Production of bioethanol from organic acid pretreated pine needle biomass using maleic acid

Parvez Singh Slathia*[1], Saniya Slathia[1], Amrit Dhar[1], Kamaldeep Boora[2] and Preeti Sharma[1]

[1]*School of Biotechnology, Shri Mata Vaishno Devi University, Kakrial, Katra, J&K, India*

[2]*School of Electrical Engineering, Shri Mata Vaishno Devi University, Kakrial, Katra, J&K, India*

Abstract

Our current world can rely on biofuels as sources of green energy. The abundance of lignocellulosic waste in our environment presents a unique potential to meet energy needs by creating biofuels. Due to its high combustible resin content, pine needles are another LB that is entirely a waste product and a cause of soil infertility as well as forest fires. Each pre-treatment method for the generation of biofuels has benefits and drawbacks, however pre -treatment by organic acids has certain advantages like low cost, less corrosive and environmentally benign. The results show that pine needles are a good and prospective source for the synthesis of bioethanol. The best results for total reducing sugar (TRS) yield are attained by applying pretreatment with organic acid i.e. maleic acid using OVAT approach with 10% biomass loading and 3% organic acid concentration with an outcome of 15.45 g/l yield using OVAT after pre-treatment. Enzymatic hydrolysis was carried out using an enzyme Hemicellulase obtained from Sigma Aldrich. Enzymatic hydrolysis increased the total reducing sugars to 30.3 g/l. Afterfermentation , the ethanol yield was found to be 12.6 g/l after 72 hours of fermentation.

Introduction:

The modern world needs to address a number of concerns, including global warming, an excessive reliance on fossil resources, and the security of food and energy. Energy use rises along with the global economy and population. But conventional fossil fuels like petroleum have a finite supply and produce greenhouse gases. Greenhouse gases (GHG) are emitted during

combustion. Future energy demands must be met by environmentally friendly and sustainable energy (Robak & Balcerek, 2018) .Over the past two decades, there has been a lot of research into renewable energy sources to replace fossil fuels due to long-term economic and environmental concerns. Moving towards green fuels, which are environmentally beneficial, more affordable than conventional fuels, and capable of meeting future energy demands, is urgently necessary in this situation (Mahapatra & Kumar, 2017).

The search for environmentally friendly ways to produce fuel and chemicals from non-fossil feedstock has garnered interest on a global scale in an effort to meet the world's energy needs and combat climate change. Biomass can effectively replace petroleum-based fuels to a considerable amount over time because it is abundant in nature. More than 70% of all renewable energy produced globally is produced from waste and biomass. It contributed roughly the same amount to total energy consumption in 2015 as coal did. The most typical method for using garbage and biomass is direct combustion. However, different liquid biofuels can be produced from such a feedstock using various thermochemical or biochemical processes and methods. The majority of today's transportation biofuels are produced using well-established technologies for producing ethanol and biodiesel from organic oils and other lipid feedstock, as well as from sugar and starch-based feedstock (Raud et al., 2019).

Biomass is a biological resource created through photosynthesis and a renewable energy source that emits nearly no CO2, making it environmentally benign. The term "biomass" refers to all biologically produced matter and all living things on the surface of the earth whose energy comes from plant resources, such as forestry, agroindustry, agricultural waste, animal waste, or human waste, and can be converted into useful solid fuels, liquid fuels, or gaseous fuels. Similar to how coal can be used for heating or generating electricity in power plants, biomass (A. Kumar et al., 2014).

The majority of lignocellulosic biomass, which includes whole plants and inedible remnants of food crop production, is found in trees, shrubs, and other plants that are not edible. These materials are a suitable bioethanol substrate since they are not likely to run out or suffer long-term degradation and are readily available in sufficient quantities. Lignin, hemicellulose, cellulose, and trace amounts of inorganic materials make up lignocelluloses. By hydrolysing

polysaccharides like hemicellulose and cellulose, simple sugars that are monosaccharides can be created. (Isroi et al., 2011)

Lignocellulosic sources are recognised as suitable feedstock since they are compatible with high yield, abundance, and minimal environmental impact. (Achinas & Euverink, 2016). They offer a cheap energy source that can be generated in big batches. Several conversion techniques, including fermentation and pyrolysis, have been used to convert lignocellulosic biomass, which is a rich source of energy. Waste products from the production of wood are utilised to warm houses and provide electricity (Singh et al., 2016).

