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Full Length Research Paper

Ethanol Production from Fresh and Dry Water Hyacinth Using Ruminant Microorganisms and Ethanol Producers.

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An investigation into the use of *Echhornia crassipes* (fresh and dried), as possible alternative and cost effective for the production of ethanol was carried out using microorganisms isolated from ruminants (ram, cow, and goat). The bacteria isolated include species of *Bacteroides* (17.39%), *Streptococcus* (8.69%), *Yersinia* (8.69%), *Lactobacillus* (4.3%), *Peptococcus* (4.3%), *Clostridium* (4.3%), *Nesseria* (4.3%), *Alcaligenes* (4.3%), *Staphylococcus* (4.7%), *Veillonella* (8.69%), and *Bacillus* (13.0%), While the fungi isolated were *Aspergillus fumigatus* (64%), *Aspergillus flavus* (11%), *Candida guilliermondii* (9%), and *Scoploriopsis Candida* (16%). Based on their capability to breakdown the cellulose in *E. crassipes*, *Bacterodes succinogenes* yielded the highest concentration of reducing sugar from fresh *E. crassipes* (9.7mmol/L) *Bacteroides convexus* yielded (7.8mmol/L) for dried *E. crassipes*. *Bacteroides ovatus* also had a significant production of reducing sugar from both fresh and dried *E.crassipes*. The least production was from *Aspagillus species*, *Clostridium* and *neiseseria* for dried and fresh *E. crassipes* respectively. Comparative fermentation of the *hydrolysates* was examined using *Saccharomyses cerevisiae* and *Zymomonas mobilis*, those were done separately and the fermented substrates were distlled, *Zymomonas mobilis* produced more ethanol than *Saccharomyses cerevisiae*, fresh *E. crassipes* produced more ethanol than the dried one. The use of *E. cassipes* for production of ethanol will go a long way in reducing dependence on fossil fuel. However, further investigation is recommended.

Keywords: Ethanol, Water Hyacinth, Ruminant Microorganisms and Cellulose

INTRODUCTION

Ethanol is a clearly colourless liquid. It is biodegradable, low in toxicity and causes little environmental pollution. It burns to produce carbon dioxide and water. Ethanol is a high- Octane fuel and has replaced lead as an octane enhancer in petrol (Ramasamy, 1998). Ethanol is an excellent multipurpose liquid fuel and chemical base which can be synthesized by variety of synthetic and fermentative processes from chemical and biological feed stocks (Odeyemi, 2001). Ethanol is primarily produced from glucose and sucrose, a wide range of other sugars can also be used but the main sources of such sugars required to produce ethanol come from fuel or energy crops and in general all non-fossil based living or dead

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organisms and organic materials that have an intrinsic chemical energy content; (Klass, 2005).

As price and availability of fossil fuel become subject of concern there is renewed interest in examining the fermentation process as means of producing all or portion of the future needs of ethanol. Hence the only carbon source known that is large enough to be use as substitute to fossil is the biomass of which aquatic plants have the benefit of not requiring fertile land to grow and thus do not take precious space away from food crops; also the harvest frequency of aquatics tends to be in the order of days where as the frequency for trees and crops are on the order of years and months. Such aquatic includes water hyacinths and algae (Hronic et al., 2007).

Water hyacinth is regarded as a nuisance plant because of it remarkable growth rate and is considered by many as an invasive pest; water hyacinth where not controlled could cover lakes and ponds entirely, this dramatically affect water flow, block sunlight from reaching native aquatic plants and starve the water of oxygen often leading to fish kill. The plants also create a prime habitat for mosquito, the classic vectors for malaria and a species of snail known to host the parasitic flatworm which causes *Schistosomiasis* (snail fever); However, the use of plants like water hyacinth (non edible) is deem necessary in the sense that the waste is of no economical value and if not well disposed it could turn into a form of hazards and a threat to the environment hence it would be beneficial if this waste material could be processed into a source of useful energy. This research had therefore utilized the water hyacinth to produce ethanol by harnessing the cellulose degrading capability of microorganism in some ruminants (enzyme hydrolysis) and employing the fermentative capability of yeast and *Zymomonas mobilis* to ferment the substrate and finally distilled the fermented substrate.

