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Screening of significant media components for production of bioprotein from coconut dregs using statistical approach

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Abstract: The production of bioprotein by coconut dregs is found to be a novel and cheaper carbon source. Media optimization for bioprotein production from coconut dregs through solid state fermentation has been developed as a one of the approaches to increase the protein production. The utilization of these coconut dregs provides as alternative substrates and also helps in solving waste disposal problems. Among the seven media components, only NH_4NO_3, $MgSO_4.7H_2O$ and $CuSO_4.5H_2O$ were found to be significantly affecting the bioprotein production.

Keywords: Bioprotein,coconut dregs, solid state fermentation and Placket-Burman design

Introduction

The significant increase in demand for livestock products in recent years in developing countries has required an increase in animal and human food supply. The importance of protein as a food nutrient cannot be ignored because its deficiency can cause various malnutrition problems. The significant increased in demand for livestock products has triggered to explore new proteinaceous food sources, with high nutritional value, economically feasible and locally available. Microbial proteins or bioprotein (protein derived from micro-organisms) nowadays, become one of the most promising breakthroughs of bioprocess innovations that can be produced using a number of different substrates from low carbon cost, high energy source and agricultural wastes (Uysal *et al*., 2002). This will certainly increase the availability of affordable quality proteins but also can reduce dependence on animal proteins.

The production of bioprotein by fermentation of coconut dregs as agricultural wastes has been found to be a novel substrate instead of using cassava and wheat flour as a cheaper carbon source. This novel substrate which is the leftover fiber from coconut milk production traditionally will be used as animal feed and finds no other application. In addition, this substrate is easily available in Malaysia, have high nutritional value, high carbohydrate percentage, and available at low cost. This discovery can beneficial in producing large quantity of nutritional bioprotein from agricultural waste which can also contribute to fulfill the protein demand of the world's ever increasing population. Abundant supply of quality protein will certainly improve the quality of human and animal life on the earth presently also in times to come (Changchui, 2002).

Aspergillus niger (ATCC 16404), used in this study for the improvement of bioprotein from coconut dregs was previously screened for its potentiality to produce maximum bioprotein, by comparing with two different microorganisms - *Saccharomyces cerevisiea* (ATCC 9080) and *Phanarochaete chrysosporium* (ATCC 24725) (Hafiza *et al*., 2011). Plackett-Burman design has been employed to screen the significant media components for production of bioprotein from coconut dregs.

Material and Methods

Collection and preparation of substrate

Coconut dregs were procured from the grocery at Bintong, Kangar was dried in an oven at 60ºC for 24 hours. The dried substrate was grained and sieved to obtain 500 mm mesh size. Coconut dregs sample was sent to UNIPEQ Sdn. Bhd. for proximate analysis.

Fungus Strain

Aspergillus niger (ATCC 16404) was obtained from the School of Bioprocess Engineering culture collection, Universiti Malaysia Perlis. The fungus was grown on potato dextrose agar (PDA) plate and sub-cultured every 2 weeks and incubated at 32ºC.

Inoculum preparation

Inoculums were prepared by washing the growing culture with 25 ml sterile distilled water. The spore suspensions were rubbed and adjust to final concentration of 10^7 spores per ml. The suspension inoculums were kept in chiller at 4ºC for further use (Jamal *et al*., 2005).

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Growth media preparation

The 70% of moisture are maintained for every 5 g substrate in a conical flask. The 70% moisture is equal to 12 ml of solution and distributed into 2 ml inoculum suspension and 10 ml of growth media solution that contain KH_2PO_4, NH_4NO_3, NaCl, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$,$CuSO_4.5H_2O$ and $ZnSO_4.7H_20$.

Solid state fermentation

Solid state fermentation was carried out in 250 ml conical flask containing 5 g sterilized coconut dregs. 10 ml of sterile growth media was added into 5 gram substrate in the flask. The flasks were aseptically inoculated with 2 ml of inoculum suspension. All the mixture was stirred using glass rod and incubated at 32ºC for 7 days.

