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APPENDICES

APPENDIX A

Appendix A

Image Processing

A1 Theory (Image-pro plus, 2002)

In this study, the image processing technique was adopted and applied as an indirect measurement of the amount of lignin removed. The principle of the technique is that when the scanner scans the image into a computer, the image is converted into a numeric form. This process is called image digitizing. The digitized image is composed of horizontal grid of very small area called picture elements, or pixels. This digital grid is called bitmap. Each pixel is identified by its location in the grid, in the form of row x and column y (x,y) just like in matrix. By convention pixel (0,0) is in the upper left hand corner of the image. When an image is digitized, each pixel is examined; its brightness is measured and quantified. The value is stored in the corresponding pixel of the image bitmap.

(0,0)	(0,1)	(0,2)		
(1,0)				
			(x,y)	

Figure A1 Example digital grid

Bit and Byte: A computer understands only 2 conditions-on and off or one or naught. Therefore, computer count number in 0 and 1, this is called binary system. A computer usually deals with 8-bit numbers, which consists of 8 ones and 8 naught. The 8-bit numbers are called bytes. Each byte represents a combination of 0/1 conditions. Therefore, eight 0/1 condition can represent 256 combinations (2^8). That is |0|0|0|0|0|0|0|0| is equal to 0 and |1|1|1|1|1|1|1|1| is equal to 255.

So a byte can be expressed in 256 ways (counting 0 to 11111111).

Depending on the capability of the hardware, 1 to 32 bits might be used to store each pixel value. The number of bits used to represent the pixel values is called pixel depth, or bits-per-pixel (BPP).

In gray scale, pixel values represent a level of grayness or brightness, ranging from completely black to completely white. In an 8-bit gray scale image, a pixel with a value of 0 is completely black and a pixel with a value of 255 is completely white. Everything in between this 2 number is a shade of gray. Although some hardware and software can handle up to 32 BBP, the 8 BPP Gray Scale images are the most common. This is because it has 1 byte per pixel depth which makes it easy to manipulate with computer and because it can be faithfully represent any gray scale image because it provides 256 distinct level of gray.

In this research work, the 8 BPP gray scale was used for the above reason. After the computer digitized the image, the image was then stored in bitmap file for true representation of the image. Other type of this files such as jpeg will distraught the image.

After the computer have the record of the gray scale for all the pixels. These data have to be manipulated into a form that can be worked with. A computer program written by Mr. Danulux was used to organize the data into the form as shown in Appendix C. From these sets of data the gray scale for all images of untreated and treated samples were calculated (see Appendix C for sample of calculation).

A2 Data collecting using image processing technique

Procedure 1. Setting the scanner to the following scanning parameter:

8 bit Gray Scale

800 dpi

Width = 1,236 pixels

Height = 1,220 pixels

No color correction

Save as bitmap file

2. Scan the image.
3. Save the image

APPENDIX B

APPENDIX B

Preparation of Buffer Solution

B1 Preparation of 0.2 M phosphate buffer pH 6.5

1. Preparation of stock solution A
    1.1 mixing 27.8 g of monobasic sodium phosphate monohydrate with distilled water
    1.2 adjusted to 1000 ml.
2. Preparation of stock solution B
    2.1 mixing 53.6 g of dibasic sodium phosphate heptahydrate with distilled water
    2.2 adjusted to 1000 ml.
3. Mixing 68.5 ml of stock solution A to 31.5 ml of stock solution B
4. Measure the pH using Orion 701A pH meter from Orion Research USA
5. Adjust the pH using stock solution A and B

B2 Preparation of 0.2 M acetate buffer pH 4.5

1. Preparation of stock solution A
    1.1 mixing 27.8 g of sodium acetate anhydrous with distilled water
    1.2 adjusted to 1000 ml.
2. Mixing 43 ml of stock solution A with 57 ml of 0.2 M acetic acid
3. Measure the pH using Orion 701A pH meter from Orion Research USA
4. Adjust the pH using stock solution A and 0.2 M acetic acid

APPENDIX C

Appendix C

Sample of Calculation

Gray Scale (x_i)	Number of pixel		weight	
	Untreat(n_{ui})	Treat(n_{ti})	$n_{ui}x_i$	$n_{ti}x_i$
0	0	0	0	0
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
84	28,609	19,365	2,403,156	1,626,660
85	26,174	21,318	2,224,790	1,812,030
86	23,511	23,254	2,021,946	1,999,844
87	21,054	24,598	1,831,698	2,140,026
88	18,774	26,190	1,652,112	2,304,720
89	16,298	27,391	1,450,522	2,437,799
90	14,120	29,156	1,270,800	2,624,240
91	12,188	30,010	1,109,108	2,730,910
92	10,461	30,649	962,412	2,819,708
93	8,724	31,234	811,332	2,904,762
94	7,120	31,684	669,280	2,978,926
.				
.				
.				
.				
.				
255				
	1,009,910	1,000,974	77,532,513	95,464,274
	$= \sum n_{ui}$	$= \sum n_{ti}$	$= \sum n_{ui}x_i$	$= \sum n_{ti}x_i$

Average gray scale of untreated wood = $\frac{\sum n_{ui}x_i}{\sum n_{ui}} = \frac{77,532,513}{1,009,910} = 76.77$

