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A Statistical and Microbial Study: Rose Flower Waste into Bioethanol

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Abstract

The increasing demand for sustainable energy sources has directed attention toward bioethanol production from lignocellulosic waste. This study explores the valorisation of *Rosa indica* flower waste as a potential feedstock for bioethanol production via submerged fermentation using yeast *Saccharomyces cerevisiae* VBG3. A systematic statistical approach was applied to optimize the fermentation parameters. Initially, the Plackett–Burman design was employed to identify significant factors influencing ethanol yield. Among the screened parameters, fructose concentration, corn steep liquor (CSL), and substrate concentration were found to significantly enhance ethanol production. These variables were further optimized using Central Composite Design (CCD) under Response Surface Methodology (RSM). The optimal levels of the selected parameters yielded a maximum bioethanol concentration of 42.12 g/L. The findings underscore the efficacy of statistical optimization in enhancing bioethanol production from rose flower waste, offering a promising avenue for waste-to-energy bioconversion.

1. Introduction

The need for energy from diverse and plentiful sources was satisfied by bioenergy. Applications for bioenergy included processing, transportation, power generation, and industrial fuel. It is included under renewable energy sources, which are made from biomass, or organic material. The biological biomass absorbs photoenergy and stores it as chemical energy. Typically, high-grade biofuels were produced from unprocessed biomass using thermal, chemical, and biochemical methods[10]. First and second-generation biofuels are the two main categories. First-generation biofuels are made from food supplies (corn and sugarcane) that are grown on agricultural land, while second-generation biofuels are made from non-food sources or byproducts. Waste products from industry, municipalities, forests, and agriculture are the main sources of feedstock for second-generation biofuels[6]. Anaerobic digestion was used to ferment the sugar in biomass and produce biofuels[5]. Biomass made of cellulose from non-food sources, like maize stover and wheat straw[3]; woodchips[8]; switchgrass[4] and municipal solid waste[7] was suggested for bioethanol production. Other materials, such as industrial waste, and marigold flowers have also been utilised as feedstock. The most crucial components of this feedstock are lignocellulose, which is present in adequate amounts[9].

It is important to improve the performance of the systems and to increase the yield of the processes without increasing the cost. The method used for this purpose is called optimization. RSM is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes in which a response of interest is influenced by several variables and the objective is to optimize this response[2]. In addition to analysing the effects of the independent variables, this experimental methodology generates a mathematical model which describes the chemical or biochemical processes[1].\

The statistical optimisation of the synthesis of bioethanol from *Rosa indica* (rose) blossoms is the main topic of this work. The influencing elements for bioethanol production were screened using Plackett Burmann Design (PBD), and the interaction between the screened factors was then investigated using Response Surface Methodology-Central Composite Design (RSM-CCD).

2. Material and methods

2.1 Substrate procurement

The floral waste material was sourced from religious establishment, Isckon temple (22˚549576" N 72˚92'2691" E), located in Anand district, Gujarat. Rosa species petals were selectively isolated from the heterogeneous floral waste matrix through manual segregation. The isolated rose petals underwent solar dehydration until gravimetric stability was achieved, as evidenced by constant mass measurements in consecutive weighing. Subsequently, the desiccated petals were mechanically pulverized to obtain a homogeneous powdered substrate. This processed rose flower waste (RFW) was then utilized as the fermentable feedstock for bioethanol production through microbial conversion pathways.

2.2 Microorganism Activation and Fermentation Media Formulation

The yeast colony *Saccharomyces cerevisiae* VBG3 (Accession no: PV809907) was immersed in Glucose yeast extract (GYE) broth and incubated at 33°C for 72 hours. The production medium was formulated as follows: 6 g% substrate concentration, 1.5 g% of fructose supplementation, 0.5 mL % corn steep liquor as a nitrogen source, 4 mL % inoculum size, pH 5.5, temperature of 30 °C, and an incubation period of 3 days.

