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Screening of medium components for glucose oxidase production by Aspergillus terreus UniMAP AA-1

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Abstract. Screening of medium components for production of glucose oxidase from *Aspergillus terreus* UniMAP AA-1 was studied using Plackett-Burman experimental design. Seven medium components with four dummy variables were studied in this experimental design. The seven medium components; $NaNO_3$, KH_2PO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, peptone, $CaCO_3$ and glucose were screened in twelve experiments as per the design. It was observed that glucose was the most influential variable followed by $NaNO_3$, $CaCO_3$ and peptone on the glucose oxidase activity; while KH_2PO_4, $MgSO_4.7H_2O$ and $FeSO_4.7H_2O$ showed negative effect on the enzyme activity.

Keywords: *Glucose oxidase, Screening, Plackett-Burman design, Aspergillus terreus*

1. Introduction

Glucose oxidase is an enzyme which catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide [1]. Glucose oxidase has broad range of applications including (i) removal of oxygen and glucose for food and bevarage preservation [2], (ii) as biofuel [3], (iii) production of gluconic acid [4] and (iv) majorly as glucose biosensor [5].

In order to increase the production efficiency, it is necessary to optimize production process of glucose oxidase. As common to enzyme production, the most crucial factors in the optimization of process is medium composition, since it affects the production in terms of cost and its productivity [6]. Hence, it is important to consider the optimization of fermentation medium in order to maximize the production efficiency and profits eventually.

Screening of significant medium components for glucose oxidase production is important prior to optimization study. Plackett-Burman design has been employed for rapid screening of large number of medium components in a minimal time and experiments. Therefore, in this work, the Plackett-Burman design has been employed to study the effects of medium components for production of glucose oxidase from a novel source, *Aspergillus terreus* UniMAP AA-1.

2. Materials and Methods

2.1. Microorganism

Aspergillus terreus UniMAP AA-1 is a new selected and identified strain, maintained at the culture collection of School of Bioprocess Engineering, University Malaysia Perlis. The culture is maintained on Malt Extract Agar (MEA) slants at 4°C.

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2.2. Production *of crude glucose oxidase*

The culture medium employed was similar to that suggested by Petruccioli et al. [7] which consisted of (g/l): $NaNO_3$, 5.0; KH_2PO_4, 1.0; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.01; peptone, 3.0; $CaCO_3$ (sterilized separately), 35; glucose (sterilized separately), 80 g/l. All growth experiments were carried out in 100 Erlenmeyer flasks with 50 ml working volume. The flasks were inoculated with 10 ml (5.17×10^7 spores/ml) of inoculums and incubated in a rotary shaker operating at 200 rpm and 30°C for 110 hours. Crude extracellular enzyme was prepared by removing the cell by centrifugation at 3000 g for 15 min. The harvested supernatant was assayed for glucose oxidase activity.

2.3. Assay of glucose oxidase activity

Glucose oxidase activity in the supernatant was measured spectrophotometrically at 500 nm wavelength using the coupled *o*-anisidine-peroxidase reaction method explained by Banker et al. [8].

2.4. Screening of media components

Plackett-Burman design has been employed to evaluate various medium components for production of glucose oxidase. All trials in Plackett-Burman design [9] were performed in triplicate and the average of glucose oxidase activity was used as response. The main effect of each variable factor was calculated as the difference between the average of measurements made at the high setting level (+) and the average of measurements observed at the low setting level (-). Plackett-Burman design is based on the first-order model:

$$Y = \beta_0 + \Sigma\beta i Xi \qquad (1)$$

where Y is the response (glucose oxidase activity), β_0 is the model intercept, β is the linear coefficient and Xi is the level of the independent variable [9]. This model does not describe interaction among factors and it is used to screen and evaluate the important factors that influence the response.

In this study, a total seven (*n*) variables (glucose, $NaNO_3$, peptone, $CaCO_3$, $MgSO_4.7H_2O$, KH_2PO_4, $FeSO_4.7H_2O$) with four dummy variables were studied in 12 trials (Table 1). The concentration for each component was set based on literature.

