studies among species of *Pycnoporus* in liquid fermentation are sparse and showed varied responses. This species has been reported to produce 22 U/l (0.022 U/ml) in a non-induced liquid growth medium and optimum induced laccase production was 1368 U/l (Pointing et al., 2000), which was higher than that reported for many other basidiomycetes cultured under similar conditions (Orth et al., 1993; Kantelinen et al., 1989; Srinivasan et al., 1995). However, it was not clear if such culture conditions were optimal for every fungi. Conversely few species are also reported to produce laccase at greater levels than those recorded for *Pyc. sanguineus* by Pointing et al. (2000) under similar growth conditions. Notable exceptions are *Trametes versicolor* (5000 U/l) (Collins and Dobson, 1997) and *Ple. sajor-caju* (4000 U/l) (Buswell et al., 1996). Further, *Pyc. cinnabarinus* has been reported to produce laccase at higher levels compared to *Pyc. sanguineus* under similar growth conditions (Eggert et al., 1996) but laccase from *Pyc. coccineus* showed relatively lower levels (Oda et al., 1991).

Pointing et al. (2000) suggested that *Pyc. sanguineus* may be grown effectively on agricultural lignocellulose residue as laccase production increased by 12 fold by the addition of wood fibres to liquid cultures without inducers. This value was also reported to be 3 fold higher than those reported for *Pyc. sanguineus* grown on *Eucalyptus grandis* wood chips. In this unoptimised study using *Pyc. sanguineus*, laccase productivity in the selected agro-residues at 5.7–7.6 U/g was higher compared to reported studies using this specie. Further, laccase productivity was high in OPFPt (7.5 U/g) compared to sawdust (5.7 U/g) and this high level was produced

for a longer period i.e. until day 15 compared to sago 'hampas' (7.6 U/g) which immediately dropped after day 11. Hence, OPFPt was selected as the substrate for laccase productivity by *Pyc. sanguineus* and the SSF was optimized.

3.2. Optimization of extraction of laccase

Laccase recovery from fermented OPFPt using different extraction temperature and medium are shown in Fig. 2. At day 10, laccase recovery was four times higher at 30.6 U/g substrate when extracted with tap water having pH 8.0 and a temperature of 25 °C compared to the recovery of 7.46 U/g substrate using sodium acetate buffer at pH 4.8 and 4 °C. However, there was no significant difference ($p < 0.001$) in laccase recovery by extraction with tap water (pH 8.0) and sodium acetate buffer (pH 4.8) extracted at 25 °C. The four types of extraction conditions tested had significant effects on laccase recovery with varying incubation time. Studies showed that laccase of *Pyc. sanguineus* from liquid growth medium was very stable at 4 °C compared to 35 °C (Pointing et al., 1998). However, optimum temperature for laccase activity of *Ple. ostreatus* was 30–35 °C (Youn et al., 1995). Similarly, optimum laccase production by *Polyporus sanguineus* (Sandhu and Arora, 1984) and *Pyc. cinnabarinus* (Schliephake et al., 2000) was carried out at 37 °C. Laccase production from *Tra. versicolor* was also successfully enhanced in a study done at 27 °C (Lee et al., 1999). Most proteins exhibit normal increased solubility with temperature elevation. Because enzymes generally show normal temperature dependence, temperature change was not often used to fractionally precipitate them. However, the differences in enzyme stability at higher temperatures are quite marked.

Laccase from *Pyc. sanguineus* was reported to have higher activity in glycine–HCL buffer at pH 3.0 compared to sodium tartrate or sodium acetate buffers at the same pH (Pointing et al., 2000). Stability of laccase activity in buffers with variable pH values showed laccase activity decreasing in the order: phosphate-buffer > sodium-acetate-buffer > citrate-buffer in a study done using *Ple. ostreatus* (Hublik and Schinner, 2000). This study showed that extraction of crude enzyme using tap water at 25 ± 2 °C gave high yields of laccase. Thus, extraction with tap water at 25 ± 2 °C was chosen as the extraction method and further studies were carried out to optimize enzyme recovery in tap water at various pH levels. In this study, the stability of laccase in tap water and the effect of storage, if any, was not investigated.