2.3 Statistical optimization

2.3.1 Plackett Burman Design

Plackett-Burmann design (PBD) is one of the statistical methods to screen out the elements influencing the response with reduction in number of experiments. The elements to be screened in PBD for bioethanol are fructose, corn steep liquor (CSL), substrate (RFW) concentration, inoculum size and incubation days. In this screening tool, the elements are examined at higher (+1) and lower (-1) values (Table 1).

Table 1: Variables assessed in PBD

Sr. No.	Variables	High (+1)	Lower (-1)
1	Fructose (%)	1.5	0.5
2	Corn steep liquor (%)	0.8	0.3
3	Inoculum size (%)	10	4
4	pH	4.5	6.5
5	Substrate concentration (%)	10	4
6	Incubation period (hours)	24	72

PBD employed to screen the media components and fermentation parameters were performed according to a design matrix created by *Minitab version 20.3*. A description of the experimental design used to screen these variables is provided in Table 2.

The following equation **(1)** determined the effect of the independent variables:

$$EtOH = \propto 0 + \sum(i = 0)^n 〚\propto i〛 + Xi \quad \textbf{(1)}$$

Where, *EtOH* is ethanol concentration in g/L, α_0 is intercept; αi is linear coefficient, X_i is the coded level of an independent factor, *n* is the number of factors and *i* is the factor number.

Table 2: Plackett Burmann Design and experimental results for Bioethanol production from RFW

Sr. No.	Fructose (%)	CSL (%)	Inoculum size (%)	Substrate concentration (%)	pH	Temperature (°C)	Incubation time (h)	Bioethanol (g/L)
1	+1	+1	-1	+1	-1	-1	-1	**32.9**
2	-1	+1	+1	-1	+1	-1	-1	18.4
3	+1	+1	-1	+1	+1	-1	+1	29.9
4	-1	+1	-1	-1	-1	+1	+1	16.6
5	-1	-1	-1	+1	+1	+1	-1	17.4
6	-1	-1	+1	+1	+1	-1	+1	17.8
7	-1	-1	-1	-1	-1	-1	-1	9.22
8	+1	-1	+1	+1	-1	+1	-1	30.22
9	+1	-1	-1	-1	+1	+1	+1	27.4
10	+1	+1	+1	-1	+1	+1	-1	31.44
11	+1	-1	+1	-1	-1	-1	+1	17.3
12	-1	+1	+1	+1	-1	+1	+1	32.3

2.3.2 Response surface methodology

After screening the factors affecting bioethanol production by PBD tool, the three factors vis., fructose, corn steep liquor and substrate concentration were further optimized using RSM-CCD in order to determine optimum concentration elevating bioethanol production by *Saccharomyces cerevisiae VBG3*. RSM requires large number of experiments to be ran to achieve optimum concentration for an enhanced bioethanol production. The level of 3 major parameters was optimized, while temperature, pH and incubation time were kept constant. In CCD, the independent variables are studied at five different levels (-α, -1, 0, +1, +α), -α and +α encoded as axial points, -1 and +1 encoded as factorial points and (0) as central point (Table 3). The experimental design with the encoded and actual values are enlisted in table 4. Bioethanol production was estimated in triplicates in all 20 experimental sets. The results underwent statistical analysis through variance analysis at a significance level of $\alpha = 0.05$. The adequacy of the model was evaluated using the coefficient of determination (R^2) and the model *p*-value. Employing response surface methodology proves beneficial in studying the influence of various factors on responses by concurrently adjusting them, thus reducing the need for numerous experiments. In order to match the data with equation (2), a multiple regression approach was applied. Equation (2) represents a second-order polynomial equation used to study bioethanol production.

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_{ii}^2 + \sum \beta_{ij} X_i X_j \qquad \textbf{(2)}$$

Where Y = the predicted response, XiXj = independent variables (Effect the response Y), β0 = constant, βi = the linear coefficient, βii = the quadratic coefficient and βij = interaction coefficient.

