

Biofuel from *D*-xylose – the Second Most Abundant Sugar

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Anil Lachke is a scientist in the Division of Biochemical Sciences of National Chemical Laboratory, Pune. His major research interests include pentose metabolism in yeasts, biotechnology for biomass utilization and biodeinking for recycling of waste paper. He takes special interest in popularization of science and technology. He likes Indian classical music, yoga and drawing.

In the biosphere we find cellulose and hemicellulose as the major polysaccharides. On acid or enzymatic hydrolysis, *D*-glucose is produced from cellulose and *D*-xylose is produced from xylans as the major sugar in the hydrolysate. Initially it was believed that yeasts do not ferment *D*-xylose to ethanol although many are capable of producing xylitol. Twenty years ago, a few yeasts that could convert *D*-xylose to ethanol were found. Ethanol is viewed as a potential fuel that is available from biomass and hence new methods to generate ethanol from hitherto inaccessible sources are gaining importance. Biotechnology for efficient utilization of lignocellulose wastes as fuels relies on the utilization of both the cellulosic as well as hemicellulosic portions of the biomass. In this article, conversion of xylose into ethanol is discussed.

Henry Ford was once asked about the fate of his automobile business if the petroleum supplies of the world run out. He exclaimed, “*we can get fuel from the sumac by road side or from weeds, sawdust almost anything. There is fuel in every bit of vegetable matter that can be fermented.... And it remains for someone to find out how this fuel can be produced commercially... Better fuel at a cheaper price than we now know.*” The optimism shown by Henry Ford must be appreciated.

Many countries are showing increasing interest in obtaining a large fraction of their energy needs from biomass. More than 25% of the cars in Brazil run on gasohol (*Box 1*) – a mixture of petrol and alcohol. They produce alcohol from molasses. Current scientific opinion seems to favour the view that the world’s fossil fuel resources are steadily diminishing. Alternative sources of energy must therefore be investigated urgently. There is no

Keywords

D-xylose, biofuel.

Box 1. Gasohol – An Alternative Fuel

Alcohol was identified as a possible replacement fuel during 1920's when there was a growing demand for gasoline and potential shortage of oil. Many automobile giants like General Motors and Ford, predicted that alcohol could be a viable fuel for vehicles. A blend of gasoline and alcohol that is referred to as 'gasohol' was tried out in various cars. However, gasohol got a bad name because of excess use or wrong choice of alcohol. Of the two types of alcohols that could be used, ethyl alcohol and methyl alcohol can be used by itself as a fuel but when mixed with gasoline, it is limited to 10% by volume. Methyl alcohol is very corrosive and this limits its use in the mixture.

The heat content of a litre of alcohol is less than gasoline and hence more alcohol is required to achieve the same power levels as that of gasoline. The penalty is decreased fuel economy and lower driving range without installing bigger fuel tanks. Another disadvantage of alcohol as a fuel is its higher volatility. During hot weather driving, the fuel vaporises easily and vapour lock in the engine can occur. This can make the engine run rough or even prevent it from running. An advantage of using alcohol as a fuel is that it mixes easily with water and prevents ice formation in cold weather. It also has a high octane rating than gasoline, which allows engine compression ratios to be increased and ignition timing to be advanced for better performance.

The successes in development of other alternate energy sources may limit the use of alcohol as a fuel, but there has been some work in using alcohol in conjunction with fuel cells to produce power for the future vehicles. Alcohol has been used as a fuel for centuries and it may well be the fuel of the future too. Currently, there is only one major fuel company in Canada, Mohawk, supplying fuel with alcohol in it. Brazil is also supplier and user of gasohol for a number of years.

doubt that in the long run we have to turn to renewable sources of food and energy.

Over 150 billion tonnes of organic substances are photosynthesized annually which consists of three major constituents, namely cellulose, hemicellulose (*Box 2*) and lignin with an average proportion of 4:3:3. The xylan (hemicellulose) content of the hardwood and the softwood lies in the range of 20-25% and 7-12%, respectively.

