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Original Research Article

Using local agricultural residues for bioethanol production under full optimized processes

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Abstract

Rice straw (RS) and rice husk (RH) were selected as a local agricultural residues for biopolymers (cellulose, hemicellulose and lignin) extraction using 2% alkaline sodium hydroxide at 90°C for almost 3 hours. The extraction process was optimized by Taguchi experimental design method. Results revealed that cellulose was 54.6 % and 52.75 % in case of RS and RH, respectively. Separate hydrolysis and fermentation (SHF) were done using *Aspergilus niger* crude enzymes and *Saccharomyces cerevisiae*, which statistically optimized with two experimental design models (Taguchi and Placket-Burman design). Maximum glucose yield from hydrolysis of the extracted cellulose RS (CRS) and cellulose RH (CRH) was 255 mg/g and 120 mg/g, respectively. Bioethanol yield from the obtained fermentable glucose of CRS and CRH were 231.8 mg/g and 269.4 mg/g, respectively. Extraction, hydrolysis and fermentation processes optimization can be an alternative sustainable development approach to utilize this abundant agricultural waste for new and renewable energy.

Keywords: Rice straw and husk, Response surface methodology, Taguchi and Placket-Burman design, Bioethanol

Introduction

In recent years, scientific research has focused on the problems of energy by finding clean, renewable, low-cost biofuel as bioethanol. Bioethanol production is based on fermentation of sugar rich substrate such as starch and cellulosic materials (Lin and Tanaka, 2006; Zabed et al., 2017). The expansion of the ethanol market has led many researchers to investigate alternative low-cost materials and methods to produce bioethanol (Achinas and Euverink, 2016). Utilizing abundant lignocellulosic materials is one possible alternative. Bioconversion of biomass into fermentable sugars and then to ethanol would be seen to be a practical sustainable approach (Chaturvedi and Verma, 2013).

Rice can be considered as the most widely distributed cereal crop in the world. It is a fundamental food and very important source of income for several tropical nations (Macauley, 2015). Massive quantity of lignocellulosic wastes is produced from rice cultivation. Rice cultivation produces over 650 million tons of rice per year, with over 800 million tons of lignocellulosic waste (mostly straw) including over 113 million tons of RH (Wood et al., 2016).

Cellulose, hemicelluloses as well as lignin are the major polymers of lignocellulosic materials. Cellulose is mainly hydrolyzed to glucose using mixture of enzymes and continually fermented to bioethanol (Karimi and Taherzadeh, 2016).

However, the presence of lignin and hemicellulose decreases the action of these hydrolytic enzymes to cellulose (Shawky et al., 2011). To increase the enzymes' accessibility, lignin should be removed, and

the complex structure should be opened up (Raghavi et al., 2016). Thus, a pretreatment step is an essential and important in the production of biofuel from lignocellulosic biomass.

Therefore, several pretreatment techniques have been improved such as alkaline and acidic methods (Loow et al., 2016). The alkaline pretreatments using sodium hydroxide are the most effective chemical pretreatment method (Shafiei et al., 2015). After pretreatment, the treated waste can be either applied to separate hydrolysis and fermentation (SHF) or simultaneously saccharification and fermentation (SSF). SHF offers different advantages and more opportunities. It enables hydrolytic enzymes to operate at higher temperature (50°C) to increase performance and allows fermentation organisms to operate at moderate temperatures (37°C) to optimize the utilization of produced sugars (Chandel et al., 2007).

Saccharomyces cerevisiae is a common microorganism used for the fermentation of hexose (Alonso-del-Real et al., 2017), while recombinant microorganisms can utilize a wider range of monosaccharaides, including pentoses, with high performance for bioethanol production from lingocellulosic biomass (Khaleghian et al., 2015). This work based on investment the agriculture residue such as RS and RH to produce bioethanol.

In order to realize the effective utilization of different lignocellulosic materials and to develop an economically viable biorefinery process, extraction of major component; cellulose, hemicellulose and lignin is essential (Vallejos et al., 2017). Bioethanol production from extracted cellulose through hydrolysis (saccharification) and fermentation processes will have strong impacts on the future economics of the biofuel and bio-based industry.

The purpose of the present study is to obtain acceptable amount of bioethanol from rice straw and rice husk cellulose as local agriculture residues using different statistical optimization designs.

Material and Methods

Chemical analysis of the plant samples

RS and RH were collected from Zagazig, Sharkya on August 2015. The collected agricultural wastes were then dried in an oven 70°C until no further weight loss and milled into (0.5-2 cm) using a grinding machine. The chemical constituent (e.g., moisture, ash, wax, crude lipids and low-molecular weight carbohydrates (LMWC)) of the initial plant samples was determined according to (AOAC, 1992). After soxhlet extraction, LMWC can be determined by dissolving in 85% ethanol for 24 h. and examined using paper chromatography according to (Jayme and Knolle, 1956). Detection of spots was attained by spraying the papers with aniline-phthalate reagent (Partridge and Beidler, 1949) and aniline-xylose. Qualitative determination of monosaccharides was applied by phenol-sulfuric acid method (Dubois et al., 1956). Thereafter, the color density was measured at wavelength 480 nm and 490 nm for pentoses and hexoses, respectively.

