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Research Note

Ethanol production efficiency of various species of cassava effluents using different inoculants

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Abstract

The produce bio-fuel (ethanol) from cassava waste water using fungal enzymes from *Aspergillus niger* and *Rhizopus oryzae*, synthetic diastase enzyme; and to compare the fermentation of the various cassava specie is reported. Ethanol was produced from cassava effluents of four varieties of cassava: Type I (NR 8082), Type 2 (TMS 92/00057), Type 3 (NR 87184) and Type 4 (TMS 30572) using amylase enzyme produced from *Aspergillus niger, Rhizopus oryzae* and synthetic diastase. Type I and III varieties produced 30% each of total distillate volume yield, while Type II and IV produced 20% each of total distillate volume yield. Treatment of cassava effluent samples with *Rhizopus oryzae* gave overall ethanol distillate volume yield of 62%, followed by *Aspergillus niger* 25% and diastase 13%. A combination of cassava effluent Type and enzyme treatment R_3 gave significantly ($p<0.05$) the largest ethanol volume of 164.0±1.0 ml with a total percentage yield of 79%. Though A_1 (*Aspergillus niger* fermentation of Cassava Type I-NR 8082), followed closely with 123.0±2.5 ml, it had a comparatively lower percentage yield of 58% compared to R_2 and R_4 with 69% and 70% total per cent yield of ethanol, respectively. Thus ,the source of biochemical enzyme could be a determining factor in the percentage yield and volume of ethanol distillate produced rather than the choice of cassava specie.

Keywords: Ethanol, cassava specie, cassava effluents, enzyme treatment

Introduction

Cassava (*Manihot esculanta Crantz*) is extensively cultivated throughout the tropics for its starchy tubers. In Nigeria, it is a very important staple food eaten popularly as *garri, fufu, amala* and *lafun*. These food types are processed after the cassava tuber is peeled, grated, dewatered and fermented. The entire process and operations are necessary due to the peculiarities of the root tubers and these cause variable losses of cassava components (Meuser and Smolnik, 1980). These processes pose a lot of hazards both to the environment and the processor (Oboh, 2004). The two important wastes generated in the course of processing of cassava root tubers include the cassava peels, cassava effluents or waste water and the whey (the liquid out of the cassava mash). No utilization of cassava effluents has been made probably because of the cyanogenic glycoside content of cassava whey (Ihekoronye and Ngoddy, 1985).

Cassava waste water (effluent) is rich in various residual carbohydrates that could be hydrolyzed and fermented to produce bio-fuel in the form of ethanol to promote cleaner environment. The present study was aimed at not only producing alcohol using enzymes obtained from fungi (*Aspergillus niger* and *Rhizopus oryzae*) and synthetic diastase

but also to compare their efficiency in terms of volume of ethanol distillate produced with respect to the cassava specie.

Materials and Methods

Materials

Cassava tubers from Agricultural Development Project (ADP) Port Harcourt, Rivers state, Nigeria, were peeled and washed with clean water and grated. The grated pulp was filled into a polyethylene bag and sealed. The bag was then pressed between two metal slabs with a screw device to remove waste water (effluent) from the cassava pulp into a clean basin. The Cassava effluent was used for this study as the fermenting syrup. Active dried yeast was purchased from a grocery store in Owerri, Imo State, Nigeria. The fungi (*Aspergillus niger* and *Rhizopus oryzae*) were donated by the Department of Microbiology, University of Calabar, Nigeria, while synthetic diastase (analytical grade) was obtained from the Federal Institute of Industrial Research Oshodi (FIRRO), Lagos, Nigeria.

Sample preparation

Freshly harvested cassava roots were peeled, washed and grated. The mashed pulp was bagged and pressed according to local processing method. The resulting cassava effluent was collected in a bowl and allowed to settle for 2 hours. Cassava starch settled at the bottom of the bowl while waste water remained atop and was decanted.

Alcohol fermentation

50g each of cassava starch left in the bowl was dissolved in 100mls of distilled water and heated to 90^0C for 10min to gelatinize the starch. On heating, the mixture changed from a whitish coloured solution to a pale-yellow viscous gel. 300ml of distilled water was added to the gelatinized starch to reduce the viscosity. The addition of water lowered the temperature of the starch slurry in preparation for saccharification. At about 26^0C, samples were inoculated (saccharified) with 12ml of mineralized *Aspergillus niger* and *Rhizopus oryzae* and 5g of synthetic diastase enzyme thereafter, respectively. The mixtures obtained were buffered with 3mls each of phosphate buffer (85% KH_2PO_4) at pH 7. Fermentation was carried out with the addition of 1.5g of yeast extract and 1g of dextrose extract. Dextrose extract was added to accelerate the fermentation process as described by Mmegwa *et al*. (1989). *De novo* enzymes in the cassava waste water have been earlier inactivated by heating to 90^0C. The experimental mixtures were left to stand under anaerobic conditions at room temperature (25±2.0°C), for 6 days at pH 7±0.2, to ferment. After the fermentation process, it was observed that the mixture separated into a clear top layer and a bottom layer which contain sediments. The liquors were filtered with Whatman No. 4 filter paper and distilled with a quick-fit distillation apparatus equipped with a Lie-big condenser and in-let cold water to obtain ethanol distillate.