The incentives for energy recovery from wastes are most attractive in the rural areas of India. Lignocellulose as a raw material for bioconversion has a role in energy production; it can prevent deterioration of the environment, and facilitate waste management (*Figure 2*). Large quantities of lignocellulosic materials in the form of agricultural and forest residues are available in

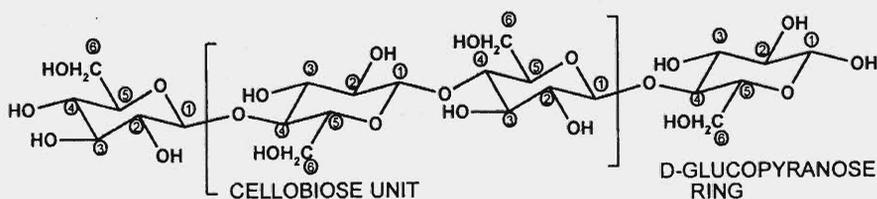
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Box 2. Hemicellulose

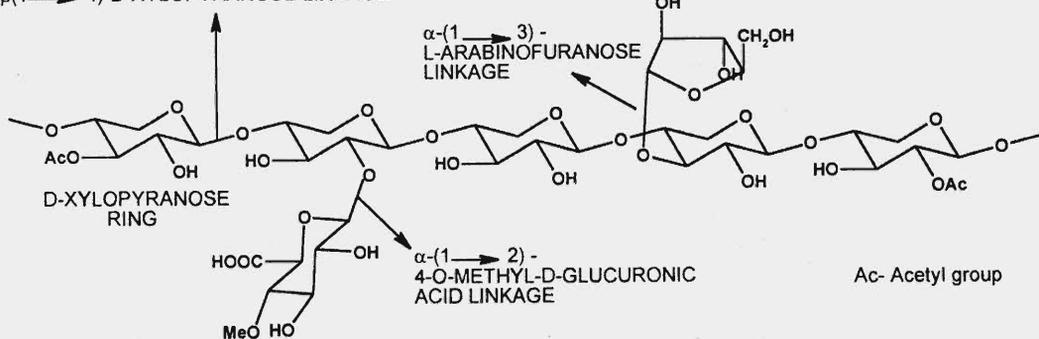
Plants contain about 6×10^{11} tonnes of hemicellulose and about 3×10^{10} tonnes are photosynthesized annually by higher plants to make over one third of their dry weight. The close association of hemicelluloses with cellulose and lignin confer rigidity to the plant cell wall.

The hemicelluloses are composed of a linear as well as branched hetero- and homopolymers of pentosans (*D*-xylose and *L*-arabinose) and hexosans (*D*-glucose, *D*-mannose and *D*-galactose). Unlike cellulose hemicelluloses show structural variability (*Figure 1*).

As compared to cellulose, hemicelluloses are low molecular weight polymers with a degree of polymerization of around 200. Native hemicelluloses like xylan is composed of 85-90% *D*-xylose and a small amount of *L*-arabinose and traces of glucuronic acid. Hemicelluloses have more branches and are less crystalline than cellulose. The glycosidic linkages between the anhydro-*D*-xylose residues in xylan are more susceptible to acid or enzymatic hydrolysis than linkages between anhydro-*D*-glucose residues in cellulose. As a result, pentose sugars can be obtained readily in hydrolysates with sufficient yields from hemicelluloses. Considering the complex structure of hemicelluloses, a diverse set of enzymes is necessary for its hydrolysis to form soluble pentose sugars. These include: Endo-1,4- β -*D*-xylanase, Exo-1,4- β -xylanase, 1,4- β -*D*-xylosidase and α -*L*-arabinofuranosidase.

Figure 1.

