Optimization Of Enzyme Pre-Treatment Variables Using Response Surface Methodology For Oil Palm Empty Fruit Bunches Soda-AQ Pulp Yield

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ABSTRACT

The major disadvantage of conventional chemical pulping processes is the consumption of large amount of energy and chemical treatments. One of the methods to decrease the utilization of energy and chemical treatments is by biopulping. However little information is available on biopulping of oil palm biomass - empty fruit bunch fibre (EFB). The main objective of this study is to determine the effect of enzyme on pulp yield. The EFB was treated with a commercial enzyme (Novozymes) at various dosage (A), pH (B) and retention times (C). The effects of enzymatic pre-treatment variables were examined and analyzed using statistical experimental design response surface methodology (RSM) utilizing the central composite design (CCD) approach. In order to facilitate the analyses, statistical software DESIGN-EXPERT of Stat-Ease, Inc. USA was used to optimize the above-mentioned three parameters. After pre-treatment, the EFB was pulped by soda-anthraquinone pulping process. Comparison was done between treated and untreated pulping. The yields were in the range of 48 to 53%. The preliminary results show that pre-treatment improved pulp yield (maximum up to 4%). The optimum conditions to produce a high screened yield at lowest dosage, natural pH and shortest retention time were as follows A = 5%, B = 6.5 pH and C = 6 hours.

Keywords: *Biopulping, enzyme pre-treatment, oil palm (Elaeis guineensis) empty fruit bunches, response surface methodology, soda pulping*

Introduction

The main purpose of soda pulping is to remove enough lignin so that the fibres can be readily separated from one another, producing a pulp. The soda, an alkaline, causes the lignin molecules to fragment into smaller segments, which dissolve as phenolate or carboxylates (Smook, 1992). The major disadvantages of soda pulping processes are that they consume large amounts of energy and chemical treatments. One of the methods to decrease utilisation of energy and chemical treatments is by biopulping. In chemical pulping, biopulping is to reduce the amount of cooking chemicals, to increase the cooking capacity, or to enable extended cooking, resulting in lower consumption of bleaching chemicals.

Enzymes and microorganism have great potential for biotechnological applications. Numerous studies have been carried out regarding the use of enzymes and microorganism for biopulping of different types of wood and nonwood pulps. However little information is available on biopulping of oil palm biomass - empty fruit bunch fibre (EFB) (Rushdan & Nurul Husna, 2007). In Rushdan and Nurul Husna (2007) preliminary study on the effect of enzyme pre-treatment – the effect of two types commercial enzyme on pulp yield, delignification and strength of EFB soda-AQ pulp. They found that Novozym, perform better than Pulpzyme in biopulping (Rushdan & Nurul Husna, 2007). This is an extended study of that study. The main objective of this study is to determine the effect of Novozymes on pulp yield.

Material and Methods

Materials

Oil palm empty fruit bunches (EFB) was collected from an oil palm mill in Selangor. The EFB were shredded, cut, washed and dried at Forest Research Institute of Malaysia (FRIM).

Enzyme Source

Commercial enzymes provided by Novozymes Malaysia Sdn Bhd.

Enzyme Treatment

For each experiment, 100 g EFB was subjected to different enzymatic treatment conditions as shown in Table 1. The range of the variables for enzymatic treatment conditions were based on the preliminary experiments conducted earlier (Rushdan & Nurul Husna, 2007). The independent variables were, concentration of enzyme used, A (5 - 10 v/v%), pH, B (6.5 - 9.5), and the incubation time, A (6 - 24 hour).

Soda-anthrAquinone Pulping Process

After pre-treatment, the EFB was pulped by soda-anthraquinone pulping process. A control pulping was done without any pre-treatment. Pulping trials were also carried out in a M/K System digester. The pulping conditions employed were:

a. maximum cooking temperature: 160°C,
b. time to maximum temperature: 90 minutes,
c. time at maximum temperature: 60 minutes,
d. EFB to liquor ratio: 1:6
e. amount of anthraquinone: 0.1% of EFB dry weight
f. amount of NaOH: 27.3% of EFB dry weight.

At the end of each digestion, the softened EFB was disintegrated for five minutes in a hydropulper, washed and screened by Somerville fractionators. The total pulp yield was calculated as the sum of the screened pulp yield and the sieves. Comparison was done between treated and untreated pulping.

Experimental Design

Response surface methodology was utilized to optimize the biopulping process and a CCD was adopted. It involves outlining the composition of the experimental process conditions subsequently used to develop the regression models. The basic CCD for k variables consists of a 2^k factorial design with each factor at two levels (+1, -1) superimposed on a star design or 2^k axial points and several repetitions at the design centre points.

