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Hydrolysis by Commercial Enzyme Mixtures of AFEX-Treated Corn Fiber and Isolated Xylans

ROBERT B. HESPELL,*,[1] PATRICIA J. O'BRYAN,[1] MOHAMMED MONIRUZZAMAN,[2] AND RODNEY J. BOTHAST[1]

*[1]Fermentation Biochemistry Research Unit, National Center for Agricultural Utilization Research, **USDA, Agricultural Research Service, Peoria, IL 61604, USA, [2]Department of Chemical Engineering, Texas A&M University, College Station, TX*

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ABSTRACT

Corn fiber is a coproduct produced during the corn wet-milling process and is similar to other high hemicellulose/cellulose-containing biomass such as grasses, straws, or bagasse, all of which represent potential fermentation feedstock for conversion into biofuels or other products. Corn fiber was subjected to ammonia-explosion (AFEX) treatment to increase degradability and then enzymatically digested with a combined mixture of commercial amylase, xylanase, and cellulase enzyme preparations. Whereas the starch and cellulose components were converted solely to glucose, oligosaccharides represented 30–40% of the xylan degradation products. This enzyme mixture also produced substantial oligosaccharides with xylans purified from corn fiber, corn germ, beechwood, oatspelt, or wheat germ. Commercial xylan-degrading enzyme preparations containing xylanase, xylosidase, and arabinosidase activities were then used alone or in varying combinations to attempt to maximize degradation of these isolated xylans of differing chemical compositions. The results showed that oatspelt and beechwood xylans were degraded most extensively (40–60%) with substantial amounts of xylose, xylobiose, and xylotriose as products depending on the enzyme combination used. Corn fiber and wheat germ xylans, which contain

*Author to whom all correspondence and reprint requests shall be addressed.

**Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

large amounts of arabinose and uronic acid sidechains, were poorly degraded and only small amounts of arabinose and xylose and large amounts of pentamer or longer oligosaccharides were produced by enzymatic degradation. The data suggest that whereas enzymatic digestion of biomass hemicellulose does not produce toxic products, the process is not effective in producing a suitable fermentable substrate stream because of the low levels of monosaccharides and high levels of oligosaccharides produced.

Index Entries: Xylans; xylanases; corn fiber; biomass; hydrolysis; biofuels ethanol; AFEX-treatment.

INTRODUCTION

In the United States, annual fuel ethanol production has increased to about 1.3 billion gallons. Currently, almost all fuel ethanol is made from corn grain as the starting feedstock. Whereas the starch fraction is converted to ethanol, the other grain fractions (e.g., protein, germ, hull/fiber) are used to produce oil and a variety of animal feed (corn gluten, corn gluten meal, distillers grains) products. Income from the sales of these products are used to offset the fermentation costs of fuel ethanol production. Consequently, corn grain currently represents one of the least expensive feedstocks for fuel ethanol fermentation. However, future uses for increased quantities of corn product feeds are questionable.

At present, the maximum practical yield of ethanol from corn grain is about 2.7 gallons/bushel, but potentially could be increased by 0.3 gallons/bushel if the corn fiber were fermented *(1)*. Corn fiber is a cellulosic biomass such as grain straws or one of a variety of agricultural residues. Over 50 yr ago, Dunning and Lathrop *(2)* recognized the potential of agricultural residues such as corncobs, oat hulls, flax shives, and so on, as large sources of potentially fermentable sugars. In their classic paper, these authors presented clearcut methods for low temperature (100–120°C), dilute sulfuric acid extraction, and hydrolysis of the hemicellulose components of biomass. Using ground corncobs as a model feedstock, 95% of the hemicellulose was removed from the biomass as product stream consisting of about 86% xylose, 9% furfural, and 0.8% glucose. In the intervening years, these results have been repeated many times by other researchers by applying the same or slightly modified dilute acid methods of Dunning and Lathrop to a wide variety of biomass sources. Recent results have shown that dilute acid hydrolysates can be made from corn fiber, corn stover, or bagasse and used in fermentations to produce ethanol *(3–5)*.

