Optimized Fermentation Process for Improved Bioethanol Production from Sweet and Bitter Cassava Processing Wastes

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Abstract: Optimization of fermentation process for 72 h was carried out to determine the effect of pH (3 – 8), temperature (30°C – 50°C) and agitation rate (200 rpm – 500 rpm) on bioethanol production and microbial count using cassava variety samples. Bioethanol yield and microbial count were highest at pH 6, temperature 35°C and agitation rate of 300 rpm. The bacterial species had highest population counts of 5. 21 x 10^5 cfu/g at pH 6, 4.62 x 10^5 cfu/g at temperature 35°C and 4.84 x 10^5 cfu/g at pH 6, 3.94 x 10^5 cfu/g at temperature 35 °C for sweet and bitter cassava varieties respectively. The fungal species had highest counts of 5.60 x 10^4 cfu/g at pH 6, 2.53 x 10^4 cfu/g at temperature 35°C and 5.20 x 10^4 cfu/g at pH 6, 2.11 x 10^4 cfu/g at temperature 35°C for sweet and bitter cassava varieties. Bioethanol yield was 56% and 44% resulting in a significant increase of 75% and 63% for the sweet and bitter cassava varieties respectively. The optimization of the fermentation process yielded maximum bioethanol and also detoxified the cassava processing wastes which are environmental pollutants.

Key words: Bioethanol, Bitter cassava, Optimized fermentation process, Processing Wastes, Sweet Cassava

Introduction

Bioethanol is produced by hydrolysis and fermentation of carbohydrate feed stocks (Nuwamanya *et al*., 2011). It is a microbiological way of converting simple sugar into ethanol and carbon dioxide (Oyeleke *et al*., 2012). Ethanol has been promoted as a solution for a variety of complex problems relating to energy and environment. Compared to fossil fuels, ethanol has the advantage of being renewable, providing cleaner burning, having high octane rating and producing no greenhouse gases. Bioethanol is a principal fuel that can be used as petrol substitute for vehicle. By blending ethanol with gasoline, the fuel mixture is oxygenated so it burns more completely and reducing pollution emissions (Oyeleke *et al.*, 2012). The most common blend is 10% ethanol and 90% petrol (E10) (Lin and Tanaka, 2006). Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the green house gas effect (Anuj *et al*., 2007).

Global climate issues, energy crisis and increase in crude oil prices are the reasons for diversion of food resource for biofuel production (Nuwamanya *et al*., 2011). Cassava (*Manihotesculenta* Crantz) a crop crucial for food security in Nigeria has become an important biofuel crop aside from its traditional role as a food crop. The high productivity and yield of cassava (Ziska *et al*., 2009), along with its ability to grow in marginal soils (Dixon *et al.*, 2002), requiring minimum labour (Chiwona – karltum *et al*., 1998) and management costs (Nuwamanya *et al*., 2011), high amounts of easily hydrolysable biomass and high content of dry matter (Kosogi *et al*., 2009) has placed it among the candidates for bioethanol production.

The peels (about 10 – 35% of weight of the tuber) are usually discarded and allowed to rot leading to foul odour and sometimes poisonous and polluted air. Vegetation and soil around the heap of peels are rendered unproductive and devastated. The liquid effluent, which contains microorganisms capable of harnessing the glucosides during the fermentation process, is released into the soil and water bodies thereby causing environmental pollution. These cassava processing wastes if harnessed properly is a potential feedstock for energy production (Obadina *et al*., 2006). The ability to produce sufficient amount of reducing sugar determines the importance of a particular feedstock for ethanol production (Agbogbo and Wenger, 2007). The low ethanol yield produced in the previous study (Obueh, 2014) from cassava wastes (32% and 27% for sweet and bitter cassava varieties respectively) was due to hydrolysis as well as the amounts of the total carbohydrate coupled with a significant portion of proteinous matter in the peels (Ballesteros *et al*., 2000). This study was therefore to optimize the fermentation process in order to improve bioethanol production from the sweet and bitter cassava processing wastes.

Materials and Methods

Sample Collection

The sweet and bitter cassava variety tubers were collected from a farm at Ekiadolor in Edo State, Nigeria. The tubers were washed and peeled. The peels were dried and ground with an electric blender. The liquid effluent from pressed fermenting cassava from a local cassava mill in Ekiadolor was collected into a clean 10 litre gallon.

