Enzymatic Hydrolysis Pretreatment for Mechanical Expelling of Soybeans

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Mechanical expelling of soybeans with enzymatic hydrolysis as pretreatment was investigated, and the process parameters were optimized by means of response surface methodology. Enzyme pretreatment enhanced both the amount of extractable oil in soybeans and oil extractability. A second-order response surface model was developed to predict the expelled oil as a function of the six process parameters investigated. The optimum was found at: Moisture content during hydrolysis, 23.00% wet basis (w.b.); enzyme concentration, 11.84% vol/wt; incubation period, 13.24 h; moisture content during pressing, 9.36% w.b.; pressing pressure, 75 MPa; and pressing time, 5.36 min. The parameters had no interactive effects on expelled oil. Pressing pressures above 75 MPa caused extrusion. Under the optimal conditions, oil expelled from dehulled cracked soybeans by static pressing at room temperature (18°C) was 63.5% of the total extractable oil. Much higher oil recovery would be expected in actual screw expellers due to dynamic pressing and higher operating temperature. Oil recovery could be further increased by adding one or more conventional pretreatments to the enzymatic hydrolysis pretreatment investigated in this study.

KEY WORDS: Bioconversion processing, enzymatic hydrolysis, mechanical expelling, oil expelling, oilseeds, oilseeds pretreatments, response surface methodology, soybean, soybean pretreatments.

Like other oilseeds, soybeans need to undergo certain pretreatment operations to facilitate extraction of oil by means of mechanical expelling or solvent extraction. The purpose is to break the cell walls and release the oil for extraction. The conventional pretreatments for soybean may include dehulling, size reduction, breaking, grinding or flaking, as well as thermal/hydrothermal treatment—cooking or steaming (1).

Enzymatic hydrolysis is another option for pretreatment of oilseeds as it opens up the oil cell walls through biodegradation. It also breaks up the complex lipoprotein and lipopolysaccharide molecules (not extractable for oil) into simple molecules releasing extra oil for extraction. Fullbrook (2) first observed that when enzymatically hydrolyzed, the crude protein isolated from melon seeds released extra oil. He further demonstrated the usefulness of enzymatic hydrolysis in the processing of ground soybean and rapeseed. He reported low energy and solvent usage, a better quality of oil, and anticipated the resulting protein to be of high nutritive value. Enhanced release of extractable oil was later shown by Bhatnagar and Johari (3) in crushed soybean, cottonseed and castor bean hydrolyzed in the presence of hexane, and by Sosulski *et al.* (4) in canola flakes. Sosulski *et al.* (4) also reported a reduction in oil extraction time, and they optimized the enzymatic hydrolysis conditions for canola.

Soybeans are conventionally subjected to solvent extraction for oil with hexane. Mechanical expelling of soybeans is not practiced commercially. Nonetheless, some research reports on mechanical expelling of soybeans are available in the literature. Singh and Agrawal (1) have examined the issue of mechanical expelling of soybeans and have professed its suitability, particularly at decentralized levels in developing countries, for soy oil recovery at lower costs. While recovering about 80–90% oil, mechanical expelling would yield a high-protein food free of hexane toxicity, making it safer for sustained human consumption. It would leave a stable, partially deoiled cake for transportation to solvent extraction plants. No work has been reported on the effect of enzymatic hydrolysis on mechanical expelling.

The objective of this research was to investigate the use of enzymatic hydrolysis as a pretreatment for mechanical expelling of soybeans and to optimize the process parameters to yield enhanced oil recovery. The parameters studied were enzyme concentration, moisture content during hydrolysis, time of hydrolysis, pressure, pressing time and moisture content during expelling. The hydrolysis parameters and expelling parameters were simultaneously optimized because of possible interactions.

