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# Addition of molasses, corn steep liquor, and rice polish as economical sources to enhance the fungal biomass production of wheat straw by *Arachniotus* sp.

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**Abstract:** This study was planned to see the additive effects of various industrial residues including molasses, rice polish, and corn steep liquor for the bioprocessing of wheat straw (WS) through solid-state fermentation (SSF) using *Arachniotus* sp. as a fermenting agent. The performance of fungal-based WS (seed culture) was evaluated in terms of the favorable changes in crude protein (CP) contents. WS (5 g) was mixed with our selected carbon and nitrogen sources in a basal medium containing optimized salt concentrations to attain the desired level of water and nutrients. An increased ($P < 0.0001$) CP value was observed at a 25:1 C:N ratio and maximum fungal protein contents (13.40%) were found by mixing 0.4 mL of molasses, 0.8 g of rice polish, and 3.0 mL of corn steep liquor in treated WS at a setting time of 48 h in SSF. It was seen that the growth rate of *Arachniotus* sp. was greatly improved by the addition of our selected amounts of carbohydrate and nitrogen sources. Hence, our current results support the use of these economical byproducts for cheap biomass production.

**Key words:** Wheat straw, molasses, rice polish, corn steep liquor, fermentation, *Arachniotus* species

## 1. Introduction

The poor availability of high-quality feed stuff and subsequent feeding of low-quality roughages to farm animals are major obstacles in the growth of the livestock sector in Pakistan. Therefore, nutritional enrichment of available feed resources is an utmost need for the improvement of livestock productivity. The major feed resources for livestock in Pakistan (in percentage share) are crop residues (46%), grazing (27%), cultivated fodders (19%), cereals and legume grains and their byproducts (6%), and oil cakes, meals, and animal protein (2%) (1). Among these, crop residues are emerging as dominant and abundantly available feed ingredients for sustainable crop-livestock systems. It is well known that crop residue such as wheat straw (WS) has a low feeding value due to its depleted protein and energy content and therefore results in poor animal intake (2,3).

To improve the nutritive quality of WS, biotechnological approaches like the use of more suitable microbes have been employed (4). These fungal species grow on moist substrates under aerobic conditions by solid-state fermentation (SSF) and have the ability to degrade fiber contents (5). The growth and development of these microbes depend upon macroelements such as carbon, hydrogen, oxygen, sulfur, phosphorus, and nitrogen, which are the components of carbohydrates, nucleic acids, and proteins. Although used as substrate for fungal growth, WS has inherently poor levels of these macroelements; therefore, supplementary addition of carbon and nitrogen sources is required for fungal mycelial growth, spore yield, and thus commercial biomass production (6).

Rice polish is a supplementary product of rice milling that is also used as a cheap source of protein, energy, vitamins, and minerals and could also be a substrate for fungi growth. Moreover, it contains a high concentration of amino acids, particularly lysine and methionine, compared to other cereals like corn and wheat (7). Rice polish contains

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as many total digestible nutrients as maize. Similar to rice polish, other economical sources like molasses and corn steep liquor (CSL) could also be used to enhance fungal biomass protein production as a cost-effective carbon and nitrogen source. Both of these ingredients are available in slurry liquid form and are widely present in the market. They are rich sources of energy and protein and are low-cost byproducts of the corn and sugar industries compared to other sources such as sucrose, glucose, and urea (8). CSL is composed of carbohydrates, peptides, essential amino acids, minerals, vitamins, and an abundance of unidentified organic and inorganic compounds (2). Enhancement in biomass protein production could be possible by using *Arachniotus* sp. and the additive effects of molasses and fermentable sugars of CSL. Hence, this study aimed to identify the cumulative effects of cheap industrial sources like molasses, CSL, and rice polish as carbon and nitrogen sources in WS via SSF processes using *Arachniotus* sp. to obtain maximum production of fungal biomass.

## 2. Materials and methods

### 2.1. Material collection

Fresh WS was procured from the Dairy Animal Training and Research Center, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, Pakistan. Molasses and CSL were purchased from Pattoki Sugar Mills Ltd. and Rafhan Maize Products, respectively. The purchased products were of quality grade and free from any fungal or bacterial impurities.

### 2.2. Chemical analysis of experimental material

Before the start of the experiments, fresh WS, CSL, rice polish, and molasses were subjected to proximate analysis on the basis of dry matter, crude protein (CP), crude fiber, ash contents, and nitrogen-free extract (9). The composition of CSL (DM = 50, CP = 35, NDF = 0, ADF = 0, ADL = 0, ash = 10, and ME = 1.8) and molasses (DM = 74, CP = 02, NDF = 0.4, ADF = 0, ADL = 0, ash = 13, and ME = 2.78) was previously analyzed (10).

