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Optimization of enzymatic saccharification for quality bio-oil from rice straw

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Abstract
The research was carried out to extract bio oil from rice straw at different parameters. The major problem in production of bio-oil is its increase in density with the passage of time. The reason for such phenomenon is the cross-linking of lignin which was bound to bio-oil after pyrolysis. The aim of this work was to reduce the lignin content in rice straw and study the effects of decreased lignin content in bio-oil. Additionally enzymatic saccharification of rice straw was also conducted after pre-treatment to estimate optimum conditions for bio-oil production. The independent variables for the optimization of enzymatic saccharification were, time (24, 48, 72 hours), Enzymatic loading ratio (5:10, 10:10, 15:10), substrate concentration (4, 5, 6% w/v) were taken into account. Designed experiments were conducted randomly to find the effect of these variables on reducing sugar, lignin and ash. The analysis of variance for each sample was calculated. The reducing sugar increased with increase in time and enzyme loading ratio while lignin content increased with increase in substrate concentration 48.1 to 51.1% w/w and 9.1 to 12.1 w/w. Optimum value of levels of independent variables obtained by compromise optimization of the responses were; 64.8 h time, 15:10 enzyme loading ratio and 4.62 substrate concentrations.

Keywords: Rice straw, lignin, ash, bio-oil and reducing sugar

1. Introduction
Lignocellulosic substrates are regarded as one of the most important sources of carbon and related carbohydrates. Its subsequent bioconversion and utilization into useful products can be regarded as better sources of energy and fuel. Rice straw consists of three main components: cellulose, hemicellulose, and lignin. The complexity of these components leads to the stability of its biomass and results in the difficulty of its enzymatic disassembly. Rice straw consists of cellulose (35–40% w/w) and hemicellulose (25–30% w/w) in close association with lignin (10–15% w/w), is one of the most abundant lignocellulosic crop residues (Thygesen *et al.* 2003).

In terms of total production, rice is the third most important grain crop in the world behind wheat and corn. As per commodity profile for rice (March 2015), world annual rice production in 2014-15 was estimated about 475 million tons. Similarly for commodity profile of rice (March 2015), India's annual rice production in 2014-15 was estimated about 103.04 million tons. Rice production in Uttarakhand in 2014-15 was estimated to about 5.5 lakh tones (Mani, 2014) [12]. Every kilogram of grain harvested is accompanied by production of 1–1.5 kg of the straw (Maiorella, 1985) [11]. It gives an estimation of about 480–720 million tons of rice straw produced per year globally and a large part of this is going as cattle feed and rest as waste. The options for the disposition of rice straw are limited by the low bulk density, slow degradation in the soil, harbouring of rice stem diseases, and high mineral content. Nowadays, field burning is the major practice for removing rice straw, but it increases the air pollution and consequently affects public health (Mussatto and Roberto, 2004) [10]. As climate change is extensively recognized as a threat to development, there is growing interest in alternative uses of agro-industrial residues for energy applications. In this context, rice straw would be a potential candidate for our future energy needs.

Bio-oil is normally derived from biomass such as rice straw. The benefit of energy generation is often offset by the increased emissions, thus restraint it value as fuel for incineration. Therefore, alternative technologies should be invented in order to transform this beneficial renewable biomass into useful products. Liquefaction of rice straw using thermo chemical conversion process such as pyrolysis offers an option for conversion of solid biomass into liquid bio-oil.

Bio-oil is easier to transport, store, and can be upgraded to improve its quality. Bio-oil is also utilized as specialty chemicals like flavourings, renewable resins and slow release fertilizers (Baratieri *et al.* 2008, Abdullah and Gerhauser 2008) [7, 4]. However, bio-oil cannot be used as a direct substitution for petroleum and chemical feedstock since it has high viscosity, high content of unstable oxygenated molecules and high density (1.2 kg/L). Therefore, upgrading of bio-oil into higher quality liquid product with wider applications is necessary. Various upgrading methods have been reported including solvent fractionation, steam reforming, hydrogenation, and catalytic cracking.

The main objective of this work is to investigate the potential of chemically pretreated rice straw as a promising endeavour for bio fuel production. The usage of alkaline chemical in the pre-treatment of rice straw and enzymatic hydrolysis to degrade lignocelluloses biomass and saccharification of cellulose is expected to alter the original biomass structure. Response surface methodology by Box–Behnken design employing the multivariate approach enables substantial improvement in the method development using fewer experiments, without wastage of large volumes of organic solvents, which leads to high analysis cost (Wani *et al*. 2012) [15] it can be used to get an optimum process conditions considering single response or multiple responses. It encompasses statistical and mathematical techniques.

2. Materials and Methods

Rice straw (*Oryza Sativa* L.) was procured from Crop Research Centre (C.R.C.) of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar for conducting the experiments. Chemicals and enzymes used in the sample preparation for the pre-treatment of rice straw and for proximate analysis chemicals were purchased from Himedia Pvt. Ltd. All the experiments were designed and conducted in the department of Post-Harvest process and Food Eng, College of Technology, Pantnagar, Uttarakhand.

