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Optimum media compositions for bioprotein production by mixed culture of Phanerochaete chrysosporium and *Candida utilis* using palm kernel cake and seaweed as substrates

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Abstract

Bioprotein is one of the useful products obtained from biotechnology invention. It is a promising replacement for the commercial fish feed supplement. In this study, the enrichment of the bioprotein content after solid state fermentation using palm kernel cake and seaweed by the white rot fungus: Phanerochaete chrysoporium and yeast: *Candida utilis* was carried out. The growth media components were selected from 11 types of media using Plackett-Burman design (hereinafter PBD) and were optimized by one-factor-at-a-time (OFAT) method with bioprotein concentration (mg/g) as the response. From the screening result using PBD, three media components, namely K_2HPO_4, $CuSO_4.5H_2O$ and $MnSO_4.H_2O$ were selected for further optimization using OFAT method because of their positive contributions to the response. The final results showed that 5.0 g/L K_2HPO_4, 3.0 g/L $CuSO_4.5H_2O$ and 0.1 g/L $MnSO_4.H_2O$ were there to be the optimum media constituents with 9.0 g/L, $MgSO_4.7H_2O$, 0.1 g/L, $CaCl_2.H_2O$, 3.0 g/L $FeSO_4.7H_2O$ and 3.0 g/L peptone as fixed compositions. At this optimum concentration, the protein increment of 11% was observed as compared to the results determined in the screening using PBD. The study revealed the benefits of using mixed cultures in improving the protein concentrations which can be used as nutritious fish feed.

Introduction

Seaweeds have become a suitable and beneficial alternative of protein sources for farmed fish due to high amount of protein as well as high production rate. Several types of seaweeds have been used by previous researchers as feed supplements and also feed substitute for a particular type of fish (Ergun *et al*., 2008; El-Tawil, 2010). The presence of vitamins, various types of minerals, proteins and lower lipid content in the seaweed have led to the use of seaweed as a supplement in human and animal food for past few decades (Wong and Cheung, 2000). The plant feed stuffs were economically viable and could be considered as an important biomass in the future aquaculture feed formulation (Tacon *et al*., 2006). Thus, they can be a low cost substitute for protein source to replace the expensive fish meal as well as limit the use of synthetic vitamins and minerals. Several studies proved that the addition of various species of seaweeds to fish diets can contribute to a positive effect on the growth of fish, body composition, feed utilization, lipid metabolism, disease resistance as well as carcass quality (Guroy *et al*., 2007; Roy *et al*., 2011; Ergun *et al*., 2008). Palm Kernel Cake (hereinafter PKC) is a protein source obtained from the leftover of the mechanical pressed nuts of palm fruit (Chong *et al*., 2008). Several important nutrients present in PKC like protein, carbohydrate and lipid showed that PKC is one of the good sources for microbial growth. Studies have been done on the use of PKC in bioconversion process to produce several products like xynalase (Kheng and Omar, 2005) and lipase (Imandi *et al*., 2010).

The use of lignin-degrading microorganisms in converting the complex carbohydrate into simple monomers (sugars) and the secretion of enzymes can enrich the produced bioprotein. *Phanerochaete chrysosporium*, which is also known as white rot fungus, belongs to a group of lignin-degrading basidomycetes. *P. chrysosporium* having the ability to degrade complex lignin due to the existence lignin peroxidase (LiP) and manganese peroxidase (MnP). The degradation of lignin is also very important in removing the physical barrier against cellulose utilization (Kanmani *et al*., 2009). *P. chrysosporium* also has the physiological capabilities and also mycelial (hyphal) mode of growth that are able to

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penetrate deeper into the substrate (Rashid *et al*., 2011). Yeast like *Candida utilis* was extensively studied for the production of bioproduct due to its case of isolation and growth on media that contains carbohydrate. Minimum energy is required in the production of bioprotein; and it has the ability to grow well at room temperature (Adoki, 2002). *C. utilis* documented to secrete pectinase, cellulose, Manganese-dependent peroxidase and xynalase enzymes, which support its ability in enriching the protein in the feedstock. Study done by Saheed (2012) found that the potentiality of protein synthesis was increased when the *P. chrysosporium* was cultivated alongside with other yeast cell *(C. uitilis)* due to their synergistic effect on solid matrix.

