

Screening of Potential Strain for Bioprotein Production from Coconut Dregs

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Abstract. The world's population is currently about 6 million people, and increasing. This increase leads to an inevitable and immediate consequence like a proportional growth in the needs for food stuffs. The natural resources available on the planet are limited and only a rational and sustainable exploitation will continue to produce enough food, of sufficient quality, to satisfy the future needs of humankind. Bioprotein production is one of the most promising breakthroughs of biotechnological innovations in animal feed industry. Due to its increasing demand, the utilization of coconut dregs as a novel substrate and cheaper carbon source for production of bioprotein is found necessary for the fulfilling animal feed industry's demand. High fiber and protein content through the incorporation of selected strain, with careful formulation through fermentation process are an important part of this exploitation, and this has, as an immediate consequence, the necessity of feed with higher nutritive value. The efficient strains, substrate and method must be used for high yield product. In this present study, screening of three different strains; *Aspergillus niger* (ATCC 16404), *Saccharomyces cerevisiae* (ATCC 9080) and *Phanerochaete chrysosporium*(ATCC 24725), was done for bioprotein production by solid state fermentation process. *Aspergillus niger* produced the highest amount of protein on the sixth day, with the amount of 427 mg/L. This study may provide a better alternative in agricultural products by converting agriculture waste to valuable and quality product bioprotein, which can be used as supplement and additive in the animal feed and food as well as in chemical and pharmaceutical industries.

Keywords: bioprotein, coconut dregs, screening, solid state fermentation.

1. Introduction

Increasing in population growth nowadays has lead most of the developing countries to the malnutrition problems due to deficiency of protein in human food and animal feed. Because of this problem, it is important to increase protein production by utilizing all available ways. This increasing world demand for food and feed protein led to the search for non-conventional protein sources to supplement the conventional protein sources. Animal feed industry also suffering from inadequate and high cost availability of conventional ingredients. Supplementation of vegetable proteins with animal proteins results in higher feeding costs. Other than that, there is also a strong competition between human beings and livestock for conventional protein source. The utilization of coconut dregs waste provides as alternative substrates and also helps in solving waste disposal problems has never been exploited. Coconut dregs are the leftover fiber from coconut milk production is traditionally used as animal food and finds no other applications. Therefore, in order to solve the problems of protein shortage and proper waste management of coconut milk production's waste, coconut dregs have been chosen as potential substrate for bioprotein production by solid state fermentation. In addition, this substrate is easily available in Malaysia and other part of the world and it also has high nutritional value, high carbohydrates content and available in low cost or no cost.

Bioprotein also called as microbial protein or single cell protein (SCP) is actually protein extracted from cultivated microbial biomass and can be produced using a number of different microorganism [1]. Protein obtain from microorganism is cheap compared to other protein sources and it also have good nutritive value [2]. It is useful because it can be used as food additive, fat binding, additive in certain chemical and pharmaceutical and also as a replacing costly conventional protein sources like animal protein [3]. Besides, it can be utilized as protein supplement of a staple diet because of high quantity of protein, carbohydrates, fatty acid, vitamins and minerals [4]. Bioprotein also source for human food and animal feeds that similar to fish protein in term of amino acids [5]. The inherent high protein content about 60 percent to 70 percent of the cell, rapid increase in cells in a short time and independence from climate conditions for growth are make bioprotein is a good source of protein compared to others [6].

There are several advantages to produce bioprotein by fermentation process compared to other process or conventional source of protein. The fermentation process is not affected by weather conditions and they can be controlled for product and quality in any geographical location [7]. Actually, there are two main types of fermentation process which are liquid state fermentation, sometimes called submerged fermentation and solid state fermentation. Solid state fermentation (SSF) can be defined as “a method of growing microorganisms in an environment of limited moisture without having free flowing water”. It is mean that in the SSF process, the microorganisms grows on a solid surface which is moistened and free access to air. This conditions shows that the growth environment of SSF is similar to natural process. In liquid state fermentation or submerged fermentation (SmF), the condition is different where “the microorganism and the substrate are present in the submerged state in the liquid medium, where a large quantity in the form of solvent is present” [8]. The SSF are fermentations at low moisture levels and the water content is about 40 percent to 80 percent while water content in submerged fermentation is more than 95 percent [9].

