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Value-Added Chemicals from Animal Manure

* S. Chen
* W. Liao
* C. Liu
* Z. Wen
* R. L. Kincaid
* J.H. Harrison
D. C. Elliott
M. D. Brown
A. E. Solana
D. J. Stevens

* Washington State University

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under Contract DE-AC06-76RL01830

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Northwest Bioproducts Research Institute – Report #1

Value-Added Chemicals from Animal Manure

Final Technical Report

S. Chen
W. Liao
C. Liu
Z. Wen
R. L. Kincaid
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D. C. Elliott
M. D. Brown
A. E. Solana
D. J. Stevens

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Richland, Washington 99352

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Final Technical Report

PNNL-14495

Submitted by

Shulin Chen, Associate Professor, Department of Biological Systems Engineering
Phone: 509-335-3743; Fax: 509-335-2722; E-mail: chens@wsu.edu
Wei Liao, doctoral candidate, Department of Biological Systems Engineering
Chaunbin Liu, postdoc, Department of Biological Systems Engineering
Zhiyou Wen, postdoc, Department of Biological Systems Engineering
Ronald L. Kincaid, Professor, Department of Animal Science
Joseph H. Harrison, Professor, Department of Animal Science
Washington State University, Pullman, WA 99164

Douglas C. Elliott, Staff Scientist, Chemical and Biological Process Development
Michael D. Brown, Senior Research Engineer, Chemical and Biological Process Development
Amy E. Solana, Research Engineer, Technology Systems Analysis Group
Don J. Stevens, Senior Program Manager, Technology Systems Analysis Group
Pacific Northwest National Laboratory, Richland, WA 99352

December 19, 2003

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Project Objective

The objective of the project proposed by Washington State University (WSU) and Pacific Northwest National Laboratory (PNNL) was to develop technology for using animal manures as feedstocks to produce value-added products. These products include medium-volume commodity chemicals, such as glycols or diols, and protein-based products, such as chemicals or feed supplements. The research focused on two aspects of this approach, including the analysis and treatment of the feedstock to produce intermediate chemical precursors and the aqueous phase conversion of these intermediates to chemicals and other value-added products.

Project Organization

This research project consisted of four tasks that provided detailed information to determine how the carbohydrate- and protein-based building blocks from manure could be recovered and converted to high-value chemical products. The first task was the development of detailed chemical information about the components in manure. The second task was evaluation of separation methods to recover the carbohydrate and protein-based chemical building blocks in the feedstock. The third task was to obtain detailed information about the aqueous phase chemistry used to convert these building blocks in the mixture solutions into chemicals. The fourth task was to analyze the availability of manures, the economic potential of the conceptual processes, and the ability of these processes to sequester carbon or produce overall carbon savings. This technical report presents the results from these four tasks, along with conclusions and suggestions for future studies. Publications and presentations from the project are also given.

Technical Report

Task 1.
Development of detailed chemical information about the components in manure.

It is estimated that about 160 million tons of animal manures are produced annually in the United States (Council for Agricultural Science and Technology, 1995). This waste is difficult to dispose of, and contributes to environmental problems such as greenhouse gas release and contamination of streams and ground water. The complex physical and chemical composition of manure makes it difficult to utilize, and limited efforts to convert manure to higher-value chemicals and energy on a commercial scale have largely been unsuccessful. As a result, manure is an abundant but under-used biomass resource for producing biobased chemicals and energy. However, creating new, innovative processes and pathways would allow this resource to be better utilized and simultaneously reduce environmental impacts. Developing an economical animal manure utilization process would also provide alternatives to manure treatment and would help increase farmers' incomes while decreasing of greenhouse gas release. Animal manures contain large quantities of lignocelluloses, polysaccharides, proteins, and other biological materials, and the ability to convert these materials into value-added products has been recognized as an attractive technology.

Utilization of manure has led to chemical analyses for a better understanding of the utilization process and insights into improving process efficiencies. However, the analyses have been limited in the elemental compositions and general classes of chemicals reported in the literature (Fontenot, et al. 1983; McCaskey, 1995). Additional information on detailed chemical compositions of animal manures, especially fiber (including cellulose, hemicellulose, and lignin) and protein contents is critical for the future utilization of manures as a biomass for producing value-added products.

The objective of this task is to provide basic information on the composition and characteristics of different types of manures. The major project activities in this task were characterization of cattle, swine, and poultry manures. Parameters analyzed included total solids; water content; fiber (cellulose, hemicellulose, lignin); crude protein and detailed amino acids composition; monosaccharide content; and elemental composition (C, N, P, K, S, Na, Ca, Mg, Cu, Zn, Fe, Al, Cr, Mo, Ni, Pb). The data presented in this report, including nutrients, minerals, fibers, and amino acid contents in diary, beef, poultry, and swine manures, can be used

to evaluate the potential of these manures as sugar and protein sources as well as their nutrient values. This work will contribute to related knowledge for manure utilization, waste management, and new American Society of Agricultural Engineers animal manure characterization standard update.

The dairy manure used for the experiments was collected at the Dairy Center of Washington State University (WSU). Manure produced at the WSU dairy center was managed by a typical flush system. The flushed manure was separated into a solid and a liquid fraction by a stationary inclined screen separator. The fresh, clean "as excreted" dairy manure (feces) samples and manure solids below the stationary screen separator were collected and stored in a refrigerator before usage. The beef, poultry and swine manures (fresh feces) were collected by WSU Puyallup Research & Extension Center from waste piles at several farms within Washington State. Additional poultry manure samples were taken at different times of storage. A shovel was used to dig into each waste pile about 2 ft, then collect the samples.

Analysis of total solids (TS), total volatile solids (TVS), total carbon, total phosphorus and total sulfur were performed using standard methods (APHA, 1998). Nitrogen and crude protein were determined using AOAC method (Association of Official Analytical Chemists, 1990). The neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of the manure were analyzed using the reflux apparatus (Goering, et al. 1970). Analysis for trace elements was done by inductively coupled plasma atomic emission spectroscopy (ICP-AES) at the University of Idaho Analytical Sciences Laboratory (Moscow, ID) using the procedures of Anderson (1996). Amino acids (AA) were analyzed by Experiment Station Chemical Laboratories at the University of Missouri-Columbia (Columbia, MO) using a Beckman 6300 AA analyzer following the AOAC (1990) procedure.

Characterization of cattle manures

Basic nutrient information of cattle manures

Traditional parameters used for animal waste characterization mainly include two physical properties, i.e., total solids (TS) and moisture content (MC), and three chemical constituents—nitrogen (N), phosphorus (P), and potassium (K). As land application of agricultural waste is the primary waste utilization procedure, and N, P, and K are the principal

components considered in the development of an agricultural waste management plan. The "as excreted" manure samples collected from dairy and beef farms were characterized. The results of the above-mentioned traditional parameters used for manure characterization and some other elements are shown in Table 1.1.

Table 1.1. Basic nutrient information for cattle manure characterization

Parameter	Content in manure (%)			Elements	Content in dry manure (%)		
	Dairy	Beef	Feedlot		Dairy	Beef	Feedlot
Water	86.61	87.44	78.39	Nitrogen	2.90	1.94	2.72
Total solids	13.39	12.56	26.61	Phosphorus	0.48	0.42	0.81
Total volatile solids	11.21	9.97	22.78	Potassium	2.86	1.44	0.92
Total fixed solids	2.18	2.59	3.83	Carbon	45.37	43.81	43.56

Fiber content in cattle manure

The information presented in Table 1.2 can satisfy the requirement of land application of cattle manure. However, it is not sufficient enough for the study of animal manure as feedstock for value-added chemicals production. More detailed information on manure composition, such as cellulose, hemicellulose, lignin, and protein, and even amino acid content, are seriously needed for biorefinery study.

The content of cellulose, hemicellulose, and lignin in manure can be determined by the analysis of NDF, ADF and ADL using the reflux apparatus (Goering, et al. 1970). NDF is usually used to estimate the total lignocellulosic materials (including cellulose, hemicellulose, and lignin), while ADF is used to estimate the content of lignin and cellulose. Hemicellulose content can be determined by the difference between them (%NDF-%ADF). The fiber composition of the cattle manures used in this study is given in Table 1.2. It is apparent that the fiber was the largest component in cattle manures, as NDF content in dairy, beef, and feedlot manure is 52.6%, 51.5%, and 41.7% of dry matter, respectively. Fiber provides the substantial resources of cellulose and hemicellulose, which can be degraded to mono-sugars and then used as feedstocks to produce value-added products like glycols and diols.

Table 1.2. Fiber content in cattle manures

Parameter	Content in manure (% dry base)		
	Dairy	Beef	Feedlot
NDF	52.6	51.5	41.7
ADF	40.4	34.1	20.3
ADL	13.0	12.2	6.1
Cellulose (=ADF-ADL),	27.4	21.9	14.2
Hemicellulose (=NDF-ADF)	12.2	17.4	21.4

Protein and detailed amino acid content in dairy manure

In addition to fiber, protein is also an important composition of animal manures. Crude protein content in dairy manure is 18.11% (dry base) as the total nitrogen in manure is 2.90% (dry base). The content of crude protein in beef and feedlot manure is 25.13% and 17.00%, respectively. In order to explore the possibility of developing protein-based products from manures, the detailed amino acid composition of cattle manures was analyzed. The results are shown in Table 1.3.

Elements content in dairy manure

The information of macro and micro element contents in manure is important for the utilization of this low value feedstock, because some elements, such as magnesium and calcium, are essential for the metabolism of microorganisms that may be used for the conversion process. The contents of calcium, magnesium, copper, zinc, iron, sulfur, aluminum, cadmium, cobalt, chromium, manganese, molybdenum, nickel, lead, and vanadium in cattle manures were analyzed. The results are presented in Table 1.4.

Characterization of poultry manures

Similar to cattle manure, samples of poultry manure collected from the house of chick starter, pullet grower, 17-40 weeks, and post-molt diet chicken were collected and analyzed. The

Table 1.3. Amino acid composition of cattle manures

Amino Acid	Content in manure (% dry base)		
	Dairy	Beef	Feedlot
Taurine	0.06	0.06	0.06
Hydroxyproline	0.08	0.19	0.02
Aspartic acid	0.73	0.82	1.00
Threonine	0.36	0.37	0.57
Serine	0.30	0.35	0.43
Glutamic acid	1.46	1.09	1.93
Proline	0.49	0.44	0.75
Lanthionine	BDL	0.01	0.02
Glycine	0.82	0.66	0.56
Alanine	0.82	0.61	0.72
Cysteine	0.14	0.12	0.23
Valine	0.49	0.44	0.64
Methionine	0.12	0.11	0.23
Isoleucine	0.38	0.33	0.5
Leucine	0.60	0.54	0.82
Tyrosine	0.15	0.21	0.36
Phenylalanine	0.32	0.32	0.58
Hydroxylysine	BDL	0.01	BDL
Histidine	0.09	0.14	0.24
Ornithine	0.03	0.02	0.03
Lysine	0.24	0.37	0.63
Arginine	0.24	0.35	0.48
Tryptophan	BDL	0.05	0.09
Total	7.92	7.61	10.89

* BDL – Below Determination Limit

results of fiber, amino acids, and elements content in these samples are listed collectively in Table 1.5.

Characterization of swine manure

Samples of swine manure collected from the house of nursery, finish, phase 2, and phase 3 pigs were collected and analyzed. The results of fiber, amino acids, and elements content in these samples are listed collectively in Table 1.6.

Table 1.4. Content of elements in cattle manures

Elements	Content in dry manure		
	Dairy	Beef	Feedlot
Calcium, %	1.2	1.06	0.69
Magnesium, %	0.55	0.3	0.34
Sodium, %	0.47	0.25	0.12
Copper, ppm	30	1.44	18
Zinc, ppm	320	0.42	87
Iron, ppm	300	590	550
Sulfur, %	0.31	250	0.21
Aluminum, ppm	140	170	210
Cadmium, ppm	BDL	BDL	BDL
Cobalt, ppm	0.89	1.2	1.6
Chromium, ppm	2.1	2.1	1
Manganese, ppm	510	600	120
Molybdenum, ppm	2.6	2.4	0.78
Nickel, ppm	9.7	10	BDL
Lead, ppm	BDL	BDL	BDL
Vanadium, ppm	4.7	5.6	BDL

* BDL – Below Determination Limit

Comparison of fiber and crude protein content in cattle, poultry, and swine manure

The diets of cattle, swine, and poultry are different, and the capability of different animal species in digestion and utilization of protein and fiber components in diet varies dramatically; as a result, the composition of cattle, swine, and poultry manure is not the same. The contents of fiber (include hemicellulose, cellulose, and lignin) and crude protein in manure are of most concern, because they are the major components that can be converted into value-added products. The fiber and protein composition of cattle, swine, and poultry manure are compared in Table 1.7.

Table 1.5. Characterization of poultry manures

Parameter	Content in dry poultry manure			
	Chick starter	Pullet grower	17-40 weeks	Post-molt diet
NDF, %	31.7	36.4	34.5	31.2
ADF, %	13.4	14.9	14.3	14.8
Lignin, %	4.9	7.2	3.2	4.1
Nitrogen, %	6.37	7.74	2.93	3.31
Taurine, %	0.21	0.21	0.06	0.04
Hydroxyproline, %	0.23	0.24	0.19	0.17
Aspartic acid, %	1.22	1.31	0.82	1.09
Threonine, %	0.59	0.74	0.37	0.55
Serine, %	0.54	0.88	0.35	0.5
Glutamic acid, %	1.64	1.95	1.09	1.48
Proline, %	0.72	1.22	0.44	0.54
Lanthionine, %	0.01	0.01	0.01	0.01
Glycine, %	2.11	2.53	0.66	1.27
Alanine, %	1.14	1.23	0.61	0.76
Cysteine, %	0.31	0.59	0.12	0.18
Valine, %	0.77	1.02	0.44	0.60
Methionine, %	0.2	0.22	0.11	0.18
Isoleucine, %	0.56	0.73	0.33	0.44
Leucine, %	0.94	1.25	0.54	0.76
Tyrosine, %	0.33	0.48	0.21	0.31
Phenylalanine, %	0.53	0.71	0.32	0.45
Hydroxylysine, %	0.02	0.02	0.01	0.01
Histidine, %	0.21	0.26	0.14	0.21
Ornithine, %	0.11	0.13	0.02	0.02
Lysine, %	0.6	0.73	0.37	0.47
Arginine, %	0.43	0.76	0.35	0.5
Tryptophan, %	0.1	0.09	0.05	0.07
Total amino acids, %	13.52	17.31	7.61	10.61
Calcium, %	4	4	9.6	6.9
Magnesium, %	0.66	0.68	0.91	0.96
Sodium, %	0.77	0.64	0.72	0.6
Potassium, %	2.7	2.5	3.9	3.8
Phosphorus, %	2.3	2.6	3.4	3.2
Copper, ppm	46	44	59	48
Zinc, ppm	480	400	500	410
Iron, ppm	79	56	190	250
Sulfur, %	0.56	0.65	0.76	0.70
Aluminum, ppm	55	84	190	220
Cadmium	BDL*	BDL	BDL	BDL
Cobalt, ppm	0.41	0.43	0.67	0.54
Chromium, ppm	0.81	1	1.7	1.5
Manganese, ppm	370	400	540	460
Molybdenum, ppm	1.5	1.5	3	3.6
Nickel, ppm	6.7	6.2	12	11
Lead, ppm	BDL	BDL	BDL	BDL
Vanadium, ppm	BDL	BDL	BDL	BDL

* BDL – Below Determination Limit

Table 1.6. Characterization of swine manures

Parameter	Content in dry swine manure		
	Nursery	Grower	Finisher
NDF, %	39.2	39.1	37.4
ADF, %	17.3	18.7	15.8
Lignin, %	4.1	5.4	2.9
Nitrogen, %	4.02	4.26	3.90
Taurine, %	0.08	0.06	0.07
Hydroxyproline, %	0.03	0.09	0.01
Aspartic acid, %	1.7	2.01	1.61
Threonine, %	0.82	1	0.77
Serine, %	0.57	0.77	0.54
Glutamic acid, %	2.3	2.54	2.15
Proline, %	0.83	0.95	0.76
Lanthionine, %	0.03	0	0.04
Glycine, %	0.95	1.13	0.84
Alanine, %	1.28	1.37	1.12
Cysteine, %	0.28	0.39	0.3
Valine, %	1.11	1.13	1.08
Methionine, %	0.4	0.49	0.37
Isoleucine, %	0.91	0.92	0.93
Leucine, %	1.4	1.65	1.34
Tyrosine, %	0.63	0.69	0.56
Phenylalanine, %	0.9	0.97	0.84
Hydroxylysine, %	BDL	0.01	BDL
Histidine, %	0.33	0.43	0.29
Ornithine, %	0.05	0.03	0.08
Lysine, %	1.1	1.33	1.13
Arginine, %	0.73	0.88	0.7
Tryptophan, %	0.13	0.15	0.1
Total amino acids, %	16.56	18.99	15.63
Calcium, %	1.6	4.2	2.9
Magnesium, %	0.61	0.86	0.62
Sodium, %	0.28	0.28	0.15
Potassium, %	1.6	1.6	1.2
Phosphorus, %	1.5	2.45	1.6
Copper, ppm	580	1786	1200
Zinc, ppm	590	2167	660
Iron, ppm	250	2566	510
Sulfur, %	0.41	0.55	0.37
Aluminum, ppm	580	620	1000
Cadmium	BDL	BDL	BDL
Cobalt, ppm	1	1.5	1.5
Chromium, ppm	33	3.7	21
Manganese, ppm	280	740	340
Molybdenum, ppm	1.1	3.8	BDL
Nickel, ppm	8.5	15	6.3
Lead, ppm	BDL	BDL	BDL
Vanadium, ppm	3.3	7.4	3.2

* BDL – Below Determination Limit

Table 1.7. Comparison of fiber and protein contents in cattle, swine and poultry manures

		Crude protein, % DM	Total fiber, % DM	Hemicellulose, % DM	Cellulose, % DM	Lignin, % DM
Cattle manures	Dairy	18.1	52.6	12.2	27.4	13.0
	Beef	12.1	51.5	17.4	21.9	12.2
	Feedlot	17.0	41.7	21.4	14.2	6.1
Swine manures	Nursery	25.1	39.2	21.9	13.2	4.1
	Grower	22.7	40.8	20.5	13.9	6.4
	Finisher	22.0	39.1	20.4	13.3	5.4
Poultry manures	Chick starter	39.8	31.7	18.3	8.5	4.9
	Pullet grower	48.4	36.4	21.5	7.7	7.2
	17-40 weeks	31.6	34.5	20.2	12.0	2.3
	Post-molt	28.0	31.2	16.4	10.7	4.1

DM = dry matter

From the results presented in Table 1.7, we can clearly see that the protein contents of poultry manures were the highest among the manure types analyzed, accounting for 1/3 to 1/2 of the dry matter. Swine manures had about 1/4 of dry matter as protein, while protein contributed to no more than 1/5 of dry matter in cattle manures. In contrast, the fiber content in cattle manures was the highest, accounting for more than half of the dry matter. Total fiber in swine and poultry manures was 40% or less of dry matter. Because of the high fiber content and low protein concentration, cattle manure is most suitable for mono-sugar production. However, the utilization of animal manures is more difficult than the utilization of other lignocellulotic biomass such as wood and straw, because of the complicated composition and substantial protein content.

