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Effect of Various Agriculture Wastes and Pure Sugars on the Production of Single Cell Protein by Penicillium Expansum

[1]M. Yakoub Khan and [2]M. Umar Dahot

[1]Institute of Biochemistry University of Sindh, Jamshoro, Pakistan
[2]Institute of Biotechnology and Genetic Engineering,
University of Sindh, Jamshoro, Pakistan

Abstract: The aim of our study was to see the effect of agricultural wastes (sugar cane baggase, orange peel, wheat straw), some plant seeds (cotton seeds, cajanus cajan seeds, and castor been), pure sugars (mannose, glucose, fructose, galactose, maltose, lactose, lactose, sucrose, starch, cellulose) along with treated rice husk medium were investigated on the production of single cell protein by *Penicillium expansum* when culture was incubated at 28 ± 2°C for 240 hours with initial pH 4.0. Initially 0.6N H_2SO_4 treated agricultural wastes as carbon source including, sugar cane baggase, orange peel, wheat straw, cotton seeds, cajanus cajan seeds, castor been and rice husk were used alone as carbon source for the production of SCP, the protein content and biomass were (15.53% and 0.3169 g/l), (9.89 % and 0.3142 g/l), (6.48 % and 0.319 g/l), (16.66 % and 0.4205 g/l), (10.94 % and 0.5344 g/l), (11.62 % 7 0.3277 g/l), and (18.25 % and 1.64 g/dl) respectively. The maximum production of protein (18.25%) and biomass (1.64 g/l) was produced when rice husk was used as carbon source for the production of SCP by *Penicillium expansum*. In next experiment acid hdrolysate of rice husk incorporated with various agricultural wastes to obtained higher yield of SCP and protein content by *Penicillium expansum*. Higher protein content (21.36%) and SCP biomass (1.92 g/l) was obtained when acid hydrolysate of rice husk cotton seeds were mixed together. Finally acid treated rice husk was incorporated along with 1.0% pure sugars including mannose, glucose, fructose, galactose, maltose, lactose, lactose, sucrose, starch and cellulose and molasses give rice protein content and biomass were (24.50% and 3.125 g/l), (28.20% and 4.953 g/l), (24.93% and 4.022 g/l), (26.25% and 4.844 g/l), (25.00% and 4.808 g/l),(26.90% and 2.988 g/l), (30.10% and 5.107 g/l), (28.00% and 4.448 g/l), (26.20% and 1.287 g/l) and (25.37% and 4.377 g/l) respectively. The maximum protein content (30.10%) and SCP biomass (5.107g/l) was obtained when acid treated rice husk was incorporated with 1.0% sucrose. Again the above experiment was repeated with different concentrations (1-10 %) of pure sugars along with acid treated rice husk for the production of SCP biomass. The high protein content (30.10%) and biomass (5.758) was produced when I.0% sucrose was used along with acid treated rice husk as carbon source for the production of SCP by *Penicillium expansum* when compared with different concentrations of pure sugars.

Key word: Single cell protein · *Penicillium expansum* · Agricultural wastes and pure sugars

INTRODUCTION

The growing shortage of protein and other protein rich food supplies has stimulated the effort in searching new and alternate source of protein rich food and feed. Single cell protein (SCP) is used as protein sources in human foods and animal feeds [1]. Single cell protein or microbial protein is the dried cells of microorganisms such as algae, bacteria, yeast molds and higher fungi grown in large scale culture systems for use as protein sources in food or feeds [2]. Initially yeast was used for human food, but then research has been directed towards using it as a protein supplement in animal feeds due to acute shortage of both soybean and fish meal in many countries [3-5]. Single cell protein (SCP) was produced in Germany during First World War. The yeast, *Sacchromyces cerevisiae* was grown with molasses as the carbon source and ammonium salts as the nitrogen source for the consumption as protein supplement [6]. Single cell protein produced by culturing different microorganisms [7,8] on different substrates such as whey [9] starch [10], cellulose [11,12], hydrocarbon [13], alcohols [14] and molasses [6].

Pakistan is an agriculture country and more than 50 million tons of agricultural waste is produced annually

Corresponding Author: M. Umar Dahot, Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan

which does not possess economical value. These wastes are disposed of by unconventional process, which creates environmental pollution. The main components of agricultural waste are cellulose, hemicellulose and lignin. These wastes can converted after hydrolysis with acids [15,16], alkali [17,18], cellulase [19,20], and steam [21] into fermentable sugars. Fermentable sugars can be used as a substrate for the growth of microorganisms and production of single cell protein. Agricultural wastes are the most abundant raw material consist cellulose as a major component, which is suitable for the growth of microorganisms and production of SCP biomass. The production of SCP by certain strains of microorganisms in agricultural waste medium under optimum cultural and nutritional conditions increases the protein content of SCP, making it an ideal supplement for animal and poultry feed. Several studies has been conducted by using agricultural waste as a substrate including Mango Kennel Meal [22], Hyacinth Bean (Lablab purpureus) [23], Ipomoea asarifolia Leaf Meal [24], Breadfruit (Treculia africana) hulls [25], Papaya (Carica papaya L.) [26], Rice straw [27], waste paper [28] and Rice bran [29] to produce single cell protein.