2.1. Drying of rice straw

The procured rice straw was dried in a hot air oven simultaneously moisture content was also evaluated. The rice straw was dried at 115˚C until it kept constant weight during estimation of moisture content (Zhang *et al.* 2008) [18]. After that the rice straw was bone dried for size reduction process.

2.2. Determination of moisture content

The moisture content of the sample was calculated using the following equation

$$\%W = [(A-B)/B] * 100 \quad (1)$$

Where:

%W = Percentage of moisture in the sample,

A= Weight of wet sample (gm), and

B = Weight of dry sample (gm)

2.3. Size reduction of rice straw

The dried rice straw was subjected to size reduction process in hammer mill. The mesh size being used was of 2 mm size. The size reduction process was done for better availability of grounded straw to alkali during pre-treatment process.

2.4. Alkali Pre-treatment

The alkali pre-treatment was used for reduction of lignin content in the sample. The alkali pre-treated samples were used for enzymatic saccharification. The rice straw was treated with 2% (w/v) NaOH solution at 85˚C for 1 hour. Then the treated rice straw was washed with water until neutral pH achieved and then oven dried.

2.5. Enzymatic saccharification

2.5.1. Preliminary experiments

Preliminary trials were conducted for subjection of enzymes to the pre-treated rice straw. The objective of this experiment was to examine the effects of one step enzyme addition and two step enzyme additions during saccharification process. The one step enzyme addition included the addition of enzymes viz. cellulase (10 FPU) and β-glucosidase (10 IU) at conditions mean to their optimum activity in individual action for 24 hours. On the other hand in two step enzyme addition the enzyme in similar amounts were added but at varied conditions as provided by manufacturer's recommendation on optimum activity of individual enzymes in the intervals of 12 hours. The substrate used in the experiment was cellulose powder (5% w/v concentration) with the two enzymes viz. cellulase and β-glucosidase. The operating conditions of each enzyme and used in one step enzyme addition are shown in Table 1.

Table 1: Operating conditions of preliminary experiments

Method	Temperature	pH
One step enzyme addition	37 ˚C	4.8
Two step enzyme addition:		
Cellulase	40 ˚C	4.5
β-glucosidase	35 ˚C	5.0

2.6. Experimental Design

Experimental design, defined as a specific set of experiments which are defined by a matrix, a matrix composed by the different level of combination variables. Response surface methodology (RSM) was used for the design and analysis of all experiments for three predicted variables at three levels. It also helps to reduce the number of experiments without affecting the accuracy of results and to decide the interactive effects of influencing factors on the response. Box-Behnken model was selected for the optimization of the process variables (Sharma 2014) [13]. Box-Behnken is a class of rotatable second order design based on the levels incomplete factorial design. This design does not contain for which all factors are simultaneously at their highest and lowest levels. So this design is useful in avoiding experiments performed under extreme conditions for which unsatisfactory results might occur (Bezzerra *et al.* 2008) [8]. The detailed values of independent and constant variables of experimental design are given in Table 2 and 3. The number of experiments (N) required for development of Box-Behenken Design using equation 2.

$$N= 2K(K-1)+C_0 \quad (2)$$

where,

N= Total no. of experiments

K = No. of variables

C_0= Centre point

In order to determine a critical point (maximum, minimum or saddle) it is necessary for the polynomial function to contain the quadratic terms. According to the equation presented below:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j + \sum_{i=1}^{n} \beta_{ii} X_i^2 \quad (3)$$

where

β_{ii}, β_0 and β_{ij} are the coefficients of regression. (i=1, 2, 3,.....n) (j=1, 2, 3,....n)

Table 2: levels of independent variables for the production of bio-oil

Independent variables	Values		
	-1	0	+1
Reaction time (hours)	24	48	72
Enzyme loading ratio	5:10	10:10	15:10
Substrate concentration (% w/v)	4	5	6

Table 3: The variables kept constant during the experiments

Variables	Values
Temperature	37 ^{0}C
pH	4.8
Buffer concentration	0.1M

2.7. Experimental Procedure
The whole experiment was conducted in three phases. Experiments were planned with a view to develop pre-treated, enzymatically hydrolyzed agricultural waste, which could be a better method for bio-oil extraction by pyrolysis process. In the first phase experiments were carried out by already optimized pre-treated conditions for rice straw for maximum breakdown of lignin. The said conditions utilized in alkali pretreatment were NaOH at 85 ^{0}C for 1 hour. The analysis of sample was done on the basis of proximate parameters which are important for the sample analysis. During the second phase, enzymatic hydrolysis was carried out and the independent variables (reaction time, enzyme ratio, substrate concentration) were optimized for best reducing sugar yield. Again, the analysis of sample was done on the basis of proximate parameters which were important for the sample analysis.

