JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

COMPARATIVE STUDY OF THREE YEAST STRAINS ON BIOETHANOL FERMENTATION OF *Pontederia crassipes* (Water Hyacinth) FEEDSTOCK

Aprajita Chauhan[1] and Yogesh Kwatra[2]
[1]Professor, Department of Chemistry
[2] Student
Sri Aurobindo College (University of Delhi) Malviya Nagar,
NewDelhi-110017, India.

Abstract: Water Hyacinth Biomass (WHB) is an ideal feedstock for bioethanol production as it is rich in hemicelluloses and cellulose while the lignin content is low. This feedstock is, therefore, a high source of reducing sugars (C_5 and C_6). Water hyacinth plants were collected from five sites selected along the river Yamuna in Delhi: A. Okhla West, B. Okhla East, C. Okhla Barrage, D. Najafgarh Drain and E. DND Flyover. As feedstock plants growing in different conditions of water quality in Delhi exhibited different sugar contents, a comparative study reveals that plants from site A (Okhla West) possessed maximum reducing sugar content.

The sugar content in plants growing in different sites was:
A>B>C>D>E

Maximum saccharification (sugar extraction) was obtained after 3% sulphuric acid pretreatment for 1.5h at 121^0C. Optimization of acid pretreatment helps in breaking strong linkages between cellulose, hemicellulose and lignin, thereby making the feedstock more accessible for further enzymatic hydrolysis.

Of the three yeast strains used, the Four Seasons Yeast(FSY) gave best results for bioethanol fermentation (0.198g/L) when used on 3% sugar hydrolysate from 1:10 WHB after 24h. Corresponding values of bioethanol production by other two yeasts were slightly lower. Studies in time course utilization of hydrolysate corroborated the results for efficiency of yeasts in the order:

FSY> Baker's Yeast> Yeast Extract.

IndexTerms- ***Pontederia crassipes* (Water Hyacinth), bio feedstock, yeast strains, bioethanol fermentation.**

I. INTRODUCTION

Covering over four million hectares of water surface in India, water hyacinth is now considered as the most noxious weed. Continuous efforts to eradicate this plant by physical means or biocontrol have not met with much success as its prolific growth far surpasses the removal strategies. Moreover, the disposal of waste biomass also poses serious problems of soil contamination. Ironically, this weed has in recent times, emerged as one of the most promising and appropriate raw materials for biofuel production and as a viable alternative to conventional fossil fuels. Water hyacinth feedstock-based bioethanol and briquettes are being exploited to produce biogas and electricity (Bhattacharya& Kumar, 2010; Ganguly et al,2012; Alvi et al,2014; Das et al,2016; Bote et al, 2020; Hirphaye et al, 2022; Ainomujuni et al, 2023 and Dandasena & Shahi, 2023). In addition, this weed has a unique absorption capacity for removal of heavy metals thereby cleansing of water bodies (Chauhan et al, 2016 and Ainomujini et al, 2023). By not being a part

of the human food chain, exploitation of the water hyacinth does not compete with the food crops and thus, does not endanger the food security.

Recent years have witnessed many advances in the pretreatment technologies for enhancing bioethanol from lignocellulosic biomass (Baig et al, 2017; Busic et al, 2018; Rezania et al,2018; Broda et al, 2022; Haldar & Purkait, 2021; Sai Kumar et al,2021; Caeser et al,2024 and Jain & Kumar, 2024). Various studies involving different pretreatment approaches have been carried out for the conversion of water hyacinth biomass into ethanol. These include pretreatment with acids, enzymatic or microbial cellulose treatments prior to saccharification and subsequent fermentation by yeast and other microbes (Das et al, 2016; Zhang et al, 2016 and Chauhan et al, 2020).

Ethanol is being extensively produced from lignocellulosic biomass derived from agricultural wastes using yeast fermentation (Irfan et al, 2014). Present investigations have been carried out with the objective of comparing the fermentation efficacy of three different yeasts as manifested by bioethanol production.

II. METHODOLOGY

2.1 Collection of Substrate

Plants of water hyacinth –*Pontederia crassipes* (earlier called *Eichhornia crassipes* (Family Pontederiaceae) were collected from five sites selected along the river Yamuna in Delhi, India: A. Okhla West, B. Okhla East, C. Okhla Barrage, D. Najafgarh Drain and E. DND Flyover. After removing the roots, the petioles and leaves were washed with tap water to remove dirt. Leaves and petioles were dried in sun for 12 hours. Many cuts were made in the plant parts to hasten drying. The sundried biomass was chopped into small pieces and oven-dried at 106°C for 6 hours. Dried plant material was ground in a mixer-grinder to reduce the particle size to few mm and filtered through a 1mm sieve. The ground material was stored in air-tight containers at room temperature and was subsequently used for saccharification and fermentation procedures.