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Task 2.
Evaluation of separation methods to recover the carbohydrate- and protein-based chemical building blocks in the feedstock.

The major activities for this task were solid/liquid separation, hydrolysis of manure, and fungus culture for cellulase production from manure.

Solid/liquid separation of manure

Animal manure is a complicated solid/liquid mixture. Apart from fiber, it contains certain amounts of protein-enriching components, including undigested protein, urine, microbe, etc. These protein-enriching components have negative effects on sugar yield because of the formation of colored materials by Maillard reactions. In addition, soluble protein in manure hydrolysate solution inhibits the catalyst in the catalytic hydrogenation of sugars. Therefore, an operation to remove protein-enriching materials from manure is necessary for producing of value-added products.

Solid/liquid separation can be an effective manure treatment method for producing nutrient-rich organic solids for multiple uses and potentially reducing odor generation. The majority of nutrients, especially most carbohydrates, are in solid states. To change these sugars into valuable materials, solid/liquid separation should be the first step for manure biorefinery. The objectives of this part of the research are to (1) examine the particle-size distribution of solid manure; and (2) investigate the nutrient distribution among liquid and solid particles of different size.

Size distribution of dairy manure solids

The size distribution of particles in dairy manure was determined by sieving the manure with a set of American Standard sieves. Manure samples were added at the top of six sieves of decreasing mesh size. The manure sample was washed through the continuous shaking sieves with mesh sizes of 2.4, 1.68, 1.19, 0.84, 0.42, and 0.125 mm one by one. Total solids of each portion of particles and the filtrate were measured. The nutrient content, including total carbon, total nitrogen and total sulfate, were also analyzed. From the results of total solids of each portion, as presented in Table 2.1, we can clearly see that dairy manure is mainly composed of

particles larger than 1.680 mm. This fraction accounts for 56.36% of total solids. Fine particles contribute 23.84% of total solids of manure passed through a 0.125-mm sieve. The amount of fiber over 1 cm is significantly larger than the amount of smaller sized material. Consequently, it is easy to remove the protein-enriching materials from fiber by sieving.

Table 2.1. Total solids of different portions after solid/liquid separation

Portion	1.680 mm	1.190 mm	0.840 mm	0.420 mm	0.125 mm	Filtrate	Total
TS/g	31.25	2.49	2.62	3.16	2.71	13.216	55.446
%	56.36	4.49	4.73	5.70	4.88	23.84	100

In addition to particle size distribution, the nutrient content of each portion was measured. Results are given in Table 2.2. The carbon content of solids with particle size larger than 0.168 mm was a bit higher compared with other five portions, of which the carbon content of each portion varied little. The existence of a certain amount of feed particles mixed with manure should contribute to the higher carbon content of solid with a particle size larger than 0.168 mm. We can also notice that the total nitrogen content of filtrate is rather high compared with other solid particles.

Table 2.2. Nutrient content distribution of dairy manure

Portion	C %	S %	N %
>1.680 mm	43	0.3421	2.405
>1.190 mm	39.64	0.3646	2.94
>0.840 mm	40.85	0.3439	3.03
>0.420 mm	41.31	0.3983	2.97
>0.125 mm	39.22	0.4569	2.963
Filtrate	37.67	0.2751	7.125

In order to study the effects of solid/liquid separation and small particle removal on acid hydrolysis, hydrolysis of manure solids after sieving operation and raw manures were compared. As shown in Table 2.3, the yield of total reducing sugar from manure solids after the sieving treatment is a little bit higher than that from raw manure. Meanwhile, the color of hydrolysate, a direct indication of Maillard byproducts content, is significantly different. It indicates that the formation of Maillard compounds from raw manure was five times over that from manure solids

after sieving treatment. Therefore, solid/liquid separation is an essential step for a manure biorefinery.

Table 2.3. Effects of solid/liquid separation and small particles removal on acid hydrolysis

	Raw manures		Manure solids after sieving treatment	
Acid concentration	1%	3%	1%	3%
Total reducing sugar (g/L)	0.597	1.054	0.811	1.075
A_{540}	0.674	0.698	0.120	0.134

Coagulation-flocculation treatment of dairy manure liquids

Solid/liquid separation is an effective manure treatment method for producing nutrient-rich organic solids for further uses. However, the liquid stream after solid/liquid separation still has a certain amount of solid particles. Coagulation-flocculation treatment has been proven to be an effective method for separating such solids. In order to develop a whole manure utilization process, the removal of suspended solids from the liquid stream after solid/liquid separation via coagulation-flocculation treatment was further studied. The flocculants used were ferric chloride-$FeCl_3.6H_2O$ having a gram molecular weight of 270.3 and alum-$Al_2(SO_4)_3.18H_2O$ with a molecular weight of 666.4.

A conventional jar test procedure (Phipps and Bird apparatus) was applied at various coagulant dosages. The filtrate obtained from the sieving operation was first diluted by distilled water to 1% total solid content before coagulation-flocculation treatment. The coagulant was added to six beakers, 800 ml each, with raw effluent and mixed at 120 rpm for 60 s. The paddle speed was adjusted thereafter to 30 rpm for the next 20 min. Observations on floc formation were made. The slow mixing was stopped, and the suspension was allowed to settle for 30 min. After settling, samples were drawn from 4 cm below water surface and checked for turbidity. The detailed results are given in Tables 2.4 and 2.5.

Table 2.4. Effects of $FeCl_3$ on nutrients removal from dairy manure liquids

$FeCl_3$, g/L	1.0	1.5	2.0	2.5	3.0
Total Suspended Solids removal, %	15.7	46.3	61.9	70.2	71.1
Total N removal, %	7.2	28.5	36.4	38.6	39.1
Total P removal, %	18.5	39.7	52.4	58.9	61.6

The results indicated that suspended solids and nutrients can be effectively removed by both ferric chloride-$FeCl_3.6H_2O$ and alum-$Al_2(SO_4)_3.18H_2O$. However, $FeCl_3.6H_2O$ is more effective in phosphorus removal, and $Al_2(SO_4)_3.18H_2O$ is more effective in suspended solid removal.

Table 2.5. Effects of $Al_2(SO4)_3$ on nutrients removal from dairy manure liquids

$Al_2(SO_4)_3$, g/L	1.0	1.5	2.0	2.5	3.0
TSS removal, %	19.2	52.1	68.4	74.3	78.5
TN removal, %	8.3	30.2	39.1	40.8	41.6
TP removal, %	10.5	30.9	44.7	47.6	50.1

Hydrolysis of manure

The focus of this part of study is on the conversion of manure fiber to mono-sugars such as glucose and xylose. Since the 1910's, various methods have been studied to hydrolyze fibrous materials, with acid hydrolysis and enzymatic hydrolysis the most commonly used methods.

Manure is a special fibrous material, which has a nitrogen content of around 2.6% that is considerably higher than the 1% of other fibrous materials such as wheat straw. Proper conditions for hydrolysis of manure, though, are potentially different from wood and straw fibers because of higher nitrogen levels and differences in composition and structure complexity. The nitrogen is in the form of indigestible forage proteins, proteins from the metabolism of rumen bacteria, and inorganic nitrogen such as urine and ammonia. During the hydrolysis, especially acid hydrolysis, ammonia and amino acids from hydrolyzed protein can react with mono-sugars in the hydrolyzed solution, and ultimately can influence the final sugar yield. Additionally, the fiber in manure has already undergone some breakdown while passing through the animal's digestive tract. As a result, the conditions and yield for acid hydrolysis of manure fiber would most likely be affected.

Little research has been reported on hydrolysis of manure fiber with high nitrogen content using either acid hydrolysis or enzymatic hydrolysis. The specific objectives of this study were to compare these two methods for manure fiber hydrolysis and find their optimal conditions in terms of sugar yields from both hemicellulose and cellulose in manure.

Acid hydrolysis

Acid hydrolysis is widely used in the paper industry to treat lignocellulosic materials. Acid first cleaves the matrix structure of fiber into cellulose, hemicellulose, and lignin, and then further reduces these polysaccharides to mono-sugars. This type of application most commonly utilizes either concentrated acid hydrolysis at low temperatures or dilute acid hydrolysis at high temperatures. Dilute acid hydrolysis benefits to eliminate the plant hazards and potential environmental problems that are concerns commonly associated with the use of concentrated acid hydrolysis, but it has a relatively low sugar yield. Compared with dilute acid hydrolysis, concentrated acid hydrolysis has advantages of high sugar yield and low reaction temperature, and negatives of high operation cost and environmental problems. In terms of finding the best method of acid hydrolysis of manure, the comparison of different acid hydrolysis procedures were compared.

1.1. Comparison of different acid hydrolysis procedures. The sample manure came from the WSU dairy center. Its composition is given in Table 2.6. Five kilograms of manure were mixed with 2.5 kg of water and blended for 1 min on the liquefy setting for size reduction.

Table 2.6. Characteristics of original manure[a]

	Original manure
Dry Matter, %	15.50 ± 0.092
Crude Protein, % dry matter	16.44 ± 0.53
NDF, % dry matter	48.27 ± 0.46
ADF, % dry matter	35.80 ± 0.14
ADL, % dry matter	13.91 ± 0.45
Cellulose (=ADF-ADL), % dry matter	21.89 ± 0.38
Hemicellulose(=NDF-ADF), % dry matter	12.47 ± 0.32
N, % dry matter	2.63 ± 0.086
C, % dry matter	45.49 ± 0.30

[a] all data are the average of triplicates with standard deviations of the means (n=3) at α=0.05.

Five individual acid hydrolysis procedures were used for the comparison: one-stage high temperature acid hydrolysis; one-stage acid hydrolysis with decrystallization; two-stage dilute acid hydrolysis; two-stage acid hydrolysis with alkaline extraction; and two-stage acid hydrolysis with decrystallization (Figure 2.1). Among these procedures, the conditions of the first stage of

the two-stage acid hydrolysis procedures were the optimal conditions for dilute acid hydrolysis of hemicellulose in the original manure (see section 1.2.1). The conditions for one-stage high-temperature hydrolysis and the second stage hydrolysis of the two-stage procedures, except the one with decrystallization, are based on the optimization of one-stage high-temperature hydrolysis (data not shown). The conditions of decrystallization were from Arkenol's process. For all procedures, sample concentration was 10% dry manure.

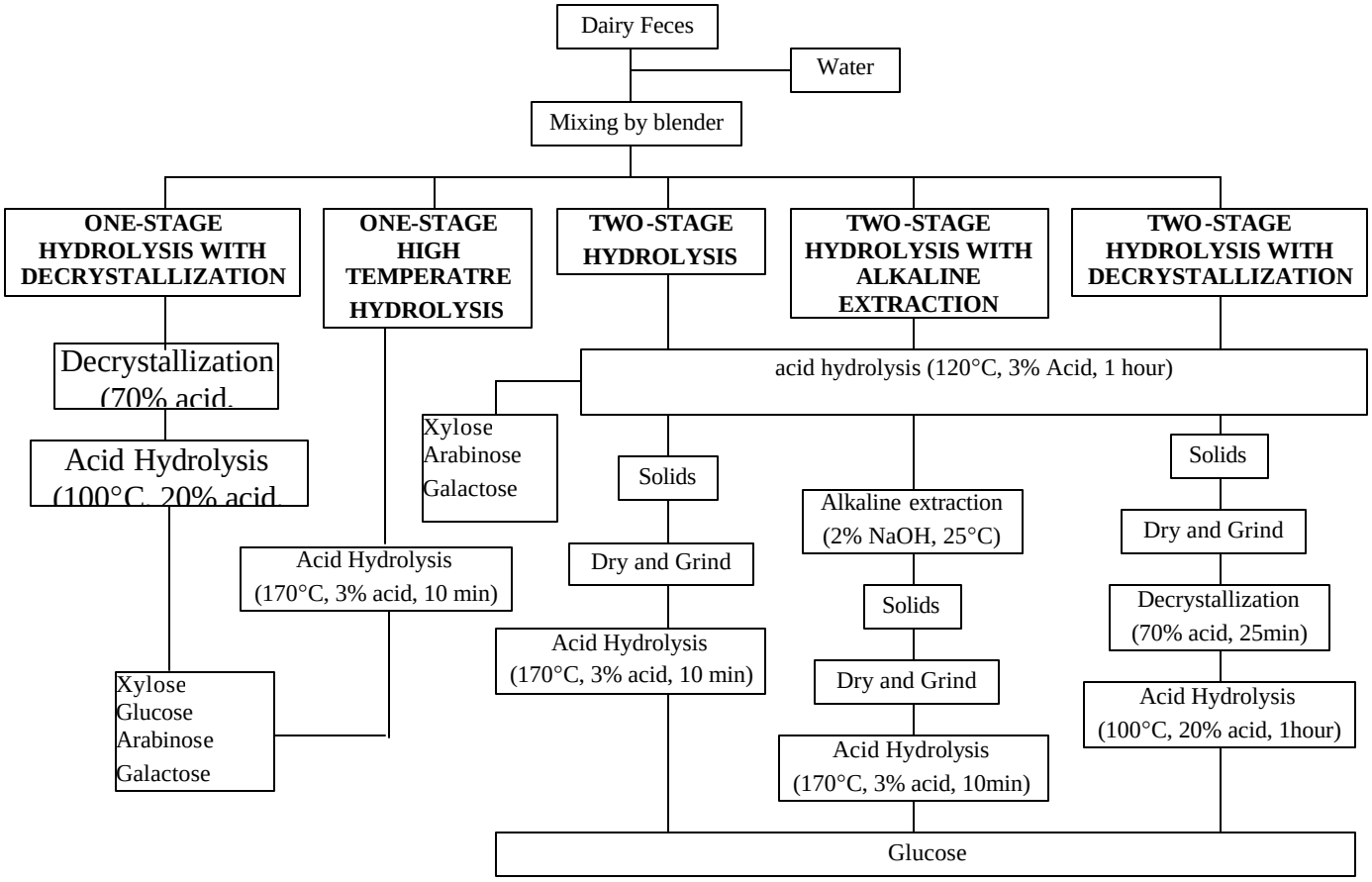


Figure 2.1. Different procedures of acid hydrolysis of dairy manure

The sugar yields of the five procedures are presented in Figure 2.2. It is apparent that two-stage hydrolysis procedures recover both cellulose sugar (glucose) and hemicellulose sugars (arabinose, galactose, and xylose). For hydrolysis of hemicellulose in manure, the first stage of two-stage procedures has an approximate 104% yield of sugars from hemicellulose, which means that hemicellulose almost completely converted to mono-sugars, while one-stage

hydrolysis with decrystallization and one-stage high-temperature hydrolysis had rather low sugar yields of 69% and 22%, respectively.

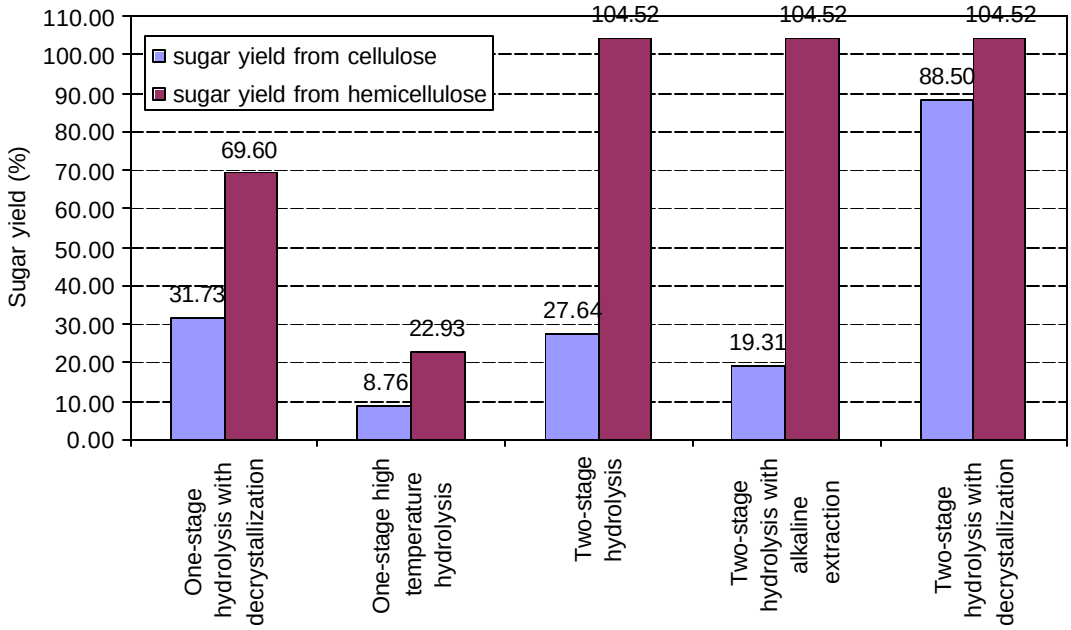


Figure 2.2. Comparison of glucose yield of different procedures

Figure 2.2 also demonstrates that various treatments did have significantly different effects on hydrolysis of cellulose in manure. Compared with alkaline treatment or high-temperature treatment, procedures with decrystallization had higher glucose yields, especially for two-stage hydrolysis with decrystallization, the glucose yield from hemicellulose reached 88% much higher than those from the other four procedures. The results also further verified that the crystal structure of cellulose in manure is the most difficult part to be attacked by acid and the critical factor to influence glucose yield of acid hydrolysis of manure.

