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ASSESSMENT OF ETHANOL PRODUCTION OPTIONS FOR CORN PRODUCTS

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Abstract

The production of ethanol from corn fiber has the potential to increase ethanol yields by a maximum of 0.3 gal/bushel in a wet-milling process. Incremental yields would be 0.13 gal/bushel from hexose, 0.1 from D*-xylose and 0.07 from* L*-arabinose, at 100% hydrolysis and fermentation efficiency. At 80% efficiency for hexose hydrolysis and fermentation, and 70% for pentose, an incremental yield of 0.22 gallons/bushel of corn is expected. Of this total, 0.1 gal/bushel would be from hexoses, 0.07 from* D*-xylose, and 0.05 from* L*-arabinose. A maximum practical incremental yield would probably fall between 0.22 and 0.3 gallons/bushel. These calculations are based on published compositional analyses of cellulose, starch, mono-saccharides, hemicellulose, protein and oil as distributed between the compartmentalized components of the corn kernel and published yield factors for hexose and pentose fermentations. Experimental yield factors for xylose (0.36 g ethanol/g xylose) and arabinose (0.34) fermenting microorganisms are lower than that for glucose (0.45–0.50), and significantly less than the theoretical yield of 0.51 g ethanol/g pentose. Nonetheless, we estimate that a wet-milling facility which currently produces 100 million gallons/year of ethanol from starch could generate an additional $4–8 million of annual income if the fiber components were processed into ethanol. Hence, advances in fiber pretreatment and pentose fermentation are likely to have a major impact on enhancing productivity of corn ethanol plants. An engineering framework for assigning economic consequences of the additional utilization of fiber is presented. Copyright © 1997 Elsevier Science Ltd.*

Key words: Ethanol, economics, yields, wet milling, dry milling, cellulosic co-products.

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NOMENCLATURE

A	yield, gallons ethanol/bushel
A_{incr}	incremental ethanol yield, gallons ethanol/bushel of corn
b	weight of corn (as is basis), lbs/bushel
d	density of 100°C ethanol at 20°C = 6.58 lb ethanol/gallon (specific gravity = 0.79)
g	theoretical D-glucose yield upon saccharification from starch = 1.111 lb D-glucose/lb starch
$g_{f,a}$	theoretical L-arabinose yield from hemicellulose based on arabinan content = 1.136
$g_{f,c}$	theoretical D-glucose yield from fiber based on cellulose content = 1.111
$g_{f,gal}$	theoretical D-galactose yield from hemicellulose based on galactan content = 1.111
$g_{f,s}$	theoretical D-glucose yield from fiber based on starch content = 1.111
$g_{f,x}$	theoretical D-xylose yield from hemicellulose based on xylan content = 1.136
m	moisture, lbs water/lb total weight
R	recovery ratio [defined in eqn (8)]
$y_{e,a}$	theoretical ethanol yield from L-arabinose (0.511 for L-arabinose)
$y_{e,g}$	theoretical ethanol yield from D-glucose = 0.511 lb ethanol/lb D-glucose
$y_{e,gal}$	theoretical ethanol yield from D-galactose (assume = $y_{e,g}$)
$y_{e,x}$	theoretical ethanol yield from D-xylose (0.511 lb ethanol/lb D-xylose)
y_f	fraction of fiber (lb fiber/lb corn, dry basis)
$y_{f,a}$	dry weight fraction of arabinan in fiber (lb araban/lb fiber, dry basis)
$y_{f,c}$	dry weight fraction of cellulose in fiber (lb cellulose/lb fiber, dry basis)
$y_{f,gal}$	dry weight fraction of galactan in fiber (lb galactan/lb fiber, dry basis)

$y_{f,s}$ dry weight fraction of starch in fiber (lb starch/lb fiber, dry basis)
$y_{f,x}$ dry weight fraction of xylan in fiber (lb xylan/lb fiber, dry basis)
y_s fraction of starch (lb starch/lb corn, dry basis)

Greek letters
η actual overall fractional hydrolysis and fermentation yield
η_{ferm} actual fractional fermentation yield
η_{hyd} actual fractional hydrolysis yield

INTRODUCTION

The technical feasibility of fermentation ethanol production from whole corn is a function of the composition of corn and the manner in which it is processed in large-scale wet or dry mills. Wet mills process corn by a series of steeping, wet-grinding and fractionation steps which result in starch-, oil-, protein- and fiber-containing streams, corn gluten meal and corn gluten feed (Fig. 1) (Voloch *et al.*, 1984; Ladisch & Svarczkopf, 1991; Matz, 1991). The germ fraction is converted into corn oil, while corn germ animal feed is derived from the residual germ. The corn fiber fraction is dried together with varying amounts of the steep waters and fermentation solids to form the protein corn gluten animal feeds. The high-protein corn gluten meal (an animal feed) is derived from the protein fraction [stream (6) in Fig. 1]. Fiber, mixed with germ cake and concentrated steep water [streams (4), (3), and (1) in Fig. 1], is sold as corn gluten feed.

A dry-milling process for alcohol production processes the whole grain, or components derived from the whole grain. Saccharification and fermentation of dry-milled corn results in ethanol and distillers' dried grains (DDG). When distillers' dried grains are combined with fermentation liquids and dried they result in distillers' dried grains with solubles

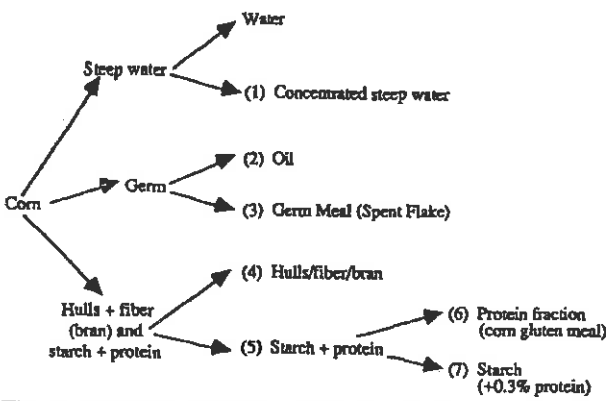


Fig. 1. Simplified diagram of starch and fiber recovery in corn wet milling [adapted from Ladisch & Svarczkopf (1991) and Hoseney (1986)].

