

**Comperatively Investigation Pretreatments of H<sub>2</sub>SO<sub>4</sub> and Ultrasound Effect for  
Corncob to Increase Available Bioenergy Potential by Response Surface  
Methodology**

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**ABSTRACT**

Bioenergy is a sort of renewable energy source, such as bioenergy, bioethanol, biogas or bioethanol, and it has widespread application in the world. The main purpose of this work is to optimize the physicochemical processes of sulfuric acid and ultrasound + sulfuric acid pretreatment application to get more energy per unit mass from the corncob. In order to optimize the best reaction conditions, a statistical experimental design method which is Box Behnken was used. The parameters such as acid concentration, temperature, reaction time, ultrasound power were selected as the factors to be optimized. The solid concentration was fixed at 1% and the particle mesh size was fixed at 0.2. In acid pretreatment, the highest total sugar concentration according to the statistical software model is 5.05 g/L in 4.74% acid concentration, 100 °C temperature and 117 min reaction time. If the concentration of reducing sugar is desired to be maximum in the acid pretreatment, a concentration of 5% acid, 80°C temperature and a reaction time of 180 minutes can be obtained. In ultrasound supported acid pretreatment application, while total sugar concentration rise up to 22%, reduced sugar concentration increased up to 70%. Finally it could be said that ultrasound assisted acid pretreatment has much more effective than the solely acid pretreatment.

**Keywords:** Corncob, renewable energy, RSM.

**INTRODUCTION**

It's expected that, in a sort time, fossil fuels have been shifted intensively to renewable sources. The unsolvable disadvantages of the fossil fuels such as energy crises, global environmental problems, fluctuation of the oil price and energy security issues enforced the countries to choose and investigate renewable energy sources which have to be appropriate the countries natural sources. Especially alternative renewable sources like biomass and biofuels from biomass have been attractive day by day (EBA, 2017).

Biomass have organic content, biological basis, unlike fossil fuels, which main composition is carbon compounds. Additionally, biomass is well known renewable source. Liquid and gas phase biofuels to produce electricity, thermal power and other some kind of chemicals as by product have been produced from biomass and other organic wastes. That's why developed and under developed countries prefer the biomass sources in a large scale (Koçar et. al., 2013).

It is suppose that the 805 of energy consumption is dependent on the fossil fuels. This

situation has caused not only the climate change but also devastate the natural energy sources. Therefore almost all countries interested in clear, ecofriendly and renewable energy sources. According to international energy agency (IEA) 14% of the worlds' total energy need was met with the biomass energy in 1998 (Adıgüzel, 2013).

Turkey was taken a decision about biofuel production by Turkey Energy Market Regulatory Authority (EMRA) at 29.07.2011 with a regulation. According to regulation, biofuel, which produced from domestic agricultural products, must be added to fuels at least 2% after 1 January 2013 and must be added at least 3% to fuels after 1 January 2013. This produced biofuels has no tax (private consumption tax - OTV) obligation.

It was suggested that if the mixing ratio of the produced bioethanol to gasoline is 3% in Turkey, the cost of petroleum import can decrease to 385 billion dollars. If the mixing ratio of produced bioethanol to gasoline is also 5%, the expenditure for petroleum import can decrease to 596 billion dollars (Polat et. al. 2009). If small or medium scale biofuel facility will set up around Niğde region, this can be contributed to economy of country. According to the data of energy ministry, Turkey consumed three times higher energy than the produced energy and 70 % of consumed energy was imported. Therefore, renewable energy sources such as biomass could be used for producing electric and thermal energy and it is attractive option.

In energy market, using of renewable raw material have been get more and more important day by day. Because of more popularity of the sustainability concept, main target is zero CO<sub>2</sub> emission for electricity production, travelling and heating. To access of main target, even if other renewable energy sources such as the sun and wind are popular today, it is problem that waiting for solution because of fluctuating according to the hours of the day and electricity production is not to be constant. For renewable energy, agricultural biomass and also animal waste is the most reliable. For example, The United States National Research Council aims to convert 10% of liquid fuels and 50% of organic chemicals into renewable biomass in 2020.

