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Research Article

OPTIMIZING LIGNOCELLULOSES SACCURIFICATION FOR BIOETHANOL PRODUCTION

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ABSTRACT
The Influence of the main pretreatment variables on fermentable sugar generation from walnut green skin is studied by using design of experiment based on concentrated acid hydrolysis. Levels for pretreatment temperature (65, 80, 90^{o}C), process time (120, 180, 240 minute), solid content (5, 10, 15%) and concentration of sulfuric acid (20, 0, 60%) were selected according to previous results. Glucose and pentose composition, as well as furfural and acetic acid were analyzed by HPLC and modeled by a quadratic equation. Mathematical model was validated by independent experiment. Optimization based on mathematical model show that maximum Glucose concentration was 12.724% in 90^{o}C, 36% acid concentration, 15% solid content and 175 min process time.

Keyword: *Walnut Green Skin, Fermentable Sugar, Hydrolysis, Pretreatment, Bioethanol*

INTRODUCTION
Finding new biomass for producing ethanol is not stopped, because this biomass is the most abundant and attractive renewable resource in many countries for the production of ethanol and it does not compete with animal feed and food industry. One of this biomass is green Walnut skin.
Walnut is one of the most important nuts that culture widely around the entire world. In the year 2007, total production of this nut was forecast at a record of 1700000 tons. China (503000 tons), USA (209000 tons), Turkey (184000 tons) and Iran (170000 tons) are its important producers. Walnut consists of 3 main parts, meat, woody skin and green skin.
Green skin consists of cellulose, hemicelluloses and lignin. The carbohydrate polymers in the green skin need to be converted to simple sugars before fermentation through a process called hydrolysis. Hydrolysis (saccharification) breaks down the hydrogen bonds in the hemicelluloses and cellulose fraction into their sugar components, pentose and hexose (Demirbas, 2005). The reaction can be catalyzed by concentration acid.Further decompositions occur during these process conditions yielding other unexpected compounds such as Furfural from pentose and Acetic acid from acetyl groups in hemicellulose. For the fermentation process, the presence of these materials in hydrolysates can hinder or prevent a subsequent fermentation step.
Time, acid concentration, biomass content and temperature are four variables that effect on sugars and inhibitor production. The effect of pretreatment on hydrolysate composition was modeled by response surface methodology by some authors for optimizing of acid pretreatment. Castru (2011) used this model to optimize dilute acid pretreatment of rapeseed straw. Similar optimizing was used by Jeong *et al.*, (2010) for extraction of hemicelluloses. Zhou *et al.*, (2009) used similar investigate for efficient hydrolysis of steam exploded of corn Stover. Optimization of H2SO4- catalyzed hydrothermal pretreatment of rapeseed straw was other research that was done by Xuebin lu and his colleague in 2009 (Luo *et al.*, 2011). The surfaces generated by models can be used to find the optimal conditions (Bezerra *et al.*, 2008).
For using a surface modeling a quadratic equation was used to evaluate the effect of these four variables. A large number of experimental designs adapted to various types' problems are available such as factorial designs (Box and Hunter, 1978). Centriod composite matrices (Atkinson, 1992) or doehlert shells (Khuri & Cornell, 1978; Doehlert, 1970). But, since concentrate of sulfuric acid was high (about 20 to 60%) and process time was long (about 120 to 240 minute) a simple method was required like Taqushi method. Four variables should be studied in 3 levels then L-9 matrix of Taqushi could be deigned. But at least

fifteen parameters should be determinedand fifteen run must be done to solve a quadratic equation with four variables. Then another L-9 Matrix in reveres level was combined with first one and run.

MATERIALS AND METHODS

Methods

Raw Material

Walnut green skin (WGS) was gathered in September of 2009 from Spidan village from North Khorasan in Iran. It was washed by distillated water, air dried, milled using vibratory disc mill (Retsch RS 100) to particle size smaller than 50 micrometers and stored in sealed plastic bags at room temperature. For the determination of the chemical composition of the WGS, preparation of the test specimens was carried out according to TAPPI T 257 om (1985) standard. Extracted materials, lignin and ash contents were determined according to TAPPI standards T 204 om (1988), T 222 om (1988), T 211 om (1988) standards, respectively. The hemicellulose and cellulose contents were determined according to WiseÕs chlorite and K.rschner-Hoffner nitric acid methods. The resulted composition of is shown in Table 1.

