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Production of Ethanol from Quetta *Pinus halepensis* by Fermentation

Awais Sattar Ghouri[1]*, Saeed Noor[1], Azeem Munawar[2] and Javeed A. Awan[3]

[1]*Department of Chemical Engineering, Faculty of Engineering, Balochistan University of Information Technology, Engineering and Management Sciences, Quetta, Pakistan*
[2]*Department of Polymer Engineering and Technology, University of the Punjab, Lahore, Pakistan*
[3]*Institute of Chemical Engineering and Technology, University of the Punjab, Lahore, Pakistan*

Abstract

Pinus halepensis is wasted in large amount in Quetta, Pakistan. The direct discharge of *Pinus halepensis* enhances environmental pollution so it is urgent demand to treat this waste *Pinus halepensis* in such a way to produce beneficial products and reduce the environmental impacts. In this work *Pinus halepensis* are first crushed in jaw crusher to a size of 212 μm (65 mesh number) and 600 μm (28 mesh number) Tyler sieve standard. The crushed powder is then converted into brix by H_2SO_4 (1N) hydrolysis at 363.15 K. The brix are then converted into ethanol by fermentation. During the experiments acid hydrolysis and various parameters such as pH of *Pinus halepensis* solutions, sugar brix, specific gravity of *Pinus halepensis* solutions and fermentation time were studied. The best conversion of *Pinus halepensis* to ethanol has been recorded at 4.5 pH. The statistical analysis was carried out in order to study the percentage contribution of different parameters and their effect on the production of ethanol.

Keywords: Fermentation; *Pinus halepensis*; Baker's yeast; Hydrolysis; Biomass; Specific gravity

Introduction

Nowadays alternate form of energy production, chemicals and fuels are being focused due to scarcity and high prices of available natural resources [1-3]. Biomass can be used as source of raw material to fulfill ongoing demand of fuel. Moreover, its side effect on ecosystem is very less. Renewability and sustainability of biomass is much more than conventional energy resources [4,5]. Particularly, ethanol has become attractive common source of bio-fuel worldwide due its clean and green emissions. Its applicability in the field of pharmaceutical, chemical, petrochemical industry is very much versatile and can be used for sustainable processes [6]. Combustion of ethanol produces less amount of undesirable emissions. As a fuel, ethanol is far better than gasoline. Due to its high compression ratio and high octane rating, it can increase the thermal efficiency of an engine.

Cellulosic material such as wheat, barley, malt, corn, sugar beets, *Pinus halepensis*, sugar cane, molasses and any sugar or starch can be used as a potential and promising source of ethanol production from fermentation process. Before the conversion of cellulosic material into ethanol, the cellulosic material is firstly physically, chemically or biologically pre-treated to reduce its particle size. This pretreatment helps to increase surface area, reaction rate and accessibility of microorganism to cellulosic material [7]. Afterwards, acid hydrolysis is followed by fermentation in the presence of any suitable microorganism, which acts as a biological catalyst [8]. Nitric acid, hydrochloric acid, Sulphuric acid and phosphoric acid are widely used to produce sugar content from biomass [9,10]. Production of ethanol from corn is very limited due to its high price [11,12]. Therefore, research is being conducted to produce ethanol from low lignin-cellulosic material such as woody biomass, agriculture residues and forest wastes. Woody biomass is the most abundant organic source on Earth, with annual production in the biosphere of about 5.64×10^{10} Mg-C [13,14]. The price of ethanol per gallon ($1.59/gallon) is less than petrol gallon ($2.05/gallon) which further necessitates the production of ethanol on urgent basis. In this work production of ethanol from *Pinus halepensis* has been conducted and a detail statistical analysis were tabulated and plotted as a function of different experimental parameters.

Experimental

Materials

Raw materials used in this process are *Pinus halepensis* and detail specification of raw material and chemicals are tabulated in Tables 1 and 2. No further purifications were made to the chemicals. Distilled Water (H_2O) was obtained from a direct Q distilled water machine.