Table 3: Experimental range and levels of the independent variables of selected components used for response surface Central Composite design

Independent variables	Coded levels				
	-α	-1	0	1	α
Fructose (g/L)	1.5	5	10	15	18.41
Malt extract (g/L)	1.3	3	5	8	9.70
Substrate concentration (g/L)	19.55	40	70	100	120.45

Table 4: The central composite design matrix employed for three independent variables

Run No.	Independent Variables			Bioethanol concentration (g/L)
	Fructose (g/L)	Corn steep liquor (g/L)	Substrate concentration (g/L)	
1	10	5.5	19.5462	28.44
2	15	8	100	34.8
3	5	3	100	29.46
4	10	5.5	70	42.12
5	1.5	5.5	70	19.55
6	10	5.5	70	42.38
7	5	3	40	24.56
8	10	5.5	70	41.34
9	10	9.70448	70	32.67
10	10	5.5	70	41.9
11	18.4	5.5	70	29.33
12	5	8	40	21.54
13	15	3	40	27.33
14	10	5.5	70	41.88
15	10	5.5	120.454	32.56
16	15	8	40	36.78
17	10	5.5	70	41.21
18	15	3	100	30.26
19	5	8	100	23.1
20	10	1.29552	70	30.44

3. Results and Discussion

3.1 Substrate preparation and fermentation media formulation

Rose flowers were manually segregated from the bulk floral waste collected from local sources. The selected rose flower waste (RFW) was subjected to preliminary cleaning by washing twice with tap water to remove loosely adhered particulate matter, followed by a final rinse with distilled water to eliminate residual impurities. The cleaned biomass was air-dried at room temperature (25 ± 2 °C) for few hours and subsequently sundried until a constant weight was achieved. The dried material was then ground using a mechanical grinder to obtain a fine powder (Fig 1). The resulting rose flower waste powder (RFWP) was sieved through a 60-mesh screen to ensure uniform particle size and stored in airtight containers at 4 °C until further use in fermentation experiments.

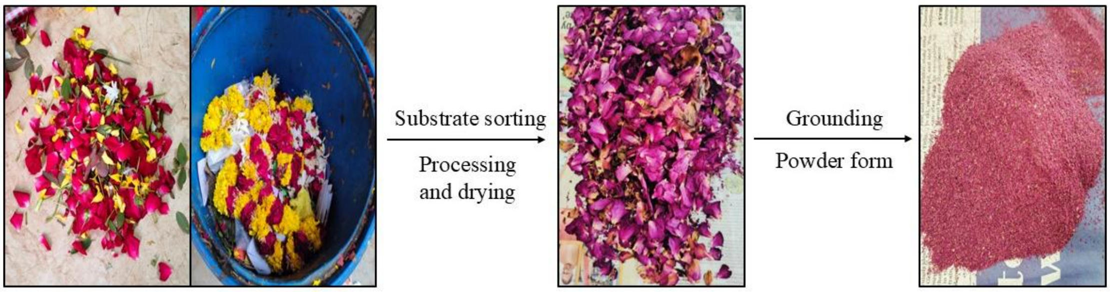

Figure 1: Substrate procurement, sorting and processing

3.2 Statistical Analysis

Rose Flower Waste Powder (RFWP) was utilized as a substrate for bioethanol production via submerged fermentation employing an active culture of *Saccharomyces cerevisiae* VBG3. Optimization of the fermentation medium and process parameters was carried out using statistical experimental design approaches. Initially, the Plackett Burman design was applied to identify the significant variables influencing ethanol production. Subsequently, the interaction effects among the screened factors were investigated and optimized using Response Surface Methodology (RSM) based on a Central Composite Design (CCD).

3.2.1 Plackett Burmann Design

To maximise process output, it is essential to methodically identify and choose the critical elements influencing the fermentation process. A small number of tests are used to screen a large number of elements, and statistical approaches are then used to identify the components that have a substantial impact on the manufacturing process. One such statistical technique used to identify the important parameters among the parameters under analysis and assess the relative significance of medium components in the fermentation process is the Plackett-Burman design (PBD)[13,11].