Based on the results of this comparison, the further study of acid hydrolysis of manure fiber focused on dilute-acid, relatively-low-temperature hydrolysis of manure hemicellulose and hydrolysis of manure cellulose with decrystallization in terms of obtaining higher yields of sugars from cellulose and from hemicellulose.

1.2. The effect of nitrogen on acid hydrolysis of manure. As mentioned in previous sections, manure has a relatively high nitrogen content, which apparently can influence sugar yields

during acid hydrolysis. There are few reports on the effects of nitrogen content on hydrolysis of manure. Therefore, the specific objectives of this part of study were to statistically: (1) study the influence of nitrogen content on hydrolysis of dairy manure and (2) determine the optimal conditions of hydrolysis in regards to acid concentration, temperature, reaction time and sample concentration to produce mono-sugars from both hemicellulose and cellulose in manure.

Fresh dairy manure, described in section 1.1, was used in this work as well, along with pretreated manure. The original manure sample (composition given in Table 2.6) was obtained using the same treatment as described in section 1.1. The pretreated manure was obtained by washing 10 kg of fresh manure three separate times with 5 kg of water and then separating out the solid using a centrifuge at 3000 rpm for 10 min. The three washings with water were enough to lower the nitrogen content of the manure by half by releasing much of the soluble nitrogen to the liquid rinse. The data are given in Table 2.7.

Table 2.7. Characteristics of pretreated manure[a]

	Pretreated manure
Dry Matter, %	13.26 ± 0.021
Crude Protein, % dry matter	8.13 ± 0.089
NDF, % dry matter	67.11 ± 0.68
ADF, % dry matter	52.23 ± 1.54
ADL, % dry matter	16.56 ± 0.65
Cellulose (=ADF-ADL), % dry matter	35.67 ± 1.43
Hemicellulose(=NDF-ADF), % dry matter	14.88 ± 0.94
N, % dry matter	1.30 ± 0.014
C, % dry matter	41.00 ± 0.88

[a] all data are the average of triplicates with standard deviations of the means (n=3) at α=0.05.

1.2.1. Dilute acid hydrolysis of hemicellulose in manure. The effects of temperature, reaction time, acid concentration, and nitrogen content on dilute acid hydrolysis of hemicellulose in manure were first carried out by a completely randomized design (CRD) with two replications of 120 treatment combinations. Five acid concentrations (1% to 5%) involving four durations (30 min to 3 hours) were studied at three different temperatures (105°C, 120°C, and 135°C) on each type of manure (original and pretreated manure). Each sample used for hydrolysis contained 2.50 grams of dry manure mixed with 47.50 grams of acid solution. After hydrolysis, each

sample was filtered with Whatman No. 5 filter paper to separate the liquid from the solid. The procedure is illustrated in Figure 2.3. The general lineal model (GLM) for analysis of variance (ANOVA) of sugar yield from hemicellulose was tested using the Statistical Analysis System (SAS 8.0) program.

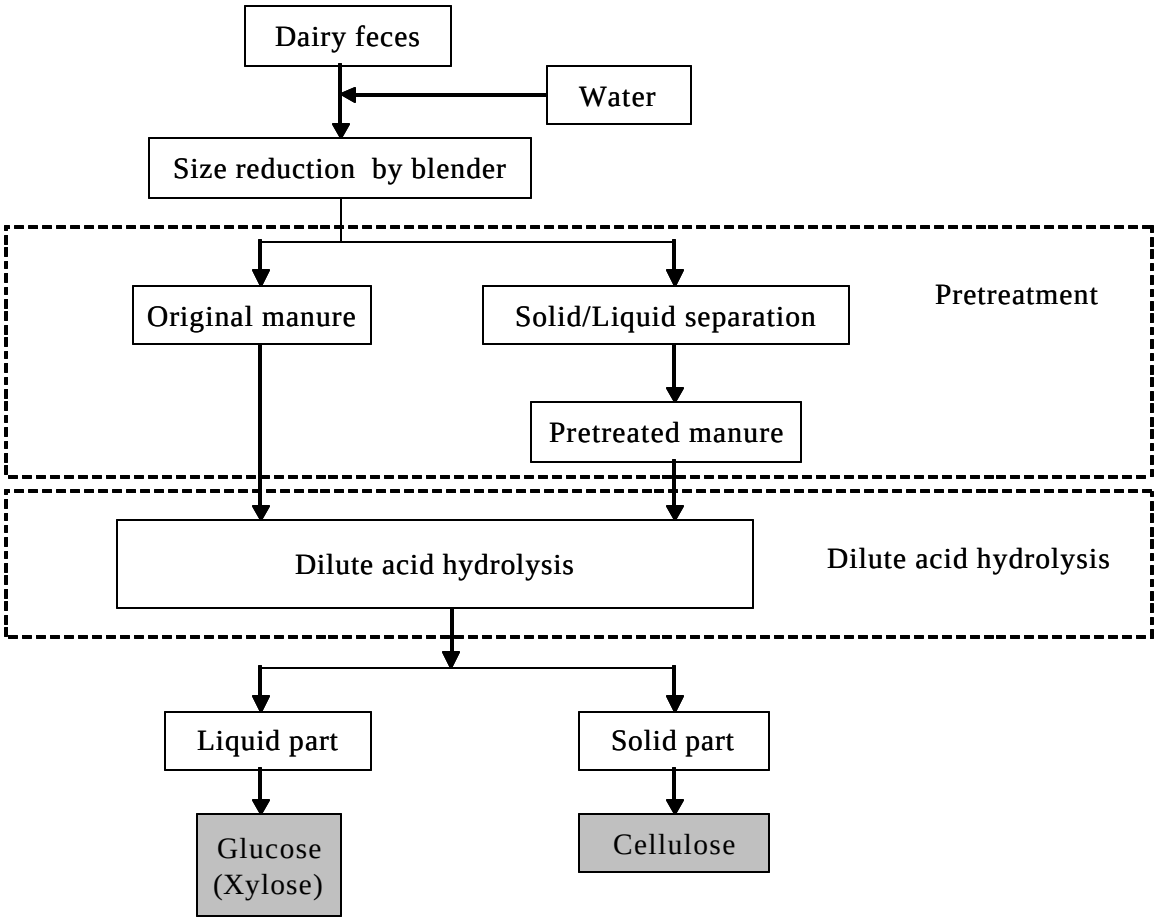


Figure 2.3. Procedure of dilute acid hydrolysis of original and pretreated manure

The effect of sample concentration was then studied based on the optimal conditions of temperature, reaction time, acid concentration, and nitrogen content. Four sample concentrations (2.5%, 5%, 7.5%, and 10%) with two replications were used by the same experimental design (CRD). The difference among the sugar yields of these samples was also analyzed using the Statistical Analysis System (SAS 8.0) program.

1.2.1.1. The effects of nitrogen content reaction time, reaction temperature, and acid concentration on hydrolysis of hemicellulose in dairy manure. The sugar yield (total amount

of mono-sugars from hemicellulose to amount of hemicellulose in the sample) from the hemicellulose hydrolysis experiments are outlined in Figures 2.4-2.6. The sugar yields of pretreated manure with low nitrogen content were overall higher than those of the corresponding hydrolysis of original manure with high nitrogen content (Figures 2.4-2.6). For instance, at the point of 2 hours, 3% acid, and 120°C, the hydrolysis of pretreated manure had a yield of 114%, while the corresponding original manure hydrolysis had only a yield of 102%. In addition, the hydrolyzed solution color of original manure was much darker than the pretreated manure (data not shown). These phenomena might have been caused by a much stronger browning reaction between reducing sugars and protein in original manure. Meanwhile, Figures 2.4-2.6 also demonstrate that hydrolysis of original manure needed more acid to reach the highest yield than did the pretreated manure. At the low acid concentration of 1%, sugar yields from hydrolysis of original manure for all three temperatures were much lower than those of the corresponding hydrolysis of pretreated manure, and sugar yields at the acid concentration of 1% did not increase too much following the increase of reaction time compared with yields at a much higher acid concentration. This means that a certain amount of acid is needed to start the hydrolysis reaction of the original manure. This is most likely because an acid-base reaction between acid and non-protein nitrogen like urine and ammonia occurred in original manure prior to hydrolysis of hemicellulose.

The sugar yield of both the original manure and the pretreated manure increased noticeably with the increased reaction time from 0.5 to 3 h and acid concentration from 1% to 5% of hydrolysis at 105°C (Figure 2.4). As temperature increased to 120°C (Figure 2.5), the yields of both manures reached their highest values of 102% and 114%, respectively. Figure 2.5 also shows that the sugar yields for both manures stopped increasing once reaction time and acid concentration were more than 2 hours and 2%, respectively. This means that side reactions such as dehydration and the browning reaction occurred very slowly at this temperature. At 135°C (Figure 2.6), the sugar yields of the pretreated manure reached its highest value at an acid concentration of 1%, which was lower than that achieved during the hydrolysis at 120°C. Also, the sugar yields of the original manure were lower than the corresponding hydrolyses at 100°C and 120°C. For both hydrolyses at this temperature, the increase of yield did level off as time progressed and acid concentration further increased, because reducing sugars were able to cause a dehydration reaction and produce byproducts under acid conditions at higher temperature.

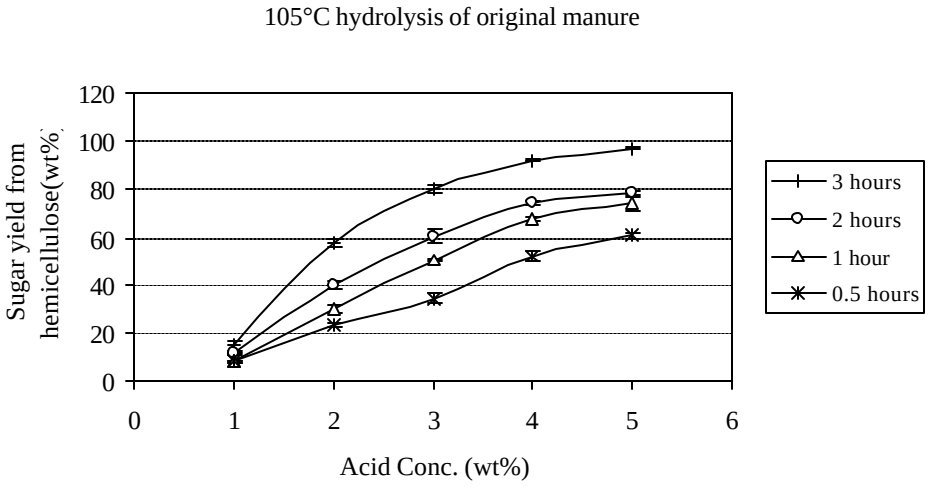


Figure 2.4(A)

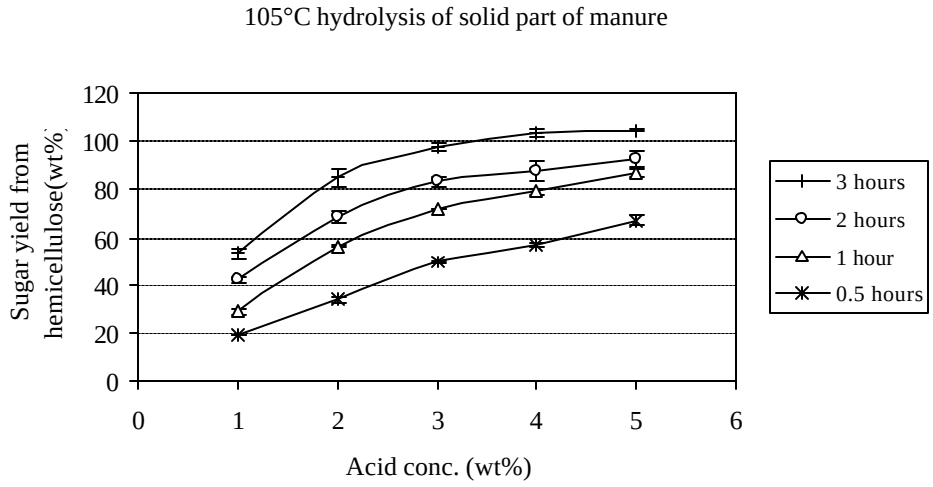


Figure 2.4(B)

Figure 2.4. Comparison of sugar yield from hemicellulose of acid hydrolysis of original manure and pretreated manure at 105°C
A: Sugar yield from hemicellulose = grams of total mono-sugars (xylose + arabinose + galactose)/grams of hemicellulose in raw material
B: Data are presented as the mean of two replicates and the error bars show the standard deviation

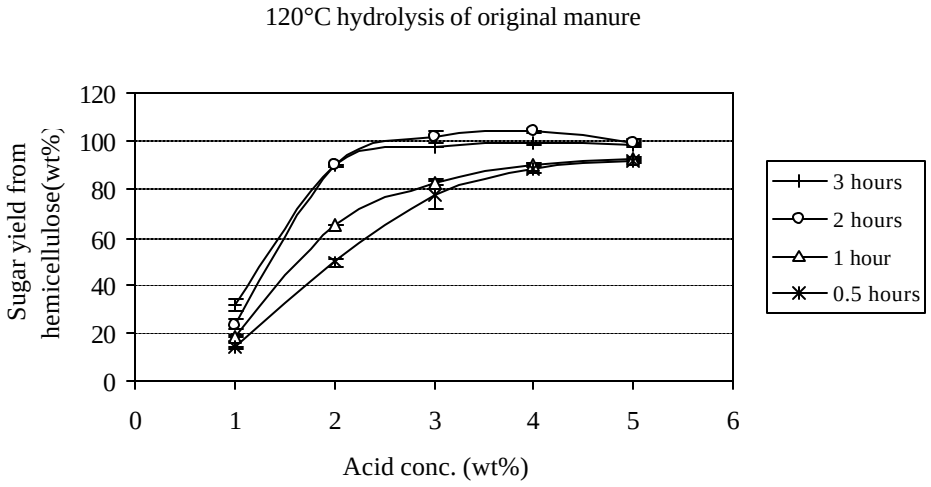


Figure 2.5(A)

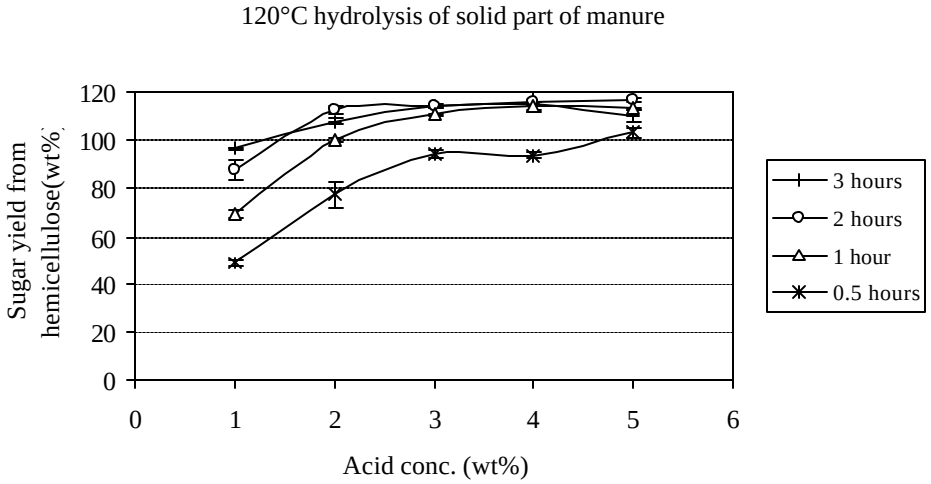


Figure 2.5(B)

Figure 2.5. Comparison of sugar yield from hemicellulose of acid hydrolysis of original manure and pretreated manure at 120 °C
A: Sugar yield from hemicellulose = grams of total mono-sugars (xylose + arabinose + galactose)/grams of hemicellulose in raw material
B: Data are presented as the mean of two replicates and the error bars show the standard deviation

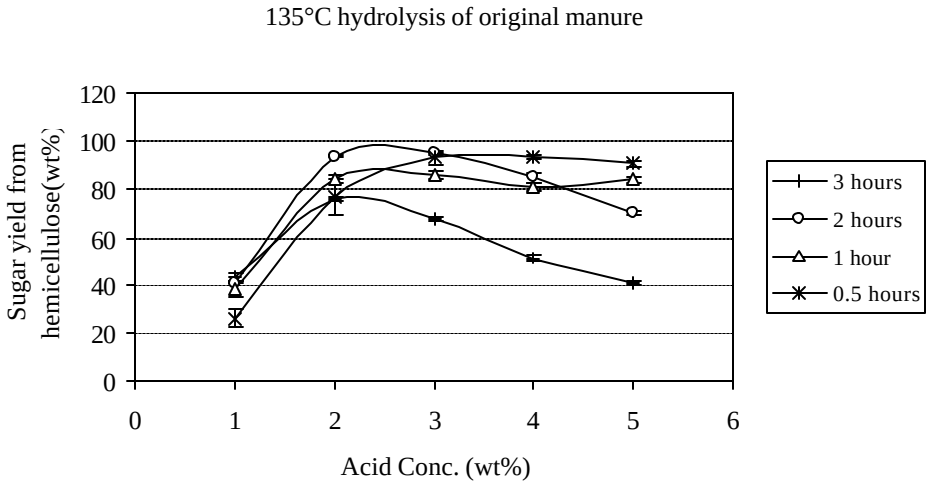


Figure 2.6(A)

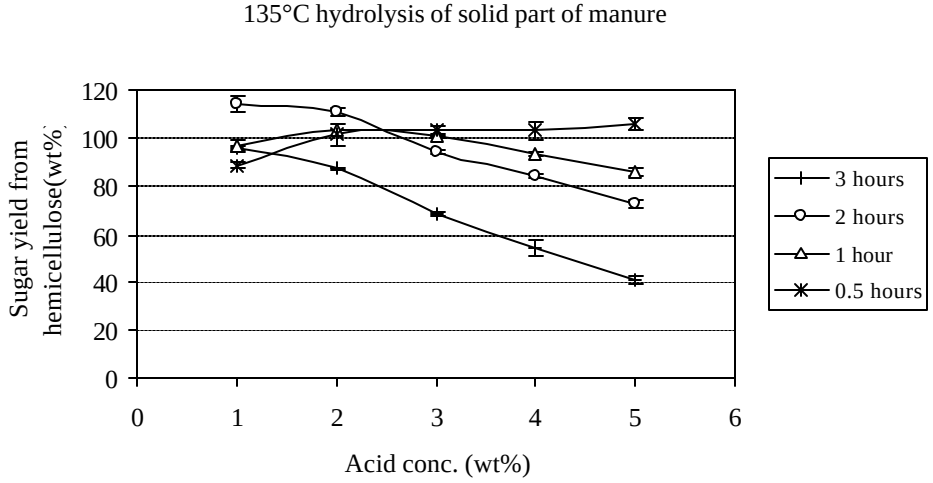


Figure 2.6(B)

Figure 2.6. Comparison of sugar yield from hemicellulose of acid hydrolysis of original manure and pretreated manure at 135°C
A: Sugar yield from hemicellulose = grams of total mono-sugars (xylose + arabinose + galactose)/grams of hemicellulose in raw material
B: Data are presented as the mean of two replicates and the error bars show the standard deviation

For instance, under these circumstances xylose can be further degraded to produce the byproduct furfural, and a browning reaction, especially for original manure, may have occurred under the same conditions.

