Dilute inorganic acid pretreatment of mixed residues of Cocos nucifera (coconut) for recovery of reducing sugar: optimization studies

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Abstract. Inorganic acids, such as sulphuric acid, hydrochloric acid, and nitric acid are widely used for the pretreatment of lignocellulosic biomass for bioenergy production. In this study, the effect of different acids on the recovery of reducing sugar from coconut residues (coir and pith) mixed in different ratios was studied. The pretreatment conditions for different acids were optimized using response surface methodology (RSM). The independent variables, such as biomass ratio, time and acid concentration were considered for the optimization studies with reducing sugar as the dependent variable. The maximum recovery of reducing sugar (45%) from mixed biomass was observed during nitric acid (NA) pretreatment. The recovery of reducing sugar was lower for hydrochloric acid (HA) and sulphuric acid (SA). The lower yield was attributed to the possible formation of sugar degradation compounds during acid pretreatment. Therefore, NA pretreatment was found suitable for mixed biomass compared to other acids. Further studies are required to understand the effect of NA pretreatment through a detailed study of liquid hydrolysate and the introduction of the saccharification process. Mixed biomass benefits the biorefinery industries for sustainable bioenergy production.

Keyword. Inorganic acid, pretreatment, reducing sugar, optimization, coconut

1 Introduction

Lignocellulosic biomass is the most abundant and promising alternative renewable resource for the production of bioenergy and valuable chemical by-products [1]. Lignocellulose is a complex structure with three polymer layers: cellulose, hemicellulose, and lignin. Lignocellulosic biomass includes agricultural residues, energy crops, wood residues and other waste. Coconut coir and pith residues of *Cocos nucifera* (Coconut) is an agricultural waste generated in higher amounts in India [2]. The annual coconut coir and pith production in India is 280,000 and 50,000 metric tonnes, respectively. Most of the generated coir and pith is considered waste and is incinerated as part of waste management. In this study, the residues of coconut were utilized for the recovery of sugar during pretreatment. Coconut residues are rich substrates for the cellulose and lignin content [3]. Lignin and hemicellulose hinder the enzymes during the saccharification process and microbial attack. Similarly, bioenergy production from lignocellulosic biomass requires three essential steps: pretreatment, saccharification, and fermentation. Pretreatment is a process of breaking the inter-and intra-hydrogen linkages present in lignin and hemicellulose structure [4]. The pretreatment process usually is performed by physical, chemical, or biological processes. Pretreatment methods under the chemical process include acid, alkaline, ionic liquid, Organosolv, Ammonia fibre expansion (AFEX) and deep eutectic solvent (DES) [5].

The current study has focused on acid pre-treatment in a dilute state to break bonds between hemicellulose and lignin to recover reducing sugar. Inorganic acids such as sulfuric acid, hydrochloric acid, and nitric acid were used to extract reducing sugar from coconut residue. Inorganic acids readily dissociate, and the hydrogen bonds are cleaved in the presence of free H^+ ions. Different inorganic acids have different effects during the pretreatment process. Therefore, the effect of different acids on the recovery of reducing sugar from coconut residues was studied. Several studies have reported the effect of acid during pretreatment using single biomass. Zhang *et al.* studied the effect of different acids under mild conditions for selective saccharification by a fast pyrolysis process. Phosphoric acid showed improved results compared to other inorganic acids [6]. Different acids have different effects on the recovery of reducing sugars. Pretreatment with acids leads to the production of inhibitory compounds such as furfural and 5-hydroxymethylfurfural (drawback). However, the percentage of such compounds varies depending on the acidity of the various acids and pretreatment conditions (time, temperature, type of acid and its concentration). The pretreatment conditions for various acids were optimized

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using the response surface methodology (RSM). The processing of one biomass does not give a stable production of bioenergy. Single biomass is not always available, so mixed biomass offers an alternative solution for the sustainable production of bioenergy. In our previous published study, the effect of different acids on coconut coir for recovery of maximum reducing sugar was investigated [7].

