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NUTRITIVE VALUE OF COTTON SEED HULLS AFTER BIOLOGICAL TREATMENTS

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In vivo dry matter digestibility of cotton seed hulls increased from 15.5 to 26.6 % due to symbiotic effect of *T. viride* and *B. polymyxa.* Further improvement in the digestibility was observed when the mixed cultures of mould and bacteria were propagated on alkali treated hulls. An improvement in the digestibility of cellulose, minerals and organic matter alongwith two to three folds increase in non-protein nitrogen was also observed.

Key words: Cotton seed hulls, Biological treatments, Nutritive value.

INTRODUCTION

Bio-conversion of lignocellulosic materials into nutritious animal feed has been studied during the last few years. Lynch *et al.* [1] observed an improvement in the nutritive value of some cellulosic materials by the propagation of fungus on these materials. Symbiotic effect of fungus and yeast was also found to be more effective in increasing the nutritive value of barley straw [2]. Degradation of cellulosic materials has been found inefficient without pretreatment [3-4]. The present studies were conducted to increase the nutritive value of cotton seed hulls by the propagation of cellulolytic micro-organisms.

MATERIAL AND METHODS

Cotton seed hulls collected from Kohi-Noor Oil Mills Ltd., Kala Shah Kaku were ground to 20 mesh size for further treatments.

Chemical treatments. Cotton seed hulls were first treated with solutions of different alkalies (NaOH, KOH, $Ca(OH)_2$, NH_4OH) of varying concentrations, keeping 20 % moisture in the substrate, and then subjected to biological treatments.

Biological treatments. Biodegradation of untreated and pretreated hulls was carried out by semi solid fermention technique [5]. Locally isolated cultures of *Chaetomium globosum, Penicillium roqueforti, Trichoderma viride* and *Bacillus polymyxa* were propagated in Reese medium containing 2 % glucose and the broth was used as inoculum. 4 Kg of the substrate containing 2 % urea was sterlized in 10 kg drum and inoculated with one litre fermented Reese medium keeping water to substrate ratio (v/w) 2 : 1. The substrate in the drum was mechanically agitated with a spindle fitted with blades (30 r.p.m.). The batch was removed after 3 days and dried at 100 ± 5°.

Analytical methods for the estimation of cellulose, nitrogen, lignin and ash contents were the same as reported elsewhere [6–9].

In vivo digestibility. In vivo rumen gestibility of the treated materials was estimated in nylon bags (in six replicates) as described by Orskove *et al.*, [10]. Results of the digestibility were analyzed statistically [11].

RESULTS AND DISCUSSION

Treatment with a single strain of mold and bacteria. In vivo digestibility of cotton seed hulls after fermentation with different cellulolytic fungi and bacteria is given in Table 1. Dry matter digestibility of the hulls increased from 15.53 to 20.97 % when *T. viride* was propagated on the substrate. The second best result was obtained when *B. polymyxa* was propagated on cotton seed hulls. The digestibility of cellulose, minerals and organic matter also increased after biological treatment. Surinder and Gupta [12] hydrolyzed bagasse using the culture filtrate of *T. viride* and found glucose, xylose, arabinose, cellobiose and other saccharides after enzymic hydrolysis, which clearly indicated degradation of cellulose.

Symbiotic effect of mold and bacteria. In vivo digestibility of cotton seed hulls was further improved when a mixed culture of fungus and bacteria was propagated on the substrate (Table 1). Maximum digestibility of dry matter, cellulose, minerals and organic matter was 26.63, 19.0, 40.4 and 25.05 % respectively when a combination of *T. viride* and *B. polymyxa* was used. Symbiotic effect of *C. globosum* and *B. polymyxa* was also found to be quite effective in increasing the digestibility of the hulls. These results are also in agreement with that of Han *et al.*, [13] and Peitersen [2] who reported that bacteria in combination with fungus and yeast utilized more cellulose as compared with fungus alone.