Table 1: The composition of WGS

Composition	Percent dry weight
Cellulose	21.5
Hemicellulose	13.25
Extractive M	18.25
Lignin	26.07
Ash	20.93

Experimental Design

Table 2: Experimental design, Experimental factor and code levels

Run	Acid concentrate. (code)	Temper -ature (code)	Solid content(c ode)	Time(c ode)	Acid concentrat. (%)	Tempera -ture (^oC)	Solid content (%)	Time(min)
1	1	1	1	1	20	65	5	120
2	1	2	2	2	20	80	10	180
3	1	3	3	3	20	90	15	240
4	1	3	1	2	20	90	5	180
5	1	2	3	1	20	80	15	120
6	1	1	2	3	20	65	10	240
7	2	1	2	3	40	65	10	240
8	2	2	3	1	40	80	15	120
9	2	3	1	2	40	90	5	180
10	2	3	2	1	40	90	10	120
11	2	2	1	3	40	80	5	240
12	2	1	3	2	40	65	15	180
13	3	1	3	2	60	65	15	180
14	3	2	1	3	60	80	5	240
15	3	3	2	1	60	90	10	120
16	3	3	3	3	60	90	15	240
17	3	2	2	2	60	80	10	190
18	3	1	1	1	60	65	5	120
sum	36	36	36	36	----	----	----	-----

Research Article

Concentrated acid hydrolysis was used to hydrolyze the WGS. The influence of temperature, acid concentration, reaction time and solid contents on fermentable sugars production was studied. Results were formulated by using quadratic equation:

$$Y = A_0 + A_1.C + A_2.T + A_3.S + A_4.t + A_5.C^2 + A_6.T^2 + A_7.S^2 + A_8.t^2 + A_9.C.T + A_{10}.C.S + A_{11}.C.t + A_{12}.T.S + A_{13}.T.t + A_{14}.S.t \quad (1)$$

This equation allows influence of each factor on responses as well as interactions among factors to be determined. This equation has 15 constant coefficients and at least 15equationswere needed to determine these coefficients by least square methods. Based on previous experience with concentrated acid hydrolysis to ensure a broad range of response, three levels for each factor were considered and walnut green skin was pretreated at 18 different operational conditions according to combined Taguchi matrix. Selected conditions were shown in Table 2. Two extra experiments were run to verify the model validity. Every experiment was run for three times and nearest results was averaged.

Concentrated Acid Hydrolysis

Dried WGS was treated with 20, 40, 60 wt% sulfuric acid in screw-capped laboratory bottles (Pyrex bottles) in hot water jacket with an electric heater and temperature controller(Figure 1) at 65, 80, 90^{o}C for 2, 3, 4 hours with agitation by a laboratory mixer. A distiller was used to prevent water evaporation. Solid to liquid ratio of 5%, 10%, 15% was applied. Once the temperature of reaction mixture reached to designed point, pretreatment time was started. At the end of each run the bottle was removed from heating jacket and put in a cool water bath., sodium hydroxide (NaOH) was used until its PH reached around 7.0 and then solids were separated by filtering, washed with distilled water and final solution reached to 1000 ml. A 200 ml sample of solution was used to analyze by HPLC.

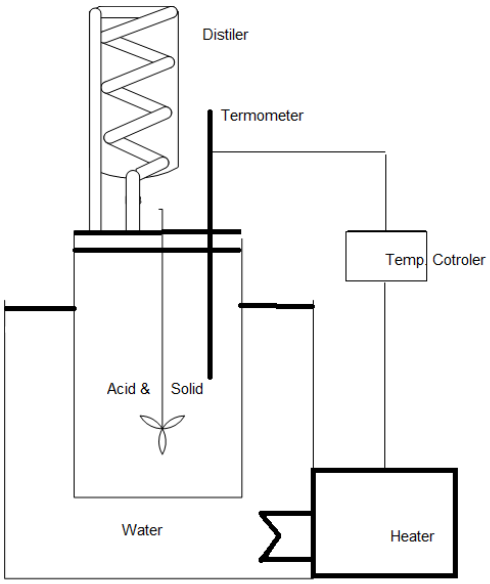

Figure 1: Experimental Setup of Hydrolysis reactor

Analytical Methods

The composition of the hydrolyzate from acid hydrolysis (Glucose, Xylose, Mannose, Arabinose, Galactose, Furfural and Acetic acid) was determined by high performance liquid chromatography (HPLC). An HPLC model JASCO was used. Glucose, Xylose, Galactose, Mannose and Arabinose were analyzed by Bio-Rad column Aminex HPX-87P and detected by RI detector at 40^{o}C and Acetic acid and Furfural analyze by Bio-Rad column Aminex HPX-87H and detected by UV detector at 210nm. To unify the response all results was divided on raw material content (gr WGS) and results were shown as percentage of gr product/gr rawmaterial (e.g. 2 % gr glucose/gr raw material).

