Utilization of Saudi Wheat Straw in the Production of Protein-rich Biomass

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Abstract. Wheat straw, a raw material abundantly available in the Kingdom of Saudi Arabia was obtained from western province of the Kingdom and was thoroughly ground to powder form. *Trichoderma viride* was selected as experimental organism to produce protein-rich biomass. Hydrolyzed ground wheat straw gave the highest protein-rich biomass. The potent nitrogen source was found NH_4NO_3 at concentration of 5mg/ml compared to other sources. The microbial biomasses obtained was found to be rich in several biochemical constituents which allowed to be used as animal fodder with cheap cost.

Keywords: Wheat straw, Microbial biomass, *Trichoderma viride*, Animal fodder.

Introduction

Wheat straw (WS), a lignocellulosic material contains cellulose, hemicellulose and lignin, is the major production crop in Saudi Arabia. The rate of production has been estimated over 2.5 million ton per year^[1,2]. The α -cellulose content of wheat straw is an excellent raw material for industrial production that it can be easily hydrolyzed into glucose which is utilized by microorganisms to produce animal fodder^[3]. Wheat straw is comparatively much cheaper substrate, was used instead of costly substrate (pure cellulose or lactose) to produce complete cellulase system. A pan bioreactor was developed for solid-state fermentation (SSF) to yield highly of complete cellulase system compared to that in the liquid-state fermentation (LSF) from WS, when

treated with 4.25% NaOH at 121°C for 1h. and mixed with Mandel's medium^[4]. A complete cellulase system is defined as one in which the ratio of β -glucosidase activity to filter paper activity (FPA) in the enzyme solution is close to 1.0. Mandel (1974) investigations on the mutant of *Trichoderma viride* led to strain QM 9136 which yielded more than 80% glucose using wheat straw as substrate^[5]. Another mutant strain of *T. reesei* MCG 80 proved to be best by producing an optimal cellulase system with wheat straw as substrate. The preparation exhibited a β -glucosidase activity to filter paper activity (FPA) ratio of about 1.0 which indicate a high potential for hydrolysis of cellulose^[6]. Gaspar *et al.* (1997), reported that wheat straw is also the best substrate to produce xylanase complex which is a cellulase free and have an optimal activity at pH 4.6-5.0 and 55-60°C on birchwood xylane^[7]. The components of WS can be hydrolyzed (except lignin) to produce sugars which can then be fermented to produce fuel ethanol. Numerous studies have been published in the last two decades indicating the importance role of wheat straw as raw material for several industrial products^[8-13]. The purpose of the present investigation is to address the utilization of wheat straw which is abundantly available in Saudi Arabia and usually wasted to produce a protein-rich biomass to be used as an animal fodder.

Materials and Methods

Collection of Wheat Straw

Wheat straw was obtained from western province of the Kingdom of Saudi Arabia. It was cut into small pieces and dried in an oven at 90°C. The dry wheat straw were thoroughly ground to powder form to use in further experiments. All chemicals and solvents used in this study were of the purest analytical grade. They were purchased from different local chemical agents available in the market.

Maintenance Medium

The medium contained the following ingredients (g/l): glucose, 10.0; peptone, 2.5; yeast extract 2.5; KH₂PO₄, 1.0; MgSO₄.7H₂O, 0.25; MnSO₄.4H₂O, 0.02; FeSO₄.7H₂O, 0.005; agar, 30.0 in 1000 distilled water. The ingredients were mixed thoroughly and the initial pH was adjusted to 6.0. The medium was poured into test tubes containing 10 ml and plugged with cotton and sterilized at 121°C for 15 min. The slants were inoculated with experimental organisms under aseptic conditions,

and incubated at 30 °C for 72 h to obtain luxuriant growth. The organisms were maintained on a slant and stored at 5°C.

Vegetative Medium

The medium contained the following ingredients (g/l): glucose, 10.0; peptone, 5.0; yeast extract 5.0; KH_2PO_4 , 1.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.25; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.025; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.005; in 1000 distilled water. The initial pH was adjusted to 6.50. Fifty milliliters of the medium was portioned in 250ml Erlenmeyer flasks which cotton plugged and sterilized at 121°C for 20 min. When the flasks attained room temperature, they were inoculated with 2 ml of the vegetative medium contained organisms. The flasks were shaken (200 rpm) at 30°C for 168 h. At the end of the fermentation process, the following parameters were determined, the final pH values, reducing sugars liberated, total biomass and total protein present in biomass.

Experimental Organism

The fermentation medium prepared above was inoculated with two experimental organisms, *Trichoderma viride* NRRL 13680 and *cellulomonas fimi* NRRL B-403. Five Erlenmeyer flasks were used for each organism. The flasks were shaken (200 rpm) at 30°C for *T. viride* and at 37°C for *C. fimi* for 168 h. At the end of the fermentation process, the final pH values, reducing sugars liberated, biomass and total protein in biomass were determined.