Total carbohydrates determination

Strong acid hydrolysis was applied to determine the total carbohydrates of RS, RH, extracted cellulose and hemicellulose as glucose (Dubois et al., 1956).

Qualitative determination

Qualitative determination were performed according to the procedure described in LMWC determination.

Quantitative determination

Quantitative determination of the monosaccharides was done according to the modified method of (Wilson, 1959). The individual chromatographic spots were cut off, split into small strips, then dumped into 4 mL eluting agents (0.7 N HCl in 80% ethanol) in test tube and shaked for complete elution. The absorbance of the resulting colored solutions was determined at 390 nm for pentoses and 490 nm for hexoses sugars using the spectrophotometer UNICO 7200. Monosaccharides were quantified using appropriate standard curves plotted under the same conditions.

Dewaxing RS and RH

The wax content was determined by immersing the cut wastes (0.5-1 cm) in a mixture of toluene and ethanol (2:1, v/v) overnight, and then filtrated, the oily residue was then recovered in a 250 mL round-bottom flask previously tarred in an oven and exactly weighed. The used solvent was eliminated at reduced pressure in a rotary evaporator and the obtained residue was separately subjected to 60°C for 16 hours in an oven for dryness then weighted.

Aqueous extraction optimization

Extraction experiments involving 5g of untreated RS (URS) and RH (URH) at static 100mL flask level (liquid to solid ratio, 20 mL/g). Alkalines (NaOH,

$Ca(OH)_2$, NH_4OH) treatment at different concentration and time of dewaxed RS and RH were statistically optimized (Table 1). The levels of optimized factors chosen were based on the suitable conditions for efficient dissolving of hemicelluloses, and lignin (weight loss %, w /w). After NaOH treatment, the reaction mixture was filtrated, the crude cellulose was washed more times with distilled water until neutralization and the filtrate was neutralized at pH 7- 6 by HCl under cooling. Filtration, the residue was hemicellulose and the filtrate has lignin. To precipitate lignin, hydrochloric acid was added reaching to a pH 1.5 of the solution. All separated residues (cellulose, hemicellulose and lignin) were dried at 105°C overnight (Ragab et al., 2014).

Infrared spectroscopy
Characterization of the extracted products was performed via FT-IR. IR spectra of cellulose, hemicelluloses and lignin were recorded between 4000-400 cm^{-1} wave number ranges.

Optimization of enzymatic hydrolysis by Taguchi method
Laboratory prepared *Aspergillus niger* (*A. niger*) crude enzymes containing FP-ase, CMC-ase and β-glucosidase were used for statistical optimize enzymatic hydrolysis of extracted cellulose (Table 2). After optimization, enzymatic hydrolysis of untreated RS and RH as well as extracted cellulose of each were conducted in 100 mL buffer solutions at pH 5.5 (0.05 M citrate buffer) using laboratory prepared *A. niger* crude enzyme and commercial cellulase enzymes (Novozyme 188).

Determination of glucose concentration
One mL of hydrolysate samples was added to 1 mL of glucose oxidase peroxidase reagent then incubated at 37°C for 10 minutes. The absorbance was measured at 546 nm. Finally, the amount of glucose was determined using a standard curve of glucose oxidase.

Fungal growth
The Fungal growth was measured in term of optical density (OD) at wavelength 540 nm using Shamidzo 2410c UV spectroscopy.

Optimization of fermentation
Fermentation media were statistically optimized (Table 3) in terms of overall bioethanol production (g/g). The fermentative microbe, *Saccharomyces cerevisiae* (*S. cerevisiae*) strain was obtained from the culture collection of the Bacteria Division, Faculty of Science, Helwan University in Egypt.

Analysis of variance (ANOVA)
ANOVA for the responses of aqueous extraction, enzymtic hydrolysis and fermentation yield was carried out according to the factors contribution by the statistical models. ANOVA determined ratio (F) and the p-value ($p< 0.05$) to know which factors were statistically significant.

Validation of the experimental model
Statistical models predicted responses lead to assign the optimum conditions. And so on, aqueous extraction, enzymatic hydrolysis and fermentation were carried out under optimized conditions.

Distillation and bioethanol determination
Pyrex distillation flask (500 mL) containing 30 mL of distilled water was inoculated with 1 mL of fermented wash. In 50 mL rounded flask (contain 30 mL of acidified potassium dichromate solution which consist of $K_2Cr_2O_7$, 33.768 g; H_2SO_4, 325 mL; H_2O, 400 mL and volume raised to 1 liter), the distillate was collected. About 20 mL of sample mixture was collected and kept in a water bath at 62.5°C for 20 minutes. The flasks were then kept in an ice bath for cooling and the volume was raised to 50 mL. Finally, 5 mL of solution was diluted with 5 mL of H_2O for measuring the OD at 600 nm using a spectrophotometer (Caputi et al., 1968). Standard curve of ethanol was plotted under similar set of conditions using different concentrations of ethanol that ranged from 2 to 12% to estimate the concentration of produced bioethanol in samples.