EXPERIMENTAL PROCEDURES

Soybrokens, 6–8 pieces from cleaned, dehulled, commercial-grade PK-262 variety soybeans, were enzymatically hydrolyzed with mixed-activity crude enzyme from *Aspergillus fumigatus* at different combinations of hydrolysis parameters (enzyme concentration, moisture during hydrolysis and incubation period). The enzyme used was reported to be more effective than mixed-activity enzymes from other micro-organisms and the specific purified enzymes such as cellulase, hemicullulase and protease (2,3). *A. fumigatus*, obtained from National Chemical Laboratories (Pune, India), was grown on wheat bran medium for optimum production of enzyme (Bhatnagar, S., unpublished data). The activity of enzyme solutions prepared in this study was 0.2–0.5 IU by the methods of Miller (5) and Mandels *et al.* (6). The hydrolyzed samples were mechanically expelled in a specially designed test cell (7) with a laboratory Carver Press at different combinations of expelling parameter values (moisture content during pressing, pressure and pressing time). The hydrolyzed sample and the expelled cake were solvent-extracted in a rapid extractor (Soxtec System HT; Tecator, Hoganes, Sweden) to determine the total extractable oil in the sample and the residual oil in cake, respectively. The difference gave the amount of oil expelled. The expelled oil data were analyzed by multiple linear regression techniques to develop the response surface model and thereby determine the optimal combination of parameters for enhanced oil recovery.

The experimental design was based on response surface methodology (RSM). It is a useful statistical technique for investigation of complex processes. It consists of a group of mathematical and statistical procedures (8) that

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TABLE 1

Experimental Design in Coded[a] Form for Response Surface Analysis

Coded variable								
X_1	X_2	X_3	X_4	X_5	X_6	Combinations	Replications	Number of experiments
0	0	0	0	0	0	1	9	9
±1	±1	±1	±1	±1	±1	32[b]	2	64
±1.733	0	0	0	0	0	2	2	4
0	±1.733	0	0	0	0	2	2	4
0	0	±1.733	0	0	0	2	2	4
0	0	0	±1.733	0	0	2	2	4
0	0	0	0	±1.733	0	2	2	4
0	0	0	0	0	±1.733	2	2	4

[a]Code "0" is for the center point of the parameter range investigated, "±1" for factorial points, and "±1.733" for augmented points; X_1, moisture content during hydrolysis; X_2, enzyme concentration; X_3, incubation period; X_4, moisture dontent during presing; X_5, pressing pressure; X_6, pressing time.
[b]Factorial points were in half replicate.

can be used to study relationships between one or more responses (dependent variables) and a number of factors (independent variables). A second-order factorial design with augmented points in six variables at five levels in half replicate was used (Table 1). The coded and uncoded parameter values for the center, factorial and augmented points of the design (8) are presented in Table 2. The levels of the parameters were carefully chosen (Smith, D.D., unpublished data) based on the limited literature available on enzymatic hydrolysis of oilseeds (2–4) and mechanical expelling of soybean with conventional pretreatments (1,9–11).

For each experiment, a sample of 50-g soybrokens was taken in a conical flask, and the moisture content was adjusted to the desired level for hydrolysis by adding appropriate amounts of water and enzyme solution commensurate with the desired concentration (Smith, D.D., unpublished data). The flask was plugged, shaken and equilibrated in a refrigerator. A 5-g sample was drawn to check the moisture content, and the rest was incubated at 45°C for the desired time period for hydrolysis. This temperature was optimum for the enzyme's activity (Bhatnagar, S., unpublished data). The hydrolyzed sample was dried in a petri dish at 104°C in an oven to inactivate the enzyme and to readjust the moisture to the desired level for mechanical expelling. A 5-g sample was drawn to check the moisture content, and 10-g sample for Soxtec extraction to determine the total extractable oil available in soybrokens after hydrolysis. A 19-g hydrolyzed sample, enough to uniformly form a 10-mm thick bed in the test cell, was then expelled on the Carver Press at the desired pressing pressure for the desired pressing time at room temperature (18°C). The expelled cake was Soxtec-extracted to determine the residual oil. The amount of oil expelled was calculated by subtracting the residual oil in the cake from the total extractable oil available in the soybrokens after hydrolysis. Unhydrolyzed soybrokens were also mechanically expelled at central point values of the pressing parameters (Table 2).

