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Optimization of combined pre-treatments on sugarcane leaves for bioethanol production

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ABSTRACT

This paper reports the comparison of an optimized pretreatment model for Reducing Sugar (RS) production from Sugarcane leaf (SL) using NaOH to depolymerize of lignocellulosic biomass. The addition variables for a pretreatment model consisted of NaOH concentrations, heating temperatures and pretreatment times in the variety of 1-2% (v/v), 30-40 °C and 1-3 days individually. After pretreatment RS was estimated to transform into energy, both of RS and the energy were optimized by Respond surface methodology (RSM) on Central composite design (CCD). The models showed high determinational coefficients (R^2) above 0.9876. Process optimization provided highest RS yield and energy of 5.632 g/L and 90.112 kJ/L. for NaOH pretreatment at condition 2% (v/v) NaOH, low temperature (40 °C) for 3 days. This report presented demonstrate that sugarcane leaf waste which is usually burnt after harvesting can be a part of renewable source for the biofuel production.

1. Introduction

Lignocellulosic biomass is a promising bioprocess substrate for the production of biofuels. Agricultural lignocellulosic plant biomass constitutes a potential source of low-cost material for the bioenergy production. Biomass is primary materials for fuel production as biofuels propose advantages over petroleum-based fuels (Demirba., 2008): (1) biofuels are obtainable from biomass materials, (2) they are represent a CO_2-cycle in combustion, (3) biofuels have a considerable environmentally friendly potential, (4) there are benefits of the environment, economy and consumers in using biofuels, and (5) they are biodegradable and contribute to sustainability. The major benefits of biofuels are presented in Table 1. (Balat., 2011). Four groups of lignocellulosic materials based on category of resource: (1) forest residues, (2) municipal solid waste, (3) waste paper, and (4) crop residue resources. Several papers on utilization of various lignocellulosic waste materials such as sorghum stalk (Manmaia et. al., 2017), sunflower stalk (Manmaib et. al., 2017), sugarcane leaf (Manmaic et. al., 2017), corn stalk (Manmaid et. al., 2018), (Balat., 2011).

Nomenclature and Abbreviation	
RS	Reducing sugar
TS	Total sugar
RSM	Response surface methodology
CCD	Central composite design
SL	Sugarcane leaf

Table 1. Major benefits of biofuels dropped from (Balat., 2011).

Economic impacts	Sustainability Fuel diversity Increased number of rural manufacturing jobs Increased income taxes Increased investments in plant and equipment Agricultural development International competitiveness Reducing the dependency on imported petroleum
Environmental impacts	Greenhouse gas reductions Reducing of air pollution Biodegradability Higher combustion efficiency Improved land and water use Carbon sequestration
Energy security	Domestic targets Supply reliability Reducing use of fossil fuels Ready availability Domestic distribution Renewability

Sodium hydroxide (NaOH) Pretreatment is the simple methods and has been widely studied in the bioconversion of lignocellulosics. NaOH treatment is very effective in increasing the digestibility of hardwood and agricultural residues with low lignin content. NaOH pretreatment offers great potential because working of NaOH pretreatment at low temperatures and presents an observable delignification capacity relative to its severity (Bali et. al., 2014). NaOH was used for delignification of the lignocellulosic biomass. In alkaline pretreatment, lignin was degradation occurs due to the breaking down of aryl ether linkages, 50–70% total linkages consist of approximately. Diaryl ethers and carbon–carbon bonds are comparatively stable. The depolymerized of ether linkages in the lignin were supported to catalyze by hydroxyl ions and thus release the soluble sodium phenolates in the liquid. The intense NaOH alkalinity reagent as a result solvation of hydroxyl groups in carbohydrates and generates the inflated effect in sugar residue. NaOH increases the fragmentation of the reagent through the capillaries. These causes supportable adsorption of the enzyme molecules onto NaOH treated biomass, thus increasing the accessibility and digestibility of the carbohydrate fraction in the biomass.

During the NaOH lignocellulosic pretreatment, past of degradable carbohydrates occurs thus reducing the sugar concentrations (Gupta and Lee., 2010).

The Response Surface Methodology (RSM) is a modeling and optimization procedure to evaluate the relations of input data on the process output. It has been applied in modeling. Optimizing relate to enhance the performance of a system, a process, or a product in order to obtain the maximum benefit from it. The term optimization has been utilized in a chemical analysis for discovering conditions at application of a process to produce the best response (Bezerra et. al., 2008).