In this study, attempts have been made to investigate the effects of different acids on different proportions of coconut coir and pith in different ratios. In addition, the pretreatment conditions such as biomass ratio, time and acid concentration were optimized using RSM. The present study investigated the potential of different acids to recover maximum reducing sugar from mixed biomass. Reducing sugar was considered the response/dependent variable for the generation of a second-order model to determine the optimum condition.

2 Materials and Methods

2.1 Collection and Characterization of Coconut residues

Coconut residues were collected separately from a coconut processing facility in the central part of Hyderabad, Telangana, India. The size of the pith sample was reduced to 1 mm using a food processor after drying in a hot air oven at 65°C for 48 h. The coir sample was soaked in liquid N2 and grounded to powder (size 1 mm) using a mortar and pestle. The samples were characterized for cellulose, hemicellulose and lignin by following the NREL standard protocol [8].

2.2 Central Composite Design – Design of Experiment

The pretreatment of coconut residues was carried out using inorganic acids such as sulphuric acid (SA), hydrochloric acid (HA), and nitric acid (NA). The pretreatment conditions such as biomass ratio (X_1), time (X_2, min) and acid concentration (X_3, w/w) were optimized using response surface methodology. The optimum conditions were determined and considered maximum reducing sugar yield after pretreatment from mixed biomass. The central compound design (CCD) was considered for three independent variables, resulting in 20 experimental runs. The biomass ratio was calculated from the expression shown in Eq (1).

$$Biomass\ ratio \ = \frac{Coconut\ coir, g}{Coconut\ pith, g} \qquad (1)$$

The CCD matrix with 20 runs includes 8 full factorial runs, 6 axial runs, and 6 central runs. The acid pretreatment was carried out for individual experimental runs to generate a second-order model for different acids. The general second-order model expression for three factors is shown in Eq (2).

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum \sum_{i<j=3}^{3} \beta_{ij} x_i x_j \qquad (2)$$

Where Y is the response, x_i and x_j are the independent variables, β_o, β_i, β_{ii}, and β_{ij} is the constant coefficient, i^{th} linear coefficient, quadratic coefficient, and ij^{th} interaction coefficient, respectively. The experimental data were analysed statistically and processed using Statistica (7.0).

2.3 Inorganic acid pretreatment

Inorganic acid pretreatment of mixture biomass (coir/pith) using sulphuric acid (SA), hydrochloric acid (HA), and nitric acid (NA). The acid pretreatment was performed in a laboratory autoclave following the conditions for each experimental run summarized in Table 1. The pretreatment temperature was fixed at 121°C to avoid the formation of sugar degradation compounds. The solids and liquid hydrolysate were separated by filtration after pretreatment. The pH of the liquid hydrolysate was neutralized to 7 and analysed for reducing sugar following the DNS protocol [9]. The yield of reducing sugar was calculated based on the expression shown in Eq. (3)

$$Y \ = \frac{RS, mg/mL}{Total\ sugar\ in\ biomass, mg/mL} \times 100 \qquad (3)$$

$$RS, mg/mL = \frac{A}{2.615} \times D \qquad (3.1)$$

where RS - reducing sugar mg/mL, A – absorbance (575 nm), D - dilution factor, 2.615 - slope value (glucose standard curve)

Table 1. Central composite design (CCD) matrix for inorganic acid pretreatment of coconut residues

Run no	Biomass ratio (X_1)	Time, min (X_2)	Acid concentration, % w/w (X_3)
1	0.47	20.1	0.70
2	0.47	20.1	1.30
3	0.47	49.9	0.70
4	0.47	49.9	1.30
5	2.11	20.1	0.70
6	2.11	20.1	1.30
7	2.11	49.9	0.70
8	2.11	49.9	1.30
9	0.25	35.0	1.00
10	4.00	35.0	1.00
11	1.00	10.0	1.00
12	1.00	60.0	1.00
13	1.00	35.0	0.50
14	1.00	35.0	1.50
15	1.00	35.0	1.00
16	1.00	35.0	1.00
17	1.00	35.0	1.00
18	1.00	35.0	1.00

Run no	Biomass ratio (X_1)	Time, min (X_2)	Acid concentration, % w/w (X_3)
19	1.00	35.0	1.00
20	1.00	35.0	1.00

3 Results and Discussion

3.1 Acid pretreatment: Optimization studies

The characterization of coconut coir and pith was performed, and the chemical composition was shown in Figure 1. The glucose content was higher in coir compared to the pith. Acid pretreatment using SA, HA, and NA was carried out for the coconut residues following the experimental condition displayed in Table 1. The reducing sugar for individual experimental runs were determined. The reducing sugar for SA, HA, and NA varied from 13-43%, 28-47%, and 18-56%, respectively. It was observed that the maximum reducing sugar yield was observed for nitric acid pretreatment.

