International Journal of Innovative Technology and Exploring Engineering (IJITEE)
ISSN: 2278-3075 (Online), Volume-9 Issue-5, March 2020

Bioethanol Production from the Pods of Delonix Regia

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Abstract: ***To replace conventional fossil fuels extensive research works are being carried out using bio-resources. In this scenario an experimental study was carried out to extract bioethanol from the seeds of the plant Delonix regia. . Delonix regia pod has several characteristics features like high cellulose and hemicelluloses content and that can be readily hydrolysed into fermentable sugar. In our present study the pods of D.regia were powdered and sterilised. The pod powder was treated with laboratory grown Sacchromyces cervisiae and allowed for fermentation. Different doses of Delonix regia pod powder was used (4, 8, 12, and 16%) to study their effect on ethanol fermentation by the yeast Saccharomyces cerevisiae. An increase in the substrate concentration was found to increase the bioethanol yield. The ethanol production was high after 24 hour of fermentation when the substrate concentration was 16%. Temperature between 30-35°C and pH4.5 are reported to be optimum for the growth of yeast and good bioethanol synthesis. HPLC analysis of the extracted sugar of the pod showed the presence of cellulose and hemicellulose. After distillation the produced bio ethanol was tested for its quality and found it well suited for fuel.***

Keywords: Delonix regia **pod, bio ethanol,** ***Sacchromyces cervisiae,*** **biofuel**

I. INTRODUCTION

In recent years several research works are being carried out to find an alternative fuel to replace the conventional fossil fuels as crude oil and the fossil fuels are finite nature (Nigam et al., 2011). So attempts are made to find the replacement with bio-ethanol, as an eco-friendly fuel .Agricultural crops such as sugarcane, maize, millet, cassava etc. are a good raw material for bioethanol. Maturation of sugars from farming items or waste plant materials, can be used to develop ethylene with steam (Mousdale et al. 2011) .Bioethanol is more useful over conventional fuels. It comes from the renewable resource like plant sources like cereals, sugar beet and maize with optimum release of greenhouse gas emissions. Since 1925 Brazil has been using bio-ethanol as a transportation fuel (Singh et al.,2009). . Bio ethanol has a higher octane number and extensive combustibility limits. Bioethanol is an oxygenated fuel with 35% oxygen, which decreases particulate and nitrogen oxides (NOx) outflows from ignition. The bioethanol mixed fuel for vehicles can essentially reduce oil utilisation and ozone fumes harming substance outflow. Recycling food and agro wastes to develop biofuels is one of the most eco-friendly processes. *Delonix regia* pod is an oil seed pod with rich carbohydrate content and this can be well used to extract oil as an alternative fuel. In the present study testing was made to find out the possibility of using *Delonix regia* pod extract as biofuel.

Revised Manuscript Received on March 30, 2020.
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II. MATERIALS AND METHODS

Collection of sample

The *Delonix regia* pod sample was collected from the garden of Prathyusha engineering college, Chennai. The collected sample was shade dried and powdered. The powdered sample was stored in a dry glass container.

Treatment of sample:

The powder (100g) was treated with 200ml of 2% H2SO4 and incubated for overnight After incubation the sample was heated and autoclaved at 121°C for 15 minutes. The heat pre-treated samples were cooled to room temperature and then the sample was filtered through activated carbon and the filtrate was collected in pure form. Finally the pH was adjusted to 4.5 for the fermentation of yeast.

Preparation of inoculums

Fifty ml of nutrient broth was taken in 250 ml conical flask and sterilized at 121°C and 15 psi pressure for 20 minutes and it was inoculated with 2ml of dense culture of *Saccharomyces cerevisiae*. The flask was incubated at room temperature for 24 hrs. To find out the optimum pH 50 ml of the hydrolysed sample was taken in four 100 ml conical flasks and 20 µl of inoculum was transferred. The flasks were adjusted to pH 3.5, 4, 4.5 and 5 respectively. To find out the optimum temperature 50 ml of the hydrolysed sample was taken in four 100 ml conical flasks and 20 µl of inoculum was transferred and kept at varying temperatures viz.,26°C, 28°C, 30°C, 32°C and 34°C respectively.