Yard and garden trash are converted to liquid biofuels, while agricultural waste and residues are burned as fuel or made into liquid biofuels. Wood waste from homes and forests is burned in thermal power plants to produce a significant amount of electricity. Specific biomassqualities become crucial during processing when lignocellulosic biomass is used to produce fuel for transportation. The primary features of interest are the calorific value of moisture content, proportions of fixed carbon and associated volatiles, residue/ash content, and alkali metal concentration. (Ibarra-Gonzalez & Rong, 2019).

Among the many lignocellulosic feedstocks available, pine needles are a significant biomass source for the production of biofuel or ethanol. The genus Pinus of the Pinaceae family includes coniferous, perennial, resinous, and native to the northern hemisphere trees known as pines. In evergreen forests, the adult green pine needle-shaped leaves make up the majority of the debris fall and are a major source of issues. Dried and falling pine needles destroy flora and wildlife on a huge scale, harming the ecosystem of the soil. Tannins from pine needles discharged into the soil may stop a number of vital agricultural microbes from growing, delaying the availability of nutrients in the soil.Tannins, which are solvent polyphenolic compounds found in pine needles, have an impact on soil nutrient management because they slow down the breakdown of organic matter and the uptake of nutrients. Additionally, a lot of dried pine leaves that are falling could start forest fires. Contrarily, PNB has a content of about 75% polysaccharides (hemicellulose, cellulose), which can be converted into monosaccharides and used as a starting point for microbial fermentation to produce energy, biomaterials, biofuel, and a variety of other commercially viable products. Therefore, using PNB as a source would not only eliminate issues

brought on by the accumulation of pine needles, but would also lead to waste being valued. (Singh et al., 2016).

Materials and Methods

Biomass collection- The pine needles were gathered from the forest areas in Tikri,Udhampur,. The dried pine needles have a brown colour. The dried pine needles were subsequently mechanically ground into a fine powder and put through a 1 mm sieve.

Physical pretreatment - The sample was taken in the forests near Tikri , Udhampur. The dried pine needles were physically crushed into a fine powder, sieved through a 1 mm sieve, and then further exposed to chemical processing.

Thermochemical pretreatment- The acid concentration, processing time and biomass loading parameters were all sought to be as efficient as possible while applying organic acid pretreatment to lignocellulosic raw material. Solutions of maleic acid , succinic acid and acetic acid were used to produce the desired acidic environment . In thermochemical pretreatment , organic acids were used to treat the biomass followed by autoclaving for 15 minutes at 121°C at 15 psi of pressure. The biomass loading for thermochemical pretreatment was 10% and 3% organic acids namely succinic acid , maleic acid and acetic acid .

Estimation of reducing sugars –Reducing sugar analysis was carried out after pretreatment of the pine needles. For the analysis of reducing sugars, the dinitrosalicylic acid method was utilised (Miller, 1959).

Enzymatic hydrolysis - For enzymatic hydrolysis, the pretreated sample was treated with hemicellulase enzyme HEMICELULAZA, Z from *Aspergillus niger*. Enzymatic hydrolysis was done using enzyme hemicellulaseat a loading of5 units/gram biomass. Firstly,thermochemical pre-treatment was given with 1% maleic acid at a biomass loading of 15% for 20 minutes. To find out the optimum pH for enzymatic hydrolysis the treatment with enzyme was done at pH 5, 6 and 7. For pH 5 citrate buffer was used whereas for pH 6 and 7 phosphate buffer was used. The samples were then incubated at 37°C at a shaking 200rpm and the readings were taken after every 24 hrs.

