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# Optimization of microbial hydrogen production using statistical experimental design from maize stalk by isolated strain

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Experimental designs have been applied for optimizing the process parameters for hydrogen production from maize stalk hydrolyzate by a newly isolated facultative strain. Using Plackett-Burman design glucose, yeast extract, malt extract, peptone, and NaCl have been identified as significant variables influencing hydrogen production and these variables are subsequently optimized using a central composite design (CCD). Box-Behnken design has been adopted for optimizing the process parameters for maximum hydrogen production. Using this statistical optimization method a maximum hydrogen yield of 0.91 mol $H_2$/mol glucose has been achieved.

**Keywords:** Hydrogen production, Facultative strain, Hydrolysate, Maize stalk, Statistical experimental design

Hydrogen energy is now considered as the most promising alternative to fossil fuels, as it has fewer side effects and has less environmental damage[1]. It is preferred to biogas or methane because hydrogen is not chemically bound to carbon and combustion provides only water[2]. Besides, hydrogen has a high energy yield of 122 KJ/g which is 2.75 times greater than the hydrocarbon fuel[3,4]. It has been reported that 50 million tons of hydrogen are traded annually worldwide with a growth rate of nearly 10% per year[5]. Currently, 90% of commercially usable hydrogen is obtained by steam reformation of natural gas apart from coal gasification and water electrolysis which are not eco friendly[6,7]. Other methods of hydrogen production are photocatalytic and biological routes. Biological hydrogen production can be achieved either via photo fermentation[8,9] or dark fermentation[10]. Though hydrogen production through photo fermentation is high, it is not applicable, as it needs continuous supply of light energy and the difficulties associated with the design of the reactor[11]. Hence, the production of hydrogen via dark fermentation seems to gain more prominence. Biomass, one of the renewable energy sources can be utilized for hydrogen production through biological means, which is a less carbon emission process[12,13]. In this context, we had isolated a facultative bacterial strain from the soil sample of maize stalk storage yard, which produces hydrogen. Maize stalk hydrolyzate was used as the sole carbon sources for the production of hydrogen. An attempt was made to improve the hydrogen production by optimization of media components and process parameter using statistical experimental design.

## Experimental Section

### Isolation and preservation of hydrogen producing strain

The strain used in this study was isolated from the soil samples of maize stalk storage yard. The media consisting of glucose, 1% (w/v); malt extract 0.1% (w/v); yeast extract, 0.2% (w/v); peptone, 0.5% (w/v); and NaCl, 0.5% (w/v)) at ***p***H 7 and temperature 30°C was used for the growth and agar slants with 1.5% (w/v) of agar was used for the maintenance of the organism. Sub culturing was carried out once in 2 weeks and the culture was stored at 4°C.

### Acid hydrolysis

Powdered maize 5g stalk is hydrolyzed with one hundred milliliters of 1% sulfuric acid for 75 min at 121°C and 15 psi in an autoclave (Hi-tech equipment, India). After hydrolysis, the hydrolysate was filtered through ordinary filter paper followed by filtration through Whattman No.1 filter paper. The filtrate was mixed thoroughly with the media and was neutralized with concentrated NaOH solution to attain a neutral ***p***H. Unless otherwise stated the concentration of maize stalk hydrolysate used in all the fermentation studies was 42.8% (v/v) equivalent to 1% (w/v) glucose.

**Batch experiments**

One hundred milliliters of sterile medium with ***p***H 7 was taken in 250 mL Erlenmeyer flask. The medium was cooled and inoculated with one day pre grown culture 5% (v/v). The fermentation was allowed to take place in a fermentation jar, which is kept in a constant temperature water bath in order to maintain constant fermentation temperatures. The released gas during the fermentation was collected in a separate jar by water displacement method. The gas sample was taken in a syringe and was loaded into gas chromatography for the qualitative assay of hydrogen. All the experimental runs were carried out in triplicate and the average value was taken.

**Analytical methods**

The gas produced during the fermentation was collected in a graduated aspirator bottle by water displacement method at regular time intervals. The percentage of hydrogen constituted in the total gas was analyzed by a gas chromatograph (AIMIL-NUCON 5765, Mumbai, India) equipped with a thermal conductivity detector (TCD) and 2.0 m (1/4 in. inside diameter) steel column filled with Porapak Q (50/80 mesh) using nitrogen as carrier gas at a flow rate of 20 mL/min. Injector, oven and column temperature was set at 150, 80 and 200°C respectively.