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Sample Preparation for Fermentation

The sweet and bitter cassava varieties were processed for fermentation. Weighed 200 g ground peels were added to 150 ml of cassava effluent in a 500 ml conical flask. Sterile distilled water was added to make up the 500ml mark of the flask and the flasks were stopped with sterile cotton wool covered with aluminum foil. The mixtures were sterilized in an autoclave at 121^0C for 15 min to gelatinize the starch. They were allowed to cool and sterile distilled water was aseptically added to make the 500 ml mark of the flask again. Sample pretreatment was carried out using α – amylase for 2 h at pH 5.5 at 100 °C and cooled to 60 °C. Then β – amylase added at pH 4.5 at 55 °C for 24 h. The samples were then centrifuged and the supernatant analyzed for reducing sugar content by the method of Miller (1959) modified by Kimaryo *et al*. (2000). The supernatant was transferred into a sterile container which acted as fermenter. Sterile distilled water was added, sterilized and cooled to 30 °C. The pH of the sample was determined by the method of AOAC (2000) using a HANNA Combo pH meter. Prepared 20% w/v *Saccharomyces cerevisiae* isolates, representing 20 ml, were aseptically inoculated into the sample(Obueh and Ikenebomeh, 2014) and fermentation of the cassava wastes took place for 72 h.

Microbiological Analyses

The bacterial and fungal counts of the substrates were determined using pour plate technique (Ezeama, 2007). Ten milliliter (10 ml) of wastes collected during the fermentation process were aseptically transferred into 90ml of sterile distilled water to give a 10^{-1} dilution and serial dilutions prepared from the suspension to give a range of 10^{-6}. From these dilutions 1ml was aseptically plated out using pour plate method for total viable counts on Nutrient Agar (Lab M Ltd UK) and total fungal counts on Potato Dextrose Agar (PDA) (Lab M Ltd UK) supplemented with 10% lactic acid and 0.5% chloramphenicol (AOAC, 2001). The colonies were observed and counted in a Techmel and Techmel USA Counter Model TT 201. The results were expressed as colony forming units per gram (cfu/g).

Optimization of Fermentation Conditions

Batch fermentation was carried out for 72 h to study the effect of pH, temperature and agitation rate on ethanol production and microbial count. The initial pH was adjusted to 3, 4, 5, 6, 7 and 8 using acetate or phosphate buffer. The effect of temperature was determined with temperature range of 30 °C, 40 °C, 45 °C and 50 °C. Adjustment of factors was done 8 hourly. Samples were aseptically collected to determine the parameters tested (Neelakandan and Usharanti, 2009). Effect of agitation rate was determined at agitation speed of 200 rpm, 300 rpm, 400 rpm and 500 rpm (Ado *et al*., 2009). The flasks were incubated with shaking at 30 °C for 72 h. Aliquots of 30 ml were collected 24 h during fermentation to determine ethanol yield and microbial cell biomass. Cell biomass was determined by centrifuging 10 ml sample, drying the cells obtained to constant weight at 60 °C (Ado *et al*., 2009). At the end of fermentation, the pH, temperature and agitation rates that produced the highest concentration of ethanol and microbial count were considered best for ethanol production. Fermentation was again carried out at these values to determine the ethanol concentration and yield.

Ethanol Concentration and Yield

The distillate collected was measured using a measuring cylinder and the ethanolconcentration was determined by measuring its specific gravity after distillation (Maiorella *et al*., 1981). The specific gravity value was used to determine ethanol concentration from a standard curve prepared using known concentrations of ethanol (Ado *et al*., 2009). Alcohol yield was read with an alcohol meter (Distillique, South Africa).

Statistical Analysis

The results were presented as mean standard values of triplicates of results. A One – Way Analysis of Variance ANOVA and student's t – test was carried out (Ogbeibu, 2005). Significant difference was accepted at $P \leq 0.05$.

