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BIOCONVERSION OF SEAWEED WITH WHITE ROT FUNGI FOR PRODUCTION OF PROTEIN ENRICHED FISH FEEDSTOCK

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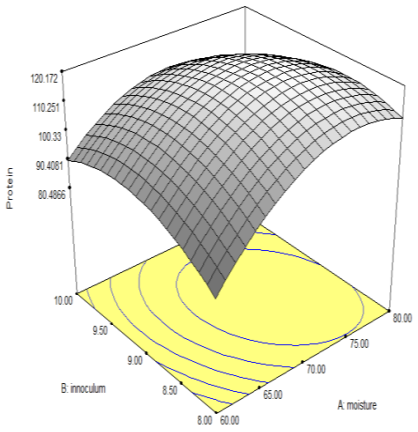
Graphical abstract



Abstract

Viable and economical source of fish feed ingredients remained one of most important factors for a successful and profitable fish production for peasant farmers and industries. Solid state bioconversion (SSB) involving *Phanerochaete chrysosporium* (*P. chrysosporium*) was conducted to enrich seaweed with protein. Optimization of processing parameters (moisture content, inoculum size and minerals) with response surface methodology (RSM) showed crude protein increased to 120.29 mg/g. Positive interaction existed among all investigated process parameters and the quadratic model describing the process was significant at $p<0.05$. The coefficient of determination –R-squared, of the model was close to unity. The optimum value of moisture content was 73.5% (v/w), 8.5% (v/w) for inoculum size and 7% (v/w) of mineral supplement. Validation of the model showed protein production falling within 10% tolerable point.

Keywords: Seaweed, Fish feed, Bioconversion, *Phanerochaete crysosporium*, Interaction

1.0 INTRODUCTION

Seaweeds by their natural composition are important algae colonizing several marine coastal areas. Many species of seaweed are normally used in their unprocessed forms in folk medicine, human and animal feeding and manure for agricultural land improvements [1]. The rheological properties of seaweed species such as gelling properties of their sulphated polysaccharides enable them to carry out biological activities for improved utilization beyond folk medicine. Ulva is a species of seaweed with bright green sheets and domiciled in marine environments and in brackish water; especially in estuaries [2]. This specie is one of the important type of seaweed found abundantly in coastal areas of several countries and contains minerals, protein and vitamins.

Utilization of *Ulva sp.* seaweed as animal feed or supplement is a daunting task since bioavailability of the nutrients embedded in the polysaccharide remained elusive due to inefficient metabolism by animals. This chemical limitation generally impedes efficient use of *Ulva* seaweed as sole animal feed. Although several species of seaweed contained valuable amino acids of immense nutritional efficacy, their release could be poor due to crosslinking within the polysaccharide matrix of the algal mass.

Bioconversion process involving fungi, bacterial and yeast remained a viable option in metabolizing polysaccharide molecules present within the matrix of seaweed. Bacteria strains are less utilized for bioconversion process since they lack specialized enzymes that can degrade algal polysaccharide membranes. Moreover, high concentration of nucleic

acids within bacterial cells limits their use as feed sources for animals. Therefore, fungi and yeast are preferred microbial classes for bioconversion of highly lignified solid matrix like seaweed. Furthermore, fungi cells harbor high protein, vitamins and low nucleic acid after successful bioconversion process [3]. *P. chrysosporium* is a filamentous fungus that can break down seaweed polysaccharides through its multi-enzyme secretion during bioconversion process [4].

Solid-state bioconversion (SSB) entails the growing of microorganisms on a solid matrix devoid of absolute aqueous medium. It has been widely studied over the years because of its economical technology, high throughput, low contamination issues and high potential of successfully converting inexpensive agro-industrial wastes, as well as plant substrates, in a large variety to value added industrial compounds [5]. When compared to the liquid media used in submerged fermentation systems, the solid media used in SSB contain less water, but they present an important gas phase between the particles. This condition favours growth and development of filamentous fungi, which have great capacity to colonize the inter-particle spaces of solid matrices [5].

The objective of this paper is to examine the conditions necessary for optimization of protein synthesis by *P. chrysosporium* using *Ulva* seaweed as carbon source.

2.0 EXPERIMENTAL

2.1 Raw Material Collection and Cleaning

Seaweeds (*Ulva* sp.) were collected from the laboratory stock of Universiti Kebangsaan Malaysia in a semi dried form. The samples were thoroughly washed with clean tap water to separate stones and dirt before oven dried at 60°C to complete dryness. Dried seaweed was then ground through 2 mm sieve size and stored in airtight container for future use.