The analysis of total extractable oil by Soxtec solvent extraction was performed after grinding the sample to a 0.5 mm particle size. Petroleum ether (boiling point 65°C) was used as solvent while keeping the oil bath temperature at 104 ± 1°C. A boiling time of 45 min and rinsing time of 60 min were used, as this combination resulted in more complete oil recovery (Smith, D.D., unpublished data). Moisture contents were determined by drying 5-g ground samples at 130 ± 2°C for 2 h in a hot-air oven (12). A Mettler balance of 120 g capacity with an accuracy of 0.0001 g was used for weighing.

RESULTS AND DISCUSSION

The total extractable oil available in raw soybrokens was 23.75% on a moisture-free basis. Soybrokens, given different enzymatic pretreatments, had an extractable oil of

TABLE 2

Coded and Uncoded Parameter Levels

	Code				
Parameter	+1.733 (augmented point)	+1 (factorial point)	0 (center point)	−1 (factorial point)	−1.733 (augmented point)
Moisture content during hydrolysis, % w.b.[a] (X_1)	28.2	26	23	20	17.8
Enzyme concentration, % vol/wt (X_2)	17.2	15	12	9	6.8
Incubation period, h (X_3)	18.9	16	12	8	5.1
Moisture content during pressing, % w.b. (X_4)	10.7	10	9	8	7.3
Pressing pressure, MPa (X_5)	72.0	64.2	53.5	42.8	35.0
Pressing time, min (X_6)	6.7	6	5	4	3.3

[a]w.b., Wet basis.

TABLE 3

Extractable Oil[a] Present in Enzymatically Pretreated Soybrokens[b]

Treatment number	Coded enzymatic hydrolysis parameters			Extractable oil, % moisture-free sample basis		
	X_1	X_2	X_3	Rep. 1	Rep. 2	Average
T1	+1.733	0	0	23.70	24.12	23.91
T2	−1.733	0	0	24.25	23.99	24.12
T3	0	+1.733	0	23.89	23.61	23.75
T4	0	−1.733	0	24.53	24.65	24.59
T5	0	0	+1.733	25.26	25.26	25.26
T6	0	0	−1.733	23.63	23.90	23.77
T7	−1	−1	−1	23.93	23.97	23.95
T8	+1	−1	−1	23.55	23.67	23.61
T9	−1	+1	−1	23.92	24.29	24.11
T10	+1	+1	−1	23.66	23.69	23.68
T11	−1	+1	+1	24.51	24.14	24.33
T12	+1	+1	+1	23.86	23.70	23.78
T13	−1	−1	+1	23.82	24.54	24.18
T14	+1	−1	+1	24.26	24.21	24.24
T15	0	0	0	25.15	25.15	25.15

[a]Determined by solvent extraction with a rapid extractor (Soxtec System HT; Tecator, Hoganas, Sweden).
[b]Extractable oil in untreated raw soybrokens was determined to be 23.75% as an average of three replications (Rep.). See Table 1 for definitions of X_1–X_3.

up to 25.26% (Table 3), showing enhancement of up to 1.51% in the release of extractable oil due to hydrolysis.

Oil expelled under different conditions of enzymatic pretreatments and mechanical expelling of soybrokens is presented in Table 4. It was in the range of 5.01 to 14.49% of moisture-free sample. Comparison of the amounts of oil expelled from untreated soybrokens to that from enzymatically treated soybrokens pressed under the same expelling conditions (Table 4, combination numbers C0 to C7) revealed that hydrolysis enhances the expelled oil by up to 2.78% of moisture-free sample. This enhancement is equivalent to about an 11.7% increase in oil recovery on total extractable oil basis. Because the release of extractable oil was enhanced by only a maximum of 1.51% (Table 3), it is evident that the enzymatic pretreatment also enhanced the oil extractability of soybrokens.

