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Bioconversion of water hyacinth lignocellulose into feed supplement by lignocellulolytic streptomyces

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ABSTRACT

The bio-conversion of *Eichhornia crassipes* (MART) (water hyacinth) into feed supplement by some *Streptomyces* species isolated from compost dump in Calabar was investigated. The proximate composition analysis revealed 14% crude protein, 16.8% crude fibre, 7% crude fat, 8% ash content and 54.2% carbohydrate. After fermentation for fourteen days, all the components decreased in composition except crude protein which increased from 14 to 21%. The toxicants were hydrocyanic acid (0.70mg), total and soluble oxalate (2.12mg, 1.21mg), phytic acid (0.274 mg) and tannins (0.038mg), per kg. All the toxicants showed a decrease in composition after fermentation. Of the four *Streptomyces* species studied for their ability to degrade water hyacinth lignocellulose, two, GS_3 and S_{22} had the highest lignocelulolytic activity. GS_3 and S_{22} produced a lignin loss of 20% and 27% respectively, carbohydrate loss of 52% and 55%, lignocellulose weight loss of 47% and 48%, crude protein production of 15% and 14%, APPL production of 0.20 gram and 0.180 gram respectively. A consortium of these two isolates was used for fermentation of water hyacinth lignocellulose into protein feed supplement. A 20% level supplementation gave weight increases and an apparent digestibility of 87.23 in rats fed with the diets. The present study demonstrates the high potentials of *Streptomyces* in converting lignocellulose waste into useful products.

INTRODUCTION

Lignocellulose is a collective name for three polymeric materials, cellulose, hemicellulose and lignin, which occur in woody plants and grasses (Wardrope 1964). In lignocellulose, lignin is the most recalcitrant and protects the cellulose and hemicellulose from enzymatic attack by some micro-organisms, thereby limiting the recycling of carbon. Many actinomycetes can clearly modify lignin but cannot mineralize it. The only organisms shown to be able to mineralize a substantial fraction of lignin are the white rot fungi (Ahmed et al, 2001). Degradation or modification of lignin is important for the efficient conversation of cellulose and hemicellulose in biomass into fuels and chemicals. In addition, the use of unsterile lignocellulose for bioremediation purposes hold promise for most cost-effective environmental clean-up endeavours. Novel lignocellulose-base applications have found functionality in textile, biological control, and medical research fields, (Malherbe and Cloete 2002).

Due to animal feed shortages and the disposal problems of waste, attempts have been made to produce microbial protein from lignocellulose wastes such as rice straw, waste banana, potato and cassava wastes, sugar cane bagasse, groundnut hulls, water hyacinth (Ibrahim and Antai 1986, Kusemiju and Akingboju 1988, Antai and Mgbomo 1993, Deobald and Crawford 2002). This is because fermentation increases the nutritional value of waste residues and improves acceptance by animals resulting in higher levels of dietary inclusion.

Water hyacinth has spread from its origin in Central and South America to tropical and sub-tropical regions of the world and have caused much menace to ships in many water ways, endangers fish life and is a dangerous water pollutant (Harley 1988). This is caused by its very prolific growth rate which is not easily controlled. Many methods, biological, chemical and mechanical have been used for control of its growth rate.

Apart from growth control method, various methods have been suggested for turning water hyacinth into useful economic products (Edewor 1988, Oladiran 1988, Akinyemiju et al 1988, Fish and Agboke 1988). The feasibility of feeding cultured fish with water hyacinth diet has been documented (Kusemiju and Akingboju 1988).

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The present study is a possible bioconversion of water hyacinth by fermentation and incorporation into feeds, as both protein supplement and feed adjunct.

MATERIALS AND METHODS

Source of lignocellulose

Lignocellulose was prepared from water hyacinth, collected from a small pond in Ikorodu, Lagos State.

Actinomycete cultures were isolated from soil samples obtained from the Botanical garden, University of Calabar.

Isolation of actinomycetes

The soil-dilution plate technique of Nuretin and Aysel (2003) was used. In brief, soil samples were pretreated with $CaCO_3$ (10:1 w/w) and incubated at 37°C for 4 days. It was then suspended in sterile Ringer solution (1/4 strength). Test tubes containing 10^{-2} dilutions of samples were placed in a water bath at 45°C, for 16 hrs to allow the spores to separate from the vegetative cells. The dilutions were inoculated on the surface of cellulose nitrate agar (an actinomycete isolation agar) to which 5µg/ml of rifampicin was added to retard fungal growth. Primary Screening for Lignocellulose Degraders (Sundman & Nase 1971)

The medium comprised of the following per litre of distilled water: Glucose 5g, Ammonium tartrate 5g, yeast extract 1g, $MgSO_4.7H_2O$ 1g, $CaCl_2.2H2O$ 0.01g, NaCl 0.1g, $FeCl_3$ 0.01g, vitamin solution 5ml, Lignocellulose 1g, Agar 20g.

