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Biomass Production of *Pleurotus sajor-caju* by Submerged Culture Fermentation

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Abstract. The effect of different carbon sources, namely, sawdust and powder of agrowastes (as such, or water soluble extracts), and inorganic/natural nitrogen sources on the biomass production of *Pleurotus sajor-caju* by submerged culture fermentation was studied. Supplementation of the fermentation medium with 2% molasses, 2% wheat spike powder, extract of 2% wheat spike powder, and corn gluten meal resulted in 12.85, 10.85, 12.35 and 13.92 g/l biomass production of *P. sajor-caju*, respectively. The fungal hyphae biomass contained 8.28% moisture, 21.18% crude protein, 1.55% fat, 3.59% ash, 2.32% crude fibre, and 63.48% nitrogen free extract.

Keywords: *Pleurotus sajor-caju*, mushrooms, submerged culture fermentation, fungal culture, fungal biomass

Introduction

Among the suggested non-conventional novel sources of proteins are bacteria, yeasts, algae and the mycelia or sporophores of higher fungi. The latter deserves special consideration since humans have consumed mushrooms throughout their evolutionary history because of their pleasing flavour. Mushrooms are not only a food delicacy, but also a rich source of proteins, carbohydrates, minerals and vitamins (Martin, 1992; Bano and Srivastava, 1962; Jenninson *et al.*, 1957). Mushroom production, as compared to other vegetable crops among the marketed foods, is highly efficient in terms of energy conversion, i.e., energy input as the nutrients supplied for growth and output in the form of biomass. Several mushrooms have simple growth patterns and growing nature, as they are able to grow on agricultural and other cellulosic industrial waste products. Many researchers have effectively and economically grown them on different solid substrates including agrowastes (Kausar and Zafar, 1999; Khan, 1982; Jaindak and Kapoor, 1976). By cultivating mushrooms suitably, these wastes can be recycled into food and the environment may be rendered less endangered by their pollution.

In Pakistan, the awareness of growing mushrooms at the cottage industry levels has increased many-folds during the last few years. It will not only help in bridging the existing protein gap, but is also expected to earn a fair amount of foreign exchange through their export. Mycelium of *Pleurotus* species has been previously grown successfully on agrowastes (Kausar *et al.*, 2003; Kausar and Zafar, 2002). The present study was carried out to determine the effect of different carbon sources, such as agrowastes in powder form, the extract of these agrowastes, and the incorporation of various inorganic/bioorganic nitrogen sources on the growth of *Pleurotus sajor-caju* in submerged culture conditions.

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Materials and Methods

Source and maintenance of stock culture. The culture of *Pleurotus sajor-caju* was obtained through the courtesy of Dr. Ralph H. Kurtzman Jr., U.S. Department of Agriculture, West Regional Research Centre, Berkley, California, USA. The stock culture was maintained on standard malt extract agar (MEA) medium in test tube slants at 3-5 °C. The inoculum was grown on MEA medium in 90 mm petri plates incubated at 25±0.5 °C, and the fungus colony was allowed to grow till the plates were completely covered with mycelial growth.

Shake flask cultures. Different carbon sources, grounded powder/sawdusts of various plant wastes, extract of these plant waste materials, and inorganic and natural (bioorganic) nitrogen sources were used as the substrates for shake flask fermentation.

In one set of experiments, 50 ml of commercially prepared potato dextrose medium (Oxoid, England) was supplemented separetely with 1 g each of glucose, sucrose, sorbitol, maltose, starch or molasses in separate 250-ml Erlenmeyer flasks. pH of each supplemented substrate medium was adjusted at 6.1. In the second set of experiments, various plant wastes, such as cotton sticks, wheat straw, wheat spikes, sawdust of 'shisham' (*Dalbergia sissoo*) and 'kikar' (*Acacia arabica*) were ground to 100 mesh size (BSS) in an electric grinder. Two gram of each material and 1 g glucose were added to 50 ml

distilled water in 250-ml Earlenmeyer flasks. In the third set of experiments, 2 g of each plant waste, as was used in the second set of experiments, were taken separately in about 50 ml of distilled water, boiled for 10 min and filtered through cloth. One gram of glucose was added to it and the volume was adjusted to 50 ml in each case. No amendment of carbon and nitrogen sources comprised the control. In the fourth experiment different inorganic and natural nitrogen sources, which included ammonium sulphate, ammonium nitrate, urea, corn gluten meal, mustard seed meal, leaf protein concentrate of alfalfa, along with 1 g glucose, were added separately to 50 ml water in each 250-ml Earlenmeyer flask.