Fermentation-Fermentation was carried out usingseparate hydrolysis and fermentation (SHF). In separate hydrolysis and fermentation(SHF) the biomass was separately treated with

hemicellulase enzyme followed by fermentation with the yeast (*Saccharomyces cerevisiae*). The biomass thermochemically pretreatedby the optimized process was subjected to enzymatic treatment for 48 hours. The filtrate and the biomass pretreated using enzyme was mixed to carry out fermentation. pH of the pretreated biomass was adjusted to 5 using 1M NaOH.Theyeast culture was inoculatedand was incubation wasdone at 30°Cunder static conditions and the ethanol assay was done after every 24 hours. The yeast inoculum was made in YEPD (Yeast Extract Peptone Dextrose) broth and incubated for 24 hours in an incubator at 30°C with 200 rpm shaking.

Results and Discussion

Physical or mechanical pretreatment helps in increasing the surface area and also decreasing the degree of polymerization and crystallinity by reducing the particle size. The physical methods are environmentally friendly as they don't produce any toxic materials . They have the benefit of increasing the size of pores.The impact of Physical /Mechanical pretreatment is shown in the figure 1 and 2.

Figure 1:- Pine Needle biomass before physical pretreatment

Figure 2:- Pine Needle biomass after Physical pre treatment

Pretreatment by organic acids- OVAT approach was used for pine needle biomass pre-treatment with organic acids i.e. succinic acid , maleic acid and acetic acid at concentration of 3% with biomass loading of 10%.Pine needle biomass pretreated with succinic acid resulted in the total reducing sugars yield of 6.3g/l . For maleic acid and acetic acid the total reducing sugar yield of 15.45g/l and 0.48 g/l was obtained, respectively shown in figure 3. On pretreatment of biomass with different organic-acids i.e. succinic acid , maleic acid and acetic acid at a biomass loading of 10%, the best reducing yield i.e. 15.45 g/l was obtained with 3% maleic acid at a biomass loading of 10%.