Average gray scale of treated wood = $\frac{\sum n_{ti}x_i}{\sum n_{ti}} = \frac{95,464,274}{1,000,974} = 95.37$

% change in average gray scale = $\frac{95.37 - 76.77}{76.77} \times 100$

$= 24.23\ \%$

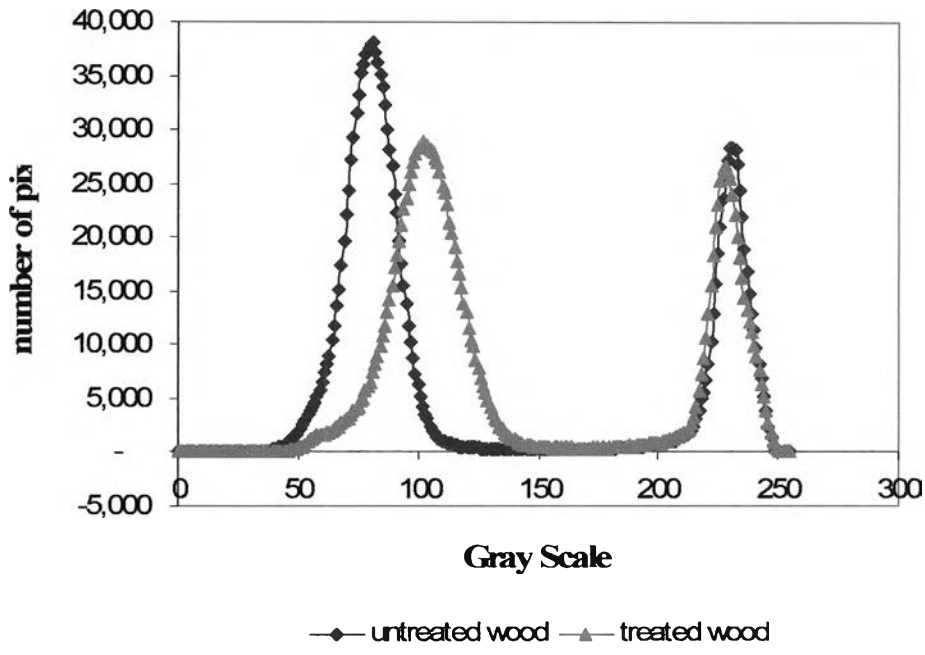

Figure C Example of Gray Scale of untreated and treated wood

APPENDIX D

Appendix D

Preliminary Experiments

Table D1 Effect of temperature on % change in gray scale

time (hour)	Temperature		
	32 degree C	45 degree C	60 degree C
0	0	0	0
0.5	5.31	5.41	8.64
1	6.10	7.54	12.98
2	7.11	12.75	18.47
4	10.17	14.96	22.13

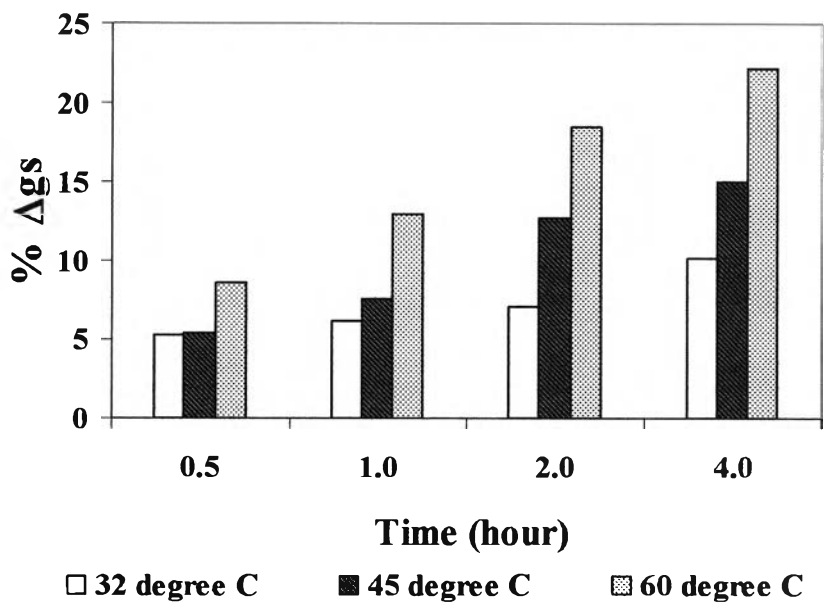

Figure D1 Effect of temperature on % change in gray scale

Table D2 Effect of pH on % change in gray scale

time (hour)	% change in gray scale		
	pH 4.5	pH 6.5	pH 9.0
1	10.35	12.98	9.02
2	14.61	18.47	13.26