Statistical analysis of sugar yields from hemicellulose showed that not only were there significant differences ($P<0.01$) caused by the effects of temperature, reaction time, acid concentration, and nitrogen content, but also significant two-way, three-way, and four-way interactions between the variables. Consequently, the main effects could not be directly interpreted. Therefore, a simple pair-wise comparison, using least square means (lsmeans) was analyzed. The resulting analysis showed that there was no significant difference ($P>0.05$) among the nine hydrolyses of pretreated manure that had an average highest yield of 114% (Table 2.8). Meanwhile, the original manure had an average highest yield of 102% from two treatments (Table 2.9), which also had no significant statistical difference ($P>0.05$). This 11% difference in yield between the two nitrogen-differing manures, combined with the color change of hydrolyzed solution mentioned earlier in the section, indicates that nitrogen content had a noticeably negative effect on the hydrolysis of manure.

Since acid-sugar separation is usually the bottleneck for utilization of the entire acid hydrolysis system, the lower the acid concentration the hydrolysis uses, the better the utilization of the sugars. Thus, the best raw material for acid hydrolysis was the pretreated dairy manure with optimal conditions of reaction time of 2 hours, acid concentration of 1%, and reaction temperature of 135°C, with hydrolysis under these conditions having a yield of 114%. When compared to acid hydrolysis of softwood hemicellulose, which used 1% w/w acid at 185°C within a steam explosion reactor to reach a yield of 85%, the method of hydrolysis for hemicellulose of dairy manure was more moderate and had a higher sugar yield (114%).

1.2.1.2. The effect of sample concentration on acid hydrolysis of dairy manure. The effect of sample concentration on acid hydrolysis was investigated in terms of increasing the sugar concentration in solution. As stated in the previous section, the optimal conditions obtained were based on a sample concentration of 5%. Although the reaction under this condition had a highest yield of 114%, the corresponding sugar concentration in solution was only 8.5 g/L, which was still low. Four sample concentrations (2.5%, 5%, 7.5%, and 10%) of pretreated manure were used for this part of study. The reaction conditions were still the optimal conditions obtained as discussed in the previous section.

Table 2.8. The highest sugar yield from hemicellulose in hydrolyzed solutions (pretreated manure) with different reaction conditions

Time (hour)	Acid conc. (%)	Temperature (°C)	Nitrogen content (%)	Mean of mono-sugar concentration from hemicellulose (g/100 dry nitrogen-lacking manure)	Mean of sugar yields from hemicellulose (%)
1	4	120	0.65	16.97	114
1	5	120	0.65	16.82	113
2	2	120	0.65	16.79	112
2	3	120	0.65	16.98	114
2	4	120	0.65	17.23	116
2	5	120	0.65	17.38	117
2	1	135	0.65	17.01	114
3	3	120	0.65	16.96	114
3	4	120	0.65	17.10	115

Table 2.9. The highest sugar yield from hemicellulose in hydrolyzed solutions (original manure) with different reaction conditions

Time (hour)	Acid conc. (%)	Temperature (°C)	Nitrogen content (%)	Mean of mono-sugar concentration from hemicellulose (g/100 g nitrogen-rich manure)	Mean of sugar yields from hemicellulose (%)
2	3	120	2.6	12.72	102
2	4	120	2.6	12.96	104

Figure 2.7 demonstrates the effect of sample concentration on sugar yield from hemicellulose and sugar concentration in solution. Sugar concentration linearly increased with sample concentration. The concentration reached the highest value of 16.5 g/L with respect to 10% sample concentration. Meanwhile, the sugar yields were around 111%. Statistical analysis showed that there are no significant differences ($P>0.05$) among these four treatments in terms of sugar yield. Since more was used, a higher sugar concentration was obtained, and the 10% sample concentration of pretreated manure was adopted as the optimal sample concentration.

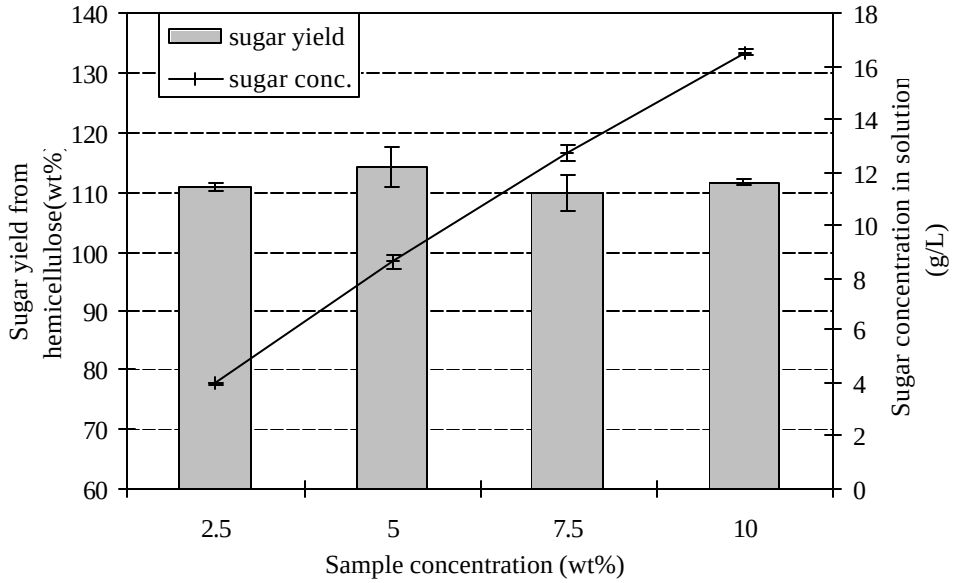


Figure 2.7. The effect of sample concentration on sugar concentration in solution and sugar yield from hemicellulose[a, b]

[a] Sugar yield from hemicellulose = grams of total mono-sugars (xylose + arabinose + galactose)/grams of hemicellulose in raw material

[b] Data are presented as the mean of two replicates and the error bars show the standard deviation

In this part of study, it was found that the nitrogen content in nitrogen-rich cellulosic material such as dairy manure is very important in the hydrolysis of hemicellulose to obtain mono-sugars. Effective removal of the nitrogen sources in this type of raw material contributed largely to an increase in the sugar yield from hemicellulose and made the reaction conditions more moderate. Meanwhile, the hydrolyzed solid had 43% dry matter of cellulose, which was much higher than the original manure and the pretreated manure (Table 2.10), and thus could supply a substantial cellulose resource for downstream glucose production.

Table 2.10. Fiber characteristics of hydrolyzed pretreated manure[a, b]

	Hydrolyzed pretreated of manure
Cellulose, % dry matter	42.67 ± 0.64
Hemicellulose, % dry matter	~ 0
Lignin, % dry matter	32.12 ± 0.97

[a] all data are the average of triplicates with standard deviations of the means (n=3) at α=0.05.

[b] The hydrolyzed pretreated manure was from the treatments of temperature of 135°C, acid concentration of 1% and reaction time of 2 hours.

1.2.2. Acid decrystallization of both hemicellulose and cellulose in manure. The dry and ground samples of original and pretreated manure were mixed with 70% acid solution by a sample/acid-solution ratio of 1:5, and then treated by the combination of two factors of acid concentration of decrystallization, and decrystallization time. Three levels of acid concentration (65%, 70%, and 75%), and four levels of decrystallization time (30, 60, 90, 120 min) were studied to compare the decrystallizations of the two manure types. All samples obtained from the decrystallization step with different combinations were diluted to the same acid concentration (20%) and same sample content (5%). The diluted samples were further hydrolyzed at 100°C for 1 hour to produce glucose, which is used to calculate sugar yield to compare different combinations (see Figure 2.8).

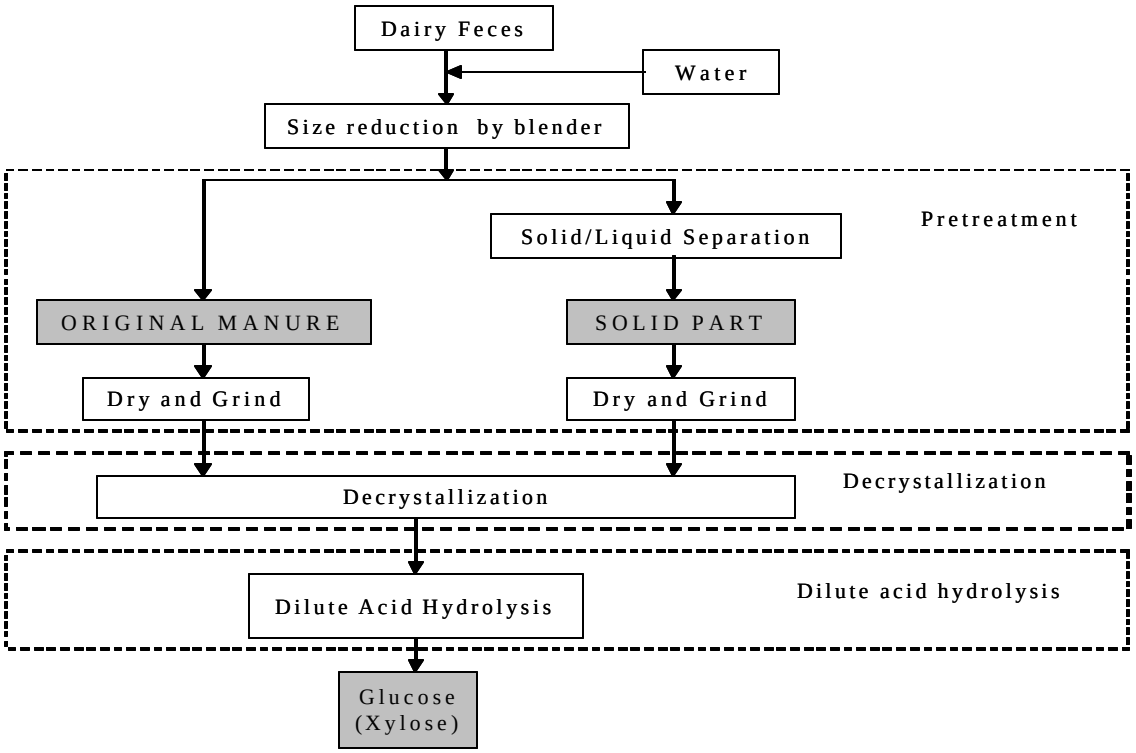
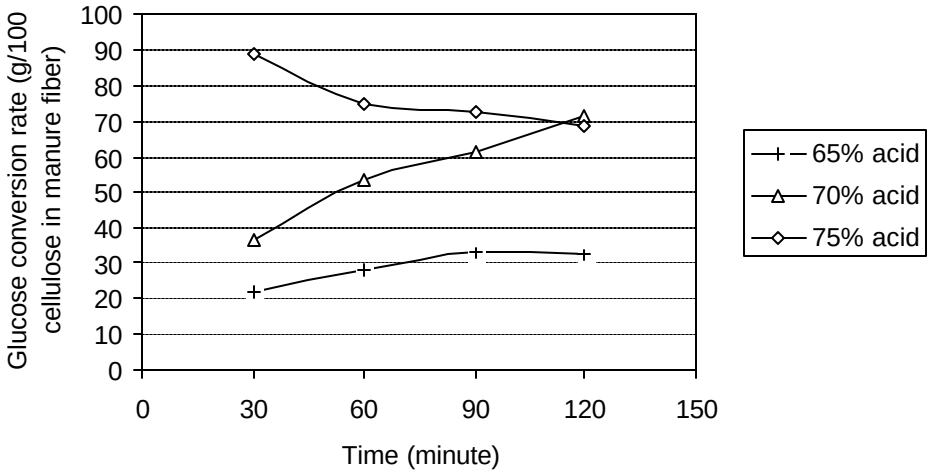
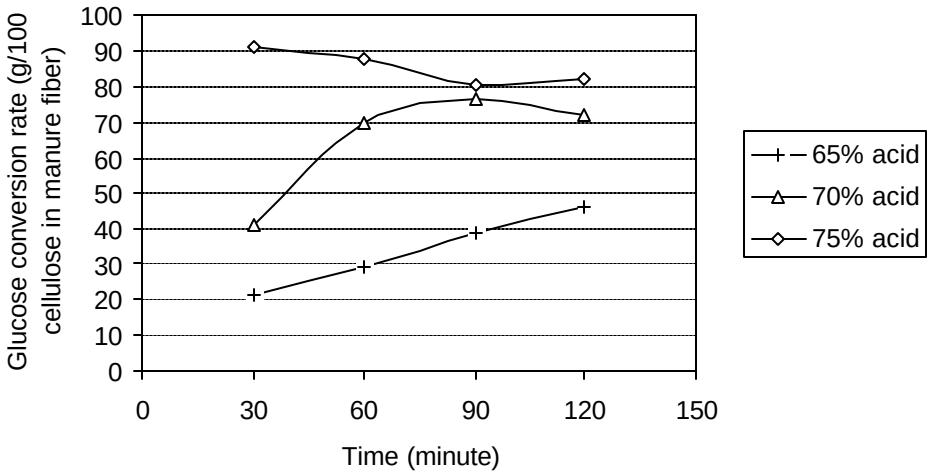


Figure 2.8. Dilute acid hydrolysis with decrystallization of different treated manures

1.2.2.1. Effects of nitrogen on glucose yield during decrystallization of manure fiber. The effects of nitrogen on decrystallization and sugar yield from cellulose are presented in Figure 2.9. At the low acid concentration of 65%, glucose yields of original manure and pretreated manure showed an increase during the period from 0.5 hours decrystallization to 2 hours



2.9(A)



2.9(B)

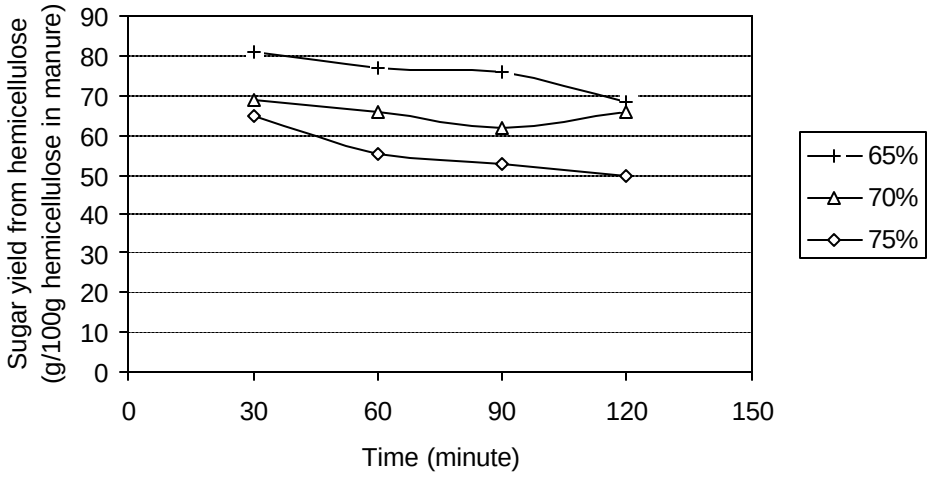
Figure 2.9. The effect of nitrogen content, acid concentration and time of decrystallizatio n on hydrolysis of cellulose(Sample-acid ratio is 1:5)
A. Decrystallization of original manure, B. Decrystallization of pretreated manure

decrystallization, and pretreated manure at this acid concentration had much higher sugar yield than original manure. Both ultimately peaked at 45% and 32%, respectively, which might be caused by that nitrogen content in original manure consuming the certain amount of acid prior to acid decrystallization of manure cellulose. This decreases the total amount of acid used for decrystallization, and further influences the efficiency of decrystallization.

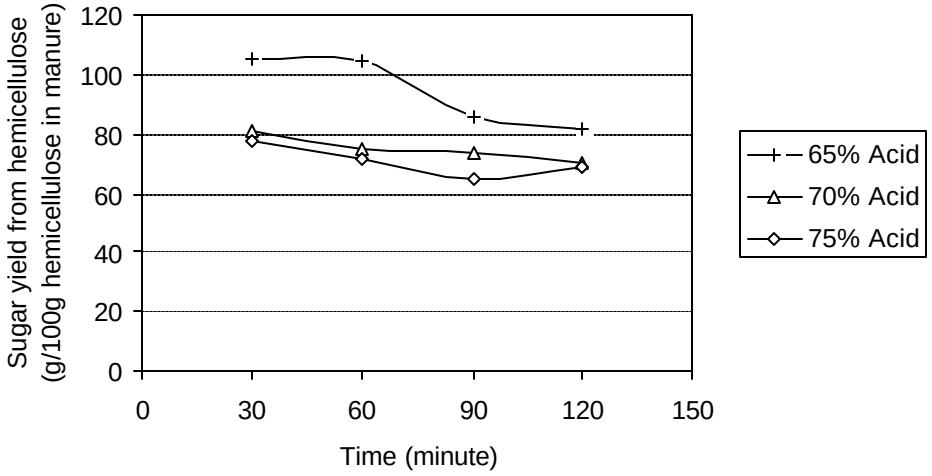
At the high acid concentration of 75%, though, the glucose yield of pretreated manure reached its maximum of 91% at 30 min of reaction time, and held roughly constant as time progressed. As a comparison, the decrystallization of original manure had a little lower yield of 88% at the same reaction time, but, following the increase of reaction time, 20% of sugar yield waseventually lost. The reason for this phenomenon might be that the browning reaction at high acid concentration and long reaction time could have easily occurred as indicated by the color of the hydrolyzed solution of the original manure, which was darker than that of pretreated manure.