The main advantages of the dilute acid treatment of biomass include the production of a soluble pentose stream that can be physically separated from the particulate residue. Secondly, a substantially increased rate of enzymatic hydrolysis of the residual cellulose portion results, presumably because of, in

large part, to the acid-induced increased fiber porosity *(6)*. On the other hand, acid treatment produces furfural that is toxic to many micro-organisms and the residual acid must be neutralized. Dunning and Lathrop *(2)* produced a clean fermentable 15% xylose stream by removing the furfural by vacuum distillation and removing the acid as a filterable calcium sulfate cake by addition of lime. However, today these procedures are not commercially practical as a result of considerable costs added to the processing in ethanol fermentations. To lower these costs, various modified procedures have been tried. For example, studies with ground wood, use of very dilute acid (0.7%) and higher temperatures (140–170°C) produces a 92% extraction of the hemicellulose with only 2% furfural, but 44% of the solubilized hemicellulose is still in the form of unfermentable oligomeric sugars *(7)*.

A number of alternative methods have been proposed and used to overcome the problems of dilute acid treatment. One of these is steam explosion, which is very efficient in fractionating wood biomass, but results in large losses of hemicellulose sugars *(8)*. A more gentle procedure is low temperature (30–80°C), ammonia fiber explosion treatment (AFEX), which effectively disrupts nonwoody biomass such as Coastal bermudagrass *(9)*, but not softwoods. Recently, the AFEX process combined with enzyme treatments has been used with corn fiber to produce sugar streams *(10)*. In this paper, it is reported in detail on the enzymatic production of sugar streams from AFEX-treated corn fiber as well as isolated corn fiber xylan and other xylans. Unlike digestion of cellulose by commercial enzyme mixtures that produces primarily monosaccharides corn fiber or isolated xylans are partially digested to varying degrees to yield some monomeric sugars and mixtures of oligomeric sugars.

MATERIALS AND METHODS

Materials

Corn fiber samples were obtained from Pekin Energy Company (Pekin, IL) and frozen until subjected to AFEX treatment or used for isolation of xylans. Oatspelt xylan was obtained from Sigma Chemical Company (St. Louis, MO), beechwood xylan from Lenzing Aktiengesellschaft (Lenzing, Austria), and wheat germ xylan from Megazyme (Sidney, Australia). Corn fiber xylan was prepared by lime extraction of corn fiber using the methods of Rutenberg and Herbst (USA Patent #2,801,955). Various commercial enzyme mixtures were obtained from different manufacturers: amylase/Spezyme AA20, glucoamylase/Spezyme GA300, and xylanase/Multifect PL from Genecor, Inc., Cedar Rapids, IA; cellulase/Cytolase 300 from Genencor, Inc., San Francisco, CA; xylanase/Multifect xylanase from Genencor, Finland; cellobiase/Novozyme 188 from Novo Labs, Wilton, CT; and xylanase/Viscozyme L from Novo Nordisk Bioindust., Danbury, CT.

Methods

AFEX-treated corn fiber was prepared using previously described methods *(9,10)*. For enzymatic digestion, air dried AFEX-treated corn fiber was suspended as 5 or 10% w/v mixtures in 20 m*M* citrate phosphate (pH 4.8) and incubated for up to 72 h at 50°C with several commercial enzyme preparations added at various levels (see Table 1). Isolated xylans were digested in a similar manner with the same enzyme mixture or with xylanase enzymes only, but the incubations were for 20 h at 37°C with four times the enzyme levels. For all digestion mixtures no pH adjustment was made during the incubations as little or no pH changes occurred.

Samples were periodically removed from corn fiber or xylan digestion mixtures, heated in boiling water for 15 min to inactivate the enzymes and then centrifuged (16,000*g*, 10 min, 22°C) to remove the remaining particulate matter. The supernatant fluids were quantitatively analyzed for reducing sugar content using the dinitrosalsilic acid (DNS) method *(11)* and for neutral sugars were determined by gas-liquid chromatography (GLC) *(12)* of their alditol acetate derivatives *(13)*. When needed, the fluid samples were hydrolyzed with trifluroacetic acid (2.0*M*, 60 min, 100°C) to convert oligomer soluble sugars into their monomeric forms.

Sugar profiles of fluid samples of corn fiber or xylan digests were also analyzed by using one or both of two high pressure liquid chromatography (HPLC) systems. One system (HPX-HPLC) consisted of Spectra Physics SP8800 equipped with Carbo-C guard column, an Aminex HPX-87H column (300 × 7.8 mm; Biorad), and a Waters 410 refractive index detector. Samples (10 µL) were injected and eluted with water at a 85°C column temperature. With the second system (PAD-HPLC), fluid samples (25 µL) were injected onto a 4 × 250 mm Carbo Pac PA100 column and eluted (1 mL/min) with a gradient of 0 to 15% sodium acetate (1 m) in 0.1*M* sodium hydroxide using a Dionex HPLC equipped with a pulsed amphometric detector (Model ED 40). The fluid samples were also qualitatively analyzed for free sugars and oligosaccharides by thin layer chromatography (TLC) using previously described standard methods *(12)*.

