

Screening for Potential Strain to Produce Bioprotein from Cheaper Carbon Source

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Abstract

Enough production of food and fodder are essential for sustainability. In times to come, the population of developed countries might be stabilized, but it will keep on increasing, in developing countries, at the rate of average four per cent. This will warrant utilization of certain carbon rich sources, which are easily available and can effortlessly be converted into food and fodder, rich in protein and other essential nutrients. The production of bioproteins (proteins derived from micro-organisms) by fermentation of cheaper carbon source is one of the most promising breakthrough of biotechnological innovations, which will certainly increase the availability of high quality affordable proteins in the world and reduce dependence on animals. Due to the increasing demand for bioproteins, the efficient strains, substrate and method must be used for high yield product.

In this study, we have made prudent efforts to produce maximum quantity of bioprotein by liquid state bioconversion of wheat flour - a cheaper carbon source having high nutritional value, high carbohydrate percentage and easily available in Malaysia. Screening of five different microorganisms - *Aspergillus niger*, *Phanerochaete chrysosporium*, *Saccharomyces cerevisiae*, *Mucor hiemalis* and *Thricoderma harzianum* - was done with fixed process conditions. Bioconversion was done in a 500 ml Erlenmeyer flask having 100 ml of wheat flour with known concentration of 2% (w/v) at a temperature of 27⁰C, agitation of 150 rpm with 2% inoculum (10⁶ spores/ml). Biomass production was recorded every day for six days and the protein content was also determined every day. From the observed results, *Mucor hiemalis* was found to be as the most potential strain with biomass of about 11.4 g/L on the fourth day of treatment. With this promising result, the amount of bioproteins could be further increased by optimizing process factors. This study may provide a better alternative in agricultural products by converting cheaper carbon source to valuable and quality product bioprotein, which can be used as supplement and additive in the animal feed as well as in chemical and pharmaceutical industries.

Key words: Liquid state bioconversion – protein - wheat flour – biomass - Mucor hiemalis.

1.0 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. To meet the demand, the production of bioproteins (proteins derived from micro-organisms) by fermentation of cheaper carbon source is one of the most promising breakthroughs of biotechnological innovations. This will certainly increase the availability of affordable proteins in the world. In

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addition to high quality, quantity will also be plentiful. It will reduce dependence on animals. This could be possible due to rapid growth rate of microorganisms and high production of bioproteins. Use of microbes as a food source may appear to be unacceptable for some people, but the idea of consumption of microbes as food for man is certainly innovative to solve the global food problem [1]. Algae, fungi and bacteria are generally used as producers of bioproteins and can be utilized as a protein supplement because they are rich in protein, carbohydrates, fatty acids, vitamins and minerals. The protein extracted from cultivated microbial biomass, can be used for protein supplementation of a staple diet by replacing costly conventional sources like soymeal and fishmeal to alleviate the problem of protein scarcity [2]. The importance of protein in food nutrient cannot be neglected. Various malnutrition problems may occur due to protein shortage. This situation has created a demand for the formulation of innovative and alternative proteinaceous food sources [2]. In addition to this, the food route represents highest immediate cash return because demand for food is huge and stable, and the technologies involved are not too sophisticated.

Due to the increasing demand for bioproteins, the efficient strains, substrate and method must be used for higher level of product. Various cheap carbohydrate sources are capable of supplying adequate calories to livestock such as wheat flour and cassava flour [3, 4]. Wheat flour had long been recognized as substrate that is full with nutrient and carbohydrate. Flour primarily consists of five nutrients: fat, minerals, moisture, starches and protein. Wheat flour could be a high quality substrate for bioprotein production due to its high carbohydrate (i.e. starch) value [5]. It is less expensive and easily available in Malaysia. Therefore, in this research project, we introduced wheat flour as a cheaper carbon source for fermentation by a suitable microorganism to produce bioprotein. Selection of potential microorganism is necessary to produce maximum quantity bioprotein by liquid state bioconversion of substrate –wheat flour. Five different microorganisms- *Aspergillus niger* (*A.niger*), *Phanerochaete chrysosporium* (*P.chrysosporium*), *Saccharomyces cerevisiae* (*S. cerevisiae*), *Mucor hiemalis*(*M.Hiemalis*) and *Thricoderma harzianum* (*T. harzianum*) were selected from lab stock for screening. The experiment was conducted with fixed process conditions and the potential strain was selected on the basis of maximum biomass production and its protein content.

2.0 Materials and Methods

2.1 Raw materials

Wheat flour, bought from the local market, is used as raw material in this study.

2.2 Microorganisms

Five different microorganisms - *A.niger*, *P.chrysosporium*, *S. cerevisiae*, *M.Hiemalis*, and *T. harzianum* were collected from lab stock at International Islamic University Malaysia, Universiti Putra Malaysia and some other sources. All strains were cultured, maintained on (PDA) slants, and stored at 4°C. Subculture was done once a month.