Hydrolyzed Ground Wheat Straw as Carbon Source

Two fermentation media were prepared, one contained hydrolyzed and the other non-hydrolyzed ground wheat straw. The media were inoculated with *T. viride* and flasks were shaken (200 rpm) at 30°C for 168 h. The above parameters were determined.

Determination of the Optimum Concentration of Hydrolyzed Wheat Straw

Five different concentrations of hydrolyzed wheat straw (10, 20, 30, 40 and 50g/l) were incorporated in fermentation medium to obtain the optimum concentration. At the end of fermentation process the previous mentioned parameters were determined.

Nitrogen Sources

Ammonium sulphate in concentration of 4.0 g/l of fermentation medium was substituted by an equivalent amount of different nitrogen sources included, NH_4Cl ; KNO_3 , NaNO_3 and NaNO_2 . The fermentation process was carried out as before and the previous parameters were determined.

Determination of Biomass

Cell biomasses of *T. viride* NRRL 13680 were determined at the end of fermentation process. The fermented media was centrifuged at 4000 rpm for 2 min to precipitate the microbial cells of *T. viride*, then, dried at 95 °C till constant weight was obtained ^[14].

Determination of Sugars Liberated in the Medium

Sugars were determined according to the colorimetric method of Somogyi^[15]. A standard curve was constructed using different concentrations of glucose.

Determination of Protein Concentration

The most widely used method for determining protein concentration is Lowry method which was used to determine protein concentration ^[16].

Results and Discussion

The results presented in Table 1 showed that the microorganism *Trichoderma viride* NRRL 13680 was selected for further studies on the basis of the results obtained in Table 1 which indicates that the organism *T. viride* produced higher percentage of protein (24.40) compared to the other organism *Cellulomonas fimi*.

Table 2 showed that the hydrolyzed ground wheat straw as carbon source produced higher protein percentage than unhydrolyzed. When different concentrations of ground wheat straw was used to obtain the optimum concentration as carbon source, the organism *T. viride* produced 30.9 mg/ml of dry cell biomass at 50 mg/ml of ground wheat straw beside the concentration of reduced sugar is the higher at the same concentration (Table 3). The results presented in Table 4 showed that NH_4NO_3 is more suitable than urea and $(\text{NH}_4)_2\text{SO}_4$ as nitrogen source for the growth of *T. viride*, and the optimum concentration was 5.0 mg/ml on the basis of percentage of protein 37.5% (Table 5). The final pH values

for fermented media in each experiment was mainly shifted to be the more acidic side due to the formation of some acidic metabolites namely organic and inorganic acids during the fermentation process. The production of protein-rich biomass by fermentation of wheat straw as carbon source by utilizing organisms decomposing cellulase such as *T. viride* allows agricultural wastes to be utilized economically to produce protein-rich animal fodder from local renewable material and in very cheap cost.

Table 1. Cultivation of *Trichoderma viride* NRRL 13680 and *Cellulomonas fimi* NRRL B-403 on ground wheat straw as carbon source.

Organism	<i>Trichoderma viride</i> NRRL 13680	<i>Cellulomonas fimi</i> NRRL B-403
Final pH value*	3.7	3.8
Sugar (mg/ml)	0.14	1.05
Biomass (mg/mg)	30.0	32.0
Total protein (mg/ml)**	0.244	0.22
% Proteins	24.400	22.00

* The initial pH value of fermentation medium was 6.5.

** (mg/mg) = (mg proteins /mg dry biomass).

Table 2. Cultivation of *Trichoderma viride* NRRL 13680 on hydrolyzed and unhydrolyzed ground wheat straw as carbon source.

Organism	<i>Trichoderma viride</i> NRRL 13680	
Wheat straw	Hydrolyzed	Unhydrolyzed
Final pH value*	5.3	5.5
Sugar (mg/ml)	0.5	0.4
Biomass (mg/mg)	37.0	32.5
Total protein (mg/ml)**	0.325	0.255
% Proteins	32.500	25.500

* The initial pH value of fermentation medium was 6.5.

** (mg/mg) = (mg proteins /mg dry biomass).

The results findings of this study agreed with numerous studies of investigation in the utilization of wheat straw as a good raw material for production of several cheap and economical products. Nabawia *et al.* (1983) found that ensiling straw, treated or untreated, caused a clear improvement in its nutritive value^[17]. Dahot and Noomrio (1996) utilized wheat straw as a carbon and energy source for the growth of *Aspergillus*

fumigatus and production of cellulase^[18]. The high content of cellulose (34%) in wheat straw makes it suitable as raw material for production of bioethanol as fuel instead of petrol which makes the net contribution to the green house effect zero^[19]. Pure vision technology (2004) has produced clean fiber from biomass and agricultural waste materials by converting wheat straw into usable pulp and paper^[20]. Wheat straw and barley straw was used for cattle, sheep, goats fattening after treatment with urea^[21].