Solid state bioconversion is a fermentation process conducted on non-soluble materials which acts as a physical support and as a source of nutrients in the absence or near absence of water (Couto and Sanroman, 2006). Solid state fermentation was proven to enrich the nutritional compound of feed supplement (Joseph *et al*., 2008; Vijayan *et al*., 2009; Khodanazary *et al*., 2013). The selection of sufficient good medium is very important in order to ensure the success of the fermentation process. A poor medium can contribute to the alteration of the types and ratios of products produced (Joseph, 2011). Therefore, the present study focused on the screening and optimization of selected media parameters by using PBD followed by one-factor-at-a-time (OFAT) methodology. Hence, a high quality feed can be produced which then overcomes the problem of expensive feed cost.

Materials and methods

Microorganisms

A mixed culture of microorganisms which were *Phanerochaete chrysosporium* (PC) (PC 2094) and *Candida utilis* (CU) (FTCC 8100) were used. PC strains were obtained from the IIUM laboratory stock while CU cultures were sourced from MARDI, Serdang, Malaysia. PC was subcultured fortnightly on a plate containing potato dextrose agar (PDA, Sigma-Aldrich, USA) for subsequent use. CU stock culture was maintained on nutrient solution containing YEPG media: yeast extract 0.3%, peptone 0.5% (Sigma-Aldrich, USA), glucose 1% (HmbG Chemicals, Germany), malt extract 0.3% (HmbG Chemicals, Germany) and distilled water. These microorganisms were incubated at 32°C to obtain luxuriant growth and for further use.

Inoculums preparation

Seven days old plate of PC was harvested and the inoculum was prepared by washing the agar plate with 25 ml of sterile distilled water. The filtered spore suspension was collected in 250 ml Erlenmeyer flask where the fungus was maintained at $7.5x10^9$ spores/ml. The inoculum for CU was made by taking 1-2 loops of cell suspension and transferred into 250ml conical flasks containing yeast, peptone and glucose (YEPG). The inoculum was incubated at 30°C and 150 rpm for 24 hrs and stored at 4°C for further use.

Substrates preparation

Sargassum fulvellum was collected from the near-shore waters of Port Dickson, Negeri Sembilan, Malaysia. The seaweed was thoroughly washed and dried in an oven at 60°C for 24 hrs. The dried seaweed was grinded to the size of 2 mm and was kept in an airtight container at 4˚C for further use. PKC was obtained from a local palm oil plantation (Sime Darby, Pulau Carrey, Banting, Selangor). It was then dried at 60°C for 72 hrs and was made to the size of 2 mm.

Solid state fermentation

A total of 20 g fermentation media was prepared in a 250 ml flask consisted of 30% substrates (seaweed and PKC) with 70% (v/w) moisture content including 6% (v/w) of inoculum, growth media and distilled water. The mixture was autoclaved at 121°C for 15 min. 6% inoculums were added and then incubated at 32°C in an incubator for 6 days. The biomass was harvested after the fermentation.

Analysis of protein concentration

Total crude protein was determined according to the Lowry method (Folin-Phenol Reagent, Sigma-Aldrich, USA) (Lowry, 1951). Gallic acid was used as a standard to determine the amount of protein.

Screening of media compositions using Plackett-Burman Design and Single Factor Optimization

Two experiments were conducted for screening of media component. Screening of media constituents using PBD was conducted as the initial step. The statistical analyses were performed using the statistical analysis package DesignExpert Software (Stat-Ease Inc., Statistic made easy, Minneapolis, MN, USA, version 6.0.8. Then, conventional single factor optimization was carried out as a pre-optimization step to get the operating range for each variable. The Plackett-Burman design (PBD) can be represented by first-order polynomial shows in Equation (1):

