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BIOCONVERSION OF BREWERS SPENT GRAINS (BSG) FOR POSSIBLE USE AS POULTRY FEED

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ABSTRACT: Analytical study was carried out to investigate the possibility of converting Brewers Spent Grains (BSG) into poultry feed. Sterile BSG samples were subjected to a 10 – day solid fermentation process using pure cultures of *Aspergillus niger* TF-4, *Saccharomyces cerevisiae* FP-4 and *Streptomyces* sp. UU-2 at room temperature (28 ± 2°C). The results of the proximate analysis revealed a general improvement in the biochemical composition of the fermented substrate, although its use as poultry feed may be hindered by the high fiber content and energy values of the resultant products. For example the crude protein content of the substrate was increased to appreciable levels but below the 19% required by broilers and 17% needed by layers. Enzymatic assay showed that none of the organisms tested possessed an "overcoming" enzymatic suite necessary for complete conversion of BSG to animal feed. However the improvement in quality of the biodegraded BSG with increase in fermentation period shows that the acceptable values of nutrients recommended for poultry nutrition may be attained if the fermentation process is prolonged using consortium of the test microorganisms. Prolong fermentation of BSG will ensure further or complete degradation of the fiber and the energy based components (fat and carbohydrate) of the substrate.

INTRODUCTION

A major problem in brewing industries in developing countries and newly industrialized countries is the management of spent grains. The disposal of spent grains on land and into water bodies has been of serious ecological hazards. The brewer's effluent and spent grains are highly acidic, sometime as low as pH 2.6 and combined waste water pH range of 3.5 - 5.2 has been reported (Zhang *et al*., 1992). The low pH may harm aquatic biota and prevent self purification of the receiving water body. Suspended solids settle on the stream bed and spoil fish breeding areas. The organic solid waste may decompose and deoxygenate the water (Saxena, 1983), while on land, the observed effect of excessive organic waste pollution include reduced plant growth, although low levels of contamination may stimulate growth (Zhang *et al*., 1992, Essien and Essien, 2006).

In some developed countries, brewer's spent grains are used in the production of biogas and bio-fertilizer (Hackenseller and Behmel, 1994). These often involve the extracellular hydrolytic enzymes activities of microorganisms. Microbial enzymes are notably produced by moulds, yeasts, bacteria and actinomycetes. These microorganisms are adapted to produce diverse and large amounts of hydrolytic enzymes *in situ* when introduced to an appropriate system. Hydrolytic enzymes include the amylases, proteases, lipases, pectinases, pullulanases, amyloglucosidases, glucomylases, cellulases, xylanases, chitinases and keratinases (Toye, 2002 and Sanchez-Porro *et al.*, 2003). These enzymes catalyzed the breakdown of different organic polymers such as starch, cellulose, protein, lipids, xylan, chitin and pectin into simpler monomeric compounds such as glucose, maltose, limit-dextrin, amino acids, esters etc for their

nutritional, biogeochemical recycling and diverse industrial benefits (Loeffler, 1986, Berry and Patterson, 1990, Crueger and Crueger, 1990, Bhat and Bhat, 1997). However, few investigations have been conducted on the possibility of converting BSG into nutrient-rich animal feed. For example BSG has been utilized as feed for cattle, poultry and hamsters (Cowling and Kirk, 1976; Zhang *et al*., 1992). In the present study attempt was made to convert BSG into poultry feed using *Aspergillus niger*, *Saccharomyces cerevisiae* and *Streptomyces* sp as the bio-degraders.

MATERIALS AND METHODS

Source of Brewer's Spent Grains (BSG) and Bio-degraders

Samples of wet distillers by-products of barley bran referred to as brewer's spent grains used in this investigation were obtained from Golden Guinea PLC, Umuahia, Nigeria. The biodegraders namely *Aspergillus niger* -TF4, *Saccharomyces cerevisiae* -FP4 and *Streptomyces* sp- UU2 were isolated from textile effluent, fresh palmwine and Uyo ultisol, respectively. The isolates were characterized and their identities confirmed according to the procedures described by Cowan (1985), Raper and Fennel (1974) and Kreger-van Rij (1984). The fungal isolates were purified and maintained on Sabouraud dextrose agar (DIFCO) plates, while pure cultures of the bacterium *Streptomyces* sp-UU2 was maintained on slightly acidic (pH 5.6) nutrient agar (DIFCO) plates. None of the bio-degraders selected have been implicated with poultry illnesses.

