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Research Paper

Brewer's Spent Grain as a Valuable Substrate for Low Cost Production of Fungal Cellulases by Statistical Modeling in Solid State Fermentation and Generation of Cellulosic Ethanol

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Abstract

Brewer's spent grain (BSG), a brewery waste was used as a substrate for cellulase production employing a laboratory isolate of *Aspergillus* sp.SS-25 under solid state fermentation as well as for cellulosic ethanol production. The cultural parameters and media components were statistically optimized for enhancing the cellulase yields employing Plackett-Burman design model. A near-optimum medium formulation for maximum cellulase production by the fungus was determined that increased the CMCase, FPase and β-glucosidase yields by 3.1, 3.6 and 2.1 folds, respectively. Predicted results showed that supplementing malt spent grain with 20% (w/w) wheat bran, 2% each of potato peels, peptone, soyabean meal, tryptone, malt extract, 0.14% NH_4SO_4, 0.002% $CoCl_2$, 0.0006% $FeSO_4$, 0.1% $MnCl_2$, 0.1% $NaNO_3$, 0.03% urea, 0.03% NH_4Cl, maintaining 70% moisture content and incubation at 30°C for 6 days induced maximum production of all the components of cellulase system yielding 295, 90 and 80 IU/g respectively for CMCase, FPase and β-glucosidase against the predicted yields of 289, 94 and 82 IU/g dry solids. The enzyme preparation worked well in degrading the cellulose fraction in steam pretreated BSG into glucose revealing 75% cellulose conversion efficiency by way of yielding 134 mg glucose/g of BSG. The fermentation efficiency of glucose was found to be 91% producing 78µl ethanol/g of BSG.

Keywords: Brewer's spent grain, Statistical modeling, *Aspergillus* sp., Cellulases, Cellulosic alcohol, solid state fermentation

Introduction

Fossil fuel depletion and restricted environmental concerns have compelled to search for alternative energy sources. Hence, focus has been placed on producing ethanol as a substitute or additive to gasoline for transportation. Ethanol production from sugarcane and cereals like wheat, corn is well known. Since these raw materials which are to be used as human food so cannot sustain long term ethanol production demands, thus the lignocellulosic residues including agricultural, industrial and kitchen waste residues offer an alternative substrate which does not compete with human food for the production of second generation cellulosic alcohol. Cellulose, the major component of these lignocellulosic residues is the primary product of photosynthesis and the most abundant renewable bioresource produced in the biosphere at 100 billion tons/ year (Zhang and Lynd, 2004). The chemical hydrolysis of cellulose involves harsh conditions of temperature and pressure in presence of high concentration of acids. On the other hand, enzyme treatment involves the eco-friendly

conditions operating at near ambient temperature and is thus gaining world-wide interest. Complete enzymatic hydrolysis of cellulose requires synergistic action of three types of enzymes, namely endoglucanase or carboxymethyl cellulase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21). The availability of cellulases is considered a major barrier to the economical production of cellulosic ethanol. So the major task to make breakthrough in the research on cellulosic alcohol is to lower the production costs of cellulases that may account for as much as 40% of the total cellulosic ethanol production. Efforts have thus been focused on screening the microorganisms that harbour novel enzymes as well as increasing enzyme yield, activity and stability (Elkins *et al*, 2010).

Substrate costs account for a major fraction of the total cost of cellulase production, and the use of cheap, abundant and easily available biomass resources as substrates can help reduce cellulase prices and the use of cheaper technologies like solid-state fermentation (SSF) can further improve the production economics. It results in an enzyme preparation, which is more concentrated and hence best, suited for biomass conversion applications (Chahal, 1985). Research on the selection of suitable substrates for SSF has mainly been centered on agro-industrial residues due to their potential advantages for organisms, which are capable of penetrating into the hardest of these solid substrates, aided by the presence of turgor pressure at the tip of the mycelium (Ramachandran *et al*, 2004).

Agricultural, agro-industrial and domestic wastes have increased as a result of rising population and industrialization (Da Silva *et al*, 2005). These represent one of the most energy-rich resources on the planet and the utilization of the same has been on the priority list of several companies around the world for developing technologies to produce some valuable products like cellulases and ethanol, and simultaneously reducing waste biomass going into landfills. Many of these residues like wheat bran, sugar cane residues, straws and meals have been extensively exploited by several workers for the production of various industrial enzymes and other metabolites, but brewer's spent grain (BSG) is one of the abundantly available residues which is underexploited. BSG is a by-product of brewing industry, rich in cellulose and non-cellulosic polysaccharides and has a strong potential to be recycled. Due to the global intense pressure towards green environmental technology, both academic and industrial researchers are putting more efforts to reduce the amount of such wastes by finding alternative uses apart from the current general use as an animal feed (Aliyu and Bala, 2011).

Several microorganisms produce cellulases (Bansal *et al*, 2011; Deswal *et al*., 2011; Gautam *et al*., 2011; Gilleran *et al*., 2010, Han *et al*., 2009, Immanuel *et al*., 2006, Juwaied *et al*., 2011; Kim and kim, 2012, Soni *et al*., 2010; Soni and Soni, 2010) but only those organisms producing substantial amounts of complete cellulase system extracellularly on simple media are of industrial importance. Extracellular cellulase production in microorganisms is highly influenced by the media components and environmental factors. Process optimization is a topic of central importance for industrial production processes, where the classical optimization method of one variable at a time (OVAT) is most frequently used. However, this strategy is not only time and effort consuming, but also lacks in a study of the interaction between variables. Thus, statistical approaches like Plackett-Burman and Response Surface methodology designs can offer cost-effective solutions for process optimization studies in biotechnology, as they provide pre-planned methods through which interaction between variables can be calculated (Haaland, 1989). The first step involves a screening experiment using a Plackett-Burman fractional factorial design to address the most significant factors affecting production among the studied variables. The second step then determines the optimum levels of the most significant variables identified in the first step through a nonlinear optimization algorithm and application of the response surface methodology.

The aim of present investigation was to evaluate the potential of brewer's spent grain, as a substrate for the production of complete cellulase system by *Aspergillus* sp. SS-25 *via* SSF as well as to study the application of Plackett-Burman design to assess the relative importance of various process variables favouring the hyper-production of the enzyme system and the subsequent exploitation of this crude preparation for bioconversion of BSG in to cellulosic alcohol.

Materials and Methods

Microorganisms

The cellulolytic fungal strain of *Aspergillus* sp. SS-25 used in the present study was isolated from the soil samples of Chandigarh city. It was grown and maintained on potato dextrose agar plates at 28°C for 4 days to allow the

development of spores and then stored at 4°C until use. The distiller's strain of *Saccharomyces cerevisiae* 360 was kindly provided by M/S Jagatjit Industries Limited, Kapurthala, India

Solid state fermentation of Brewer's spent grain for the production of cellulase system

The cellulase production was carried out under solid state conditions in 250 ml Erlenmeyer flasks containing 5g brewers spent grain moistened with 5 ml of distilled water. The flasks were autoclaved and inoculated in triplicate with 2.5ml of fungal spore suspension (2.8×10^7 spore/ml) and incubated at 30°C in stationary state for 96 h. The enzymes were extracted by adding 100 ml of deionised water to each flask and churning the contents in a laboratory blender. The contents were then filtered through a metallic sieve, and the solid residue was pressed to release remaining liquid. The suspension from each flask was centrifuged at 10,000×g for 10 min at 4°C after separating the mycelium by decanting and the supernatant analysed for various cellulase activities. The yields were expressed as IU/g dry solids.