Biological treatment of sodium hydroxide treated hulls. Dry matter digestibility of 4 % sodium hydroxide treated hulls was 29.21 % after propagation of *T. viride* and 30.85 % with *B. polymyxa* (Table 2). It increased to 40.5 % by the propagation of mixed culture of *T. viride* and *B. polymyxa* on 4 % sodium hydroxide treated cotton seed hulls. This improvement seems to be due to breaking of the bond between lignin and structural polysaccharides

Table 1. *In vivo* digestibility and chemical composition of cotton seed hulls after biodegradation.

Treatment	Digestibility after 48 hrs* (% age) Dry matter	Cellulose	Minerals	Organic matter	Composition** (% age) Ash	Nitrogen	Cellulose	Lignin	Dry matter
Cotton seed hulls (as such)	15.53 ± 1.20	18.25 ± 1.23	40.78 ± 1.05	14.69 ± 1.25	2.50	1,25	42.83	27.79	86.99
Chaetomium globosum	19.93 ± 1.74	26.73 ± 1.77	49.09 ± 1.65	17.77 ± 1.65	3.65	2.12	55.37	29.05	86.73
Penicillium roquefortie	18.66 ± 2.05	25.68 ± 1.05	48.88 ± 1.71	16.99 ± 1.08	3.40	2.04	54.44	28.73	86.66
Trichoderma viride	20.97 ± 1.87	28.01 ± 2.02	55.69 ± 1.11	20.22 ± 1.88	3.87	2.02	57.53	28.75	86.27
Bacillus polymayxa	20.69 ± 1.09	26.36 ± 1.79	47.64 ± 1.76	25.80 ± 1.01	3.08	2.83	49.78	29.95	87.77
Penicillium roquefortie + *B. polymyxa*	20.68 ± 1.75	16.61 ± 1.23	29.92 ± 1.11	18.37 ± 1.35	3.01	2.43	51.95	29.61	87.07
Chaetomium globosum + *B. polymyxa*	23.85 ± 1.34	18.08 ± 1.72	38.99 ± 1.12	18.88 ± 1.22	3.12	2.37	52.99	29.49	87.33
Trichoderma viride + *B. polymyxa*	26.63 ± 1.21	19.01 ± 1.50	40.40 ± 2.08	25.05 ± 2.05	3.61	2.69	54.05	29.35	86.72

* Average of six replicates along with standard deviation; ** Average of three replicates.

Table 2. *In vivo* digestibility and chemical composition of sodium hydroxide treated cotton seeds hulls after biodegradation.

Treatment	Digestibility after 48 hrs* (% age) Dry matter	Cellulose	Minerals	Organic matter	Composition** (% age) Ash	Nitrogen	Cellulose	Lignin	Dry matter
1.0% NaOH+*T.viride*	20.72 ± 3.17	30.25 ± 1.24	58.88 ± 1.69	16.20 ± 2.72	7.17	2.15	59.80	30.75	86.37
+*B.polymyxa*	22.73 ± 1.28	18.23 ± 1.79	53.64 ± 1.70	21.43 ± 1.35	4.83	2.74	52.40	29.87	86.53
+*T.viride*+*B.polymyxa*	21.74 ± 1.98	20.26 ± 1.82	55.76 ± 3.02	20.05 ± 2.05	4.83	2.72	53.84	30.17	86.63
2.0% NaOH+*T.viride*	21.82 ± 2.56	32.22 ± 1.39	63.25 ± 1.11	16.22 ± 2.72	8.04	2.09	55.24	30.69	87.21
+*B.polymyxa*	20.28 ± 1.62	17.46 ± 2.20	55.62 ± 2.62	18.02 ± 2.28	6.25	3.01	52.55	29.73	86.21
+*T.viride*+*B.polymyxa*	26.26 ± 1.69	29.30 ± 2.85	65.16 ± 2.73	24.05 ± 1.70	6.25	2.65	52.83	30.75	86.32
3.0% NaOH+*T.viride*	27.61 ± 0.94	33.19 ± 2.73	72.60 ± 0.18	33.16 ± 2.22	9.30	2.23	56.79	30.88	88.01
+*B.polymyxa*	24.79 ± 3.07	21.64 ± 2.19	82.66 ± 0.76	24.40 ± 2.68	7.45	2.79	54.15	30.69	86.67
+*T.viride*+*B.polymyxa*	29.94 ± 1.17	35.81 ± 3.28	72.87 ± 2.30	26.49 ± 2.27	9.53	2.69	53.14	30.95	85.69
4.0% NaOH+*T.viride*	29.21 ± 2.62	29.15 ± 3.51	68.68 ± 3.90	28.08 ± 3.55	9.41	2.28	57.02	30.73	87.63
+*B.polymyxa*	30.85 ± 0.94	18.33 ± 2.74	66.28 ± 1.42	27.31 ± 0.81	8.53	2.64	52.50	30.95	86.92
+*T.viride*+*B.polymyxa*	40.50 ± 2.02	37.96 ± 4.37	80.24 ± 3.24	39.76 ± 2.27	9.53	2.69	53.14	30.95	85.69
5.0% NaOH+*T.viride*	23.95 ± 1.50	35.72 ± 1.26	75.09 ± 3.51	32.32 ± 3.48	11.82	2.17	58.93	30.09	87.59
+*B.polymyxa*	23.53 ± 1.28	18.97 ± 4.70	69.38 ± 0.96	25.96 ± 1.38	11.91	2.89	53.91	31.06	87.04
+*T.viride*+*B.polymyxa*	32.80 ± 0.91	17.41 ± 1.89	85.70 ± 1.24	26.73 ± 1.10	11.77	2.65	53.68	31.33	87.01