An RSM for expelled oil as a function of enzymatic hydrolysis and expelling parameters was developed by employing a multiple regression technique. A linear model and second-order models with and without interaction terms were tested for their adequacy to describe the response surface by using Fisher's F-test at 95% confidence level (Smith, D.D., unpublished data). The second-order model without interaction terms best described the response surface (Table 5). The lack of fit of the second-order model with interaction terms (calculated F-value of 1.82 being lower than table F-value of 2.15) showed that the investigated parameters had no interactions among them. The response function developed was:

$$Y_E = -127.169 + 5.339\,X_1 + 3.456\,X_2 + 0.723\,X_3 + 4.978\,X_4 + 0.304\,X_5 + 6.585\,X_6 - 0.116\,X_1^2 - 0.146\,X_2^2 - 2.694 \times 10^{-2}\,X_3^2 - 0.266\,X_4^2 - 1.438 \times 10^{-3}\,X_5^2 - 0.614\,X_6^2 \quad [1]$$

where Y_E = oil expelled, as % of total soybroken sample pretreated by enzymatic hydrolysis, moisture-free basis; X_1 = moisture content during hydrolysis, % wet basis (w.b.); X_2 = enzyme concentration, % vol/wt of sample; X_3 = incubation period, h; X_4= moisture content during pressing, % w.b.; X_5 = pressing pressure, MPa; and X_6 = pressing time, min.

Representative-predicted three-dimensional response surfaces for expelled oil, as a function of two parameters at a time while maintaining the other parameters at their center-point values, are shown in Figure 1. The effects of all parameters except pressure were similar. The expelled oil first increased with increasing parameter value and then decreased, indicating the existence of an optimum within the parameter ranges investigated. Pressure, however, continually increased the expelled oil, indicating a possible theoretical optimum beyond the investigated range.

The optimum parameter values for maximum expelled oil were calculated by partially differentiating Equation 1 with respect to each parameter and equating to zero. These optima were: X_1 = 23.00% w.b., X_2 = 11.84% vol/wt, X_3 = 13.24 h, X_4 = 9.36% w.b., X_5 = 105.78 MPa and X_6 = 5.36 min. The optimum of all the parameters except pressing pressure was close to the center point value. The optimum pressing pressure of 105.78 MPa was beyond the range of investigation requiring further experimental verification. Also, such a high level of pressure could result in extrusion of soybrokens instead of expelling the oil and, if so would require experimental confirmation of a practical limit on pressing pressure. Experimental verification did reveal extrusion at the optimum pressing pressure of 105.77 MPa. The extrusion point of soybrokens pretreated by enzymatic hydrolysis at optimum conditions and pressed at optimal moisture content was found to be of the order of about 77.60 MPa. Hence, a pressure of 75 MPa was considered to be the safe maximum limit at the optimal conditions of the rest of the parameters. Thus, the optimum parameter values for maximum amount of expelled oil from soybrokens pretreated by enzymatic hydrolysis were: moisture content during hydrolysis (X_1) = 23.00% w.b.; enzyme

TABLE 4

Oil Expelled[a] Under Different Experimental Conditions of Enzymatic Hydrolysis and Pressing