The test agar was coloured green, and the disappearance of lignin was indicated by clear (yellow) zones under, and or around the growth of lignin-decomposing actinomycetes.

Confirmation of lignocellulose degradation was carried out by growing the isolates on mineral salt medium containing lignocellulose, agar and yeast extract. Isolates which showed substantial clear zones were further examined for abilities to cause lignocellulose weight loss, lignin loss and carbohydrate loss over a 4 week incubation period.

The actinomycetes were characterized and identified as *Streptomyces* species based on the scheme of Cross and McIver (1968).

Lignocellulose preparation

The water hyacinth (whole plant) was cut into small pieces and oven dried at 70°C for 24 hours. After drying, they were ground in a mechanical grinder to pass a 500µm sieve. Before use as substrate, the lignocellulosic material was extracted with hot water at 80°C until all the water soluble components were removed. Ten changes of water were used in sequence with benzene: ethanol (1:1) reflux for 30 minutes and decanted. This procedure was carried out twice. It was then washed in ethanol (hot) for 30 min refluxing to remove benzene. The ethanol was filled to the same volume as the benzene: ethanol. This was decanted and the procedure repeated twice. The material was then washed into distilled water in a beaker with stirring magnet. It was heated to 70°C and stirred for 10 minutes. This was decanted and this procedure was carried out three times. The material (extractive-free lignocellulose) was dried in the oven at 50°C for 1 hours.

Characterization of lignocellulose degrading actinomycetes

The isolates, which showed substantial clear zones from the screen tests were further examined for their ability to cause lignocellulose weight loss using the dampened lignocellulose culture system (Antai and Crawford 1981).

In this method, 500 ± 0.1mg lignocellulose was placed in cotton-plugged Erlenmeyer flasks and sterilized by autoclaving for 2 hours. Each flask was inoculated with actinomycete spores. (2mls suspended in mineral salts plus 1% of yeast extract). Control flasks had no organisms. The flasks were incubated in a humid incubator for 4 weeks at 30°C, and afterwards analysed.

Lignocellulose weight loss, lignin and carbohydrate content The lignocellulose weight loss, was determined gravimetrically by subtracting the final weight from the original weight.

Lignin content was determined by the Klason procedure. One ml of concentrated H_2SO_4 was added to 50mg of lignocellulose. After autoclaving for 1 hour the content was filtered and oven dried at 50°C. The difference in weight of sound and decayed lignocellulose residues represented the lignin loss.

Carbohydrate content was analyzed from the Klason supernatants by determining the amount of glucose present from a standard curve.

Crude protein content

Protein (N x 6.25) was determined according to the standard Kjedahl method of AOAC (1990).

Acid precipitable polymeric Lignin

This was carried out using the modified procedure of Njoku and Antai (1989). Forty (40) ml of distilled water was added to the 500mg lignocellulose and steamed at 100°C for 1 hour. It was filtered and APPL was recovered from the filtrate by acidifying with 0.4ml of Conc HCl. The resultant precipitate was recovered by centrifugation at 3000 rpm in centrifuge tubes for 45mins. The precipitates were dried at 50°C for 48 hrs and weighed

Pretreatment and fermentation of lignocellulosic substrate

One part of lignocellulose substrate was ground to pass 0.25 inch screen and mixed with two parts of sodium hydroxide solution to give an alkaline treatment level of 4% dry weight, before being allowed to stand at room temperature (28°C) for 1 hour. The lignocellulose was then neutralized with 1.5N HCl and fortified with $(NH_4)_2 SO_4$ (1.4%N), i.e 2 grams per 30 grams of substrate.

The pretreated lignocellulose in 30g quantities were weighed into sterile plastic containers and moistened with mineral salts medium. Five mls of the inoculum was spread carefully over the entire surface of the mash. The mouth of the containers were covered with cheese cloth fastened to the container with rubber band and left to ferment for 5 days. At the end of fermentation, part of the fermented lignocellulose was analysed while the remaining part was mixed with other ingredients for diet formulation.

Feeding trials and measurements

The method of Umoren *et al.* (1997) was used. Weaning male albino rats obtained from the Animal House of the Department of Biological Sciences, University of Calabar were used for the study. Rats were 21 days old and weighed from 22 to 28g. They were distributed into groups to achieve weight equalization. Rats were caged in individual stainless steel metabolic cages and distributed into eight groups of five rats each. Eight experimental diets were formulated. These included the control diet (casein group), the basal or non protein diet (meant to adjust the protein content of the test diet to zero), and six other diets (Table 5). Feeding lasted for 21 days. Feed and water were provided *ad libitum*. During the stabilization period, urine and faeces were collected on daily basis, stored in screw-capped plastic containers at 4°C and later pooled according to each group of rats and diet at the end of the balance period. Rats were weighed at the beginning and at the end of the experiment. Fecal and urinary nitrogen were determined using the Kjedahl method (AOAC 1990).

