OPTIMIZATION OF FERMENTATION PARAMETERS USING RESPONSE SURFACE METHODOLOGY FOR BIOHYDROGEN PRODUCTION FROM URBAN WASTE

Annam Renita ANTONY[1]*, Narendra KUMAR[2], Sunitha SALLA[3]

Biohydrogen was prepared by batch fermentation and the parameters for anaerobic fermentation under mesophilic condition were optimized using Design Expert Software version 7.0.0. Central composite design of response surface methodology was used to optimize parameters like weight of substrate, weight of culture and time. The substrate used was food waste and the culture were a mixed culture obtained from cow dung and industrial effluent. Biohydrogen was obtained at optimized parameters of 96 hours with 125 g of food waste and 125 g of cow dung. The coefficient of regression 0.9979 and the adjusted R^2 value of 0.9956 is in good agreement with predicted R^2 value of 0.9957. The investigation was done by dark fermentation and the composition of gas produced was determined using gas chromatography which confirmed the presence of biohydrogen of 26.87% yield.

Keywords: food waste, biohydrogen, dark fermentation, optimization, RSM-CCD

1. Introduction

The need for a clean energy resource has become crucial in the wake of global warming incidents. Fuel cells are ecofriendly and hydrogen fuel cells are efficient in operation [1]. Apart from hydrogen being used for fuel cells it is also used for synthesis for various chemicals, cryogenics and for welding. Conventional methods for production of hydrogen are energy intensive and cost consuming. Bio-hydrogen production methods like fermentation and photolysis of water are environmental friendly methods [2-4]. Microorganism production method of hydrogen is considered to be cost-effective [5]. Anaerobic or photosynthetic microorganism can be used for bio hydrogen like *Thermoanaerobacterium* and *Clostridium* sp. [6-11], *Escherichia coli* [12,13] *E. aerogenes* [14], aerobic bacteria and *Enterobacter cloacae* [15]. In anaerobic processes, biomass is converted to fatty acids, which are further converted to acetate and hydrogen, which gets finally converted into methane [16]. Production of hydrogen can be enhanced if methanogenesis phase is eliminated. This could

[1]* Sathyabama University, India, contact author, e-mail: reniriana@gmail.com
[2] Sathyabama University, India, e-mail: gnaren22@gmail.com
[3] Sathyabama University, India, e-mail: sunithasalla@gmail.com

be achieved by elimination of hydrogen consumers which can be achieved by operating system at shorter retention time [17].

According to the United Nations Development Programme, up to 40% of the food produced in India is wasted as per 2016 statistics. Utilization of waste not only addresses solid waste management but serves also as a potential resource for biohydrogen production. Hence this paper deals with management of food waste by dark fermentation method. Various natural sources can be used for mixed culture like acclimated sludge [18,1], sewage sludge [19-21], soil [22,23] and anaerobically digested sludge [24-27].

2. Materials and Methods

Food waste was collected from canteen of Sathyabama Institute of Science and Technology, Chennai, India. It was dried at 105°C for 24 hours and incinerated at 550°C for one hour to determine the total solids and volatile solids as per American Public Health Association (APHA) standard method. Cow dung was collected from some cattle shed nearby the institute premises. The industrial effluent was collected from Aavin dairy outlet, Chennai, India.

The food waste was segregated according to the amount found: 62% cooked waste rice, 21 % vegetables and 15% dal. Biohydrogen was produced by dark fermentation using mixed culture under anaerobic conditions. Oxygen was removed using vacuum pump. The food waste was crushed and mixed with cow dung in various ratios along with mixed consortium supplied by industrial effluent. The microorganism loading was a mixed culture of cow dung and industrial effluent in mesophilic conditions. Mixed consortia were preferred over single as it eliminates the need for sterilization. *Bacillus safensis, Bacillus cereus, Bacillus subtilis, Lysinibacillus xylanilyticus* and *Bacillus licheniformis* have been identified in cow dung [28, 29]. Three batch reactors of 5 L capacity under anaerobic condition were studied for different pH initially. Batch reactor 1 had a loading rate of (1:1) food waste and cow dung slurry at pH 4, Batch reactor 2 had a loading rate of (1:1) food waste and cow dung slurry at pH 5 and Batch reactor 3 had a loading rate of (1:1) food waste and cow dung slurry at pH 6 to determine the optimum pH of operation.