Parameters combination number	Coded parameters[b]						Oil expelled, % moisture-free sample
	X_1	X_2	X_3	X_4	X_5	X_6	
C0 (untreated)	—	—	—	0	0	0	9.40 ± 0.01(3)
C1	0	0	0	0	0	0	11.54 ± 0.25(9)
C2	+1.733	0	0	0	0	0	9.80 ± 0.01
C3	−1.733	0	0	0	0	0	9.13 ± 0.16
C4	0	+1.733	0	0	0	0	8.41 ± 0.09
C5	0	−1.733	0	0	0	0	8.88 ± 0.21
C6	0	0	+1.733	0	0	0	12.18 ± 0.05
C7	0	0	−1.733	0	0	0	10.27 ± 0.23
C8	0	0	0	+1.733	0	0	12.39 ± 0.08
C9	0	0	0	−1.733	0	0	11.54 ± 0.10
C10	0	0	0	0	+1.733	0	14.49 ± 0.25
C11	0	0	0	0	−1.733	0	9.68 ± 0.09
C12	0	0	0	0	0	+1.733	11.84 ± 0.27
C13	0	0	0	0	0	−1.733	9.79 ± 0.11
C14	−1	−1	−1	−1	−1	−1	5.79 ± 0.15
C15	+1	−1	−1	−1	−1	+1	6.31 ± 0.08
C16	−1	+1	−1	−1	−1	+1	6.98 ± 0.00
C17	+1	+1	−1	−1	−1	−1	5.89 ± 0.28
C18	−1	−1	+1	−1	−1	+1	7.88 ± 0.22
C19	+1	−1	+1	−1	−1	−1	7.38 ± 0.72
C20	−1	+1	+1	−1	−1	−1	6.42 ± 0.12
C21	+1	+1	+1	−1	−1	+1	8.73 ± 0.12
C22	−1	−1	−1	+1	−1	+1	8.89 ± 0.16
C23	+1	−1	−1	+1	−1	−1	5.01 ± 0.05
C24	−1	+1	−1	+1	−1	−1	5.59 ± 0.03
C25	+1	+1	−1	+1	−1	+1	5.49 ± 0.43
C26	−1	−1	+1	+1	−1	−1	7.29 ± 0.25
C27	+1	−1	+1	+1	−1	+1	7.81 ± 0.08
C28	−1	+1	+1	+1	−1	+1	7.45 ± 0.28
C29	+1	+1	+1	+1	−1	−1	6.81 ± 0.05
C30	−1	−1	−1	−1	+1	+1	12.29 ± 0.10
C31	+1	−1	−1	−1	+1	−1	10.25 ± 0.25
C32	−1	+1	−1	−1	+1	−1	8.32 ± 0.36
C33	+1	+1	−1	−1	+1	+1	8.45 ± 0.22
C34	−1	−1	+1	−1	+1	−1	12.08 ± 0.02
C35	+1	−1	+1	−1	+1	+1	10.02 ± 0.36
C36	−1	+1	+1	−1	+1	+1	8.49 ± 0.23
C37	+1	+1	+1	−1	+1	−1	9.46 ± 0.00
C38	−1	−1	−1	+1	+1	−1	8.34 ± 0.02
C39	+1	−1	−1	+1	+1	+1	12.62 ± 0.23
C40	−1	+1	−1	+1	+1	+1	12.17 ± 0.25
C41	+1	+1	−1	+1	+1	−1	10.99 ± 0.07
C42	−1	−1	+1	+1	+1	+1	8.08 ± 0.29
C43	+1	−1	+1	+1	+1	−1	8.57 ± 0.14
C44	−1	+1	+1	+1	+1	−1	11.74 ± 0.05
C45	+1	+1	+1	+1	+1	+1	12.11 ± 0.00

[a]Amounts of oil recovered by mechanical expelling, expressed as percent of moisture-free soybean sample weight. All averages of two experiments, except for C0 (3 reps.) and C1 (9 reps.). Replications, reps.
[b]X_1 = Moisture content during hydrolysis, X_2 = enzyme concentration, X_3 = incubation period, X_4 = moisture content during pressing, X_5 = pressing pressure, and X_6 = pressing time.

TABLE 5

Analysis of Variance of Second-Order Response Surface Model Without Interaction Terms[a]

Sources of variation due to:	Sum of squares	Degrees of freedom	Mean sum of squares	F-value (calculated)
Regression	176.268	12	14.689	7.94
Residual	59.179	32	1.849	
Total	235.447			

[a]Standard error of the estimate = 1.3599; R-value = 0.8652; F-value with degrees of freedom (12,32) 0.95 = 2.09.