2.2 Acid Pretreatment

Sulfuric acid pretreatment of the substrate was carried out according to the procedure already outlined in an earlier investigation (Chauhan *et al.* 2020). 3% sulfuric acid pretreatment yielded maximum sugars. The hydrolysate was cooled and vacuum filtered with Whatmann filter paper No. 1 to remove unhydrolysed material. The hydrolysate was neutralized with 10N NaOH and subjected to sugar analysis. Optimum conditions were ascertained for maximum sugar extraction. However, for subsequent fermentation experiments, three different concentrations of water hyacinth biomass (WHB) viz., 1:10, 1:15 and 1:20w/v (powder: sulfuric acid) were used along with two different concentrations of sulfuric acid (2% and 3%).

2.3 Detoxification of Acid Hydrolysate

The acid hydrolysate was heated at 60^0C for 15 min. to reduce the concentration of volatile components. Volume lost due to heating was replaced by equal amount of heated distilled water. Over limed the mixture with $Ca(OH)_2$ up to pH 10 along with 0.1% sodium sulfite. The mixture was filtered to remove insolubles and subsequently re- acidified to pH 6.0 with 1N H_2SO_4.The filtrate was concentrated and used for fermentation experiments.

2.4 Fermentation and Ethanol Formation

Yeast (*Saccharomyces cerevissiae*) was obtained from three sources. Commercial Baker's yeast (BY) was obtained from the local market. Yeast extract (YE) was obtained from CDH. "Four Seasons" instant dry yeast (FSY) was provided by Kothari Fermentation and Biochem Ltd. The Primary Inoculum consisted of a combination of Culture medium and yeast cells. First, yeast cells (1g/100mL) were introduced into 50.0mL of culture medium containing yeast extract (10g/L) peptone (20g/L) and glucose (20g/L). The inoculum was then incubated for 24h at 30^0C.The detoxified acid hydrolysate was supplemented with yeast extract (1g/L), $(NH_4)_2SO_4$ (2g/L) and $MgSO_4$ (1.0g/L) to make a solution. For fermentation experiments the inoculum to solution ratio used was 1:10.

Samples were spectrophotometrically analyzed for ethanol (according to Caputi et al (1968) and reducing sugar content by Miller's 3,5-dinitrosalicylic acid --DNS method (Miller, 1959) at the beginning and at the end of 4,8,12,16.20,24 and 36 hours.

All experiments were performed in triplicates (n=3), values are represented as Mean ± S.D

III. RESULTS AND DISCUSSION

Sugar content in Water hyacinth stem and leaves from different sites is in the following order:

Site A>B>C>D>E

Since optimization of dilute acid pretreatment conditions yields more cellulose and hemicellulose- derived sugars from site A, plants from Site A were chosen for bioethanol production.