Results

The effect of varying pH on microbial count during the optimized fermentation process is presented in Table 1. The bacterial counts increased gradually from pH 3 to 6 and then declined from pH 7 to 8 during each fermentation time. At pH 3, the bacterial counts increased slightly from 0 – 72 h of fermentation and decreased at pH 8 at 72 h of fermentation. The highest increase at the end of fermentation was at pH 6 with 5.21 x 10^5 cfu/g for the sweet cassava variety and 4.84 x 10^5 cfu/g for the bitter cassava variety. There was gradual increase for the fungal counts at pH 3 to 6. At pH 3 and 4, there was decrease in fungal counts from 48 h of fermentation for both varieties. At pH 5 to 7, there was increase in the fungal counts throughout the fermentation time. The fungal counts decreased from 24 h of fermentation with no change in the counts at pH 8. The fungal counts were highest at pH 6 with 5.60 x 10^4 cfu/g for the sweet cassava variety and 5.20 x 10^4 cfu/g for the bitter cassava variety. The highest concentration of ethanol produced at pH 6 was 163 cm^3 and 120 cm^3 for the sweet and bitter cassava varieties respectively (Figure 1). During fermentation at 0 – 72 h of fermentation, the bacterial counts increased from 30 °C – 35 °C for both cassava varieties with decline from 40 °C – 50 °C (Table 2). Highest values for the bacterial counts was recorded at 35 °C at the end of fermentation with 4.62 x 10^5 cfu/g for the sweet cassava variety and 3.94 x 10^5 cfu/g for the bitter cassava variety. The fungal counts increased during fermentation with decrease at the end of fermentation for 45 – 50 °C. The fungal counts had highest values at 35 °C with 2.53 x

10^4 cfu/g and 2.11 x 10^4 cfu/g for the sweet and bitter cassava varieties respectively. The concentration of ethanol was highest at 35 °C with 119 cm^3 for the sweet cassava variety and 102 for the bitter cassava variety (Figure 2). In table 3 is presented the effect of agitation rate on ethanol production during optimized fermentation process. Reducing sugar converted at the end of fermentation was highest at 300 rpm with 52.81% for sweet cassava variety and 45.37% for bitter cassava variety. The lowest reducing sugar converted was at 500 rpm for both cassava varieties. The cell dry weight (growth of microorganisms) was highest in the sweet cassava variety than the bitter cassava variety. Cell dry weight reduced as agitation rate increased during fermentation of the cassava varieties. At 72 h of fermentation, ethanol concentration was highest at 300 rpm with volume of 143 cm^3 for sweet cassava variety and 125 cm^3 for bitter cassava variety.

Table 1: Effect of pH on Microbial Population during Optimized Fermentation Process

Microbial Count	Variety	Fermentation Time (h)	pH 3	4	5	6	7	8
Bacterial Count	**Sweet**	**0**	2.31×10^{5a}	2.43×10^{5a}	2.49×10^{5a}	2.51×10^{5a}	$2.14x10^{5a}$	2.07×10^{5a}
	Bitter		2.11×10^{5a}	2.27×10^{5a}	2.31×10^{5a}	2.36×10^{5a}	1.90×10^{5a}	1.69×10^{5a}
	Sweet	**24**	2.78×10^{5a}	2.85×10^{5a}	2.86×10^{5a}	2.95×10^{5a}	$2.36x10^{5a}$	2.19×10^{5a}
	Bitter		2.63×10^{5a}	2.72×10^{5a}	2.74×10^{5a}	2.76×10^{5a}	2.06×10^{5a}	1.92×10^{5a}
	Sweet	**48**	2.80×10^{5a}	3.12×10^{5a}	3.95×10^{5a}	4.07×10^{5a}	$2.38x10^{5a}$	2.23×10^{5a}
	Bitter		2.65×10^{5a}	2.95×10^{5a}	3.12×10^{5a}	3.91×10^{5a}	2.18×10^{5a}	2.01×10^{5a}
	Sweet	**72**	2.82×10^{5a}	3.46×10^{5a}	4.94×10^{5a}	5.21×10^{5a}	$2.42x10^{5a}$	1.87×10^{5a}
	Bitter		2.68×10^{5a}	3.16×10^{5a}	4.44×10^{5a}	4.84×10^{5a}	2.25×10^{5a}	1.61×10^{5a}
Fungal Count	**Sweet**	**0**	$1.40x10^{4a}$	1.30×10^{4a}	1.50×10^{4a}	1.60×10^{4a}	1.50×10^{4a}	1.30×10^{4a}
	Bitter		1.10×10^{4a}	1.10×10^{4a}	1.20×10^{4a}	1.40×10^{4a}	1.40×10^{4a}	1.00×10^{4a}
	Sweet	**24**	$2.10x10^{4a}$	2.30×10^{4a}	2.40×10^{4a}	2.60×10^{4a}	2.10×10^{4a}	1.50×10^{4a}
	Bitter		1.70×10^{4a}	2.10×10^{4a}	2.20×10^{4a}	2.40×10^{4a}	2.00×10^{4a}	1.10×10^{4a}
	Sweet	**48**	$1.80x10^{4a}$	2.10×10^{4a}	4.20×10^{4a}	4.40×10^{4a}	2.90×10^{4a}	6.00×10^{3a}
	Bitter		$1.20x10^{4a}$	1.90×10^{4a}	4.00×10^{4a}	4.40×10^{4a}	2.60×10^{4a}	3.00×10^{3a}
	Sweet	**72**	$7.00x10^{3a}$	1.50×10^{4a}	4.90×10^{4a}	5.60×10^{4a}	3.20×10^{4a}	6.00×10^{3a}
	Bitter		$6.00x10^{3a}$	1.20×10^{4a}	4.80×10^{4a}	5.20×10^{4a}	2.90×10^{4a}	3.00×10^{3a}