RESULTS AND DISCUSSION

The composition of hydrolysates in terms of fermentable sugars and inhibitors is summarized in Table 3.

Table 3: Composition of liquied phase (hydroysate) after concentrate acid pretreatment of WGS

Run	Glucose (%)	Pentose (%)	Furfural (%)	Acetic acid (%)
1	2.5	0.28	0.35	2.3
2	5.6	1.99	1.9	4.3
3	4.7	1.4	1.6	3.4
4	4.9	1.72	1.5	3.7
5	5.7	0.7	2.1	4.2
6	8.5	2.9	3.5	6.6
7	6.7	2.1	0.7	5.8
8	4.4	1.5	0.5	4.9
9	7.4	1.6	0.8	6.0
10	6.7	1.8	0.3	5.1
11	7.9	0.8	0.9	5.6
12	6.7	1.7	0.5	5.6
13	*0*	*0*	*0*	2.5
14	*1.2*	*0*	*0*	3.1
15	*0.5*	*0.14*	*0.49*	4.85
16	*1.6*	*0*	*0.6*	3.6
17	*1.7*	*0.24*	*0.13*	4.4
18	*4.4*	*1.6*	*1.9*	6.6

Mathematical Model, Factors and Responses

The study of pretreatment performance by concentrated acid was addressed by performing the experimental design in which process temperature (T, ^{o}C), process time (t, min), solid content (S, %) and acid concentration(C, %), as detailed before, were retained as factors and glucose concentration (Gl, %), pentose concentration (P,%), furfural concentration (F, %) and acetic acid concentration (Ac, %) were considered as response (Y).

Table 4: Coefficient of mathematical model Eq.(1)

	Glucose	Xylose	Furfural	Acetic acid
A_0	-25.93	-2.757	-6.286	-1.045
A_1	0.7059	0.177	-0.0596	0.26786
A_2	0	0	0	0
A_3	-4.23	-0.809	-1.763	-0.5010
A_4	0.399	0.03867	0.1663	-0.0158
A_5	-0.00915	-0.0019	0.0017	-0.0035
A_6	0.0027	0.00025	0.000526	0.000752
A_7	0.0905	0.0108	0.03365	0.01876
A_8	-0.0005	0.00004	-0.00023	0.000143
A_9	0.000156	-0.00037	-0.00029	0.000387
A_{10}	-0.001153	-0.00086	-0.00127	-.00126
A_{11}	-0.00047	-0.000068	-0.000838	-0.000015
A_{12}	0.03045	0.00871	0.01381	0.000673
A_{13}	-0.00236	-0.00054	-0.00095	-0.0004
A_{14}	0.000569	0.00028	0.000553	0.0011

The static interpretation of results was formulated by equation 1, which allows the influence of each factor on the response as well as interactions among factors to be determined, according to constant coefficients A_i (Castro *et al.*, 2011; Caqueret *et al.*, 2008). Table 4 summarizes the model constant coefficients obtained from ANOVA table for different responses. Figure 2 shows a good agreement between predicted and experimental values.

