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*Review*

# Lignocellulose for ethanol production: A review of issues relating to bagasse as a source material

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**Fossil fuels reservoirs have been declared to serve mankind's needs more for a very limited time period. This notion has already initiated scientific search for alternatives. Amongst renewable resources for emerging biotechnological strategies to produce high energy-less volume fuels, cellulose is the most abundantly synthesized but stable carbohydrate of the biosphere. Cellulose has earlier been taken into account for chemical/biological saccahrification and subsequent biological conversion of the monomeric sugars to ethanol. The stable nature of the substrate and some of the monomeric products' fermentation difficulties have been the major hardles. But because of its ubiquitous nature and being the most abundantly available renewable resource, research on the utilization of cellulose for obtaining the biofuel has continued and has been representing by diverse fields. Following the recognition of different bacteria and yeasts and various kinetics of the process involved in its saccahrification, the substrate is increasingly being worked out by different laboratories. Biotechnological endeavors are in fact reshaping the economics of different countries. Production of high grade sweeteners with low caloric values by the contemporary biotechnological processes is likely to influence the conventional sucrose production negatively. The raw material for sugar industry would be available for other products such as ethanol. At present, cellulosic waste of such industries for example, sugarcane bagasse, may be targeted for sacharification and ethanologenesis. Various aspects regarding the nature of the cellulosic substrate and its potential for obtaining the biofuel are covered in this review.**

**Key words:** Biofuel, cellulose and ethanol, xylose fermentation, environmental rehabilitation.

## INTRODUCTION

Ever increasing human population density and the desire for higher life standards, demanding more and more comforts have necessitated large scale exploitation of fossil fuel-derived energy resources, in the recent centuries. By the end of the last century, it had been speculated that human population may exceed in figure of 8 billions in the beginning of this century (Smith, 1996). Owing to the facts of ever increasing number of consumers and rapidly depleting resources of fossil fuels, scientists have rightly sensed that to feed and provide other requirements to such dense population at a reduced environmental cost is a real target for future biotechnological improvements. Fueling both the humans and the required mechanical engines necessitates various developments in the agricultural and energy sectors. Plant biomass, due to its abundant and regenerative nature has been targeted for supply of a sustainable energy resource. Various sections of this review are concerned with this alternative to fossil fuels.

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## PLANT BIOMASS – AN ALTERNATIVE TO PETROLEUM

Irrespective of environmental deterioration, the supply of fossil fuels is being consumed due to its combustion. About a quarter century before, Detroy and St Julian (1983) stressed that petroleum can no longer be relied upon as a stable, economical raw material for provision of energy and industrial chemicals. They indicated the possibility of plant biomass as a desirable alternative material to petroleum because of its renewability. Indeed, biofuels represented by biologically produced alcohols, gasses and oil represent renewable energy resources, unlike petroleum, coal and nuclear fuels. Rising energy and environmental problems have led to increased interest in the production from diverse routes and resources and utilization of alcohols as fuel (Von et al., 1994; Lawford et al., 2001; Atiyeh and Duvnjak, 2002; Dien et al., 2004; Taherzadeh and Karimi, 2007). Ethanol has been used as biofuel in the United States, Europe and Brazil. In Brazil industrial scale, ethanol is produced from sugarcane for blending with gasoline. In the U.S, corn is used for ethanol production and is then blended with gasoline to produce gasohol (Wheals et al., 1999; Enger and Smith, 2002). Besides being a renewable fuel made from plants, with high octane at low cost, ethanol is a much cleaner fuel than petrol. Ethanol blends dramatically reduce emissions of hydrocarbons, (major sources of ground level ozone formation), cancer-causing benzene and butadiene, sulphur dioxide and particulate matter. Moreover, ethanol blends can be used in all petrol engines without modifications (Miller, 2003; Steven and Ronald, 2004).

