Journal of Biochemical
& Microbial Technology

Srinivasarao B et al., J Biochem Microb Technol 2013, 1(1): 8–15
http://dx.doi.org/10.14312/2053-2482.2013-2

Original research

Open Access

Ethanol production from cashew apple juice using statistical designs

Srinivasarao B[1,2,4*], Ratnam BVV[3], Subbarao S[4], Narasimharao M[4,5] and Ayyanna C[4,5]

[1] KIMS Foundation and Research Centre, Minister Road, Secunderabad - 500003, India
[2] Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar- 522510, India
[3] Department of Neurology, Johns Hopkins University School of Medicine, Pathology 233/Meyer 222, 600 North Wolfe Street, Baltimore, MD 21287, USA
[4] Center for Biotechnology, Department of Chemical Engineering, College of Engineering, Andhra University, Visakhapatnam-530003, India
[5] Al-Ameer College of Engineering and Information Technology, Gudilova, Anandapuram, Visakhapatnam-531173, India

Abstract

Statistical experimental designs were carried out for enhancing the ethanol production from cashew apple juice after obtaining the biochemical analysis of cashew apple juice. The biochemical analysis of the cashew apple juice was as follows: 79.2 % moisture, 12.5 % total sugars, 4.9 % reducing sugars, 0.16 % protein, 0.2 % fat, 1.91 % tannin and 1.13 % ascorbic acid. Cashew apple juice can be preserved at -18oC for several months. The Plackett - Burman design was used initially to screen seven nutritional parameters which are critical and important for growth of *Saccharomyces cerevisiae* as well as enhancing ethanol production. Among them three parameters were found to have a significant effect on ethanol production and these three elements were further optimized using central composite design (CCD) by response surface methodology. The optimal values of three variables obtained for maximum production of ethanol was (g/l): ammonium chloride 0.45, magnesium sulfate 0.08 and dipotassium phosphate 0.21 and the predicted ethanol production was 61.34 g/l. With the predicted optimal values, experiments conducted in the laboratory yielded an ethanol production of 59.80 g/l equal to 93.62% of its theoretical yield. Statistical experimental designs such as Plackett-Burman design and CCD are critical and important for rapid screening and determining significant nutritional parameters and then optimizing them for enhancing the ethanol production.

Keywords: *Saccharomyces cerevisiae*; cashew apple juice; ethanol; response surface methodology; Plackett-Burman design; central composite design

Introduction

Ethanol is an important organic compound used as solvent in laboratories, industries, and households. Recently, the use of ethanol as an alternative fuel has received much attention as a solution for many problems caused by insufficient gasoline fuel obtained from depleting petroleum stocks. Ethanol can be blended with petrol or used directly in dedicated engines, taking advantage of the higher octane number and higher heat of vaporization; furthermore, it is an excellent fuel for future advanced flex fuel hybrid vehicles [1]. Nearly all fuel ethanol is produced by fermentation of sucrose in Brazil or by corn glucose in the USA; however, these raw material bases will not be sufficient to satisfy the international demand [2].

Ethanol is produced from various substrates such as saccharine, starchy and cellulose materials. Selection of suitable and cheap substrate is an important cost component for industrial ethanol production. Agricultural based substrates are more economical and alternative substrates for the production of ethanol. The cashew apple is an agricultural based product which has a high concentration of reducing sugars [3]. Cashew apple, a product of the fruit of the cashew tree *(Anacardium*

***Corresponding author:** Srinivasarao B, KIMS Foundation and Research Centre, Minister Road, Secunderabad - 500003, India, Email: srinumicros@yahoo.co.in

Received 23 April 2013 *Revised* 12 June 2013 *Accepted* 19 June 2013 *Published* 28 June 2013

Citation: Srinivasarao B, Ratnam BVV, Subbarao S, Narasimharao M, Ayyanna C (2013) Ethanol production from cashew apple juice using statistical designs. J Biochem Microb Technol 1:8-15. doi:10.14312/2053-2482.2013-2

occidentale), is a resilient and fast-growing evergreen tree that can grow in tropical and subtropical countries and the production is far greater in areas with a distinct wet and dry season, such as its native range in India, America, Brazil, Nigeria and East Africa. A four year old tree can produce 100 to 140 kg of cashew apple per year. The cashew apple is very sour and astringent until fully ripe when it becomes edible. The fruit reaches the maturity stage, the cashew apple and its nut fall to the ground (Figure 1), and are collected daily. After collection, the nut is removed from the apple (Figure 2). A negligible portion of apple is consumed by the harvesters but the major portion of the apple goes as waste. Utilization of such waste is an economical and an alternative source for ethanol production.

