CHAPTER V

DATA ANALYSIS AND RESULTS

This chapter presents statistical and non-statistical findings from the data collected in the chapter IV. The data in chapter IV are presented in appendix G. Analysis of variant (ANOVA) are used to analyze the data. The ANOVA results are presented in appendix H. Referring to research Questions in chapter I and II, the analysis and outcomes are organized and presented as follows.

1. Examining the effect of each independent variables, xylanase, laccase, hydrogen peroxide and time, on lignin removal from teak veneer.
2. Examining the effect of the combination of different levels of xylanase, laccase, hydrogen peroxide and time, on lignin removal from teak veneer.

This chapter also presented the extended research and its findings.

According to preliminary experiments, shown in Appendix D, all experiments concerning hydrogen peroxide were conducted at 60°C and pH 6.5.

5.1 Examining the Effect of Each Independent Variables, Xylanase, Laccase, Hydrogen Peroxide and Time, on Lignin Removal from Teak Veneer.

5.1.1 Hydrogen peroxide experiment

The data from this 2x3 factorial experimental design for hydrogen peroxide is presented in Table G1 in Appendix G. The average amount of lignin removed from 3 replicates, as indicated by % change in gray scale (% Δgs), using hydrogen peroxide at 2% and 10% and time 0.5, 1 and 2 hour is presented in Table 5.1 and in graph in Figure 5.1.

These results clearly show the effect of H_2O_2 concentration and time on the amount of lignin removed. As H_2O_2 increases from 2% to 10%, the amount of lignin removed is increase. The effect of time on lignin removal can be seen clearly from these table and graph. As time progress, the amount of lignin removal increases.

However, as can be seen from the graph in Figure 5.1, in the beginning, from 0 to 0.5 hour, the rate of lignin removal is higher than that of other time interval. That is as time proceeds; although, the amount of lignin removed increases but the rate of lignin removed decreases. This may be extrapolated to say that after a certain time has passed, increasing the time will not result in significant change in the amount of lignin removed.

Statistical analysis using analysis of variant (ANOVA) also indicated that hydrogen peroxide has significant effect on lignin removal (F=1,444.914, p=0.000). Time also has significant effect on lignin (F=566.424, p=0.000). The combination effect of hydrogen peroxide and time also has significant effect on lignin removal (F=69.096, p=0.000). The ANOVA results for H_2O_2 and time system are in Table H1 in Appendix H.

Table 5.1 Effect of hydrogen peroxide and time on % change in gray scale

Time (hours)	% change in gray scale (% Δgs)	
	2% (w/v) H_2O_2	10%(w/v) H_2O_2
0	0	0
0.5	4.37	8.64
1	6.42	12.98
2	9.10	18.47

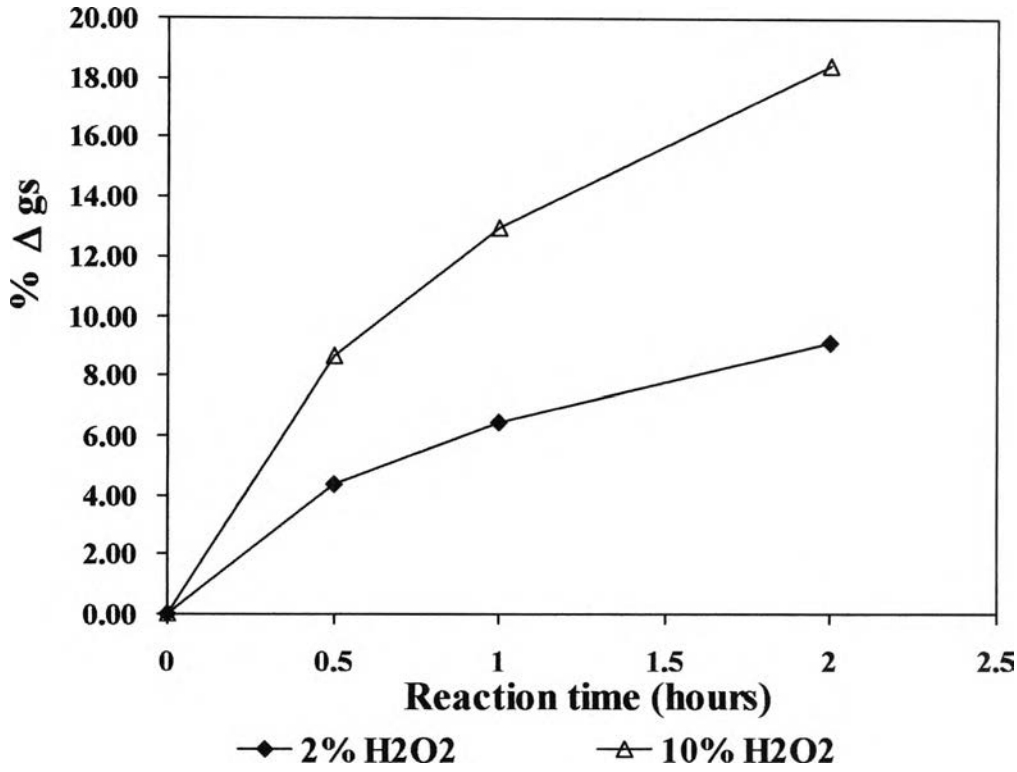


Figure 5.1 Effect of hydrogen peroxide and time on % change in gray scale.

5.1.2 Xylanase experiment

The data from the 3x3 factorial experimental designs for xylanase is presented in Table G2 in Appendix G. The average amount of lignin removed (% Δgs) from 3 replicates using xylanase at 0.25, 0.50 and 1.00 u/ml and at time 0.5, 1 and 2 hour is presented in Table 5.2 and in graph in Figure 5.2. From these table and figure, the following findings were observed.

1. The data of 0.50 and 1.00 u/ml xylanase concentration show similar results. The average lignin removals at each time level from these 2 data sets are very close to each other. The 0.25 u/ml xylanase concentrations on the other hand show the obvious difference in the average amount of lignin removed from the other 2 data sets at the 1 and 2 hour level but not on the 0.5 hour level.

2. As can be seen from Figure 5.2, the effect of time, at 0.5 and 1 hour level when view from the data sets are rather scatter, however, when view from the average amount of removed lignin data set, the data show some pattern. The data at 2 hour level of time show stronger effect of time.

3. The obtained data show the effect of xylanase on the amount of lignin removed to be negative, meaning that the xylanase make the teak veneer sample darker. This phenomenon may be explained by that as xylanase remove xylan it may exposed the underneath lignin to view, hence the darker color. Similar line of reasoning are found in Kenealy and Jeffries that xylanase could be removing xylan that blocks access to the lignin thus allow bleaching agent better access to lignin (Kenealy and Jeffries, 2001).

The results from analysis of variant (ANOVA), presented in Table H2, show that the xylanase concentration has no significant effect on lignin removal as measured by % change in gray scale. The ANOVA results show time to have significant effect on lignin removal ($F=4.471$, $p=0.027$). However, the combination effect of xylanase and time has no significant effect on lignin removed.

From statistical and non statistical findings, conclusion can be drawn that xylanase alone will not remove lignin from teak veneer.

All these findings suggest further investigation in the ability of xylanase in cleaving xylan and in how xylanase can be used to aid in removing lignin from teak veneer.

Table 5.2 Effect of xylanase and time on % change in gray scale

Time (hour)	% change in gray scale (% Δgs)		
	0.25 xylanase (u/ml)	0.5 xylanase (u/ml)	1.00 xylanase (u/ml)
0.5	-1.03	-0.98	-1.12
1	-0.49	-1.25	-1.16
2	-1.33	-2.07	-2.17

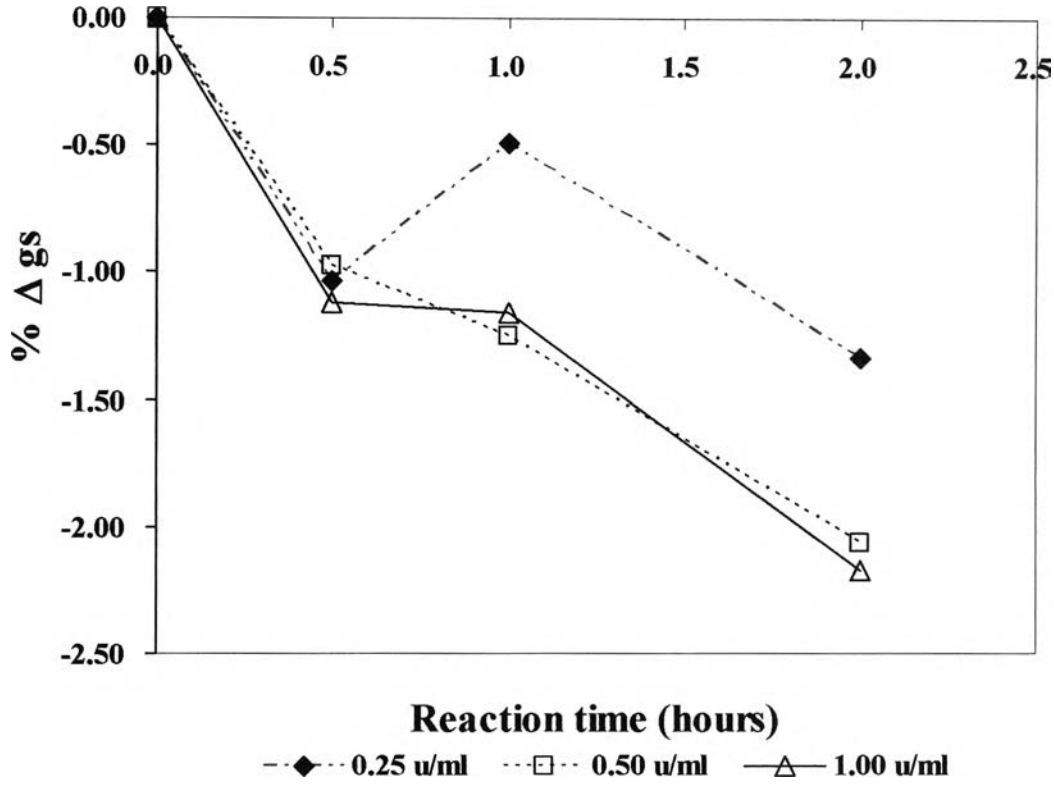


Figure 5.2 Effect of xylanase and time on % change in gray scale

5.1.3 Laccase experiment

The data from the 3x3 factorial experimental designs for laccase is presented in Table G3 in Appendix G.

