APPENDIX A: ANALYTICAL TECHNIQUES

DETERMINATION OF SOLUBLE PROTEIN

Reagents

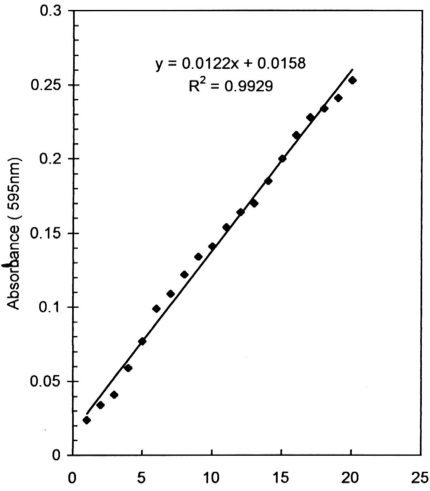
Coomassie Brilliant Blue G-250 (100mg) was dissolved in 50ml 95% ethanol. To this solution, 100ml 85% (w/v) phosphoric acid was added. The resulting solution was made upto a final volume of 1L.

Procedure for preparation of protein calibration plot

Bovine Serum Albumin (BSA) solution containing 10 to 100 μg protein in a volume upto 0.1ml was pipetted into 12 x 100 mm test tubes. The volume in the test tube was adjusted to 0.1ml with appropriate buffer. Then 5.0ml of protein reagent was added into the test tubes and the contents mixed either by inversion or vortexing. The absorbance at λ =595 nm was measured after 2 min and before 1h in a 3.0 ml cuvette against a reagent blank prepared from 0.1 ml of the appropriate buffer and 5.0 ml of protein reagent. The weight of the protein was plotted against the corresponding absorbance resulting in a standard curve.

Protein assay of test solution

Solution containing protein in a volume of up to 0.1 ml was pipetted into 12 x 100 mm test tubes. The volume in the test tubes was adjusted to 0.1 ml with appropriate buffer and the protein content was measured following the procedure described above for protein standard curve. The amount of protein in the test solution was calculated by using the protein standard graph with the following formula:



Protein Calibration Plot

$$\text{Soluble protein (mg/ml)} = \frac{(\text{Final absorbance} - 0.0158)}{0.0122} \times \frac{1}{0.2\ \text{ml}} \times \frac{1\ \text{mg}}{1000\ \mu\text{g}}$$

XYLANASE ACTIVITY

Reagents

1% (w/v) suspension of xylan in 50 mM sodium citrate buffer (pH 4.8).To prepare the substrate, heat 1% (w/w) suspension of xylan into buffer to boiling point on a heating magnetic stirrer. Cool the suspension with continuous stirring overnight. Dinitrosalicylic (DNS) acid reagent, Rochelle salt (40%),50 mM sodium citrate buffer, pH 4.8.

Dinitrosalicylic acid (DNS)

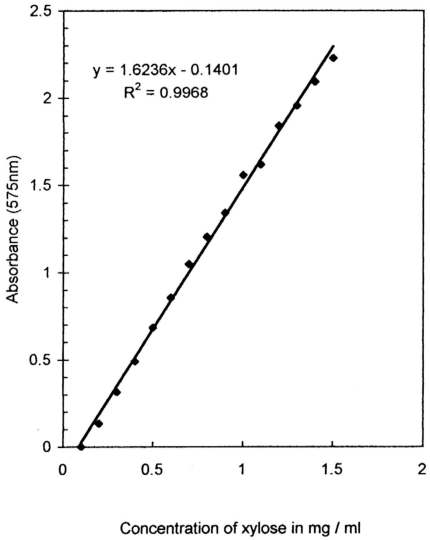
The DNS reagent used contained 1% (w/v) dinitrosalicylic acid, 0.2 % (w/v) phenol, 0.05% sodium sulfite and 1 % NaOH (Miller, 1959). Large batches of reagent was prepared without sulfite and stored in a dark bottle at 4°C. Appropriate amount of sodium sulfite was added to aliquots just prior to the time when the reagent was to be used.