Apparatus and experimental setup

In this work ethanol is being produced from two different mesh size of Quetta *Pinus halepensis* i.e., 212 μm (65 mesh number) and 600 μm (28 mesh number) Tyler sieve standard by acid hydrolysis method followed by fermentation. Initially, *Pinus halepensis* is crushed in jaw crusher to a size of 212 μm (65 mesh number) and 600 μm (28 mesh number) Tyler sieve standard. After that we put the 5 ml H_2SO_4 (1N) and 15 ml Distilled water in test tube and shake it for at least 2 minutes. After shaking we put 0.4 g *Pinus halepensis* powder into the test tube and heat the contents of test tube at 90°C for about 2 hours. During heating process we continuously shake the test tube. After 2 hours the *Pinus halepensis* is converted into sugar brix. After acid hydrolysis we cool the solution below 20°C. For this purpose we take the water in 1000 ml beaker and put the test tube in it. After two hours the contents

Chemical Name	CAS No	Purity %	Supplier
Sulphuric Acid (H_2SO_4)	7664-93-9	99%	Sigma-Aldrich
Sodium Hydroxide (NaOH)	1310-73-2	≥ 97.0%	Sigma-Aldrich

Table 1: CAS numbers, purities, and suppliers of materials.

***Corresponding author:** Sattar Ghouri A, Engineering and Management Sciences, Department of Chemical Engineering, Faculty of Engineering, Balochistan University of Information Technology, Quetta, Balochistan, Pakistan, Tel: 00923337863457; E-mail: awais159@outlook.com

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Contents	Amount
Lignin	30.9
Cellulose	39.3
Hemicellulose	21.3
Ethanol extracts	7.3
Net calorific value (kJ kg^{-1})	18,782
Moisture (wt. %)	8.9
Analysis on dry basis	54.2
C (wt. %)	6.8
H (wt. %)	0.3
N (wt. %)	-
S (wt. %)	0.17
Cl (wt. %)	0.8
Ash content (wt. %) O% by difference (wt. %)	38.7

Table 2: Specification of material (*Pinus halepensis*) used [17].

of the test tube becomes cool down. The temperature of the solution is determined by thermometer. Specific gravity (SG) is determined with the help of hydrometer whereas sugar brix is determined from the following Brix formula [15]:

$$\text{Brix} = [\{(182.4601 \times SG - 775.6821) \times SG + 1262.7794\} \times SG - 669.5622]$$

Finally after filtering the contents of test tube, fermentation of sugar brix into ethanol is carried out at pH=4-5. Sodium hydroxide (NaOH) was intermittently added in order to maintain pH at desired value. After 24 hours the percentage of alcohol is being duly checked by SB038-spectrophotometer. The equipment used in the process of ethanol production from Quetta *Pinus halepensis* are beakers, test tubes, graduated cylinder, thermometer, digital weight balance, magnetic agitator, pH paper, hydrometer, pipette, spectrophotometer. The schematic process description of ethanol production from Quetta *Pinus halepensis* is shown in Figure 1.