Turkey's renewable energy consuming ratio in energy consumption is 13% between 2000 and 2013, these ratios fluctuated about 9-10% between 2004 and 2013. As share of biomass energy in energy production was 14 % in 1990, these share dropped back 3% in 2012, because total energy consumption was increased enormously (Akçiçek, 2015).

Biomass includes various kind of source such as agricultural waste, wood industry wastes, animal livestock waste or agro-industrial wastes. Biomass cannot utilize directly microorganisms and it has extremely low degradability, due to the heterogeneity and crystallinity properties of the lignocellulosic structure in biomass (Datara et. al. 2007). Consequently there is a need to improve pre-treatment process on lignocellulosic structure to convert it into a usable structure for microorganisms. In this study, due to increase production efficiency in biofuel production and be able to use microbial conversion technique, experiments were carried out chemical pretreatment (H<sub>2</sub>SO<sub>4</sub>). Corncob is valuable agricultural waste, whose content is including 30-35 % hemicellulose, 40-45 % cellulose and 10-20 % lignin. Converting this agricultural waste, which has a very low economic value, to a value-added product such as biofuels is more important as a renewable resource (Sheng & Marquis, 2006). However, due to its lignin content, it is

very difficult to be converted into fermentable sugar. Thus it must be applied either chemical or biological pretreatment (Mosiera et. al., 2005).

Experimental design methods using in experimental studies is very effective method to find factors and their effect way and also to make the optimization of parameters. The Box–Behnken experimental method is a three-level fractional factorial design and it used to determine the effect of factors in the experimental zone. This design is a combination of a two-level factorial design with an incomplete block design. In this study, it was used Box-Behnken experimental design for optimization pretreatment. In this study, acid pretreatment was applied corn cob by Box-Behnken.

## MATERIALS AND METHODS

The raw material of Corncob was collected from local market and air dried. After chipping and rendering, corncob was sieved by a 0.2 mesh screen and stored for future use at + 4 °C.

Box-Behnken experimental method used to optimize the factors which are time, temperature and acid ratio for the response of total sugar concentration and reduced sugar concentration. The independent variables, levels and their values are shown in Table 1. and 5. center point added to experimental design in without Ultrasound experiments. Table 2. Indicate the experimental design setup for ultrasound experiments. All experiments were made with 2 replicate and all analyses was made in 3 replicated. The ranges of factors were selected based on previous experiences.

**Table 1.** Independent variables of design, coded view and values of factors without US

STD order	Acid %	Temperature °C	Time (min)	Acid %	Temperature °C	Time (min)
1	-1	-1	0	1	50	105
2	+1	-1	0	5	50	105
3	-1	+1	0	1	100	105
4	+1	+1	0	5	100	105
5	-1	0	-1	1	75	30
6	+1	0	-1	5	75	30
7	-1	0	+1	1	75	180
8	+1	0	+1	5	75	180
9	0	-1	-1	3	50	30
10	0	+1	-1	3	100	30
11	0	-1	+1	3	50	180
12	0	+1	+1	3	100	180
13	0	0	0	3	75	105
14	0	0	0	3	75	105
15	0	0	0	3	75	105
16	0	0	0	3	75	105
17	0	0	0	3	75	105

In experiments working volume of the experiment was 250 ml in glass bottle. Initial solid concentration is 1%. Ultrasound experiments made with temperature controlled shaker at 200 rpm. Ultrasounds experiments made with Hielscher UP400S. All samples firstly

filtered through filter paper and then centrifuged with 10.000 rpm at 10 minute, finally supernatant used for the total sugar and reduced sugar analyzed. For total sugar amount was calculated according to approach of Dubois, 1956 (Dubois et. al., 1956) and reduces sugar concentration was determinated using with Dinitrosalisilik acid (DNS) method (Miller, 1959). Design expert software (trial version) was used for experimental design.