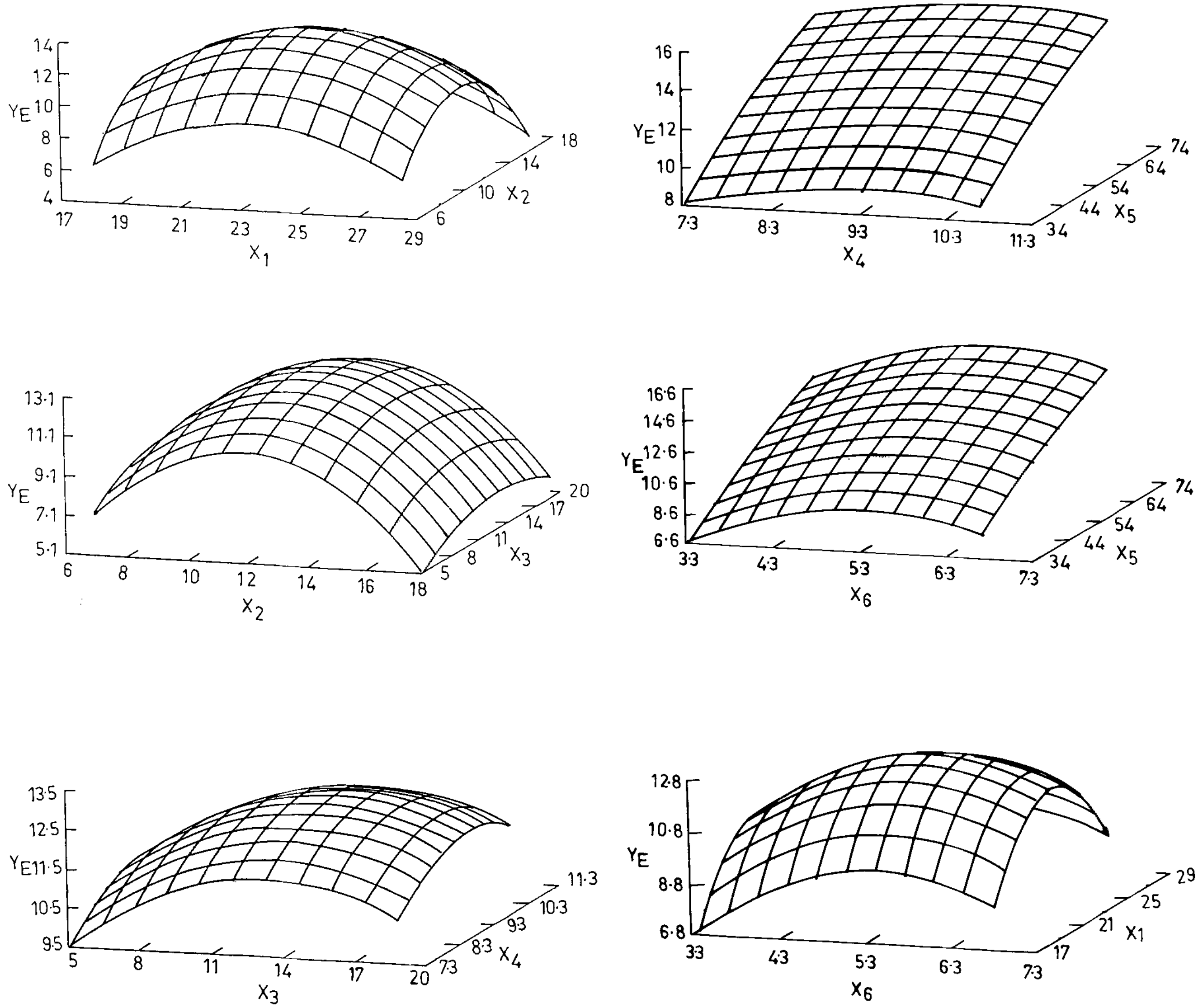

FIG. 1. Representative response surfaces of expelled oil. Y_E = oil expelled, X_1 = moisture content during hydrolysis, X_2 = enzyme concentration, X_3 = incubation period, X_4 = moisture content during pressing, X_5 = pressing pressure and X_6 = pressing time.

concentration (X_2) = 11.84% vol/wt of sample; incubation period (X_3) = 13.24 h; moisture content during pressing (X_4) = 9.36% w.b.; pressing pressure (X_5) = 75.0 MPa; and pressing time (X_6) = 5.36 min.

The oil expelled at the above optimal conditions was 15.08% of hydrolyzed sample on a moisture-free basis. This corresponded to an oil recovery of 59.76% of the total extractable oil after hydrolysis and 63.50% of the total extractable oil in the raw untreated soybrokens. The oil recovery is expected to be much higher in actual screw expellers because of higher operating temperature and dynamic pressing, as against the static pressing at room temperature (18°C) employed in this study. Further, oil recovery could be increased by adding one or more of the conventional pretreatments (grinding/flaking and heating/steaming) to the enzymatic hydrolysis pretreatment investigated in this study.

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