2.2 Inoculums Preparation and Solid State Bioconversion (SSB)

Laboratory cultures of *P. chrysosporium* fungi were grown on Potato dextrose agar (PDA) plates at 32°C for 5-7 days until the fungi covered the plates. The plates were washed with 25 ml sterilized distilled water using L-shaped rod then poured into sterilized flask. Excess inoculum was stored at 4°C for further use. The fungi strain was subcultured every fortnight to keep it in good shape. SSB was carried out in 250 ml Erlenmeyer flasks with 30% solid substrate and 70% liquid containing mineral supplements. Inoculums were added after sterilization of media at 121°C for 15 mins in an autoclave.

2.3 Protein Assay

Protein content of the sample was determined using folin phenol reagent method [6]. 100 mg of sample was dissolved in 1M NaOH and allowed to stand for 24 h. samples were centrifuged at 6000 rpm for 15 mins and supernatant was assayed for protein using bovine serum albumin (BSA) for standard curve and absorbance was read at 660 nm.

2.4 Response Surface Methodology (RSM)

Central composite design (CCD) a form of RSM is a powerful statistical technique useful for the evaluation of the numerical relationship existing between experimental variables in relation to the response. The process entails: (i) generation of statistical design for the experiment, (ii) calculating relevant coefficients in the mathematical model, (iii) predicting the response direction and validating the suggestions of the model [7]. Statistical optimization of the protein synthesis by RSM was performed using Design expert 6.0.8 software. Central composite design (CCD) was used to determine the optimal level of the selected factors. The high and low levels of the factors were; moisture content (60% and 80%), inoculum size (7% and 10%) and mineral content (6% and 8%). The software, based on CCD, generated fifteen experiments with five center points (Table 1). Experiments were conducted as per the combination of the selected factors and the response (protein content) was tabulated. The experimental result was fitted into the second order polynomial equation adopting multiple regression procedure. The regression equation takes the format described in equation 1:

$$Y = \beta_0 + \beta_1 A_1 + \beta_2 B_2 + \beta_3 C_3 + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (1)$$

where Y is the predicted response (Protein (mg/g)), β_0 is a constant; β_1, β_2 and β_3 are the linear coefficients; β_{11}, β_{22} and β_{33} are the model squared coefficients and β_{12}, β_{13} and β_{23} are the interaction coefficients. Data generated was analyzed with the statistical software – Design Expert 6.0.8 (Stat Ease Inc., Minneapolis, USA). Multiple regression equation developed was evaluated using analysis of regression coefficient, ANOVA (analysis of variance), P-values and t-test. The coefficient of determination (R^2) measured the quality of fit of the model to experimental results.

Table 1 Actual values of process parameters for protein synthesis optimization

Run	Moisture content (%)	Inoculum size (%)	Mineral content (%)
1	70.00	10.41	7.00
2	70.00	9.00	8.41
3	55.86	9.00	7.00
4	70.00	9.00	7.00
5	60.00	8.00	6.00
6	60.00	10.00	8.00
7	70.00	9.00	7.00
8	70.00	7.59	7.00
9	84.14	9.00	7.00
10	70.00	9.00	5.59
11	80.00	10.00	6.00
12	70.00	9.00	7.00
13	70.00	9.00	7.00
14	80.00	8.00	8.00
15	70.00	9.00	7.00

3.0 RESULTS AND DISCUSSION

3.1 Optimization of Process Parameters for Protein Enrichment

Central composite design (CCD) was adopted to estimate the optimum process conditions necessary for improved protein synthesis by *P. chrysosporium* under SSB. Moisture content, inoculum size and mineral content affected protein enrichment. Results obtained showed a general dependence of crude protein synthesis on alteration of the parameters (Table 2). The regression model necessary for high protein production followed the quadratic form with independent variables A, B and C (Equation 2):

$$\text{Protein} = +118.26 + 0.000A + 0.19B - 4.14C - 19.88A^2 - 11.87B^2 - 11.17C^2 - 0.086AB + 1.66AC + 3.81BC \quad (2)$$

The results of analysis of variance (ANOVA) generated after statistical analysis of the data (Table 3) showed that the model was highly significant with probability value (p -value) of 0.002 at $p<0.05$ and F-value of 10.88. The adequate precision, which is a measure of signal-to-noise ratio of 8.46 was high showing that the signal within the model is adequate enough to cancel the effects of the noise. Similarly, the evaluation of the regression equation of the software gave a coefficient of determination -R-Squared (R^2), which tested the fitness of the experimental design. The resultant R^2 (0.9732) showed that 97.32% of the tested independent variables correlated very well with one another.