$$Y = \beta 0 + \sum \beta i\, xi \qquad (1)$$

In which, the response (bioprotein concentrations, mg/g) was represented by Y while β0, βi and xi is the model coefficient, the linear coefficient and the level of the independent variable respectively. Eleven possible media affecting the amount of protein concentrations in this study, including Sucrose (HmbG Chemicals, Germany), Wheat flour, Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) (Merck, Germany), Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) (Sigma-Aldrich, USA), Iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$) (HmbG Chemicals, Germany), Potassium dihydrogen phosphate (KH_2PO_4) (Merck, Germany), Dipotassium hydrogen phosphate (K_2HPO_4) (HmbG Chemicals, Germany), Peptone, Calcium chloride monohydrate ($CaCl_2.H_2O$) (Sigma-Aldrich, USA), Copper (II) sulphate pentahydrate ($CuSO_4.5H_2O$) (Sigma-Aldrich, USA) and Manganese (II) sulphate monohydrate ($MnSO_4.H_2O$) (Sigma-Aldrich, USA), were used. The 12- Run on PBD were used in studying these factors.

Single Factor Optimization (OFAT)

Based on the PBD results, three variables were selected for (OFAT) studies, which were: K_2HPO_4, $CuSO_4.5H_2O$ and $MnSO_4.H_2O$. This method is important to determine the possible optimum range for the three media in order to maximize the bioprotein production. In this study, the single factor optimization of each media was carried out in triplicate at five levels of experiments.

Results and discussion

Selection of media constituents using Plackett-Burman design

From the results, the main effects of variables (β) were on bioprotein production involving sucrose, wheat flour, $MgSO_4.7H_2O$, $NH_4H_2PO_4$, $FeSO_4.7H_2O$, KH_2PO_4, K_2HPO_4, peptone, $CaCl_2.H2O$, $CuSO_4.5H_2O$ and $MnSO_4.H_2O$ were found to be -32.94, -14.00, 9.47, -4.84, -4.43, -17.81, 35.30, -1.44, 4.84, 18.73 and 25.53 respectively. The difference between the average of the measurements made at high (+1) and low (-1) level of the factors was the estimation of the main effect of each variable on bioprotein production as shown in Figure 1.

From Figure 1, it can be depicted that five media show positive effects on the bioprotein production of the biomass produced after the fermentation process. The most positive effect is shown by K_2HPO_4 followed by $MnSO_4.H_2O$, $CuSO_4.5H_2O$, $MgSO_4.7H_2O$ and $CaCl_2.H_2O$. The positive effect is an indicator that the increment of the concentration of this variable from low to high level will increase the bioprotein production. On the contrary, the other media like sucrose, wheat flour, $NH_4H_2PO_4$, $FeSO_4.7H_2O$, KH_2PO_4 and peptone showed negative effects. Table 1 shows the maximum bioprotein concentration which was obtained at Run 4 that is about 186.197 mg/g while Run 2 shows the lowest amount of bioprotein after the fermentation process.

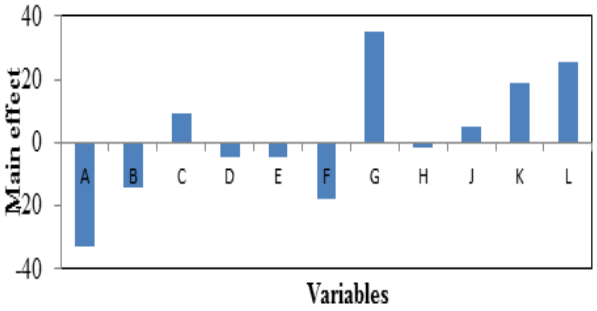

Figure 1. Main effects of medium constituents in Plackett Burman experimental results on protein content (A, Sucrose; B, Wheat flour; C, $MgSO_4.7H_2O$; D, $NH_4H_2PO_4$; E, $FeSO_4.7H_2O$; F, KH_2PO_4; G, K_2HPO_4; H, Peptone; J, $CaCl_2.H_2O$; K, $CuSO_4.5H_2O$ and L, $MnSO_4.H_2O$).

Analysis of variance (ANOVA) results consisting of Fisher-variation value (F-value) and p-value obtained from DesignExpert Software shown in Table 2 were used as a confirmation of the significance of the variables studied. The results showed that sucrose, KH_2PO_4, K_2HPO_4, $CuSO_4.5H_2O$ and $MnSO_4.H_2O$ are significant variables since the value of the confidence level above 95% and p-value is less than 0.005. The R-squared (regression coefficient) is 0.9936, which indicates that the model is successfully predicts the response.

