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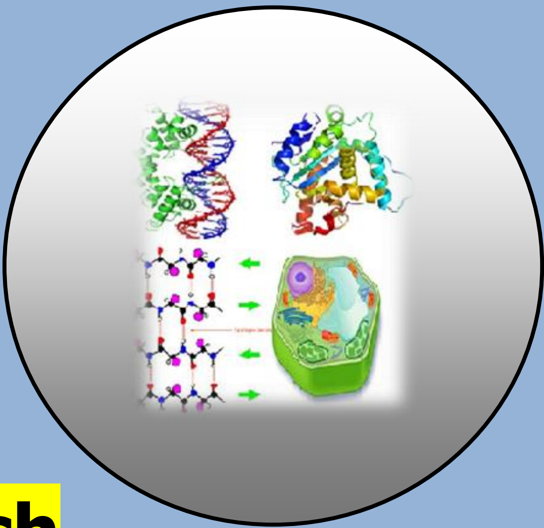

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RESEARCH PAPER

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Bioethanol Potential of Water hyacinth and Sugar cane Leaves: Estimation of the Total Reducing Sugar Yield

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ABSTRACT

The study investigated the total reducing sugar yield of water hyacinth and sugar cane leaves which could be converted into ethanol. The water hyacinth and sugar cane leaves were collected and subjected to pretreatment (rinsing, drying, blending and sieving). The pretreated water hyacinth and sugar cane leaves were separately hydrolysed in acid media (3%v/v H2SO4, 3%v/v HCl, 3%w/v Oxalic acid) and alkaline media (3%w/v KOH, 3%w/v NaOH). The hydrolysed samples were filtered; the reducing sugar yield for each hydrolysate was estimated using the 3, 5 dinitrosalicyclic acid method. The results from the study showed that both samples could yield a maximum value of between 40 to 100% of fermentable sugars after hydrolysis (1000mg/g for water hyacinth and 472mg/g for sugar cane leaves. There was significant difference (P=.05) in the sugar yield between the two samples when treated with 3% v/v H2SO4, 3%v/v HCl, 3%w/v Oxalic acid , 3%w/v KOH and 3%w/v NaOH. The value of sugar concentration of water hycianth treated with the reagents were significantly higher than that of sugar cane leaves, the highest being in water hycianth hydrolysed with 3% KOH (567.11+/ 10.83mg/g) and the least value of sugar yield was in water hyacinth hydrolysed with 3% w/v oxalic acid (66.43+/-0.00mg/g). Both water hyacinth and sugar cane leaves could be used as alternative sources of bioethanol especially water hyacinth. This would create a panacea to the problem that water hyacinth constitutes in the water ways and also create wealth and reduce the demand on sugarcane, maize and other staple food used as sources of bioethanol.

Keywords: Bioethanol, Water hyacinth, Sugar cane leaves, Hydrolysis, Hydrolysate and Reducing Sugar Yield.

INTRODUCTION

Energy consumption has increased steadily over the last century as the world population is increasing and more countries have become industrialized (Hu, et al, 2008). The global oil availability is practically fixed because the natural formation of petroleum is a process which takes a very long time and it is not replaced at the rate at which it is being extracted (SathvikVarma et al., 2013). The use of conventional fuels as primary energy source has led to scarcity in fuel, climate change, environmental degradation and human health problems (Patil et al., 2013). Combined with the dependency on increasingly scarce fossil fuels, there is a growing interest in alternative renewable fuel source. Consequently there has been emphasis on plant biomass as a source of fermentable sugar which can serve as an alternative to fossil fuel.

Bioethanol is considered as an effective fuel produced from plant biomass (Mukhopadhyay, et al. 2008). It is a renewable fuel that is becoming increasingly important as panacea for the problem of depleting oil reserves, rising crude oil prices and greenhouse effect (Hu et al., 2008). Bioethanol emits 85% less green-house gases compared to gasoline (Mete et al., 2002).