Figure 1 Harvested cashew apples with nuts

Figure 2 Cashew apples ready for experiment

Enhancing of ethanol production involved two important tools such as screening of nutritional parameters and optimization of the selected parameters. In the first step for screening, several research studies showed that the Plackett-Burman design was used for screening of fermentation parameters such as production of alpha-galactosidase by *Aspergillus niger* in solid state fermentation system [4] and production of ethanol from cellulosic biomass by *Clostridium thermocellum* in submerged fermentation [5]. The Plackett - Burman design employs a design that allows testing of the largest number of factor effects with the least number of observations resulting the factors which are more significant on the response. In the second step of optimizing the traditional 'one factor at a time' technique used for optimizing a multivariable system is time consuming and misses alternative effects between components. It also requires carrying out a number of experiments to determine the optimum levels which are not correct. Avoiding of these drawbacks, recently several researchers have been employed different statistical experimental design methods in bioprocess optimization. Among them, response surface methodology (RSM) using a central composite design (CCD) is a suitable method for identifying the effect of individual variables and optimum conditions for a multivariable system efficiently. CCD was used previously for optimization of fermentation conditions/parameters for the production of ethanol from sago starch [6], from Palmyra jaggery [7], finger millet medium [8, 9], rapeseed straw [10] and optimization of medium constituents for the production of citric acid from pineapple waste [11].

In the present study, biochemical data of cashew apple juice were also presented because the biochemical components are critical and important sources for the growth of *Saccharomyces cerevisiae* in the fermentation medium as well as for ethanol production. Further studies were conducted for screening and optimization of nutritional parameters for enhancing the ethanol production by *Saccharomyces cerevisiae* from cashew apple juice using statistical experimental designs. Substrate is the main cost component for industrial ethanol production and it is essential that ethanol production should be carried out with cheaper substrates [12, 13]. In the natural environment, plant and biological materials are good sources for alcohol production since they are renewable, economical, cheaper and easily available raw materials [14–17].

Materials and methods

Substrate

The fresh cashew apple juice is slightly yellowish, transparent, astringent *flavoured*, typical of the aroma, which is extracted from cashew apples. The cashew apples are collected from farms of Tadepalligudem, West Godavari District, A.P., India.

Biochemical analysis of cashew apple juice

The substrate was biochemically analyzed for moisture content, protein, fat, initial pH, total sugars, reducing sugars, tannin and ascorbic acid. All these tests were conducted using standard protocols.

Microorganism and growth conditions

Saccharomyces cerevisiae NCIM 3090, obtained from the National Chemical Laboratory, Pune, India, maintained on a nutrient agar slant at 4°C, was used in this study. The nutrient agar medium had the following composition (%w/v): Glucose 1.0, malt extract 0.3, yeast extract 0.3, peptone 0.5 and agar-agar 2.0. The pH of the medium was adjusted to 6.4-6.8 and incubated at 30oC for 48h.

Fermentation conditions

The experiments were carried out in 5 l (4 l working volume) laboratory bioreactor (B-lite, Sartorious Pvt. Ltd., Mumbai, India), with computer controlled and recorded parameters. The bioreactor was sterilized at 121°C for 15 min. in an autoclave after addition of 4 l of cashew apple juice and cooled to room temperature. The cultivation was started by inoculating 4% (v/v) seed culture (exponential phase) into a sterile medium in aseptic conditions. The cultural condition was controlled at 30°C, pH 5.0 (using 0.1 M HCl/NaOH). As a source the nutrients the salts chosen for the study with their concentration ranges were as follows (g/l): $ZnSO_4$ 0.005 to 0.015, $MnSO_4$ 0.002 to 0.006, NH_4Cl 0.2 to 0.6, $FeSO_4$ 0.02 to 0.06, $MgSO_4$ 0.05 to 0.015, K_2HPO_4 0.1 to 0.3 and $CaCl_2$ 0.002 to 0.006. Stock solutions of these salts were prepared and added to the production medium before autoclaving as per the experimental design. All runs were carried out in duplicate and the average of the ethanol produced on the second day was considered.