**Table 2.** Independent variables of design, coded view and values of factors with US

STD order	US Kj/kgDM	Acid %	Time (min)	US Kj/kgDM	Acid %	Time (min)
1	-1	-1	0	100	1	17,5
2	+1	-1	0	60	3	17,5
3	-1	+1	0	60	3	17,5
4	+1	+1	0	100	5	17,5
5	-1	0	-1	60	3	17,5
6	+1	0	-1	100	3	5
7	-1	0	+1	20	3	30
8	+1	0	+1	60	3	17,5
9	0	-1	-1	20	3	5
10	0	+1	-1	20	5	17,5
11	0	-1	+1	20	1	17,5
12	0	+1	+1	60	5	30
13	0	0	0	60	3	17,5
14	0	0	0	60	1	30
15	0	0	0	60	1	5
16	0	0	0	60	5	5
17	0	0	0	100	3	30

## RESULT and DISCUSSION

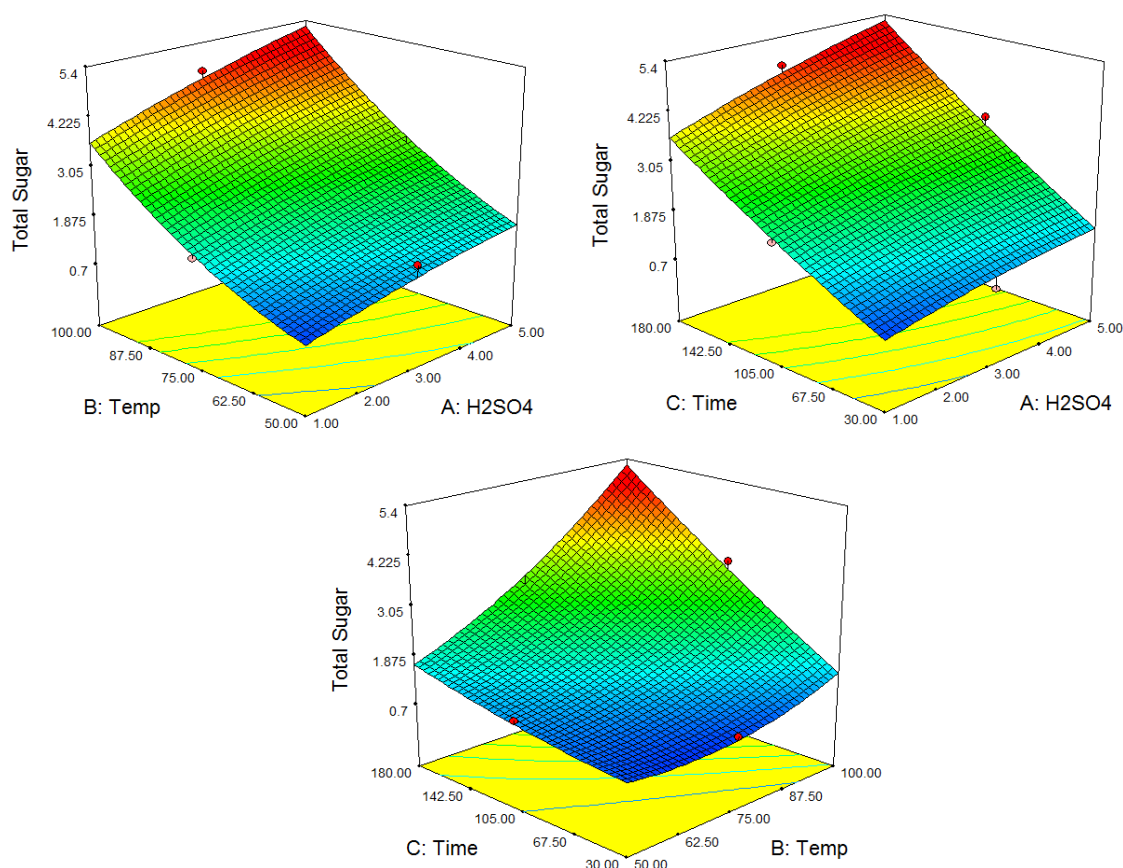
Box-Behnken experimental design method was used to optimize the factors of acid ratio, temperature and time to find maximize for total sugar concentrations from the corn cob. Initial solid concentration of corn cob is 1% (w/v). High level, low level of the factors and the response of the total sugar concentrations was shown at the Table 3.

### *Acid Pretreatment*

From the software, Box Behnken model is significant according to ANNOVA test and value of the model is 0.95. Maximum conversation ratio is about 47% according to experimental model.