Protein recovery

The fermentation samples in the flasks were dried for 24 hours at 60ºC-70ºC. Dried samples were added with 50 ml phosphate buffer and macerated in pestle mortar. The mixtures were centrifuged at 8000 rpm for 20 min. The supernatant obtained were kept in the refrigerator for further analysis of protein concentration using Bradford method.

Screening of significant media components

Screening of media components was carried out using Plackett-Burman design were generated by using the statistical analysis package Design-Expert Software (Stat-Ease Inc., Statistic made easy, Minneapolis, MN, USA, version 7.1.5). This design is useful in screening of the most important factor from a list of candidate factors in a minimal numbers of run compared to two-level fractional design (Burden, 1995). This design requires that the frequency of each level of a variable should be equal and that in each test the number of high and low variables should be equal. The effects of changing the other variables cancel out while determining the effect of particular variable (Rosenberg, 2005).

Plackett-Burman experimental design was based on the first order model:

$$Y = \beta_0 + \Sigma \beta_i X_i \quad (1)$$

Where Y is the response (bioprotein production mg/L), β_0 is the model intercept and β_i is the variable estimates. The factor with confidence level above 95% is considered the most significant factor that affects the bioprotein production. A total of eleven parameters including seven media components (Table 1) and four dummy variables were screened in twelve runs. All the runs were performed in triplicate and the average of observation was used as the response of the design. Normally, four dummy variables will provide an adequate estimate of the error (Rajendran *et al*., 2007).

The parameters were KH_2PO_4, NH_4NO_3, NaCl, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $CuSO_4.5H_2O$ and $ZnSO_4.7H_20$. Dummy 1, Dummy 2, Dummy 3 and Dummy 4 also included in this screening process. All the parameters were prepared in two levels which are -1 and +1. The -1 indicates low level and + 1 indicates high level.

Table 1. Plackett-Burman design for evaluation of seven media components with actual values and along with observed result

Run	A	B	C	D	E	F	G	Bioprotein (mg/L)
1	0.70	0.10	0.20	0.15	0.00	0.20	0.20	350.7
2	0.30	0.10	0.20	0.05	0.20	0.20	0.00	310.7
3	0.30	0.30	0.20	0.05	0.20	0.20	0.20	321.0
4	0.70	0.30	0.20	0.05	0.00	0.00	0.20	366.7
5	0.70	0.30	0.00	0.15	0.20	0.20	0.00	363.7
6	0.70	0.10	0.00	0.05	0.20	0.00	0.20	364.3
7	0.30	0.10	0.00	0.05	0.00	0.00	0.00	318.0
8	0.30	0.30	0.00	0.15	0.20	0.00	0.20	277.3
9	0.70	0.10	0.20	0.15	0.20	0.00	0.00	327.3
10	0.70	0.30	0.00	0.05	0.00	0.20	0.00	383.0
11	0.30	0.10	0.00	0.15	0.00	0.20	0.20	336.7
12	0.30	0.30	0.20	0.15	0.00	0.00	0.00	294.7

Variables are listed in alphabetical order (%, w/v): A: NH_4NO_3, B:KH_2PO_4, C:NaCl, D: $MgSO_4.7H_2O$, E: $FeSO_4.7H_2O$, F:$CuSO_4.5H_2O$ and G: $ZnSO_4.7H_2O$

Result and Discussion

Proximate analysis

Proximate analysis of the new potential substrates (coconut dregs) has been donned in order to know the content of protein, fat, carbohydrate, ash, moisture, energy and crude fiber before the solid state fermentation. Proximate analysis results on coconut dregs shows that it contains (%w/v) proteins (3.5), carbohydrate (56.5), ash (2.2), moisture (7.3), crude fibre (24.1) and energy (515Kcal / 100g)

Screening of Media Composition

Aspergillus niger was selected as the potential strain for the bioprotein production from coconut dregs using the Plackett-Burman design to evaluate the significant of seven media constituents for bioprotein production (Table 1). The highest protein concentration obtained was 383 mg/L while the lowest protein concentration obtained 277.3 mg/L. Analysis of variance (ANOVA) result was presented

in Table 2. The R-squared for this experiment is 0.9409. From Table 2, only NH_4NO_3, $MgSO_4.7H_2O$ and $CuSO_4.5H_2O$ are significant parameters since the parameters show the confidence level above 95% and p-value less than 0.0500. The main effect for each variable was estimated and graphically presented in Figure 1 which revealed NH_4NO_3 has the most positive effects on the production of bioprotein followed by $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$. This positive effect means that this parameter will increase the bioprotein production by increasing their concentration from low to high level. On the other hand, NaCl, KH_2PO_4, $FeSO_4.7H_2O$ and $MgSO_4.7H_2O$ have negative effect on bioprotein production.