This study focused on optimization of the reducing sugar production from sugarcane leaves using low-thermal and diluted NaOH pretreatment techniques which includes heat pretreatment, time of pretreatment and NaOH concentration. The responsible effects of input parameters on the product of reducing sugars are examined. Furthermore, an initial assessment of the use of these sugars for estimation into energy output of reducing sugar production.

2. Materials and methods

2.1 Materials preparation

Sugarcane leaf (SL) was collected from during harvest from the farm of Program in Agronomy, Faculty of Agricultural Production, Maejo University, Chiang Mai, Thailand (18° 8' 98" N 99°0' 13" E). The first drying step of the sugarcane leaf, it was dried by sunlight and ambient temperature for 3 days until moisture in the leaf was evaporated and reduced size by a cutting machine. The second drying step, the leaf was milled again to a size less than 1 mm by a blender then dried at 60 ºC for 48 h in a hot air oven and stored in plastic bags at room temperature until further using adopted from (Moodley and Bosco., 2017).

2.1 Experimental design

The CCD response surface design was used to generate 29 runs with varied inputs of NaOH concentration, heating temperature and pretreatment time of 1-2%, 30-40 °C and 1-3 days respectively in Table 2. This experimental work considered the following control-lable process parameters to investigate their influence on the surface roughness: Temperature (A), NaOH concentrations (B) and Time (C).

Table 2. Reducing sugar process and energy estimation parameters and their values used for experiment

Factors	Symbols	Unit	-1	0	1
Temperature	A	°C	30	35	40
NaOH	B	%	1	1.5	2
Time	C	days	1	2	3

2.3. Reducing sugar extraction

5 g of powdered sugarcane leaves were transferred into 50 ml beaker, then 15 ml of NaOH addition solid to liquid ratio of 1:3 at varied concentrations as specified in Table 6. (1.0, 1.5 and 2.0% (v/v)) were added into these beakers and covered by Aluminum foil. Heating was achieved by placing these substrates into a hot air oven. The holding temperature (30, 35 and 40 °C) and pretreating time set points (1, 2 and 3 days) were maintained as specified in the experimental designs were showed in Table 2. After finished pretreatment processes 20 ml of distilled water were added into the beakers and determined reducing sugar yields by the 3,5-dinitrosalycylic acid method using glucose as the standard (Marcos, 1959).

2.4. Analytical methods

2.4.1. Reducing sugar estimation

1 ml of the sample and 2 ml of 3,5-dinitrosalicylic acid (DNS) reagent were dispensed in clean test tubes and mixed by a vortex. The mixture was heated in a boiling water bath for 15 mins at 90 °C. It was allowed to cool, after which 4 ml of distilled water was added. The absorbance was measured with a UV-Spectrophotometer at 540 nm, using blank as control (Marcos, 1959).

2.4.1. Energy calculation

In generally, 1 g glucose can produce 16 kJ of energy. In this research used glucose as the standard for reducing sugar estimation. In consequence, reducing sugar yields can assume that it is glucose concentration and transform to energy directly was indicated in Equation 1. (Thangavelu et. al., 2014).

$$Energy\ (kJ/L) = Reducing\ sugar\ yield\ (g/L) \times 16 \quad (1)$$

3. Results and discussion

3.1. Composition of sugarcane leaf

The analysis of raw sugarcane leaves indicated that the hemicellulose, cellulose and lignin components were 28.28%, 44% and 10.04% respectively ((Moodley and Bosco., 2015).

3.2. Final equation in terms of coded and actual factors

Experimental conditions in terms of coded and actual factors of reducing sugar yields and energy from each of the examined NaOH is shown in Table 2. The experimental data were used to generate models for reducing sugar production and transformation to energy with the following equations relating the yield of reducing sugar and energy to the input parameters were showed in Equation 2-5:

$$\text{Coded reducing sugar} = 2.627 + 0.136A + 0.697B + 0.948C + 0.065AB + 0.455AC + 0.408BC - 0.056A^2 - 0.0869B^2 - 0.342C^2 + 0.115ABC + 0.209A^2B - 0.024A^2C + 0.485AB^2 \quad (2)$$