Humprey and Lee (1997) stated that with the ever increased price and dwindling supply of crude oil, ethanol fermented from grains and other renewable organic resources is in close competition with synthetic ethanol produced from ethylene; in 1984, 150 million gallons of ethanol were produced synthetically in the United States, and around 500 million gallons of ethanol were produced by fermentation close to 1.1 billion gallons produced in 1992. Ethanol is widely used as partial gasoline replacement in U.S and in other part of the world such as Canada. It can also be used in a variety of crocking, heating and lighting appliances. Fuel ethanol that is produced from corn has been used in gashol or oxygenated fuels since 1980, ethanol that is blended directly with gasoline in a mix of 10% ethanol and 90% gasoline is called gashol. Recently, the US automobile manufacturers have announced plans to produce significant number of flexible - fueled vehicle that can use ethanol blend - E85 (85% ethanol and 15% gasoline by volume). Demand for ethanol E85 has grown from 144,000 gallons in 1992 to 2 million gallons in 1998. (Humprey and Cariatas, 2007). The largest single use of ethanol is as a motor fuel and fuel additive. However, the largest national fuel ethanol industries exists in Brazil (gallons sold in Brazil contains at least 20% ethanol and anhydrous ethanol is also use as fuel in more than 90% of new cars sold in the country.

The research was aimed at producing ethanol from both fresh and dried water hyacinth (*Echhornia crassipes*) by harnaissing the cellulose degrading capability of some ruminant microorganisms and the use of ethanol producers.

MATERIALS AND METHODS

Eichhonia crassipes (water hyacinth) was collected from Maimasukka River Sokoto North Local Government Area Sokoto State. Three water hyacinth plants were sundried and processed into powder using morta and pestle and were passed through sieve to remove the large debris. Another three of the plant were cut into small pieces and blended with a blender and passed through a sterile muslin cloth to hydrolyse the water hyacinth.

Determination of physico – chemical qualities of fresh and dried water hyacinth

200 grams of both fresh and dried samples of water hyacinth were analyzed in Agric
Lab Usmanu Danfodiyo University Sokoto for determination of moisture, ash, lipid, fibre, nitrogen, and carbohydrate using methods described for soil and plant analysis (1979) and methods by Bakare (1985) and the results indicate in table 4.

Hydrolysis of water hyacinth

Conical flasks were labeled according to the number of bacterial and fungal isolates identified with cellulose hydrolyzing capability (obtained from rumen of ruminant) and each flask prepared in duplicate. For the fresh water hyacinth 200ml solution of the processed samples was added in each conical flask, while for the dried sample 10g were added in the each conical flask followed by 200ml of distilled water and shaken vigorously to mixed. The conical flask were plugged with cotton wool and foil paper and sterilized at 1210c for 30min. The solutions were allowed to cool and were inoculated with a good growth of the corresponding bacterial and fungal isolates excluding the control. The solutions containing fungal isolates were incubated at room temperature for 5 days. While those containing bacterial isolates were incubated in an incubator at 370c for 5 days (Okusanmi 2008).

Determination of reducing sugar concentration

After 5 days of incubation, the samples were filtered and the presence of reducing sugar in each filtrate was detected using benedict's test. *Spectrphotometry* was applied to find the concentration of reducing sugar in each sample.

Into 5ml of each sample, 2ml of Benedict's reagent were added. The resulting mixture was placed in a boiling water bath for 5min. Positive result gave rise to brown colouration (Plummer, 1971).

From a stock solution of 20mmol/L, standard glucose concentration of 0mmol/L, 2.5mmol/L 5mmol/L 7.5mmol/L and 10mmol/L were prepared and 5ml of each was put in a test tube. 2ml of Benedict's reagent were then added to each tube and placed in a boiling water bath for 5min. Absorption of each content was read with spectrophotometer at 477nm. The readings were used to plot a graph of absorbance against concentration. The blank i.e (0mmol/L) which is 10ml distilled water and 2ml Benedict reagent was used to zero the spectrophotometer. In line with this glucose in samples was determined i.e by adding 2ml Benedict's reagent in 5ml of each filtrate and the mixture placed in a boiling water bath for 5min, absorption of a portion of each mixture was then read using spectrophotometer at 477nm, and the glucose concentration was extrapolated from the standard plot.

Fermentation

The filtrates obtained (i.e after hydrolysis) were sterilized and inoculated with 10g of baker's yeast (*Saccharomyces cereviseae*) which was obtained from the Sokoto Central Market i.e for half of the conical flasks, according to the number of isolates. The rest conical flasks were inoculated with *Z. mobilis* from the stock: Both flasks were incubated at 300c for 7 days.

