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Original Research Article

Optimization of nutrient media for glucoamylase production by Plackett-Burman design using *Aspergillus niger*

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ABSTRACT

Background: Glucoamylase is an important enzyme produced by various bacterial and fungal species as an intracellular or extra cellular enzyme. It is used to treat gastrointestinal issues, autoimmune diseases, inflammation, psoriasis, allergies, eczema, emphysema and asthma, and thus, supplements are often included in complexes for digestive disorders and also applications in different industries.

Methods: Natural ingredients are screened for glucoamylase production by stationary culture of solid-state fermentation technique and Plackett-Burman design microorganism used *Aspergillus niger*. Eight nutrient medias are designed and from this glucoamylase isolated from the medium by centrifugation at 1500 rpm and assayed by spectrophotometric method.

Results: high glucoamylase levels are found in nutrient media VI with optimized physical conditions like percentage inoculum- 2%, pH- 6, incubation temperature- 40°C upon incubation for 4 days.

Conclusions: High amount of glucoamylase is produced in the media VI due to the presence of high amounts of sucrose and optimum concentration of potato, peptone, wheat bran and yeast extract.

Key words: Wheat bran, Plackett-Burman design, Centrifugation

INTRODUCTION

Enzymes

Enzymes are produced intercellular or extracellular based on the location of enzyme extraction process is different. Intra-cellular enzymes are the enzymes produced inside a cell. To isolate such components cell wall disruption is done by mechanical methods (sonication) or by chemical methods (sodium dodecyl sulphate or lysozyme) followed by centrifugation. Extra cellular enzymes are secreted into the broth they are separated by filtration or centrifugation, in the filtrate or supernatant enzyme is present.

Glucoamylase

It is a natural compound found in many natural sources like plants (Corn, wheat, milo, sorghum, tapioca, barley, rice & potatoes), Animals (pancreas and salivary glands) and also in microorganisms (*Aspergillus* species, *Rhizopus* spcs).¹ It is used as manage symptoms of osteoarthritis, alleviate joint pain, and promote overall joint flexibility and mobility and also in different industries specially food industries.²

METHODS

Plackett-Burmann design serves as a valuable tool in nutrient media designing This statistical technique, helps

to optimize nutrient combinations.³ By carefully selecting a subset of crucial factors from a larger set, the method helps in pinpointing the key ingredients that yield the desired outcome. With its ability to explore numerous

variables simultaneously and with limited experiments, the Plackett-Burmann method serves as a culinary compass, guiding the creation of nutritionally balanced and enticing concoctions.

Table 1: Plackett-Burman design.

Trails	Variables								Optical density
	A	B	C	D	E	F	G	H	
1	H	H	H	H	L	L	L	L	T-(SB+EB)
2	H	H	H	L	L	L	L	H	T-(SB+EB)
3	H	H	L	L	L	L	H	H	T-(SB+EB)
4	H	L	L	L	L	H	H	H	T-(SB+EB)
5	L	L	L	L	H	H	H	H	T-(SB+EB)
6	L	L	L	H	H	H	H	L	T-(SB+EB)
7	L	L	H	H	H	H	L	L	T-(SB+EB)
8	L	H	H	H	H	L	L	L	T-(SB+EB)

Classical methods of media optimization involve changing one independent variable while keeping others constant, becoming impractical for multiple variables. The Plackett-Burman design offers a solution by identifying crucial variables through a series of experiments. It evaluates X-1 variables using X experiments (X being a multiple of 4, e.g., 8, 12). Unused factors and those with negligible effects are represented as dummy variables to estimate experimental error variance. This streamlined approach is a powerful tool for efficiently optimizing complex systems with numerous variables. This approach streamlines the optimization process by identifying significant variables efficiently and enabling informed decision-making with fewer experiments. Plan of this method shown in (Table 1).

ranked by importance, guiding further detailed studies for optimal values. Randomized trials enhance accuracy. Concentration of the enzyme produced is estimated by using spectrophotometer optical density is measured. From the Plackett-Burman design optimized nutrient media selected based on high OD values. For this media physical parameters are optimized includes pH, temperature, inoculum level and incubation temperature. For all individual physical parameters 5-7 combinations are made the parameter which has shown highest OD values is said to optimum parameter. With all these parameter glucoamylase produced and for this concentration of enzyme is calculated and reported. Organism used is *Aspergillus niger* for glucoamylase production (Figure 1).



Figure 1: *Aspergillus niger* growth pattern.