2.7.1. Reducing sugar
The DNS method for estimating the concentration of reducing sugars in a sample was originally invented by the author (Miller, 1959). Reducing sugars have the property to reduce many of the reagents. A reducing sugar is one that in a basic solution forms an aldehyde or ketone. The aldehyde group of glucose converts 3, 5-dinitrosalicylic acid (DNS) to 3-amino-5-nitrosalicylic acid, which is the reduced form of DNS. Water is used up as a reactant and oxygen gas is released during the reaction. The formation of 3-amino-5-nitrosalicylic acid results in a change in the amount of light absorbed, at wavelength 540 nm. The absorbance measured using a spectrophotometer is directly proportional to the amount of reducing sugar (Miller, 1959).

2.7.2. Ash estimation
Samples of air dried, ground (0.5 mm) lignocellulosic biomass (0.7 g each) were boiled with 5 mL of 72% w/w H_2SO_4 solution for 4.5 hours in order to hydrolyze the cellulose and hemicellulose. The suspension remaining after the above treatment was filtered through a crucible and the solid residue dried at 105 ^{0}C for 24 hours and weighed (W_1). The residue was then transferred to a pre-weighed dry porcelain crucible and heated at 600 ^{0}C for 5 hours. After cooling down, it was weighed (W_2) and ash content (%) was determined (Ververis *et al.* 2007) [14].

2.7.3. Lignin estimation
The lignin can be estimated gravimetrically by klason or 72% w/w H_2SO_4 Method. The most widely used method for lignin determination by chemists is probably the simplest and overall the most reliable, despite its limitations. Samples were digested with 72% w/w sulphuric acid, then with dilute sulphuric acid, to hydrolyze and solubilise the polysaccharides; the insoluble residue were dried and weighed as lignin (Liu, 2004). Following from ash estimation, acid insoluble lignin was then calculated by the difference (W_1-W_2) (Ververis *et al.* 2007) [14].

3. Results and Discussion
3.1. Effect of independent variables on reducing sugar
Experimental data in Table 4 shows that in case of enzymatic pretreatment, the reducing sugar ranged from 25.1 to 35.2 g/L. Maximum reducing sugar was found 35.2 g/L for experiment number 17, with the combination of time 48 hours, enzyme loading ratio 10:10 and substrate concentration 5% w/v. Minimum reducing sugar was found 25.1 for experiment number 3, with the combination of time 48 hours, enzyme loading ratio 5:10 and substrate concentration 4% w/v. It can be concluded that enzyme activity for an optimum time proves highly significant in release of reducing sugar.

3.2. Effect of independent variables on lignin
Experimental data in Table 4 shows that in case of enzymatic pretreatment, the lignin ranged from 7.7 to 10.8% w/w. Maximum lignin was found 10.8% w/w for experiment number 9, with the combination of time 24 hours, enzyme loading ratio 10:10 and substrate concentration 6% w/w. Minimum lignin was found 7.7% w/w for experiment number 5, with the combination of time 48 hours, enzyme loading ratio 15:10 and substrate concentration 4% w/v. Lignin showed no significant reduction by enzymes. Lignin was directly proportional to substrate concentration.

3.3. Effect of independent variables on ash
Data tabulated in Table 4 shows that ash content ranged from 7.8 to 9.8% w/w. Maximum ash was found 9.8% w/w for experiment number 11 with the combination of time 48 hours, enzyme loading ratio 5:10 and substrate concentration 6% w/w. Minimum ash was observed 7.8 w/w for experiment number 5 with the combination of time 48 hours, enzyme loading ratio 15:10 and substrate concentration 4% w/v. Losses of ash content could be concluded from activity from enzymes because of conversion of cellulose to reducing sugar.

Table 4: Experimental data of independent parameters for enzymatic saccharification

	Factor1	Facto r2	Factor3	Response1	Response3	Response4
Run	Time	E L R	Subs. Conc.	R. S.	Lignin	Ash
	hr	IU:FPU	%w/v	g/L	%w/w	%w/w
1	-1	-1	0	28.8	10.2	9.2
2	-1	0	-1	26.1	8.4	9
3	0	-1	-1	25.1	8.5	8.4
4	1	0	-1	27.9	8.1	7.9
5	0	1	-1	27.1	7.7	7.8
6	-1	1	0	27.9	9.7	8.4

7	1	-1	0	27.1	9.6	8.8
8	0	0	0	34.8	9.5	9.1
9	-1	0	1	25.8	10.8	9.1
10	0	1	1	30.8	10.6	9.4
11	0	-1	1	25.6	9.4	9.8
12	1	1	0	32.3	9.7	8
13	0	0	0	35	10.5	8.9
14	0	0	0	34.1	10	8.9
15	1	0	1	29.3	10.5	9.6
16	0	0	0	34.6	9.7	8.8
17	0	0	0	35.2	9.2	8.9

3.4. Statistical analysis of dependent variables

3.4.1. Reducing sugar

The statistical analysis of reducing sugar was given in Table 5. The model of reducing sugar was found highly significant (P<0.01) because it had higher F-value (44.63). It was also observed that the effect of independent variables on reducing sugar was highest at quadratic level due to highest calculated F-value (99.15) followed by linear level and interactive level.