Distillation and determination of ethanol concentration

After fermentation the samples were distilled to separate ethanol from water. After this further re-extraction was done using *soxhelet* apparatus after which the volume of ethanol for each sample is taken using a measuring cylinder and the sample poured into sample bottles and labeled. Determination of ethanol concentration was done using spectrophotometric method in accordance with Zuru et al (2005) This was based on a reaction between ethanol and a dye (acidic Potassium dichromate) which gives a characteristic colour change that was measured spectrophotometrically. A standard curve was constructed by mixing different concentrations of absolute ethanol prepared by serial dilution followed with addition of 2ml each of acidic Potassium dichromate. The concentrations were prepared as follows.

a) 2ml of the ethanol were diluted to 10ml in a 10ml cylinder.

b) 2ml of (a) were diluted to 10ml

c) Then 0ml, 2ml, 4ml, 6ml, 8ml, 10ml were taken from (b) and each diluted to 10ml to produce 0% 0.2%, 0.4%, 0.6% 0.8% and 1%. 2ml of acidic potassium dichromate were added into each. The mixtures were heated in a boiling water bath for 5min to allow colour development. The absorbance value for each concentration was determined using spectrophotometer. The values were used to construct a standard curve for ethanol. However, 2ml each of the fermented sample (after distillation) were separately put in a marked test tube and each diluted to 10ml, from this dilution 2ml were taken and diluted to 10ml then 5ml each were taken and diluted to 10ml, 2ml acidic potassium dichromate were added to each, and the mixtures were heated for 5 minutes to allow colour development, after cooling the absorbance value for each sample was taken, and the concentration of ethanol produced in each sample was extrapolated from the standard curve. The actual concentration in mol/dm3 of ethanol in each sample was determined by multiplying the extrapolated value with dilution factor 100 divided by 1000cm3 (equivalent to 1dm3) To detect the percentage of ethanol in each sample the extrapolated concentration(s) was substituted in the following formula.

$$\% \text{ ethanol} = \text{Concentration reading} \times \frac{\text{Dilution factor}}{\text{Volume used}}$$

(Mendham *et al,* 2003).

RESULT AND DISCUSSION

Treatment of both fresh and dry water hyacinth with cellulose degrading microorganisms result in substantial increase in the concentration of reducing sugar when compared with the controls that were not treated with cellulose degrading microorganisms as indicated in (table 1 and 2).

Bacteroids Succinagenes yielded the highest concentration for fresh water hyacinth, in both tables i.e 11.6 and 6.4 mol/dm3 respectively as against 6.0 and 5.7mol/dm3 for dry water hyacinth. However, *Bacteroids Convexus* yielded the highest concentration of reducing sugar on hydrolysis of dry water hyacinth, 8.2 and 7.5mol/dm3 as against 4.8 and 5.2 for fresh water hyacinth.

Equally *Bacteroides ovatus* yielded a significant amount of reducing sugar from both fresh and dry water hyacinth 7.9mol/dm3 and 6.7mol/dm3 respectively (Table 1). This indicated that *Bacteroides* species are better hydrolyzing organisms; and among which *Bacteroides Succinogines* is the best for hydrolysis of fresh water hyacinth; other bacterial specie that averagely hydrolysed

TABLE 1 Result of fresh and dry water hyacinth hydrolysis by bacterial and fungal isolates and estimation of ethanol produced from fermentation with *S. cerevisiae*