Enzyme Assays

Complete cellulase complex was measured in terms of endo-β-1, 4-glucanase, exo-β-1, 4-glucanase and β-glucosidase activities (Mandels *et al*, 1976) and the same have been expressed in terms of CMCase, FPase and β-glucosidase activities using CMC, Whatmann filter paper strips and salicin, respectively, as the substrates in 0.1 M acetate buffer, pH 4.0 and determining the micromoles of glucose liberated/min at 50°C using dinitro salicylic acid reagent (Miller,1959).

Statistical optimization of cellulase production by Plackett-Burmann design

Cellulase production is highly influenced by many factors including media components and environmental parameters. For screening the effect of these parameters on enzyme production, 27 different process variables were chosen and examined, in one block, at two levels using first order Plackett-Burman factorial design:

$$Y = \beta o + \Sigma \beta i Xi$$

Where, Y is the response, βo is the model intercept, βi is the linear coefficient, and Xi is the level of the independent variable. Table 1 represents the experimental design matrix in which 27 assigned variables, with their levels as depicted in Table 2, were screened in 28 trial experimental runs. The respective responses for the three enzyme activities observed in trials are shown in Table 3.

Table 1: Randomized Plackett-Burman experimental design for evaluating factors influencing CMCase, FPase and β-glucosidase production.

Run	Urea (X_1)	NH_4SO_4 (X_2)	KH_2PO_4 (X_3)	Peptone (X_4)	Yeast Extract (X_5)	Meat Extract (X_6)	Soyabean meal (X_7)	Tryptone (X_8)	$CaCl_2$ (X_9)	$MgSO_4$ (X_{10})	$CoCl_2$ (X_{11})	$ZnSO_4$ (X_{12})	Wheat Bran (X_{13})	$FeSO_4$ (X_{14})	Water (X_{15})	Tween 80 (X_{16})	$MnCl_2$ (X_{17})	Malt Extract (X_{18})	Incubation Time (X_{19})	Tween 20 (X_{20})	Inoculum size (X_{21})	SDS (X_{22})	Potato Peels (X_{23})	$MnSO_4$ (X_{24})	NH_4Cl (X_{25})	$NaNO_3$ (X_{26})	NaCl (X_{27})
1	-1	+1	-1	+1	-1	-1	-1	+1	-1	-1	+1	+1	+1	-1	+1	-1	+1	+1	-1	-1	-1	-1	+1	+1	+1	+1	+1
2	+1	-1	-1	-1	-1	+1	+1	-1	-1	+1	+1	-1	-1	+1	+1	+1	+1	-1	-1	-1	-1	+1	+1	-1	+1	+1	+1
3	+1	-1	+1	+1	-1	+1	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1	-1	-1	+1	-1	-1	+1	-1	+1	-1
4	+1	+1	+1	-1	-1	-1	+1	+1	-1	+1	-1	-1	+1	-1	-1	-1	-1	+1	+1	+1	-1	+1	+1	-1	-1	+1	+1
5	-1	+1	+1	-1	+1	+1	+1	-1	+1	+1	+1	+1	-1	-1	-1	-1	+1	+1	-1	+1	-1	-1	+1	-1	+1	-1	-1
6	+1	-1	+1	-1	+1	+1	-1	+1	+1	-1	+1	+1	+1	+1	+1	-1	-1	-1	+1	-1	-1	+1	+1	-1	-1	+1	-1
7	-1	-1	+1	-1	-1	+1	-1	+1	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1
8	-1	+1	-1	-1	-1	+1	-1	-1	+1	+1	+1	-1	+1	-1	+1	+1	-1	+1	+1	-1	+1	+1	+1	+1	-1	-1	-1
9	+1	+1	-1	-1	+1	+1	+1	+1	-1	-1	-1	-1	+1	+1	-1	+1	+1	+1	+1	-1	-1	-1	-1	-1	+1	+1	-1
10	-1	-1	+1	-1	+1	-1	-1	-1	+1	+1	-1	+1	+1	+1	-1	+1	-1	+1	-1	-1	-1	+1	-1	+1	+1	+1	+1
11	-1	+1	+1	+1	+1	-1	+1	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1	-1	-1	+1	+1	-1	-1	+1	-1	-1
12	+1	+1	-1	+1	+1	-1	-1	+1	+1	+1	+1	+1	-1	-1	-1	+1	+1	-1	+1	-1	-1	+1	-1	-1	-1	-1	+1
13	-1	+1	+1	+1	-1	+1	-1	+1	+1	-1	-1	-1	-1	+1	+1	+1	+1	+1	-1	+1	-1	+1	-1	-1	-1	+1	-1
14	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
15	+1	-1	+1	+1	+1	-1	+1	-1	+1	-1	-1	-1	+1	-1	+1	+1	+1	+1	-1	-1	+1	-1	+1	-1	-1	-1	+1
16	+1	-1	-1	+1	-1	-1	-1	-1	+1	+1	+1	-1	+1	+1	-1	-1	+1	+1	+1	+1	+1	-1	-1	-1	+1	+1	-1
17	+1	-1	+1	+1	+1	+1	-1	-1	-1	-1	+1	-1	-1	-1	+1	-1	-1	+1	+1	+1	-1	+1	-1	+1	+1	-1	+1
18	+1	+1	+1	-1	-1	-1	-1	+1	+1	-1	+1	-1	-1	+1	-1	+1	-1	-1	-1	+1	+1	-1	+1	+1	+1	-1	+1
19	-1	-1	-1	+1	+1	-1	+1	+1	+1	+1	-1	-1	-1	-1	+1	+1	-1	-1	+1	+1	-1	-1	+1	+1	+1	+1	-1
20	+1	+1	+1	-1	-1	-1	+1	-1	+1	-1	-1	+1	-1	-1	+1	-1	+1	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1
21	-1	-1	+1	+1	-1	-1	+1	-1	-1	-1	+1	+1	+1	+1	-1	+1	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1
22	+1	+1	-1	+1	+1	+1	-1	-1	-1	-1	-1	+1	+1	-1	-1	+1	-1	-1	-1	+1	+1	+1	+1	-1	+1	+1	-1
23	+1	-1	-1	-1	+1	-1	-1	+1	-1	+1	-1	+1	-1	+1	+1	-1	+1	+1	-1	+1	+1	+1	+1	+1	-1	-1	-1
24	-1	-1	-1	-1	+1	+1	+1	+1	+1	-1	+1	-1	+1	-1	-1	-1	+1	-1	-1	+1	+1	+1	-1	+1	-1	+1	+1
25	+1	+1	-1	+1	-1	+1	+1	-1	+1	+1	-1	+1	+1	+1	+1	-1	-1	-1	-1	+1	-1	-1	-1	+1	-1	-1	+1
26	-1	+1	-1	-1	+1	-1	+1	-1	-1	-1	+1	+1	-1	+1	+1	+1	-1	+1	+1	+1	+1	-1	-1	-1	-1	+1	+1
27	-1	+1	+1	+1	+1	+1	-1	-1	-1	+1	-1	-1	-1	+1	-1	-1	+1	-1	+1	-1	+1	-1	+1	+1	-1	+1	+1
28	-1	-1	-1	+1	-1	+1	+1	+1	+1	-1	-1	+1	-1	+1	-1	-1	-1	+1	+1	-1	+1	+1	+1	-1	+1	-1	+1