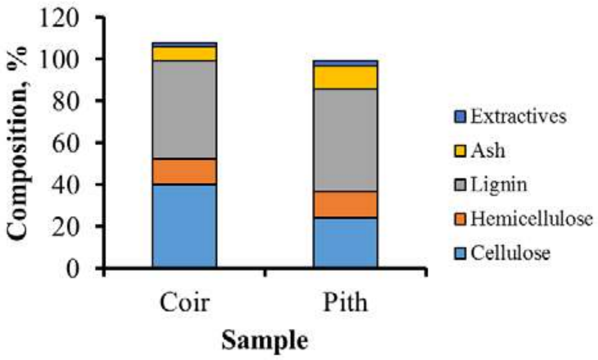


Figure 1. Chemical composition of coconut coir and pith

The second order model for SA, HA, and NA was generated by multiple regression analysis of the experimental data obtained for different experimental runs. The second order model for SA, HA, and NA was shown in Eq (4), Eq (5), and Eq (6), respectively

$$Y_{SA} = -38.28 - 4.88X_1 + 1.762X_2 + 57.22X_3 - 1.03X_{11} - 0.021X_{22} - 33.58X_{33} - 0.0789X_{12} + 13.09X_{13} + 0.1928X_{23} \quad (4)$$

$$Y_{SA} = -17.83 + 9.93X_1 + 1.17X_2 + 37.96X_3 - 0.567X_{11} - 0.011X_{22} - 13.08X_{33} - 0.095X_{12} - 1.21X_{13} - 0.0013X_{23} \quad (5)$$

$$Y_{NA} = -57.3 + 21.92X_1 + 0.75X_2 + 92.1X_3 - 5.23X_{11} + 0.0026X_{22} - 33.46X_{33} + 0.0037X_{12} + 2.07X_{13} - 0.445X_{23} \quad (6)$$

The generated models were tested for their adequacy and significance by (a) coefficient of determination (R^2), (b) analysis of variance (ANOVA), (c) lack of fit, and (d) residual plots. The value of R^2 is dependent upon the variance between the observed data versus predicted data. A low variance between these data shows that the model is statistically significant. A second-order model with R^2 greater than 0.8 is significant with observed data closer to the predicted data [10]. The R^2 value for SA, HA, and NA was greater than 0.95 showing the model was statistically significant. The predicted and observed data plot for SA, HA, and NA was shown in Figure 2.