To find out the optimum load of substrate concentration 50 ml of the hydrolysed sample was taken in four 100 ml conical flasks and 20 µl of inoculum was transferred and kept at varying concentrations (i.e) 4%, 8%, 12% and 16% respectively.

The fermentation process in different pH, temperature and substrate concentration was found out by estimating the glucose content by DNSA method and the formation of ethanol was tested by Potassium dichromate test at 24 hour intervals for four days and optimum pH was determined

Medium optimization

The medium composition was prepared using standard protocol (Table 1).For testing the fermentation of yeast *Delonix regia* pod was hydrolyzed and pre-treated with fifty ml broth (g/L) (Nacl-0.5, yeast-l) in order to convert the sugars into ethanol using the enzymes released by *Saccharomyces cerevisiae.* The pH of the solution was maintained at 4.5 by adding 0.1 M NaCl. This enabled the growth of yeast. Before inoculating the yeast the medium was completely sterilized. One ml of the inoculum was transferred to the fermentation medium and the fermentation was carried out at a temperature of 30˚C. Contamination was prevented by sealing the flask. Three different doses were prepared and incubated. After 3 days of fermentation samples were taken from the different conical flask at an interval of 4 h to find out the growth kinetics of yeast. (Fig.1). After the fermentation process, triple distillation process was carried out to separate ethanol.

Estimation of glucose by DNSA method:

The hydrolyzed pod samples (0.8&1.0 ml)were taken in test tubes . Using distilled water the volume was made up to 1 ml. and incubated at room temperature for 5 minutes. Followed by this DNS reagent was added and mixed well. The test tubes were kept in water bath at 95°C for 10 minutes and then cooled in cold water. After cooling , 40% Na-K tartrate was added to stop the reaction. Using 1 ml of water in the place of sample blank was prepared. With the increasing concentration of glucose (i.e. 0.2, 0.4, 0.6, 0.8 and 1 mg/ml) 1 ml of standard solutions were prepared. The bright red color appeared in the samples was measured at 540 nm against the blank. The concentration of glucose in the samples was estimated by comparing with blank using standard graph (Manickam et al. 2008).

Ethanol assay

In the test tubes 1 ml of different concentration ethanol was taken and made up to 5 ml using distilled water and 5 ml of chromic acid was added. The test tubes were incubated in a water bath at 60oC for 20 min. After the appearance of reddish brown colored reaction the absorbance was determined at 584nm using a spectrophotometer. Ethanol concentration was determined according to Caputi et al. (1968).

High performance liquid chromatography

Using HPLC method the major sugars present in Delonix regia pod was estimated according to Montesano et al., (2016).

- **Gas chromatography:** Using gas chromatography the concentration of each compounds was measured.

III. RESULTS AND DISCUSSION

Quantitative analysis of compounds

Effect of pH

In the present study, the pods of *Delonix regia* were powdered and the phytochemicals present in it was estimated using HPLC and GLC. HPLC analysis of the extracted sugar of the pod showed the presence of cellulose and hemicellulose (Fig.4 & Table5). The reducing sugar present in the extract helped in the formation of bio-ethanol. The pH needed to enhance bio-fuel synthesis was 4.5 is (Table 2). The reducing sugar is shown in (Fig.2). T0 measure reducing sugar the various concentration of glucose were plotted against control at OD at 540nm by the method of DNS and the slope was determined to be 1ml/mg

The concentration of ethanol and OD was determined at 584nm. The unknown ethanol concentration was also determined. Temperature has profound effect on ethanol fermentation as reported by Kopsahelis et al. (2007). Temperature between 30-35°C is reported to be optimum for the growth of yeast .The temperature above 35°C was found to inhibit yeast growth (Gray et. al., 1942). Different doses of *Delonix regia* pod powder was used (4, 8, 12, and 16%) to study their effect on ethanol fermentation by *Saccharomyces cerevisiae.* An increase in the substrate concentration was found to increase the bioethanol yield. The ethanol production was maximum at 24 hours of fermentation when the substrate concentration was 16% (Table 4). .

