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Pentoses and Lignin

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Utilization of Xylose by Bacteria, Yeasts, and Fungi

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Hemicellulosic sugars, especially D-xylose, are relatively abundant in agricultural and forestry residues. Moreover, they can be recovered from the hemicelluloses by acid hydrolysis more readily and in better yields than can D-glucose from cellulose. These factors favor hemicellulosic sugars as a feedstock for production of ethanol and other chemicals. Unfortunately, D-xylose is not so readily utilized as D-glucose for the production of chemicals by microorganisms. The reason may lie in the biochemical pathways used for pentose and hexose metabolism. Different pathways are employed by prokaryotes and eukaryotes in the initial stages of pentose assimilation. Transport and phosphorylation possibly limit the overall rate of D-xylose utilization. The intermediary steps of pentose metabolism are generally similar for both bacteria and fungi, but substantial variations exist. Phosphoketolase is present in some yeasts and bacteria able to use pentoses. Regulation of the oxidative pentose phosphate pathway occurs at D-glucose-6-phosphate dehydrogenase by the intracellular concentration of NADPH. Regulation of nonoxidative pentose metabolism is not well understood. In some

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yeasts and fungi, conversion of D-xylose to ethanol takes place under aerobic or anaerobic conditions with rates and yields generally higher in the former than in the latter. Xylitol and acetic acid are major byproducts of such conversions. Many yeasts are capable of utilizing D-xylose for the production of ethanol. Direct conversion of D-xylose to ethanol is compared with two-stage processes employing yeasts and D-xylose isomerase.

1 Introduction

Hemicellulosic sugars in acid hydrolysates of hardwoods and agricultural residues could become important feedstocks for the production of ethanol and other chemicals by microbial processes. Several factors favor their use: they are relatively abundant in a variety of common lignocellulosic residues; they can be recovered by mild acid hydrolysis; and new microbiological processes are being developed for their conversion.

Within the past 2 years, several significant findings have advanced the prospects for production of ethanol and other chemicals from D-xylose. First, yeasts, which were previously considered unable to ferment[1] 5-carbon sugars, have now been shown

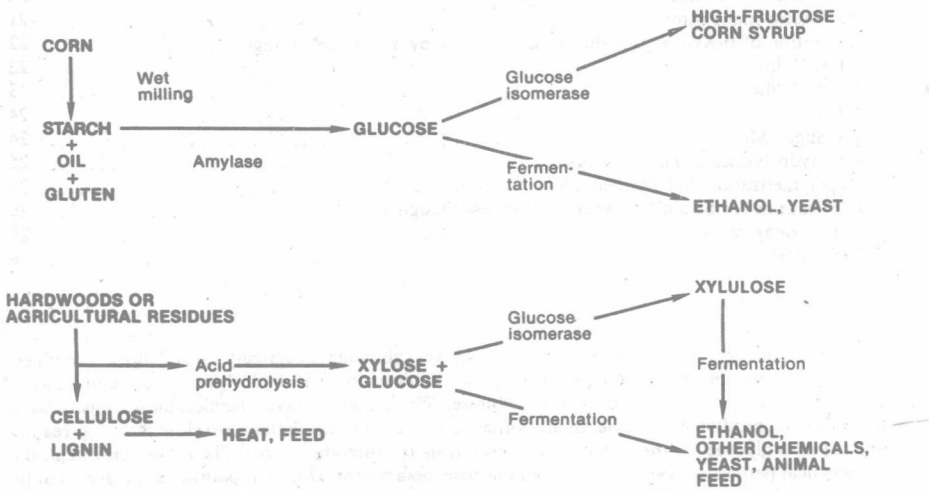

Fig. 1. Comparison of ethanol production from grain and lignocellulosic residues. (M 151 671). For definition see footnote

[1] The term "fermentation" and its various derivatives is used herein to refer to dissimilatory metabolic processes through which an organic substrate is converted into oxidized and reduced products without a net overall change in the oxidation state.