The average amount of lignin removed, as indicated by the % change in gray scale, using laccase at 0.05, 0.25 and 1.00 u/ml and at time level of 0.5, 1 and 2 hour is presented in Table 5.3 and in graph in Figure 5.32.

Statistical finding from ANOVA, the results of which are presented in Table H3, indicate that laccase concentration has no significant effect on % change in gray scale but time has (F=5.301, p=0.015). The combination effect of laccase and time does not have significant effect on % change in gray scale. The observation of graph Figure 5.3 show similar results.

The data show similar effect as the xylanase data that is the laccase also darkening the teak veneer. In pulp bleaching, laccase was found to cause an initial darkening of pulp (Paice et al., 1995, cited in Kenealy and Jeffries, 2003). Bajpai and Riva indicated that laccase alone does not degrade lignin; its oxidation reaction catalyzed by the enzyme leads instead to further polymerization of lignin (Bajpai, 1999; Riva, 2006).

Therefore, from statistical and non statistical finding, conclusion can be made that laccase has significant effect on gray scale but laccase concentration has no significant effect. The effect as can be seen from graph in Figure 5.3, however darken the veneer. Therefore, laccase alone will not remove lignin from teak veneer.

Table 5.3 Effect of laccase and time on % change in gray scale

Time (hour)	% change in gray scale (% Δgs)		
	0.05 laccase (u/ml)	0.25 laccase (u/ml)	1.00 laccase (u/ml)
0.5	-1.46	-1.54	-0.92
1	-2.17	-1.79	-1.33
2	-2.44	-2.53	-2.41

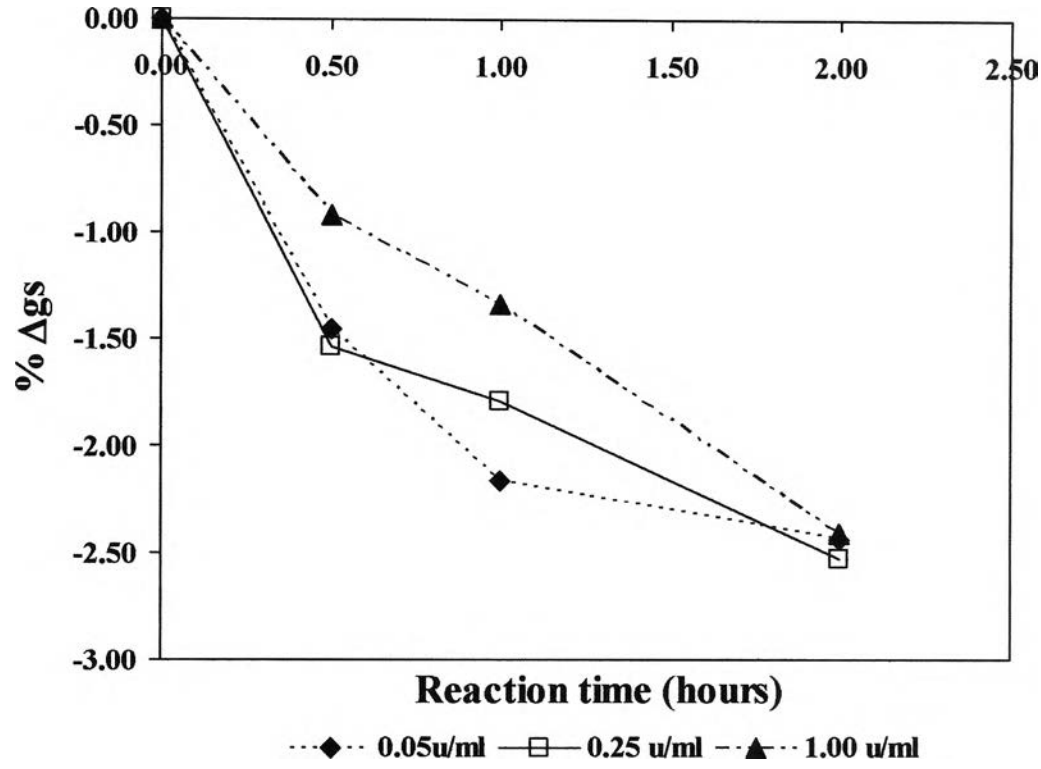


Figure 5.3 Effect of laccase and time on % change in gray scale

5.1.4 Section summary

This section present the examinations of the effect of xylanase, laccase, H2O2 and time on % change in gray scale. The study shows that H_2O_2 provides the best results. The concentration of xylanase or laccase was shown to have insignificant effect on the % change in gray scale. However the presence of either enzyme should have significant effect on the outcome.

5.2 Examining the Effect of the Combination of Different Levels of Xylanase, Laccase, Hydrogen Peroxide and Time, on Lignin Removal from Teak Veneer.

After the effect of each independent variable was determined, the effects of the combination of each independent variable are examined.

5.2.1 Hydrogen peroxide, xylanase and time

The data from this 2x3x3 factorial experimental design are collected and presented in Table G4 in Appendix G. The average amount of lignin removed, as measured in % change in gray scale, using 2 levels of H_2O_2 (2% and 10%), 3 levels of xylanase concentration (0.25, 0.50 and 1.00 u/ml) and 3 levels of time (0.5, 1 and 2 hour) are presented in Table 5.4 and in graphical representation in Figure 5.4 and 5.5.

The effect of the chosen xylanase concentration, as can be observed from Table 5.4, Figure 5.4 and Figure 5.5, show some differences in % change in gray scale between combinations. However these differences between each level of combination are not big enough to make a desirable difference in % change in gray scale to justify the use of the enzyme xylanase.

Table 5.4 Effect of xylanase, H_2O_2 and time on % change in gray scale

Time (hour)	H_2O_2 (%)	% change in gray scale (% Δgs)		
		0.25 xylanase (u/ml)	0.5 xylanase (u/ml)	1.00 xylanase (u/ml)
0.5	2	3.98	4.14	4.31
	10	9.52	9.70	10.73
1	2	6.83	7.05	7.52
	10	13.25	12.81	14.38
2	2	8.02	8.25	8.67
	10	17.32	17.20	17.58

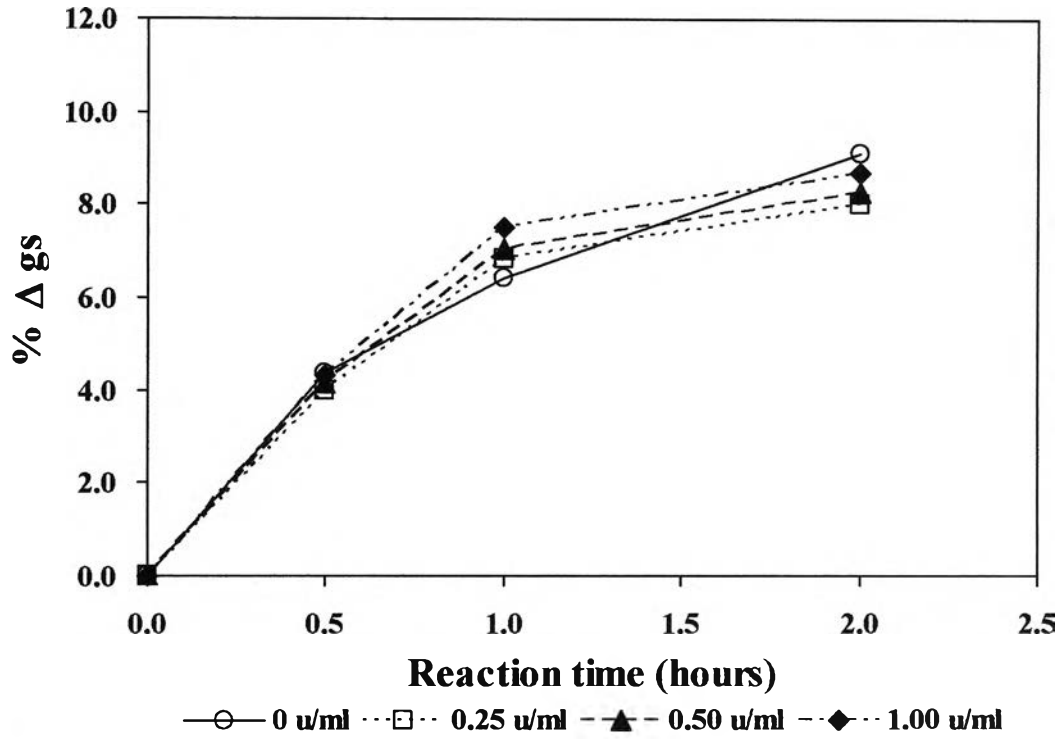


Figure 5.4 Effect of xylanase and time at 2% H_2O_2 on % change in gray scale

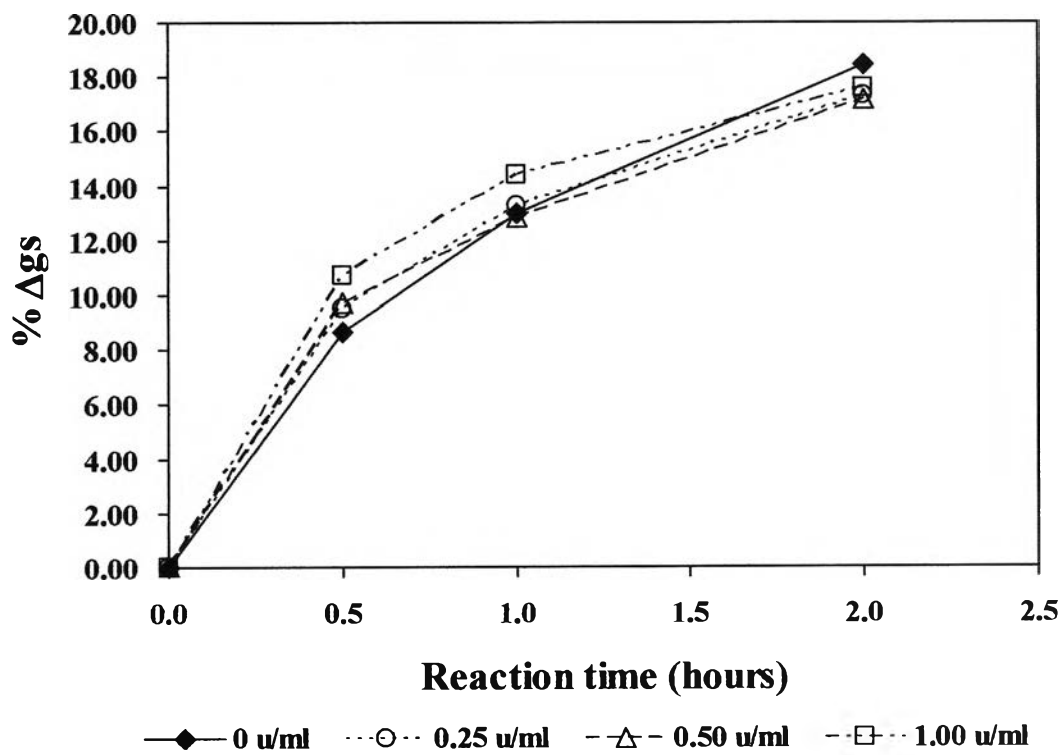


Figure 5.5 Effect of xylanase and time at 10% H_2O_2 on % change in gray scale

The ANOVA results, presented in Table H4 of Appendix H, show that H_2O_2, xylanase and time each has significant effect on the dependent variable (F=1,237.024, p=0.000), (F=5.233, p=0.01) and (F=276.096, p=0.000) respectively. These findings are different from the results for single independent variable which statistically state that xylanase does not have any significant effect on the dependent variable. This could mean that the presence of H_2O_2 effect the performance of xylanase.