Table 5: Analysis of variance for Plackett Burmann Design of bioethanol production by *S. cerevisiae* VBG3

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	649.830	92.833	5.63	0.057
Linear	7	649.830	92.833	5.63	0.057
fructose	1	274.946	274.946	16.68	0.015
CSL	1	148.403	148.403	9.01	0.040
Inoculum size	1	16.427	16.427	1.00	0.375
Substrate concentration	1	134.402	134.402	8.16	0.046
pH	1	1.203	1.203	0.07	0.800
Temperature	1	74.202	74.202	4.50	0.101
Incubation time	1	0.247	0.247	0.01	0.909
Error	4	65.915	16.479		
Total	11	715.746			

Table 5 shows the p values of the variables, revealed by ANOVA. The variables' p values were thought to be important indicators of an increased ethanol content. The current study found that the elements that significantly increased the production of bioethanol were *substrate concentration, fructose, and corn steep liquor*, all of which had reduced p-values and positive co-efficient values. The rest of the factors i.e., inoculum size and incubation days had a higher p-value higher than 0.05. The inoculum volume of 4 % and 3 days of incubation were found to be sufficient to achieve bioethanol concentration. Both of the factors were considered nonsignificant as an elevated concentration of inoculum volume, pH and more days of incubation had no effect on improvement in ethanol production, thus were considered as nonsignificant variables in the PBD experiment. The maximum concentration of bioethanol achieved from PBD screening was *32.9 g/L*. Fructose, Corn steep liquor and substrate concentration were chosen as influencing factors for the increase in bioethanol production and the fermentation process was further optimized by adopting RSM-CCD to study the interaction among the significant components and also determine their optimal levels for bioethanol production.

3.2.2 Response Surface Methodology

The central composite design was utilised in order to investigate the neighbouring interaction between the important components and attain their optimal values. Twenty trials were conducted in the current investigation in order to produce a quadratic model with two concentration levels for each component. To investigate the effects of combining these components, experiments were conducted at various doses. The central composite design matrix in Table 4 displays different combinations of the three independent variables, resulting in varying values of the dependent variable. The design shows variation as well as enhancement in the production of bioethanol, which corresponds to the synergistic effect of all the considered components within their defined range.

The regression equation was solved by looking at the response surface contour plots and surface plots, and the ideal values of the selected variables were reached, leading to an estimate of the level of bioethanol concentration following analysis of variance for the planned trials.

Table 6: Analysis of variance for response surface quadratic model of bioethanol production by *S. cerevisiae* VBG3

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1050.04	9	116.67	234.95	< 0.0001	significant
A-Fructose	161.46	1	161.46	325.14	< 0.0001	
B-CSL	5.12	1	5.12	10.31	0.0093	
C-Substrate concentration	15.06	1	15.06	30.32	0.0003	
AB	68.27	1	68.27	137.48	< 0.0001	
AC	3.80	1	3.80	7.64	0.0200	
BC	8.51	1	8.51	17.13	0.0020	
A^2	527.12	1	527.12	1061.48	< 0.0001	
B^2	179.81	1	179.81	362.10	< 0.0001	
C^2	219.79	1	219.79	442.61	< 0.0001	
Residual	4.97	10	0.4966			
Lack of Fit	3.95	5	0.7902	3.89	0.0810	not significant
Pure Error	1.01	5	0.2029			
Cor Total	1055.01	19				

The Model f-value of 234.95 implies that model is significant (Table 6). P-values less than .05000 indicate model terms are significant. In this case A, C, AB, BS, A2, B2 and C2 are significant model terms.

