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Optimization of Process Parameters for the Production of Single Cell Biomass of *Candida utilis* in Solid State Fermentation

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Abstract: In the present study *Candida utilis was* employed for the production of single cell protein (SCP) from wheat bran using solid state fermentation in 250ml Erlenmeyer flask. Various cultural conditions were also optimized for the maximum yield of cell biomass of *Candida utilis.* Maximum cell biomass was obtained when wheat bran was supplemented with 2% molasses as carbon and 0.25% ammonium nitrate as nitrogen sources and 250mg/L of Biotin as inducer at pH 6.5. The incubation period of 4 days was found suitable for maximum cell biomass of *Candida utilis* with 10% (v/v) inoculum size. The protein contents (48%) were also estimated in *Candida utilis* biomass which indicated its potential ability to improve the nutritional problems of protein deficiency associated malnutrition.

Key words: Single cell protein · *Candida utilis* · solid state fermentation

INTRODUCTION

Now days in this world the sources of food are limited and the human being are in the search of new resources of food. The use of microorganism is certainly innovative I dea to solve the global food problem. Therefore, the production of microorganism for food industry is the main concern for the industry and scientific society.

Food grade yeasts is another solution to overcome the deficiency of food and also used as sources of high nutritional value proteins, vitamins and enzymes. It can also be implies in the health food industry as nutritional supplements, as food additives, conditioners and flavoring agents, for the production of microbiology media, as well as livestock feeds. Yeasts are also involved for the production of specific types of fermented foods like cheese, bread, sourdoughs, fermented meat and vegetable products, vinegar, etc. Yeast is very significant in food technology as well as in human nutrition to cover the demands in a world of low agricultural production and rapidly increasing population makes the production of food grade yeasts, as alternative sources of protein, extremely important. As we know that large part of the earth's population is suffering from malnourished, due to poverty and inadequate distribution of food. Scientists are concerned whether the food supply can keep up with the pace of the world population increase, with the increasing demands for energy, the ratio of land area required for global food supply or production of bioenergy, the availability of raw materials, as well as the maintenance of wild biodiversity and they also trying to discover the alternative source of food [1,2].

In the production of food grade yeast various factors such as carbon and nitrogen sources, temperature, pH of growth medium, phosphorus and potassium primarily influenced the fermentation process [3]. To make the yeast production process cost effective prior to commercial scale production optimizations of these parameters are essential.The utilization of agriculture waste material such as rice husk, wheat bran and fruit wastes for the production of microbial protein would serve to supplement the available traditional protein sources [4].

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Certain microorganism such as algae, certain bacteria, yeasts, moulds and higher 'fungi' that can be used as protein supplement for both human food and animal feed are used as dried cell mass called single cell protein [5]. The yeast *Candida* and *Saccromycis* sp. were extensively used as a microbe of choice for production of single cell protein because of its case of isolation and growth on carbohydrate containing media. And, also its energy requirements were considered to be minimal as, it grew very well at room temperature [6]. For the production of SCP developed microorganism can be used to ferment some of the vast amounts of waste materials, such as wheat straw wood and wood processing wastes, food cannery and food processing wastes and residues from alcohol production [7]. Thus the benefits of SCP production extended from the production of food to the preservation of the environment [8].

Considering these facts, attempt has been made to produce the protein rich biomass of *Candida utilis* from fruit waste and agriculture waste material. The food grade yeast production on commercial scale can be used to overcome the protein deficiency in animal feed and also help full to improve the protein contents of animal feed.

MATERIALS AND METHODS

Substrate: Wheat bran was purchased from local market of Lahore city, Pakistan and was used as a substrate in the present study.

Microorganism: *Candida utilis* obtained from microbiology laboratory of PCSIR laboratories, complex Lahore, was used as microorganism for the production of single cell protein. The strains was grown on PDA slant (Oxoid) for 96 h at 30°C. The culture were then preserved at 4°C and further shifting on the PDA slant at the interval of 15 days to keep them viable.

Inoculum Preparation: For the production of single cell protein, inoculum was prepared by adding 5ml sterilized distilled water in culture slants. With the help of inoculum loop the surface of the slant was gently rubbed to make it almost homogeneous. Fresh inoculum was prepared for each the parameter under investigation.