The result from ANOVA also show that the combination of xylanase and H_2O_2, xylanase and time, and xylanase, H_2O_2 and time, do not have any significant effect on the dependent variable. Only the combination of H_2O_2 and time is statistically significance (F=24.560, p=0.000). These findings concur with graphical finding.

From statistical and non statistical findings, conclusion can be made that although there are some differences between all the chosen combination of H_2O_2, xylanase and time, the differences are too small to justify the use of xylanase. However, as mention above, the ANOVA results indicate that xylanase in the xylanase- H_2O_2 system has significant effect on the dependent variable.

These findings and the findings in section 5.1.2 still suggest further investigation as stated in section 5.1.2

5.2.2 Hydrogen peroxide, laccase and time

The data from this 2x3x3 factorial experimental design are collected and presented in Table G5 in Appendix G. The average amount of lignin removed form 3 replicates, as measured in % change in gray scale, using 2 levels of H_2O_2 (2% and 10%), 3 levels of laccase concentration (0.05, 0.25 and 1.00 u/ml) and 3 levels of time (0.5, 1 and 2 hour) are presented in Table 5.5 and in graphical representation in Figure 5.6 and 5.7.

The effects of the chosen laccase concentration on the % change in gray scale, the dependent variable, are observed from Table 5.5, Figure 5.6 and Figure 5.7. These data show some differences in the % change in gray scale between combinations. Two observations can be made from these data. First the differences between each level of

combination are not big. Second, the effect is in a way that it is darkening the teak veneer.

Table 5.5 Effect of laccase, H_2O_2 and time on % change in gray scale

Time (hour)	H_2O_2 (%)	% change in gray scale (% Δgs)		
		0.05 laccase (u/ml)	0.25 laccase (u/ml)	1.00 laccase (u/ml)
0.5	2	4.82	4.43	4.79
	10	8.74	7.41	7.63
1	2	6.31	5.89	5.96
	10	11.53	11.52	11.90
2	2	8.03	8.19	8.58
	10	15.81	16.34	17.32

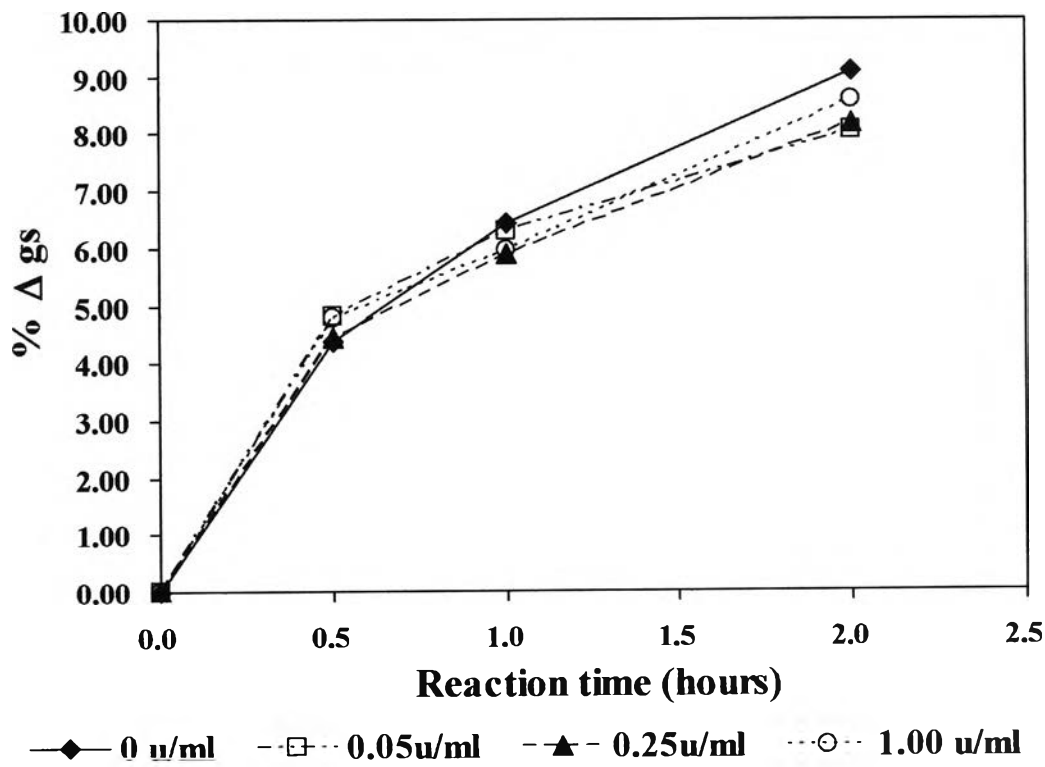


Figure 5.6 Effect of laccase and time at 2% H_2O_2 on % change in gray scale

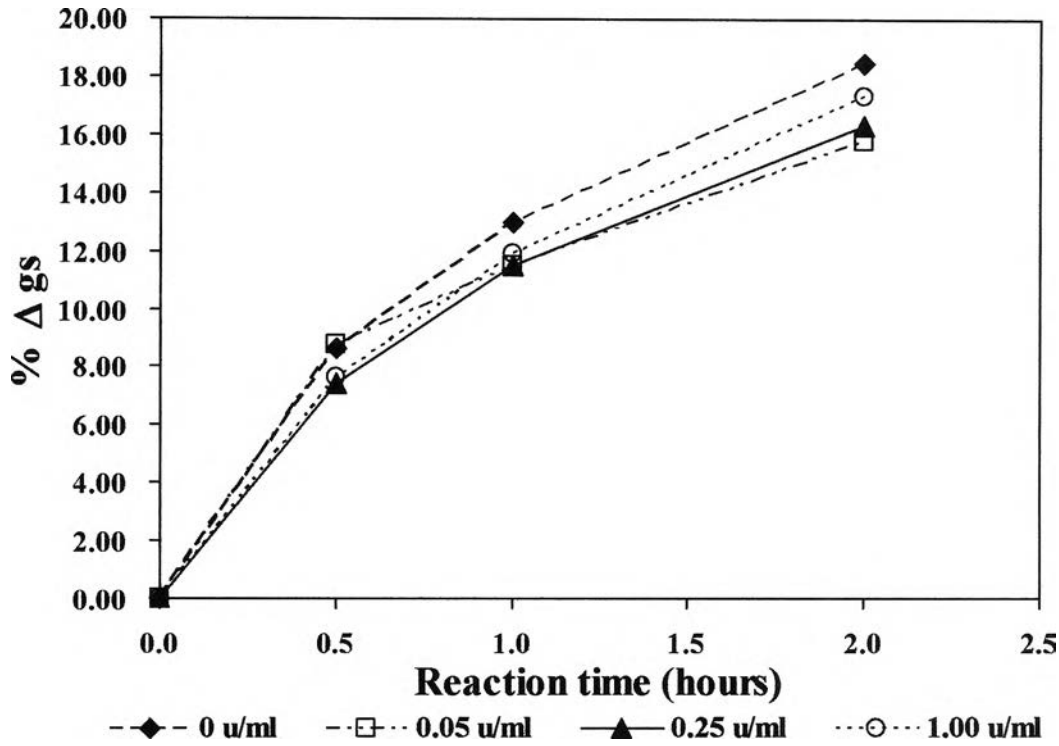


Figure 5.7 Effect of laccase and time at 10% H_2O_2 on % change in gray scale

The ANOVA results are presented in Table H5 of Appendix H. The results show that laccase, H_2O_2, and time each have significant effect on the dependent variable (F=9.101, p=0.001), (F=4,403.841, p=0.000) and (F=1,681.985, p=0.000) respectively. These findings are different from the results of laccase single independent variable which indicate that laccase alone will not have any significant effect on the dependent variable. The combination effect of laccase- H_2O_2 does not have a significant effect on the dependent variable. The results from ANOVA show that combination of laccase-time, H_2O_2 – time and laccase- H_2O_2 – time do have significant effect on the dependent variable (F=10.499, p=0.000) , (F= 279.206 , p=0.000) and (f=4.968, p=0.003) respectively. The effect, however, is a negative effect, that is the combination will reduce lignin removal ability when compare with the sole hydrogen peroxide; therefore, it is undesirable. The findings suggest that H_2O_2 may have effect on laccase and may influence it to have an effect on % change in gray scale.

From statistical and non statistical findings, it can be concluded that laccase has undesirable effect on the dependent variable.

5.2.3 Xylanase, laccase and time

The data from this 3x3x3 factorial experimental design are collected and presented in Table G6 in Appendix G. The average amount of lignin removed from 3 replicates, as measured in % change in gray scale, using 3 levels of laccase concentration (0.05, 0.25 and 1.00 u/ml), 3 levels of xylanase concentration (0.25, 0.50 and 1 .00u/ml) and 3 levels of time (0.5, 1 and 2 hour) are presented in Table 5.6 and in graphical representation in Figure 5.8, 5.9 and 5.10..

The ANOVA results are presented in Table H6 in Appendix H. The results show that each of all 3 independent variables, xylanase, laccase and time, has significant effect on the dependent variable, ($F=6.536$, $p=0.003$), ($F=4.647$, $p=0.014$) and ($F=99.948$, $p=0.000$) respectively. However, none of the combinations, xylanase-laccase, xylanase-time, laccase-time, or xylanase-laccase-time, has any significant effect on the % change in gray scale.

