SINGLE CELL PROTEIN PRODUCTION BY PENICILLIUM JAVANICUM FROM PRETREATED RICE HUSK

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SUMMARY: The growth of a strain of Penicillium javanicum on pretreated rice husk was studied by batchwise fermentation in shake flasks. The rice husk was pretreated with various concentrations of acids/alkalis and enzyme. It was observed that the growth on perchloric acid treated rice husk was higher. However nitrogen and protein content was found higher in fungal biomass when sulphuric acid treated rice husk was used as a substrate for the growth of Penicillium javanicum.

Key Words: Penicillium javanicum.

INTRODUCTION

Most of the developing countries of the world have been facing malnutrition problem. The deficiency of protein in human food and animal feed is well recognized due to the rapid growth of population. It has been reported that in Pakistan the protein gap would continue to increase unless well planned measures are adopted to handle the situation (1). It is therefore, important to increase protein production by utilizing all the available ways and means. The increasing world demand for food and feed protein spurred the search for non-conventional protein sources to supplement the available protein source. A great deal of interest has been focused on the potential of agricultural wastes to microbial protein or single cell protein. The impetus behind single cell protein production lies partly in the need for more protein and partly in the commercial increase in the economic advantages gained by substitution of microbial protein for the convantional protein supplements used in livestock feeding.

Several studies have involved various fungal species in producing single cell protein from waste celluloses and fungal mycelial biomass is an acceptable source of edible protein (2-5). Rice husk is a paddy field waste and contains minerals (ash matter), Carbohydrate (cellulose, non-cellulose), nitrogen and lipid. The object of the present work is to examine the efficiency of pretreated (Chemical and enzymatic) rice husk as a medium for the growth of *Penicillium javanicum* and production of single cell protein.

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MATERIALS AND METHODS

Strain: Penicillium javanicum was isolated and purified from *Lawsonia inermis* leaves samples. The stock culture was maintained on agar slants, containing (gL^{-1}) dextrose 20.0; peptone 10.0; agar 20.0 and distilled water. The ingredients were thoroughly mixed and kept in culture tubes sterilized at 1.5 kg/cm^2 for 20 minutes. The sterilized slants were inoculated with *Penicillium javanicum* and incubated at 27°C to obtain luxuriant growth.

Inoculum: A spore suspension was prepared by adding sterile water to stock culture to get $80x10^6$ spores/ml.

Preparation of substrate from rice husk: Rice husk was used as a substrate for the growth of microorganism and production of single cell protein. Rice husk (lignocellulosic material) was degradated to simple components (fermentable sugars) by chemical and enzymatic treatment, the details of the procedures are as under.

Chemical treatment: 10.0 G of rice husk was mixed with various concentrations (0.15, 0.30, 0.45, and 0.60 N) of acids (sulphuric acid and perchloric acid) and alkali (sodium hydroxide and ammonium hydroxide). These mixture were frequently agitated on flame for one hour, maintaining the level of solution constant. After cooling at room temperature, the slurry was autoclaved at 1.5 kg/cm^2 for 30 minutes. The autoclaved slurry was cooled at room temperature and unsolubilized rice husk was removed by filtration through suction pump. The filtrate of solubilized rice huck was incorporated into the culture medium as a carbon and energy source. The loss in weight of rice husk sample was determined after drying at 110°C to constant weight.

Enzymatic treatment: 10 G of rice husk was mixed with 800 ml of various concentrations (16.5; 33.0; 49.5 and 66.0 mg) of cellulose (From Trichoderma viride 4.4 units/mg of Sigma Chemicals). The rice husk and enzyme mixture was incubated in shaking water bath (Gallenkamp) at 37°C for over night. The mixture was then autoclaved at 1.5 Kg/cm^2 for 20 minutes. The autoclaved slurry was cooled at room temperature. The unsolubilized rice husk was removed by filtration through suction pump. The filtrate of solubilized rice husk was incorporated into the culture medium as a carbon and energy source. The loss in weight of rice husk sample was determined after at 110°C to constant weight.