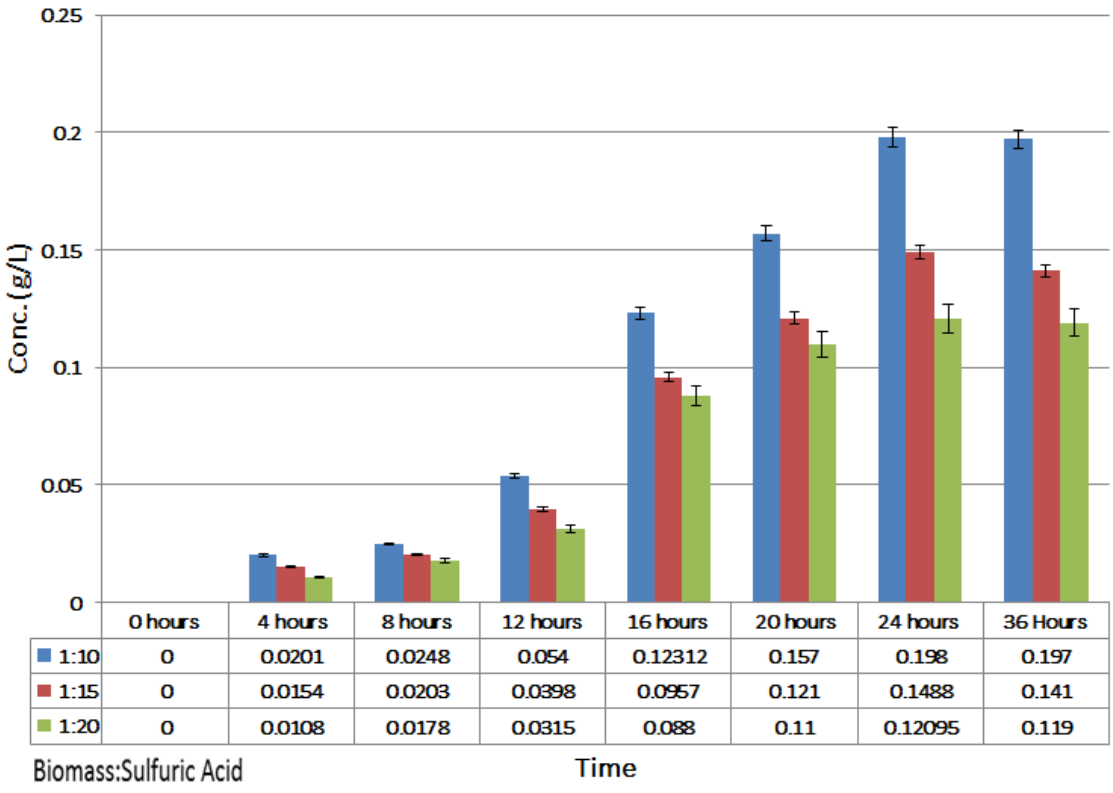
3.1 Fermentation and Ethanol Production

In the present studies, yeast from three different sources were used, viz.,Four Seasons Yeast, Commercial Baker's Yeast and Yeast Extract Powder.

Using the Four Seasons Yeast, there was a gradual increase in ethanol formation for the first twelve hours. Thereafter, a spurt was observed which reached a peak production level at 24h (Fig. 1). However maximum ethanol production (0.198g/L) was recorded when 3% sugar hydrolysate from 1:10 WHB was used (Fig.1). The corresponding values of ethanol from 2% hydrolysate with the same biomass concentration after 24h were 0.170g/L. Both the treatments had a nearly equal ethanol production after 36h too. There was a corresponding gradual decline in the sugar utilization after 36 hours of fermentation.

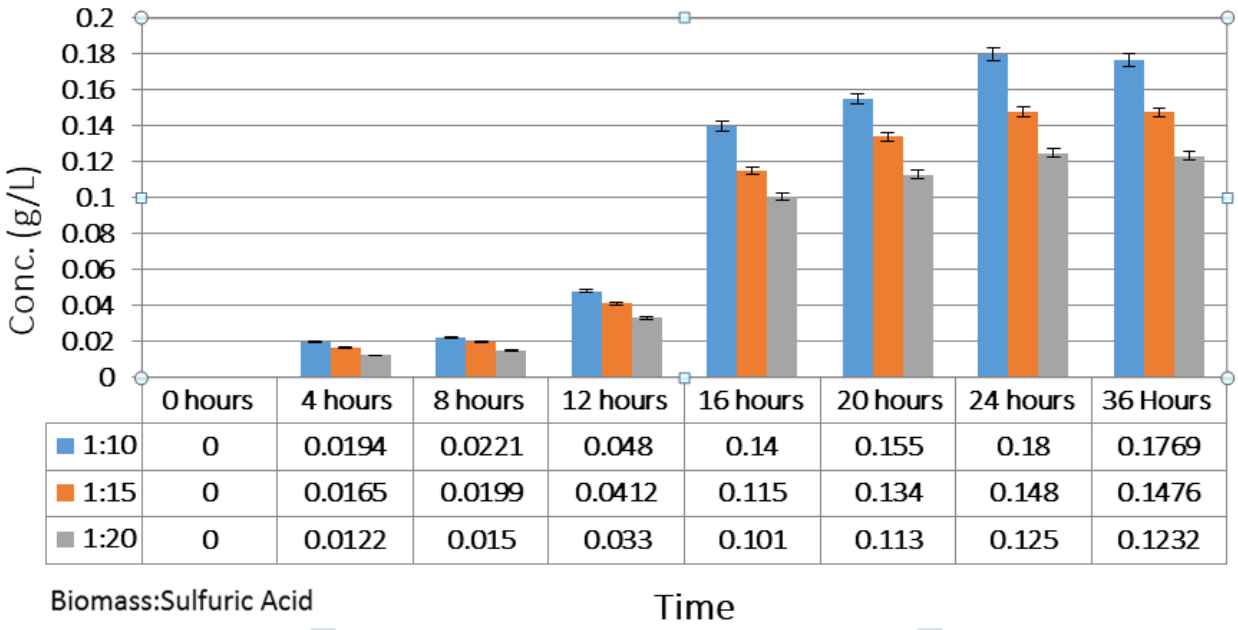
A similar pattern of ethanol formation was observed while using Baker's yeast. Amount of ethanol formed under corresponding conditions with 3% hydrolysate was 0.18g/L (24h) and 0.176g/L respectively (Fig.2). In both these experiments there was a gradual utilization of reducing sugar hydrolysate recording a minimum at 36h. With 2% hydrolysate and 1:10 biomass concentration, maximum amount of 0.168g/L ethanol was formed after 24 h which gradually decreased a little at 36h.

