CHAPTER FIVE

ENZYME PRODUCTIVITY DURING LIGNINOCELLULOSIC DEGRADATION UNDER OPTIMIZED PARAMETERS

INTRODUCTION

Laccase, a lignolytic enzyme is reported to have role in the detoxification of polyphenolic compounds (Shannon and Bartha 1988). When the laccase extract of *Geotrichum candidum* was added to soil containing pollutants such as 4- methylphenol, the pollutant was incorporated into the soil humic acids. Here it was immobilized and thus it was not leached from the contaminated soil into ground water

In SSF of 'hampas' with suitable nitrogen level and improved inoculum, laccase was the prominent enzyme produced by *Pycnoporus sanguineus*. Maximum laccase activity of 18.05 U/g of substrate was recorded with 0.76%N (w/w) and a 10% 3-week-old inoculum on the 15^{th} day of SSF of 'hampas'.

The aim of this investigation was:

1. to obtain a fermentation profile of pH, soluble protein and various enzyme activities during SSF on sago 'hampas' at optimized parameters of *Pycnoporus saguineus*.

MATERIALS AND METHODS

Substrate

Sago 'hampas' was obtained as in Chapter 3. pp. 47.

Inoculum

Pycnoporus sanguineus was used as the spawn culture.

Fermentation

SSF was carried out as described in Chapter 3 pp. 47 except with the following changes:

i. Inoculum age: 10 % (w/w) 3-week-old 'Koji' culture.

ii. Nitrogen level: 0.76% (w/v).

iii. Fermentation period: 20 days.

Triplicate flasks were sampled randomly on day 0, 5, 10, 15, 20.

Analysis

The crude culture extract was used to determine pH, soluble protein content, xylanase, lignin peroxidase and laccase activity using standard methods as described in Appendix A. Analysis of variance was done to compare the fermentation profile of present study (after optimization) with the fermentation profile (before optimization) of Chapter 3. The analysis of variance was carried out for data on day 10 when most of the maximum activities were recorded (Appendix C).

	pH		Protein		Xylanase		Lignin Peroxidase		Laccase	
Day	Unopt	Opt	Unopt	Opt	Unopt	Opt	Unopt	Opt	Unopt	Opt
0	4.03	4.3	0.002	0.014	3.45	2.09	0.83	0.823	0.6	0.165
5	4.09	4.5	0.018	0.060	4.47	3.95	0.85	1.473	3.4	5.15
10	4.28	4.6	0.043	0.133	5.26	6.36	1.31	0.993	13.65	18.35
15	4.66	4.6	0.065	0.104	5.02	5.62	1.07	0.965	14.35	17.35
20	4.36	4.5	0.044	0.074	4.35	5.10	0.99	0.027	13.0	11.5

Unopt : Unoptimized
Opt : Optimized

Table 5.1: Variation in SSF of sago *hampas* with *P.sanguineus* using unoptimized and optimized parameters

RESULTS AND DISCUSSIONS

pH variation

The pH changes during SSF of 'hampas' are shown in (Fig 5.1). (Appendix C. Table 32). The pH was significantly ($P < 0.05$) affected under optimized parameters.

The initial value of pH was 4.3. There was a similar trend of gradual rise in pH, which peaks on day 10 of fermentation. However, SSF in this study produced maximum pH of 4.6 at day 10 compared to maximum pH on day 15 of unoptimized study previous study (Table 5.1). This value was not different from the unoptimized study (Table 5.1). Though there was not much mycelial growth, other contaminants such as bacteria may have grown during the course of fermentation. Their metabolism may have caused the hydrolysis of urea and the liberation of high levels of ammonium ions thereby increasing the pH values. The pH did not vary from day 10-15. As the fermentation proceeded, the fungal mycelia used up ammonium ions, and this may have caused a slight decrease in the pH values.

Freitag and Morell (1992) studying enzyme activities of a white-rot fungus reported that the complex enzyme systems involved in the substrate degradation could be responsible for maintaining the pH of the substrate at a suitable range for fungal colonization. At the end of the fermentation a pH of 4.5 recorded which was higher than the pH of 4.4 recorded in the unoptimized study on the 20th day of fermentation. This difference in pH is not significant and it can be considered as no change (Table 5.1).

