Optimization of [Amim]Cl Pretreatment Conditions for Maximum Glucose Recovery from Hybrid *Pennisetum* by Response Surface Methodology

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Because of a complex chemical ultra-structure of lignocellulosic biomass, pretreatment is a necessary step for its conversion into bio-ethanol. In the present study, pretreatment conditions using the ionic liquid (IL) 1-allyl-3-methylimidazolium chloride ([Amim]Cl) were optimized for a relatively new model energy crop, hybrid *Pennisetum* (*P. americanum* × *P. purpureum*) to maximize the yield of fermentable sugars (glucose). The design of experiment programs employed a central composite design (CCD), with variables of temperature (102 to 187 °C), retention time (0.5 to 5.5 h), and solids loading (2 to 15 wt%). These factors were further optimized using response surface methodology (RSM). The proposed quadratic model to predict the glucose recovery from hybrid *Pennisetum* was verified by variance analysis (ANOVA). The model displayed high F and R^2 values, indicating that it could be successfully used to identify the relationship among the independent variables studied. A maximum glucose recovery of 72.2% was found with temperature conditions of 139 °C, 2.97 h retention time, and 9.1 wt% solids loading.

Keywords: Hybrid Pennisetum; Pretreatment; Ionic liquids; Optimization; Response surface methodology; Central Composite Design

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INTRODUCTION

Recently the miss-match between ever-growing energy demands and diminishing fossil fuel reserves has become increasingly prominent (Luque *et al*. 2008). Among the sustainable energy alternatives to fossil fuels, bio-fuels produced from biomass have been considered to be pre-eminent. Relative to traditional grain materials, lignocellulosic biomass (*e.g.*, corn stalk, wheat straw, and switchgrass) is regarded as one of the most promising materials for the production of fuels (Jorgensen *et al*. 2007). As a relatively new candidate biomass, the herbaceous energy grass hybrid *Pennisetum* has attracted increasing research because of its various advantages, such as resistance to salinity and drought, suitability for marginal land quality, high productivity, low nutritional requirements, environmental benefits, and multipurpose uses (Bai *et al*. 1994; Chen *et al*. 2014).

Hybrid *Pennisetum* is one of the most prolific renewable cellulose resources, containing 36.04±1% cellulose, 20.11±1% hemicelluloses, and a relatively low content of lignin, 8.89±1% (Fan *et al*. 2012). The dissolution and subsequent hydrolysis of the cellulose are basic pre-requisites to produce bio-ethanol (Tadesse and Luque 2011). However, cellulose in the form of micro-fibrils with radii of 2 to 3 nm are enclosed within a rigid protective sheath of lignin and hemicelluloses to form a cell wall (Tadesse and Luque 2011; Brandt *et al*. 2013) that is intransigent to saccharification and hydrolysis

efforts. This is a natural circumstance because the rigid protective sheath and complex structure of lignocellulosic biomass make it resistant to biological and chemical degradation (Himmel *et al*. 2007). Therefore, efficient pretreatment before saccharification is key to unlocking its intrinsic recalcitrance by increasing porosity to accelerate enzyme accessibility (Zhang *et al*. 2009).

Pretreatment is a primary stage in an efficient and economic conversion of lignocellulosic biomass into bioethanol. Quite a number of physical, biological, and chemical methods have been proposed (Yang and Wyman 2008). However, various issues remain. For instance, physical pretreatment suffers from relatively poor performance, high-energy requirements, and high capital costs (Hendriks and Zeeman 2009), whereas a controllable and sufficiently rapid system has not yet been found for biological pretreatment (Chandra *et al*. 2007). To date, chemical pretreatment is considered to be the most promising option (Yang and Wyman 2008). A few chemical solvents show solubility for cellulose, such as dimethyl sulfoxide/tetra-N-butyl ammonium fluoride; N,N-dimethyl acetamide/lithium chloride; and N,N-dimethyl formamide/nitrous tetraoxide (Wang *et al*. 2012). However, all of them have drawbacks, such as poisonous gas production, high toxicity, difficulty in down-stream recovery, and unrecyclability (Zheng *et al*. 2009). Compared to traditional cellulose solvents, ionic liquids (ILs) have low melting points, low volatility and toxicity, high thermal and chemical stability, and an environmentally benign nature. Therefore, they have attracted increasing research for the dissolution and subsequent hydrolysis of lignocellulosic biomass (Zhao *et al*. 2009; Sun *et al*. 2011). For example, 1-allyl-3-methylimidazolium chloride ([Amin]cl), 1-butyl-3-methylimidazolium chloride ([Bmim]Cl), and 1-ethyl-3-methylimidazolium acetate ([Emim]Ac) are able to effectively dissolve cellulose (Feng and Chen 2008). With the addition of an anti-solvent (water, ethanol, or acetone), the dissolved cellulose can be easily regenerated (Zhu *et al*. 2006). The regenerated cellulose exhibits significantly increased porosity and reduced crystallinity, which enhances its digestibility (Kuo and Lee 2009). Among different ILs, [Amim]Cl, which possesses an asymmetrical structure, shows better solubility for cellulose samples than ILs having symmetrical structures (Liu *et al*. 2012).

