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Effect of Selected Azotobacter Bacterial Strains On the Enrichment of Cassava Waste during Solid State Fermentation

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Target audience: Local farmers, Livestock researchers, microbiologist

Abstract

The effect of three different strains of Azotobacter bacteria in solid substrate fermentation on cassava waste was evaluated. The substrate was incubated at 30°c for 10 days after inoculation with the Azotobacter bacteria species. One non-inoculated batch was incubated as a reference. Percentages of the initial and final proximate contents were analysed. The experiment was carried out in quadruplicate. Inoculation of cassava waste with species of Azotobacter bacteria significantly increased the levels of the crude protein content to a mean (0.58 ±SE) of 11.3% of the initial value. Among the tested species Azotobacter agilii increased the crude protein level most effectively, followed by Azotobacter vinelandii and least for Azotobacter beijerinckii. The increased crude protein was significantly correlated (r = 0.86) with the extent of fermentation. The non-protein nitrogen content increased significantly (P<0.25) to a mean of 5.23%. while the total nitrogen followed similar trend. The hemicellulose content was higher for Azotobacter agilii and Azotobacter vinelandii compared with Azotobacter beijerinckii while the lignin content was lowest for Azotobacter Vinelandii and Azotobacter agilii than the control sample. The energy level was increased in the bacteria treated substrates than the control sample. It is concluded that both incubation and bacterial activity are instrumental in increasing the potential protein, hemicellulose and energy content of cassava waste during solid substrate fermentation and the efficacy depends on the species of bacterial used.

Keyword: Azotobacter bacteria, cassava waste, biochemical changes, nitrogen fractions, solid state fermentation.

Description of Problem

Cassava waste (consisting of the rinds, some pulp and small tubers) is a major by-product of cassava processing industry. The rind is about 18% of the whole tuber. The waste is poor nutritionally with crude protein of between 2.3 - 5.0%, crude fibre 8.0% and either extract 1.5%. The limitation in the use of cassava waste could be related to its poor protein content as well as the presence of hydrocyanic acid (HCN). However, the HCN content could be reduced by soaking, boiling, fermentation, sun-drying and shredding (1) while the poor protein content could be improved by protein supplementation. Supplementation with plant or animal protein source (s) could be costly while supplementation with free atmosphere nitrogen through the action of some free nitrogen fixing bacteria thereby enriching the waste or supplying nitrogen to the waste is a welcome approach. For many years. Azotobacter species of bacteria are known and considered vital in the nitrogen economy of field soil, (2) but its importance in enhancing the poor nitrogen content of agricultural waste residues for livestock is scanty. Hence the thrust of the study was to evaluate the contribution of three selected free

nitrogen bacteria strains to the changes in the nitrogen and other proximate content of cassava waste by solid substrate fermentation.

Materials and Methods

(i) Sources of Inoculum and Media Used.

The method of Sprent and Sprent (3) which enables Azotobacter to form colonies on the surface of soil was used. Soil sample of sufficient quantity was filled into small petri-dish (1cm deep, 5cm diameter) and was supplied with 1-2g of both Ca Co_3 and Mannitol. After mixing, 4 drops of a 10% solution of both K_2HPO_4 and $MgSO_4.7H_20$ were added. Subsequently, enough water was added to prepare a soil paste that was not water saturated (the soil should not be water logged so a to prevent the growth of *Clostridium pasterianum*). The mixture was put at the bottom of the petri-dish and the surface smoothed with a knife. The dish was then placed (without cover) into a larger petri-dish on a wet filter paper or cotton wool so as to ensure a moist atmosphere. The larger dish was covered such that the lid did not touch the soil surface. After a 7 day incubation at 30°C, the mucoid colonies of Azotobacter developed on the soil surface.

(ii) Purification of Azotobacter colony

Azotobacter bacterial colony was then purified on Mannitol agar consisting of the following.

Mannitol, 10g; K_2HPO_4 1g; $MgS0_4.7H_20$, 0.5g; Agar, 20.0g; trace element solution 0.1ml, distilled water, 1 litre and yeast extract, 0.002%.

The trace elements consist of AICL, 1g; K1, 05g; KBR, 0.5g LiCL, 0.5g; $Mncl_2 4H_20$, 7.0g, H_3B0_3, 11.0g; $Zncl_2$ 1.0g; $Cucl_2$,1.0g $Nicl_2$ 1.0g; $Cocl_2$; 5.0g; $Sncl_2$ $2H_20$, 0,05g, $Bacl_2$ 1.0g; $Na_2M_o0_4$, 0.5g; $NaVo_3H20$, 0.1g, selenium salt, 0.5g.