BIOETHANOL FROM CELLULOSIC BIOMASS

According to Hamidreza, Amin & Alireza (2012), bioethanol can be produced from sugar (sugar beet, sugar cane, sweet sorghum), starch (corn, barley, ray, wheat, potatoes, cassava) and cellulose (willows, poplar, straw, corn stover, switchgrass, bagasse). Hence, the large scale of bioethanol production needs substantial amount of cultivable land. Sugar easily ferments to ethanol but starch must be converted to sugar (Hydrolysis) then to ethanol. Lignocellulosic feedstock is considered an attractive raw material for bioethanol because of its availability in large quantities at low cost not only for the liquid transportation fuel but also for the production of chemicals and materials (Farrell, et al., 2006). Besides terrestrial plants, aquatic plants are also promising renewable resources. Cellulosic bioethanol is made from structural material, lignocellulose, which constitutes much of the mass of plants.

WATER HYACINTH (*Eichhornia crassipes*)

Water hyacinth (*Eichhornia crassipes*) is a free floating aquatic perennial plant or hydrophyte of fresh water ecosystem. It is found at the surface of rivers, lakes canals and ponds and may root in the mud of shallow waters. Water hyacinth possess a broad, thick, glossy, ovate leaves, it may rise above the surface of the water as much as 1 meter in height. The leaves are 10-20cm high, and float above the water surface. They have long, spongy and bulbous stalks. The feathery, freely hanging roots are purple-black. An erect stalk supports a single spike of 8-15 conspicuously attractive flowers, mostly lavender to pink in colour with six petals (Anjanabha & Kumar, 2010).

According to Sullivan & Wood (2012), water hyacinth is one of the fastest growing plants known, each plant can produce thousands of seeds each year, and these seeds can remain viable for more than 28years. In the developing world, it is used in traditional medicine and even used to remove toxic elements from polluted water bodies. Water hyacinth tolerates an annual temperature between 21oC -27.2oC and its pH tolerance is between 5.0 -7.5.

It is an invasive species which invade fresh water habitats and is listed along with some of the worst weeds (Anjanabha & Kumar, 2010). It is very difficult to eradicate by physical, chemical, and biological means, and a substantial amount is spent on their control annually throughout the world. It is also a very sturdy species.

It cause blockage of irrigation channels affecting the flow of water to fields, get entangled with motorboat rotors, making fishing difficult, and almost makes any place inhabitable and inaccessible (Sullivan & Wood, 2012). They may block hydroelectric turbines causing enormous damage, which are vital for economy and green environment (Farell et al., 2006).
The disadvantage of this fresh water plant can be harnessed, as water hyacinth has useful properties which include its low lignin (10%) and moderately high cellulose content (25%) Poddar, Mandal & Benerjee, (1991).
Vidya & Kumari (2013) stated that as lignin cannot be converted into sugars, their degradation is a high energy process. Water hyacinth has low lignin, which means the cellulose and hemicellulose are more easily converted to fermentable sugar thus resulting in enormous amount of utilizable biomass for the biofuel industry.

SUGAR-CANE LEAVES

Sugarcane (*Saccharum officinarum*), is a species of tall perennial true grasses of the genus *Saccharum*, tribe Andropogoneae. Sugarcane has stout jointed fibrous stalks that are rich in sugar and measure two to six meters (6 to 19 feet) tall. The main product of sugarcane is sucrose, which accumulates in the stalk internodes. Sucrose, extracted and purified in specialized mill factories, is used as raw material in human food industries or is fermented to produce ethanol. Ethanol is produced on a large scale by the Brazilian sugarcane industry (Macedo, et al. 2008), and yet the world demand for sugar is the primary driver of sugarcane agriculture.
According to Peter (2000), A full grown sugarcane plant will have about 75% sugarcane stalks and the leaves and tops will be around 25%. Sugarcane leaf which contains sugar in form of cellulose is a rich cellulosic material. Thus ethanol can be extracted from sugarcane leaves by taking advantage of its rich cellulosic matter and the large biomass. Extraction of ethanol from sugar cane leaves will not affect food supply nor will it Interfere negatively with sugar based food or juice extracted from stalks of sugarcane plant (Michael & Matthew 2006). The lignin content of Sugar cane leaves is stated to be about 25.80% and a cellulose content of about 40.84) average composition of the biomass of sugarcane leaves (Food and Agriculture Organization of the United Nations (FAO) 2012).