Analytical methods

Ethanol was estimated by GC in which a flame ionization detector and a stainless steel column (2.0 m length, 3.0 mm I.D.) packed with Porapak-Q (50-80 mesh, manufactured by Nucon Engineers, India) were used. The column oven was operated isothermally at 150°C and the detector and injection ports were kept at 170°C. Nitrogen was used as the carrier gas at a flow rate of 30 cm^3/min and the combustion gas was a mixture of hydrogen and air [7]. Sugar was determined using Miller's method [18].

Statistical experimental designs for screening and optimization

The Plackett-Burman experimental design is a factorial design which is used in this study to demonstrate the relative importance of medium supplements. Seven independent variables (Table 1) in nine combinations were organized according to the Plackett-Burman design matrix (Tables 2 and 3). For each variable, a low (-1) and a high (+1) levels were tested. All trials were performed in duplicate and the mean of the response was considered. Using the data, Pareto chart was generated which revealed the most significant parameters that could contribute to ethanol formation.

Table 1 Variables for screening using PBD

Variable	*(-) Level*	*(+) Level*
Zinc sulfate ($ZnSO_4$) (g/l)	0.005	0.015
Manganese sulfate ($MnSO_4$) (g/l)	0.002	0.006
Ammonium chloride (NH_4Cl) (g/l)	0.2	0.6
Ferrous sulfate ($FeSO_4$) (g/l)	0.02	0.06
Magnesium sulfate ($MgSO_4$) (g/l)	0.05	0.15
Dipotassium hydrogen phosphate (K_2HPO_4) (g/l)	0.1	0.3
Calcium chloride ($CaCl_2$) (g/l)	0.002	0.006

Table 2 The Plackett-Burman design matrix representing the coded values for 7 independent variables.

Run	*$ZnSO_4$*	*$MgSO_4$*	*$CaCl_2$*	*$FeSO_4$*	*$MnSO_4$*	*K_2HPO_4*	*NH_4Cl*
1	-1	-1	-1	1	1	1	-1
2	-1	-1	1	1	-1	-1	1
3	-1	1	-1	-1	1	-1	1
4	-1	1	1	-1	-1	1	-1
5	1	-1	-1	-1	-1	1	1
6	1	-1	1	-1	1	-1	-1
7	1	1	-1	1	-1	-1	-1
8	1	1	1	1	1	1	1
9	-1	-1	-1	-1	-1	-1	-1

Table 3 The Plackett-Burman experimental design matrix for screening of important variables for ethanol production.

Run	*$ZnSO_4$ (g/l)*	*$MnSO_4$ (g/l)*	*NH_4Cl (g/l)*	*$FeSO_4$ (g/l)*	*$MgSO_4$ (g/l)*	*K_2HPO_4 (g/l)*	*$CaCl_2$ (g/l)*	*Ethanol (g/l)*
1	0.005	0.002	0.2	0.06	0.15	0.3	0.002	49.05
2	0.005	0.002	0.6	0.06	0.05	0.1	0.006	52.13
3	0.005	0.006	0.2	0.02	0.15	0.1	0.006	54.01
4	0.005	0.006	0.6	0.02	0.05	0.3	0.002	46.57
5	0.015	0.002	0.2	0.02	0.05	0.3	0.006	54.23
6	0.015	0.002	0.6	0.02	0.15	0.1	0.002	46.41
7	0.015	0.006	0.2	0.06	0.05	0.1	0.002	53.28
8	0.015	0.006	0.6	0.06	0.15	0.3	0.006	40.12
9	0.005	0.002	0.2	0.02	0.05	0.1	0.002	59.36

CCD [19] was used in the optimization of ethanol production by the three significant variables given by PBD namely, ammonium chloride (X_1, g/l), magnesium sulfate (X_2, g/l) and dipotassium hydrogen phosphate (X_3, g/l) which were shown in Table 4. Ethanol concentration (Y, g/l) was used as the dependent output variable. For statistical calculations the variables X_i were coded as x according to Equation (1)

$$x_i = \left(X_i - \bar{x}_i\right) / (\Delta x_i) \ (= 1,2,3,\ldots\ldots\ldots, k) \quad \text{------------ (1)}$$

Where x_i is the dimensionless value of an independent variable, X_i is the real value of an independent variable, $\bar{x}_i$ is the real value of an independent variable at the center point, and Δx_i is a step change.