## ETHANOL FERMENTATION

Ethanologenic fermentation is the conversion of sugars into carbon dioxide and ethyl alcohol. Regarding the provision of sugars for ethanol fermentation, it is pertinent to note that, development of several novel sweeteners, many times sweeter than sucrose could ultimately lead to a reduction in the traditional sugar market for sugarcane and sugar beet. In this way, these economics predominately in developing countries could experience severe financial and employment discretion with alternatives difficult to find (Smith, 1996). Ethanol fermentations meant to generate biofuel would then be amongst the considered alternatives for the sugar mills. Sugars may also be derived from starches and cellulosic materials in addition to black strap molasses, a by-product of cane sugar manufacture. Once simple sugars, the monometric units are formed, enzymes from yeasts and bacteria can readily ferment them to ethanol. Presently, most widely used sugar source for ethanol fermentation is blackstrap molasses, which contains about 35 to 40% sucrose, along with of 15 to 20% invert sugars such as glucose. The latter, a major though not sole substrate for the most familiar fermentation pathway, may emerge in different forms of starchy commodities/molasses either by diatase present in sprouting grains or by microbial amylases.

## CELLULOSE AND BIOETHANOL

A large number of both bacterial and fungal species has been well documented for ethanol fermentations from glucose and fructose. Recently, Sharifia et al. (2008) have discussed that yeast in the form of the fungus *Mucor indicus* showed faster ethanol production with an average productivity of 0.90 g/l h from glucose, fructose and inverted sucrose, than the filamentous form with an average productivity of 0.33 g/l h. However, dietry requirements appear to restrict the exploitation of starchy materials and /or available sugars for obtaining bio-ethanol at a level to replace the fossil fuels, supply. Cellulosic material is a source of carbohydrate monomers and converted to sugars, generally by the action of mineral acids. Dietry requirements of familiar carbohydrates could discourage large scale biofuel generation from them. Abiet having technologically difficult boundaries the abundantly present cellulosic material has been conceived by contemporary biotechnologists for bioethanol generation, for instance, Wheals et al. (1999) while discussing commercial viability of fuel ethanol had described that, there will only be sufficient, low-cost ethanol if lignocellulose feedstock is also used. Similarly, Farrell et al. (2006) have explained that, large-scale use of ethanol for fuel will almost certainly require cellulosic technology. Recently, Wu et al. (2006), while assessing the energy and emission benefits of cellulosic biomass for the U.S. transportation sector in the years 2015 and 2030 have revealed that, cellulosic biomasses E85 (mixture of 85% ethanol and 15% gasoline by volume), Fisher-Tropsch diesel and dimethyl ether offer substantial savings in petroleum (66 to 93%) and fossil energy (65 to 88%) consumption on a per mile basis. These authors have strongly suggested that, integrated heat and power co-generation by means of gas turbine combine cycle is a crucial factor in the energy savings and emission reduction. Any biotechnological development requires a constant and abundant supply of raw material. The cost of raw material has a decisive status for establishing any biotechnological process. Cellulose may serve a major source of feed stock for various biotechnological processes including generation of fuels.