				Con. of reducing sugar		Vol of ethanol produced (ml)		Conc of ethanol (mol/dm^3)		% of ethanol	
S/No	Sample		Organism	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
1	CG_4	ARB	*Homofermentative Lasctobacillus*	6.8	5.4	5.0	15.0	0.09	0.037	45%	18.5%
2	AR_2	ARB	*S. Alattae*	5.8	6.0	3.5	15.0	0.032	0.024	16%	12%
3	BC_3	AN	*Bacteroids convexus*	4.8	8.2	19.0	27.0	0.063	0.034	32.5%	17%
4	BC_4	AN	*Yersinia intermedia*	6.6	5.6	3.5	31.0	0.02	0.037	10%	18.5%
5	CG_1	AN	*Yersinia enterocolitica*	4.8	7.2	21.0	13.0	0.018	0.030	40.5%	15%
6	CG_2	AN	*Chlosteridium Hystoliticum*	3.7	4.5	32.0	35.0	0.146	0.036	73%	18%
7	AR_2	AN	*Bacteroides succinogenes*	11.6	6.0	20.0	32.0	0.036	0.029	18%	14.5%
8	BC_2	ARB	*Nesseria Haemolysans*	6.3	3.8	7.0	14.0	0.023	0.023	11.5%	11.5%
9	BC_4	ARB	*Streptococcus Spp*	5.9	6.3	22.0	13.0	0.062	0.027	31%	13.5%
10	CG_4	AN	*Bacteroides ovatus*	7.9	6.7	14.0	22.0	0.022	0.032	11%	16%
11	Dark green col		*Aspergillus fumigatus*	5.5	4.2	19.0	10.0	0.019	0.030	95%	15%
12	Light green col		*Aspergillus flavus*	2.3	4.4	3.0	13.0	0.036	0.033	18%	16.5%
13	Control			2.0	2.2	3.0	2.0	0.005	0.00	2.5%	0%

KEY:

CG = Sample C from Goat
AR = Sample A from Ram
BC = Sample B from Cow

* The number 1, 2, 4, 5 represent various colonies from the sample
ARB = Aerobic
AN = Anaerobic

fresh water hyacinth includes *H.lactobacillus*, *Y.intermedia and A.fumigatus* (table 1 and 2). On the other hand from the two tables, apart from *B.convexus*; other good hydrolyzing organisms for dry water hyacinth are *S.alattae and Y.enterocolitica*. Ethanol concentration was initially expressed in percentage going by the work done, but the percentage were further standardized in mol/dm3 by multiplying the results of extrapolated percentage by dilution factor (100) divided by 1000cm3. For the fresh water hyacinth fermented with *S. cerevisiae*, sample pretreated with *A. fumigatus, C.histolyticum* and *Homofermentative lactobacillus* had the highest concentrations of ethanol being 0.19mol/dm3, 0.146mol/dm3 and 0.09mol/dm3 respectively. Followed by samples pretreated with *Bacteroides covextus* and *Streptococcus* which yielded 0.063mol/dm3 and 0.062mol/dm3. Other samples had lower concentrations. (Table 1) For fresh water hyacinth fermented with *Z. mobilis* samples pretreated with *Bacteroides succinogenes* had the highest concentration of ethanol (0.190mol/dm3) followed by samples pretreated with *Bacteroides spp I* and *C. hystolyticum* that yielded 0.184mol/dm3, 0.174mol/dm3 respectively, sample pretreated with *Bacteroides ovatut* also recorded 0.126mol/dm3, most of the samples yielded above 0.1mol/dm3 while the rest produced moderate concentrations above 0.05mol/dm3, but sample pretreated with *S. arlettae* had the lowest concentration of 0.039mol/dm3 (Table 2).

The concentration of ethanol obtained from fermentation of dry water hyacinth with *S. cerevisiae* is also indicated in Table 1, *Homofermentative Lactobacillus* and *Yersinia intermedia* had the highest concentrations of 0.037mol/dm3 each followed by 0.036mol/dm3 from pretreatment with *C. hystolyticum*. Other samples yielded below 0.035mol/dm3 with the least being 0.024 from *S. arlettae*.

However, from Table 2 the fermentation of dry samples with *Z. mobilis* yielded a little bit more ethanol with the highest being 0.045mol/dm3 from sample pretreated with *Bacteroides ovatus* followed by 0.44mol/dm3, 0.041mol/dm3 and 0.040mol/dm3 from samples pretreated with *S. arlattae; C. histolyticum* and *A. fumigatus* respectively; the least concentration of 0.023mol/dm3 was recorded from sample pretreated with *A. flavus*.