Three enzymatic variables, which are most likely to affect the pulp yield produced from soda-AQ pulping, were identified and investigated by the CCD. These variables were: (1) dosage (*A*), (2) pH (*B*) and (3) retention times (*C*). The experimental design matrix with both the coded and real variables is shown in Table 1, where the former is calculated by Esq. (1) – (3) below:

$$A_{code} = (A - 7.5\ \%)/2.5\ \% \qquad (1)$$
$$B_{code} = (B - 7)/1.5 \qquad (2)$$
$$C_{code} = (C - 15\ \text{h})/9\ \text{h} \qquad (3)$$

Each independent variable had 3 levels which were -1, 0 and +1. A total of 26 different combinations (including five replicates of the centre point each sighed the coded value 0) were chosen in random order according to a CCD configuration for three factors (Cochran & Cox, 1957). The experimental design in the coded (x) and actual (X) levels of variables is shown in Table 1. The responses function (y) measured was pulp yield. The values of responses obtained allow the calculation of mathematical estimation models for each response, which were subsequently used to characterize the nature of the response surface. All statistical analyses were carried out using the statistical software, DESIGN EXPERT® of Stat-Ease, Inc., USA.

Table 1 : The pre-treatment's conditions of 100 g (o.d) EFB

Biopulping variables						
No	Coded			Values		
	A	B	C	A (%)	B	C (h)
1	-1.00	-1.00	-1.00	5	6.5	6
2	1.00	-1.00	-1.00	10	6.5	6
3	-1.00	-1.00	-1.00	5	6.5	6
4	1.00	-1.00	-1.00	10	6.5	6
5	-1.00	1.00	-1.00	5	9.5	6
6	1.00	1.00	-1.00	10	9.5	6
7	-1.00	1.00	-1.00	5	9.5	6
8	1.00	1.00	-1.00	10	9.5	6
9	-1.00	-1.00	1.00	5	6.5	24
10	1.00	-1.00	1.00	10	6.5	24
11	-1.00	-1.00	1.00	5	6.5	24
12	1.00	-1.00	1.00	10	6.5	24
13	-1.00	1.00	1.00	5	9.5	24
14	1.00	1.00	1.00	10	9.5	24
15	-1.00	1.00	1.00	5	9.5	24
16	1.00	1.00	1.00	10	9.5	24
17	-2.00	0.00	0.00	2.5	8	15
18	2.00	0.00	0.00	12.5	8	15
19	0.00	0.00	0.00	7.5	8	15
20	0.00	0.00	0.00	7.5	8	15
21	0.00	-2.00	0.00	7.5	5	15
22	0.00	2.00	0.00	7.5	11	15
23	0.00	0.00	-2.00	7.5	8	0
24	0.00	0.00	2.00	7.5	8	33
25	0.00	0.00	0.00	7.5	8	15
26	0.00	0.00	0.00	7.5	8	15

Note: A – dosage (w/w based on oven dried EFB), B - pH and C - retention times (h)

Results and discussion

Statistical Analysis

The experimental values for pulp yield under different treatment conditions are presented in Table 2. The regression coefficients for the second order polynomial equations and results for the linear, quadratic and interaction terms are presented in Table 3. The statistical analysis indicates that the proposed model was adequate, possessing no significant lack of fit and with very satisfactory values of the R2 for all the responses. The R2 values for pulp yield was 0.965. The closer the value of R2 to the unity, the better the empirical model fits the actual data.

Table 2: Yield for Soda-AQ Pulping

Std	Run	Dosage (w/w based on oven dried EFB)	pH	Retention Times (h)	Yield (%)
19	1	7.5	8.00	15.00	51.1
21	2	7.5	5.00	15.00	52.5
5	3	5.0	9.50	6.00	51.56
13	4	5.0	9.50	24.00	51.09
6	5	10.0	9.50	6.00	50.36
18	6	12.5	8.00	15.00	52.72
23	7	7.5	8.00	-3.00	51.13
24	8	7.5	8.00	33.00	49.71
16	9	10.0	9.50	24.00	53.2
26	10	7.5	8.00	15.00	50.18
4	11	10.0	6.50	6.00	50.99
14	12	10.0	9.50	24.00	50.37
15	13	5.0	9.50	24.00	51.69
2	14	10.0	6.50	6.00	52.02
12	15	10.0	6.50	24.00	50.55
1	16	5.0	6.50	6.00	53.22
22	17	7.5	11.00	15.00	51.89
7	18	5.0	9.50	6.00	50.24
8	19	10.0	9.50	6.00	51.26
17	20	2.5	8.00	15.00	50.46
9	21	5.0	6.50	24.00	48.63
3	22	5.0	6.50	6.00	51.26
25	23	7.5	8.00	15.00	50.17
10	24	10.0	6.50	24.00	47.65
20	25	7.5	8.00	15.00	49.57
11	26	5.0	6.50	24.00	50.32

The smaller the value of R2 the less relevant the dependent variables in the model have to explain of the behaviour variation (Little & Hills, 1978; Mendenhall, 1975). The probability (p) values of all regression models were less than 0.000, with no lack-of-fit.