Results and Discussion

Chemical composition and total carbohydrates
Chemical composition as %w/w: LMWC, lipids, wax, ash and moisture were (7.35, 2.15, 5.85, 17.65 and 12.41) and (4.20, 5.45 12.55, 14.20 and 9.20) for RS and RH, respectively.

Optimization of cellulose extraction by Taguchi method
The impact of four factors on the extraction process was tested by Taguchi experimental design (Byrne and Taguchi, 1987) in 9 runs (Table 1). The response

values in terms of weight loss (% w/w) were chosen for the optimization of weight loss (% w/w) by extraction process (Table 4). The variation of weight loss (% w/w) ranging from 0% to 0.45 % corresponding to the combined effect of the four factors in their specific ranges. The experimental results imply that these factors at optimum level are strongly support the weight loss. A maximum weight loss of RS was 0.39 %, which detected in run (expt. 4) with a combination of 4% NaOH for two hours and temperature (90°C) with the best response (Table 4). When the best response for RH 0.45 % was observed in run (Expt. 4) under the same condition (Table 4).

ANOVA analysis of the Taguchi model experiments (runs)

The two models of RS and RH are significant according to the F-value of 67.08 and 218.98, respectively. Table 5 showed the significance of different model terms which determined according to the values of "Prob> F" (significant, $P < 0.05$, not significant, $P > 0.01$).

Model evaluation

A common and simple approach to evaluate models is to compare predicted vs. actual values. The "Pred R-Squared" of 0.8874 is in reasonable agreement with the "Adj R-Squared" of 0.9764. Predicted vs. actual plot showed how the model predicts over the range of data.

Response surface methodology (RSM)

Cube graph showed the effect of interaction between the significant factors on cellulose extraction. Maximum RS weight loss was obtained using NaOH as a solvent with 2% concentration for 2.9 hours and temperature of 90°C (Figure 1a). In RH model, the same conditions were applied for 3 hours (Figure 1b).

Experimental model validation

The model was validated by carrying out the best-predicted extraction process parameters. The validation of the experimental model was executed by determining weight loss of RS (%, w/w) was 38.00% which almost equal to predicted 40.73 % and 48.10% of RH which almost equal to predicted 47.25%.

Aqueous extraction of defatted plant material

Alkali treatment disrupts the cell wall by dissolving (lignin, hemicelluloses as well as silica), swelling cellulose in addition to decreasing the crystallinity of cellulose (Brodeur et al., 2011). Cellulose; hemicelluloses and lignin were extracted with good yield for both agriculture residues under study. Interestingly, it was noticed that RS has high cellulose content 54.6% (w/w). In contrast, RS have low hemicellulose and lignin content 19.10%, 11.95% when RH has 52.75% cellulose, 20.20% hemicellulose and only 10.85% of lignin content. Therefore, optimization was effective in obtain higher portion of cellulose using low concentration of NaOH compared to (Ragab et al., 2014) (RS, 44%, RH, 43% using 4% NaOH).

Ash content for extracted crude cellulose, hemicelluloses and lignin

Ash content for all the extracted crude fractions was determined. It was noted that all samples have different ash content as (% w/w) hemicellulose RS (HRS), 18.5; hemicellulose RH (HRH), 21.1; CRH, 10.8; CRS, 10.4; lignin RH (LRH), 9.1; lignin RS (LRS), 7.1. It show that cellulose have median ash content which reflect on total carbohydrate estimation. Also, removal of ash (dissolved with hemicellulose and lignin) could facilitate the enzymatic hydrolysis (Bin and Hongzhang,2010).

Determination of total carbohydrates

The total carbohydrate content was determined for different residues after hydrolysis. Strong hydrolysis by sulfuric acid was done. The total carbohydrates of RS and RH were 37.2% and 41.4%, respectively but percent content was low so that maybe some of them lost during dewaxing processes. Ratios of glucuronic acid, glucose, arabinose and xylose were determined according to paper chromatography analysis. Strong acid hydrolysis using H_2SO_4 indicated just glucose due to over hydrolysis for arabinose and xylose