Table 5: Analysis of variance for reducing sugar

Source	DF	SS	MS	F-value
Model	9	218.59	24.29	44.63*
Linear	3	28.04	9.34	17.30*
Quadratic	3	160.62	53.54	99.15*
Interactive	3	12.58	4.19	7.76**
Residual error	7	3.81	0.54	
Lack of fit	3	3.1	1.03	N.S.
Total	16	222.4	24.83	

, * and ******* represent significant values at1, 5 and 10%

It was assumed that the increase in the concentration of reducing sugar improves the quality of bio-oil from the enzymatically saccharified rice straw. The reason for the above assumption arises from the fact that monomeric sugars results in higher quality of bio-oil compared to polymeric sugars. From Fig. 1 at linear level, observed the effect of time on reducing sugar at optimum point of enzyme loading ratio 1.0 (15:10) and at substrate concentration -0.38 (4.62% w/w) for producing quality improved bio-oil. It depicted that reducing sugar increased rapidly with increased level of time from -1 to 0.5 while it is constant with respect to time. Afterwards reducing sugar decreased with increased time and this point quality of bio-oil could decrease and similar readings were supported by Hsu *et al.* 2008.

From Fig. 2 at linear level, observed the effect of enzyme loading ratio on reducing sugar at optimum point of time 0.7 (64.8 hours) and at substrate concentration -0.38 (4.62% w/w) for producing quality improved bio-oil. It depicted that reducing sugar increased rapidly with increased level of enzyme loading ratio from -1 to 0.5 while it is constant with respect to enzyme loading ratio. Afterwards reducing sugar decreased with increased enzyme loading ratio and this point quality of bio-oil could decrease. From Fig. 3 at linear level, observed the effect of substrate concentration on reducing sugar at optimum point of time 0.7 (64.8 hours) and at enzyme loading ratio 1 (15:10) for producing quality improved bio-oil. It depicted that reducing sugar increased rapidly with increased level of substrate from -1 to 0.25 while it is constant with respect to substrate concentration. Afterwards reducing sugar decreased with increased substrate and this point quality of bio-oil could decrease. Fig. 4 shows the variation of reducing sugar with time and enzyme loading ratio at optimum point of substrate concentration -0.38 (4.62%, w/v) in interactive level of the model. The reducing sugar increased with increase in the level of time and after some time it decreased steeply from -1(24 hr) to 1(72hr) while reducing sugar increases with increase in the level of enzyme loading ratio. Fig. 5 shows the variation of reducing sugar with substrate concentration and enzyme loading ratio at optimum point of time 0.7 (4.62%, w/v) in interactive level of the model. The reducing sugar increased with increase in the level of substrate concentration and after some time it decreased steeply from -1 (4% w/v) to 1 (6% w/v) while reducing sugar increases with increase in the level of enzyme loading ratio.