Table 4 Independent variables in the experimental plan for CCD.

Variables	*Coded levels*				
	-1.632	-1	0	1	1.632
NH_4Cl (g/l), X_1	0.073	0.2	0.4	0.6	0.726
$MgSO_4$ (g/l), X_2	0.018	0.05	0.10	0.15	0.181
K_2HPO_4 (g/l), X_3	0.036	0.1	0.2	0.3	0.363

A 2^3-factorial CCD, with six axial points ($a = \sqrt{3}$) and six replications at the center points (no=6) leading to a total number of 20 experiments was employed (Table 5) for the optimization of the constituents of fermentation. The second degree polynomials (Equation 2) were calculated with the statistical package (Stat-Ease Inc, Manneapolis, MN, USA) to estimate the response of the dependent variable.

$$Yi = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad \ldots\ldots\ldots(2)$$

Where Y_i is the predicted response, X_1, X_2, X_3 are independent variables, $\beta 0$ is offset term, $\beta 1$, $\beta 2$, $\beta 3$ are linear effects, $\beta 11$, $\beta 22$, $\beta 33$ are squared effects and $\beta 12$, $\beta 13$, $\beta 23$ are interaction terms.

Results and discussion

The biochemical analysis of the cashew apple juice was as follows: 79.2 % moisture, 12.5 % total sugars, 4.9 % reducing sugars, 0.16 % protein, 0.2 % fat, 1.91 % tannin and 1.13 % ascorbic acid. These values are compared with those from the literature [20, 21] and they are found to be in good agreement. These values also indicate the cashew apple juice contains the essential biochemical components required for the growth of Saccharomyces cerevisiae in the fermentation medium and can be directly used for the ethanol production with any pre-treatment.

The results of Plackett-Burman design used to screen and identify the significant nutritional parameters among the selected were listed in Table 3 and Pareto graph (Figure 3) showing the effect of various nutritional parameters on ethanol production.

NH_4Cl, $MgSO_4$ and K_2HPO_4 were shown as significantly enhancing the yield whose probability values are above 0.05. Supplementation of the rest of the elements to the substrate may slightly contribute but not significant to product formation. Hence their probability values are under 0.05 and hence they may be avoided.

RSM is a sequential procedure with an initial object to lead the experimenter rapidly and efficiently to the general vicinity of the optimum. Since the location of the optimum is unknown prior to the running of the RSM experiments, it makes sense to use a design that provides equal precision of estimation in all directions, in other words ratability is a very important property in the selection of an RSM. 20 experimental runs were carried out according to three variables CCD. As per the design, various combinations of the three parameters selected from PBD and used along with the results were summarized in Table 5. A quadratic equation was fitted to the data obtained as in Table 5, using multiple linear regressions available in STATISTICA 6 software (Equation 3). The significance of each coefficient was determined by

Table 5 Experimental design and results of central composite design.

Run No.	X_1		X_2		X_3		Ethanol (g/l)	
	Coded value	*Actual value*	*Coded value*	*Actual value*	*Coded value*	*Actual value*	*Experimental*	*Predicted*
1	-1	0.2	-1	0.05	-1	0.1	40.87	41.83
2	-1	0.2	1	0.15	1	0.3	43.61	47.85
3	1	0.6	-1	0.05	1	0.3	51.39	53.80
4	1	0.6	1	0.15	-1	0.1	39.25	41.49
5	0	0.4	0	0.10	0	0.2	59.62	59.63
6	0	0.4	0	0.10	0	0.2	59.62	59.63
7	-1	0.2	-1	0.05	1	0.3	41.57	39.53
8	-1	0.2	1	0.15	-1	0.1	47.96	45.76
9	1	0.6	-1	0.05	-1	0.1	55.94	51.90
10	1	0.6	1	0.15	1	0.3	48.53	47.78
11	0	0.4	0	0.10	0	0.2	59.62	59.63
12	0	0.4	0	0.10	0	0.2	59.62	59.63
13	-1.632	0.073	0	0.10	0	0.2	40.56	40.06
14	1.632	0.726	0	0.10	0	0.2	48.05	48.22
15	0	0.4	-1.632	0.018	0	0.2	49.86	51.61
16	0	0.4	1.632	0.181	0	0.2	51.98	49.90
17	0	0.4	0	0.10	-1.632	0.036	44.72	46.67
18	0	0.4	0	0.10	1.632	0.363	52.21	49.93
19	0	0.4	0	0.10	0	0.2	59.62	59.63
20	0	0.4	0	0.10	0	0.2	59.62	59.63