### 2.3. Organism, inoculation, and culture conditions

A certified pure culture of *Arachniotus* sp. was obtained from the stock culture of the National Institute for Biotechnology and Genetic Engineering in Faisalabad and maintained on Czapek's agar slants (11). This study was conducted at the Buffalo Research Institute in Pattoki. Inoculum was prepared using fungus raised in a sporulation medium and the spores of *Arachniotus* sp. were transferred aseptically to three autoclaved conical flasks of 250 mL containing 25 mL of inoculum medium. The pH of the medium was adjusted at 4.00 ± 0.2 with 1 N $H_2SO_4$. The flasks were incubated in the orbital shaker for 72 h at 28 °C and an agitation rate of 100–120 rpm. Concentration of *Arachniotus* sp. spores in the inoculum was adjusted to $10^6$–$10^7$ spores/mL by diluting the suspension with sterile distilled water. A homogeneous suspension of the organism, freshly prepared for each experiment, was used as an inoculum for the growth medium to investigate the various fermentation conditions.

### 2.4. Solid-state fermentation

The used WS (5 g) was added into duplicate Erlenmeyer flasks as SSF substrate and adjusted at a substrate-to-water ratio of 1:2 on the 4th day of incubation at 28 °C. The different salt concentrations in the growth medium were as follows: $MgSO_4.7H_2O$ (0.050%), $CaCl_2$ (0.075%), $KH_2PO_4$ (0.150%), and urea (0.15%). Inoculum (1 mL) of the spore suspension ($10^6$–$10^7$ spores/mL) of *Arachniotus* sp. was added to the growth medium of each flask to obtain the maximum fungal protein. Crude protein contents of samples were tested according to the AOAC (9).

### 2.5. Optimization of carbon-to-nitrogen ratios

Different C:N ratios, i.e. 0:1, 5:1, 10:1, 15:1, 20:1, 25:1, and 30:1, were tested to determine the optimum proportion of carbon (in the form of carbohydrate) required by the fermenting agent to convert the nitrogen into crude protein for maximum biomass protein production under predetermined optimum conditions.

### 2.6. Determination of optimum level and setting time for the addition of molasses

For the addition of molasses in the growth medium during fermentation, five levels were tested, i.e. 0, 0.2, 0.4, 0.6, and 0.8 mL/5 g of substrate, to obtain maximum biomass protein production. These were harvested after 96 h under predetermined culture conditions. The optimum level of molasses was determined for use in the subsequent experiments.

Once the C:N ratio and optimum level of molasses were determined, the optimum incubation time at which the molasses would be added during the fermentation process was explored. For this purpose, three times were tested, i.e. 48 h, 60 h, and 72 h, and fermentation was eventually terminated at 96 h. The optimum incubation time was selected and used in the subsequent experiments.

### 2.7. Addition of rice polish

Five levels of rice polish were tested, namely 0, 0.2, 0.4, 0.6, and 0.8 g/5 g of substrate, to obtain maximum biomass protein production and harvested after a predetermined fermentation period. The optimum level of rice polish was used in subsequent experiments.

### 2.8. Determination of optimum level and setting time for the addition of corn steep liquor

For the addition of CSL in the growth medium during fermentation, six levels were tested, i.e. 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL/5 g of substrate, in fermentation medium under predetermined optimum fermentation conditions

to obtain the maximum yield of biomass protein. Three incubation times were then assessed, namely 48 h, 60 h, and 72 h, and all flasks were harvested after 96 h of fermentation. The optimum incubation time was selected and used in the subsequent experiments.

### 2.9. Statistical analysis

The collected data were analyzed according to a completely randomized design using the general linear models procedure of SAS software (12) and were presented as mean ± root mean square error (RMSE). Treatments were compared with linear and quadratic polynomial contrast. The level of significance was set at $P < 0.05$.

## 3. Results and discussion

Maximum fungal protein contents were found in treated WS by optimizing the concentration levels of $MgSO_4.7H_2O$, $CaCl_2$, $KH_2PO_4$, and urea at 0.05%, 0.075%, 0.15%, and 0.15%, respectively, during 4 days of incubation at 28 °C (13).