* Average of six replicates along with standard deviation; ** Average of three replicates.

Table 3. *In vivo* digestibility and chemical composition of potassium hydroxide treated cotton seed hulls after biodegradation.

Treatment	Digestibility after 48 hrs* (% age)				Composition** (% age)				
	Dry matter	Cellulose	Minerals	Organic matter	Ash	Nitrogen	Cellulose	Lignin	Dry matter
1.0% KOH+*T.viride*	17.73 ± 1.11	30.41 ± 2.60	26.06 ± 0.78	17.38 ± 1.08	5.01	2.50	55.73	28.88	87.63
+*B.polymyxa*	18.45 ± 3.16	19.33 ± 0.96	30.19 ± 2.97	17.82 ± 3.23	3.78	2.60	54.94	29.05	86.55
+*T.viride*+*B.polymyxa*	23.05 ± 1.09	31.11 ± 1.21	35.55 ± 1.22	21.11 ± 1.23	5.61	2.55	54.98	29.65	87.52
2.0% KOH+*T.viride*	17.60 ± 0.95	27.69 ± 1.05	45.22 ± 3.51	18.46 ± 0.96	6.36	2.89	54.81	28.93	87.91
+*B.polymyxa*	16.74 ± 1.46	17.09 ± 1.38	41.79 ± 2.61	15.31 ± 1.01	5.36	3.01	53.09	29.11	86.52
+*T.viride*+*B.polymyxa*	26.08 ± 1.05	34.41 ± 1.08	41.06 ± 1.99	24.04 ± 1.56	7.85	2.78	54.08	29.88	87.05
3.0% KOH+*T.viride*	20.40 ± 1.87	29.49 ± 3.28	77.26 ± 4.20	20.40 ± 1.87	8.73	2.75	54.88	28.99	87.95
+*B.polymyxa*	18.76 ± 1.01	28.87 ± 1.25	63.70 ± 1.96	15.51 ± 1.01	6.13	3.20	53.11	29.01	86.45
+*T.viride*+*B.polymyxa*	28.06 ± 0.82	37.77 ± 1.69	53.61 ± 1.37	26.13 ± 1.73	8.26	2.80	53.01	29.67	87.21
4.02% KOH+*T.viride*	26.67 ± 1.67	40.75 ± 4.27	81.19 ± 1.04	25.62 ± 2.92	10.44	2.87	53.99	29.35	87.63
+*B.polymyxa*	25.14 ± 0.86	24.14 ± 3.17	67.64 ± 1.15	19.18 ± 0.91	10.52	3.20	53.19	29.35	86.63
+*T.viride*+*B.polymyxa*	32.76 ± 2.24	45.51 ± 0.69	78.75 ± 1.09	30.08 ± 1.08	12.04	2.82	53.81	29.58	87.37
5.0% KOH+*T.viride*	23.15 ± 1.40	37.08 ± 1.37	69.05 ± 1.55	23.15 ± 1.40	12.83	2.79	54.05	29.77	87.56
+*B.polymyxa*	23.69 ± 1.05	27.83 ± 1.28	77.01 ± 1.47	17.87 ± 0.42	12.19	3.20	53.22	29.89	86.61
+*T.viride*+*B.polymyxa*	28.39 ± 2.75	35.77 ± 1.51	71.77 ± 2.87	26.39 ± 3.65	14 18	2.85	53.72	30.12	87.62

* Average of six replicates along with standard deviation; ** Average of three replicates.