Future processes will increasingly make use of organic materials that are renewable in nature and/or occur as low value, valueless or negative value wastes and which presently cause environmental pollution. Currently, more than ten times more energy is generated annually by photosynthesis than is consumed by mankind. On a wordwide basis, land plants produce 24 tonnes of cellulose per person per year (Smith, 1996). Definitely, lignocellulose is the most abundant and renewable natural resource available to humanity throughout the world. It has been documented that, massive technological difficulties such as expensive energy-demanding pretreatment processes have to be overcome before economic use may be made of the plentiful renewable resource. Following its chemical and/or enzymatic hydrolysis, soluble sugar products of cellulose can then be converted to form ethanol, butanol, acetone, single cell protein and methane, etc. (Smith, 1996; Nelson and Cox, 2000; Anderson et al., 2005). Hill et al. (2006) have recently described that, negative environmental consequences of fossil fuels and concerns about petroleum supplies have spurred the search for renewable transportation biofuels, but to be a viable alternative, a biofuel should provide a net energy gain, have environmental benefits, be economically competitive and be producible in large quantities without reducing food supplies. These authors have reported that, even if all the U.S. corn and soybean productions were dedicated to produce the biofuels, it would only cover 12% of gasoline demand and 6% of diesel. Thus, fuels such as cellulosic ethanol produced from low-input biomass grown on agriculturally marginal land or from waste biomass could provide much greater supplies and environmental benefits than food-based biofuels. Similarly, Farell et al. (2006), while evaluating some representative analyses of fuel ethanol have concluded that, studies reporting negative net energy incorrectly ignored various co-products and used some obsolete data. These workers further stressed that, it is well clear that large scale use of ethanol for fuel will almost certainly require cellulose technology. Likewise, Taherzadeh and Kairimi (2007) have recently indicated that lignocelluloses can be expected to be major feedstocks for ethanol production in the future. Evans (2005) has explained that, being 50% of the total dry matter of plant, cellulose is potentially a huge renewable energy store and vast amounts of this material are routinely thrown away. However, until recently, the prospect of realizing this potential fuel source was viewed difficult and expensive. The combination of cellulase-resistant links and its close association with lignin discourage its large-scale hydrolysis to sugars. Energy involved in rendering various cellulosic materials into acceptable from had been considered a major limiting factor. Nevertheless, contemporary technologies employying whole organism and isolated enzymes appear to be promising to make the commercial processing of cellulose to alcohol a reality. Evans (2005) has also commented that, earlier technical difficulties in cellulose fermentation are likely to be overcome and that the first plant to extend this technology will shortly begin its operation.

## CELLULOSE-COMPOSITIONAL ASPECTS

It is not only glucose, the familiar sugars both for the fermentation scientists and microorganism as well. Rather other monomeric sugars, the xylose is representing from 20 to 40% of the contents of different cellulosic materials (Bicho et al., 1988). Economic ethanol fermentations of cellulose are required to use this pentose sugar along with glucose following the saccaharification of the fibrous matter. Majority of the reported microbial diversity is capable of utilizing glucose only. Previously reported scarcity of xylose fermenting pathways in the microorganisms has been discouraging for cellular materials to be employed for economic ethanol generation. However, recently naturally occurring as well as genetically engineered xylose fermenting microorganisms have also been well documented (Govindaswany and Vane, 2006; Sonderegger et al., 2004; Toivari et al., 2001; Jeppsson et al., 2002; Chaudhary and Qazi, 2006). It has been however, reported variously that glucose is preferred by fermenting microorganisms capable of fermenting both categories of the monosacchrides that is, the glucose and xylose. In such cases, glucose depletion within a fermenting sub-strate may allow for xylose utilization (Govindaswamy and Vane, 2006). Many strategies can be attempted to overcome co substrate inhibition of xylose consumption by glucose. For instance, incase of microbial consortia, first, the glucose be utilized and then, the residual be attempted with xylose utilizers. Microorganisms capable of co-utilization appear promising. For instance, Karhumaa et al. (2006) have reported simultaneous co-utilization of xylose and arabinose in recombinant strains of *Saccharomyces cerevisiae*. This is well clear that, econo-mic ethanol generation from lignocelluloses requires maximum utilization of all the diverse sugars monomers derived through any feasible saccharification process.

Responding to the earlier referred situations requires isolation and construction of microorganisms capable of fermenting glucose and xylose at appropriate levels. Fermenting microbes that would prefer xylose or be incapable of glucose utilization may find increasing utilization along with glucose utilizers for the process developments employing various types of fermenters. For example, in case of homogenously mixed bioreactors, glucose and xylose utilizers may be inoculated at appropriately different times. Alternatively, in a plug flow reactor, the two types of the microorganism can be allowed to work at appropriate different locations. In the latter case, a fermented matter in which glucose has been used maximally would serve as feed stock for xylose-fermenting microorganisms. Mutants or genetically modified organisms with derived characteristics would be required in many cases meant to develop processes for obtaining ethanol from cellulose.