An efficient and economic pretreatment process is affected by factors such as pretreatment temperature, solids loading, retention time, particle size, biomass type, and moisture (Badgujar and Bhanage 2015). These factors have a complex interaction with each other; therefore, optimization of the pretreatment process is important for improving the performance of the hydrolysis of biomass. The traditional 'one-factor-at-a-time approach' is time-consuming. Furthermore, the interactions between independent variables are not considered. Response surface methodology (RSM) is a statistical technique for modeling and analysis of the interactions of designated independent variables on final response (Kleijnen 2008). It has been widely used in fields such as food process operations (Altan *et al*. 2008), new product development, biotechnology, media composition, and bio-processing (Xin and Saka 2008). Many types of lignocellulosic biomass, including sugarcane bagasse, wheat straw, oil palm frond, and sago waste, have been pretreated by ILs and optimized by RSM to achieve efficient and economic conversion of lignocellulosic biomass into fermentable sugars (Fu and Mazza 2011; Yoon *et al*. 2012; Lee *et al*. 2013). For IL pretreatment optimization, temperature, retention time, and solids loading are considered to have significant effects on biomass digestibility (Tan *et al*. 2011; Fu and Mazza 2011). However, the parameters of pretreatment processing of hybrid *Pennisetum* with [Amim]Cl have not been optimized, and are thus the focus of this study.

The objectives of the current study were as follows: (1) to evaluate and optimize the effect of [Amim]Cl pretreatment parameters (temperature, solids loading, and retention time) to obtain the maximum glucose recovery using RSM and a factorial central composite design (CCD); and (2) to develop empirical predictive models for glucose recovery from hybrid *Pennisetum* under [Amim]Cl pretreatment.

EXPERIMENTAL

Materials and Chemicals

Hybrid *Pennisetum* was collected from an experimental field of the Beijing Academy of Agricultural Sciences (China), containing 33.64% cellulose, 18.05% hemicelluloses, and 11.03% lignin. It was milled with a FZ120 plant shredder (Truelab, Shanghai, China) and then sieved to 40 to 60 mesh. A comprehensive extraction process with toluene/ethanol (2:1, v/v) was performed in a Blst-250SQ Soxhlet apparatus (Bilon, Shanghai, China) for 5 h and oven-dried at 45 °C for 24 h. The extractives-free samples were stored at 4 °C in a sealed bag. The ionic liquid 1-allyl-3-methylimidazolium-chloride ([Amim]Cl) was purchased from the Lanzhou Institute of Chemical Physics (Lanzhou, China). Commercial cellulase (Celluclast 1.5 L) from *Tricoderma reesei* was purchased from Novozymes (China). All chemicals used were obtained from Sigma Chemical Co. (Beijing, China).

Methods

Design of experiments

Response surface methodology (RSM) is a collection of mathematical and statistical technique for designing experiments, developing regression models, analyzing effects of variables, and optimizing response curves (Ruangmee and Sangwichien 2013). In this study, the effect of three independent variables on the response was investigated using RSM and CCD. Three independent variables, *i.e.*, pretreatment temperature (X_1, 102 to 187 °C), retention time (X_2, 0.5 to 5.5 h), and solids loading (X_3, 2 to 15 wt%), were studied at five levels with three repetitions at the central point and two replicates at the axial and factorial points (Table 1). For each independent variable, the highest and lowest levels were selected based on the results achieved in preliminary tests. The central composite rotatable designed (CCD) matrix was generated with the aid of Design Expert 8.0.6 software (STAT-EASE Inc., Minneapolis, MN).

Table 1. Coded and Decoded Values for Each Variable of the Central Composite Rotatable Design

Variable	Coding	Unit	Coded Levels of the Experimental Factors				
			−2	−1	0	1	2
Temperature	X_1	°C	102	120	145	170	187
Retention time	X_2	h	0.48	1.5	3	4.5	5.52
Solids loading	X_3	wt%	2.27	5.0	9	13	15.73

A total of 20 experiment designs were randomly performed and consisted of eight trials for factorial design, six trials for axial points, and six trials for repetitions of the

central point. Three replicate confirmation experiments were conducted using the complete experimental design matrix shown in Table 2.