Table 4. *In vivo* digestibility and chemical composition of calcium hydroxide treated cotton seed hulls after biodeoradation.

Treatment	Digestibility after 48 hrs* (% age)				Composition** (% age)				
	Dry matter	Cellulose	Minerals	Organic matter	Ash	Nitrogen	Cellulose	Lignin	Dry matter
0.5% $Ca(OH)_2$+*T.viride*	18.99 ± 0.98	16.47 ± 2.74	29.52 ± 3.30	18.66 ± 1.01	4.60	2.28	51.37	27.95	88.25
+*B.polymyxa*	19.84 ± 1.57	17.94 ± 0.88	44.26 ± 2.42	18 72 ± 1.42	4.75	2.40	50.40	28.07	89.61
+*T.viride*+*B polymyxa*	21.03 ± 1.1[illegible]	22.34 ± 1.34	32.42 ± 2.09	20.42 ± 1.10	4.82	2.60	52.71	29.11	86.65
1.0% $Ca(OH)_2$+*T.viride*	18.79 ± 0.73	20.07 ± 1.86	29.61 ± 2.15	19.28 ± 1.07	5.12	2.27	52.73	27.99	88.01
+*B.polymyxa*	21.02 ± 1.01	24.26 ± 3.13	43.06 ± 4.57	19.29 ± 1.96	5.19	2.24	51.40	28.15	88.25
+*T.viride*+*B.polymyxa*	22.13 ± 0.91	18.91 ± 0.59	47.94 ± 2.97	22.23 ± 0.89	5.73	2.68	52.89	29.49	86.77
1.5% ($Ca(OH)_2$+*T.viride*	22.60 ± 1.99	23.80 ± 2.34	37.17 ± 2.86	26.24 ± 2.21	8.09	2.49	51.61	28.35	88.12
+*B.polymyxa*	25.62 ± 1.00	31.86 ± 3.97	53.71 ± 1.14	23.70 ± 0.98	6.90	2.43	51.79	28.01	89.09
+*T.viride*+*B.polymyxa*	26.76 ± 2.04	21.03 ± 1.26	43.92 ± 2.24	23.27 ± 2.20	8.17	2.75	52.99	30.09	86.72
2.0% $Ca(OH)_2$+*T.viride*	25.46 ± 0.97	31.16 ± 1.57	46.85 ± 0.84	24.83 ± 3.40	7.04	2.47	53.30	28.67	87.73
+*B.polymyxa*	24.21 ± 1.22	27.46 ± 3.35	54.79 ± 0.79	21.90 ± 1.21	6.82	2.45	50.10	28.44	89.31
+*T.viride*+*B.polymyxa*	30.38 ± 1.93	32.45 ± 1.97	61.05 ± 1.06	29.22 ± 2.28	7.48	2.78	52.16	29.99	86.65
2.5% $Ca(OH)_2$+*T.viride*	26.39 ± 1.27	26.75 ± 1.35	41.42 ± 1.38	23.69 ± 1.25	7.81	2.55	50.16	28.73	88.06
+*B.polymyxa*	28.97 ± 1.56	29.68 ± 1.25	50.43 ± 1.53	28.32 ± 1.36	6.84	2.45	52.00	28.79	88.36
+*T.viride*+*B.polymyxa*	32.43 ± 2.81	35.76 ± 2.04	64.60 ± 3.69	30.92 ± 2.23	7.86	2.80	52.33	30.75	87.05

* Average of six replicates along with standard deviation; ** Average of three replicates.

Table 5. *In vivo* digestibility and chemical composition of ammoniated cotton seed hulls after biodegradation.