Rochelle salt solution (40%)

Dissolve about 400 g of AR grade Rochelle salt (potassium sodium tartarate tetrahydrate crystals $C_4H_4KNaO_6.4H_2$) in 1L of distilled water.

Procedure

About 1.8 ml substrate solution was mixed with 0.2 ml of enzyme solution. The mixture was mixed and incubated for 1 h at 40° C in a water bath with moderate shaking. The reducing sugars released in the reaction was determined using the DNS method. The standard used was pure xylose.



Xylose Calibration Plot

Calculation of Unit of Activity

The unit of enzyme activity was μmol reducing sugar released per min. Correction was made for absorbance due to back ground color in the enzyme black. By using the standard line for xylose, the corrected absorbance was converted to enzyme activity units. Finally, the activity in the original sample was calculated by multiplying activity units by the dilution factor using the following formula:

$$\text{Xylanase (U/ml)} = \frac{(\text{Final absorbance}+0.1401)}{1.6326} \times \frac{\text{dilution factor}}{0.2\text{ml}} \times \frac{1}{60\text{ min}} \times \frac{1000\mu\text{g}}{1\text{mg}} \times \frac{1\mu\text{mol}}{150.13\mu\text{g}}$$

LACCASE ACTIVITY

Reagents

0.1mM syringaldazine (4-Hydrxy- 3, 5-dimethoxybenzaldehyde azine) in 50% ethanol. The substrate was dissolved in 50% ethanol after 13 h of stirring. 50mM sodium citrate buffer, ph 4.8.

Procedure

0.2ml enzyme solution was mixed with 3.0ml buffer at room temperature. Then 0.2ml portion of 0.1 mM syringaldazine was added and mixed with a vortex mixer. The initial rate of color change was measured on a spectrophotometer at wave length $\lambda = 525$nm.

Calculation of Unit of Activity

One unit was defined as the enzyme producing one unit of absorbance change/min./g substrate. Laccase activity in the culture filtrate was calculated as follows:

$$\text{Laccase activity (U/ml)} = \text{Final absorbance} \times \frac{\text{dilution factor} \times 1\text{ min}}{0.2\text{ ml}}$$

LIGNIN PEROXIDASE ACTIVITY

Reagents

1M tartaric acid:

15g tartaric acid was dissolved in 100ml. of distilled water to make 1M solution.

Vertaldehyde stock solution:

The stock vertaldehyde was prepared by dissolving 20mg of vertaldehyde (2.3-Dimethoxybenzaldehyde) in 100ml with water.

0.5mM hydrogen peroxide stock solution:

first a stock of 1M H_2O_2 was prepared by taking 5.66ml. of H_2O_2 and topping it up to 100ml with distilled water to give 0.5mM solution of H_2O_2.

2 mM vertryl alcohol solution:

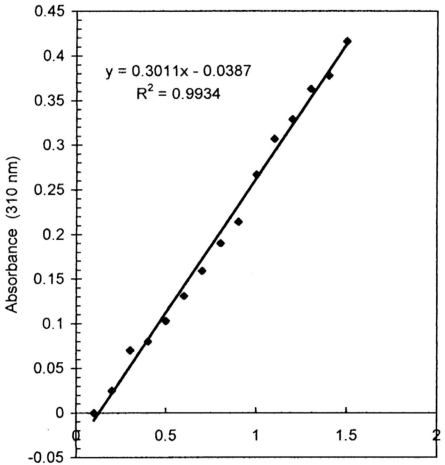
0.4ml of stock solution was taken and was topped up to 100ml with distilled water to give 2 mM vertryl alcohol solution.

0.5mM hydrogen peroxide solution:

0.5ml of stock solution was taken and topped up to 100ml with distilled water to give 0.5mM hydrogen peroxide solution.

Procedure

In the test tubes different aliquots of the stock solution were taken in the volume from 0.1ml till 2.0ml. and mixed with 3.0ml of 100mM sodium tartarate buffer pH 3.0. To this solution 0.5ml. of 0.5 mM H_2O_2 was added and the concentration of vertaldehyde was measured at 310 nm after 5 min. In the blank no vertaldehyde was added and the test tube had 3.2ml of 100mM sodium tartarate buffer pH 3.0. A standard was plotted by taking the absorbance verses concentration.



