International Journal of Applied Agricultural Research
ISSN 0973-2683 Volume 6 Number 2 (2011) pp. 99-105

http://www.ripublication.com/ijaar.htm

Studies on Bioethanol Production using *Gardenia erubescens* Fruits as Substrate

***[1]Ibrahim A.D., [1]Jumare Fatimah I., [1]Manga S.B., [1]Rabah A.B. and [2]Karaye I.U.**

[1]Department of Microbiology, Usmanu Danfodiyo University, Sokoto. Nigeria
[2]Department of Biological Sciences, Botany Unit, Usmanu Danfodiyo University, Sokoto, Nigeria
**Corresponding Author E-mail: aid4life@yahoo.com*

Abstract

A study on bioethanol production from *G. erubescens* fruits was conducted. The ethanol concentration was determined using Uv-visible spectrophotometer. Production of ethanol from *G. erubescens* fruits hydrolysate seeded with *Saccharomyces cerevisiae* was determined at 1, 2, 3, 4 and 5 days of fermentation. The highest ethanol yield of 33.89% was obtained on the second day of fermentation. This concentration is appreciable thereby suggesting that *G erubescens* could be harnessed as substrate for large scale production of ethanol after further feasibility studies and validation.

Keywords: A. niger, Bioethanol, Gardenia erubescens, Hydrolysis, S. cerevisiae.

Introduction

An important benefit regarding bio ethanol utilization as a bio fuel is the fact that it can create economic growth in rural areas and industry (Mojovic et al., 2009). Several reports published in recent years have tried to access the effects of bio ethanol production on job creation (Mojovic et al., 2009). One of them claims that the ethanol industry has created 700,000 jobs in rural areas of Brazil, (Macedo, 1995), while other predict the creation 20,000 jobs in bio fuel industry in US by 2017, 100,000 jobs in EU by 2020 and 600,000 jobs in China by 2020 (Kearney,2006). Most developed countries today are dependent on imported out and recent global energy price

increases have been economically detrimental for these nation. According to UN – energy, no country in modern time has substantially reduced poverty in the absence of access to energy (UN – energy, 2007).

Many agro wastes have been exploited in pilot studies for, ethanol production, such waste include rice husk, rice bran, corn cob, groundnut shell, maize coat, maize stalk, banana stalk, (Zakpaa et al., 2009); and some plants such water hyacinth (Yerima et al., 2008). Though there has not been any report on the industrial utilization of such waste, for large scale production. However, in developed countries, different agricultural products such as maize in the United State of America, sugar cane in Brazil, Palm oil in Malaysia, has been exploited for large scale production of bio ethanol (Mojovic et al., 2009). Unfortunately, in Nigeria and other part of the world all these are vital food sources among the poor and wealthy citizens. Therefore, their utilization is not feasible. In India and Mali, *Jatropha* has been exploited for large scale ethanol production which requires large hectares of land for its cultivation to meet industrial demand.

In Nigeria, land is a valuable resource so dedicating such large hectares of land for *Jatropha* cultivation will have an adverse effect on agricultural produce thereby exposing Nigeria citizens to food shortage and possibly starvation. So far to my knowledge university of Ilorin and National institute for chemical technology, Zaria, has committed land for jatropha cultivation and till date none of them has cultivated this plant to meet industrial demand. This makes it paramount to search for other rich source of carbohydrate that could be exploited for large scale ethanol production and not effecting human in any way.

Gardenia erubescens is a shrub or very small tree to 3 m high, branching from near the base of the savannah or Savannah wood land common in places from Senegal to Northern Nigeria and in Ubangi, Sudan and Uganda. The wood is yellow, hard and compact and tough. It is abundantly found on ground and on mountains surrounding Zuru area of Kebbi State, Nigeria, and its environments. It fruits are consumed by some groups. It is most enjoyed by its consumers. One other problem faced by the rural dwellers who harvest the fruit for sale, is its low sales and sometimes, their supply is more than it demands. This project is aimed at: utilizing *Gardenia erubescens* fruits as substrate for ethanol production and to quantify the amount of ethanol produced.

Materials and Methods

Collection and preparation of fruit samples

Fruits of *Gardenia erubescens* were purchased in Rikoto Market in Zuru Local Government Area of Kebbi State, Nigeria. The *Gardenia erubescens* fruits were cut into two pieces, the seeds removed, dried and collected in a polythene bag. The samples were brought to Microbiology laboratory of Usmanu Danfodiyo University for studies on bioethanol.

Yeast inoculum preparation

The yeast inoculum was prepared as described by Scholar and Benedikte (1999) and Suh et al. (2007). One gram of dry baker's yeast purchased from Kumasi central market was grown on Yeast Peptone Dextrose (YPD) agar plate at 30oC for 48 h to activate the yeast and check for contaminates For inoculum, a loopful of the yeast colony from a 2 day old agar plate was transferred into 5 ml sterile distilled water to form a suspension. Zero point one (0.1) – 0.5ml of this suspension having spore concentration of approximately 2.6 x 10^6 to 1.3 x 10^7 cells/ml, was used as inoculum for the fermentation.