Table 2. Experimental Design Matrix Showing both Coded and Actual Values of Variables, as well as Observed and Predicted Responses

Run	Coded and actual values of variables			Glucose recovery (%)	
	Temperature X1, (°C)	Retention time X2, (h)	Solids loading X3, (wt%)	Observed	Predicted
1	120(-1)	4.5(1)	13(1)	28.3	31.46
2	145(0)	3(0)	15.73(1.682)	40	35.26
3	145(0)	5.52(1.682)	9(0)	37	33.84
4	145(0)	3(0)	9(0)	72.6	71.45
5	145(0)	3(0)	9(0)	65.8	71.45
6	102.96(-1.682)	3(0)	9(0)	35.8	34.47
7	145(0)	3(0)	9(0)	73.2	71.45
8	145(0)	3(0)	9(0)	67.8	71.45
9	187.04(1.682)	3(0)	9(0)	13.2	10.03
10	145 (0)	3(0)	2.27(-1.682)	33	33.24
11	120(-1)	1.5(-1)	5(-1)	32.5	32.8
12	170(1)	1.5(-1)	5(-1)	17.1	17.12
13	145(0)	3(0)	9(0)	75.2	71.45
14	170(1)	1.5(-1)	13(1)	24	27.17
15	145(0)	0.48(-1.682)	9(0)	37.5	36.16
16	120(-1)	1.5(-1)	13(1)	36.4	37.75
17	170(1)	4.5(1)	5(-1)	18.8	20.64
18	120(-1)	4.5(1)	5(-1)	39.1	39.12
19	170(1)	4.5(1)	13(1)	15.2	18.08
20	145(0)	3(0)	9(0)	73.3	71.45

Ionic liquid pretreatment

The first step of the pretreatment process was dissolution of material by [Amim]Cl. Various solids loading amounts of hybrid *Pennisetum* (2 to 15 wt%) were prepared by mixing the shredded hybrid *Pennisetum* powder with [Amim]Cl in screw-capped Synthware tubes (Synthware, Beijing, China). The pretreatment was performed in a preheated oil bath with a stirring rate of 450 rpm at various temperatures and reaction times. The conditions for biomass dissolution are given in Table 2. At the end of the reaction time, deionized water (90 °C) was added as an anti-solvent to the hybrid *Pennisetum*/[Amim]Cl slurry with an agitation rate of 250 rpm for 1 h to regenerate biomass. The mixture was centrifuged at 5000 rpm (Sigma, Beijing, China) to separate the solid (regenerated biomass) and liquid (IL and water) phases. The precipitate (regenerated biomass) was washed thoroughly with hot deionized water several times until a colorless supernatant was obtained. It has been reported that ILs are not detected in the colorless supernatant using Fourier transform infrared (FTIR) measurement (Arora *et al*. 2010). The precipitate (regenerated biomass) was freeze-dried to avoid the recrystallization of cellulose and collected for subsequent enzymatic hydrolysis (Cao *et al*. 2014).

Enzymatic hydrolysis

The enzymatic hydrolysis reaction was carried out in 25-mL stoppered conical flasks. First, 0.2 g of pretreated sample was suspended in 10 mL of 50 mM citrate acid-sodium citrate buffer (pH 4.8) at an enzyme (Celluclast 1.5 L, activity 84.5 FPU/g) loading of 20 FPU/g substrate. The flasks were incubated at 50 °C in an air bath shaking incubator

(ZWY-2102C, Shanghai, China) at 150 rpm for 72 h. After hydrolysis, the samples were kept in oven at 105 °C for 5 min to inactivate the enzyme.

The glucose concentrations from the enzymatic hydrolysates of pretreated samples were quantitatively analyzed by HPLC (Agilent 1200, USA) with a Bio-Rad Aminex HPX-87H analytical column and a refractive index detector. The mobile phase was 5 mM sulfuric acid solution with a volume flow rate of 0.6 mL/min. The column temperature was set at 35 °C. The temperature of RID was 50 °C, and the injection volume was 10 μL.

Under the optimal conditions, the yield of pretreatment samples relative raw materials was 59.32%. The yield of glucose was defined as the percentage of glucose produced from pretreated samples, which was calculated using Eq. 1 as proposed by Watanabe (2010).

$$\text{Yield}(\%) = \frac{\text{Amount of glucose produced}}{\text{Amount of Hybrid Pennisetum}} \times 100 \quad (1)$$

Statistical analysis

The results of the enzymatic hydrolysis experiments were analyzed using RSM (Design Expert 8.0.6 software). The experimental data were fit using a second-order polynomial quadratic equation to evaluate the effects of the variables in terms of linear, quadratic, and interactions on the response (glucose recovery), and determine the optimum process conditions. The polynomial quadratic model for the response, as shown in Eq. (2), was employed,

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

where y is the predicted response, β_0 is the constant coefficient, β_i is the i^{th} linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the ij^{th} interaction term, and X_i and X_j are the independent variables studied.

Each coefficient in Eq. 2 was computed and the interaction effects of the process variables on the response were obtained. The significance of the coefficients was checked by variance analysis (ANOVA). The quality of fitness of the polynomial quadratic model was expressed by the coefficient of determination, R^2, and the statistical significance was determined by the *F* test. Using the same program, three-dimensional plots were drawn to illustrate the effects of the interactions of pretreatment variables on glucose recovery. Optimization (maximizing glucose recoveries) of the fitted polynomial was determined by numerical optimization in Design Expert 8.0.6. Three additional replicates were performed to verify the validity of the optimum values predicted by the program.