Results and Discussion

In this work, particularly specific gravity and brix of *Pinus halepensis*, ethanol production and effect of pH on ethanol production have been studied for two different mesh size of Quetta *Pinus halepensis* i.e., 212 µm (65 mesh number) and 600 µm (28 mesh number) Tyler sieve standard. Specific gravity and brix of *Pinus halepensis* as function of heating time for 212 µm (65 mesh number) and 600 µm (28 mesh number) Tyler sieve standard by doing Acid Hydrolysis at 363.15 K is presented in Figures 2 and 3 respectively. Formation of ethanol (volume %/20 ml sol.) by fermenting *Pinus halepensis* brix for 212 µm (65 mesh number) and 600 µm (28 mesh number) Tyler sieve standard is presented in Figures 4 and 5 respectively. Ethanol (volume %/20 ml sol.) production as a function of solution pH is presented in Figure 6. It is also observed that the fermentation process is affected with the concentration of sugar, time of fermentation, temperature of fermentation and pH of *Pinus halepensis* solution. In this work control over pH is duly monitored with the help of pH paper. The best conversion of *Pinus halepensis* to ethanol has been recorded at 4.5 pH. The contents of test tube with a hole (so that oxygen can enter and bacteria can grow) is left for 24 hours after adding prescribed amount i.e., 0.4 g of baker's yeast (*Saccharomyces cerevisiae*) in test tube after maintaining the pH and temperature at desired value. The quality and characteristics of baker's yeast (*Saccharomyces cerevisiae*) is tabulated in Table 3.

Conclusions

Experimental work was restricted to those variables which can affect the overall process of ethanol production. Results have been determined on specific ranges; however, there are still a number of other variables which can potentially affect the process. It was concluded by the experiment that production of ethanol is increased by increasing mesh number. Agitations do have influence on production of ethanol. We just proceeded by limiting our process between the ranges of temperature in which microorganism (bacteria) growth in fermentation is secured. The yeast selection is of major importance as it determines the allowance in other variables and the whole process of fermentation. Nutrients are being added to the solution of *Pinus halepensis* having Baker's yeast. The nutrients act as food for yeast, helping them to grow and guaranteeing their survival. The faster the growth rate of yeast, the lesser will be the time taken by fermentation. So one can easily perform experiments on the amount and type of

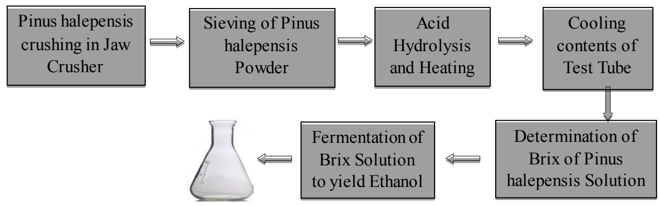

Figure 1: Schematic process description of ethanol production from Quetta *Pinus halepensis*.

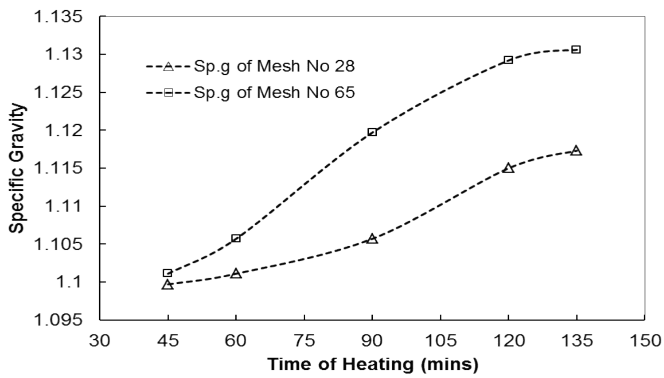

Figure 2: The effect of heating time on specific gravity of *Pinus halepensis* solution, where (□) shows experimental data points of mesh number 65 and (Δ) shows experimental data points of mesh number 28 and dotted lines show the trend lines.

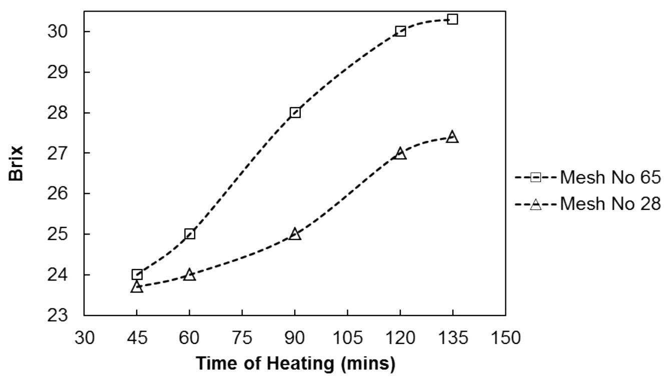

Figure 3: The effect of heating time on the production of Brix, where (□) shows experimental data points of mesh number 65 and (Δ) shows experimental data points of mesh number 28 and dotted lines show the trend lines.