As Figure 1a it is clear that the maximum total sugar concentration is 4.71 g/L when the temperature is 100 °C and H<sub>2</sub>SO<sub>4</sub> ratio is 5%. As shown in Figure while both temperature and acid ratio decrease total sugar concentration decline to 0.48 g/L from 4.71 g/L. According the Figure1a, the effect of temperature is more significant than the acid ratio change. Figure 1b show the effect of both time and acid ratio. As Figure 1a maximum total sugar concentration is 4.71 g/L when the time is 180 min and acid ratio in high level. The factor of time has clearly significant effect on the total sugar production. Interaction effect of both time and temperature effect is clearly high as Figure 1c. The graph shows that

exponential increase of total sugar concentration up to 4.71 g/L Brodeur *et.al* (2011) reported that if the lignocellulosic content is lower than the corn cab it's probably the low level of temperature is enough to degradation. Produced total sugar amount is linked with the lignocellulosic content. From Barışık *et.al.* (2016) the effect acid is enlarge when the temperature get higher. According to report from Barışık *et.al.* (2016) pretreatment was applied to straw samples with Box-Behnken method. The optimum process conditions for maleic acid were, 210°C, 1.08% acid concentration and 19.8 min; for succinic acid 210 °C, 5% acid concentration and 30 min; for oxalic acid 210 °C, 3.6% acid concentration and 16.3 min. The ethanol concentrations obtained at optimum conditions were 12.9, 10.3 and 12.9 g/L for maleic, succinic and oxalic acid pretreatments, respectively. These results indicate that, organic acids decomposition of lignocellulosic structure is effectively. On the other hand, maleic and oxalic acids show to be more effective compared to succinic acid. In this study which it was H<sub>2</sub>SO<sub>4</sub> pretreatment and corn cob as substrate, optimum temperature 100°C was determined which was the highest temperature of experimental value. According design, model is significant and R<sup>2</sup> value is 0.88 for reduced sugar production. Reduced sugar concentration can reach up to maximum 2.1 g/L. The independent variables values of the maximum reduced sugar concentration is similar with the conditions of maximum total sugar concentration, which are 5% acid ratio, 180 minute and 100 °C.