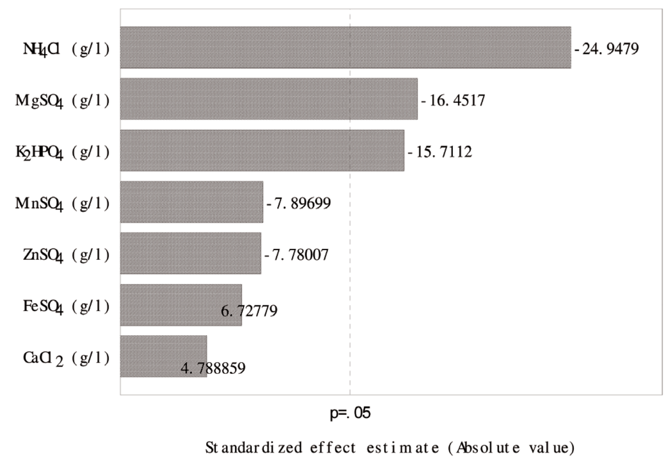

Figure 3 Pareto graph showing the effect of various nutritional parameters on ethanol production based on the results of PBD.

student's t-test and p-values which are listed in Table 6. The larger the magnitude of the t-value and the smaller the p-value, the more significant is the corresponding coefficient [22]. The best model for maximizing ethanol production by response surface methodology was the following quadratic polynomial model.

$Y_i = 59.6379 + 2.4998\,X_1 - 0.5218\,X_2 + 0.9983\,X_3 - 5.8103\,X_1^2 - 3.3297\,X_2^2 - 4.2503\,X_3^2 - 3.5850\,X_1X_2 + 1.0475\,X_1X_3 + 1.0975\,X_2X_3$ ……….. (3)

The goodness of the model can be checked by different criteria. The coefficient of determination, R^2 is 0.9297, implies that 92.97 % of the sample variation in the ethanol production is attributed to the independent variables. The corresponding analysis of variance (ANOVA) is presented in Table 7. The predicted levels of ethanol production from cashew apple juice medium using the above equation are given in Table 8 along with experimental data. The predicted optimum levels of NH_4Cl, $MgSO_4$ and K2HPO$_4$ were obtained by applying the regression analysis to the Equation (3). The predicted and experimental ethanol production at the optimum levels of the medium constituents were also determined

Table 6 Model co-efficient estimated by multiple linear regression.

Term	*Coefficient*	*Value*	*Std. error*	*t-value*	*p-value*
Constant	b_0	59.6379	1.0933	54.5458	1.00E-14
NH_4Cl	X_1	2.4998	0.7362	3.3952	0.00687*
$MgSO_4$	X_2	-0.5218	0.7362	-0.7087	0.49463
K_2HPO_4	X_3	0.9983	0.7362	1.3559	0.20494
NH_4Cl x NH_4Cl	X_1^2	-5.8103	0.7398	-7.8537	0.00001*
$MgSO_4$ x $MgSO_4$	X_2^2	-3.3297	0.7398	-4.5007	0.00111*
K_2HPO_4 x K_2HPO_4	X_3^2	-4.2503	0.7398	-5.7451	0.00011*
NH_4Cl x $MgSO_4$	$X_1 X_2$	-3.5850	0.9505	-3.7715	0.00362*
NH_4Cl x K_2HPO_4	$X_1 X_3$	1.0475	0.9505	1.1020	0.29622
$MgSO_4$ x K2HPO4	$X_2 X_3$	1.0975	0.9505	1.1546	0.27501

*p ≤ 0.05 indicating that the factors are significant.

(Table 8) by using Equation (3). The F values for models and for each of the response variables were calculated by dividing the mean square due to model variance by that due to error variance.