Table 2 Optimization of process parameters by central composite design

Run	Moisture content (%)	Inoculum size (%)	Mineral content (%)	Protein content (%)
1	70.00	10.41	7.00	90.00
2	70.00	9.00	8.41	85.28
3	55.86	9.00	7.00	73.72
4	70.00	9.00	7.00	120.29
5	60.00	8.00	6.00	89.46
6	60.00	10.00	8.00	78.41
7	70.00	9.00	7.00	120.29
8	70.00	7.59	7.00	89.46
9	84.14	9.00	7.00	73.72
10	70.00	9.00	5.59	97.00
11	80.00	10.00	6.00	78.90
12	70.00	9.00	7.00	122.00
13	70.00	9.00	7.00	120.29
14	80.00	8.00	8.00	73.72
15	70.00	9.00	7.00	118.00

Table 3 ANOVA for Response Surface Quadratic Model

Source	P-Value
Model	0.0020
A	0.0114
B	0.7976
C	0.1137
A^2	0.0003
B^2	0.0021
C^2	0.0008
AB	0.1505
AC	0.1242
BC	0.0671
R-Squared	0.9732
Adj R-Squared	0.9249

However, the adjusted R-Squared value of 0.9249 (92.49%) was very close as expected signifying the robustness of the model in predicting the optimum values of the processing parameters was reported [8]. Apart from the model, factors A, A^2, B^2, C^2 and BC were significant from the resulting ANOVA generated from the software. This showed that those variables significantly contributed to the optimization of product synthesis.

3.2 Effects of Interaction Among Optimized Parameters on Protein Production

Response surface methodology (RSM) was used to detect the optimum points of tested independent variable in such a manner that the targeted response is maximized [9]. The kind of interactions existing among the three optimized parameters in improving protein synthesis was illustrated using response surface plot (Figure 1-3). The 2D contour plots and 3D response surface described the degree of interactions leading to optimum product synthesis between the two parameters [10]. The maximum value of predicted production was represented by the innermost surface confined to smallest ellipse in the contour plot. The elliptical shape of the contour plot of interactions between independent variables showed their perfect relationship for product optimization [11, 12].

3.3 Effects of Interaction Between Moisture Content and Inoculum Size on Protein Synthesis

Moisture content and inoculum size are important parameters involved in the bioconversion of any organic material. Moisture content measures the amount of water molecules that can be transported within the void space of substrate bed while inoculum size offers the amount of viable cells that can grow on the substrate. The interaction between moisture content and inoculum size on protein production showed that increasing the concentration of both parameters resulted in the improvement of product synthesis (Figure 1). However, at their elevated levels (>70% moisture and >9% inoculum size), product formation declined. The maximum production of protein (110.25 mg/g) was recorded at the point of intersection of major and minor axis of the ellipse.

3.4 Effects of Interaction Between Moisture Content And Minerals Content on Protein Synthesis

The interaction between moisture content and minerals on protein production showed that increasing the concentration of both parameters affects protein synthesis positively (Figure 2). General observation showed that mineral content was highly optimized compared with moisture content. This could be attributed to efficient metabolism of the substrate through higher mass transfer within substrate bed [13]. However, both parameters demonstrated perfect synergism in high product synthesis. The maximum production of protein was recorded at 7% mineral and 70% moisture content.

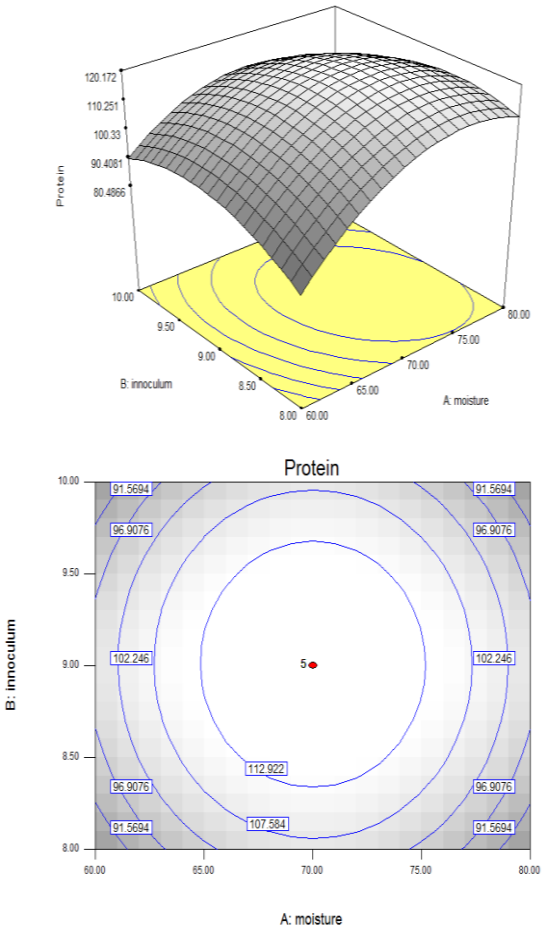


Figure 1 Response surface and contour plot of effect of interaction between moisture content and inoculum size on protein synthesis