The amount of biohydrogen gas produced in each batch reactor was determined using a glass syringe at a retention time of 300h. Batch reactor 1 had a hydrogen yield of 35% followed by reactor 2 with 25% and reactor three with 12%. The operating pH was decided to be 4 which is in confirmation with earlier studies that the optimal pH for ethanol-type fermentative bacteria ranges from 4.0 to 4.5 [30]. Research has proved that by increasing pH, the ability of bacteria in producing hydrogen increases but decreases at higher pH levels showing the importance of varying pH [31-39]. Hence by maintaining the pH constant at 4, the important parameters like substrate concentration, cow dung loading and retention time effect was studied by design expert software 7.0.0. Twenty experimental runs were done in triplicate to ensure the reproducibility at ± 5%. The coded value for the experimental design is given by Table-1 for the parameters of weight of substrate, weight of inoculum and time.

Table 1.

Variables used in the experimental design matrix

Study Type	Response Surface	Runs	20
Initial Design	Central Composite	Blocks	No Blocks
Design Model	Quadratic		

Factor	Name	Units	Low		High		Mean	Std. Dev.
			Coded	Actual	Coded	Actual		
A	Cow dung	g	-1	50	1	200	125	61.97572
B	Rice	g	-1	50	1	200	125	61.97572
C	Time	h	-1	72	1	120	96	19.83223

Response	Name	Obs	Analysis	Minimum	Maximum	Mean	Model
Y1	H_2	20	Polynomial	2.47	27.1	17.864	Quadratic

The empirical formula to find the optimized bio hydrogen yield is given by equation 1:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta 4AB + \beta 5AC + \beta 6BC + \beta_7 A^2 + \beta 8B^2 + \beta 9C^2 \quad (1)$$

Where Y is the yield of biohydrogen and coded values for variables are A, B and C. AB, AC and BC are the cross products and A^2, B^2 and C^2 are the squared values of coded variables. Since the percentage of rice is higher in food waste, for the purpose of design, food waste factor is termed as rice.

3. Results and discussion

The biochemical characterization of food waste and cow dung is indicated in Table-2, which was done on a daily basis for a period of 300 h. APHA method was followed for the determination of total solids and volatile solids to estimate hydraulic retention time. These data are used for optimization by design expert software employing Response Surface Methodology (RSM).

Table 2.

Biochemical characterization of food waste and cow dung

Components	Total Solids (TS) %	Volatile Solids (VS) %	VS/TS (%)
Food Waste	29	21	72.41
Cow Dung	19.1	15.4	80.63

The design matrix as suggested by the software is based on Box-Wilson model given by Table 3. The highest yield of biohydrogen obtained is 26.87% as compared to the predicted yield of 28.03%. It can be inferred that the variance is only 1.10% which predicts the model's fitness with the experiments. Lack of fit is not significant which is evident from the Analysis of Variance (ANOVA) Table 4. The F value is 146.73 and p value of < 0.0001 implies the model's significance of A^2, B^2 and C^2 model terms.

Table 3.

Central Composite Design matrix

Run No	Factor 1 A:Cow Dung	Factor 2 B: Food waste	Factor 3 C:Time	Response % H_2	
	(g)	(g)	h	actual	predicted
1	200	200	72	25.91	25.98
2	125	-1.13446	96	11.01	10.92
3	125	125	96	26.47	26.36
4	125	125	136.363	12.12	12.06
5	200	200	120	19.8	19.81
6	125	125	96	27.1	26.36
7	125	125	96	26.87	28.03
8	-1.13446	125	96	13.48	13.26
9	125	125	96	25.1	26.36
10	50	50	72	19.58	19.73
11	125	125	96	26.09	26.36
12	125	251.1345	96	20.17	20.03
13	200	50	72	5.71	5.74
14	50	200	72	13.08	13.23
15	125	125	96	26.5	26.36
16	125	125	55.63697	19.8	19.64
17	200	50	120	2.47	2.48
18	50	200	120	7.35	7.48
19	251.1345	125	96	11.87	11.87
20	50	50	120	16.8	16.89

Table 4.

ANOVA and regression coefficient estimate

Source	Sum of Squares	Df	Mean Square	F Value	P value Pro>F	
Model	1167.274	9	129.697	483.67	< 0.0001	significant
A	2.319	1	2.319	8.648	0.0148	
B	100.16	1	100.16	373.53	< 0.0001	
C	69.355	1	69.355	258.64	< 0.0001	
AB	357.51	1	357.51	1333.27	< 0.0001	
AC	0.0882	1	0.0882	0.3829	0.5790	
BC	4.234	1	4.234	15.790	0.0026	
A^2	343.02	1	343.02	1279.227	< 0.0001	
B^2	213.40	1	213.40	795.85	< 0.0001	
C^2	199.14	1	199.14	742.66	< 0.0001	
Residual	2.681	10	0.2681			
Lack of Fit	0.1817	5	0.036	0.0726	0.9940	not significant
Pure Error	2.499	5	0.49995			
Cor Total	1169.955	19				
R^2-0.9979 Adj R^2-0.9956 Pre R^2-0.9957						

$$PercentageYield = 26.36 - 0.41A + 2.7B - 2.25C + 6.685AB - 0.105AC - 0.7275BC - 4.878A^2 - 3.848B^2 - 3.717C^2 \quad (2)$$