1.2.2.2. Effects of nitrogen on hydrolysis of hemicellulose during acid decrystallization of manure fiber. Figure 2.10 demonstrates the effects of nitrogen and acid concentration of decrystallization on hydrolysis of hemicellulose. The sugar yields from hemicellulose of pretreated manure were much higher than the corresponding yield from original manure, which indicates that the same browning reaction also took place between hemicellulose sugars and protein-nitrogen. The highest hemicellulose-sugar yields of hydrolysis of original manure and pretreated manure are 80% and 106%, respectively, which were obtained at 65% of acid concentration and 30 min of reaction time. The 26% difference in the hemicellulose-sugar yield was caused by the nitrogen-related reaction (browning reaction). Compared to the decrystallization of manure cellulose, nitrogen content has much larger impact on hemicellulose-sugar yield during decrystallization of manure fiber. Meanwhile, hemicellulose-sugar yields of both manure decreased following the increase of acid concentration. This phenomenon suggests that these sugars (xylose, arabinose and galactose) from hemicellulose should react with protein-nitrogen much more dramatically at higher acid concentration.

The results presented here indicated that nitrogen did influence the sugar yield during decrystallization of manure. Furthermore, a higher acid concentration and shorter time of decrystallization appear beneficial for obtaining the highest glucose yields from cellulose of pretreated manure, while a lower acid concentration and shorter time of decrystallization were better for deriving the highest hemicellulose-sugar yields from pretreated manure. However, considering the composition of manure fiber, dominated by cellulose, glucose yield from cellulose must be the main concern once the conditions of decrystallization are optimized. Therefore, the optimal conditions for decrystllization of manure are 30 min of reaction time and 75% of acid concentration. Furthermore, the pretreated manure has a much higher cellulose and hemicellulose content than the original manure, and consequently produces the higher



2.10(A)



2.10(B)

Figure 2.10. The effect of nitrogen content, acid concentration and time of decrystallization on hydrolysis of hemicellulose (Sample-acid ratio is 1:5)
A. Decrystallization of original manure, B. Decrystallization of pretreated manure

sugar concentrations (6 g xylose/L, 16 g glucose/L) in the hydrolyzed solution (Figure 2.11). Therefore, the pretreated manure was chosen as the raw material to fulfill the acid decrystallization.

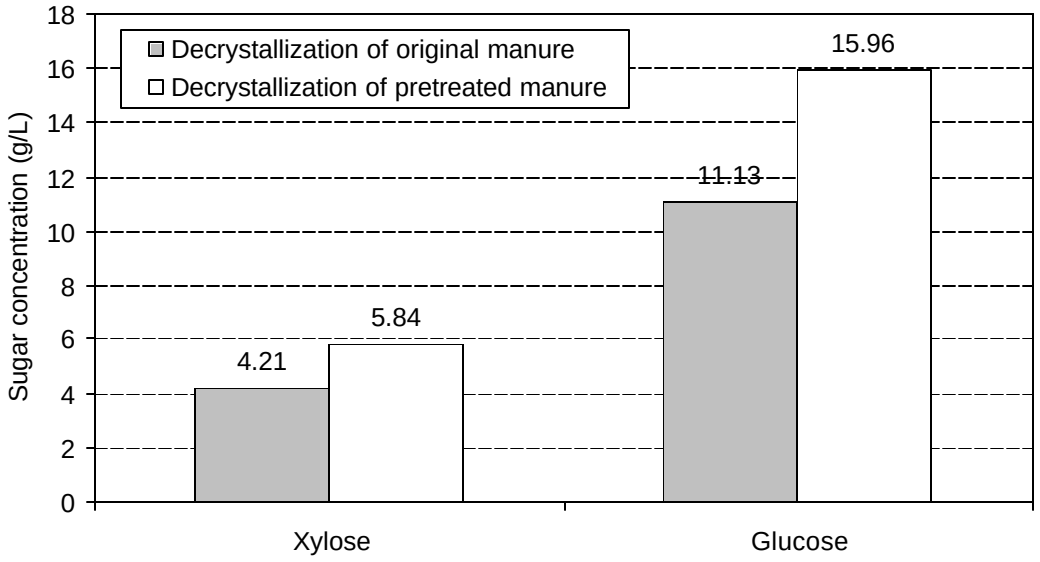


Figure 2.11. Comparison of sugar concentrations of hydrolyzed solution of decrystallization of original manure and pretreated manure

Sections 1.2.1 and 1.2.2 showed that nitrogen content has a large influence on either acid hydrolysis of hemicellulose in manure fiber or acid decrystallization of manure fiber. Pretreated manure has been proven to diminish the effect of nitrogen on sugar production in both hydrolyses. Moreover, the study also concludes that the conditions of low acid concentration, relatively high temperature, and moderate reaction time (1%, 135°C, 2 hours) can offer the highest hemicellulose-sugar yield, while the condtions of high acid concentration and short decrystallization time are good for achieving the highest glucose yield from manure cellulose. Overall, since the decrystallization procedure has not only the highest glucose yield but also a rather high hemicellulose-sugar yield, combining with that cellulose is the biggest component in manure. The decrystallized pretreated manure was chosen as the substrate for the following studies.

1.3. Dilute acid hydrolysis of decrystallized pretreated manure

1.3.1. The effect of sample-acid ratio on decrystallization. Before the dilute acid hydrolysis of decrystallized pretreated manure was studied further, the effect of sample-acid ratio on decrystallization was studied in terms of increasing the sample concentration. Three sample-acid

ratios of 1:5, 3:5, and 5:5 were tested under the optimal decrystallization acid concentration of 75%.

Figure 2.12 demonstrates the effect of sample-acid ratio on the decrystallization process. The results show that the ratios of 1:5 and 3:5 were much better than the ratio of 5:5 on glucose yield, and there was no difference between the two ratios (Figure 2.12A). Figure 2.12B shows

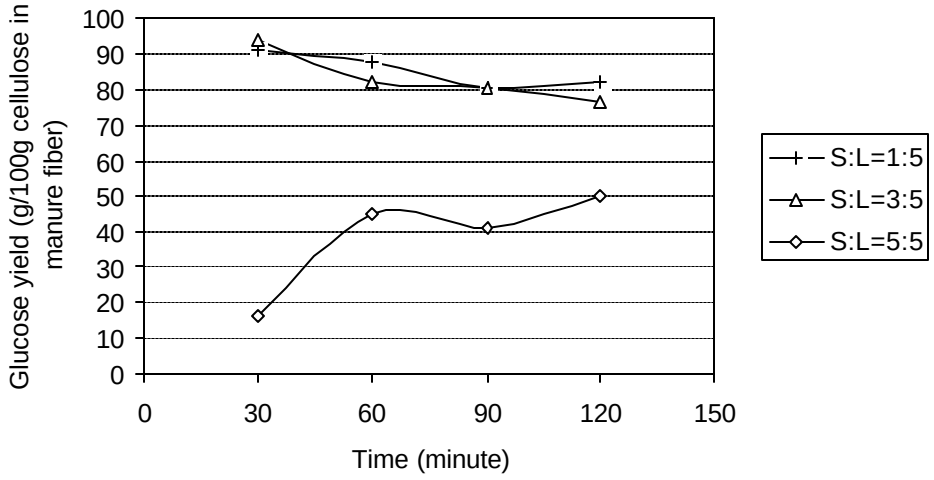


Figure 2.12(A)

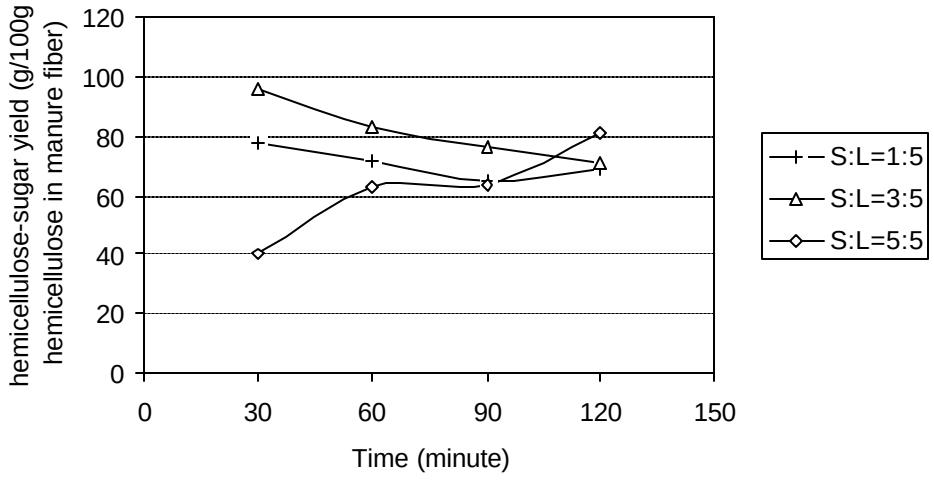


Figure 2.12(B)

Figure 2.12. The effect of sample-acid ratio of decrystallization of solid part of dairy manure at 75% acid concentration *S:L means total amount of sample to total amount of acid solution. (A) The effect on cellulose in manure. (B) The effect on hemicellulose in manure.

that the ratio of 3:5 was coincidently the best for the decrystallization of hemicellulose among the three ratios. The higher ratio of sample to acid was used, since more cellulose and hemicellulose for hydrolysis were supplied. Thus, the ratio of 3:5 combined with 30 min of decrystallization time and 75% of acid concentration was the optimal condition for the decrystallization step of this procedure.

1.3.2. Dilute acid hydrolysis of decrystallized pretreated manure. The following dilute acid hydrolysis was studied by changing reaction temperature and time under a fixed sample concentration of 10% and acid concentration of 12.5%. Three levels of temperature (100°C, 120°C and 135°C) and six levels of time (10, 20, 30, 60, 120, 180 min) were used.

Figure 2.13 demonstrates that sugar yields of dilute acid hydrolysis varied with different reaction temperatures and time. Glucose yield and hemicellulose-sugar yield both show that the low temperature under dilute acid condition did not convert much of cellulose and hemicellulose to sugars, although the sugar yields did increase following the increase of reaction time, and also the higher the temperature, the more rapid the sugars are consumed by the side reactions (browning reaction, dehydration reaction). This means that shorter time and higher temperature are beneficial for increasing sugar yields, especially for glucose yield. The 135°C and 10-min treatment had the highest glucose yield of 84% (Figure 2.13A), achieving a glucose concentration of 26 g/L. Hemicellulose-sugar yield and concentration reached the highest value of 91% and 13 g/L, respectively, at the point of 120°C and 10 min (Figure 2.13B).

Considering the composition of manure fiber, with much higher cellulose content (around 26%) than hemicellulose (around 12%) (Table 2.6), the glucose yield has the priority. The optimal conditions for dilute acid hydrolysis were chosen as 10% of sample concentration, 12.5% of acid concentration, 135°C of reaction temperature and 10 minutes of reaction time following decrystallization. At this point, dilute acid hydrolysis of hemicellulose produced an 80% sugar yield and 11g/L hemicellulose-sugar, which is relatively close to the highest value (91% and 13g/L).

Finally, comparing sugar yields at the optimal conditions with 90% glucose yield and 80% hemicellulose-sugar yield of 20% acid hydrolysis of decrystallized pretreated manure at 100°C (Figures 2.9 and 2.10), hemicellulose-sugar yield of dilute acid hydrolysis was higher than that of 20% acid hydrolysis. Glucose yield of dilute acid hydrolysis was very close to that

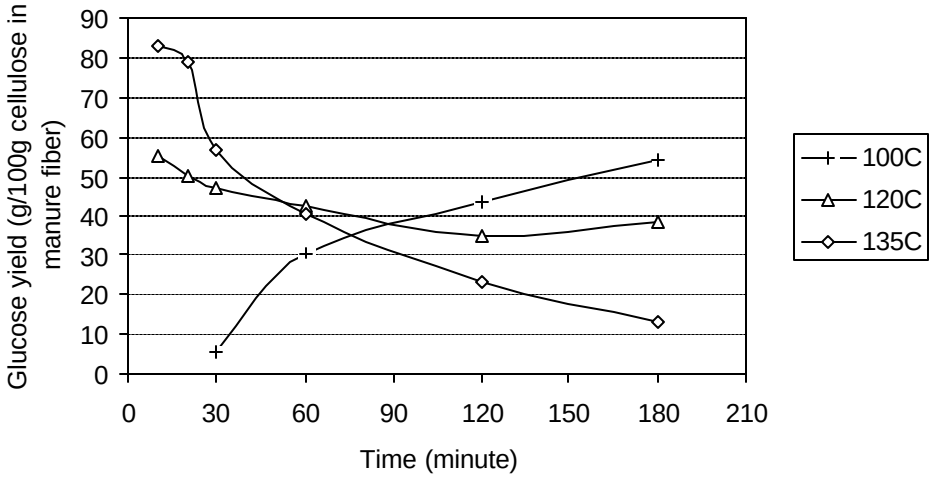


Figure 2.13(A)

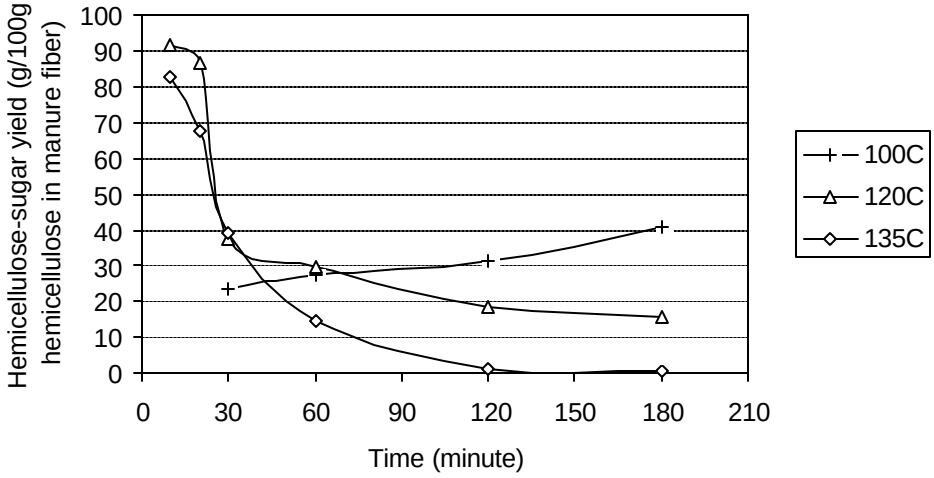


Figure 2.13(B)

Figure 2.13. Effect of different reaction temperatures and time on glucose yield (13A) and hemicellulose-sugar yield (13B) of dilute acid hydrolysis of decrystallized pretreated solid part of dairy manure

of 20% acid hydrolysis. However, the lower the acid concentration, the more environmentally friendly the process is. The shorter time of dilute acid hydrolysis under higher temperature may be able to make the continuous process of acid hydrolysis of decrystallized pretreated manure possible.

Enzymatic hydrolysis

Enzymatic hydrolysis has attracted increased attention as an alternative to acid hydrolysis for degrading lignocellulosic materials because it is highly specific, it has mild reaction conditions (pH around 5 and temperature less than 50°C), and does not create a corrosion problem. Cellulase is used to carry out this task. A typical cellulase consists of endo-1,4-β-D-glucanase (E.C.3.2.1.4), exo-1,4-β-D-glucanase (E.C. 3.2.1.91), and β-glucosidase (E.C.3.2.1.21). The endo-glucanase attacks cellulose to create free chain-ends, the exo-glucanase degrades the molecule by removing cellobiose from the free chain-end, and the β-glucosidase produces glucose by breaking down the cellobiose. Unfortunately, cellulase is only capable of degrading cellulose because the matrix of hemicellulose and lignin around cellulose in lignocellulosic materials functions to prevent cellulase from attacking cellulose. Thus, different pretreatments must be used to break down the matrix structure in order to make cellulase more effective.

The objectives of this part of the study were to (1) determine the optimal conditions of enzymatic hydrolysis of manure fiber, and (2) study the effect of pretreatment on enzymatic hydrolysis using the optimal conditions of the enzyme, and further develop an enzymatic hydrolysis process with proper pretreatment of dairy manures to produce mono-sugars. Three pretreatments of the dairy manure were performed: dilute acid pretreatment of solid part of manure, sodium chlorite pretreatment of solid part of manure, and solid part of manure.

1.4. Optimization of enzymatic hydrolysis of manure without hemicellulose. The manures were firstly treated by diluted sulfuric acid before hydrolyzed by cellulase enzymes. The objective of this procedure is to remove the hemicellulose barrier. The flow chart for the whole procedure is shown in Figure 2.14. The original manure was used for this study. Manure fiber with hemicellulose was obtained using dilute acid hydrolysis with the optimal conditions in Table 2.9. The acid-treated manure slurry was washed by hot tap water until a pH around 5.0. The solid residues were dried at 100°C before being ground by a mortar. The particles were screened through 20# US standard sieve, the opening of which is less than 840 μm. The characteristics of manure fiber without hemicellulose were listed in Table 2.10.

Commercial enzyme solutions, Celluclast-1.5L and Novozyme-188 (Sigma), were used for the production of glucose from the pretreated manure. Hydrolysis was performed in 125

Erlenmeyer flasks containing a 50-ml mixture of buffer solution (pH 4.8) and manure particles. The flasks were incubated in an orbital shaker (175 rpm).