3.5 Effects of Interaction Between Inoculum Size and Minerals on Protein Synthesis

The interrelationship between minerals and inoculum size on protein production showed a positive correlation in improving protein synthesis by *P. chrysosporium* over the 7 days bioconversion period (Figure 3). The two parameters showed similar trend in protein enrichment of the substrate base. The maximum production of protein was recorded at the point of intersection of the two parameters (7% mineral and 9% inoculum). This result was consistent with the opinion of other workers where inoculum level was paramount to product formation [14].

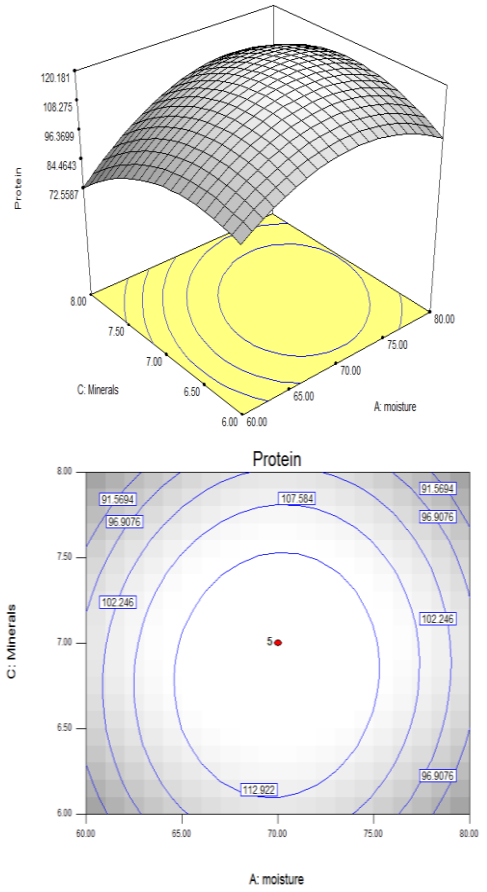


Figure 2 Response surface and contour plot of effect of interaction between moisture content and minerals on protein synthesis

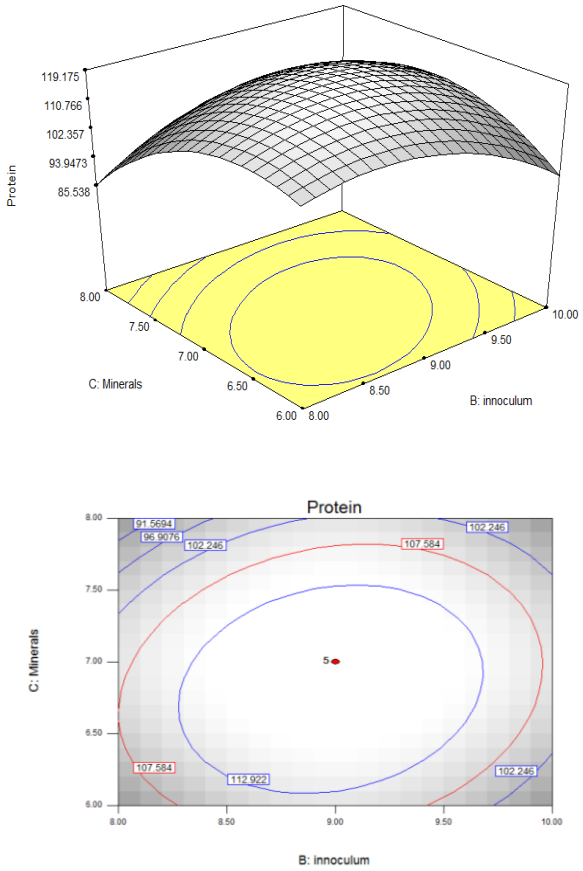


Figure 3 Response surface and contour plot of effect of interaction between inoculum size and mineral content on protein synthesis

3.6 Model Solutions and Validation of CCD

Validation of model that explains the optimum points of investigated parameters is an important step to finalize the amount of participating parameters in the substrate bed. Based on the results generated from interactions of media parameters, several numerical solutions were suggested by the software with desirability of 100%; signaling adequate prediction of product formation. The quadratic model generated by the software suggested solutions with corresponding predicted protein values. Two of the solutions were experimentally determined and the result obtained showed congruency between the predicted and experimental results. Hence, the model was adjudged validated (Table 4).