Based on the main effects, F-value and p-value, K_2HPO_4, $CuSO_4.5H_2O$ and $MnSO_4.H_2O$ were selected for further optimization due to their positive and significant effects on the bioprotein concentration. Growth of microorganisms can be triggered by the presence of primary sources as nitrogen, phosphorus and potassium. The screening results from the PBD showed that the highest positive effect was shown by K_2HPO_4 as a good source of phosphorus and also potassium. Study done by (Rathnasabapathy *et al*., 2009) on the screening of media for glutathione production involving *Candida utilis* has included K_2HPO_4 and KH_2PO_4 in the variables tested. The other media that showed positive effect were the minerals from various sources including copper, magnesium, manganese, calcium, sulfur and chloride. These minerals are very important for the growth of microorganisms although required in trace

Table 1. Plackett–Burman design showing actual and coded values for the screening of media with responses

Run	A	B	C	D	E	F	G	H	J	K	L	Bioprotein production (mg/g)
1	-	-	-	-	-	-	-	-	-	-	-	133.395
2	+	+	+	-	+	+	-	+	-	-	-	72.256
3	-	+	+	+	-	+	+	-	+	-	-	146.364
4	-	-	+	+	+	-	+	+	-	+	-	186.197
5	+	-	-	-	+	+	+	-	+	+	-	137.101
6	+	+	-	+	-	-	-	+	+	+	-	103.752
7	+	+	-	+	+	-	+	-	-	-	+	138.027
8	-	-	-	+	+	+	+	+	+	-	+	135.248
9	+	-	+	-	-	-	+	+	+	-	+	174.155
10	+	-	+	+	-	+	-	-	-	+	+	131.542
11	-	+	-	-	-	+	+	+	-	+	+	179.713
12	-	+	+	-	+	-	-	-	+	+	+	173.537

The level of variables are given in (g/l); (A, Sucrose (0-60); B, Wheat flour (0-40); C, $MgSO_4.7H_2O$ (3-9); D, $NH_4H_2PO_4$ (0-0.05); E, $FeSO_4.7H_2O$ (3-7); F, KH_2PO_4 (5-9); G, K_2HPO_4 (0.5-1.5); H, Peptone (3-7); J, $CaCl_2.H_2O$ (0-0.01); K, $CuSO_4.5H_2O$ (0-0.01) and L,$MnSO_4.H_2O$ (0-0.01)).

amount. Minerals like calcium (Ca^+ ion) is crucial in the process of tip growth for fungal hyphae and also other eukaryotic-walled cells (Jackson and Heath, 1993). According to Jellison *et al.* (1997), a small amount of copper is very important for the growth of fungi and can act as a metal activator of various fungal enzymes like oxidases and play an important role in the synthesis of pigments. Other minerals, like manganese, also play an important role in the growth of microorganisms. In the growth of white rot fungi like *Phanerochaete chrysosporium*, manganese is required for the regulatory control of many types of proteins during the secondary metabolism.

Organic nitrogen was found to be an important source that can contribute to the increase in the produced bioprotein. In the study, the organic sources, which were peptones, had a negative effect towards the bioprotein production therefore, it was kept at low level (-1) based on the range decided in the design of the Plackett-Burman. The mineral like $FeSO_4.7H_2O$ was also maintained at low level (-1) due to its negative effect on the response. Three media were omitted from the study, sucrose and wheat flour (carbon sources) as well as $NH_4H_2PO_4$ (inorganic nitrogen) as they exhibit negative effect in which the low level (-1) is zero. Hence, it shows that the microorganisms' growth does not depend on these media in order to maximize the bioprotein production. The PBD also showed that both carbon

Table 2. Analysis of Variance (ANOVA) table for evaluating the significant of variables on bioprotein production

Source	Sum of Square	Degree of freedom	Mean Square	F Value	p-value Prob > F
Model	11938.35	9	1326.483	34.70769	0.0283*
A	3254.55	1	3254.55	85.15593	0.0115*
B	587.853	1	587.853	15.38129	0.0593
C	269.0089	1	269.0089	7.038669	0.1175
E	58.76623	1	58.76623	1.53763	0.3407
F	951.2248	1	951.2248	24.88898	0.0379*
G	3739.204	1	3739.204	97.83701	0.0101*
J	70.20801	1	70.20801	1.837006	0.3081
K	1052.771	1	1052.771	27.54595	0.0344*
L	1954.764	1	1954.764	51.14678	0.0190*