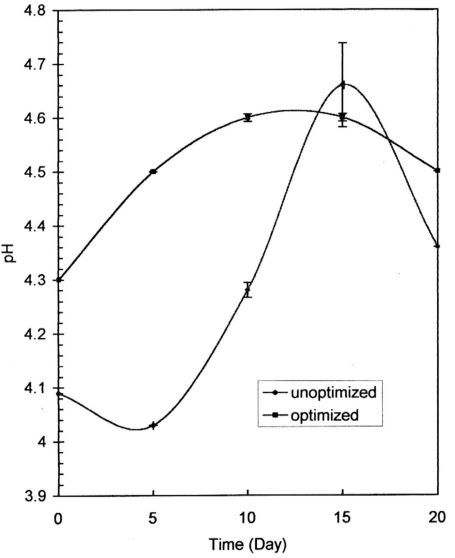

Fig 5.1:Variation of pH in crude culture extract during SSF of sago 'hampas' with *P. sanguineus*

Soluble protein

The soluble protein content during SSF of 'hampas' is shown in (Fig 5.2). (Appendix C. Table 33). The soluble protein content was significantly ($P < 0.05$) affected under optimized parameters (Appendix C. Table 13).

Gradual rise in soluble protein content during first 10 days of SSF was observed. The peak in soluble protein content was observed much earlier in fermentation at 10 day. In the earlier study, maximum values were obtained on day 15 (Table 5.1). The maximum soluble protein content of 0.13 mg/ml recorded was 1.9 times higher when compared to the maximum value of 0.071 mg/ml obtained in the unoptimized study. There was, however, a decline in soluble protein content from day 10 onwards to 0.074 mg/ml at the end of fermentation. This value was same as the maximum value recorded in the previous study (Table 5.1).

It was thus shown that under optimized parameters higher levels of soluble protein, was produced. This may include the enzymes such as xylanase, laccase and lignin peroxidase responsible for the substrate degradation over a longer period of SSF.

Xylanase activity

The xylanase activities during SSF of 'hampas' are shown in (Fig 5.3). (Appendix C. Table 34). The optimized parameters had a significant ($P < 0.05$) effect on xylanase activity (Appendix C. Table 14).

The xylanase activity showed a gradual increase during the 10 days of fermentation period. The peak on day 10 was a maximum value of 6.36 U/g

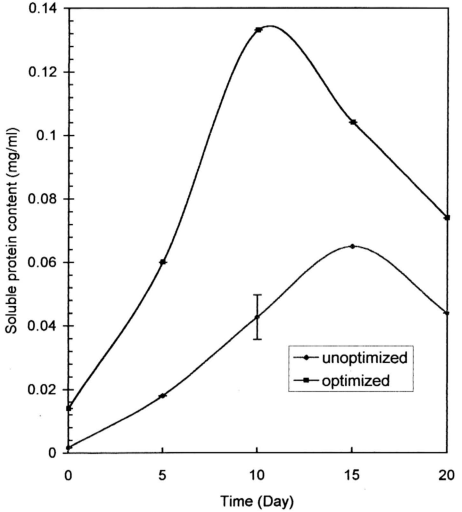

Fig 5.2 :Soluble protein content in crude culture extract during SSF of sago'hampas' with *P.sanguineus*

of substrate. This value was approx. 1.2 times higher then the maximum value of 5.6 U/g of substrate recorded in the unoptimized study (Table 5.1) at day 30. Decline in the xylanase activity which dropped to 5.10 U/g of substrate from 10 day onwards was observed. This could be due to inhibition (Bastawde, 1992). This value was 0.75 U/g of substrate higher compared to the value of 4.4 U/g of substrate obtained in the unoptimized study (Table 5.1) on day 20^{th} of fermentation.

Lignin peroxidase activity

The lignin peroxidase activities of *Pycnoporus sanguineus* during SSF of 'hampas' are shown in Fig 5.4. (Appendix C. pp. Table 35). Statistical analysis revealed that there was no significant ($P > 0.05$) difference on the lignin peroxidase activity under optimized parameters. (Appendix C. Table 15). From Fig 5.4 the lignin peroxidase activity with an initial value of 0.8 U/g of substrate rapidly increased during the SSF and reached a maximum value of 1.5 U/g of substrate. This value recorded was 0.16 U/g of substrate more compared to the maximum value of 1.31 U/g of substrate recorded in the unoptimized SSF study (Table 5.1). The peak in lignin peroxidase activity was observed at day 5 in this study; However, in the unoptimized study maximum lignin peoxidase was obtained on day 10. From day 5 onwards the lignin peroxidase activity declined with a value of 1.313 U/g of substrate towards the end of the fermentation period. This value was 1.3 times higher than the value of 0.99 U/g of substrate recorded in the unoptimized study (Table 5.1) on the 20^{th} day of the fermentation. The lignin peroxidase activity was generally low.