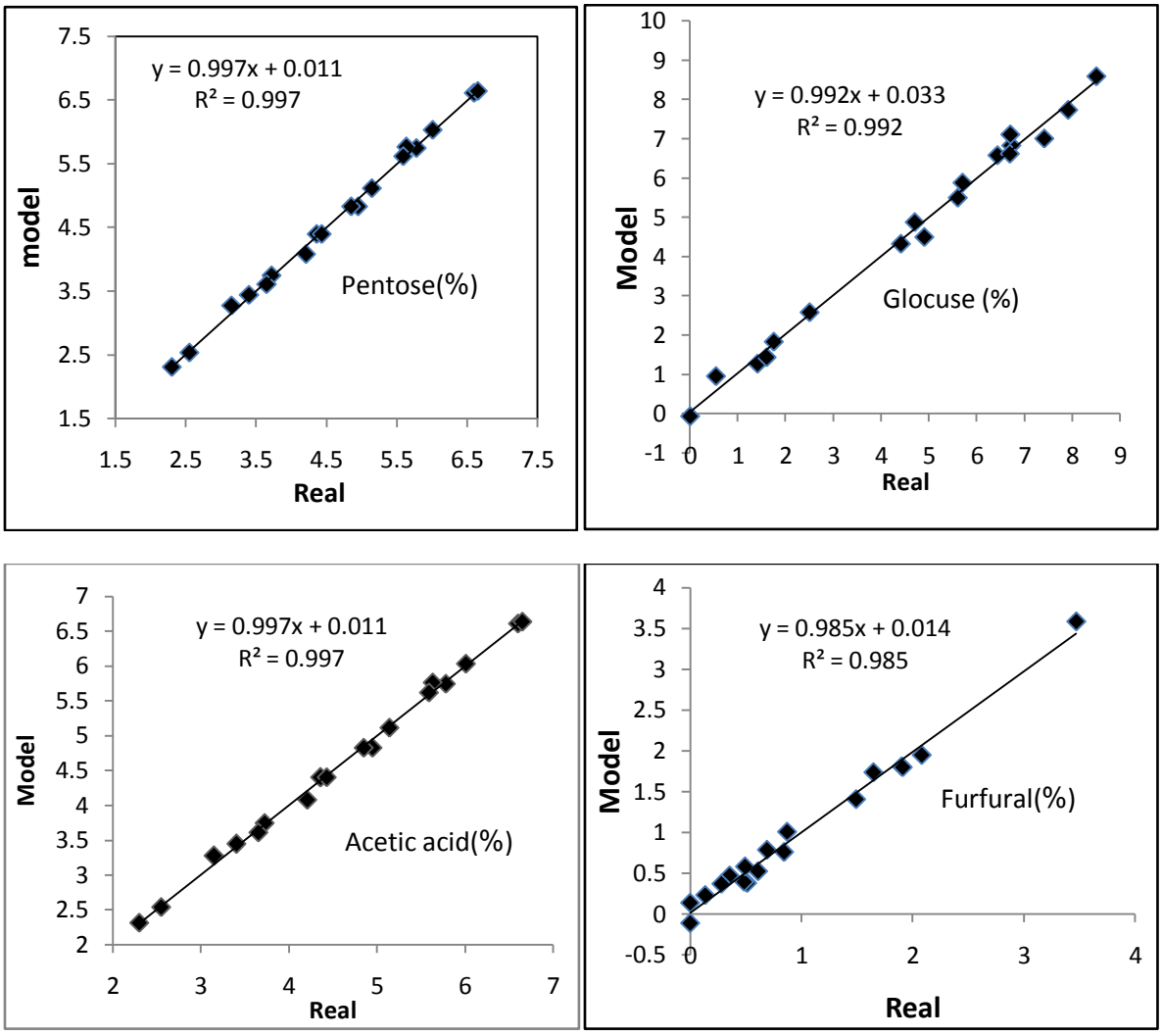

Figure 2: Predicted versus experimental values for liquid phase after pretreatment

Using the Model to Interpret the Effect of Hydrolysisconditions

The influence of temperature (T) and acid sulfuric concentration (C) in glucose yield is depicted in fig. 3. As can be seen in this figure the influence of acid concentration differs from the influence of temperature on glucose production. Surface shown in Fig. 3 shows that the maximum point is outside of the experimental region and that it is necessary to displace the experimental range to attain it. While acid concentration was a determining factor for glucose production, glucose concentration increases with temperature very slightly in studied range. At low levels of both temperature and acid WGS remained almost untreated. Glucose concentration rise with acid concentration, reached to maximum in medium and decrease in high level of acid. In constant condition (acid concentration 40%, solid content 8.2% and process time 167 min) the glucose concentration increase 11% from minimum temperature (65 ^{o}C) to medium (80^{o}C), this increase repeat from 80^{o}C to 95^{o}C (5.88, 6.56 and 7.33% respectively). In constant

condition (process temperature 80°C, solid content 8.2%, and process time 167 min) when acid concentration rise from 20% to 40%, glucose yield increase 44% but in the second increase glucose concentration decrease 65% from acid concentration 40% to 60% (4.54%, 6.56% and 1.26% respectively). Glucose concentration in 6th run was the maximum value 3.5% and in 13th run was the minimum (0%).

