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XYLOSE FERMENTATION ANALYSIS

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ABSTRACT

Development of a process to convert xylose to ethanol will significantly improve the economics for biochemical conversion of lignocellulosic resources to liquid fuels. The industrial yeasts currently used to ferment glucose to ethanol are unable to ferment xylose. However, alternate yeast strains, bacteria, fungi, and a xylose isomerase-yeast combination have been identified which can carry out this conversion.

In this paper, we examined the conversion of xylose to ethanol and the effects of key process variables associated with xylose conversion on the overall economics of a wood-to-ethanol plant. For the plant design considered, the maximum potential reduction in the price of ethanol derived from utilization of xylose was $0.42 per gallon from a base case price of $1.65 per gallon. The current performances of several yeasts, fungi, bacteria, and a xylose isomerase-yeast combination were examined. P. stipitis or C. shehatae appear to be capable of reducing the ethanol cost by $0.29 per gallon, or 70% of the maximum potential reduction. To equal this performance, other types of biocatalysts must have a xylose to ethanol yield of 70% and be capable of producing ethanol at a concentration greater than 2 to 2.5%. With this bench mark established, future work in biocatalyst development should be centered on those biocatalyts which show promise of significantly exceeding this mark.

XYLOSE FERMENTATION ANALYSIS

INTRODUCTION

Wood is an attractive feedstock for ethanol production because it is available at low cost and in large quantities. The primary constituents of wood are cellulose, hemicellulose, and lignin. Cellulose, the most abundant constituent, comprising about 50% of the dry weight, is a source of glucose. The abundance of cellulose has provided incentive for research aimed at improving the hydrolysis of cellulose to glucose and the subsequent fermentation of glucose to ethanol for fuel. However, economical use of wood for liquid fuel production depends upon utilization of the hemicellulose and lignin components as well.

The hemicellulose component of hardwood represents about 25% of the dry weight of wood, with D-xylose as the major sugar constituent. Unfortunately, conventional yeasts cannot ferment xylose to a fuel, and the xylose was assumed to be sent to costly waste disposal or burned as boiler fuel. However, over the past few years, several yeasts, fungi and bacteria have been discovered that can ferment xylose. In addition, xylose isomerase can be used to produce xylulose from xylose, and the xylulose can then be fermented to ethanol with certain yeasts. All of these processes offer a means for producing ethanol from hemicellulose hydrolyzates.

In this paper, we studied the conversion of xylose-to-ethanol and examined the effects of key process variables associated with xylose conversion on the overall economics of a wood to ethanol plant. The key variables examined are: yield, maximum ethanol concentration the fermentation organism can produce, and xylose conversion capital cost per annual gallon of ethanol produced from xylose. The relationship between the key process variables and overall plant economics was used to assess the impact that various yeast, fungi, bacteria, or a combination of xylose isomerase and yeast might have on the economics of wood-to-ethanol.

PROCESSES CONSIDERED

A base case, in which xylose was not fermented, and alternative cases, where xylose was fermented, were examined. All cases were based on a feed of 73, 831, kg hr^{-1} (162,729 lbs/hr) of dry wood. For the base case and both alternates, wood is partially hydrolyzed via a dilute acid pretreatment step. The main products are cellulose, lignin, and xylose. A liquid stream, containing xylose, is separated from cellulose and lignin and then neutralized. After removing gypsum, the neutralized xylose stream is ready for further processing. For the base case and alternates, the neutralized liquid stream contains 60 g L^{-1}, xylose, which seems to be the highest concentration that can reasonably be obtained using dilute acid pretreatment without a xylose concentrating step.

In the base case, the neutralized xylose stream is combined with the cellulose and lignin solids, and the slurry is sent to a simultaneous saccharification and fermentation (SSF) step where ethanol is produced from cellulose. Following SSF, lignin is removed, and the remaining solution is sent to distillation, where the ethanol is separated from water and xylose. The lignin is dried and sent to the boiler as fuel. The water/xylose stream is concentrated, and the xylose and other organics are sent to the boiler as fuel.