Table 3: The Regression Coefficients for the Second Order Polynomial Equations

Sequential Model Sum of Squares

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Mean	67405.86	1	67405.86			Suggested
Linear	5.367446	3	1.789149	1.079198	0.3784	
2FI	11.68487	3	3.894956	2.985503	0.0570	Suggested
Quadratic	6.156326	3	2.052109	1.762269	0.1948	
Cubic	3.623079	4	0.90577	0.724209	0.5920	Aliased
Residual	15.00843	12	1.250703			
Total	67447.7	26	2594.142			

□I+"Sequential Model Sum of Squares"□0+: Select the highest order polynomial where the

additional terms are significant and the model is not aliased.

Lack of Fit Tests

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Linear	21.73166	11	1.975605	1.474227	0.2652	
2FI	10.04679	8	1.255849	0.937134	0.5243	Suggested
Quadratic	3.890463	5	0.778093	0.580625	0.7147	
Cubic	0.267384	1	0.267384	0.199526	0.6638	Aliased
Pure Error	14.74105	11	1.340095			

□I+"Lack of Fit Tests"□0+: Want the selected model to have insignificant lack-of-fit.

Model Summary Statistics

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	1.287575	0.128285	0.009414	-0.2472	52.18311	
2FI	1.142201	0.407559	0.220472	-0.18144	49.43177	Suggested
Quadratic	1.079106	0.554698	0.304215	-0.23472	51.66084	
Cubic	1.118348	0.641291	0.25269	-0.6639	69.61799	Aliased

□I+"Model Summary Statistics"□0+: Focus on the model maximizing the "Adjusted R-Squared"

and the "Predicted R-Squared".

Effects of Enzyme Concentration, pH and Time

The effect of different enzyme treatment conditions on the pulp yield is reported (Table 1) by the coefficient of the second order polynomials. To aid visualization, the response surfaces for pulp yield is shown in Figure 1,2 and 3. Figure 1 shows the contour map for the effect of the independent variables on the pulp yield. As shown in Table 3, pulp yield was positively related to the linear effect of enzyme concentration ($p < 0.001$), pH ($p < 0.05$) and incubation time ($p < 0.001$) and the quadratic terms of these variables were not found to be significant resulting in a linear increase in pulp yield with enzyme concentration at all temperatures (Figure 1a). It can be seen in Table 3 that there is an interaction effect between enzyme concentration and incubation time on filterability. At the lowest level of incubation time, the pulp yield was found to increase rapidly with an increase in enzyme concentration (Figure 2). At the highest level of incubation time, the pulp yield increase to a certain level and then increase at a slower rate owing to the contribution by the interaction term ($p < 0.01$) of enzyme concentration and incubation time (Figure 2).

Optimization

Figure show the optimum conditions of the pre-treatment process to yield maximum filterability pulp yield. It was noted that the optimum conditions for clarification were slightly different. There are a number of combination of response function can been determined (Fig.). The process variables for best combination of response function are enzyme concentration 0.084%, pH, and incubation time 80min. The response functions were calculated from the final polynomial, and the response was pulp yield.