From non statistical findings the following results are observed. The concentration of laccase, when observed at constant xylanase concentration, do have some effect on the dependent variable, like the statistical results indicate, but the direction of the effect is not certain. Time has obvious effect on the dependent variable. The combination of the 2 enzymes still results in the darkening of the teak veneer. Further investigation on other enzymes doses may be helpful.

Table 5.6 Effect of xylanase, laccase and time on % change in gray scale

Time (hour)	xylanase (u/ml)	% change in gray scale (% Δgs)		
		0.05 laccase (u/ml)	0.25 laccase (u/ml)	1.00 laccase (u/ml)
0.5	0.25	-0.91	-0.94	-1.15
	0.50	-1.12	-1.27	-0.94
	1.00	-0.97	-0.81	-1.04
1	0.25	-0.95	-1.93	-1.24
	0.50	-1.33	-2.34	-1.79
	1.00	-2.12	-1.96	-2.15
2	0.25	-1.87	-2.52	-2.37
	0.50	-2.41	-2.65	-2.39
	1.00	-2.61	-2.69	-2.48

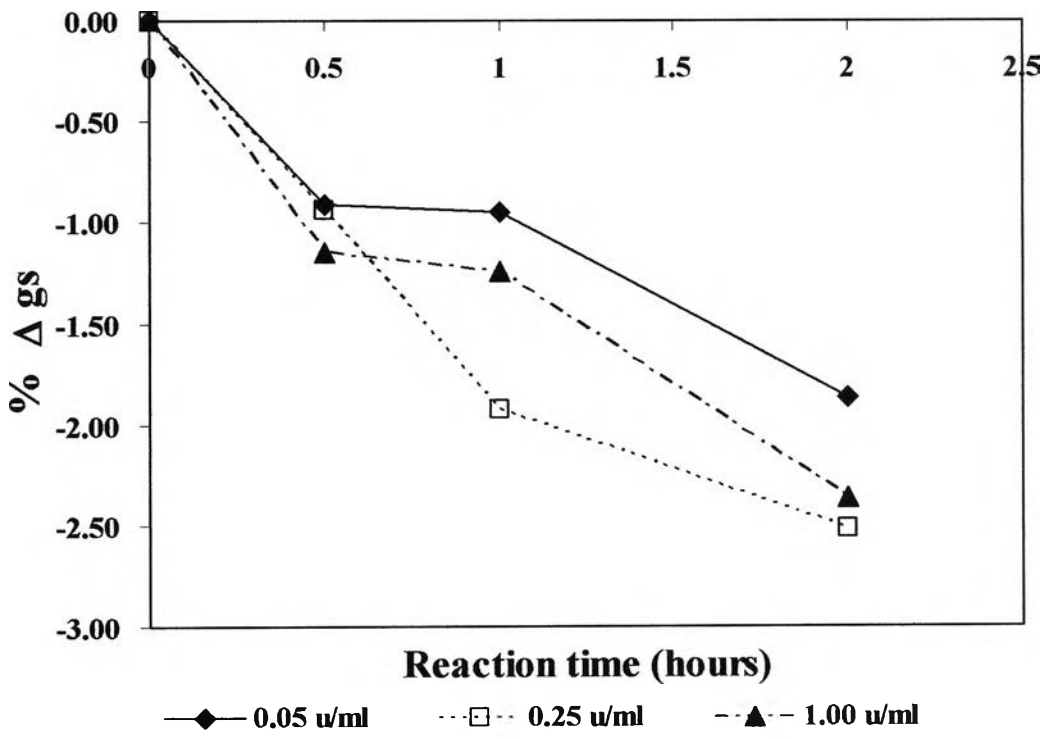


Figure 5.8 Effect of laccase and time at 0.25u/ml xylanase on % change in gray scale

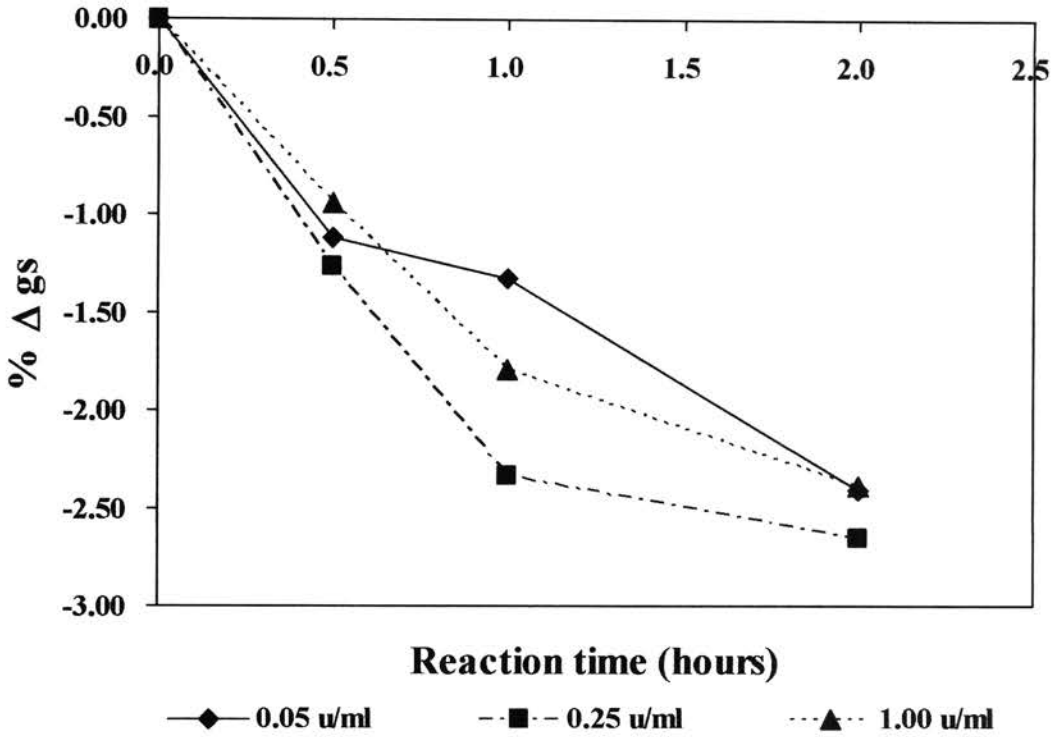


Figure 5.9 Effect of laccase and time at 0.50u/ml xylanase on % change in gray scale

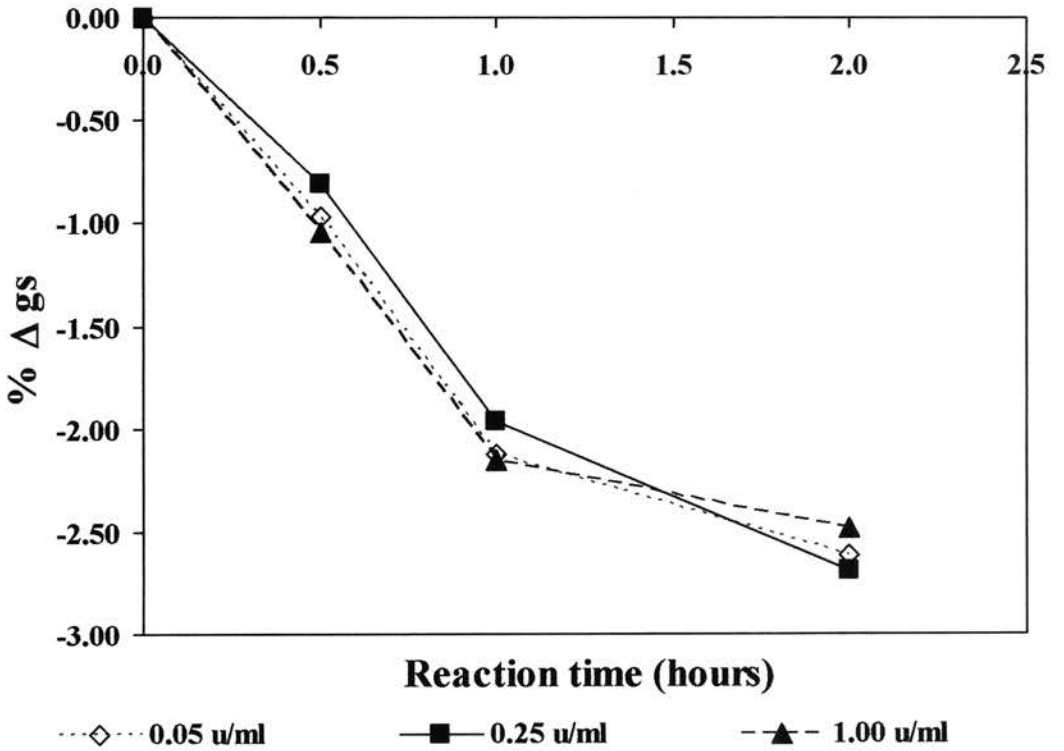


Figure 5.10 Effect of laccase and time at 1.00u/ml xylanase on % change in gray scale

5.2.4 Section summary

This section presented the examination of H_2O_2, xylanase, laccase and time in combinations as outlined in the experimental design in chapter IV. When xylanase was used in combination with H_2O_2 some their combinations can slightly improve the function of H_2O_2 but the difference in % change in gray scale is not significance enough to justify the use of xylanase. Furthermore, some of these combinations will reduce lignin removal ability when compared with the sole H_2O_2. In case of laccase, adding laccase to H_2O_2 cause the % change in gray scale to decrease.

5.3 Conclusions and suggestions.

In examining the analysis results from the individual variable effect and the combination of variable effect, the following conclusions were made.

1. Xylanase and laccase, either separately or in combination with each other, will not remove lignin.
2. H_2O_2 provides the best results in removing lignin.
3. Time has a significant effect on lignin removal. The longer the time used in treatment the more lignin can be removed. However, the rate of lignin removed declined with time
4. The mixture of xylanase at 0.25 u/ml or higher and H_2O_2 will remove lignin but the improvement is not significance enough to justify its use. Moreover some of these combinations will reduce lignin removal ability when compared with the sole H_2O_2.
5. Laccase when used in combination with H_2O_2 will lower the performance of H_2O_2

From these conclusions, the following suggestions are made.

1. Further investigation to verify the ability of xylanase to cleave xylan at the specified condition and time.
2. Further study to include H_2O_2 concentrations outside the area covered in the factorial experimental design established in chapter IV.

3. Further study to extend the times to outside those covered in the experimental design established in chapter IV.

4. Further study on how xylanase can be used to improve H_2O_2 performance.

5. Further study on how laccase can be used to improve H_2O_2 performance.

6. From the above results, select the reasonable combinations, conditions and methods of application that are thought to be likely to improve H_2O_2 performance and verify them.