Three surface and contour plots are shown to represent the interaction effects of the substrate, inoculums and retention time on biohydrogen yield. Fig.1 shows the interaction of weight of cow dung and food waste on yield. This plot shows the 3D representation of factors cow dung (grams), food waste (grams) and the yield percentage (%). The marked point at the centre shows the optimum point which shows the maximum yield is almost 30%. According to the graph we can infer that, as the cow dung and food waste the yield percentage is found to be increasing. But after certain point the yield percentage is found to be decreasing, as the cow dung and food waste is increased. The optimum values for the cow dung and food waste is found to be 125 grams and 125 grams. Fig. 2 shows the effect of interaction of time and food waste on biohydrogen yield. According to the graph we can infer that, as the time and food waste amount increases, the yield percentage is found to be increasing. But after certain point the yield percentage is found to be stable even as the parameters time and food waste are increased. The optimum values for the time and food waste is found to be 96 hrs and 200grams.

Fig.3 shows the interrelation of factors time (hrs), cow dung (grams) and the yield percentage (%). The marked point at the centre shows the optimum point for the speed and time (min). The maximum yield of biohydrogen is 28%. The optimum values for the time and cow dung is found to be 96 hrs and 125 grams.

The model was validated at the optimum conditions proposed by software and yield obtained was 28.2% biohydrogen yield.

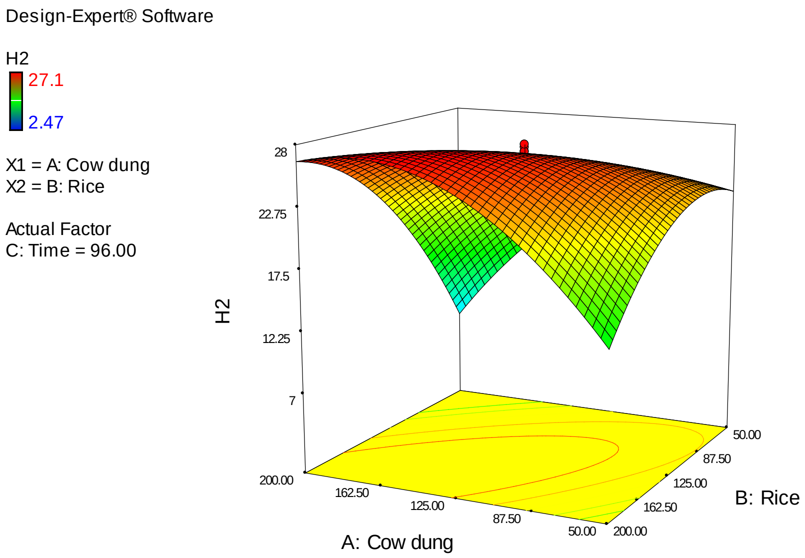

Fig.1. Surface and contour plot of biohydrogen yield for time of 96 hrs.

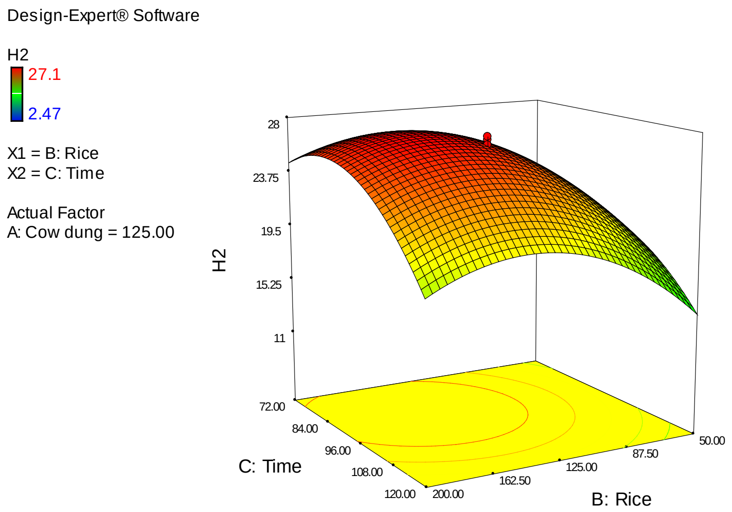

Fig.2. Surface and contour plot of biohydrogen yield for cow dung amount of 125 g