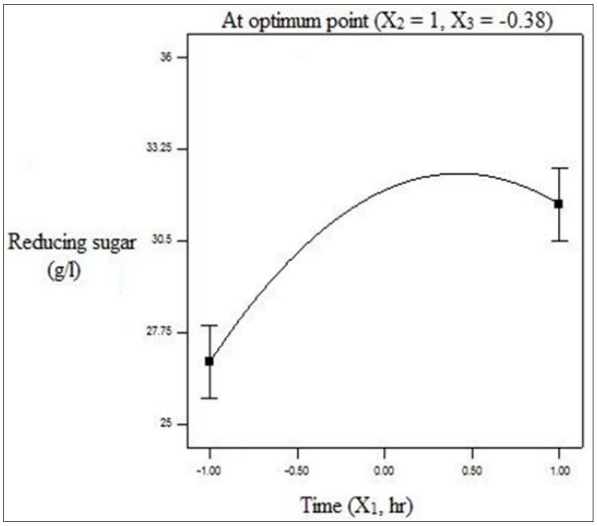

Fig 1: Effect of time on reducing sugar

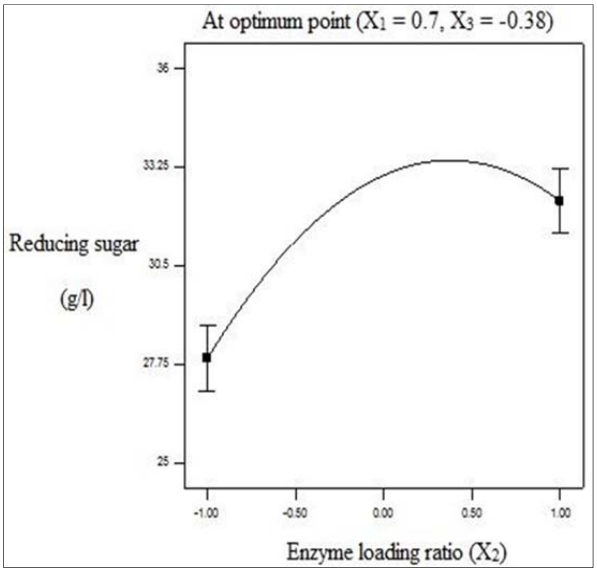

Fig 2: Effect of enzyme loading ratio

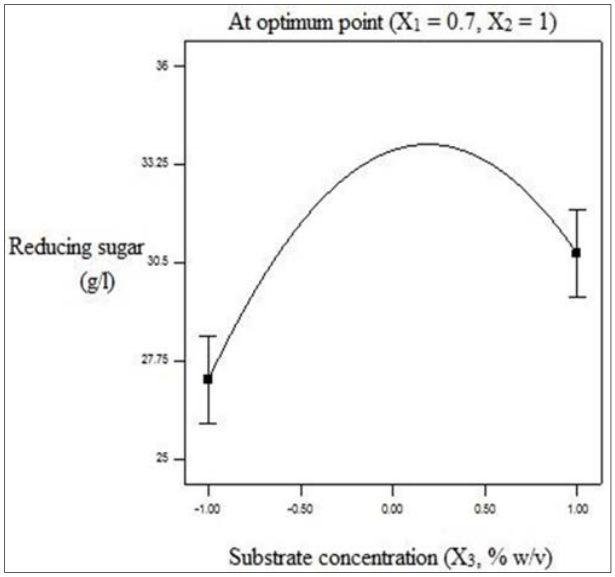

Fig 3: Effect of substrate concentration on reducing sugar

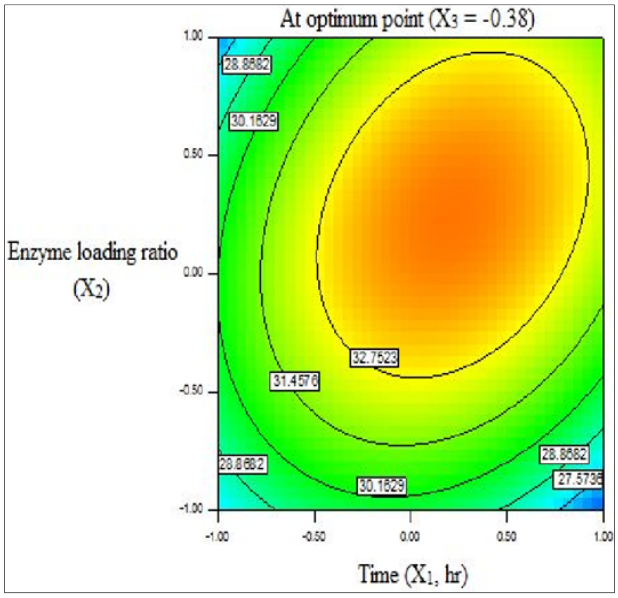

Fig 4: Effect of enzyme loading ratio on reducing sugar

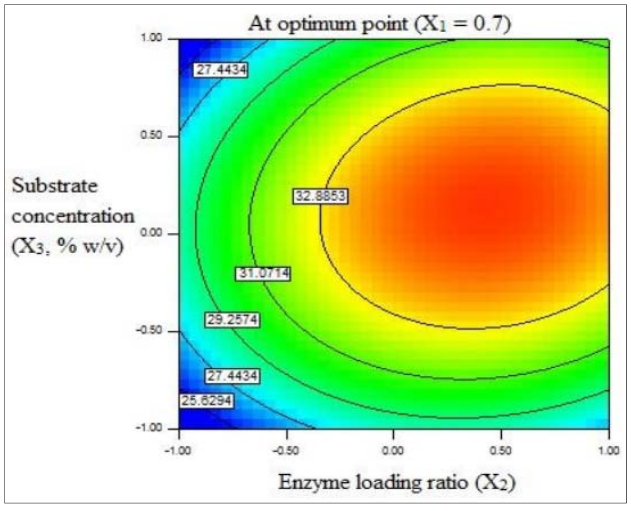

Fig 5: Effect of enzyme loading ratio and substrate concentration on reducing sugar

3.4.2 Lignin content

The statistical analysis of lignin was given in Table 6. The model of lignin was found highly significant ($P< 0.01$) because it had higher F-value (7.87). It was also observed that the effect of independent variables on lignin was highest at linear level due to highest calculated F-value (18.47) followed by quadratic level and interactive level.

Table 6: Analysis of variance for lignin

Source	DF	SS	MS	Fvalue
Model	9	12.07	1.34	7.87*
Linear	3	9.43	3.14	18.47*
Quadratic	3	1.57	0.52	3.05
Interactive	3	1.09	0.36	2.11
Residual error	7	1.19	0.17	
Lack of fit	3	0.21	0.068	N.S.
Total	16	13.26	1.51	

*, ** and *** represent significant values at1, 5 and 10%

It was observed that the increase in the lignin degradation in enzymatically saccharified rice straw could increase the quality of bio-oil. The reason for the above assumption arises from the fact that lignin cross linking occurs during storage of bio-oil results in lower quality of bio-oil. From Fig. 6 at linear level, observed the effect of substrate concentration on lignin at optimum point of enzyme loading ratio 1 (15:10) and at time 0.7 (64.8 h) for producing quality improved bio-oil. It depicted that lignin increased regularly with increased level of time from -1 to 1 while it is constant with respect to enzyme loading ratio. More of the substrate concentration analyzed provided for the increased lignin content. Fig. 7 shows the variation of lignin with substrate concentration and enzyme loading ratio at optimum point of time 0.7 (64.8 h) at interactive level of the model. The lignin increased steeply with increase in the level of substrate concentration (X_3, %, w/v) and the lignin is reduced with increasing enzyme loading ratio.