The Plackett-Burman design in the table showcases a concise approach for optimizing eight variables. Equal frequency of each level within a column and balanced high-low variables in each trial are required. The design cancels out variable interactions, aiding the determination of each variable's individual effect. Dummy variables are treated similarly, measuring experimental precision and analytical error. This method identifies crucial variables,

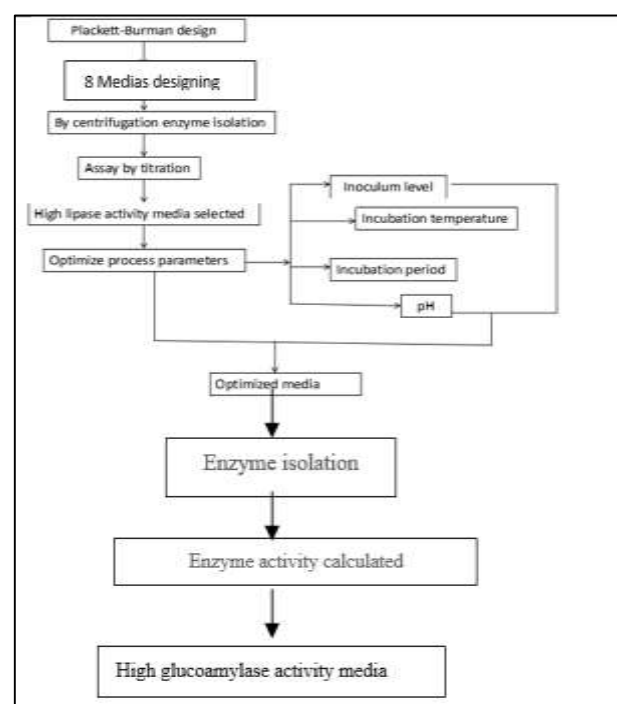


Figure 2: Study procedure.

Table 2: Eight nutrient media composition according to Plackett-Burmann design.

Wheat bran (%)	Peptone (%)	Potato starch (%)	Yeast extract (%)	Sucrose (%)	K ₂ HPO ₄ (%)	Lysine (%)	(NH ₄) ₂ SO ₄ (%)	Distilled water (ml)
5	0.2	1	0.5	1	0.2	0.35	0.14	100
5	0.4	1	0.2	3	0.1	0.35	0.14	100
5	0.4	6	0.2	1	0.2	0.1	0.14	100
2	0.4	6	0.5	1	0.1	0.35	0.14	100
5	0.2	6	0.5	3	0.1	0.1	0.14	100
2	0.4	1	0.5	3	0.2	0.1	0.14	100
5	0.2	6	0.2	3	0.2	0.35	0.14	100
2	0.2	6	0.2	1	0.2	0.35	1	100

Classification

Domain: Eukaryota, Kingdom: Fungi, Phylum: Ascomycota, Subphylum: Pezizomycotina, Class: Eurotiomycetes, Order: Eurotiales, Family: Trichocomaceae, Genus: *Aspergillus*. Species: *niger*, Binomial name: *Aspergillus niger*.

Generally, it is produced as mold on onions sometimes effects other crops like grapes and peanuts. Rarely it causes diseases in humans lung disease if inhaled in large amounts, causing aspergillosis and otomycosis, contributing to ear infections and potential ear canal damage. Industrial has many applications for production of valuable enzymes like glucoamylase and pectinases, magnetic isotope-incorporated macromolecules for NMR analysis.

**Figure 3: *Aspergillus niger* in optimized media.**

Procedure

In order to optimize the nutrient media by using plackett-burman design as taking different concentration of variables as high level and low level is shown in (Table 2). From the different media enzyme has to be isolated and assayed by using spectrophotometric method shown in (Table 3). Different parameters like incubation temperature, incubation period, inoculums level and initial pH has to be optimized.³⁻⁵ All the chemicals used in the

present study were of pure quality grade; medium constituents were of bacteriological grade (Hi media, Bombay, India). Materials used potato starch, wheat bran, peptone, lysine, yeast extract, Sucrose and other salts are dipotassium hydrogen phosphate and ammonium sulphate.

Microorganism

The fungal species *Aspergillus niger* isolated by screening was used in our present study. The isolates were maintained on wheat bran and ground nut cake. Generally, 96 hr old cultures were used for the preparation of the inoculum. Composition of Potato Detrose Agar medium (PAD) (%W/V): Potato starch-20 gm, Detrose-2 gm, Agar -2.5 gm, Distilled water-up to 50 ml, pH-6. Both inoculum and production media were selected based on the earlier studies. Both the media and all the constituents in common except the substrate.