5.4 Extended Research

In this section, the area outside the boundary of the established experimental design in chapter IV was further investigated. The research follows the suggestion presented in section 5.3. All experiments performed in this extended research use the same research method, sample selection criteria, sample preparation procedure, experimental procedure and data collecting method as in chapter IV.

5.4.1 Xylanase

In the case of xylanase, this is a special case since xylanase function is not to remove lignin directly but to do so indirectly by cutting xylan which in turn will help free lignin from the matrix of cellulose-xylan-lignin.

Due to the fact that no work has been done on teak veneer; therefore, experimenters do not have previous work to rely on in determining the suitable amount of enzymes to be used in experiments. Other works are in the area of delignification of pulps, the nature of which is entirely different from veneers. Therefore, experiments were performed to determine whether the amount of xylanase used in this study can actually reduce xylan in teak veneer into xylose at the specified xylanase concentration, temperature and time, and if it can, to determine the relationship between the amount of xylanase used and xylose produced.

In order to do so, 25 experiments and 20 experiments each with 3 replicates were performed at room temperature and at 60 °C respectively using 5 levels of xylanase concentration (0.05, 0.25, 0.50, 1.00 and 2.50 u/ml) and at 6 levels of time (15 min, 0.5, 1, 2, 4 and 24 hours). The experimental procedure established in section 4.5.5 was followed. The image data were collected and processed. The solutions obtained from these experiments were analyzed to determining the amount of xylose presented. The method used in determining xylose content is Nelson-Somogyi (Nelson, 1994) described in Appendix D.

The data from image analysis are collected and presented in Table 5.7 and Figure 5.11 for room temperature and in Table 5.8 and Figure 5.12 for 60°C data. The

xylose determination data are collected in Table 5.9 and Figure 5.13 for data at room temperature and in Table 5.10 and Figure 5.14 for data at 60°C.

Figure 5.11 and 5.12 show the effect of xylanase on the % change in gray scale. The time scale was extended to 24 hours, the veneer was shown to be darker than any other points. With the exception of 0.05 u/ml xylanase all other concentrations show some fluctuation with time.

Table 5.7 Effect of xylanase concentration and time on the average % change in gray scale (room temperature)

Time (hours)	% change in gray scale (% Δgs)				
	0.05 xylanase (u/ml)	0.25 xylanase (u/ml)	0.50 xylanase (u/ml)	1.00 xylanase (u/ml)	2.5 xylanase (u/ml)
0.00	0.00	0.00	0.00	0.00	0.00
0.50	-0.18	-1.03	-0.98	-1.12	-1.27
1.00	-1.05	-0.49	-1.25	-1.16	-0.88
2.00	-1.61	-1.33	-2.07	-2.17	-1.63
4.00	-1.89	-1.52	-1.44	-1.25	-1.82
24.00	-2.30	-3.20	-3.21	-2.83	-2.96

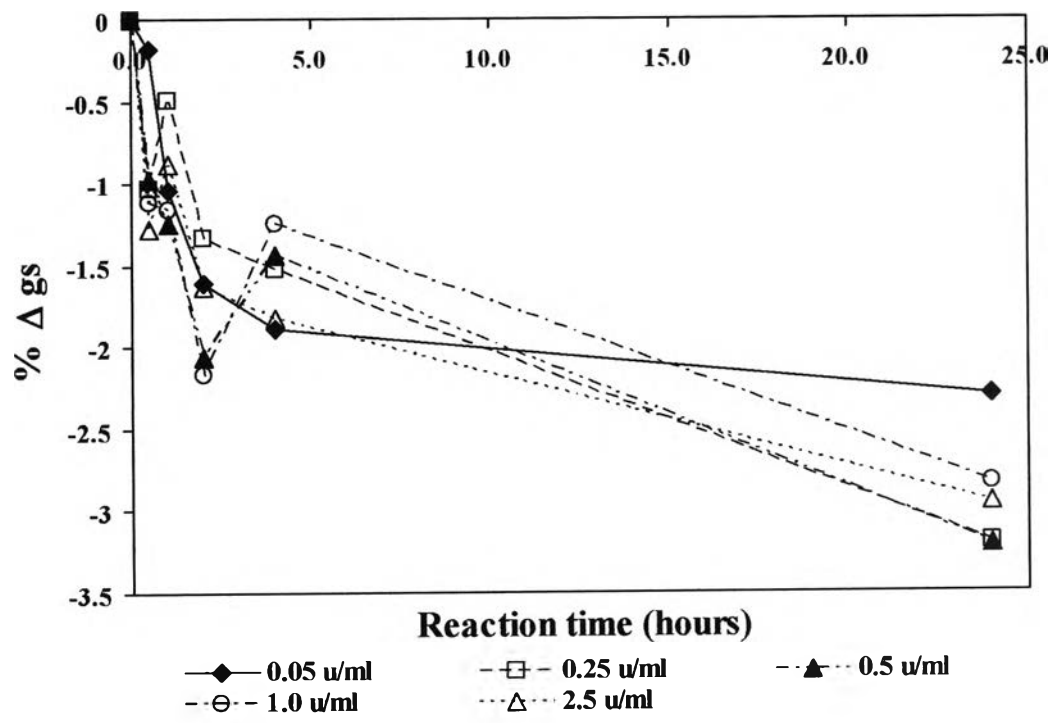


Figure 5.11 Effect of xylanase concentration and time on the average % change in gray scale (room temperature)

Table 5.8 Effect of xylanase concentration and time on the average % change in gray scale (60°C)

Time (hours)	% change in gray scale (% Δgs)			
	0.05 xylanase (u/ml)	0.25 xylanase (u/ml)	0.50 xylanase (u/ml)	1.00 xylanase (u/ml)
0.00	0.00	0.00	0.00	0.00
0.50	-1.32	0.62	0.58	0.89
1.00	-1.45	-1.59	0.67	0.70
2.00	-1.57	-3.10	-2.40	0.46
4.00	-1.85	-0.33	-0.91	0.08
24.00	-3.99	-3.32	-3.38	0.23

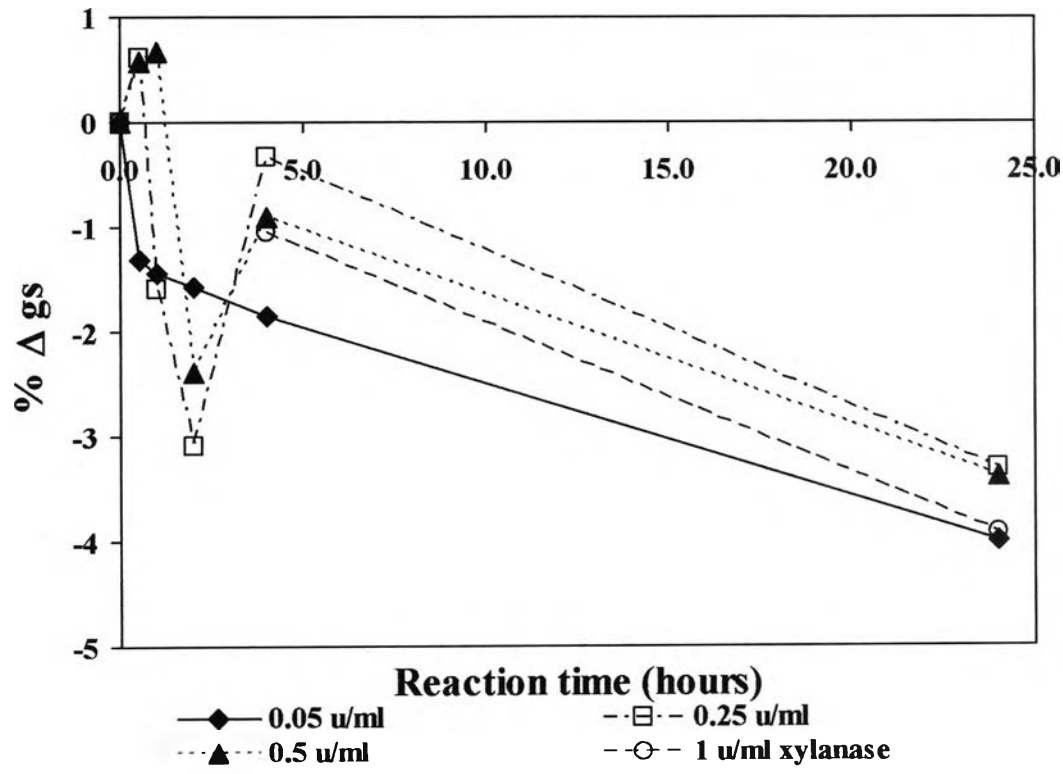


Figure 5.12 Effect of xylanase concentration and time on the average % change in gray scale (60°C)

The xylose data in Table 5.9 and 5.10, and in Figure 5.13 and 5.14, show the production of xylose over the wide range of xylanase concentration and time which cover the study. Thus verify that xylanase can cleave xylan at the specified concentration and time. Statistical analysis using ANOVA supports these findings. The ANOVA results are presented in Table H7 and H8 in Appendix H. The data show that higher xylanase concentration and higher time yield more xylose. Also xylanase performs better at 60 °C than at 32 °C.

Once it was determined that xylanase can actually cut xylan in teak veneer at as low concentration as 0.05 u/ml, at time as short as 15 min and at room temperature, the next step is to find out how xylanase can be used to improve H_2O_2 performance.