Before mixing the above constituents together, the pH of each solution was adjusted to pH below 7.1. The total final volume was 3.6 litres and the pH of the total final volume was adjusted to between 3-4 with hydrochloric acid (HCL). The purified colony started developing on the fifteenth day of inoculation of the Mannitol agar.

(iii) Identification of Isolates

Both cultural and microscopic characteristics of the organisms were done by observing. Shape colony, optical characteristics, pigmentation, edge of colony, consistency and colony surface. Gram staining, motility and catalase tests were done while the bacteria were identified and differentiated using Bergey,s (4) method.

(iv) Preparation of Substrate

Cassava waste was collected from a gari processing industry in Ilorin, Kwara State. The wastes destined for the bacterial growth was treated with ethanol (70% v/v) for 60 seconds. Subsequently, the waste was autoclaved at 121°c for 15 minutes

Inoculation

Inoculation of the waste was similar for all microbial cultures thus; One Sixth of the pure culture contain in a petri-dish was used to inoculate 50g each of the well sterilized substrate and water incubated at ambient temperature for 3-4 weeks until the bacterial growth covered the substrate (Rolz *et al* (5)

There were four treatments based on the different species of the bacterial (4) and a control. Treatment codes are shown in Table 1. Control treatment was uninoculated substrate which had received the same sterilization and were incubated as described . The extent of growth was monitored semi-quantitatively, using a subjective classification scale from 0 (no visible growth) to 5 (completely covered). The experiment was carried out 5 times.

Chemical Analysis

The proximate composition and fibre fractions of the untreated and treated cassava wastes were determined according to the method of AOAC (6) while the NPN content was calculated by the subtraction of true protein from the total crude protein. Other nitrogen fractions was determined by the method of Licitra *et al* (7). All data collected were validated statistically by a completely randomized design model and treatment means separated by Duncan (8) multiple range test.

Results and Discussion

Bacterial growth was encouraging in the inoculated samples and absent in the un-inoculated sample. The disinfection and the

Table 1: Analytical data of Original Sample (Cassava Waste)

Component	% dry weight
Moisture	8.25
Crude Protein	3.50
Crude Fibre	10.75
Ether Extract	1.22
Nitrogen free extract	63.72
Lignin	7.25
Hemicellulose	7.75
Organic Matter	70.31
Energy	4:75

Table 2: Biochemical Changes in the composition of cassava waste incubated with *Azotobacter Agilii*

Component	%	% Loss	%Gain	% of Original sample
Moisture	15.25	-	7.0	84.85
Crude Protein	13.50	-	10.0	285.71
Crude Fibre	10.95	-	0.25	2.33
Ether Extract	1.24	-	0.22	1.64
Nitrogen free extract	68.23	-	4.51	7.08
Lignin	3.25	4.00	-	55.17
Hemicellulose	10.30	-	2.55	32.90
Organic Matter	75.25	-	4.94	7.03
Energy	5.90	-	1.15	34.21

Table 3 Biochemical Changes in the composition of cassava waste incubated with *Azotobacter vinelandil*

Component	%	% Loss	%Gain	% of Original sample
Moisture	12.00	-	3.75	45.45
Crude Protein	12.25	-	8.75	250.00
Crude Fibre	11.15	-	0.40	3.72
Ether Extract	1.24	-	0.22	1.64
Nitrogen free extract	65.30	-	1.58	2.48
Lignin	4.30	2.95	-	40.69
Hemicellulose	9.25	-	1.50	19.35
Organic Matter	72.50	-	2.19	3.11
Energy	4.78		0.03	0.63
Average	21.42	2.95	2.02	40.79

Table 4 Biochemical Changes in the composition of cassava waste incubated with *Azotobacter beijerinckii*

Component	%	% Loss	%Gain	% of Original sample
Moisture	17.25	-	9.00	1.09
Crude Protein	8.75	-	5.25	150.00
Crude Fibre	11.20	-	0.45	4.19
Ether Extract	1.26	-	0.04	3.28
Nitrogen free extract	70.12	-	6.40	10.04
Lignin	5.10	2.15	-	14.84
Hemicellulose	8.90	-	1.15	14.84
Organic Matter	80.15	-	9.84	14.00
Energy	6.30	-	1.55	32.63
Average	-	2.15	3.74	-

Table 5 : Comparison of the effect of Azotobacter species on the biochemical composition of cassava waste (%)