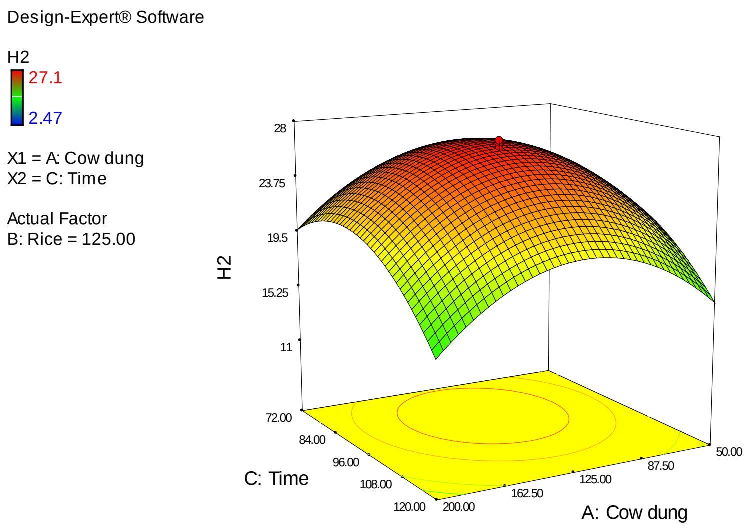

Fig.3.Surface and contour plot of biohydrogen yield for food waste amount of 125 g.

A glass syringe was used to measure biohydrogen yield. The composition of the gas was analyzed by gas chromatograph (model Shimadzu). Helium was used as the carrier gas at a flow rate of 1 ml/min – 2ml/min at a pressure of 30kPa to 100kPa. Fig. 4 shows the chromatograph obtained from batch reactor which was analyzed for the peaks of biohydrogen

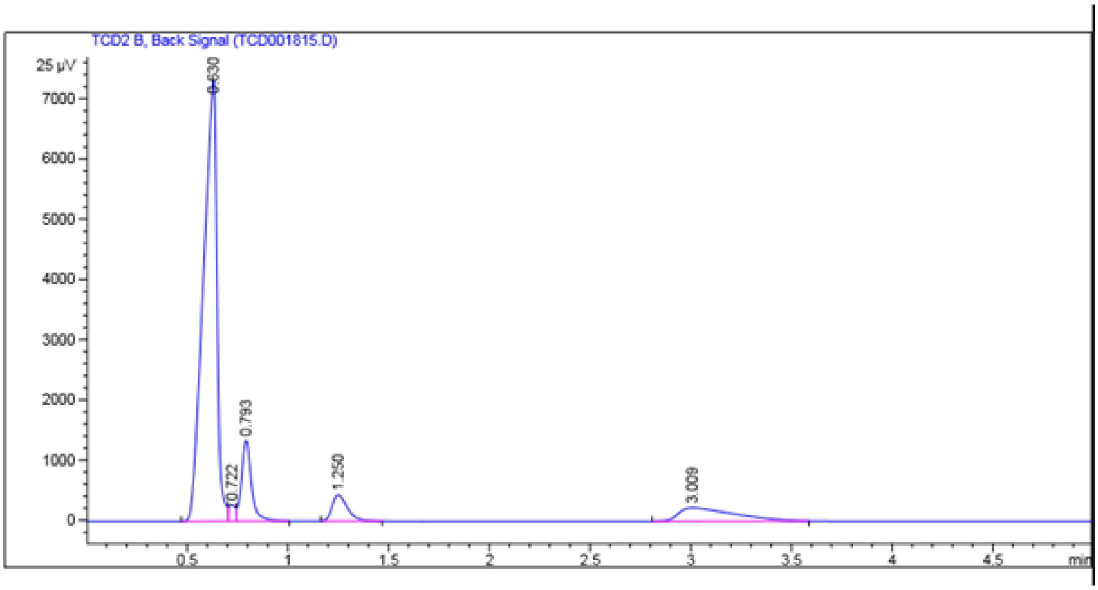

Fig. 4. Gas chromatograph of biohydrogen peak.

The area of a peak refers to the amount of desired component in sample. The area of a peak in gas chromatography is proportional to the product of its height and its width at half height. From the values of h and w, the area of the hydrogen peak was found to be 231.83 mm^2 .

4. Conclusions

From the research work, it can be concluded that biohydrogen can be produced from cooked food waste under anaerobic conditions. The process parameters like the substrate, inoculums and retention time on biohydrogen yield were optimized using response surface methodology that confirmed a hydraulic retention time of 96 h. Since clean energy is the need of the hour to combat pollution, bio hydrogen from food waste is a promising resource for fuel cells.

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