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Bioprotein Production from Oil Palm Empty Fruit Bunch by *Aspergillus Niger*

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Abstract. The increasing global demand for food and feed products has led to the search for alternative protein sources to supplement the conventional protein source. Therefore, in this study the utilization of oil palm empty fruit bunch as potential substrate for bioprotein production is being explored. The objective of this study is to investigate the capability of empty fruit bunch as substrate and to identify the important parameters in producing bioprotein by solid state bioconversion of *Aspergillus niger*. A solid state fermentation process was carried out aerobically in conical flasks with the working mass of 20 g each at 32°C for 7 days. The substrates were initially pretreated with 1% NaOH and added with essential minerals prior to inoculation of 2% (mass/mass) *A. niger*. The screening of process parameters was done using 2-Level Factorial design which consists of temperature, substrate concentration and inoculums size. Analysis of protein has shown that the protein content in EFB has increased 1.6 folds after fermentation. Among the parameters studied, temperature and substrate concentration were found to be significantly affecting the bioprotein production.

Keywords: Aspergillus niger, Bioconversion, Empty fruit bunch, Single cell protein, Solid state fermentation.

1. Introduction

The productivity of livestock mainly depends on feeds with high and balanced nutritional composition. However, good quality of feeds required higher costs due to some limitations in the raw materials of feed and competition between human nutrition [1]. This has led to the search and development for non-conventional protein sources which can overcome this problem. Hence, bioconversion of agricultural waste contents of high protein sources as a valuable product has become the alternative way to generate high nutritional ingredients of animal feeds [2].

In Malaysia, agricultural sector is one of the largest industries. Each year, this sector has produced tremendous amounts of wastes or by-products which made them available at low cost and all year round. However, most of these by-products and wastes are lacking in nutrients such as protein and vitamins and are rich in fibre with low digestibility [1]. The addition of micro-ingredients are essential to improve the nutritional content of the low cost raw material and the other advance process making the final prices of feeds even higher. In order to overcome this problem, microbial usage has been exploited to convert the wastes into bioprotein. This bioprotein contains high nutritional value with higher digestibility, do not compete with food for human consumption, economically feasible and locally available [3].

Currently, bioprotein is produced using different microorganisms including fungi, bacteria and algae [2]. The usage of fungi is most common due to their capability to propagate on agricultural wastes within a short period and ability to produce high protein content in their biomass [2]. The enhancement of yield for bioprotein production can be achieved by selection of potential strain, substrate and optimum condition [4].

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In this study, the empty fruit bunch (EFB) from palm oil industries was chosen as a potential substrate. The selection of this substrate is mainly due to their availability and can be obtained at a cheaper cost. In order to enhance the protein content in EFB, this waste is fermented together with fungi, *A. niger*.

This study aims to investigate the potential of EFB for microbial protein enrichment prior to screening of process parameters. The potential of EFB as substrate for bioprotein production at different process condition is determined based on its protein content after fermented with *A. niger*. This result will be used in the future for process optimization studies.

2. Materials & Methods

2.1. Collection and Preparation of Substrate

EFB was obtained from Norstar Palm Oil Mill Sdn. Bhd. The substrate was washed and dried in an oven at 60^{o}C for 24 hours. The dried substrate was ground and sieved to obtain 500 mm mesh size and was pretreated using 1 % NaOH at 90^{o}C for 1 hour as described by [5] with some modifications. Then, the pretreated substrates was washed until the pH is neutral (pH 7) and dried in an oven at 60^{o}C for 24 hours. The substrates were kept in air-tight container until further use.

2.2. Preparation of Inoculums

The *Aspergillus niger* was obtained from School of Bioprocess Engineering culture collection and was grown on potato dextrose agar (PDA) at 32^{o}C. Inoculums were prepared by washing the growing culture with 25 ml of sterile distilled water. The spore suspensions were rubbed and adjusted to final concentration of 10^7 spores per ml. The suspension inoculums were kept in chiller at 4^{o}C until further use.

2.3. Growth Media Preparation

Growth media was prepared as a supplement for fungi growth. The growth media contain mineral solution which included 0.2% of KH_2PO_4, 0.5% of NH_4NO_3 and 0.1% each NaCl, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$. The solution was autoclaved prior usage.