Measurements

The feed intake and protein intake data were collected and calculated. The protein efficiency ratio, net protein retention, protein retention efficiency, net protein utilization, feed efficiency and apparent digestibility were similarly calculated according to the method of Pellet and Young (1980).

RESULTS

Table 1 shows the proximate composition of water hyacinth as follows: 14% crude protein, 16.8% crude fibre, 7.0% crude fat, 8.0% ash content and 54.2% carbohydrate. As a result of fermentation, the crude protein increased from 14 to 21%.

Table 2 shows that the toxicant composition were quite low. Even the low composition was further reduced by fermentation such that hydrocyanic acid was reduced by 64.3%.

Total oxalate and soluble oxalate by 47.3 and 46.3% respectively, phytic acid was reduced by 42.1% while tannin was reduced by 44.7%, after 120 hours of incubation.

Table 1. Proximate Composition of Water hyacinth

COMPONENT	% COMPOSITION	
	UF	F
Crude protein	14.10	21
Crude Fibre	19.8	18
Crude Fat (ether extract)	3.5	4.7
Ash Content	27.0	29.3
Carbohydrate	25.2	20.50

UF: Unfermented, F: Fermented after pretreatment with NaOH. Fermentation lasted 14 days.

Table 2. Effect of Fermentation on toxic Components of *Eichhornia crassipes*

Toxicants mg/kg	Fermentation (hrs						
	0	24	48	72	96	120	% Reduction
Hydrocyanic acid	0.70	0.66	0.48	0.46	0.45	0.25	64.3
	±0.05	±0.15	±0.02	±0.13	±0.05	±0.12	
Tannin	0.038	0.035	0.029	0.026	0.023	0.021	44.7
	±0.01	±0.01	±0.13	±0.013	±0.01	±0.10	
Phytic acid	0.38	0.34	0.030	0.28	0.25	0.22	42.1
	±0.03	±0.05	±0.16	±0.15	±0.11	±0.01	
Oxalate (Total)	2.92	2.01	1.6	1.58	1.55	1.54	47.3
	±0.10	±0.15	±0.13	±0.15	±0.02	±0.15	
Oxalate (Soluble)	1.21	0.85	0.76	0.73	0.68	0.65	46.3
	±0.12	±0.12	±0.12	±0.15	±0.07	±0.16	

Values are mean ± SD based on 3 replicates

Table 3. Effect of fermentation of water hyacinth by *Streptomyces* spp based on measurement of lignocellulose degradative parameters

Streptomyces spp	Parameters %				
	LWL	CPP	LL	CL	APPL
CD6	23±0.6	10±0.1	15±1.3	58±1.5	70
CD7	15±1.3	6±1.2	18±1.4	50±0.5	90
CD3	47±1.0	15±0.5	17±0.4	54±1.2	200
SS22	48±0.8	14±2.2	16±0.5	56±1.1	180
GS3&S22	58±0.5	25±0.1	30±1	68±1	250

The lignocellulytic activity of four *Streptomyces* species were measured after 12 weeks fermentation and the results are recorded on Table 3. The isolates giving the best lignolytic activity were GS_3 and S_{22} respectively as follows; lignocellulose weight loss, 47 and 48%, crude protein production, 15 and 14%, lignin loss, 17 and 16%, carbohydrate loss, 52 and 55%, and APPL production, 0.20 and 0.18 grams.

A consortium of GS_3 and S_{22} gave results that were better than results obtained from the monocultures (GS_3 or S_{22}). The results were 58%, 25%, 30%, 68% and 250mg for lignocellulose weight loss, crude protein production, lignin loss, carbohydrate loss and APPL production respectively (Table 3).

Table 4 shows the correlation between the period of incubation and the various parameters of lignocellulose degradation. The correlation between the various parameters and the period of incubation ranged from 0.8053515 to 0.9967569. These data showed positive correlation between the length of fermentation and the parameters studied.

Table 6 shows the response of rats to diets containing fermented water hyacinth lignocellulose. When compared with the control diet, it was shown from the protein quality indicators that the rats accepted the feed.

Fermentation lasted for 12 weeks., APPL was measured in mg/gm. LWL = lignocellulose weight loss, CPP = crude protein production, LL = lignin Loss, CL = carbohydrates loss, APPL = APPL production, Data = means ± SD of 3 replicates.

Table 4. Correlation between incubation period, Lignin weight loss and crude protein production from water hyacinth by different *Streptomyces* species.

