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Optimizing Bio-ethanol Production from *Striga hermonthica* Using Yeast (*Saccharomyces cerevisiae*) as a Fermenting Agent

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Abstract: Global warming, urban pollution and depletion of fossil fuels have been driving for looking alternative energy sources, especially those derived from biomass. Production of bio-ethanol from lingocellulosic materials is providing a long-term sustainable for fuel supply. *Striga hermonthica*, a parasitic weed plant is one of cheap source of lignocellosic materials to serve as feedstock for bio-ethanol production. With the objective of evaluating its potential for bio-ethanol production, different concentrations (10g, 20g, 30g, and 40g) of *Striga hermonthica* treated with 1% diluted sulfuric acid and untreated were subjected to batch fermentation for 16 days with 0.5% and 1% yeast inoculums. Percent of bio-ethanol production, cell density and reducing sugars were measured at an interval of 4 days starting from the beginning. Results of these study showed that ethanol production was observed starting from the 4th day of fermentation, but its amount peaked 28.05% from 40g substrate with 1% inoculum on the 12th day of fermentation, and declined on 16th days (20.24%) from the same substrate concentration. Pretreated substrate showed significantly higher ethanol production than untreated. In agreement with ethanol production, cell density and reduction in reducing sugar were observed in the same pattern. Compared ethanol production between untreated substrates yield of 21.31%, and treated substrates yielded of 28.05%. Overall, this study showed that acid pre-treatment, inoculum concentration, fermentation period and substrate concentration affect the amount of bio-ethanol production. Finally, it can be concluded that the production of bioethanol from *Striga hermonthica* is economically and environmentally viable. Extensive use of this harmful weed for bioethanol production may have twofold advantages, viz. reduction of its negative impact on crop productivity and generation of bio-ethanol.

Keywords: Bio-ethanol, Distillation, Fermentation, Pretreatment, *Saccharomyces cerevisiae*, *Striga hermonthica*

1. Introduction

Bio-ethanol is one of the liquid fuels found to have an efficient calorific content capable of being used in automobiles [15]. It is derived from renewable feedstock sources such as sugary, starchy and lignocellulosic biomass. Among the three major types of raw materials, production of bio-ethanol from sugary and starchy materials is easier as compared to lingo-cellulosic materials since it requires additional technical challenges such as pretreatment [14]. Using bio-ethanol as a fuel decreases fossil fuel consumption and increases energy supply security [10].

`Bio-ethanol is a liquid obtained by distillation of fermented sugar by different yeast and bacterial species. The yeast *Saccharomyces cerevisiae* and facultative bacterium *Zymomonas mobilis* are better candidates for industrial alcohol production. *Zymomonas mobilis* was recombinant bacterial species that possesses advantages over *S.cerevisiae* with respect to bio-ethanol productivity. However, bio-ethanol is produced commercially by yeast [13]

Many countries have been using bio-ethanol as alternative to petrochemicals like diesel and petrol by blending it with gasoline in fixed proportion. In Ethiopia, the blending of Ethanol with Benzene was started in September 2008 with 5% Ethanol and 95% benzene [12]. Using ethanol blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission. Fossil fuel is depleting day by day throughout the world. This limitation along with the

problem of Green House Gas (GHG) emissions leads findings for alternative energy that are environmentally and commercially feasible

The production of bio-ethanol from comparatively cheaper source of raw materials using efficient fermentative microorganisms is the only possible way to meet the great demand for bio-ethanol in the present situation of energy crisis. As a result, alternative biomass sources such as agricultural and municipal wastes, and other lignocellulosic materials from non-food plants being sought as alternatives source [11]

Moreover, *Striga* is parasitic weed that grows on the roots of cereal and legume crops in dry, semi-arid, and harsh environments of tropical and subtropical Africa, Arabian Peninsula, India, and a small part of USA. In Ethiopia, especially eastern region, grain yield loss for susceptible sorghum varieties was estimated to be 59% due to the infection of *Striga* weed species [2]. Extensive use of this harmful weed for bioethanol production may have twofold advantages, viz. reduction of its negative impact on crop productivity and generation of bio-ethanol production. Therefore, the research was initiated to production of bio-ethanol from *Striga hermonthica* with following general and specific objectives.