pH	TTA*	Moisture Content	Enterobacteriaceae	Total coliforms	Faecal coliforms	Enterococci	LAB
4.3 ± 1.6	35.3 ± 0.22	68.9 ± 0.17	1.4 ± 0.1	<1	<1	2.3 ± 0.1	3.6 ± 0.08

*Total tirable acidity

Table 3: pH, TTA, Moisture content and microbial counts of baker's yeast (*Saccharomyces cerevisiae*) [18].

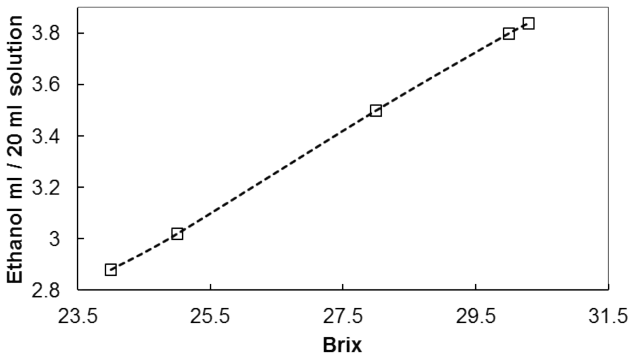

Figure 4: The formation of ethanol (Volume %/20 ml sol.) by fermenting *Pinus halepensis* brix, where (□) shows experimental data points of mesh number 65 and dotted lines show the trend lines.

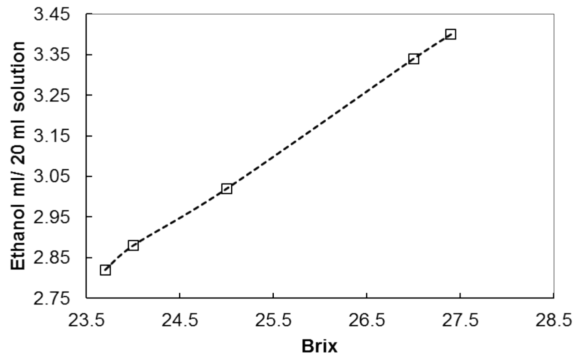

Figure 5: The formation of ethanol (Volume %/20 ml sol.) by fermenting *Pinus halepensis* brix, where (□) shows experimental data points of mesh number 28 and dotted lines show the trend lines.

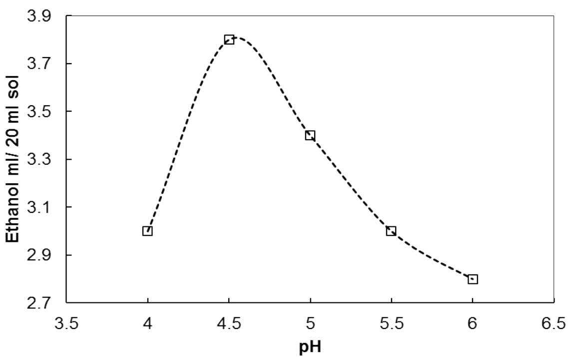

Figure 6: The ethanol production as a function of solution pH, where (□) shows experimental data points measured in this work and dotted line shows the trend line.

nutrients used, which are commonly nitro phosphate and urea etc. In this work it was shown that *Pinus halepensis* can be a better source of ethanol production as its abundance in different regions of world are enormous especially in Pakistan where it is being waste on daily basis. Although fermentation of *Pinus halepensis* (woody biomass) results in lower concentration of ethanol but continuous and significant R&D can assist in developing better and easiest way for production of ethanol from *Pinus halepensis* by altering reaction conditions, microorganism (a recombinant strain of Zymomonas mobilis can be a best option) and equipment design [16]. In this work main focus was to explore hidden potential of *Pinus halepensis* from the perspective of biofuel, which are mostly discarded directly into environment leading to pollution. Moreover very less work has been done on *Pinus halepensis* for ethanol production.