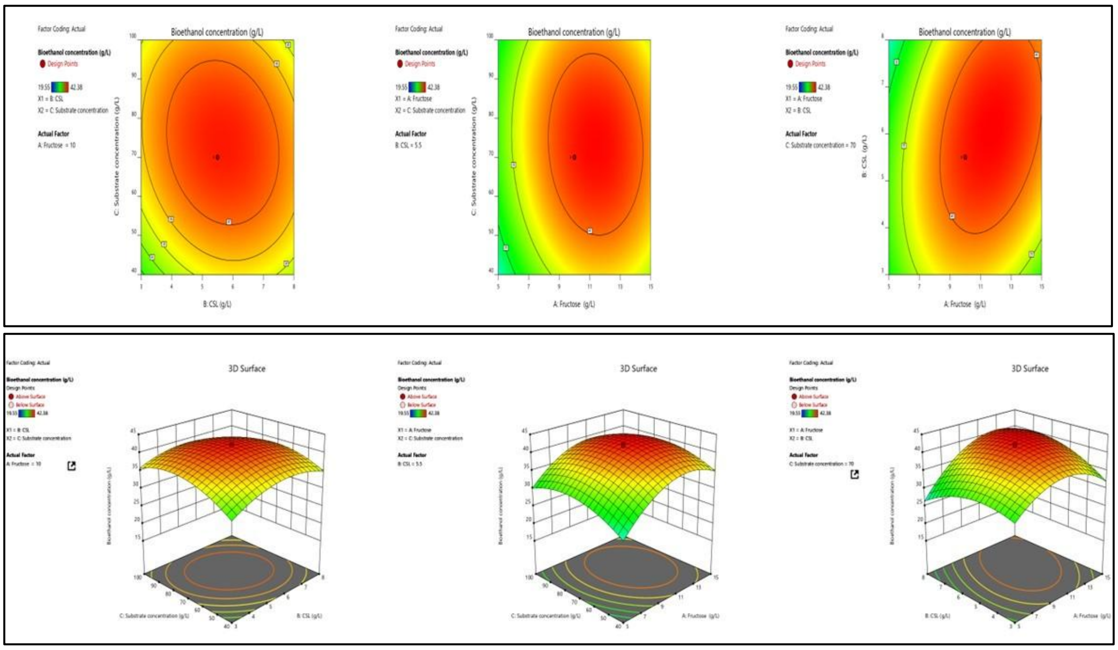

Figure 2: Contour and response surface graph showing interaction effects between concentration of the independent variables taken into consideration (Fructose, CSL and Substrate concentration)

Fructose and *corn steep liquor* were the most significant variables found to elevated bioethanol production as indicated in contour and surface plots (Fig 2). Maximum bioethanol production was achieved upto *42.12 g/L*. Bioethanol production of 50.25 g/L was achieved upon process optimization employed using RSM-Box Behnken Design from potato waste by, while 83 g/L of bioethanol was produced from sweet sorghum juice by *Saccharomyces cerevisiae*[12,15]. Using RSM-CCD for bioethanol production from kitchen garbage resulted in 30.3 g/L of bioethanol production[14].

3. Conclusion

The present study demonstrated the potential of *Rosa indica* flower waste as an effective lignocellulosic substrate for bioethanol production via microbial fermentation using *Saccharomyces cerevisiae* VBG3

(Accession no: PV809907). The application of statistical tools, namely Plackett–Burman Design and Central Composite Design within Response Surface Methodology, proved successful in identifying and optimizing the key parameters influencing ethanol yield. Fructose concentration, CSL, and substrate concentration were found to be the most significant factors. Through the optimized conditions, a maximum bioethanol concentration of 42.12 g/L was achieved, confirming the viability of this approach. This work not only promotes sustainable waste management but also supports the development of eco-friendly biofuels, contributing to the advancement of renewable energy technologies.