The characteristics of the sugar compounds present in the pod of *D.regia* were studied using HPLC (Table.5). The retention time for Cellulose and Hemicellulose were found to be 4.062 and 5.311 respectively and the peak area (%) was found to be 78.254 and 21.746. The growth kinetics graph plotted for biomass concentration and time showed the growth curve of three different medium (Fig.6). The graph plotted for biomass and substrate utilisation showed that an increase in ethanol production decreased the substrate concentration due to the utilization by yeast extract (Fig.7).

The concentration of bioethanol increased significantly after 24 hour period of fermentation (Fig.8). As the fermentation continues the accumulation of intermediate co-products tend to inhibit or slow down fermenting process of yeast activity. Zakpaa et al., (2009) reported that as toxic compounds such as lignin residues, acids and aldehydes accumulated in the fermentation medium the concentration of bioethanol tend to decrease. Also, as the fermentation period increases some quantity of bioethanol may be lost due to the volatility of ethanol. Also inability of yeast to ferment glucoses in *Delonix regia* may be another factor for low bioethanol concentration. After anaerobic fermentation of the yeast, distillation process was carried out thrice to get pure form of ethanol. For single distillation **eighty** ml of bioethanol was produced in the process of single distillation of 250ml of fermented sample within a time period of 1 h.

For double distillation forty five ml of bioethanol was produced in the process of double distillation of 250ml of fermented sample within a time period of 35 minute and for triple distillation fifteen ml of bioethanol was produced in the process of double distillation of 250ml of fermented sample within a time period of 10 min. For bio-ethanol confirmation five ml of the sample was taken in a test tube and 1% of iodine solution was added and then diluted with sodium hydroxide in drops. When the colour of the iodine changed the tubes were warmed gently in water bath and yellow participate obtained. It indicated the presences of ethanol, and the bio-ethanol properties were shown in the Table.7. Bioethanol appeared clear and colourless .The bioethanol was found highly soluble in water and poorly soluble in fats and oils. The density was found to be 0.789g/ml 4.The boiling point was78 °C and flash point was 27 °C.

Table.1 Composition of medium

FLASK 1	FLASK 2	FLASK 3
Yeast extract(2ml)+ NaCl (0.5g)+hydrolysed sample (20ml)	Glucose(2ml)+NaCl (0.5g)+hydrolysed sample (20ml)	Peptone extract(2ml)+NaCl (0.5g)+hydrolysed sample (20ml)

Table.2 Effect of pH on fermentation

S. No	pH	Absorbance at 584nm	Conc. Of ethanol (mg/ml)
1	3.5	0.313	0.18
2	4	1.26	0.76
3	4.5	1.98	1.0
4	5	0.83	0.52
5	5.5	0.43	0.39

Table.3 Effect of temperature on fermentation

S. NO	Temperature	Absorbance at 584nm	Conc. of ethanol (mg/ml)
1	26	0.318	0.18
2	28	0.815	0.46
3	30	1.863	1.02
4	32	1.693	0.94
5	34	0.453	0.24

Table.4 Effect of substrate concentration on fermentation

S. No	Concentration of sample (g/100ml	Absorbance at 584nm	Conc. of ethanol (mg/ml)
1	4	0.623	0.35
2	8	0.985	0.55
3	12	1.83	1
4	16	1.95	1.09

Table.5 HPLC analysis of D.regia pod extract

Peak No	Retention time	Compound name	Molecular formula	Molecular weight	Peak area (%)
1	**4.062**	**cellulose**	C6H10O5	324.29g/mol	78.254
2	**5.311**	Hemicellulose	C5H10O5	150 g/mol	21.746