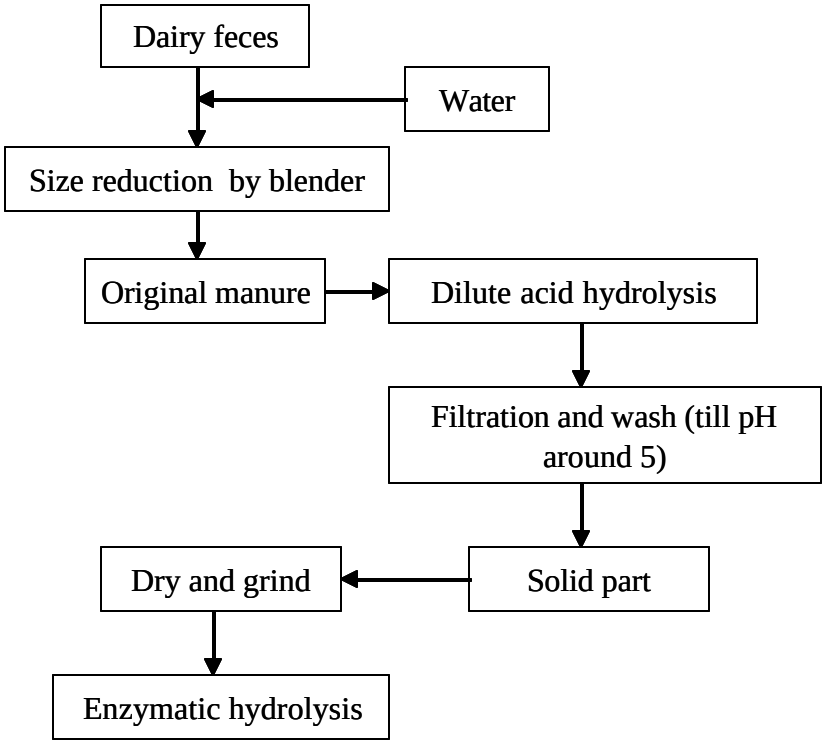


Figure 2.14. Flow chart of animal manure pretreatment for enzymatic hydrolysis

1.4.1. Effects of enzyme loading. Celluclast-1.5L was first used as single enzyme for hydrolysis. The substrate concentration was fixed at 5% (50 g/L). As shown in Figure 2.15, both the glucose concentration and yield increased with Celluclast-1.5L loading from 100 IU/L (international unit per liter) to 650 IU/L and leveled off from 650 IU/L to 1000 IU/L.

Celluclast-1.5L contains high levels of cellulase; however, the β-glucosidase activity of the enzyme is relatively low. To enhance the concentration and yield of glucose, Novozyme 188, which contains high levels of β-glucosidase activity, was added to the enzymatic solution. The results indicate that the highest glucose concentration and yield were obtained at 650 IU/L of filter paper activity and around 250 IU/L of β-glucosidase activity. The ratio of enzyme to substrate at this enzyme loading was 13 IU/g manure.

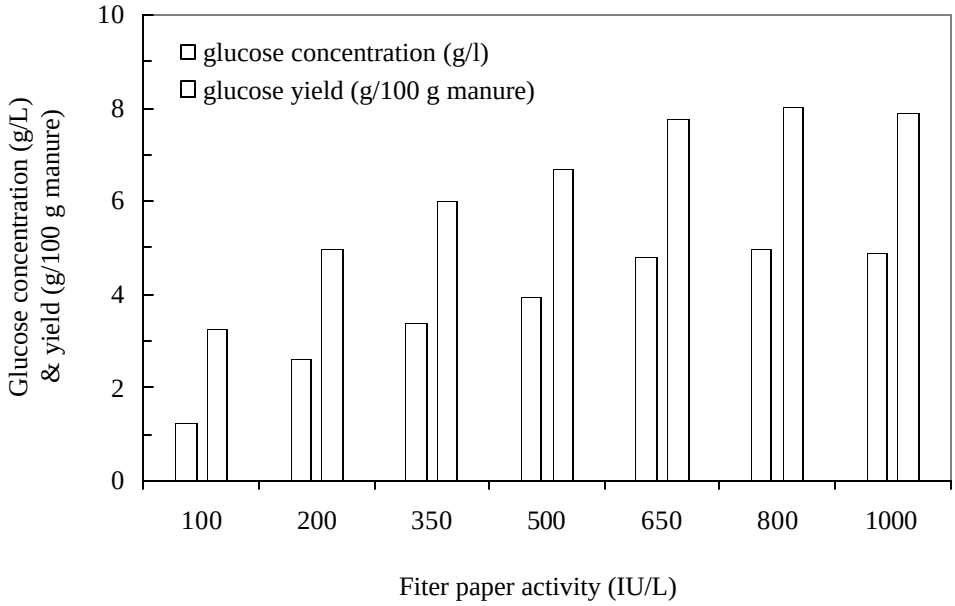
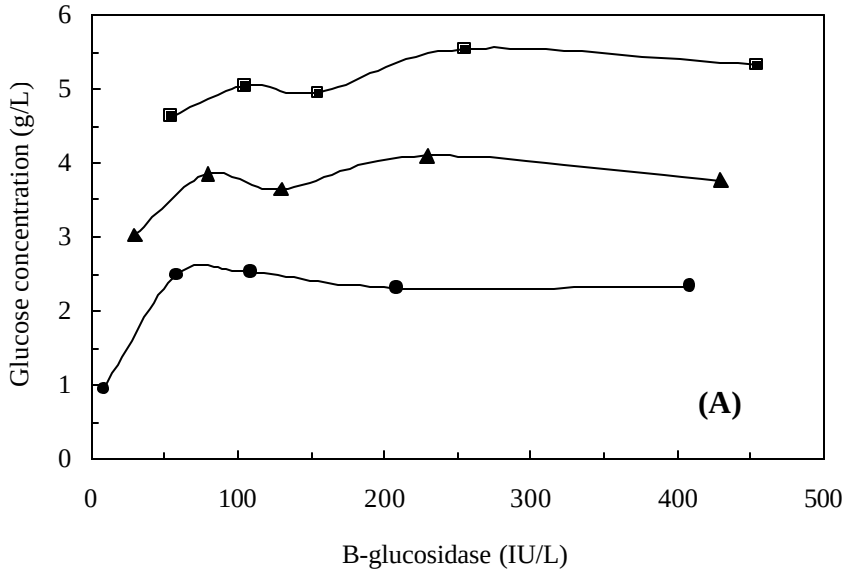


Figure 2.15. Effects of cellulase activity (filter paper activity) on the glucose concentration and yield from manure hydrolysis.

The effects of β-glucosidase activity on the hydrolysis were investigated at three cellulase levels, i.e., 100 FPU/L, 350 FPU/L and 650 FPU/L (FPU is filter paper units or international units of filter paper activity). As shown in Figure 2.16, both glucose concentration and yield were lower when β-glucosidase was less than 100 IU/L. When the activity exceeded 100 IU/L, further addition did not improve glucose concentration and yield. Figure 2.16, also shows that the highest glucose concentration and yield was obtained at 650 IU/L of filter paper activity and around 250 IU/L of β-glucosidase activity, such enzyme loadings were thus, employed in the following works.

1.4.2. Effects of pH and temperature. The effects of temperature (T) and pH on enzymatic hydrolysis were investigated by a central composite design. This statistical method has proven to be efficient in the optimization of enzymatic hydrolysis processes. The glucose concentration of runs 1-8 in Table 2.11 were the means of two duplicates, while the central point was run in triplicate (run 9-11) and its standard deviation was 0.18 g/L. The results showed a high reproducibility, as the deviation of each run was less than 5%.

Glucose concentration in Table 2.11 was correlated as a function of T and pH (coded value) (Eq. 1) by the NCSS 2000 software. The resulting equation was:



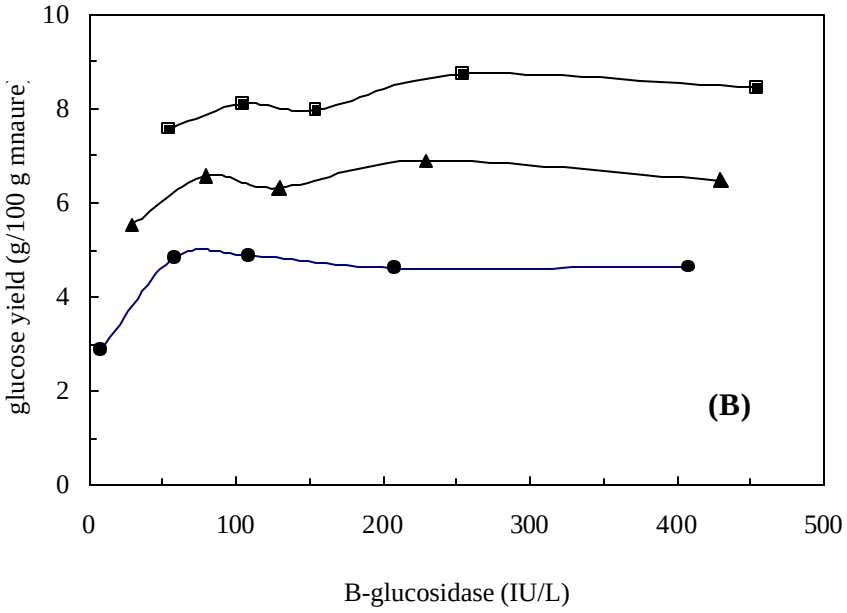


Figure 2.16. Effect of β-glucosidase loading on glucose concentration (A) and yield (B) after 120 hr at different cellulase levels (FPU/L) (■: 100 FPU/L; ▲: 350 FPU/L; ●: 650 FPU/L). The presented β-glucosidase activities are the sum of Celluclast 1.5 L and Novozyme 188.

$$glucose(g/L) = 5.28 - 0.34 \cdot T + 0.14 \cdot pH - 0.42 \cdot T^2 - 0.65 \cdot pH^2 - 0.21 \cdot T \cdot pH \qquad \textbf{(1)}$$

The correlation coefficient (R^2) of the above equation was 0.90. F-tests showed that the model had a significance of 98% ($P<0.02$). These data indicated that the model was reliable in reflecting the relationship between T and pH with glucose concentration.

Table 2.11. Central composite design of temperature (T) and initial pH with the glucose concentration as response

Run	Variables				Response
	T		pH		glucose (g/L)
	Coded unit	Real value	Coded unit	Real value	
1	-1	43°C	-1	4.3	4.99
2	-1	43°C	+1	5.3	4.39
3	+1	53°C	-1	4.3	3.89
4	+1	53°C	+1	5.3	4.13
5	-1.41	41°C	0	4.8	4.80
6	+1.41	55°C	0	4.8	3.85
7	0	48°C	-1.41	4.1	3.35
8	0	48°C	+1.41	5.5	4.38
9	0	48°C	0	4.8	5.48
10	0	48°C	0	4.8	5.28
11	0	48°C	0	4.8	5.11

Analysis of variance

Source of variation	Sum of square	Degree of freedom	Mean square	*F*-value [a]
Regression	3.931	5	0.786	6.36
Residual	0.618	5	0.124	
Total	4.549	10		
R^2	0.864			

[a] the *p* value is less than 0.05

The response surface of glucose concentration as a function of T and pH (in coded unit) is shown in Figure 2.17. The plot is hump shaped with a clear peak within the experimental

range investigated. Using the NCSS 2000 software, the exact optimal T and pH values (in coded unit) were obtained as -0.39 and 0.04, which corresponded to real values of 46°C and pH 4.8, respectively. Equation 1 predicted the maximum glucose concentration as 5.36 g/L, and was verified by the experimental data (5.56 g/L) obtained under the optimal conditions. It was also found that the value of response did not fall steeply when the variables changed slightly from their best values (Figure 2.17). This is a desired property for the hydrolysis process, because it means that the process will be robust to slight error or fluctuation in T and pH during operation.

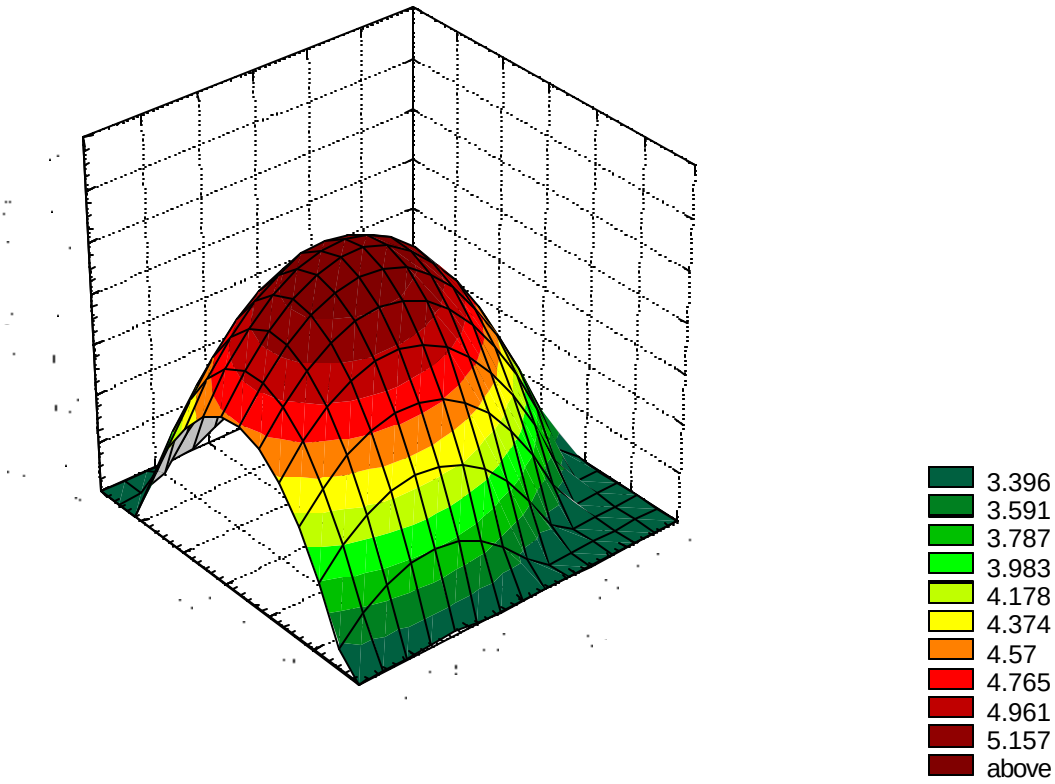


Figure 2.17. Three-dimensional surface plot of glucose concentration as a function of T and pH

1.4.3. Effects of substrate concentration. The effects of substrate concentration on enzymatic hydrolysis were investigated with two sets of experiments, one at fixed enzyme concentration, the other at fixed enzyme to substrate ratio. For each experimental run, glucose concentration and yield showed an opposite trend, with glucose increasing, and yield decreasing with increasing substrate concentration (Figure 2.18). Similar results have also been observed for other lignocellulosics such as softwood, weeds, and bagasse. When enzyme concentration was