Culture medium: Culture medium was used for growth of *Penicillium javanicum* as reported by Burrel *et al.* (6), without altering chemical composition containing the following reagents (per liter solution), Fumaric acid, 2.0 g, $(NH_4)_2SO_4$, 2.5 g; $KH_2PO_4.2H_2$, 1.0 g; $MgSO_4$, 0.5 g; (NH_4) $Fe(SO_4)_2.12H_2O$, 0.2 mg; $ZnSO_4.7H_20$, 0.2 mg; $MnSO_4.H_2O$:, 0.1 mg; thiamine hydro-chloride 0.1 mg and soluble extract of 10 g rice husk. The final volume of culture medium was adjusted to liter and pH was maintained to 6.0.

Cultivation condition: 100 ml of culture media supplemented with rice husk solubilized filtrate was taken in a 250 ml conical flask plugged with cotton wool and autoclaved at 1.5 Kg/cm^2 for 20 minutes. The sterilized media cooled at room temperature were inoculated with 1.0 ml of *Penicillium javanicum* spores. These flasks were incubated in an orbital cooled shaking incubator (Gallenkamp) at 25+ 2 adjusted at 200 rev m^{-1}. The culture broth was separated from mycelium after an interval of 48 hours incubation period by filtration through Whatman No 1 filter paper.

Determination of Ph values: The initial and the final Ph values of culture broth were determined using WPA pH meter (WPA Scientific Instrument).

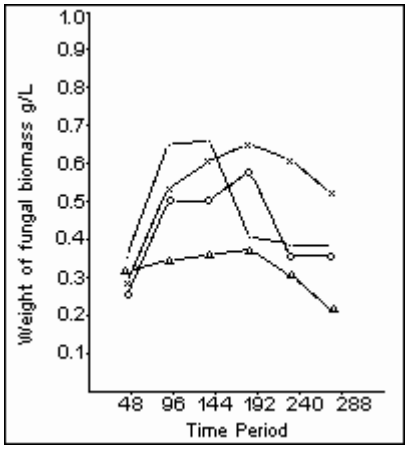
Determination of mycelial biomass: The quantity of the mycelium was noted after washing with distilled water and drying at 105-110°C in a hot oven until a constant weight was obtained.

Determination of crude protein: The total nitrogen content of the dried biomass was estimated by conventional Kjeldhal's method (7). The crude protein values were obtained by multiplying the total nitrogen content by 6.25.

RESULTS AND DISCUSSION

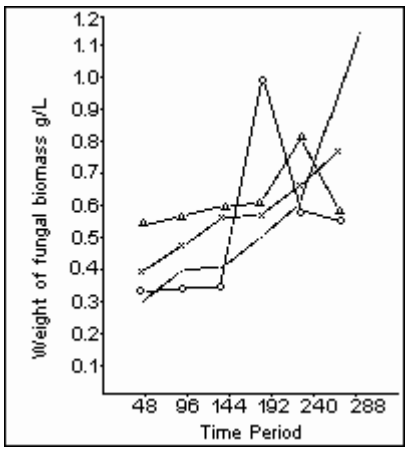
The fungi was obtained in pure culture from *Lawsonia inermis* leave sample. The fungi was related mainly to the generic nomenclature Penicillium known as *Penicillium javanicum*. This fungi is more active when grown on the medium containing sugar as a carbon source for biosynthesis of single cell protein.

Figure 1: Production of single cell protein by *Penicillium javanicum* on sulp- huric acid treated rice husk in bath culture supplemented with mineral medium.



o-o : 0.15 N; .-. : 0.3 N; x-x : 0.45 N; Δ-Δ : 0.6 N.

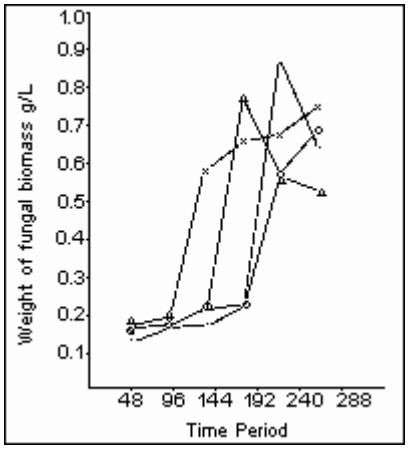
Figure 2 : Production of single cell protein by *Penicillium javanicum* on perchloric acid treated rice husk in bath culture supplemented with mineral medium.



o-o : 0.15 N; .-. : 0.3 N; x-x : 0.45 N; Δ-Δ : 0.6 N.