Table.6 Biomass and substrate of profile yeast

S.NO	Time (h)	Biomass concentration(mg/ml)			Substrate concentration (mg/ml)		
		FLASK1	FLASK2	FLASK3	FLASK1	FLASK2	FLASK3
1	0	0.05	0.04	0.07	1.18	1.16	1.14
2	4	0.10	0.08	0.11	1.06	1.02	1.03
3	8	0.157	0.14	0.18	0.90	0.88	0.85
4	12	0.25	0.22	0.30	0.65	0.69	0.63
5	16	0.34	0.32	0.47	0.31	0.37	0.26
6	20	0.66	0.44	0.70	0.13	0.10	0.10
7	24	0.79	0.58	0.91	0.06	0.03	0.05

Table.7 Bioethanol Properties

S NO	Properties	Remarks/ Units
1	Appearance	Bioethanol is clear and colorless
2	Solubility	Bioethanol is highly soluble in water but poorly soluble in fats and oils
3	Density 0.789g/ml 4	0.789g/ml
4	Boiling point	78 °C.
5	Flash point	27 °C

Fig.1 Anaerobic fermentation of Bio-ethanol production

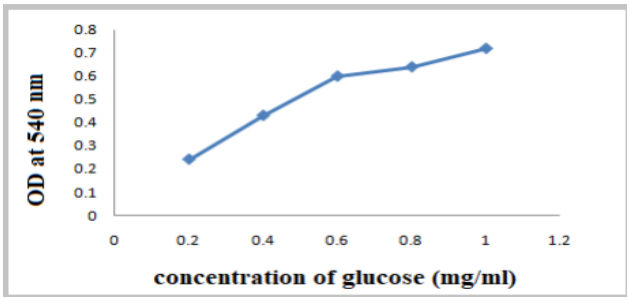

Fig.2 Standard Chart for glucose

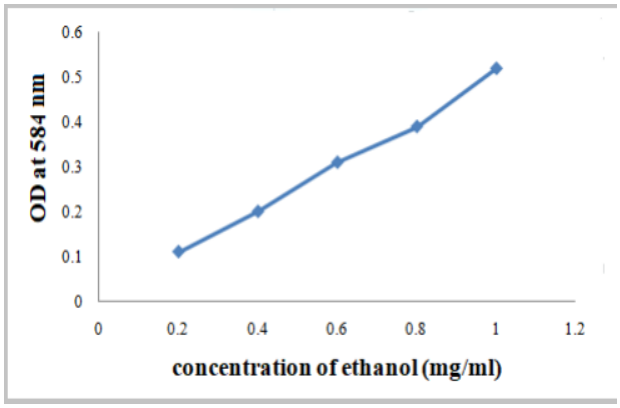