**Experimental design and optimization**

***Plackett Burman design***

Plackett–Burman experimental design[14] is based on the first order model:

$$Y = \beta o + \Sigma \beta i + Xi \quad \text{... (1)}$$

where, Y is the response, $\beta_o$ is the model intercept and $\beta_i$ is the linear coefficient, and $X_i$ is the level of the independent variable. The purpose of the first optimization step was to identify which ingredients of the medium have a significant effect on hydrogen production. Based on the Plackett–Burman experimental design each factor was prepared in two levels: -1 for low level and +1 for high level. Fifteen variables were screened in 20 experimental runs. The statistical software package Minitab version 15.0 was used to analyze the experimental data.

***Central composite design***

The screened medium components affecting hydrogen production were optimized using CCD[15,16]. According to this design:

$$N = 2^n + 2n + n_o \ldots \quad \text{... (2)}$$

where n, the number of independent variable; $n_o$, the number of repetitions of the experiment at the center point. For statistical analysis the variables $X_i$ are coded as $x_i$ according to the equation:

$$Xi = \frac{Xi - Xo}{\delta X} \quad \text{. ...(3)}$$

where $x_i$ is the dimensionless coded value of the variable $X_i$, $X_O$ the value of the $X_i$ at the center point, and $\delta X$ is the step change.

**Box–Behnken design**

In order to optimize the critical factors for enhanced hydrogen production, a three-variable Box–Behnken design[17] with three replicates at the center point was applied. For statistical calculations, the relation between coded values and actual values are described using the following equation:

$$X_i = (A_i - A_o)/\Delta A \quad \text{... (4)}$$

where, $X_i$ is a coded value of the variable, Ai the actual value of the variable, $A_0$ the actual value of the Ai at the center point, and $\Delta A$ the step change of variable. For predicting the optimal point, a second-order polynomial function was fitted to correlate the relationship between variables and response. The quadratic equation for the variables was as follows:

$$Y = \beta o + \Sigma \beta i Xi + \Sigma \beta ii Xi^2 + \Sigma \beta ij Xi Xj \quad \ldots (5)$$

where, Y is the predicted response; $\beta_0$ a constant; $\beta_i$ the linear coefficients; $\beta_{ii}$ the squared coefficients; and $\beta_{ij}$ the cross-product coefficients. The software Minitab 15.0 was used for the regression and graphical analysis of the experimental data obtained.

## Results and Discussion

**Isolation of microorganism**

Soil samples obtained from maize stalk storage yard were serially diluted and plated on nutrient agar, which resulted in eight morphologically distinct colonies. Individual colonies were screened based on high hydrogen yielding capacity and the strain with high hydrogen production rate was selected for further studies.

**Identification of important medium constituents using Plackett Burman design**

A total of 15 medium components including carbon, nitrogen and mineral sources were screened using Plackett Burman design through 20 experimental runs. The experimental design matrix for screening of important variables for hydrogen production is shown in Table 1. The resulting effects of the variables on the responses, the associated t-value and significant levels are shown in Table 2. A p-value less than 0.05 for the four variables: glucose, peptone, yeast extract, malt extract and NaCl indicate that they were significant. These variables had a confidence level above 95% in comparison to other variables and thus

Table 1 — Twenty run Plackett-Burman design matrix for fifteen variables with the experimental and predicted $H_2$ yields

| Runs | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ | $X_{13}$ | $X_{14}$ | $X_{15}$ | Hydrogen yield (mol $H_2$/mol )glucose) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | Exp. | Pred. |
| 1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 0.670 | 0.7229 |
| 2 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | 0.023 | 0.0170 |
| 3 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | 0.262 | 0.2919 |
| 4 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | 0.719 | 0.6671 |
| 5 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 0.232 | 0.2720 |
| 6 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 0.673 | 0.6560 |
| 7 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | 0.638 | 0.6550 |
| 8 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | 0.432 | 0.4069 |
| 9 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 0.673 | 0.6411 |
| 10 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | 0.021 | 0.0150 |
| 11 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | 0.564 | 0.5939 |
| 12 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 0.696 | 0.6512 |
| 13 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 0.139 | 0.1641 |
| 14 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | 0.720 | 0.6949 |
| 15 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | 0.531 | 0.5629 |
| 16 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 0.464 | 0.4192 |
| 17 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 0.022 | 0.0759 |
| 18 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | -1 | 1 | -1 | 0.494 | 0.4641 |
| 19 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 1 | -1 | -1 | -1 | -1 | 1 | 0.696 | 0.7279 |
| 20 | -1 | 1 | -1 | 1 | -1 | 1 | 1 | 1 | 1 | -1 | -1 | 1 | 1 | -1 | 1 | 0.464 | 0.5020 |