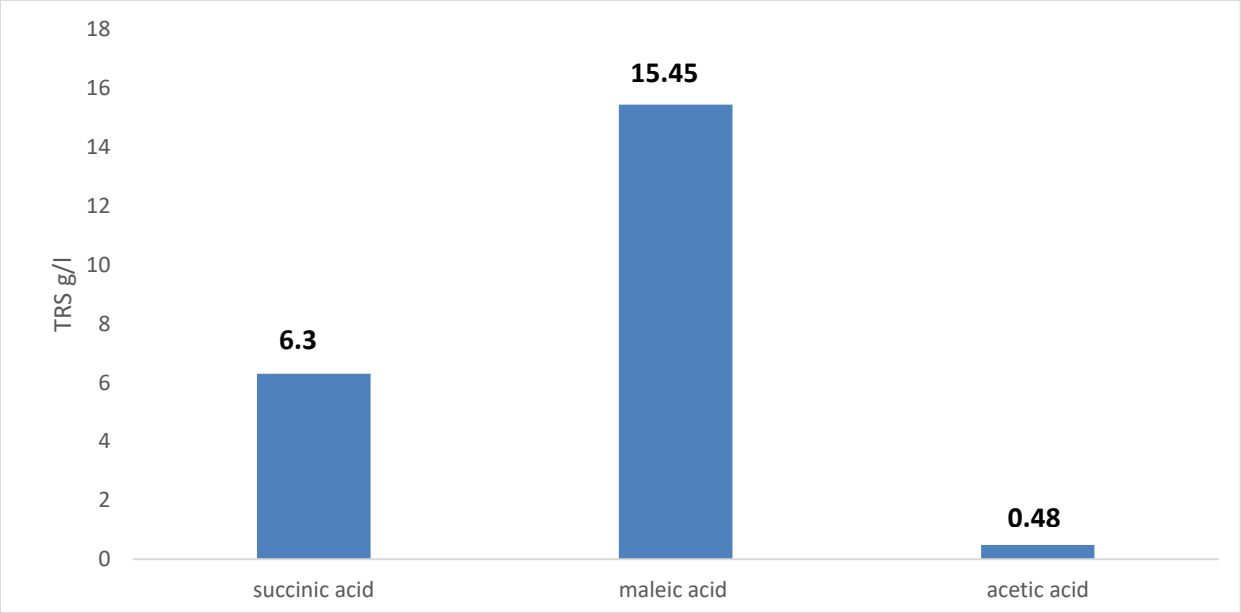

Fig 3:- Reducing sugar yields obtained in organic acid pretreatment

Enzymatic hydrolysis- It was done to further increase the reducing sugar yield treated with buffers. The thermochemically pre-treated biomass was subjected to enzymatic hydrolysis. The enzyme was used in the concentration of 5U/g. 1% pine needle biomass was first treated with 10 ml buffer with pH 5,6 and 7 (pH 5– Citrate buffer, pH 6, 7 –phosphate buffer). The PNB was then treated with the Hemicellulase enzyme and was incubated at 37°C temperature under shaking of 200rpm. Before incubation , the sample was taken and OD readings were taken at 0 hours. After this, we ran the experiment and hydrolysed the pre-treatment biomass. The TRS yield was determined after 24 hours and subsequently after 48 hours and 72 hours using DNS method. The highest TRS yield was recorded after 48 hours with Citrate buffer (pH-5). It was observed that after 48 hours of enzymatic hydrolysis, the TRS yield was calculated and it was almost double (two-fold) of the TRS yield before enzymatic hydrolysis.

Fermentation-In order to determine the ethanol content after fermentation, the dichromate test was used.Fermentation of thermochemically and enzymatically pre-treated biomass was carried out with yeast (*Saccharomyces cerevisiae*) in SHF mode.The yield of ethanol obtained in fermentation experiments is shown in figure The maximum yield of ethanol was found to be 12.6

g/l after 72 hours offermentation.Ethanol yield showed increase till 72 hours after which it reduced.