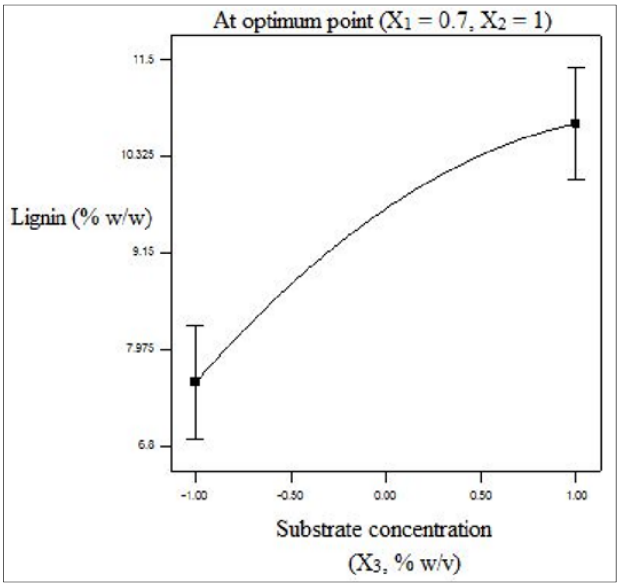

Fig 6: Effect of substrate concentration on lignin

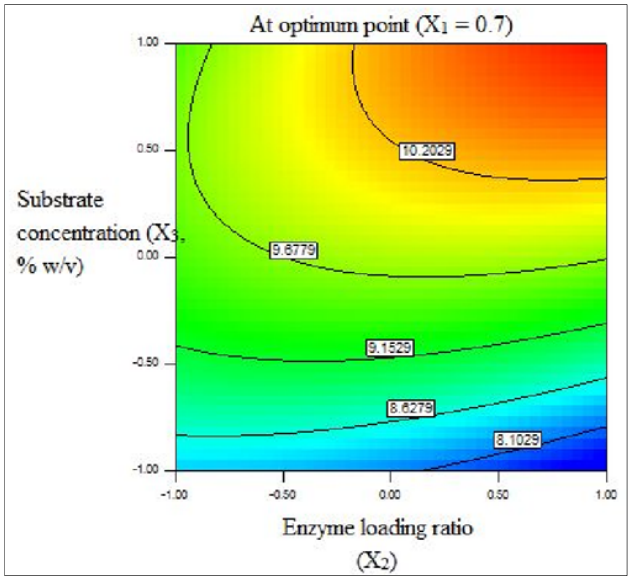

Fig 7: Effect of enzyme loading ratio and substrate concentration on lignin

3.4.3. Ash content

The statistical analysis of ash content for the bio-oil was given in Table 7. The model of ash was found highly significant ($P < 0.01$) because it had higher F-value (13.69). It was also observed that the effect of independent variables on ash was highest at linear level due to highest calculated F-value (33) followed by interactive and quadratic level.

Table 7: Analysis of variance for ash

Source	DF	SS	MS	F-value
Model	9	4.89	0.54	13.69*
Linear	3	3.96	1.32	33*
Quadratic	3	0.273	0.091	2.27
Interactive	3	0.65	0.21	5.25
Residual error	7	0.28	0.04	
Lack of fit	3	0.23	0.077	N.S.
Total	16	5.17	0.58	

*, ** and *** represent significant values at1, 5 and 10%

From Fig. 8 at linear level, it was observed that ash decreases with increase level of time at optimum values of enzyme loading ratio 1 (15:10), time 0.7 (64.8 h). From Fig. 9 at linear level, it was observed that ash decreases steeply with increase in level of enzyme loading ratio and then decreases at optimum values of time 0.7 (64.8 h), enzyme loading ratio 1 (15:10).From Fig. 10 at linear level, it was observed that ash increases steeply with increase in level of substrate concentration -0.38 (4.62%, w/v) at optimum values of time 0.7 (64.8 h), enzyme loading ratio 1 (15:10). From Fig. 11 shows the variation of ash with time and substrate concentration at optimum point of enzyme loading ratio 1 (15:10) at interactive level of the model. The ash decreased with increase in the level of time from -1(24 h) to 1(72h) while ash increased in the level of substrate concentration.