Table 5.9 Effect of xylanase concentration and time on xylose produced (room temperature)

Time (hours)	average xylose concentration (μg/ml)				
	0.05 xylanase u/ml	0.25 xylanase u/ml	0.50 xylanase u/ml	1.00 xylanase u/ml	2.50 xylanase u/ml
0.00	0.00	0.00	0.00	0.00	0.00
0.25	12.23	6.55	10.64	13.45	23.13
0.50	16.37	16.41	18.49	19.79	29.60
1.00	22.02	25.18	24.71	27.46	32.09
2.00	21.93	24.54	28.74	40.62	48.36
4.00	28.88	36.62	46.72	52.88	63.67
24.00	46.54	45.59	49.74	60.89	70.60

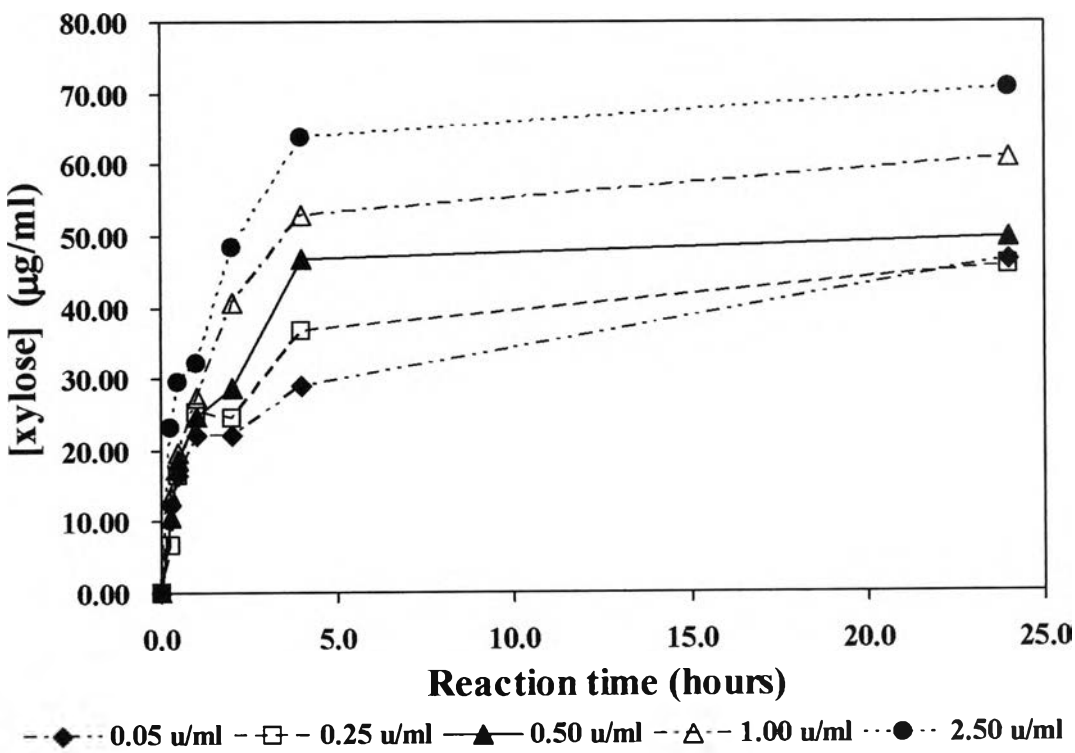


Figure 5.13 Effect of xylanase concentration and time on xylose produced (room temperature)

Table 5.10 Effect of xylanase concentration and time on xylose produced (60°C)

Time (hours)	average xylose concentration (μg/ml)			
	0.05 xylanase u/ml	0.25 xylanase u/ml	0.50 xylanase u/ml	1.00 xylanase u/ml
0.00	0.00	0.00	0.00	0.00
0.50	26.38	32.93	33.15	39.66
1.00	34.60	37.93	36.95	39.78
2.00	38.11	44.89	52.64	57.03
4.00	38.00	53.47	53.38	59.64
24.00	53.23	55.29	71.58	73.51

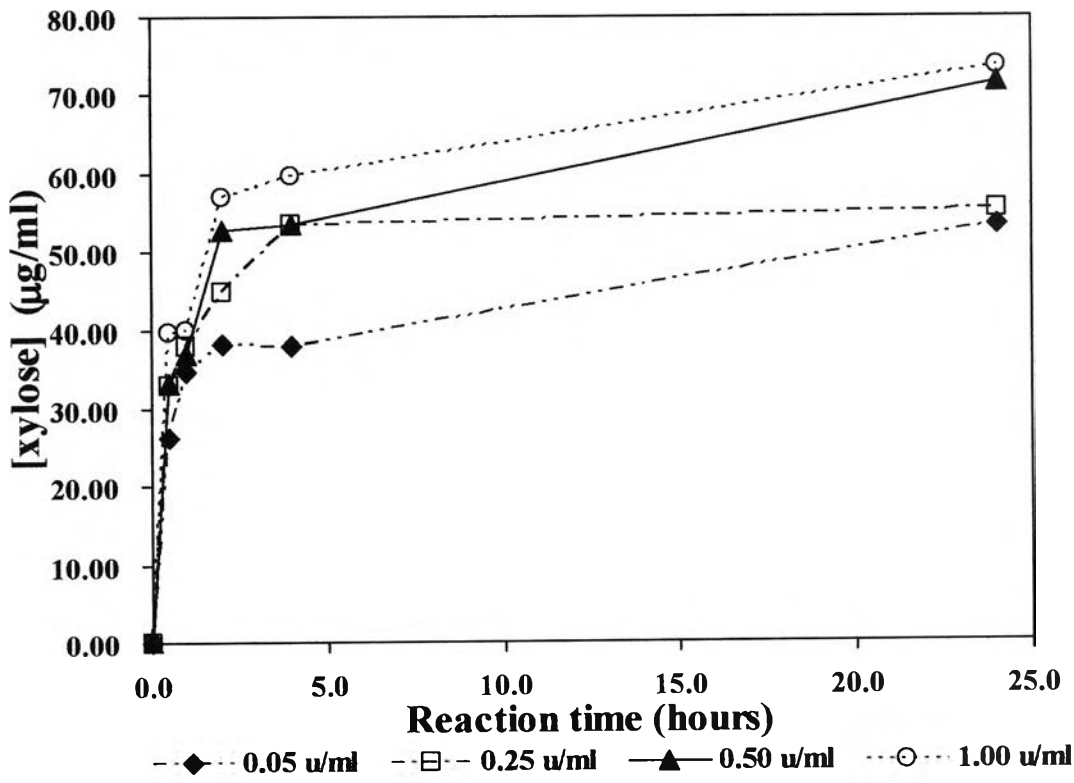


Figure 5.14 Effect of xylanase concentration and time on xylose produced (60°C)

5.4.2 Hydrogen peroxide

The H_2O_2 was investigated further to include 5, 8, 15 and 20% and the time used was expanded to include 4 hour. The amount of lignin removed, as indicated by % change in gray scale, using different level of H_2O_2 concentration and time is presented in Table 5.11 and shown in Figure 5.15. By comparing Figure 5.15 with Figure 5.1, it can be seen that this expanded set of data still show a significant effect of H_2O_2 concentration and time. The ANOVA results, presented in Table H9 of Appendix H, support this finding.

ANOVA results in Table H10 show that except for the comparison between 8% and 10% hydrogen peroxide, all data are significantly different from each other. Direct observation from graph, Figure 5.15, found that the 8% and 10% hydrogen peroxide are intertwined with one another. The % changes in gray scale of 15% hydrogen peroxide, although significantly difference, are not much higher than that of the 8% and 10% hydrogen peroxide. The 20% hydrogen peroxide gave the best result, but the concentration is too high. As can be seen from Table 5.11 and Figure 5.15, double the amount of hydrogen peroxide used does not double the % change in average gray scale. The % change in gray scale at 2% H_2O_2 are much lower than any other concentration. The highest change in gray scale was reported at 11.54% at time of 4 hour. Therefore, due to poor results in % change in gray scale, 2% H_2O_2 was excluded from further experiments. From these findings, since the purpose of this study is to reduce the amount of chemical used, the 5%, 8% and 10% hydrogen peroxide were selected for further study.

Table 5.11 Effect of hydrogen peroxide and time on % change in gray scale

Time	% change in gray scale (% Δgs)					
(hours)	2%(w/v) H_2O_2	5%(w/v) H_2O_2	8%(w/v) H_2O_2	10%(w/v) H_2O_2	15%(w/v) H_2O_2	20%(w/v) H_2O_2
0	0	0	0	0	0	0
0.5	4.37	6.23	10.56	8.64	12.63	12.94
1	6.42	9.87	13.04	12.98	14.96	20.05
2	9.10	13.34	16.89	18.47	19.32	23.13
4	11.54	18.41	22.69	22.13	23.04	27.48

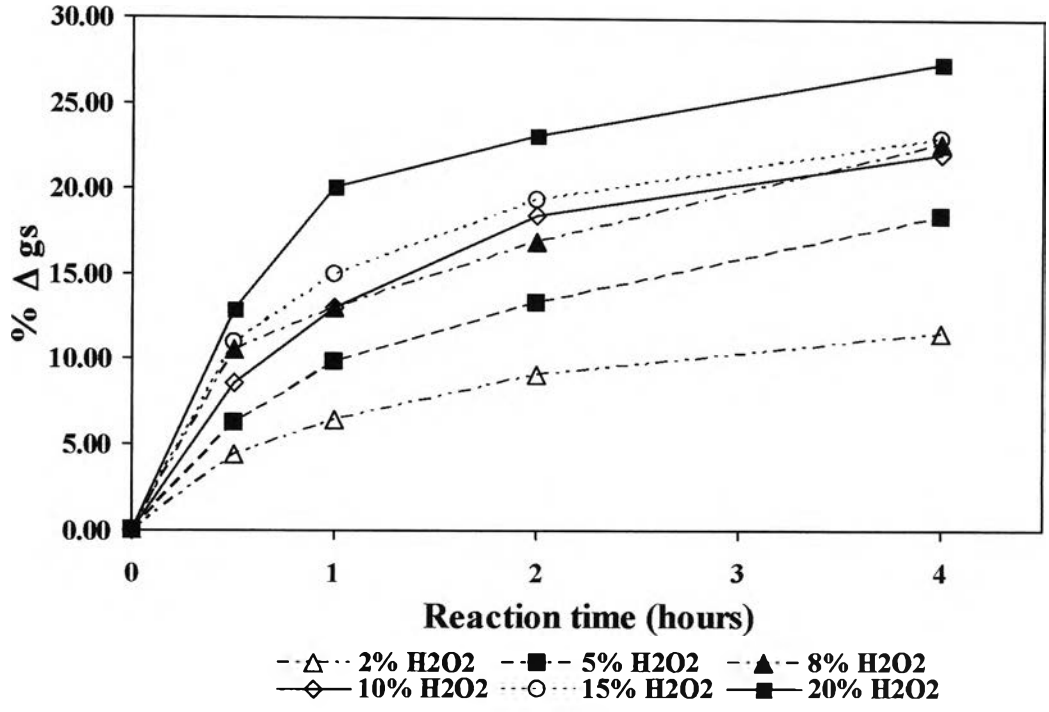


Figure 5.15 Effect of hydrogen peroxide and time on % change in gray scale

5.4.3 Further study on xylanase, laccase and H_2O_2

The study so far shows that H_2O_2 yield the best result. It also shows that xylanase and laccase effect the performance of H_2O_2, the effect, however, is sometimes undesirable. It shows that without H_2O_2, xylanase and laccase darken the teak veneer. Finally, it was found that xylanase can actually cut the xylan at the designed concentration, time and condition.

