BIOSYNTHESIS OF SINGLE CELL BIOMASS OF *CANDIDA UTILIS* BY SUBMERGED FERMENTATION

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ABSTRACT: *Candida utilis* was employed for the production of single cell protein (SCP) from fruit waste extract (FWE) using submerged fermentation in shake flask. Different cultural conditions were optimized for the maximum yield of cell biomass. Maximum yield of cell biomass appeared when fruit waste extract was supplemented with soluble starch as carbon and ammonium nitrate as nitrogen sources at pH 6.0. The incubation period of 4 days was found suitable for maximum *Candida utilis* biomass with 4% (v/v) inoculum size. The protein contents (49%) were also estimated in *Candida utilis* biomass which indicated its potential for feed supplement in both animals and humans to improve protein deficiency associated malnutrition.

Key words: Single cell biomass, *Candida utilis*, Submerged fermentation.

INTRODUCTION

Many developing countries where major nutritional problems exist produce materials rich in carbohydrates to provide bulk food. In doing so these low-cost non-conventional agro-industrial residues are produced in heavy amounts. Such wastes accumulate up to 50 million ton in Pakistan including fruit processing waste (Anonymous., 1988). The fruit waste can be utilized as a substrate of choice to produce single cell protein by fermentation process. The protein obtained from the microorganisms is not only cheap but also provide balanced nutrition and is a potential supplemental source for feeding poultry, livestock and humans (Singh *et al*., 1991; Pacheco *et al*., 1997).

Various factors such as carbon and nitrogen sources, temperature, pH of growth medium, phosphorus and potassium are prime need of any fermentation process (Pirt, 1975). The utilization of fruit wastes for production of microbial protein would serve to supplement the available traditional protein sources (Moo-Young, 1977). Single cell protein is dried cells of microorganisms such as algae, certain bacteria, yeasts, moulds and higher fungi that can be used as protein supplement for both human food and animal feeds (Landgraf, 1989). The yeast *Candida* sp. was used as a microbe of choice for production of single cell protein because of its ease of isolation and growth on carbohydrate containing media. Similarly, its energy requirements were considered to be minimal as it grew very well at room temperature (Adoki, 2002). For the production of SCP developed microbes can be used to ferment some of the vast amounts of waste materials, such as straws, wood and wood processing wastes, food, cannery and food processing wastes and residues from alcohol production (DaSilva *et al*., 1992). Thus the benefits of SCP production extended from the production of food to the preservation of the environment (Bunker, 1966).

Considering these facts, attempt has been made to produce the protein rich biomass of *Candida utilis* from fruit waste. The biomass can be used as protein supplement in animal feed to improve its protein contents.

MATERIALS AND METHODS

Substrate: Fruit waste including orange juice pulp and banana skin was obtained from the juice corner situated at main boulevard Gulshan Ravi Lahore. The extract of fruit waste was prepared by the method of Adoki (2007). The fruit waste suspension (10% w/v) was passed through the muslin cloth to remove solids leaving the fruit waste extract (FWE) which was used as substrate in the present study.

Microorganism: *Candida utilis* (PCSIR-2) procured from PTCC (Pakistan Type Culture Collection), PCSIR laboratories, complex Lahore, was used for the production of *Candida utilis* biomass.

Inoculum preparation: The inoculum of *Candida utilis* was prepared by the method of Adoki (2007).

Fermentation Technique: Submerged fermentation was carried out in shake flasks in a growth medium comprising of (%) K_2HPO_4 (0.25), $MgSO_4$ (0.05), carbon source (0.5) and nitrogen source (0.25) as supplement. The pH of the media was adjusted to 6 with 1N

NaOH/HCl before sterilization at 121°C for 15 min. After sterilization the media was inoculated with 1ml suspension of *Candida utilis.* The inoculated flasks were incubated at 30 ˚C with the agitation speed of 120rpm for 4 days. After fermentation, biomass was separated by centrifugation (5000 rpm, 10 ˚C for 10 minutes). The pellet containing the cell mass was dried at 40 ˚C in an incubator for 24 hrs and cell free broth was used for further analysis.

OPTIMIZATION OF PROCESS PARAMETERS

Effect of Fermentation Period: Effect of fermentation period was studied by harvesting samples at 24, 48, 72, 96 and 120hrs to check the maximum yield of biomass at the mentioned incubation period

Effect of Inoculum Size: Different concentrations of inocula sizes i.e. 2, 3, 4, 5 and 6% v/v were used to analyze for the maximum production of single cell biomass of *Candida utilis.*

Effect of Initial pH of medium: Effect of different initial pH values *viz*. 4, 5, 6, 7 and 8 of growth media was checked before sterilization to enhance the maximum production of biomass of *Candida utilis.*

Effect of Temperature: Different incubation temperatures i.e. 20, 25, 30, 35, and 40°C were used to check the maximum production of single cell biomass.

Effect of different Carbon sources: The Fruit Waste Extract was supplemented with different carbon source such as glucose, sucrose, soluble starch, maltose and wheat bran each at 0.5 % w/v to check the maximum production of biomass.