AIM

The main aim of this work is to estimate the total reducing sugars of two types of feedstock, namely water hyacinth and sugar cane leaves in order to compare which feedsctock is best suited for bioethanol production.

MATERIAL AND METHODS

The process will involve the use of pretreatment (rinsing, drying, blending and sieving), hydrolysis (by both acid and alkali), Reducing sugar test using Dinitrosalicyclic acid test, measurement of the absorbance with a UV-Visible Spectrophotometer and calculation of the Reducing Sugar Yield.

SAMPLE COLLECTION

Fresh Water Hyacinth plants were collected from Majidun River Ikorodu lagos, a major tributary of the Lagos lagoon. Samples of water hyacinth were collected 10m apart to north, west, east and south. Sugarcane leaves were collected at Ipetumodu, Ife north, Osun state. The leaves were collected 10m apart to north, south, west and east.

PRE-TREATMENT

The samples were initially sundried for five days and then oven dried at a temperature of 105oC for 60 min in order to get rid of the residual moisture. The oven dried water hyacinth and sugar cane leaves were cut into pieces and grinded in a grinder to reduce the particle size. The samples were sieved separately using a 0.4cm sieve, to get a large surface area which will give effective exposure and contact with the hydrolyzing agent. The dried, blended and sieved water hyacinth and sugar cane leaves were then stored in an air tight container at room temperature until used.

HYDROLYSIS AND ESTIMATION OF TOTAL REDUCING SUGAR YIELD

Hydrolysis was carried out in a (250 mL) Erlenmeyer flasks by mixing 3g of the dried water hyacinth per 100 mL of (3% v/v) of HCl, H2SO4 ,C2H2O4 and 100mL of3% w/v KOH and NaOH. The mixtures were autoclaved at 121 °C, 15 min and further cooled down to room temperature. The hydrolysates were filtered using Whatman filter paper to remove the unhydrolysed materials. Each filtrate was collected and analyzed for the reducing sugar yield by using DNS test (Mamata et al., 2013). The procedure was repeated for the dried, blended and sieved sugar cane.

Total reducing sugar was estimated using dinitrosalicylic acid (DNSA) reagent (Miller, 1959). The 3,5- Dinitrosalicyclic acid (DNSA) detects the presence of free C= O of reducing sugars during the reaction, the aldehyed group is oxidized to glucose, the dinitrosalicyclic acid is then reduced to complex which has a maximum absorbance at 540 nm.

RESULTS AND DISCUSSION

Water Hyacinth samples and sugar cane leaves were hydrolysed using different acids (Sulphuric acid, Hydrochloric acid and oxalic acid) and alkalis (Sodium hydroxide, Potassium hydroxide). The result obtained (Table 1) revealed that for the hydrolysis of sugarcane leaves, the sample hydrolysed with 3%w/v potassium hydroxide gave the highest reducing sugar yield (176.89mg/g) and the least was obtained in sample hydrolysed with oxalic acid (67.22mg/g).

Table 1. Sugar Yield achieved after Hydrolysis of Sugarcane leaves.

Reagent	Absorbance	Sugar Yield (mg/3ml)	Sugar yield (mg/100mL) (in 3g dry weight	Sugar Yield (mg/g) dry weight
3% v/v H_2SO_4	**0.646**	**12.58**	**419.33**	**139.78**
3%v/v HCl	**0.766**	**14.92**	**497.33**	**165.78**
3% w/v oxalic	**0.311**	**06.05**	**201.67**	**67.22**
3% w/v KOH	**0.817**	**15.92**	**530.67**	**176.89**
3% w/v NaOH	**0.758**	**14.77**	**492.33**	**164.11**

The reducing sugar yield was calculated using the following equations and data;

The standard solution of glucose was prepared by dissolving 0.3g of glucose D in 100mls of solution.