(DDGS) as the major feed byproducts (LeBlanc *et al.*, 1988; Ladisch & Svarczkopf, 1991; Matz, 1991; Hohmann & Rendleman, 1993).

The improvements in cellulose conversion technology have yet to make ethanol from cellulose processes economical, although significant progress continues to be made (Lynd *et al.*, 1991; Wyman, 1994; Stone & Lynd, 1994). It has been suggested that improvements in cellulose hydrolysis technology may find practical use in the wet-milling industry for the processing of fiber fractions to ethanol (Ladisch & Svarczkopf, 1991; Voloch *et al.*, 1984). Consequently, incremental reductions in net substrate costs are possible, thereby decreasing the cost of ethanol from corn processing. This hypothesis would suggest that ethanol production from both corn and cellulose will benefit from on-going research, although the cost of ethanol from cellulose could decrease more rapidly than that for ethanol from corn. Another look at the possibilities of corn fiber conversion to ethanol seemed useful, given the improvements in microbial fermentation of D-xylose to ethanol (Slininger *et al.*, 1987; 1991; Sanromán *et al.*, 1994; Hahn-Hägerdal *et al.*, 1993; 1994; Schaaff-Gerstenschläger *et al.*, 1994; du Preez, 1994; Jeffries & Kurtzman, 1994), cellulose pretreatment (Kohlmann *et al.*, 1993; Wyman, 1994; Weil *et al.*, 1994) and activity of cellulases (Woodward, 1991; Kohlmann *et al.*, 1996; Ladisch *et al.*, 1992).

This paper reviews the structure, composition and ethanol production potential of hemicellulose and cellulose components found in corn grain. The theoretical yields, based on cellulose and hemicellulose composition, are higher than maximum practical yields, due to incomplete fractionation of starch and fiber from other corn constituents in wet-milling processes and incomplete conversion of starch and/or other fiber components in dry-mill fermentation systems. It should be noted that modern wet mill and dry-mill processes already have efficiencies of up to 95% in recovering ethanol from starch, hence improvements in these technologies, while economically significant, are likely to occur in an incremental fashion.

BACKGROUND

The corn kernel

Structure

The corn seed is referred to as the kernel (Kiesselbach, 1980). The cross-section of a mature kernel is shown in Fig. 2. The four main parts of a corn kernel are: the tip cap, hull, endosperm and germ (Inglett, 1970). The tip cap is at the extreme base of the kernel and is composed of the pedicel tissue which originally joined the kernel to the cob. Usually the kernel separates from the cob at the base of the tip cap (Wolf *et al.*, 1952a).

The hull comprises the outer fibrous covering and consists of the pericarp, seed coat and the hilar layer (Wolf *et al.*, 1953; 1952b; 1952c). The endosperm consists of an outermost layer called the aleurone layer and an inner starchy endosperm (Wolf *et al.*, 1952c). The outer aleurone cells contain mainly oil and protein and the inner storage tissue is rich in starch and protein (Wolf *et al.*, 1952a). The germ is embedded in the lower portion of the endosperm and consists of the embryo and the scutellum (Wolf *et al.*, 1952d).

Composition of corn kernel parts (dry weight basis)

The average composition of a corn kernel and the distribution of these components in the four structural parts of the corn kernel is shown in Table 1 (Earle *et al.*, 1946; Watson, 1987). Most of the starch and protein is contained in the endosperm, while the germ contains most of the lipids and soluble sugars. Over 50% of the fiber (hemicellulose+cellulose +lignin) is present in the hull and germ fractions.

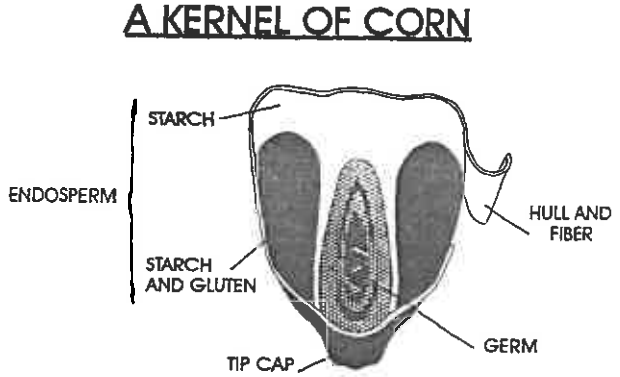


Fig. 2. Schematic diagram of a kernel of corn [reproduced from Rutenberg (1987)].

Composition of a theoretical bushel of corn (as is basis)

The analysis presented here defines a theoretical bushel of corn as the weight of starch, sugars, hemicellulose, cellulose and other components which would be recovered if fractionation of the corn into its individual molecular or polymer components was 100% efficient. While 100% efficiency of fractionation does not occur in a practical corn-processing plant, an analysis using this basis gives insight into the maximum theoretical yields which could be expected. The as is basis refers to the 15.5% equilibrium moisture content of corn at 21°C and 80% relative humidity (Shove, 1970). The components of a theoretical bushel of corn at this equilibrium moisture content are summarized in Table 2 and include 33.93 lb of starch and 8.68 lb of water.

Theoretical maximum ethanol yield obtainable from corn

The weights of the various fractions of a theoretical bushel, given in Table 2, can be used to calculate the maximum achievable yield of ethanol. This assumes that the components are fractionated, saccharified to monosaccharides and fermented with 100% efficiency, and that D-glucose and all other hexoses, as well as D-xylose and L-arabinose are fermentable to ethanol. The basis of this calculation differs from the usual calculation of theoretical ethanol yield based on saccharification and fermentation yields since it includes the efficiency of fractionating the components of corn as well.