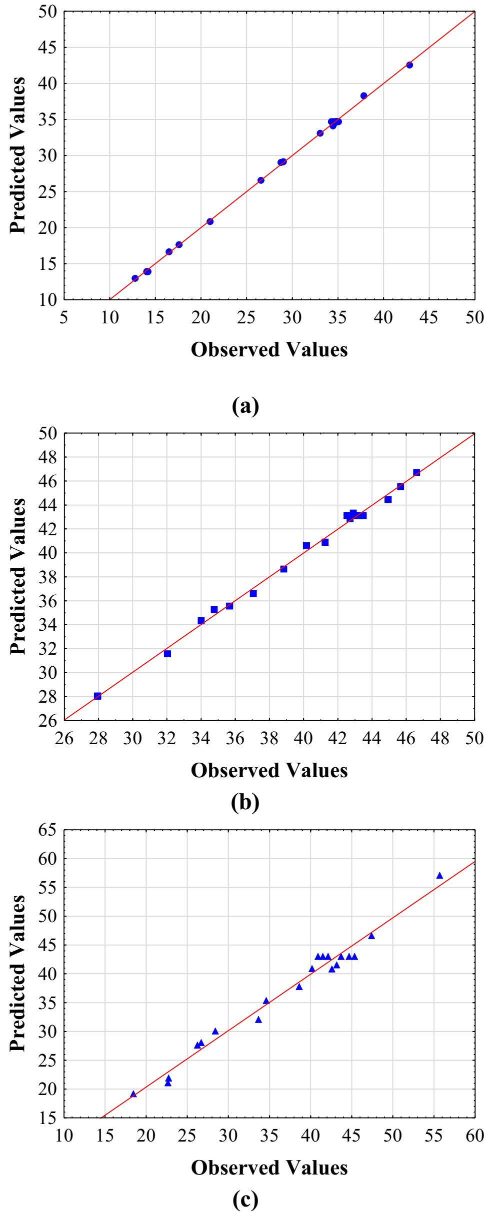


Fig 2. Predicted and observed data plots for the different second-order models. (a) Sulphuric acid (SA); (b) Hydrochloric acid (HA); (c) Nitric acid (NA)

The ANOVA performed for SA, HA, and NA second-order model showed that the model was significant (Table 2). The significance of the model was depicted by a p-value < 0.05. Furthermore, the model's significance and adequacy were examined through the insignificance of lack-of-fit (p-value > 0.05). Lack of fit usually describes the relationship between the dependent and independent variables. It was observed that the lack of fit for all the models was insignificant. Therefore, it is clear that the second-order models were significant. Further significance and adequacy of the second-order model were investigated through residual plots. The residual values are the difference between the predicted and observed values.

Table 2. Analysis of variance (ANOVA) for different second order model

Parameter	SS	df	MS	F	P
Sulphuric acid (SA)					
X_1	19.07	1	19.07	264.63	0.00
X_{11}	23.03	1	23.03	319.67	0.00
X_2	275.37	1	275.37	3822.06	0.00
X_{22}	314.20	1	314.20	4361.02	0.00
X_3	719.87	1	719.87	9991.70	0.00
X_{33}	132.96	1	132.96	1845.47	0.00
X_{12}	13.69	1	13.69	190.06	0.00
X_{13}	150.61	1	150.61	2090.52	0.00
X_{23}	5.81	1	5.81	80.65	0.00
Lack of Fit	0.78	5	0.16	2.17	0.21
Pure Error	0.36	5	0.07		
Total SS	1600.9	19			
Hydrochloric acid (HA)					
X_1	150.67	1	150.67	1354.87	0.00
X_{11}	7.17	1	7.17	64.47	0.00
X_2	98.49	1	98.49	885.66	0.00
X_{22}	89.07	1	89.07	800.91	0.00
X_3	101.89	1	101.89	916.24	0.00
X_{33}	19.26	1	19.26	173.18	0.00
X_{12}	19.90	1	19.90	178.96	0.00
X_{13}	1.29	1	1.29	11.57	0.02
X_{23}	0.00	1	0.00	0.00	0.96
Lack of Fit	1.64	5	0.33	2.95	0.13
Pure Error	0.56	5	0.11		
Total SS	477.62	19			
Nitric acid (NA)					
X_1	59.65	1	59.65	18.33	0.01
X_{11}	610.55	1	610.55	187.60	0.00
X_2	757.57	1	757.57	232.78	0.00
X_{22}	4.67	1	4.67	1.44	0.28
X_3	235.30	1	235.30	72.30	0.00
X_{33}	126.70	1	126.70	38.93	0.00
X_{12}	0.08	1	0.08	0.02	0.89
X_{13}	3.40	1	3.40	1.05	0.35
X_{23}	29.82	1	29.82	9.16	0.03
Lack of Fit	23.06	5	4.61	1.42	0.36
Pure Error	16.27	5	3.25		
Total SS	1843.7	19			

X_1, X_2, and X_3 – Main terms; X_{11}, X_{22}, and X_{33} – Quadratic terms; X_{12}, X_{13}, and X_{23} – Interaction terms; SS – sum of squares; df – degree of freedom; MS – mean sum of square

The residual plots for SA, HA, and NA second-order models were plotted and shown in **Figure 3**. The residual graph is plotted with residual values and predicted values on a vertical and horizontal axis. The model is considered significant when the residual values are scattered randomly without forming any patterns. The residual plots showed that the model was significant since the residual values were scattered randomly. The overall observation of the models showed that the SA, HA, and NA models were significant for the selected levels.