Fermentation Technique: Ten grams of substrate (wheat bran) were taken in each Erlenmeyer flask of 250 ml capacity. The substrate was moistened with the mineral medium in 1:1(w/v) (%): K_2HPO_4 (0.25), $MgSO_4$ (0.05), Soluble starch (0.5), Peptone (0.25) NH_4NO_3 (0.25). The initial pH of the medium was adjusted to 6 with 0.1 N HCl/HaOH. Before sterilization at 121 ºC and 15.0 lbs/inch^2 pressure for 15 min. After sterilization, the media was inoculated with 1 ml of spore suspension containing 1x10^7 spores ml^{-1} kept for incubation at 30±1ºC for four days in static condition.

Optimization of Production Parameters: Various production parameters such as fermentation period (24, 48, 72, 96 and 120hrs), initial medium pH (4, 4.5, 5, 5.5, 6, 6.5, 7 and 7.5), inoculum size (5,10,15,20,25 and 30%), incubation temperature (20,25,30,35 and 40°C), different molasses concentrations (0.5,1,1.5,2,2.5,3,3.5 and 4%) and different inducers (Pyridoxin, Biotin, Isnsitol and Calcium pentathionate) were optimized by *Candida utilis* in solid state fermentation using wheat bran as substrate.

Analytical Procedure

Proximate Analysis of Substrate: In proximate analysis of wheat bran crude protein content was determined by Kjeldhal method. Ash, crude fiber and fat were determined using the standard methods out lined in AOAC [9].

Soluble Protein Estimation: Soluble protein was estimated by the method as described by Lowery *et al.* [10] using BSA as a standard protein.

Statistical Analysis: The data was subjected to statistical analysis for the determination of significance by using ANOVA [11].

RESULTS AND DISCUSSION

Compositional Analysis of Substrate: The data in table 1 showed the compositional analysis of wheat bran (substrate). Moisture, ash and fat content of wheat bran were 7.39, 6.61 and 2.67 %, respectively. Amount of crude protein and crude fiber were12.61 and 6.50 %, respectively while the amount of nitrogen free extract was 60.53%. da-Silveira and Badiale-Furlong [12] reported that wheat bran comprised of 9.4, 13.8, 5.2, 60.1, 6.3 and 5.2% of moisture, proteins, lipids, carbohydrates, ash and fiber content, respectively. The utilization of wheat bran for production of microbial protein would serve to supplement the available traditional protein sources [13].

Table 1: Chemical composition of substrate (Wheat bran)

S.No.	Constituent	(%)
1	Moisture	7.39±.21
2	Ash	6.61±.34
3	Crude protein	12.61±.33
4	Crude fiber	6.50±.26
5	Nitrogen free extract	60.53±.50
6	Fat	2.67±.20

Effect of Initial Ph of Media: Different initial pH values were used to check the optimum pH value for maximum yield of the biomass. The results of present study showed (Fig. 1) that yield of biomass increased from pH 4 to 6 and optimum production was observed at 6.5 yielding soluble protein of 3.13 mg/ml and 48.01 ± 1.32 % of crude protein. Further increase in initial medium pH decline in protein production was observed which were in good agreement with the work of Halasz and Radomie [14]. *Candida sp*. was capable of growth over a wide pH range of 3.0 to 6.2 [15]. Gbologade [16] obtained maximum production of *Lepiota procera* biomass at pH of 6.5. Initial pH of 7 produced maximum protein (30.54%) of *A.niger* and pH 5.5 in case of *Chaetomimum sp.* (29.39% protein content) [17]. Where as Rosma and Ooi [18] obtained maximum yield of *Candida utilis* pH 4.5. In another study, Rajoka *et al* [19] and Munawar *et al* [20] also produced maximum biomass of *Candida utilis* at pH 6.0. Li *et al*. [21] optimized the cultural conditions for production of yeast biomass using bamboo as a substrate and reported optimum pH of 6.1. pH greatly affected the true protein production from cultures of *Candida utilis* and *Saccharomycopsis fibuliger* using Potato Processing Waste water as medium [22].

Effect of Fermentation Period: To optimize the proper fermentation period, a set of flasks with solid state substrate were inoculated with 1% inoculum and incubated at 30º C for 24hrs, 48hrs, 72hrs, 96hrs and 120hrs. Among these various tested fermentation periods, 96hrs was found suitable for maximum production of proteins (3.22±0.225 mg/ml soluble protein and 47.6 ±1.41% crude protein) as shown in Fig. 2. Munawar *et al* [20] also reported similar findings. Li *et al* [21] optimized fermentation period of 69h was best maximum cell biomass production of *candida utilis*. Ravinder *et al.* [23] studied effect of fermentation period on the production of mutant *Aspergillus oryzae* SCP from deoiled rice bran and obtained maximum SCP after72 hrs. Similarly, Adoki [6] studied the cultural characteristics of *Candida sp*. in waste conversion for single cell protein enriched feed supplement production and obtained maximum SCP after 72hrs of fermentation.