β (1 \rightarrow 4)-*D*-XYLOPYRANOSE LINKAGE



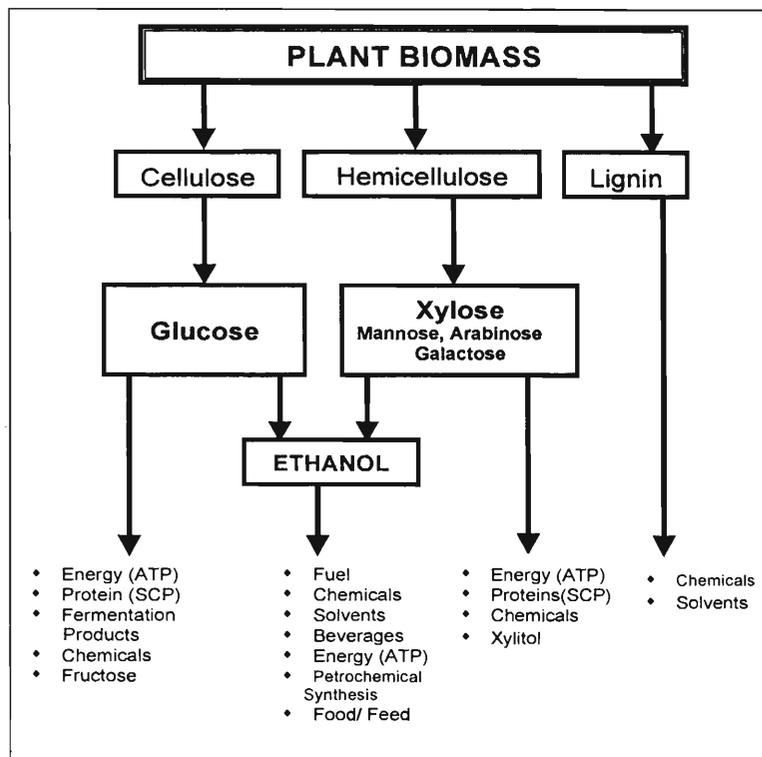


Figure 2.

India. For example, India's production of wheat straw and rice straw is over 200 million tonnes per year. Although straw has low protein content it is rich in fermentable carbohydrates. Also, hemicellulose rich waste materials like corncob or coconut pith or coconut shell are valuable renewable resources (Tables 1 and 2). In addition, cultivation of cereals generates a lot of waste residues which is underutilized.

Hydrolysis of Cellulosics

The ultimate product of cellulose hydrolysis is glucose; a well-known fermentable six-carbon sugar while the hemicellulosic fraction gives *D*-xylose, a five-carbon sugar. Thus, *D*-xylose is the second most abundant sugar in nature and a potential feedstock for generating food and fuel. Cost analysis has indicated that economically viable bioconversion processes require quantitative conversion of cellulosic as well as hemi-cellulosic sugars. Hemicellulosic sugars (*D*-xylose) can be obtained more

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Table 1. Composition of selected ligno-cellulosic materials.

(% Dry weight – subject to variation.)

BIOMASS	CELLULOSE	HEMI-CELLULOSE	LIGNIN
Rice straw	37	24	14
Wheat straw	31	29	18
Bamboo	40	20	20
Subabul	33	20	NA
Mesta wood	35	18	NA

readily with better yield of 80-90% from xylan by acid or enzymatic hydrolysis than *D*-glucose from cellulose. Utilization of *D*-xylose for the commercial production of valuable chemicals like ethanol, acetic acid, 2, 3-butanediol, acetone, isopropanol and *n*-butanol using microorganisms is thus important for enhancing the economic viability of lignocellulose utilization.

For a long time, it was believed that yeast could not ferment *D*-xylose although many strains can ferment *D*-xylulose, the keto isomer of *D*-xylose. In 1914, a famous microbiologist Kluyver in his PhD thesis mentioned that, "...only a small amount of xylose was available. Therefore, I did not routinely include xylose in my fermentation experiments. Although many fungi can assimilate this sugar there are so many reports on their inability to ferment that omission of xylose was not considered to be serious. The small amounts of xylose available was used in a few experiments with selected yeasts strains... all results were negative."

Table 2. Various sugars in agricultural residues.

(% Dry weight subject to variation.)