Variability in corn composition based on industrial data, as well as differences between industry and laboratory data, have been thoroughly examined and reported (Eckhoff *et al.*, 1993). The bases shown in Table 3 are within the ranges reported by Eckhoff

Table 1. Distribution of components in corn kernel parts (dry weight basis)

Part	Component (% by weight)[a,b]							Percentage of total, by weight[c,d]		
	Starch	Soluble sugars	Hemicellulose+ cellulose+ lignin	Lipids	Protein	Ash	Balance	Low	High	Basis
Percent corn kernel (dry wt)[e,f]	71.7	2.6	8.1[g]	4.3	10.3	1.4	1.6	—	—	100
Tip cap	0.1	0.7	0.1	0.7	0.8	0.9	—	0.8	1.1	0.9
Hull (bran)	0.5	1.0	51.0	1.1	2.0	2.9	—	4.4	6.2	5.3
Endosperm	98	28.2	27.0	14.5	74.8	16.5	—	79.7	83.5	81.9
Germ	1.4	70.2	16.0	83.7	22.4	79.7	—	10.2	14.1	11.9
Total	100	100.1	94.1	100	100	100	—	95.1	104.9	100

[a]Data from Earle *et al.* (1946).
[b]Watson (1987), p. 77; defined as '% kernel fiber'.
[c]Watson (1987), p. 61.
[d]Data from Earle *et al.* (1946).
[e]Data from Watson (1987), p. 72.
[f]Earle *et al.* (1946).
[g](5.5%+2.4%+0.2%).

Table 2. Distribution of corn composition per theoretical bushel of corn (as is basis includes moisture)

Component	Weight (lb/bushel)	Theoretical maximum ethanol (gallons/bushel)[a]
Starch	33.93	2.928
Soluble Sugars	1.23	0.095
Hemicellulose	2.59	0.228
Cellulose	1.16	0.100
Lignin	0.08	—
Protein	4.87	—
Fat	2.04	—
Ash	0.66	—
Water	8.68	—
Balance	0.76	—
Total	56	3.351

[a]1 Bushel = 56 lb corn at 15.5% moisture.
1 Gallon ethanol = 3.785 l = 6.58lb of 100% ethanol at 20°C (density = 0.79) (Perry *et al.*, 1984).

et al. (1993) although the starch composition (at 67.5%) is higher than the value of laboratory results at 64.8%. This research also shows that there is significant variability between different laboratory studies due to factors including: the low starch content of the hybrid being milled; poor initial corn quality; errors in performing the rather complex sequence of operations which make up the laboratory milling procedure; and laboratory steep times (36–48 h), which are longer than the current industrial norm of 24–36 h. (Eckhoff *et al.*, 1993). The reader is referred to the work of Eckhoff and Tso (1991), Eckhoff *et al.* (1993), Fox and Eckhoff (1993) and co-workers for a detailed explanation of the sources of variability in fractionating the components of corn in a wet-milling process. Given this variability, it was difficult to identify a single corn composition representative of all wet mills. Consequently, we chose to use the data reported by Matz (1991) (Table 3), which is similar to the range of compositions based on industrial yield data, as well as the careful work and experimental simulations of Fox and Eckhoff (1993) and Eckhoff *et al.* (1993). This composition gives a measure of fractionation efficiency when compared to that of a theoretical bushel of corn. For example, 100% efficient recovery of starch would correspond to 33.93 pounds/bushel or 71.7% of the corn on a dry weight basis. A starch composition of 67.5% would indicate 94.1% recovery. The term theoretical bushel of corn is used to emphasize the need for an internally consistent basis for estimating ethanol yield.

The equation which relates alcohol yield to starch composition is given by

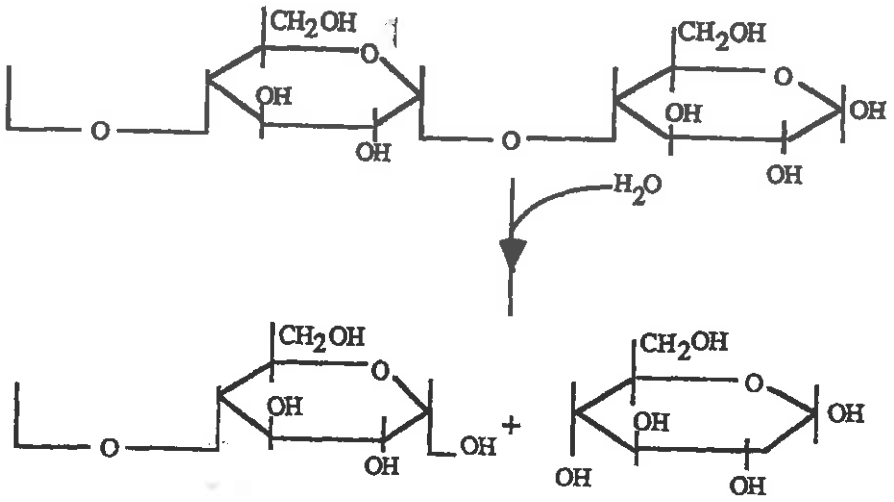
$$A = \frac{(b)(1-m)(y_s)(g)(y_{e,g})}{(d)} \qquad (1)$$

A = gallons ethanol/bushel; b = weight of corn (as is basis), lb per bushel; m = moisture, lb water/(lb total weight); y_s = fraction of starch (lb starch/lb corn, dry basis); g = theoretical D-glucose yield upon saccharification from starch = 1.111 lb D-glucose/lb starch; $y_{e,g}$ = theoretical ethanol yield from D-glucose = 0.511 lb ethanol/lb D-glucose; d = density of 100% ethanol at 20°C = 6.58 lb ethanol/gallon (specific gravity = 0.79). The factor for the theoretical yield of D-glucose upon saccharification from starch, [g, in eqn (1)] is obtained by accounting for the weight gain due to the water which is added across the glycosidic ether bonds, as represented by

Table 3. Summary of industrial and laboratory yield data for corn wet milling [adapted from Eckhoff *et al.* (1993)]

Milling fraction	Industrial			Laboratory			Basis[b]
	Anderson & Watson (1982)	May (1987)	Anderson (1963)	Eckhoff *et al.* (1993)	Eckhoff & Tso (1991)	Watson (1987)	Matz (1991)
Starch, %	67.5	66.0	64.3	64.8	67.3	63.7	67.5
Solubles	7.5	7.0	N/A	7.0	6.2	7.6	7.6
Fiber	11.5	13.0	12.8	9.9	8.8	9.5	9.5
Germ	7.5	7.9	N/A	7.0	6.0	7.3	7.5[a]
Gluten	5.8	5.7	14.5	9.9	9.8	11.3	5.8
Recovery, %	99.8	99.6	91.6	98.6	98.1	99.4	97.9
Steep time, h	N/A	N/A	N/A	36	48	48	—

[a]Sum of germ cake and crude oil, 3.64 and 3.86%.
[b]Data from Livezey (1982), p. 503, as quoted from Matz (1991).
Sources cited by Eckhoff *et al.* (1993).