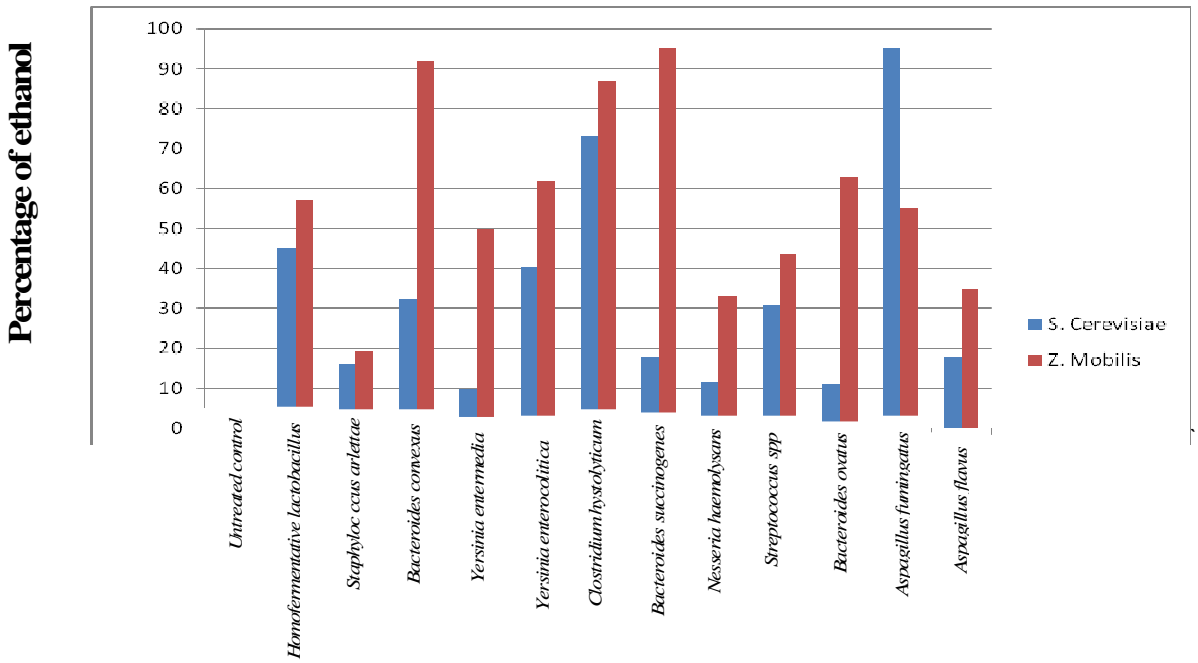
Figure 1 shows the percentage of ethanol in the distillates for fresh water hyacinth as per isolate used for the pretreatment, and with regards to the organisms used for fermentation i.e *S. cerevisiae* and *Z. mobilis*. Sample pretreated with *A. fumigatus* had the highest percentage of ethanol on fermentation with *S. cerevisiae* (95%) followed by 73%, 45% and 40.5% from samples pretreated with *C. hystolyticum, Homofermentative lactobacillus* and *Yersinia enterocolitica* respectively, while the rest of the samples yielded lower percentages. However, on fermentation with *Z. mobilis*, sample pretreated with *Bacteroides succinogenes* had the

TABLE 2 Result of fresh and dry water hyacinth hydrolysis by bacteria and fungal isolates and estimation of ethanol produced from fermentation with Z.*mobilis*

			Con. of reducing sugar		Vol of ethanol produced (ml)		Conc of ethanol (mol/dm^3)		% of ethanol	
S/No	Sample	Organism	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
1	CG_4 ARB	*Homofermentative Lasctobacillus*	7.4	5.6	8.6	23.0	0.115	0.038	57%	19%
2	AR_2 ARB	*S. Alattae*	5.0	6.4	6.0	18.0	0.039	0.044	19.5%	22%
3	BC_3 AN	*Bacteroids convexus*	5.2	7.5	5.0	22.0	0.184	0.030	92%	15%
4	BC_4 AN	*Yersinia intermedia*	5.0	7.2	22.0	23.0	0.1	0.035	50%	17.5%
5	CG_1 AN	*Yersinia intercolitica*	7.1	6.9	15.0	11.0	0.124	0.026	62.%	3%
6	CG_2 AN	*Chlosteridium Hystoliticum*	5.9	5.4	20.0	16.0	0.174	0.041	87%	20.5%
7	AR_2 AN	*Bacteroides succinogenes*	6.4	5.7	20.0	29.0	0.190	0.028	95%	14%
8	BC_2 ARB	*Nesseria Haemolysans*	4.2	5.9	5.0	34.0	0.066	0.022	33%	11%
9	BC_4 ARB	*Streptococcus Spp*	10.2	4.3	6.0	18.0	0.087	0.027	43.5%	13.5%
10	CG_4 AN	*Bacteroides ovatus*	4.9	7.8	15.0	15.0	0.126	0.045	63%	22.5%
11	Dark green col	*Aspergillus fumigatus*	6.0	3.7	10.0	33.0	0.11	0.040	55%	20%
12	Light green col	*Aspergillus flavus*	10.4	3.6	5.0	24.0	0.070	0.023	35%	11.5%
13	Control		2.5	2.2	2.5	2.0	0.005	0.00	2.5%	0%