Historically, the cultivations on solid substrate became the earliest form of industrial fermentation and the first patent being granted in 1984. At that time, the solid state fermentation only suitable for a small number of fermentations usually for food or food associated products [10]. For example, in Asia, the solid state fermentation has traditional presence in the production of tempeh and soy sauce. Over the last few years, a lot of research has been done to prove that solid state fermentation was available for reprocessing and reuse of different agricultural and food wastes for the conversion of valuable and nutritive products such as enzyme, citric acid, single cell protein, protein enriched feed and protein rich biomass [11]. Therefore, in this present study, coconut dregs waste was introduced as a new potential substrate for fermentation by a suitable microorganism to produce bioprotein. Selection of potential microorganism is necessary to produce maximum quantity bioprotein by solid state bioconversion of substrate. The three microorganisms were collected from School of Bioprocess Engineering Culture Collection, Universiti Malaysia Perlis. 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. Materials and Methods

2.1. Collection and Preparation of Substrates

Coconut dregs were procured from the grocery at Bintong, Kangar. Coconut dregs were dried in an oven at 60°C for 24 hours. The dried substrate was grained and sieved to obtain 500 mm mesh size.

2.2. Microorganism Strain

The microorganisms used to ferment coconut dregs included *Phanerochaete chrysosporium* (ATCC 24725), *Aspergillus niger* (ATCC 16404) and *Saccharomyces cerevisiae* (ATCC 9080) were obtained from School of Bioprocess Engineering culture collection, Universiti Malaysia Perlis. These microorganisms were grown on the potato dextrose agar (PDA) plate and sub-cultured every 2 weeks and incubate at 32°C.

2.3. Inoculum Preparation

Inoculums were prepared by washing the growing culture with 25 ml sterile distilled water. The spore suspensions were rubbed and adjust to final concentration of 10^7 spores per ml. The suspension inoculums were kept in chiller at 4°C for further use.

2.4. Growth media preparation

The 70 percent of moisture are maintained for every 5g substrate in one conical flask. The 70 percent moisture is equal to 12 ml of solution which divided into 2 ml inoculum suspension and 10 ml of growth media solution that contain KH_2PO_4 , NH_4NO_3 , NaCl , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$.

2.5. Screening of Microorganisms

Screenings of microorganisms were carried out to determine the best and potential microorganism that could produce highest bioprotein by using coconut dregs as a substrate and applying the solid state fermentation method. Each microorganism was screened using the same quantity of substrate, constant media composition and process condition. The fermentation medium composed of 5 gram substrate, 2 ml of inoculums suspension and 10 ml of growth media that contain 0.2 percent KH_2PO_4 , 0.5 percent NH_4NO_3 and 0.1 percent of each NaCl , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. All the samples of different inoculums suspension were statically incubated at 32°C in a laboratory incubator for 7 days.