In the alternatives where xylose is fermented, the neutralized xylose stream is converted to ethanol via xylose fermentation utilizing a yeast, bacteria, fungi, or combined enzyme-yeast system. The resulting ethanol solution is then combined with the cellulose and lignin solids. The remainder of the process is as described in the base case, except that there is no xylose to be sent to the boiler.

The design of the prehydrolysis section was as described by Torget et al. (Torget, 1987). The liquid-solid separation step, neutralization, and gypsum removal were carried out as described in a process evaluation study by Badger Engineers, Inc. (Badger, 1984). The xylose conversion unit and the xylose isomerase unit were designed using batch reactors. A study by Raphaell Katzen and Associates (Katzen, 1978) was utilized in the design of the xylose conversion unit. The design for the SSF process and the remainder of the plant was as described by Wright et al. (Wright, 1987).

Capital cost estimates were produced with the ICARUS computer aided cost estimating program and have an accuracy of $\pm$10% for a completely defined process (ICARUS, 1987). Therefore, the overall accuracy of the cost estimate stems from the uncertainties in the process design and performance, not the estimating technique. To rapidly assess the relationships among the various steps of the process, a mathematical model was developed on Lotus 1-2-3 to calculate material and energy balances, capital and operating costs, and the ethanol selling price.

The design presented should not be viewed as that from a real operating plant but as our best estimate of current technology. While the model accurately reflects the sensitivity of the process to the key variables associated with xylose conversion unit, uncertainty in the basic design means that the absolute ethanol selling price cannot be accurately estimated. Therefore, although great care was exercised in preparing the model and economics, caution must be used when comparing the results of this study to other authors who may have used different cost estimating, economic methodologies, or other technologies.

RESULTS

Effects of Key Xylose Conversion Variables on Wood-to-Ethanol Economics

For the base case, in which none of the xylose is converted to ethanol, the cost of ethanol is $1.65/gal. For the alternate cases, the cost of ethanol varies with yield, maximum allowable ethanol concentration, and xylose conversion capital cost per annual gallon of ethanol produced from xylose. The percent theoretical yield was varied between 20% and 100%, where 100% theoretical is 0.51 g ethanol/g xylose. For a given yield, the maximum allowable ethanol concentration was varied between the maximum possible for the yield and lower values by varying the amount of dilution water added to the 6% xylose feed stream. The range of maximum allowable ethanol concentration was 1% to 3%, with three percent being the highest that can be achieved with the 6% xylose feed stream.

The capital cost per annual gallon is a function of fermentation time which, for a given yield and equipment configuration, is a function of volumetric productivity. The capital cost per annual gallon for a conversion plant which includes a xylose isomerase unit and that for a plant without the enzyme unit varies between about $0.25 and $1.00 for reasonable fermentation times. Accordingly, in this study, the xylose conversion capital cost per annual gallon of ethanol from xylose was varied between $0.25 and $1.00. However, it was found that changes in the capital cost per annual gallon have relatively minimal impact on the cost of ethanol. The difference between $0.25 capital cost per annual gallon and $1.00 per annual gallon is only about $0.05 per gallon of ethanol.

The effect of changes in the yield and allowable ethanol concentration on the cost of ethanol is shown in Tables IA and IB. For Table IA, the capital cost per annual gallon was $0.25 and the ethanol concentration in the SSF unit was limited to 4.5%, which is in accord with the current average experimental results for this process. In certain instances where the concentration of ethanol from xylose conversion was high, it was necessary to add dilution water to SSF in order to keep the ethanol concentration at 4.5%. Because adding water to SSF has a negative effect on the overall economics, we also looked at the situation where the SSF process was not limited by any particular ethanol concentration. These results are shown in Table IB.