KEY:

CG = Sample C from Goat
AR = Sample A from Ram
BC = Sample B from Cow
* The number 1, 2, 4, 5 represent various colonies from the sample
ARB = Aerobic
AN = Anaerobic



Microbial innoculants
Figure 1 Percentage of ethanol for fresh water hyacinth

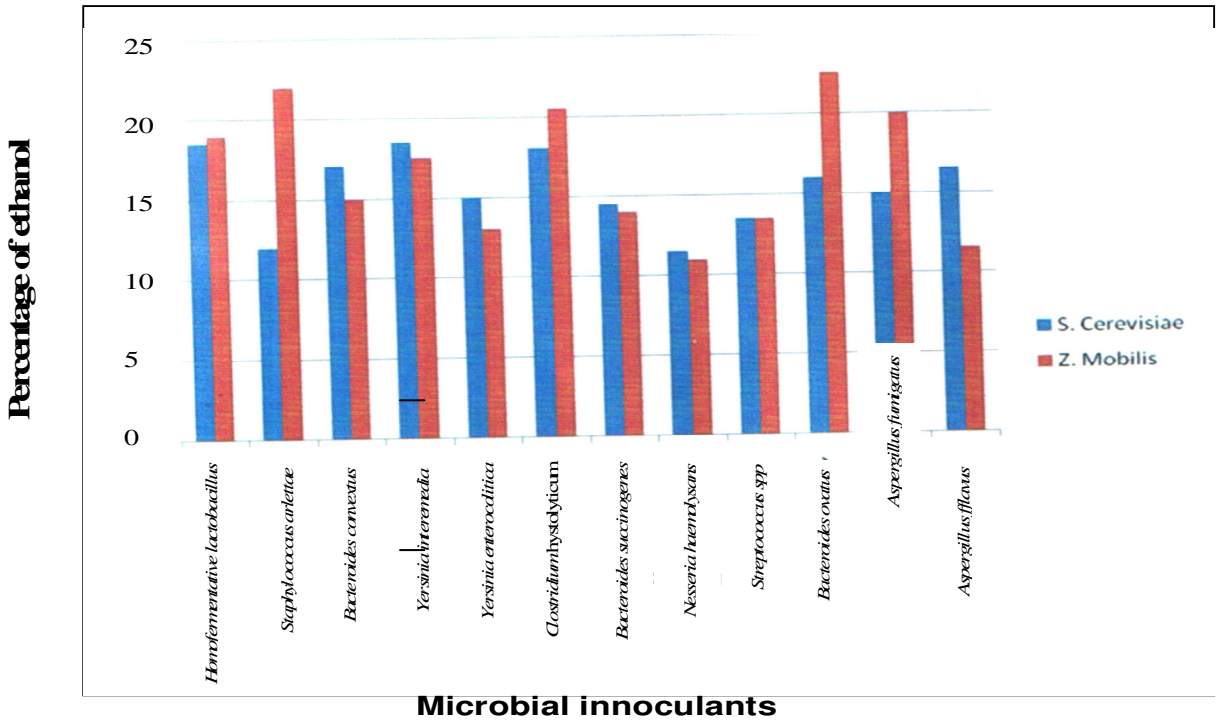


Figure 2 Percentage of ethanol for fresh water hyacinth

TABLE 3 Results of proximate analysis of fresh and dry water hyacinth:

SAMPLE	% MOISTURE	% ASH	% FAT	%FIBRE	%NITROGEN	% CP	% CHO
FRESH	86.000	2.500	1.500	1.000	0.154	0.963	94.040
DRY	5.000	42.500	3.000	15.000	0.550	3.410	36.090

CP= Crude Protein
CHO=Carbohydrate

highest percentage (95%) followed by 92%, 87%, 63% and 62% from samples pretreated with *Bacteroides convexus*, C. *hystolyticum*, *Bacteroides ovatus* and *Yersinia enterocolitica* respectively, other samples had moderate percentages while only three samples yielded lower concentrations.