On day 10 of SSF, laccase recovery was 50% higher at 46.5 U/g substrate using tap water at pH 5.0 as extraction medium at 25 ± 2 °C compared to the recovery of 30.6 U/g substrate obtained using tap water at pH 8.0 (Fig. 3). There was however, no significant difference ($p < 0.001$) in laccase recovery between pH 5.0 and pH 4.0. However, the various level of pH tested caused a significant difference ($p < 0.001$) in laccase recovery with incubation time.

Laccase recovery seemed to be more enhanced in an acidic condition compared to alkaline medium (pH 7 and 8). Several workers have also reported that pH optimum for laccase activity is in the acidic region (Bollag and Leonowicz, 1984; Pointing et al., 2000). Elsewhere, optimum laccase activity from *Pyc. cinnabarinus* was achieved between pH 4.4 and 5 (Eggert et al., 1996; Schliephake et al., 2000). The low pH value has also been reported to be favorable for the release of laccase by *Pol. sanguineus* (Sandhu and Arora, 1984). It was suggested that the recovery at low pH may be physiologically more significant and depends on the natural habitats of the laccase producing fungi. Fungi growing in acidic environments come in contact with various acidic plant phenols or pesticides, and the lower pH optima of

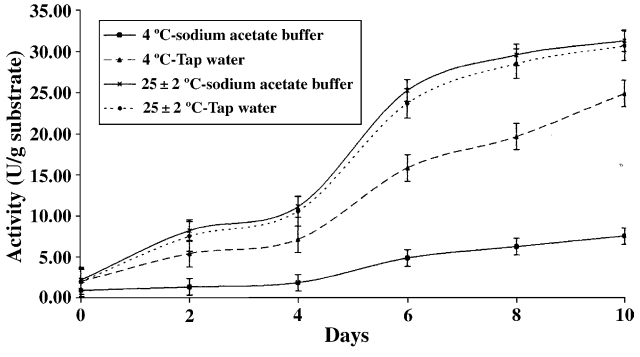

Fig. 2. The effect of extraction temperature and medium on laccase recovery in SSF of OPFPt using *Pyc. sanguineus*. SSF conditions: 18 days old inoculum, 10% inoculum density, 0.46% N, 25 ± 2 °C incubation temperature.

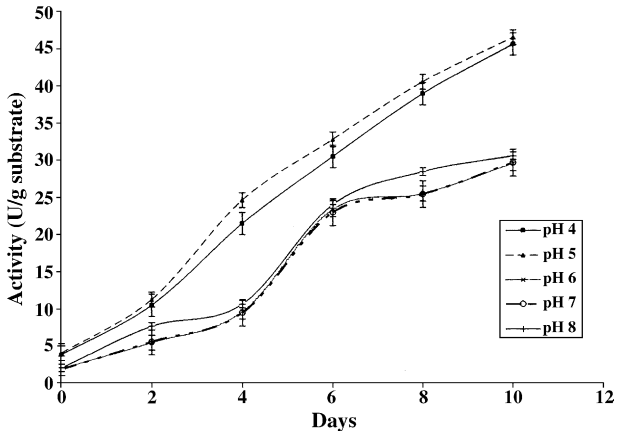

Fig. 3. Effect of pH of tap water medium on laccase recovery from fermented OPFPt using *Pyc. sanguineus*. SSF conditions: 18 days old inoculum, 10% inoculum density, 0.46% N, 25 ± 2 °C incubation temperature.

the laccase allows a more effective oxidation of toxic compounds (Leonowicz et al., 1978). However, maximum laccase activity of *Ple. ostreatus* was obtained at pH 5.8 and this activity has been preserved in extreme alkaline environment of pH 10 which is very unusual and has not yet described for other laccases (Hublik and Schinner, 2000).