Fig.3 Standard chart for Ethanol Estimation

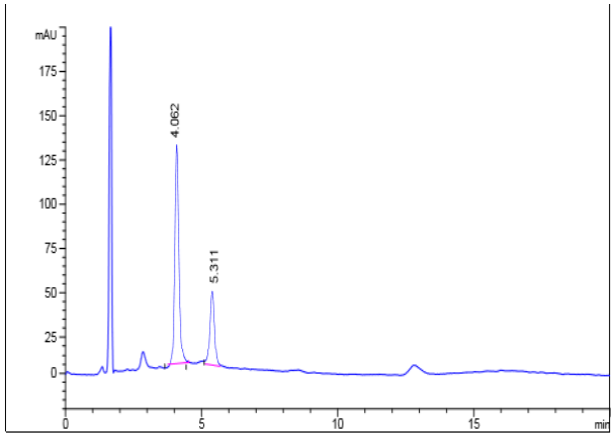

Fig.4 Analysis of extracted sugar HPLC

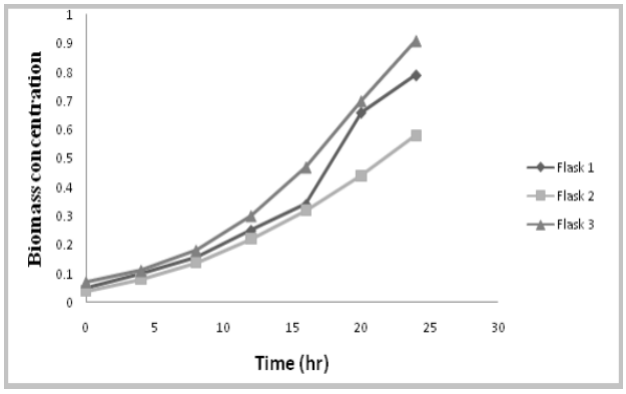

Fig.5 Growth curve chart

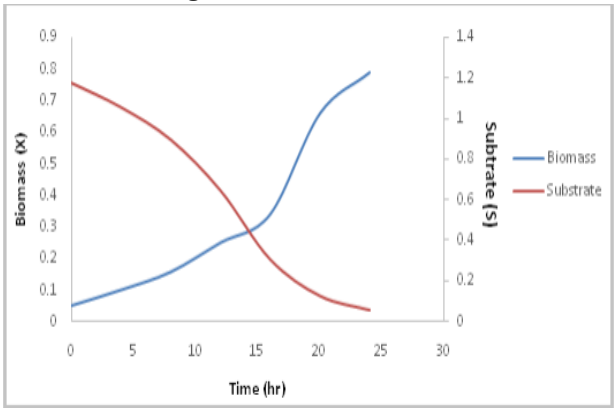

Fig.6(A) Biomass and substrate (yeast extract medium)

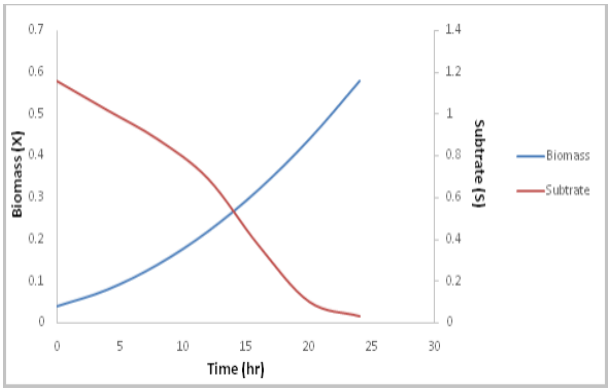

Fig.6(B) Biomass and substrate (glucose)

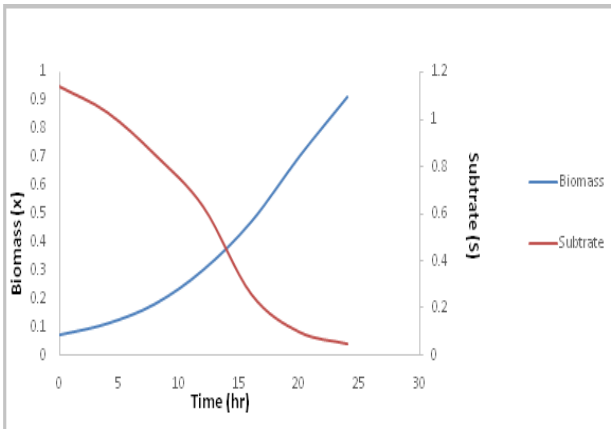

Fig.6(C) Biomass and substrate (peptone extract medium)

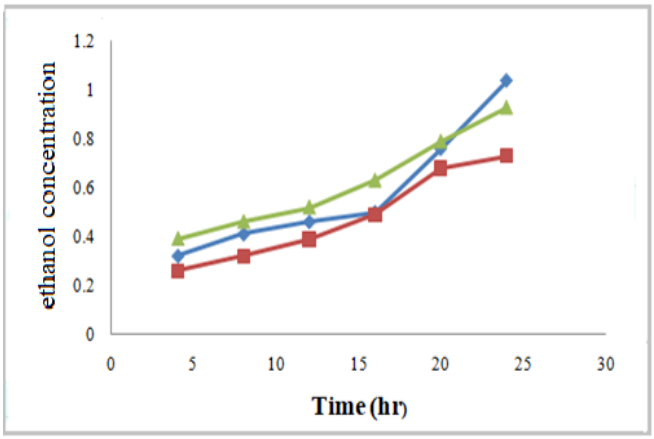

Fig.7 Growth kinetics

IV. CONCLUSION

The study proved that the *Delonix regia* pod as a potent raw material for the production of bioethanol. However, optimization of hydrolysis process and environmental conditions are required for an industrial application. So further improvement in the process can be carried out to enhance the purity of ethanol.