to utilize the pentulose D-xylulose under anaerobic conditions [1-5]. Since D-xylulose can be formed from D-xylose through the action of glucose isomerase (actually xylose isomerase) [6,7], processes have been developed employing two-stage isomerization and fermentation [8-12]. Second, several yeasts, particularly those belonging to the genera *Pachysolen* [13-15] and *Candida* [16-18], have been shown to convert D-xylose to ethanol under aerobic and anaerobic conditions. Besides these recent findings, it is known that certain fungi, particularly *Fusarium lini* [19-23], are capable of converting D-xylose to ethanol; and various bacteria can form several potentially useful products such as ethanol, acetic acid, 2,3-butanediol, acetone, isopropanol, and n-butanol from D-xylose [24-29]. Finally, unlike the fermentation of sugar from grains, utilization of pentoses derived from forestry and agricultural residues for the production of chemicals does not decrease food supplies; indeed, by virtue of the production of microbial biomass and unutilized sugars, such processes can supplement animal feed resources (Fig. 1).

This review attempts to examine recent microbiological findings in relation to the previous understanding of D-glucose fermentation and pentose metabolism. It briefly examines the availability of hemicellulosic sugars — particularly D-xylose — in lignocellulosic residues, reviews aspects of pentose metabolism and metabolic regulation of fermentative processes, and discusses some recent research progress on aerobic and anaerobic conversions of D-xylose to ethanol by yeasts and bacteria. The 2,3-butanediol fermentation and the butanol/acetone/ethanol fermentations are reviewed in other chapters of this volume.

1.1 Distribution of Pentoses in Lignocellulosic Residues

Hemicelluloses are widely distributed, major components of lignocellulosic materials comprised of neutral sugars, uronic acids, and acetal groups, all present as their respective anhydrides (e.g., the anhydride of D-xylose is xylan). As the anhydrides, hemicellulosic sugars average about 26% of the dry weight of hardwoods,[2] and 22% of softwoods and about 25% of several major agricultural residues. Pectin, ash, and protein account for variable fractions in lignocellulosic materials, whereas cellulose (anhydro D-glucose) and lignin make up the balance (Table 1).

The xylan and arabinan contents of hemicelluloses vary with the plant species. The xylan content of hardwoods is generally much higher than that of softwoods, ranging between 11% and 25% in the former and between 3% and 8% in the latter [30-32]. Hemicelluloses in hardwoods contain appreciable amounts of D-xylose, D-mannose, acetyl, and uronic acid. The acetyl content ranges between 3% and 4.5% in hardwoods and between 1% and 1.5% in softwoods; uronic acid (as the anhydride) ranges between 3% and 5% in both hardwoods and softwoods [30]. In conifers, the predominant hemicellulosic sugar is D-mannose, which, as mannan, averages about 11% of the total dry weight [32]. Whereas the xylan content of softwoods is lower than in hardwoods, the lignin content is higher. The predominant hemicellulosic sugar of agricultural residues is D-xylose. The xylan content of corn residues varies

[2] The word "hardwoods" refers to broad-leafed trees (angiosperms) and has nothing to do with the hardness of the woods. Similarly, "softwoods" refers to coniferous trees (gymnosperms).

from about 17% in the leaves and stalks to 31% in the cobs, but, on the average, it comprises about 24% of the total dry weight of corn stover (L. H. Krull, personal communication). The chemistry of the hemicelluloses of grasses has been reviewed recently [35].

Table 1. Proximate composition of various biomass resources

	% of total dry weight									
	Glucan	Galactan	Mannan	Arabinan	Xylan	Hemicellulosic sugars[a]	Hemicellulose[b]	Cellulose[c]	Lignin[d]	Ref.
Hardwoods	50	0.8	2.5	0.5	17.4	26.2	34	45	21	30–32)
Softwoods	46	1.4	11.2	1.0	5.7	22.3	28	43	29	32–35)
Wheat straw	35	0.7	0.4	4.4	19	28.5	—	31	14	33–34)
Corn stalks	36.5	1.1	0.6	2.1	17.2	27.5	—	30	—	33–34)
Soybean residue	38	1.8	2.4	1.0	12.5	18.7	—	37	—	33–34)