Residue	XYLOSE	ARABINOSE	GLUCOSE	OTHERS
Corn cobs	65	10	25	-
Wheat straw	58	9	28	3
Flax straw	65	13	1	21
Bagasse	60	15	25	-

Several yeasts like *Candida polymorpha* and *Pichia miso* can aerobically convert *D*-xylose to xylitol as the major product. The conversion efficiency can be as high as 90%. This finding is also encouraging since xylitol a sugar alcohol, is a natural sweet-

ener present in small quantities in a wide variety of fruits and vegetables. Xylitol does not form acid and may be used clinically as a sugar substitute for diabetic patients. Xylitol is frequently used in chewing gums and toothpaste. Several other yeasts are also known to convert *D*-xylose to xylitol but the conversion efficiency is only 50%.

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Production of Ethanol from Xylose by Biological Fermentation

The metabolic steps involved in the fermentation of six-carbon sugars have extensively been studied when compared to five carbon sugars. The initial biochemical steps in *D*-xylose fermentation are the isomerization of *D*-xylose to *D*-xylulose, the corresponding keto isomer (Figure 3). In bacteria, *D*-xylose isomerase produces *D*-xylulose from *D*-xylose. In yeast, two enzymatic steps involving reduction and oxidation carry out the same conversion. The NADPH-linked *D*-xylose reductase converts *D*-xylose to xylitol. In the next step, xylitol is converted to *D*-xylulose by NAD-linked xylitol dehydrogenase.

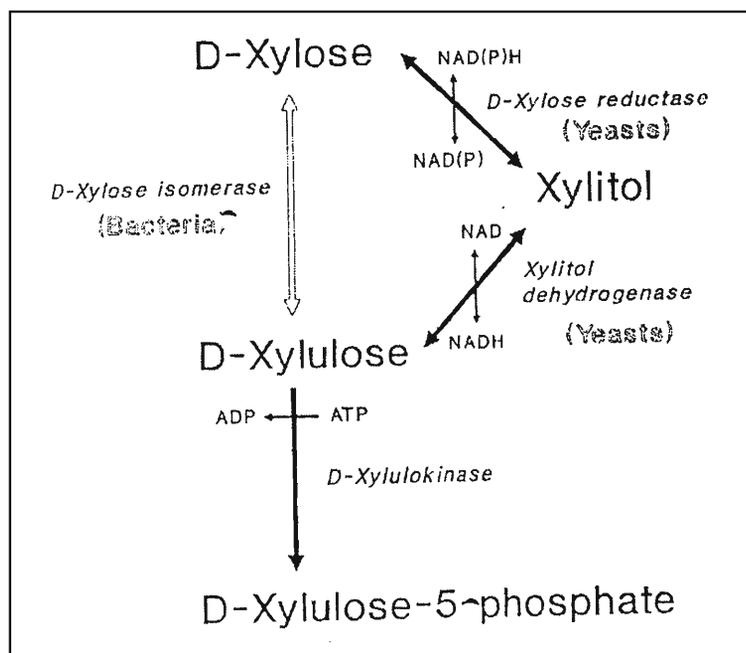


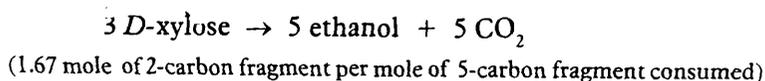
Figure 3.

The main problem in the use of bacteria is attributed to their low ethanol tolerance and the formation of products other than ethanol.

Phosphorylation of *D*-xylose is then catalyzed by *D*-xylose kinase, which is a chief driving reaction in the pathway. Beyond this point, much of the pathway is assumed from biochemical studies with other organisms. In one route, *D*-xylose kinase proceeds by way of epimerase, isomerase, transketolase and transaldolase to form glyceraldehyde-3-phosphate and fructose-6-phosphate. Glyceraldehyde-3-phosphate is an intermediate, both in the pentose phosphate pathway and the Embden Meyerhof Parnas pathway. The requirement for the induction of these enzymes and their proportion, as well as the availability of the necessary cofactors are thought to be the major factors causing the inefficiency of pentose fermentation.