Production of Ethanol/Seeding with *S. cerevisiae* for Ethanol Production

After hydrolysis for 6, 7 and 8 days, the broth was filtered and the hydrolysate was autoclaved at 121^{o}C for 15 minutes. The sterile hydrolysate was allowed to cool at room temperature and seeded with 1%, 2% and 3% inoculum of *S. cerevisiae.* This form the basis for labelling the flask as A, B and C respectively and were incubated at 40 ^{o}C, 35 ^{o}C and room temperature for 1, 2, 3, 4 and 5 days to monitored ethanol production.

Distillation

The fermented broth was filtered using filter paper. Each sample was weighed into Microkjeldahl flasks and was heated at 78 ^{o}C on the Microkjeldahl apparatus until the solution turned colourless.

Quantification of Ethanol Produced

Ethanol concentrations of the fermentation were determined at 1,2,3,4 and 5 days. One (1ml) mile of ethanol sample was poured into a test tube, 7ml of distilled water were added and 2ml of acidified potassium dichromate were added. It is then heated at 40mls in water bath. The absorbance read at 580nm using UV-visible spectrophotometer.

Result

Production of bioethanol from *Gardenia erubescens* fruit hydrolysate seeded with *S. cerevisiae* for 1day was evaluated and result presented in Table 1. From the result, the hydrolysate from 1:2 substrate water concentrations (A1) had the highest concentration of ethanol 1.75% and 1:3 substrate water concentration (B1) of 8 days had the least concentration 0.07%.

Production of ethanol from *Gardenia erubescens* fruit hydrolysate seeded with *S. cerevisiae* for 2 day was evaluated and result presented in Table 2. From the result, the hydrolysate from 1:4 substrate water concentrations (C2) had the highest concentration of ethanol 15.25% and 1:3 substrate water concentration (B2) of 8 days had the least concentration 0.08%.

Production of ethanol from *Gardenia erubescens* fruit hydrolysate seeded with *S. cerevisiae* for 3 day was evaluated and result presented in Table 3. From the result, the hydrolysate from 1:4 substrate water concentrations (C3) had the highest concentration of ethanol 1.44% and 1:3 substrate water concentration (B3) of day 8 had the least concentration 0.07%

Production of ethanol from *Gardenia erubescens* fruit hydrolysate seeded with *s cerevisiae* for 4 day was evaluated and result presented in Table 4. From the result, the hydrolysate from 1:4 substrate water concentrations (C4) had the highest concentration of ethanol 5.10% and 1:3 substrate water concentration (B4) of day 6 had the least concentration 0.10%

Production of ethanol from *Gardenia erubescens* fruit hydrolysate seeded with *s cerevisiae* for 5day was evaluated and result presented in Table 5. From the result, the hydrolysate from 1:2 substrate water concentrations (A5) had the highest concentration of ethanol 3.74% and 1:3 substrate water concentration (B5) of day 8 had the least concentration 1.53%

Table 1: Result of Ethanol Produced from *Gerdenia erubescens* fruits using Uv-visible Spectrophotometer Seeded with *Saccharomyces cerevisiae* for 1 day.

Samples	Time of Hydrolysis (days)	Concentration (%)
A_1	6	1.75
A_1	8	0.95
B_1	6	0.20
B_1	8	0.077
C_1	6	1.71
C_1	8	1.61
TOTAL YIELD		6.29

Table 2: Result of Ethanol Produced from *Gerdenia erubescens* fruits using Uv-visible Spectrophotometer Seeded with *Saccharomyces cerevisiae* for 2 days.

Samples	Time of Hydrolysis (days)	Concentration (%)
A_2	6	1.86
A_2	8	1.17
B_2	6	0.28
B_2	8	0.08
C_2	6	15.25
C_2	8	15.25
TOTAL YIELD		33.89

Table 3: Result of Ethanol Produced from *Gerdenia erubescens* fruits using Uv-visible Spectrophotometer Seeded with *Saccharomyces cerevisiae* for 3 days.

Samples	Time of Hydrolysis (days)	Concentration (%)
A_3	6	0.89
A_3	8	0.70
B_3	6	0.28
B_3	8	0.07
C_3	6	1.44
C_3	8	0.82
TOTAL YIELD		4.20

Table 4: Result of Ethanol Produced from *Gerdenia erubescens* fruits using Uv-visible Spectrophotometer Seeded with *Saccharomyces cerevisiae* for 4 days.