pH of each variation of the culture medium was noted and adjusted to 6.1. The flasks were plugged and sterilized at 15 psi for 15 min. Four inoculum plugs (6 mm) were transferred from petri plate cultures grown seperately to the fermentation medium in the flasks and the inoculated flasks were shaken in a rotary shaker at 250 rpm at 25±0.5 °C for five days (with and without light). pH of the medium was noted at the termination of fermentation period. Mycelial biomass, in the form of pellets, was separated by filtration on preweighed and dried Whatman filter paper No. 4 and dried overnight at 80 °C, followed by weighing to calculate its yield (g/l).

Biomass determination. The dry cell mass was gravimetrically determined by using 80 mesh stainless steel sieve discs (30 mm dia) placed onto a porcelain Buckner funnel. 50 ml of the fermented broth, containing the fungal biomass and insoluble particles of non-consumed plant wastes, was filtered through the stainless steel sieve disc to retain thick biomass. The strained biomass was thoroughly washed with warm (40 °C) and acidified distilled water to remove the adhered insoluble substrate particles, and the organic and inorganic contents of the fermentation medium. The biomass was quantified gravimetrically according to the method described by Trinci (1969).

Proximate composition of biomass. Mycelial biomass was ground to 100 mesh size. The proximate composition of the biomass, namely, moisture, crude proteins, fat, ash and crude fibre, was determined according to AOAC (2000). Nitrogen free extract (NFE) of the samples was calculated as below:

NFE (%) = 100 - (moisture + proteins + fat + ash + crude fibre)

Statistical analysis. The data were statistically evaluated using analysis of variance based on completely randomized design and the difference in the mean value was tested by Duncan's multiple range test (Steel *et al.,* 1996).

Results and Discussion

The effect of different carbon sources on the biomass production of *Pleurotus sajor-caju* in submerged fermentation is shown in Table 1. Biomass production varied from 5.50 to 12.85 g/l. Maximum and minimum being when molasses and sucrose were used as the carbon sources, respectively. Biomass production in the presence of starch (10.07 g/l) and maltose (11.13 g/l) was in agreement with the findings of Nair and Kaul (1988) and Sakamoto *et al*. (1978). Significantly higher (p = 0.01) biomass production in the presence of molasses as the carbon source might be due to the presence of some additional growth promoting factors such as minerals and vitamins (Hubble, 1984). Changes in pH (except when starch and sucrose were supplemented in the medium), and crude proteins showed non-significant differences when different carbon sources were used.

Biomass production of *P. sajor-caju* was significantly higher (10.08 g/l) when powder of wheat spike was added to the fermentation medium. Other sawdust/powdered plant wastes (cotton sticks, cotton locules, 'shisham', 'kikar' and wheat straw) produced 4.73, 4.80, 2.20, 3.15 and 8.50 g/l fungal biomass, respectively (Table 2). It is evident that

Table 1. The effect of different carbon sources on the biomass and crude protein production of *Pleurotus sajor-caju*

Carbon source (2%)	Initial pH of the medium	Final pH of the medium^{+}	Biomass weight (g/l)	Crude protein (%)
Molasses	5.42	6.50	12.85**	20.81
Starch	6.01	6.88	10.07**	19.75
Maltose	6.14	6.37	11.13**	20.87
Sorbitol	6.19	6.34	8.64*	19.50
Sucrose	6.12	7.32	5.50	21.01
Glucose	6.20	6.46	6.16^{NS}	21.5

** = highly significant; * = significant; NS = non-significant with respect to mean value; + = at the termination of fermentation period after 5 days

Table 2. The effect of different plant waste powders and sawdust on the biomass and crude protein production of *Pleurotus sajor-caju*

Plant waste	Initial pH of the medium	Final pH of the medium[+]	Biomass weight (g/l)	Crude protein (%)
Cotton sticks powder	7.18	5.35	4.73[*]	20.05
Cotton locules powder	7.58	4.21	4.80[*]	21.30
'Shisham' wood sawdust	5.21	2.28	2.02	19.95
'Kikar' wood sawdust	6.53	3.44	3.15[NS]	20.95
Wheat straw powder	6.38	3.41	8.54[**]	21.35
Wheat spikes powder	6.48	3.53	10.08[**]	19.85