$$\text{Actual reducing sugar} = -60.848 + 2.599Temp. + 64.387NaOH - 0.853Time - 2.403(Temp. \times NaOH) + 0.089(Temp. \times Time) - 0.795(NaOH \times Time) - 0.026Temp.^2 - 13.93NaOH^2 - 0.342Time^2 + 0.046(Temp. \times NaOH \times Time) + 0.0168(Temp.^2 \times NaOH) - 0.001(Temp.^2 \times Time) + 0.388(Temp. \times NaOH^2) \quad (3)$$

$$\text{Coded energy} = 42.039 + 2.171A + 11.149B + 15.168C + 1.036AB + 7.280AC + 6.520BC - 0.897A^2 - 1.390B^2 - 5.473C^2 + 1.840ABC + 3.351A^2B - 0.384A^2C + 7.761AB^2 \quad (4)$$

$$\text{Actual energy} = -973.649 + 41.585Temp. + 1030.233NaOH - 13.637Time - 38.449(Temp. \times NaOH) + 1.427(Temp. \times Time) - 12.720(NaOH \times Time) - 0.407Temp.^2 - 222.878NaOH^2 - 5.473Time^2 + 0.736(Temp. \times NaOH \times Time) + 0.268(Temp.^2 \times NaOH) - 0.015(Temp.^2 \times Time) + 6.209(Temp. \times NaOH^2) \quad (5)$$

Table 3. ANOVA and variance analysis for respective regression models and model terms

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value	Remark
Model	29.080	13	2.24	91.71	< 0.0001	significant
A-Temperature	0.1104	1	0.1104	4.53	0.0503	
B-NaOH	2.910	1	2.91	119.45	< 0.0001	
C-Time	5.390	1	5.39	221.07	< 0.0001	
AB	0.0336	1	0.0336	1.38	0.2591	
AC	1.660	1	1.66	67.91	< 0.0001	
BC	1.330	1	1.33	54.47	< 0.0001	
A^2	0.0216	1	0.0216	0.8857	0.3616	
B^2	0.0519	1	0.0519	2.13	0.1652	
C^2	0.8047	1	0.8047	32.99	< 0.0001	
ABC	0.1058	1	0.1058	4.34	0.0548	
A^2B	0.1503	1	0.1503	6.16	0.0254	
A^2C	0.002	1	0.002	0.0811	0.7798	
AB^2	0.8067	1	0.8067	33.07	< 0.0001	
Residual	0.3659	15	0.0244			
Lack of Fit	0.0017	1	0.0017	0.0662	0.8007	not significant
Pure Error	0.3641	14	0.026			
Cor Total	29.45	28				

df: degrees of freedom, F-value: Fisher-Snedecor distribution value, P-value: probability value.

Table 4. ANOVA and variance analysis for respective regression models and model terms

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value	Remark
Model	7444.83	13	572.68	91.71	< 0.0001	significant
A-Temperature	28.27	1	28.27	4.53	0.0503	
B-NaOH	745.85	1	745.85	119.45	< 0.0001	
C-Time	1380.41	1	1380.41	221.07	< 0.0001	
AB	8.59	1	8.59	1.38	0.2592	
AC	423.99	1	423.99	67.9	< 0.0001	
BC	340.08	1	340.08	54.46	< 0.0001	
A^2	5.53	1	5.53	0.8859	0.3615	
B^2	13.29	1	13.29	2.13	0.1652	
C^2	206.01	1	206.01	32.99	< 0.0001	
ABC	27.08	1	27.08	4.34	0.0548	
A^2B	38.49	1	38.49	6.16	0.0253	
A^2C	0.5056	1	0.5056	0.081	0.7799	
AB^2	206.53	1	206.53	33.08	< 0.0001	
Residual	93.66	15	6.24			
Lack of Fit	0.441	1	0.441	0.0662	0.8006	not significant
Pure Error	93.22	14	6.66			
Cor Total	7538.5	28				

df: degrees of freedom, F-value: Fisher-Snedecor distribution value, P-value: probability value.