The earlier mentioned approach appears to play a major part in addressing the largest environmental issue of our time, energy and waste. Energy extraction accompanied by environmentally safe disposable of a cellulosic waste is likely to render the process economically feasible. A large number of point and non-point/plant biomass loads, result in higher biological oxygen demand (BOD) values of various affected water systems. Processing such wastes for ethanol fermentation is highly appealing to reduce the pollutants (Evans, 2005). In Brazil, sugar cane cultivation and its dependent sugar industry is well developed. Consequently, a huge amount of bagasse is generated. It consists mainly of 37% cellulose, 28% hemicellulose and 21% lignin (Bon, 1996). We, in our laboratory, have recently started to mobilize the cellulosic portion of the industrial waste for yielding the sugars, which are then fermented to ethanol. The material has been targeted for two reasons. It is a waste of the sugar industry and secondly, it contains a few percent of free sugars. The latter may serve as breakfast to activate microorganisms representing inoculum. A reasonable number of cellulose saccharifying and/or ethanologenic bacteria as well yeast have been isolated and characterized (Saeed AAWS, 2005; Chaudhary and Qazi, 2006). Conver-ting cellulose to ethanol is accomplished in essentially four stages, namely:

### Acid/microbial hydrolysis

This process breakdown the cellulose into slurry of sugars in acid water and solid lignin particles. While reviewing acid-based hydrolysis processes for ethanol from lignocellulosic material, Taherzadeh and Karimi (2007) have summarized that, concentrated-aid processes are operated at low temperatures and give high sugar yield. On the other hand, dilute-acid processes are operated at high temperature and give low sugar yield. Both categories of the hydrolysis results however, into equipment corrosion. In a more recent communication the same author (Taherzadeh and Karimi, 2008) have reviewed different pretreatments for lignocellulosic wastes and discussed effects of milling, irradiation, microwave, steam explosion, ammonia fiber explosion (AFEX), supercritical $CO_2$ and its explosion, alkaline hydrolysis, liquid hot-water pretreatment, organosolv processes, wet oxidation, ozonolysis, dilute and concentrated-acid hydrolyses and biological treatment on improvement in ethanol and /or biogas production.

### Acid recovery

Following acid hydrolysis, sugary liquid is separated from the previous stage and the process acid is recovered partly and reused. Van Groenestijin et al. (2006) have claimed that recovery of sulphuric acid in the form of $H_2S$ from anaerobic waste water treatment is the overall cost for ethanol production.

### Fermentation

The derived sugars are fermented by yeast and/or bacteria into alcohol. As already indicated, fermentation of lignocellulosic hydrolyztes requires employing microorganism capable of utilizing monomeric sugars and to resist inhibitory products of hydrolyses process as well.

### Distillation

It is required to collect the market grade ethanol. Economizing these four phases necessitates understanding and optimizing the diversity of the processes involved. Fortunately, a large number of efforts in this regards are being continuously reported in the literature (Ballesteros et al., 2001; Negro et al., 2003; Anderson et al., 2005). The latter authors have described that, crops such as switch grass, bermudagrass or napiegrass have the capacity to produce large quantities of lignocellulose for biofuel. To facilitate use of lignocellulosic material for ethanol, it will be necessary to determine cost efficient pretreatments to enhance the conversion to fermentable sugars.

## CONCLUSION

Considering process kinetics is very important. Microorganisms tend to disturb the optimum conditions provided to them due to their own growth and metabolic activities. Furthermore, feed back inhibition is an important limiting factor for both saccharification and ethanologenic levels. Thus, microorganisms with a wide range of activities are to be worked out. Prompt recovery of hydrolysis yield as well as fermentation yields is indicated. Different bacteria and yeast have varying levels of ethanol tolerance; many find increasing applications for processes developed for different lignocellulosic materials. Here, thermophilic ethanologenic microorganisms become important as the product recovery can be achieved at elevated temperatures, while it does not stop or influence negatively the fermentation process. Finally, it is a requirement for large scale microbial decontamination of the process material for controlled microbial hydrolysis and subsequent fermentation. This is surely an energy demanding activity. A plant design that has recently been reported will work by intensifying solar radiations to provide heat for decontaminating the substrate and enabling cellulose substrate to be hydrolyzed as well as fermented to ethanol by thermophilic microorganisms (Chaudhary and Qazi, 2007). The pro-posed plant has technically been designed; its outcome is likely to be reported soon.

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