### 3.1. Effects of various C:N ratios on fungal biomass production

The C:N ratio is an important factor that regulates the economics of biological processes. Supplementation of carbon and nitrogen is required as important nutrients for fungal growth that are used for microbial metabolism during the fermentation process. Optimum concentrations of these nutrients are required to increase growth performance and fungal biomass. Hence, it is necessary to maintain an accurate composition of growth media for an effective fermentation process and to keep the C:N ratio within the desired range. A linear significant progressive increase in CP contents ($P < 0.001$) was observed with increasing levels of C:N ratio, i.e. 0:1 (3.94%), 5:1 (3.99%), 10:1 (4.38%), 15:1 (4.65%), 20:1 (4.70%), and 25:1 (4.81%), and contents decreased at a 30:1 (4.59%) C:N ratio (Table). Therefore, a maximum level of CP percentage (4.81%) was observed at the 25:1 C:N ratio. A significant enhancement in microbial biomass protein production was previously observed at a 30:1 C:N ratio in 6% (w/v) corn stover using sequential culture fermentation of *Arachniotus* sp. and *Candida utilis* (14). Hence, determination of the optimal C:N ratio is key in harvesting maximum microbial biomass protein (15).

### 3.2. Effects of adding molasses for maximum biomass production

Molasses could be favorably used as a cheaper source of carbohydrates in fungal biomass protein production instead of sucrose and glucose. Cane molasses is commonly available on the market. It is a byproduct of sugar manufacturing that has been used as a carbon source for the enrichment of substrates and economical microbial biomass production during SSF by using *Arachniotus* sp. (16,17). An optimized level of cane molasses was previously found to be 1.5% for a maximum level of 26.25% CP content from the filtered pressed cake of sugar cane (18). Similarly, 1% molasses supplementation was

**Table.** Production of biomass protein from wheat straw at different levels of C:N ratio, molasses, rice polish, and CSL and addition of molasses and CSL at different incubation hours. All values of protein contents (%) are mean ± RMSE. Data represent the average of two samples, each analyzed in duplicate. Significantly ($P < 0.05$) higher CP values within the treatment group are represented in bold. Root mean square error (RMSE) and linear (L) effects of different C:N ratios, molasses, rice polish, and CSL on CP contents (%) of biomass. Quadratic (Q) effect of C:N ratios, molasses, rice polish, and CSL on CP contents (%) of biomass. Means with different superscripted letters represent a significant difference between the treatment groups ($P < 0.05$). The level of significance was set at $P < 0.05$.

| | Parameter | | | | | | | | | Statistics | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Carbon-to-nitrogen ratio | 0:1 | 5:1 | 10:1 | 15:1 | 20:1 | 25:1 | 30:1 | RMSE | P-value | L | Q |
| | Crude protein (%) | 3.94 | 3.99 | 4.38 | 4.65 | 4.70 | **4.81** | 4.59 | 0.181 | <0.0001 | <0.0001 | 0.7509 |
| 2. | Levels of molasses (mL) | 0 | 0.2 | 0.4 | 0.6 | 0.8 | -- | -- | RMSE | P-value | L | Q |
| | Crude protein (%) | 4.70 | 4.76 | **4.92** | 4.65 | 4.43 | -- | -- | 0.179 | 0.022 | 0.037 | 0.010 |
| 3. | Rice polish (g) | 0 | 0.2 | 0.4 | 0.6 | 0.8 | -- | -- | RMSE | P-value | L | Q |
| | Crude protein (%) | 4.92 | 6.13 | 6.29 | 6.51 | **7.60** | -- | -- | 0.205 | <0.0001 | <0.0001 | 0.675 |
| 4. | Levels of CSL (mL) | 0 | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | 3.0 | RMSE | P-value | L | Q |
| | Crude protein (%) | 7.55 | 8.86 | 9.90 | 10.93 | 11.81 | 12.14 | **13.45** | 0.183 | <0.0001 | 0.183 | <0.000 |
| | Setting time (h) for molasses | 0 h | 48 h | 60 h | 72 h | -- | -- | -- | RMSE | P-value | | |
| 5. | Crude protein (%) | $4.70^{ab}$ | **$4.98^{a}$** | $4.87^{ab}$ | $4.59^{b}$ | -- | -- | -- | 0.133 | 0.007 | | |
| | Setting time (h) for CSL | 0 h | 48 h | 60 h | 72 h | -- | -- | -- | RMSE | P-value | | |
| 6. | Crude protein (%) | $7.65^{c}$ | **$13.40^{a}$** | $12.52^{b}$ | $12.42^{b}$ | -- | -- | -- | 0.221 | <0.0001 | | |