Glucose($X_1$); Peptone($X_2$); Beef extract($X_3$); Malt extract($X_4$); Yeast extract($X_5$); KCl($X_6$); NaCl($X_7$); $NH_4Cl$($X_8$); $ZnCl_2$($X_9$); $KH_2PO_4$($X_{10}$); $K_2HPO_4$($X_{11}$); MnSO4.$7H_2O$($X_{12}$); $MgSO_4.7H_2O$($X_{13}$); $ZnSO_4.7H_2O$($X_{14}$); and $FeSO_4.7H_2O$($X_{15}$)

Table 2 — Estimated effects and coefficients of the Plackett–Burman design

| Terms | Variable | Low level-1(g/l) | High level+1(g/l) | Effect | t | P |
|---|---|---|---|---|---|---|
| Constant | | | | | 25.01 | 0.000 |
| $X_1$ | Glucose | 20 | 40 | -0.2627 | -7.19 | 0.002 |
| $X_2$ | Peptone | 1 | 5 | 0.2067 | 5.66 | 0.005 |
| $X_3$ | Beef Extract | 0.5 | 2.5 | 0.0625 | 1.71 | 0.162 |
| $X_4$ | Malt extract | 1 | 3 | -0.2257 | -6.18 | 0.003 |
| $X_5$ | Yeast extract | 2 | 10 | -0.1131 | -3.10 | 0.036 |
| $X_6$ | KCl | 1 | 9 | -0.0381 | -1.04 | 0.356 |
| $X_7$ | NaCl | 1 | 5 | 0.1535 | 4.20 | 0.014 |
| $X_8$ | $NH_4Cl$ | 1 | 9 | -0.0195 | -0.53 | 0.622 |
| $X_9$ | $ZnCl_2$ | 0.5 | 5 | 0.0533 | 1.46 | 0.218 |
| $X_{10}$ | $KH_2PO4$ | 0.05 | 0.5 | 0.0469 | 1.28 | 0.269 |
| $X_{11}$ | $K_2HPO4$ | .5 | 2.5 | 0.0855 | 2.34 | 0.079 |
| $X_{12}$ | $MnSO_4.7H_2O$ | 0.5 | 5 | -0.807 | -2.21 | 0.092 |
| $X_{13}$ | $MgSO_4.7H_2O$ | 0.1 | 1 | -0.0533 | -1.46 | 0.218 |
| $X_{14}$ | $ZnSO_4.7H_2O$ | 0.1 | 5 | -0.0013 | -0.04 | 0.973 |
| $X_{15}$ | $FeSO_4.7H_2O$ | 0.1 | 1 | -0.0881 | -2.41 | 0.073 |

were considered to be significant for hydrogen production. These significant variables were further optimized by central composite design involving RSM.

**Optimization of the selected medium constituents using Central Composite Design**

The nutrient components glucose, peptone, malt extract, yeast extract and NaCl were selected for further optimization by RSM. A CCD was used for studying the interaction of these variables within the range of -2 to +2 in relation to hydrogen production shown in Table 3. **C**CD experimental design with 52 experimental runs was employed for the optimization of the parameters.

The results obtained from the CCD experiments were fitted to the following second order polynomial equation to represent the hydrogen production adequately.