[a] Reported as anhydrides;
[b] Includes acetyl- and uronic-acid residues;
[c] Residual glucan following acid prehydrolysis;
[d] Analyzed as Klason lignin (acid-insoluble)

Overall, it would appear that the high hexose (D-glucose plus D-mannose) content of softwoods would favor their utilization as fermentation feedstocks. Presently, however, most softwood residues find their way into pulping operations because of the favorable fiber characteristics of conifer species. In contrast, hardwood residues have much less value for paper production and are generally burned for the generation

Table 2. Estimated and projected total agricultural residues in the United States [36, 37]

Material	Quantity	
	1980	2000
	10^6 ODT	
Corn residue	100	142
Wheat straw	101	87
Soybean residue	98	159
Other grains	57	74
Other agricultural products	28	35
Totals	385	497

of process heat. Most wood residues and agricultural residues are not generally collected at the time of harvest. Branches, leaves, tops, and roots of trees are left in the forest when the merchantable bole is taken for pulp or lumber production; only 50 to 75% of the tree is removed during harvest [39-40]. More residues are generated during milling and pulping operations. A certain proportion of agricultural residues (about 1 ton per acre) are left in the soil to maintain tilth and prevent erosion [33]. A notable exception is sugarcane bagasse. In this instance, the residues are recovered at the sugar mill where they are burned to provide heat for sugar processing. Such collection greatly facilitates economic utilization. Wood mill residues hold a similar advantage.

Of all biomass resources in the United States, low-grade hardwoods and agricultural residues are the two largest available components (Tables 2 and 3). Each possesses

Table 3. United States forest biomass

Category	Quantity	
	Total (Ref.)	Available (Ref.)
	10^6 ODT a^{-1}	
Harvest sites:		
Above ground	160 [38]	110 [41]
Stumps and roots	50 [39]	
Residues:		
Mills (wood)	65 [39]	12 [39,41]
(bark)	18 [39]	8 [39]
Urban tree removals	70 [38]	35[a]
Land clearing	20 [38]	10[a]
Commercial forest lands:		
Surplus growth	270 [40]	150[a]
Annual mortality	95 [38]	50[a]
Noncommercial forest lands:		
Reserved	25 [41]	0
Unproductive	27 [41]	0
Totals	800	375

[a] Author's estimate

characteristics that favor its utilization. Low-grade hardwoods are abundant in the southeastern United States and, aside from direct combustion, have few commercial uses. They can be harvested on a year-round basis using presently available technology. Some hemicellulosic sugars are available as a byproduct of hardboard and insulation board manufacture; others are available as a byproduct formed during the manufacture of sulfite and dissolving pulps.

Significant quantities of wood residues are combusted for the production of steam [42]; wood and bagasse supplied $4 \sim 6 \times 10^{14}$ kJ to the United States' energy budget in 1980. Even though the hemicellulose comprises up to 30% by weight of these materials, it has only about two-thirds of the heat value of the lignin. Hence

it is possible to remove the hemicellulosic sugars for use as a fermentation feedstock and combust the cellulose and lignin residues with relatively little loss of the energy value from the original feedstock.

1.2 Recovery of Hemicellulosic Sugars

Unlike cellulose, which is impermeable even to water, hemicellulose has a relatively open structure. This molecular architecture facilitates diffusion of acid into the polymer and speeds hydrolysis. Moreover, hemicellulose facilitates its own hydrolysis: Acetyl groups are readily hydrolyzed off, and the resulting acetic acid catalyzes the partial depolymerization of the hemicellulose.

In general, hemicellulosic sugars can be recovered with milder treatment and in better yield than can glucose from cellulose [43]. Research at the Forest Products Laboratory in Madison, Wisconsin, has shown that more than 80% of the D-xylose can be recovered from southern red oak (*Quercus falcata*) wood chips through dilute sulfuric acid hydrolysis; in contrast, it is unlikely that more than 50% of the total D-glucose can be recovered from the residual cellulose in a second-stage acid hydrolysis, which must be carried out at higher temperature (Fig. 2). The significance of this finding is that about half of the sugars produced from wood by a two-stage acid hydrolysis process are from the hemicellulose.