Table 2: Levels of independent variables used for medium optimization in Plackett-Burman design

Variables	Levels	
	Low (-1)	High (+1)
X_1:Urea	0	1.5 mg
X_2:NH_4SO_4	0	7.0 mg
X_3:KH_2PO_4	0	100 mg
X_4:Peptone	0	10 mg
X_5:Yeast extract	0	100 mg
X_6:Meat extract	0	100 mg
X_7:Soyabean meal	0	100 mg
X_8:Tryptone	0	100 mg
X_9:$CaCl_2$	0	1.5 mg
X_{10}:$MgSO_4$	0	1.5 mg
X_{11}:$CoCl_2$	0	0.01 mg
X_{12}:$ZnSO_4$	0	0.01 mg
X_{13}:Wheat bran	0	1.0 mg
X_{14}:$FeSO_4$	0	0.03 mg
X_{15}:Water	5	12 ml
X_{16}:Tween 80	0	0.01 mg
X_{17}:$MnCl_2$	0	0.5 mg
X_{18}:Malt extract	0	100 mg
X_{19}:Incubation time	3 days	6 days
X_{20}:Tween 20	0	0.01 mg
X_{21}:Inoculum size	1 ml	2.5 ml
X_{22}:SDS	0	0.6 mg
X_{23}:Potato peels	0	100 mg
X_{24}:$MnSO_4$	0	0.5 mg
X_{25}:NH_4Cl	0	1.5 mg
X_{26}:$NaNO_3$	0	5.0 mg
X_{27}:NaCl	0	1.5 mg

Table 3: Responses observed in CMCase, FPase and b-glucosidase productivities in randomized Plackett-Burman experimental runs.

Run	Response 1CMCase (U/g)	Response 2FPase (U/g)	Response 3β-glucosidase (U/g)
1	254	70	59
2	234	68	57
3	146	72	26
4	186	76	44
5	160	30	30
6	177	52	73
7	170	56	58
8	184	52	63
9	185	76	49
10	95	12	25
11	189	96	52
12	146	52	21
13	208	60	49
14	118	52	15
15	202	34	64
16	152	52	35
17	182	68	61
18	143	76	20
19	204	56	71
20	205	10	68
21	175	48	46
22	139	28	28
23	205	52	46
24	135	72	26
25	207	12	52
26	214	48	65
27	156	52	31
28	180	34	41

Statistical analysis of data

The software package, Design-Expert trial version 8 from Stat-Ease which provides highly efficient design of experiments was employed. Multiple linear regression analysis was carried out to estimate t-values, p-values to evaluate the significance of experimental design and to screen out the factors affecting enzyme production.

Bioconversion of Brewer's spent grain into cellulosic alcohol with cellulases produced in-house

The cellulase system produced as above was evaluated for its potential use in the bioconversion of cellulose fraction in the BSG into glucose and the subsequent fermentation into cellulosic alcohol. The cellulose content in BSG was analyzed by Crampton and Maynard method (1938).

Ten g of oven dried BSG dispensed in 250 ml Erlenmeyer flask containing 40 ml distilled water was steam pretreated at 15 psi for 30 min. This was cooled and supplemented with crude enzyme preparation from *Aspergillus* sp. SS-25 and 0.1 M acetate buffer, pH 4.5 to make a total volume of 75 ml with enzyme to solid ratio of 14 IU/g, 6 IU/g and 9 IU/g for CMCase, FPase and â-glucosidase respectively. The mixture was incubated at 50°C in a water bath shaker at 150 rpm for 72 h. The samples were withdrawn at regular intervals of 24 h to determine the extent of cellulose hydrolysis by measuring the glucose concentrations, after centrifugation at 5000 rpm for 10 min, by glucose oxidase-peroxidase method (Morin and Prox, 1973). The hydrolyzed sample was inoculated with 7.5 ml suspension of actively

grown culture of *Saccharomyces cerevisae* made in distilled water and incubated at 30°C for 48 h in static state and the alcohol content was estimated by spectrophotometric method (Caputi *et al*,1968).All analysis was done in triplicate and data were expressed in terms of 100% dry matter basis. The results of enzymatic hydrolysis have been depicted in terms of cellulose conversion and glucose yield where as the results of fermentation have been expressed in terms of ethanol yield.

Cellulose conversion as percentage of the theoretical glucose yield obtained from the equation which involves the transfer of cellulose to sugar $(C_6H_{10}O_5)_n + {}_nH_2O$ ® $(C_6H_{12}O_6)_n$ was computed by using the following formula:

Cellulose conversion (%) = [Glucose]/ (1.11× f × [biomass]) × 100

where [Glucose] is the glucose concentration (g), [Biomass] is dry BSG concentration used in enzymatic hydrolysis; f is the cellulose fraction in dry biomass (g/g) and 1.11 is the factor that corresponds to the mass balance of the conversion of cellulose to sugar. Glucose yield has been expressed in terms of mass of glucose produced/ mass of untreated BSG.

Fermentation efficiency (%) = [ml of alcohol actually produced]/ [0.64 × Glucose (g)] × 100

[0.64 ×Glucose (g)] is the factor that corresponds to the mass balance of conversion of 1 g of glucose into ethanol (ml). Alcohol yield has been expressed in terms of volume of alcohol produced/mass of untreated BSG.

Results and Discussion

Due to the global intense pressure to reduce the amount of industrial wastes by finding alternative uses apart from the current general use as an animal feed, this residues was used as the substrate for the growth and cellulase production by *Aspergillus* sp. SS-25. The strain of *Aspergillus* sp. SS-25 colonized well on Brewer's spent grain, simply moistened with water, and produced an appreciable yield of all the cellulase components including CMCase (93.8 IU/g), FPase (25 IU/g), β-glucosidase (38 IU/g). Although several fungi have been evaluated for their ability to degrade cellulosic substrates, those belonging to genera *Trichoderma* and *Aspergillus* have been studied in detail for their cellulase production potential (Kumar *et al*, 2008). Besides *Trichoderma viride*, the other mesophilic strains producing cellulases are *Fusarium oxysporium, Piptoporus betulinus, Penicillium echinulatum, P. purpurogenum, Aspergillus niger, A. fumigates* and *Chaetomium erraticum* (Kumar *et al*., 2008; Martins *et al*., 2008, Soni and Soni, 2010). The cellulases from *Aspergillus* usually have high β-glucosidase activity but lower endoglucanase levels, whereas *Trichoderma* has high endoglucanase and exoglucanase but lower β-glucosidase levels, and hence has limited efficiency in cellulose hydrolysis. Many earlier studies have reported the production of cellulases from fungi in submerged as well as solid state fermentations, employing expensive substrates including pure carbohydrates, and complex nutritional composition. Solid-state fermentation (SSF) is receiving a renewed surge of interest, primarily because of increased productivity and prospects of using a wide range of agro-industrial residues for cellulase production (Alam *et al*.,2009, Sun *et al*.,2010; Verma *et al*., 2011; Xin and Geng, 2010). In a SSF process, the solid substrate not only supplies the nutrients to the microbial culture growing in it but also serves as an anchorage for the cells.