Lignin peroxidase Calibration Plot

Lignin peroxidase assay

0.2ml of enzyme solution was mixed with 3.0ml of 100mM sodium tartarate buffer pH 3. To this solution 0.5mM vertryl alcohol solution was added. The contents wee mixed with vortex. The reaction was started by adding 0.5ml of 0.5mM H_2O_2 and the oxidation of vertryl alcohol to vertaldehyde was measured at 310nM after 5 min. The substrate blank and the enzyme blank were also treated in the same manner. In the substrate 0.2ml of 100mM sodium tartarate buffer pH 3.0 was added in place of the enzyme solution and in the enzyme blank 0.5ml of 100mM sodium tartarate buffer pH 3.0 was added in place of the substrate solution.

Calculation of unit of activity

A vertaldehyde standard plot was used to give the concentration of vertaldehyde produced by oxidation of vertryl alcohol (Tien and Kirk 1984). One unit of enzyme (U) is defined as 1μmol vertaldehyde released /min/gm of substrate. Lignin peroxidase was measured by recording the increase in absorbance at 310nm

$$\text{Lignin peroxidase (U/ml)} = \frac{(\text{final absorbance} + 0.0387)}{3.0107} \times \frac{\text{dilution factor}}{0.2\ \text{ml}} \times \frac{1}{5\ \text{min}} \times \frac{1000\mu\text{g}}{1\text{mg}} \times \frac{1\mu\text{mole}}{166.18\mu\text{g}}$$

APPENDIX B: BUFFERS AND MEDIA

1. BUFFERS

a. Sodium citrate buffer (0.5 M, pH 4.8)

To prepare 1L of 1M solution of sodium citrate, dissolve 210.0 g of citric acid monohydrate in 750 ml of distilled water. Adjust the pH to 4.3 with sodium hydroxide pellets (approx. 50-60 g). Dilute to 1L and adjust the pH to 4.5 with sodium hydroxide if necessary. This is stock solution of 1M sodium citrate buffer, pH 4.5. Dilute twenty times to get 0.05 M sodium citrate buffer solution with a final pH of 4.8.

b. Sodium tartarate buffer (100mM)

2.3 g of sodium tartarate was dissolved in 100ml of distilled water to make 100mM solution. The pH of this solution was set to 3.0 using 1 M tartaric acid.

2. MEDIA

a. *Potato Dextrose Agar (PDA)*

Suspend 39 g of PDA and 2 g of Agar No.3 (Oxoid) in 1L distilled water and boil to dissolve completely. Autoclave for 20 min at 15 psi, 115° C. Cool medium to 45-50°C and dispense into Petri dishes. Final pH should be 5.6 ± 0.2 at 25°C.

APPENDIX C: DATA AND STATISTICAL TABLES

Table 1: Parameter variation in SSF with 0.38% (w/v) of Urea and 10% (w/w) 2 week old inoculum.

Time (Day)	pH	Protein	Xylanase	Lignin peroxidase	Laccase
0	4.03	0.002	3.45	0.83	0.6
5	4.09	0.018	4.47	0.85	3.4
10	4.28	0.043	5.26	1.31	13.65
15	4.66	0.065	5.02	1.07	14.35
20	4.36	0.044	4.35	0.99	13.0
25	4.53	0.071	4.93	0.95	10.75
30	4.56	0.050	5.64	0.83	5.85

Notes: Soluble protein content is expressed as mg/g sago 'hampas'.
Xylanase, lignin peroxidase and laccase activities are expressed as U/g sago 'hampas'.

Table 2: Parameter variation in SSF with different nitrogen levels and 10% (w/w) 2week old inoculum.

Time (Day)	Nitrogen level	pH	Protein	Xylanase	Lignin peroxidase	Laccase
15	0.19%	4.4	0.023	3.39	1.01	0.65
	0.38%	4.5	0.046	4.87	0.97	14.35
	0.57%	4.5	0.048	6.01	1.05	14.70
	0.76%	4.9	0.108	2.82	0.84	15.20

Notes: Soluble protein content is expressed as mg/g sago 'hampas'.
Xylanase, lignin peroxidase and laccase activities are expressed as U/g sago 'hampas'

Table 3: Parameter variation in SSF with different inoculum age, inoculum densities and 0.76% (w/v) of Urea.