In the case of corn residues the situation is even more extreme. Cellulose and hemicellulose each make up approximately 30% of the total dry weight of the material. Through dilute (0.8%–1.2%) sulfuric acid hydrolysis carried out at relatively

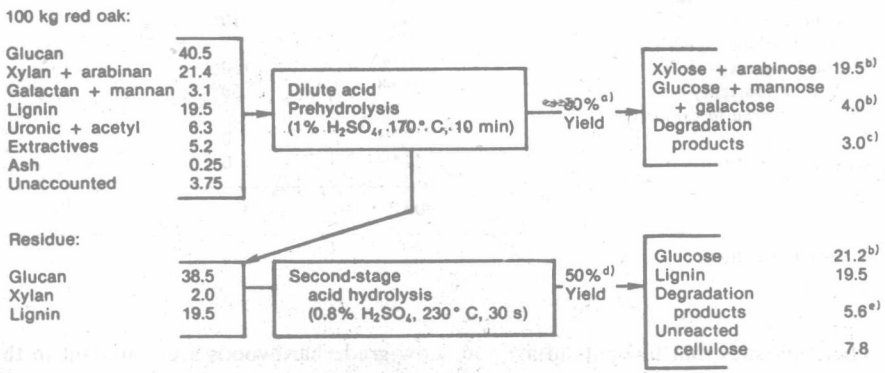

Fig. 2. Dilute acid hydrolysis of southern red oak (*Quercus falcata*). Data are taken from pilot- and bench-scale experiments at the Forest Products Laboratory, Madison, Wis.

[a] Prehydrolysis yield is based on the amount of D-xylose recovered versus the potential amount present as the anhydride polymer, xylan, in the original feedstock.

[b] Products are expressed as free sugars.

[c] Degradation products are mainly furfural.

[d] Second-stage acid hydrolysis yield is based on the amount of D-glucose recovered versus the potential amount in the prehydrolysis residue.

[e] Degradation products are mainly hydroxymethyl furfural. (M 151672)

mild temperature (100 C), more than 90% of the hemicellulosic sugars can be recovered by pressing and washing after 30 min [43]. Yields of D-glucose from the residual cellulose in a second, necessarily more drastic, hydrolysis are appreciably lower.

Two-stage dilute acid hydrolysis has been developed to enhance the recovery of pentose sugars [44], and the partial acid hydrolysis that releases the pentoses has been found to be favorable as a pretreatment for subsequent enzymatic saccharification of the residual cellulose [45]. One of the principal disadvantages of acid hydrolysis is that, even under relatively mild conditions, appreciable amounts of furfural and hydroxymethylfurfural are formed from D-xylose and D-glucose. These dehydration products are generally toxic to yeasts [46]. However, the concentration of furfural decreases during fermentation of glucose by *Saccharomyces* [47], indicating that detoxification by the organism is possible.

While it is conceivable that economical enzymatic or chemical processes might be developed to hydrolyze the cellulosic residue in the second stage, utilization of this residue for sugar production is not always essential for the economic practicability of a first-stage hydrolysis. As mentioned above, dilute sulfuric acid hydrolysis to remove hemicellulosic sugars would still leave a significant fuel component. It is also possible that acid-prehydrolyzed wood might be incorporated into cellulosic products.

For the purposes of this review, it is sufficient to note that pentoses are presently available as byproducts of industrial processes such as fiberboard manufacture and sulfite pulping, and that supplies of these sugars could be significantly increased. Conversion of pentoses to ethanol and other chemicals can be accomplished through microbial processes. To better understand the rate-limiting step(s) in this conversion, the biochemistry of D-xylose utilization is treated in the following section.

2 D-Xylose Metabolism

The biochemical mechanisms of D-xylose metabolism are quite different from those for D-glucose. Whereas D-glucose is metabolized by the Embden-Meyerhoff-Parnas pathway, D-xylose metabolism proceeds by way of the pentose phosphate pathway (PPP). Following transport into the cell, D-xylose is either isomerized or reduced, then reoxidized to form D-xylulose. This sugar is then phosphorylated, isomerized, and rearranged to form a metabolic pool of phosphorylated 3-, 4-, 5-, 6-, and 7-carbon sugars at equilibrium within the cell. The PPP interacts with the Embden-Meyerhoff-Parnas pathway and other parts of intermediary metabolism. D-Glucose can enter the PPP through either oxidative or nonoxidative reactions. Intermediates can exit the PPP through the formation of nucleic acids, aromatic amino acids, lipids, and other metabolic end products.