Effect of different Nitrogen sources: Different nitrogen sources, organic (urea, peptone, meat extract, yeast extract and lab-lamco powder) and inorganic (ammonium nitrate, ammonium sulphate, ammonium dihydrogen phosphate, ammonium chloride, ammonium phosphate and ammonium citrate) nitrogen sources were supplemented to the growth media, each at 0.25% w/v to check the maximum production of biomass.

ANALYTICAL PROCEDURE

Compositional Analysis of Substrate: The moisture, ash and crude fat contents of the substrate were determined by the methods of AOAC (2005).

Total Protein Estimation: Total Protein in the fermented broth was estimated by the method of Lowry *et al.* (1951) and nitrogen content of dry biomass of the *Candida utilis* was estimated by micro-Kjeldhal method (AOAC., 2005).

Total Carbohydrates Estimation: Total carbohydrates in the cell free extracts were estimated according to the method described by Dubois *et al.* (1956) using phenol sulphuric acid method.

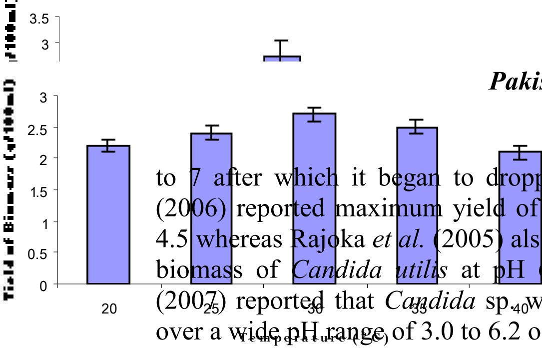
RESULTS AND DISCUSSION

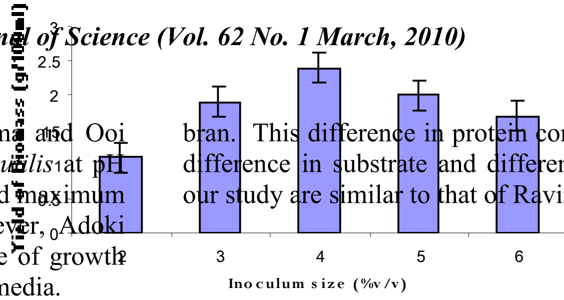
Compositional Analysis of Substrate: The proximate analysis of fruit waste (banana and orange) showed a composition of 81% moisture, 5.75%ash, 3.90% crude fats, 3.82% crude protein and 40.34% total carbohydrates (Table 1). Nazem *et al.* (2007) studied the nutritive value of the orange pulp and found that it consisted of 79% moisture, 6.5% ash, 6.2% crude fats and 6.8% crude protein. Essien *et al.* (2005) reported on mould growth and biomass production using waste banana skin that consisted of 78.4% moisture, 13.44% ash, 11.60% crude fats, 7.87% crude protein and 59.51% carbohydrates. Due to the mixture of the banana and orange waste these proximate composition do not match with the optimum proximate composition of the fruit waste observed in present study. The results of the chemical analysis of fruit waste extract was comparable with the work of Nazem *et al.* (2007) and Essien *et al.* (2005). All these results indicated that that fruit waste can be used as a potential substrate for microbial growth due to its reasonable carbohydrates contents.

Effect of Fermentation Period: Maximum production of biomass was found after four days of incubation as shown in the Figure 1. Ravinder *et al.* (2003) studied the production of mutant *Aspergillus oryzae* SCP from deoiled rice bran and obtained maximum SCP after 3 days. Similarly, Adoki (2002) studied the cultural characteristics of *Candida* sp. in waste conversion for single cell protein enriched feed supplement production and found maximum SCP after 3 days of fermentation. The result shows some variation which might had existed due different nature of the strain isolated from different habitats.

Effect of Inoculum Size: Different inocula sizes viz., 2, 3, 4, 5 and 6% v/v were studied as shown in the figure 2 which indicated that maximum yield of *Candida utilis* biomass was obtained with the 4% (v/v). Different workers obtained maximum biomass production with different inoculum size. Rajoka *et al.* (2005) reported maximum yield of *Candida utilis* biomass with 10% (v/v) inoculum size on rice bran and Ravinder *et al.* (2003) obtained maximum *Aspergillus oryzae* biomass with 3% (v/v) inoculum size on deoiled rice bran.

Effect of Initial pH of medium: Optimum pH for maximum biomass yield was found 6.0 as shown in the Figure 3 having a value of 2.61g/100ml.The results showed that yield of biomass increased from pH 4 to 6 Halasz and Radomie (1991) have reported that the yield of yeast biomass increased by increasing the pH from 4





to 7 after which it began to dropped.. Rosma and Ooi (2006) reported maximum yield of *Candida utilis* at pH 4.5 whereas Rajoka *et al.* (2005) also produced maximum biomass of *Candida utilis* at pH 6.0. However, Adoki (2007) reported that *Candida* sp. was capable of growth over a wide pH range of 3.0 to 6.2 of growth media.