Streptomyces species	Carbohydrate Loss	Lignocellulose weight loss	Lignin loss	Crude protein produced	APPL production
CD_6	0.9885208	0.9767328	0.9967569	0.940709	0.91129565
CD_7	0.9823791	0.9852689	0.9833708	0.957841	0.9797959
GS_3	0.971225	0.8053515	0.9785885	0.976088	0.94559449
S_{22}	0.9785242	0.8813242	0.9708911	0.978589	0.93810268

Table 5. Composition of diets used for the feeding experiment

Ingredients	Casein (control)	Basal (non-protein diet)	Test Diet 1	Test diet II	Test diet III
Corn starch	68.64	64.75	61.78	54.91	48.05
Casein	11.12	-	11.12	11.12	11.12
Glucose	-	5	-	-	-
Sucrose	-	10	-	-	-
Lignocellulose	-	-	6.86	13.72	20.59
Non-nutritive cellulose	5	5	5	5	5
Corn oil	10	10	10	10	10
Vitamin premix	2	2	2	2	2
Oyster shell	1	1	1	1	1
Bone meal	2	2	2	2	2
Niacin	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100

Table 6. Responses of rats to diets containing fermented water hyacinth lignocelluloses

Diets	Protein quality indicators									
	Feed intake	Weight gain (g)	Protein intake (g)	Nitrogen intake (g)	PER	NPR	PRE	NPU	Feed efficiency	Apparent digestibility
Casein (control)	73.11 ±0.01	30.12 ± 0.02	7.31 ±0.11	1.17 ± 0.10	4.12 ±0.1	4.98 ± 0.01	79.67 ± 0.05	0.97 ± 0.01	2.24 ±0.11	90.59 ±0.05
Test Diets with fermented water hyacinth										
10%	54.50 ± 0.5	28.60 ± 0.1	5.45 ± 0.6	0.87 ± 0.1	5.25 ± 0.2	4.04 ± 0.5	64.53 ± 0.6	1.36 ± 0.1	1.99 ± 0.1	87.33 ± 0.1
20%	58.83 ± 0.1	30.05 ± 0.08	5.88 ± 0.1	0.99 ± 0.5	5.11 ± 0.5	4.09 ± 0.5	65.68 ± 0.4	1.37 ± 0.3	1.99 ± 0.5	87.36 ± 0.8
30%	52.29 ± 0.02	26.20 ± 0.1	5.23 ± 0.5	0.84 ± 0.6	5.01 ± 0.1	3.81 ± 0.3	60.94 ± 0.2	1.51 ± 0.5	1.96 ± 0.1	86.90 ± 0.6

Values are means ± S.D based on 10 rats per group.

DISCUSSION

The present study shows that in the unfermented form, water hyacinth had a crude protein of 14% which was raised to 21% after fermentation and pretreatment with NaOH. Though high in carbohydrate (25.2%), it was observed that fermentation reduced the carbohydrate content to 20.5% after 14 days.

Another desirable feature of water hyacinth for use as feeding stuff is its low level of toxicants (Table 2). The reduction of toxic components (hydrocyanic acid and tannin) by fermentation, had been previously demonstrated on cassava peels by Ofuya and Obilor (1993). This low level of toxicity is an indication that there be will no toxic side effects when water hyacinth is fed to animals.

Fermentation also aided the degradation of water hyacinth lignocellulose leading to the production of intermediates such as APPL (acid-precipitable polymeric lignin) and reducing sugars. A weight loss was also observed on the lignocellulose from 15 to about 58% depending on the length of fermentation. Lignin loss ranged from 15 to 30%. The results are in consonance with earlier report by Antai and Crawford (1983) on their studies on grass lignocellulose degradation.

Another effect of fermentation was an increased net protein utilization value for the diet supplemented with fermented water hyacinth. The value for the control diet averaged 0.97 whereas that of the diet supplemented with water hyacinth lignocellulose diet were higher (up to 1.51) at 30% inclusion level. The feed intake was slightly lower for the water hyacinth lignocellulose – supplemented diet, control diet, and water hyacinth lignocellulose diet. This is probably due to the fact that the rats were not yet used to the diet.

Uriyapongson and Toaprayoon (1994) fed lambs with water hyacinth ensiled with yeast and showed that the ensiled substrate had higher digestibility than the dried substrate. Malaya *et al.* (1995)

showed that two fungi, *P.ostreatus* and *P.sajor-caju* were capable of converting water hyacinth biomass into protein-rich food or feed. This was also confirmed by Das and Karim (1995) who studied the use of degraded lignin in water hyacinth lignocellulose for animal feed production using *P. ostreatus*. The present study used two *Streptomyces* spp GS_3, and S_{22} for the bioconversion of water hyacinth lignocellulose into feed. From the study, the protein quality indicators of the diets compared favourably with that of the control (protein) diet showing that the lignocellulose feed supplement was accepted by the rats in the formulated diets.

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