STUDIES ON BIOCONVERSION VI. RECYCLING BAGASSE TO PROTEIN RICH BIOMASS

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SUMMARY: Media prepared using Molasses treated with potassium ferrocyanide on inoculation with Aspergillus oryzae *yielded 27.4 g biomass and 9.6 g protein / litre broth. Untreated molasses media produced scanty growth and yielded 8 g biomass with 2.7 g protein / litre broth. Fifty gram bagasse pretreated with alkali (Ca0) when added to treated molasses produced 42 g biomass with 15 g protein / litre broth, whereas bagasse pretreated with water yielded 40 g biomass with 11.2 g protein / litre broth on dry weight basis. Nutritive value of bagasse with an initial value of 4% crude protein, enhanced by 22.4% and 30% on treatment with water and alkali and subsequent fermentation using* A. oryzae. *Thus net gain of 18.4% (4.6 fold) and 26% (6.5 fold) of protein on initial protein in bagasse on dry weight basis has been obtained respectively. Pretreatment of bagasse with alkali before fungal inoculation is, therefore, suggested for upgrading biomass protein.*

Key Words: Bagasse, water and alkali treatment, biomass, protein enhancement, aspergillus oryzae.

INTRODUCTION

Pakistan, fourth largest sugar cane producing country, generates annually 5.4 million tones of bagasse (2), eighty percent being used for producing fuel, the remaining bulk (2 million tones approx) containing 50-55% cellulose, can possibly be recycled and put to beneficial use instead of merely burning. Seventy percent of population in Pakistan suffers from protein deficiency and malnutrition. Scarcity of protein rich leguminous grazing sites coupled with feeding cheap and protein deficient materials, result in slow growth and low productivity of farm animals. Feeding high protein diet is quite expensive and, therefore, not feasible. Feeding bagasse residues directly to animal has limitations due to low digestability, and poor protein content. Nutritive value and digestability of bagasse can, however, be significantly improved through microbial degradation. De Menezes (8) converted bagasse into protein using *Myrothecium verrucaria*, *Trichoderma viride* and *Geotrichum* sp. Jahuri (4-6) reported protein production from agricultural waste using fungi. Fermentation of bagasse for improving its digestability as animal feed was also carried out by Nigam *et al.* (9, 10).

Based on earlier reported information on synthesis of fungal protein from molasses (3) and on other studies relating to recycling waste (7) the present work was initiated to enhance the nutritive value of bagasse, employing simple techniques at farm level for feeding animal.

MATERIALS AND METHODS

Cane molasses having 45% sugar concentration was diluted with water, boiled and filtered to remove impurities. It was further treated with potassium ferrocyanide [$K_4Fe(CN)_6$] to remove metallic iron in the form of complex salts (13). Finally,

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sugar concentration was adjusted to 6% adding required quantity of water.

Bagasse was dried in oven at 105°C to constant weight, cut into 1-2 cm pieces before employing any treatment. Chemical analysis of bagasse has been reported in earlier communication (7). Fifty g bagasse was soaked overnight in 250 ml water at room temperature. In another treatment 50 g bagasse was soaked overnight in 250 ml water which was earlier made alkaline to pH 7.5 by adding sufficient amount of calcium oxide.

Media used in this study had the following composition: $(NH_4)_2SO_4$ 1.2%; KH_2PO_4 0.3%; $CaCO_3$ 1.0%; molasses (diluted to 6% sugar concentration) treated or untreated as required. Spores from nine days old culture of *A. oryzae* grown on Czapeck's Dox agar medium slant were suspended in 200 ml distilled water. Ten ml of this suspension / litre media was used for inoculation.

To observe response of treated and untreated molasses to growth of fungi, experiments were conducted in five 1-litre, Erlenmeyer flasks, each containing 200ml broth having pH of 4.5. Flasks were inoculated and incubated at 28-30°C for 3 days. Another set of experiment to enhance nutritive value of bagasse under unsterilized conditions was conducted in open plastic pans having 22 cm dia, x 11 cm height. Each pan contained 50 g bagasse and one litre molasses media, and pH adjusted to 4.5. To expose maximum surface area to aeration, depth of media was maintained at 1 inch level in the pan.

Entire experiment was triplicated and average values calculated. Bagasse, degraded at 28-30°C forming myclial mat, which was harvested after three days incubation. Biomass (mycelium/bagasse) thus obtained was washed with water, dried in oven at 55-60°C to constant weight, powdered and analyzed. Biomass yield is defined as weight of produce obtained per litre broth, and also on % sugar initially added to the media. Total nitrogen determined as described in Official Method of Analysis using semi microkjeldhal method, and crude protein derived by multiplying nitrogen with factor 6.25.

RESULTS AND DISCUSSION

Data obtained on inoculating treated and untreated media with *A. oryzae*, after 3 days incubation is given in Table 1. Molasses treated with $K_4Fe(CN)_6$ and designated as A-2 yielded thick mycelial mat weighing 27.4 g/litre broth (dry weight) with 35% crude protein. Mycelial mat obtained on untreated molasses and designated as A-1 was sparse in texture and weighed 8 g/litre broth (dry weight) with 34% protein. Development of scanty mycelial

Table 1: Effect of potassium ferrocyanide on molasses, with regard to protein and biomass production on inoculation with A. Oryzae.

	Untreated Molesses A-1	Treated Molasses A-2
Biomass (g) litre	8	27.4
% Crude protein	34	35
Protein (g) litre broth (based on dry biomass)	2.7	9.6
% yield of protein based on sugar added	4.5	16

Table 2: Effect of Addition of water and alkali treated with bagasse to potassium ferrocyanide treated molasses media with regard to protein and biomass production on inoculation with *A. Oryzae*.