Composition of inoculum medium

Composition of inoculum medium was; peptone-5 g/l, Meat extract-1 g/l, Yeast extract-2 g/l, Sodium chloride-5 g/l, Agar-15 g/l, Water-100 ml, pH-7.0±0.2, Storage temperature-2-8°C.

Composition of production medium

Composition of production medium was; Wheat bran-2%, Peptone-0.4%, Potato starch-6%, Yeast extract-0.5%, Sucrose-3%, K₂HPO₄ -0.2%, Lysine-0.1%, (NH₄)₂SO₄ -0.14%, pH-6, Water-100 ml. The medium was prepared and 100 ml quantities were distributed into 250 ml EM flask and sterilized by autoclaving. Inoculums were prepared from fully grown slant culture. Five ml of sterile distilled water was added to slant, growth contents was scrapped, by a sterile needle and transferred this suspension into 50ml of inoculums medium and incubated at 37°C for 48 hrs. Cultivation of the isolates: The selected isolates were subcultured production medium and incubated at 37°C for 3 days. Preparation of inoculums: Five ml of sterile distilled water was added to two-day-old culture, the growth contents were scrapped and transferred into 50 ml of inoculums medium contained in 250 ml

conical flasks. The flasks were incubated at 37°C for 48 hrs shown in (Figure 3).

Fermentation

Total 5% level of 48 hr inoculums was transferred into 100ml of production medium containing wheat bran, peptone, potato starch, sucrose, lysine, yeast extract, (NH₄)₂SO₄, and KH₂SO₄ in 250 ml EM flasks. The flasks were incubated at 37°C for 4 days.⁷⁻¹⁰ The broth was separated and assayed for glucoamylase. A strain of *Aspergillus niger* which showed production of extra cellular enzyme¹¹ was employed in the present study to determine the most suitable production medium for the fermentative production of glucoamylase by submerged fermentation process on stationary culture (Table 3).^{11,12}

Table 3: OD values for eight nutrient media.

Media	OD value
1	-0.24
2	0.22
3	0.17
4	0.24
5	0.11
6	0.3
7	0.21
8	0.15

After incubating the eight medias for 5 days enzyme is isolated by filtration followed centrifugation supernatant is collected and optical density is determined spectrophotometrically, along with substrate blank and enzyme blank using formula:

$$OD = T - (SB + EB)$$

Where Odis optical density; T is test OD value; SB is substrate blank OD value and EB is enzyme blank OD value.

From (Table 4) it was found that media 6 shows highest OD value so more enzyme was produced. To this media all the process parameters has to be optimized as inoculum level, media pH, incubation temperature and period.5 Inoculum level (IL): To optimize inoculum level different dilutions of initial inoculum media is diluted with sterile distilled water as 0.5 ml-2.5 ml. Five dilutions are made and the results are shown in (Table 4, Figure 4). Media pH: To optimize pH media 6 pH is adjusted in the range 2-8 with sterile 5M NaOH and dil. HCl, results are shown in (Table 4, Figure 5). Incubation temperature: To optimize incubation temperature media 6 is incubated at different temperatures ranges from 30°C to 50°C. Five readings are taken and the results were shown in (Table 4, Figure 6). Incubation period: To optimize incubation period media 6 is incubated for different periods 1 to 7 days. Seven readings are taken and the results were shown in (Table 4, Figure 7). Graphs were plotted for all the physical parameters shown in (Figure 4-7).

RESULTS

Table 3 shows the measured Optical Density value for all the designed eight nutrient medias, optimized media was found as media VI with 0.3 OD value. physical parameters were also optimized and the results were shown in Table 4 and shown with graphs in the Figures 4-7.

Table 4: OD values for optimized physical parameters.

Inoculum level		Media pH		Incubation temperature		Incubation period	
IL ml	OD	pH	OD	°C	OD	Day	OD
0.5	0.7	1	0.05	30	0.98	2	0.04
1	0.73	2	0	35	1.11	3	0.04
1.5	0.57	3	0.10	40	1.32	4	0.06
2	0.63	4	1.22	45	1.19	5	0.08
2.5	0.41	5	0.98	50	0.89	6	1.11
--	--	6	0.77	--	--	7	0.92
--	--	7	0.09	--	--	8	0.68



Figure 4: Inoculum level.