The objective of this section is to examine the principal steps of D-xylose metabolism in prokaryotes and eukaryotes with the aim of elucidating those reactions that might limit the overall rate of D-xylose utilization. More general aspects of pentose and pentitol metabolism have been reviewed elsewhere [48,49].

2.1 Transport

Transport across the cell membrane is the first step in the metabolism of D-xylose or any other nutrient; as in the cases of D-glucose, it can limit the overall rate of utilization [50]. Sugar transport can occur by at least three mechanisms: passive (or physical) diffusion, facilitated diffusion, or active transport. Active transport can be further classified into chemiosmotic, direct energization, and group translocation mechanisms [51]. These processes are not mutually exclusive and two or more may function in a single organism.

Passive diffusion is the simplest and slowest process. It requires a substantial concentration gradient and progresses by diffusion of the solute across the plasma membrane. Only small, lipid-soluble molecules such as glycerol or ethanol are transported by this mechanism to any appreciable extent. The rate of transport for most sugars by passive diffusion is probably negligible, but the transport of acyclic polyols (erythritol, xylitol, ribitol, D-arabinitol, mannitol, sorbitol, and galactitol) has been reported to occur by such a mechanism in *Saccharomyces cerevisiae*. As is characteristic of passive diffusion, the process is independent of pH, uncoupling agents, and uranyl ions, and the initial rate of transport increases with the solute concentration [52].

Facilitated diffusion, like passive diffusion, requires no metabolic energy, employs a concentration gradient, and attains an equilibrium of sugar concentrations inside and outside the cell. Unlike passive diffusion, however, it is mediated by a carrier protein and hence exhibits specificity toward the substrate molecule. Although the rates of both passive and facilitated diffusion increase with increasing sugar concentrations, facilitated diffusion is distinguished by the fact that the system becomes saturated at some concentration, resulting in a maximum rate of transport. Other characteristics of facilitated diffusion are that similar sugars competitively inhibit transport, that uranyl ions specifically inhibit transport and that facilitated diffusion exhibits counter transport. Facilitated diffusion is used by many types of eukaryotic cells for transport of sugars.

Active transport mechanisms, like facilitated diffusion, are mediated by carrier proteins, and hence exhibit the properties of saturability, substrate specificity, and specific inhibition, but the processes require metabolic energy and can transport sugars against a concentration gradient. Metabolic energy can be provided by establishing a membrane potential as in the chemiosmotic mechanism, by the hydrolysis of adenosine 5′-triphosphate (ATP) as in the direct energization mechanism, or by the transfer of phosphate from phospho*enol*pyruvate (PEP) to the sugar substrate as in the group translocation mechanism.

2.1.1 Bacteria

Bacteria generally employ active transport mechanisms for the uptake of sugars and other nutrients. Transport of D-xylose across the cell membrane of *Escherichia coli* is linked to the movement of protons, as evidenced by a rise in pH in the extracellular medium upon addition of D-xylose to an energy-depleted suspension of cells [53]. This evidence supports the chemiosmotic symport mechanism proposed by Mitchell [54] in which protons and D-xylose are transported together across the

cell membrane. In *E. coli*, accumulation of [^{14}C] D-xylose is inhibited by various uncoupling agents such as tetrachlorosalicylamide (TCS), 2,4-dinitrophenol (DNP) and carbonylcyanide *m*-chlorophenylhydrazone (CCCP), which destroy the proton gradient. Neither sodium fluoride, which prevents PEP formation by the enolase reaction, nor arsenate, which drastically reduces the intracellular concentration of ATP, inhibits D-xylose transport, implying that it is energized by a chemiosmotic mechanism and not by directly energized or PEP-phosphotransferase mechanisms [53]. The PEP-phosphotransferase system is not involved in the uptake of D-xylose and xylitol by *Staphylococcus xylosus* and *Staphylococcus saprophyticus* either [55], but pentitols (ribitol and xylitol) are transported by a substrate-specific PEP-phosphotransferase system in *Lactobacillus casei* [56,57]. In contrast to D-xylose transport in which an energized membrane state is employed, the D-ribose transport system of *E. coli* is apparently coupled to the hydrolysis of ATP [58,59].