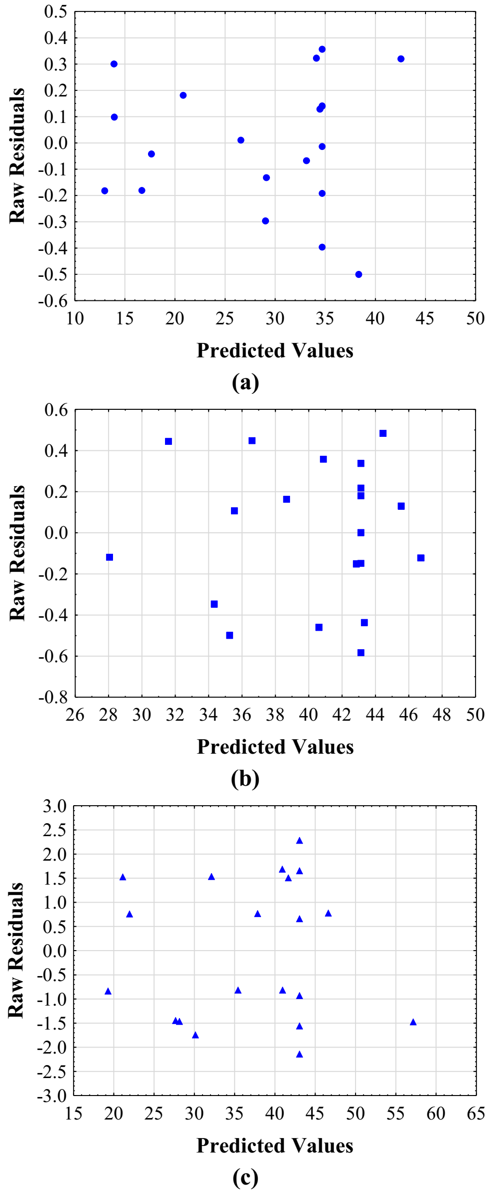
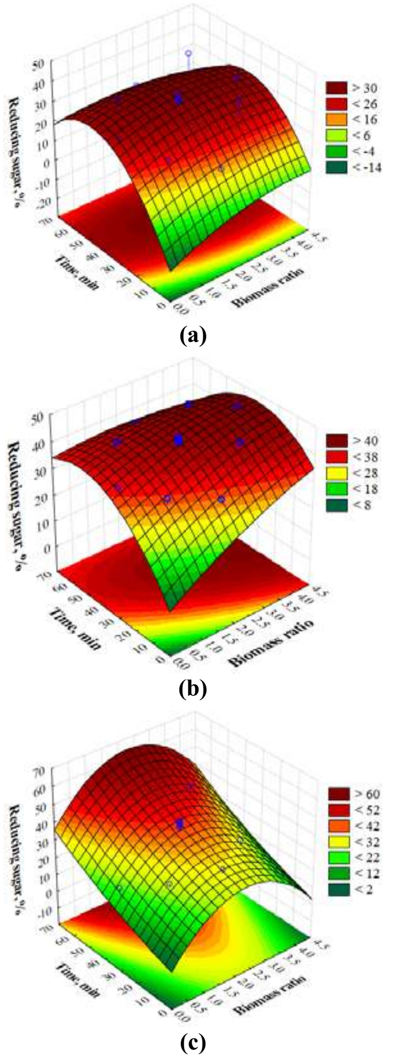


Fig 3. Residual plots for a different second-order model. (a) Sulphuric acid (SA); (b) Hydrochloric acid (HA); (c) Nitric acid (NA)

3.1.1 Interaction effect of biomass ratio and time

The interaction effect of biomass ratio and time for all the models were shown as a three-dimensional surface plot with the contour in Figure 4. Two variables were varied, with the third variable condition fixed at a center run. It was observed that the model predicted reducing sugar yield in the range of 6-30%, 8-40%, and 2-60% for the SA, HA, and NA models, respectively. The reducing sugar recovery from mixed biomass of coconut residues increased as the time increased for SA and HA models. On the other hand, the reducing sugar yield increased for the NA model when the biomass ratio levels were in the mid-range and higher reaction time. Therefore, it is understood that the recovery of reducing sugar from mixed biomass is more dependent on reaction time followed by biomass ratio.