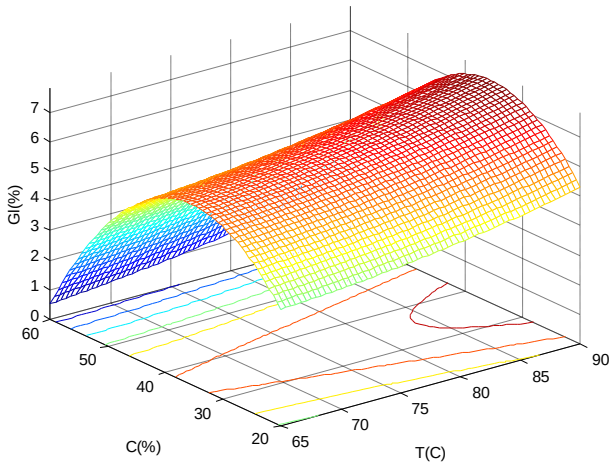

Figure 3: Response surface for glucose recovery in liquid phase as a function of acid concentration and temperature according to the model

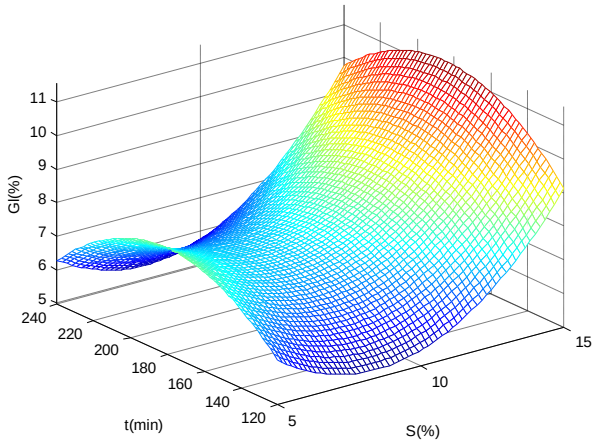

Figure 4: Response surface for glucose recovery in liquid phase as a function of solid content and time according to the model

The surface shown in Figure 4 presents a saddle point as a critical point. The saddle point is an inflexion point between a relative maximum and a relative minimum. As can be conducted from fig. 4, in studied reaction time in constant condition from 120 min to 180 min, glucose concentration increase 37% but in next step, from 180 min to 240 min decrease 24% (5.24, 7.18 and 5.42% respectively). It means that glucose degraded during time. While solid content increase from 5% to 10% glucose concentration decrease 10%, an increase about 51% visible when solid content reached to 15 % (8.05, 7.33 and 11.13 respectively).

Similar analysis could be down for pentose, but it was preferred to address furfural. Furfural in GWS hydrolysis is a byproduct of xylan decomposition. Fig. 5 and fig.6 show result of model for furfural. In studied ranges, both of temperature and acid concentration increased furfural concentration. In constant condition (acid concentration 35%, solid content 8.2% and process time 167 min), from minimum

temperature (65°C) to medium (80°C) furfural concentration increase 71% and this increase become 44% in the next range, 80°C to90°C(0.438, 0.75 and 1.08% respectively). In the constant condition temperature 80°C, solid content 8.2% and process time 167 min, while acid concentration rise from 20% to 40%, furfural concentration decrease from 1.525% to 0.58% (94% lost), this value reached to 14% decrease when acid concentration change from 40 to 60% (furfural concentration 0.58 and 0.44% respectively).

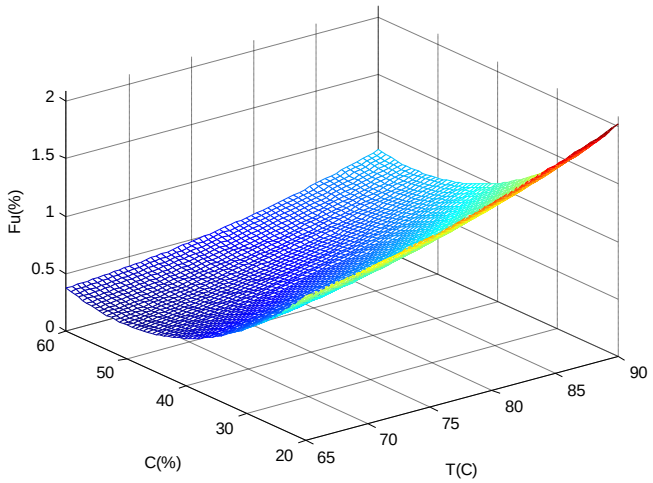

Figure 5: Response surface for furfural production in liquid phase as a function of acid concentration and temperature according to the model

In figure 6, it can be seen that in constant condition, glucose concentration increase from 120 to 180 min and 180 to 240 minute. It means that xylan decompose during hydrolysis process to its byproducts. The surprising results was seen when solid content change from 5 to 10% in constant condition. In this step, furfural concentration decrease 10% and then increase from 10% to 15% about 147% (furfural concentration 1.178, 1.05 and 2.6 %, respectively).

