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A BRIEF REVIEW ON SUNN HEMP AS THE SOURCE OF ENHANCED BIOETHANOL PRODUCTION

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Abstract

The Sunhemp is converted to products such as bioethanol, building materials, car components, bio-plastics, paper, and absorption materials. Bioethanol production in India is a novel event that was initiated by the government to minimize the gap between the country's crude oil consumption and production. Bioethanol is produced from Sun hemp biomass to reduce both consumption of crude oil and also environmental pollution. Bioethanol is used as the mixed fuel in the gasoline engine due to its high octane number and low cetane number. As a feed stock, Sunhemp biomass is sustainable and renewable with the potential to replace a diversity of fossil based products within the bioenergy sector. This paper reviews the status of Sunn hemp in India, available pretreatment techniques, fermentation and purification of bioethanol based on the concept of fractional conversions.

Keywords: Sunn Hemp, Pretreatment, Fermentation, Purification, Bioethanol, Bioenergy.

1. Introduction
1.1 Sunn Hemp

When Sunn hemp (*Crotalaria juncea*L) is grown as a summer crop, it is able to produce over 2,268Kg of biomass and over 45.5 Kg of nitrogen per acre. It is required only 8 to 12 weeks for its well growth and receive the above quantities. When the Sun hemp is grown as a winter crop, the soil characteristics will be improved. The relatively short period between crop harvest in the summer and planting the following winter will result optimum biomass production of the cover crop. Sunn hemp is an excellent alternative to other feed stocks to produce bioethanol because of its rapid growth and relatively short growing season requirement.

1.2 Origin of Sunn hemp

The Sunn hemp has been grown in India since the beginning of agriculture but, it has been utilized as a livestock feed, green manure, and anon-wood fiber crop. It has also been grown as a soil-improving crop in Bangladesh and Brazil. The research results of Sunn hemp were shown to be excellent in improving soil conditions (Cook & White, 1996).The Sunn hemp (*Crotalaria juncea*L.)is well growing and is an important species of the *Crotalaria* genus. All plants belong to *Crotalaria* are good at producing biomass and also fixing nitrogen. They are grown on droughty soil with low fertility as it is an additional advantage of it.

1.3 Characteristics of Sunn hemp

The Sunn hemp is used as a soil improvement crop in the tropics because of its ability to produce large amounts of biomass in period of time such as 60 to 90 days. It has good potential to build organic matter levels and sequester carbon due to its short period of growth. When

Sunn hemp is used as a cover crop, it will reduce soil erosion, conserve soil water, recycle plant nutrients and improve soil properties. Other uses of Sunn hemp biomass are production of paper fiber, forage and as alternative fuel crop (Rotar& Joy,1983).

1.4 Organic Matter Builder

The cover crop studies results obtained by Rotat and Joy (1983) were reproduced by Mansoer et al. (1997) at the same conditions. The biomass was produced at an average of 2,359Kg in 9 to 12weeks over a two-year study at two locations in Alabama. Thiswas compared with 1,950Kg per acre with hairy vetch and1,996Kg with crimson clover (Mansoer et al., 1997). Ground cover from the tropic sun averaged approximately 96 percent at mowing and approximately 65 percent soil coverage 16 weeks later (mid-April). This residue provides erosion control and promote increases in organic matter accumulations in conservation tillage systems.

1.5 Sunn Hemp as a Nitrogen Producer and Potential Crop

A report from a research Mansoer et al. (1997) showed that at an average of 57 Kg nitrogen in 9 to 12 weeks and concluded that, a crop such as small grain should follow Sunn hemp to utilize the symbiotically produced nitrogen irrespective of season.

As an alternative to winter cover crops, the Sunn hemp is a symbiotic nitrogen producing legume and as an organic matter builder. To produce nitrogen and biomass, warm weather is required for a period of 8 to 12 weeks and Sunn hemp is well suited to southern climates than northern (McKee & Enlow, 1931). Sunn hemp is also used in rotations with kenaf for non-wood fiber production as Kenaf is susceptible to root knot nematodes but, Sunn hemp is resistant. Sunn hemp is used after early season vegetables, tobacco and small grains or other winter crops in the summer or after corn in southern climates and prior to winter small grains.