Qualitative and quantitative analysis of carbohydrate content

In order to obtain more information about the chemical composition of crude cellulose, hemicelluloses and lignin of RS and RH extracts, they were subjected to complete strong acid hydrolysis followed by qualitative separation and then quantitative determination using paper chromatography. Strong acid hydrolysis was done by 80% sulfuric acid and cellulose contents were 62.4% and 64.6% for RS and RH, respectively. In case of HRS and HRH, strong acid hydrolysis was caused over hydrolysis so that the total carbohydrate content was low; 71.7% and 74.8%,

respectively. Presence of carbohydrates traces in lignin (RS, 3.1%; RH, 3.3%) means the extraction method was effective resulting precipitation in some of cellulose and hemicelluloses together with lignin, also cellulose has traces gained from hemicelluloses and lignin. Table 6 showed monosaccharide constituents (D-glucuronic acid, D-glucose, L-arabinose and D-xylose) of acid hydrolysis for cellulose, hemicellulose and lignin extracted from RS and RH. It was clear that cellulose still has hemicelluloses (L-arabinose and D-xylose) because pure cellulose should have just repeating units of D-glucose. Hemicellulose has glucose, mainly xylose and arabinose. Lignin has glucose, xylose and arabinose (lignin has some of soluble cellulose and hemicelluloses) (Lupoi et al., 2015). The results obtained from acid hydrolysis and ash content indicated that cellulose, hemicelluloses and lignin crudes may be still has traces or due to hydrolysis conditions, cellulose and lignin in hemicelluloses, cellulose and hemicelluloses in lignin and silica in all components. So that, crude cellulose and hemicelluloses could be purified using alkaline system to gain nearly pure cellulose (α-cellulose) which was not necessary in this study.

FT-IR spectroscopy analysis of extracted components

The FT-IR analysis was done mainly to identify functional groups present in CRS, CRH, HRS, HRH, LRS, LRH and untreated residues. The fingerprint region between 3500 cm^{-1} and 650 cm^{-1} comprises bands assigned to the main components of RS and RH, such as cellulose, hemicellulose and lignin. In general, some differences were detected in the spectrum in terms of intensity of the band and disappearance of bands after alkaline extraction (Figure 2). CRS, CRH, HRS and HRH spectra showed an absence of bands at 1570 cm^{-1} and 1624 cm^{-1} which indicated that these components were delignified completely after the extraction with NaOH. CRS spectrum showed an increase in the intensity of the transmission percentage observed at 3732 cm^{-1}, 3004 cm^{-1} and 2772 cm^{-1} that indicated that more specific functional groups in CRW than CRH.

Optimization enzymatic hydrolysis

The response values in terms of reducing sugars produced, chosen for optimization process showed the efficiency of hydrolysate production ranging from (mg/g) 14.35 to 230.31 corresponding to the combined effect of the four factors in their specific ranges. The experimental results suggest that these factors at optimum level strongly support the hydrolysate production. A maximum reducing sugars produced form both CRS and CRH was 230.31 mg/g and 221.8 mg/g, which observed in run 5 with a combination of 20 IU of enzymes with 3% (w/v) of substrate at 120 rpm and temperature (50ºC) with the best response (Table 7).

ANOVA analysis of the Taguchi model experiments (runs)

The two models of CRS and CRH were significant according to the F-value of 14.18 and 14.48, respectively. Table 8 showed the significance of different model terms which determined according to the values of "Prob> F" (significant, $P < 0.05$, not significant, $P > 0.01$).

Model evaluation

Convergence of predicted and actual values showed how the applied model predicted over the range of data. R^2_{pred} and R^2_{adj} for both CRS (0.6148 and 0.8682) and CRH (0.5890 and 0.8708) models were in acceptable agreement.

RSM

The effect of the interaction between key variables was displayed by RSM. Maximum reducing sugars production from CRS predicted was 268.04 (mg/g) with 25.36 IU of enzymes, 1.05 % (w/v) of substrate at 121.8 rpm and 49.3ºC. CRH was predicted to produce 241.647 mg/g of reducing sugars using 21.32 IU of enzymes, 2.67 % (w/v) of substrate at 103 rpm and 48ºC. The data was further validated under these conditions.

Experimental model validation

The model was validated by carrying out the best-predicted extraction process parameters. The validation of the experimental model was executed by determining the amount of reducing sugars produced (mg/g) from CRS was 263.88 almost equal to predicted 268.04 and 331.74 from CRH almost equal to predicted 241.647.

Enzymatic hydrolysis of extracted cellulose and untreated wastes

The efficiency of crude enzyme *A. niger* and commercial enzymes on saccharification of substrates was investigated. Figure 3 showed that reducing

sugars production from untreated wastes, and extracted cellulose, which increased with increasing hydrolysis time and beyond a hydrolysis time, it became almost constant. The maximum reducing sugars were (mg/g) URS, 105.55; CRS, 263.88; URH, 126.19; CRH, 222.69, which obtained at crude enzyme loading. On the other hand, commercial enzymes showed high reducing sugars yield as (mg/g) URS, 214.36; CRS, 583.41; URH, 169.28; CRH, 335.87. Figure 3 indicated that it is very difficult to obtain high yield of reducing sugars from the lignocellulosic residues without pretreatment because lignin present in the plant cell wall which hinders the enzyme action (Jeya et al., 2009).

Glucose estimation of hydrolyzed cellulose

The maximum glucose yield obtained from hydrolysis the crude enzyme of both CRS and CRH was 0.63825 g with 25.5% hydrolysis rate and 0.2886 g with 11.5% hydrolysis rate, respectively.