Table 7 ANOVA for the entire quadratic model

Source of variation	*Sum of squares (SS)*	*Degrees of freedom (df)*	*Mean squares (MS)*	*F value*	*Probe>F*
Model	956.848	9	106.316		
Error	72.281	10	7.2281	14.708	0.0068
Total	1029.129	19			

Coefficient of determination (R^2) = 0.9297

Table 8 Optimum values of fermentation parameters and the experimental and predicted ethanol production

Variables	*Optimum values*	*Ethanol (g/l)*	
		Experimental	*Predicted*
NH_4Cl	0.4585		
$MgSO_4$	0.0892	59.80	61.34
K_2HPO_4	0.2125		

The parity plot (Figure 4) showed a satisfactory correlation between the experimental and predicted values (obtained from Eq. 3) of ethanol production, wherein, the points cluster around the diagonal line which indicated the optimal fit of the model. The 3D response surface plots described by the regression model were drawn to illustrate the effects of the independent variables, and combined effects of each independent variable upon the response variable (Figures 5 to 7). Figure 5 illustrates the 3D response surface based on the Yi response against NH_4Cl (X_1) and $MgSO_4$ (X_2) with K_2HPO_4 maintained at 0.2 g/l. An increase in NH_4Cl with a simultaneous increase in $MgSO_4$ has led to an initial increase in ethanol production till they reached their optimal values. The data obtained by varying concentrations of NH_4Cl (X_1) and K_2HPO_4 (X_3) keeping $MgSO_4$ at 0.10 g/l, were plotted in Figure 6. It shows that an increase in NH_4Cl with a simultaneous increase in K_2HPO_4 resulted in an increase in production of ethanol. And an increase beyond a limit has affected the production. Figure 7 shows the response surface plot illustrating the effect of $MgSO_4$ (X_2) and K_2HPO_4 (X_3) on ethanol production keeping NH_4Cl at 0.4 g/l. A typical response surface plot reveals that ethanol production is lower at lower as well as higher levels of both the nutrients and at a certain optimal value the yield is high. The optimum values for maximum ethanol production obtained were 0.45 g/l of ammonium chloride, 0.08 g/l of magnesium sulfate and 0.21 g/l of dipotassium hydrogen phosphate. A final run was carried out maintaining these critical values of parameters and the ethanol production obtained was 59.80 g/l which was very much close to the predicted value.

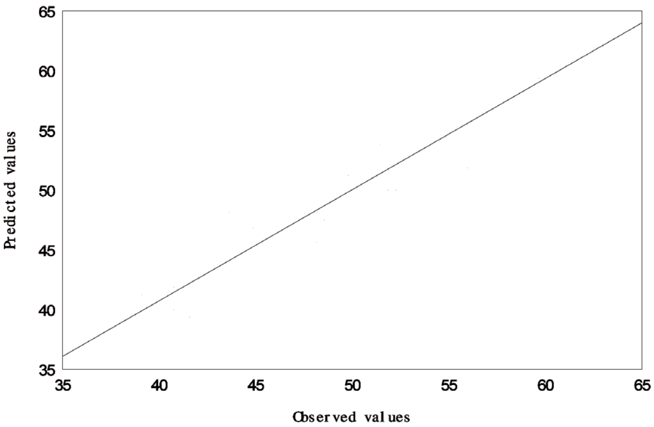

Figure 4 Parity plot showing the distribution of experimental vs. predicted of ethanol production.

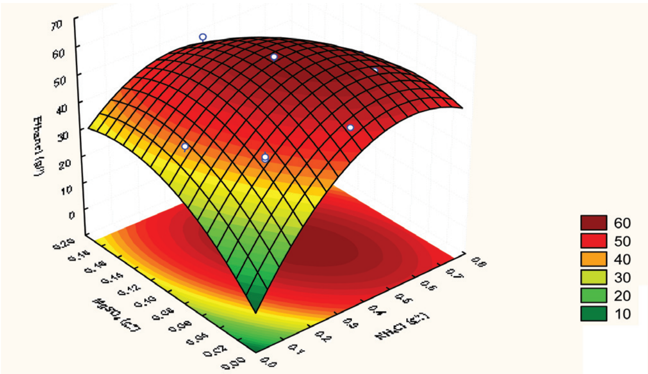

Figure 5 Response surface and contour plot of ammonium chloride (X_1) vs magnesium sulfate (X_2) on ethanol production (dipotassium hydrogen phosphate was kept constant at 0.2 g/l).