Fermentation with yeast extract showed maximum ethanol production by 3% hydrolysate at 1:10 biomass concentration (0.171g/L) after 24h (Fig.3), whereas 0.156g/L ethanol was formed with 2% hydrolysate under similar conditions. Ethanol formation declined in both the cases at 36h. A similar pattern of sugar utilization was observed while using both hydrolysate concentrations.



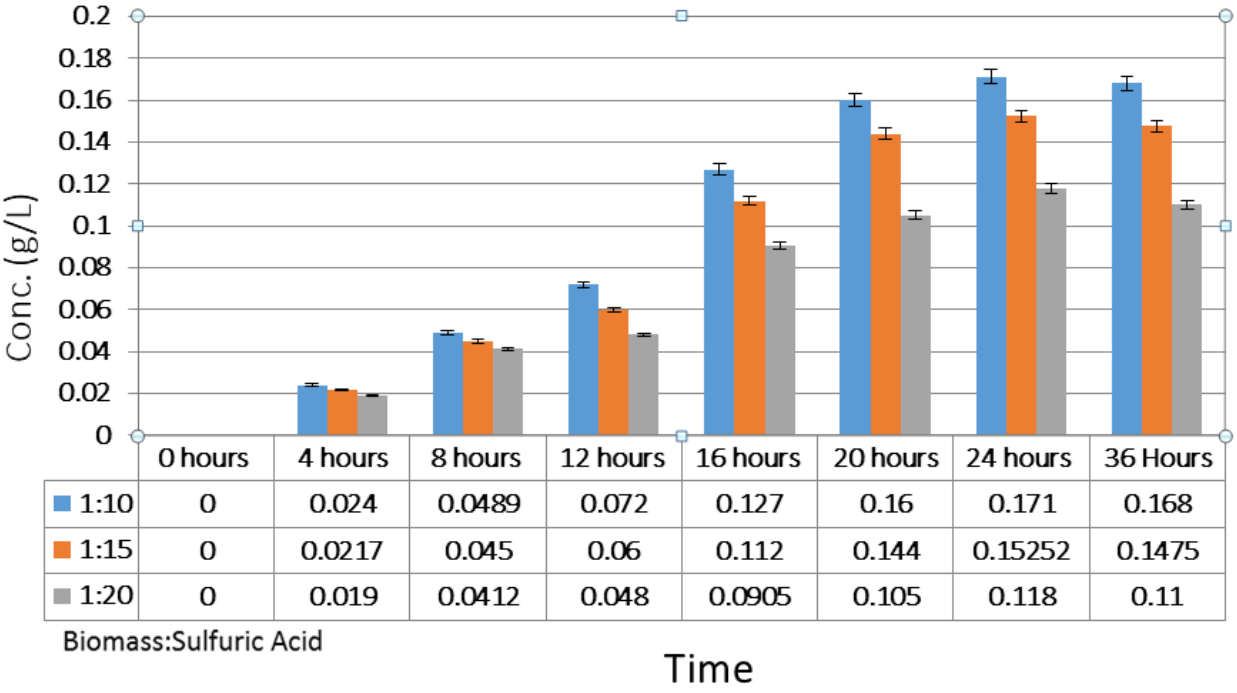
Biomass:Sulfuric Acid	0 hours	4 hours	8 hours	12 hours	16 hours	20 hours	24 hours	36 Hours
1:10	0	0.0201	0.0248	0.054	0.12312	0.157	0.198	0.197
1:15	0	0.0154	0.0203	0.0398	0.0957	0.121	0.1488	0.141
1:20	0	0.0108	0.0178	0.0315	0.088	0.11	0.12095	0.119

Fig. 1: Formation of ethanol from 3% reducing sugar hydrolysate by 0.2% Four Seasons Yeast.



	0 hours	4 hours	8 hours	12 hours	16 hours	20 hours	24 hours	36 Hours
1:10	0	0.0194	0.0221	0.048	0.14	0.155	0.18	0.1769
1:15	0	0.0165	0.0199	0.0412	0.115	0.134	0.148	0.1476
1:20	0	0.0122	0.015	0.033	0.101	0.113	0.125	0.1232

Fig. 2: Formation of ethanol from 3% reducing sugar hydrolysate by 0.2% Bakers Yeast.