2.3 Inoculum preparation

Inoculum preparation (spore suspension) was done according to the method suggested [6]. Cultures grown on PDA medium in petri dishes at 32⁰C for 7 days were transferred into

Erlenmeyer flask (250ml) containing 100 ml of sterile distilled water. It was shaken in a rotary shaker at 150 rpm for 24 hours. The suspended fungal cultures were filtered by Whatman #1 filter paper and the filtrate was used as inoculum after measuring its concentration (spores mL⁻¹) by Haemocytometer. Sterilization was done prior to addition of inoculum.

2.4 Screening

Screening was done in order to determine the potential strain for the maximum production of bioprotein. All selected strains were screened under controlled process conditions in 500 ml of Erlenmeyer flask having 2 % (w/v) of wheat flour. All experiments were conducted in a rotary shaker for six days by incubating at a temperature of 30⁰C, agitation of 150 rpm with 2% inoculum (10⁶ spores/ml). Biomass was recorded (three replicates) on 2nd, 4th and 6th days.

2.5 Total Protein Determination (Folin-Phenol Reagent)

The following reagents are used: Phenol, sodium sulphate, sodium-potassium tartarate (Rochelle salt), sodium tartarate, NaCO₃, NaOH, CuSO₄ 5H₂O, Folin reagent [7].

2.6 Biomass Analysis

The biomass was filtered by vacuum filtration and washed three times with 20 ml of distilled water. Before taking the weight of the biomass, it was transferred into an aluminum disk and dried in an oven at 103°C-105°C for one hour followed by cooling in desicator to balance the temperature and weight [8].

3.0 Results and Discussion

3.1 Evaluation of Potential Microorganism

The images of each strain (*A. niger*, *M. hiemalis*, *P. chrysosporium*, *S. cerevisiae* and *T. harzianum*) cultured on PDA plate are shown in Figure-1. Screening was done to determine the best microorganism that can produce highest protein and maximum amount of biomass by utilizing wheat flour.



(a) *A. niger*



(b) *P. chrysosporium*

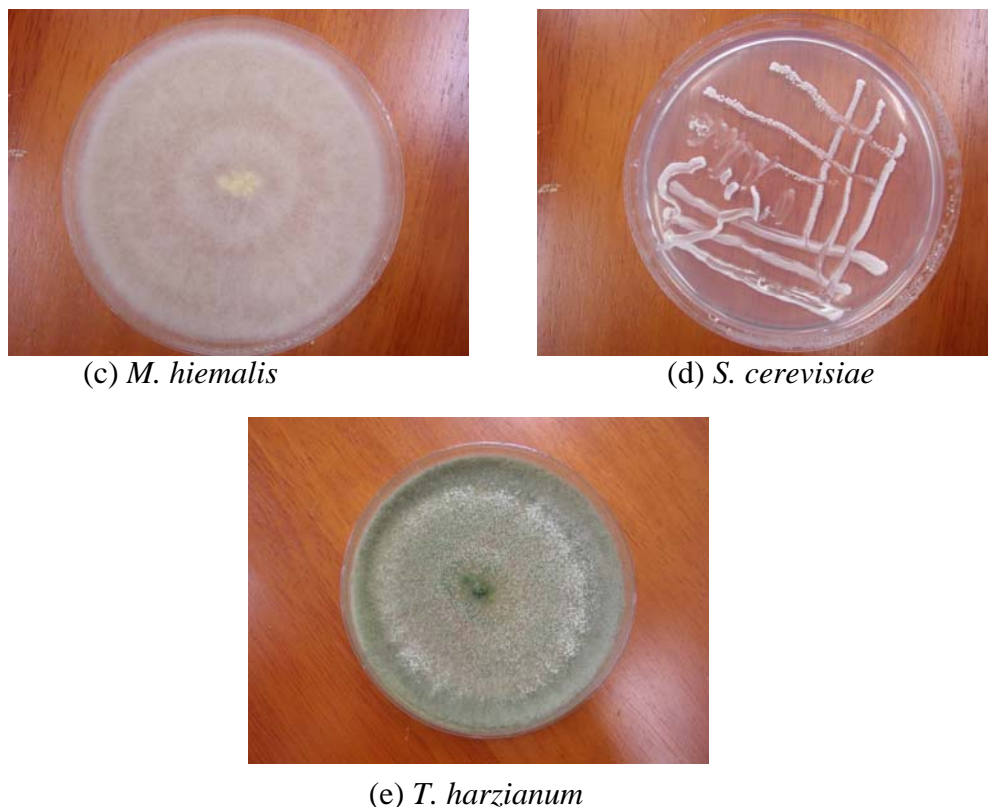


Figure-1 Culture of microorganisms on PDA plate

3.2 Biomass production

Biomass concentration was one of the parameter used to evaluate the potentiality of microorganisms. The fermentation was conducted for six days and each experiment was replicated three times. Biomass was determined on second, fourth and sixth days of the fermentation process. The concentration of biomass on different days of treatment period is shown in Figure-2. All strains did not give similar trend for dried biomass concentration. *T. harzianum* and *M. hiemalis* obtained the optimum biomass of 10.7 g/L and 11.4 g/l respectively on 4th day with an increase from day 2 to day 4 and then decreased on 6th day. This result indicated that these strains were already in exponential phase on day 4. After that they entered the death phase. *A. niger* showed a continuous growth until the last day of fermentation but the amount of biomass was lowest as compared to others. On the other hand, dried biomass of *S. cerevisiae* decreased gradually along the screening period from 7.9 g/L to 6.5 g/L and 6.0 g/L on day 2, 4 and 6 respectively. *P. chrysosporium* showed good growth of 9.1 g/l on the sixth day of fermentation. This biomass concentration could not be considered as highest biomass concentration because this strain did not reach maximum growth yet and were still in growth phase. Considering the growth curve in batch fermentation, generally, the biomass should increase exponentially as the cell is growing and when the cells enter the decline phase or death phase, biomass will decrease [9].