found to be optimum for protein enrichment of rice polish under SSF with *Arachniotus* sp., wherein the true protein of rice polish was increased from 10.93% to 17.13% (19). Moreover, a 1-mL molasses level was found to be optimum for maximum biomass production in a mixed culture of *Arachniotus* sp. and *Candida utilis* as fermenting agents carrying out SSF of 5 g of rice bran (20). Our results revealed that biomass CP contents (4.92%) were higher at a 0.4-mL level of molasses during SSF of WS with *Arachniotus* sp. while the CP level decreased with further addition of molasses from 0.6 mL (CP: 4.65%) to 0.8 mL (CP: 4.43%) (Table). The decrease in CP contents could be the result of reverse osmosis that might be due to the higher sugar and mineral contents in molasses or perhaps due to lower enzyme production with the addition of molasses at higher concentrations (21). Our results are therefore in agreement with previous findings and showed the potential use of molasses as a source of carbohydrates for biomass production from a cheap substrate like WS.

### 3.3. Effects of setting time for the addition of molasses in growth medium for maximum biomass production

Results revealed that biomass protein (4.98%) was higher ($P < 0.001$) when molasses was added to the growth medium at 48 h of the fermentation process (Table). For the addition of 1% molasses, the setting time was also previously found to be 48 h for maximum yield of biomass protein using rice polish with *Arachniotus* sp. (19) or its mixed culture with *C. utilis* using rice bran as a substrate in SSF (14).

### 3.4. Influence of rice polish addition on biomass protein production

The effects of adding rice polish at 48 h for the growth of *Arachniotus* sp. was determined under predetermined conditions. For this purpose, five levels of rice polish were tested to obtain maximum production of biomass protein and harvested under predetermined fermentation conditions. Among the five levels tested (0, 0.2, 0.4, 0.6, and 0.8 g/5 g of substrate), maximum biomass protein production (7.60%) was achieved when rice polish was added at 0.8 g/5 g of substrate to the growth medium (Table). Similarly, rice bran was found to be a more suitable substrate for *Aspergillus niger* at a 6% w/v substrate concentration (22). This could be due to its ability to support fermentation activity owing to the presence of starch, vitamin B, and other mineral components. Moreover, 1% rice bran deoiled cake was used as a substrate for *Aspergillus oryzae* and *Trichoderma reesei* for the production of enzymes at an incubation period of 5 days, which demonstrated the technical feasibility of this substrate for optimal enzyme production (23). Similarly, the addition of rice polish also resulted in high (17.33%) biomass production using *Arachniotus* sp. (24). The divergence among the results might be due to the difference in substrate and type of fermentative organism resulting in a different tolerance for minerals in the growth medium.

### 3.5. Effect of corn steep liquor supplementation on biomass production

CLS in nitrogen sources is a widely available, low-cost byproduct of the corn industry. It is the least expensive nitrogen source and would be beneficial for the production of cheap fungal biomass. Maximum production of CP (13.45%) was realized when the CSL level was maintained at 3 mL (Table). A significant ($P < 0.0001$) quadratic increasing trend was observed in the CP percentage at varying levels of CSL, i.e. 0 mL (7.55%), 0.5 mL (8.86%), 1.0 mL (9.90%), 1.5 mL (10.93%), 2.0 mL (11.81%), 2.5 mL (12.14%), and 3.0 mL (13.45%). Similar findings were observed in a previous study in which an increasing trend in biomass protein production was observed from 0.5% to 2.0% CSL by using filter press cake (mud) as the substrate (18). Additionally, rice polish resulted in high biomass production with the addition of 5% CSL using *Arachniotus* sp. (24). This difference might again be due to the difference in the type of substrate and the mode of SSF adopted.

### 3.6. Effect of setting time for the addition of corn steep liquor for maximum biomass production

Among the three tested times of incubation (48 h, 60 h, and 72 h), results revealed that biomass protein (13.40%) was improved at 48 h ($P < 0.0001$) and then declined from 60 h (12.52%) to 72 h (12.41%) (Table). These results are in accordance with previous findings, whereby maximum biomass production was observed at a setting time of 48 h for CSL and then dropped at 60 h and 72 h using rice polish under SSF with *Arachniotus* sp. (19). This means that early supplementation supported growth while the addition of CSL at a later stage might be fruitless due to the exhaustion of the substrate.

The current results revealed that optimized conditions such as C:N ratio (25:1), setting time (48 h), level of molasses (0.4 mL), level of rice polish (0.8 g), and setting time (48 h) and level (3.0 mL) of CSL in growth medium led to a maximum fungal protein yield of 13.40% from WS. These results demonstrate the potential for molasses and CSL as sources of cheap carbohydrate and nitrogen and thus support the use of these economical byproducts for cheap biomass production from WS at the industrial level.

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