Research Article

Application of Box–Behnken design for the optimization of cellulase production under solid-state fermentation

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Received: 26 September 2019 / Accepted: 23 November 2019 / Published online: 30 November 2019

Abstract
Lignocellulosic biomass offers an encouraging possibility to satisfy future energy demand since it is an abundant and potential carbon source for the production of a wide range of fuels and value-added chemicals. Most of the proposed processes are currently unable to make economically viable conversion due to incomplete utilization of the biomass feedstock. Optimization of significant process conditions plays a very crucial role in the development of efficient and cost-effective bioconversion processes. The present paper describes the Box–Behnken design based optimization to evaluates the effect of process parameters. A set of combinations of parameter viz. temperature (30 °C), pH (5), inoculum dosages (0.56 g/L), particle size (850 µm), moisture percentage (76) and incubation period (6 days) was found quite effective on cellulase production by *Aspergillus niger* under wheat bran based solid-state fermentation. It also evaluates the utility of dairy industry waste (whey) and starch hydrolysates in cellulase production.

Keywords Wheat bran · Box–Behnken design · Whey · Starch hydrolysates

1 Introduction

Lignocellulosic biomass appears to be the most promising, economical and highly renewable natural resource in the world and it serves as prominent and sustainable feedstocks for bioconversion [1, 2]. Lignocellulosic biomass mostly refers to agricultural post-processing residues such as wheat bran, rice straw, wheat straw, bagasse [3–6]. India is an agricultural land that produced a large magnitude of lignocellulosic wastes which can be utilized for the production of value-added products such as industrial enzymes or enzyme-based products. Renewable resources handle the global energy crises in a sustainable and eco-friendly manner. However, extensive research is required for the commercialization of lignocellulose mediated biotransformation process [7, 8].

Wheat bran is separated from the wheat kernel by the milling process and chemically it consists of cellulose and hemicellulose polysaccharides (approximately 38%), starch (approximately 19%), protein (approximately 18%) and lignin (approximately 6%). It also contains a significant amount of phenolic acids such as ferulic and *p*-coumaric acid [9–11]. It is envisaged that the utilization of both starch and hemicellulosic/cellulosic part of wheat bran would greatly expedite potential applications in lignocellulosic biomass utilization. It has also been considered as a good supporting substrate probably due to the presence of various available nutrients, good porosity, suitable particle size and consistency required for fungal anchorage and enzyme excretion [3, 5]. Its texture remains loose in moist conditions, thereby provides a large surface area with increased water holding capacity [12]. Wheat bran serves as an excellent carbon source without any supplementary carbon or nitrogen source for the production of lignocellulolytic enzymes. The presence of starch, protein, and soluble oligosaccharides improves its microbial utilization.

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The soluble cello-oligosaccharides compositions of wheat bran were proved to be one of the most significant factors for cellulase production and it can also be utilized without any prior pretreatment [3, 13].

Various approaches in response surface methodology (RSM) have been employed for screening and optimization of different process parameters during the fermentation process [14]. Experimental design based statistical tools is the most recent approaches in bioprocess optimization. Response surface methodology (RSM) is an assortment of mathematical and statistical tools, used for designing experiments and achieving optimum conditions of independent variables/factors for desirable responses [15, 16]. RSM based on Box–Behnken experimental design has been widely used in various bioprocess experiments to optimize process parameters [16, 17]. Whey is a liquid by-product of the dairy industry that remains after the manufacturing process of cheese, chhana, paneer and casein. It is a serious pollutant and imposes a very high BOD of 30,000–50,000 mg/L. Discarding of whey creates a significant loss of potential nutrients and energy. On the other hand, it has also been noticed seriously by the environmentalists, due to its potent polluting strength. The bioconversion of lactose sugar present in whey to valuable products has been actively explored and could be served as a substrate for fermentation [18–20].

The key carbohydrate reserve in plants is starch (a polymer of glucose) composed of two main fractions, amylose and amylopectin. Amylose is highly hydrophilic, due to containing more hydroxyl groups. Dextrin is the intermediate product of acidic or enzymatic hydrolysis process. The starch molecule initially breaks into shorter glucose units (oligosaccharides) and further broken down into maltose and finally maltose breaks into glucose [21, 22]. The amylose content of the starch granules varies with starch sources. It has also been observed that large potato starch granules have a higher amylose content than small granules [23].

Starch utilization is widely used as an ingredient for the food and pharmaceutical industry, especially for the food industry, as substitutes, mixtures, thickening agents, and fillers [22]. It has been demonstrated that granuler size of rice starch was significantly smaller than that of the corn starch. Wheat starch shows physical and chemical properties different from potato starch. It contains higher amounts of fat mainly lysophospholipids forming gelatinous complexes with amylose, proteins and arabinoxylans [24].