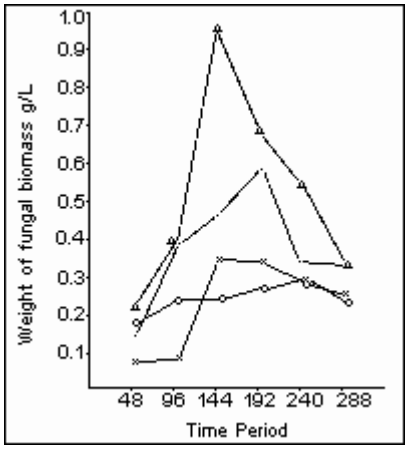
Rice husk is a paddy field waste which contains variable ingredients (mineral matter, carbohydrate, nitrogen and lipid) and these may be used as a carbon and energy source for the growth of fungi in the production of single cell protein. Rice husk was decomposed to fermentable sugars by chemical and enzymatic methods. These fermentable sugars were supplemented with mineral medium for the growth of *Penicillium javanicum* and single cell protein production. Figures 1 and 2 show the single cell protein production behavior with various concentrations (0.15; 0.30; 0.45 and 0.60 N) of sulphuric acid and perchloric

Figure 3: Production of single cell protein by *P. javanicum* on hydroxide acid treated rice husk in bath culture supplemented with mineral medium.



o-o : 0.15 N; .-. : 0.3 N; x-x : 0.45 N; Δ-Δ : 0.6 N.

Figure 4: Production of single cell protein by *P. javanicum* on - sulphuric acid treated rice husk in bath culture supplemented with mineral medium.



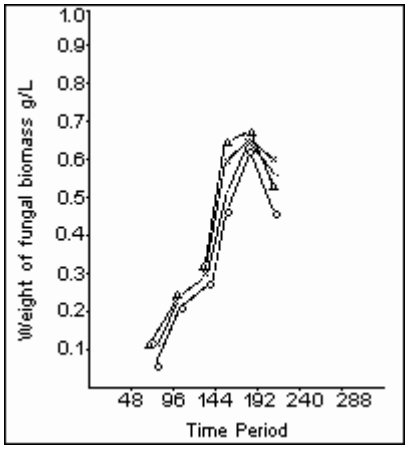
o-o : 0.15 N; .-. : 0.3 N; x-x : 0.45 N; Δ-Δ : 0.6 N.

acid pretreated rice husk medium. Maximum fungal biomass production occurs at 144 and 288 hours on 0.3 N sulphuric acid and perchloric acid pretreated rice husk medium respectively.

Penicillium javanicum was grown in the fermentation medium containing rice husk pretreated with various concentrations (0.15; 0.30; 0.45 and 0.60 N) of ammonium hydroxide and sodium hydroxide as a sole carbon source for the formation of single cell protein. The results obtained are presented in Figures 3 and 4. It is clear that maximum fungal biomass was obtained at 240 and 144 hours on 0.3 N ammonium hydroxide and 0.60 N sodium hydroxide pretreated rice husk medium respectively. It was observed that maximum fungal biomass was achieved at various time periods when *Penicillium javanicum* was grown on chemically (H_2SO_4; $HClO_4$; NH_4OH and NaOH) pretreated rice husk medium. A shift of pH to higher side was also noted during the growth of *Penicillium javanicum* on different pretreated rice husk medium (unpublished data). This finding is in accordance with the observations of other workers in this regard (8-12). They have concluded that medium pH, C/N ratio, N/P ratio, medium composition, environmental and nutritional conditions exert a great influence on the growth of microorganisms as well as protein formations and accumulation in the biomass.