Enzyme activities were measured by using previously described assays. Xylanase was determined as the release of reducing sugars (DNS method) from soluble oatspelt xylan *(14)* with one unit defined as the release of one µmole sugar/min. Xylosidase and arabinofuranosidase activities were determined by release of p-nitrophenol from p-nitrophenyl glycosides *(15)* with one unit being defined as one µmole nitrophenol released/min. Protein levels of samples were estimated using the methods of Lowry et al. *(16)* with cytochrome C as the standard.

RESULTS

Previous studies have shown that AFEX-treatment of corn fiber increases its susceptibility to enzymatic degradation *(10)*. Corn fiber con-

Table 1
Enzymatic Analysis of Commercial Enzyme Mixtures

Enzyme Mixture	Enzyme Units[a]	Xylanase			Arabinosidase		Xylosidase	
		Units[b]	S.A.[c]	Used[d]	S.A.	Used	S.A.	Used
Amylase (Spezyme™ AA20)	20,000	3	1.6	2	0.001	1	0.002	2
Glucoamylase (Spezyme™ GA300)	300	116	1.4	7	0.002	8	0.003	13
Cellulase (Cytolase 300™)	132	1.4	21.2	106	0.048	4	0.330	9
Cellobiase (Novozyme 188™)	250	455	10.7	104	0.078	45	0.030	17
Xylanase (Multifect® PL)	6,600	869	72.4	139	1.17	2	0.440	1
Xylanase (Multifect® xylanase)	8,000	481	458.6	---	0.16	---	1.44	---
Xylanase (Viscozyme® L)	100	276	16.7	---	0.56	---	0.03	---

[a]Primary enzyme activity as units (as defined by manufacturer)/ml of enzyme preparation, except cellulase where value is units/mg dry weight.

[b]U/mL enzyme mixture except cellulase where value is units/mg dry weight.

[c]Specific activity defined as units/mg protein.

[d]Units added per 100 mL of AFEX-treated corn fiber digest.

sists of about 20% starch, 20% cellulose, and 30% hemicellulose. Thus, a mixture of commercial enzyme preparations was used to completely digest AFEX-treated corn fiber (Table 1). As expected, the designated xylanase preparations had the highest xylan-degrading enzyme levels and predictably the cellulase and amylase preparations had little of these activities (Table 1). The cellobiase (β-glucosidase) preparation had considerable contaminating xylanase, xylosidase, and arabinofuranosidase activities. The "xylanase" activity of each of these preparations however, does not represent "true" xylanase activity, but reflects the sum total of all enzyme activities (e.g., xylanase, xylosidase, arabinosidase, and so on) that produce reducing sugar equivalents from a given xylan. Thus, when each of these preparations was evaluated or beechwood xylan, 4-methylglucuronoxylan, or corn fiber xylan as the substrate, about a three- to four-fold difference in xylanase activity level was observed compared to the activity with oatspelt xylan (data not shown). These changes were positive or negative, depending on the particular xylan and enzyme preparation used.

Enzymatic digestion of 5% suspensions of AFEX-treated corn fiber resulted in a rapid degradation of this material as measured by release of reducing sugars. About 50% of the fiber appeared as reducing sugars in the digestion fluid within 3 h of incubation and about 66% within 12 h, but little increase occurred after this time (Table 2). Analysis of free sugars by GLC of alditol acetate sugar derivatives yielded a similar pattern, but total amount of sugars recovered was only 50 to 65% of the levels estimated by DNS reducing sugar assay. However, subjecting the fluid samples to trifluroacetic

Table 2
Digestion of AFEX-treated Corn Fiber with Commercial Enzyme Mixture[a]

Time (hr)	Reducing sugars (mg/ml)	TFA treatment[b]	Neutral Sugars (mg/ml)[c] Total	Arabinose	Xylose	Galactose	Glucose
3	26.7	-	16.0	1.4	0.8	0.1	13.6
		+	21.2	4.0	4.5	0.7	11.5
12	30.9	-	16.2	1.5	1.2	0.2	13.3
		+	26.6	5.1	6.9	1.1	13.2
24	32.0	-	17.3	1.6	1.3	0.2	14.1
		+	28.9	5.5	7.9	1.1	13.9
48	32.8	-	20.3	2.1	1.7	0.3	16.0
		+	27.6	5.5	7.3	1.0	14.7
72	30.7	-	19.3	2.0	1.8	0.3	15.0
		+	41.5	9.7	12.3	1.4	16.1

[a]A 5% (wt/vol) suspension of AFEX-treated corn fiber was digested with a mixture of amylase, glucoamylase, cellulase, cellobiose, and xylanase enzymes (*see* Table 1) and fluid-associated, released carbohydrates were determined with time.