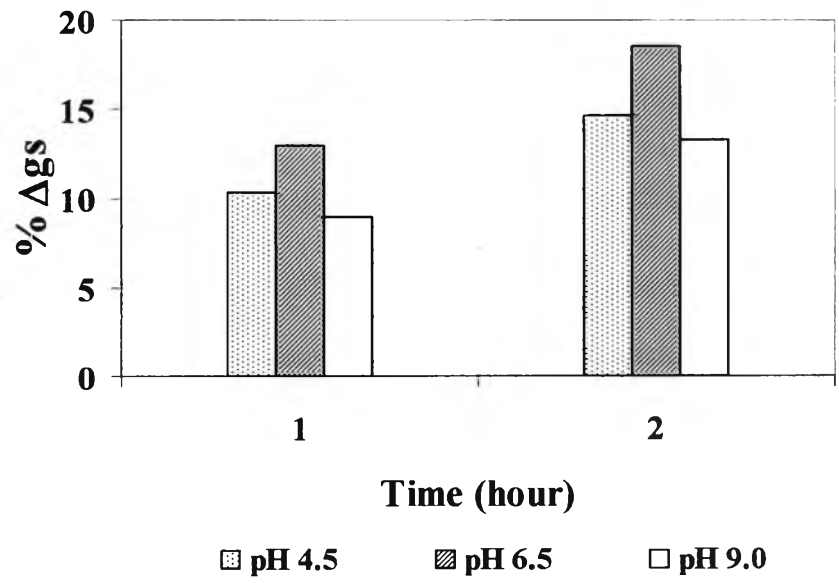

Figure D2 Effect of pH on % change in gray scale

APPENDIX E

Appendix E

Enzymatic Assay of Laccase

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 6.5 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, and Sigma Prod. No. P5379. Adjust to pH 6.5 at 30°C with 1 M KOH.)

B. 0.216 mM Syringaldazine Solution
(Prepare 3 ml in absolute methanol using Syringaldazine, Sigma Prod. No. S-7896.)

C. Laccase Enzyme Solution (Immediately before use, prepare a solution containing25 - 50 units/ml of Laccase in cold deionized water.)

PROCEDURE:

1. Pipette (in millilitres) the following reagents into suitable cuvettes:

	Test	Blank
Deionized Water	------	0.50
Reagent A (Buffer)	2.20	2.20
Reagent C (Enzyme Solution)	0.50	------

2. Equilibrate to 30°C. Monitor the A_{530nm} until constant, using a suitably thermos tatted spectrophotometer. Then add:

Reagent B (Syringaldazine)	0.30	0.30

3. Immediately mix by inversion and record the increase inA530nm for approximately 10 minutes. Obtain the530nm/minute using the maximum linear rate for both theTest and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{530nm} / \text{min Test} - \Delta A_{530nm} / \text{min Blank})(df)}{(0.001)(0.5)}$$

df = Dilution factor

0.001 = the change in A530nm/minute per unit of Laccase at pH 6.5 at 30°C in a 3 ml reaction mix

0.5 = Volume (in millilitre) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

UNIT DEFINITION:

One unit will produce a ΔA_{530nm} of 0.001 per minute at pH 6.5 at 30°C in a 3 ml reaction volume using syringaldazineas substrate.

FINAL ASSAY CONCENTRATION:

In a 3 ml reaction mix, the final concentrations are 73 mM potassium phosphate, 0.02 mM syringaldazine, 10% methanol, and 12.5 - 25.0 units laccase.

APPENDIX F

Appendix F

Enzymatic Assay of Xylanase

1. Weigh 20 mg of xylanase use 4-digit balance.
2. Add 100 ml of 0.02 M acetate buffer pH 4.5
3. Take 20 µl of this solution.
4. Add 20 ml of 0.02 M acetate buffer pH 4.5
5. Add xylan 0.1000 g.
6. Incubate at 30°c for 10 minutes.
7. Take 1 ml of this solution.
8. Follow Nelson-Somogyi method, detail in appendix D.

APPENDIX G

Appendix G

Experimental Data

Table G1 H_2O_2 experiment

H_2O_2	Time (hour)	% change in gray scale	Sample weight (gram)		
			untreated	treated	difference
2%	0.5	4.7	0.3284	0.3180	0.0104
		4.51	0.3294	0.3180	0.0114
		4.89	0.3097	0.3018	0.0079
	1	6.58	0.3264	0.3124	0.0140
		6.2	0.3175	0.3087	0.0088
		6.49	0.3232	0.3115	0.0117
	2	8.87	0.2962	0.2839	0.0123
		9.18	0.3146	0.2989	0.0157
		9.26	0.3197	0.3046	0.0151
10%	0.5	7.96	0.3032	0.2925	0.0107
		8.98	0.2956	0.2848	0.0108
		8.98	0.2927	0.2805	0.0122
	1	12.86	0.3130	0.3005	0.0125
		13.44	0.2836	0.2730	0.0106
		12.64	0.2864	0.2755	0.0109
	2	18.2	0.3247	0.3065	0.0182
		18.72	0.3019	0.2854	0.0165
		18.48	0.3151	0.2989	0.0162