	0 hours	4 hours	8 hours	12 hours	16 hours	20 hours	24 hours	36 Hours
1:10	0	0.024	0.0489	0.072	0.127	0.16	0.171	0.168
1:15	0	0.0217	0.045	0.06	0.112	0.144	0.15252	0.1475
1:20	0	0.019	0.0412	0.048	0.0905	0.105	0.118	0.11

Fig. 3: Formation of ethanol from 3% reducing sugar hydrolysate by 0.2% Yeast extract.

Studies in time course of utilization of hydrolysate sugar and ethanol formation revealed that maximum efficiency of ethanol formation is exhibited by Four Seasons Yeast at 3% hydrolysate followed by Baker's Yeast and Yeast Extract respectively at the same concentration (Figs 4, 5, 6 and 7).

Further studies are underway to make a comprehensive insight regarding the comparison of microbial and fungal inocula for second generation water hyacinth feedstock-based bioethanol production. Using strains of *Aspergillus niger* as a source of cellulase enzyme, much better recovery of fermentable sugars is possible by saccharification of water hyacinth substrate (see Alvi et al, 2014 and Bhatia et al, 2019). This will help in making the bioconversion economically more sustainable (see Haldar & Purkait, 2021 and Shakila Begum et al, 2024).

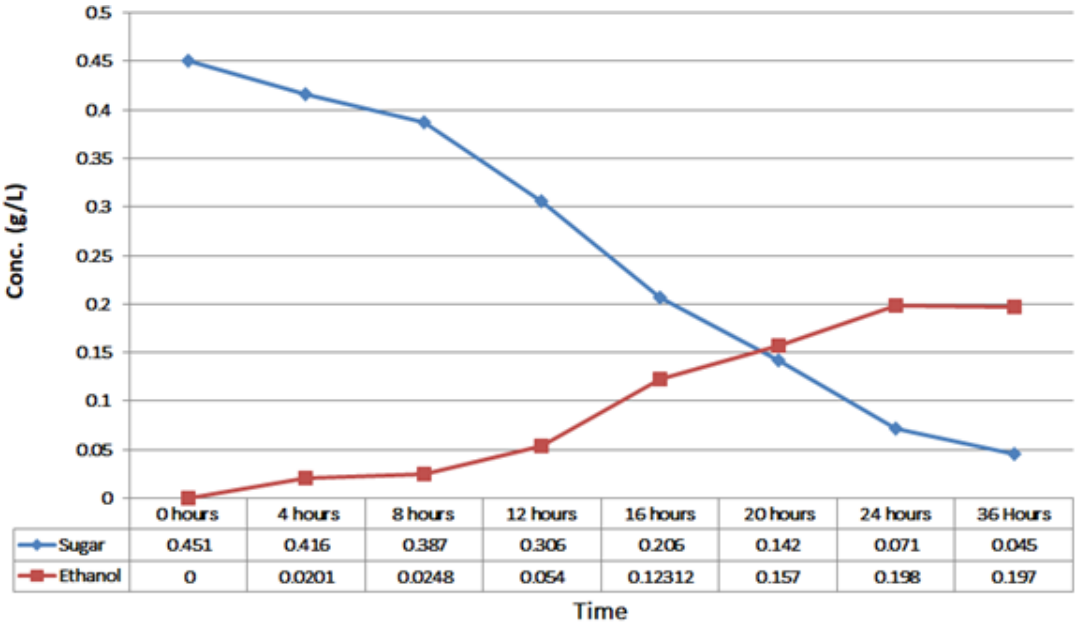


Fig 4: Time Course of Utilization of 3%Reducing Sugar Hydrolysate and Ethanol formation by 0.2% Four Seasons Yeast