Component	Control (T)	Azotobacter Agili (T_2)	Azotobacter Vinelandii	Azotobacter beijerinckii (T_4)	Significance ± SE
Moisture	8.25[a]	15.25[b]	12.00[c]	17.25[d]	0.58*
Crude Protein	3.50[a]	13.00[b]	12.25[b]	8.75c	0.29*
Crude Fibre	10.75	1.95	11.15	11.20	0.59[NS]
Ether Extract	1.22	1.24	1.24	1.26	0.16[NS]
NFE	63.72	68.23	65.30	70.12	3.22[NS]
Lignin	7.25[a]	3.25[b]	4.30[0]	5.'10[d]	0.17*
Hemicellulose	7.75[a]	10.30[b]	9.25[b]	8.90[c]	0.20*
Organic Matter	70.23[a]	75.25[b]	72.50[c]	80.15[d]	3.45*
Energy	4.75[a]	5.90[b]	4.78[a]	6.30[c]	0.21*

a,b,c,d, values with different superscript in each row are significantly ($P < 0.50$) different
NS Not Significant ($P > 0.50$)

Table 6: Nitrogen Fractions of Azotobacter treated cassava waste

Component	Control (T)	Azotobacter Agili (T_2)	Azotobacter Vinelandii	Azotobacter beijerinckii (T_4)	Significance ± SE
Non Protein Nitrogen	8.05[a]	5.80[b]	4.23[c]	3.10[d]	1.12*
Total Nitrogen	0.560[a]	2.080[b]	1.960[c]	1.40[c]	0.78*
Acid detergent Insoluble nitrogen	0.040[a]	0.20[b]	0.14[b]	0.11[b]	0.15*
Neutral detergent	0.015[a]	0.18[b]	0.10[c]	0.09[c]	0.03*

a,b,c,d, values with different superscript in each row are significantly different ($P < 0.05$)
NS Not Significant

autoclaving of the samples prevented to some extent the growth of unwanted microbes. Table 1 gives a summary of the untreated cassava waste proximate composition (%DM, %CP, %EE, %CF) which is consistent with the values obtained in literature (1,9) Results of inoculation showed that the waste was of good inoculating material. The crude protein significantly increased ($P < 0.05$) to 13% (SE ± 0.29) after fermentation by *Azotobacter* bacteria strains. The crude protein after incubation (treatment 4 was significantly higher than those of control treatment (1) with pH 0.05 for treatments, 2.3 and 4 indicating that incubation alone led to increment of CP level in all the samples treated. The effects of the bacterial species (treatment 1-4) on the crude protein levels of the substrates could be differentiated statistically ($p < 0.05$) as follows *Azotobacter vielandii* and the least was recorded for *Azotobacter beijerinkii*. It has been observed earlier (10) that *Azotobacter* group of *bacteria* has the ability

to fix large amount of nitrogen (as much as 10mg/g of organic substrate respired). This group are the principal agents of aerobic nitrogen fixation in soil and water (2). The increase in crude protein level was significantly correlated (r= 0.74) with energy level due probably to the production of gummy extracellular polysaccharides by the bacterial species (giving their colonies of a mucoid appearance)

The NPN content was between 3.4 and 5.8%. The NPN component as reported by Licitra *et al* (7) was metabolically closer to soluble protein. This protein fraction is of vital importance to ruminant animals since it can only be digested in the lowergut. The NPN reported here was higher than the values reported for fungus treated waste paper, corn cobs, cotton wastes and untreated corn silage respectively. The levels of dry-matter as well as the proportion of the lignin were significantly different ($p< 0.05$) after the different treatments. The higher drymatter of *Azotpobacter beijerinckii* and *Azotobacter agilii* was consistent with report in literature (12).

As the level of the ether extract in all the samples remained constant, this component was not influenced by the processing method. As the energy level increased in the treated samples the higher level of the energy in the *Azotobacter* treated sample may be explained by higher crude and other proximate component of the samples.

The hemicellulose content of the treated samples was significantly higher than the control due probably to the large percentage of hemicellulose content in the bacterial (2).

However, the low lignin. like material of the bacteria (A. *agilii* and *A. vinelandii*) could have contributed to the slightly lignin content of the treated samples.

The culture conditions during the experiment resembled average in situ conditions. The growth and nitrogen fixing of these bacteria may be favoured according to the environmental conditions (temperature, pH and moisture). In conclusion, it is noteworthy that both incubation and bacterial growth were instrumental in increasing the crude protein, the hemicellulose and the energy content in cassava waste.

The effectiveness varied, between the specie of bacteria and was also associated with the environmental condition. From the perspective of increasing the crude protein, the hemicellulose and the energy contents of cassava waste, *Azotobacter agilii* was most successful of the tested strains followed by *Azotobacter vinelandii* and *Aziotobacter beijerinckii.*

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