Biodegradation Study

The BSG samples were oven dried at 30^{o}C and then sterilized by autoclaving at 121^{o}C before fermentation. A modified solid state fermentation technique described by Singh *et al*., (1988) and Brimala *et al* (1994) was adopted. In this procedure 30g of sterile BSG samples were aseptically transferred to sterile 500 ml Erlenmeyer flasks, and inoculated separately with 10ml spore/cell (10^6 cells/ml) suspension obtained from 7 day old pure cultures of the test organisms. The inoculated flasks in replicates of three were allowed to ferment for 10 days at room temperature (28±2^{o}C). An un-inoculated flask containing 30g of sterile BSG served as the control. During fermentation sub-samples were taken 5cm below the surface with a sterile spoon, on the 3rd and 10th day of fermentation. The samples were transferred to sterile conical flasks and taken to the analytical laboratory.

Proximate Analyses of Biodegraded BSG and Enzyme Assay of Bio-degraders

The method for the determinations of dry matter, crude protein (Kjeldahl), crude fat, crude fibre, ash and total carbohydrate as well as the ascorbic acid content were those recommended by AOAC as described by Horwitz (1980) and Ranjhan and Krishna (1981).

The enzymatic potential of the bio-degraders were examined. The medium to detect protease production by *A. niger* and *S. cerevisiae* contained nutrient agar (DIFCO) to which gelatin was added as the protein source (0.4% final concentration) (Anagnostakis and Hankin, 1975). After incubation of inoculated plates and growth of the fungi, a saturated solution of ammonium sulphate was poured over the agar surface to enhance visibility of the zones of proteolysis. For the production of protease by the bacterial (*Streptomyces* sp) culture the medium described by Martley *et al.*, (1970) was used, except that the plate count agar (DIFCO) was substituted for standard methods agar (DIFCO). In this test protease production is seen as a precipitate of para-casein around the colonies. The medium described by Sierra (1975) with sorbitan monolaurate as the lipid source was used to detect lipase production for both bacteria and fungi. A precipitate of the calcium salt of the liberated fatty acid or a clear zone around colonies indicates lipase activity. Excretion of amylase was detected on a medium composed of nutrient agar (DIFCO) to which soluble starch was added (0.2% final concentration). After growth, an

iodine solution was poured over the agar surface to indicate degradation of the starch (Anagnostakis and Hankin 1975). The ability of the test organisms to produce cellulase was determined by the production of clearance zones in cellulose agar column using the method of Reutella and Cowling (1966).

The enzymatic potential of the test organisms was graded as very high, high or low based on observable response of the organism to test procedure.

RESULTS AND DISCUSSION

Proximate composition of the fermented BSG presented in Table 1 revealed an improvement in the nutrient contents of spent grains over time, as a result of biodegradation. The results showed a general increase in the crude protein content of the substrate to appreciable levels but below the 19% required by broilers and 17% needed by layers (Portsmouth, 1978, Sastry and Thomas, 1980). The increase in protein content of the fermented BSG may be partly due to the high protein content of microbial biomass (Singh *et al,* 1988), which in this case may have been imparted into the substrate as a result of the growth and proliferation the microbial degraders. It may also be as a result of the low proteolytic potential of the bio-degraders. This allows for the selective utilization of carbohydrates and fat as the major energy and carbon source leaving the protein content of the substrate underutilized. Proteins are essential constituent of muscle, blood, egg and feathers in birds. Excess proteins are usually broken down, some being used for energy purposes and the remainder being excreted via the feaces.

The fat content of the BSG was affected more positively by the activities of *A. niger* and *S. cerevisiae* than that of *Streptomyces* sp. Fat in its degraded form (fatty acids) is absorbed by birds and it provides fowl with its second source energy. High levels of fats sometimes cause digestive upsets and interfere with the utilization of other feed nutrients by birds (Rajhan *et al,* 1974, Portsmouth, 1978, Udedibe and Mba, 1994). When provided with the required amount of fat, birds will utilize linoleic and oleic acids in the substrate for health and productive reasons (Portsmouth, 1978). The activities of the bio-degraders also improved the ash content of the BSG, with *A. niger* giving the highest level of mineralization within 10 days of fermentation. The high ash content of the fermented BSG is an indication of high mineral component of the degraded grains. An appropriate level of minerals plays an important role in bird's health because they are essential for the maintenance of body processes. Portsmouth (1978) reported that birds skeleton contain mostly calcium and phosphorus while potassium, iodine and iron are found mainly in the muscle, thyroid gland and blood respectively. Although microbial degradation of BSG increases mineral content of the substrate its special effect on the quality of the mineral elements was not analyzed. However, by degradation necessary minerals may be provided by the process and at the required concentrations if supplemented by sources of calcium, phosphorus, iron, iodine, manganese, zinc and selenium which represent the major minerals required by birds (Rajhan *et al,* 1974).