*$P < 0.05$,
$R^2 = 0.9147$, Adj $R^2 = 0.8484$

sources were not important for the growth of microorganisms used. This might be due to the type of substrates used in the study (seaweed and palm kernel cake) which had been proven previously to have the ability in providing carbon sources for microorganisms' growth. Jamal *et al.* (2009) have reported the importance of carbon source (pineapple skin soluble) to *Phanerochaete chrysosporium* for optimum bioprotein production after fermentation. Gad *et al*. (2010) also found that the supplementation of carbon source (*Opuntia ficus indica* waste) is important for the growth of microorganisms.

Determination of the optimum range of media compositions by One-factor-at-a-time (OFAT) method

Three variables, K_2HPO_4, $CuSO_4.5H_2O$ and $MnSO_4.H_2O$, were selected for one-factor-at-a-time (OFAT) studies. This is due to the positive main effect given from the screening results after Plackett-Burman experiment and also the significance of these variables towards the model studied. The OFAT method is important to determine the optimum level of constituents for the three media in order to maximize the bioprotein production of the final product after fermentation process and then identifying the range and also the centre point for the next optimization process. In this study, the influences of the three media was conducted in a series of experiments with other media like $MgSO_4.7H_2O$, $CaCl_2.H_2O$, $FeSO_4.7H_2O$ and peptone were maintained at 9.0 g/L, 0.1 g/L, 3.0 g/L and 3.0 g/L respectively.

Results from the screening process using PBD determined the range for $CuSO_4.5H_2O$ concentrations

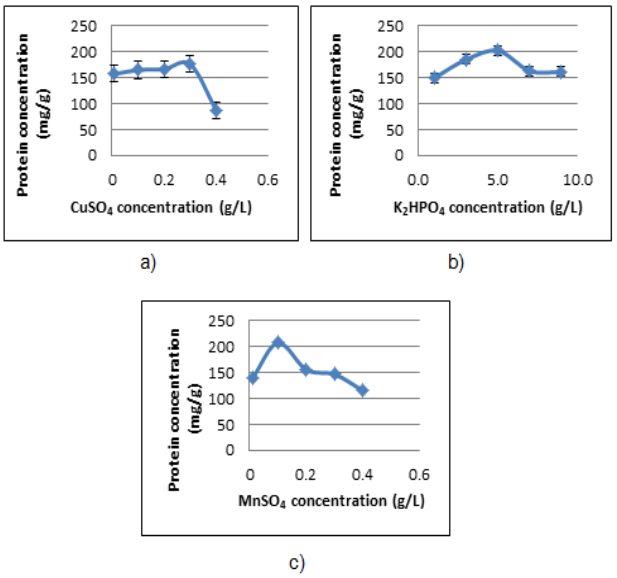

Figure 2. (a) Effect of $CuSO_4.5H_2O$ on protein concentration; (b) Effect of K_2HPO_4 on protein concentration (c) Effect of $MnSO_4.H_2O$ on protein concentration.

(0.01 to 0.4 g/L) that were conducted in the OFAT procedure. Triplicate experiments were conducted and it was observed that lower level of $CuSO_4.5H_2O$ was favorable in producing high amount of bioprotein concentrations 176.0 mg/g compared to the high level as depicted in Figure 2(a). It was proven by a significant decrement in the protein concentration, when the concentration of $CuSO_4.5H_2O$ was increased to 0.4 g/L. The highest protein concentration was obtained at 0.3 g/L of $CuSO_4.5H_2O$ concentration. Hafiza *et al.* (2012) has reported that $CuSO_4.5H_2O$ was among the important media that significantly influencing the bioprotein production from coconut dregs.

Five levels of K_2HPO_4 in a range of 1.0 to 9.0 g/L were studied in triplicates by maintaining the concentrations at 0.3 g/L $CuSO_4.5H_2O$ (from Figure 2(a)). Based on the result, it was observed that the variation of K_2HPO_4 concentrations affects the level of bioprotein concentrations at the end of the fermentation process with the highest bioprotein concentration (201.95 mg/g) was found to be at a level of 5.0 g/L K_2HPO_4 as shown in Figure 2(b). This result was supported by Jamal *et al*. (2012), in which the liquid state fermentation of *Klebsiella pneumoniae* WMF02 on sludge palm oil has required an optimum level of 4.0 g/L K_2HPO_4 to achieve maximum biosurfactant production.