Effect of Inoculum Size: The effect of different inoculum size on growth of SCP production was studied as shown in fig. 3. Different inoculum size viz., 5% to 30%v/v were applied in growth medium and maximum protein was obtained with the 10% (v/v) inoculums size producing 3.12±.05 mg/ml of soluble protein and 47.4±0.99 % of crude protein respectively. Fungal spore loading of 108 to the orange waste medium produced the protein content of 39.65% of *Chaetomium sp* and 30.47% of *A.niger* [17]. Inoculum size of 7.5% (v/v) was suitable for maximum production of protein content from *Candida utilis* [21]. Rajoka *et al.* [24] working on kinetics of batch single cell protein production from rice polishing with *Candida utilis* in continuously aerated tank reactors obtained maximum yield of *Candida utilis* biomass with 10% (v/v) inoculum size on rice bran. Oshoma and Ikenebomeh [25] studied on the production of *Aspergillus niger* biomass from rice bran and used 2% (v/v) inoculum size to obtained maximum production of the biomass. Maximum biomass of *Aspergillus oryzae* was obtained with 3% (v/v) inoculum size on deoiled rice bran [23]. Fruit waste extract also yield a maximum cell biomass of *Candida utilis* with 4% inoculum size [20].

Effect of Temperature: Figure 4 represents the effect of incubation temperature on SCP production. Maximum protein production was observed at 30º C which was 3.00±0.23 mg/ml soluble protein and 45.78±2.78 % crude protein. Similar results were reported by Adoki [26] working on factors affecting yeast growth and protein yield production from orange, plantain and banana wastes processing residues using *Candida* sp. Incubation temperature of 25°C produced maximum protein content for *Chaetomium sp* and *A.niger* [17]. The production of SCP by *Candida utilis* at 25-35°C temperature range revealed that there was a sudden increase from 25-35°C in the protein production. It was also observed that protein production decrease as temperature increase up to 35 °C which was significant as one of the requirements of human microbial pathogens is that they must be capable of growth at 37°C, this result presumably indicates the safety of the use of this organism for animal feed supplement production.

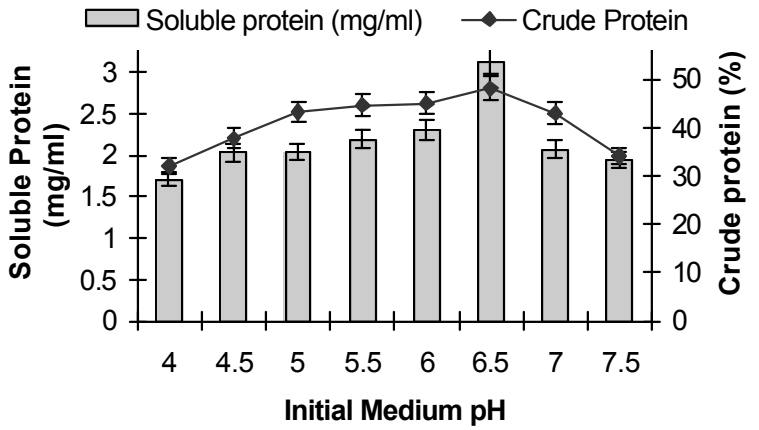

Fig. 1: Effect of initial medium pH on crude protein and soluble protein production from wheat bran using *Candida utilis* under solid state cultivation. Results represent the mean of duplicate analysis and bar indicates ± standard deviation which differs significantly at $p \leq 0.05$.

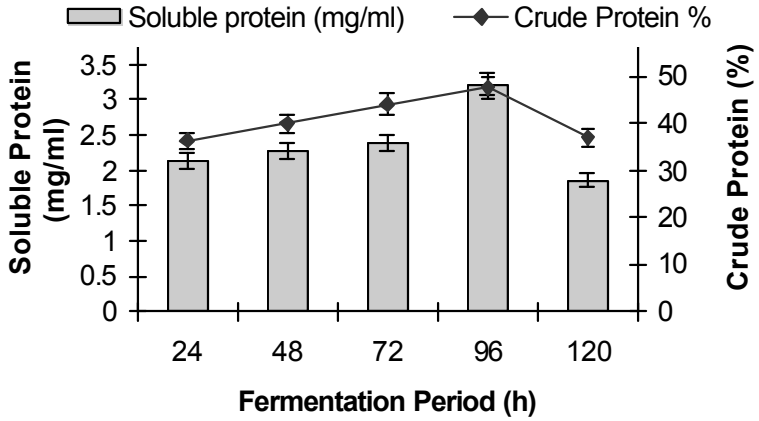

Fig. 2: Effect of fermentation period on crude protein and soluble protein production from wheat bran using *Candida utilis* under solid state cultivation. Results represent the mean of duplicate analysis and bar indicates ± standard deviation which differ significantly at $p \leq 0.05$.