A number of agro-waste residues including wheat bran (Bansal *et al*., 2011; Deswal *et al*., 2011) sugarcane bagasse, palm kernel cake (Lee *et al*., 2011) empty palm fruit bunch fiber (Alam *et al*., 2009, Kim and kim, 2012), soya flour and ground nut cake, sweet potato and peanut meal residue (Yang and Wang, 1999), sugar cane baggase (Mekala *et al*, 2008; Youssef and Bereka, 2009), sugarcane beet pulp (Nasab and Nasab, 2010), apple pomace (Sun *et al*., 2010), pea peel (Verma *et al*., 2011), coconut oil cake, ground nut oil cake and olive oil cake (Ramachandran *et al*, 2004) have already been tried for the cultivation of microorganisms to produce industrial enzymes. Brewers spent grain (BSG) is one such residues which has gained attention for the production of enzymes under SSF (Francis *et al*., 2003, Sim and Oh, 1990; Xiros *et al*., 2008) by acting as a substrate and growth medium for microorganisms capable of utilizing the complex carbohydrates present in them. It is the major by-product of the brewing industry, representing around 85% of the total by-products generated.BSG is a lignocellulosic material containing about 17% cellulose, 28% non-cellulosic polysaccharides, chiefly arabinoxylans and 28% lignin (Aliyu and Bala, 2011).

Statistical evaluation of parameters affecting cellulase production by *Aspergillus* sp. SS-25 employing Plackett-Burman design

A set of 27 independent variables, designated as X_1, X_2, X_3 $X_{27,}$ examined in the present study with their respective

responses on cellulase yields are shown in Tables 1-3. The main effects of the examined variables on CMCase, FPase, β-glucosidase production were calculated as the difference between the average measurements made at higher level (+1) and low level (-1) of that factor, as represented in Fig.1a-c. Water content in the medium was found to have the maximum positive effect on CMCase production followed by the presence of soyabean meal (X_7) and potato peels (X_{23}) while KH_2PO_4(X_3), yeast extract (X_5) and $CaCl_2$ (X_9) exerted significant inhibitory effect (Fig 1a). On the other hand, Tryptone (X_8) and $CoCl_2$(X_{11}) had an appreciable promotory effect while $ZnSO_4$(X_{12}) and $CaCl_2$ (X_9) caused pronounced inhibition of FPase synthesis (Fig. 1b) while â-glucosidase productivity was enhanced with moisture content(X_{15}), the presence of wheat bran(X_{13}), soyabean meal(X_7), potato peels(X_{23}) in the medium and the incubation time (X_{19}) (Fig. 1c). In each model, some regression coefficients were found to be unnecessary having p values > 0.05 suggesting their insignificance. Thus, by neglecting the insignificant terms, the final model equations for CMCase, Fpase and β-glucosidase activities in terms of coded factors may be written as:

$$+176.88 + 2.41 \times X1 + 7.27 \times X2 - 5.78 \times X3 + 4.58 \times X4 - 6.19 \times X5 - 0.86 \times X6 + 10.36 \times X7 + 3.78 \times X8 - 5.55 \times X9 - 3.03 \times X10 + 1.03 \times X11 - 1.83 \times X13 + 3.26 \times X14 + 25.71 \times X15 - 2.12 \times X16 + 7.90 \times X17 + 5.55 \times X18 + 2.86 \times X19 - 3.89 \times X21 - 0.80 \times X22 + 8.88 \times X23 + 1.20 \times X25 + 2.12 \times X26 + 2.06 \times X27$$

$$+52.34 - 0.34 \times X1 + 0.34 \times X2 + 0.63 \times X3 - 0.37 \times X5 + 11.94 \times X8 - 9.20 \times X9 + 0.34 \times X10 + 8.80 \times X11 - 11.20 \times X12 + 0.23 \times X13 + 0.34 \times X14 + 0.37 \times X16 + 0.23 \times X18 - 0.34 \times X22 - 0.37 \times X23 - 0.37 \times X24 - 0.37 \times X26 - 0.23 \times X27$$

$$\textbf{β-glucosidase} = +45.51 + 0.50 \times X1 - 0.52 \times X2 + 0.63 \times X3 + 0.22 \times X5 + 0.44 \times X6 + 3.86 \times X7 - 0.20 \times X8 - 1.80 \times X10 - 0.25 \times X11 + 0.13 \times X12 + 2.57 \times X13 + 0.21 \times X14 + 14.29 \times X15 + 0.29 \times X16 + 0.16 \times X17 + 1.26 \times X18 + 6.33 \times X19 - 0.47 \times X20 - 0.97 \times X21 - 0.7 \times X22 + 2.47 \times X23 + 0.38 \times X24 + 1.23 \times X25 + 1.37 X26 - 1.03 \times X27$$

Where 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}, X_{16}, X_{17}, X_{18}, X_{19}, X_{20}, X_{21}, X_{22}, X_{23}, X_{24}, X_{25}, X_{26}, X_{27} are urea, NH_4SO_4, KH_2PO_4, peptone, yeast extract, meat extract, soyabean meal, tryptone, $CaCl_2$, $MgSO_4$, $CoCl_2$, wheat bran, $ZnSO_4$, $FeSO_4$, moisture content, Tween 80, $MnCl_2$, malt extract, incubation time, Tween 20, inoculum

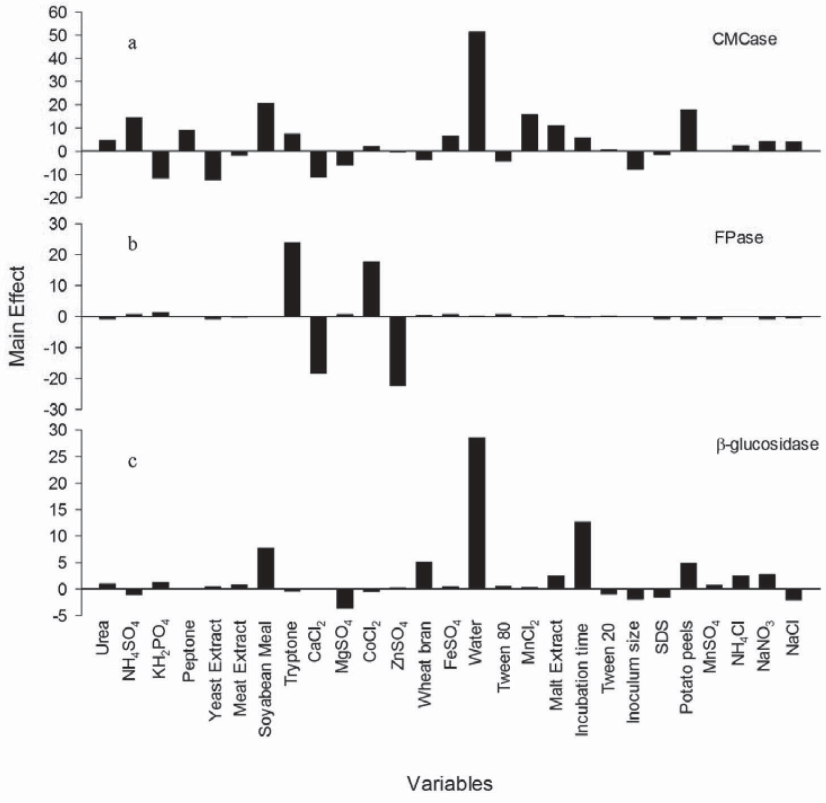

Fig. 1 Effect of various parameters on the production of CMCase (a), FPase (b) and b-glucosidase (c) production.

size, SDS, potato peels, $MnSO_4$, NH_4Cl, $NaNO_3$, NaCl respectively.