[b]Sampled hydrolyzed (+) or not hydrolyzed (–) with trifluroacetic acid prior to neutral sugar derivatization.

[c]Determined by alditol acetate derivatization procedures.

acid hydrolysis prior to derivatization increased the total recovered sugars dramatically. Whereas the recovered glucose levels remained about the same, the levels of arabinose and xylose increased two- to three-fold (Table 2). Enzymatic digestions of 10% corn fiber suspensions produced similar results, with the expected greater amounts of sugars were released.

The aforementioned results suggested much (30–40%) of the corn fiber digestion products could be in the form of oligosaccharides. Thus, TLC analysis of the fluids taken after 12 or 72 h of incubation was done and showed the presence of arabinose, glucose, xylose, xylobiose, xylotriose, and material that did not migrate from the origin (data not shown). PAD-HPLC and HPX-HPLC analyses of these same fluids yielded similar results, with numerous higher oligomer peaks. The levels of monomeric sugars determined by HPX-HPLC were slightly higher than those determined by GLC procedures (data not shown). In addition, TLC and HPX-HPLC analyses confirmed the presence of only monomeric sugars in trifluroacetic acid hydrolyzed samples.

The presence of oligosaccharides indicated the enzyme mixture used to digest AFEX-treated corn fiber apparently did not completely degrade the corn fiber xylan component. To gain insights on the effectiveness of this enzyme mixture for xylan degradation alone (i.e., in the absence of starch, cellulose, or other fiber components), various isolated xylans of differing

Table 3
Digestion of Isolated Xylans with Commercial Enzyme Mixture[a]

Xylan	Reducing sugars (mg/ml)	Mono- and oligosaccharides (mg/ml)[b] Total	Arabinose	Glucose	X	X_2	X_3	X_4
Corn fiber	21.8	12.2	5.6	2.4	4.1	0.0	0.0	0.0
Corn germ	46.1	30.3	10.2	0.0	18.3	0.4	1.1	0.3
Oatspelt	59.3	30.0	4.2	0.0	9.3	11.7	3.4	1.4
Beechwood	35.3	28.2	0.7	0.0	16.4	8.4	1.8	0.9
Wheat germ	51.9	27.8	7.9	0.2	9.5	8.1	1.9	0.4

[a]Isolated xylan (100 mg/mL) suspensions were digested for 20 h with a mixture of amylase, glucoamylase, cellulase, cellobiase, and xylanase enzymes used at 4 times the levels used with AFEX-corn fiber digests (*see* Table 1).

[b]Determined by PAD-HPLC. X, X_2, X_3, X_4 = xylose, xylobiose, xylotriose, xylotetraose, respectively.

chemical composition were incubated with mixture and the products determined (Table 3). Based on reducing sugars released, about 21–60% of the initial amounts of xylans were digested, with corn fiber xylan digested the least, and oatspelt xylan the most. In all cases, the HPX-HPLC and PAD-HPLC analyses indicated significant amounts of oligosaccharides were formed. These oligomers were particularly evident with digestion of corn fiber and corn germ xylans, which also yielded arabinose and xylose as products. Wheat germ and corn germ xylans have an arabinose/xylose ratio of about 0.7 to 0.8 (Hespell, unpublished data) and thus, the presence of arabinose as a major digestion product (Table 3) was not unexpected. Xylose, xylobiose, and xylotriose were significant products with digestion of beechwood, oatspelt, and wheat germ xylans.