1.6 Sun Hemp in India

The Sunn hemp (*Crotalaria juncea*L.) is a native of India and sunn hemp fibre is used in making cordage, fishing net and paper. The fibre pulp sunn hemp is also used in currency note paper and cigarette paper manufacturing. Genus Crotolaria consists more than 200 species distributed in tropical and sub-tropical regions. From India, it was introduced to other countries and as per some investigators, Myanmar is the place of origin of the crop, because it is found there in its wild state. The crop cannot withstand low temperatures and frost condition. It is also grown in rabi season, where winter temperature is mild i.e. in southern region. It is cultivated in areas with a well distributed rainfall of 400-1000 mm throughout the crop growth, with high relative humidity (60-85%) and a temperature ranging from 20-35^{o} C (Tripathi et al., 2012). Ram and Singh(2011) reported that, it is one of the green manure crops suited to almost all parts of the India. Sunn hemp has ability to fix atmospheric nitrogen, to add organic matter to the soil, suppressing weeds and to reduce soil erosion. One of the important reasons for reduced popularity of Sunn hemp is unavailability of good quality seeds (Chittapur and Kulkarni, 2003). Some research has been done aiming at standardization of different factors for seed production in many crops, but most of the agronomic practices have still not been standardized for seed crop of Sunn hemp.

Spacing will affect seed yield of different crops, growth rate and crop yield as a result of inter plant competition for different inputs needed for growth and development. The practice of topping has proved to be effective in increasing the yield levels of different crops (Bhattacharjee and Mitra, 1999; Sajjan et al., 2002; Jagannatham et al., 2008; Singh et al., 2011). Very less information on spacing along with topping is available in seed crop of Sunnhemp. Tripathi*et al.*, (2013) have studied and reported the effect of spacing on growth characteristics and yield attributes, effect of topping on growth characteristics and yield attributes and concluded that the seed crop of Sunn hemp at the spacing of 30 × 10 cm coupled with topping at 30 days is effective in increasing the seed yield of Sunn hemp.

2. Methods

2.1 Sunn Hemp Pretreatment

Sunn hemp plant can be cultivated and harvested between 9 to 12 weeks after plantation shown in figure 1. The biomass can be washed to remove soil residues and air dried to obtain a constant weight. Combination of sonication with alkaline pretreatment shows greater efficiency and biodegradability of lignocellulose material in lignin removal and increase in cellulose yield. In enzymatic hydrolysis, 0.25N NaOH treated biomass can be subjected to time interval for sonication. Sonicated NaOH treated biomass can be autoclaved at 121˚C for 20 min. Pretreated biomass can be recovered by filtration and washed with deionized water to take off excess alkali and dissolved byproducts and dried till it attains constant weight (Verma et al. 2013).

Figure 1: Sunn hemp cultivated for acid and alkali hydrolysis at the Department of Biotechnology, NIT Warangal, India.

2.2 Physical Methods

By using physical methods, waste materials will be comminuted and yield is increased. Some physical methods like chipping, grinding and milling are used to reduce cellulose crystallinity and reduction that facilitates the access of cellulases to the biomass surface increasing the conversion of cellulose. Final particle size and biomass characteristics are considered to calculate energy requirements for the mechanical comminution of lignocellulosic materials. Cellulose reactivity can be increased towards enzymatic hydrolysis by mechanical pretreatment methods and these methods are unattractive due to their high energy and capital costs (Ghosh &Ghose, 2003). Pyrolysis is also used as a physical method for pretreatment of lignocellulosic biomass as cellulose rapidly decomposes when it is treated at high temperatures.

2.3 Physical–Chemical Methods

In physical–chemical methods such as steam explosion the use of saturated steam at high pressure causes auto hydrolysis reactions and part of the hemicellulose and lignin are converted into soluble olygomers. The residence time, temperature, chip size and moisture content are the main factors affect steam explosion pretreatment. Shahbazi et al., (2005) studied and reported to consider the combined action of both temperature and time over the performance of steam explosion pretreatment under acidic conditions. The use of very small particles in herbaceous waste due to the economic feasibility of the process (Ballesteros et al., 2002). This method is recognized as one of the most cost-effective methods for hardwood and agricultural residues, but is less efficient for soft wood. According to these authors, this pretreatment is a promising method for increasing the overall yield during bioethanol production from other lignocellulosic biomass sources.