Paired variables (sweet and bitter cassava varieties) for each varying pH with the same superscript are not significantly different at$P>0.05$.

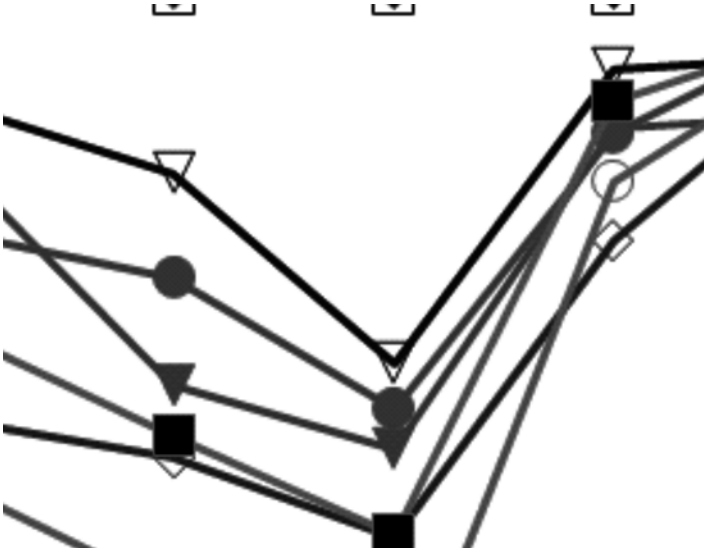

Figure 1: Amount of Ethanol Produced at Different pH by Sweet Cassava (SC) and Bitter Cassava (BC) Varieties

Table 2: Effect of Temperature on Microbial Population during Optimized Fermentation Process

