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Research Note

Comparative study of the efficacy of fungal and diastase induced fermentation of cassava effluents in production of ethanol

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Abstract

The yield of conversion of absolute ethanol (A% by weight) from four varieties of cassava roots namely: NR 8082 – Type I, TMS 92/00057- Type II, NR 87184 – Type III and TMS 30572 – Type IV- were determined using two fungi (*Aspergillus niger and Rhizopus oryzae*) and synthetic diastase as sources of hydrolytic enzymes. Results showed that Type II cassava produced significantly ($p<0.05$) the highest ethanol percentage by weight (Alc 9.5% by wt) and a best yield of 46%. Sample A_2 combination of enzyme treatment of Type II with *Aspergillus niger*, had significantly the highest ($p<0.05$) ethanol percentage by weight followed by A_3, A_1,D_2/R_2, D_4, R_3, D_1, A_4 and R_4 as the least. *Aspergillus niger* had the high yield of total percentage of conversion (62%) of cassava to ethanol concentration, 22% and 16% respectively for diastase and *Rhizopus oryzae*.

Keywords: Cassava, waste water, ethanol, amylolytic enzymes.

Ethanol is generally produced by fermentation of sugar, cellulose or converted starch. In Nigeria, West Africa, local production of ethanol from maize, guinea corn, millet, other starchy substrate and cellulose abound. Cassava root has very little protein, 5% sugars and 30% starch (O'Hair, 1995 and FoodMarket Exchange.com, 2006) making it one of the richest fermentable substances for the production of alcohol. Nigeria is a large producer of cassava (O'Hair, 1995) which is readily available in Nigeria because of the need for its various utilization as *gari*, *fufu* flour/dough, *tapioca*, *lafun*, *kpokpogari*. The abundance and availability of cassava make it suitable for use in ethanol production.

The yield of conversion of absolute alcohol (concentration of alcohol) from cassava roots depends on the variety of cassava and the method of manufacture (FoodMarketExchange .com, 2006). This study aimed at comparing the yield of conversion of absolute alcohol from four varieties of cassava waste water (by-product arising from cassava processing) using amylolytic enzymes from three different sources namely- fungi (*Aspergillus niger* and *Rhizopus oryzae*) and synthetic diastase.

Materials and Methods

Cassava waste water (effluents) used for this study were obtained from Agricultural Development

Project (ADP) Port Harcourt, Rivers state, Nigeria. Active dried yeast was purchased from the grocery store. The other micro-organisms were collected from the department of microbiology, University of Calabar and Rivers State University of Science and Technology, Port Harcourt (RUST) while the synthetic diastase was from the Federal Institute of Industrial Research Oshodi (FIRRO), Lagos, Nigeria. The chemical (synthetic diastase) was of analytical grade.

Sample preparation

Freshly harvested cassava roots were washed and grated. The mash was bagged and pressed. The resulting liquor (cassava whey/effluent) was collected. The waste water was kept for about 2 hours which gave ample time for the cassava starch in the waste water to settle. The water was decanted.

Waste water from four varieties of cassava roots were treated similarly. 50g of each of the cassava starch in the waste water was dissolved in 100 ml of distilled water and made to volume according to the method of Wang (2002). Samples were inoculated with 12 ml of mineralized *Aspergillus niger* and *Rhizopus oryzae* and 5g of diastase was respectively. 3 mls of phosphate buffer at pH 7 was also added. 1.5g of yeast extract and 1g of Sabraud dextrose extract were added as supplements. After the 144th (6 days), steam distillation was carried out and the ethanol distillate was obtained.

The alcohol content of distillates was determined. Refractometer readings were taken to obtain the refractive index and ethanol concentration (Alc % by wt) extrapolated.

Statistical Analysis

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

Hypothesis 1, H_o: Cassava Type I = cassava Type II = cassava Type III = cassava Type IV. H_1: At least one gives the best yield of concentration of alcohol.

Hypothsis II, H_o: Enzymic treatment with *Aspergillus niger* = *Rhizopus oryzae* = Diastase. H_1: At least one enzyme is most effective.

Hypothesis III, 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. Ethanol concentration (A% by wt) of Waste water from cassava varieties (Type), enzymes and combinations of Type and enzyme treatments

	(A% by wt)			
Type/Sample Treatments	**I**	**II**	**III**	**IV**
Aspergillus niger (A)	3.90±0.25	9.75±0.44	5.35±0.32	2.1±0.25
Rhizopus oryzae (R)	0.38±0.31	2.90±0.25	2.10±0.25	0.13±0.14
Diastase (D)	2.10±0.25	2.90±0.25	0.55±0.13	1.65±0.39

Means ± Standard deviation

Table 2. Cassava varieties (Type) percentage yield and total percentage best yield.

	(%)			
Type/Sample Treatments	**I**	**II**	**III**	**IV**
Aspergillus niger (A)	18	46	26	10
Rhizopus oryzae (R)	7	46.6	38	2
Diastase (D)	28	41	8	23
% Best yield	17.6	46	24	11.6

*Expressed as percentages of means.

Table 3. Alcohol concentration (A%) percentage yield from treatments of different cassava varieties (Type) and percentage conversion by enzymes.