RESULTS AND DISCUSSION

Model Development

The experimental data shown in Table 2 were fitted into the quadratic polynomial equations to predict the responses and determine the regression coefficients of the polynomial equation. The following models describe the glucose recovery in terms of coded factors (Eq. 3), and actual factors (Eq. 4); where A is the pretreatment temperature (°C), B is the retention time (h), and C is the solids loading (wt%). Similar equations have been reported for solvent-ionic liquid [Bmim]Cl pretreatment of palm (Tan *et al*. 2011), aqueous ionic liquid pretreatment of wheat straw (Fu and Mazza 2011), alkali-extrusion

pretreatment of big bluestem (Karunanithy and Muthukumarappan 2011), alkaline peroxide pretreatment of wheat straw (Qi *et al*. 2009), and alkali-extrusion pretreatment of rice husk (Bazargan *et al.* 2015).

$$\text{Glucose recovery} = 71.45 - 7.26 * A - 0.69 * B + 0.60 * C - 0.70 * A * B + 1.28 * A * C - 3.15 * B * C - 17.39 * A^2 - 12.89 * B^2 - 13.15 * C^2 \quad (3)$$

$$\text{Glucose recovery} = -595.27 + 7.72 * \text{Temperature} + 41.33 * \text{Time} + 14.67 * \text{Solid loading} + 1.86e^2 * \text{Temperature} * \text{Time} + 1.27e^2 * \text{Temperature} * \text{Solid loading} - 0.525 * \text{Time} * \text{Solid Loading} - 0.027887 * \text{Temperature}^2 - 5.73 * \text{Retention time}^2 - 0.82119 * \text{Solid loading}^2 \quad (4)$$

The significance of the developed models was analyzed by ANOVA and is shown in Table 3. The 'Prob > F' values were used to check the significance of the effects of each independent variable and quadratic and interaction term on the response. As indicated in Table 3, the '*F*' values of each model term suggest that A, BC, A^2, B^2, and C^2 had significant effects ($P < 0.05$) on the glucose recovery from hybrid *Pennisetum* from the [Amim]Cl pretreatment. It has been reported that A, C, B^2, C^2, AB, and AC had significant effects on the glucose recovery from palm under [Bmim]Cl pretreatment (Tan *et al*. 2011). However, the significance of independent variable C was not observed in this study. The effects of B and C terms on glucose recovery were not significant because their 'Prob > F' values are greater than 0.1. Although not significant, those factors could not be eliminated from the response model to maintain model hierarchy because the quadratic effects of B and C are significant (Tan *et al*. 2011).

The effects of the three independent variables on glucose recovery were analyzed using the regression equation. As observed from Eq. 4, pretreatment temperature, retention time, and solids loading all had a positive influence. However, all of the quadratic terms had a negative influence on glucose recovery from hybrid *Pennisetum* under [Amim]Cl pretreatment. This phenomenon showed that when the three variables were high, the quadratic terms dominated the glucose recovery. These findings did not agree well with previous studies, which indicated that some quadratic terms have a positive influence on glucose recovery (Fu and Mazza 2011; Tan *et al*. 2011). The reasons for this difference might be that a larger range of independent variables was used in this study or because of the different solvation properties between ILs and substrates (Xie *et al*. 2012). The magnitude of the coefficient of pretreatment temperature was also lower than that of the retention time and solid loading (Eq. 4). From the coefficent perspective, the effect of pretreatment temperature on glucose recovery was not as significant as retention time and solid loading. But, as can be seen from Table 3, the effect of pretreatment temperature (A) on glucose recovery was more significant than retention time (B) and solid loading (C). This contradictory result was reasonable because the pretreatment temperature value was higher than the retention time value and solids loading value, in Eq. 4.Similar results were reported for oil palm frond (Tan *et al*. 2011).

Effects of the Interactions of Pretreatment Variables on Glucose Recovery

Along with linear and quadratic terms, interactional terms also contributed to glucose recovery. The effects of the interactions of pretreatment variables on the production of

glucose were analyzed by RSM. Three-dimensional response surface and contour plots were generated to illustrate the interactions between any two variables. This was done by evaluating two variables at a time while fixing the remaining variable at a central level, as shown in Fig. 1(a)–(c).

Effect of pretreatment temperature and retention time

The effect of the interaction between pretreatment temperature and retention time on glucose recovery in enzymatic hydrolysis with a constant solids loading of 9 wt% is shown in Fig. 1(a). As the retention time and pretreatment temperature was increased from 0.5 to 4.5 h and 110 to 170 °C, respectively, the glucose recovery increased almost linearly, from 11.4% to 75.2%. Additionally, under an optimal retention time of 2.97 h, the glucose recovery increased from 47.8% to 72.2% with increasing pretreatment temperature. The glucose recovery exceeded 65% when the temperature was increased to 125 °C. It can be inferred that when the pretreatment temperature was higher than 125 °C, cellulose from hybrid *Pennisetum* was easily dissolved by [Amim]Cl and then hydrolyzed to glucose. This was consistent with previous studies in that the swelling and dissolution of cellulose in ILs are temperature-dependent (Xu *et al*. 2010). Thus, a higher pretreatment temperature may accelerate the rate of heat transfer between ILs and cellulose to destabilize the H-bonding network and enhance cellulose dissolution by solvation (Kilpeläinen *et al*. 2007; Xu *et al*. 2010; Badgujar and Bhanage 2015). Similar results showed that the glucose yield of bagasse pith in cellulase hydrolysis for 72 h had not yet reached its peak when the pretreatment temperature was 80 °C, while it gradually increased as pretreatment temperature increased to 120 °C (Wang *et al*. 2015). However, the opposite trend was observed when the reaction temperature was over the optimum temperature of 139 °C, which may have been due to simultaneous carbohydrate degradation when the temperature was too high (Swatloski *et al*. 2002; Sun *et al*. 2009; Brandt *et al*. 2010; Wang *et al*. 2011;).