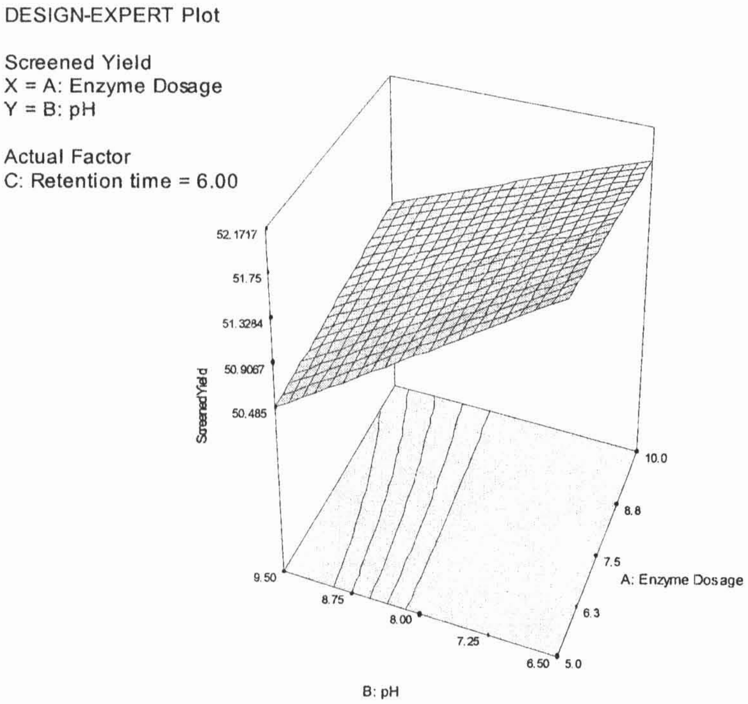


Figure 1 : Screened yield for ph VS enzyme dosage

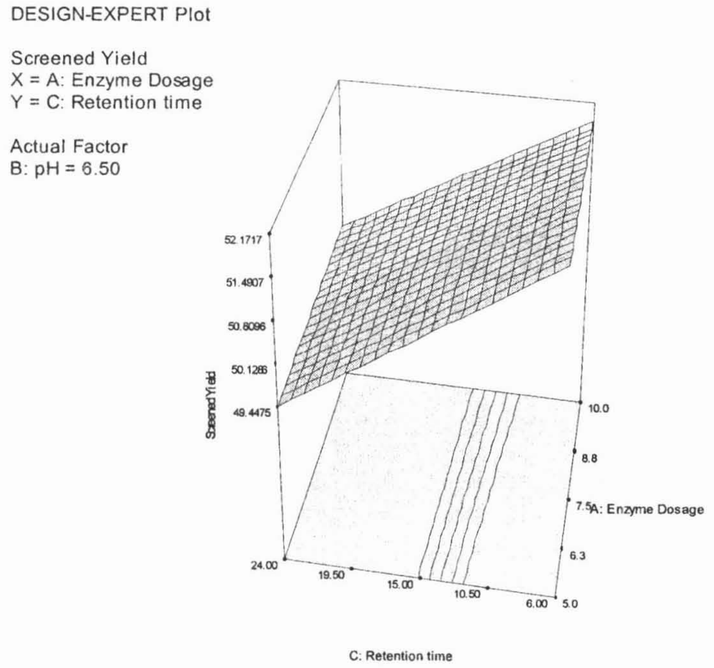


Figure 2: Screened yield for retention time VS enzyme dosage