	A_2	(A_2w)	(A_2a)
Bagasse (g) litre	-	50 g pretreated with water	50 g pretreated with alkali
Biomass (g)/ litre broth	27.4	40	42
% Crude protein	35	28	35.6
Protein (g)/ litre broth (based on dry biomass)	9.6	11.2	15
% yield of protein based on sugar added	16.0	18.70	25
% yield of biomass based on sugar added	46	67	70

mat on A-1 may be due to presence of impurities and metallic ions in the crude molasses which may have exerted an inhibitory effect on growth and development of *A. oryzae*. Removal of iron and other impurities, through precipitation with $K_4Fe(CN)_6$ forming insoluble complex material, improved biomass production. Percent protein yield based on dry matter obtained from A-1 (34%) and A-2 (35%) was almost the same. However, yield of protein calculated and compared on the basis of dry biomass produced / litre broth showed that 9.6 g protein / litre (16% based on initial sugar in molasses) and 2.7 g protein / litre (4.5% based on initial sugar in molasses) is produced on A-2 and A-1 respectively. Results obtained on treated molasses (A-2) are in agreement with results reported earlier by Husain *et al* (3). The present study was conducted at pH 4.5 and a thick sporic biomass of *A. oryzae* was harvested in three days, much before any other microorganism could develop on the susbtrate. The

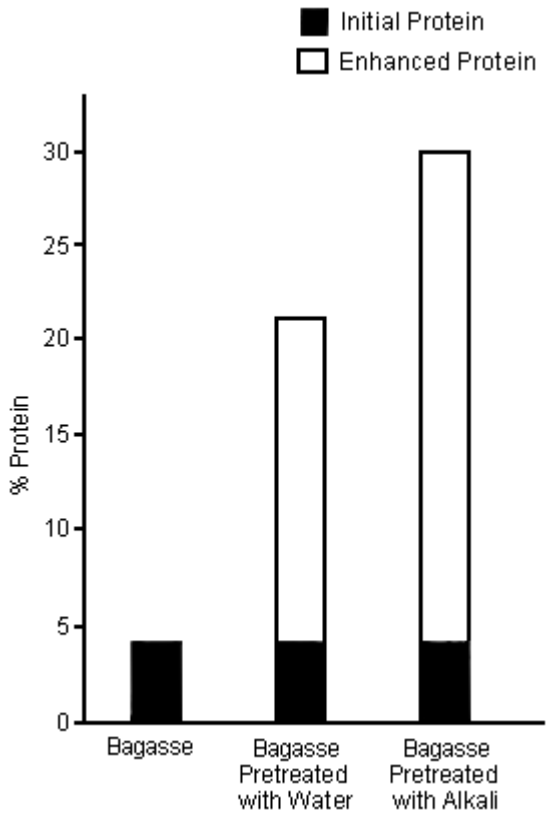

Figure 1: Protein values in initial bagasse, and on addition of pretreated bagasse (with water and alkali separately) to molasses madia inoculated with *A. oryzae.*

object of growing the fungus at acidic pH was to provide farmers an open pan simple technology to obtain biomass with reduced changes of contamination and save cost on sterilization and transportation, besides making protein rich biomass readily available for farm animals. Media A-2 was, therefore, selected for enhancing the nutritive value of bagasse.

Enhancement of protein from water (A_2w: Media with water treated bagasse) and alkali (A_2a: Media with alkali treated bagasse) treated bagasse is compared in Table 2. Dry biomass of almost equal quantity i.e. 40 and 42 g/litre broth respectively was obtained from (A_2w) and (A_2a) but difference in yield of protein i.e. 28% from (A_2w) and 35.6% from (A_2a) was quite distinct. Pretreatment of bagasse with mild alkali (calcium oxide) resulted in earlier swelling and softening of lignocellulosic bond making it easily accessible to microbial enzyme resulting in its subsequent breakdown and further utilization for building microbial protein. Thus 15 g protein / litre media (25% based on sugar in molasses) was obtained from alkali treated (A_2a) bagasse as compared to 11.2 g / litre (18.6% protein based on sugar in molasses) from water treated (A_2w) bagasse. An increase of 1.6 and 5.4 g protein / litre broth from (A_2w) and (A_2a) when compared with A-2 alone though does not appear to be significant, basic requirements of animal feed like fiber, cellulose, bulk are made available in sufficient amount beside enrichment of protein in the resulting biomass from bagasse.

From nitrogen content of bagasse earlier reported (7) 4% crude protein was calculated in untreated bagasse. On pretreatment of bagasse with water and alkali and subsequent inoculation with *A. oryzae*, protein value enhanced by 22.4% and 30% (on dry wt. basis) with a net gain of 18.4% and 26% respectively (Figure 1) thus in three days, 4.6 and 6.5 fold protein enhancement was achieved. Results obtained in (A_2a) showing 30% protein are better than that obtained in (A_2w) with 22.4% protein, likely due to partial degradation of water treated bagasse. It may, therefore, be presumed that pretreatment with alkali may be more useful for upgrading protein value of bagasse, and biomass obtained on employing alkali treatment will have better digestability than treatment with water. Chemical means for synthesizing and upgrading

crop protein though available, residual chemicals remaining after synthesis in the feed may show side effects at later stages in animals, and hence microbial upgrading of bagasse is more desirable.

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