3.3. Optimization of laccase productivity during SSF of OPFPt by *Pyc. sanguineus*

Laccase activity during SSF of OPFPt using different inoculum age and density are shown in Figs. 4 and 5 respectively. The inoculum age had a significant effect on the laccase productivity ($p < 0.05$). A similar trend was observed for all levels of inoculum tested. There was a gradual rise in laccase productivity which peaked at day 10 of fermentation for all the three different ages of inoculum (Fig. 4). However, SSF using 4 weeks old inoculum had the highest laccase productivity of 46.6 U/g substrate at day 10 compared to 2 weeks and 6 weeks old inoculum. This maximum laccase productivity, however, was not different from the previous SSF done using 18 days old inoculum with optimised extraction technique at day 10. However, when a 6 weeks old inoculum was used, the laccase productivity was 45% lower at day 10 compared to 4 weeks old inoculum. Optimum laccase productivity using a 4 weeks old inoculum was also reported by Kumaran et al. (1997) and Ling (1994) using *Ple. sajor-caju* from SSF of sago 'hampas' and OPFPt respectively.

Laccase activity increased more rapidly with 30% inoculum density and peaked at day 8 with 48.7 U/g substrate compared to 10% and 20% densities which peaked at day 10 (Fig. 5). However, there was a decline

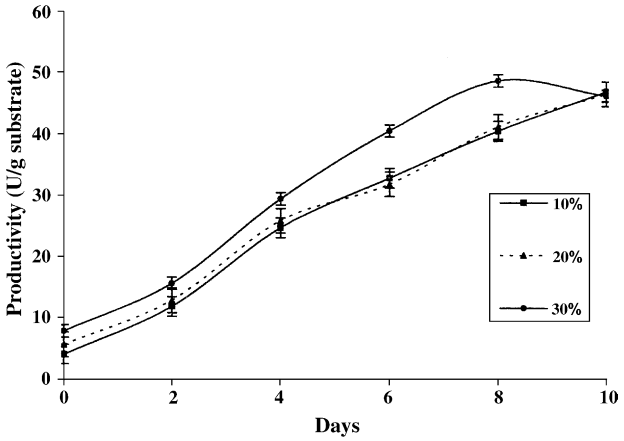

Fig. 5. Effect of various inoculum density on laccase productivity during SSF of OPFPt using *Pyc. sanguineus*. SSF conditions: 4 weeks old inoculum, 0.46% N supplementation, 25 ± 2 °C incubation temperature. Extraction method: tap water at pH 5 and 25 ± 2 °C temperature.

in laccase activity after day 8 with 30% inoculum density and this may be due to enzyme inhibition (Bastawde, 1992). Similar trend was reported by Kumaran et al. (1997) using *Ple. sajor-caju* on sago 'hampas'.

A gradual increase in laccase productivity with all four levels of nitrogen tested during the first four days of fermentation was observed (Fig. 6). Maximum yield was observed much earlier in fermentation using 0.92% N and 1.15% N on day 6 compared to day 8 of SSF for 0.46% N and 0.69% N. However, maximum laccase productivity of 46.5 U/g substrate was obtained with 0.92% N which was maintained till day 10. There was however, a decline laccase productivity with supplementation of 1.15 N which dropped to 37.9 U/g substrate after day 6 of fermentation. An increase in