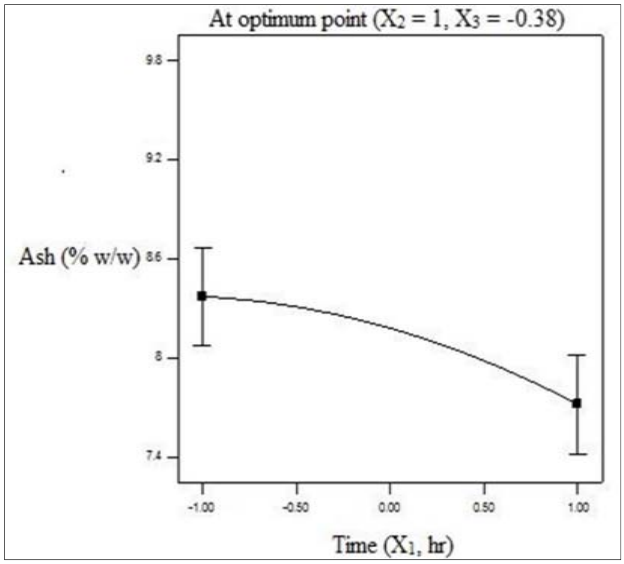

Fig 8: Effect of time on ash

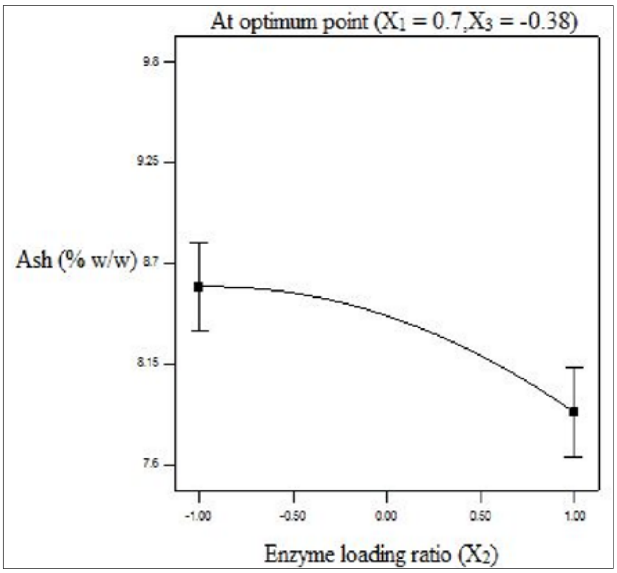

Fig 9: Effect of enzyme loading ratio on ash

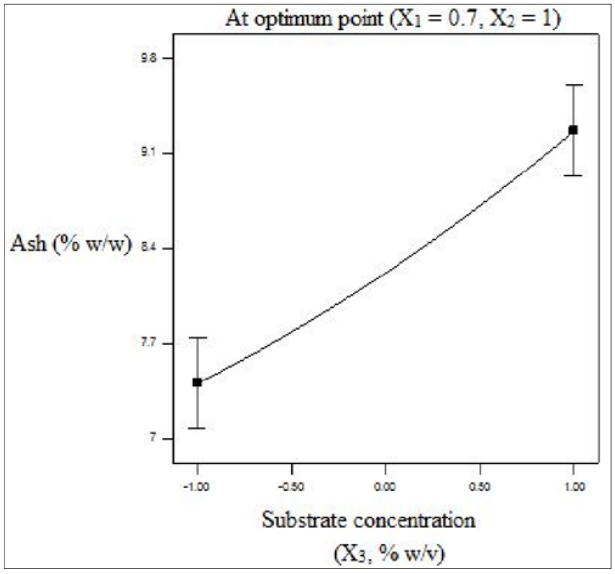

Fig 10: Effect of substrate concentration

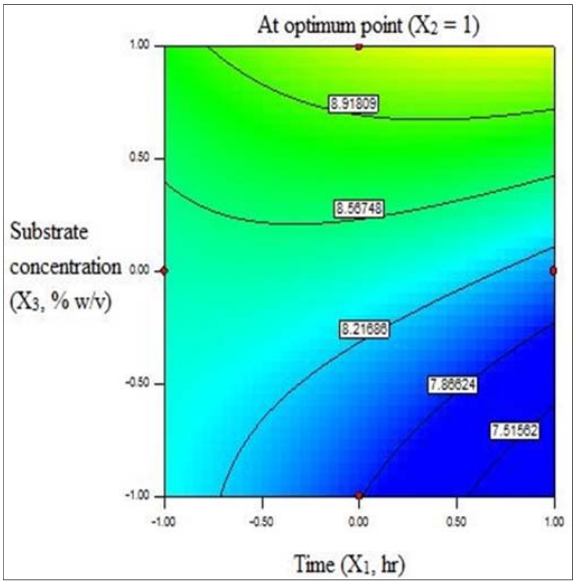

Fig 11: Effect of time and substrate concentration on ash

3.4.4 Optimum results of independent variables

The Optimized results of independent variables for the enzymatic saccharification of rice straw to improve the quality of bio-oil which are given in Table 8.

