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Evaluation of sugar content and bioethanol potentials of some freshwater biomass

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Abstract: An evaluation of sugar content and bioethanol potential of some freshwater biomass namely; *Eichhornia crassipes* (Water Hyacinth) *Pistia stratiotes* (Water Lettuce) and *Salvinia molesta* (Water Fern) was carried out in a batch hydrolysis and fermentation processes. Determinations of xylose and glucose content were achieved using phloroglucinol and Dinitrosalicylic assay respectively. While the amount of glucose in *E. crassipes, P. stratiotes and S. molesta* were 0.08, 0.07 and 0.04 g/L, that of xylose were found to be 0.11, 09 and 0.07 g/L respectively. The results of analysis of biofuel potential show that maximum ethanol yield of 25 cm^3 was observed in *E. crassipes*s, 25 cm^3 in *P. stratiotes* and 20 cm^3 was found in *S. molesta* after 21days of fermentation and this gave a corresponding mean yield of 18.3 cm^3, 17.6 cm^3 and 15.0 cm^3 respectively. The study concludes that the sugar content in the freshwater biomass determined the amount of ethanol yield. While there are no significant differences in the bioethanol production potential between the three biomass samples, *E. crassipes*s and *P. stratiotes* have higher yield than *S. molesta.*

Keywords: Freshwater, Biomass, Bioethanol, Fermentation

1. Introduction

Freshwater biomass is aquatic weed which interfere with the use of water and constitute a nuisance to the environment and human welfare (Uka *et al*., 2009). Some freshwater biomass such as *Eichhornia crassipes* (water hyacinth) *Pistia stratiotes* (water lettuce) and *Salvinia molesta* (water fern), have invaded freshwater ecosystem especially in Northern Nigeria, causing considerable socio-economic problems. These invasive aquatic weeds affect biodiversity as well as water quality and have become a source of concerns to ecologists and fishermen in Nigeria. Several attempts which include mechanical, chemical and biological remedies were made to eradicates and control their growth to a manageable level, however these efforts were not very successful because of the plants prolific rate of reproduction (Chukwuka and Uka, 2007).

Global depletion of energy supply due to the unsustainable consumption and the associated environmental problems of fossil fuel utilization have prompted the research on alternative energy sources (Bentley, 2002). One of such innovative approaches has been the conversion of biomass into fuel ethanol. Production of bioethanol provides several advantages over fossil fuel. Utilization of abundant and inexpensive sources of biomass resources would control the spread and curb their negative effects, result in the reduction of greenhouse gas emission and ensure energy security (Lin and Tanaka, 2006).

The freshwater weeds; *Eichhornia crassipes* (waterhyacinth) *Pistia stratiotes* (water lettuce) and *Salvinia molesta* (water fern) are fast growing aquatic plants widely distributed throughout the world. These tropical plants can cause infestations over large areas of water and consequently lead to series of Ecological problems including; reduction in biodiversity, blockage of watercourses, depletion of dissolved oxygen, alteration of water chemistry and causing environmental pollution (Malik, 2007). More recently, attention has been focused on the potentials and constrains of using freshwater biomass

for variety of applications. Their application as animal fodder and means of metal remediation has been reported (Campbell and Doswald, 2009). The prospect of converting aquatic weeds to biogas and bioethanol is ongoing in some developing countries such as India (Singhal and Rai, 2003).

The aquatic weeds are monocotyledons and naturally grown vegetation, preferably perennials, high cellulose with low lignin content per unit volume of dry matter, easily degradable and do not compete with arable crop plant for space, lights and nutrients. While the weeds resist pests, insects and diseases, they are also not prone to genetic pollution by cross breeding with cultivated food crops (Gressel, 2008). The low lignin content means that cellulose and hemicelulose could be easily converted to fermentable sugars resulting in enormous amount of utilizable biomass for the biofuel industry (Masami *et al.,* 2008). The aim of this paper therefore, is to assess the sugar content and evaluate the bioethanol potentials of *Eichhornia crassipes* (water hyacinth) *Pistia stratiotes* (water lettuce) and *Salvinia molesta* (water fern).

2. Materials and Methods

The fresh water Biomass: *Eichhornia crassipes, Pistia stratites* and *Salvinia molesta* were sampled from Ahmadu Bello University Dam and Hanwa Dam within Kaduna State Nigera. The aquatic plants were thoroughly washed with tap water to remove adhering dirt and were chopped into small pieces using sharp knife. The plants were dried separately in an oven at 105^0C for six hours and subsequently pulverized using motar and pestle (Galbe and Zacchi, 2007).