Table G2 Xylanase experiment

Xylanase (u/ml)	Time (hour)	% change in gray scale	Sample weight (gram)		
			untreated	treated	difference
0.25	0.5	-1.42	0.2425	0.2385	0.0040
		-1.19	0.2736	0.2681	0.0055
		-0.49	0.2374	0.2329	0.0045
	1	-1.71	0.2765	0.2728	0.0037
		-0.41	0.2366	0.2319	0.0047
		0.65	0.2672	0.2608	0.0064
	2	-0.80	0.2968	0.2914	0.0054
		-0.84	0.2412	0.2359	0.0053
		-2.36	0.2750	0.2683	0.0067
0.5	0.5	-0.42	0.3001	0.2949	0.0052
		-1.26	0.3095	0.3040	0.0055
		-1.25	0.2453	0.2410	0.0043
	1	-1.79	0.2619	0.2568	0.0051
		-1.70	0.2493	0.2450	0.0043
		-0.25	0.2724	0.2680	0.0044
	2	-1.93	0.2496	0.2445	0.0051
		-1.88	0.2597	0.2499	0.0098
		-2.39	0.3062	0.2987	0.0075
1	0.5	-1.53	0.2704	0.2647	0.0057
		-1.60	0.2454	0.2408	0.0046
		-0.22	0.2633	0.2581	0.0052
	1	-1.05	0.2458	0.2412	0.0046
		-1.72	0.2589	0.2530	0.0059
		-0.70	0.2717	0.2657	0.0060
	2	-2.45	0.2997	0.2907	0.0090
		-1.92	0.2728	0.2654	0.0074
		-2.14	0.2401	0.2367	0.0034

Table G3 Laccase experiment

laccase (u/ml)	time	% change in gray scale	Sample weight (gram)		
			untreated	treated	difference
0.05	0.5	-1.88	0.2984	0.2930	0.0054
		-1.97	0.3018	0.2962	0.0056
		-0.52	0.3112	0.3059	0.0053
	1	-1.99	0.3045	0.2985	0.0060
		-2.32	0.3046	0.2985	0.0061
		-2.19	0.2890	0.2829	0.0061
	2	-2.48	0.3055	0.2968	0.0087
		-2.27	0.2573	0.2502	0.0071
		-2.56	0.2989	0.2903	0.0086
0.25	0.5	-2.64	0.2444	0.2394	0.0050
		-0.56	0.2860	0.2800	0.0060
		-1.42	0.3121	0.3057	0.0064
	1	-3.19	0.2978	0.2919	0.0059
		-1.12	0.2574	0.2519	0.0055
		-1.07	0.3151	0.3073	0.0078
	2	-2.92	0.3151	0.3073	0.0078
		-2.16	0.3151	0.3073	0.0078
		-2.50	0.2953	0.2878	0.0075
1	0.5	-1.29	0.2514	0.2465	0.0049
		-1.70	0.2965	0.2908	0.0057
		0.24	0.2533	0.2483	0.0050
	1	-0.85	0.2569	0.2518	0.0051
		-2.12	0.3103	0.3035	0.0068
		-1.03	0.2965	0.2909	0.0056
	2	-2.86	0.3016	0.2950	0.0066
		-2.42	0.2737	0.2667	0.0070
		-1.94	0.3052	0.2964	0.0088

Table G4 H_2O_2, xylanase and time experiment

H_2O_2	Xylanase (u/ml)	time	% change in gray scale	Sample weight (gram)		
				untreated	treated	difference
2%	0.25	0.5	3.5	0.3042	0.2963	0.0079
			4.07	0.3249	0.3168	0.0081
			3.73	0.2817	0.2724	0.0093
	0.25	1	7.03	0.3137	0.3018	0.0119
			6.07	0.3269	0.3172	0.0097
			7.40	0.3223	0.3101	0.0122
	0.25	2	7.39	0.3092	0.2985	0.0107
			8.25	0.3122	0.2974	0.0148
			8.42	0.3188	0.3068	0.0120
	0.5	0.5	4.52	0.3221	0.3127	0.0094
			4.10	0.3121	0.306	0.0061
			3.81	0.2858	0.2773	0.0085
	0.5	1	6.88	0.3160	0.3049	0.0111
			6.99	0.3374	0.3289	0.0085
			7.46	0.3047	0.2972	0.0075
	0.5	2	8.46	0.3095	0.2991	0.0104
			8.28	0.3330	0.3143	0.0187
			8.02	0.2846	0.2713	0.0133
	1	0.5	3.51	0.3110	0.3008	0.0102
			3.95	0.3208	0.3122	0.0086
			5.46	0.3028	0.2937	0.0091
	1	1	6.79	0.2873	0.2778	0.0095
			8.49	0.3123	0.3008	0.0115
			7.27	0.3125	0.3039	0.0086
	1	2	8.54	0.3298	0.3129	0.0169
			7.92	0.3169	0.3050	0.0119
			9.55	0.3055	0.2902	0.0153
10%	0.25	0.5	9.05	0.2969	0.2856	0.0113
			9.96	0.3082	0.2968	0.0114
			9.55	0.3000	0.2895	0.0105
	0.25	1	13.28	0.3311	0.3185	0.0126
			13.17	0.3108	0.3024	0.0084
			13.29	0.3298	0.3203	0.0095

H_2O_2	Xylanase (u/ml)	time	% change in gray scale	Sample weight (gram)		
				untreated	treated	difference
	0.25	2	17.47	0.2941	0.2795	0.0146
			16.74	0.3189	0.3019	0.0170
			17.76	0.3133	0.2961	0.0172
	0.5	0.5	9.06	0.3070	0.2961	0.0109
			10.09	0.3245	0.3118	0.0127
			9.96	0.3305	0.3284	0.0021
	0.5	1	13.76	0.3140	0.2990	0.0150
			11.66	0.2863	0.2765	0.0098
			13.00	0.2835	0.2739	0.0096
	0.5	2	17.13	0.3098	0.2977	0.0121
			17.71	0.3081	0.2928	0.0153
			16.75	0.3004	0.2845	0.0159
	1	0.5	10.76	0.2814	0.2713	0.0101
			9.03	0.3127	0.3044	0.0083
			12.39	0.3326	0.3227	0.0099
	1	1	14.68	0.2952	0.2830	0.0122
			14.39	0.2876	0.2764	0.0112
			14.06	0.2725	0.2628	0.0097
	1	2	16.51	0.3912	0.2738	0.1174
			18.17	0.3079	0.2921	0.0158
			18.06	0.3092	0.2980	0.0112