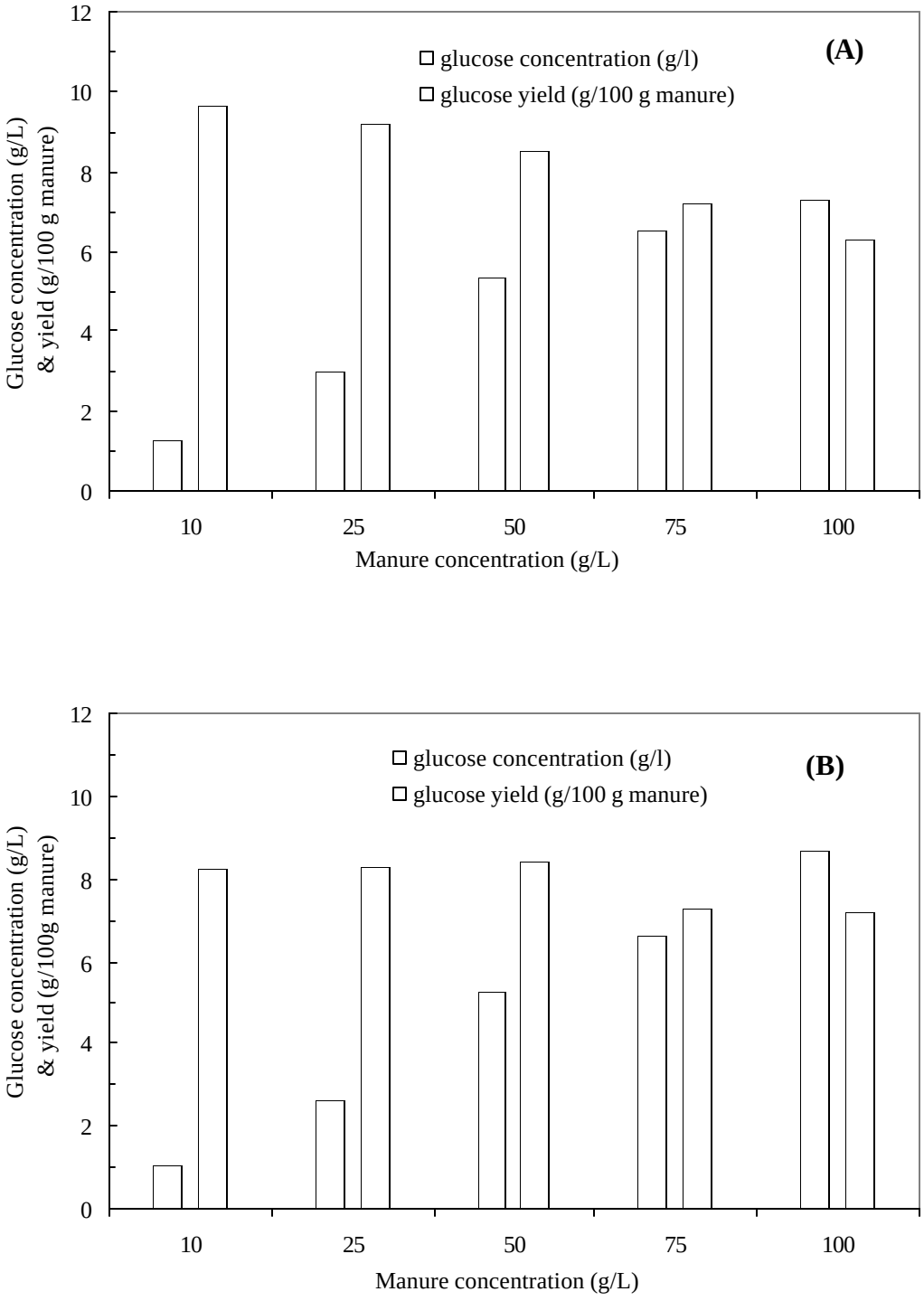


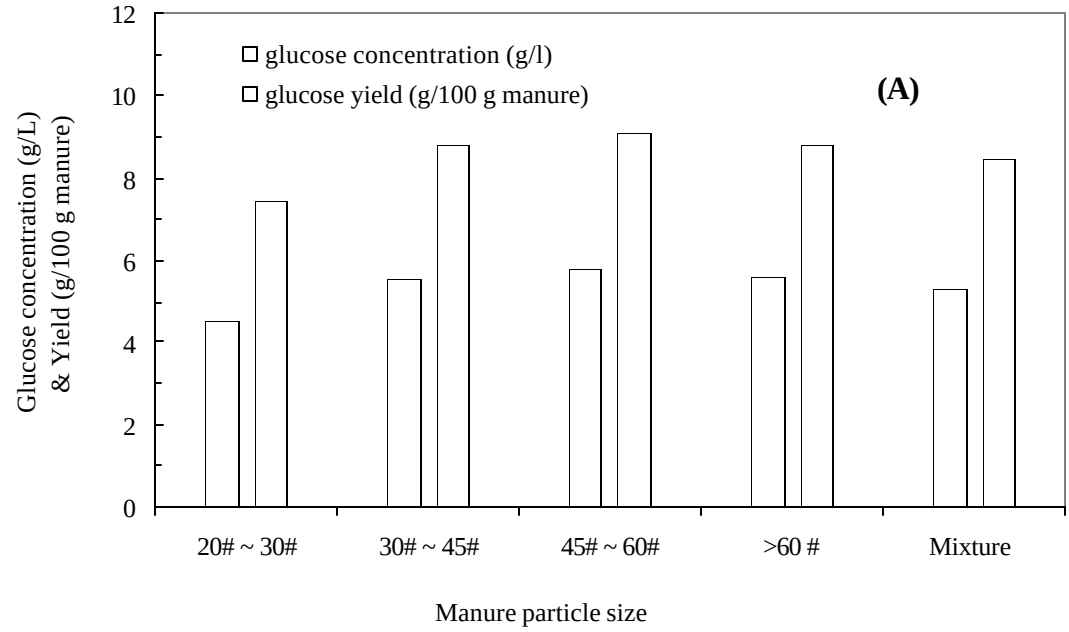
Figure 2.18. Effects of substrate (manure) concentration on enzymatic hydrolysis of manure. (A) enzyme loadings were fixed at 650 IU/L of filter paper activity and 250 IU/L of β-glucosidase activity. (B) enzyme loadings were varied to maintaining the ratio enzyme to substrate at 13 FPU/g manure.

fixed, increasing substrate concentration resulted in more cellulose available for hydrolysis. However, the amount of enzyme was not proportionally increased, and, as a result, more intact cellulose was present in the system, which led to a low glucose yield. End-product inhibition or insufficient hydrolysis time for the additional cellulose are other possible explanations for why high substrate concentration resulted in lower yield.

When the ratio of enzyme to substrate was fixed, sufficient enzyme could be supplied with increased substrate concentrations. As a result, glucose yield remained almost constant within the substrate concentration ranging from 10 to 50 g/L (Figure 2.18B). However, further increases in substrate concentration (50-100 g/L) resulted in a lower glucose yield. This may be due to the end-product inhibition caused by the high concentration of glucose. Based on the above results, it was found that 50 g/L of substrate favored both glucose concentration and glucose yield, so this concentration was used in subsequent experiments.

1.4.4. Effects of manure particle size and surfactant. In the enzymatic hydrolysis process, the size of the substrate particles is an important parameter for the hydrolysis efficiency because it influences the contact of enzyme to substrate. In addition, some non-ionic surfactants have been found to enhance the hydrolysis, as the interfacial energy may have some impact on the transfer between solid substrate and soluble enzyme as well as the sugars produced. These two parameters were investigated.

The glucose concentration and yield by hydrolyzing each sized substrate are presented in Figure 2.19. The mixture containing all sized material was taken as control. Hydrolysis was performed under either surfactant-free or 2% Tween-80 added systems. As shown in the figure, the particles ranging from 840-590 μm resulted in a low glucose concentration and yield, probably due to the low surface area available for enzyme attaching. Decreasing the particle size from 840-590 micron to 590-350 micron enhanced glucose yield by 29%. However, further decreases in the particle size resulted in almost no change in glucose yield, suggesting that the size of particles was not a limiting factor when below 590 micron. Glucose yield of the control (mixture) was in agreement with the theoretical value calculated by weighting the average of glucose yield for each particle size. It was found that for each particle size investigated, the addition of Tween-80 enhanced glucose yield by at least 20% (Figure 2.19), suggesting the beneficial effects of this surfactant on enzymatic hydrolysis.



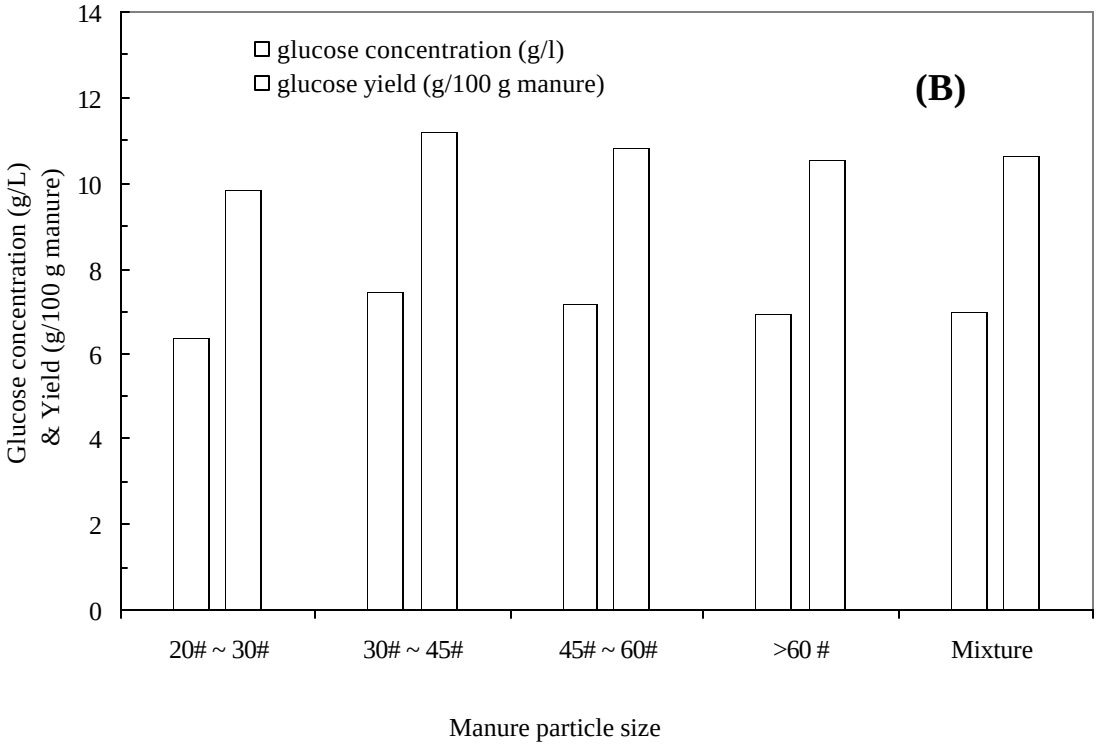


Figure 2.19. Effects of particle size and Tween-80 addition on the glucose concentration and yield. (A) Tween-80 free. (B) 2% tween-80 (w/v). All manure particles were passed through 20# US Standard sieve (840 µm opening), then screened by 30# (590 µm), 45# (350 µm), and 60# (250 µm) sieves serially. The mixture is all size of particles passing through 20# sieve.

The enzymatic hydrolysis of cellulosic materials is a heterogeneous reaction, with soluble enzymes attaching to solid cellulose and converting it into soluble sugars. The particle size (i.e., the surface area) of the substrate is an important parameter for the hydrolysis because it influences the contact between enzyme and substrate. In addition, modifying the interfacial energy may have some impact on the transfer of enzymes and soluble sugar between the solid substrate and bulk solution. It has been reported that the improved cellulose conversion with the addition of surfactant is due to reduction of cellulase adsorption to the lignin part of the substrate, as the hydrophobic interaction of surfactant with lignin occurs at the substrate surface. Such interaction releases the non-specifically bound enzyme. Non-ionic surfactants such as tween-80 and polyoxyethylene glycol are often used for enhancing the hydrolysis, while anionic surfactants have inhibitory effects.

In conclusion, the optimal conditions of enzymatic hydrolysis of manure fiber without hemicellulose were determined as 13 FPU/ml cellulase, 5 IU/ml β-glucosidase, 50 g/L substrate (<590 μm of particle size), with supplementation of 2% Tween-80 under pH 4.8 and 46°C. Under such conditions, glucose yield achieved 11.32 g/100 g manure (26 g/100 g cellulose in manure).

1.5. Enzymatic hydrolysis of manure fiber with different treatments. Based on the results in previous section, the effects of major fiber components (lignin, hemicellulose) on enzymatic hydrolysis of manure fiber were studied in terms of developing an enzymatic hydrolysis process with proper treatment of manure to produce mono-sugars.

Two methods were applied to perform the treatment: dilute acid treatment (3% acid, 120°C and 2 hours, which are the optimal conditions of acid hydrolysis of hemicellulose in original manure from section 1.2.1) to remove hemicellulose and sodium chlorite treatment (3% sodium chlorite, 1% acetic acid, 70°C and 1 hour) to remove lignin. Four different treated samples were ready to be enzymatically hydrolyzed: original manure fiber, manure fiber without hemicellulose, manure fiber without lignin, and manure fiber without both hemicellulose and lignin. The samples were then hydrolyzed using the optimal conditions noted in the previous section except for different concentration levels of enzyme. The flowchart is presented in Figure 2.20.

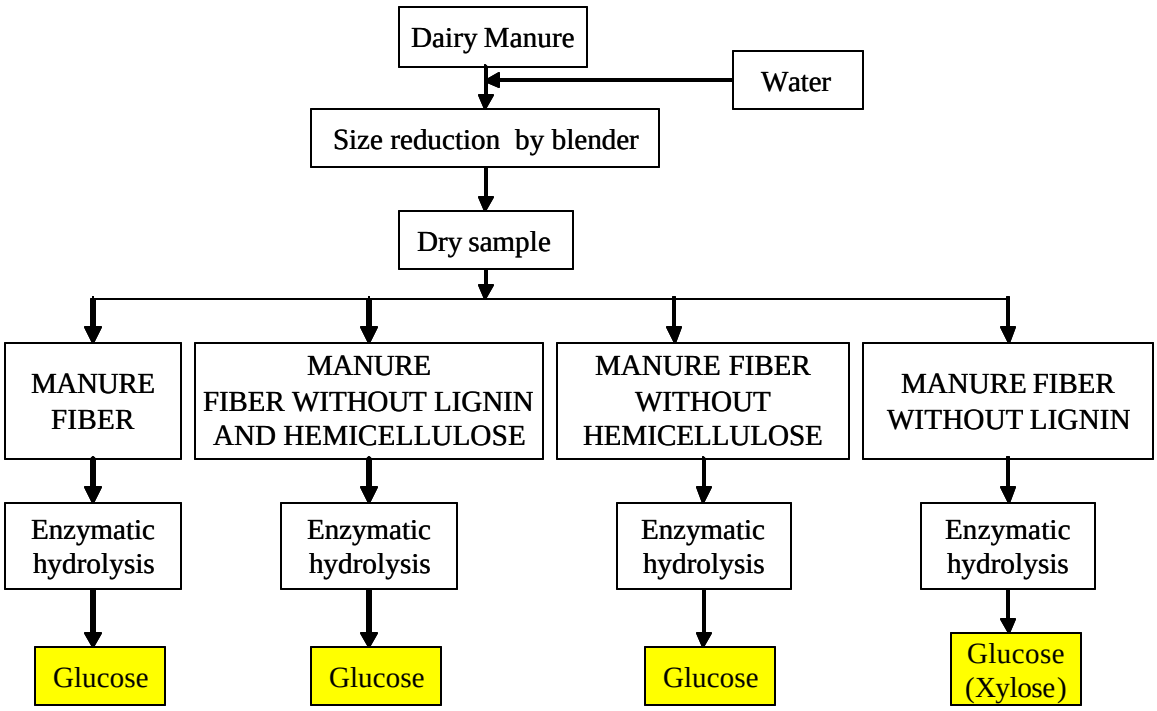


Figure 2.20. Enzymatic hydrolysis of four different treated manure fibers

Figure 2.21 shows the cellulose content of manure fibers with various treatments. Compared with original manure, the treatments increased the cellulose content significantly

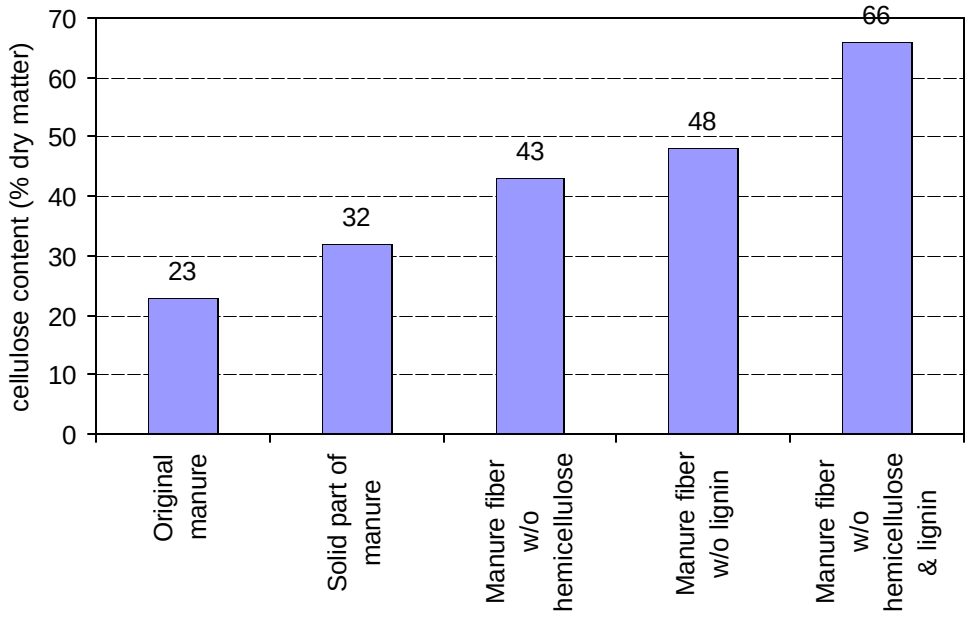


Figure 2.21. Cellulose content of manure fibers with various treatments

indicating that treated manure supplied more substance to be hydrolyzed by the enzyme. The sample from the treatment of dilute acid hydrolysis combined with sodium chlorite treatment, which had no lignin and hemicellulose in it, had the highest cellulose content of 66%, which is supposed to produce more glucose.

1.5.1. Enzymatic hydrolysis of pretreated manure. Figure 2.22 shows a comparison of enzymatical hydrolysis of different pretreated types of manure samples at an enzyme level of 500 FPU/L (including 500 FPU/L cellulase and 192 IU/ml β-glucosidase). It shows that enzyme hydrolysis of manure fiber without hemicellulose, manure fiber without lignin, and manure fiber without both lignin and hemicellulose had much higher conversion rates than the original manure fiber. Also, manure fiber without both hemicellulose and lignin had the highest glucose yield of about 42% at 72 hours of reaction time. The result indirectly demonstrates that either lignin or hemicellulose may prevent the enzyme from attacking the cellulose to produce glucose. Thus, further studies on how lignin and hemicellulose influence the final sugar yield are discussed in the following sections.

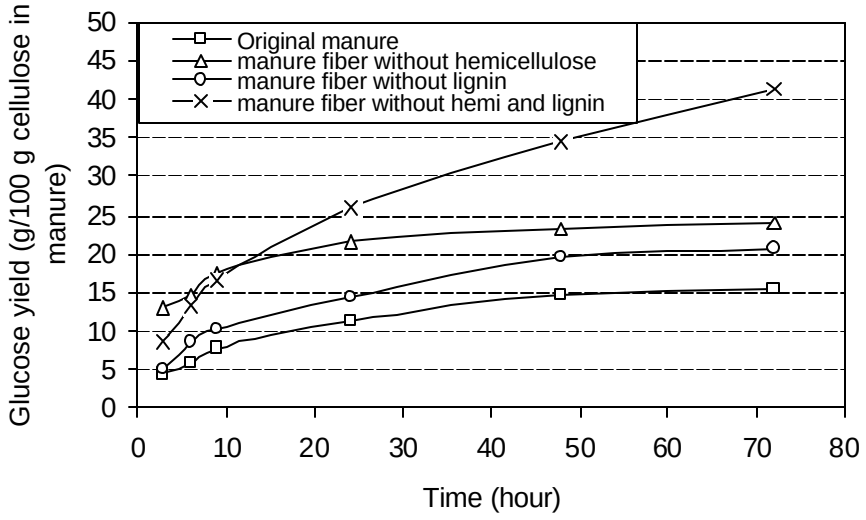


Figure 2.22. Comparison of enzymatic hydrolysis of different pretreated manure samples

1.5.2. Enzymatic hydrolysis of manure fiber without hemicellulose. Figure 2.23 demonstrates the effect of different enzyme loadings on glucose yield of enzymatic hydrolysis of manure fiber without hemicellulose. Glucose yield increased as enzyme loading and reaction

time kept increasing. The amount of glucose produced increased sharply within the first 24 hours. After 24 hours, all three enzyme loadings had no further significant increases with increase of time. Maximum glucose yield was obtained at after 24 hours for each level of enzyme concentration. An enzyme level of 650 FPU/L had the highest glucose yield at almost 30%.