Table 5. Summary of ANOVA and regression analysis for respective responses

Models	Std. Dev.	Mean	C.V. %	R^2	Adjusted R^2	Predicted R^2	Adeq Precision
Reducing sugar	0.1562	2.390	6.53	0.9876	0.9768	0.9155	47.2930
Energy	2.500	38.290	6.53	0.9876	0.9768	0.9155	47.2930

The adequacy of the developed models was examined by analysis of variance (ANOVA) and the results are presented in Table 3-4. The model F-values of 91.71 of reducing sugar and energy respectively suggests the models were significant. There are only a 0.01% chance that an F-value this large could occur due to noise. In addition, the significance of two models were also evaluated based on the p-value, since a p-value less than 0.050 are significant. In also two models case B, C, AC, BC, C^2, A^2B, AB^2 are significant model terms. Values greater than 0.100 indicate the model terms are not significant. In Table 2-3 the models showed p-values less than 0.050 respectively. The p-value lower than 0.05 indicate that these models are statistically significant at 95% (Kassim and Bhattacharya., 2016).

The Lack of Fit F-value of reducing sugar and energy are 0.070 suggests the Lack of Fit is not significant relative to the pure error. There are 80.07% and 80.06% chance that a Lack of Fit F-value this large could occur due to noise are non-significant lack of fit are good.

Table 5. showed the std. dev., mean, percent of C.V., R2, adjusted R2, predicted R2 and adeq precision of the reducing sugar and energy. In this case, the value of std. dev. and mean are 0.1562 and 2.500, 2.390 and 38.290.

Both of the reducing sugar and energy there are identical percent of C.V., R^2, adjusted R^2 and predicted R^2 as 0.9876, 0.9768, 0.9155 and 47.2930. The predicted R^2 of 0.9155 was agreed with the adjusted R^2 of 0.9768 because the difference is less than 0.200 and the adeq precision measures the signal to noise ratio. Two ratios of 47.293 indicates an adequate signal. Two models can be used to navigate the design space showed in Table 5.

In pretreatment optimization study of Kassim and Bhattacharya in 2016 for *Tetraselmis suecica* and *Chlorella* sp. were using variant alkalis as KOH and NaOH. Two substances were selected based on the efficiency of producing high reducing sugar concentration. Analysis of variance (ANOVA) for the response surface methodology model for testing the effect of the parameters significance on the reducing sugar production. The R^2 value of the model of T. *suecica* and *Chlorella* sp. were 0.96 (Adjusted R^2 = 0.93) and 0.92 (Adjusted R^2 = 0.85). The R^2 values of two models close to 1, model-predicted evaluation of reducing sugar concentration was suggesting the model provide satisfactory result of both microalgal biomass. The ANOVA indicated that *p*-value for *T. suecica* and *Chlorella* sp. were less than 0.05 ($p < 0.05$), suggesting that all the parameters tested have significant effect on reducing sugar production.

3.3. Model of observed and predicted values

An optimization was performed to the reducing sugar yield, as shown in Table 5. According to the prediction model a reducing sugar yield of 5.640 g/L and the observation model a reducing sugar yield of 5.632 g/L, under optimal conditions of 2% NaOH pretreated at 40 °C for 3 days, respectively.

An optimization was performed to the energy, as shown in Table 6. According to the prediction model an energy of 90.240 kJ/L and the observation model a reducing sugar yield of 90.211 kJ/L, under optimal conditions of 2% NaOH pretreated at 40 °C for 3 days, respectively.

Figure 1a and Figure 1b presented the normal probability graph of residuals is also plotted for reducing sugar and energy. It is seen that the residuals are falling in a straight line, which indicates that the errors are normally distributed. Figure 2a and Figure 2b the comparison of predicted values of the reducing sugar and energy with that of experimental values. The experimental and predicted values are in good understanding with each other, which assures the correctness of the information generated. The obtained model can predict reducing sugar yield and energy with the coefficient of determination of 98.76%

Both of reducing sugar and energy are interactive effect between factor A and B (Figure 3. a,b and 4. a,b) , factor A and C (Figure 3. c,d and 4. c,d) and factor B and C (Figure 3. e,f and 4. e,f) of the low-thermal and NaOH pretreatment parameters was examined using the response surface graphs. It was observed that NaOH concentration and pretreatment time and pretreatment temperature have a linear relationship on reducing sugar and energy. When these parameters were varied from their lower to higher levels, so did the reducing sugar concentration increase from 0.500 g/L to 5.632 g/L and energy increase from 8.064 kJ/L to 90.112 kJ/L. The condition of low reducing sugar concentration and energy at 30 °C, 1% (v/v) NaOH for 1 day and high reducing sugar concentration and energy at 40 °C, 2% (v/v) NaOH for 3 days. The interactive effects of factors on reducing sugar production and energy estimation were presented by 2D and 3D response surface graphs against any two independent factors while keeping other variables at its central level It can be seen that when a factors is fixed at its central level, the interactive effects of the other factors on reducing sugar reduces and energy. In addition, the maximum yield of reducing sugar and energy cannot be achieved at the central level.