2.4 Chemical Methods

Chemical methods use different chemical agents such as acids, alkalis, ozone, peroxide and organic solvents in the pretreatment of lignocellulosic biomass. With the use of dilute sulfuric acid hydrolysis technique has been developed with high reaction rates and improved cellulose hydrolysis. When costs are compared, costs of dilute acid pretreatment are higher than the steam explosion process (Sun and Cheng, 2002). The dilute acid pretreatment of corn stover was studied at pilot plant level using high solid loads and obtained a xylose yield of 77% at 190°C (Schell et al.2003). Dilute acid pretreatment is also accomplished in a two-stage way like depolymerization stage of hemicellulose at 140°C for 15 minutes is carried out in order to avoid the formation of furan compounds and carboxylic acids, followed by a second stage at 190°C for 10 minutes to make cellulose more accessible to enzymatic hydrolysis (Saha et al., 2005). Due to addition of dilute bases on the biomass in alkaline treatment, the internal surface will be increased by swelling, decreased polymerization degree and crystallinity, destruction of links between lignin and other polymers, and breakdown of lignin. The efficiency of physical methods depend on the lignin content of the biomass (Sun and Cheng, 2002). The utilization of bases, such as sodium hydroxide or solvents such as bioethanol or biomethanol allows the dissolution of lignin, but their costs are so high that these methods are not competitive at large scale (Lynd et al., 1999).

2.5 Low Pressure Steam Explosion Technology

Ghosh and Ghose (2003) reported the model process for the production of bioethanol at Indian Institute of Technology in Delhi, India. Two pretreatment steps were involved in this technology : (a) Steam explosion for xylose production and (b) Solvent pretreatment for delignification of biomass. In steam explosion technology, the crystalline structure of lignocellulose can be broken through chemical effects and mechanical forces and hemicellulose is hydrolyzed in this pretreatment by adding acids derived from acetyl groups at high temperatures (Narasimhulu et al., 2017). Mosier et al., (2005) reported that the lignin was redistributed and removed from the material and steam explosion of biomass will open up the fibers and make the biomass polymers more accessible for subsequent processes, i.e. fermentation, hydrolysis or densification processes. In this process, biomass was treated with hot steam at a temperature in the range of 180 to 240 °C under pressure in the range of 1 to 3.5 MPa followed by an explosive decompression of the biomass that resulted in a rupture of the biomass fibers rigid structure. The sudden pressure release defibrillated the cellulose bundles and resulted in a better accessibility of the cellulose for enzymatic hydrolysis and fermentation. Lower environmental impact, less hazardous process chemicals, and greater potential for energy efficiency are the advantages of steam explosion process when compared to other pretreatment technologies (Narasimhulu et al., 2017).

2.6 Combined Pretreatment Methods

There are limitations of steam explosion method like partial destruction of xylan, limited lignin removal, incomplete disruption of the lignin-carbohydrate matrix and lignin redistribution on the cellulose surfaces (Chen et al., 2008). Wingren et al., (2004) suggested a two-step steam explosion pretreatment by solubilizing hemicellulosic in the first step at low temperature and the cellulose fraction is then subjected to a second pretreatment step at temperatures higher than 210°C. Galbe and Zacchi (2007) reported that an economic evaluation is needed to determine the effectiveness of an additional steam explosion step and it is necessary to combine other methods with steam explosion to get the optimum pretreatment effect on lignocellulosic biomass. Zho et al., (2009) reported that alkaline pretreatment provides an effective delignification and chemical swelling of the fibrous cellulose.

2.7 Biological Methods

Expensive equipment, chemicals and intensive energy consumption are required in physical and chemical pretreatment methods and biological pretreatment by solid fermentation employs microorganisms that degrade lignocellulosic biomass at mild conditions without special requirements for equipment. Low energy requirements and mild environmental conditions are the main advantages of biological pretreatment method over physical and chemical pretreatment methods. Most of these processes are slow, thus limiting its application at industrial level. Lee (1997) reported that the main microorganisms producing lignin degrading enzymes and indicates the fermentation processes for producing them by both submerged culture and solid-state fermentation.

2.8 Enzymatic Hydrolysis of Biomass

Enzymatic hydrolysis can be performed on raw biomass and pretreated Sunn hemp biomass with free and immobilized enzymes. Hydrolysis experiments can be conducted for 48 hrs in 0.1M sodium acetate buffer with different pH values and at different temperatures. Every 12 hrs interval, the samples can be taken and estimated for reducing sugars using DNS method. Cellulose hydrolysis yield can be calculated by using the below equation. The value 0.9 can be used in the equation as a correction factor for hydration.

$$\text{Cellulose hydrolysis \%} = \frac{\text{reducing sugar concentration X total reaction volume X 0.9}}{\text{Amount of cellulose added}} \text{ X } 100$$