The D-xylose transport of *E. coli* is relatively specific as indicated by the fact that L-arabinose, D-ribose, D-lyxose, xylitol, and D-fucose fail to promote pH changes in D-xylose-induced cells [53]. The specificity of D-xylose transport is somewhat lower in *Salmonella typhimurium*. In this organism, L-arabinose accumulates in D-xylose-induced cells and D-xylose accumulates in L-arabinose-induced cells. Xylitol and L-arabinose compete against D-xylose uptake, but D-arabinose, D-lyxose, and L-lyxose do not [60].

2.1.2 Yeasts and Fungi

In yeasts, D-xylose transport can occur by either facilitated diffusion or active processes. In *Saccharomyces cerevisiae*, D-xylose is nonmetabolizable and it appears to be transported by a facilitated diffusion process [61]. Transport of D-xylose is apparently related to D-glucose transport because, in the presence of D-glucose, the influx of D-xylose or D-arabinose is more rapid under anaerobic than under aerobic conditions. In the absence of D-glucose, the rate of entry of these two sugars is identical under either condition [61].

Transport of D-xylose by eukaryotes has been most extensively studied in *Rhodotorula* where it occurs by an active process [62–70]. Under aerobic conditions, D-xylose is accumulated against a concentration gradient of 1,000 with an apparent K_m of 2 mM. Under anaerobic conditions, transport is blocked, indicating that respiration is essential for transport in these obligately aerobic yeasts [61]. Alcorn and Griffin [62] have reported the presence of at least two carriers for D-xylose in the membrane of *Rhodotorula gracilis* (= *R. glutinis*). The carrier exhibiting a low K_m (high affinity) is repressed in rapidly growing cells and derepressed by starvation. Several hexoses competitively inhibit D-xylose transport, but the low K_m carrier exhibits greater specificity [62]. There is a single common system for D-xylose and D-galactose, but another distinct system for D-fructose. The transport of D-glucose has a special position in that D-glucose blocks all other systems observed, although D-glucose itself is transported by one of the systems [64]. D-xylose competitively inhibits the transport of D-glucosamine, indicating a single system for these two sugars [69]. Transport of nonmetabolizable monosaccharides by *R. gracilis* is partially inhibited by raising the temperature and greatly inhibited by uncouplers of oxidative phosphorylation [63]. Rotenone, antimycin A, potassium cyanide, sodium azide,

oligomycin, dicyclohexylcarbodiimide (DCCD), DNP and CCCP all inhibit active transport in yeasts [68].

The uptake of monosaccharides and polyols by *R. gracilis* is accompanied by proton transport. Addition of D-xylose, D-glucose, 3-0-methyl-D-glucose or D-galactose (but not melibiose) to aqueous suspensions of *R. gracilis* causes a rapid increase in the extracellular pH. Similar but slower responses can be demonstrated with the addition of xylitol or ribitol to induced, but not uninduced, cells. The membrane potential, essential for proton mediated transport, is demonstrable by the intracellular accumulation of liquid-soluble cations and is strongly pH-dependent [67]. Höfer and Misra [65] have demonstrated that the intracellular steady-state concentration of D-xylose changes with the pH of the external medium and that the alteration is reversible. This reversibility can be explained by assuming that the carrier affinity is reversibly changed with external pH. At pH 8.5, the gradient vanishes and no D-xylose transport is demonstrable. The stoichiometry of H^+ and D-xylose uptake, determined under various physiological conditions, is one H^+ per sugar molecule taken up. The half-saturation constants of H^+ and sugar uptake are also similar. These data strongly support the hypothesis that D-xylose transport in *R. gracilis* is energized by an electrochemical gradient of H^+ across the plasma membrane and functions by the H^+ symport mechanism [65].