Effect of Temperature: Maximum yield of *Candida utilis* biomass was obtained at temperature 30°C. The results (Fig. 4) also showed that yield of biomass increased from incubation temperature of 20°C to 30°C and then began to decrease as the temperature increased. Rajoka *et al.* (2005) reported that the yield of *Candida utilis* biomass increased up to 35°C and then began to decrease. Oshoma et al (2005) obtained maximum yield of *Aspergillus niger* biomass at 30°C using rice bran as substrate.

Effect of different carbon sources: All the carbon sources, except maltose enhanced biomass yield of *Candida utilis* and highest dry biomass (2.07g/100ml) was observed with soluble starch followed by glucose as shown in Figure 5. The utilization of glucose reported by Adoki (2007) (on orange, plantain and banana processing residues) and Kurbanoulu (2001) (on horn hydrolysate) supports results of the present study.

Effect of different nitrogen sources: Among all the organic nitrogen sources investigated, supplementation of media with peptone gave highest biomass yield of 2.06g/100ml [Fig. 6(a)]. Gbolagade (2006) reported maximum production of biomass of *Lepiota procera* (a Nigerian edible higher fungus) in submerged liquid culture when peptone was used as a organic nitrogen source. Similarly, among various inorganic nitrogen sources, ammonium nitrate (NH_4NO_3) gave maximum yield (2.96g/100ml) of *Candida utilis* biomass [Fig. 6(b)]. Adoki (2007) and Rajoka *et al*. (2005) (on rice polishing) reported maximum yield of *Candida utilis* biomass with ammonium nitrate among different inorganic nitrogen sources.

Table 1: Proximate composition of the fruit waste

Sr. #	Composition	(%)
1	Moisture	12
2	Ash	5.75
3	Crude fat	3.90
4	Crude protein	3.82
5	Total carbohydrates	40.34

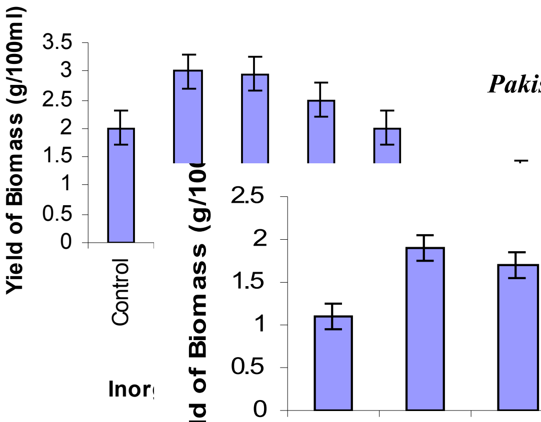
Protein estimation in biomass: Total crude protein contents of 49% was estimated in *Candida utilis* biomass. Rajoka *et al.* (2005) reported 32.75% total crude protein in *Candida utilis* biomass cultivated on rice bran. Ravinder *et al.* (2003) reported 43% total crude protein content in biomass of *A. oryzae* cultivated in deoiled rice bran. This difference in protein contents might be due to difference in substrate and different strains. Findings of our study are similar to that of Ravinder *et al.* (2003).

Figure 1: Effect of different fermentation periods on the biomass of *Candida utilis.*

Figure 2: Effect of different inoculum sizes on the biomass of *Candida utilis.*

Figure 3: Effect of different initial pH values on the biomass of *Candida utilis.*

Figure 4: Effect of different inoculum sizes on the biomass of *Candida utilis.*



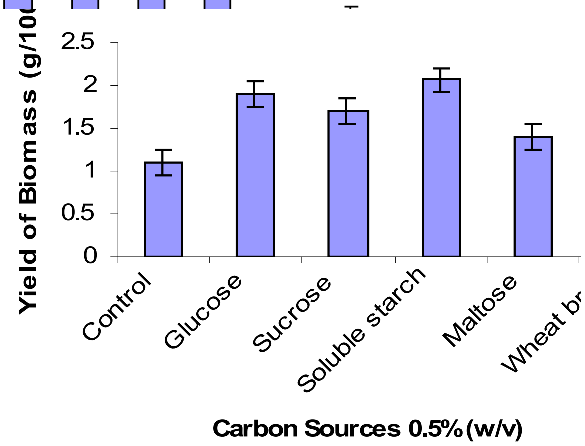


Figure 5: Effect of different carbon sources on the biomass of *Candida utilis*.

Figure 6: Effect of different organic (a) and inorganic (b) nitrogen sources on the biomass of *Candida utilis.*

Conclusion: Maximum yield of cell biomass was obtained when fruit waste extract supplemented with soluble starch as a carbon source and ammonium nitrate as nitrogen source at pH 6.0 for 4 days with 4% (v/v) of inoculum size The level of crude protein of 49% obtained from the dry biomass of *Candida utilis* produced in the present study indicated its potential for feed supplement in animals.

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