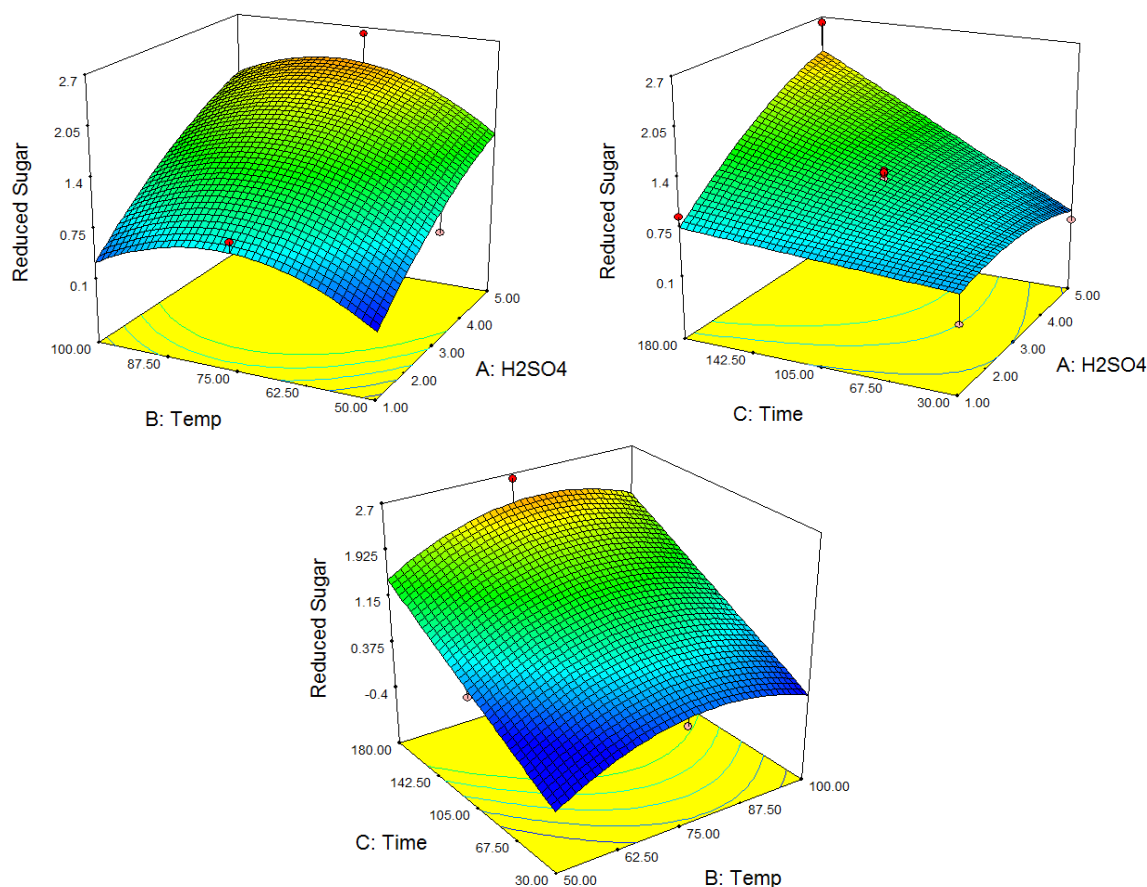


**Figure 1.** Response graphichs of total sugar (g/L) for box behinkein experimental design a) temperature (°C) and acid ratio (%), b) time (min) and acid ratio (%) c) time (min) and temperature (°C) interactions.

In order to effective pretreatment for acid pretreatment, it requires high temperature and pressure and also dilute acid pretreatment is the most effective process for lignocelluloses. End of this process, lower degradation products is generated than concentrated acid pretreatments (Kumar et. al., 2009). The report from Saha et. al was indicate that the effects of temperature (140, 160 and 180 °C) on dilute H<sub>2</sub>SO<sub>4</sub> (0.00, 0.25 and 0.50%, v/v) pretreatment of wheat straw for 15 min and subsequent enzymatic saccharification (45 °C, pH 5.0, 72 h) were evaluated. Dilute acid (0.5%, v/v) pretreatment at 180 °C for 15 min and after enzymatic saccharification using the commercial cellulase and a β-glucosidase preparation produce 576 mg total sugars per gram dry solid of wheat straw (75% yield) (Saha et. al., 2005).

From the graphichs acid ratio is significantly effected the reduced sugar concentration as Figure 2a. Chemical pretreatment has become one of the most promising methods to improve the biodegradability of cellulose by removing lignin and/or hemicelluloses, and to decrease the degree of polymerization (DP) and crystallinity of the cellulosic component in lignocelluloses (Mtui, 2009; Agbor et. al., 2011) generally dilute acids concentration are ranging from 0.1% to 2% (w/v) in the acid pretreatment methods. Gütsch et. al. (2012) were studied three different acids (acetic (0.02–0.15 M), oxalic (0.01–0.1 M) and sulfuric acid (0.01–0.1 M)) for their catalytic activity during the pretreatment of Eucalyptus globulus wood. In this study, the reactor was heated up to prehydrolysis temperature (120–200 °C) within minimum heating time (approximately 30 min), the temperature was maintained for the prehydrolysis time (10–120 min). Result of this study, conversion ratio was up to 95 % in experiment condition for Eucalyptus globulus wood. Yu et al. (2012) indicate that a total xylose recovery of 79.6% for sweet sorghum bagasse in a step-change flow rate reactor (184 °C, 20 ml/min, 8 min, and 10 ml/min, 10 min) and a total xylose recovery of 84.4% for eucalyptus wood chips in the batch stirred reactor (184 °C, 5% w/v, 18 min). Another study from Wang et al. (2009) reported up to 40% of cellulose conversion, when spruce wood chips were used for pretreatment at 180 °C with an acid concentration of 1.84% followed by disk milling. Though, acid methods is less attractive due to the formation of inhibitory compounds, equipment corrosion, toxic nature, and high operational and maintenance costs. In this study, conversion ratio was determined as 47% for total sugar and % 21 for reduced sugar which maximize condition was 5 % H<sub>2</sub>SO<sub>4</sub> and 100 °C in 180 min.