Time (Day)	Inoculum age and size	pH	Protein	Xylanase	Lignin peroxidase	Laccase
15	2-week					
	5%	4.9	0.093	2.31	0.93	10.8
	10%	5.1	0.120	2.89	1.01	15.35
	3-week					
	5%	5.08	0.153	4.48	1.17	17.30
	10%	4.9	0.221	5.06	1.77	18.05
	4-week					
	5%	4.7	0.113	3.33	1.01	13.30
	10%	4.9	0.160	3.60	1.63	14.05

Notes: Soluble protein content is expressed as mg/g sago 'hampas'.
Xylanase, lignin peroxidase and laccase activities are expressed as U/g sago 'hampas'

Table 4: Parameter variation in SSF with 0.76% (w/v) of Urea.and 10% (w/w) 3 week old inoculum.

Time (Day)	pH	Protein	Xylanase	Lignin peroxidase	Laccase
0	4.3	0.014	2.09	0.823	0.165
5	4.5	0.060	3.95	1.473	5.15
10	4.6	0.133	6.36	0.993	18.35
15	4.6	0.104	5.62	0.965	17.35
20	4.5	0.074	5.10	0.027	11.5

Notes: Soluble protein content is expressed as mg/g sago 'hampas'.
Xylanase, lignin peroxidase and laccase activities are expressed as U/g sago 'hampas'

Table 5: ANOVA of the effect of nitrogen levels on soluble protein content of the culture filtrate.

One-Way Analysis of Variance

Data

0.02	0.02	0.03	0.04	0.06	0.04	0.05	0.05	0.04	0.12	0.09	0.11

Level codes

0.19	0.19	0.19	0.38	0.38	0.38	0.57	0.57	0.57	0.76	0.76	0.76

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	.0114250	3	.0038083	35.154	.0001
Within groups	.0008667	8	.0001083		

Total (corrected) .0122917 11
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
0.19	3	.0233333	X
0.38	3	.0466667	X
0.57	3	.0466667	X
0.76	3	.1066667	X

Contrast	Difference	+/-	limits
0.19-0.38	-0.02333		0.01960*
0.19-0.57	-0.02333		0.01960*
0.19-0.76	-0.08333		0.01960*
0.38-0.57	0.00000		0.01960
0.38-0.76	-0.06000		0.01960*
0.57-0.76	-0.06000		0.01960 *

* denotes a statistically significant difference

Table 6: ANOVA of the effect of nitrogen levels on xylanase activity of the culture filtrate.

One-Way Analysis of Variance

Data

4.04	3.54	3.10	3.89	5.66	5.67	6.96	4.89	6.96	3.58	3.34	1.90

Level codes

0.19	0.19	0.19	0.38	0.38	0.38	0.57	0.57	0.57	0.76	0.76	0.76

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	20.318025	3	6.7726750	7.685	.0097
Within groups	7.050667	8	.8813333		

Total (corrected) 27.368692 11
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
0.76	3	2.9400000	X
0.19	3	3.5600000	XX
0.38	3	5.0733333	XX
0.57	3	6.2700000	X

Contrast	Difference	+/-	limits
0.19-0.38	-1.51333		1.76810
0.19-0.57	-2.71000		1.76810 *
0.19-0.76	0.62000		1.76810
0.38-0.57	-1.19667		1.76810
0.38-0.76	2.13333		1.76810*
0.57-0.76	3.33000		1.76810*

* denotes a statistically significant difference

Table 7: ANOVA of the effect of nitrogen levels on lignin peroxidase activity of the culture filtrate.