Standard solution of glucose = 0.3g (300mg) of glucose was in 100mls

in 3mL gives = $\frac{300 \times 3}{100} = 9.0$ mg

The standard concentration of glucose = 9.0mg/3mL of glucose solution.
Absorbance of standard (glucose) = 0.462A
The concentration of reducing sugar in 3mL of each sample was estimated using,

$$\text{Unknown sugar yield of sample (mg/3mL)} = \frac{O\Delta y \times 9.0}{O\Delta x}$$

Where, $O\Delta y$ = Absorbance of sample solution
$O\Delta x$ = Absorbance of standard glucose solution

From the value obtained from mg/3mL, mg/100mL was deduced and since the 100mL of solution contained 3g of dried sample then mg/g of dried sample was also deduced from the value obtained.

The result on water hyacinth hydrolysis (Table 2) revealed that the highest reducing sugar content was produced in the biomass hydrolysed with 3%w/v KOH (212.11mg/g) and the least was obtained in the hydrolysate of water hyacinth and oxalic acid (24.89mg/g).

Table 2. Sugar Yield after Hydrolysis of Water hyacinth.

Reagent	Absorbance	Sugar Yield (mg/3ml)	Sugar yield (mg/100mL) dry (in 3g of dry weight	Sugar Yield (mg/g dry weight)
3% v/v H_2SO_4	0.735	14.32	477.33	159.11
3%v/v HCl	0.910	17.73	591.00	197.00
3% w/v oxalic	0.115	02.24	74.67	24.89
3% w/v KOH	0.980	19.09	636.33	212.11
3% w/v NaOH	0.903	17.59	586.33	195.44

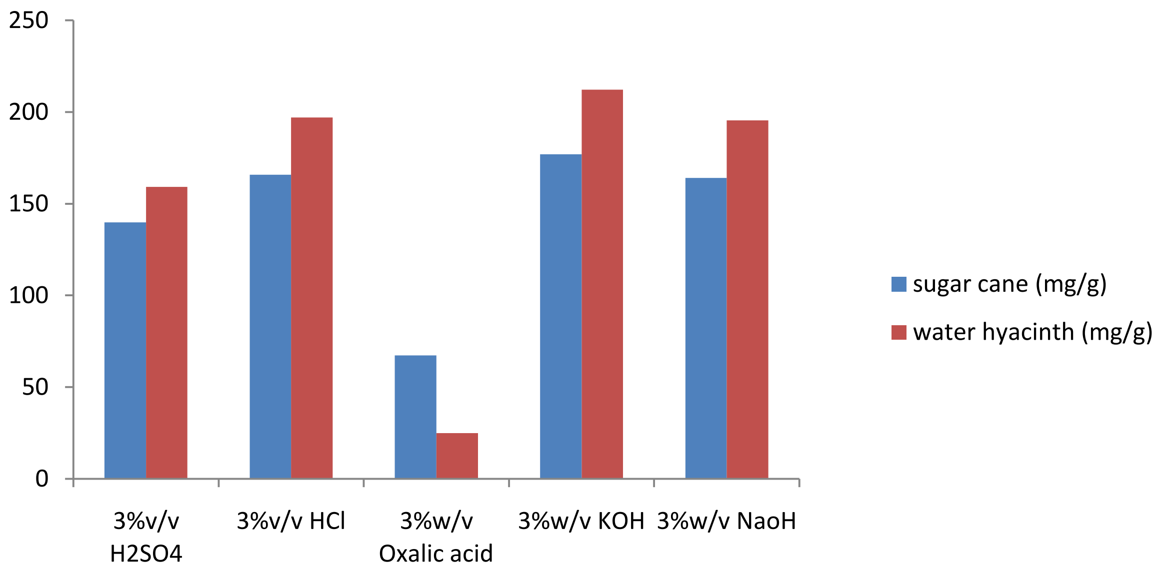

Fig 1. Comparative yield of reducing sugar from sugarcane leaves and water hyacinth using different reagents. (3 mL of Conc. H2S04 in 100 mL solution, 3 mL of conc. HCl in 100 mL solution, 3 g of oxalic acid in 100 mL solution, 3 g of KOH in 100 mL solution and 3 g of NaOH in 100 mL solution.