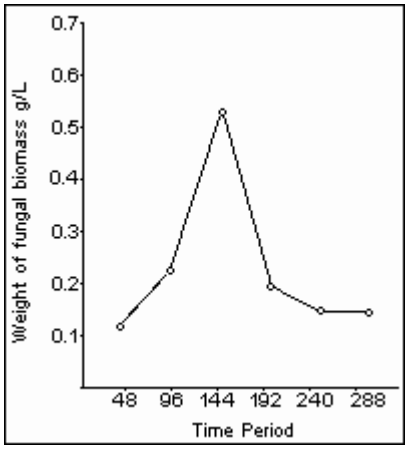
Figure 5 presents the effect of different concentrations (16.5, 33.0, 49.5 and 6.0 mg) of cellulose treated rice husk on the growth and production of single cell protein. It is evident that maximum yield of single cell protein was achieved at 240 hours when rice husk pretreated with 49.9 mg cellulase. It was also recorded that 240 hours time period was found optimum for single cell protein production on various concentrations of cellulose treated rice husk medium. The behavior of single cell protein production by *Penicillium javanicum* on cellulose treated rice husk medium is different in respect of time period than acid or alkali treated rice husk medium. It may be concluded that pure sugars were produced during the decomposition of rice husk with cellulose where as phenols and their derivatives are produced with acids and alkalis.

Figure 5: Production of single cell protein by *P. javanicum* on - cellulose treated rice husk in bath culture supplemented with mineral medium.



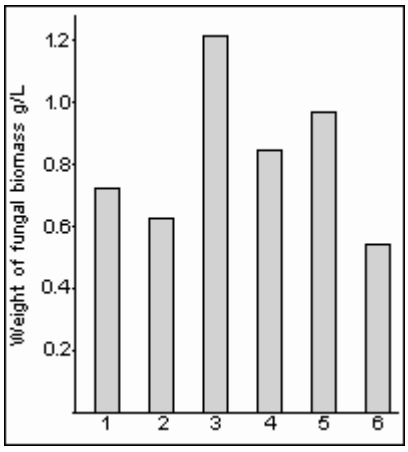
o-o : 0.15 N; .-. : 0.3 N; x-x : 0.45 N; Δ-Δ : 0.6 N.

Figure 6: Production of single cell protein by *Penicillium javanicum* on water treated rice husk in bath culture supplemented with mineral medium.



o-o : 0.15 N; .-. : 0.3 N; x-x : 0.45 N; Δ-Δ : 0.6 N.

Figure 7: Comparison of pretreated rice husk for the maximum production of single cell protein by *Penicillium javanicum* at different time periods.



1) 66 mg cellulose for 240 hours, 2) 0.3 NH_2SO_4 for 144 hours, 3) 0.3N $HCLO_4$ for 288 hours, 4) 0.3 N NH_4oh for 240 hours, 5) 0.6 NaOH for 144 hours, 6) Distilled water for 144 hours.

The effect of water treated rice husk on the growth of *Penicillium javanicum* and production of single cell protein is shown in Figure 6. It is observed that maximum yield of single cell protein was obtained at 144 hours and declined sharply on prolong incubation. The declination in single cell protein production after 144 hours may be due to the exhaustion of carbon source and this is in concurrence with the finding of other workers in case of single cell protein production by microorganisms (13-15).

Table 1: Comparison of protein contents of *Penicillium javanicum* mycelia derived from fermentation of pretreated rice husk using the following culture conditions. Initial pH 6.0, Temperature 26± 2 Co, Fermenter adjusted at 200 rpm.

Substrate	Time (hour)	% of Nitrogen	% of Protein
0.6 NaOH pretreated rice husk	144	0.31	1.94
0.3 NH_4OH pretreated rice husk	240	0.17	1.06
0.3 N H_2SO_4 pretreated rice husk	144	2.92	18.25
0.3 N $HCLO_4$ pretreated rice husk	288	2.68	16.75
Rice husk treated with distilled water	144	0.58	3.62
Rice husk treated with 66 mg cellulase	240	0.43	2.68

The results shown in Figure 7 demonstrate the comparison of single cell protein formation by *Penicillium javanicum* on rice husk pretreated with acid/alkali/enzyme and water. It seems that single cell protein yield is higher in case of perchloric acid treated rice husk medium than sulphuric acid, ammonium hydroxide, sodium hydroxide, cellulose and water.

Table 1 shows the comparison of protein content of single cell protein to *Penicillium javanicum* grown on rice husk pretreated with acid/alkali/enzyme and water. It is observed that protein content (18-25%) is higher in sulphuric acid treated rice husk medium than perchloric acid, ammonium hydroxide, sodium hydroxide, enzyme and water treated rice husk. This observation is in agreement with previous report in case of single cell protein production by Penicillium expansum (16).

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