Samples	Time of Hydrolysis (days)	Concentration (%)
A_4	6	2.46
A_4	8	0.44
B_4	6	0.33
B_4	8	0.10
C_4	6	5.10
TOTAL YIELD		8.43

Table 5: Result of Ethanol Produced from *Gerdenia erubescens* fruits using Uv-visible Spectrophotometer Seeded with *Saccharomyces cerevisiae* for 5 days.

Samples	Time of Hydrolysis (days)	Concentration (%)
A_5	6	3.74
A_5	8	1.53
B_5	6	1.56
B_5	8	1.53
C_5	6	2.12
C_5	8	2.12
TOTAL YIELD		12.60

Discussion

Production of ethanol from *Gardenia erubescens* fruit hydrolysate seeded with *Saccharomyces cerevisiae* was determined at 1, 2, 3, 4 and 5 days after inoculation.

The highest ethanol concentration was obtained on day 2 with 33.89% followed by 12.6%, 8.93% and 6.29% on day 5, 4, 1 respectively. And the least was 4.2% on day 3. The result of ethanol obtained on the 1st day could be attributed at fact that a yeast cells are probably at their lag phase trying to synthesized necessary enzymes for their metabolism thereby converting the readily available sugar to ethanol (Nester et al., 2004). The highest concentration may represent the maximum tolerable limit of ethanol by the yeast cells. Ethanol has been reported as a well known inhibitor of the growth of micro organism due to its effect on the mitochondrial of yeast cell and some enzyme such as hexokinase and dehydrogenase (Ibeas and Jimenez, 1997). Never the less, some strains of the yeast *Saccharomyces cerevisiae* show tolerance and can adapt to high concentration of ethanol (Alexandre et al., 1994).

The variation observed after the 3rd, 4th and 5th days may be attribute error that might have occurred during distillation and possible quantitatively ethanol concentration. Comparison to similar works in literature is difficult because ethanol concentration was not cited and they differ in either in type of pretreatment if any and detoxification, substrate concentration, fermentation strain, temperature or mode of operation which affects the final ethanol concentration as reported in work by Olofsson et al. (2008). The results suggest that *Gardenia erubescens* fruit could be exploited as raw materials for ethanol production thereby creating jobs and maximizing profit.

Conclusion

The studies on the production on bioethanol from *G. erubescens* fruit gave the highest cumulative ethanol yield of 33.89% after seeding the hydrolysate with *S. cerevisiae* for 2 days. This concentration is appreciable thereby suggesting that *G erubescens* could be harnessed for large scale production of ethanol after further feasibility studies and validation.

References

[1] Alexandre H, Rovsseaux, I. and Charpentier. C., (1994) Ethanol, Adaptation Mechanism in *Saccharomyces Cerevisiae*. *Biotechnol. Appl. Biochem.* 20: 173-183.

[2] Ibeas, J. I. and Jimenez, J. (1997), Mitochondrial DNA loss caused by ethanol in *Saccharomyces* flour yeast *Appl. Environ Microbial* 63:7-12.

[3] Macedo, I. C. (1995). In converting Biomass to liquid fuel: Making ethanol from sugarcane in Brasil, Sau paulo, Brasil

[4] Mojović, L. Pejin, D., Grujić, V., Markov, S., Pejin, J. Rakin, M., Vukašinović, M., Nikolić, S. and Savić, D. (2009). Progress in the production of bioethanol on starch-based feedstocks. *Chemical Industry and Chemical Engineering Quarterly* 15(4) 211−226

[5] Nester EW, Anderson DG, Roberts CE, Pearsall NN, Nester MT (2004). Dynamics of prokaryotic growth In: Microbiology: A human perspective.4th ed. McGraw-Hill, New York. pp. 87-108.
[6] Olofsson K., Bertiloson M. Liden G. (2008) A short review on SSF an interesting process option for ethanol production from lignocellucosic fedstocks *Biotechnol for Biofuel* 1(7):1-14.
[7] Scholar J, Benedikte W (1999). Practical fermentation: a guide for schools and colleges. The society for general microbiology. www.ncbe.reading.ac.uk.
[8] Suh S, Zhang N, Nguyen N, Gross S, Blackwell M (2007). Lab manual for yeast study. Mycology lab. Louisiana state university.
[9] UN Energy (2007) Sustainable Bioenergy A framework for decision makers.
[10] Yerima, M. B., Maccido D. A., Jodi S. M., Farouq, A. A., Kolawole R. S., Ibrahim, A. D; Almustapha M. N., and Umar A. F (2008) Effect of organic nitrogen on Bioethanal production from water Hyaanta (Eichhornic Crossipes). *Nig. J. Biotech* 19:81-83.
[11] Zakpaa H. D. Mak-Con A. cos Mensah, E.E and Johnson F. S (2009) Production of bioethanol from corncobs using *Aspengillus and saccharomyces Cervistal* in simultaneous saccharification and Fermentation. African Journal of Biotech 3(13):3018-3022.