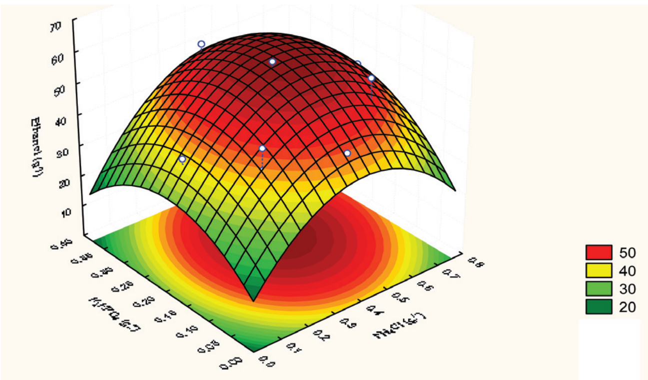

Figure 6 Response surface and contour plot of ammonium chloride (X_1) vs dipotassium hydrogen phosphate (X_3) on ethanol production (magnesium sulfate was kept constant at 0.10 g/l).

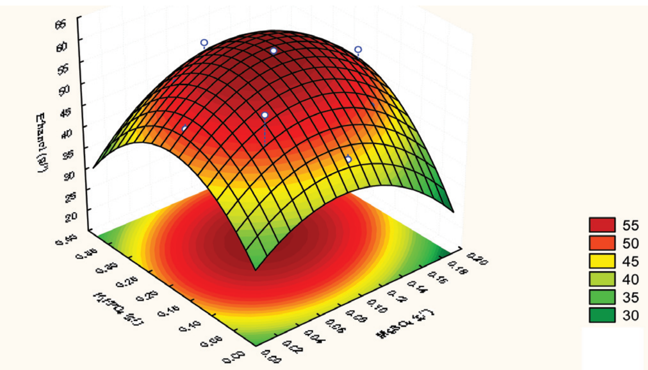

Figure 7 Response surface and contour plot of magnesium sulfate (X_2) vs dipotassium hydrogen phosphate (X_3) on ethanol production (ammonium chloride was kept constant at 0.4 g/l).

Conclusions

The biochemical analysis of the cashew apple juice was as follows: 79.2 % moisture, 12.5 % total sugars, 4.9 % reducing sugars, 0.16 % protein, 0.2 % fat, 1.91 % tannin and 1.13 % ascorbic acid. These values indicate that the cashew apple juice can be directly used for ethanol production without any pre-treatment. Cashew apple juice can be preserved at -18^{o}C for several months. The result of the present study using response surface methodology proves undoubtedly the closeness of the experimental result and theoretically predicted values, which reflected the accuracy and applicability of RSM to optimize the process for maximum production of ethanol using Saccharomyces cerevisiae NCIM 3090 in a bioreactor. The screening of 7 parameters in the Plackett - Burman design was done, out of which three parameters were found to contribute significantly to ethanol production. A central composite design was used to identify exactly the optimal concentrations of three parameters that can result in optimal yields of ethanol. 20 experimental runs were carried out according to the chosen experimental design and a quadratic equation was fitted. An R^2 value of 0.9297 was obtained which indicates that 92.97 % variability could be explained by the model. The predicted ethanol production was 61.34 g/l and a final run with the obtained optimal values was carried out which resulted ethanol production of 59.80 g/l equal to 93.62% of its theoretical yield.

Conflicts of Interest

The authors wish to express that they have no conflict of interest

References

[1] Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund MF, Lidén G, Zacchi G (2006) Bio-ethanol--the fuel of tomorrow from the residues of today. Trends Biotechnol 24:549–556.

[2] Rosillo Calle, F, Cortez LAB (1998) Towards ProAlcohol II - A review of the Brazilian bioethanol programme. Biomass and Bioenergy 14:115–124.

[3] Pinheiro AD, Rocha MV, Macedo GR, Gonçalves LR (2008) Evaluation of cashew apple juice for the production of fuel ethanol. Appl Biochem Biotechnol 148:227–234.

[4] Srinivas MRS, Nagin Chand, Lonsane BK (1994) Use of Plackett-Burman design for rapid screening of several nitrogen sources, growth / product promoters, minerals and enzyme inducers for the production of alpha-galactosidase by *Aspergillus niger* MRSS 234 in solid state fermentation system. Bioprocess Biosyst Eng 10:139–144.