The Plackett-Burman design (PBD) has been frequently used for screening process variables that make the greatest impact on a process (Plackett and Burman, 1946). It is a set of small and efficient experimental design, which is very powerful, widely applicable and especially well suited for biotechnology research and development (Haaland, 1989). PBD have been recently employed by many research groups aiming to enhance cellulase yields utilizing different agroindustial substrates (Jabasingh and Nachiyar, 2011; Mekala *et al*., 2008; Youssef and Bereka, 2009). A consideration in the choice of the PBD in screening studies is the ratio of the number of experiments to be conducted to the number of variables being studied. This design allows for the study of k= (N-1)/ (L-1) factors, each with L levels with N experimental trials. The usefulness of the design lies in the fact that in determining the effects of one variable, the net effect of changing other variables cancel out so that the effect of each variable on the system can be independently determined. A number of indicators including the p-value, R^2, standard deviation and predicted sum of square (Press) values, are generally used to check the adequacy of the models. The statistical significance of the ratio of mean square variation due to regression and mean square residual error is tested using the analysis of variance (ANOVA) which is a statistical technique that subdivides the total variation in a set of data into component parts associated with specific sources of variation for the purpose of testing hypotheses on the parameters of the model (Huiping *et al*, 2007).The associated p-values are used to estimate whether F values are large enough to indicate statistical significance. The larger F values corresponding to CMCase1759.71, FPase 4337.52, β-glucosidase 23356.54, observed in the present study, indicate the significance of the models with p-values < 0.05(Tables 4-6).The value *S/N*, which is a measure of the adequate precision is 188.28 for CMCase, 263.834 for FPase and 510.362 for β-glucosidase. A value greater than 4 is desirable in support of the fitness of the model (Muthukumar *et al*., 2003). The coefficient of variation

Table 4: Statistical analysis of Plackett-Burmann design showing sum of squares, coffiecient values, t-test, F-value, p-value and confidence level for each variable affecting CMCase activity after backward elimination regression analysis.

Variables	Sum of squares	Coefficients	t-test	F-value	p-value	Confidence level(%)
Model	33614.45	176.88	1040.47	1759.71	< 0.0001	99.99
X_{-1}:Urea	162.14	2.41	14.27	203.71	0.0007	99.93
X_2:NH_4SO_4	1480.20	7.27	43.12	1859.72	< 0.0001	99.99
X_3:KH_2PO_4	935.97	-5.78	-34.29	1175.94	< 0.0001	99.99
X_4:Peptone	587.94	4.58	27.17	738.69	0.0001	99.99
X_5:Yeast extract	1071.14	-6.19	-36.68	1345.78	< 0.0001	99.99
X_6:Meat extract	20.57	-0.86	-5.08	25.84	0.0147	98.53
X_7:Soyabean meal	3007.01	10.36	61.46	3777.99	< 0.0001	99.99
X_8:Tryptone	400.21	3.78	22.42	502.82	0.0002	99.98
X_9:$CaCl_2$	862.98	-5.55	-32.92	1084.24	< 0.0001	99.99
X_{10}:$MgSO_4$	257.66	-3.03	-17.92	323.72	0.0004	99.96
X_{11}:$CoCl_2$	29.66	1.03	6.10	37.26	0.0088	99.12
X_{13}:Wheat bran	93.78	-1.83	-10.85	117.82	0.0017	99.83
X_{14}:$FeSO_4$	298.06	3.26	19.35	374.48	0.0003	99.97
X_{15}:Water	18506.16	25.7	152.48	23251.06	< 0.0001	99.99
X_{16}:Tween 80	125.77	-2.12	-12.57	158.01	0.0011	99.89
X_{17}:$MnCl_2$	1748.84	7.90	46.87	2197.23	< 0.0001	99.99
X_{18}:Malt extract	863.16	5.55	32.93	1084.47	< 0.0001	99.99
X_{19}:Incubation time	229.15	2.86	16.96	287.91	0.0004	99.96
X_{21}:Inoculum size	424.77	-3.89	-23.10	533.68	0.0002	99.98
X_{22}:SDS	17.95	-0.8	-4.74	22.55	0.0177	98.23
X_{23}:Potato peels	2206.47	8.88	52.65	2772.20	< 0.0001	99.99
X_{25}:NH_4Cl	40.56	1.20	7.13	50.95	0.0057	99.43
X_{26}:$NaNO_3$	125.50	2.12	12.55	157.67	0.0011	99.89
X_{27}:NaCl	118.81	2.06	12.21	149.28	0.0012	99.88

Std.Dev.= 0.89, R^2 = 0.9999, Mean =176.88, Adj R^2 =0.9994, C.V.% =0.50, Pred. R^2 = 0.9938, PRESS =208.00, Adeq. Precision = 188.28

Table 5: Statistical analysis of Plackett-Burmann design showing sum of squares, coffiecient values, t-test, F-value, p-value and confidence level for each variable affecting FPase activity after backward elimination regression analysis.

Variables	Sum of squares	Coefficients	t-test	F-value	p-value	Confidence level(%)
12095.47	52.34	707.29	4337.52	< 0.0001	99.99	
X_1:Urea	3.29	-.34	-4.60	21.25	0.0013	99.87
X_2:NH_4SO_4	3.29	.34	4.60	21.25	0.0013	99.87
X_3:KH_2PO_4	11.06	.63	8.45	71.41	< 0.0001	99.99
X_5:Yeast extract	3.86	-.37	-4.99	24.93	0.0007	99.93
X_8:Tryptone	3993.69	11.94	160.55	25778.95	< 0.0001	99.99
X_9:$CaCl_2$	2369.92	-9.20	-123.68	15297.64	< 0.0001	99.99
X_{10}:$MgSO_4$	3.29	.34	4.60	21.25	0.0013	99.87
X_{11}:$CoCl_2$	2168.32	8.80	118.30	13996.33	< 0.0001	99.99
X_{12}:$ZnSO_4$	3512.32	-11.20	-150.57	22671.74	< 0.0001	99.99
X_{13}:wheat bran	1.46	.23	3.07	9.44	0.0133	99.67
X_{14}:$FeSO_4$	3.29	.34	4.60	21.25	0.0013	99.87
X_{16}:Tween 80	3.86	.37	4.99	24.93	0.0007	99.93
X_{18}:Malt extract	1.46	.23	3.07	9.44	0.0133	98.67
X_{22}:SDS	3.29	-.34	-4.60	21.25	0.0013	99.87
X_{23}:Potato peels	3.86	-.37	-4.99	24.93	0.0007	99.93
X_{24}:$MnSO_4$	3.86	-.37	-4.99	24.93	0.0007	99.93
X_{26}:$NaNO_3$	3.86	-.37	-4.99	24.93	0.0007	99.93
X_{27}:NaCl	1.46	-.23	-3.07	9.44	0.0133	98.67

Std.Dev. = 0.39, R^2 = 0.9999, Mean = 52.34, Adj R^2 = 0.9997, C.V. % = 0.75, Pred. R^2 = 0.9989, PRESS = 13.50, Adeq Precision = 263.834

Table 6: Statistical analysis of Plackett-Burmann design showing sum of squares,co-ffiecient values, t-test, F-value, p-value,confidence level for each variable affecting β-glucosidase activity after backward elimination regression analysis.