The present paper portrays the Box–Behnken design based optimization of different process parameters such as temperature, pH, moisture percentage, particle size, inoculum dosages and incubation period for cellulase production by *Aspergillus niger* under wheat bran based solid-state fermentation. It also describes the utility of dairy industry waste (whey) and various starch hydrolysates in cellulase induction and production process.

2 Materials and methods

Chemicals and reagents used to conduct the experimental work were of Himedia, Sigma Aldrich, and Merck make. *Aspergillus niger* NCIM 777 was procured from National Chemical Laboratory, Pune, India. Wheat bran was collected from the local market. Separate sets of batch experiment were performed in 250 mL Erlenmeyer flasks containing sieved wheat bran biomass, which was impregnated with the following production media in (g/L) Urea, 0.3; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $MgSO_4{\cdot}7H_2O$, 0.3; Peptone, 1.0; Tween 80, 0.2; $FeSO_4{\cdot}7H_2O$, 0.005; $MnSO_4{\cdot}7H_2O$, 0.0016; $ZnSO_4{\cdot}7H_2O$; 0.0014; $CaCl_2{\cdot}2H_2O$; $CoCl_2{\cdot}6H_2O$, 0.02. Basal salt media soaked wheat bran beds were autoclaved, cooled and then inoculated with a specific volume of liquid *A. niger* pellets. Based on different sets of combinations of process parameters, the production flasks were placed in an incubator. Another set of batch experiments was also performed to investigate the effect of whey and starch hydrolysates. Each wheat bran bed containing production flasks inoculated with culture solution to study the effect of whey and starch hydrolysates were placed in an incubator at optimized conditions.

2.1 Experimental design

To optimize different process parameters for cellulase production, Box-Bhenken Design (BBD) was used for optimization work. Three different levels were studied for each independent variable. A total of 13 experiments (Different sets of combinations) were conducted for three independent variables and cellulase activity obtained in terms of FPase (IU/mL) was taken as a dependent variable (*Y*1). Design based experiments were performed in 250 mL Erlenmeyer flask containing wheat bran biomass as a solid bed. The independent variables used were process temperature (°C) (*X*1), process pH (*X*2), inoculum dosages (*X*3) and their levels were mentioned in Table 2. After getting the optimized process conditions, another trail was run of 13 experiments with other three independent variables. Now the independent variables used in the trail were particle size of wheat bran (µm) (*X*1), moisture percentage (*X*2), the incubation period (*X*3) and their levels were mentioned in Table 5. The relation between independent and dependent variables was illustrated by the following equation.

$$Y1 = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 11X1 + \beta 22X2 + \beta 33X3 + \beta 12X1X2 + \beta 13X1X3 + \beta 23X2X3$$

$Y1$ was the response; $X1$, $X2$ and $X3$ were the independent variables; $\beta 0$ was the intercept; $\beta 1$, $\beta 2$ and $\beta 3$ were linear coefficients; $\beta 11$, $\beta 22$ and $\beta 33$ were square coefficients; and $\beta 12$, $\beta 13$ and $\beta 23$ were interaction coefficients.

2.2 Preparation of starch hydrolysates

Acid pretreatment of starch was carried out by using 2%, 5%, and 10% HCl (v/v) solution. 10 g of wheat, potato and rice starch powdered biomass were taken separately, further 40 mL of diluted HCl solution with specific strength was added to maintain the slurry of about 25%. Afterwards, starch slurries were subjected to steam treatment under a pressure of 15 psi at 121 °C for 1 h and 3 h time duration. The treated starch slurries were used in the production medium as pure hydrolyzates.

2.3 Lactose analysis

The lactose content of whey was estimated by the spectrophotometric method [25]. The absorbance of the sample was determined at 540 nm.

2.4 Inoculum development

Aspergillus niger NCIM 777 was procured from National Chemical Laboratory (NCL), Pune, India. For inoculum development separate experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of potato dextrose broth (PDB) medium (In g/L peeled potato, 200; dextrose, 20; and yeast extract, 0.1) in which 5 loopfull cultures of *Aspergillus* spores were added and shaken at 180 rpm at 30 °C in an incubator shaker for 3 to 4 days [26]. A definite volume of prepared cultures in broth suspension was used as inoculum for further production studies.

2.5 Dry weight determination

The cell dry weight of *Aspergillus* suspensions was determined by the procedure used by Verma et al. [4]. The determination of fungal growth by cell dry weight was expressed as the mean of three independent readings.

2.6 Preparation of production media

Three types of production medium were used for production studies. (1) Normal basal salt media was used for production studies having the following constituents (g/L): urea, 0.3; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $MgSO_4{\cdot}7H_2O$, 0.3; peptone, 1.0; Tween 80, 0.2; $FeSO_4{\cdot}7H_2O$, 0.005; $MnSO_4{\cdot}7H_2O$, 0.0016; $ZnSO_4{\cdot}7H_2O$; 0.0014; $CaCl_2{\cdot}2H_2O$; $CoCl_2{\cdot}6H_2O$, 0.02. (2) Modified basal salt media, In which 15, 30 and 50% (v/v) whey were incorporated in the earlier described production media separately. (3) Modified basal salt media, in which 2 and 5% (v/v) dosages of 2 and 5% HCl treated potato, wheat and rice starch hydrolysates solution were incorporated in the earlier described production media separately.