[5] Balusu R, Paduru RM, Seenayya G, Reddy G (2004) Production of ethanol from cellulosic biomass by *Clostridium thermocellum* SS19 in submerged fermentation: screening of nutrients using Plackett-Burman design. Appl Biochem Biotechnol 117:133–141.

[6] Bandaru VV, Bandaru SR, Somalanka SR, Mendu DR, Imandi SB, et al. (2007) Optimization of fermentation parameters to enhance the production of ethanol from palmyra jaggery using *Saccharomyces cerevisiae* in a batch fermentor. Appl Biochem Biotechnol 143:224–235.

[7] Ratnam BVV, Narasimha Rao M, Damodara Rao M, Subbarao S, Ayyanna C (2003) Optimization of fermentation conditions for

the production of ethanol from sago starch using response surface methodology. World J. Microbiol Biotechnol 19: 523–526.

[8] Pradeep P, Krishna Goud GK, Reddy OVS (2010) Optimization of very high gravity (VHG) finger millet (Ragi) medium for ethanolic fermentation by yeast. Chiang Mai J Sci 37: 116–123.

[9] Pradeep P, Reddy OVS, Mohan PR, Sanghoon KO (2012) Process optimization for ethanol production from very high gravity (VHG) finger millet medium using response surface methodology. Iranian journal of biotechnology 10:168–174.

[10] López-Linares J. C, Romero, I Cara, C Ruiz, E Castro E, et al. (2013) Experimental study on ethanol production from hydrothermal pretreated rapeseed straw by simultaneous saccharification and fermentation. J Chem Technol Biotechnol doi: 10.1002/jctb.4110.

[11] Imandi SB, Bandaru VV, Somalanka SR, Bandaru SR, Garapati HR (2008) Application of statistical experimental designs for the optimization of medium constituents for the production of citric acid from pineapple waste. Bioresour Technol 99:4445–4450.

[12] Lee JM, Woodward J (1983) Properties and application of immobilized beta-D-glucosidase coentrapped with Zymomonas mobilis in calcium alginate. Biotechnol Bioeng 25:2441–2451

[13] Elisson A, Hofmeyr JHS, Pedler S, Hahn-Hagerdal B (2001) The xylose reductase/xylitol dehydrogenase/xylulokinase ratio affects product formation in recombinant xylose-utilizing *Saccharomyces cerevisiae*. Enzyme Microb Technol 29:288–297.

[14] Obisanyo MO, Aina JO, Oguntimehin GB (1987) Production of wine from mango (Mangifera indica L.) using *Saccharomyces* and *Schizosaccharomyces* species isolated from palm wine. J Appl Bacteriol 63:191–196.

[15] Kristine W, Galbally I, Ye Y, Hooper M (1998) Emissions of volatile organic compounds 25 (primarily oxygenated species) from pasture. J Geophys Res 103:10605–10619.

[16] Lamanna MS, Goldstein AH (1999) In situ measurements of C 2-C 10 volatile organic compounds above a Sierra Nevada ponderosa pine plantation. J Geophys Res 104: 21247–21262.

[17] Ezeronye OU (2004) Nutrient utilization profile of *Saccharomyces cerevisiae* from palm wine in tropical fruit fermentation. Antonie Van Leeuwenhoek 86:235–239.

[18] Miller GL (1959) Use of DNS reagent for determination of reducing sugars. Anal Chem 31:426–438.

[19] Box GEP, Wilson KB (1951) On the experimental attainment of optimum conditions. J R Statist Soc 13:1–38.

[20] Sivagurunathan P, Sivasankari S, Muthukkaruppan SM (2010) Characterization of cashew apple *(Anacardium occidentale)* fruits collected from Ariyalur District. J Biosci Res 1:101–107.

[21] Mohanthy S, Ray P,Swain M. R, Ray R. C (2006) Fermentation of cashew *(Anacardium occidentale L.)* “Apple” into wine. J. Food Process Preserv 30:314–322.

[22] Imandi S. B, Bandaru V. R, Somalanka S. R,Garapati H. R (2007) Optimization of medium constituents for the production of citric acid from byproducts using Doehlert experimental design. Enzyme Microb Technol 40: 1367–1372.