In addition, the glucose recovery increased substantially from 37.5% to 72.2% when the retention time was increased from 0.5 h to 2.97 h at the optimal pretreatment temperature (139 °C). This result indicated that extended retention time of the biomass material with ILs can boost dissolution by efficient mass transfer/diffusion (Zavrel *et al*. 2009; Andanson *et al*. 2014). However, glucose recovery followed a slowly decreasing trend when retention time was prolonged over 2.97 h, which might be due to excessive retention time. A similar finding for excessive retention time on the pretreatment of lignocellulosic palm using [Bmim]Cl was also reported (Tan *et al*. 2011).

Effect of pretreatment temperature and solids loading

The interaction effect of pretreatment temperature and solids loading on the response observed by RSM is shown in Fig. 1(b), at a fixed retention time of 3 h. With a maximum solids loading of 15 wt%, the glucose recovery increased slowly from 16.6% to only 30%, as the pretreatment temperature increased. High solids loading does not only lead to heat and mass transfer limitation and nonuniformity of the system, but also promotes the formation of inhibitory compounds, which will inhibit subsequent cellulose hydrolysis (Kootstra *et al*. 2009; Noureddini and Byun 2010). Moreover, the same trend was observed when the solids loading was reduced to 3 wt%. It revealed that lower solids loading did not enhance the final glucose recovery, a result consistent with a previous study (Tan *et al*. 2011). The glucose recovery increased to a maximum and then decreased as the solids loading and pretreatment temperature increased from 3 to 15 wt% and 110 to 170 °C, respectively. The maximum glucose recovery (72.2%) was obtained under the conditions

of a solids loading of 9.06 wt% and pretreatment temperature of 139 °C.

With the optimal pretreatment temperature (139 °C), glucose recovery increased rapidly as the solids loading was increased from 3 to 9 wt%. Similarly, Tan *et al*. (2011) found that with a solids loading of 10 wt%, the glucose recovery increased substantially as pretreatment temperature increased. This revealed that higher solids loading did enhance the final glucose recovery. However, it has been reported that lower biomass loadings could accelerate cellulose dissolution (Sun *et al*. 2009; Wang *et al*. 2011). This difference might reveal no obvious correlation between the IL pretreatment conditions for maximum glucose recovery and IL dissolving conditions for maximum cellulose dissolution. The inconsistency might be due to the degradation of cellulose in ILs with low solid loading, difference in the characteristics of the biomass, or solvation properties of ILs (Badgujar and Bhanage 2015).

Effect of retention time and solids loading

The effect of interaction between retention time and solids loading on glucose recovery is shown in Fig. 1(c). As illustrated, the extremely low predicted value was obtained at low solids loading and short retention time. With an increase in retention time from 0.5 to 2.9 h, the predicted value of glucose increased almost linearly from -0.9% to 71.4% as the solids loading increased from 3 to 9 wt%. In practice, a negative value should not be found. In this three-dimensional response surface, a negative value demonstrated that the pretreatment conditions of low solids loading and short retention time were undesirable. Similar results were reported by Fu and Mazza (2011), which was a significant comprehensive prediction of pretreatment parameters for glucose recovery.

Thus, with optimal retention time, appropriately raising the solids loading can increase the economic feasibility of the [Amim]Cl pretreatment of hybrid *Pennisetum* and also has the potential to reduce reactor size during pretreatment. Increasing solids loading also leads to a more concentrated product stream, thereby increasing the efficiency of downstream processing while decreasing the operating costs (Kootstra *et al*. 2009).

Response Surface Model Evaluation

The responses of the predicted values and observed values along with coded and actual variables are presented in Table 2. The relationship between the observed values and predicted values is shown in Fig. 2. The predicted values of the response from the developed model agreed well with the observed ones in the range of the operating variables. The similarities of the predicted and observed responses reflect the goodness of fit.

The ANOVA for the statistical significance of the quadratic model is shown in Table 3. A model is deemed significant if its 'Prob > F' value (*p*-value) is smaller than 0.05 and its F-value is relatively high, suggesting only a 5% chance that a 'Model F-value' could occur due to confounders (Ruangmee and Sangwichien 2013). In this model, the F-value of 65 was higher when compared to that of similar models, such as Ruangmee and Sangwichien (2013) and Tan *et al*. (2011), with *F*-values of 36.76 and 13.3, respectively. This indicated that the model was highly significant. Moreover, the 'Prob > F' value was less than 0.0001, which implied that the regression equation was sufficient to explain the response. The lack of fit confirms the failure of the model to represent data in the experimental area at points, which are not included in the quadratic equation. The insignificant lack of fit further proves the goodness of the model in representing the reactions.