$$Y= 0.789-0.0038X_1 +0.001X_2+0.015X_3+0.0038X_4-0.009X_5-0.027X_1^2 - 0.018X_2^2 - 0.033X_3^2 - 0.019X_4^2 - 0.021X_5^2 - 0.018X_1X_2 + 0.006\ X_1X_3 + 0.012\ X_1X_4 + 0.005\ X_1X_5 + 0.014\ X_2X_3 + 0.014\ X_2X_4 - 0.005\ X_2X_5 - 0.008\ X_3X_4 - 0.006\ X_3X_5 + 0.0068\ X_4X_5\ . \quad \ldots (6)$$

where (Y) is the predicted response, ($X_1$) concentration of glucose, ($X_2$) peptone, ($X_3$) malt extract, ($X_4$) yeast extract and ($X_5$) NaCl. The analysis of variance of the quadratic regression model calculated are listed in Table 4, which contain one constant, five linear, five quadratic and ten interaction terms. The significance of each coefficient was determined by p-values and is also listed in Table 4. The analysis of variance of the regression model demonstrates that the model is highly significant. The goodness of the fit of the model was confirmed by the determination coefficient ($R^2$). The correlation coefficient, $R^2$, was 0.987, revealing that 98.7% of experimental data of the hydrogen production were compatible with the data predicted by the model. The predicted optimum values obtained for the maximum production of hydrogen as determined from the second degree polynomial equation were: glucose, 19.25g/L; peptone, 5.64g/L; malt extract, 1.64g/L; yeast extract, 3.16g/L and NaCl, 4.312g/L. At the optimum medium concentration maximum hydrogen production of 0.791mol $H_2$/mol substrate was obtained.

**Optimization of process parameter using Box-Behnken design**

The Box-Behnken design was applied to optimize the important process parameters, namely, initial ***p***H of the medium, temperature and fermentation time. The design matrix which consists of 15 experimental runs was constructed, in order to arrive at a second order polynomial equation to predict the hydrogen fermentation system shown in Table 5. Multiple regression analysis was used to analyze the data and the polynomial equation derived from regression analysis is as follows:

Table 3 — Coded and uncoded value of medium components used for central composite design

| Independent variables | Symbols | Coded levels | | | | |
|---|---|---|---|---|---|---|
| | | -2 | -1 | 0 | +1 | +2 |
| Glucose, (g/L) | $X_1$ | 10 | 15 | 20 | 25 | 30 |
| Peptone, (g/L) | $X_2$ | 1 | 3 | 5 | 7 | 9 |
| Malt extract, (g/L) | $X_3$ | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 |
| Yeast extract, (g/L) | $X_4$ | 1 | 2 | 3 | 4 | 5 |
| NaCl, (g/L) | $X_5$ | 1 | 3 | 5 | 7 | 9 |

Table 4 — Results of the regression analysis of second order polynomial model for the optimization of hydrogen production obtained in the screening experiment

| Term constant | Regression coefficient | Std. deviation | t-statistics | p-value |
|---|---|---|---|---|
| Intercept | 0.788605 | 0.002844 | 277.299 | <0.001 |
| $X_1$ | -0.003651 | 0.001375 | -2.655 | 0.012 |
| $X_2$ | 0.001536 | 0.001375 | 1.117 | 0.272 |
| $X_3$ | 0.015171 | 0.001375 | 11.035 | <0.001 |
| $X_4$ | 0.003857 | 0.001375 | 2.805 | 0.009 |
| $X_5$ | -0.009275 | 0.001375 | -6.746 | <0.001 |
| $X_1X_1$ | -0.027689 | 0.001182 | -23.433 | <0.001 |
| $X_2X_2$ | -0.018950 | 0.001182 | -16.038 | <0.001 |
| $X_3X_3$ | -0.033250 | 0.001182 | -28.140 | <0.001 |
| $X_4X_4$ | -0.019656 | 0.001182 | -16.635 | <0.001 |
| $X_5X_5$ | -0.021863 | 0.001182 | -18.503 | <0.001 |
| $X_1X_2$ | -0.018875 | 0.001600 | -11.799 | <0.001 |
| $X_1X_3$ | 0.006250 | 0.001600 | 3.907 | 0.001 |
| $X_1X_4$ | 0.012125 | 0.001600 | 7.579 | <0.001 |
| $X_1X_5$ | 0.005250 | 0.001600 | 3.282 | 0.003 |
| $X_2X_3$ | 0.014438 | 0.001600 | 9.025 | <0.001 |
| $X_2X_4$ | 0.014938 | 0.001600 | 9.337 | <0.001 |
| $X_2X_5$ | -0.005437 | 0.001600 | -3.399 | 0.002 |
| $X_3X_4$ | -0.008313 | 0.001600 | -5.196 | <0.001 |
| $X_3X_5$ | -0.006188 | 0.001600 | -3.868 | <0.001 |
| $X_4X_5$ | 0.006812 | 0.001600 | 4.258 | <0.001 |