One-Way Analysis of Variance

Data

1.08	0.98	0.97	1.27	0.89	0.79	0.96	1.21	0.99	0.83	0.82	0.87

Level codes

0.19	0.19	0.19	0.38	0.38	0.38	0.57	0.57	0.57	0.76	0.76	0.76

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	.0768333	3	.0256111	1.175	.3781
Within groups	.1743333	8	.0217917		

Total (corrected) .2511667 11
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
0.76	3	.8400000	X
0.38	3	.9833333	X
0.19	3	1.0100000	X
0.57	3	1.0533333	X

Contrast	Difference	+/-	limits
0.19-0.38	0.02667		0.27802
0.19-0.57	-0.04333		0.27802
0.19-0.76	0.17000		0.27802
0.38-0.57	-0.07000		0.27802
0.38-0.76	0.14333		0.27802
0.57-0.76	0.21333		0.27802

Table 8: ANOVA of the effect of nitrogen levels on laccase activity of the culture filtrate.

One-Way Analysis of Variance

Data

0.45	0.95	0.65	8.3	21.05	13.8	9	21.3	13.85	18.25	17.6	9.75

Level codes

0.19	0.19	0.19	0.38	0.38	0.38	0.57	0.57	0.57	0.76	0.76	0.76

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	447.27729	3	149.09243	5.862	.0204
Within groups	203.45500	8	25.43188		

Total (corrected) 650.73229 11
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
0.19	3	.683333	X
0.38	3	14.383333	X
0.57	3	14.716667	X
0.76	3	15.200000	X

Contrast	Difference	+/-	limits
0.19-0.38			9.49785*
0.19-0.57			9.49785*
0.19-0.76			9.49785*
0.38-0.57			9.49785
0.38-0.76			9.49785
0.57-0.76			9.49785

* denotes a statistically significant difference

Table 9: ANOVA of the effect of Inoculum age on soluble protein content of the culture Filtrate.

One-Way Analysis of Variance

Data

0.11	0.13	0.11	0.25	0.20	0.19

Level codes

2-Weeks	2-Weeks	2-Weeks	3-Weeks	3-Weeks	3-Weeks

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	.0140167	1	.0140167	24.029	.0080
Within groups	.0023333	4	.0005833		

Total (corrected) .0163500 5
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
2-Weeks	3	.1166667	X
3-Weeks	3	.2133333	X

Contrast	Difference	+/-	limits
2-Weeks - 3-Weeks	-0.09667		0.05477*

* denotes a statistically significant difference

Table 10: ANOVA of the effect of inoculum age on xylanase activity of the culture filtrate.

One-Way Analysis of Variance

Data

2.74	2.94	3.34	4.69	5.28	5.81

Level codes

2-Weeks	2-Weeks	2-Weeks	3-Weeks	3-Weeks	3-Weeks

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	7.6162667	1	7.6162667	37.405	.0036
Within groups	.8144667	4	.2036167		

Total (corrected) 8.4307333 5
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
2-Weeks	3	3.0066667	X
3-Weeks	3	5.2600000	X

Contrast	Difference	+/-	limits
2-Weeks - 3-Weeks	-2.25333		1.02330*

* denotes a statistically significant difference

Table 11: ANOVA of the effect of inoculum age on lignin peroxidase activity of the culture filtrate.

One-Way Analysis of Variance

Data

0.97	1.17	1.01	2.41	1.47	1.19

Level codes

2-Weeks	2-Weeks	2-Weeks	3-Weeks	3-Weeks	3-Weeks

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	.6144000	1	.6144000	2.929	.1622
Within groups	.8392000	4	.2098000		

Total (corrected) 1.4536000 5
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
2-Weeks	3	1.0500000	X
3-Weeks	3	1.6900000	X

Contrast	Difference	+/-	limits
2-Weeks - 3-Weeks	-0.64000		1.03872

Table 12: ANOVA of the effect of inoculum age on laccase activity of the culture filtrate

One-Way Analysis of Variance

Data

15.45	16.65	14	18.1	17.75	18.3

Level codes

2-Weeks	2-Weeks	2-Weeks	3-Weeks	3-Weeks	3-Weeks

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	10.800417	1	10.800417	11.750	.0266
Within groups	3.676667	4	.919167		

Total (corrected) 14.477083 5
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
2-Weeks	3	15.366667	X
3-Weeks	3	18.050000	X

Contrast	Difference	+/-	limits
2-Weeks - 3-Weeks	-2.68333		2.17418*

* denotes a statistically significant difference

Table 13: ANOVA of the effect of optimizing parameters on soluble protein content of the culture filtrate.