General objective of the study

To determine the amount of bio-ethanol production from *Striga hermonthica* by a batch culture using *S. cerevisiae.*

Specific objectives of the study are

1) To identify the optimum substrate concentration for maximum bio ethanol production
2) To determine the cell density and reducing sugar concentrations at different fermentation time
3) To assess the effect of acid pre-treatment on the rate of ethanol production

2. Materials and Methods

2.1. Description of the Study Area

The experiment was conducted in Botanical Science laboratory, Department of Biology at Haramaya University which is located at latitude of 9º26’ N, longitude of 42º03’E and altitude of 1980 m.a.s.1 [9].

2.2. Substrate Preparation

Fresh *Striga hermonthica* plants were collected from eastern Hararghe, babile district farmlands and brought to the laboratory. The plants were washed using tap water, and chopped and dried in oven at 60±3°C for 24 h prior to pre-treatment. Different substrate amount (10g, 20g, 30g and 40g) was then be pretreated with 100ml of 1 % (v/v) H_2SO_4 add to each gram of substrate and mixture was shaken and autoclaved for 15 minutes, at 121°C

2.3. Inoculums Preparation

Inoculums were prepared from dried baker's yeast, *Saccharomyces cerevisiae* that was purchased from Neway plc. 0.5% and 1% inoculums concentration was prepared from 0.5g and 1g of yeast *(S. cerevisiae)* in 99.5 and 99ml of distilled water respectively and 10ml of the solutions was used as inoculums volume for batch fermentation process [5].

2.4. Preparation of Nutrient Solution

Nutrient supplements were prepared by adding 0.1g KH_2PO_4, 0.5g $CaCl_2$, 0.5g $MgSO_4$, 0.1g Na_2SO_4 and 0.1g $(NH_4)_2SO_4$ per liter of the culture solution [8, 7].

2.5. Experimental Design

The experimental design was based on CRD factorial and eight treatment combination with three replications for each treatment and control.

2.6. Fermentation Processes

The fermentation process was allowed for 16 days at 30°C and production of bio-ethanol was estimated at the of 4th, 8th, 12th, and 16th day

2.7. Determination of Cell Density (Biomass)

Cell density was measured by dry weight method at 4^{th}, 8^{th}, 12^{th} and 16^{th} day of fermentation using spectrophotometer (Humas Think HS 3300, Korea) at 600 nm absorbance [8, 4, 7]

2.8. Estimation of Total Reducing Sugar

The amount of reducing sugar in the fermenting sample broth was estimated spectrophotometriclly at 540nm following the method used by Nelson (1944) using D-glucose as standard. A fermenting sample (0.05ml) was mixed with 0.35ml citrate buffer (pH=6.5) and 0.6ml of Dinitrosalicylic acid (DNS) and then the mixture was boiled for 5 minutes immediately to stop the reaction [6]

2.9. Quantitative Estimation of Bio-ethanol

25ml of the fermented sample was taken into 500ml Pyrex distillation flask containing 30ml of distilled water and distilled and collected in 250ml flask [3]. About 20ml of distillate were kept in a water bath maintained at 6o°C for 20 minutes and cooled to room temperature. Five ml of this solution was diluted with 5ml of distilled water for measuring the optical density at 600nm using spectrophotometer [1]. A standard curve was prepared under similar set of conditions by using standard solution of ethanol containing 0 to 20% (v/v) [13]

2.10. Data Analysis

Microsoft offices excel spreadsheet and Statistical Package for Social Studies (SPSS) version 17 is used to data analysis

3. Results and Discussion

3.1. Amounts of Ethanol Production from Fermenter

Effects of acid pre-treatment, substrate and inoculums concentrations and fermentation period on ethanol production

were observed and results were indicated in table 1. The results revealed that, acid pre-treated substrate produce more ethanol than untreated substrate in all the substrate and inoculums concentration. Moreover, acid-treated substrates significantly ($p<0.05$) produce higher (28.05) amount of ethanol than untreated substrates(21.31%) at the concentration of 40g substrate inoculated with 1% yeast at 12th day of fermentations.