2.7 Preparation of raw material

Wheat bran biomass was dried then ground and sieved with a mesh screen. The ground raw material was used as a solid substrate for cellulase production studies.

2.8 Solid state fermentation process

A separate set of fermentation experiments was carried out in 250 mL Erlenmeyer flasks containing sieved wheat bran as the carbon source, which was impregnated with the earlier discussed production medium. Raw material soaked with normal as well as modified basal salt medium was autoclaved, cooled, and then inoculated with a specific volume of PD broth culture solution of *A. niger*, after that the autoclaved and inoculated flasks were placed in an incubator.

2.9 Extraction and assay of enzyme

For the extraction of the crude enzyme, distilled water was added to the fermented samples (in a 1:5 proportion) in Erlenmeyer flasks, and the extraction was done after shaking in a shaker at 150 rpm for 1 h. The sample was then filtered and the extract obtained was centrifuged at 6000 rpm. The resulting supernatant was stored and used as a crude enzyme source. All extractions were conducted in duplicate.

2.10 Total cellulase activity (filter paper activity) and CMCase activity

Filter paper (FPA) and carboxymethyl cellulase (CMCase) activity were determined by the method recommended by Ghose [27].

3 Results and discussion

3.1 Utilization of wheat bran biomass in cellulase production

Wheat bran due to its nutritional content and large surface area serves to be an excellent carbon source without any

supplementary carbon source for the production of lignocellulolytic enzymes [13]. XRD and FTIR pattern of wheat bran also suggest that cellulose present in wheat bran is easily available for microbial attack. A lower percentage of lignin may also provide a fruitful conditions for the easier uptake of cellulose and other inducers required for cellulase production [28].

Separate sets of batch experiments were performed for the cellulase production studies by *A. niger*. *Aspergillus* grew well in a wheat bran based solid bed as observed in Fig. 1b.

To study the magnified view of *Aspergillus* growth under wheat bran bed, scanning electron microscopic studies of *Aspergillus* treated wheat bran sample were carried out. Wheat bran bed had both rough and smooth types of surfaces with layered structural morphology as shown in the SEM micrograph (Fig. 2a). The magnified view of *Aspergillus* treated wheat bran bed is shown by SEM micrograph (Fig. 2b, c). Vast microbial growth was observed at the surface of raw material, a number of distinctive spores were seen at the bed, as they are utilizing raw material as a carbon source (Fig. 2).

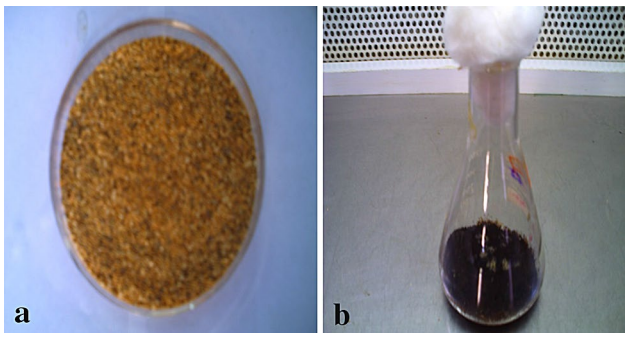

Fig. 1 Grounded and sieved wheat bran (**a**), growth of *Aspergillus* under wheat bran solid bed (**b**)

3.2 Optimization of physical and chemical parameters for cellulase production by *Aspergillus niger*

To resolve the much operative and best conditions of process parameters, statistical optimization methodology has been used. Design based optimization for bioprocess could overcome the constraints of conventional methods and has been proved to be a dominant approach for the optimization of cellulase production. The present section evaluates the Box–Behnken based design experiments for cellulase production by *A. niger*. Temperature, pH, initial moisture percentage of the substrate, inoculum dosages of fungal strains, raw materials particle size, incubation time were recognized as the most influential physical and chemical parameters. A Box–Behnken design was used to explore the interactive effect of these parameters and to attain an optimum. The base points for the design were selected from a single parameter study (data not shown). A summary of the variables and their variation levels is given in Tables 1 and 2.