4. Conclusions

In this study focus on bioconversion of lignocellulosic biomass (SL) to reducing sugar by a novel NaOH, time and temperature, after SL was pretreated, It releasing of high reducing sugar and estimating of energy on CCD, The models of reducing sugar (g/L) and energy (kJ/L) were significant at 98.76%. Maximum reducing sugar yield (5.632 g/L) and energy (90.112 kJ/L) at at 40 ºC, 2% (v/v) NaOH for 3 days.

Table 6. Factors selection of reducing sugar and energy from sugarcane leaf

Run	Factor 1	Factor 2	Factor 3	Reducing sugar (g/L)		Energy (kJ/L)	
	A: Temperature (ºC)	B: NaOH (%)	C: Time (Days)	Observed	Predicted	Observed	Predicted
1	40	1	1	0.932	0.936	14.912	14.976
2	40	2	3	5.632	5.640	90.112	90.240
3	40	2	1	1.829	1.830	29.264	29.280
4	40	1.5	2	2.612	2.710	41.792	43.360
5	35	1.5	1	1.422	1.340	22.752	21.440
6	35	1.5	3	3.522	3.230	56.352	51.680
7	30	1	1	0.500	0.504	8.000	8.064
8	35	1.5	2	2.742	2.630	43.872	42.080
9	35	1.5	1	1.100	1.340	17.600	21.440
10	35	1	2	1.922	1.840	30.752	29.440
11	35	1.5	1	1.505	1.340	24.080	21.440
12	40	1.5	2	2.812	2.710	44.992	43.360
13	30	1.5	2	2.465	2.440	39.440	39.040
14	30	1	3	0.853	0.857	13.648	13.712
15	35	1.5	3	3.051	3.230	48.816	51.680
16	35	2	2	3.112	3.240	49.792	51.840
17	35	1.5	2	2.579	2.630	41.264	42.080
18	35	2	2	3.310	3.240	52.960	51.840
19	35	1.5	2	2.502	2.630	40.032	42.080
20	40	1.5	2	2.712	2.710	43.392	43.360
21	30	1.5	2	2.343	2.440	37.488	39.040
22	35	1.5	3	3.142	3.230	50.272	51.680
23	30	1.5	2	2.514	2.440	40.224	39.040
24	35	2	2	3.305	3.240	52.880	51.840
25	30	2	1	1.598	1.600	25.568	25.600
26	30	2	3	3.121	3.120	49.936	49.920
27	35	1	2	1.968	1.840	31.488	29.440
28	35	1	2	1.656	1.840	26.496	29.440
29	40	1	3	2.645	2.650	42.320	42.400