In an attempt to obtain a higher monosaccharide content of the digestion products and more extensive xylan degradation, three different commercial xylanase preparations were used alone and in combination to digest xylans of varying chemical composition (Tables 4 and 5). With corn fiber xylan, there was little synergism with enzyme mixtures. This arabinoglucuronoxylan was poorly digested, with only about 25% converted to monosaccharides and the remaining portion was mainly in the form of large (pentamers or greater) oligosaccharides (Table 4). In contrast, oatspelt xylan was highly degraded (60% or more), particularly with mixtures of two or more enzyme preparations, to sugars and to short oligosaccharides such as xylobiose and xylotriose. Viscozyme L degraded oatspelt xylan poorly and seemed to negatively affect degradation by the other xylanase preparations (Table 4). This effect appeared to also occur with degradation of beechwood and wheat germ xylans (Table 5). Beechwood xylan (basically a homopolymer of xylose) was almost completely degraded by Multifect PL or Multifect xylanase enzyme preparations. Up to 50% of this xylan was converted to xylose with substantial amounts of xylobiose and xylotriose also being formed. Wheat

Table 4
Digestion of Corn Fiber or Oatspelt Xylan with Commercial Xylan-hydrolyzing Enzyme Mixture[a]

Xylan-Xylanases	Reducing Sugars (mg/ml)	Mono- and oligosaccharides (mg/ml)							
		Total[b]	Arabinose	Glucose	X	X_2	X_3	X_4	X_5+
Corn fiber									
PL	18.8	11.9	4.5	3.2	4.2	0.0	0.0	0.0	55.6
MX	11.6	10.4	3.5	3.4	1.9	1.5	0.0	0.0	0.0
VL	14.2	9.6	2.3	1.5	5.8	0.0	0.1	0.1	67.3
PL+MX	16.4	12.2	4.5	4.5	3.2	0.1	0.0	0.0	0.0
PL+VL	18.2	13.8	5.1	3.6	5.0	0.0	0.0	0.1	69.9
MX+VL	20.20	12.3	4.4	2.4	4.7	0.0	0.0	0.1	68.9
PL+MX+VL	20.10	12.8	4.7	3.4	4.6	0.0	0.0	0.1	63.8
Oatspelt									
PL	34.4	30.5	4.2	1.0	13.4	9.1	2.8	0.0	24.0
MX	36.2	22.7	2.4	0.4	14.5	4.8	0.6	0.0	16.4
VL	25.4	17.3	1.7	1.0	1.3	7.0	6.3	0.0	67.3
PL+MX	35.4	33.6	3.9	0.9	20.0	7.8	1.1	0.0	23.9
PL+VL	37.2	29.1	3.7	1.0	9.4	10.3	4.7	0.0	69.9
MX+VL	36.4	31.0	3.4	0.8	16.4	8.9	1.4	0.0	68.9
PL+MX+VL	36.9	31.6	3.7	0.9	17.1	8.4	1.5	0.0	63.9

[a]Xylan suspensions (50 mg/mL) digested for 20 h with Multifect PL (PL, 8.7 U/mL), Multifect xylanase (MX, 13.4 U/mL), Viscozyme L (VL, 2.8 U/mL) alone or in combination.

[b]Does not include oligomers of X_5 or greater (X_5^+).

germ xylan, an arabinoxylan, was usually degraded to the extent of only about 20 to 30%, with arabinose and xylose as major products (Table 5). Most of the degradation products were oligomers that gave HPX-HPLC and PAD-HPLC peaks that appeared near around the xylopenteaose peak.

DISCUSSION

Enzymatic digestion of corn fiber using a mixture of commercial hydrolytic enzyme preparations resulted in the conversion of the starch and cellulose components into glucose as the only recovered sugar product (Table 2). On the other hand, the hemicellulose or xylan component, was converted into arabinose, xylose, plus oligomeric sugar products that constituted about 30% or more of the total recovered products. The incomplete digestion of the corn fiber xylan probably was not related to the presence of the other fiber polysaccharides, such as starch or cellulose, since these appeared to be completely degraded by the amylases and cellulases in the enzyme preparation. Secondly, isolated xylans were degraded to varying extents to sugars and oligosaccharides by this enzyme mixture (Table 3), with corn fiber being degraded the least. This type of variation in xylan

Table 5
Digestion of Beechwood or Wheat Germ Xylan with Commercial Xylan-hydrolyzing Enzyme Mixture[a]