Examination of Tables IA and IB shows that when the maximum allowable ethanol concentration for xylose conversion is limited at 1%, the cost of ethanol is generally not appreciably lower than the base case, no matter what the yield. In fact, in some instances the cost is higher. At ethanol concentrations of 1.5% or greater, there is an improvement in ethanol cost relative to the base case for all values of yield. The lowest ethanol cost is $1.23 per gallon for the case of 3% maximum allowable ethanol, 100% theoretical yield, $0.25 capital cost per annual gallon of ethanol, and no limitation on SSF ethanol concentration. This is a decrease of 25% or $0.42 per gallon from the base case of $1.65 per gallon.

The data in Table IB shows that a 1% improvement in yield at 3% maximum ethanol concentration can have an average positive impact on the cost of ethanol of 0.37 cents per gallon whereas a 1% improvement in yield at 1% maximum ethanol can have an average negative impact of 0.18 cents per gallon. Improvements in ethanol tolerance only result in improvements in the cost of ethanol. For a change in maximum allowable ethanol concentration from 10 g/L to 30 g/L at 100% theoretical yield, the improvement is 0.22 cents per gallon for every 1% improvement in maximum allowable ethanol concentration. From this, one can see that maximum allowable ethanol concentration and yield are important parameters affecting the economics and that the effects of improvements in yield can be positive or negative depending on the maximum allowable ethanol concentration.

The unexpected result that an improvement in yield can have a negative as

TABLE IA.

ETHANOL COSTS (CENTS/GALLON) AS A FUNCTION OF KEY PROCESS VARIABLES
(Ethanol concentration for SSF set at maximum of 4.5%)

$0.25 CAPITAL COST PER ANNUAL GALLON

Percent Theoretical Yield		Maximum Ethanol Concentration (%) 1.00%	1.50%	2.00%	2.50%	3.00%
	20%	157.27	157.28	157.28	157.28	157.28
	30%	155.76	153.51	153.51	153.51	153.51
	40%	157.84	149.94	150.01	150.01	150.01
	50%	159.68	146.53	146.73	146.73	146.73
	60%	161.32	145.42	143.58	143.67	143.67
	70%	162.79	145.41	140.62	140.77	140.80
	80%	164.12	145.36	137.83	138.00	138.10
	90%	165.32	145.28	135.21	135.39	135.51
	100%	167.80	145.18	134.30	132.93	133.06

TABLE IB.

ETHANOL COST (CENTS/GALLON) AS A FUNCTION OF KEY PROCESS VARIABLES
(No restriction on SSF ethanol concentration)

$0.25 CAPITAL COST PER ANNUAL GALLON

Percent Theoretical Yield		Maximum Ethanol Concentration (%) 1.00%	1.50%	2.00%	2.50%	3.00%
	20%	153.39	153.10	153.10	153.10	153.10
	30%	155.76	147.81	147.81	147.81	147.81
	40%	157.84	145.29	142.90	142.90	142.90
	50%	159.68	145.38	138.33	138.33	138.33
	60%	161.32	145.42	137.27	134.06	134.06
	70%	162.79	145.41	136.49	131.05	130.06
	80%	164.12	145.36	135.74	129.87	126.31
	90%	165.32	145.28	135.01	128.74	124.49
	100%	167.80	145.18	134.30	127.65	123.16

well as a positive effect on economics merits closer examination. If the maximum ethanol concentration is limited to 1%, we see that increasing the yield from 30% theoretical toward 100% theoretical actually increases the cost of ethanol. Moreover, for a maximum allowable ethanol concentration of 1.5%, we see that the cost of ethanol is, for all practical purposes, independent of yield from 50% to 100% of theoretical. Only when the maximum ethanol concentration is 2% or greater, do we always see a positive effect with increasing yield. This phenomena can be explained by the fact that at low allowable ethanol concentration it is necessary to add appreciable amounts of dilution water to the feed stream in order to keep the final ethanol concentration below the allowable concentration for a given yield. As yield improves, we are required to add even more dilution water to achieve the improved yield while keeping the ethanol concentration below the maximum allowable value. Unfortunately, although the economics are improved by a better yield, the overall economics are hindered by the addition of water since all downstream processes will have to have larger capacity, and greater operating expenses are incurred to heat, cool, and pump the extra water. At 1% maximum allowable ethanol, the negative effects of adding water generally outweigh the positive effects of improved yield.