Optimization of fermentation process results of PBD

The influence of 11 factors on the fermentation process was tested by Plackett Burman design (PBD) (Plackett and Burman, 1946) in 12 runs (Table 3). The response values in terms of bioethanol production (%, w /w) chosen for optimization by fermentation process. Table 9 showed the rate of bioethanol production (%, w /w) ranging from 5.82% (in run 10) to 17.77 % (in run 4) in case of CRS and from 3.73% (run 11) to 9.13 % (run 9) for CRH hydrolysate. Because of variance in the results and in order to increase the bioethanol yield, the experiment factors were optimized.

Statistical analysis

The R^2_{pred} of 0.7916 and 0.7750 are in acceptable agreement with the R^2_{adj} of 0.9682 and 0.9656 for CRS and CRH hydrolysate models, respectively. Table 10 showed ANOVA of CRS and CRH hydrolysate models, which indicate the significance of variables. It was observed that the significant effects were only six factors: nitrogen source conc., aeration, agitation, Temp., O.D. and Na_2HPO_4. Rest of the variables did not have a significant effect on bioethanol production.

Model evaluation

All predicted and actual points were closed to the fitted line that means the model prediction was highly effective (Ohtani, 2000).

RSM

RSM define the optimum conditions for bioethanol production. Maximum bioethanol production from CRS hydrolysate predicted was 20.519% (g/g) with 4 g/L yeast extract concentration, pH 5.7, 34.5^{o}C, aeration of 29.9, O.D. of 2 , 7.69 g/L of K, 0.5 g of micro-elements, 4g/L ammonium, and 0.4g/L Na_2HPO_4. CRH hydrolysate produced 25.76% (g/g) of ethanol with 4 g/L yeast extract, pH 6.2, 35^{o}C, aeration of 28.76, O.D. of 2 , 0.6 g/L of K, 0.5 g of micro-elements, 0.4g/L ammonium, and 4g/L Na_2HPO_4 at shaked conditions of 192 rpm.

Validation

Fermentation of CRS and CRH hydrolysate were carried out with the optimized conditions and the glucose was completely assimilated in 36 h and 48 h, respectively. It was noticed that bioethanol yield of 23.18% (w/w) was almost identical to the CRS model predicted value (Figure 4a) and 26.94% in case of CRH hydrolysate (Figure 4b). The bioethanol yield from CRS and CRH hydrolysate was 69.2% and 52.84% of theoretical value (0.51 g/g glucose), respectively with specific bioethanol productivity of 231.8 mg/g and 269.4 mg/g.

Table 1: Matrix layout of the L9 Taguchi model design

Run	A Solvent	B Concentration (%)	C Time (h)	D Temperature (ºC)
1	NaOH	2	1	90
2	$Ca(OH)_2$	2	2	30
3	NH_4OH	6	2	90
4	NaOH	4	2	55
5	$Ca(OH)_2$	4	3	90
6	NH_4OH	4	1	30
7	$Ca(OH)_2$	6	1	55
8	NH_4OH	2	3	55
9	NaOH	6	3	30

Table 2: Matrix layout of the L9 Taguchi model design for RS and RH

Run	A Amount of enzymes (IU)	B Shaked rate (rpm)	C Temperature (ºC)	D Amount of substrate (% w/v)
1	30.00	120	42.50	1.00
2	20.00	120	35.00	2.00
3	10.00	135	50.00	2.00
4	30.00	150.00	42.50	1.00
5	20.00	120	50.00	3.00
6	20.00	150.00	50.00	2.00
7	30.00	150.00	35.00	3.00
8	20.00	135	42.50	3.00
9	10.00	135	35.00	1.00

Table 3: Factors (parameters) and levels in PBD for fermentation process

Factors	Levels	
	Low level	High level
A. Nitrogen source type	Yeast extract	Peptone
B. Nitrogen source conc.	0 g/L	4 g/L
C. Aeration	10%	30%
D. Agitation	0 rpm	200 rpm
E. Temperature	25 ºC	35 ºC
F. pH	5.5	7.0
G. O.D.	0.5	2.0
H. KH_2PO_4	0 g/L	8 g/L
J. Microelements	0 mg/L	80 mg/L
K. Ammonium sulphate	0 g/L	4 g/L
L. Na_2HPO_4	0 g/L	4 g/L

Table 4: Response values of L9 Taguchi design

Runs	1	2	3	4	5	6	7	8	9
RS	0.29	0.10	0.15	0.39	0.11	0.04	0.19	0.27	0.17
RH	0.30	0.00	0.23	0.45	0.10	0.16	0.19	0.43	0.19

Table 5: ANOVA table for RS and RH Taguchi model

Source	Sum of Squares		df		Mean Square		F Value		p-value Prob> F	
	RS	RH	RS	RH	RS	RH	RS	RH	RS	RH
Model	0.12	0.17	5	5	0.024	0.034	67.08	218.98	0.0028	0.0005
A-Type	0.052	0.080	2	2	0.026	0.040	73.77	255.69	0.0028	0.0004
B-Concentration	6.000E-004	2.400E-003	1	1	6.000E-004	2.400E-003	1.71	15.36	0.2823	0.0296
C-Time	1.500E-004	8.167E-004	1	1	1.500E-004	8.167E-004	0.43	5.23	0.5600	0.1064
D-Temperature	0.065	0.088	1	1	0.065	0.065	185.71	562.95	0.0009	0.0002