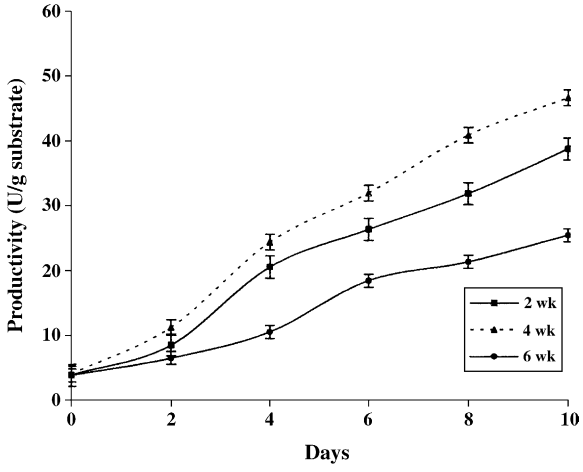

Fig. 4. Effect of various ages of inoculum on laccase productivity during SSF of OPFPt using *Pyc. sanguineus*. SSF conditions: 10% inoculum density, 0.46% N supplementation, 25 ± 2 °C incubation temperature. Extraction method: tap water at pH 5 and 25 ± 2 °C temperature.

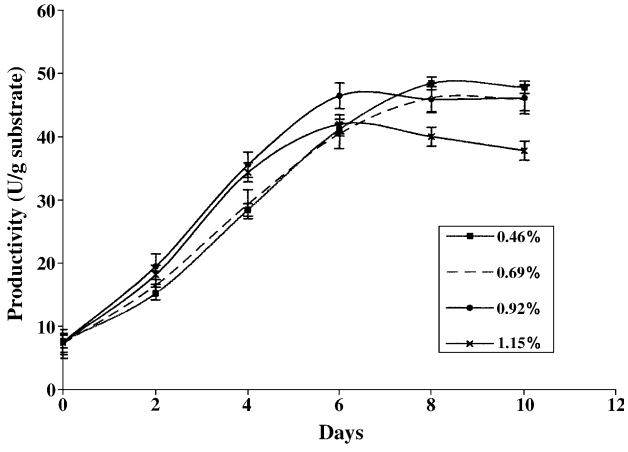

Fig. 6. Effect of various level of nitrogen supplementation of on laccase productivity during SSF of OPFPt using *Pyc. sanguineus*. SSF conditions: 4 weeks old inoculum, 30% inoculum density, 25 ± 2 °C incubation temperature. Extraction method: tap water at pH 5 and 25 ± 2 °C temperature.

nitrogen content had a significant effect ($p < 0.01$) on laccase production. It was also reported that urea stimulated fungal growth when it made up to 40–50% of total nitrogen in the substrate (Raimbault and Alazard, 1980). There was a reduction in laccase productivity after the peak, and this may be due to inhibition which suggests the existence of an optimum nitrogen level for enzyme production by *Pyc. sanguineus*. Optimum laccase production of 1368 U/l by *Pyc. sanguineus* was reported by Pointing et al. (2000) in a culture condition of high carbon and low nitrogen medium. Slight repression of laccase production was also noticed at high nitrogen levels. Similarly, laccase of *Pyc. cinnabarinus* was also reported to be slightly repressed by high nitrogen levels (24 mM) (Eggert et al., 1996). In contrast, laccase activity was only detectable in *Pha. chrysosporium* under a high nitrogen level (24 mM) (Srinivasan et al., 1995). This study also showed slight repression in laccase productivity at a high nitrogen level of 1.15%.

4. Conclusions

Pycnoporus sanguineus was the chosen fungi in this study as suggested and supported by the study done by Pointing et al. (2000). This study revealed high laccase productivity during SSF of sago 'hampas' and OPFPt compared to sawdust with maximum activity of 7.60 U/g substrate, 7.52 U/g substrate and 5.68 U/g substrate respectively. Laccase recovery was fourfold higher using extraction with tap water at pH 8.0 and at 25 ± 2 °C compared to extraction with sodium citrate buffer at 4 °C. Although extraction using tap water with adjusted pH of 5 gave higher enzyme recovery, the stability of the enzyme has yet to be studied. The yield of laccase was about eightfold higher using a 30% (w/w) of 4 weeks old inoculum with 0.92% (v/w) of nitrogen supplementation of the substrate compared to the initial cultivation conditions of 10% of 18 days old inoculum and 0.46% N level of substrate.