Fig 1. *Infestation of water body by Water Hyacinth (E. crassipes)*

2.1. Determination of Glucose Content

Glucose content of the samples was achieved by measuring 3 cm^3 of dinitrosalicylic assay (DNS) reagent and added to 3 cm^3 of hydrolysate sample in a lightly capped test tube (to avoid loss of liquid due to evaporation). The mixture was heated at 90^0C for 5-15 minutes to develop the red brown color. 1 cm^3 of 40% Potassium Sodium Tartrate (Rochelle salt) solution was added to stabilize the color. This was then cooled to room temperature. Absorbance was recorded for the resultant solution using a spectrophotometer at 540nm (Miller, 1959).

2.2. Determination of Xylose Content

Xylose content was determined using the phloroglucinol assay. The reagent consisting of 0.5 g of phloroglucinol, 100 cm^3 of glacial acetic acid and 10 cm^3 of HCl. Stock standard sugar containing xylose was prepared by dissolving 1g D-xylose powder in saturated benzoic acid solution and it was used for the preparation of calibration curve. 2 cm^3 of the plant sample hydrolysate each was mixed with 0.5 cm^3 of the reagent in a cuvette and subsequently heated at 100^0C for 4 minute in a water bath. It was then cooled down to room temperature in water and the absorbance was recorded at 540nm using a spectrophotometer (Eberts *et al.,* 1979; Johnson *et al.,* 1984).

2.3. Hydrolysis

10 g of each dried pulverized plant sample was weight separately using electronic weighing balance and placed into a 250 cm^3 conical flask, 10% sulfuric acid was added and made up to 150 cm^3. The mixture was autoclaved at 121^0C for 15 minutes and was then filtered using whatman filter paper to remove the unhydrolysed materials (Carvelheiro *et al.,* 2008).

2.4. Hydrolysate Detoxification and Fermentation

The hydrolysate of each Biomass sample was heated to 60^0C (for dissolution) then basified with NaOH by adding 2.0 g starting with 0.5 g at interval and measured with a pH meter till it reaches pH 9.0 - 9.5. 1.0 g of $Ca(OH)_2$ was added to the solutions to detoxity harmful materials present in the hydrolysate and filtered to remove insoluble residues. The filterate was used as fermentable sugars (Martinez *et al.,* 2000). 2.0 g of peptone water was added to the previously detoxified hydrolysate and the pH was adjusted to 5.6 by adding 10% sulfuric acid (H_2SO_4). The medium was sterilized by autoclaving at 121^0C for 15mins. The yeast (*Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis)* were inoculated into the medium and fermented by incubating for 3 weeks at 30^0C (Standbury and Whittaker, 1984). The fermented medium was aliquoted after 7 days, 14 days and 21 days interval and distilled to assay ethanol content.

Ethanol content was determined by Dichromate assay: 7.5 g of potassium dichromate was dissolved in dilute sulfuric acid and the final volume was adjusted to 250 cm^3 with deionized water; and the maximum absorbance was recorded at 590nm with a multiwavelenths spectrophotometer.

3. Results and Discussion

The results of glucose and xylose content in *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia molesta* as indicated by their concentration is presented in table 1. The concentrations of glucose in *E. crassipes, P.stratiotes* and *S.molesta* were 0.08, 0.07 and 0.04 g/L respectively. This indicate that *S. molesta* has the least concentration than the other two sample. Similarly, the amount of xylose sugar were found to be 0.11, 09 and 0.07 g/L in *E. crassipes, P.stratiotes* and *S.molesta* respectively. The values of sugar concentration detected in the three aquatic weeds differs slightly. The highest value was recorded in *E.crasipes* which may be attributed to high potential yield in ethanol production.

Table 1: *Sugar Content in the Three Freshwater Biomass Samples*

S/N	Freshwater Biomass Sample	Glucose Concentration (g/L)	Xylose Concentration (g/L)
1	*Eichhornia crassipes*	0.08	0.11
2	*Pistia stratiotes*	0.07	0.09
3	*Salvinia molesta*	0.04	0.07

Ethanol Content of the Aquatic Weeds

The quantity of ethanol produced from *Eicchornia crassipes, Pistia stratiotes* and *Salvinia molesta* after fermentation with *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* aliquoted for 7, 14 and 21 days interval with the calculated mean are presented in table 2. The results show a progressive increase in the amount of ethanol production with time from 7 to 21 days. *E. crassipes* and *P. stratiotes* hydrolysates fermented with *S. cerevisiae* and *S. carlsbergensis* have higher quantity of ethanol, yielding 20 cm^3 and 18 cm^3 at day 14. However, *Salvinia molesta* recorded a lower quantity of 15 cm^3 on day 14 (see table 2). Similarly, *Eichhornia crassipes, Pistia stratiotes* and *Salvinia molesta* yielded the same quantity of ethanol distillate of 10 cm^3 on day 7. *Eichhornia crassipes* fermented with only *Saccharomyces cerevisiae* has the highest quantity of Ethanol distillate of 9 cm^3 on the 21 day followed by *Pistia stratiotes* which has 8 cm^3 and lowest quantity of 7 cm^3 was found in *Salvinia molesta.*