Various sets of combinations have been used for cellulase production by *A. niger*. It was observed in Table 3 as well as Figs. 3 and 4 that temperature (30 °C), pH (5)

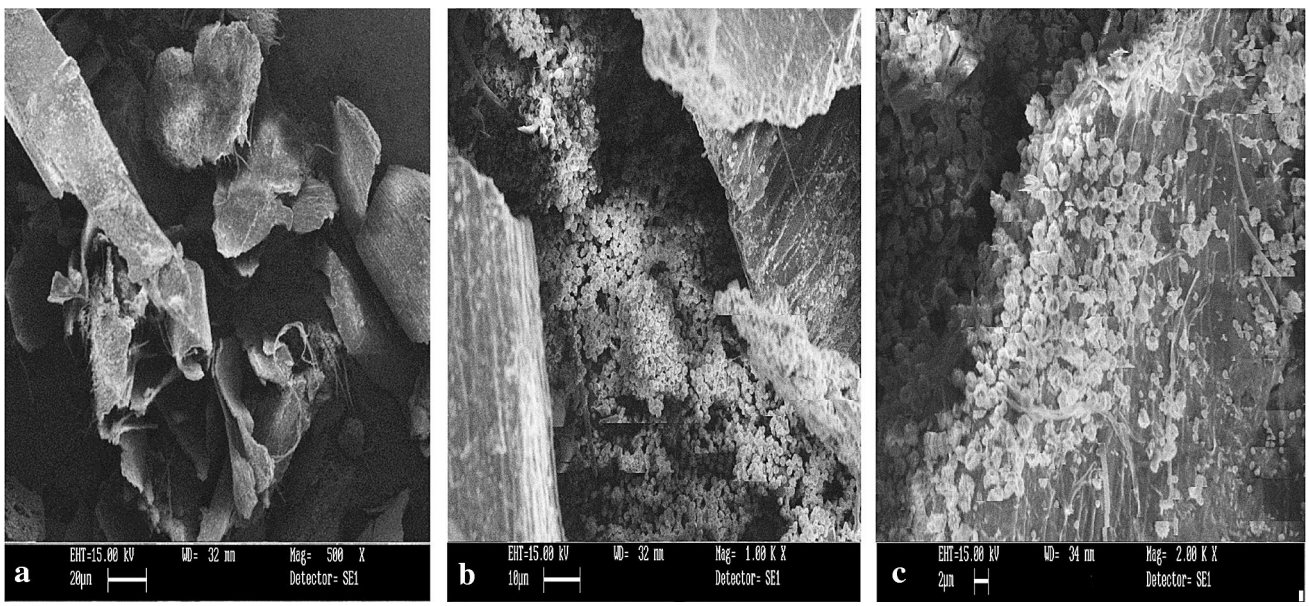

Fig. 2 Scanning electron micrograph (SEM) of *Aspergillus* treated wheat bran bed at ×500 magnification (**a**), at ×1000 (**b**), at ×2000 (**c**)

Table 1 Variables used in Box–Behnken design for the optimization of process parameters such as temperature, pH and inoculum dosages

Factor	Basic level	Variation interval	Value of the factor	Coded value
Temperature (°C)	30	5	25	–
			30	0
			35	+
pH	5	2	3	–
			5	0
			7	+
Inoculum dosages	0.56	0.24	0.32	–
			0.56	0
			0.80	+

Table 2 Variables used in Box–Behnken design for the optimization of process parameters such as particle size, moisture percentage and incubation period

Factor	Basic level	Variation interval	Value of the factor	Coded value
Particle size (μm)	850	400	450	–
			850	0
			1250	+
Moisture percentage (%)	76	5	71	–
			76	0
			81	+
Incubation period (days)	6	3	3	–
			6	0
			9	+

and inoculum dosages (0.56 g/L) was found a quite operative set of combinations for cellulase activity (0.980 IU/mL) produced by *A. niger* as compared to other sets of combinations.

As observed in Fig. 3 that higher cellulase activity region lies in the center of the graph represents significantly better optimization of process parameters.

It was also observed in Fig. 4 that the rate of decrement in enzyme activity was somewhat lower towards (> and < 30 °C), pH (< 5) and inoculum dosages (> and < 0.56 g/L).

The experimental and predicted values of cellulase activity (FPA) are somehow nearly closer to each other which represents the nearly good correlation as observed from Table 3 and Fig. 5).

Distinctive sets of permutation have been used for cellulase production by *A. niger*. It was observed from Table 4 and Fig. 6 that particle size (850 μm), moisture (76%), and incubation period (6 days) was found a quite efficient set of combinations for cellulase activity (1.29 IU/mL) produced by *A. niger* as compared to other.

As observed in Fig. 6 that the higher cellulase activity region lies in the center of the graph which proves the significantly better optimization of process parameters.

It was also observed from Fig. 7 that rate of decrement in enzyme activity was somewhat lower towards smaller particle size (< 850 μm), higher moisture percentage (> 76) and higher incubation period (> 6 days).

The observed and predicted values of cellulase activity are extremely close to each other which signifies the very fine correlation between them as observed from Table 4 and Fig. 8. In each sets total of 10 coefficients are generated as observed from Table 5.