The Inferential Statistics of the result using Analysis of Variance (ANOVA) of Mean values Table 3 shows the analysis of variance in sugar content of sugarcane leaves and water hyacinth. The result reveal that there was significant (P=.05) difference in the sugar concentration between the two samples when 3% V/V H2SO4, 3% V/VHCl,3% V/V oxalic acid, 3% V/V KOH and 3% V/V NaOH were used. The value of H2SO4, HCl, KOH and NaOH was significantly higher (P =.05in water hyacinth than in sugar cane while sugar cane had significantly (P = .05) higher sugar content yield in oxalic acid than water hyacinth.

Table 3. T-test statistics of Reducing Sugar Yield Mean Values.

Medium	Sugar cane (mg/g)	Water hyacinth (mg/g)
3%v/v H_2SO_4	139.78 ± 0.1467^b	159.11 ± 0.0600^a
3%v/v HCl	165.78 ± 0.0267^b	197.00 ± 0.0667^a
3%w/v oxalic acid	67.22 ± 0.0133^a	24.89 ± 0.1267^b
3%w/v KOH	176.89 ± 0.0933^b	212.11 ± 0.0667^a
3%w/v NaOH	164.11 ± 0.0267^b	195.44 ± 0.0600^a

a,b Means within each row with different superscript are significantly different(P=0.05)

DISCUSSION

According to Beer's Lambert law the absorbance of a substance is directly proportional to the concentration and path length. The results of the study showed that 3% w/v potassium hydroxide KOH gave the highest value of reducing sugar from water hyacinth and sugarcane leaves. This implies that it is the most effective of the five reagents used in hydrolysis to convert the cellulose in plants to reducing sugars. This corroborates the report of Narayan, et al. (2013) and Masami & Urano (2008) that KOH is a very effective reagent in chemical hydrolysis of cellulose. The oxalic acid yielded the least sugar content from both water hyacinth and sugar cane leaves. This could be due to the fact that it is a weak acid and therefore dissociates partially in solution. Furthermore, there is significant difference in the sugar content yield of water hyacinth and sugar cane leaves (P=.05) when hydrolysed with the acids and bases used in the study. Water hyacinth yielded significantly higher sugar content than sugarcane leaves in all the hydrolysis except in the hydrolysis with oxalic acid where sugarcane had a significantly higher yield of sugar. This could be as a result of the low lignin content of water hycianth compared to sugar cane leaves. Lignin is not easily degraded during hydrolysis and it hinders the hydrolysis of cellulose to simple sugars (Buddhiporn & Kongkiattikajorn, 2010; Mishima et al 2008). The results of this study therefore support the reports of Mako & Akinwande 2012; Mamta et al. 2013 and Muhammad et al 2012, that water hyacinth has a high energy potential via its sugar content as a source of bioethanol. Moutta et al. 2012 also stated in their own reports that sugar cane leaves though having relatively high lignin content, could be hydrolysed under optimum conditions into simple sugars which could then be fermented into ethanol. From the results displayed in tables 1,2 and 3, the highest reducing sugar yield of was 0.567g per g of dry biomass of water hyacinth (Table 2) and 0.472g per gram of dry biomass of sugarcane leaves (Table 1). This result is close to the report given by Abraham & Kurup (1996) and Kaari et al. (2011).

CONCLUSION

This investigation has revealed that aquatic weeds such as water hyacinth and sugarcane leaves could be used as alternative source of bioethanol since they yield significant amount of reducing sugar which can be further processed into ethanol by microorganisms.

From the investigation water hyacinth is a better biomass than sugarcane leaves in terms of the concentration of sugar that would be produced. Potassium hydroxide (3% w/v KOH) is a most effective reagent for chemical hydrolysis of the cellulosic biomass.

Sugar cane leaves can be harnessed and put into productive use rather than burning them after harvesting the sugar canes. Water hyacinth has been labeled as a nuisance to our water ways, however the advantage of its abundance can be harnessed not forgetting the fact that its growth is continuous throughout the year. The use of water hyacinth as a source of bioethanol will provide an alternative means to disposing off this unwanted aquatic weed, this will also help generate income for the country and also shift the attention of the country from the conventional use of fossil fuel to bioethanol.

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