Table 5 — Three level Box-Behnken design matrix for the optimization of significant process parameters

| Run No. | ***p***H | Temperature | Time | Hydrogen yield (mol $H_2$/mol glucose) | |
|---|---|---|---|---|---|
| | | | | Exp. | Pred. |
| 1 | 0.00 | 0.00 | 0.00 | 0.889 | 0.888 |
| 2 | -1.00 | 0.00 | -1.00 | 0.700 | 0.701 |
| 3 | -1.00 | 0.00 | 1.00 | 0.685 | 0.683 |
| 4 | 0.00 | 0.00 | 0.00 | 0.886 | 0.888 |
| 5 | 1.00 | 0.00 | 1.00 | 0.812 | 0.812 |
| 6 | 0.00 | 1.00 | 1.00 | 0.747 | 0.748 |
| 7 | 1.00 | 1.00 | 0.00 | 0.785 | 0.785 |
| 8 | 0.00 | 1.00 | -1.00 | 0.715 | 0.713 |
| 9 | 0.00 | 0.00 | 0.00 | 0.888 | 0.888 |
| 10 | -1.00 | 1.00 | 0.00 | 0.680 | 0.681 |
| 11 | -1.00 | -1.00 | 0.00 | 0.660 | 0.660 |
| 12 | 1.00 | 0.00 | -1.00 | 0.764 | 0.766 |
| 13 | 1.00 | -1.00 | 0.00 | 0.752 | 0.751 |
| 14 | 0.00 | -1.00 | 1.00 | 0.698 | 0.700 |
| 15 | 0.00 | -1.00 | -1.00 | 0.707 | 0.706 |

$$Y=0.887+0.049A+0.0137B+0.007C+3.25AB+0.015AC+0.01BC-0.07A^2 - 0.09B^2 - 0.07C^2 \quad \ldots (7)$$

where, Y, predicted hydrogen potential: A, pH; B, time and C, temperature respectively.

The goodness of fit of the model depends on the multiple correlation coefficients, $R^2$. The $R^2$ value obtained from the ANOVA was 0.9998, which implies that the variation of 99.98% hydrogen production is attributed to the independent variables. The parameter estimate and the corresponding p-value ($p<0.5$) (Table 6) suggest that all the linear, square and interactive terms of ***p***H, time and temperature have a significant effect on hydrogen production. $R^2$ value closes to 1 denotes better correlation between the observed and predicted value. Three dimensional surface plots are drawn to determine the optimum values and the interactive effect of the three process parameters. Figure 1(a) represents the interaction between time and ***p***H. The shape of the response surface curves showed a maximum hydrogen production obtained when both the factors lies between -0.5 and 1 level. Figure 1(b) represents the interaction between temperature and ***p***H. Maximum hydrogen production was recorded at ***p***H 7 and temperature of 34.5°C. The optimal values of ***p***H and temperature obtained here are in accordance with that obtained for Souparno et al for a defined microbial consortium producing hydrogen from molasses[18]. Further increase or decrease of ***p***H or temperature negatively affects the yield of hydrogen. Figure 1(c) represents the interaction between temperature and time and it follows the same trend obtained previously. The experimental results suggest that the maximum values of hydrogen yield (0.89 mol $H_2$/mol