The negative effects of limiting SSF ethanol concentrations to 4.5% are seen by comparing the values in Tables IA and IB for 2.5% and 3.0% maximum allowable ethanol concentration. For the case of 100% theoretical yield, 3% maximum allowable ethanol concentration, the SSF limitation increases the ethanol cost by about $0.10, which is 24% of the maximum potential cost reduction due to xylose utilization.

It might also be noticed that at low yields the costs of ethanol for 2.5% and 3.0% maximum allowable ethanol are identical. This is due to the fact that under these particular conditions actual concentrations are always less than 2.5% because of the limitation of the starting xylose concentration at 6% and no additional dilution water is required for the 3.0% over the 2.5% case.

Biocatalyst: Performance Data

As indicated previously, the key performance parameters associated with the conversion of xylose to ethanol are: yield, maximum allowable ethanol concentration, and volumetric productivity, which, for a given yield and equipment configuration, is a function of capital cost per annual gallon of ethanol from xylose. As discussed above, yield and maximum allowable ethanol concentration are the most important.

The performance parameters, associated with various biocatalysts and biocatalyst combinations were obtained from the literature. Although a comprehensive literature search was done to obtain this data, it is possible that some data was missed. Nevertheless, the data obtained is representative. All data obtained was for batch fermentation. Where the performance data included yield, final ethanol concentration, and fermentation time or volumetric productivity, the corresponding cost of ethanol was determined. These costs were obtained by incorporating the performance data into the LOTUS 1-2-3 economic model. If the fermentation time, or volumetric productivity was not given in the literature, the fermentation time was assumed to be 48 hrs for purposes of determining ethanol cost. Since fermentation time has a minimal affect on the cost of ethanol, the value assumed is not critical. An overall average ethanol cost was calculated for each type of biocatalyst using all costs determined for the particular type of biocatalyst. In addition, an average cost of ethanol was calculated for certain types of biocatalysts using only costs associated with performance data where the initial xylose concentration was close to the feed concentration of 60 g L^{-1} used in this study. A summary of the ethanol costs associated with each type of biocatalyst is given in Table II.

A considerable amount of data exists for three types of xylose fermenting yeast, i.e, P. tannophilus, C. shehatae, and P. stipitis. For these yeasts, it appears that C. shehatae and P. stipitis are better than P. tannophilus. Data for other yeasts is scanty, but none of these yeasts performed better than the average performance of either C. shehatae or P. stipitis. The reduction in ethanol cost by converting xylose to ethanol using C. shehatae and P. stipitis is about $0.29 per gallon, which is about 70% of the maximum possible reduction of $0.42 per gallon.

Data for fungi, bacteria, and SFIX is limited and, therefore, it is difficult to assess their economic performance with the same degree of confidence as can be done with yeasts. However, in general, the data for these biocatalysts does not suggest that they are currently capable of attaining the performance of the best yeasts.

DISCUSSION

This study has shown that for the wood-to-ethanol plant described, conversion of xylose to ethanol in a batch system results in a maximum reduction in the price of ethanol of $0.42 from a base case cost of $1.65 per gallon. Three key process variables associated with xylose conversion that impact the economics of a wood to ethanol plant are yield, maximum allowable ethanol concentration, and volumetric productivity. This study has shown that yield and maximum allowable ethanol concentration are the most important, while volumetric productivity has a relatively small impact.