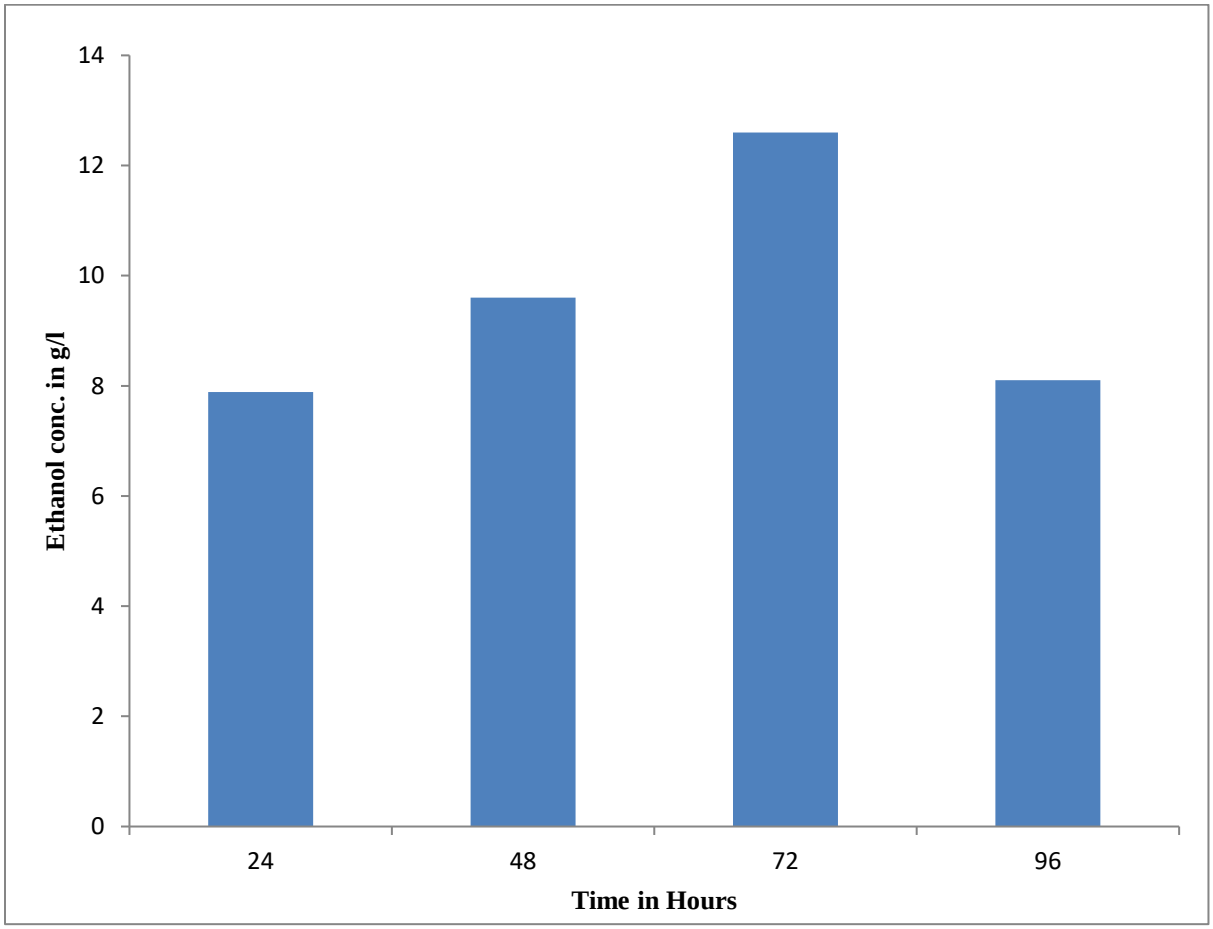

Figure 4: Ethanol yield obtained in fermentation experiments

References:

1. Abdeshahian, P., Dashti, M. G., Kalil, M. S., &Yusoff, W. M. W. (2010). Production of biofuel using biomass as a sustainable biological resource. *Biotechnology*, *9*(3), 274-282.
2. Achinas, S., &Euverink, G. J. W. (2016). Consolidated briefing of biochemical ethanol production from lignocellulosic biomass. Electronic Journal of Biotechnology, 23, 44–53. https://doi.org/10.1016/j.ejbt.2016.07.006

3. Al-Battashi, H. S., Annamalai, N., Sivakumar, N., Al-Bahry, S., Tripathi, B. N., Nguyen, Q. D., & Gupta, V. K. (2019). Lignocellulosic biomass (LCB): a potential alternative biorefinery feedstock for polyhydroxyalkanoates production. *Reviews in Environmental Science and Bio/Technology*, *18*, 183-205.

4. Alexopoulou, E.; Papatheohari, Y.; Christou, M.; Monti, A. Origin, description, importance, and cultivation area of kenaf. In *Kenaf: A Multi-Purpose Crop for Several Industrial Applications*;
5. Bajpai, P., & Bajpai, P. (2016). Structure of lignocellulosic biomass. Pretreatment oflignocellulosic biomassfor biofuel production, 7-12.
6. Bhutto, A. W., Qureshi, K., Harijan, K., Abro, R., Abbas, T., Bazmi, A. A., Karim, S., & Yu, G. (2017). Insight into progress in pre-treatment of lignocellulosic biomass. Energy, 122, 724–745. https://doi.org/10.1016/j.energy.2017.01.005
7. Chandra, R., Takeuchi, H., & Hasegawa, T. (2012). Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. Renewable and Sustainable Energy Reviews, 16(3), 1462–1476. https://doi.org/10.1016/j.rser.2011.11.035
8. Chiaramonti, D., Prussi, M., Ferrero, S., Oriani, L., Ottonello, P., Torre, P., &Cherchi, F. (2012). Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. Biomass and Bioenergy, 46, 25–35. https://doi.org/10.1016/j.biombioe.2012.04.020
9. De, P. P. D. (2022). Development of a Process for Efficient Enzymatic Hydrolysis of Pine Needles for Enhanced Sugar Production : A Key Step for Second Generation Ethanol Development of a Process for Efficient Enzymatic Hydrolysis of Pine Needles for Enhanced Sugar Production . March.
10. Ebringerová, A., Hromádková, Z., & Heinze, T. (2005). Hemicellulose. *Polysaccharides I: Structure, characterization and use*, 1-67.
11. Ewa Langer, ... Marta Lenartowicz-Klik, inPlasticizers Derived from Post-Consumer PET, 2020
12. Ferreira, J. A., &Taherzadeh, M. J. (2020). Improving the economy of lignocellulose-based biorefineries with organosolv pretreatment. Bioresource Technology, 299(October 2019), 122695. https://doi.org/10.1016/j.biortech.2019.122695