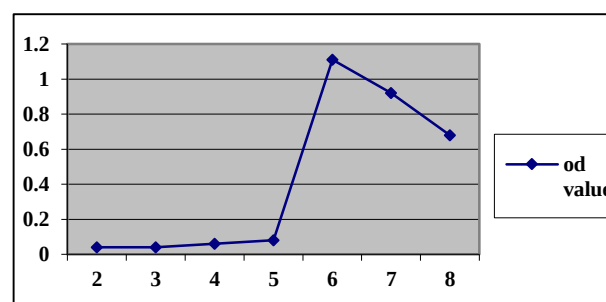


Figure 5: Media pH.

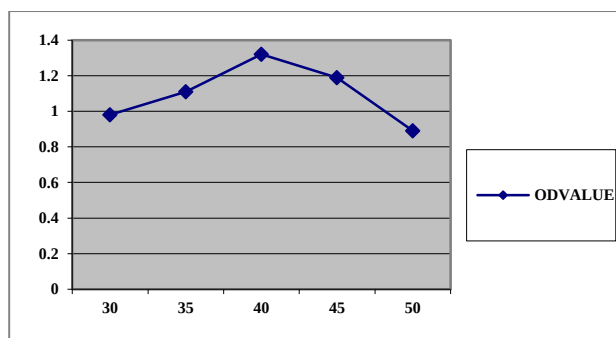


Figure 6: Incubation temperature.

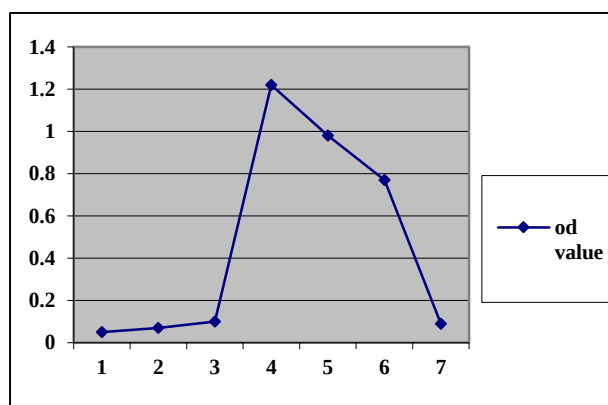


Figure 7: Incubation period.

DISCUSSION

In the present study for production of glucoamylase *Aspergillus niger* used.¹ A Plackett–Burman Factorial Design of 16 experiments was conducted to assess the influence of nine factors on the production of lipases by filamentous fungi.² We conducted similar kind of work is same Plackett-Burman Factorial Design but with 8 experiments and eight variables were compared for the production of glucoamylase.

The optimized conditions for glucoamylase production by *Aspergillus niger*, as determined through factorial design and response surface techniques, underscore the significance of pH and bed thickness in tray. The pivotal role of bed thickness was evident, with the highest glucoamylase production achieved at pH 4.5 and a bed thickness within the range of 2.0-4.2 cm. Additionally, productivity was maximized at pH 4.5, with an optimal bed thickness ranging from 4.4 to 7.5 cm.¹¹

Comparing these findings with the results obtained in our study, which involved the design of a high enzyme-producing media, the concentrations of substrates were determined as sucrose (3%), potato starch (1%), wheat bran (2%), peptone (0.4%), yeast extract (0.5%), lysine (0.1%), and ammonium sulfate (0.14%). The exploration of physical parameters revealed that an inoculum level of 1 ml, a media pH of 5, an incubation temperature of 40°C,

and a period of 6 days were optimal for enzyme production.

Optimum glucoamylase production was observed on wheat bran supplemented with 1%, (w/w) starch, 0.25%, (w/w) urea at pH 6, 100%, (v/w) initial moisture and 30°C after incubation 120 hrs.^{12,13} In the present study highest yield found wheat bran -2% with sucrose-3% and potato starch-1% and in the place of urea NH_4SO_4 is used and optimized pH is 5, for incubation period 6 days (144 hrs).

CONCLUSION

High enzyme producing media is designed and the concentrations of substrates are found as sucrose-3%, potatostarch-1%, wheat bran-2%, peptone-0.4%, yeast extract-0.5%, lysine-0.1%, ammonium sulphate-0.14%. Physical parameters are also screened and found to be inoculum level-1ml, media pH-5, Incubation temperature-40°C and period- 6 days.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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