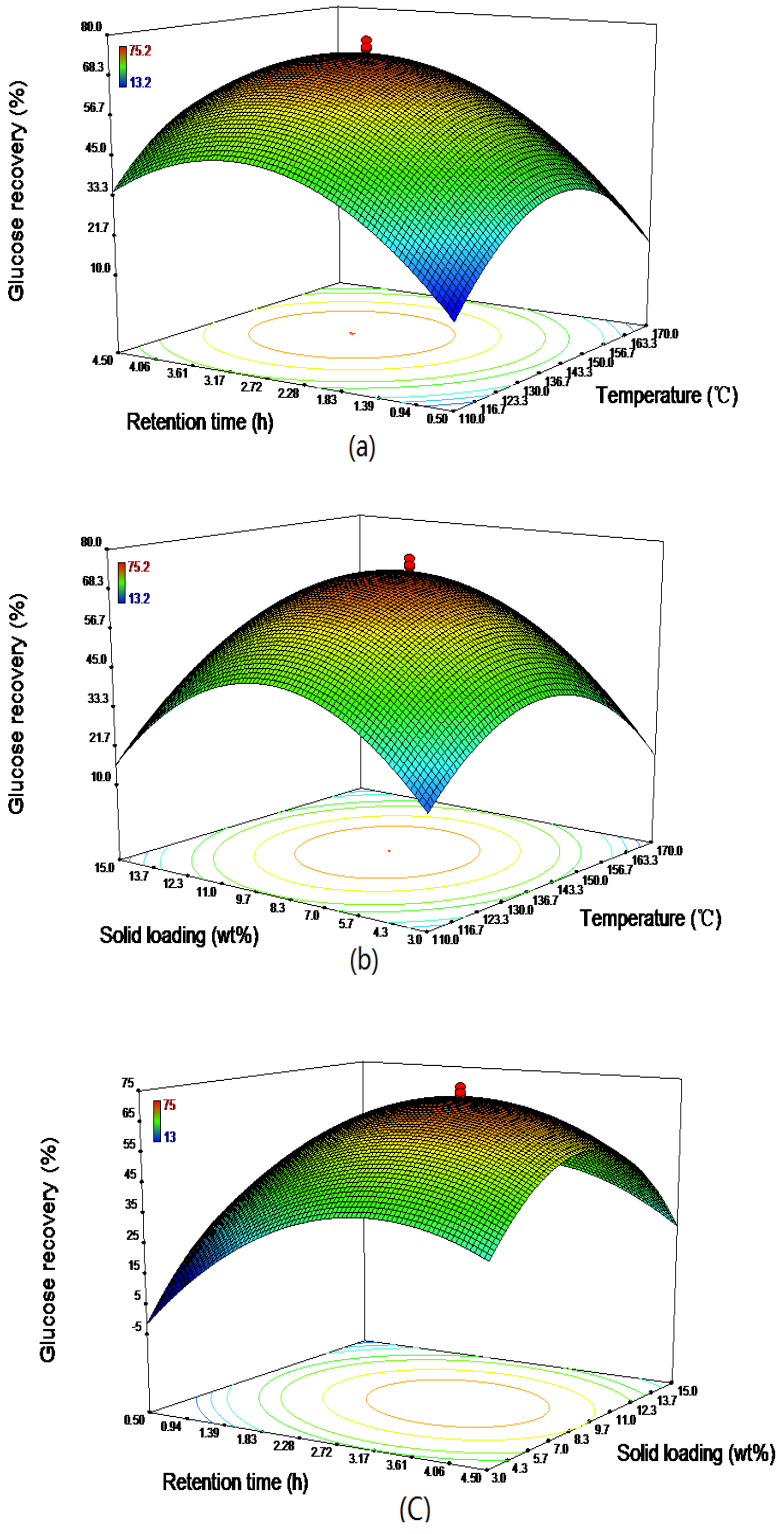

Fig. 1. Interaction effect of two independent variables on glucose recovery (other factors fixed at the center point: 145 °C, 3 h, and 9 wt%)