It can be concluded that optimization was found quite effective, as well as in most of the cases experimental and

Table 3 Comparative experimental and predicted values of cellulase (FPA) activity (IU/mL) achieved by *Aspergillus* under different sets of combinations

Medium code	T (°C)	pH	Inoculum dosages	FPA (IU/mL) (E)	T (°C)	pH	Inoculum dosages	FPA (IU/mL) (P)
A	25	3	0.56	0.518	−1	−1	0	0.345249
B	25	5	0.32	0.646	−1	0	−1	0.79221
C	25	5	0.80	0.578	−1	0	1	0.136993
D	25	7	0.56	0.442	−1	1	0	0.400984
E	30	3	0.32	0.622	0	−1	−1	0.613322
F	30	3	0.80	0.554	0	−1	1	−0.12464
G	30	5	0.56	0.980	0	0	0	0.695444
H	30	7	0.32	0.583	0	1	−1	0.639056
I	30	7	0.80	0.476	0	1	1	−0.0309
J	35	3	0.56	0.578	1	−1	0	0.049683
K	35	5	0.32	0.544	1	0	−1	0.549388
L	35	5	0.80	0.646	1	0	1	−0.20332
M	35	7	0.56	0.510	1	1	0	0.113418

T temperature (°C), *E* experimental, *P* predicted

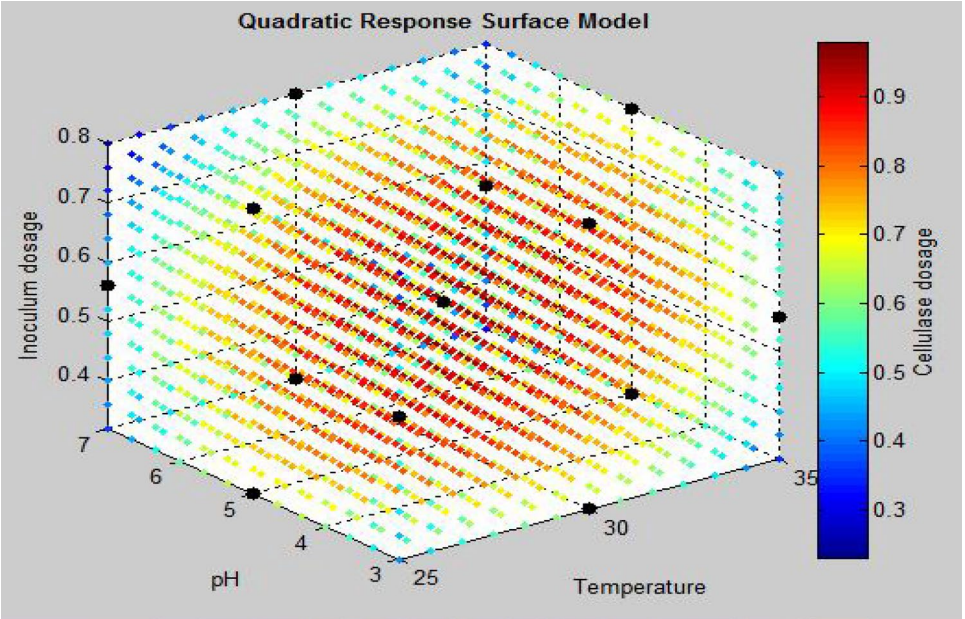

Fig. 3 Quadratic response surface model with cellulase activity (IU/mL) attained by *Aspergillus* as a response of three parameters temperature, pH and inoculum dosage. Cellulase dosage represents the cellulase activity (IU/mL)

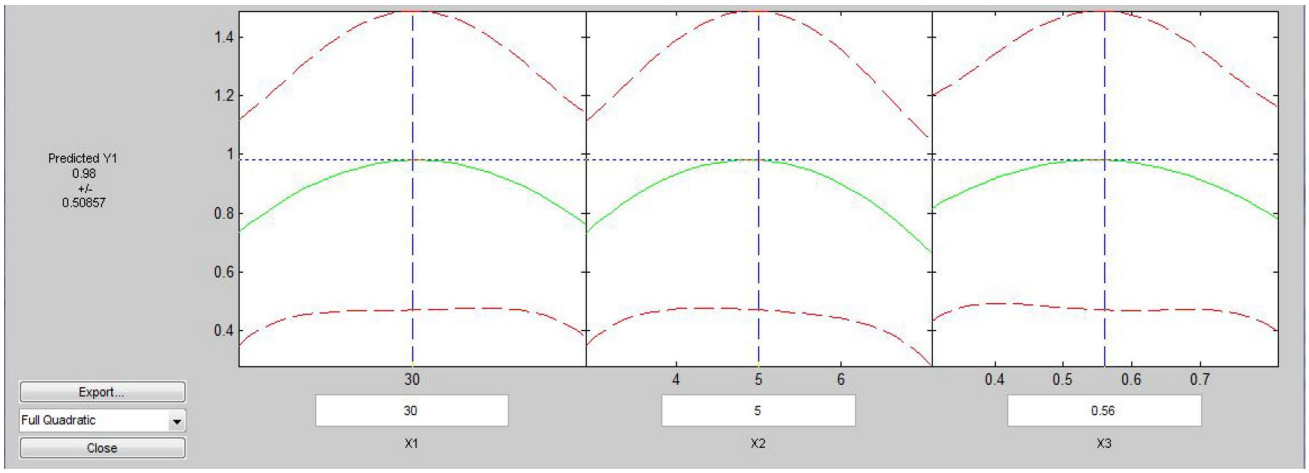

Fig. 4 Cellulase activity achieved by *Aspergillus* as a response (Y1) of three parameters temperature (X1), pH (X2) and inoculum dosage (X3)