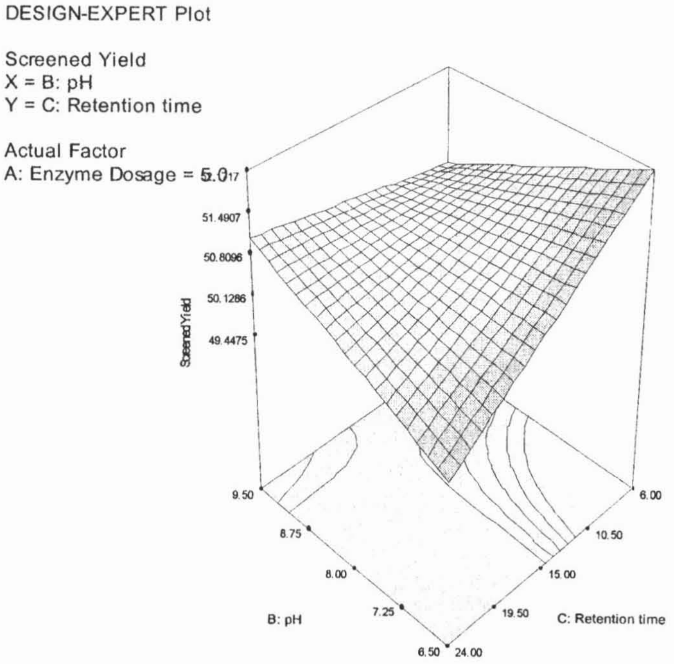
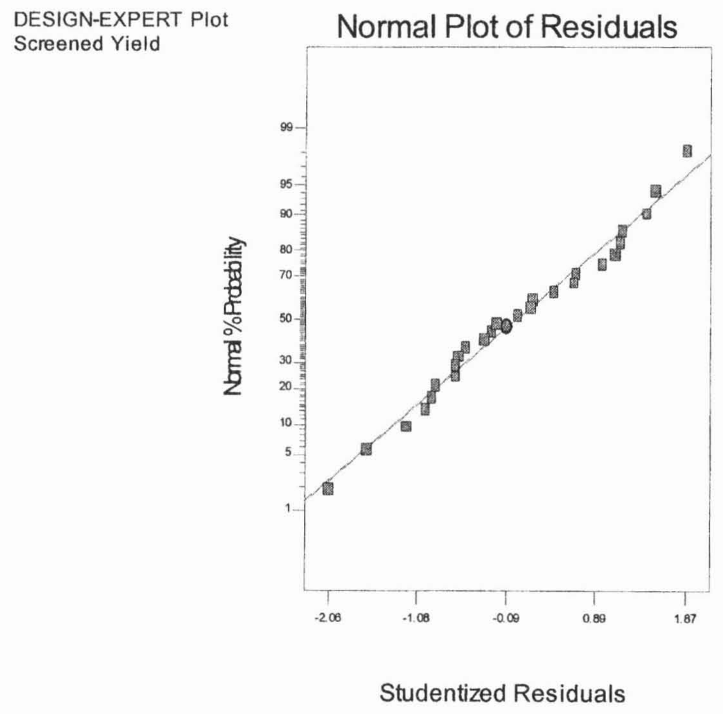
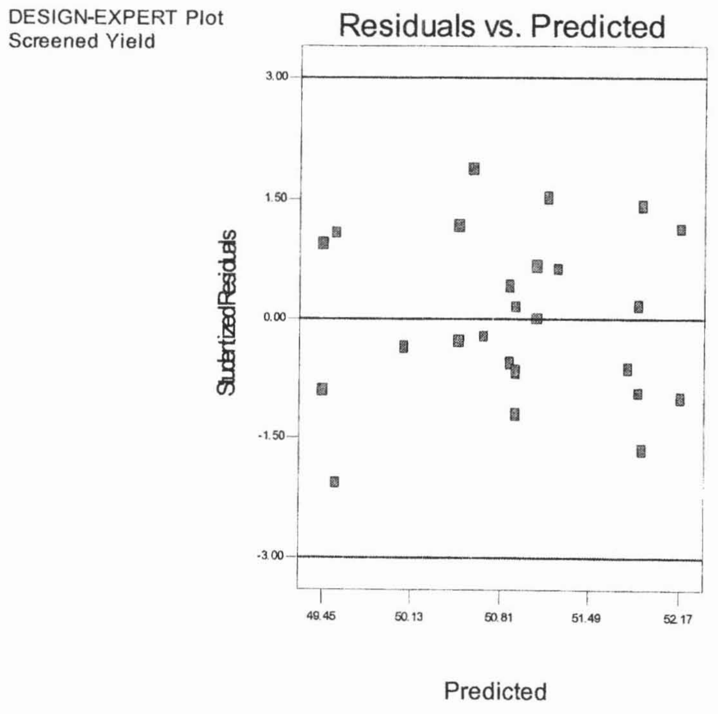
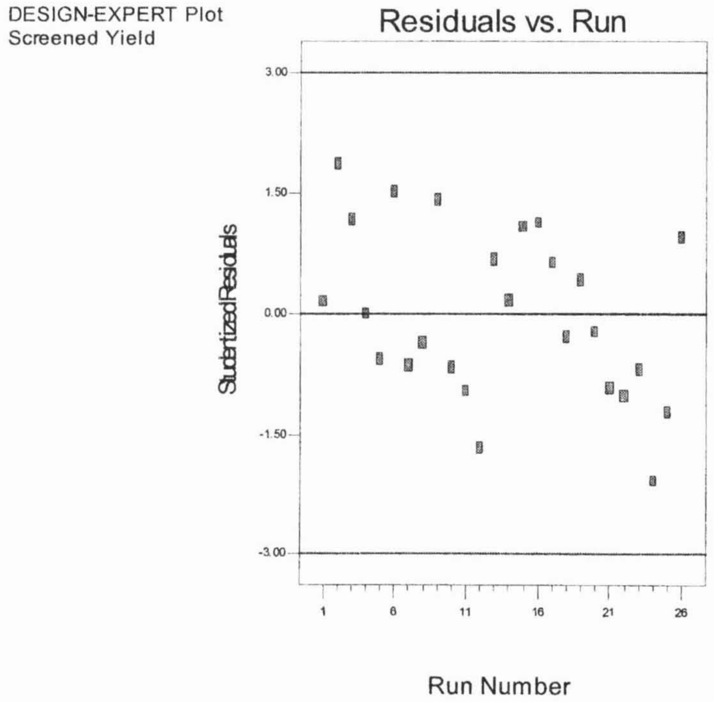
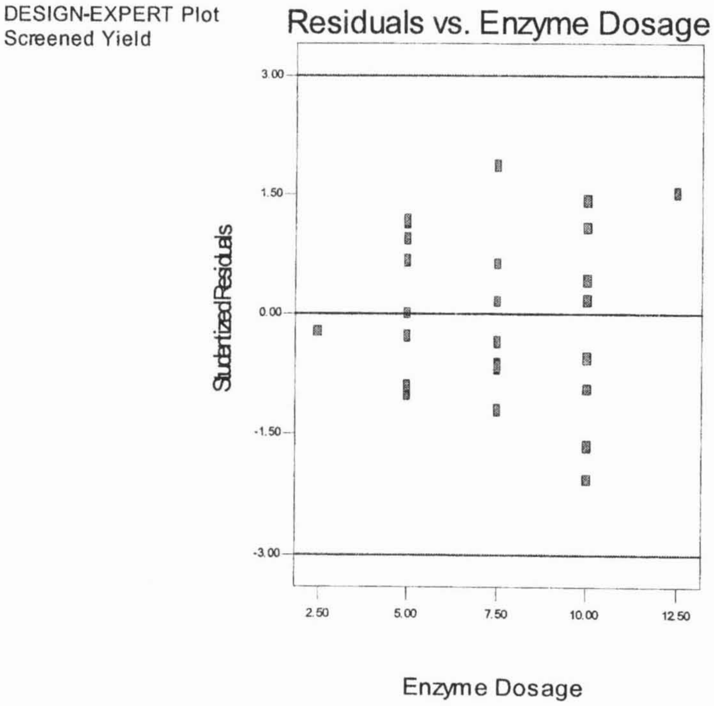