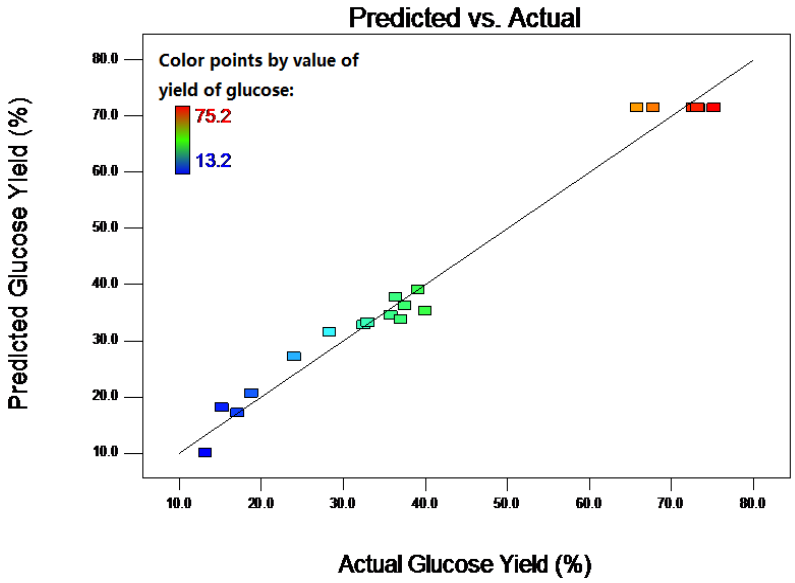

Fig. 2. Predicted glucose yield *vs*. actual glucose yield

Furthermore, the ANOVA for the fitness of the quadratic model is presented in Table 4. The coefficient of determination (R^2) is always between 0 and 1. The closer to 1.0 the R^2 is, the stronger the model is and the better it predicts the response value. Generally, a quadratic model with an R^2 value higher than 0.90 is considered to have a very high correlation (Haaland 1989). As can be seen from Table 4, the R^2 value of the model was 0.9832, which further suggested that the quadratic polynomial equations were suitable to adequately represent the relationships among the independent variables. However, a high R^2 value does not always mean that the fitness of the quadratic model is good. This is because R^2 will increase with the addition of a variable, regardless of whether the additional variable is significant or not (Xin and Saka 2008). Hence, the coefficient of adjusted determination (Adj-R^2) and the coefficient of predicted determination (Pred-R^2) were calculated to check the adequacy of the model. The Adj-R^2 rectifies the R^2 value for the sample size and for the number of terms. The Adj-R^2 is markedly smaller than R^2 if there are many variables in the model and a sample size that is not too large. In this model, the high value of Adj-R^2 (0.9681) implies a high significance of the model. The Pred-R^2 (0.9194) is in reasonable agreement with the adjusted determination coefficient, which is also satisfactory to verify the fitness of the model.

Another measure used to evaluate the precision degree of the model is the coefficient of variation (CV), represented as a percentage. It is the ratio of standard error to the mean values. In general, the CV should not be greater than 10% (Linko *et al*. 1984; Cadoche and Lopez 1989). Consequently, the value of CV, as shown in Table 4, revealed that the experiments conducted are precise and reliable. "Adeq Precision" measures the ratio of signal to noise. A larger ratio denotes better prediction; as a general rule, a ratio greater than 4 is desirable. In this regression model, the ratio of 22.661 suggests an adequate signal, which implies that the model can be used to navigate the design space (Liu *et al*. 2010). The residuals from the least squares are a significant tool for estimating the model adequacy. Additionally, the normal probability was checked by plotting the normal probability plot of the internally studentized residuals (Li *et al*. 2007). The nonlinear internally studentized residuals not only indicate that the model is against underlying

assumptions, but they also suggest that the error terms are not normally distributed (Alireza *et al*. 2015).

As shown in Fig. 3, a linear normal distribution of the observed data was verified. The plot of residuals versus the predicted response is shown in Fig. 4. The residual plots of the model exhibited randomly scattered data. This result indicates the adequacy of the quadratic models and good predictions of the response (Mu *et al*. 2006).