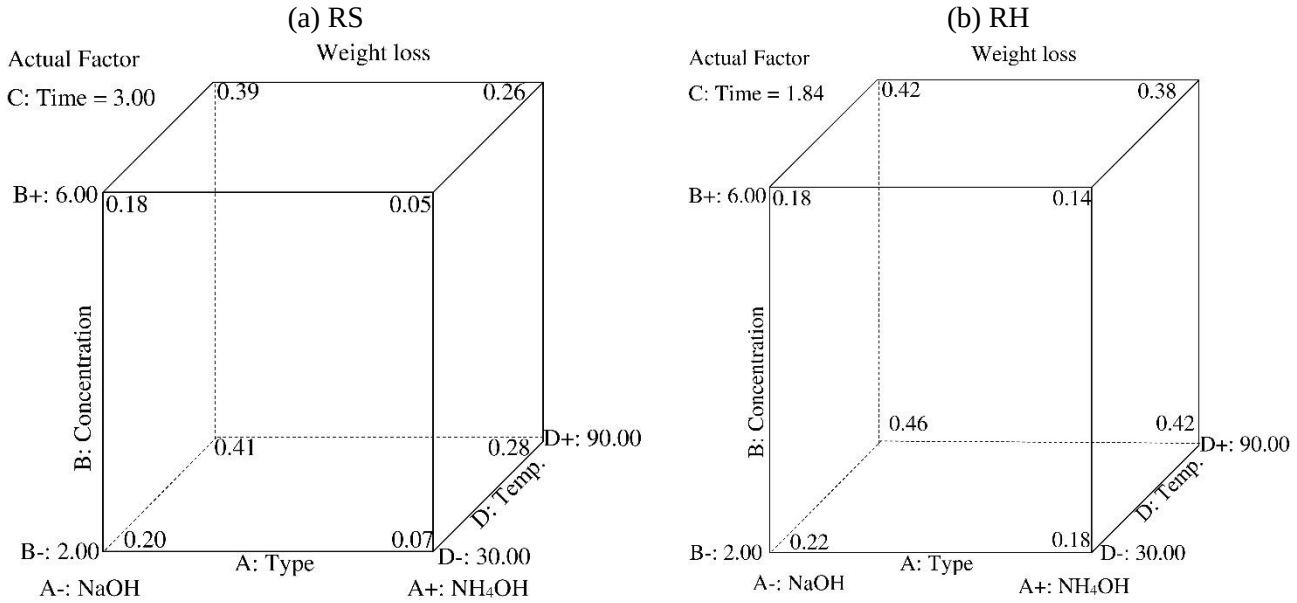

Figure 1: Response surface graph on cellulose extraction.

Table 6: Monosaccharide constituents of Acid hydrolysis (sulfuric acid) for cellulose, hemicellulose and lignin extracted from RS and RH by NaOH 2% and 90°C.

Monosaccharide constituents	Monosaccharide constituents of acid hydrolysis (% w/w)					
	CRS	CRH	HRS	HRH	LRS	LRH
D-glucuronic acid	t	t	t	t	t	t
D- glucose	100	100	100	100	t	100
L-arabinose	t	t	t	t	t	t
D-xylose	t	t	t	t	t	t

Table7: Response values of L9 Taguchi design.

Runs	1	2	3	4	5	6	7	8	9
RS	221.35	38.21	129.67	150.50	230.31	201.14	51.78	137.32	25.45
RH	207.45	28.35	111.65	129.85	221.80	185.50	36.75	102.90	14.35