Table 2. Statistical analysis of Plackett-Burman design for each variable

Media Source	Main Effect	F value	p-value	Confidence Level (%)
NH_4NO_3	24.78	55.06	0.0007	99.93
NaCl	-5.99	3.22	0.1327	86.73
$MgSO_4.7H_2O$	-9.44	8.00	0.0368	96.32
$FeSO_4.7H_2O$	-7.12	4.55	0.0860	91.40
$CuSO_4.5H_2O$	9.79	8.60	0.0325	96.75
$ZnSO_4.7H_2O$	1.61	0.23	0.6503	34.97

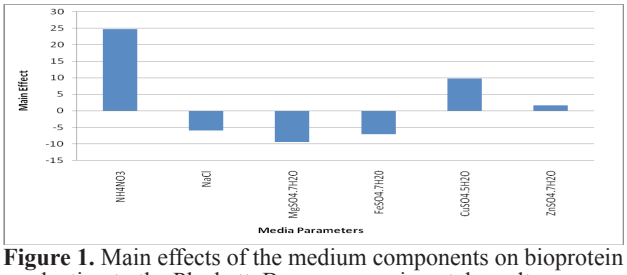

Figure 1. Main effects of the medium components on bioprotein production to the Plackett–Burman experimental results

Inorganic nitrogen source of ammonium nitrate (NH_4NO_3) shows the highest positive effect on bioprotein production. This result is is in agreement with the findings by Roberta Correia *et al*. (2007) who study the effect of nitrogen supplementation on protein enrichment of pineapple waste (PW). They found that 2.5% nitrogen source supplementation enriched protein levels of PW from 16 to 22% during solid state bioprocessing by *S. cerevisiae* (Roberta *et al*., 2007). In another study on single cell protein production by *Aspergillus niger* in solid state fermentation of rice bran also shows improvement in biomass yield (expressed in term of total protein yield) when supplemented with sodium nitrate as nitrogen source (Nupama and Pogaku, 2001). This nitrogen source contributed to the highest confidence level of 99.93 percent which proved that it is important for optimization of bioprotein production.

Figure 1 shows $CuSO_4.5H_2O$ as a copper source for the fungus has the second highest positive effect on bioprotein production. Increasing this source of the mineral in the media will result in high bioprotein yield. Jamal *et al.* (2007) studies on optimization of media composition for the production of bioprotein from pineapple skins reported that the parameter such as $FeSO_4$, $ZnSO_4$, $CuSO_4$ and NaCl also found to be significant media components for bioprotein production (Jamal *et al*., 2007).

$MgSO_4.7H_2O$ shows the largest negative effect on bioprotein production. This magnesium source is important but required in small quantities because it shows the negative effect on bioprotein production, which is in agreement with Jamal *et al*. (2007) where the magnesium source from $MgSO_4.7H_2O$ shows significant level but required in small quantity. Other parameters are not significant for bioprotein production because of their lower confidence level than 95 percent. $MgSO_4.7H_2O$, NaCl and $FeSO_4.7H_2O$ are to be maintained at low amount in order to increase bioprotein yield and the excessive used could inhibit the microorganism growth

Conclusions

NH_4NO_3, $MgSO_4.7H_2O$ and $CuSO_4.5H_2O$ were identified by Plackett-Burman design as important parameters for improving the production of bioprotein from coconut dregs. Since the coconut dregs was successfully utilized for the enrichment of protein in product, there is possibility of converting this agricultural waste to proteinaceous feed or food and at the same time contribute to the proper waste management. This study also can improve the availability of protein and fulfill the protein demand. Therefore, it can be conclude that the statistical design of Plackett-Burman offers efficient methodology to identify the significant factors that will be considered in the next stage of optimization.

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