The next research question is how to make xylanase and laccase help improve the performance of H_2O_2. Since the function of xylanase is to cut xylan not lignin, and since the removal of too much xylan can expose the underneath lignin which will darken the veneer; therefore, further study in the area of low xylanase concentration should be explored. Furthermore, the ANOVA results in Table H2 indicate that concentration of xylanase does not have a significant effect on the % change in gray scale; therefore, from economic point of view, the low concentration of xylanase should be employed. The decision made from this two reasons leads to the framework for the next iteration of experiments. First the mixture of xylanase and hydrogen

peroxide was studied. The 0.05 u/ml xylanase concentration was selected to study with 10% H_2O_2.

5.4.3.1 Mixture of xylanase and hydrogen peroxide

In this part the experiments with 0.05 u/ml xylanase and 10% H_2O_2 mixtures were performed at 60°C and pH 6.5 for 4 hours using the procedure established in chapter IV. The results were compared in Table 5.12 to 10%, 15% and 20% H_2O_2 concentration at the same condition.

Table 5.12 Effect of 0.05u/ml xylanase on H_2O_2 performance

Method of treatment	Average %change in gray scale
0.05u/ml xylanase+10% H_2O_2	24.34
10% H_2O_2	22.13
15% H_2O_2	23.04
20% H_2O_2	27.48

The mixture result is about 10% improvement over the 10% H_2O_2 performance. The result is even better than the 15% H_2O_2 but still can not match that of 20% H_2O_2.

5.4.3.2 Sequent experiments

Reexamination of Table H2 and H4 reveal that even though the concentration of xylanase in single component experiment does not have any significant effect on % change in gray scale but the concentration of xylanase in the xylanase-H_2O_2 mixture does have a significant effect on % change in gray scale. This should mean that H_2O_2 influence xylanase to have effect on % change in gray scale. Some effects may result in an increase in % change in gray scale; some may result in a decrease in % change in gray scale, (see Figure 5.4 and 5.5). Therefore, the next research step in the experiment is to remove the xylanase-H_2O_2 interaction effect and used them in sequence. Furthermore, since the function of xylanase is to cut xylan and cutting

xylan may expose the underneath lignin; that is, while H_2O_2 is removing lignin; xylanase may keep exposing the new layer of lignin. Therefore, the use of xylanase and H_2O_2 in sequence should be able to prevent this. Referring to the finding in section 5.4.1, xylanase can reduce xylan at room temperature and at as short a time as 15 minutes.

From these findings and the fact that laccase should be further investigated, the next iteration of experiments was designed to be the treatment of veneer samples with xylanase at designed concentrations for half an hour at room temperature and pH 4.5, followed by the treatment with H_2O_2 or laccase-H_2O_2 mixture at 60°C and pH 6.5 for 4 hours. The results are presented in Table 5.13. The findings from Table 5.13 are as follows.

Comparison within 0.05u/ml xylanase results

1. The 10% H_2O_2 sequence set yield the best result; follow by 8% and 10% H_2O_2 respectively.
2. Within the 10% H_2O_2 sequence set, laccase at 0.05 u/ml concentration and below yield better results than laccase at higher concentration. Laccase at 0.05 u/ml yields the best result at 26.40 % change in gray scale, which is the improvement of 19.3% over the 10% H_2O_2.
3. For 8% H_2O_2 sequence set, the best result occurs at 0.05 u/ml laccase concentration at 25.14 % change in gray scale, which is 10.8% improvement over the 8% H_2O_2. The rest of the laccase concentration yield results that are not significantly difference from one another.
4. H_2O_2 at 10% response more to xylanase treatment than H_2O_2 at 8%.
5. For 5% H_2O_2 sequence set, the best result of 21.51% change in gray scale occurs at 0.03 u/ml laccase concentration. This result is 16.8% improvement over the 5% H_2O_2. The rest of the laccase concentration yield results that are not significantly difference from one another.

Table 5.13 Experimental data on sequential experiments

wood treatment	laccase (u/ml)	% change in gray scale					
		xylanase 0.05 u/ml			xylanase 0.03 u/ml		
		5%H_2O_2	8%H_2O_2	10%H_2O_2	5%H_2O_2	8%H_2O_2	10%H_2O_2
X to H_2O_2 + Lac 0 u/ml	0.00	19.57	22.60	25.11	19.52	23.47	24.91
X to H_2O_2 + Lac 0.01 u/ml	0.01	20.10	22.84	25.52	20.89	23.63	24.36
X to H_2O_2 + Lac 0.03 u/ml	0.03	21.51	23.89	25.40	21.29	24.47	24.16
X to H_2O_2 + Lac 0.05 u/ml	0.05	20.61	25.14	26.40	20.93	25.11	21.21
X to H_2O_2 + Lac 0.25 u/ml	0.25	19.02	22.29	23.52	20.92	23.42	23.19
X to H_2O_2 + Lac 1.00 u/ml	1.00	20.40	23.06	23.96	18.10	22.86	22.21

6. Using xylanase and laccase, the 5% H_2O_2 sequence set result of 21.51% comes very close to the 10% H_2O_2 result of 22.13% while using only half the H_2O_2 requirement.
7. When laccase was not used, the best result of 25.11% change in gray scale occurs at 10% H_2O_2. This result is a 13.5% improvement over the 10 % H_2O_2.
8. The best result for the 0.05 u/ml xylanase treatment is 26.40 % change in gray scale. This result comes from the use of the mixture of 10% H_2O_2 and 0.05 u/ml laccase.

Comparison within 0.03u/ml xylanase results

1. The 10% H_2O_2 and 8% H_2O_2 sequence set yield the results that are not significantly difference with the exception of the result at 0.05 u/ml laccase concentration that the 8% H_2O_2 yield 25.11 % change in gray scale while the 10% H_2O_2 yield only 21.21% change in gray scale.
2. For 10% H_2O_2 sequence set, the best result range of 24.16 to 24.91 % changes in gray scale occurs at laccase concentration range of 0.00 to 0.03 u/ml.
3. The 5% H_2O_2 sequence set yield the lowest % change in gray scale. The best result of 21.29 % change in gray scale occurs at 0.03 u/ml laccase. This result is 15.6% improvement over the 5% H_2O_2.
4. Using xylanase and laccase, the 5% H_2O_2 sequence set result of 21.29% comes very close to the 10% H_2O_2 result of 22.13% while using only half the H_2O_2 requirement.
5. The best result for the 0.03 u/ml xylanase treatment is 25.11 % change in gray scale. This result comes from the use of the mixture of 10% H_2O_2 and 0.05 u/ml laccase.

Comparison between 0.05 u/ml and 0.03 u/ml xylanase data

For 5% H_2O_2 and 8% H_2O_2 sequence sets, the difference between 0.05 and 0.03 u/ml xylanase is insignificance. However, for 10% H_2O_2 at laccase concentration

of 0.05 u/ml or lower, the results are different. The difference is highest at laccase concentration of 0.05 u/ml.

The use of xylanase enzymes in sequence provides the following benefits. First xylanase can then be operated at different condition. Second the contact time for xylanase can be shorten

The results from sequent experiments are summarized in table 5.14 together with the results from H_2O_2 experiments.

Table 5.14 Results summarization

Method of treatments	%Δgs	% improvement over 10% H_2O_2
10% H_2O_2	22.13	-
A	24.34	10%
B	25.11	13.5%
C	26.40	19.3%
D	25.14	13.6%
E	21.51	-2.8%
15% H_2O_2	23.04	-
20% H_2O_2	27.48	-
30% H_2O_2	24.98	-

Note: %Δgs = % change in gray scale

The values of %Δgs represent the average values

A: 0.05 u/ml xylanase + 10% H_2O_2

B: 0.05 u/ml xylanase → 10% H_2O_2

C: 0.05 u/ml xylanase → 10% H_2O_2 + laccase 0.05u/ml

D: 0.05 u/ml xylanase → 8% H_2O_2 + laccase 0.05u/ml

E: 0.05 u/ml xylanase → 5% H_2O_2 + laccase 0.03u/ml

Table 5.15 Comparison of market teak veneer and research teak veneer

Sample	Gray scale		Method of treatment	% Δ gs	Observed by SEM
	Untreated wood	Treated wood			
Market	72	92	ECF	27	Figure5.21
	75	93	ECF	23	
	80	95	ECF	18	
Grade AAA teak veneer	101	-	-	-	
	114	-	-	-	
This research sample					
A1	80	102	T1	27	Figure5.17
A2	75	95	T2	26	Figure5.19
A3	75	92	T3	22	

Note: %Δgs = % change in gray scale

The values of %Δgs represent the individual values

T1 = 0.05 u/ml xylanase → 10% H_2O_2 + 0.05u/ml laccase

T2 = 0.05 u/ml xylanase → 10% H_2O_2

T3 = 10% H2O2

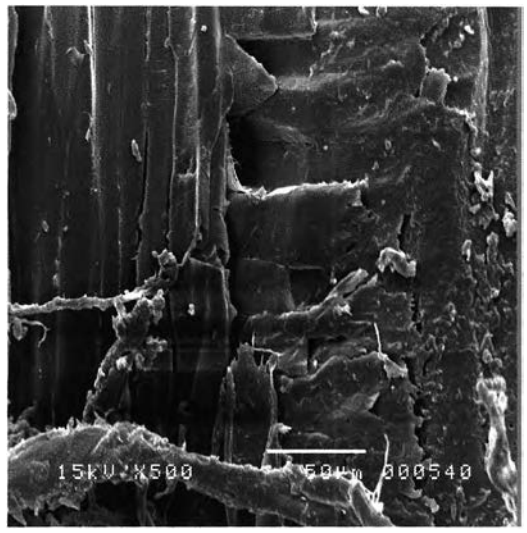
ECF = elemental chlorine free

5.5 Surface Examination

The purpose of this research is to improve the color quality of teak veneer with minimal damage to its texture. Therefore, the surface of the sample should be examined to determine the extent of damage the treatment has done. The scanning electron microscope (SEM) was chosen. Sixteen treated samples from various experiments, 1 untreated sample, and 1 treated sample from local market were selected and scanned using SEM. Their images were examined and compared. The samples of their images are presented in Figure 5.16 to 5.21.

In examining each image from the treated sample from research experiment and comparing them to the untreated wood, it was found that no damage had been detected. Examining the market sample, however, found some rough surface which may be due to the chemical treatment

Therefore, conclusion was made that, the damage to the texture of veneer, if there is any, is undetectable.