			Temperature (°C)				
Microbial Count	**Variety**	**Fermentation Time (h)**	**30**	**35**	**40**	**45**	**50**
Bacterial Count	**Sweet**	**0**	1.53×10^{5a}	1.84×10^{5a}	1.12×10^{5a}	9.10×10^{4a}	8.10×10^{4a}
	Bitter		1.33×10^{5a}	1.65×10^{5a}	1.02×10^{5a}	8.10×10^{4a}	5.60×10^{4a}
	Sweet	**24**	1.83×10^{5a}	2.31×10^{5a}	1.41×10^{5a}	1.42×10^{5a}	9.50×10^{4a}
	Bitter		1.61×10^{5a}	2.12×10^{5a}	1.22×10^{5a}	9.70×10^{4a}	8.00×10^{4a}
	Sweet	**48**	2.93×10^{5a}	3.84×10^{5a}	2.10×10^{5a}	1.76×10^{5a}	1.51×10^{5a}
	Bitter		2.46×10^{5a}	3.16×10^{5a}	2.01×10^{5a}	1.42×10^{5a}	1.10×10^{5a}
	Sweet	**72**	3.12×10^{5a}	4.62×10^{5a}	2.31×10^{5a}	1.97×10^{5a}	1.63×10^{5a}
	Bitter		2.71×10^{5a}	3.94×10^{5a}	2.16×10^{5a}	1.60×10^{5a}	1.51×10^{5a}
Fungal Count	**Sweet**	**0**	8.50×10^{3a}	9.10×10^{3a}	6.00×10^{3a}	5.10×10^{3a}	3.10×10^{3a}
	Bitter		6.60×10^{3a}	7.90×10^{3a}	4.30×10^{3a}	3.50×10^{3a}	2.90×10^{3a}
	Sweet	**24**	9.30×10^{3a}	1.13×10^{4a}	7.80×10^{3a}	6.20×10^{3a}	2.70×10^{3a}
	Bitter		7.40×10^{3a}	1.02×10^{4a}	6.30×10^{3a}	4.50×10^{3a}	1.70×10^{3a}
	Sweet	**48**	1.32×10^{4a}	1.68×10^{4a}	9.30×10^{3a}	3.20×10^{3a}	2.10×10^{3a}
	Bitter		1.14×10^{4a}	1.42×10^{4a}	7.40×10^{3a}	2.10×10^{3a}	1.10×10^{3a}
	Sweet	**72**	1.74×10^{4a}	2.53×10^{4a}	9.80×10^{3a}	2.40×10^{3a}	1.90×10^{3a}
	Bitter		1.65×10^{4a}	2.11×10^{4a}	8.20×10^{3a}	1.60×10^{3a}	1.00×10^{3a}

Paired variables (sweet and bitter cassava varieties) for each varying temperature with the same superscript are not significantly different at P>0.05.

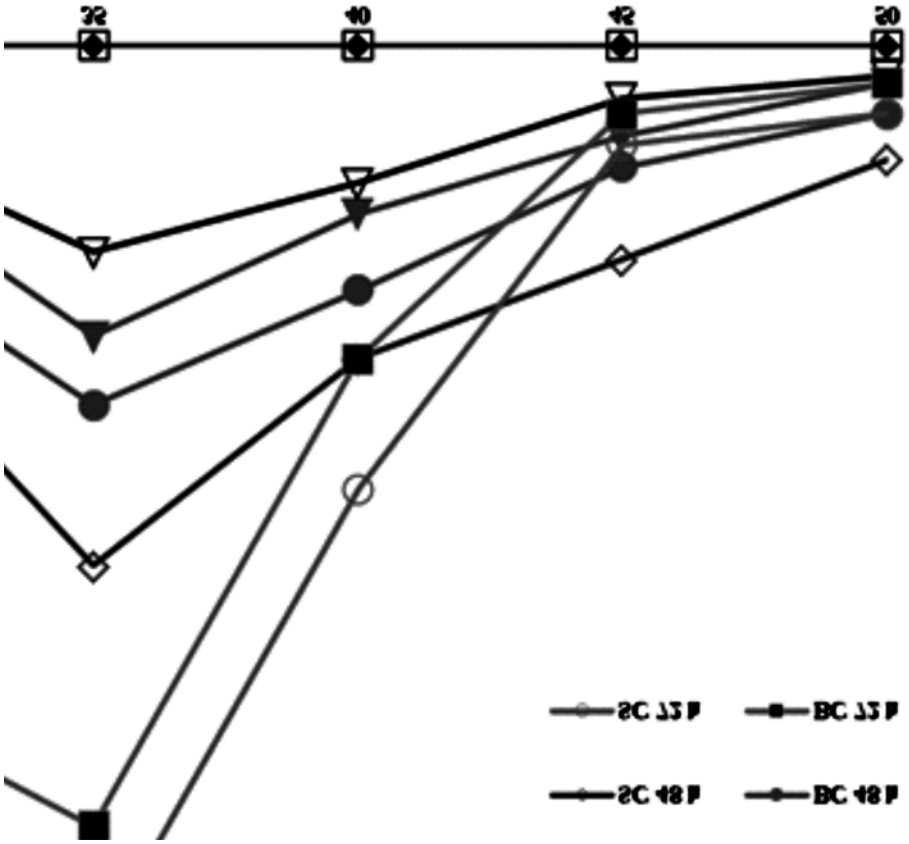

Figure 2: Amount of Ethanol Produced at Different Temperature by Sweet Cassava (SC) and Bitter Cassava (BC) Varieties