(a) RS (b) RH

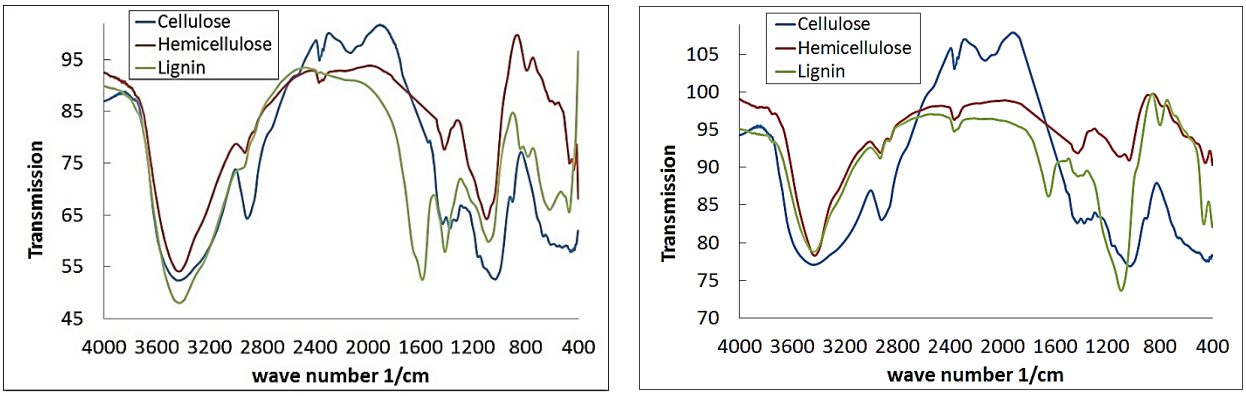

Figure 2: **FT-IR spectra of cellulose, hemicellulose and lignin.**

Table 8: ANOVA table for CRS and CRH Taguchi model

Source	Sum of Squares		df		Mean Square		F Value		p-value Prob> F	
	RS	RH	RS	RH	RS	RH	RS	RH	RS	RH
Model	46134.50	45892.31	4	4	11533.62	11473.08	14.18	14.48	0.0125	0.0120
A-Amount of enzyme	11317.21	11153.74	1	1	11317.21	11153.74	13.91	14.08	0.0203	0.0199
B-shaking rate	2987.70	3863.05	1	1	2987.70	3863.05	3.67	4.88	0.1278	0.0918
C-Temperature	39968.06	39241.72	1	1	39968.06	39241.72	49.13	49.52	0.0022	0.0021
D-amount of substrate	626.43	880.40	1	1	626.43	880.40	0.77	1.11	0.4298	0.3513
Residual	3254.25	3169.60	4	4	813.56	792.40				
Cor Total	49388.74	49061.91	8	8						

Table 9: **Response values of PBD design.**

Runs	1	2	3	4	5	6	7	8	9	10	11	12
RS	12.98	16.02	09.40	17.76	11.19	11.18	11.55	09.60	15.90	05.82	09.73	14.06
RH	05.96	05.05	05.15	05.32	04.24	04.30	08.14	05.15	09.13	08.29	03.73	07.46

Table 10: ANOVA table for CRS and CRH hydrolyste PBD model.

Source	Sum of Squares		df		Mean Square		F Value		p-value Prob> F	
	RS	RH	RS	RH	RS	RH	RS	RH	RS	RH
Model	126.38	35.47	9	9	14.04	3.94	38.17	35.33	0.0258	0.0278
B-Nitrogen source conc.	13.52	11.72	1	1	13.52	11.72	36.76	105.06	0.0261	0.0094
C-Aeration	19.83	4.17	1	1	19.83	4.17	53.89	37.38	0.0181	0.0257
D-Agitation	19.28	3.39	1	1	19.28	3.39	52.39	30.37	0.0186	0.0314
E-Temperature	18.85	3.97	1	1	18.85	3.97	51.24	35.57	0.0190	0.0270
G-O.D.	13.21	2.61	1	1	13.21	2.61	35.89	23.36	0.0267	0.0402
H- KH_2PO_4	0.20	0.052	1	1	0.20	0.052	0.53	0.47	0.5419	0.5642
J-Microelements	2.27	0.35	1	1	2.27	0.35	6.18	3.18	0.1308	0.2167
K-Ammonium sulphate	2.63	0.58	1	1	2.63	0.58	7.16	5.21	0.1159	0.1499
L-Na_2HPO_4	36.59	8.63	1	1	36.59	8.63	99.46	77.33	0.0099	0.0127
Residual	0.74	0.22	2	2	0.37	0.11				
Cor Total	127.12	35.70	11	11						