Determination of Ethanol Content by Refractometry.

The alcohol content (as % ethanol) of each test production was determined using a handheld Westward refractometer (Grainger, UK), according to the method of Colin and Julian (2004). In this method, the Brix (or % sugar) was determined in the mixture before and after fermentation at 20°C. The difference in sugar content was multiplied by 4 to afford the sugar content. The value of sugar content obtained was converted to an SG value and extrapolated to the corresponding alcohol content (Colin and Julian, 2004).

Statistical Analysis

Multiple comparisons were made for different parameters of samples, treatments and/or both samples and treatments. Analysis were carried out for significant differences by analysis of variance (ANOVA), using the statistical package (SPSS). Hypothesis was raised at 0.05 level of significance.

Hypothesis 1

H_o: Cassava Type I (NR 8082) = cassava Type 2 (TMS 92/00057) = cassava Type 3 (NR 87184) = cassava Type 4 (TMS 30572), where Types 1, 2, 3 and 4 are different cassava species.

H_1: At least one gives the highest alcohol distillate volume yield.

Hypothsis 2

H_o: Enzymic treatment with *Aspergillus niger* = *Rhizopus oryzae* = Diastase.

H_1: At least one enzyme is most effective.

Hypothesis 3

H_o: Each cassava Type and enzyme combination is as effective as the other.

H_1: At least one combination is different from the rest.

Results and Discussion

Table 1 shows ethanol volume (ml) output by direct measurement from each cassava variety treated with either *Rhizopus oryzae*, *Aspergillus niger* or diastase enzyme. R_3 (combination of Type 3 inoculated with *Rhizopus oryzae*) gave significantly ($p<0.05$) highest volume of ethanol yield. All *Rhizopus oryzae* (R) treated samples gave consistently higher

Table 1: Volume (ml) of Ethanol distillate with respect to Treatment

Treatment	Ethanol Volume(%)			
Type of Cassava (species)	I	2	3	4
Aspergillus niger (A)	123.0±2.5 ml	12.3±0.6 ml	19.0±2.5 ml	16.8±0.6 ml
Rhizopus oryzae (R)	76.5±2.7 ml	95.0±7.5 ml	164.0±1.0 ml	96.5±6.3 ml
Diastase (D)	13.0±5.0 ml	31.0±1.3 ml	24.0±3.8 ml	25.5±6.2 ml

Values are mean ± SD; n=3 and mean = SEM
Note
1 = Cassava Type I (NR 8082)
2 = Cassava Type 2 (TMS 92/00057)
3 = Cassava Type 3 (NR 87184)
4 = Cassava Type 4 (TMS 30572).

Table 2: % Distillate yield with respect to % conversion by enzyme

	(Ethanol A%)				
Type of Cassava species	I	2	3	4	% Conversion by enzyme
Aspergillus niger (A)	72	7	11	10	25
Rhizopus oryzae (R)	18	2	38	22	62
Diastase (D)	14	33	26	27	13

*Expressed as percentages of means.
1, 2, 3 and 4 as specified in Table 1
% Conversion = Initial cassava effluent – final cassava effluent x 100/Initial cassava effluent

Table 3: Cassava variety (Type) with respect to Ethanol content (%yield).

	(Ethanol Content A%)			
Sample(Type)/Treatments	I	2	3	4
Aspergillus niger (A)	58	9	9	12
Rhizopus oryzae (R)	36	69	79	70
Diastase (D)	6	22	12	18
% best yield	30	20	30	20

*Expressed as percentages of means.
1, 2, 3 and 4 as specified in Table 1

ethanol volumes compared to *Aspergillus niger* (A) treated samples, except for sample A_1 which gave a significantly high ethanol volume yield.