Table 8: Optimum results for the enzymatic saccharification

Independent variables	Coded level	Actual level
Time (X_1, hr)	0.7	64.8
Enzyme loading ratio (X_2)	1.0	15:10
Substrate concentration (X_3, %, w/v)	-0.38	4.62

4. Conclusions

The present study was aimed for removal of lignin and saccharification of rice straw for quality improvement of bio-oil. In the proposed work, lignin removal was achieved upto 10% and saccharification resulted in high yields of reducing sugar. The enzymatic saccharification released reducing sugar may have multiple fates viz. Bio-ethanol, Biogas, Bio-oil, Bio-diesel. Reducing sugar yields describe its effects from all three independent variables. Moreover interactive effects were also seen in both numerical and graphical analysis. Lignin showed responses from substrate concentration. Although little effects were observed from both time and enzyme loading ratio. Ash showed linear effects from all three independent variables. Hence the future scope of this study could show a path towards sustainable, renewable and pollution free energy source.

5. References

1. Arvanitoyannis IS, Kassaveti A. Current and potential uses of composted olive oil waste. International Journal of Food Science and Technology, 2007a; 42(3):281-295. doi: 10.1111/j.1365-2621.2006.01211.
2. Ates F, Putun E, Putun AE. Fast pyrolysis of sesame stalk: yields and structural analysis of bio-oil. Journal of Analytical and Applied Pyrolysis. 2004; 71:779-790.
3. Ayse E, Putun E, Apaydın, Ersan Putun. Rice straw as a bio-oil source via pyrolysis and steam pyrolysis. Energy. 2004; 29:2171-2180.
4. Abdullah N, Gerhauser H. Bio-oil derived from empty fruit bunches. Fuel. 2008; 87:2606-2613.
5. Baig MMV, Baig MLB, Baig MIA, Yasmeen Majeda. Saccharification of banana agro-waste by cellulolytic enzymes. African Journal of Biotechnology. 2004; 3(9):447-450.
6. Bak Jin Seop, Ja Kyon Ko, Young Hwan Han, Byung Cheol Lee, In-Geol Choi, KyoungHeon Kim. Improved enzymatic hydrolysis yield of rice straw using electron beam irradiation pretreatment. Bioresource Technology. 2008; 100:1285-1290.
7. Baratieri M, Baggio P, Fiori L, Grigiante M. Biomass as an energy source: Thermodynamic constraints on the performance of the conversion process. Bioresource Technology. 2008; 99:7063-7073.
8. Bezerra MA, Santelli ER, Oliviera PE, Villar LS, Escaliera AE.RSM as a volumetric tool for optimization in analytical chemistry. Talanta. 2008; 76:965-977.
9. BinodParameswaran, Raveendran Sindhu, Reeta Rani Singhania, Surender Vikram, Lalitha Devi, Satya Nagalakshmi. Bioethanol production from rice straw: An overview. Bioresource Technology. 2010; 101:4767-4774.
10. Mussatto SI, Roberto IC. Optimal experimental condition for hemicellulosic hydrolyzate treatment with activated charcoal for xylitol production. Biotechnology Progress, 2004; 20:134–139.
11. Maiorella BL. Ethanol. In: Moo-Young, M. (Ed.), Comprehensive Biotechnology. Pergamon Press, Oxford, 1985; 861–914.
12. Mani SC. Status Paper on Rice in Uttarakhand. Rice Knowledge Management Portal (RKMP). Directorate of Rice Research Hyderabad, 2014.
13. Sharma A. Optimisation of fermentation parameters an blend ratio for the development of under-utilized crops based idli batter: Thesis M.Tech GBPUAT Pantnagar, 2014, 198.
14. Ververis CK, Georghiou D, Danielidis DG, Hatzinikolaou P, Santas R, Santas V. Corleti. Cellulose, hemicelluloses, lignin and ash content of some organic materials and their suitability for use as paper pulp supplements, Bioresource Technology, 2007; 98:296-301.
15. Wani Tanveer A, Ajaz Ahmad, Seema Zargar, Nasr Y KhalilandIbrahim, Darwish A. Use of response surface methodology for development of new microwell-based spectrophotometric method for determination of atrovastatin calcium in tablets. Chemistry Central Journal. 2012; 6:134.
16. Zanariah CW, Ngah CW, Yahya MA. Optimisation of digestion method for determination of arsenic in shrimp paste sample using atomic absorption spectrometry. Food Chem. 2012; 134(4):2406-10.
17. ZhengJi-lu. Bio-oil from fast pyrolysis of rice husk: Yields and related properties and improvement of the pyrolysis system. Journal of Analytic and Applied Pyrolysis. 2007; 80(1):30-35.
18. Zhang Qiu Zhuo, Wei Min Cai. Enzymatic hydrolysis of alkali-pretreated rice straw by Trichoderma reesei ZM4-F3. Biomass and bioenergy. 2008; 32:1130-1135.
19. Zhang YHP, Ding SY, Mielenz JR, Cui JB, Elander RT, Laser M. Fractionating recalcitrant lignocellulose at modest reaction conditions. Biotechnology and Bioengineering. 2007; 97:214-223.