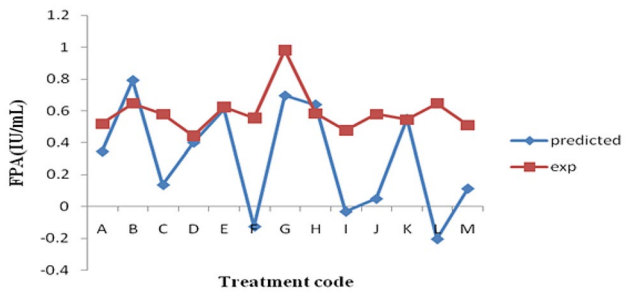

Fig. 5 Comparative graph of experimental and predicted values of cellulase activity (FPA)

predicted values were very close to each other, representing the better correlation between them.

3.3 Effect of dairy waste (whey) on cellulase production under wheat bran based solid-state fermentation

To investigate the effect of dairy waste whey on cellulase production under wheat bran based solid-state fermentation, a separate set of experiments have been performed using 15%, 30% and 50% (v/v) whey as carbon source.

Table 4 Comparative experimental and predicted values of cellulase activity (IU/mL) attained by *Aspergillus* under a different sets of combinations

Particle size	Moisture percentage	Incubation period	FPA (IU/mL) (E)	Particle size	Moisture percentage	Incubation period	FPA (IU/mL) (P)
450	76	3	0.672	−1	0	−1	0.680729
850	71	3	0.567	0	−1	−1	0.60796
850	81	3	0.672	0	1	−1	0.712898
1250	76	3	0.567	1	0	−1	0.647129
450	71	6	0.735	−1	−1	0	0.75069
450	81	6	0.840	−1	1	0	0.855565
850	76	6	1.290	0	0	0	1.333828
1250	71	6	0.630	1	−1	0	0.71709
1250	81	6	0.735	1	1	0	0.821965
450	76	9	0.798	−1	0	1	0.815566
850	71	9	0.696	0	−1	1	0.74286
850	81	9	0.801	0	1	1	0.847673
1250	76	9	0.683	1	0	1	0.781966

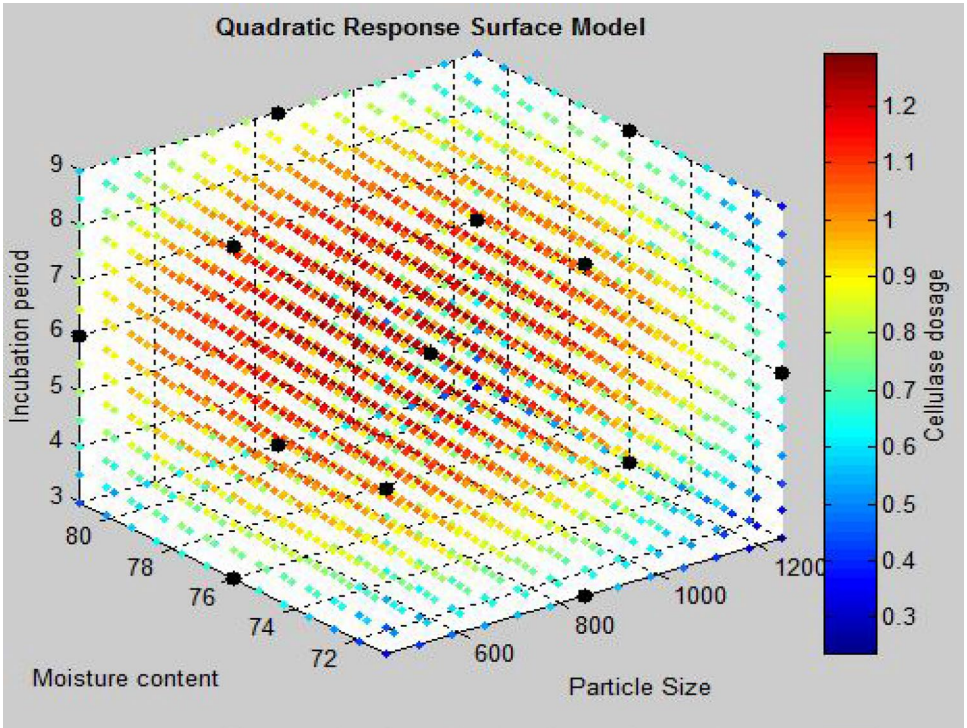

Fig. 6 Quadratic response surface model with cellulase activity (FPA) attained by *Aspergillus* as a response of three parameters temperature, pH and inoculum dosage. Cellulase dosage represents the cellulase activity (IU/mL)