For the dry water hyacinth fermented with *S. cerevisiae* sample pretreated with *Homofermenttive lactobacillus* and sample pretreated with Yersinia intermedia yielded the highest percentage of 18.5% each, followed by samples pretreated with *C. hystolyticum*, *Bacteroides convexus* and *A. flavus* which recorded 18%, 17% and 16.5% respectively, other sample yielded lower percentages than the former. Dry water hyacinth fermentated with *Z. mobilis* yielded a bit more concentration than that fermented with *S. cerevisae* the highest percentage was obtained from sample pretreated with *Bacteroides ovatus* (22.5%) followed by 22%, 20.5% and 20% from samples pretreated with S. arlettae, C. *hystolyticum* and *A. fumigatus*. Other samples had a concentration of below 20% figure (2).

The fresh water hyacinth had a high amount of carbohydrate from the proximate analysis conducted, this accounted for almost 94.04% (table 3) this serve as abundant source of sugar for conversion to ethanol. Hence the main reason why most of the conical flasks containing fresh water hyacinth yielded more concentration(s) of reducing sugar and in turn had high concentration and high percentages of ethanol as against the dry sample (see Table 2 and 3). Although some samples of fresh water hyacinth i.e 2, 3 and 6 (in table 2) and 2,3 and 8 in (Table 3) yielded low concentration(s) of reducing sugar and low ethanol volume when compared with samples from dry water hyacinth. The difference in the concentration of reducing sugar yielded among samples could be due to the reason stated by *Colombato* et al (2002) that it is generally recognized that there are differences between the bacterial species in their ability to generate cellulosic material, some bacteria has a thin cell coat that possess firmly bound *cellulase* and hence adhere tightly to the plant cell material, while in some bacteria, the *cellulase* is released from the cell coat which

is thick and hence they adhere loosely to the plant cell wall. On the other hand some samples i.e sample 8 (Table 2) 1,7,9 and 11 from Table 3 yielded high concentration(s) of reducing sugar but low ethanol volume as against the dry sample; likewise samples, 5 and 9 (Table 2) yielded low concentration of reducing sugar but higher volume of ethanol when compared with samples from dry water hyacinth, this could be due to certain factors that could affect fermentation e.g stress; whereby if the concentration of sugar is higher than the desired level, the activity of the fermenting organism will slow down there by yielding less product, similarly where two or more stress factors meets such as bacterial infection, mycotoxins and nutrient level, this affects the fermenting organism and if the organism cannot tolerate the environment it will die (Verbelen et al 2006). Higher or lower temperature could affect the fermenting organism by killing or slowing down it's activity and this could occur through power fluctuations.

As the study used the potential of bacterial and fungal isolate obtained from rumen of ram, cow and goat to digest cellulosic materials of water hyacinth (fresh and dry). Bacteria was found to be the most predominant, because up to nine bacterial specie isolated from ruminants (ram, cow, and goat) were found to hydrolysed cellulose (Table 1). This agrees with findings of Bhat (2000) that, bacteria are the most numerous of the rumen inhabitants, there are 60 – 100 species regarded as normal flora and although each species can only undertake a few specific tasks, the bacteria as a whole are capable of degrading all the constituents of plant based diet. The raw data for ethanol percentages was subjected to statistical analysis i.e paired T- test using mini tab and the results revealed that there is significant difference at ($P<0.05$) at 95% confidence level between percentage ethanol produced from fresh water hyacinth by Z. mobilis and that produced by *S. cerevisiae*. With *Z. mobilis* producing more percentages. But there is no significant difference between percentage ethanol produced from dry water hyacinth by *Z. mobilis* and that produced by *S. cerevisiae*. However, there is a significant difference between percentage ethanol produced from fresh water hyacinth by *Z. mobilis* and that produced from dry water hyacinth by *Z. mobilis*. There is also a significant difference between ethanol produced from fresh water hyacinth by *S. cerevisiae* and ethanol produced from dry water hyacinth by *S. cerevisiae*.

RECOMMENDATION

Government should invest heavily in research that focus on utilization of wastes (digested food from rumen) and agro menance such as the water hyacinth for beneficial purposes like ethanol production , as this could convert waste to wealth, and also ensure food security than grains usage,

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