(2)

The theoretical yield of ethanol upon fermentation of D-glucose derives from the glycolytic pathway, where 1 mole D-glucose forms 2 moles pyruvate and ultimately 2 moles of ethanol (molecular weight = 46) and 2 moles of CO_2 (molecular weight = 44). Consequently, the yield factor is

$$y_{e,g} = \frac{|2 \text{ g moles ethanol}|\frac{46 \text{ g}}{\text{gmole}}|}{|1 \text{ g mole glucose}|\frac{180 \text{ g}}{\text{gmole}}|} = \frac{92}{180} = 0.511 \quad (3)$$

Similarly, yields for other hexoses are assumed to follow this relationship (i.e. for D-galactose, $y_{e,gal} = 0.511$). For D-xylose, L-arabinose or other pentoses, for calculation purposes, it is assumed that six pentose molecules give the same ethanol yield as five D-glucose molecules.

Consequently,

$$y_{e,x} = \frac{|5 \times 2 \text{ moles ethanol}|\frac{46 \text{ g}}{\text{gmole}}|}{|6 \text{ mole xylose}|\frac{150 \text{ g}}{\text{gmole}}|} = 0.511 \quad (4)$$

and similarly $y_{e,a} = 0.511$ for L-arabinose. However, the yield of pentoses from pentosan which makes up hemicellulose is 1.136 (= 150/132 = molecular weight of pentose per molecular weight of anhydropentoses which make up pentosans). The density of alcohol at 20°C is used in the calculations. The fuel-grade ethanol is required to contain less than 1.25 mass% of water (ASTM Standard D4806-93a). A lower water content is necessary in some cases to avoid phase separation of gasoline–ethanol at very low temperatures. The ethanol yields are computed on a water-free (neat) basis for purposes of this calculation framework.

An example of a calculation for ethanol yield from the starch of one bushel of corn is given in eqn (5) by using the appropriate values from eqns (3)–(4) in eqn (1):

$$A = \left(56\frac{\text{lb wet corn}}{\text{bushel}}\right) \times \left(1\text{-}0.155\frac{\text{lb dry corn}}{\text{lb wet corn}}\right) \times \left(0.717\frac{\text{lb starch}}{\text{lb dry corn}}\right) \times \left(1.111\frac{\text{lb glucose}}{\text{lb starch}}\right) \times \left(0.511\frac{\text{lb ethanol}}{\text{lb glucose}}\right) \times \left(\frac{1 \text{ gallons ethanol}}{6.58 \text{ lb ethanol}}\right)$$

$$= 2.928 \text{ gallons/bushel} \quad (5)$$

This value represents a theoretical (maximum) yield, since not all of the starch in wet-milled or dry-milled corn is recovered in the starch stream. Further, the efficiency for fermentation of D-glucose to ethanol is less than 100%.

Analogous calculations for the sugar, hemicellulose, and cellulose fractions yield the results summarized in Table 2. The fiber is taken to contain 25.2% D-xylose, 3.8% D-galactose and 18.4% L-arabinose (Watson, 1987), all of which are fermentable. The other sugars which make up the remaining polysaccharides in the fiber, principally uronic acid (4-O-methyl-glucuronic acid), are assumed to be unfermentable.

Fermentable sugar content of fiber

Wet milling (Fig. 1) results in 4.49 pounds fiber (dry basis) per bushel of corn (Table 4) or 9.5% of the dry weight of the corn (Table 3). The sum of cellulose, hemicellulose and lignin is 8.1% of the dry weight of corn (Table 1), or 3.83 lb per bushel of corn (Table 2). We assume all the fiber is derived from the hull and endosperm, which contain 83% of the hemicellulose, cellulose and lignin ((51+27)/94.1 × 100 = 0.83, Table 1). Hence, the fiber is assumed to contain about 83% or 3.18 lb

Table 4. Yields for wet milling per bushel of corn based on Table 3 (basis below shows moisture)

Product/ byproduct	lbs/ bushel	Theoretical yield (gallons/bushel)
Starch	31.94	2.757
Solubles	3.60	unknown
Fiber	4.49	0.302
Germ cake	1.72	—
Gluten meal	2.75	—
Crude oil	1.83	—
Other	0.99	—
Water	8.68	—
Total	56	3.059

Derived from data of Matz (1991), as shown in Table 3.

$(=3.83\times0.83)$ of hemicellulose, cellulose and lignin in the theoretical bushel of corn of Table 2.

The pericarp consists of 67% hemicellulose, 23% cellulose, and 0.1% lignin (see Table 7, p. 77, in Watson, 1987) which gives 48% D-xylose, 35% L-arabinose, 7% D-galactose and 7–12% uronic acid upon hydrolysis (Wolf *et al.*, 1953). An analogous breakdown of the hemicellulose fraction in the endosperm is not available. Hence, the composition of pericarp was used to calculate the values given in Table 5, with the hemicellulose, cellulose and lignin content in fiber being assumed to exist in the same proportions in both the endosperm and pericarp. The starch and protein components were assumed to be the same as given by Voloch *et al.* (1984).