References

1. Anjum, M. F., Tasadduq, I., & Al-Sultan, K. Response surface methodology: A neural network approach. *European Journal of Operational Research*, ***101*****(1)**, 65-73 **(1997)**. https://doi.org/10.1016/S0377-2217(96)00232-9.
2. Baş, D., & Boyacı, İ. H. Modeling and optimization I: Usability of response surface methodology. *Journal of food engineering*, ***78*****(3)**, 836-845 **(2007)**. https://doi.org/10.1016/j.jfoodeng.2005.11.024.
3. Dhiman, S. S., David, A., Braband, V. W., Hussein, A., Salem, D. R., & Sani, R. K. Improved bioethanol production from corn stover: role of enzymes, inducers and simultaneous product recovery. *Applied Energy*, ***208***, 1420-1429 **(2017)**. https://doi.org/10.1016/j.apenergy.2017.09.013.
4. Ghaderi, H., Moini, A., & Pishvaee, M. S. A multi-objective robust possibilistic programming approach to sustainable switchgrass-based bioethanol supply chain network design. *Journal of cleaner production*, ***179***, 368-406 **(2018)**. https://doi.org/10.1016/j.jclepro.2017.12.218.
5. Khammee, P., Ramaraj, R., Whangchai, N., Bhuyar, P., & Unpaprom, Y. The immobilization of yeast for fermentation of macroalgae Rhizoclonium sp. for efficient conversion into bioethanol. *Biomass Conversion and Biorefinery*, ***11***, 827-835 **(2021)**. https://doi.org/10.1007/s13399-020-00786-y.
6. Manmai, N., Unpaprom, Y., & Ramaraj, R. Bioethanol production from sunflower stalk: application of chemical and biological pretreatments by response surface methodology (RSM). *Biomass Conversion and Biorefinery*, ***11***, 1759-1773 **(2021)**.
7. Miezah, K., Obiri-Danso, K., & Kádár, Z. Municipal solid waste management in a low income economy through biogas and bioethanol production. *Waste Biomass Valori* ***8****: 115–127* **(2017)**. https://doi.org/10.1007/s12649-016-9566-5.
8. Mosina, N. L., Krishna, S. B. N., Ramnath, L., & Govinden, R. Screening, Production and Partial Characterization of Xylanases from Woodchips Fungi with Potential Application in Bioethanol Production. *Current Trends in Biotechnology and Pharmacy*, ***11*****(4)**, 414-423 **(2017)**.
9. Park, J. H., Jin, M. H., Lee, Y. J., Song, G. S., Choi, J. W., Lee, D. W., ... & Kim, J. G. Two-in-one fuel synthetic bioethanol-lignin from lignocellulose with sewage sludge and its air pollutants reduction effects. *Energies*, ***12*****(16)**, 3072 **(2019)**. https://doi.org/10.3390/en12163072.
10. Sahu, O. Appropriateness of rose (*Rosa hybrida*) for bioethanol conversion with enzymatic hydrolysis: Sustainable development on green fuel production. *Energy*, *232*, 120922 **(2021)**. https://doi.org/10.1016/j.energy.2021.120922.
11. Singh, V., Haque, S., Niwas, R., Pasupuleti, M., & Tripathi, C. K. M. Strategies for fermentation medium optimization: an in-depth review. *Frontiers in microbiology*, **7**, 227613. 542 **(2017)**. https://doi.org/10.3389/fmicb.2016.02087.
12. Suresh, T., Sivarajasekar, N., Balasubramani, K., Ahamad, T., Alam, M., & Naushad, M. Process intensification and comparison of bioethanol production from food industry waste (potatoes) by ultrasonic assisted acid hydrolysis and enzymatic hydrolysis: Statistical modelling and optimization. *Biomass and Bioenergy*, **142**, 105752 **(2020)**. https://doi.org/10.1016/j.biombioe.2020.105752.
13. Vaidya, R., Vyas, P., & Chhatpar, H. S. Statistical optimization of medium components for the production of chitinase by *Alcaligenes xylosoxydans*. *Enzyme and Microbial Technology*, **33(1)**, 92-96. 539 **(2003)**. https://doi.org/10.1016/S0141-0229(03)00100-5.
14. Wang, Q., Ma, H., Xu, W., Gong, L., Zhang, W., & Zou, D. Ethanol production from kitchen garbage using response surface methodology. *Biochemical Engineering Journal*, **39(3)**, 604-610 **(2008)**. https://doi.org/10.1016/j.bej.2007.12.018.
15. Yu, J., Zhang, X., & Tan, T. **(**. Optimization of media conditions for the production of ethanol from sweet sorghum juice by immobilized *Saccharomyces cerevisiae*. *Biomass and bioenergy*, **33(3)**, 521-526. 549 **(2009)**. https://doi.org/10.1016/j.biombioe.2008.08.020.