One-Way Analysis of Variance

Data

0.05	0.04	0.03	0.12	0.14	0.14

Level codes

2-Weeks	2-Weeks	2-Weeks	3-Weeks	3-Weeks	3-Weeks

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	.0130667	1	.0130667	112.000	.0005
Within groups	.0004667	4	.0001167		

Total (corrected) .0135333 5
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
2-Weeks	3	.0400000	X
3-Weeks	3	.1333333	X

Contrast	Difference	+/-	limits
2-Weeks - 3-Weeks	-0.09333		0.02449*

* denotes a statistically significant difference

Table 14: ANOVA of the effect of optimizing parameters on xylanase activity of the culture filtrate.

One-Way Analysis of Variance

Data

5.46	5.43	5.43	6.34	6.60	6.92

Level codes

2-Weeks	2-Weeks	2-Weeks	3-Weeks	3-Weeks	3-Weeks

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	2.0886000	1	2.0886000	49.318	.0022
Within groups	.1694000	4	.0423500		

Total (corrected) 5
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
2-Weeks	3	5.4400000	X
3-Weeks	3	6.6200000	X

Contrast	Difference	+/-	limits
2-Weeks - 3-Weeks	-1.18000		0.46669*

* denotes a statistically significant difference

Table 15: ANOVA of the effect of optimizing parameters on lignin peroxidase activity of the culture filtrate.

One-Way Analysis of Variance

Data

2.20	0.89	0.86	0.81	1.17	1.03

Level codes

2-Weeks	2-Weeks	2-Weeks	3-Weeks	3-Weeks	3-Weeks

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	.1472667	1	.1472667	.476	.5350
Within groups	1.2367333	4	.3091833		

Total (corrected) 1.3840000 5
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
3-Weeks	3	1.0033333	X
2-Weeks	3	1.3166667	X

Contrast	Difference	+/-	limits
2-Weeks - 3-Weeks	0.31333		1.26097

Table 16: ANOVA of the effect of optimizing parameters on laccase activity of the culture filtrate.

One-Way Analysis of Variance

Data

18.4	18.25	18.4	14.2	15.15	11.66

Level codes

2-Weeks	2-Weeks	2-Weeks	3-Weeks	3-Weeks	3-Weeks

Confidence level: 95 Range test: LSD

Analysis of variance

Source of variation	Sum of Squares	d.f.	Mean square	F-ratio	Sig. level
Between groups	32.853600	1	32.853600	20.136	.0109
Within groups	6.526400	4	1.631600		

Total (corrected) 39.380000 5
0 missing value (s) have been excluded.

Multiple range analysis

Method level	95% LSD count	Average	Homogeneous groups
3-Weeks	3	13.670000	X
2-Weeks	3	18.350000	X

Contrast	Difference	+/-	limits
2-Weeks - 3-Weeks	4.68000		2.89671 *

* denotes a statistically significant difference

Table 17: Data of standard deviation for pH under unoptimized parameters.

Day	Activity	Std. Dev
0	4.09	0
5	4.03	0
10	4.28	0.014
15	4.66	0.078
20	4.36	0
25	4.53	0.021
30	4.56	0.035

Std. Dev = standard deviation

Table 18: Data of standard deviation for soluble protein under unoptimized parameters.

Day	Activity	Std. Dev
0	0.00172	0
5	0.018	0
10	0.0427	0.007
15	0.065	0
20	0.044	0
25	0.071	0.007
30	0.05	0

Table 19: Data of standard deviation for xylanase under unoptimized parameters.

Day	Activity	Std. Dev
0	3.45	0.071
5	4.47	0.141
10	5.26	0
15	5.02	0.071
20	4.35	0
25	4.93	0
30	5.64	0.141

Table 20: Data of standard deviation for lignin peroxidase under unoptimized Parameters.

Day	Activity	Std. Dev
0	0.83	0
5	0.85	0
10	1.31	0
15	1.07	0
20	0.99	0.141
25	0.95	0
30	0.83	0

Table 21: Data of standard deviation for laccase under unoptimized parameters.