The theoretical yield of alcohol from fiber is given in eqn (6). This is defined as an incremental yield since the ethanol derived from fiber would be added to the major part derived from the starch fraction. The equation is

Table 5. Fiber composition and ethanol yield

Component	Basis, %	Percentage by weight (as hydrolyzed monosaccharides)	lb	lb as monosaccharide	Theoretical yield (gallons ethanol/bushel)
Starch[a]	11.4	12.7	0.512	0.569	0.044
Hemicellulose[b,c]	52.7	(59.8)			
D-Xylose	25.2	(28.7)	1.131	1.285	0.100
L-Arabinose	18.4	(20.9)	0.826	0.939	0.073
D-Galactose	3.8	(4.2)	0.170	0.189	0.015
Uronic acid	5.3	(6.0)	0.238	0.271	—
Cellulose	18.1	(20.1)	0.813	0.903	0.070
Lignin	0.1	—	0.005	—	—
Protein	11.3	—	0.507	—	—
Balance	6.4	—	0.287	—	—
Total	100	—	4.49	4.156	0.302

[a]Voloch *et al.* (1984) (as obtained from Watson, 1987).
[b]Watson (1987).
[c]Wolf *et al.* (1953).

$$A_{\text{incr}}=\frac{(b)(1-m)(y_f)\{(y_{f,s}g_{f,s}+y_{f,c}g_{f,c})y_{e,g}+y_{f,x}g_{f,x}y_{e,x}+y_{f,gal}g_{f,gal}y_{e,gal}+y_{f,a}g_{f,a}y_{e,a}\}}{(d)} \tag{6}$$

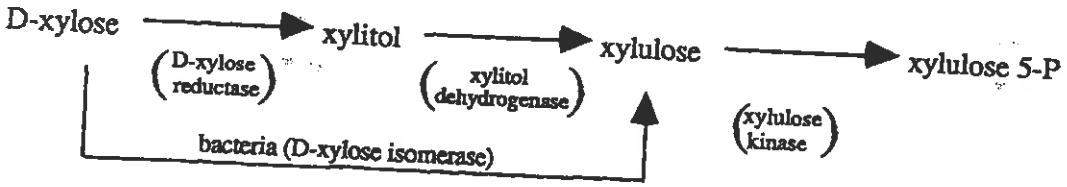
where A_{incr} = incremental ethanol yield, gallons of ethane/bushel of corn; $g_{f,s}$ = theoretical D-glucose yield from fiber based on starch content = 1.111; $g_{f,c}$ = theoretical D-glucose yield from fiber based on cellulose content = 1.111; $g_{f,x}$ = theoretical D-xylose yield from hemicellulose based on xylan content = 1.136; $g_{f,gal}$ = theoretical D-galactose yield from hemicellulose based on galactan content = 1.111; $g_{f,a}$ = theoretical L-arabinose yield from hemicellulose based on arabinan content = 1.136; $y_{e,gal}$ = theoretical ethanol yield from D-galactose (assume = $y_{e,g}$); y_f = fraction of fiber (lb fiber/lb corn, dry basis); $y_{f,gal}$ = dry weight fraction of galactan in fiber (lb galactan/lb fiber, dry basis); $y_{f,s}$ = dry weight fraction of starch in fiber (lb starch/lb fiber, dry basis); $y_{f,c}$ = dry weight fraction of cellulose in fiber (lb cellulose/lb fiber, dry basis); $y_{f,x}$ = dry weight fraction of xylan in fiber

(lb xylan/lb fiber, dry basis); $y_{t,a}$ = dry weight fraction of arabinan in fiber (lb arabinan/lb fiber, dry basis) and b, m and d are as defined for eqn (1) and the other parameters are as defined in the Nomenclature.

Xylose and arabinose fermentation

The theoretical increase in ethanol yield from fermenting the D-xylose and L-arabinose from fiber in corn grain is significant (Table 5). Potentially 33% of the incremental ethanol can be derived from the D-xylose fraction of the fiber and 24% from the L-arabinose fraction. The yield and productivities of D-xylose fermentation are lower than hexose fermentation with the major difference between hexose and pentose metabolism being that all pentoses have to be shuttled through the pentose phosphate pathway, PPP (Hahn-Hägerdal *et al.*, 1993). Consequently, there is significant impetus to continue to develop microorganisms which ferment both D-glucose (derived from cellulose) and D-xylose and L-arabinose (from hemicellulose) to ethanol.

The D-xylose fermentation proceeds as shown: (Slininger *et al.*, 1987).



Microbes which can ferment D-xylose directly must be improved in regard to ethanol tolerance, and tolerance to potential inhibitors like acetic acid present in the hydrolyzates. They must also be improved in regard to yield and production and those microorganisms which can use xylulose or xylitol must be altered or have D-xylose isomerase added so that D-xylose can be converted to xylulose and fermented.

Saccharomyces cerevisiae, the standard D-glucose fermenter which exhibits favorable ethanol production and tolerance, ferments xylulose but not D-xylose. The yeasts, *C. shehatae* and *P. stipitis*, are thought to have the highest ethanol productivities from D-xylose, the latter having the advantage of not producing xylitol (Ho *et al.*, 1991). Fungi (*Fusarium* spp. and *Paecilomyces* spp.) are of special interest in simultaneous saccharification and fermentation (SSF) processes, although overall ethanol productivities are thought to be generally lower than for other yeast- or bacterial-based systems. In simultaneous saccharification fermentation systems there is an additional need for cellulases and xylanases to provide the necessary D-glucose and D-xylose, respectively.

Yeasts for xylose fermentation

Advances in the D-xylose fermentation rates and yields for recombinant yeasts and, particularly, *S. cerevisiae* are likely to have a more immediate impact than recombinant bacteria on processes which utilize corn and have corn fiber available. Yeasts not only ferment sugars to ethanol, but the yeast cells can also be recovered in various forms for use as an animal feed. However, recombinant yeasts will need to be tested for approval of their use in animal feeds.

Numerous yeast species have been examined and ethanol yields have ranged from 0.29 to 0.44 g/g D-xylose (Kotter & Ciriacy, 1993). The early work by Gong *et al.* (1981) showed that several yeast species could ferment xylulose to significant amounts of ethanol. In addition, these workers showed *S. cerevisiae* produced ethanol from xylose when supplemented with D-xylose isomerase (to convert D-xylose to xylulose) from any one of a number of microbial sources. More recent work by Lastick *et al.* (1990) has confirmed these results, but indicates that the cost of the added enzyme is significant. A possible means to reduce costs may be to use recombinant yeasts which carry out this conversion.