The purpose of this study was to increase the single cell protein biomass and protein content by using of rice husk as a carbon source alone and along with other agricultural wastes and pure sugars by *Penicillium expansum* using shake flask submerged fermentation system. Rice husk, sugar cane baggase, orange peel, wheat straw, cotton seeds, *Cajanus cajan* seeds and castor been seeds are the most abundant and locally available agricultural wastes which contains variable ingredients such as carbohydrates that may used as carbon source for the growth of fungi in the production of single cell protein. These agricultural wastes after acid hydrolysis were converted to fermentable sugars and were supplemented with minerals for the growth of *Penicillium expansum* to produce single cell protein biomass.

MATERIALS METHODS

Materials

Substrate: Rice husk of Iri-Pak 6 was used throughout the study and was collected from Larkana Rice Mill, District Larkana Sindh.

Microorganism: *Penicillium expansum* strain CMI 39761 was obtained from the Department of Botany, University of Glasgow, U.K. and was used in this study. The stock culture was maintained on agar slant, containing (G / L) Dextrose: 20; peptone 10; agar; 20 and distilled water. The ingredients were thoroughly mixed and kept in culture tubes and sterilized at 1.5 kg/cm^2 for 20 minutes. The sterilized slants were inoculated with *Penicillium expansum* and incubated at 27°C to obtain luxuriant growth.

Methods: Preparation and degradation of agricultural waste with H_2SO_4 treatment: 10 grams of agricultural waste was mixed with 800 ml of 0.6N H_2SO_4. These mixtures were frequently agitated on flame for one hour, maintaining the level of solution. After cooling at room temperature, the slurry was autoclaved 1.5 Kg/cm^2. The autoclaved slurry was cooled at room temperature and unsulubilized waste was removed by filtration through suction pump. The filtrate of solubilized agricultural waste was incorporated into the culture medium as a carbon source [30].

Culture Medium: Culture medium was used for the growth of Penicillium expansum as reported by Burrel et.al [31], without altering chemical composition.

Cultivation Condition: 100 ml of culture media supplemented with rice husk soluble filtrate alone and along with other agriculture wastes extracts and sugars were taken in 500 ml conical flasks plugged with cotton wool and autoclaved at 1.5Kg/cm^2 for 20 minutes. The sterilized media cooled at room temperature were incubated with 2.0 ml of Penicillium expansum spores 50x10^{6}ml. Pure sugars were sterilized separately and added asceptically. These flasks were incubated in an orbital cooled shaking incubator (Gallenkamp) at 26±2°C adjusted at 200rev/min. The culture broth was separated from mycelium after a desired time period by filtration through Whatman no. 1filter paper. Final pH and extracellular protein were checked from culture broth.

Determination of Final pH Values: The final pH value of the culture broth was determined using WPA pH meter (WPA Scientific Instruments)

Determination of Mycelial Biomass: The quantity of the mycelial biomass was noted after washing with distilled water and drying at 105-110 °C.

Determination of Protein: Nitrogen content of mycelial biomass was determined by Kjeldhal method [32]. The crude protein values were obtained by multiplying the nitrogen content. by 6.25. The extracellular protein content of the culture broth was determined by the method Lowry *el al*., [33] using bovine albumin as a standard.

RESULTS AND DISCUSSION

Rice husk is an agricultural waste, which contains different ingredients as shown in Table 1. Rice husk contains major amount of carbohydrates, small amount of protein, lipid and ash. Carbohydrate, protein and lipid of rice husk supposed to be used as a carbon and nitrogen source for the growth of *Penicillium expansum* and production of mycelial biomass.

Table 2 represents the results of total biomass weight and the percentage of protein of mycelial biomass of *Penicillium expansum* grown on 0.6N H_2SO_4 pretreated agricultural wastes. The higher amount of single cell protein (1.64 g/L) with higher percentage of protein content (18.25%) was produced by *Penicillium expansum* when grown on 0.6N H_2SO_4 pretreated rice husk.