Xylan-Xylanases	Reducing Sugars (mg/ml)	Mono- and oligosaccharides (mg/ml)							
		Total[b]	Arabinose	Glucose	X	X_2	X_3	X_4	X_5+
Beechwood									
PL	23.1	21.6	1.4	1.7	12.3	4.9	1.3	0.0	4.6
MX	40.3	41.3	0.7	1.6	25.9	9.0	4.1	0.0	6.6
VL	20.4	21.3	0.0	0.9	2.2	8.7	6.5	3.1	2.2
PL+MX	36.5	32.1	0.9	2.1	20.2	7.1	1.8	0.0	5.8
PL+VL	24.5	25.3	1.3	1.8	10.4	8.3	3.5	0.0	5.2
MX+VL	35.5	40.4	0.1	2.0	21.9	9.5	2.8	4.1	8.4
PL+MX+VL	36.4	34.3	0.8	1.8	17.9	8.1	2.2	3.5	7.2
Wheat germ									
PL	20.0	16.3	4.8	1.6	6.9	2.5	0.4	0.1	21.3
MX	17.0	11.9	3.4	0.9	7.6	0.0	0.0	0.0	23.0
VL	17.6	16.6	2.8	1.6	2.0	4.8	1.6	3.8	22.9
PL+MX	20.7	12.9	4.1	1.0	7.7	0.0	0.0	0.1	20.2
PL+VL	22.7	16.8	4.8	1.8	6.0	3.4	0.7	0.1	22.7
MX+VL	22.4	14.2	3.7	1.7	7.4	1.3	0.0	0.1	24.1
PL+MX+VL	22.5	14.5	4.0	1.6	7.4	1.5	0.0	0.0	21.5

[a]Xylan suspensions (50 mg/mL) digested for 20 h with Multifect PL (PL, 8.7 U/mL), Multifect PL (PL, 8.7 mL), Multifect xylanase (MX, 13.4 U/mL) Viscozyme L (VL, 2.8 U/mL) alone or in combinations.

[b]Does not include oligomers of X_5 or greater (X_5^+).

degradation is not unusual and also can be seen with xylans during fermentation by cultures of xylanolytic bacteria *(17,18)*.

Unlike starch or cellulose, xylans are chemically quite complex and require numerous enzymes with varying substrate specificities for complete degradation. The production of oligosaccharides and incomplete degradation of xylans is partially because of their structure. Corn fiber xylan is poorly degraded (Tables 3 and 4) and structural analyses suggest that over 70% of the xylose backbone residues have one or more arabinose, 4-O-methylglucuronic acid, or other sidechains *(19)*. As a result, there are few regions in corn fiber xylan where several contiguous xylose residues are unsubstituted. Many xylanases, for example, those of *Clostridium thermolactium (20)*, require such regions for attachment and cleavage. Frequently xylanonlytic activity is highly enhanced in the presence of arabinose sidechains-removing activity (e.g., arabinfuranosidase) because of the resulting lower steric hinderance *(21,22)*. Uronic acid sidechains can also inhibit xylanase activity, but some xylanases require them for substrate recognition *(23)*. Besides these xylan structural impediments, the sequence of enzyme attack and enzyme catalytic rates are likely the other major factors regulating xylan degradation and product formation.

Previous studies have shown with enzymes purified from *Trichoderma reesi* that solubilization of wood xylans depended greatly on xylan structure and the particular combination of enzymes used in the digestion mixture *(924)*. Similarly, the degradation of glucuronoarabinoxylans of varying composition by purified *Aspergillus awamori* enzymes was 10–42%, depending on the combination of enzymes (endoxylanase, xylosidase, or acetyl xylan esterase) used *(25)*. Similar results were obtained by us with combinations of crude commercial enzyme mixtures and various xylans (Tables 3 and 4), but the extent of degradation was generally greater (20–70%). Taken together, these studies clearly indicate several limitations to enzymatic saccharification of biomass xylans to sugar streams for use ethanol or other fermentations. First, the types and proportions of enzymes in the preparation may have to be altered to match the particular xylan composition for each biomass. Secondly, sequential enzyme treatments (e.g., arabinosidase then xylanase then xylosidase) of the biomass may be needed to maximize degradation.

The loss of potential fermentable biomass sugar as oligomeric sugars and/or costs of enzyme digestions would seem to suggest this may not be a practical saccharification procedure. Dilute acid treatment of biomass may be a viable route for xylan sugar streams for fermentation. Such streams can be readily separated from the residual particulate cellulosic material, but in most published studies the sugar streams have not been examined for fermentability. Acid treatments need to be carefully optimized to limit production of toxic products/furfural and of oligomeric sugars. An alternative strategy to employ may be to use natural or genetically engineered xylanolytic microorganisms that effectively produce ethanol (or the desired bioproduct) directly from intact biomass or intact xylans that have been chemically extracted from biomass.

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