Table 3: Effect of Agitation Rate on Ethanol Production from Sweet and Bitter Cassava Varieties in Optimized Fermentation Process

Agitation Rate (rpm)	Reducing Sugar (%)				Cell Dry Weight (g/100ml)				Volume of Ethanol (cm^3)	
	Sweet Cassava Variety		Bitter Cassava Variety		Sweet Cassava Variety		Bitter Cassava Variety		Sweet Cassava Variety	Bitter Cassava Variety
	BF	AF	BF	AF	BF	AF	BF	AF		
200	63.21	28.32	61.29	30.14	1.7	1.3	1.8	1.1	118	92
300	63.21	10.40	61.29	15.92	1.6	1.4	1.8	1.2	143	125
400	63.21	37.11	61.29	40.16	1.7	0.9	1.7	0.5	58	42
500	63.21	39.36	61.29	45.34	1.6	0.4	1.5	0.3	40	36

BF= Before Fermentation AF= After Fermentation

In Table 4 is presented the properties of ethanol produced when fermentation of the processing wastes of the sweet and bitter cassava varieties occurred naturally and during the optimized fermentation process at pH 6, temperature 35 °C and agitation rate of 300 rpm. At the end of fermentation, the volume of ethanol produced was 183 cm^3, specific gravity 0.8321 and mass 152.27 g for the sweet cassava variety after 63.18% reducing sugar was converted. The volume of ethanol produced was 145 cm^3, specific gravity 0.9240 and was 133.98 for the bitter cassava variety after 40.10% reducing sugar converted. This value of the reducing sugar was higher than that produced for the normal fermentation process with 22.12% for the sweet cassava variety and 16.12% for the bitter cassava variety of the peel and effluent wastes. The actual ethanol yield was 56% and 44% for the sweet and bitter cassava varieties respectively. There was an increase of 75% for the sweet cassava variety with initial 32% yield and increase of 63% for the bitter cassava variety with initial 27% yield.

Theoretical yield was 57.7% for the sweet cassava variety and 46.6% for the bitter cassava variety. This is different from the initial 35.3% and 30.5% for the sweet and bitter cassava varieties respectively. The fermentation efficiency was 97% for the sweet cassava variety and 94% for the bitter cassava variety. The values were higher than the initial 91% for the sweet cassava variety and 89% for the bitter cassava variety.

Table 4: Properties of Ethanol Produced from the Processing Wastes of Sweet and Bitter Cassava Varieties

Parameter	Natural Fermentation		Optimized Fermentation	
	Sweet Cassava Variety	Bitter Cassava Variety	Sweet Cassava Variety	Bitter Cassava Variety
Volume (cm^3)	51	28	183	145
Specific Gravity	0.9504	0.9586	0.8321	0.9240
Mass (g)	48.47	26.84	152.27	133.98
Reducing Sugar Converted (%)	22.12^a	16.12^b	63.18^b	40.10^a
Actual Yield (%)	32^b	27^a	56^b	44^a
Theoretical Yield (%)	35.3	30.5	57.7	46.6
Fermentation Efficiency (%)	91	89	97	94

Values are means (n =3). Means with the same superscript along the same row are not significantly different ($p>0.05$)

Discussion

The mode of fermentation through optimization of cultural conditions could determine the concentration of ethanol produced (Tijani *et al.*, 2012). Just as Akponah and Akpomie (2012), Ado *et al.* (2009) and Olofsson *et al.* (2008) reported that temperature and pH of fermentation affected ethanol production, results obtained in this study showed significant variations in the ethanol produced at various pH, temperature and agitation rates. The pH variations showed that pH value of 6.0 was the optimal pH for ethanol production. This was because it was the pH at which the microbial species grew best and the probably most favourable for their fermentative activities (Akponah and Akpomie, 2012; Benerji *et al.*, 2010)

The decrease in the content of reducing sugars contributed to the production of lactic acid during the fermentation process. The decrease in reducing sugar concentration in this study could be explained by the activities of the total fermentative microflora which metabolized and converted them into energy for their growth and to organic acids (Abodjo-Kakou *et al.*, 2010). From the results, the reducing sugars were significantly degraded faster in the roots of the sweet

cassava variety fractions than in the bitter cassava variety fractions leading to higher percentage of reducing sugar reduction. This is due to the lower cyanogenic glycosides level in the sweet cassava variety (Rainbault, 1995). Also, the higher microbial count in the sweet cassava variety accounted for the higher consumption of soluble sugars.