Day	Activity	Std. Dev
0	0.6	0
5	3.4	0.141
10	13.65	0.707
15	14.35	0
20	13	0
25	10.75	0
30	5.85	0.424

Table 22: Data of standard deviation for pH using different nitrogen levels.

%age Urea	0.19	0.38	0.57	0.76
Activity	4.4	4.5	4.5	4.9
Std. Dev	0.021	0.007	0.028	0.057

Table 23: Data of standard deviation for soluble protein using different nitrogen levels.

%age Urea	0.19	0.38	0.57	0.76
Activity	0.023	0.046	0.048	0.108
Std. Dev	0	0.007	0	0

Table 24: Data of standard deviation for xylanase using different nitrogen levels.

%age Urea	0.19	0.38	0.57	0.76
Activity	3.39	4.87	6.01	2.82
Std. Dev	0.311	0.007	0	0.17

Table 25: Data of standard deviation for lignin peroxidase using different nitrogen levels.

%age Urea	0.19	0.38	0.57	0.76
Activity	1.01	0.97	1.05	0.84
Std. Dev	0.007	0.071	0.021	0

Table 26: Data of standard deviation for laccase using different nitrogen levels.

%age Urea	0.19	0.38	0.57	0.76
Activity	0.65	14.35	14.7	15.2
Std. Dev	0.141	0	0	0.46

Table 27: Data of standard deviation for pH using different inoculum ages with different (w/w) densities.

Week	Activity of **5%** (w/w) density	Std. Dev	Activity of **10%** (w/w) density	Std. Dev
Two	4.9	0.007	5.1	0
Three	5.08	0.028	4.9	0.014
Four	4.7	0.007	4.9	0.071

Table 28: Data of standard deviation for soluble protein using different inoculum ages with different (w/w) densities.

Week	Activity of **5%** (w/w) density	Std. Dev	Activity of **10%** (w/w) density	Std. Dev
Two	0.093	0	0.12	0
Three	0.153	0	0.211	0
Four	0.113	0	0.16	0

Table 29: Data of standard deviation for xylanase using different inoculum ages with different (w/w) densities.

Week	Activity of **5%** (w/w) density	Std. Dev	Activity of **10%** (w/w) density	Std. Dev
Two	2.231	0.011	2.89	0.013
Three	4.48	0.003	5.06	0.037
Four	3.33	0.006	3.6	0.001

Table 30: Data of standard deviation for lignin peroxidase using different inoculum ages with different (w/w) densities.

Week	Activity of **5%** (w/w) density	Std. Dev	Activity of **10%** (w/w) density	Std. Dev
Two	0.93	0.014	1.01	0.021
Three	1.17	0	1.77	0.198
Four	1.01	0.057	1.63	0.127

Table 31: Data of standard deviation for laccase using different inoculum ages with different (w/w) densities.

Week	Activity of **5%** (w/w) density	Std. Dev	Activity of **10%** (w/w) density	Std. Dev
Two	10.8	1.45	15.35	0.849
Three	17.3	0.177	18.05	0.141
Four	13.3	1.096	14.05	0.318

Table 32: Data of standard deviation for pH under optimized parameters.

Day	Activity	Std. Dev
0	4.3	0
5	4.5	0
10	4.6	0.007
15	4.6	0.007
20	4.5	0

Table 33: Data of standard deviation for soluble protein under optimized parameters.

Day	Activity	Std. Dev
0	0.014	0
5	0.06	0
10	0.133	0
15	0.104	0
20	0.074	0

Table 34: Data of standard deviation for xylanase under optimized parameters.

Day	Activity	Std. Dev
0	2.09	0
5	3.95	0
10	6.36	0.212
15	5.62	0.071
20	5.1	0.141

Table 35: Data of standard deviation for lignin peroxidase under optimized Parameters.

Day	Activity	Std. Dev
0	0.0823	0
5	1.473	0.071
10	0.993	0.141
15	0.965	0
20	1.313	0.141

Table 36: Data of standard deviation for laccase under optimized parameters.

Day	Activity	Std. Dev
0	0.165	0
5	5.15	0
10	18.35	0
15	17.35	0.141
20	11.5	0