Table 1. *Ethanol production from Striga hermonthica using S.cerevisiae (mean ± SD, n=3).*

Substrate (gm)	Treated untreated	Ethanol produced (%)at different fermentation period			
		4th day	8th day	12th day	16th day
A	Treated	6.54±0.03^{dK}	8.16±0.02^{bK}	11.57±0.01^{aK}	8.24±0.02^{bI}
	Untreated	5.09±0.03^{dK}	7.87±0.08^{cL}	9.23±0.05^{aL}	7.61±0.02^{cJ}
B	Treated	8.13±0.07^{cI}	10.14±0.04^{bJ}	12.10±0.03^{aJ}	11.04±0.01^{bH}
	Untreated	7.63±0.02^{cJ}	8.02±0.09^{Ak}	9.03±0.08^{aL}	8.58±0.07^{bI}
C	Treated	9.89±0.01^{dG}	12.44±0.07^{cH}	17.46±0.03^{aH}	13.32±0.03^{Be}
	Untreated	8.51±0.06^{eI}	11.77±0.04^{dI}	15.04±0.07^{aI}	12.80±0.04^{bE}
D	Treated	10.37±0.01^{eF}	13.56±0.06^{cG}	18.08±0.03^{aG}	14.91±0.01^{bF}
	Untreated	9.07±0.02^{eH}	12.77±0.04^{cH}	17.00±0.02^{aG}	13.93±0.03^{bG}
E	Treated	13.09±0.02^{dD}	16.51±0.02^{bE}	20.06±0.06^{aE}	16.15±0.02^{bD}
	Untreated	12.04±0.07^{eD}	14.96±0.05^{cF}	18.66±0.05^{aF}	15.02±0.04^{cF}
F	Treated	14.49±0.01^{dB}	17.64±0.05^{bC}	21.95±0.07^{aD}	19.08±0.04^{bB}
	Untreated	13.49±0.01^{eC}	15.04±0.06^{cD}	19.50±0.06^{aE}	16.54±0.07^{cE}
G	Treated	15.02±0.05^{eB}	20.25±0.07^{cB}	26.07±0.04^{aB}	19.35±0.06^{cC}
	Untreated	12.72±0.09^{eE}	17.01±0.06^{cD}	20.61±0.05^{aE}	17.26±0.02^{bB}
H	Treated	15.72±0.02^{dA}	21.74±0.03^{bA}	28.05±0.02^{aA}	20.24±0.04^{bA}
	Untreated	14.04±0.02^{eB}	18.37±0.01^{cD}	21.31±0.03^{aC}	17.88±0.02^{bB}

Note: Means followed by different capital letter in column are significantly different at 5% level of significance. A=10g + 0.5% yeast, B=10g + 1% yeast, C=20g + 0.5% yeast, D=20g + 1% yeast, E=30g + 0.5% yeast, F=30g + 1% yeast, G=40g + 0.5% yeast and H=40g + 1% yeast.

3.2. Amounts Reducing Sugar Yield

Concentration of reducing sugar were measured on the 4th day of fermentation was found to be significantly higher in both acid pretreated and untreated substrates and found to be decline with increasing days of fermentation (Table 2).