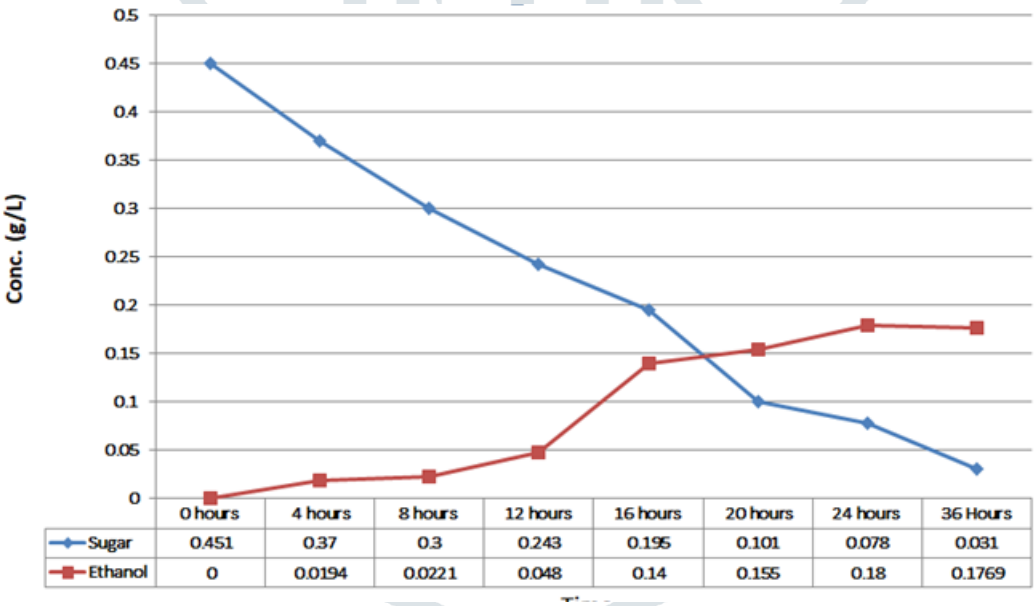
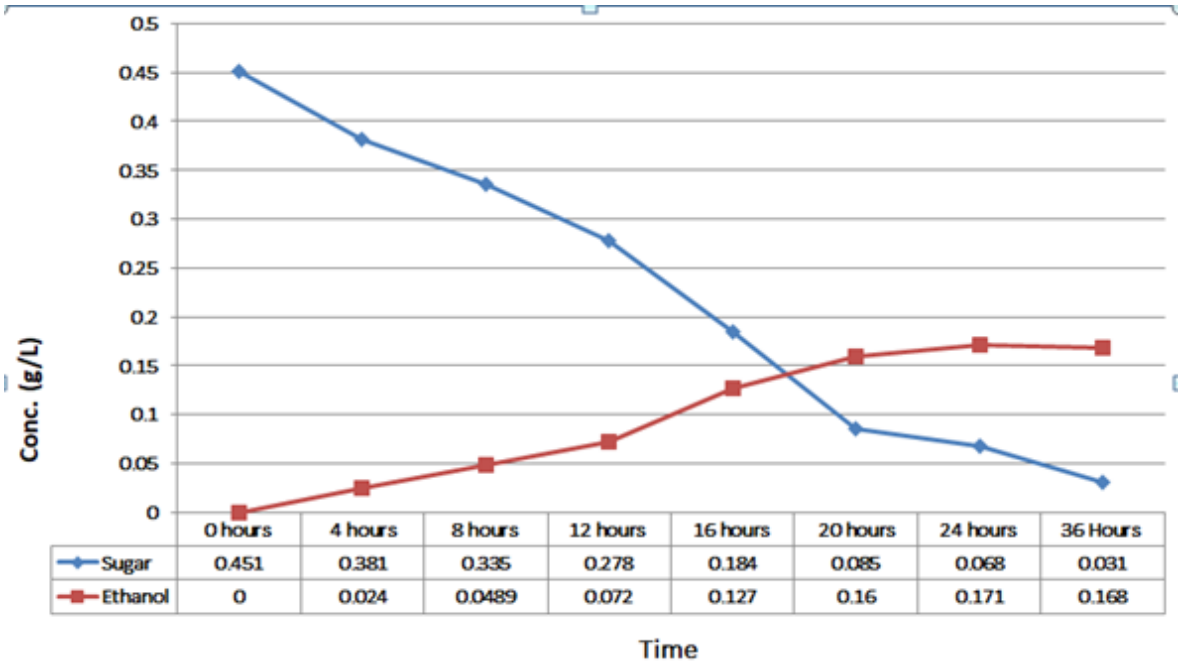
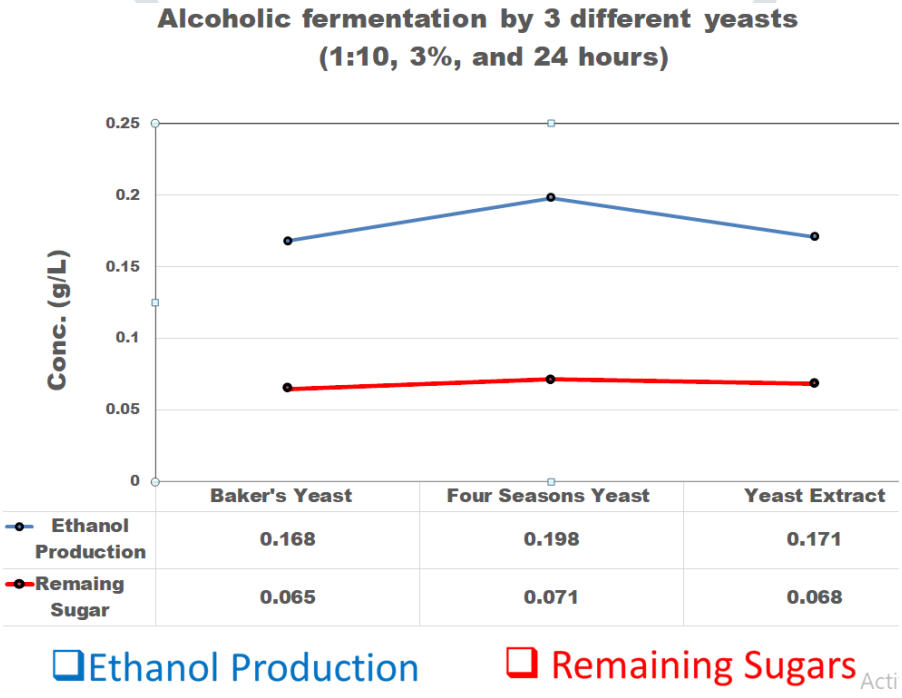