Rhizopus oryzae (Sample R_3) gave a significantly ($p<0.05$) highest distillate volume (164.0±1.0 ml) of ethanol (Table 1) corresponding to 79% ethanol content (Table 3) compared to all the treatments experimented in this study, probably because of more fermentable sugar content of Type 3 cassava variety, suggesting a possible relationship between specie, total fermentable sugar, volume of production and probably the type of enzyme treatment. There was a tie in the % best yield, for Types I and 3 (30%), and Types 2 and 4 (20%) probably because they are close varieties of each other (Table 3). The high ethanol distillate volume yield obtained from R_3 treatment (164.0±1.01 ml) and A_1 treatment (123.0±2.50 ml) respectively (Table 1), is consistent with the suggestion by Pandey (1999), that though several micro-organisms including bacteria, yeast and fungi may be capable of producing amylase, fungal species of *Aspergillus niger* and *Rhizopus oryzae* have notably been exploited for the production of amylases in submerged or solid state fermentation. Generally, *Rhizopus oryzae* treated samples gave higher volumes of ethanol distillates and %conversion (62%) compared to *Aspergillus niger* treated samples (25%) (Table 2). Thus, this study showed that *Rhizopus oryzae*, has a higher conversion efficiency of

cassava starch to sugars, which ultimately gave higher ethanol content than *Aspergillus niger.*

R_3 with a significantly ($p<0.05$) highest volume of ethanol distillate was followed by $A_1>R_4$, $R_2>R_1>D_2>D_4> D_3> A_3> A_4> D_1> A_2$. Treatment A_2 gave the least ethanol distillate volume (Table 3) and from the SPSS computations. Variability in the degree of ethanol yield by the various combinations of enzymes and treatments could be attributed to the ease of the hydrolysis by the enzymes and to some extent, on the peculiarities of the different varieties of cassava used. This is evident from the percentage (%) yield ties of the cassava varieties (Table 3).

Though all the enzyme treatments contained amylases that catalyzed the hydrolysis of the α- 1,4 glycosidic linkages of the polysaccharide starch chain in the cassava effluents, they did so in varying degrees and at varying points to give varying yields. The á-amylase of *Rhizopus oryzae* acts with a slightly different action pattern, and is more aggressive in the hydrolysis of starch, yielding mostly maltose and some oligomers. The *amyloglucosidase* (EC 3.2.1.3) from *Aspergillus niger* catalyses the breakdown of malto-oligosaccharides to glucose by breaking off glucose units from the non-reducing end of the polysaccharide chain. Thus, this process has found wide application in the food industry including its use for the saccharification of liquid starch (Pandey, 1999). On the other hand, synthetic diastase also catalyses the hydrolysis of á-1,4 glycosidic linkages of polysaccharides to yield dextrin, oligosaccharide, maltose and D-glucose. Synthetic diastase has broad substrate specificity and ability to hydrolyze starch and this property makes fungal diastase an ideal enzymatic supplement for optimum fermentation (Enzymeindia.com, 2006). However, *Aspergillus niger* and *Rhizopus oryzae* are the preferred fungal strains because they are from microbial sources and are safer and cheaper (Pandey, 1992). Fungal enzymes are comparatively cheaper and safer options in food processing and drug production than the use of synthetic diastase (Prapulla *et al*., 1999).

Conclusion

This study showed that amylolytic enzymes from *Rhizopus oryzae* may be considered the most suitable for the optimum production of ethanol when high quantity of ethanol or better efficiency is needed. The study also suggests that, the choice of enzyme rather than cassava variety may be the determining factor in the production of cassava ethanol .

References

Enzymeindia.com 2006. Advanced Enzyme Technologies where Enzyme is Life. www.enzymeindia. Com/enzymes/fungal-lipase.

Ihekoronye, A.I and Ngoddy, P.O. 1985. Integrated Food Science and Technology for the Tropics. Mcmllian Publishers, London. Pp 30–35.

Meuser, F. and Smolnik, H.D.1980. Starch Starke. *Cassava Processing*, **32**:116–122.

Mmegwa, S. V. A., Balogh, E. and Ngoddy, P. O.1989. Formulated Palm wine: Comparative Fermentation Studies. *Nig. Food J.* **6:**35-42.

Oboh, G. 2004. Management of Occupational Hazards Associated with Traditional Methods of Cassava Processing in Ogun, Ondo and Oyo States, Nigeria. In : Proceedings of a Workshop on Promotion of Improved Management Technology Aimed at Reducing Occupational and Environmental Harzards Associated with Traditional Cassava Processing in Ogun and Ondo States. Organized by Institute of Agricultural Research & Training, Obafemi Awolowo University, Ile-Ife, Nigeria. pp. 11-20.

Pandey, A., Selvakumar, P., Soccol, C.R. and Singh –Nee Nigam, Poonam 1998 .Solid State Fermentation for the Production of Industrial Enzymes. *J Biom Sci*. **77**:149-162.

Prapulla, S.G., Subheprada V., Kaworth, N. G.1999. Microbial Production of Oligosaccharides. *Adv App Microb*., **47**:270-337.

Colin, E W and Julian, D.C. Jones 2004. *Handbook of Laser Techn and Applic.* Taylor & Francis. pp. 1738-1740.