Table 2. *Reducing sugar concentration (mg/ml) measured at 540nm (values are Mean ±SD, n=3).*

Substrate(gm)	Treat/Untreat	Reducing sugar concentration(ml) at different fermentation period			
		4th day	8th day	12th day	16th day
A	Treated	7.93±0.035^{aG}	6.72±0.058^{bH}	4.0±0.023^{cF}	2.67±0.064^{dG}
Untreated		7.25±0.077^{aH}	6.53±0.077^{bH}	4.41±0.031^{cE}	3.31±0.040^{dE}
B	Treated	7.07±0.023^{aH}	5.44±0.018^{bG}	3.0±0.062^{cG}	3.18±0.058^{dH}
Untreated		6.24±0.031^{aI}	5.27±0.031^{bG}	3.20±0.049^{cG}	3.21±0.017^{dH}
C	Treated	9.96±0.045^{aE}	9.30±0.046^{bD}	5.0±0.042^{cD}	4.24±0.072^{dC}
Untreated		9.81±0.036^{aE}	9.18±0.027^{bD}	5.92±0.062^{cC}	4.31±0.071^{dC}
D	Treated	9.30±0.013^{aF}	8.95±0.044^{bE}	6.0±0.085^{cC}	3.68±0.031^{dE}
Untreated		9.19±0.017^{aF}	8.31±0.045^{bF}	6.46±0.055^{cC}	3.39±0.064^{dC}
E	Treated	11.32±0.036^{aC}	9.61±0.017^{bC}	7.06±0.072^{cB}	5.19±0.020^{dB}
Untreated		11.19±0.010^{aC}	9.26±0.026^{dD}	7.15±0.031^{cB}	5.25±0.067^{dB}
F	Treated	9.95±0.010^{aE}	8.30±0.013^{bF}	5.80±0.010^{cD}	4.31±0.015^{dD}
Untreated		9.0±0.023^{aF}	8.21±0.055^{bF}	5.92±0.053^{cD}	4.59±0.059^{dD}
G	Treated	12.53±0.040^{aB}	10.4±0.049^{bA}	7.16±0.071^{cB}	5.69±0.020^{dA}
Untreated		10.69±0.049^{aC}	10.01±0.040^{Bb}	7.47±0.018^{cA}	4.68±0.022^{dA}
H	Treated	12.75±0.058^{aA}	9.93±0.017^{bE}	4.44±0.060^{cE}	3.02±0.013^{dF}
Untreated		10.55±0.070^{aD}	8.18±0.053^{bF}	4.5±0.032^{cE}	3.03±0.022^{dF}

Note: Means followed by different small letters in row are significant at 0.05 probability levels. Means followed by different capital letter in column are significantly different at 5% level of significance. A= 10g + 0.5% yeast, B=10g + 1% yeast, C= 20g + 0.5% yeast, D= 20g + 1% yeast, E= 30g + 0.5% yeast, F= 30g + 1% yeast, G= 40g + 0.5% yeast and H= 40g + 1% yeast

3.3. Cell Density Concentrations at Different Fermentation Period

The results showed that yeast biomass was increased in all the substrate concentration from 4thday to 12thday of fermentation period. However, after 12thday of fermentation the cell biomass decreased (Table 3). This may be due to high alcohol content or cyto-toxicity of ethanol or decrease in fermentable sugar. The maximum cell density (6.03mg/ml) was obtained from 40g of acid pretreated substrate with 1% of yeast inoculums on 12th day of fermentation. However; after 12th day of fermentation period cell biomass found to decline

Table 3. *Cell Density fermented at different fermentation period (Values are Mean ±SD, n=3).*