Active transport, at least for hexoses, also seems to be present in *Candida parapsilosis* [71] and *Candida guilliermondii* [72]. Uptake of D-xylose has also been studied in *Penicillium* and *Fusarium* species [73]. Other aspects of sugar transport in yeasts have been recently reviewed [51].

2.2 Conversion of D-Xylose to D-Xylulose-5-Phosphate

Once inside the cell, D-xylose is first converted to D-xylulose and then phosphorylated. A basic difference seems to exist between prokaryotes and eukaryotes in the initial metabolism: Bacteria generally employ an isomerase to convert D-xylose to D-xylulose [6,7,25], whereas yeasts and fungi carry out the same conversion through a two-step reduction and oxidation [74] (Fig. 3). A few exceptions to this generalization have, however, been reported [75,76]. Conversion of D-xylose to D-xylulose is apparently a critical step in yeasts. It has long been recognized that roughly half of all yeasts are capable of assimilating D-xylose under aerobic conditions [77], but, until recently, none were known to utilize D-xylose anaerobically. In contrast, the ketoisomer, D-xylulose, is used anaerobically by many yeasts [1–4,10], despite the fact that it is not found extracellularly in nature. The relative ease with which D-xylulose

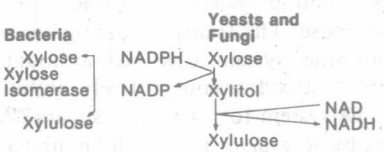

Fig. 3. Yeasts and bacteria generally employ different pathways for D-xylose assimilation

is utilized by yeasts suggests that the reductive/oxidative conversion of D-xylose to D-xylulose is regulated or rate-limiting in these organisms. The fact that many yeasts utilize D-xylose under aerobic conditions further suggests that this regulation is related to aerobic metabolism. Numerous bacteria employing D-xylose isomerase exhibit diverse patterns of D-xylose utilization under both aerobic and anaerobic conditions and yield a variety of end products [11,25–29]. The utilization of D-xylulose by bacteria has not been studied except in the case of *Zymomonas mobilis*, which did not use D-xylulose [10].

2.2.1 Isomerization

D-xylose isomerase, which catalyzes the reversible isomerization of D-xylose to D-xylulose or of D-glucose to D-fructose, has been well studied [78–84] and recently reviewed [6,7]. Comments herein will be limited to physiological aspects of importance to pentose utilization and to recent applications in the fermentation of D-xylose (via D-xylulose) by yeasts.

In the presence of D-xylose isomerase, at equilibrium, about 16% of the sugar is isomerized to D-xylulose [79]. This value increases with increasing temperature [12] and the presence of borate [12,79]. Maximum conversion (80%) of 1.0 M D-xylose to D-xylulose is observed in the presence of 0.2 M sodium tetraborate. Temperature and pH have no significant effect on the equilibrium value in the presence of this compound [12]. Temperature and pH optima for D-xylose isomerase depend on its source, and range between 50 and 90 C and pH 6.0 and 9.5, respectively [7]. Some D-xylose isomerases require cobalt for activity; almost all require Mg^{++}. Stability and half-life in commercial preparations depend greatly on the manner of immobilization [6,7,82]. Both D-xylose isomerase and D-xylulokinase are specifically induced by D-xylose in *Klebsiella pneumoniae* (= *Aerobacter aerogenes*) [85] and in other bacteria [7,55,60]. In *Staphylococcus*, utilization of D-xylose is inhibited by the presence of xylitol, due to the inhibition of D-xylose isomerase [55].

In *Klebsiella pneumoniae*, aldoses are isomerized and pentitols are dehydrogenated to form the corresponding pentuloses (Fig. 4). D-xylulose is the product of D-xylose, D-arabitol, D-lyxose, and xylitol. Other pentoses and pentitols form D- or L-ribulose or L-xylulose. These sugars are phosphorylated by kinases and the products D-ribose-5-phosphate, D- or L-ribulose-5-phosphate and L-xylulose-5-phosphate converge on D-xylulose-5-phosphate via isomerase and epimerases [48].