Table G5 H_2O_2, laccase and time experiment

H_2O_2	Laccase (u/ml)	time	% change in gray scale	Sample weight (gram)		
				untreated	treated	difference
2%	0.05	0.5	4.57	0.2763	0.2659	0.0104
			5.06	0.2627	0.2589	0.0038
			4.82	0.2959	0.2866	0.0093
	0.05	1	6.36	0.2414	0.2396	0.0018
			6.08	0.3333	0.3198	0.0135
			6.30	0.2616	0.2522	0.0094
	0.05	2	7.72	0.2785	0.2730	0.0055
			8.28	0.2870	0.2755	0.0115
			8.10	0.2952	0.2867	0.0085
	0.25	0.5	4.34	0.2632	0.2569	0.0063
			4.48	0.2658	0.2545	0.0113
			4.48	0.2684	0.2546	0.0138
	0.25	1	5.75	0.2473	0.2356	0.0117
			5.63	0.2663	0.2556	0.0107
			5.72	0.2971	0.2885	0.0086
	0.25	2	8.07	0.2663	0.2556	0.0107
			8.30	0.2904	0.2796	0.0108
			8.21	0.2315	0.2205	0.0110
	1	0.5	4.75	0.2581	0.2435	0.0146
			4.78	0.3204	0.3085	0.0119
			4.84	0.3250	0.3159	0.0091
	1	1	5.92	0.2452	0.2339	0.0113
			6.23	0.2579	0.2489	0.0090
			6.02	0.2889	0.2796	0.0093
	1	2	8.18	0.2845	0.2726	0.0119
			8.94	0.2935	0.2821	0.0114
			8.62	0.2725	0.2606	0.0119
10%	0.05	0.5	8.69	0.2917	0.2870	0.0047
			10.47	0.3080	0.3035	0.0045
			8.66	0.2275	0.2133	0.0142
	0.05	1	11.42	0.2546	0.2478	0.0068
			11.69	0.2911	0.2846	0.0065
			11.47	0.2472	0.2369	0.0103
	0.05	2	23.03	0.1997	0.1918	0.0079
			15.32	0.2312	0.2228	0.0084

H_2O_2	Laccase (u/ml)	time	% change in gray scale	Sample weight (gram)		
				untreated	treated	difference
10%	0.05	2	15.79	0.2369	0.2247	0.0122
	0.25	0.5	7.52	0.2577	0.2524	0.0053
			7.32	0.2760	0.2715	0.0045
			7.38	0.2362	0.2208	0.0154
	0.25	1	9.73	0.2355	0.2281	0.0074
			11.8	0.2893	0.2835	0.0058
			11.57	0.2616	0.2523	0.0093
	0.25	2	19.31	0.3192	0.3098	0.0094
			15.40	0.3037	0.2937	0.0100
			16.35	0.3279	0.3145	0.0134
	1	0.5	10.91	0.3170	0.3123	0.0047
			11.98	0.2103	0.2062	0.0041
			7.64	0.2896	0.2828	0.0068
	1	1	11.90	0.2929	0.2870	0.0059
			13.04	0.2955	0.2886	0.0069
			11.81	0.2484	0.2369	0.0115
	1	2	16.61	0.3023	0.2929	0.0094
			17.69	0.3179	0.3088	0.0091
			17.65	0.2458	0.2358	0.0100

Table G6 xylanase, laccase and time experiment

Xylanase (u/ml)	Laccase (u/ml)	time	% change in gray scale	Sample weight (gram)		
				untreated	treated	difference
0.25	0.05	0.5	-1.20	0.2399	0.2346	0.0053
			-0.53	0.2551	0.2524	0.0027
			-1.00	0.2585	0.2556	0.0029
	0.05	1	-1.23	0.2622	0.2601	0.0021
			-1.06	0.2550	0.2517	0.0033
			-0.56	0.2512	0.2475	0.0037
	0.05	2	-2.59	0.2288	0.2274	0.0014
			-2.08	0.2625	0.2611	0.0014
			-0.93	0.2574	0.2555	0.0019
	0.25	0.5	-0.64	0.2634	0.2612	0.0022
			-1.42	0.2445	0.2423	0.0022
			-0.75	0.2910	0.2874	0.0036
	0.25	1	-1.96	0.2462	0.2434	0.0028
			-2.08	0.2494	0.2434	0.0060
			-1.74	0.2890	0.2723	0.0167
	0.25	2	-2.85	0.2505	0.2427	0.0078
			-2.31	0.2640	0.2619	0.0021
			-2.40	0.2488	0.2464	0.0024
	1	0.5	-1.42	0.2780	0.2739	0.0041
			-1.48	0.2480	0.2442	0.0038
			-0.55	0.2759	0.2701	0.0058
	1	1	-1.63	0.2457	0.2390	0.0067
			-0.93	0.2932	0.2898	0.0034
			-1.17	0.2774	0.2745	0.0029
	1	2	-2.75	0.2449	0.2372	0.0077
			-2.61	0.2834	0.2754	0.0080
			-1.75	0.2774	0.2700	0.0074
0.5	0.05	0.5	-1.28	0.2474	0.2440	0.0034
			-1.10	0.2422	0.2361	0.0061
			-0.98	0.2775	0.2738	0.0037
	0.05	1	-1.46	0.2834	0.2735	0.0099
			-1.78	0.2457	0.2366	0.0091
			-0.74	0.2440	0.2366	0.0074
	0.05	2	-2.32	0.2669	0.2562	0.0107
			-2.55	0.2689	0.2602	0.0087