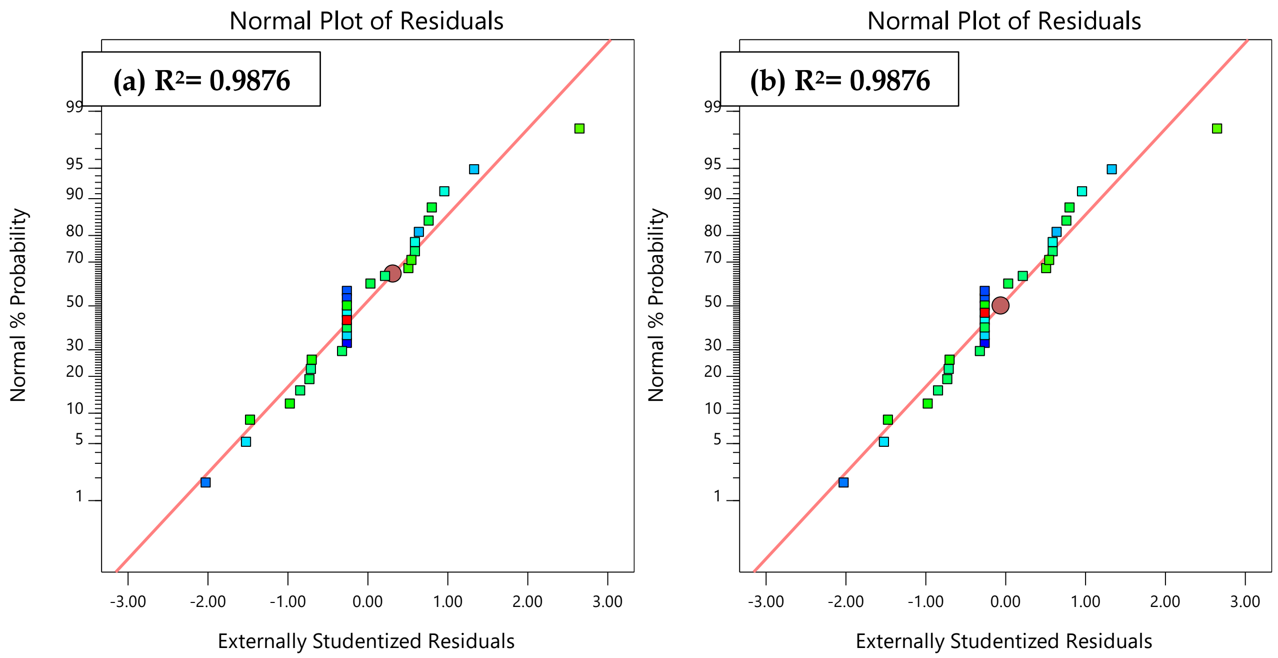

Figure 1. (a). Normal probability plot residuals for the reducing sugar, (b). Normal probability plot residuals for the energy

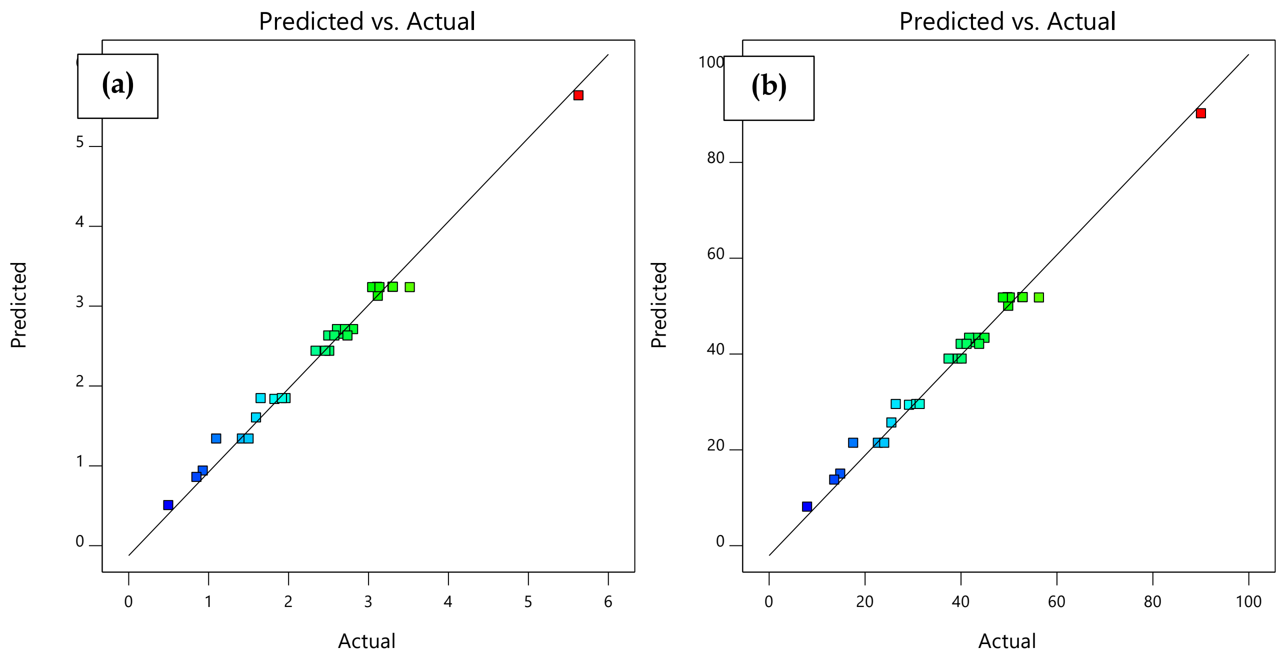

Figure 2. (a). Comparison of experimental and predicted values of RSM model for the reducing sugar, (b). Comparison of experimental and predicted values of RSM model the energy