It has been observed in Table 6 that *Aspergillus* strain showed better activity under 30% whey containing wheat bran as compared to other dosages of whey. The maximum cellulase activities (IU/mL) in terms of FPA and CMCase attained by *A. niger* were 1.47 ± 0.08 and 11.02 ± 0.05 under 30% whey containing wheat bran based solid-state fermentation. Lactose sugars present in the whey may provide a favorable conditions for better cellulase activity for *Aspergillus* strain [29].

3.4 Effect of starch hydrolysates on cellulase production under wheat bran based solid-state fermentation

To examine the role of starch hydrolysates in cellulase production under wheat bran based solid-state fermentation, a separate set of experiments were executed. Starch pretreatments were performed by using 2 and 5% HCl with the pretreatment time of 1 h. Maximum cellulase

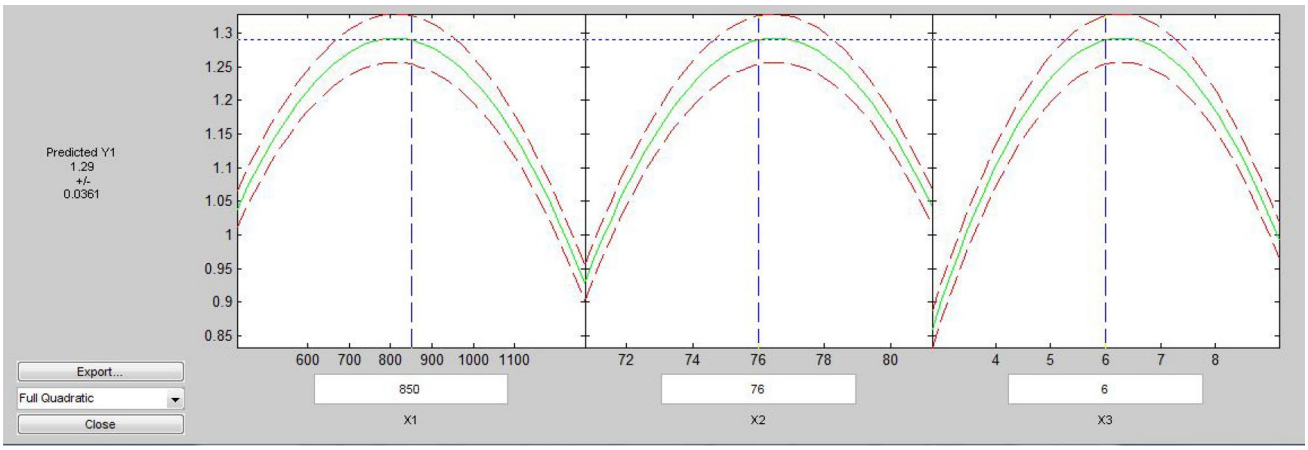

Fig. 7 Cellulase activity achieved by *Aspergillus* as a response (Y1) of three parameters particle size (X1), moisture percentage (X2) and incubation period (X3)

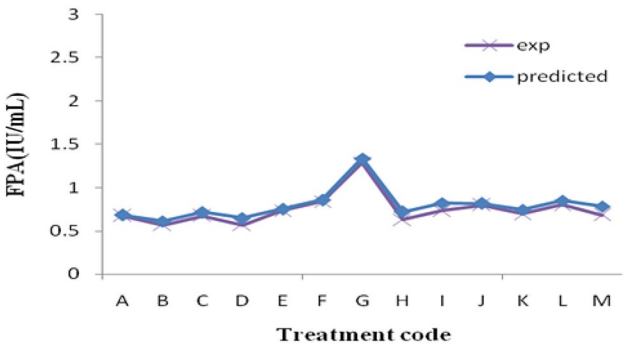

Fig. 8 Comparative graph of experimental and predicted values of cellulase activity (FPA)

Table 6 Comparative cellulase activity (IU/mL) achieved by *Aspergillus* strain under different concentrations of whey containing wheat bran based SSF at 30 °C and pH 5

Dairy industry waste (Whey)	*Aspergillus niger*	
	FPA (IU/mL)	CMCase (IU/mL)
BSM + WB (without whey)	1.29 ± 0.03	10.10 ± 0.05
Whey		
15% (v/v)	1.31 ± 0.06	10.53 ± 0.03
30% (v/v)	1.47 ± 0.08	11.02 ± 0.05
50% (v/v)	1.41 ± 0.03	10.62 ± 0.09