Genetic engineering of xylose-fermenting microorganisms

Much of the recent work to improve pentose fermentation to ethanol has been in the development of genetically engineered microorganisms. Thus far, the most promising ethanolgenic bacteria are *E. coli*, *Z. mobilis*, and *K. oxytoca*, with the latter being able to ferment sugar oligomers. The ethanolgenic yeast *S. cerevisiae*, if able to ferment D-xylose, might also have the advantage of high ethanol tolerance and high resistance to inhibitors.

Genetic engineering research began with attempts to permit the direct fermentation of D-xylose and other pentoses to ethanol. Two fundamental molecular approaches have evolved for the development of superior ethanolgenic bacteria and yeast that can ferment pentose and hexose sugars and their dimers and trimers. The first approach is to genetically engineer the ability to make ethanol into microbes that normally use multiple substrates. The second approach is to genetically engineer the ability to use multiple substrates into microbes that normally make ethanol (Bothast, 1994).

In the case of *E. coli*, the uptake and channeling of D-xylose into the glycolytic pathway existed, but the pyruvate decarboxylase enzyme in the pathway between pyruvate and ethanol was lacking. Ingram *et al.* (1987) successfully cloned genes encoding essen-

tial enzymes of the fermentative pathway for ethanol production from *Zymomonas mobilis* and inserted them into *E. coli*. This conferred high ethanol production capability into this bacteria, resulting in a D-xylose-fermenting microorganism which gave ethanol.

Lindsay *et al.* (1995) isolated mutant strains of recombinant *E. coli* which do not use D-glucose effectively but retain the ability to metabolize pentoses such as L-arabinose and D-xylose with independent transport systems. These strains have been used in a sequential fermentation. First the bacteria ferment pentose sugars to ethanol in the presence of high D-glucose concentrations, after which yeast is added to the remaining D-glucose in order to obtain more ethanol in a second fermentation.

Recently, the complementary approach of introducing the D-xylose assimilation and pentose phosphate pathway genes from *E. coli* into *Z. mobilis* has been reported and in this case ethanol production is more efficient because less substrate is used for biomass formation (Zhang *et al.*, 1995a). The yields, thus far, range from 0.15 to near the theoretical value of 0.51 g ethanol/g D-xylose. Zhang *et al.* (1995b) surveyed numerous industrial microorganisms by comparing their known metabolic characteristics to a weighted list of fermentation performance criteria. *Zymomonas mobilis* and *Lactobacillus* species were identified as promising microorganisms for metabolic engineering. Obtaining near theoretical values makes the development of fermentation systems based on recombinant bacteria attractive. Beall *et al.* (1991) stated that additional advantages include higher rates of conversion, greater efficiency, higher final product concentration, lower inocula concentration and greater ability to ferment various sugars. A perceived disadvantage with *E. coli* or *K. oxytoca* systems is that they must be grown at neutral pH, thus providing an environment which is more likely to facilitate growth of contaminating microorganisms (Ishizaki *et al.*, 1994; Wyman, 1994).

Xylose fermentation yields

Fermentation of hexose- and pentose-containing hydrolysates from red oak and eucalyptus, as well as spent sulfite liquor, have given yields of 0.33 to 0.40 g ethanol/g sugars. When D-xylose was tested alone, yields ranged from 0.29 to 0.43 g ethanol/g D-xylose. On this basis, we chose 0.36 g/g D-xylose as a reasonable, current yield factor. This is about 70% of the theoretical yield ($= 0.35/y_{e,x} \times 100$), where the theoretical yield, $y_{e,x}$, is calculated by eqn (4).

Fermentation of L-arabinose to ethanol

L-arabinose constitutes 10–25% of the pentoses derived from some hemicelluloses and, hence, is a major source of fermentable sugar. However, L-arabinose-fermenting microorganisms are less prevalent than those which can ferment D-xylose. D-xylose-fermenting yeasts were discovered in the 1980s but, to date, no yeasts have been found that ferment L-arabinose to ethanol in significant quantities.

Recently, Dien *et al.* (1996) screened 116 different yeasts for the ability to ferment L-arabinose and found four species able to ferment the sugar. Although these yeasts produced low ethanol concentrations (with *Ambrosiozyme monospora* producing the maximum of 4.1 g/l), they are potential candidates for mutational enhancement of L-arabinose fermentation. The bacterium *Erwinia chrysathemi*, which can tolerate 4% ethanol concentrations, has been considered as a candidate for genetic engineering because this microbe ferments L-arabinose (Hahn-Hägerdal *et al.*, 1993). Recombinant *Klebsiella oxytoca* strain P2 carrying *pdc* and *adh B* genes from *Zymomonas mobilis* was shown to produce 0.34 g ethanol/g sugar when grown in L-arabinose alone. The yield was 0.43 g ethanol/g sugar in a 1:2:1 mixture of L-arabinose, D-xylose and D-glucose. Substrate utilization was best for D-glucose, followed by L-arabinose and D-xylose (Bothast *et al.*, 1994).

Cost of xylose fermentation using recombinant bacteria

The cost to implement an ethanolgenic system for willow-wood hydrolysates using a recombinant strain of *E. coli* has been presented by von Sivers *et al.* (1994). They estimate that this system gives a total ethanol yield of 92% of the theoretical value. Costs for hydrolysis, hydrolysate detoxification, fermentation, production of cell mass and labor and maintenance steps resulted in a total production cost of $0.48/l, which is not yet competitive with the current market price of $0.35/l. Potential was seen to decrease the cost of the detoxification step, which currently comprises 22% of the total cost.