From the Figure 2b, acid ratio is not significantly effect the reduced sugar concentration solely, but on the other hand when acid ratio was analysed with the factor of temperature and time reduced sugar production was obviously changed in positive way. From the Figure 2c, interaction with time and temperature factors are clearly important to reduces sugar concentration. Highest reduced sugar amount of about 2.0 g/L was observed at the mid point of temperature which is 75 °C and at 180 min reaction time. Numerical optimization of model for reduced sugar production indicate that to maximize the reduced sugar production, which is 2.218 g/L, the factors of temperature, acid ratio and time value have to be 75 °C, 5% and 180 min respectively.



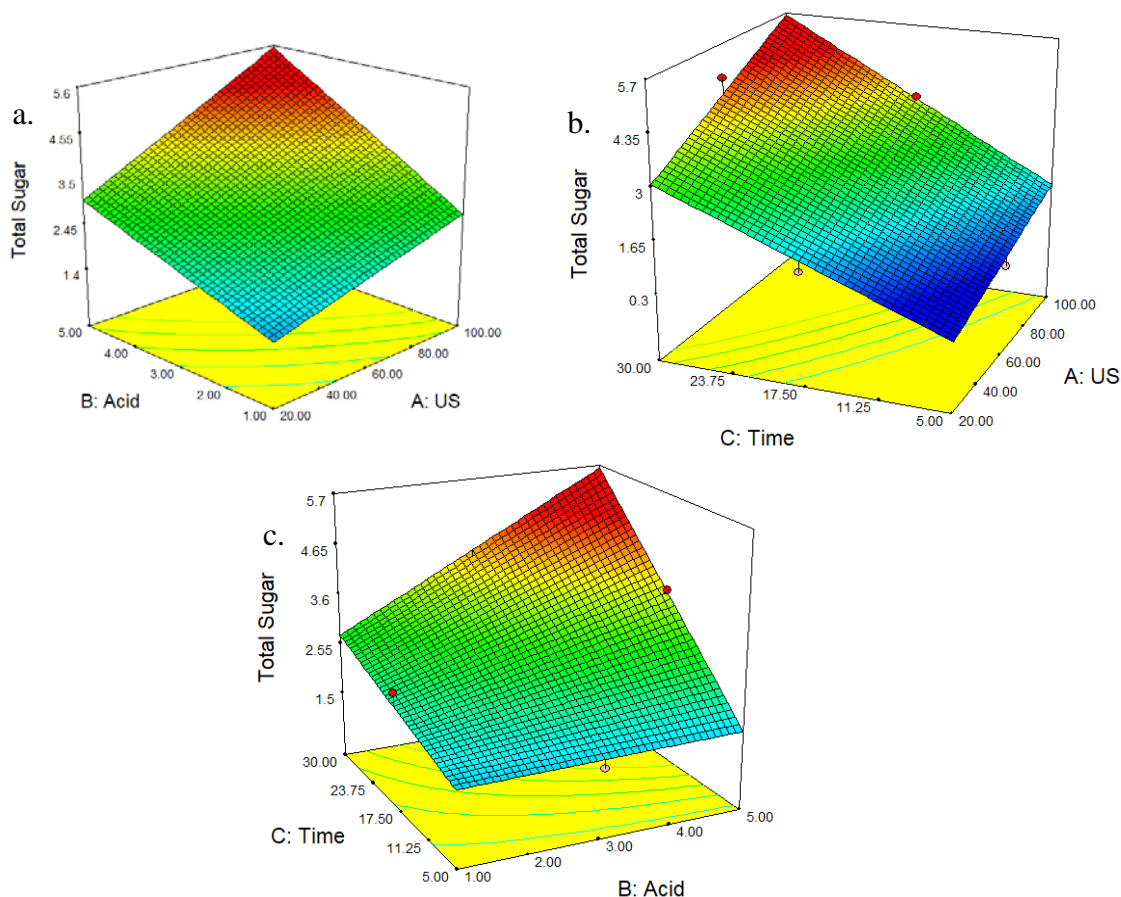
**Figure 2.** Response graphs of reduced sugar (g/L) for box behinkein experimental design a) temperature (°C) and acid ratio (%), b) time (min) and acid ratio (%) c) time (min) and temperature (°C) interactions.