Fig 5: Time Course of Utilization of 3%Reducing Sugar Hydrolysate and Ethanol formation by 0.2% Bakers Yeast



	0 hours	4 hours	8 hours	12 hours	16 hours	20 hours	24 hours	36 Hours
Sugar	0.451	0.381	0.335	0.278	0.184	0.085	0.068	0.031
Ethanol	0	0.024	0.0489	0.072	0.127	0.16	0.171	0.168

Fig 6: Time Course of Utilization of 3%Reducing Sugar Hydrolysate and Ethanol formation by 0.2% Yeast Extract.



	Baker's Yeast	Four Seasons Yeast	Yeast Extract
Ethanol Production	0.168	0.198	0.171
Remaing Sugar	0.065	0.071	0.068

Fig. 7: Alcoholic fermentation by three different yeasts (1:10,3%, and 24 hours)

IV. ACKNOWLEDGEMENT

The author thanks University of Delhi for awarding the Innovation Project SAC-301. Thanks, are also due to the Principal, Sri Aurobindo College for providing the necessary facilities.

REFERENCES

[1] Ainomujuni,S., Latinga,P.O. and Sebbit,A. 2023DOI: http://doi.org/10.26480/acmy.01.2023.01.07. Bioethanol and briquette production as an alternative control measure of water hyacinth on Lake Victoria. Acta Chemica Malaysia, 7(1): 1-7. DOI:http://doi.org/10.26480/acmy.01.2023.01.07

[2] Alvi, S.S.M., Ali , M.N., Mohiuddin , M., Khan,M.M. and Khan,M.M. 2014. Eichhornia crassipes - a potential substrate for biofuel production. International Journal of Current Microbiology and Applied Sciences, 3(10): 618-627.

[3] Baig MZ, Dharmadhikari SM, Ismail S. 2017.Technological processes for conversion of lignocellulosic biomass to bioethanol. Journal of Pure and Applied Microbiology, 11(4):1863- 1881. doi: 10.22207/JPAM.11.4.27

[4] Bhattacharya,A. and Kumar, P. 2010. Water hyacinth as a potential biofuel crop. Electronic Journal of Environmental,Agricultural and Food Chemistry, 9 (1): 112-122.

[5] Bhatia, .L., Vijay Kumar,G. and Chandel, A. 2019. Scalable Technologies for Lignocellulosic Biomass Processing into Cellulosic Ethanol. Pp. 73-90. In: Horizons in Bioprocess Engineering. R. Pogaku (ed.), Springer Nature Switzerland AG D.O.I:10.1007/978-3-030-29069-6_5.

[6] Bote,M.A., Naik, V.R. and Jagadeeshgoud,K.B. 2020. Review on water hyacinth weed as a potential biofuel crop to meet collective energy needs. Materials Science for Energy Technologies, 3 :397–406. https:// doi.org/10.1016/j.mset.2020.02.003.

[7] Broda, M., Yelle, D.J.and Serwa´nska, K.2022. Bioethanol production from lignocellulosic biomass— challenges and solutions. Molecules, 27(24): 8717. https://doi.org/ 10.3390/molecules27248717

[8] Bušić,A., Kundas,S. Morzak ,G., Belskaya ,H., Šantek,M.I., Komes ,K. Novak ,S. and Šantek, B. 2018. Bioethanol Production from Renewable Raw Materials and Its Separation and Purification: A Review. Food Technology and Biotechnology, 56(3): 289-311. doi: 10.17113/ftb.56.03.18.5546.