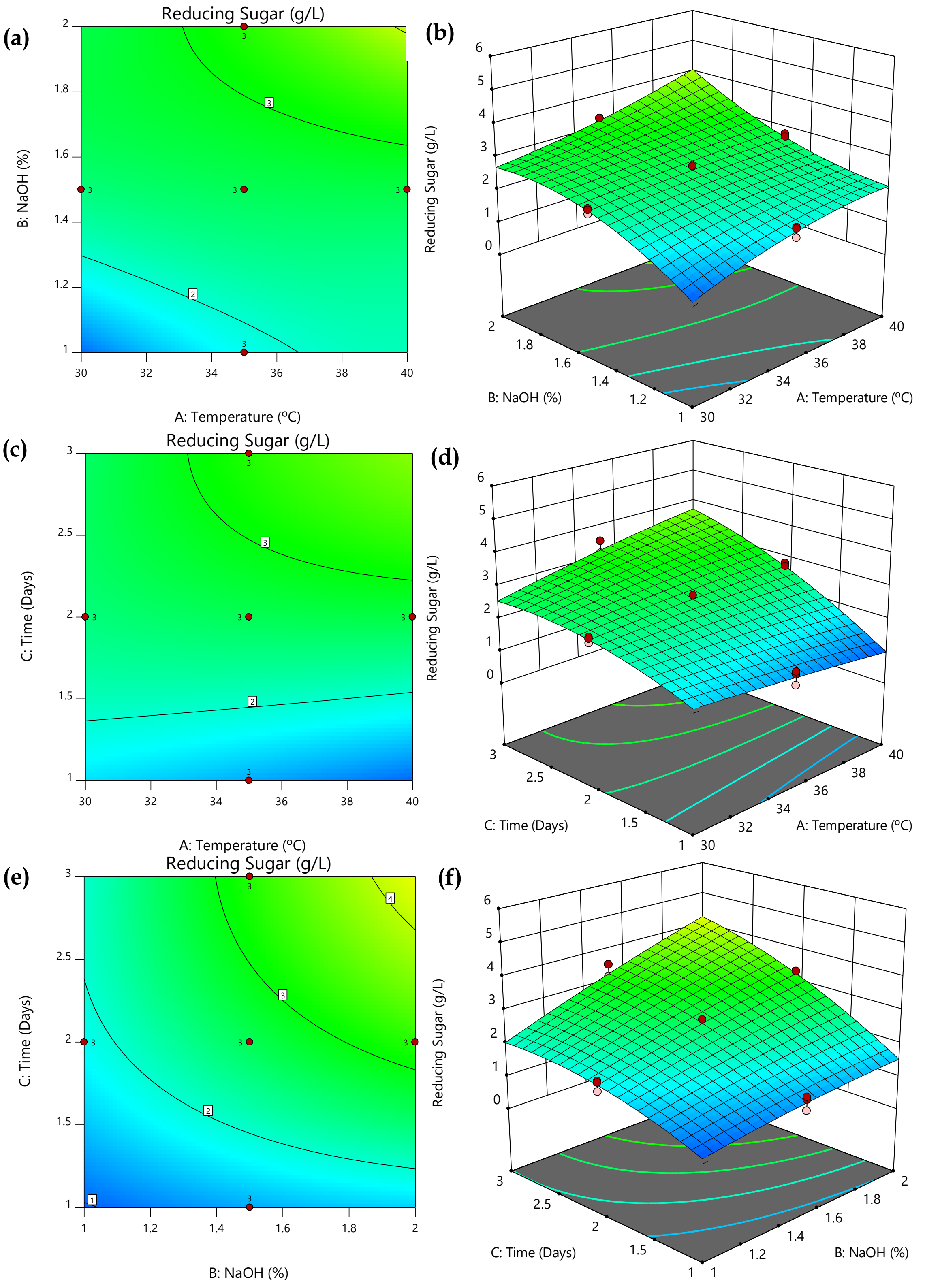

Figure 3. (a,b) Effect of factor A and B on reducing sugar, (c,d) Effect of factor A and C on reducing sugar, (e,f) Effect of factor B and C on reducing sugar