### *US+Acid Pretreatment*

The second series of pretreatment processes were carried out with ultrasound and acid. The results of optimization study of pretreatment studies with ultrasound and sulfuric acid are explained in the following graphs. The change in both total sugar and reducing sugar concentration values has been studied.

The Figure 3a shows the total sugar concentration depending on the acid concentration of and ultrasound power change. A growing in total sugar concentration for increased ultrasound power and an increase in total sugar concentration for ascending acid concentrations were observed. A much larger rise in sugar concentration is seen when the common effect of both factors is considered. The highest total sugar concentration was 5.6 g/L. This value is obtained at the point where the acid concentration and ultrasound power are maximum. Figure 3b is a graph showing the effect of time factors and ultrasound power factors. From the graph, similar to the previous graph, the increase in ultrasound power also resulted in an rise in total sugar concentration. When the time factor is examined, the total sugar concentration obtained with the elongation of the reaction time is also increased. when the effect of both factors was examined, the total sugar concentration reached to much higher levels and it had a value of 5.7 g/L. The graph of acid concentration and reaction time is shown in Figure 3c. It is clearly

shown in the graph that the acid concentration and the reaction time together have an effect on the total sugar concentration on the positive side. Factors, when examined alone, are not as high as the effects they have created, although there is an increase in total sugar concentration



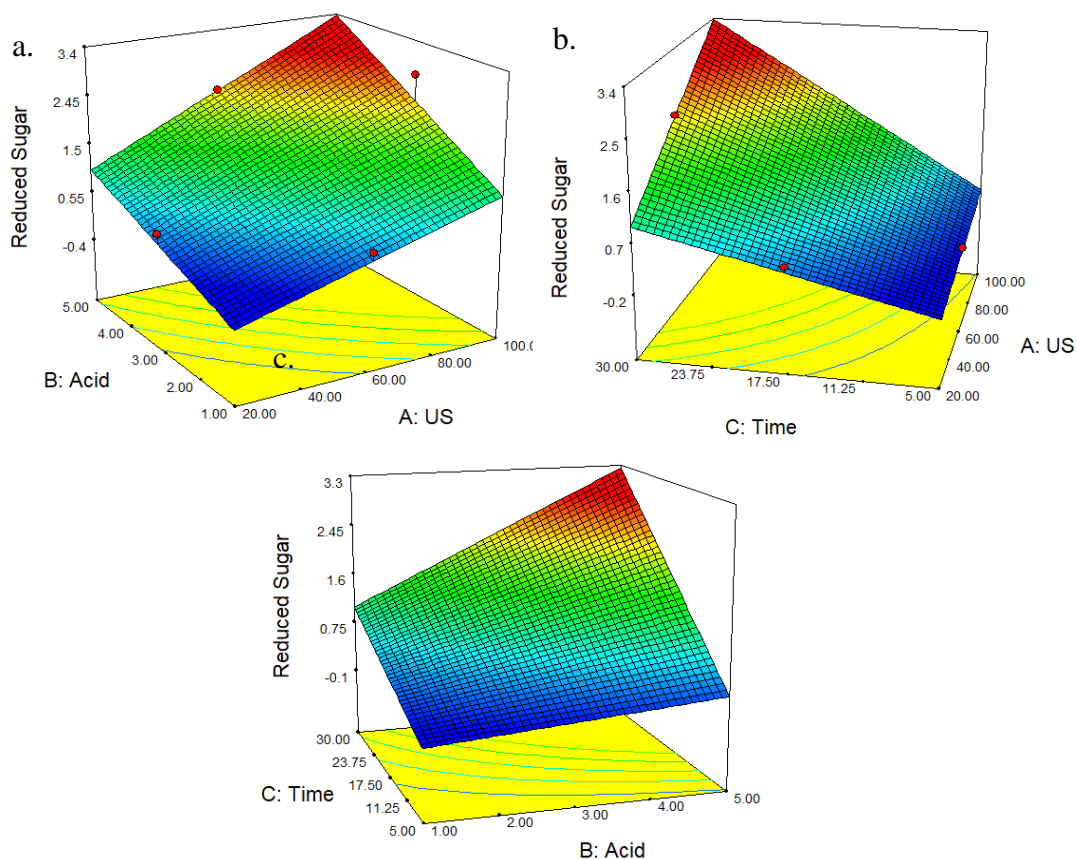
**Figure 3.** TS concentration optimization (g/L) for Box Behnken experimental design a) US power and acid ratio (%), b) time (min) and US power c) time (min) and acid ratio (%), interactions.