REFERENCES

1. G. O. Young, "Synthetic structure of industrial plastics (Book style with paper title and editor)," in *Plastics*, 2nd ed. vol. 3, J. Peters, Ed. New York: McGraw-Hill, 1964, pp. 15–64.
2. W.-K. Chen, *Linear Networks and Systems* (Book style). Belmont, CA: Wadsworth, 1993, pp. 123–135.
3. H. Poor, *An Introduction to Signal Detection and Estimation*. New York: Springer-Verlag, 1985, ch. 4.
4. B. Smith, "An approach to graphs of linear forms (Unpublished work style)," unpublished.
5. E. H. Miller, "A note on reflector arrays (Periodical style—Accepted for publication)," *IEEE Trans. Antennas Propagat.*, to be published.
6. J. Wang, "Fundamentals of erbium-doped fiber amplifiers arrays (Periodical style—Submitted for publication)," *IEEE J. Quantum Electron.*, submitted for publication.
7. C. J. Kaufman, Rocky Mountain Research Lab., Boulder, CO, private communication, May 1995.
8. Y. Yorozu, M. Hirano, K. Oka, and Y. Tagawa, "Electron spectroscopy studies on magneto-optical media and plastic substrate interfaces(Translation Journals style)," *IEEE Transl. J. Magn.Jpn.*, vol. 2, Aug. 1987, pp. 740–741 [*Dig. 9th Annu. Conf. Magnetics* Japan, 1982, p. 301].
9. M. Young, *The Techincal Writers Handbook*. Mill Valley, CA: University Science, 1989.
10. (Basic Book/Monograph Online Sources) J. K. Author. (year, month, day). *Title* (edition) [Type of medium]. Volume(issue). Available: http://www.(URL)
11. J. Jones. (1991, May 10). Networks (2nd ed.) [Online]. Available: http://www.atm.com
12. (Journal Online Sources style) K. Author. (year, month). Title. *Journal* [Type of medium]. Volume(issue), paging if given. Available: http://www.(URL)

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Publications:

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2. **Hellen Sathya, D.J**., Turakhia, A.M., Kumar, M.A., Balaji, N., Selvanaveen, S., Vinodhini, G. and Seenuvasan, M., 2017. Bioethanol from saccharificed lignocellulosic rich Aloe vera rinds using Saccharomyces cerevisiae MTCC 4779. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, *39*(13), pp.1347-1352.
3. Suresh, D., Sridhar, P., **Sathya, D**., Kumar, M.A., Seenuvasan, M. and Dhandapani, R., 2013. A complete study on biodegradation of Reactive Red C2G29 using newly isolated *Acinetobacter sp*. *Research Journal of Engineering and Technology*, *4*(4), pp.246-250.
4. Seenuvasan, M., Sanjayini, S.J., Kumar, M.A., Vinodhini, G., **Hellen Sathya, J**. and Kumar, V.V., 2017. Cellulase-mediated saccharification of lignocellulosic-rich pseudostem of Musa cavendish for bio-ethanol production by Saccharomyces cerevisiae MTCC 4779. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, *39*(6), pp.570-575.

Research work : Biofuels and Environmental Biotechnology
Ongoing work are Extraction of natural dyes from flower waste and leaves
Green synthesis of copper and iron nanoparticle.
Membership : Nil
Awards
1. Participated in National level Conference at AVIT, Chennai on August 2019 and my students won first place in oral presentation for miniproject work on 'Extraction of Natural Dyes from African tulip (*Spathodea campanulata*) for dyeing fabrics'.
2. Best paper award rewarded to 'Utilization of *Delonix regia* pods for the production of Bioethanol' in International Conference held at Cresent University, Chennai on Oct-2019. Working as an academician, Assistant Professor my contribution includes 2 publications in international journals and 2 publication in national level journals and 1 conference publications. I have been an organizer of National conference, workshop and Faculty Development Programmes.

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