[9] Ceaser,R., Montané,D., Constantí,M.and Medina ,F.2024. Current progress on lignocellulosic bioethanol including a technological and economical perspective. Environment, Development and Sustainability, 1-46. https://doi.org/10.1007/s10668-024-04792-2.

[10] Caputi, Jr. A., M. Ueda and Brown T. 1968.Spectrophotometric determination of ethanol in wine. American Journal of Enology and Viticulture, 19: 160- 165.

[11] Chauhan, A., Singh, V.K., Lahiry, S. and Kwatra, Y.2016. Phytoremediation potential of water hyacinth along the Delhi stretch of river Yamuna. Intrnational Journal of Environmental Sciences, 7 (3): 881-887. (ISSN: 0976-4402).

[12] Chauhan, A., Singh, Vinay Kr. and Kwatra, Y. 2020. Saccharification of Water Hyacinth Biomass for Bioethanol Production--Optimization of Sulfuric Acid Pre-treatment. Journal of Biofuels,11(1):1-8. (Print ISSN 0976-3015, Online ISSN- 0976-4763). DOI: 10.5958/0976-4763.2019

[13] Dandasena,T.K. and Shahi,S. 2023. A renewable biofuel-bioethanol: A review. Journal of Advanced Zoology, 44 (S3): 1698:1706. DOI:10.17762/jaz.v44iS3.2388

[14] Das, A., Ghosh, P., Paul,T., Ghosh, U., Pati,B.R. and Mondal ,K.C.2016. Production of bioethanol as useful biofuel through the bioconversion of water hyacinth (Eichhornia crassipes). Biotechnology, 6:1-9. DOI 10.1007/s13205-016-0385-*y*

[15] Ganguly A., Chatterjee P. K. and Dey A. 2012. Studies on ethanol production from water hyacinth-A review. Renewable and Sustainable Energy Reviews, 16: 966-972.

[16] Haldar, D. and Purkait, M.K. 2021. A review on the environment-friendly emerging techniques for pretreatment of lignocellulosic biomass: Mechanistic insight and advancements. Chemosphere, 264(2). https://doi.org/10.1016/j.chemosphere.2020.128523

[17] Hirphaye, B.Y., Mezgebe, F.H., Tura, A.M., Fanta, G.M. 2022. Bioethanol production potential of water hyacinth (Eichhornia crassipes) as alternative energy feedstocks. Research Square, 1-17. doi: 10.21203/ rs.3.rs-2356408/v1

[18] Irfan,M., Nadeem,M,and Syed,Q. 2014. Ethanol production from agricultural wastes using Sacchromyces cervisae. Brazilian Journal of Microbiology, 45(2):457-465.

[19] Jain,S.and Kumar ,S.2024. Advances and challenges in pretreatment technologies for bioethanol production: A comprehensive review. Sustainable Chemistry for Climate Action 5: 1-23. https://doi.org/10.1016/j.scca.2024.100053.

[20] Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31:426-428.

[21] Rezania,S., Md Din,M.F., Taib,S.M., Mohamad,S.E., Dahalan,F.A., Kamyab,H. ,Darajeh,N. and Ebrahim ,S.S. 2018. Ethanol production from water hyacinth (Eichhornia crassipes) using various types of enhancers based on the consumable sugars. Waste and Biomass Valorization, 9: 939–946. DOI 10.1007/s12649-017-9883-3

[22] Sai Kumar,A.,Siva Sankar,S. Godlaveeti,S.K., Kumar,D., Dheiver,S., Prasad,R., Chandrasekhar,N.B., Nguyen,T.H.C. and Van Le,Q. 2021. Recent Developments of Bioethanol Production. In: Bioenergy Research: Evaluating Strategies for Commercialization and Sustainability, 1st ed.(Eds Neha Srivastava and Manish Srivastava) Ch 9 (pp.175-208). John Wiley & Sons Ltd. DOI: 10.1002/9781119772125.

[23] Shakila Begam,M., Anusha Boorani, E., Akilandeswari,P. and Pradeep,B.V. 2024. Bioethanol production from water hyacinth (Eichhornia crassipes) using different microbial inoculants. Journal of Pure and Applied Microbiology1,8(1):323-331. https://doi.org/10.22207/JPAM.18.1.16

[24] Zhang,Q.,Weng, C.,Huang, H., Achal, V. and Wang,D. 2016. Optimization of bioethanol production using whole plant of water hyacinth as substrate in simultaneous saccharification and fermentation process. Frontiers in Microbiology, 6:1-9. https://doi.org/10.3389/fmicb.2015.01411