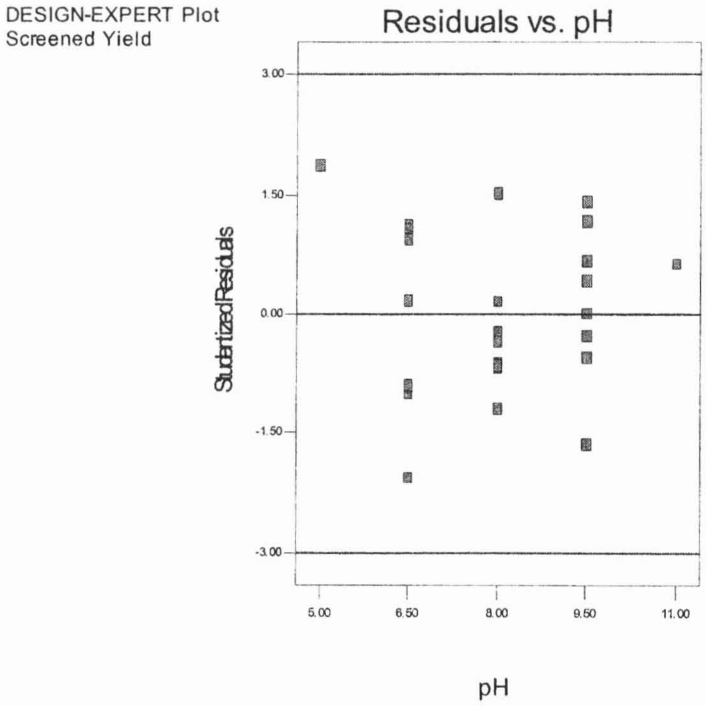
Figure 3 : Screened yield for ph VS retention time

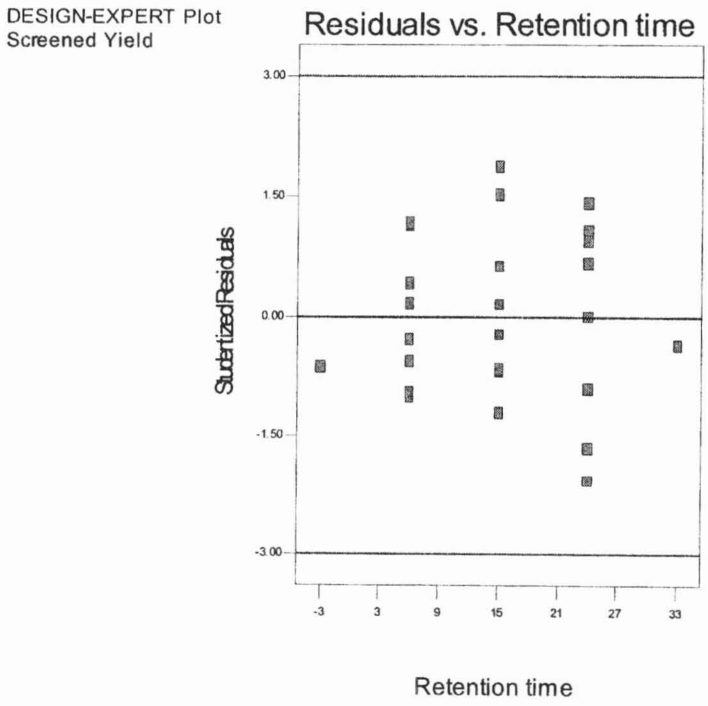

DESIGN-EXPERT Plot
Screened Yield
Normal Plot of Residuals
Normal % Probability
99
95
90
80
70
50
30
20
10
5
1
-2.06
-1.08
-0.09
0.89
1.87
Studentized Residuals

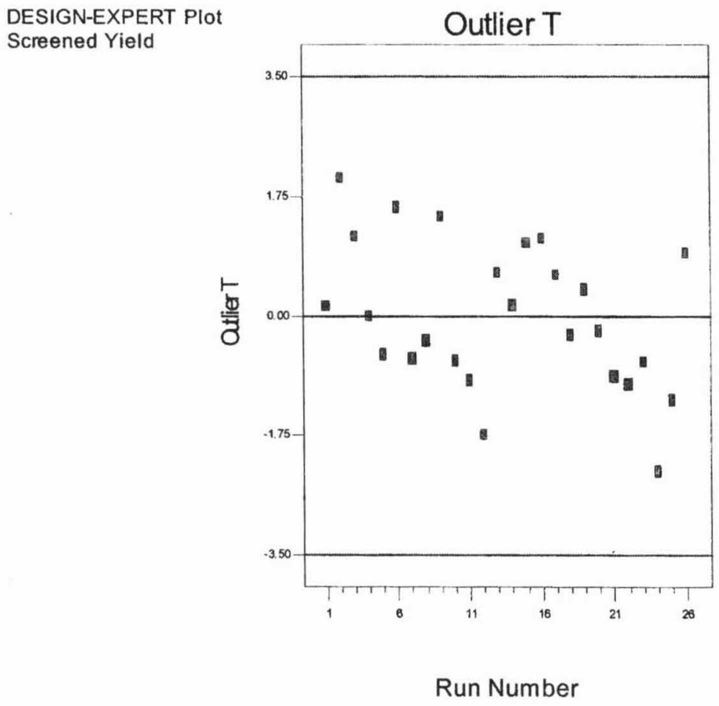

DESIGN-EXPERT Plot
Screened Yield
Residuals vs. Predicted
Studentized Residuals
3.00
1.50
0.00
-1.50
-3.00
49.45
50.13
50.81
51.49
52.17
Predicted