Yield has importance because at a given wood feed rate, each increase in yield translates directly into an increase in revenue. Maximum allowable ethanol concentration has importance because if the allowable concentration is not high enough, it is necessary to add dilution water to the feed stream to the xylose conversion unit in order to achieve the maximum possible yield that the biocatalyst is capable of. Unfortunately, while the addition of water permits the maximum possible yield to be achieved, it has a negative impact on all the other units downstream of the xylose conversion unit. Addition of water increases the size of SSF, distillation, the concentration unit, and the waste treatment unit. Moreover, the load on the utility systems also increases. Volumetric productivity is of minor importance because it only impacts the size of the xylose conversion unit which is a relatively small percentage of the total capital cost of the plant.

Various biocatalysts were examined for their potential performance in converting xylose to ethanol. Of the xylose fermenting yeasts, P. stipitis and C. shehatae appear to be best, since they are currently capable of achieving 70% of the maximum possible ethanol cost reduction. To equal this

TABLE II.

SUMMARY OF ETHANOL COSTS ASSOCIATED WITH EACH TYPE OF BIOCATALYSTS

BIOCATALYST	RANGE OF ETHANOL COST ($/GAL)	AVERAGE ETHANOL COST ($/GAL)	AVERAGE ETHANOL COST WHERE INITIAL XYLOSE IS ~60g L^{-1} ($/GAL)
Yeast			
Pachysolen-tannophilus	1.30-1.62	1.48	1.41
Candida Shehatae	1.26-1.49	1.36	1.35
Pichia Stipitis	1.26-1.64	1.37	1.36
Other Yeasts	1.37-1.65		
Fungi			
Fusarium Oxysporum VTT-D-80134	1.28	1.28	1.28
Fusarium F5	1.31-1.43	1.36	
Paecilomyces Sp NF1	1.30-1.40	1.32	1.40
Bacteria	1.41-1.63	1.52	
SFIX	1.28-1.55	1.40	

performance, other types of biocatalysts must have a yield of 70% and be capable of producing an ethanol concentration greater than 2 to 2.5%. With this bench mark established, all future work in biocatalyst development for xylose conversion to ethanol should be geared toward exceeding this performance. Specifically, future work should be centered on those biocatalysts which show promise of achieving 90% yield and producing 3% ethanol.

RECOMMENDATIONS

The current performance of the best xylose fermenting yeasts (P. stipitis and C. shehatae) warrants a detailed investigation of the possible use of these biocatalysts in converting xylose to ethanol. Specifically, a study should be conducted to examine the engineering questions surrounding the utilization of these organisms. For example, questions concerning the control of oxygen under microaerophilic conditions need to be addressed.

Additional performance data should be obtained for those biocatalysts which show promise of achieving 90% yield and producing 3% ethanol. Researchers should be encouraged to obtain data which can be readily used to evaluate microbe performance. Yield, maximum allowable ethanol concentration, volumetric productivity, and specific productivity as functions of pH, temperature, etc., are desired information.

Because maximum allowable ethanol concentration can have a major impact on economics, it would be desirable to examine engineered processes for maintaining low ethanol concentrations in a fermenter while the feed stream has a relatively high substrate concentration. Such processes would improve the performance of SSF, which could limit the potential of the xylose conversion unit due to its current experimental ethanol tolerance of 4.5%.

In the SFIX process, xylose isomerase and the xylulose fermenting yeast are placed in the same reactor. A problem is that the enzyme and yeast have different pH optima. An engineering study should be conducted to compare the SFIX process with alternate process configurations which allow xylose isomerase and the yeast to operate at their optimal pH values. For example, it may be effective to carry out xylose isomerization in a separate reactor from the fermentation. The unisomerized xylose would eventually be recycled back to the isomerization reactor. This process would permit the enzyme and yeast to operate at their optimal pH values. Alternately, it may be possible to put the enzyme and yeast in the same reactor and oscillate the pH between the optimum for the enzyme and the optimum for the yeast or solve the enzyme-yeast pH compatibility problem by ionic immobilization, etc.

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