The minimal increase at 0 – 72 h of fermentation at pH 3 could be as a result of the inhibitory effect of bacterial growth due to the acidic pH (Klaenhammar, 1993). The decline in bacterial count at pH 8 for both sweet and bitter cassava varieties at 72 h of fermentation could be attributed to the fact that lactic acid bacteria which are known to persist till the end of fermentation performs better in acidic environment and are responsible for acid production during cassava fermentation process (Oyewole, 1990). The decrease in fungal count at pH 3 and 4 from 48 h of fermentation could be due to the inhibitory effect of the acid produced by the lactic acid bacteria (Tetchi *et al.*, 2012). The higher the final conversion of reducing sugar, the more the quantity of ethanol produced in fermentation by *Saccharomyces cerevisiae.* The use of *Saccharomyces cerevisiae* for fermentation in this study agreed with the study of Oboh and Elusiyan (2007) who reported that *Saccharomyces cerevisiae* is the most effective yeast in bringing about efficiency in fermentation process.

The pH and temperature of the growth medium played an important role by inducing morphological changes in the microorganisms and in their ability to secrete enzymes for their activities. The change in the pH and temperature observed during growth of the microorganisms therefore affected product stability in the medium (Senthilkulmar *et al.,* 2012). Effect of pH and temperature from this study indicated that the microbial enzyme activity was high at pH 3 - 6 and 30^0C - 35^0C with maximum activity at pH 6 and temperature 35^0C. The cell dry weight due to the growth of the microorganisms reduced with increase in agitation rate because the reducing sugar hydrolysate that served as the carbon source was low at higher agitation rates inhibiting normal cell growth (Ado *et al.*, 2009). The increase in reducing sugar concentration by the action of the microbial flora present led to increased ethanol production at 300 rpm.

The percentage reducing sugar converted to ethanol was used to determine the theoretical yield of the ethanol. One gram of glucose used in fermentation will yield 0.511 g of ethanol(Maiorella *et al.,* 1981)

$$C_6H_{12}0_6 \longrightarrow 2CH_3CH_20H + 2C0_2$$

180 g of ethanol = 2x46 g of ethanol

180 g of ethanol = 92 g of ethanol

Thus 1 g glucose = 0.511 g of ethanol

$$\text{Theoretical yield (\%)} = \frac{\text{reducing sugar x 0.511 g}}{\text{actual yield}}$$ (Maiorella *et al.,* 1981).

The theoretical yield ethanol from the cassava substrates were comparable to the actual yield obtained. The alcohol fermentation efficiency or yield in percent depended on the ability of the yeast to utilize a particular feedstock based on their characteristics and compositional differences (Nuwamanya *et al.,* 2011).

$$\text{Fermentation efficiency (\%)} = \frac{\text{actual yield x 100}}{\text{Theoretical yield}}$$ (Ocloo and Ayernor, 2010)

Optimization of the fermentation process attributed to 75% and 63% increase in the ethanol yield for sweet and bitter cassava varieties respectively. Optimized fermentation process therefore was very efficient in enhancing microbial activity which improved the percentage of reducing sugar converted thereby producing maximum ethanol yield from the sweet and bitter cassava varieties respectively after 72 h of fermentation.

Conclusion

The efficiency of starch conversion to ethanol during fermentation of the processing wastes of the two cassava varieties was enhanced by optimization of the fermentation conditions. Optimization of the fermentation process by varying pH, temperature and agitation rate on ethanol yield and productivity has the capability of increasing the ethanol production. Emphasis should be therefore be placed on efficient utilization of cassava processing wastes, which are the cassava peel and effluent to produce energy in form of bio – ethanol. The simultaneous need for energy and food can be taken into cognizance without compromising the environment. The use of cassava ethanol as bio-energy is therefore a means of controlling environmental pollution.

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