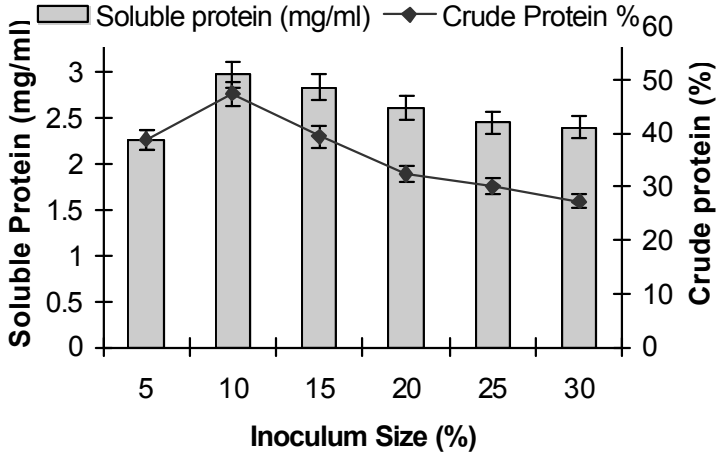

Fig. 3: Effect of different inoculum size on crude protein and soluble protein production from wheat bran using *Candida utilis* under solid state cultivation. Results represent the mean of duplicate analysis and bar indicates ± standard deviation which differs significantly at $p \leq 0.05$.

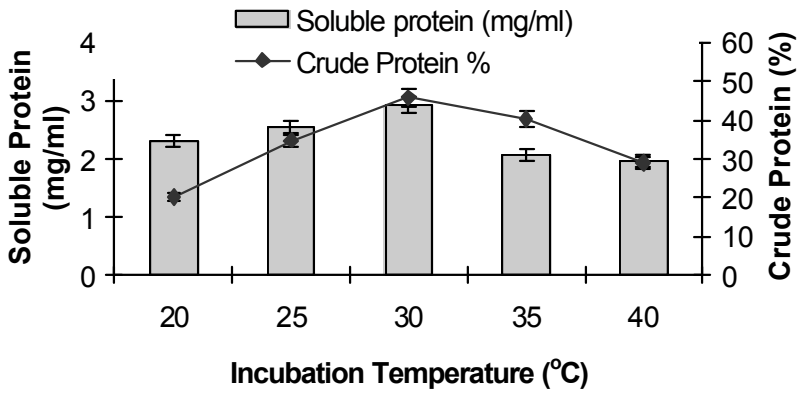

Fig. 4: Effect of different incubation temperatures on crude protein and soluble protein production from wheat bran using *Candida utilis* under solid state cultivation. Results represent the mean of duplicate analysis and bar indicates ± standard deviation which differs significantly at $p \leq 0.05$.

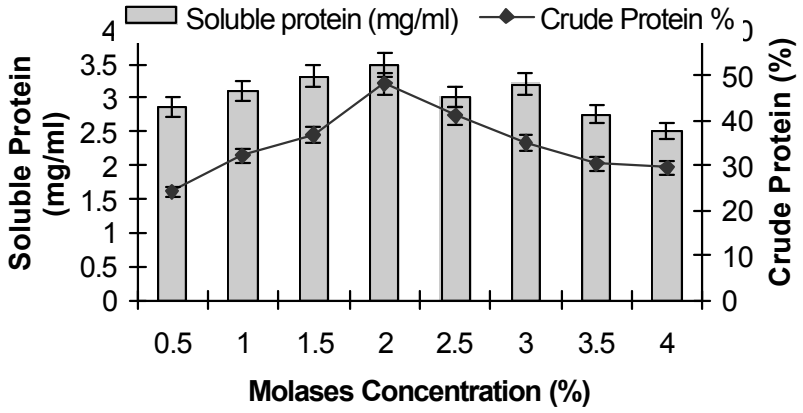

Fig. 5: Supplementation of different concentration of cane molases on crude protein and soluble protein production from wheat bran using *Candida utilis* under solid state cultivation. Results represent the mean of duplicate analysis and bar indicates ± standard deviation which differs significantly at $p \leq 0.05$.