2.9 Production and Purification Bioethanol From Sunn Hemp Fermentation

It is the biological process and used to convert the hexoses and pentoses into bioethanol by a microorganisms, such as bacteria, yeast, and fungi. The conversion reaction for hexoses (C_6) and pentoses (C_5) is as follows:

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$---(3), $3C_6H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$ ---(1)

The theoretical maximum yield of broth hexoses and pentoses is 0.511 kg ethanol and 0.489 kg CO_2 per kg sugar. The overall theoretical bioethanol yield at 20°Cis 0.719 and 0.736 liters per kg of glucan (and/or other 6C structures) and xylan (and/or other 5C structures), respectively. *S. cerevisiae*, the yeast is used for first generation ethanol production and it cannot metabolize xylose. Other yeasts and bacteria are under investigation to ferment xylose and other pentoses into bioethanol. Pretreatment and hydrolysis of lignocellulosics release specific inhibitors, for example, furans, such as furfural and 5-hydroxymethylfurfural (5-HMF), and phenols, such as 4-hydroxybenzaldehyde (4-HB), vanillin, and syringaldehyde, that need to be dealt with to operate hydrolysis and fermentation under optimum conditions and maximum conversion.

3. Purification

Typical ethanol concentrations from second generation feed stock are in the range of 3–6 vol% only, very low in comparison with 12 to 15 vol% obtained from 1st generation feedstock. Due to the higher water content of the broth, additional distillation efforts are required. Different process improvements, including energy pinch, very high gravity fermentation, and hybrid processes, are described in detail by Kang et al. (2014).

Conclusion

Conversion of lignocelluloses from Sunnhemp to bioethanol holds great potential due to the wide spread abundance, availability and relatively low cost of cellulosic materials. The economical and environmentally-friendly development of bioethanol from Sunhemp lignocellulose requires highly efficient process integration. The successes on pretreatment, enzymatic hydrolysis, fermentation, and separation of bioethanol have been achieved over the past few decades. New ideas like biorefinery and the concept of oriented conversion of classified composition, have been proposed and practiced in bioethanol plants using lignocellulose as raw material. By an intelligent combination of pretreatment, hydrolysis, fermentation and product separation, the maximum efficiency and benefits of the process can be achieved due to the simultaneous production of manyhigh-value co-products with bioethanol from Sunhemp.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

i. Ballesteros, I., Oliva, J.M., Negro, M.J., Manzanares, P., &Ballesteros, M., 2002. Enzymic hydrolysis of steam exploded herbaceous agricultural waste (Brassica carinata) at different particule sizes. *Process Biochemistry38*, pp. 187–192.

ii. BhattacharjeeAK,&Mitra BN., 1999. Jute seed productivity and its quality as influenced by suppression of apical dominance. In: Palit et al, eds.*Jute and Allied Fibres: Agriculture and Processing*. India: CRIJAF Publication, pp. 177-185.

iii. Chen HZ, Han YJ, &Xu J., 2008. Simultaneous saccharification and fermentation of steam exploded wheat straw pretreated with alkaline peroxide. *Process Biochem,* Volume 43, pp. 1462–6.

iv. Chittapur BM, &Kulkarni SS, 2003. Effect of sowing dates on the performance of sunnhemp. *J. Maharashtra Agric. Univ.*, 28(3), p. 331-331.

v. Cook C.G. & G.A. White., 1996. Crotalaria juncea: A potential multipurpose fiber crop. In:J.Janick, ed.*Progress in new crops*. Arlington, VA.: ASHS Press, pp. 389-394.

vi. Galbe M, &Zacchi G., 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. *Biofuels*, Volume 108, pp. 41–65.

vii. Ghosh, P.,&Ghose, T.K., 2003. Bioethanol in India: recent past and emerging future. *Advances in Biochemical Engineering/Biotechnology* Volume 85, pp. 1–27.

viii. Jagannatham J, Rajendra KB, Sreelatha T, &RajabapaR.V., 2008. Effect of topping on seed yield and fibre yield of mesta, sabdariffa vs cannabinus. In: Palit et al, eds.*Abstracts of Papers: International Symposium on Jute and Allied Fibres Production, Utilization and Marketing held at Kolkata, India*. Kolkata, India: CRIJAF Publication, p. 121.

ix. Lee, J., 1997. Biological conversion of lignocellulosic biomass to ethanol. *Journal of Biotechnology*Volume56, pp. 1–24.