Doran *et al.* (1994) report on the economics of a simultaneous saccharification–fermentation process using recombinant *Klebsiella oxytoca* together with Genencor's Spezyme CE to saccharify bagasse to cellobiose which the recombinant microorganism can metabolize. The ethanol yield of 40 g/l was 72% of the theoretical yield. The enzyme is required because both ethanol production and yield were found to be limited by the degree of saccharification. The cost of the enzyme/l ethanol at a yield of 20 g ethanol/g cellulose was listed between $0.25 and $0.50. Significant improvements are obviously needed to decrease this cost by 10 times.

Recovery of dilute ethanol

Regardless of the process, ethanol concentrations produced from fiber-derived sugar streams are likely to be of the order of 4–5%. Consequently, energy costs for recovering dilute aqueous alcohol from such streams could be higher than that for the 8–10% ethanol concentrations currently processed in grain alcohol plants. The energy trade-offs in

recovering dilute alcohol can be moderated, however, by improved separations technology, for example, a process which combines distillation with adsorption, is discussed by Gulati *et al.* (1995), where concentration of 5% ethanol to a dried, fuel-grade product may be achievable at an energy input of about 20000 Btu/gallon. This approach is viable if an inexpensive adsorbent can be identified.

Impact of advances in pentose fermentation on lignocellulose processing to ethanol

The impact of D-xylose fermentation (to ethanol) for other lignocellulosic biomass materials is even greater, since more than 25% of the dry weight of lignocellulosic residues are typically xylans (Ladisch, 1989), and the economic fermentation of D-xylose to ethanol is necessary for development of a fuel ethanol industry based on the use of cellulosic residues.

Cellulosic biomass can be converted to short-chain sugar polymers, called cellodextrins, by cellulase enzymes. The cellodextrins are hydrolyzed to cellobiose and ultimately glucose. The ability of yeast to ferment cellodextrins depends on β-glucosidase to hydrolyze cellobiose to glucose. However, this enzyme is inhibited by the end product, D-glucose (Ladisch *et al.*, 1977; Gong *et al.*, 1977). Recently, Skory & Freer (1995) have isolated and cloned a *Candida wickerhamii* gene encoding a unique extracellular β-glucosidase which is highly resistant to D-glucose inhibition. The introduction of this gene into *S. cerevisiae* would produce a genetically engineered strain that could yield a cellulase system that demonstrates lower glucose inhibition and therefore could ultimately improve ethanol productivity. Wyman (1994) recommends that simultaneous saccharification/fermentation systems should be favored for ethanol production from cellulose because of the potential of such systems to lower cost. The direct fermentation of glucose to ethanol moderates glucose inhibition of the cellulase and reduces tankage by combining hydrolysis and fermentation in the same vessel.

Discussion

Theoretical bushel of corn

We define a theoretical bushel of corn as a bushel of corn where fractionation of the starch, cellulose, hemicellulose, lignin, oil and protein components can be achieved with 100% efficiency and 100% recovery. Table 2 shows that such a theoretical bushel would yield 33.93 lb of starch and subsequently 2.928 gallons of ethanol/bushel from the starch alone. When starch, hemicellulose and cellulose components are considered together, the maximum ethanol obtainable from such a bushel would be 3.351 gallons. This is calculated by using eqns (1) and (2) and assumes a 100% pretreatment and hydrolysis efficiency in converting the cellulose and hemi-celluloses to sugar and/or 100% efficiency in the D-glucose, D-xylose and L-arabinose fermentation to ethanol. Of the total yield, 6.8% is from the pentose fermentation (assumes that the hemicellulose is 100% pentoses—which it is not).

Incorporating efficiencies of hydrolysis and fermentation

Fermentation and saccharification are not 100% efficient. Incorporating an overall hydrolysis and fermentation yield in eqn (1) gives

$$A = \frac{(b)(1-m)(y_s)(g)(y_{e,g})}{(d)} \times \eta \qquad (7)$$

where

η = overall hydrolysis and fermentation yield

$$= (\eta_{hyd})(\eta_{ferm})$$

With $\eta = 0.90$, i.e. $\eta_{hyd} \times \eta_{ferm} \simeq 0.95 \times 0.95$, the ethanol yield from starch alone in a bushel of corn is 2.64 gallons and the overall yield based on starch, hemicellulose and cellulose is 3.02 gallons. For starch, y_s is 0.717 ($= 33.93/(56-8.68)$) based on data from Table 2. This represents a realistic estimate of the maximum amount of ethanol obtainable from a bushel of wet-milled corn.

Wet-milling yields

A wet-milled bushel of corn is defined as a bushel of corn where the individual components are fractionated into starch, germ cake, fiber, gluten meal, crude oil and solubles by an industrial wet-milling process. Table 3 shows that such a bushel would yield 31.94 lb of starch and subsequently a maximum of 2.757 gallons of ethanol (at $\eta = 1$) or 2.48 gallons (at $\eta = 0.9$). If the recovery ratio (R) is defined as the starch yield in wet milling versus the maximum starch obtainable from a theoretical bushel of corn, then

$$R = \frac{\text{starch from a wet-milled bushel of corn}}{\text{starch from a theoretical bushel of corn}} \qquad (8)$$

Hence, a hypothetical wet-milling process, represented by Table 3, would have an $R = 0.941 (31.94/33.93)$, i.e. would recover 94.1% of the starch as a potentially fermentable substrate.

Incremental yield from fiber

The fiber currently produced in a wet-milling process is mixed with evaporated steep water and sometimes mixed with the cake left over after the oil has been expelled from the germ. This mixture is sold as corn gluten feed and is a combination of streams (1), (3) and (4) in Fig. 1 (Ladisch & Svarczkopf, 1991). If the fiber stream is instead diverted to the production of ethanol, a theoretical incremental maximum of 0.30 gallons of ethanol can be obtained per wet-milled bushel (Table 5). This would imply

an increase of 11%, which would translate to 32–58 ¢/bushel [0.30 gallons/bushel × $1.08 to $1.95/gallon (Ladisch & Schwandt, 1992)]. D-xylose fermentation alone would account for 33% of this incremental yield. With the current microbial technology, $\eta = 0.70$ appears to be realistic and that would make 0.07 gallons/bushel attainable from D-xylose. L-arabinose fermentation would account for 0.047 gallons/bushel (if $\eta = 0.067$) and the fermentation of hexoses would give an incremental yield of 0.1 gallons/bushel (at $\eta = 0.80$). Hence, current fermentation technology would allow a maximum incremental yield of 0.217 gallons ethanol/bushel from utilization of the fiber. With current average yields of 2.5 gallons ethanol/bushel, this would imply an increase of about 8.8% in the ethanol yield, which would translate to 23–42 ¢/bushel [0.217 gallons/bushel × $1.08 to $1.95/gallon (Ladisch & Schwandt, 1992)]. Capital investments and operational costs associated with fiber-conversion processes are likely to be greater than those for incorporating the fiber into gluten feed. Hence, the extra income which might be generated will be partially offset by extra processing costs.