Xylanase (u/ml)	Laccase (u/ml)	time	% change in gray scale	Sample weight (gram)		
				untreated	treated	difference
0.5	0.05	2	-2.36	0.2750	0.2683	0.0067
	0.25	0.5	-0.99	0.2767	0.2739	0.0028
			-1.14	0.2419	0.2355	0.0064
			-1.67	0.2836	0.2684	0.0152
	0.25	1	-2.45	0.2997	0.2907	0.0090
			-3.03	0.2506	0.2476	0.0030
			-1.53	0.2736	0.2659	0.0077
	0.25	2	-2.58	0.2529	0.2444	0.0085
			-2.92	0.3043	0.2950	0.0093
			-2.45	0.3009	0.2982	0.0027
	1	0.5	-1.05	0.2444	0.2398	0.0046
			-0.93	0.2922	0.2854	0.0068
			-0.84	0.2412	0.2359	0.0053
	1	1	-1.39	0.2560	0.2511	0.0049
			-1.93	0.2496	0.2445	0.0051
			-2.06	0.2428	0.2381	0.0047
	1	2	-2.13	0.2674	0.2617	0.0057
			-2.58	0.2637	0.2581	0.0056
			-2.46	0.2793	0.2695	0.0098
1	0.05	0.5	-1.23	0.2690	0.2641	0.0049
			-1.18	0.2657	0.2596	0.0061
			-0.49	0.2374	0.2329	0.0045
	0.05	1	-1.91	0.2705	0.2641	0.0064
			-2.34	0.2520	0.2481	0.0039
			-2.12	0.3103	0.3035	0.0068
	0.05	2	-2.85	0.2619	0.2584	0.0035
			-2.34	0.2520	0.2481	0.0039
			-2.64	0.2444	0.2394	0.0050
	0.25	0.5	-0.85	0.2569	0.2518	0.0051
			-0.52	0.3112	0.3059	0.0053
			-1.07	0.3151	0.3073	0.0078
	0.25	1	-1.85	0.2997	0.2966	0.0031
			-2.10	0.2903	0.2821	0.0082
			-1.94	0.3052	0.2964	0.0088
	0.25	2	-2.67	0.2939	0.2857	0.0082

Xylanase (u/ml)	Laccase (u/ml)	time	% change in gray scale	Sample weight (gram)		
				untreated	treated	difference
			-2.86	0.3193	0.3099	0.0094
			-2.55	0.3047	0.2953	0.0094
1	1	0.5	-0.68	0.2509	0.2464	0.0045
			-1.42	0.3121	0.3057	0.0064
			-1.03	0.2965	0.2909	0.0056
	1	1	-2.32	0.3046	0.2985	0.0061
			-2.15	0.3150	0.3039	0.0111
			-1.99	0.3045	0.2985	0.0060
	1	2	-2.41	0.2462	0.2398	0.0064
			-2.86	0.3016	0.2950	0.0066
			-2.98	0.2485	0.2336	0.0149

APPENDIX H

Appendix H

ANOVA Results

Table H 1

Test of Between-Subjects Effects

Dependent Variable: % reduce in gray scale

source	Type III Sum of square	df	Mean square	F	Sig.
Corrected model	383.236[a]	5	76,647	543.191	.000
Intercept	1798.800	1	1798.800	12747.905	.000
H_2O_2	203.885	1	203.885	1444.914	.000
time	159.851	2	79.926	566.424	.000
H_2O_2 * time	19.500	2	9.750	69.096	.000
Error	1.693	12	.141		
Total	2183.730	18			
Corrected Total	384.929	17			

a. R Squared = .996 (Adjusted R squared = .994)

Table H 2

Test of Between-Subjects Effects

Dependent Variable: % reduce in gray scale

source	Type III Sum of square	df	Mean square	F	Sig.
Corrected model	6.700[a]	8	.837	1.703	.166
Intercept	44.776	1	44.776	91.069	.000
xylanase	1.532	2	.776	1.558	.238
time	4.396	2	2.198	4.471	.027
xylanase * time	.771	4	.193	.392	.811
Error	8.850	18	.492		
Total	60.326	27			
Corrected Total	15.550	26			

a. R Squared = .431 (Adjusted R squared = .178)

Table H 3

Test of Between-Subjects Effects

Dependent Variable: % reduce in gray scale

source	Type III Sum of square	df	Mean square	F	Sig.
Corrected model	7.811[a]	8	.976	1.709	.164
Intercept	91.595	1	91.595	160.366	.000
laccase	1.152	2	.576	1.009	.384
time	6.055	2	3.028	5.301	.015
laccase * time	.603	4	.151	.264	.897
Error	10.281	18	.571		
Total	109.687	27			
Corrected Total	18.092	26			

a. R Squared = .432 (Adjusted R squared = .179)