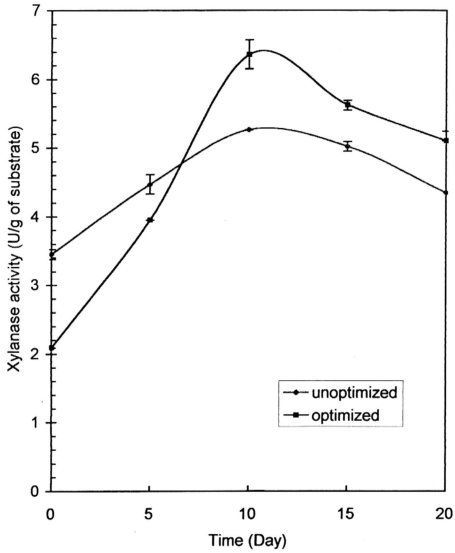

Fig 5.3 :Xylanase activity in crude culture extract during SSF of sago 'hampas' with *P. sanguineus*

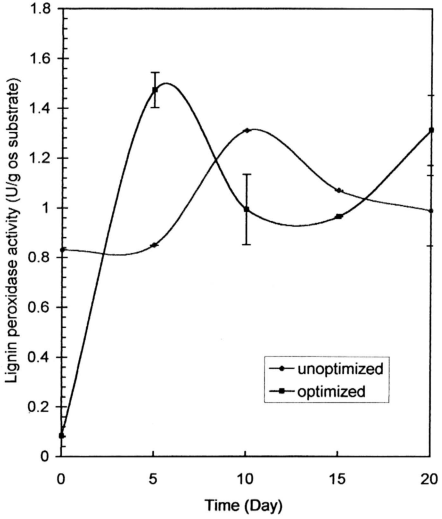

Fig 5.4 :Lignin peroxidase activity in crude culture extract during SSF of sago 'hampas' with *P. sanguineus*

Laccase activity

The laccase profiles of *Pycnoporus sanguineus* in SSF cultures of sago 'hampas' are shown in Fig 5.5. (Appendix C. Table 36). The optimized parameters had a significant effect ($P < 0.05$) on the activity of laccase (Appendix C. Table 32).

Laccase activity increased more rapidly during the SSF and peaked with a value of 18.35 U/g of substrate compared to the maximum value of 14.35 U/g of substrate recorded in the unoptimized study (Table 5.1) The maximum laccase activity recorded in this study was approx.1.3 times higher when compared to the maximum value recorded in the previous study (Table 5.1). The peak in laccase activity was observed much earlier in this study that was at day 10 while in the unoptimized study the peak was on day 15. There was a rapid decline in the laccase activity that dropped to 11.5 U/g of substrate at the end of the fermentation.

A general trend was seen where the enzyme activities increased rapidly and peaked and then decreased slightly towards the end of the fermentation period. Prolonging the SSF period did not result in any laccase increase. The profile of laccase followed that of protein and xylanase where the maximum activities were recorded on 10 day of fermentation.

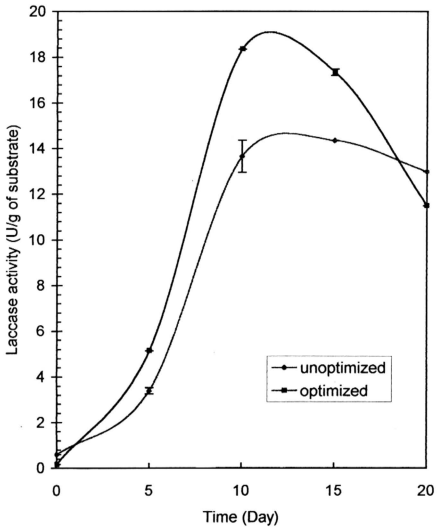

Fig 5.5 : Laccase activity in crude culture extract during SSF of sago 'hampas' with *P. sanguineus*

CONCLUSION

In this study, the optimization of selected physiological parameters for SSF of sago 'hampas' was carried out. The information obtained showed that *Pyc. sanguineus* had an advantage in rapidly colonizing the sago 'hampas' and produced high quantities of laccase. This was possible with a mature 10 % (w/w) 3-week-old inoculum, a nitrogen level of 0.76% (w/v) and an incubation time of 10 days. A maximum laccase activity of 18.35U/g of substrate was recorded during SSF of 'hampas' with *Pyc. sanguineus*.

This study showed that enzyme productivity was enhanced by using the optimized parameters. From the study, it was observed that the 3-week-old inoculum at 10% (w/w) density produced higher enzyme yield compared to the other inoclum ages of different densities (w/w) tested. Increase in levels of nitrogen supplementation also showed significant effect on the enzyme productivity. A maximum xylanase and laccase productivity using optimized parameters was also reported by Renuvathani (2002) during growth of *Pyc. sanguineus* on OPFPt using a 30% (w/w) of a 4-week old inoculum.