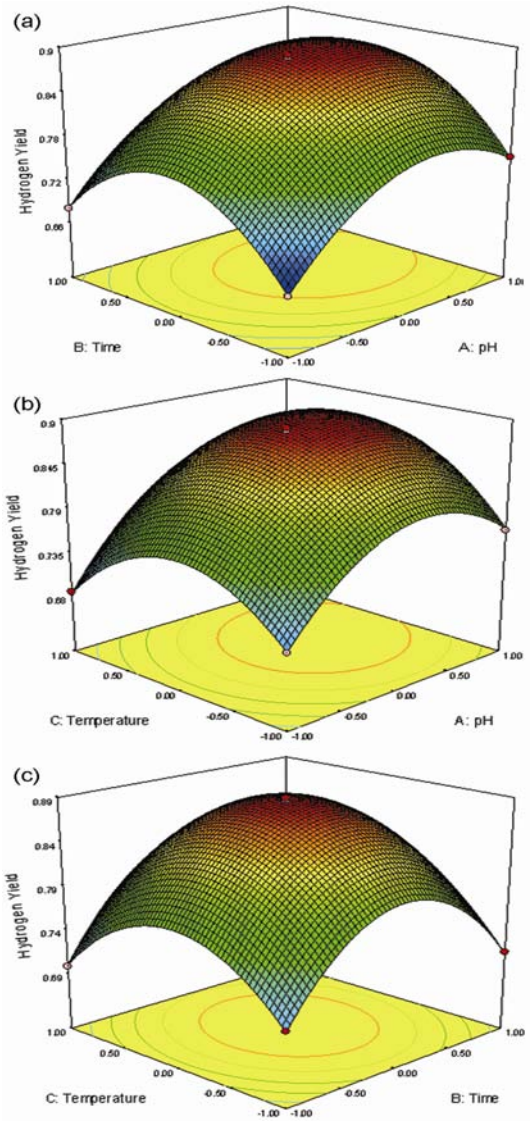


Fig. 1 (a-c) — 3D plots showing the interactive effects between the significant process parameters on hydrogen yield in batch fermentation

Table 6 — Results of the ANOVA for the quadratic model

| Source | Sum of squares | Degrees of freedom (DF) | Mean square | F value | P-value Prob > F |
|---|---|---|---|---|---|
| Model | 0.12 | 9 | 0.013 | 3331.77 | < 0.0001 |
| ***p***H (A) | 0.019 | 1 | 0.019 | 4878.74 | < 0.0001 |
| Temperature (B) | $1.513\times10^{-3}$ | 1 | $1.513\times10^{-3}$ | 392.13 | < 0.0001 |
| Time (C) | $3.920\times10^{-4}$ | 1 | $3.920\times10^{-4}$ | 101.63 | < 0.0001 |
| AB | $4.225\times10^{-5}$ | 1 | $4.225\times10^{-5}$ | 10.95 | 0.0129 |
| AC | $9.923\times10^{-4}$ | 1 | $9.923\times10^{-4}$ | 257.25 | < 0.0001 |
| B | $4.202\times10^{-4}$ | 1 | $4.202\times10^{-4}$ | 108.95 | < 0.0001 |
| C | | | | | |
| AA | 0.022 | 1 | 0.022 | 5678.61 | < 0.0001 |
| BB | 0.039 | 1 | 0.039 | 9981.91 | < 0.0001 |
| CC | 0.023 | 1 | 0.023 | 6079.10 | < 0.0001 |
| Residual | $2.700\times10^{-5}$ | 7 | $3.857\times10^{-6}$ | | |
| Lack of fit | $1.900\times10^{-5}$ | 3 | $6.333\times10^{-6}$ | 3.17 | 0.1473 |
| Pure error | $8.000\times10^{-6}$ | 4 | $2.000\times10^{-6}$ | | |
| Total | 0.12 | 16 | | | |

glucose) were obtained for the runs with the central points. The optimum values obtained by solving the second degree polynomial equation are as follows: ***p***H, 7.0; temperature, 34.5°C and fermentation time, 42.5 h.

## Conclusion

The present study optimized the media components and process parameters for the production of hydrogen using the newly isolated facultative strain. A suitable maize stalk hydrolysate in the fermentation medium is essential to get higher hydrogen yields; however, an excessively high or low concentration of maize stalk hydrolysate affects the growth of the organism which reduce the yield. The results also show the use of cheaper lignocellulosic substrate for fermentation, thus contributing to the reduction in the cost of production medium. Five variables have been identified by Plackett Burman design on significant for hydrogen production. These variables are further optimized using CCD. The optimum medium constituents of the fermentation medium are as follows: glucose, 19.25 g/L; yeast extract, 3.046 g/L; malt extract, 1.64 g/L; peptone, 5.640 and NaCl, 4.312 g/L. Box-Behnken design is adopted to identify optimum values of process parameters that bring maximum hydrogen production. The optimized values of process parameters for hydrogen production are as follows: ***p***H, 7.0; temperature, 34.5°C and fermentation time, 42.5 h. At these conditions highest hydrogen yield of 0.91 mol $H_2$/mol glucose has been achieved, when maize stalk hydrolysate (equivalent to 1.5% (w/v)) is used as the carbon source. This shows that the microorganism used in this study possess a high hydrogen producing capacity under optimal conditions.

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