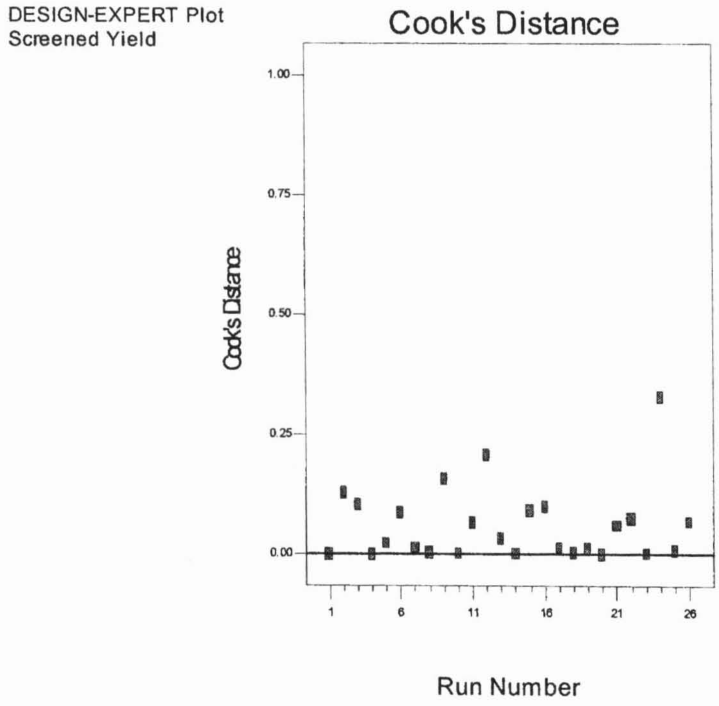

DESIGN-EXPERT Plot
Screened Yield
Residuals vs. Run
Studentized Residuals
3.00
1.50
0.00
-1.50
-3.00
1
6
11
16
21
26
Run Number

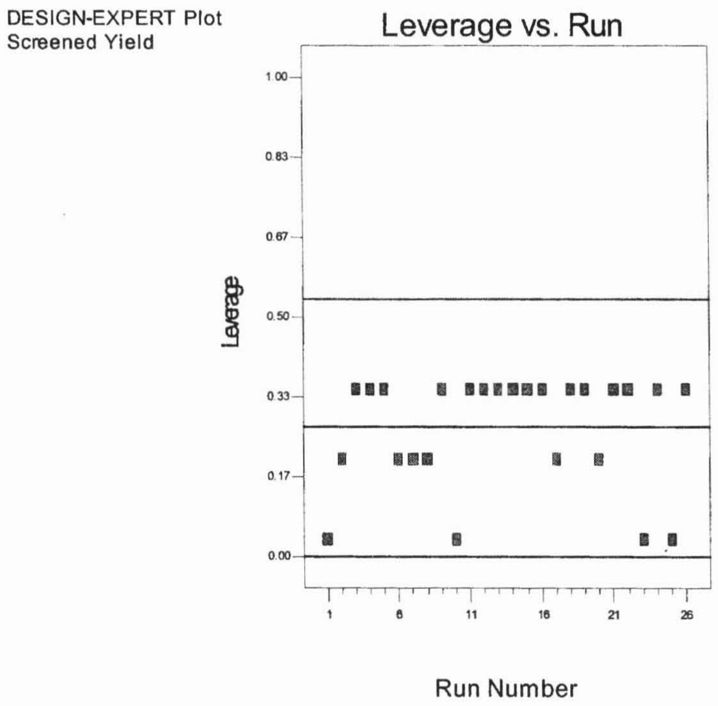

DESIGN-EXPERT Plot
Screened Yield
Residuals vs. Enzyme Dosage
Studentized Residuals
3.00
1.50
0.00
-1.50
-3.00
2.50
5.00
7.50
10.00
12.50
Enzyme Dosage