Table H 4

Test of Between-Subjects Effects

Dependent Variable: % reduce in gray scale

source	Type III Sum of square	df	Mean square	F	Sig.
Corrected model	1013.475[a]	17	59.616	109.024	.000
Intercept	5475.462	1	5475.462	10013.375	.000
xylanase	5.723	2	2.861	5.233	.010
H_2O_2	676.423	1	676.423	1237.024	.000
time	301.947	2	150.974	276.096	.000
xylanase * H_2O_2	.935	2	.467	.855	.434
xylanase* time	.696	4	.174	.318	.864
H_2O_2 * time	26.860	2	13.430	24.560	.000
xylanase * H_2O_2* time	.892	4	.223	.408	.802
Error	19.685	36	.547		
Total	6508.622	54			
Corrected Total	1033.161	53			

a. R Squared = .981 (Adjusted R squared = .972)

Table H 5

Test of Between-Subjects Effects

Dependent Variable: % reduce in gray scale

source	Type III Sum of square	df	Mean square	F	Sig.
Corrected model	838.196[a]	17	49.306	494.531	.000
Intercept	4539.700	1	4539.700	45532.758	.000
laccase	1.815	2	.907	9.101	.001
H_2O_2	439.071	1	439.071	4403.841	.000
time	335.394	2	167.697	1681.985	.000
laccase * H_2O_2	.073	2	,036	.365	.697
laccase * time	4.187	4	1.047	10.499	.000
H_2O_2 * time	55.675	2	27.837	279.206	.000
laccase * H_2O_2* time	1.981	4	.495	4.968	.003
Error	3.589	36	.100		
Total	5381.485	54			
Corrected Total	841.785	53			

a. R Squared = .981 (Adjusted R squared = .972)

TableH6

Test of Between-Subjects Effects

Dependent Variable: % reduce in gray scale

source	Type III Sum of square	df	Mean square	F	Sig.
Corrected model	36.241[a]	26	1.394	9.721	.000
Intercept	247.783	1	247.783	1728.046	.000
xylanase	1.874	2	.937	6.536	.003
laccase	1.333	2	.666	4.647	.014
time	28.663	2	14.331	99.948	.000
xylanase * laccase	1.282	4	.320	2.235	.077
xylanase * time	1.397	4	.349	2.435	.058
laccase * time	.846	4	.211	1.474	.223
xylanase* laccase * time	.847	8	.106	.739	.657
Error	7.743	54	.143		
Total	291.767	81			
Corrected Total	43.984	80			

a. R Squared = .824 (Adjusted R squared = .739)

Table H7 ANOVA results on effect of xylanase concentration on xylose produced xylanase single component experiment (room temperature)

Source of variation	Sum of Squares	df	Mean Square	F	P
Between group	3743.415	4	935.854	3.921	.006
Within group	19810.519	83	238.681		
Total	23553.933	87			

Table H8 ANOVA results on effect of xylanase concentration on xylose produced xylanase single component experiment (room temperature)

Source of variation	Sum of Squares	df	Mean Square	F	P
Between group	17876.044	5	3575.209	51.633	.000
Within group	5677.889	82	69.243		
Total	23553.933	87			

TableH9

Tests of Between-Subjects Effects

Dependent Variable: %reduce_in_grey_scale

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2910.619[a]	27	107.801	142.198	.000
Intercept	20925.228	1	20925.228	27602.113	.000
H2O2	1202.594	6	200.432	264.387	.000
time	1626.301	3	542.100	715.076	.000
H2O2 * time	81.724	18	4.540	5.989	.000
Error	42.454	56	.758		
Total	23878.301	84			
Corrected Total	2953.073	83			

a. R Squared = .986 (Adjusted R Squared = .979)