Table 1: Proximate chemical composition of rice husk

Components	Rice husk
Total nitrogen	0.47%
Protein crude (N x 6.25)	2.94%
Lipid (Ether extract)	1.15%
Cellulose	12.88%
Non-cellulose	14.26%
Lignin	46.60%
Ash	15.38%
Silica	12.20%
Sodium	219.47mg/ T.ash
Potassium	112.47 mg/T.ash
Calcium	40.28 mg/T.ash
Magnesium	5.12 mg/T.ash
Phosphorus	159.9mg/T.ash

Table 2: Effect of different agricultural waste material (Pretreated with 0.6N H_2SO_4) as a carbon source on the production of single cell protein by *Penicillium expansum* when cultural grow for 240 hours with initial pH 4.0 at 28 ± 2°C

		Wt. of Biomass	Broth protein	Reducing Sugar	% of protein
Agricultural wastes	Final pH	G/Litre	mg/ml	mg/ml	(N x 6.25)
Sugar cane Baggase	5.13	0.3169	0.32	0.45	15.53
Orange peel	5.03	0.3142	0.40	0.58	09.89
Wheat straw	5.80	0.3190	0.32	0.37	06.48
Cotton seeds	6.80	0.4205	0.24	0.20	16.66
Cajanus cajan seeds	6.02	0.5344	0.35	1.26	10.94
Castor been	6.68	0.3277	0.33	0.085	11.62
Rice husk	7.60	1.64	0.25	0.86	18.25

Table 2: Effect of different agricultural waste material (Pretreated with 0.6N H_2SO_4), along with 0.6N H_2SO_4 pretreated rice husk as a carbon source on the production of single cell protein by *Penicillium expansum* when cultural grow for 240 hours with initial pH 4.0 at 28 ± 2°C

		Wt. of Biomass	Broth protein	Reducing Sugar	% of protein
Agricultural wastes	Final pH	G/Litre	mg/ml	mg/ml	(N x 6.25)
Sugar cane Bagasse	6.24	1.8350	0.38	0.80	20.42
Orange peel	5.53	1.7665	0.44	1.00	20.42
Wheat straw	5.74	1.4620	0.35	0.50	16.57
Cotton seeds	6.92	1.9200	0.28	0.25	21.36
Cajanus cajan seeds	5.69	1.8700	0.37	1.55	20.95
Castor been	6.87	1.7680	0.34	0.12	19.43

Table 3: Effect of pure sugars as a carbon source along with 0.6N H_2SO_4 pretreated rice husk on the production of single cell protein by *Penicillium expansum* when cultural grow for 240 hours with initial pH 4.0 at 28 ± 2°C

		Wt. of Biomass	Broth protein	% of protein
Pure Sugars	Final pH	G/Litre	mg/ml	(N x 6.25)
Mannose	5.10	3.125	0.72	24.50
Glucose	5.42	4.953	0.74	28.20
Fructose	5.52	4.022	1.32	24.93
Galactose	6.94	4.844	0.44	26.25
Maltose	5.44	4.808	0.84	25.00
Lactose	5.17	2.988	1.12	26.90
Sucrose	5.49	5.107	1.20	30.10
Starch	5.77	4.448	0.42	28.00
CM-cellulose	6.64	1.287	0.48	26.20
Molasses	5.70	4.377	2.00	25.37

Table 4: Effect of glucose concentration along with 0.6N H_2SO_4 pretreated rice husk on the production of single cell protein by *Penicillium expansum* when cultural grow for 240 hours with initial pH 4.0 at 28 ± 2°C

		Wt. of Biomass	Broth protein	% of protein
Glucose Concentration used	Final pH	G/Litre	mg/ml	(N x 6.25)
1.0 %	5.5	5.250	0.325	28.20
2.0 %	3.9	6.930	0.360	27.10
3.0 %	3.4	9.170	0.400	26.70
4.0 %	3.3	10.039	0.440	24.60
5.0 %	3.3	11.712	0.500	23.70
6.0 %	3.2	14.350	0.500	17.50
7.0 %	3.1	14.370	0.600	16.60
8.0 %	3.1	12.390	0.630	16.50
9.0 %	3.1	11.720	0.470	16.00
10.0 %	3.1	10.460	0.400	16.00

The results of the effect of 0.6N sulphuric acid pretreated rice husk along with other acid pretreated agricultural wastes on the production of single cell protein by *Penicillium expansum* are given in Table 3. The results clearly indicates that higher amount of mycelial protein biomass (1.92 g/L) and percentage of protein content (21.36) of single cell protein is produced by *Penicillium expansum* when grown on 0.6N H_2SO_4 pretreated rice husk along with cotton seeds.

Table 4 shows the effect of 0.6N H_2SO_4 pretreated rice husk along with 1% pure sugars on the production of single cell protein *Penicillium expansum*. The maximum production of single cell protein (4.953) is achieved when *Penicillium expansum* grows on 0.6N H_2SO_4 pretreated rice husk along with glucose (Table 4). However, higher percentage of protein (30.10 %) was found in single cell protein when *Penicillium expansum* grown on 0.6N H_2SO_4 pretreated rice husk incorporated with sucrose. It is concluded that single cell protein production by fungus depends on the growth substrate or media composition (30,34).

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