Treatment	Digestibility after 48 hrs* (% age)				Composition** (% age)				
	Dry matter	Cellulose	Minerals	Organic matter	Ash	Nitrogen	Cellulose	Lignin	Dry matter
5.0% ammonia+*T.viride*	28.06 ± 0.73	30.13 ± 1.81	51.30 ± 1.70	26.69 ± 1.43	4.44	4.54	53.12	28.05	89.32
5.0% ammonia+*B.polymyxa*	26.59 ± 1.18	20.51 ± 1.09	52.47 ± 1.74	24.87 ± 1.69	4.51	4.88	51.57	28.29	90.09
5.0% ammonia+*T.viride+ B. polymyxa*	31.51 ± 0.77	24.96 ± 1.59	48.19 ± 2.12	30.52 ± 1.08	4.49	5.05	56.88	28.45	90.21

* Average of six replicates along with standard deviation; ** Average of three replicates.

by sodium hydroxide which rendered the substrate more susceptible to the action of rumen micro-organisms. Shah *et al.*, [14] reported an improvement in the digestibility of alkali treated rice straw after fermentation with a mixed culture of *Penicillium requefortie* and *B. polymyxa.*

Biological treatment of potassium hydroxide treated hulls. In vivo dry matter digestibility of 4.0 % potassium hydroxide treated hulls was 26.67 % after hydrolyzed with *T. viride* and 25.14 % with *B. polymyxa* (Table 3). It increased to 32.76 % when mixed cultures of *T. viride* and *B. polymyxa* was grown on 4.0 % alkali treated hulls. The digestibility of cellulose, minerals and organic matter was also improved by the fermentation of potassium hydroxide treated substrate. Improvement in the digestibility of various alkali treated crop residues with cellulose degrading microbes had already been reported by various workers [15-16].

Biological treatment of calcium hydroxide treated hulls. Results mentioned in Table 4 show the effect of different cellulolytic micro-organisms on calcium hydroxide treated cotton seed hulls. Dry matter digestibility of 2.5 % calcium hydroxide treated hulls was 26.39 % after treatment with *T. viride* and 28.97 % with *B. polymyxa.* Propagation of mixed culture of *T. viride* and *B. polymyxa* on 2.5 % calcium hydroxide treated cotton seed hulls increased the digestibility to 32.43 %.

Biological treatment of ammonia treated hulls. In vivo dry matter digestibility of 5 % ammoniated hulls, after biodegradation with *T. viride* and *B. polymyxa* , was 28.06 and 26.59 % respectively (Table 5). It increased to 31.5 % when a combination of *T. viride* and *B. polymyxa* was propagated on ammoniated hulls. The digestibility of minerals, cellulose and organic matter was also significantly improved by the combined action of ammonia and biological treatment. Our results are in agreement with the results of Bellamy [17] who reported that digestibility of ammoniated cellulose increased after fermentation. Han and Callihan [18] also observed an increase in the digestibility of ammoniated rice straw after fermentation.

Effect of treatments on the chemical composition of cotton seed hulls. The chemical composition of cotton seed hulls after bio-degradation is given in Table 1. It is clear from these results that cellulose and lignin contents increased when cotton seed hulls were incubated with different cellulolytic micro-organisms. Han and Anderson [16] also reported an increase in cellulose and lignin contents when cellulolytic micro-organisms were propagated on rye grass. This increase in cellulose and lignin contents in the fermented product was attributed to the inability of the micro-organisms to use these compounds. It is evident from these results (Table 1) that ammonical nitrogen contents increased from 1.25 to 2.83 % after fermentation. This increase in nitrogen was due to the presence of nitrogenous compounds which were added in the medium before fermentation. Treatment of cotton seed hulls with different concentrations of sodium hydroxide and then biodegraded also resulted in a two fold increase in nitrogen (Table 2). An increase in ash, cellulose, and lignin contents was also observed by this treatment. Similar results were also obtained by the fermentation of potassium hydroxide or calcium hydroxide treated substrates (Tables 3 and 4). Biodegradation of ammoniated hulls showed that the nitrogen contents increased by three to four folds (Table 5). An increase in lignin and cellulose contents was also noticed after the fermentation of ammoniated hulls.

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