BIOPROTEIN PRODUCTION FROM AGRICULTURAL WASTE AND CHEAPER CARBON SOURCE: FULFILLING THE HOPE FOR MILLIONS

Parveen Jamal*, Md. Fahrurrazi Tompang, Md. Zahangir Alam, Mohamed Ismail Abdul Karim, and Hamzah Mohd. Salleh

Bioenvironmental Engineering Research Unit (BERU), Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, P.O.BOX 10, 50728 Kuala Lumpur, Malaysia

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(jparveen@iiu.edu.my)

The necessity for exploring unconventional, non-agricultural means of food production, especially of proteins, has created a demand for the formulation of innovative and alternative protein rich food sources having a high nutritional value which are non competitive with food for human consumption, economically feasible and locally available. Thus, the production of bioproteins (proteins derived from micro-organisms) by fermentation of agricultural waste products is one of the most promising approaches of biotechnological innovations for increasing the availability of proteins. In addition to high quality, quantity will be more comparable to farm animals due to rapid growth rate of microorganisms and high production of bioproteins. Due to the increasing demand for bioproteins, the efficient strains, substrate and method must be used for high yield product. Moreover, increasing concern about pollution that occurs from agricultural and industrial waste has stimulated interest in converting waste materials into commercially valuable products. Therefore, this study emphasized on microbiological transformation of pineapple waste, which contains valuable components like sucrose, glucose, fructose and other nutrients. *Phanerochaete chrysosporium* PC-13(PC2094) was used as potential strain after screening various microorganism and agricultural wastes.

The objectives of the study were first; to identify the potential substrate from the selected agricultural wastes and additional carbon source (wheat flour), second; to optimise media and process conditions in shake flask and bioreactor respectively to obtain maximum production, and third; to determine the composition of amino acids in the bioprotein as well as to test the quality of effluent by conducting chemical oxygen demand and heavy metal test.

In order to achieve the objectives, initially five different microorganisms- *Aspergillus niger, Phanerochaete chrysosporium, Saccharomyces cerivisiea, Mucor hiemalis and Trichoderma harzianum* were screened for their potential to produce maximum biomass and protein content. Bioconversion of various agroindustrial wastes like honeydew, pineapple and durian skins as carbon source was carried out under liquid state bioconversion by using a potential and safe strain, *Phanarochaete chrysosporium* PC-13(PC2094). Further screening of the potential substrate (pineapple waste) was done by conducting proximate analysis and experimental studies of the substrates as fermentation media under two conditions; with additional nutrients and without additional nutrients. Design-Expert 6.0.8 software was used to select the suitable designs

for optimisation of media composition. All experiments were carried out in shake flask using two different statistical designs namely Plackett-Burman, to screen out the significant media constituents and subsequently a Face Centered Central Composite Design to optimise the selected constituents. All experiments were performed in triplicate to ensure accuracy and precision. Further development for optimisation of process conditions using the optimum media compositions was done in bench-top bioreactor using half-fractional factorial design to obtain maximum production. Amino acid analysis was carried out by High Performance Liquid Chromatography (HPLC) to determine the amino acid composition of the final product-bioprotein. Chemical oxygen demand (COD) removal and heavy metal removal test were done to check the quality of effluent based on the standard set by Department of Environmental (DOE), Malaysia. Analysis results for all experimental data were generated by using Design Expert 6.0.8 software.

From the screening studies, pineapple skin was found to be a potential carbon source due to its higher amount of carbohydrate and bioprotein production compared to honeydew and durian skins. Two-steps optimization strategy based on statistical experimental designs were carried out to evaluate the impact of various media components on growth of PC2094 and bioprotein production, by liquid state bioconversion utilizing pineapple skins as substrate. A two-level Plackett-Burman design was employed first where 11 variables were studied for their influence on bioprotein production. Pineapple skins soluble sugar (PSS) content, KH_2PO_4 and $NH_4H_2PO_4$ were found to be the most significant variables for improving bioprotein production. A three-level Face Centered Central Composite Design (FCCD) was further employed for improving the Bioprotein content. The significant parameters were varied in the optimisation study, in which their optimal values and interaction effects were studied. A mathematical model was developed to show the effects of each medium component and their combinatorial interaction on bioprotein production. In response to the bioprotein production, all three parameters had shown significant effect on their linear, quadratic and interactions terms. The coefficients of the response surface model were evaluated by regression analysis and tested for their significance. The coefficient of determination (R^2) of the model was 0.9994, and the model F value was 1547.68, which indicates the model to be suitable to represent adequately the real relationship among the parameters used. The importance of using carbon and nitrogen sources were found to be essential in providing a suitable growth media for *P. chrysosporium*. An experimental run was carried out to validate the model and to determine the maximum amount of bioprotein by using the optimal value of 1.0% (w/v) PSS, 0.10% (w/v) KH_2PO_4 and 0.25% (w/v) $NH_4H_2PO_4$. The maximum production of bioprotein obtained was 514.2 g/kg on the fifth day of fermentation and it was found to be 0.02% more than the experimental run obtained at similar media compositions of FCCD. The observed yield of 51.7 g/g-substrate and productivity of 121.93g/g-substrate/day was obtained after the validation experiment. Optimisation of process conditions by using half-fractional factorial design from Design Expert 6.0.8 software, in 2 liter bench-top fermentor has identified agitation, aeration and temperature as the significant factors responsible for producing 772.17 g/kg-substrate of bioprotein at 150 rpm, 1.0 vvm and 37°C respectively. Eighteen essential amino acids were found in the bioprotein produced. COD was found to be removed by 91% throughout the fermentation period. Heavy metals like lead (Pb), chromium (Cr VI), cadmium (Cd) and zinc (Zn) were shown to have concentration below the permissible discharge limit set by Department of Environmental, Malaysia. A 45 L bioreactor was designed and fabricated for scale up of bioprotein production. Performance of the experiment was evaluated on the basis of biomass

production, total protein, and total carbohydrate. Removal of chemical oxygen demand and heavy metals presence were evaluated for biosafety. We can say with utmost certainty that this study provides a better alternative in agricultural products by converting cheaper carbon source to valuable and quality bioprotein, which can be used as supplement and additive in the animal feed and food as well as in chemical and pharmaceutical industries.