The general improvement in the nutritional quality of the fermented BSG may be attributed to enzymatic activities of the bio-degraders. Results showed that the test organisms exhibited variable levels of proteolytic, cellulolytic lipolytic and amylolytic potentials (Table 2). During fermentation the protease elaborated by the degraders act on the protein content of the BSG and reduces it to its component amino acids. The lipase reduces crude fat to fatty acids and glycerol, cellulase reduces cellulose, a major component of BSG to its component sugars while the amylase catalyses the assimilation of the sugars by the biodegraders.

Table 1: Proximate properties (%) of the biodegraded BSG after fermentation with *Aspergillus niger* -TF4, *Saccharomyces cerevisiae* -FP4 and *Streptomyces* sp- UU2

Nutrient	*Recommended concentration.	Control		*A. niger* - TF4		*S. cerevisiae*- FP4		*Streptomyces* sp- UU2	
		0 day	3rd day	10th day	3rd day	10th day	3rd day	10th day	3rd day	10th day
Moisture	-	65.20	67.74	68.32	72.96	70.96	65.34	63.46	64.18	69.20
Crude protein	19.00	3.40	2.80	2.91	4.50	5.01	4.98	5.68	4.64	5.34
Crude fat	3.00	5.00	5.02	4.97	3.83	3.54	3.42	2.94	4.82	4.71
Ash content		3.00	4.59	5.21	9.10	9.25	7.19	8.40	8.70	8.89
Crude fibre	5.00	17.21	17.00	16.48	14.99	11.68	14.2	13.81	16.71	16.51
Carbohydrate	-	70.29	69.88	69.24	70.51	67.52	70.21	69.10	65.11	64.46
Energy value (Kcal/kg)	0.0025	348.76	335.90	333.33	334.51	321.98	331.54	325.62	322.38	321.59

Values are mean of three determinations *(Portsmouth, 1978)

Aspergillus niger showed a high cellulolytic, amlolytic and lipolytic capabilities but low proteolytic activity. *Saccharomyces cerevisiae* demonstrated very high lipolytic and amylolytic activities, a high proteolytic activity but low cellulolytic potential. While the *Streptomyces* sp - UU2 strain used exhibited a very high cellulolytic potential, a high proteolytic potential but low lipolytic and amylolytic activities. The deficiency in the nutritional quality of the bio-degraded BSG may be ascribed to the variation in the enzymatic potential of the bio-degraders. None of the organisms tested possess an "overcoming" enzymatic suite necessary for complete conversion of BSG to animal feed.

However their activities resulted in increase in moisture content of BSG, especially in grains degraded by the bacterium with little effect on the crude fibre and energy values of the substrate. The same was noticed in BSG degraded by the fungi. Very high moisture content is unacceptable in poultry feeds, although moisture is necessary for digestion and maintenance of homoeothermy in birds (Portsmouth, 1978, Sastry and Thomas, 1980). Feeds with high energy value usually have low fiber values (Portsmouth, 1978) but biodegraded BSG provide a food substrate rich in carbohydrate and fiber contents. Both components however are of very little value to poultry because food passes through the digestive tract and bacteria do not play a major role in digestion as they do in animals (Portsmouth, 1978). The same author reported that if fiber is fed at high levels it may be impossible for the birds to consume sufficient food to obtain adequate nourishment. The decrease in fiber content of the fermented BSG over time is therefore a desirable quality of poultry feed (Portsmouth, 1978).

Table 2: Enzymatic potential of the bio-degraders

Enzyme activity	*S. cerevisiae* – FP4	*A. niger* – TF4	*Streptomyces sp* – UU2
Amylolytic activity	+++	+++	+
Cellulolytic activity	+	+++	+++
Lipolytic activity	+++	++	+
Proteolytic activity	++	+	++

Footnote: +++ = very high activity, ++ - high activity, + = low activity, - = no activity

CONCLUSION

The bioconversion of spent grains would yield a nutritionally valuable feed for poultry, however, its utilization is limited by the high fiber and energy values of the resultant product (McCance and Widdowson, 1960). The research has shown that a longer fermentation period will ensure complete bioconversion of the carbohydrate and lipid content contents of BSG, and thus a reduction in fiber content. The results will be much better if the degradation is done by a consortium of the test organisms to ensure a complete degradation of the substrate and check excess moisture accumulation in the resultant feed. However the effect of prolonged fermentation on the amino acid properties of the feed is uncertain and deserves attention before BSG is recommended for poultry nutrition.

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