Variables	Sum of squares	Coefficients	t-test	F-value	p-value	Confidence level(%)
Model	7968.33	45.51	2068.63	23356.54	< 0.0001	99.99
X_1:Urea	6.91	.50	22.50	506.38	0.0020	99.80
X_2:NH_4SO_4	7.62	-.52	-23.63	558.63	0.0018	99.82
X_3:KH_2PO_4	11.13	.63	28.55	815.29	0.0012	99.88
X_5:Yeast extract	1.41	.22	10.17	103.54	0.0095	99.05
X_6:Meat extract	5.39	.44	19.88	395.30	0.0025	99.75
X_7:Soyabean meal	417.57	3.86	174.92	30599.57	< 0.0001	99.99
X_8:Tryptone	1.08	-.20	-8.88	78.88	0.0124	98.76
X_{10}:$MgSO_4$	90.97	-1.80	-81.64	6666.37	0.0001	99.99
X_{11}:$CoCl_2$	1.75	-.25	-11.30	127.87	0.0077	99.23
X_{12}:$ZnSO_4$	0.45	.13	5.74	32.98	0.0290	97.10
X_{13}:Wheat bran	185.19	2.57	116.49	13570.90	< 0.0001	99.99
X_{14}:FeSO4	1.29	.21	9.72	94.53	0.0104	98.96
X_{15}:Water	5718.00	14.29	647.31	419000	< 0.0001	99.99
X_{16}:Tween 80	2.31	.29	13.02	169.60	0.0058	99.42
X_{17}:MnCl2	0.74	.16	7.36	54.18	0.0180	98.20
X_{18}:Malt extract	44.13	1.26	56.86	3233.51	0.0003	99.97
X_{19}:Incubation time	1123.07	6.33	286.87	82297.64	< 0.0001	99.99
X_{20}:Tween 20	6.18	-.47	-21.27	452.56	0.0022	99.78
X_{21}:Inoculum size	26.44	-.97	-44.01	1937.67	0.0005	99.95
X_{22}:SDS	17.36	-.79	-35.67	1272.45	0.0008	99.92
X_{23}:Potato peels	170.78	2.47	111.86	12514.32	< 0.0001	99.99
X_{24}:$MnSO_4$	3.98	.38	17.06	291.29	0.0034	99.96
X_{25}:NH_4Cl	42.29	1.23	55.66	3098.79	0.0003	99.97
X_{26}:$NaNO_3$	52.86	1.37	62.23	3873.18	0.0003	99.97
X_{27}:NaCl	29.44	-1.03	-46.44	2157.19	0.0005	99.95

Std.Dev. = 0.12, R^2 = 1.0000, Mean = 45.51, Adj R^2 = 1.0000, C.V. % = 0.26, Pred. R^2 = 0.9993 , PRESS = 5.35, Adeq Precision = 510.362

(CV) indicates the degree of precision with which the treatments are compared. Usually, the higher the value of CV, the lower is the reliability of experiment. In the present study, a lower values of CV for all the three models including 0.50 for CMCase, 0.75 for FPase and 0.26 for β-glucosidase indicate a greater reliability of the experiments performed. The analysis showed that the form of the models chosen to explain the relationship between the factors and the responses is correct. Further, the "Adj R-Squared" values of 0.9994 for CMCase, 0.9997 for FPase and 1.000 for β-glucosidase were found to be very close to "Pre R-Squared values of 0.9938, 0.9989, 0.9993 respectively (Tables 4-6). A t-test of an individual effect allows an evaluation of the probability of finding the observed effect purely by chance and some investigator have found that confidence level greater than 70% are acceptable (Stowe and Mayer, 1966).Thus in this case variables with confidence levels exceeding 99% were considered as significant. Moreover, the quality of fit for the factorial model equation was expressed by the coefficient of determination R^2, which was 0.99 for CMCase and FPase and 1.000 for β-glucosidase model.

Model Validation

In order to evaluate the accuracy of statistical experimental models of Plackett-Burman design, attempts were made to formulate individual media for maximizing the CMCase, FPase and β-glucosidase yields using different variables having significant effect on the individual enzyme components. Point optimization for CMCase production attempted with Design Expert using X_1, X_2, X_4, X_7, X_8, X_{11}, X_{13}, X_{14}, X_{15}, X_{17}, X_{18}, X_{19}, X_{21}, X_{23}, X_{25}, X_{26}, X_{27} at the levels of 0 .03%, 0.14%, 2.0%, 2.0%, 2.0%, 0.0002%, 20%, 0.0006%, 70%, 0.01%, 2.0%,6 days, 2.8×10^7 spores, 2.0%, 0.03% and 0.1% in 5 g brewer's spent grain based medium predicted the yield of 292.172 IU/g. To validate the optimum concentrations, an experiment with the above specified conditions was performed and the result was 290 IU/g which is 0.75% less than the predicted value. Point type optimization for FPase production was carried out using X_2, X_3, X_8, X_{10}, X_{11}, X_{13}, X_{14}, X_{15}, X_{16}, X_{18}, X_{19}, X_{21} at the levels of 0.14%, 0.20%, 2.0%, 0.03%, 0.0002%, 20%, 0.006%, 70%, 0.0002%, 2.0%, 3 days, 2.8×10^7 spores, in 5g brewer's spent grain based medium predicted the yield of 98.45 IU/g. Experiment validation with the above specified conditions was performed and the result was 94 IU/g which is 4.53% less than the predicted value. Similarly, point optimization for β-glucosidase production attempted using X_3, X_6, X_7, X_9, X_{13}, X_{14}, X_{15}, X_{16}, X_{17}, X_{18}, X_{19}, X_{21}, X_{23}, X_{24}, X_{25}, X_{26} at the levels of 0.2% , 2.0%, 2.0%, 0.03%, 20%, 0.006%, 70%, 0.0002%, 2.0%, 6 days, 2.8×10^7 spores, 2%, 0.01%, 0.03% , 0.1% in 5g brewer's spent grain based medium predicted the yield of 86.17 IU/g. Again a validation experiment with the above specified conditions was performed and the result was 85 IU/g which is 1.46% less than the predicted value.

Since the optimal media conditions for the entire three enzyme components were different which is not cost effective, so attempts were made to formulate a common media for all the three components. The point type optimization for formulating a common medium was attempted with Design Expert using the variables X_1, X_2, X_4, X_7, X_8, X_{11}, X_{13}, X_{14}, X_{15}, X_{17}, X_{18}, X_{19}, X_{21}, X_{23}, X_{25}. Figure 2 (a–c) depicts the isoresponse contour and 3D plots showing the interactions between soyabean meal (X_7) and tryptone (X_8) for the optimization of CMCase, FPase and β-glucosidase production. From the plots, it was easy and convenient to understand the interactions between the two variables and also to locate the optimum levels. Each curve represents an infinite number of combinations of two test variables with the other variables maintained at constant level. The response surface plot obtained as a function of soyabean meal versus tryptone concentration indicated that the production of CMCase and β-glucosidase improved with the increase in soyabean meal concentration which had no effect on FPase production. Tryptone concentration improved the yields of both CMCase and FPase while slightly decreased the β-glucosidase yield. Maximum production of CMCase, FPase and β-glucosidase amounting to 289.43, 93.65 and 81.85 IU/g was predicted in 5 g brewer's spent grain based optimized medium containing different variables including X_1 (urea, 1.5 mg), X_2 (NH_4SO_4, 7.0 mg), X_4 (peptone, 100 mg), X_7 (soyabean meal, 100mg), X_8 (tryptone, 100 mg), X_{11} ($CoCl_2$, 0.01 mg), X_{13} (wheat bran, 1 g), X_{14} ($FeSO_4$, 0.03mg), X_{15} (water, 12 ml), X_{17} ($MnCl_2$, 0.50 mg), X_{18} (malt extract, 100 mg), X_{23} (potato peels, 100 mg), X_{25} (NH_4Cl, 1.5 mg), X_{26} ($NaNO_3$, 5.0 mg) inoculated with 2.8×10^7 spores, incubated at 30°C in stationary state for 6 days. To validate the optimum concentrations, an experiment with the above specified conditions was performed and the resulting yields were 295, 90 and 80 IU/g for CMCase, FPase and β-glucosidase respectively which are quite close to the predicted values. This high degree of accuracy obtained confirms the validity of the model with minor discrepancy due to the slight variation in experimental conditions. Statistical evaluation