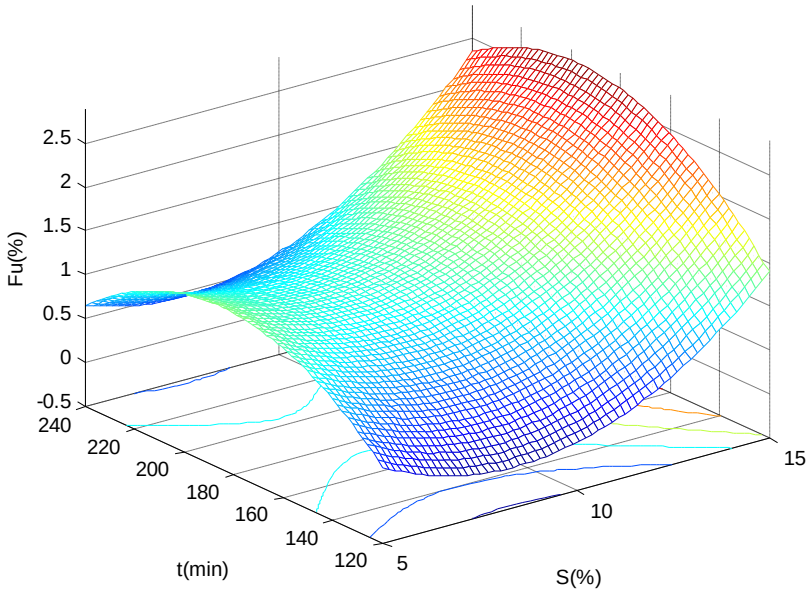

Figure 6: Response surface for furfural production in liquid phase as a function of solid content and time according to the model

Evaluation Model Validity

To evaluate model validity, tow different experiment was designed. Experiment and results were shown in table 5. As can be seen in this table, the model could be a good model when error was less than 5% except one for furfural.

Table 5: Result of model check

Acid concentration (%)	Temperature (^oC)	Solid content (%)	Time (min)	Comparison	Glucose (%)	Furfural (%)	Acetic acid (%)	Pentose (%)
40	80	10	180	Model	6.71	0.725	4.92	1.39
				Real	6.99	0.437	4.81	1.35
40	90	15	240	Model	10.11	2.28	8.02	3.04
				Real	10.62	2.26	8.01	3.04

Model Optimization

The mathematical model that was developed from experimental results is able to predict the operational conditions that should be used in pretreatment to optimize model response. It is possible to calculate the optimal point through the first derivate of the mathematical function. It is necessary to solve the four first grade system formed to find best condition. Table 6 summarizesthese conditions. Best condition was maximum glucose with minimum inhibitors. In optimize condition, mathematical model calculate condition to reach maximum glucose. Other extractive material was calculated in these conditions.

Table 6: Optimized pretreatment conditions according to mathematical model

Acid concentration (%)	Temperature (^oC)	Solid content (%)	Reaction time (min)	Glucose (%)	Furfural	Acetic acid (%)	Pentose (%)
36	90	15	175	12.72	3.178	6.44	2.92

Conclusion

This work confirm that walnut green skin can be considered as a suitable feed stock for sugar generation as a first step toward fuel ethanol production. Concentrated acid hydrolysis helps us to reach the fermentable sugar in normal conditions. This process could be model by a four variable quadratic equation. These four variables were temperature, acid concentration, solid content and process time. Model allows adjusting these variables to reaches optimum condition for maximum glucose concentrations. This conditions base on model was acid concentration 36%, solid content 15%, temperature 90^{o}C and process time 175 minute. In this condition glucose concentration reached to 12.72%. Whereas optimum condition for solid content and temperature were in upper point of experimental selected range, it is possible to reach to higher glucose concentration by change in these variables.

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