2.4. Solid State Fermentation

Solid state fermentation was carried out in 250 ml flask with the working mass of 20 g. For the growth profile determination, the moisture content was maintained at 70% containing 68% of growth media and 2% of inoculums. The substrate concentration was maintained at 30% (w/v). Samples were prepared in duplicates and statically incubated at 32^{o}C for 7 days. For the screening of process parameters, different percentage of moisture content was used depending on the substrate concentration and inoculums size as in Table 1. Samples were incubated at various temperatures (Table 1) for 4 days.

2.5. Total Protein Determination

The fermentation products were daily withdrawn for analysis. The samples were dried for 24 hours at 60^{o}C. Dried samples were added with 50 ml of water and incubated at 4^{o}C, overnight. The mixtures were then centrifuged at 8000 rpm for 20 minutes. The supernatant obtained were kept in the refrigerator for further analysis. The protein content in the supernatant was analysed using Lowry method.

2.6. Screening of Process Parameters

2- Level Factorial design was carried out for screening of process parameters using the statistical software package Design Expert Software (Stat-Ease Inc., Statistic made easy, Minneapolis, MN, USA, version 7.1.5) and the statistical analysis of experiment data was also performed using this software. The screening process was done in duplicates involving three parameters which were temperature, substrate concentration and inoculums size as shown in Table 1. All the parameters were prepared in two levels which are -1 and +1. The -1 indicates low level and +1 indicates high level.

3. Results & Discussion

3.1. Growth Profile Determination

The fermentation was performed at constant temperature, inoculum size and substrate in order to study the effect of fermentation time. The fermentation was carried out for seven days at 32°C. The reducing sugar and protein was analyzed daily. The highest glucose concentration produced is on day 3 as shown by the reducing sugar profile in Figure 1. Increasing glucose concentration observed during the first three days was due to the hydrolisis of cellulose to sugar by *Aspergillus niger*. The depletion of sugar concentration starting from day 3 showed that the sugar was being consumed by the *A. niger* to produce bioprotein.

Table 1: 2-Level Factorial design for screening of process parameters with actual values and observed result.

Standard	Factor 1	Factor 2	Factor 3	Protein (mg)
	A: Substrate Concentration	B: Temperature	C: Inoculum size	
	%(w/v)	°C	(% w/v)	Actual
1	40	30	10	0.2632
2	30	35	5	0.1199
3	50	25	15	0.1961
4	50	35	15	0.0272
5	50	25	15	0.1614
6	30	35	15	0.1327
7	50	25	5	0.1584
8	30	35	15	0.1320
9	30	25	15	0.1931
10	50	35	5	0.1094
11	50	35	5	0.1207
12	50	35	15	0.0279
13	30	25	15	0.1765
14	30	25	5	0.1305
15	40	30	10	0.2036
16	30	35	5	0.1719
17	30	25	5	0.1735
18	50	25	5	0.1795

The protein concentration of fermented EFB was gradually increased from 0.181 mg of protein after first day fermentation to the maximum concentration of 0.2971 mg of protein after 4 days of fermentation. The protein concentration gradually decreased after 5 days of fermentation and at day 7 the protein concentration dropped to 0.1493 mg. This has showed that the protein concentration of EFB has increased 1.6 fold after 4 days of fermentation.

It has been known that the growth of fungi is mainly depends on the carbon, nitrogen and inorganic sources as their nutritional sources and the main nutrient are carbon sources such as cellulose and hemicelluloses [6]. According to Mahlia et al. [7], EFB contain 48.8% of carbon and 0.2% of nitrogen. Since EFB consist of sufficient amount of carbon and nitrogen, this factor contributes significantly to their capability in bioprotein production.

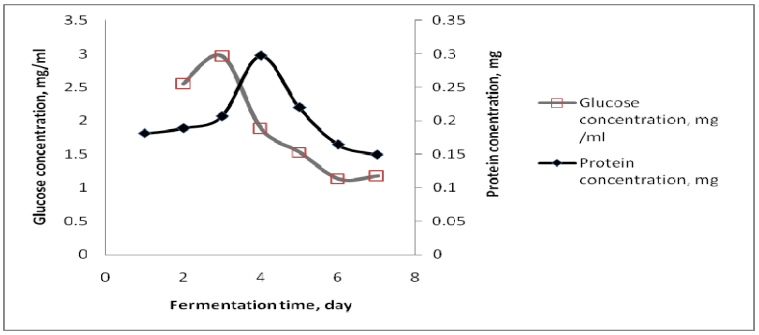

Fig. 1: Profile of bioprotein production and reducing sugar consumption.