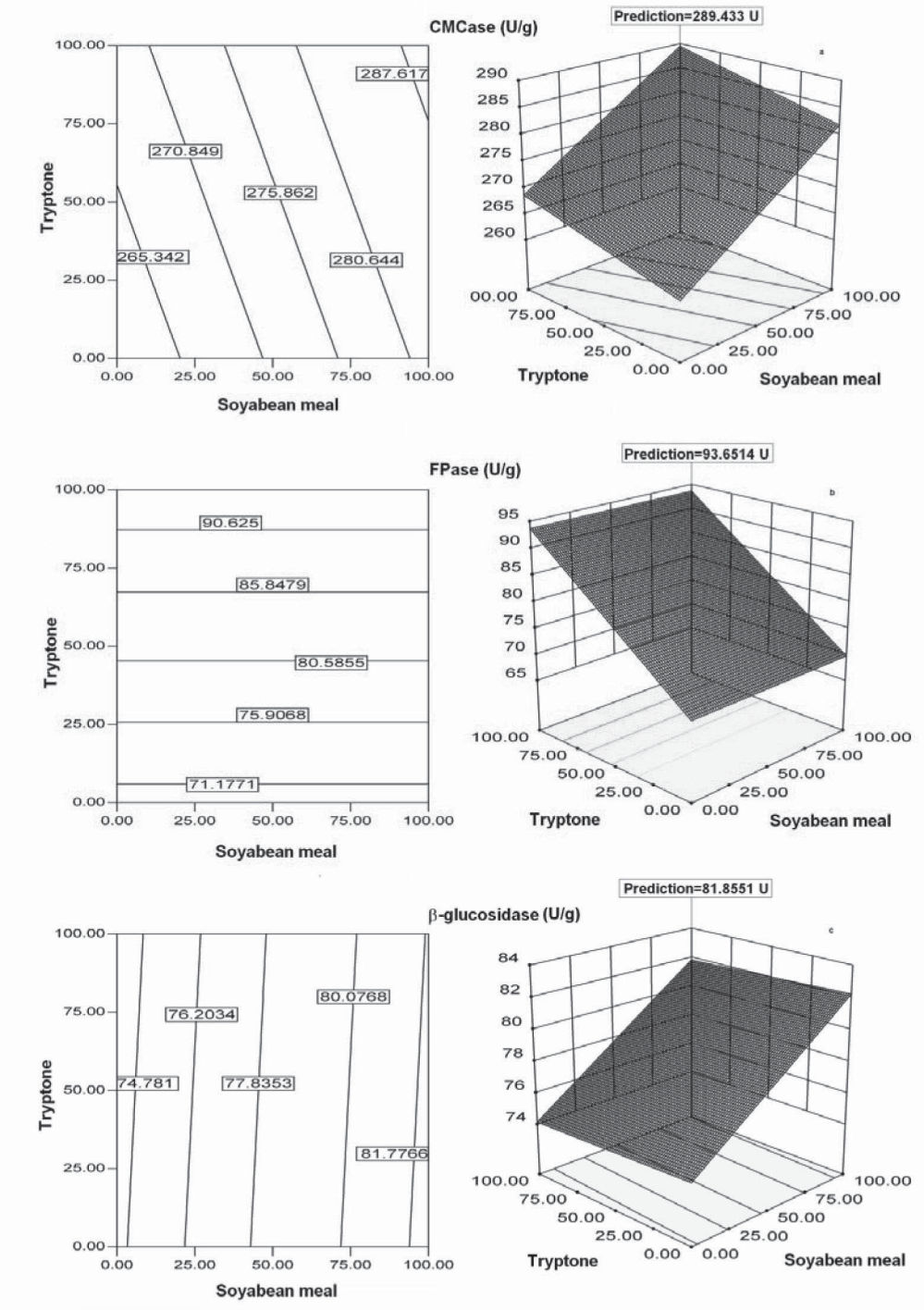

Fig. 2. Contour and three dimensional response surface plots representing CMCase (a), FPase (b) and β-glucosidase (c) yield from solid state cultures of *Aspergillus* sp. SS-25 as affected by cultural conditions.

of culture conditions thus enhanced the productivities of complete cellulase system to an appreciable amount. Earlier reports utilizing brewers spent grain as a substrate for cellulase production resulted in enzyme yields that are comparatively low (Xiros *et al*., 2008).

Various research groups have tried to improve cellulase yield employing various technologies. Although exact comparison cannot be made between these, as most of the reports are for endoglucanase component of cellulase system. The present study focused upon enhancing the yields of complete cellulase system by employing statistical tools to design economically viable media promoting the enzyme yield. The comparison of cellulase yields of *Aspergillus* sp. SS-25 under SSF with some of the earlier reports (Table 7) showed that the fungus tested in this study produced fairly good amount of complete cellulase system and thus has the potential for commercial exploitation in the production of second generation biofuels. Moreover, this is probably the first study where three different Plackett-Burman designs have been studied with respect to different enzyme components with the purpose of designing a common medium for obtaining maximum possible responses of three enzyme components.

Bioconversion of Brewer's spent grain into cellulosic alcohol with cellulases produced in-house

The crude cellulase preparation from *Aspergillus* sp. SS-25 worked very well in the hydrolysis of steam pretreated BSG as evident from gradual rise in the levels of glucose observed after 24, 48 and 72 h of reaction. The glucose was also effectively fermented by *Saccharomyces cerevisae* 360 revealing 91% fermentation efficiency (Table 8). The time course of enzyme action and glucose formation indicated that the rate of saccharification was highest during the first 24 h of reaction revealing the release of 4.17 mg glucose/g of BSG/h of reaction accumulating 13.33 mg of sugar/ml showing the cellulose conversion of 56.31% revealing the glucose yield of 100 mg/g of BSG at the end of this period. The rate of reaction decreased during the next 24 h exhibiting glucose release at 1.04 mg/g/h raising the glucose level to 16.66 mg/ml after cellulose conversion of 70.38 and glucose yield of 125 mg/g of BSF at the end of 48 h of degradation. Further incubation demonstrated the glucose formation of 0.38 mg/g/h revealing the total sugar concentration of 17.87/ml with total conversion efficiency of 75% with an overall sugar yield of 134 mg/g after 72 h of enzyme reaction. The data on glucose fermentation at every stage revealed around 91% fermentation efficiency accumulating 7.76, 9.70 and 10.4 µl of ethanol/ml demonstrating the ethanol yield of 58.5, 72.8 and 78.0 µl/g of BSG after 24, 48 and 72 h of fermentation (Table 8).