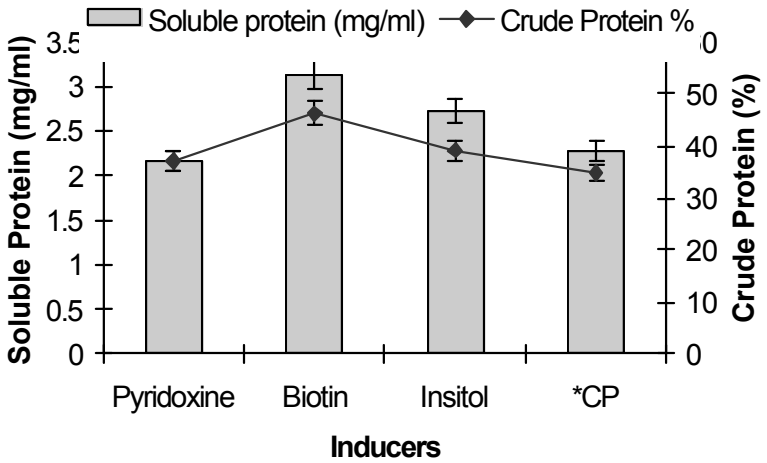

Fig. 6: Effect of different inducers on crude protein and soluble protein production from wheat bran using *Candida utilis* under solid state cultivation. Results represent the mean of duplicate analysis and bar indicates ± standard deviation which differ significantly at $p \leq 0.05$.
*CP: Calcium pentathionate.

Similar result was also reported by Adoki [26]. Lemal *et al* [22] observed that at 32°C, 34% more carbon was consumed than that of 27°C while working on production of *Candida utilis* and *S. fibuliger* on potato processing waste water. Temperature of fermentation medium is one of the critical factors that have a profound influence on the production of end product [24]. At 25°C, 20.5g/L crude protein was observed in which further increased to 27.5 g/L at 35°C. Increased or decreased temperature other than optimum value decreased protein production during fermentation process [27]. Normally high temperature can cause inactivation of enzymes of the metabolic pathway while low temperature may not permit flow of nutrient across cell membrane, resulting in high demand for maintenance energy. Rosma and Ooi [18] also observed these results during the investigation of effect of agitation speed and aeration rate on the production of *C.utilis* growing on pineapple waste material, while operating temperature and agitation speed at 30°C and 900 rpm.

Supplementation of Molasses in Media: Cane molasses is a low cost substrate which can be used for the production of microbial biomass protein for animal feed supplements [28,29]. Supplementation of different concentrations of molasses to the medium was also investigated for obtaining maximum yield of SCP. Results showed (Fig. 5) that maximum protein production (3.49±0.19 mg/ml soluble protein and 48.1±2.48 % crude protein) was observed at 2.0 %(w/v) molasses in solid state fermentation media. Further, increased in molasses concentration decreased biomass production. In batch cultivation of yeast high sugar concentration in the culture can result in catabolite repression, which inhibits respiratory enzymes and increases ethanol production [30] Ahmad *et al.* [31] reported that supplementation of molasses at 1% favored maximum cell biomass of *Candida utilis* and *Arachiniotus sp*. This result indicated that *C. utilis* was unable to convert all the sugar into biomass. Rajoka *et al*. [24] reported maximum cell biomass (2.88 g/L) in molasses based medium.

Effect of Inducers: Different inducers like Pyroidoxin, Biotin, Inositol and Calcium pentathionate at the concentration of 250mg/L was supplemented to enhance the protein content of *Candida utilis*. Results indicated (Fig. 6) that among all these inducers biotin enhanced the protein level of 3.14±0.03 mg/ml soluble protein and 46.19±1.09 % crude protein. Chandra *et al*. [32] worked on L-asparginase production by wild strain of *Alcaligenes faecalis* and reported that supplementation of media with biotin increased 40.88% biomass production but with less enzyme activity.

In conclusion, the growth rate of *Candida utilis* was maximum wheat bran was supplemented with 2% molasses as an additional carbon and 0.25% ammonium nitrate as nitrogen sources and 250mg/L of Biotin as inducer at pH 6.5 for 4 days with 10% of inoculum size. The level of crude protein of 48% obtained from the dry biomass of *Candida utilis* produced in the present study indicated its potential to provide feed supplement in animals.

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