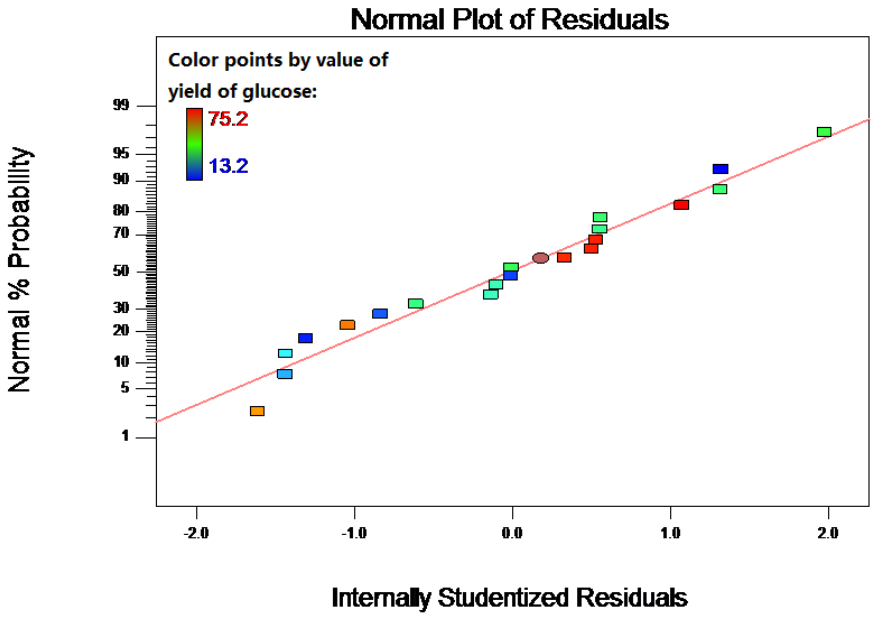

Fig. 3. Normal probability plot of residual errors for glucose yield

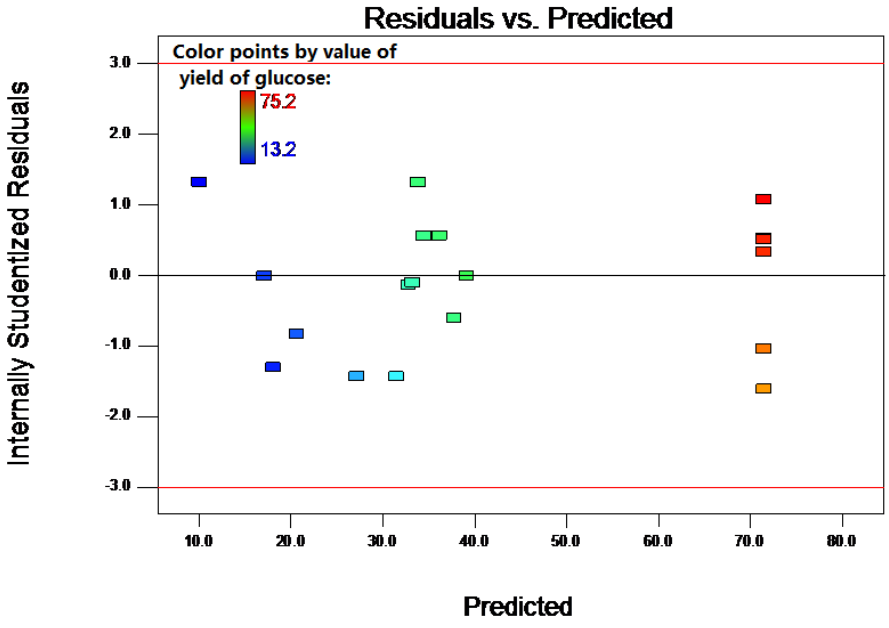

Fig. 4. Plot of internally studentized residuals *vs.* predicted response

Table 3. ANOVA Table for the Quadratic Model

Source	Sum of squares	DF [a]	Mean Square	F-value	Prob > F	
Model	8592.37	9	954.71	65	<0.0001	Significant
A	702.69	1	3475.5	49.06	<0.0001	
B	6.53	1	553.12	16.72	0.5201	
C	4.89	1	520.42	15.73	0.5767	
AB	3.92	1	0.25	0.00074	0.6167	
AC	13.01	1	15.68	0.47	0.3689	
BC	79.38	1	51.01	1.54	0.0424	
A2	4359.77	1	4378.04	132.32	<0.0001	
B2	2392.77	1	2607.88	78.82	<0.0001	
C2	2492.26	1	2506.08	75.74	<0.0001	
Residual	330.86	10	33.09			
a: Degree of freedom						

Table 4. Fitness of Quadratic Models

	Prob > F			Prob > F	
Pred-R^2	0.9194		Adj-R^2	0.9681	
Adeq precision	22.661		R^2	0.9832	
CV (%)	9.17	Lack of fit		0.4259	Insignificant

Optimization and Model Validation

Optimization has been defined as enhancing a system's performance to obtain the maximum benefit (Bezerra *et al*. 2008). Design Expert 8.0.6 software was used to predict the optimal conditions for the [Amim]Cl pretreatment. In the pretreatment process, an optimum yield of 72.2% of glucose was predicted using the quadratic model at pretreatment conditions of 2.97 h, 139 °C, and 9.06 wt% solids loading. To validate the optimum conditions, three replicate confirmation experiments were conducted, and the results are shown in Table 5.

Table 5. Verification Experiments at Optimum Conditions

	Response (yield of glucose, %)
Experimental value	
First replicate	68.35
Second replicate	71.50
Third replicate	70.85
Average	70.23
Predicted value by statistical model	72.21
Error	1.98

An average yield of 70.2% of glucose was obtained, which was consistent with the predicted values. These data show that the experimental values obtained were in agreement with the values calculated from the quadratic model developed using the software. Therefore, the current model is useful for predicting glucose recovery from hybrid *Pennisetum* under [Amim]Cl pretreatment.

CONCLUSIONS

1. [Amim]Cl pretreatment parameters were optimized for enhanced glucose recovery from the underutilized biomass hybrid *Pennisetum* by the proposed quadratic model. Under the temperature of 139 °C, retention time of 2.97 h, and solid loading of 9.06 wt-%, an optimal glucose recovery of 72.21% was obtained, which was confirmed by validation.
2. Statistical analyses revealed that the pretreatment temperature had a more significant effect on sugar recovery than retention time and solid loading.
3. The interactional effects of retention time and solid loading were greater than the interactional effects of temperature and solid loading on glucose recovery.
4. Compared to the interaction of retention time and temperature, that of temperature and solid loading has greater effect on glucose recovery.

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