Approximately 1.4 billion gallons of fuel ethanol are currently being produced from starch derived from corn, with more than 60% obtained through wet-milling. If relatively impure and less expensive sugars from fiber cellulose hydrolysis were available, the starch could be diverted from alcohol production to dextrose and high fructose corn syrup production in times of tight sugar or corn supplies. This would suggest another important use of corn fiber for fermentation alcohol production.

Byproduct credits

Although the utilization of the starch streams from corn wet-milling is efficient, opportunities would appear to exist for increasing the practical yield of ethanol by applying cellulose conversion and pentose fermentation technology to a wet-milling operation. This scenario is, however, more complex than it might at first appear, since byproduct credits of gluten feed and gluten meal recouped an average of 48.9% of the corn cost (or $1.33/bushel) over the 6-year period from 1981 to 1987 (LeBlanc *et al.*, 1988). The corn gluten feed accounted for about 30% of this credit. Ethanol production resulting from utilization of fermentable sugars in the fiber and germ flour would create the need to market a new high-protein byproduct which would result if the bran, germ flour and fiber were partially removed through hydrolysis and fermentation.

Example

An example is presented here for a mid-sized plant producing about 100 million gallons of ethanol per year. It would process nearly 40 million bushels of corn/year. A recovery ratio of 94.1% would yield 1.277×10^9 lb/year of starch (corresponds to value given in Table 4). At a 90% overall hydrolysis and fermentation efficiency, this would translate to 99.1×10^6 gal ethanol/year, or $109 million (assuming the average price of ethanol to be $1.10/gallon).

Value of fiber in gluten feed

The wet milling of 40 million bushels of corn per year would also result in 179.6×10^6 lb/year of fiber (at 4.49 lb fiber/bushel of corn, Table 4). The fiber has a composition as given in Table 5 and contains 11.3% protein. It is mixed with the germ cake and concentrated steep water [streams (3) and (1) in Fig. 1] and sold as corn gluten feed containing nearly 22% protein (Ladisch & Svarczkopf, 1991). The germ cake produced is 68.8×10^6 lb/year (at 1.72 lb/bushel, Table 4) and hence the amount of corn gluten feed produced is at least 248.4×10^6 lb/year. If the gluten feed were sold at $103/ton or 4.67 ¢/lb (LeBlanc *et al.*, 1988), the returns would be $11.6 million/year. A complicating factor in this analysis is the large year-to-year variation in byproduct credits as a fraction of the corn cost.

Value of corn fiber to ethanol

If the plant converts the corn fiber to ethanol instead of combining it as corn gluten feed, the incremental ethanol yield from the fiber would be 8.9×10^6 gallons/year or $9.83 million/year. This assumes an overall hydrolysis and fermentation efficiency of 70% ($= 0.9 \times 0.78$) for the pentoses, 80% for the cellulose, 80% for the galactose in the hemicellulose and 80% for the glucose in starch. The fiber content remaining after the conversion would then be 60.6×10^6 lb/year (using the hydrolysis efficiencies and Table 5). The remaining fiber would contain approximately 3.6% starch, 38.0% hemicellulose, 0.3% lignin, 33.5% protein and 5.7% cellulose, with 18.9% other components making up the balance. The protein content in this residual fiber is nearly three times more. If combined with germ cake, the processed fiber would give at least 129×10^6 lb of high-protein gluten feed. Assuming this sells for the same price of 4.67 ¢/lb, the returns are $6.04 million/year. Assuming that the corn gluten meal sells for $236/ton or 10.7 ¢/lb (LeBlanc *et al.*, 1988), and that the higher protein gluten feed sells for 7.7 ¢/lb, the returns would be $9.96 million/year. A comparison of the two processing options (fiber as the sole byproduct versus partial hydrolysis/fermentation of the fiber) shows that the conversion of fiber to ethanol could increase income for a mid-sized plant from 4.0 to 8.2 million $/year.

Effect of processing parameters

Several processing parameters would have an influence on the economic impact. If the hydrolysis efficiency of the polysaccharides is improved, it would result in a higher conversion to the monosaccharide and an increased incremental ethanol yield. The weight of the residual fiber would

decrease but the net revenue would still be higher (1 lb of polysaccharide yields 7.6 ¢ worth of ethanol, at $\eta = 80\%$, its value in 1 lb of fiber as gluten feed is only 4.7 ¢). An increase of 2% in the overall efficiency would result in an extra 0.18 ¢/lb of polysaccharide. It would also lead to an increase in the protein content of the residual fiber and a possible increase in the value of the gluten feed. Microbiological advances are likely and will result in a higher fermentation efficiency of the pentoses. This would also increase incremental ethanol yields without affecting the amount of residual fiber content, thus resulting in a higher marginal revenue. Similarly, improvements in methods of fiber recovery would also have a favorable impact.

CONCLUSIONS

Improved technology which enables fiber conversion to be integrated into a limited number of corn ethanol plants could have a significant impact, since 10 plants account for about 75% of the ethanol produced in the U.S. On this basis, alone, the combination of fiber pretreatment, hydrolysis and fermentation has the potential to add over 70 million gallons of ethanol production, while providing test beds for new cellulose conversion technologies. The framework presented in this paper enables estimates to be generated which will help assess the potential impact of future developments in cellulose pretreatment and pentose fermentation of corn fiber streams.

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