Table 2: *Amount of Ethanol Produced (ml) after fermentation days using Saccharomyces carlsbergensis and Saccharomyces cerevisiae in synergy and independent organism .*

Aquatic weeds	Fermenting organism	Yield (cm^3)			Mean Yield			% yield (cm^3)
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21	
Eichhornia crassipes	*Saccharonmyces cerevisae. Saccharomyces carlsbergensis*	10	20	25	18.3	20	40	50
Eichhornia crassipes	*Saccharonmyces cerevisae.*	-	-	9		18	-	-
Eichhornia crassipes	*Saccharomyces carlsbergensis*	-	-	10				
Salvinia molesta	*Saccharonmyces cerevisae. Saccharomyces carlsbergensis*	10	15	20	15	20	30	40
Salvinia molesta	*Saccharonmyces cerevisae.*	-	-	7				
Salvinia molesta	*Saccharomyces carlsbergensis*	-	-	8		16		-
Pistia stratiotes	*Saccharonmyces cerevisae. Saccharomyces carlsbergensis*	10	18	25	17.6	20	36	50
Pistia stratiotes	*Saccharonmyces cerevisae.*	-	-	8		14	-	-
Pistia stratiotes	*Saccharomyces carlsbergensis*	-	-	9		16	-	-

The same trend was observed in the fermentation of *Eicchornia crassipes* using only *Saccharomyces carlsbergensis* where the highest ethanol distillate of 10 cm^3 was recorded after 21 days of fermentation followed by *Pistia stratiotes* 9 cm^3 and the lowest quantity was found in *Salvinia molesta* which yield 8 cm^3. *Eichhornia crassipes*, *Salvinia molesta* and *Pistia stratiotes* fermented with the two organisms has the same percentage yield of ethanol on day 7 with 20% each and there is a slight variation on day 14 with *Eichhornia crassipes* having the highest percentage yield of 40% followed by *Pistia stratiotes*, 36% and the lowest percentage of 30% yield was found in *Salvinia molesta.* Both *Eichhornia crassipes* and *Pistia stratiotes* has the same highest percentage ethanol yield on day 21 of 50% compared to *Salvinia molesta* which has 40%. *Eichhornia crassipes* has the highest means of 18.3 cm^3 followed by *Pistia stratiotes* with 17.6 cm^3 and the lowest mean value of 15.0 cm^3 was found in *Salvinia molesta*.

This results indicate that *E.crassipes* had the highest ethanol quantity while lowest yield was recorded in *S.molesta*. Using Analysis of variance (ANOVA), the result shows that there is no significant difference between ethanol yield by *Eichhornia crassipes, Pistia stratiotes* and *Salvinia molesta*.

Successful bioconversion of lignocelluloses from local material to bioethanol has been achieved using two sequential steps; acid hydrolysis and yeast fermentation. Hydrolysis to break down complex sugar and lignin is essential and overliming to detoxify harmful substances in the hydrolysate is of paramount important when compared to method of Ackerson *et al*., (1981). Since, furfural a byproduct of xylose degradation is generated as a consequence of acid hydrolysis and acetic acid is produced as one of the principal components of hemicelluloses hydrolysate.

The sugar concentration of the aquatic weeds ranging from 0.4 g/L Glucose of *S. molesta* to 0.11 g/L xylose of *E. crassipes* disagree with Mukhapadhyay and Chatterjee (2010). While higher concentration of sugar (18.28 g/L) was reported, the elevated value could be due to higher biomass loading of 40 g/L compared to 10g/L used in this study. However, the highest ethanol yield of 3.0 g/L obtained in this study agrees with the results obtained by Mukhophadhyay and Chatterjee (2010).

4. Conclusion

The glucose and xylose are the major sugar constituent of the fresh water biomass; *Eicchornia crassipes, Pistia stratiotes and Salvinia molesta* analysed in this study for bioethanol production. While there are no significant differences in the bioethanol production potential between the three biomass samples, *E. crassipes*s and *P. stratiotes* have higher yield than *S. molesta.* The study concludes that all the aquatic weeds have significant bioethanol potential.

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