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References

1. Amidon TE, Wood CD, Shupe AM, Wang Y, Graves M, et al. (2008) Conversion of woody biomass to chemicals, energy and materials. J Biobased Mater Bioenerg 2: 100-120.
2. Lokhorst A, Wildenborg T (2005) Introduction to CO2 geological storage: Classification of storage options. Oil Gas Sci Technol 60: 513-515.
3. Wang Y, Liu SJ (2014) Kinetic Modeling of Ethanol Batch Fermentation by Escherichia Coli FBWHR Using Hot-Water Sugar Maple Wood Extract Hydrolyzate as Substrate. Energies 7: 8411-8426.
4. Zhao Y, Wang Y, Zhu JY, Ragauskas AJ, Deng YL (2008) Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature. Biotechnol Bioeng 99: 1320-1328.
5. Matthiesen J, Hoff T, Liu C, Pueschel C, Rao R, et al. (2014) Functional carbons and carbon nanohybrids for the catalytic conversion of biomass to renewable chemicals in the condensed phase. Chin J Catal 35: 842-855.
6. Cazetta ML, Celligoi MAPC, Buzato JB, Scarmino IS (2007) Fermentation of molasses by Zymomonas mobilis: effects of temperature and sugar concentration on ethanol production. Bioresour Technol 98: 2824-2828.
7. Zhu JY, Pan XJ (2010) Woody biomass pre treatment for cellulosic ethanol production: Technology and energy consumption evaluation. Bioresour Technol 101: 4992-5002.
8. Qureshi N, Dien BS, Liu S, Saha BC, Hector R, et al. (2012) Genetically engineered Escherichia coli FBR5: Part I. Comparison of high cell density bioreactors for enhanced ethanol production from xylose. Biotechnol Prog 28: 1167-1178.
9. Yang F, Afzal W, Cheng K, Liu N, Pauly M, et al. (2015) Nitric acid hydrolysis of miscanthus giganteus to sugars fermented to bioethanol, J Biotechnol Bioprocess Eng 20: 304-314.
10. Alvarez CES, Miranda JL, Castro MR, Perez G, Verdin MA, et al. (2013) Alkaline pretreatment of Mexican pine residues for bioethanol production. Afr J Biotechnol 12: 4956-4965.
11. Chen H, Liu S (2013) Conversion of distillers' grain to chemicals, liquid fuel and materials. J Bioprocess Eng Biorefin 2: 85-93.
12. Liu SJ, Lu HF, Hu RF, Shupe A, Lin L, et al. (2012) A sustainable woody biomass biorefinery. J Biotechnol Adv 30: 785-810.
13. Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: Integrating terrestrial and oceanic components. Sci 281: 237-240.
14. Girio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, et al. (2010) Hemicelluloses for fuel ethanol: A review. Bioresour Technol 101: 4775-4800.
15. Gramelspacher Homebrew (2015) Belgian Pilsner - All Grain.
16. Jeon YJ, Xun Z, Rogers PL (2010) Comparative evaluations of cellulosic raw materials for second generation bioethanol production. Appl Micobiol 51: 518-524.

17. Font R, Conesa JA, Molto J, Munoz M (2009) Kinetics of pyrolysis and combustion of pine needles and cones. J Anal Appl Pyrol 85: 276-286.

18. Reale A, Di Renzo T, Succi M, Tremonte P, Coppola R, et al. (2013) Microbiological and Fermentative Properties of Baker's Yeast Starter Used in Breadmaking. J Food Sci 78: 1224-1231.