Table 3. Cultivation of *Trichoderma viride* NRRL 13680 on different hydrolyzed ground wheat straw concentrations as carbon source.

Hydrolyzed ground wheat straw (mg/ml)	Final pH value*	Reducing sugars (mg/ml)	Dry biomass (mg/ml)	Total Proteins (mg/mg**)	% Proteins
0.0	6.4	0.0	0.0	0.0	0.0
10	4.9	0.50	5.6	0.297	29.7
20	5.2	0.60	10.7	0.303	30.3
30	5.5	0.65	18.5	0.314	31.3
40	5.4	0.60	27.0	0.300	30.0
50	5.4	0.70	30.9	0.254	25.4

* The initial pH value of fermentation medium was 6.5.

** (mg/mg) = (mg proteins /mg dry biomass).

Table 4. Cultivation of *Trichoderma viride* NRRL 13680 on different nitrogen sources on using hydrolyzed ground wheat straw as carbon source.

Nitrogen source*	Final pH value**	Reducing sugars (mg/ml)	Dry biomass (mg/ml)	Total Proteins (mg/mg***)	% Proteins
(NH ₄) ₂ SO ₄	4.5	0.20	18.3	0.335	33.0
NH ₄ NO ₃	4.2	0.22	21.5	0.354	35.4
NH ₄ CL	4.1	0.10	19.5	0.314	31.4
NaNO ₃	7.1	0.17	18.5	0.292	29.2
KNO ₃	7.4	0.21	19.4	0.285	28.5
NaNO ₂	8.7	0.17	18.7	0.291	29.1
Urea	8.7	1.9	21.3	0.344	34.4

* Equivalent amounts of nitrogen sources were added to the fermentation medium.

**The initial pH value of fermentation medium was 6.5.

***(mg/mg) = (mg proteins /mg dry biomass).

Table 5. Cultivation of *Trichoderma viride* NRRL 13680 on different NH_4NO_3 concentrations on using hydrolyzed ground wheat straw as carbon source.

NH_4NO_3 (mg/ml)	Final pH value*	Reducing sugars (mg/ml)	Dry biomass** (mg/ml)	Total Proteins (mg/mg***)	% Proteins
0.0	6.4	0.0	0.0	0.0	0.0
1.0	5.5	0.12	23.3	0.124	12.4
2.0	5.2	0.15	21.7	0.215	21.5
3.0	4.9	0.20	20.8	0.203	30.5
4.0	4.3	0.23	18.5	0.350	35.0
5.0	4.7	0.24	17.5	0.375	37.5
6.0	4.5	0.23	18.4	0.370	37.5
7.0	4.5	0.24	18.2	0.360	36.5

* The initial pH value of fermentation medium was 6.5.

** Dry biomass = Residual wheat straw + microbial biomass.

*** (mg/mg) = (mg proteins /mg dry biomass).

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الاستفادة من قش القمح السعودي في إنتاج البروتين غني الكتلة الحيوية

وديعة بنت صالح بكر

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المستخلص. أخذ بعض قش القمح والمتوفر بكميات كبيرة في المملكة العربية السعودية من المنطقة الغربية وتم طحنه جيدًا حتى أصبح على شكل مسحوق ناعم (بودرة). أخذت تركيزات مختلفة منه لدراسة تأثيرها على نمو فطر *Trichoderma viride* NRRL 13680 وعلى إنتاجها للبروتين وحيد الخلية. وقد أوضحت النتائج أن هناك زيادة بمقدار ٩٪ في الكتلة الحيوية للفطر *T. viride* عن بكتيريا *Cellulomonas fimi* NRRL B-403 وعلى ذلك تم استخدام هذا الفطر. وقد وجد أن استخدام قش القمح المطحون المحلل كمصدر كربوني ينتج كتلة حيوية بمقدار ١٢,١٦٪ أعلى من قش القمح غير المحلل، كما وجد أن المركب النيتروجيني NH_4NO_3 ذو تركيز ٥ جم/مل هو أفضل مصدر نيتروجيني للبكتيريا من المصادر النيتروجينية الأخرى.

وعند تحليل مكونات الكتلة الحيوية الناتجة من استخدام قش القمح المطحون المحلل الذي أدى إلى زيادة ملحوظة في نمو الفطر وتمثيله للسكريات وإنتاجه للبروتين وحيد الخلية. وجد أنها تحتوي على المواد الغذائية الأساسية والتي يمكن استخدامه كعلف حيواني ذو تكلفة مادية قليلة جدًا مقارنةً بأعلاف الأغذية الأخرى.