Table 1
Plackett-Burman design of 11 variables with coded value along with the observed result

Run	A	B	C	D	E	F	G	H	J	K	L	Glucose oxidase activity (U/ml)
1	(+)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(+)	(-)	2.823
2	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	0.289
3	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	0.57
4	(-)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(+)	(+)	0.95
5	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	0.459
6	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	0.07
7	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(-)	0.508
8	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)	1.013
9	(-)	(-)	(+)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(-)	0.715

10	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	0.285
11	(+)	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(-)	(+)	(+)	0.814
12	(+)	(-)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(+)	1.31

Variables are listed in alphabetical order and their levels are given in (%w/v), A; $NaNO_3$ (0.1-1.0), B; KH_2PO_4 (0.01-0.1), C; $MgSO_4.7H_2O$ (0.01-0.1), D; $FeSO_4.7H_2O$ (0-0.01), E; Peptone (0.1-1.0), F; $CaCO_3$ (0-4.0), G; Glucose (1.0-10) and H, J, K, L; Dummy variables

3. Result and Discussion

Plackett-Burman design has been employed to evaluate the significant of seven media constituents for glucose oxidase production (Table 1). The main effect for each variable was estimated and graphically present in Figure 1 which revealed that, glucose, $NaNO_3$, peptone, and $CaCO_3$ have positive effects on glucose oxidase production. The result showed the fungus requires glucose as a carbon source and $NaNO_3$ and peptone as nitrogen source to grow. Besides, the result is in good agreement with the studies done by Hatziuikolaou & Macris [10] who reported that glucose is the principal inducer of glucose oxidase gene. $NaNO_3$ as inorganic nitrogen source has been reported to have a stimulating effect on glucose oxidase production [11, 12]. Besides, the result support the findings by Miron et al.,[13] that nitrate is more adequate and preferable than peptone as nitrogen source for production of glucose oxidase. Apart from that, the result also agreed with the studied on the effects of $CaCO_3$ on glucose oxidase production by Liu et al., [14] and Hamid et al.,[15]. They found that the addition of $CaCO_3$ induced the production of glucose oxidase and the degree increased with the concentration of $CaCO_3$. On the other hand, KH_2PO_4, $MgSO_4.7H_2O$, and $FeSO_4.7H_2O$ have negative effects on the production of glucose oxidase. The main effect with a negative sign indicates that low concentration of these medium components is nearly optimum for production of glucose oxidase. The observations also are in agreement with the studies done by Hamid et al. [15], who reported that the addition of $MgSO_4.7H_2O$ in the medium strongly inhibit the production of glucose oxidase. Furthermore, the result demonstrates a direct influence of Mg^{2+} ion deficiency on the increment in glucose oxidase activity. Again, this result is in agreement with the findings by Lavollay and Laborey (1941) who showed that Mg^{2+} deficiency in the medium stimulates the formation of riboflavin by fungus like *A.niger* since glucose oxidase is a flavin enzyme [16]. In addition to that, the result also indicates that $FeSO_4.7H_2O$ has a negative effect on the production of glucose oxidase. This result is in agreement with Nakamatsu et al. [17]who observed that $FeSO_4$ was rather inhibitory for the enzyme production and the effect was partially reversed by the addition of $MgSO_4$.

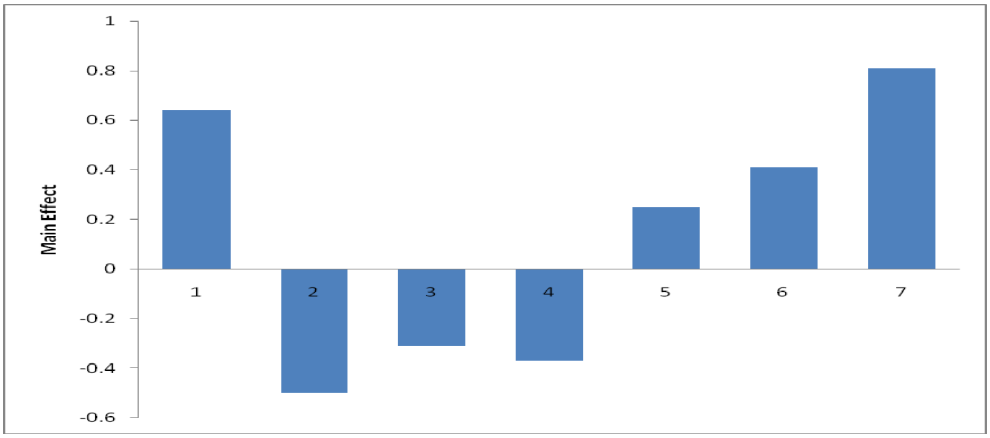

Figure 1. Main effects of the medium constituents on glucose oxidase production to the Plackett–Burman experimental results (1, $NaNO_3$; 2, KH_2PO_4; 3, $MgSO_4.7H_2O$; 4, $FeSO_4.7H_2O$; 5, peptone; 6, $CaCO_3$ and 7, glucose)

4. Conclusion

Plackett-burman design has been used to screen the significant medium components for production of glucose oxidase from a novel source of *Aspergillus terreus* UniMAP AA-1. Glucose, $NaNO_3$ and $CaCO_3$ were identified by Plackett-Burman design as important parameters for improving glucose oxidase production from *Aspergillus terreus* uniMAP AA-1. Further studies on the media optimization will be conducted using Central Composite design (CCD) for obtaining optimum conditions of these parameters.

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