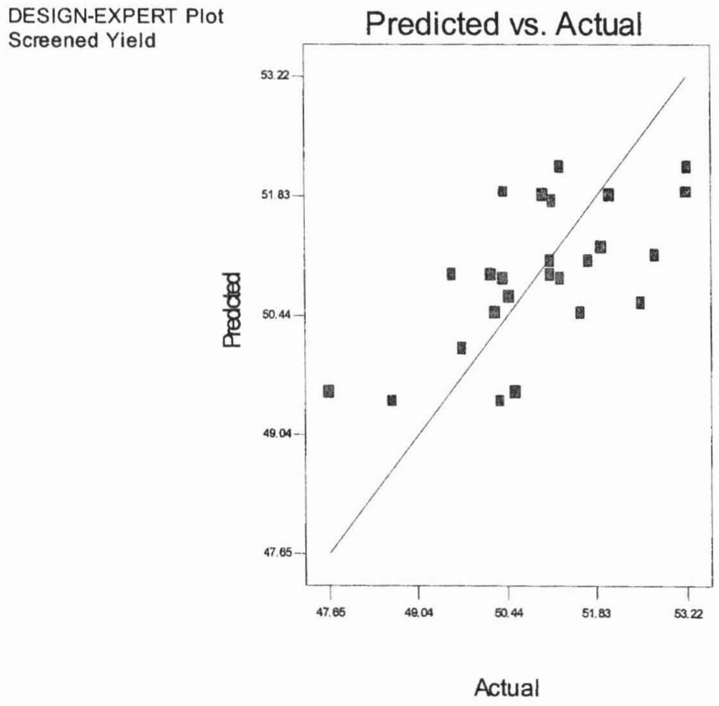

DESIGN-EXPERT Plot
Screened Yield
Residuals vs. pH
Studentized Residuals
3.00
1.50
0.00
-1.50
-3.00
5.00
6.50
8.00
9.50
11.00
pH

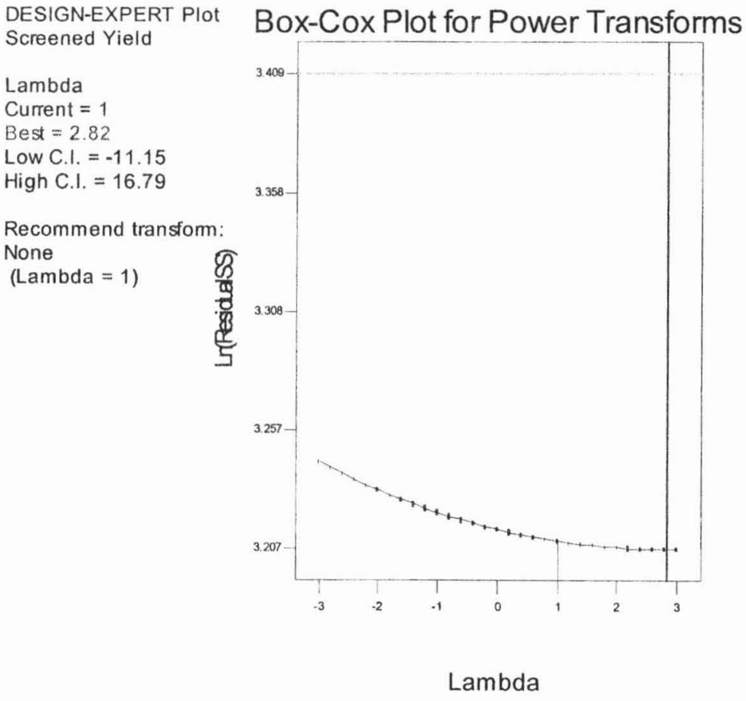

DESIGN-EXPERT Plot
Screened Yield
Residuals vs. Retention time
Studentized Residuals
3.00
1.50
0.00
-1.50
-3.00
-3
3
9
15
21
27
33
Retention time


DESIGN-EXPERT Plot
Screened Yield
Outlier T
3.50
1.75
0.00
-1.75
-3.50
Outlier T
1
6
11
16
21
26
Run Number


DESIGN-EXPERT Plot
Screened Yield
Cook's Distance
1.00
0.75
0.50
0.25
0.00
Cook's Distance
1
6
11
16
21
26
Run Number


DESIGN-EXPERT Plot
Screened Yield
Leverage vs. Run
Leverage
1.00
0.83
0.67
0.50
0.33
0.17
0.00
1
6
11
16
21
26
Run Number


DESIGN-EXPERT Plot
Screened Yield
Predicted vs. Actual
Predicted
53.22
51.83
50.44
49.04
47.65
47.65
49.04
50.44
51.83
53.22
Actual



Final Equation in Terms of Coded Factors:

Screened Yield	=
50.91692	
0.12125	* A
0.162917	* B
-0.42708	* C
0.176875	* A * B
0.105625	* A * C
0.829375	* B * C

Final Equation in Terms of Actual Factors:

Screened Yield	=
61.12644	
-0.39925	* Enzyme Dosage
-1.16667	* pH
-0.57414	* Retention time
0.047167	* Enzyme Dosage * pH
0.004694	* Enzyme Dosage * Retention time
0.061435	* pH * Retention time

Conclusion

The yields were in the range of 48 to 53%. The preliminary results show that pre-treatment improved pulp yield (maximum up to 4%). The optimum conditions to produce a high screened yield at lowest dosage, natural pH and shortest retention time were as follows A = 5%, B = 6.5 pH and C = 6 hours.

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