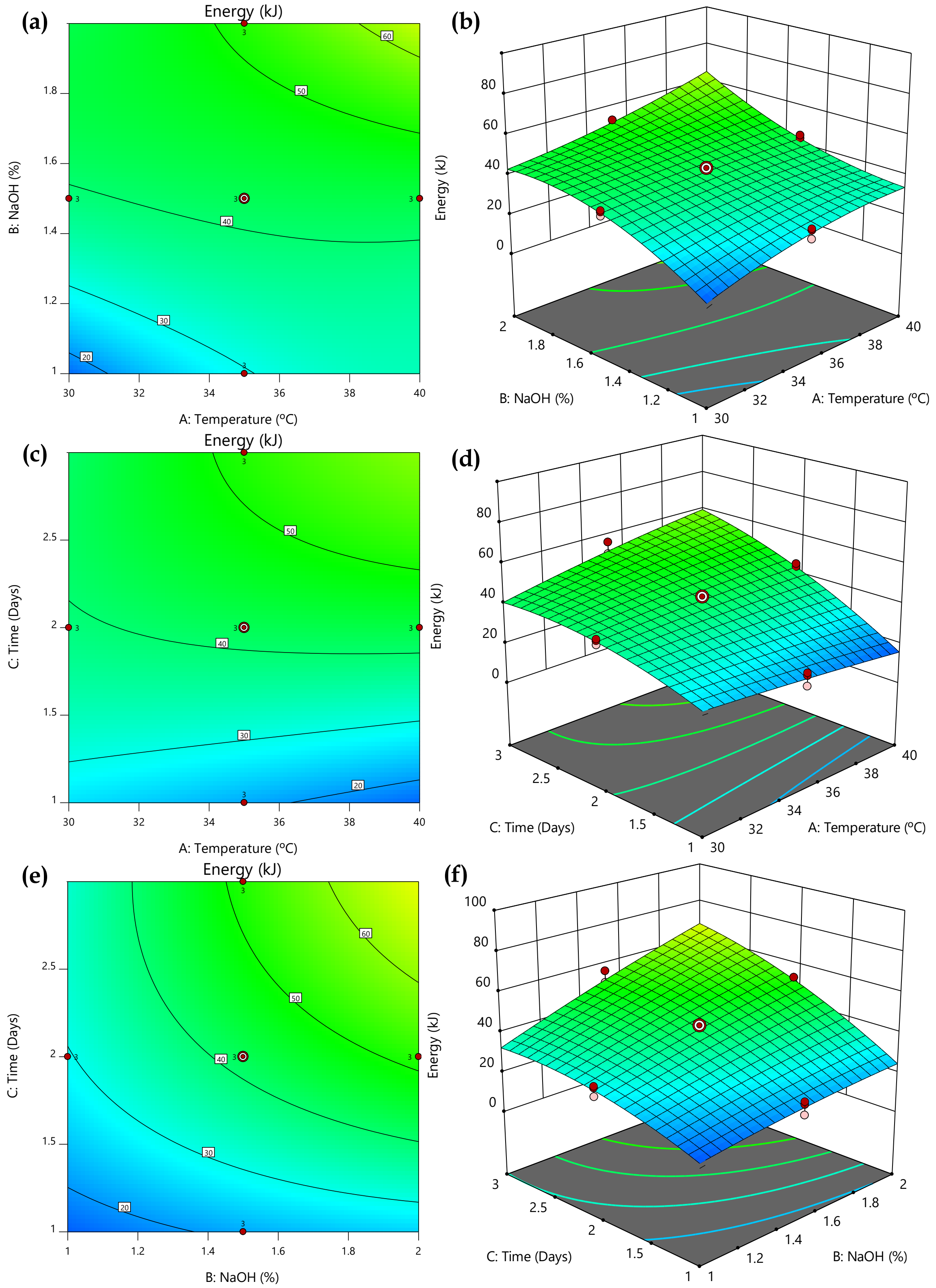

Figure 4. (a,b) Effect of factor A and B on energy, (c,d) Effect of factor A and C on energy, (e,f) Effect of factor B and C on energy

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