In our study total sugar concentration rise up to 22% with support of US application like the other studies explained below; In the study conducted by Ebringerová and Hromádková, the effect of ultrasound on the hemicellulosic extraction of the corn cob was studied. In this study, in the first stage direct extraction in the H<sub>2</sub>O<sub>2</sub>-alkali treatment, in the second stage H<sub>2</sub>O<sub>2</sub>-alkali with ultrasound was applied. According to the results obtained, it was determined that the total carbohydrate content increased by 10-40% in all ultrasound results compared to the results of the treatment where only H<sub>2</sub>O<sub>2</sub>-alkali treatment was performed (Ebringerová and Hromádková, 2002). Jacquemin et al. (2012) carried out that it was aimed to develop an environmentally friendly process by combining the hemicelluloses of wheat straw and dandruff into fractionated and technical aspects (yield, purity) combined with environmental characterizations (water consumption, carbon dioxide emissions). Plus, ultrasound effect was investigated in the study. According to the obtained results, the content of arabionase sugar increased by 34.1% with ultrasound applications. Hromádková et al (1999). determined that xylan extraction from corn cobs increased to 21.9% and 36.8% xylan contents when alkali extraction was supported by



ultrasound application. Zhang et. al. (2008) compared the differences of raw material structure and subsequent saccharification rate before and after ultrasonic pretreatment of lignocellulosic biomass. They pointed out that the ultrasonic vibration energy is too low to change the surface conformation of the raw material particle. Ultrasound pretreatment, the lignin degradation and enzymatic saccharification rates are effectively improved.

Diagrams of the changes in reducing sugar concentrations in the optimization studies are given in Figure 4. Optimized factors in these experiments are the acid concentration, ultrasound power and reaction time. From the Figure 4a, an increase in the concentration of reducing sugar with an increase in acid Concentration and ultrasound strength was observed, which increased to 3.4 g / L. Similarly, Figure 4b indicate that the duration of the reaction and ultrasound power change. It is seen that the concentration of reducing sugar at the maximum levels of the factors also reaches the maximum level. Reduced sugar concentrations obtained due to changes in reaction time and acid concentration are shown in Figure 4c. The longest reaction time was 30 minutes and the maximum acid concentration was 5%, which cause the highest level of reducing sugar, up to 3.3 g/L.



**Figure 4** RS concentration optimization (g/L) for Box Behnken experimental design a) US power and acid ratio (%), b) time (min) and US power c) time (min) and acid ratio (%), interactions.

## CONCLUSION

Chemical pretreatment is used for lignocellulosic material which is agricultural waste, agro-industrial waste such as sugar beet, corn cob and sugar cane, forestry residues. The chosen pretreatment is so important because of different properties waste. Moreover, some studies have been reported chemical, biochemical, biological and advanced application such as AFEX pretreatment, which become interesting for industrial applications.

In this work, H<sub>2</sub>SO<sub>4</sub> acid hydrolysis pretreatment was applied to decompose the lignocellulosic and cellulosic structure of corncob in order to maximise the sugar conversion by RSM method of Box Behnken. Different factors of temperature, acid concentration and reaction time was applied to optimization of factors to maximize sugar and reduced sugar concentration. According the model solutions, the maximum total sugar concentration of 5.05 g/L can be obtained at the conditions of 4.74% acid concentration, 99.55 °C and 177 min. and also the reduced sugar concentration can be reach up to 2.20 g/L.

According to results from this study it clear that the ultrasound power can increase the total sugar and reduced sugar concentration, 22% and 70% respectively, from the corncob to improve the bioavailability of the biomass to produce more efficient biofuel production for per mass unit raw material of corncob.

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