TableH10

Multiple Comparisons
Dependent Variable: %change in gray scale
LSD

(I) H2O2	(J) H2O2	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
2.00	5.00	-6.7783(*)	.35546	.000	-7.4904	-6.0663
	8.00	-7.9558(*)	.35546	.000	-8.6679	-7.2438
	10.00	-7.6958(*)	.35546	.000	-8.4079	-6.9838
	15.00	-9.2008(*)	.35546	.000	-9.9129	-8.4888
	20.00	-13.0400(*)	.35546	.000	-13.7521	-12.3279
	30.00	-10.7975(*)	.35546	.000	-11.5096	-10.0854
5.00	2.00	6.7783(*)	.35546	.000	6.0663	7.4904
	8.00	-1.1775(*)	.35546	.002	-1.8896	-.4654
	10.00	-.9175(*)	.35546	.012	-1.6296	-.2054
	15.00	-2.4225(*)	.35546	.000	-3.1346	-1.7104
	20.00	-6.2617(*)	.35546	.000	-6.9737	-5.5496
	30.00	-4.0192(*)	.35546	.000	-4.7312	-3.3071
8.00	2.00	7.9558(*)	.35546	.000	7.2438	8.6679
	5.00	1.1775(*)	.35546	.002	.4654	1.8896
	10.00	.2600	.35546	.468	-.4521	.9721
	15.00	-1.2450(*)	.35546	.001	-1.9571	-.5329
	20.00	-5.0842(*)	.35546	.000	-5.7962	-4.3721
	30.00	-2.8417(*)	.35546	.000	-3.5537	-2.1296
10.00	2.00	7.6958(*)	.35546	.000	6.9838	8.4079
	5.00	.9175(*)	.35546	.012	.2054	1.6296
	8.00	-.2600	.35546	.468	-.9721	.4521
	15.00	-1.5050(*)	.35546	.000	-2.2171	-.7929
	20.00	-5.3442(*)	.35546	.000	-6.0562	-4.6321
	30.00	-3.1017(*)	.35546	.000	-3.8137	-2.3896
15.00	2.00	9.2008(*)	.35546	.000	8.4888	9.9129
	5.00	2.4225(*)	.35546	.000	1.7104	3.1346
	8.00	1.2450(*)	.35546	.001	.5329	1.9571
	10.00	1.5050(*)	.35546	.000	.7929	2.2171
	20.00	-3.8392(*)	.35546	.000	-4.5512	-3.1271
	30.00	-1.5967(*)	.35546	.000	-2.3087	-.8846
20.00	2.00	13.0400(*)	.35546	.000	12.3279	13.7521
	5.00	6.2617(*)	.35546	.000	5.5496	6.9737
	8.00	5.0842(*)	.35546	.000	4.3721	5.7962
	10.00	5.3442(*)	.35546	.000	4.6321	6.0562
	15.00	3.8392(*)	.35546	.000	3.1271	4.5512
	30.00	2.2425(*)	.35546	.000	1.5304	2.9546
30.00	2.00	10.7975(*)	.35546	.000	10.0854	11.5096
	5.00	4.0192(*)	.35546	.000	3.3071	4.7312
	8.00	2.8417(*)	.35546	.000	2.1296	3.5537
	10.00	3.1017(*)	.35546	.000	2.3896	3.8137
	15.00	1.5967(*)	.35546	.000	.8846	2.3087
	20.00	-2.2425(*)	.35546	.000	-2.9546	-1.5304

Based on observed means.
* The mean difference is significant at the .05 level.

APPENDIX I

Appendix I

Determination of Xylose Content

Chemicals

1. Alkaline copper reagent from Merck, Inc.
2. Arsenomolybdate reagent from Merck, Inc.

Apparatus

UV-2450 UV-visible spectrophotometer from Shimadzu

Nelson's Test for Reducing Sugar

1. Measure 1 ml. of sample into test tube
2. Add 1.0 ml. of 0.02 M acetate buffer to test tube
3. Add 1.0 ml. of alkaline copper reagent
4. Mix well then boil for 10 min.
5. Cool the sample down by put it in a bowl of water
6. Add 1.0 ml. of Arsenomolybdate
7. Mix well
8. Using spectrophotometer, measure absorbance at 660 nm.
9. Using standard curve, presented in Figure I1, calculate the corresponding xylose concentration.

Table I1 Data for determination of standard curve

xylose concentration (μg/ml)	Absorbance
0	0
5	0.182
10	0.388
15	0.524
20	0.743
25	0.961

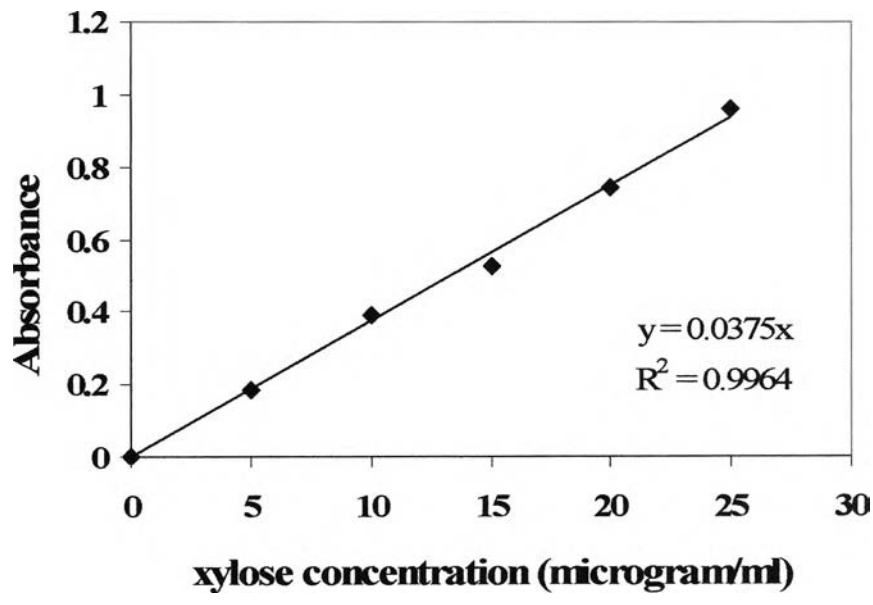

Figure I1 Standard curve for determination of xylose concentration

VITAE

Miss Bhavadee Iamtasna was born on June 25, 1958 in Bangkok, Thailand. She graduated from high school in 1976 from Triam Udom Suksa School in Bangkok. She received a Bachelor of Science degree in Chemical Engineering from the Faculty of Science, Chulalongkorn University, Bangkok, Thailand in 1980. She received a Master of Science degree in Chemical Engineering from the Department of Chemical Engineering, College of Engineering, University of South Carolina, USA in 1986.