	(%)				
Type/Sample Treatments	**I**	**II**	**III**	**IV**	**Percentage conversion by enzymes**
Aspergillus niger (A)	61	60	67	54	62
Rhizopus oryzae (R)	6	20	26	3	16
Diastase (D)	33	20	7	43	22

*Expressed percentages of means.

Results of Table 1 and Table 3 show that treatment with *Aspergillus niger* gave the highest ethanol concentrations while Table 2 shows that Type II (46% yield) was from the cassava sample with the highest value.

Sample A_2 (Table 1) being significantly ($p<0.05$) the best combination of sample and treatment followed by A_3, A_1, D_2/R_2, D_4, R_3, D_1, A_4 and R_4 as the least in terms of effective sample and treatment combination for the production of the strength of ethanol (ethanol concentration /value -A%) showed great variability. This could be due to the amounts of fermentable sugar the cassava samples have as well as the ease of the hydrolysis by the enzymes from different sources using *Aspergillus niger*, *Rhizopus oryzae* and synthetic diastase, accordingly. Along this line, Type IV samples D_4, A_4, R_4 in Table I and Table 2 with the lowest percentage ethanol content could be because of its high content of cellulose and hemicellulose which bind the carbohydrates and make them unavailable for hydrolysis by amylase enzymes (Ehlers, 1979) from the samples. Thus, the strength of ethanol (ethanol concentration) is dependent on the Type of cassava specie.

Aspergillus niger had the highest percentage conversion (62%, Table 3) of cassava species showing good yield efficiency. Statistical analysis of treatment of samples showed that *Aspergillus niger* treatment was followed by treatment with diastase and lastly *Rhizopus orzyae.* Quadrate *et al.* (1962) had earlier reported a similar trend whereby there was a 35.8% and 30.3% conversion of cooked cassava starch to maltose by amylase and diastase respectively showing a higher conversion of cooked starch by microbial amylase than by diastase. Results are in line with those reported by Rasper *et al*, (1974) who found a very low conversion of native starch to maltose using malt diastase (<0.02%) but higher conversion levels using alpha amylase and fungal amyloglucosidase (4.7% and 4.4%) respectively. Results also agree with those of Toope *et al.* (1983) that the use of enzyme is a better alternative to chemical addition due to the production of some undesirable end products after the chemical synthesis.

Unlike bacterial alpha- amylase that liquefies starch by randomly attacking the alpha bonds, fungal alpha-amylase produced by *Aspergillus niger* saccharifies cassava starch by attacking the second linkage from the non-reducing terminals (i.e C4 end) of the straight segment resulting in the splitting of two glucose units (maltose) at a time. The bond breakage is thus more extensive in saccharifying enzymes than in liquefying enzymes (Wang, 2002). The extensive bond breakage accounts for the higher conversion ability of *Aspergillus niger.*

Furthermore, a single strain of the fungus, *Aspergillus niger* is capable of producing both α - amylase and glucoamylase -amylogucosidase enzymes (Pandey *et. al.,* 1998) though the degree may vary. It is likely, therefore, that after the extensive bond breaking by the fungal α- amylase of *Aspergillus niger* to give maltose (2- glucose units), glucoamylase catalysis the breakdown of maltose. It decomposes starch finally to glucose by tearing off glucose units from the non-reducing end thereby saccharifying the liquid starch. Results are in line with the findings of Prapulla *et al.* (1999) that starch hydrolysis using fungal enzymes to produce simple saccharides is comparatively better and cheaper than the use of any synthetic source or process (in this case, synthetic diastase). *Aspergillus niger* with significant ($p<0.05$) highest ethanol concentration yield (Table 1 and Table 3) suggest that the strength of ethanol would also be dependent on the enzymatic treatment applied apart from the type of cassava variety used.

Conclusion

The strength of ethanol (A% by wt) is dependent on the cassava variety and the source of enzyme used. The microbial enzyme sources were better alternatives to chemical enzymes.

References

Ehlers, G. M. 1979. Use of fungal amylase and amyloglucosidase in the production of high dextrose syrup. Novo. Literature B. 3276-G. B.

FoodMarketExchange 2006. Application of cassava in fermented products. http//www.foodmarketexchange.com/datacenter/product/feedstuff/tapioca/detail/dcpift…1/25.

O'Hair, Stephen K. 1995. Cassava. New crop factsheet. Tropic research and education center, university of Florida. http//www.hort.purdue.edunewcrop/crops/cropfactsheet…2001.

Pandey, A., Selvakumar, P., Soccol, C.R. and Singh – Nee Nigam, Poonam 1998. Solid State Fermentation for the Production of Industrial Enzymes. Current science. *Journal of biomedical sciences*. **77**(1): 149 – 162.

Prapulla, S. G., Subhaprada, V. and Kaworth, N. G. 1999. Microbial production of oligosaccharide. *Advance applied microboiology*. **47**: 270 – 337.

Quadrat -I-Khuda, M., De H. N. and Desnath, J. C. 1962. Studies on indigenous starches of Pakistan. Part II Pakistan *Journal of Scientific and Industrial Research*, **5**: 30 – 34.

Toope, F., Simon, E. S., Bendarkski, M.D., Whitesides, G. A. 1983. Enzyme catalysed synthesis. *Carbohydrate Science*. **18**: 72-76.

Wang, N. S. 2002. Starch hydrolysis by amylase. Department of Chemical Engineering, University of Maryland. College park, MD 20742-2222. ENCH.485. Experiment No. 5.