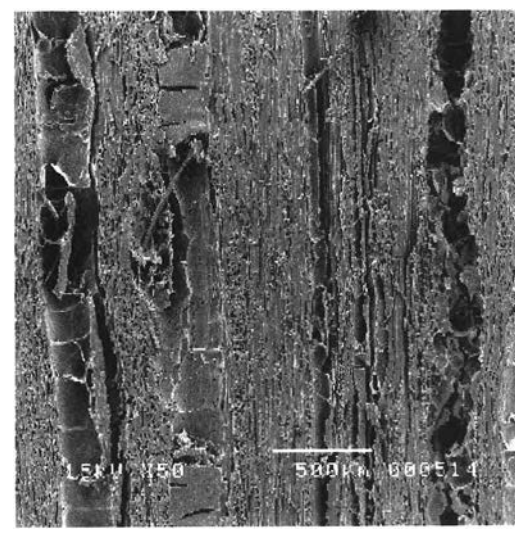


a. Untreated wood

b. Wood treated with xylanase 0.05 u/ml at 32°C for 30 min, then treated with 10%H_2O_2 and laccase 0.05 u/ml at 60°C for 4 hours

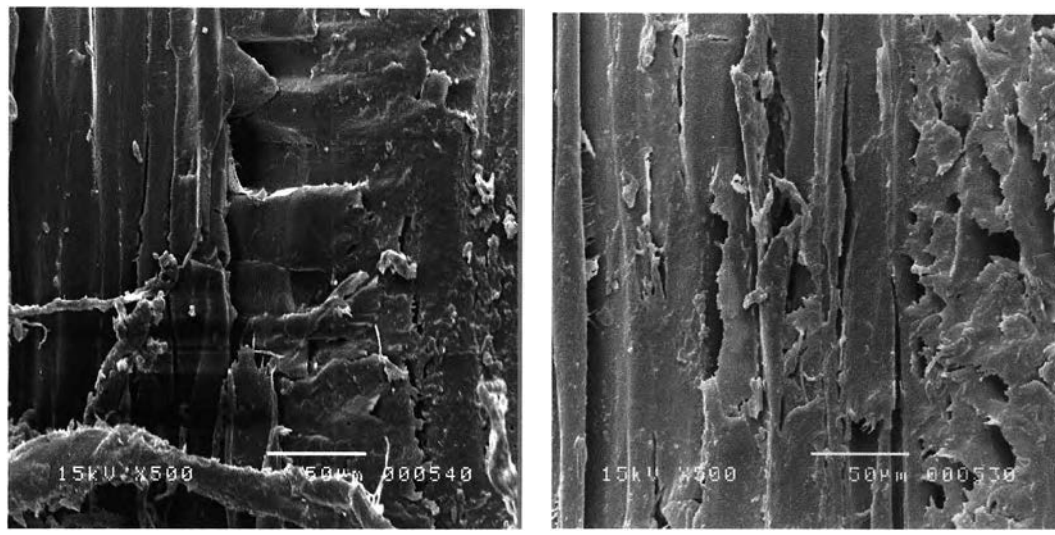
Fig 5.16 Scanning electron microscope of untreated wood and wood treated with xylanase 0.05 u/ml at 32°C for 30 min, then treated with 10%H_2O_2 and laccase 0.05u/ml at 60°C for 4 hours (x500)

a. Untreated wood



b. Wood treated with xylanase 0.05 u/ml at 32°C for 30 min, then treated with 10%H_2O_2 and laccase 0.05 u/ml at 60°C for 4 hours

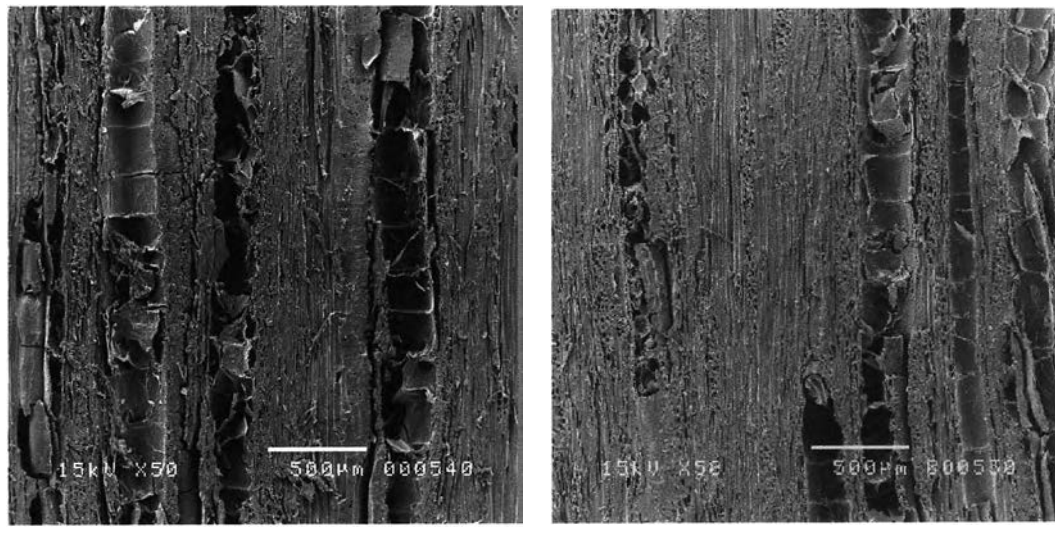
Fig 5.17 Scanning electron microscope of untreated wood and wood treated with xylanase 0.05 u/ml at 32°C for 30 min, then treated with 10%H_2O_2 and laccase 0.05u/ml at 60°C for 4 hours (x50)



a. Untreated wood

b. Wood treated with xylanase 0.05 u/ml at 32°C for 30 min, then treated with 10%H_2O_2 at 60°C for 4 hours

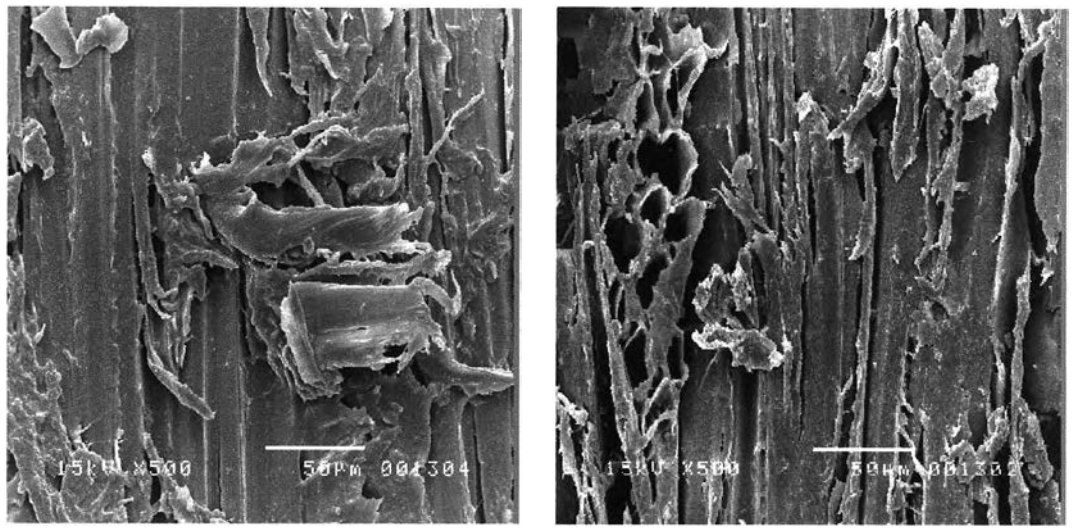
Fig 5.18 Scanning electron microscope of untreated wood and wood treated with xylanase 0.05 u/ml at 32°C for 30 min, then treated with 10%H_2O_2 at 60°C for 4 hours (x500)



a. Untreated wood

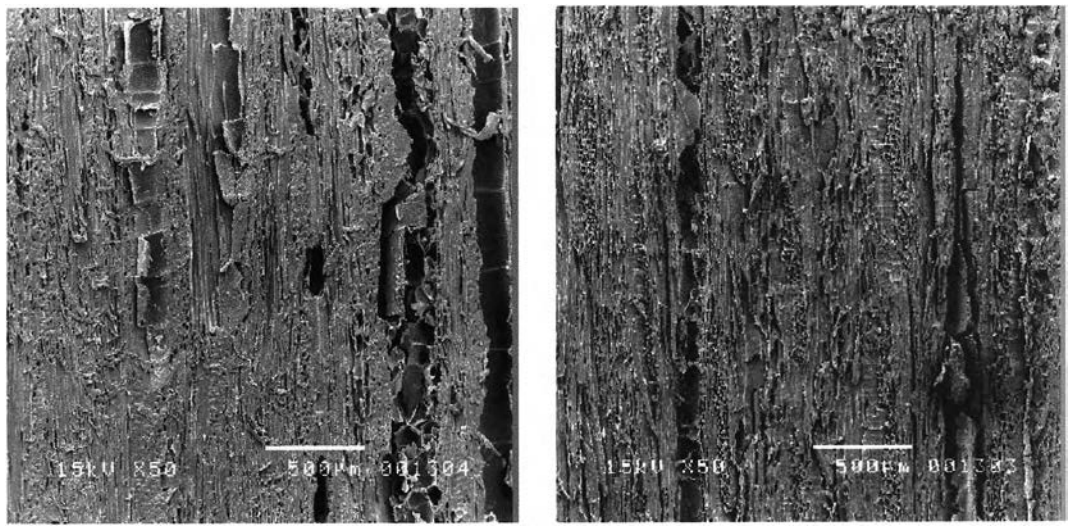
b. Wood treated with xylanase 0.05 u/ml at 32°C for 30 min, then treated with 10%H_2O_2 at 60°C for 4 hours

Fig 5.19 Scanning electron microscope of untreated wood and wood treated with xylanase 0.05 u/ml at 32°C for 30 min, then treated with 10%H_2O_2 at 60°C for 4 hours (x50)



a. Untreated wood b. Wood treated with chemical

Fig 5.20 Scanning electron microscope of untreated wood and wood treated with Elemental chlorine free chemical (x500) (market wood)



a. Untreated wood b. Wood treated with chemical

Fig 5.21 Scanning electron microscope of untreated wood and wood treated with Elemental chlorine free chemical (x50) (market wood)

5.6 Chapter Summary

This chapter presents results and data analysis from factorial experimental design and from the extended research. The conclusion and suggestion for the factorial experimental design are presented in section 5.3. For extended research, important findings are discovered and presented in section 5.4.3.2. The optimum results of the extended research are summarized in Table 5.14. Table 5.15 compares the % change in gray scale from 3 different treatments and the veneer samples from elemental chlorine free method. The scanning electron microscope reveals no detectable damage for research samples.