Few lignocellulosic residues including sugarcane bagasse, wheat straw, corn stover, spruce, brewer's spent grain

Table 7: Comparison of cellulase yields by SSF among different fungi.

Microorganism	Substrate	CMCase(IU/g)	FPase(IU/g)	β-glucosidase(IU/g)	Reference
Aspergillus niger KK2	Rice straw	129	19.5	100	Li *et al*, 2007
Aspergillus niger	Wheat bran	333	15	30	Bansal *et al*, 2011
Aspergillus terrus ATCC74135	Rice straw	351.96	410.76	16.37	Rabelo *et al*, 2009
Fomitopsis sp.RCK2010	Wheat bran	71.6	3.4	53.67	Deswal *et al*, 2011
Fusarium oxysporum	Corn stover	304	-	0.140	Kovacs *et al*, 2009
Fusarium oxysporum BAFS 768	Olive mill residue	10	1.2	0.10	Lynd *et al*, 1999
Humicola lanuginose	Beet pulp	1.7	-	46.8	Lin and Tanaka, 2006
Myceliophthora sp.	Wheat bran	26.6	0.74	3.83	Silverstein *et al*, 2007

Table 8: Glucose and alcohol yields obtained after enzymatic hydrolysis and fermentation of steam pretreated brewer's spent grain

Time(hr)	Rate of glucose formation (mg/g/h)	Glucose (mg/ml)	*Cellulose conversion (%)	Glucose yield (mg/g)	Ethanol (µl/ml)	Fermentation efficiency (%)	Ethanol yield (µl/g)
0	0	0	0	0	0	0	0
24	4.17	13.33	56.31	100	7.76	90.9	58.2
48	1.04	16.66	70.38	125	9.70	91.0	72.8
72	0.38	17.87	75.45	134	10.4	90.9	78.0

*On the basis of 16% cellulose content in BSG

and municipal solid waste have been worked upon by several workers for enzymatic bioconversion, with commercial or in-house produced cellulases, into glucose employing various pretreatment protocols including acid, alkali and steam [Kovacs *et al*, 2009; Li *et al*, 2007; Rabelo *et al*, 2009; Soni *et al*, 2010). Following pretreatment, plant cell wall polysaccharides are more susceptible to enzymatic hydrolysis that breaks them into monomeric sugars that can be fermented into ethanol (Lynd *et al.*, 1999). Depending on the type and effectiveness of the pretreatment method, complete hydrolysis takes 24-48 h (Lin and Tanaka, 2006).

There has been little research on conversion of BSG to bioethanol. Steam explosion to liquefy grains, followed by bioethanol production by simultaneous saccharification and fermentation (SSF) has been described (Shindo and Tachibana, 2006). SSF involved saccharification of liquefied grains with commercial enzymes cocktail containing glucoamylase, cellulase and hemicellulase with simultaneous yeast fermentation. The highest ethanol concentration of. 48g/L was achieved after 4 days fermentation with co-culture of *Saccharomyces cerevisiae* and *Pichia stipitis*. The hydrolysis of steam pretreated BSG carried out in the present study using in-house produced cellulases revealed appreciable degree of hydrolysis of cellulose into glucose. The highest glucose yield equivalent to 134 mg/g of the BSG was observed after 72 h of enzymatic bioconversion exhibiting 75% cellulose conversion efficiency. The glucose was also effectively fermented by *Saccharomyces cerevisae* 360 and produced ethanol yield of 78µl/g of BSG revealing 91% fermentation efficiency. The hydrolysis yields achieved in the present study was quite comparable to the already published reports on various agro waste residues (Table 9).In a study on enzymatic conversion of cotton stalks a maximum cellulose conversion of 60.8% was achieved after alkali pretreatment (Silverstein *et al*, 2007). In a similar study, three different steam-pretreated lignocellulosic substrates including spruce, wheat straw and sugarcane bagasse were used for enzymatic hydrolysis using commercial as well as laboratory produced enzymes and achieved the highest glucose yields of 29, 65 and 55% in case of spruce, wheat straw and sugarcane bagasse respectively (Kovacs *et al*., 2009). In another study, the alcohol production from BSG after hydrolysis of cellulose as well as hemicelluloses fractions with a commercial mixture of cellulases and hemicellulases and fermenting the pentoses and hexoses with *Pichia stipitis* and *Kluyveromyces marxianus* resulting the ethanol conversion yields of 0.32 and 0.23 g ethanol/g substrate respectively (White *et al*., 2008). In a study on the production of hemicellulases and cellulases on BSG using *Fusarium oxysporum* also capable of fermenting both xylose and glucose, the yield of 106 g ethanol per kg of dry BSG was obtained in a consolidated system (Xiros and Christakopoulos, 2009). In the present study the glucose yields achieved in steam pretreated BSG, appear to be quite significant taking into consideration the targeting of cellulose component alone as compared to several published reports.

Conclusion

Optimization of media components and other cultural parameters is a significant concern in developing a suitable bioprocess for complete cellulase production. The present study addresses this issue and attempts have been made to design an economically viable media capable of generating

Table 9: Comparison of cellulose hydrolysis (%) obtained in the present study with other published reports

Fungus	Substrate	Cellulose hydrolysis (%)	Reference
T. viride	Potato peels	79.0	Chen *et al*,2007
T. viride	Carrot peels	73.0	Chen *et al*,2007
T. viride	News Paper	14.0	Chen *et al*,2007
A. niger + T. reesei	Corn cobs	79.5	Chen *et al*,2007
A.niger + T. reesei	Maize straw	81.2	Chen *et al*, 2008
A. awamori + T. reesei	Sugarcane bagasse	80.0	Gottschalk *et al*, 2010
A. niger + T. reesei	Water hyacinth	71.0	Aswathy *et al*, 2009
A. niger + T. reesei	Corn stover	79.6	Fang et al,2012
A. heteromorphus + T. reesei	Rice straw	84.0	Singh and Bishnoi 2012
Aspergillus sp. S_4B_2F	Sugarcane bagasse	67.0	Soni *et al*, 2010
Aspergillus sp. SS-25	Brewer spent grain	75.0	Present study

appreciable yields of complete cellulase system using a low cost brewers spent grain as a substrate under solid state fermentation. Statistical optimization of cultural conditions using Plackett-Burman experimental designs, resulted in almost 2.1-3.6 fold increase in the enzyme activities of different components of cellulase system. Hence incorporation of statistical tools proved to be useful and powerful in designing a cheaper media for cellulase production thereby providing a major lead to lower down the cost of cellulases that are really an important class of enzymes from industrial point of view. Further, the use of on-site produced enzymes proved to be quite effective in hydrolyzing 75% cellulose into glucose after a mild steam pretreatment which could be further fermented into ethanol revealing the yield equivalent to 78 ml/kg of BSG suggesting the generation of additional cellulosic alcohol from this industrial waste. As the recovery of this much of cellulosic ethanol, separately from BSG, may not be economically viable, it is suggested that the crude enzyme preparation from *A. niger* SS-25 may be additionally used during mashing process for the simultaneous saccharification of starch and cellulose for augmenting the glucose and thus the ethanol recovery from malt. Enzyme productivities from BSG can be further enhanced by using the response surface methodology (RSM) which uses the combinatorial interactions of significant process variables while the ethanol yields may be further enhanced if the hemicellulose fraction is also hydrolysed and the pentoses released are fermented using suitable organisms.

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