2.6. Solid State Fermentation

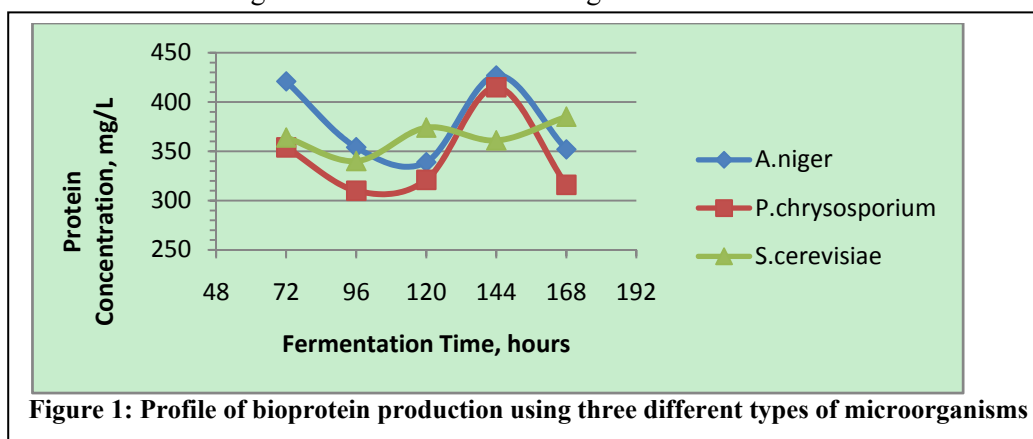
Solid state fermentation was carried out in 250 ml conical flask containing 5g sterilized coconut dregs. 10 ml of sterile growth media was added into 5 gram substrate in the flask. Then, the flasks were aseptically inoculated with 2 ml of inoculums suspension. All the mixture was stirred using glass rod and incubated at 32°C in a laboratory incubator for 7 days.

2.7. Protein Recovery

The fermentation samples in the flasks were dried for 24 hours at 60°C - 70°C . Dried samples were added with 50 ml phosphate buffer and macerated in pestle mortar. The mixtures were centrifuged at 8000 rpm for 20 minutes. The supernatant obtained were kept in the refrigerator for further analysis of protein concentration using Bradford method.

3. Results and Discussion

Screening of the best strains to produce highest bioprotein was carried out at the same media and process condition. The fermentation for three different microorganisms was carried out for seven days and the protein produced was analyzed starting on the third day of incubation until the seventh days. The production profiles for each microorganism were illustrated in Figure 1.0.



From Figure 1.0, it can be observed that the protein productions by each strain slightly differ from each other. The maximum protein concentration was obtained on the sixth day (144 hours) of incubation using *Aspergillus niger*. *Aspergillus niger* is one of the industrially important fungi that classified as deuteromycetes [12]. This microbe has been widely used for single cell protein production [13]. The maximum protein concentration obtained is 427 mg/L while the lowest protein concentration is 310 mg/L obtained by fermentation with *Phanerochaete chrysosporium* on the fourth day of incubation. Both of the strains showed the similar trend of bioprotein production and higher than *S.cerevisiae*.

Even though *S. cerevisiae* shows a gradual increase through this seven days as compared to other strains, we could not select it as a potential strain due to an unexpected protein concentration after seventh day. The objective of this present study is to obtain maximum bioprotein production by utilizing different types of strain. Therefore, *A. niger* was more preferable in this case because this strain produced highest protein concentration. This result is in agreement with the findings by Eutace A Iyayi and Dorothy M. Losel (2001) [15]. They found that *A. niger* produce significant bioprotein from cassava by-products as compare to *S. cerevisiae*. Besides, Jamal et al. also found that bioprotein from *A. niger* using wheat flour as cheaper carbon source showed highest protein concentration on sixth day [9]. Apart from that, according to chemical, microbiological and toxicological analysis, bioprotein from *Aspergillus niger* proved to be a potentially good animal feed [1].

In conclusion, the screening process was done as the first step in determination of the best strains in term of bioprotein produced using the coconut dregs as a new potential substrate. *A. niger* showed remarkable result and hence the strain will be selected for the next media optimization study using statistical approach. The performance of the potential strain will satisfactory during media optimization and optimization of the process conditions and this study this study is under progress and hopefully the amount of bioprotein production can be further increase. This study may provide the competitiveness protein source that may have good nutritive value, however upon their amino acid composition.

4. Acknowledgements

The authors acknowledge the research grant provided by the Universiti Malaysia Perlis (UniMAP)

5. References

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