Table 4 Validation of the model describing optimized process

Experiment	Moisture content (%)	Inoculum size (%)	Mineral content (%)	Protein predicted (mg/g)	Protein observed (mg/g)
1	63.5	8.9	6.6	105.1	109.5
2	67.4	8.5	7.0	112.8	114.4

4.0 CONCLUSION

Through this study, the levels of process parameters necessary for improved protein synthesis by *P. chrysosporium* were elucidated. The response surface methodology greatly described the optimum levels of investigated parameters resulting in highly significant quadratic model. Theoretical values and the experimental results correlated positively during model validation.

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References

[1] [1]. Felix, S. 2011. Seaweed (*Ulva reticulata*) Based Fermented Marine Silage Feed Preparation under Controlled Conditions for Penaeus monodon Larval Development. *Journal of Marine Science: Research & Development*.

[2] [2]. Burtin, P. 2003. Nutritional value of seaweeds. *Electronic Journal of Environmental, Agricultural and Food chemistry*. 2(4): 498-503.

[3] [3]. Dhanasekaran, D., Lawanya, S., Saha, S., *et al.* 2011. Production of Single Cell Protein from Pineapple Waste Using Yeast. *Innovative Romanian Food Biotechnology*. (8).

[4] [4]. Gad, A. S., Hasan, E., Abd El Aziz, A. 2010. Utilization of Opuntia Ficus Indica Waste for Production of Phanerochaete Chrysosporium Bioprotein. *Journal of American Science*. 6(8).

[5] [5]. Rodríguez-Jasso, R. M., Mussatto, S. I., Sepúlveda, L., *et al.* 2013. Fungal Fucoidanase Production by Solid-State Fermentation in a Rotating Drum Bioreactor Using Algal Biomass as Substrate. *Food and Bioproducts Processing*. 91(4): 587-94.

[6] [6]. Lowry, O. H., Rosebrough, N. J., Farr, A. L., *et al.* 1951. Protein Measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry*. 193(1): 265-75.

[7] [7]. Myers, R. H., Montgomery, D. C., Anderson-Cook, C. M. 2009. *Response Surface Methodology: Process And Product Optimization Using Designed Experiments*. John Wiley & Sons.

[8] [8]. Tijani, Jamal P., Alam M., *et al.* 2012. Optimization of Cassava Peel Medium to an Enriched Animal Feed by the White Rot Fungi Panus Tigrinus M609RQY. *Int Food Res J*. 19(2): 427-32.

[9] [9]. Jamal, P., Alam M., Fairoza, N. 2008. Process Optimization for Bioprotein Production from Cheaper carbon source. In: 4th *Kuala Lumpur International Conference on Biomedical Engineering 2008*. Springer. 889-92.

[10] [10]. Srikanth, S., Swathi, M., Tejaswini, M., *et al.* 2013. Statistical Optimization Of Molasses Based Exopolysaccharide And Biomass Production By Aureobasidium Pullulans MTCC 2195. *Biocatalysis and Agricultural Biotechnology*.

[11] [11]. Lim, J. Y., Yoon, H.-S., Kim, K.-Y., *et al.* 2010. Optimum Conditions for the Enzymatic Hydrolysis of Citron Waste Juice Using Response Surface Methodology (RSM). *Food Science and Biotechnology*. 19(5): 1135-42.

[12] [12]. Alam, M. Z., Jamal, P., Nadzir M. M. 2008. Bioconversion of Palm Oil Mill Effluent for Citric Acid Production: Statistical Optimization of Fermentation Media and Time by Central Composite Design. *World Journal of Microbiology & Biotechnology*. 24(7): 1177-85.

[13] [13]. Ruqayyah, Jamal P., Alam M. Z., *et al.* 2014. Application of Response Surface Methodology for Protein Enrichment Of Cassava Peel As Animal Feed By The White-Rot Fungus Panus Tigrinus M609RQY. *Food Hydrocolloids*.

[14] [14]. Irfan M., Nazir M. I., Nadeem M., *et al.* 2011. Optimization of Process Parameters for the Production of Single Cell Biomass of *Candida utilis* in Solid State Fermentation.