** = highly significant; * = significant; NS = non-significant with respect to mean value; + = at the termination of fermentation period after 5 days

Table 3. The effect of extracts of different plant wastes on the biomass and crude protein production of *Pleurotus sajor-caju*

Plant waste extract used	Initial pH of the medium	Final pH of the medium [+]	Biomass weight (g/l)	Crude protein (%)
Cotton sticks	7.15	6.70	7.61[*]	20.01
Cotton locules	7.55	4.21	8.25[**]	20.38
Sawdust of 'shisham'	5.56	4.44	3.51	19.35
Sawdust of 'kikar'	6.21	5.90	5.46[*]	20.85
Wheat straw	6.97	5.35	9.78[**]	21.05
Wheat spikes	6.62	4.93	12.35[**]	19.75

** = highly significant; * = significant; + = at the termination of fermentation period of 5 days

wheat spike powder was the best for promoting mycelial growth of *P. sajor-caju*. Changes in the pH of fermentation medium varied from 3.41 to 5.35. The lower pH values, 3.44, 3.41 and 3.53, indicated that polysaccharides of 'kikar', wheat straw and wheat spikes were easily biodegraded to monosaccharides and then oxidized to carboxylic acids. Crude protein contents (19.85-21.35%) of the biomass produced on various sources showed non-significant differences. Addition of extracts of sawdust and powder of the six agrowastes studied, produced 3.51 to 12.35 g/l of biomass, maximum being in the presence of the extract of wheat spikes (12.35g/l) followed by wheat straw as 9.78 g/l (Table 3). Similar observations have been reported by Schiesser *et al.* (1989) with wheat straw. Comparatively higher production in the presence of extracts of sawdust and powders of plant wastes clearly indicated better availability of polysaccharides and other growth promoting ingredients in agrowaste materials than in the case of sawdusts of 'kikar' and 'shisham' as such, in which these are present in complex forms. The crude protein contents in both cases showed non-significant differences (Tables 2 and 3).

The effect of supplementing inorganic and natural nitrogen sources on biomass production of *P. sajor-caju* varied from 8.15 to 13.92 g/l (Table 4). Maximum biomass production was observed in the presence of corn gluten meal (13.92 g/l), followed by leaf protein concentrate (12.24 g/l), showing non-significant differences. The lower biomass production in the presence of ammonium sulphate (8.15 g/l) and ammonium nitrate (10.58 g/l) indicated their lower growth promoting quality as compared to the natural nitrogen sources. Urea and mustard seed meal (MSM) inhibited biomass production, which might be due to the liberation of ammonia gas by decomposition of urea and the presence of toxic factors, such as glucosinolates in MSM (Niazi and Shah, 1988). Similar observations have been reported by Kausar (1988).

The proximate composition of the mycelium biomass of *P. sajor-caju* is presented in Table 5, which shows that it contained 21.18% crude protein, 1.55% fat, 3.59% ash, 2.32% crude fibre, and 63.48% nitrogen free extract (NFE). Proteins, fat, ash and crude fibre contents of the mycelium biomass were in line with that reported for *P. ostreatus*, but significantly lower ($p = 0.01$) than that of the sporophores of *P. sajor-caju* grown on the solid substrate of rice straw, whereas the NFE contents were higher (Kausar and Iqbal, 1994).

Table 4. The effect of different nitrogen sources on the biomass and crude protein production of *Pleurotus sajor-caju*

Nitrogen sources[+]	Biomass weight (g/l)	Crude protein (%)
Ammonium sulphate	8.15	20.80
Ammonium nitrate	10.58[*]	21.25
Corn gluten meal	13.92[**]	20.18
Leaf protein concentrate	12.24[**]	21.87

+ = 1% nitrogen level; pH of the medium was adjusted to 6.1; ** = highly significant; * = significant

Table 5. Proximate composition of *Pleurotus sajor-caju* grown in submerged and surface culture medium

Constituents	Submerged culture medium (%)	Solid state medium[+] (%)
Moisture	8.28	10.25
Crude protein	21.18	30.50
Fat	1.55	1.81
Ash	3.59	12.32
Crude fibre	2.32	11.30
Nitrogen free extract (NFE)	63.48	34.07

+ = Kausar (1988)

It is concluded that fermentation medium containing extracts of wheat spikes and corn gluten meal are most appropriate for biomass production of *P. sajor-caju* in submerged culture fermentation.

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