3.2. Screening of Process Parameters Affecting Bioprotein Production

The screening of process parameters was done according to 2-Level Factorial design to evaluate the significant process parameters for bioprotein production (Table 1). Analysis of variance (ANOVA) result was presented in Table 2. The R-squared for this experiment is 0.8753. From Table 2, the significant parameters are temperature and substrate concentration since the parameters showed the confidence level of above 95% and p-value less than 0.0500.

Table 2: Main effects of process parameters on bioprotein production

Process Parameter	Main Effect	F-value	p-value	Confidence level (%)
Temperature	-0.066	30.79	0.0004	99.96
Substrate Concentration	-0.031	6.89	0.0276	97.24
Inoculum Size	-0.015	1.51	0.2498	75.02

The main effect for each variable was estimated and graphically presented in Figure 2 which revealed that the temperature has the most negative effects on the bioprotein production followed substrate concentration. This negative effect indicated that these parameters will increase the bioprotein production by decreasing their temperatures and concentrations.

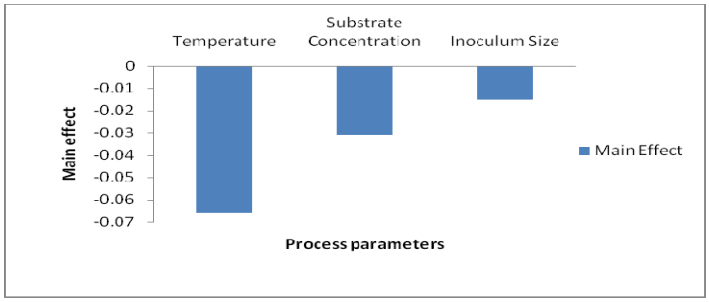

Fig. 2: Main effects of process condition on bioprotein production

Temperature is one of the most important parameters that determines the success of SSF system. Table 1 depicted that the production of bioprotein was maximum at 30^{o}C with protein concentration of 0.2632 mg. The lowest protein produced was 0.0272 mg at temperature of 35^{o}C. Figure 2 shows that temperature gave negative effect in the production of bioprotein. This indicated that the lower the temperature, the higher protein concentration will be produced. According to Pietikainen et al. [8], the optimum growth temperature for fungi is between 25-30^{o}C. This observation was in agreement with those reported by Singh et al. [9], who showed that the highest protein produced by *A. niger* was in the temperature range of 25-30^{o}C. As shown in Table 2, temperature contributed to the highest confidence level of 99.96% which proved that it is significant in producing a higher amount of bioprotein.

Figure 2 shows that besides the temperature, substrate concentration also provide a significant effect to the bioprotein production. Substrate concentration play a crucial role in SSF. Mostly, the substrate concentration used for SSF is around 50-55% which equivalent to moisture content of 45-50% [10]. In this study, the highest bioprotein produced was at condition of 40% substrate concentration and 60% moisture content. According to Kheng and Ibrahim [11], it is expected that the rate of water absorbed by different substrates varies from one substrate to another. This is possible explaination for the variation of the substrate concentration and moisture content in the production of bioprotein using different substrates. Thus, it is concluded that the degree of hydration of the substrate plays an important role on the growth of fungi.

In this study the inoculum size was found to be an insignificant factor for bioprotein production due to their confidence level of less than 95%. Therefore, the inoculum size is to be maintained at low level for optimization process.

4. Conclusion

This study aimed to explore the capability of EFB as a substrate for bioprotein production. Based on the experimental results, the solid state bioconversion has successfully increased the protein content of the fermented EFB from 0.181 mg to 0.2971 mg. After the screening of process parameters, temperature and substrate concentration were identified by 2-Level Factorial design as important parameters for improving the production of bioprotein from EFB. Hence this result highlighted the potential of EFB as a substrate for bioprotein producer in the future.

5. Acknowledgement

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