(a)

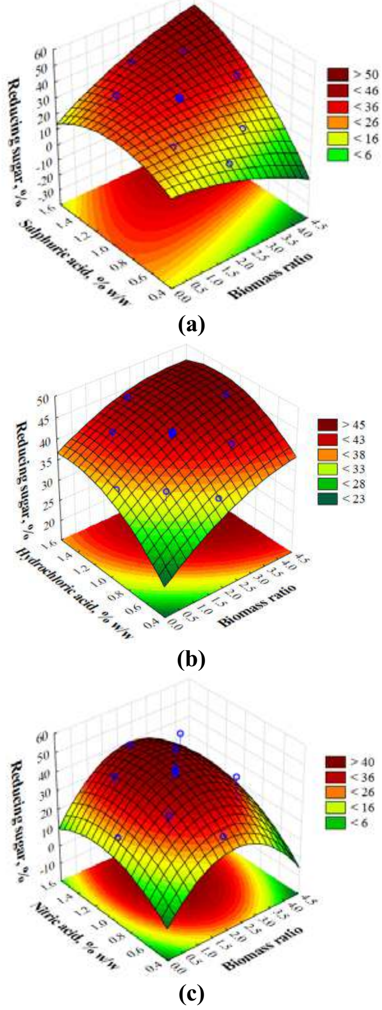
(b)

(c)

Figure 4. Three-dimensional response surface plot for time versus biomass ratio. (a) Sulphuric acid (SA); (b) Hydrochloric acid (HA); (c) Nitric acid (NA)

3.1.2 Interaction effect of Biomass ratio and acid concentration

The interaction effect of biomass ratio and acid concentration for all the models was shown as a three-dimensional surface plot with the contour in Figure 5. The model predicted reducing sugar yield in the range of 6-50%, 23-45%, and 6-40% for the SA, HA, and NA models, respectively. The reducing sugar recovery from mixed biomass of coconut residues after SA and HA pretreatment increased for the conditions of acid concentration and biomass ratio at the far-end range. On the other hand, the reducing sugar yield increased for the NA model when the biomass ratio and acid concentration levels were in the mid-range. Therefore, it was understood that the recovery of reducing sugar from mixed biomass increases when the biomass ratio and acid concentration interact.



(a)

(b)

(c)

Figure 5. Three-dimensional response surface plot for acid concentration versus biomass ratio. (a) Sulphuric acid (SA); (b) Hydrochloric acid (HA); (c) Nitric acid (NA)

3.1.3 Interaction effect of acid concentration and time

The interaction effect of acid concentration and reaction time for all the models were shown as a three-dimensional surface plot with the contour in Figure 6. The model predicted reducing sugar yield in the range of 6-40%, 13-45%, and 6-60% for the SA, HA, and NA models, respectively. The reducing sugar recovery from mixed biomass of coconut residues after SA and HA pretreatment increased at mid-range pretreatment conditions. On the other hand, the reducing sugar yield increased for the NA model when the acid concentration and time parameters were maintained at mid and far-range, respectively. Therefore, the recovery of reducing sugar from mixed biomass increased when the reaction time and acid concentration interacted for SA and HA models. However, the interaction between acid concentration and reaction time was insignificant under initial conditions. The far-end range of parameters resulted in an increase in reducing sugar yield due to interaction.