The range of 0.01 to 0.4 g/L for the concentrations of $MnSO_4.H_2O$ was also obtained from the results of the screening procedure using PBD. The experiments were conducted in triplicate and the maximum protein concentration of 207.50 mg/g was found at 0.1 g/L of $MnSO_4.H_2O$ as shown in Figure 2(c). The protein concentration was decreased with the increment of $MnSO_4.H_2O$ concentration. Manganese (II) sulphate is one of the important trace elements that were involved in the screening process of the fermentation media. The various concentrations of $MnSO_4.H_2O$ used in a previous study done by Tijani *et al.* (2011) that showed the optimum reading of protein (56.59 mg/g) was at 0.05 g/kg $MnSO_4.H_2O$ which are almost similar with the results found in this study.

Conclusion

The present study concluded that PBD has been successful in identifying three independent variables namely K_2HPO_4, $CuSO_4.5H_2O$ and $MnSO_4.H_2O$ as significant factors in improving the bioprotein content of the biomass produced after the solid state fermentation. Further optimization on the process parameters should be conducted in a future study to find the optimum conditions in which the microorganisms was able to produce high level of protein. 5.0 g/L K_2HPO_4, 3.0 g/L $CuSO_4.5H_2O$ and 0.1 g/L $MnSO_4.H_2O$ was found to be the optimum media constituents with 9.0 g/L, $MgSO_4.7H_2O$, 0.1 g/L, $CaCl_2.H_2O$, 3.0 g/L $FeSO_4.7H_2O$ and 3.0 g/L peptone as fixed compositions. At this optimum concentration, the bioprotein content was found to be increased by 11% compared to the results determined in the screening using PBD.

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References

Adoki, A. 2002. Culture characteristics of Candida sp in waste conversion: implications for single-cell-protein-enriched feed supplement production. Journal of Applied Sciences and Environmental Management 6(2): 49-58.

Chong, C.H., Zulkifli, I. and Blair, R. 2008. Effects of dietary inclusion of palm kernel cake and palm oil, and enzyme supplementation on performance of laying hens. Asian-Australasian Journal of Animal Sciences 21(7): 1053-1058.

Couto, S.R. and Sanroman, M.A. 2006. Application of solid-state fermentation to food industry- A review. Journal of Food Engineering 76(3): 291–302.

El-Tawil, N.E. 2010. Effects of green seaweeds (*Ulva* sp.) as feed supplements in red tilapia (*Oreochromis* sp.) diet on growth performance, feed utilization and body composition. Journal of the Arabian Aquaculture Society 5(2): 179-194.

Ergün, S., Soyutürk, M., Güroy, B., Güroy, D. and Merrifield, D. 2008. Influence of *Ulva* meal on growth, feed utilization, and body composition of juvenile Nile tilapia *(Oreochromis niloticus)* at two levels of dietary lipid. Aquaculture International 17(4): 355-361.

Gad, A.S., Hasan, E.A. and Abd El Aziz, A. 2010. Utilization of Opuntia ficus indica waste for production of Phanerochaete chrysosporium bioprotein. Journal of American Science 6(8): 208-216.

Guroy, B.K., Cirik, S., Guroy, D., Sanver, F. and Tekinay, A.A. 2007. Effects of *Ulva rigida* and *Cystoseira barbata* meals as a feed additive on growth performance, feed utilization, and body composition of Nile Tilapia, Oreochromis niloticus. Turkish Jornal of Veterinary Animal Science 31(2): 91-97.

Hafiza, S., Ahmad Anas, N.G. and Nor Hidayah, B. 2012. Screening of significant media components for production of bioprotein from coconut dregs using statistical approach. International Food Research Journal 19: 499-502.

Imandi, S.B., Karanam, S.K. and Garapati, H.R. 2010. Optimization of media constituents for the production of lipase in solid state fermentation by *Yarrowia* lipolytica from palm Kernel cake *(Elaeisguineensis)*. Advances in Bioscience and Biotechnology 1: 115-121.