Substrate Treatment	Treated untreated	Cell density observed from fermented *S.hermonthica* at 600nm (mg/ml) at different fermentation period			
		4th day	8th day	12th day	16th day
A	Treated	1.77 ± 0.032^{cD}	2.21 ± 0.040^{bF}	2.41 ± 0.018^{aC}	2.00 ± 0.017^{cF}
	Untreated	1.69 ± 0.080^{cE}	2.02 ± 0.083^{bF}	1.9 ± 0.054^{aD}	1.64 ± 0.050^{cF}
B	Treated	2.45 ± 0.049^{cC}	2.81 ± 0.073^{bD}	3.44 ± 0.027^{aC}	3.01 ± 0.054^{bD}
	Untreated	2.38 ± 0.022^{cC}	2.79 ± 0.013^{bD}	2.52 ± 0.032^{aC}	2.13 ± 0.015^{bD}
C	Treated	1.91 ± 0.022^{cD}	2.45 ± 0.027^{bE}	4.0 ± 0.018^{aC}	3.11 ± 0.044^{aC}
	Untreated	1.88 ± 0.040^{cD}	2.35 ± 0.027^{bE}	3.12 ± 0.058^{aC}	2.12 ± 0.025^{aC}
D	Treated	2.69 ± 0.074^{cB}	3.15 ± 0.027^{bC}	4.53 ± 0.015^{aB}	3.86 ± 0.023^{cD}
	Untreated	2.67 ± 0.047^{cB}	3.09 ± 0.018^{bC}	3.6 ± 0.013^{aB}	3.0 ± 0.067^{bD}
E	Treated	1.85 ± 0.037^{cD}	3.87 ± 0.018^{aA}	5.4 ± 0.017^{bC}	4.37 ± 0.089^{bB}
	Untreated	1.80 ± 0.085^{cD}	3.85 ± 0.059^{aA}	4.0 ± 0.064^{bC}	3.9 ± 0.013^{bB}
F	Treated	2.79 ± 0.013^{cA}	3.50 ± 0.037^{bB}	5.9 ± 0.031^{aA}	3.52 ± 0.031^{cE}
Untreated	2.74 ± 0.044^{cA}	3.39 ± 0.064^{bB}	4.01 ± 0.058^{aA}	3.38 ± 0.015^{cE}	
G	Treated	1.87 ± 0.033^{dD}	2.82 ± 0.015^{cD}	5.80 ± 0.050^{aB}	4.38 ± 0.010^{bB}
Untreated	1.85 ± 0.038^{dD}	2.76 ± 0.013^{cD}	4.23 ± 0.030^{aB}	4.39 ± 0.017^{bB}	
H	Treated	2.79 ± 0.013^{cA}	3.57 ± 0.023^{bB}	6.03 ± 0.010^{aA}	4.75 ± 0.028^{bA}
Untreated	2.75 ± 0.040^{cA}	3.92 ± 0.030^{aA}	4.97 ± 0.049^{aA}	4.73 ± 0.022^{bA}	

Note: Means followed by different capital letter in column are significantly different at 5% level of significance. A= 10g + 0.5% yeast, B=10g + 1% yeast, C= 20g + 0.5% yeast, D= 20g + 1% yeast, E= 30g + 0.5% yeast, F= 30g + 1% yeast, G= 40g + 0.5% yeast and H= 40g + 1% yeast

4. Summary, Conclusion and Recommendation

4.1. Summary

Bio-ethanol has been identified as the mostly used bio-fuel worldwide since it significantly contributes to the reduction of crude oil consumption and environmental pollution. It can be produced from various types of feed-stocks such as sucrose, starch, lignocellulosic and algal biomass through fermentation process by microorganisms. Since *Striga hermonthica* is the most dangerous parasitic weed plant that affects crop productivity. *Striga* is parasitic weed that grows on the roots of cereal and legume crops in dry, semi-arid, and harsh environments of tropical and subtropical Africa. Therefore, the research was initiated to produce bio-ethanol from *Striga hermonthica*.

The finding of present study revealed that *Striga hermonthica* exhibited significant result for the production of bio-ethanol. The amount of bio-ethanol production depended on substrates concentration and effect was statistically significant at $p<0.05$.

Finally, it can be concluded that the production of bio-ethanol from *Striga hermonthica* is economically and environmentally viable. Beside that production of bio-ethanol from *Striga hermonthica* is important for producing agriculture high yield by reducing its impacts of crops and the produced ethanol can be a good substitute of Petrol.

4.2. Conclusion

Fossil fuel is depleting day by day throughout the world. This limitation along with the problem of Green House Gas (GHG) emissions leads findings for alternative energy that are environmentally and commercially feasible. Bio-ethanol is one of the liquid fuels found to have an efficient calorific content capable of being used in automobiles and that have the ability to fulfill needed of world energy crisis by using as alternative energy that are environmentally and commercially feasible. It is derived from renewable feedstock sources such as sugary, starchy and lignocellulosic biomass (material). However, sugary and starchy biomasses are used mainly as food for humans and animal consumption that when used for energy production, the rapidly increasing world population will face food crises. As a result, alternative biomass sources such as agricultural and municipal wastes, and other lignocellulosic materials from non-food plants being sought as alternatives source.