Data are reported as mean ± standard deviation based on the repeated trails

BSM basal salt media, *WB* wheat bran

Table 5 Coefficient values under different sets of condition

Aspergillus niger	
Trail 1	Trail 2
Temp, pH, inoculum dosages	Particle size, moisture percentage, incubation period
Coefficients	Coefficients
− 8.51818	− 65.765
0.489417	0.002848
0.63000	1.68554
2.171528	0.463271
0.0002	− 5.14E−19
0.035417	− 2.08E−06
− 0.02031	− 1.61E−16
− 0.00847	− 1.75E−06
− 0.06409	− 0.01102
− 2.86241	− 0.03672

activities (IU/mL) in terms of FPA and CMCase attained by *A. niger* were 1.57 ± 0.08 and 12.45 ± 0.06 under 2% acid hydrolyzed rice starch hydrolysate (5%v/v) based solid- state fermentation, which may be due to release of some dimeric sugars such as cellobiose and sophorose in the acid hydrolysates starches. Cellobiose response regulator (ClbR) of *Aspergillus aculeatus* has been confirmed to accelerate the expression of cellulases [30]. It was also observed from Table 7 that fungal strains produce significantly higher cellulase activities under 2% HCl treated wheat starch hydrolysate based fermentation medium as compared to 5% HCl treated one, which may be due to the fact that upon under higher strength-based acid treatment, starch is over hydrolyzed and other byproducts may be generated, which might have served as inhibitors for cellulase production [31].

When compared the effectiveness of starch hydrolysates for *Aspergillus* strain than it has been observed that *A. niger* performed better under rice hydrolysate

Table 7 Comparative cellulase activities (IU/mL) achieved by *Aspergillus* strain under various starch hydrolysates incorporated wheat bran based SSF at 30 °C and pH 5

Starch hydrolysates (SH)	*Aspergillus niger*	
	FPA (IU/mL)	CMCase (IU/mL)
BSM + WB (without SH)	1.29 ± 0.03	10.10 ± 0.05
2% HCl WSH		
2% (v/v)	1.41 ± 0.03	11.17 ± 0.02
5% (v/v)	1.53 ± 0.06	11.87 ± 0.07
5% HCl WSH		
2% (v/v)	1.20 ± 0.11	9.70 ± 0.05
5% (v/v)	1.18 ± 0.02	9.51 ± 0.13
2% HCl PSH		
2% (v/v)	1.38 ± 0.09	10.70 ± 0.09
5% (v/v)	1.41 ± 0.03	11.21 ± 0.11
5% HCl PSH		
2% (v/v)	1.16 ± 0.07	9.32 ± 0.05
5% (v/v)	1.13 ± 0.02	9.21 ± 0.03
2% HCl RSH		
2% (v/v)	1.43 ± 0.13	11.59 ± 0.09
5% (v/v)	1.57 ± 0.08	12.45 ± 0.06
5% HCl RSH		
2% (v/v)	1.22 ± 0.04	9.83 ± 0.03
5% (v/v)	1.20 ± 0.03	9.67 ± 0.07

Data are reported as mean ± standard deviation based on the repeated trails

WSH wheat starch hydrolysate, *PSH* potato starch hydrolysate, *RSH* rice starch hydrolysate

containing wheat bran based fermentation. Potato starch hydrolysate was found less effective for cellulase activity enhancement as compared to wheat starch. It can be concluded that rice, as well as wheat starch hydrolysate, were found quite effective for cellulase induction capability. The acid hydrolysis of starch considerably changes the structural and functional properties of starch. Amorphous regions are hydrolyzed favorably during acid hydrolysis [32].

When compared to the induction capabilities of whey and starch hydrolysates perceived from Tables 6 and 7. It has been observed from experimental evidence that starch hydrolysate has a better ability for induction as compared to whey. This advocate that oligosaccharides and dimeric sugars provoke much better cellulase production as compared to lactose by *A. niger*. We can also suggest that raw material composition also affects the performance of starch hydrolysates.

4 Conclusions

Utilizing lignocellulosic waste materials as agricultural residues, forestry wastes and other low-cost biomass can significantly reduce the cost of production. In the present investigation, Box–Behnken design was used to optimize the process parameters for cellulase production by *A. niger* under wheat bran based solid-state cultivation. It is an applicable and trustworthy tool for finding the optimal process parameters. Various starch hydrolysates, as well as dairy industry waste (whey), were used in cellulase production. Higher cellulase activity attained by *A. niger* under 30% (v/v) whey as well as 5%(v/v) of 2% HCl treated potato starch containing media. The utilization of waste materials in cellulase production would be a cost-effective, efficient, environment-friendly and sustainable approach.

Acknowledgements Authors gratefully acknowledged the support and facilities provided by Indian Institute of Technology, Roorkee, India for scanning electron microscopic (SEM) and other analysis. They also acknowledged the Ministry of human resource and development (MHRD) India, for providing fellowship to carry out present research work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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