The presence of D-xylose isomerase has been reported in *Candida utilis* [75] and *Rhodotorula gracilis* [76]. Horitsu, Sasaki, and Tomoyeda [86] have also identified and partially purified an L-arabinose isomerase from *C. utilis*. The D-xylose isomerase of *C. utilis* was produced adaptatively when the organism was grown in a medium containing D-xylose. After partial purification, the enzyme was found to have a pH optimum of 6.5, a temperature optimum of 70 C, and a requirement for divalent cations, particularly Mn^{++}, Co^{++}, and Mg^{++} [75]. The D-xylose isomerase could be separated from the L-arabinose isomerase of *C. utilis* by ion-exchange chromatography on DEAE Sephadex A-50. The L-arabinose isomerase showed a pH optimum of 7.0, a temperature optimum of 60 C and stimulation of activity by Mn^{++} [86]. In the case of *R. gracilis*, Höfer, Betz, and Kotyk [76] concluded that induction of D-xylose isomerase was necessary for D-xylose metabolism. The assay for enzyme

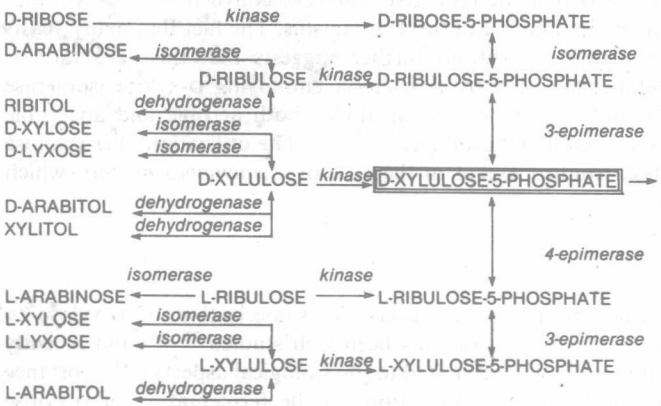

Fig. 4. Pathway of pentose and pentitol conversion to D-xylulose-5-phosphate in *Klebsiella pneumoniae* [48]. (M 151 527)

activity was based on the disappearance of D-xylose from cell-free extracts of induced cells. No NADH or NADPH was added to the reaction mixture, and xylitol was not utilized as a carbon source even though it was taken up by the cells. These findings supported their conclusion that D-xylose isomerase and not D-xylose reductase was necessary for D-xylose assimilation in *R. gracilis*. All other investigations have indicated that these and other yeasts and filamentous fungi employ the reductive/oxidative route for D-xylulose formation [74]; consequently, these reports of D-xylose isomerase in yeasts require further confirmation.

2.2.2 Reduction, Oxidation, and Polyol Formation

The reduction of D-xylose to xylitol is catalyzed by alditol: NADP 1-oxidoreductase, also known as aldose reductase [87–92]. The purified enzyme is active with a wide variety of sugars but is most active on those in which the hydroxyl group at carbon-2 is in the D-glycero configuration [89]. An enzyme with preference for the alternative configuration has also been described [93]. During purification, multiple aldose reductases are found [92]; sometimes with various different substrate and coenzyme specificities [94]. In virtually every instance, however, the enzyme catalyzing reduction of D-xylose to xylitol in yeasts and fungi has a specificity for NADPH [74,88–96]. The reoxidation of xylitol to D-xylulose is catalyzed by xylitol: NAD 2-oxidoreductase [74,88,91,97,98]. The reaction is readily reversible, and NADH oxidation occurs with a number of ketoses including D-xylulose, D-fructose, and D-ribulose. The purified enzyme shows no activity with NADP(H) [97].

Evidence for the existence of the reductive/oxidative pathway in fungi and yeasts has been presented for four species of *Aspergillus*, and three species of *Penicillium*, and for *Rhizopus nigricans*, *Gliocladium roseum*, *Byssochlamys fulva*, *Myrothecium verrucaria*, *Neurospora crassa* [74], *Sclerotinia sclerotiorum* [91], *Oospora lactis* [88], *Cephalosporium chrysogenus* [99], *Candida albicans* [100], *Candida pulcher-*