This project was prepared on title Optimization of Bio-ethanol production from *Striga hermonthica* using yeast (*Saccharomyces cerevisiae*) as a fermenting agent. The substrate used for this research *Striga hermonthica* plants were collected from eastern Hararghe, babile district farmlands. Moreover, *Striga* is parasitic weed that grows on the roots of cereal and legume crops in dry, semi-arid, and harsh environments of tropical and subtropical Africa, Arabian Peninsula, India, and a small part of USA. In Ethiopia, especially eastern region, grain yield loss for susceptible sorghum varieties was estimated to be 59% due to the infection of *Striga* weed species [8]. Extensive use of this harmful weed for bioethanol production may have twofold advantages, viz. reduction of its negative impact on crop productivity and generation of bio-ethanol production

4.3. Recommendation

Based on the findings of the experiments, the following recommendations were suggested

1) To check the bio-fuel quality of *Striga hermonthica* HPLC too.

2) Further study is very important to describe how absolute bio-ethanol can be produced from *Striga hermonthica* by using Fed batch and continuous fermentations for inclusive use of substrate.

References

[1] Chisti, Y. 2007. Biodiesel from microalgae. *Biotechnology advances*, 25 (3): 294-306.

[2] Galbe, M. and Zacchi, G. 2002. Review of the Production of ethanol from softwood. *Applied Microbiology and Biotechnology,* 59 (6): 618.

[3] Gunasekaran, P., Chandra R. K. 1999. Ethanol fermentation technology *Zymomonas mobilis. Current Science,* 77 (1): 56-68.

[4] Gupta, Nirbhay, (2009). Hydrolysis of Lignocellulosic Materials for Ethanol Production, National Institute of Technology, Rourkela.

[5] Haramaya University. 2003. Sorghum Improvement Project Progress Report for 2002. Haramaya University Research Center.

[6] Hsieh. W. 2002. Engine Performance and Pollutant Emission Of and Si Engine Using Ethanol Gasoline Blended Fuels. *Atmospheric Environment,* 36: 403-41.

[7] Ministry of Finance and Economic Development (MoFED). 2010. Ethiopia Country Report on the Implementation of the Brussels Program of Action (BPOA). Addis Ababa.

[8] Parker, C. and Riches, C. R. 1993. Parasitic weeds of the world: biology and control. *CAB International*.

[9] Prasad, S., Singh, A. and Joshi, H. C. 2007. Ethanol as an alternative fuel from agricultural, industrial and urban residues. *Resources, Conservation and Recycling*, 50 (1): 1-39.

[10] Sadasivam, S and Manickam, A. 1996. Biochemical methods 2nd edition, New Age International Ltd. Publisher, New Delhi, 179-186.

[11] Saha. L. B, Iten. Ma. Cotta and Y. V. Wu. 2005. Dilute Acid and Enzymatic Pretreatment of Lignocellulosic residues, biodegradation and bioconversion by fungi. *Biotechnology Advanced Journal,* 27 (2): 185-194.

[12] Summer, J., Georgierva, B. and Ahring, K. 2004. Bioconversion of banana wastes to ethyl alcohol. Biochemistry Translation, 32-283.

[13] Yoswathana, N and Phuriphipat, P. 2010. Bioethanol Production from Rice Straw. *Energy Research Journal,* 1: 26-31.

[14] Zhang. D and Wu. J. C., (2012). Optimization of dilute acid-catalyzed hydrolysis of oil palm empty fruit bunch for high yield production of xylose. *Chemical Engineering Journal, volume. 181-182, pp. 636–642.*

[15] Zhang. P, Whistler. R. L, Be Miller. J. N, & Hamaker. B. R., (2005). Banana starch Production, physicochemical properties, and digestibility a review. 59: 443 -458.