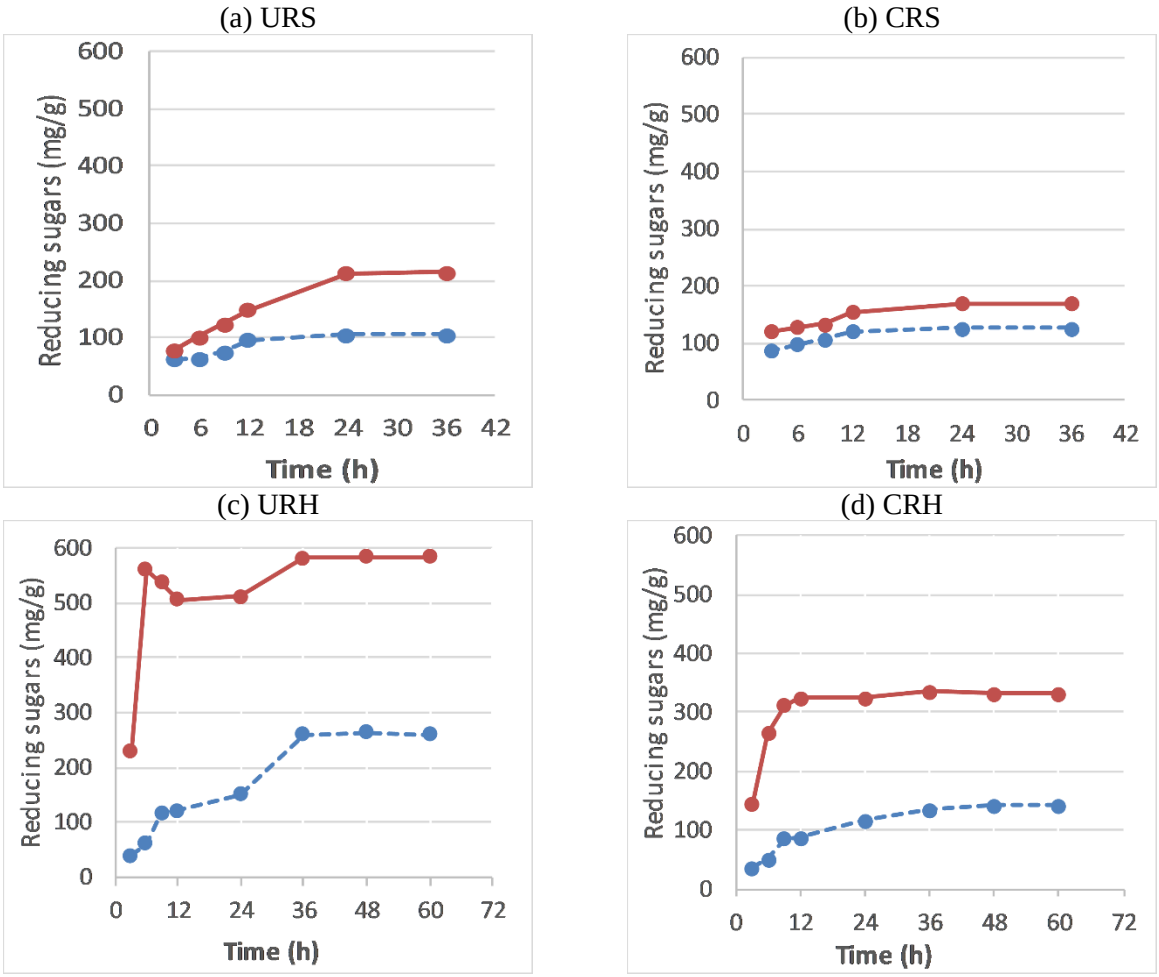

Figure 3: production of reducing sugars by commercial enzymes (—) and crude enzymes (---)

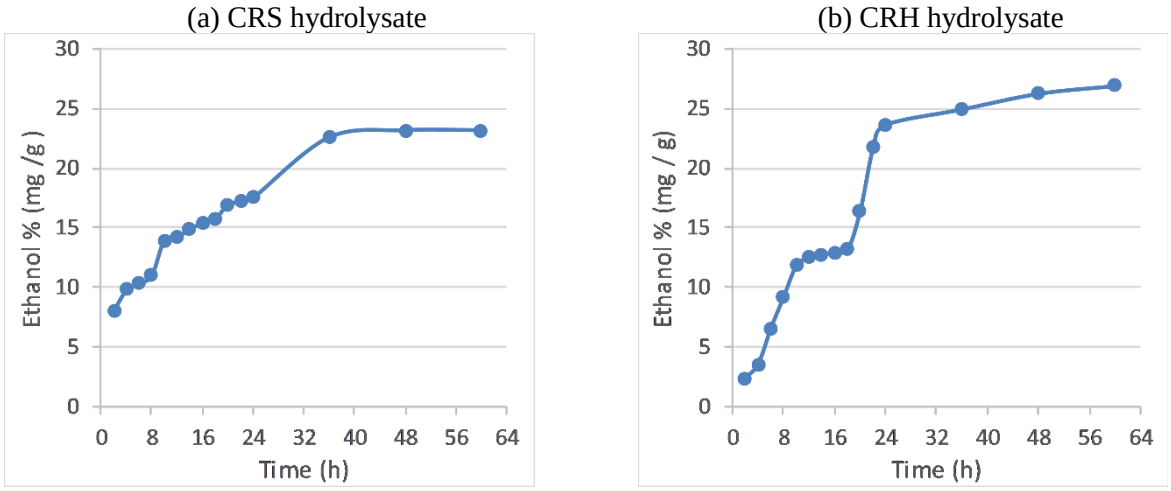

Figure 4: Bioethanol production.

Conclusion

In order to obtain acceptable yield from rice straw and rice husk, fractionation, hydrolysis as well as fermentation processes were statistically optimized using design expert software. Extraction using NaOH 2% at 90ºC and organic solvents were optimized by Taguchi experimental design method. 54.60 % and 52.75% cellulose can be extracted from RS and RH. Maximum glucose yield of 255 mg/g and 120 mg/g was obtained from the extracted CRS and CRH after optimized enzymatic treatment using *Aspergillus niger* laboratory prepared enzyme. Separate hydrolysis and fermentation using *Saccharomyces cerevisiae* obtained 177.69 mg/g, 91.34 mg/g and bioethanol obtained from CRS and CRH, respectively. After Placket Burman model statistical optimization, bioethanol yield was increased to 231.8 mg/g, 269.4 mg/g, respectively, i.e., 30.45% and 194.91%.

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