Acknowledgement

We would like to thank Ministry of Science, Technology and Environment for their generous grant: IRPA 09-02-03-675 and University of Malaya for this study.

References

Avneesh, D.S., Abdullah, N., Vikineswary, S., 2003. Optimization of extraction of bulk enzymes from spent mushroom compost. Journal of Chemical Technology and Biotechnology 78, 743–752.

Bastawde, K.B., 1992. Xylan structure, microbial xylanases, and their mode of action. World Journal of Microbiology and Biotechnology 8, 353–368.

Bollag, J.M., Leonowicz, A., 1984. Comparative studies of extracellular fungal laccases. Applied Environmental Microbiology 48, 849–854.

Bourbonnais, R., Paice, M.G., Freiermuth, B., Bodie, E., Borneman, S., 1997. Reactivities of various mediators and laccases with kraft pulp and lignin model compounds. Applied Environmental Microbiology 63, 4627–4632.

Buswell, J.A., Cai, Y.J., Chang, ST., Perberdy, J.F., Fu, S.Y., Yu, H.S., 1996. Lignocellulolytic enzyme profiles of edible mushroom fungi. World Journal of Microbiology and Biotechnology 12, 537–542.

Chew, T.Y., Shim, Y.L., 1993. Management of sago processing wastes. In: Yeoh, B.G. et al. (Eds.), Waste Management in Malaysia: Current status and prospects for bioremediation. A monograph prepared by the Environmental Biotechnology Research Group of the National Working Group on Biotechnology. Ministry of Science, Technology and the Environment, Kuala Lumpur, Malaysia, pp. 159–167.

Collins, P.J., Dobson, A.D.W., 1997. Regulation of gene transcription in *Trametes versicolor*. Applied Environmental Microbiology 63, 3444–3450.

Collins, P.J., Kotterman, M.J.J., Field, J.A., Dobson, A.D.W., 1996. Oxidation of anthracene and benzo[*a*]prene by laccase from *Trametes versicolor*. Applied Environmental Microbiology 62, 4563–4567.

Eggert, C., Temp, U., Eriksson, K.E., 1996. The lignolytic system of the white rot fungus *Pycnoporus cinnabarinus*: purification and characterization of the laccase. Microbiology 62, 1151–1158.

Harkin, J.M., Obst, J.R., 1973. Syringaldazine, an effective reagent for detecting laccase and peroxidase in fungi. Experientia 29 (4), 381–387.

Hublik, G., Schinner, F., 2000. Characterization and immobolization of the laccase from *Pleurotus ostreatus* and its use for continuous elimination of phenolic pollutants. Enzyme and Microbial Technology 27, 330–336.

Johannes, C., Majeherczyk, A., Hutterman, A., 1996. Degradation of anthracene by laccase of *Trametes versicolor* in the presence of different mediator compounds. Applied Microbiology and Biotechnology 46, 313–317.

Kantelinen, A., Hatakka, A., Viikari, L., 1989. Production of lignin peroxidase and laccase by *Phlebia radiata*. Applied Microbiology and Biotechnology 31, 234–239.

Kawai, S., Asukai, M., Ohya, N., Okita, K., Ito, T., Ohashi, H., 1999. Degradation of a non-phenolic β-O-4 substructure of *Coriolus versicolor* in the presence of 1-hydroxybenzotriazole. FEMS Microbiology Letters 170, 51–57.

Kumaran, S., Sastry, C.A., Vikineswary, S., 1997. Laccase, cellulase and xylanase activities during growth of *Pleurotus sajor-caju* on sago 'hampas'. World Journal of Microbiology and Biotechnology 13, 43–49.

Lee, I.-Y., Jung, K.-H., Lee, C.H., Park, Y.H., 1999. Enhanced production of laccase in *Trametes versicolor* by the addition of ethanol. Biotechnology Letters 21, 965–968.