13. Flores, E.M.M.; Cravotto, G.; Bizzi, C.A.; Santos, D.; Iop, G.D. Ultrasound-assisted biomass valorization to industrial interestingproducts: State-of-the-art, perspectives and challenges.Ultrason. Sonochem. 2021, 72, 105455. [PubMed]

14. Gomez, L. D., Steele-King, C. G., & McQueen-Mason, S. J. (2008). Sustainable liquid biofuels from biomass: the writing's on the walls doi:10.1111/j.1469 8137.2008.02422.x. New Phytologist, 178(3), 473–485. http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2008.02422.x
15. Ibarra-Gonzalez, P., & Rong, B. G. (2019). A review of the current state of biofuels production from lignocellulosic biomass using thermochemical conversion routes. Chinese Journal of Chemical Engineering, 27(7), 1523–1535. https://doi.org/10.1016/j.cjche.2018.09.018
16. Isroi, M. R., Syamsiah, S., Niklasson, C., Nur Cahyanto, M., Lundquist, K., &Taherzadeh, M. J. (2011). Biological pretreatment: Review. BioResources, 6(4), 5224–5259.
17. Jędrzejczyk, M., Soszka, E., Czapnik, M., Ruppert, A. M., & Grams, J. (2019). Physical and chemical pretreatment of lignocellulosic biomass. In Second and Third Generation of Feedstocks: The Evolution of Biofuels. https://doi.org/10.1016/B978- 0-12-815162-4.00006-9
18. Karimi, K., Kheradmandinia, S., &Taherzadeh, M. J. (2006). Conversion of rice straw to sugars by dilute-acid hydrolysis. *Biomass and Bioenergy*, *30*(3), 247-253.
19. Karp, E. M., Resch, M. G., Donohoe, B. S., Ciesielski, P. N., O'Brien, M. H., Nill, J. E., Mittal, A., Biddy, M. J., & Beckham, G. T. (2015). Alkaline Pretreatment of Switchgrass. ACS Sustainable Chemistry and Engineering, 3(7), 1479–1491. https://doi.org/10.1021/acssuschemeng.5b00201
20. Kim, J. S., Lee, Y. Y., & Kim, T. H. (2016). A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresource Technology, 199, 42–48. https://doi.org/10.1016/j.biortech.2015.08.085
21. Kim, S. and Dale, B. E. Biomass and Bioenergy, 2004, 26, (4), 361–375,Fuel and Energy Abstracts

22. Singh, S., Anu, Vaid, S., Singh, P., & Bajaj, B. K. (2016). Physicochemical pretreatment of pine needle biomass by design of experiments approach for efficient enzymatic saccharificatio. Journal of Materials and Environmental Science, 7(6), 2034–2041.
23. Slathia, P. S., Raina, N., Kiran, A., Kour, R., Bhagat, D., & Sharma, P. (2020). Dilute acid pretreatment of pine needles of Pinus roxburghii by response surface methodology for bioethanol production by separate hydrolysis and fermentation. *Biomass Conversion and Biorefinery*, *10*, 95-106.
24. Smith, E. L., Abbott, A. P., & Ryder, K. S. (2014). Deep Eutectic Solvents (DESs) and Their Applications. Chemical Reviews, 114(21), 11060–11082.