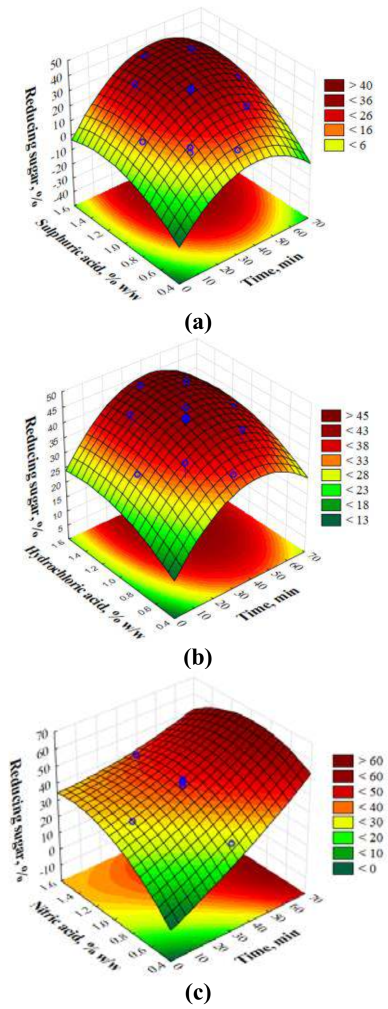


Figure 6. Three-dimensional response surface plot for time versus acid concentration. (a) Sulphuric acid (SA); (b) Hydrochloric acid (HA); (c) Nitric acid (NA)

3.2 Validation of the optimum conditions

The optimum conditions for the acid pretreatment were determined by the steepest ascent method to achieve maximum reducing sugar. The optimum conditions for SA, HA, and NA pretreatment are summarized in Table 3. The optimum conditions were validated by performing the studies using the optimum conditions thrice. Repetition of the studies using optimum conditions lowers the variance between the experimental data. It was observed that the optimum conditions for three acid pretreatments were almost similar. However, the maximum reducing sugar was obtained for nitric acid pretreatment. The maximum reducing sugar yield of 45% was observed for NA pretreatment, followed by HA (41.44%) and SA (36.55%) pretreatment. Different acids had a different effect on mixed biomass on recovery of reducing sugar. The variation in reducing sugar yield could be attributed to the strength of the acids. The pKa of sulphuric acid, hydrochloric acid, and nitric acid is -10, -5.9, and -1.5, respectively. Among the selected acid, nitric acid is the weakest due to its lower acidity strength.

Table 3. Optimum conditions determined for acid pretreatment of mixed biomass.

Parameter	SA			HA			NA		
	O_P	Y_P	Y_O	O_P	Y_P	Y_O	O_P	Y_P	Y_O
Biomass ratio	2.125	34.7%	36.55±1.92%	2.05	43.02%	41.44±0.73	2	43.13%	45.11±2.2%
Time, min	35			40			30		
Acid concentration w/w	1			1.05			1.1		

The lower yield of reducing sugar for sulphuric acid and hydrochloric acid was ascribed to the formation of sugar degradation compounds at 120°C. Furfural and 5-hydroxymethylfurfural are formed due to pretreatment at harsh conditions, such as longer reaction time, higher temperature, acid concentration, and so on [11]. During pretreatment, sulphuric acid and hydrochloric acid could degrade the sugar to degradation compounds, such as furfural and 5-hydroxymethylfurfural [12]. Since nitric acid has lower acid strength, the loss of sugar during pretreatment was lower. A study reported a maximum reducing sugar from sugarcane bagasse after sulphuric acid pretreatment compared to hydrochloric acid and nitric acid [13]. The results in this study were contradictory to the previously reported studies. However, the present study was performed for mixed biomass, and the selected biomass has higher lignin content than sugarcane bagasse. Another study reported the influence of sulphuric acid pretreatment on sugarcane bagasse for the recovery of reducing sugar and ethanol production [14]. Another study reported that acids with free H^+ ions break the inter-and intra-hydrogen bonds in lignin and hemicellulose [15]. Therefore, it was understood that the strength of acids affects the recovery of reducing sugar from lignocellulosic biomass. Furthermore, a detailed study is required to recover complete sugar from mixed biomass through the saccharification process.

4 Conclusion

The effect of different inorganic acids on the recovery of reducing sugar from mixed biomass (coir/pith) was studied. A reducing sugar recovery of 45%, 41.44%, and 36.55% was obtained from nitric acid, hydrochloric acid, and sulphuric acid pretreatment, respectively. The recovery of reducing sugar increased in NA>HA>SA. The results showed that different acids with similar optimum conditions showed different recovery of reducing sugar. Furthermore, a detailed study is necessary to analyse the liquid hydrolysate for furan compounds and introduce the saccharification process to completely recover reducing sugar.

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