Leonowicz, A., Grzywnowicz, K., 1981. Quantitative estimation of laccase forms in some white-rot fungi using syringaldazine as a substrate. Enzyme and Microbial Technology 3, 55–58.

Leonowicz, A., Trojanowski, J., Orlicz, B., 1978. Induction of laccase in basidiomycetes: apparent activity of the inducible and constitutive forms of the enzymes with phenolic substrates. Acta Biochimia Poloniae 25, 369–378.

Ling, S.K., 1994. Biochemical changes associated with growth of *Pleurotus sajor-caju* on oil palm frond parenchyma tissue. Master of Biotechnology Thesis. University of Malaya.

Oda, Y., Adachi, K., Aita, I., Ito, M., Aso, Y., Igarashi, H., 1991. Production and properties of laccase secreted by *Pycnoporus coccineus*. Agricultural Biology and Chemistry 55, 1393–1395.

Orth, A.B., Royce, D.J., Tien, M., 1993. Ubiquity of lignin degrading peroxidases among various wood-degrading fungi. Applied Environmental Microbiology 59, 4017–4023.

Pandey, A., Soccol, C.R., Mitchell, D., 2000. New developments in solid state fermentation. I. Bioprocesses and products. Process Biochemistry 35, 1153–1169.

Pointing, S.B., 2001. Feasibility of bioremediation using white-rot fungi. Applied Microbiology and Biotechnology 57, 20.

Pointing, S.B., Jones, E.B.G., Vrijmoed, L.L.P., 2000. Optimization of laccase production by *Pycnoporus sanguineus* in submerged liquid culture. Mycologia 92, 139–144.

Pointing, S.B., Vrijmoed, L.L.P., Jones, E.B.G., 1998. A qualitative assessment of lignocellulose degrading enzyme distribution in marine fungi. Botanica Marina 41, 293–298.

Raimbault, M., Alazard, D., 1980. Culture method to study fungal growth in solid fermentation. European Journal of Applied Microbiology and Biotechnology 9, 199–209.

Sandhu, D.K., Arora, D.S., 1984. Laccase production by *Polyporus sanguineus* under different nutritional and environmental conditions. Experientia 41, 355–356.

Schliephake, K., Mainwaring, D.E., Lonergan, G.T., Jones, I.K., Baker, W.L., 2000. Transformation and degradation of the diazo dye Chicago Sky Blue by a purified laccase from *Pycnoporus cinnabarinus*. Enzyme and Microbial Technology 27, 100–107.

Sigoillot, J.C., Herpoel, I., Frasse, P., Moukha, S., Lesage-Meesen, L., Marcel, A., 1999. Laccase production by a monokaryotic strain of *Pycnoporus cinnabarinus* derived from a dikaryotic strain. World Journal of Microbiology and Biotechnology 15, 481–484.

Srebotnik, E., Hammel, K.E., 2000. Degradation of nonphenolic lignin by the laccase/1-hydroxybenzotriazole system. Journal of Biotechnology 81, 179–188.

Srinivasan, C., D'Souza, T.M., Boominathan, K., Reddy, C.A., 1995. Demonstration of laccase in the white-rot basidiomycete *Phanerochaete chrysosporium* BKM-F1767. Applied Environmental Microbiology 61, 4274–4277.

Vikineswary, S., Sanar Kumaran, K., Ling, S.K., Dinesh, N., Shim, Y.L., 1997. Solid substrate fermentation of agro-residues for value-added products—the Malaysian experience. In: Wise, D.L. (Ed.), Global Environmental Biotechnology. Kluwer Academic Publishers, London.

Youn, H.-D., Kim, K.-J., Maeng, J.-S., Han, Y.-H., Jeong, I.-B., Jeong, G., Kang, S.-O., Hah, Y.C., 1995. Single electron transfer by an extracellular laccase from white-rot fungus *Pleurotus ostreatus*. Microbiology 141, 393–398.