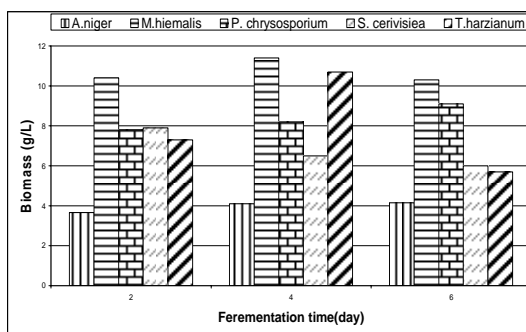


Figure-2 Biomass concentration varies with fermentation time

3.3 Protein concentration

The concentration of protein in biomass during the sixth day of fermentation period is shown in Figure-3. The protein productions by each strain slightly differ from each other. The fermentation time for maximum production of bioprotein was different for every microorganism. *A. niger* and *P. chrysosporium* showed highest concentration on sixth day, *M. hiemalis* and *T. harzianum* on fourth day while *S. cerevisiae* on second day of fermentation. The strain *M. hiemalis* showed the highest protein concentration of 11.6 g/kg and 11.4 g/kg on second and fourth day of fermentation respectively as compared to other strains. Although two strains, *A. niger* and *P. chrysosporium* had an increasing trend until the last day (sixth day), but the protein concentration was not as high as *M. hiemalis* on fourth day. Even though *S. cerevisiae* had highest biomass concentration of 9.5 g/Kg on the second day itself as compared to other strains, we could not select it as a potential strain due to a decrease in concentration after that. One of the objectives of this project is to obtain maximum concentration of bioprotein. Therefore, *M. hiemalis* was more preferable in this case because this strain produced highest biomass as well as high concentration of protein.

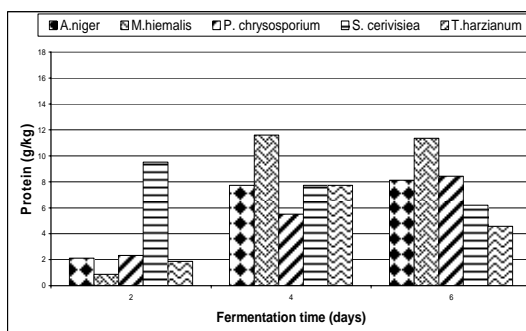


Figure-3 Protein concentration varies with fermentation time

3.4 pH variation

The values of pH for all the samples with different microorganisms can be seen in Figure-4. All five microorganisms showed a decreasing trend in the pH values. The variation in pH is different for every strain. The lowest pH was observed for *Aspergillus niger*. On the day 6, the measured pH of *A. niger* was 1.98. It was expected so because *A. niger* had been widely used in citric acid production [10]. This pH is strongly acidic and not suitable for bioprotein or food production. For *P. chrysosporium*, it was less acidic on the last day as compared to other microorganisms. *T. harzianum*, *M. hiemalis* and *S. cerevisiae*, the value of pH was

almost same on the day 6. On the fourth day, the pH of *M. hiemalis* was 4.5 and it seems to be suitable for maximum biomass production and protein concentration.

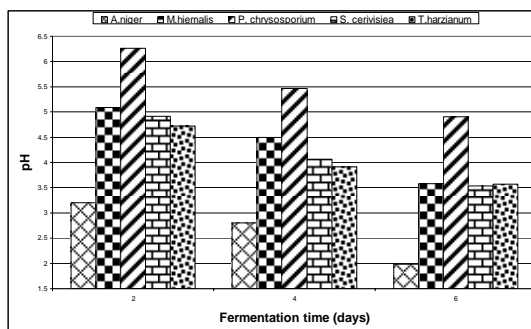


Figure-4 pH varies with fermentation time

4.0 Conclusion

Screening was done to select the best strains for bioprotein production by evaluating the strains in terms of total concentration of biomass and bioprotein produced. From the observed results, *Mucor hiemalis* was identified as the most potential strain for the maximum bioprotein production having a concentration of about 11.598 g/Kg on the fourth day of treatment. The maximum biomass of 11.4 g/L was also obtained on fourth day of fermentation of wheat flour. The study for the optimization of the process conditions is under progress, and hopefully the amount of bioproteins can be further increased. This study may provide a better alternative by converting cheaper carbon source into useful and valuable bioproteins, which can largely be used as supplement and stabilizer in food and feed as well as an additive in the chemical and pharmaceutical industries.

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