In the last 20 years, yeasts such as *Pachysolen tannophilus*, *Candida shehatae* and *Candida tropicalis* have been identified for ethanol production from *D*-xylose in appreciable yields. Conversion of pentose sugars to ethanol in the absence of oxygen is limited to only a small number of yeasts, although many yeasts can assimilate these sugars aerobically. Some molds such as *Fusarium*, *Rhizopus* and *Mucor* are known to ferment *D*-xylose to ethanol. Many bacteria are able to utilize xylose; especially members of the genera *Clostridium* and *Bacillus* (*B. macerans*) can carry out mixed fermentation in which ethanol is a major end-product. The main problem in the use of bacteria is attributed to their low ethanol tolerance and the formation of products other than ethanol.

The rates and yields of ethanol from *D*-xylose depend upon several process variables such as *D*-xylose concentration, nitrogen source, aeration and agitation. *C. shehatae* is an efficient fermenter of *D*-xylose but it is not able to utilize nitrate as the sole nitrogen source. In case of *Pachysolen tannophilus*, oxygen is required for cell growth but not for ethanol production. *P. tannophilus* yields 0.34 g ethanol per gram of pentose. *D*-xylose is assumed to follow the stoichiometry:



The rate of formation as well as final yields of ethanol from *D*-xylose fermentation is still much lower than commercial glucose fermentation rates. The ethanol production from *D*-xylose is lower than the theoretical yields. On mass basis, 0.51 g alcohol should be obtained from 1 g of *D*-xylose. One of the main reasons for the low yield is the formation of other fermentation products, such as xylitol, acetate, etc. The improvement in the final yield as well as specific rates of ethanol formation has been achieved by strain improvement through mutation studies. The biochemical basis as well as rate limiting steps during *D*-xylose utilization by yeasts are being studied in many laboratories including National Chemical Laboratory in Pune. These studies may facilitate the design of an appropriately controlled fermentation system and it also serves as a basis for obtaining genetically engineered strains.

The fermentation of *D*-xylose is practicable only where it is a by-product of lignocellulose processing.

All the parameters influencing the fermentation process have not been fully evaluated so far. Variables including aeration, pH of the medium, C:N ratio, various additives and respiratory inhibitors are manipulated in order to achieve high ethanol yields from pentose sugars. It is expected that the production of at least 5-6% (w/v) ethanol will be attained. The fermentation of *D*-xylose is practicable only where it is a by-product of lignocellulose processing. Adaptation of yeasts for fermentation using waste streams or hydrolysates is another essential feature necessary for process development.

Efforts Towards Increasing the Ethanol Yield

We have carried out enzymatic saccharification of various agricultural residues of Indian origin. For this purpose, an enzyme preparation containing the cellulase/xylanase enzyme complex produced by *Sclerotium rolfii* was used. The resulting mixture of the 6-carbon and 5-carbon sugars was fermented using *Saccharomyces cerevisiae* and *Candida shehatae*, separately and together. The co-culture system gave improved ethanol yields.

We have also carried out *D*-xylose fermentation in the presence of synthetic aluminosilicates of mineral faujasite structure, zeo-

The genetically engineered yeast produces at least 30% more ethanol from a given amount of plant materials.

lites of X and Y type or a combination thereof. Incorporation of 1% Na-Y or Na-Ca-X (w/v), in the fermentation medium increased the yield of ethanol by 8 to 15%.

Research on the role that microorganisms play in the ethanologenesi s from pentose sugars is still in the developmental stage. US researchers at Purdue University (Indiana) have developed a genetically modified yeast, which can efficiently ferment cellulose to ethanol, a breakthrough which could slash the cost of production of ethanol from renewable feed stocks. The yeast is effective at cofermenting glucose and xylose from lignocellulosic biomass into ethanol. The genetically engineered yeast produces at least 30% more ethanol from a given amount of plant materials. The goal is to make ethanol less expensive than gasoline. It is too early to speculate on the commercial significance of the second most abundant sugar available in nature.

Nevertheless, the hydrocarbons of the future need no longer be fossil fuels to be unearthed and processed, but might well be available continuously from our rooftops or gardens. While we wait for the day when we can simulate controlled fusion that generates the energy of the sun and stars, we might also find new ways of harnessing solar energy. As Robert Frost said.

“ We dance round in a ring and suppose.
But the secret sits in the middle and knows!”

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Suggested Reading

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