Jackson, S.L. and Heath, I.B. 1993. Roles of calcium ions in hyphal tip growth. Microbiological Reviews 57(2): 367-382.

Jamal, P., Tompang, M.F. and Alam, M.Z. 2009. Optimization of media composition for the production of bioprotein from pineapple skins by liquid state bioconversion. Journal of Analytical Sciences 9(17): 3104-3109.

Jamal, P., Wan Nawawi, W.M.F. and Alam, M.Z. 2012. Optimum medium components for biosurfactant production by *Klebsiella pneumoniae* WMF02 utilizing sludge palm oil as a substrate. Australian Journal of Basic and Applied Sciences 6(1): 100-108.

Jellison, J., Connolly, J., Goodell, B., Doyle, B., Illman, B., Fekete, F. and Ostrofsky, A. 1997. The role of cations wood by the in the biodegradation of brown rot fungi. International Biodeterioration and Biodegradation 39(2-3): 165-179.

Joseph, I., Paul Raj, R. and Bhatnagar, D. 2008. Effect of solid state fermentation on nutrient composition of selected feed ingredients. Indian Journal of Fisheries 55(4): 327-332.

Joseph, S.P. 2011. Process optimization for mass production of marine yeast *Candida* sp. S 27 and its nutritional characterization, p. 205. Kochi, India: Cochin University of Science and technology, PhD thesis.

Kanmani, P., Karuooasamy, P., Pothiraj, C. and Arul, V. 2009. Studies on lignocellulose biodegradation of coir waste in solid state fermentation using *Phanerocheate chrysosporium* and *Rhizopusstolonifer*. African Journal of Biotechnology 8(24): 6880-6887.

Kheng, P.P. and Omar, I.C. 2005. Xylanase production by a local fungal isolate, *Aspergillus niger* USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate. Songklanakarin Journal of Science and Technology 27(2): 325-336.

Khodanazary, A., Hajimoradloo, A. and Ghorbani, R. 2013. Influence of solid-state fermentation on nutritive values and enzymatic activities of AnchovyKilka (*Clupeonellaen grauliformis* Svetovidov, 1941) meal by using different microorganisms. International Research Journal of Applied and Basic Sciences 4(8): 2357-2367.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry 193(1): 265-275.

Rashid, S.A., Ibrahim, D. and Omar, I.C. 2011. Protein utilization of palm kernel cake for the production of mannanase by an indigenous filamentous fungus, *Aspergillus niger* USM F4 under solid substrate fermentation. The Internet Journal of Microbiology 9(1): 1-19.

Rathnasabapathy, C.S., Basha, S.M. and Dhanasekar, R. 2009. Enhanced production of glutathione from *Candida utilis* using palm jaggery. International Journal of ChemTech Research 1(4): 1137-1144.

Roy, S.S, Chaudhuri, A., Mukherjee, S., Chauduri, S.H. and Pal, R. 2011. Composite algal supplementation in nutrition of *Oreochromis mossambicus*. Journal of Algal Biomass Utilization 2(1): 10-20.

Saheed, O.K. 2012. Bio-protein production by solid state bioconversion using banana peel as carbon source. Kuala Lumpur, Malaysia: International Islamic University Malaysia, MSc. Thesis

Tacon, A.G.J., Hasan, M.R. and Subasinghe, R.P. 2006. Use of fishery resources as feed inputs for aquaculture development: trends and policy implications. FAO Fisheries Circular. No. 1018. Rome, FAO. 99.

Tijani, I.D.R., Jamal, P., Alam, M.Z. and Mirghani, E. 2011. Valorization of cassava peels by the white rot fungus Panus tigrinus M609RQY. Australian Journal of Basic and Applied Sciences 5: 808-816.

Vijayan, H., Joseph, I. and Raj, R.P. 2009. Biotransformation of tuna waste by co-fermentation into an aquafeed ingredient. Aquaculture Research 40: 1047-1053.

Wong, K.H. and Cheung, P.C.K. 2000. Nutritional evaluation of some subtropical red and green seaweed. Part I-proximate composition, amino acid profiles and some physic-chemical properties. Food Chemistry 71:475 -4.