x. Lynd, L.R., Wyman, C.E., &Gerngross, T.U., 1999. Biocommodity engineering. *Biotechnology Progress*Volume15, pp. 777–793.

xi. Manasa P, ParamjeetSaroj, Nisha G.,&Narasimhulu K., 2017. A Brief Review on Technologies for the production of Bioethanol from lignocellulosic biomass.*International Journal of Advanced Research in Engineering Technology & Science*, 4(5), pp. 6-15.

xii. Mansoer, Z., 1993. *Release of nitrogen from 'Tropic Sun' Sunn hemp (Crotalaria junceaL.) residue under no-till and conventional systems*.s.l.: s.n.

xiii. Mansoer, Z., Reeves, D.W.,Wood, C.W., 1997. Suitability of sunn hemp as an alternative late-summer legume cover crop. *Soil Sci. Soc. Am. J.,* Volume 61, pp. 246-253.

xiv. McKee, R. &Enlow., C.R., 1931. Crotalaria, a new legume for the South. Washington DC.:USDA Circular 137.

xv. Mosier N, Wyman C, Dale B, Elander R, Lee YY, &Holtzapple M, 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *BioresourTechnol*, Volume 96, pp. 673–86.

xvi. Kang, Q., Appels, L.,Baeyens, J., Dewil, R., Tan, T., 2014. Energy-efficient production of cassava-based bio-ethanol. *Advances in Bioscience and Biotechnology*, 5(12), pp. 925–939.

xvii. Ram H, &Singh G., 2011. Growth and seed yield of sunnhemp genotypes as influenced by different sowing methods and seed rates. *World J. Agric. Sci*.7(1), pp. 109-112.

xviii. Rotar, P.P. & Joy, R.J., 1983. *Tropic Sun' sunnhemp (Crotalaria junceaL)Res. Ext. Ser. 36*. Honolulu: Hawaii Inst. Trop. Agric. and Human Resour.,Univ. of Hawaii.

xix. Saha, B.C., Iten, L.B., Cotta, M.A., &Wu, Y.V., 2005. Dilute acid pretreatment, enzymatic saccharification, and fermentation of rice hulls to ethanol. *Biotechnology Progress*Volume 21, 816–822.

xx. Sajjan AS., Shekaragouda M., &BadanuVP., 2002. Influence of apical pinching and pod picking on growth and seed yield of okra. *Karnataka J. Agric. Sci.*, 15(2), p. 367-372.

xxi. Schell, D.J., Farmer, J., Newman, M., &McMillan, J.D., 2003. Dilute sulfuricacid pre-treatment of corn stover in pilot-scale reactor. Investigation of yields, kinetics, and enzymatic digestibilities of solids. *Applied Biochemistry and Biotechnology,* 105 (1–3), pp. 69–85.

xxii. Shahbazi, A., Li, Y., Mims, M.R., 2005. Application of sequential aqueous steam treatments to the fractionation of softwood. *Applied Biochemistry and Biotechnology,*pp.973–987.

xxiii. Sun Y. C, 2002. *Enzymatic hydrolysis of rye straw and Bermuda grass for ethanol production. PhD thesis, Biological and AgriculturalEngineering*. North Carolina: North Carolina State University.

xxiv. Tripathi MK, Chaudhary B, Bhandari HR, &Harish ER, 2012. Effect of varieties, irrigation and nitrogen management on fibre yield of sunnhemp. *J. Crop. Weed* 8(1), pp. 84-85.

xxv. Tripathi MK, Chaudhary B, Singh, S.R., &Bhandari HR., 2013. Growth and yield of sunnhemp (*Crotalaria juncea*L.) as influenced by spacing and topping practices. *African Journal of Agriculture Research,* 8(28), pp. 3744-3749.

xxvi. Verma ML, Barrow CJ, &Puri M, 2013.Nanobiotechnology as a novel paradigm for enzyme immobilisation and stabilisation with potential applications in biodiesel production. *Appl. Microbiol. Biotechnol.,*Volume 97, pp. 23–39.

xxvii. Wingren A, Söderström J, Galbe M, &Zacchi G.,2004. Process considerations and economic evaluation of two-step steam pretreatment for production of fuel ethanol from soft wood. *Biotechnol. Prog.*,Volume 20, pp. 1421–9.

xxviii. Zhao X, Peng F, Cheng K, &Liu D., 2009. Enhancement of the enzymatic digestibility of sugar cane bagasse by alkali–peracetic acid pretreatment. *Enzyme Microb. Technol.*, Volume 44, pp. 17–23.