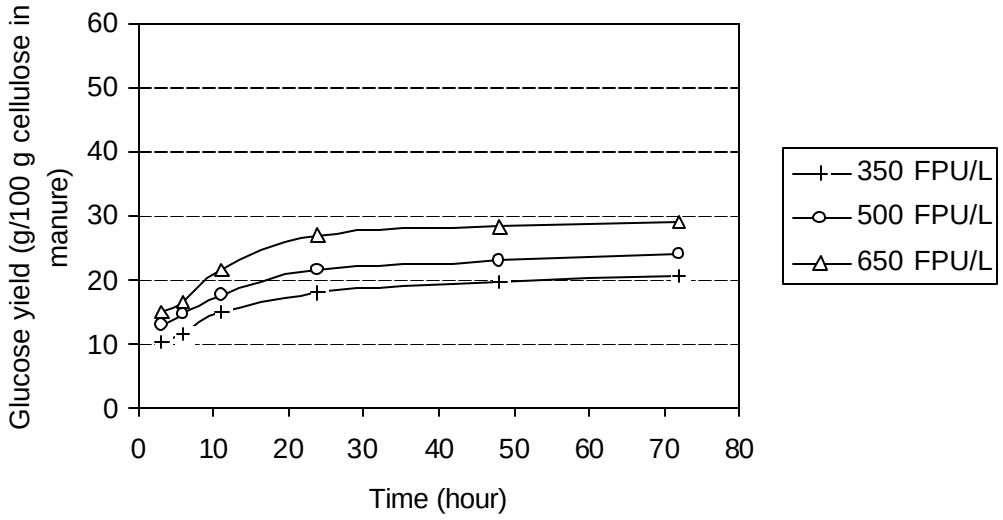


Figure 2.23. The effect of different enzyme loadings on the enzymatic hydrolysis of manure fiber without hemicellulose

1.5.3. Enzymatic hydrolysis of manure fiber without lignin. Figure 2.24 presents the effect of different enzyme loadings on enzymatic hydrolysis of manure fiber without lignin. The results of glucose conversion rate of manure fiber without lignin are similar to manure fiber without hemicellulose. The glucose yield increased as enzyme loading and reaction time increased. Maximum glucose was produced at 72 hours and an enzyme level of 650 FPU/L had the highest glucose yield of about 26%, which is a little lower than that of hydrolysis of manure fiber without hemicellulose. Also, compared to hydrolysis of manure fiber without hemicellulose, hydrolysis of fiber without lignin required a much longer time, about 3 days, to reach a higher glucose yield.

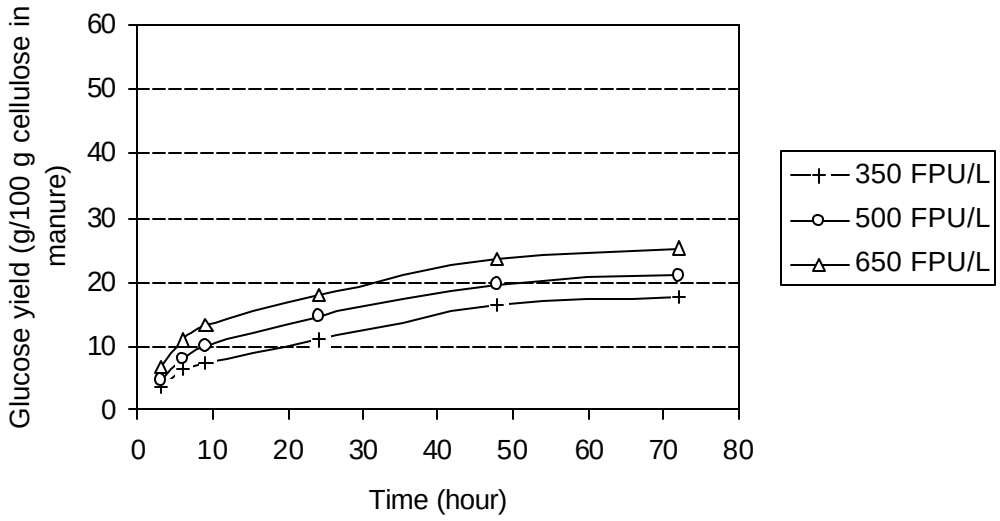


Figure 2.24. The effect of different enzyme loading on glucose conversion rate of enzymatic hydrolysis of manure fiber without lignin

1.5.4. Enzymatic hydrolysis of pretreated manure without both lignin and hemicellulose.

The enzymatic hydrolysis of manure fiber without lignin and hemicellulose showed vast improvement in glucose yield. The glucose yield of 52% at a reaction time of 144 hours and cellulase units of 650 FPU/L was much higher than previous yields, although it needed a much longer time (Figure 2.25). This means that either hemicellulose or lignin existing in the fiber has a negative effect on enzymatic hydrolysis of cellulose in manure fiber. Therefore, finding an environmentally-friendly way to pretreat the manure to effectively remove both lignin and hemicellulose will benefit the enzymatic hydrolysis of cellulose in manure fiber.

In summary, either hemicellulose or lignin has a negative effect on enzymatic hydrolysis of cellulose in manure fiber. After removing both lignin and hemicellulose, enzymatic hydrolysis of manure cellulose can reach a rather higher glucose yield of more than 50% without further treatment such as decrystallization to break down the crystal structure of cellulose. Consequently, some moderate environmentally-friendly treatments (neither acid treatment nor chlorite treatment) such as metal-H_2O_2 treatment, which is reported to be able to degrade both hemicellulose and lignin in wood, should be studied on treatment of manure fiber to make further enzymatic hydrolysis of manure fiber more practicable and effective.

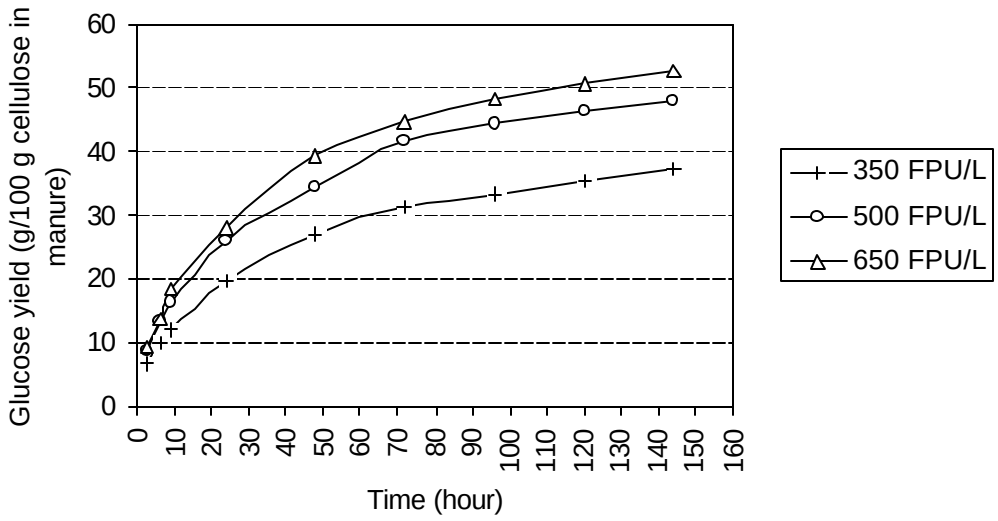


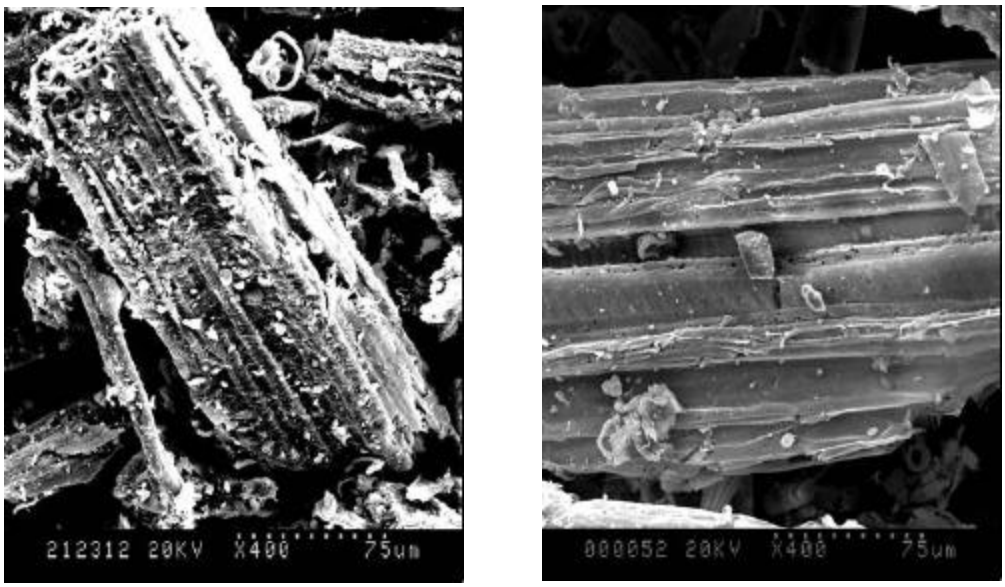
Figure 2.25. The effect of different enzyme loading on glucose conversion rate of enzymatic hydrolysis of manure fiber without lignin and hemicellulose

Structure change of manure fiber during hydrolysis

Manure samples before and after hydrolysis were observed using scanning electronic microscopy to understand what occurred on the structure of the manure fiber during hydrolysis.

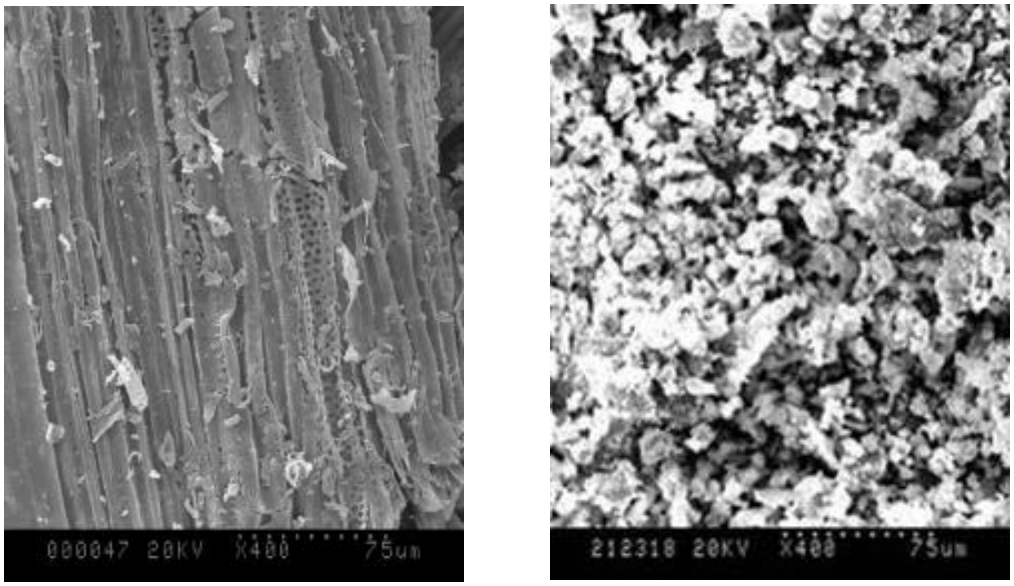
The difference in fiber structure between original manure and pretreated manure is shown in Figure 2.26. The texture of original manure fiber was much rougher than that of fiber in pretreated manure, which means there were more substances attached to the surface of the main structure of the fiber in original manure than the pretreated manure. Chemical composition comparisons of both samples showed that most of these substances were most likely nitrogen-related materials such as proteins (Tables 2.6 and 2.7).

Figure 2.27a presents a very clear view of the main structure of pretreated manure fiber after removing hemicellulose using dilute acid hydrolysis as discussed in section 1.2.1. Compared with Figure 2.26b, the striations on the surface of manure fiber are thinner, so more substances were washed from the manure fiber. In addition, fiber data for the hydrolyzed pretreated manure (Table 2.10) showed that there was almost no hemicellulose left in the hydrolyzed solid. However, the main structure of manure fiber was not destroyed by dilute acid, which means most of crystal structure of cellulose was still in the hydrolyzed solid part with



A B

Figure 2.26. Scanning electron microscope of dairy manure (400x)
A: Original dairy manure
B: Pretreated dairy manure (after washing)

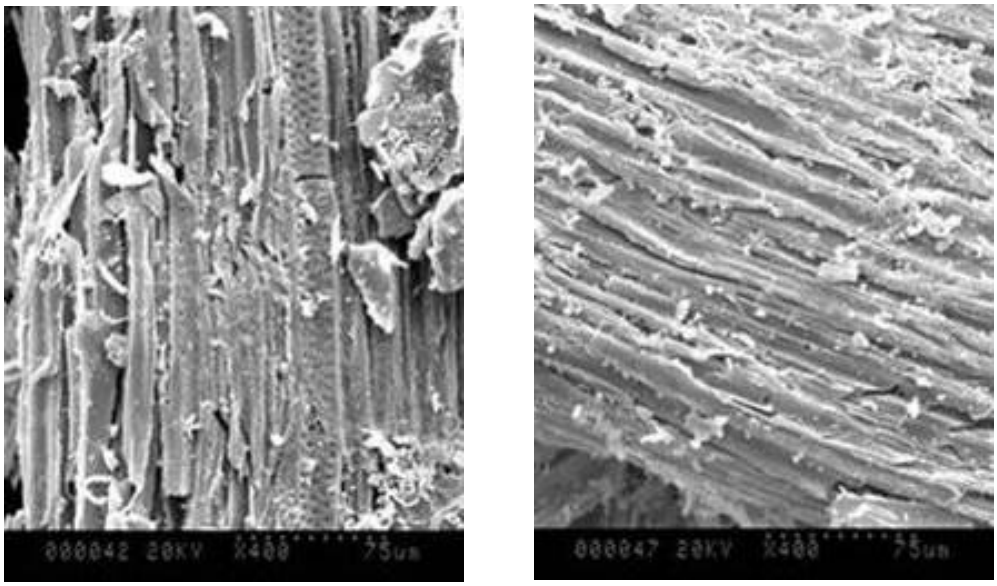


A B

Figure 2.27. Scanning electron microscope of acid hydrolysis of pretreated manure (400x)
A: Solid of dilute acid hydrolysis of pretreated manure (2 hours, 1% sulfuric acid, 135°C)
B: Solid of dilute acid hydrolysis with decrystallization of pretreated manure

respect to low glucose concentration in the hydrolyzed solution. Figure 2.27b demonstrates that, after acid decrystallization, manure fibers turned into some amorphous powders. At the same time, the glucose yield was greatly increased (see section 1.2.2). The results qualitatively prove that most of hemicellulose is attached on the surface of crystal structure of cellulose in manure fiber and is relatively easy to be hydrolyzed by dilute acid, while the back-bone structure of manure fiber is apparently composed of cellulose, which only can be degraded to produce glucose using some decrystallization method (such as concentrated acid used in this project).

Structure changes of enzymatic hydrolysis of manure are presented in Figure 2.28. The only difference between the two samples was that the striations on the surface of manure fibers after hydrolysis were thinner. Considering the relatively high glucose yield of 52%, the main structure might be partially degraded, though the main structure of manure fiber was not completely destroyed like in acid decrystallization of manure fiber (Figure 2.27).



A B

Figure 2.28. Scanning electron microscope of hydrolysis of enzymatic hydrolysis of manure (400X) A: Solid part of manure without hemicellulose and lignin (before enzymatic hydrolysis) B: Solid part of manure without hemicellulose and lignin (after enzymatic hydrolysis)

Comparison of hydrolysis methods

The results from the study of different hydrolyses of manure indicate that acid hydrolysis with decrystallization is the best among the procedures studied in this project. Decrystallization with 75% acid concentration, 3:5 sample to acid ratio, and 30 min of reaction time, followed by dilute acid hydrolysis with 12.5% acid and 10% sample at 130°C for 10 min were the optimal conditions producing 26 g/L glucose at a conversion rate of 84% and 11 g/L xylose at a conversion rate of 80%. Compared to acid hydrolysis of woods, manure has much higher nitrogen content, which makes some side-reactions such as browning reaction, dehydration occurred more easily. Thus, pretreatment of manure is a critical step in obtaining the higher sugar yield. In this particular case, acid hydrolysis with decrystallization of pretreated dairy manure had a conversion rate (84%) very close to the hydrolysis of sweetchip (conversion rate of 85%).

For enzymatic hydrolysis, some treatments are necessary to improve sugar production. The highest sugar yield was from enzymatic hydrolysis of manure fiber without hemicellulose and lignin. The optimal conditions were 650 FPU/L cellulase, 250 IU/L β-glucosidase, and 50 g/L substrate at pH 4.8 and 46°C. The glucose conversion rate under the optimal conditions reached 52%, which was more than half of the conversion rate of acid hydrolysis with decrystallization. Although the conversion rate of enzymatic hydrolysis was not as high as acid hydrolysis with decrystallization, it is still a promising method for producing sugars from lignocellulosic materials, since enzymatic hydrolysis is moderate and environmentally friendly. Moreover, the comparison also indicates that decrystallization is the critical step in trying to improve glucose yield. This suggests that finding a non-acid decrystallization method may help enzymatic hydrolysis in effectively increasing sugar yield.

Sugar recovery from hydrolysate

The purpose of this part of study was to find an economically viable manure hydrolysate purification method that can satisfy the purity requirement of the following hydrogenation step.

As reported earlier, acid hydrolysis with decrystallization is the most effective method for manure hydrolysis. Therefore, the manure hydrolysate used in this part of study was prepared using this method. Clean dairy manure solids obtained by washing the manure with water were

treated by 70% H_2SO_4 for decrystyllization. The mixture was then diluted with water, and hydrolyzed at 100°C for 1 hour. The detailed procedure for hydrolysate preparation is shown in Figure 2.29.

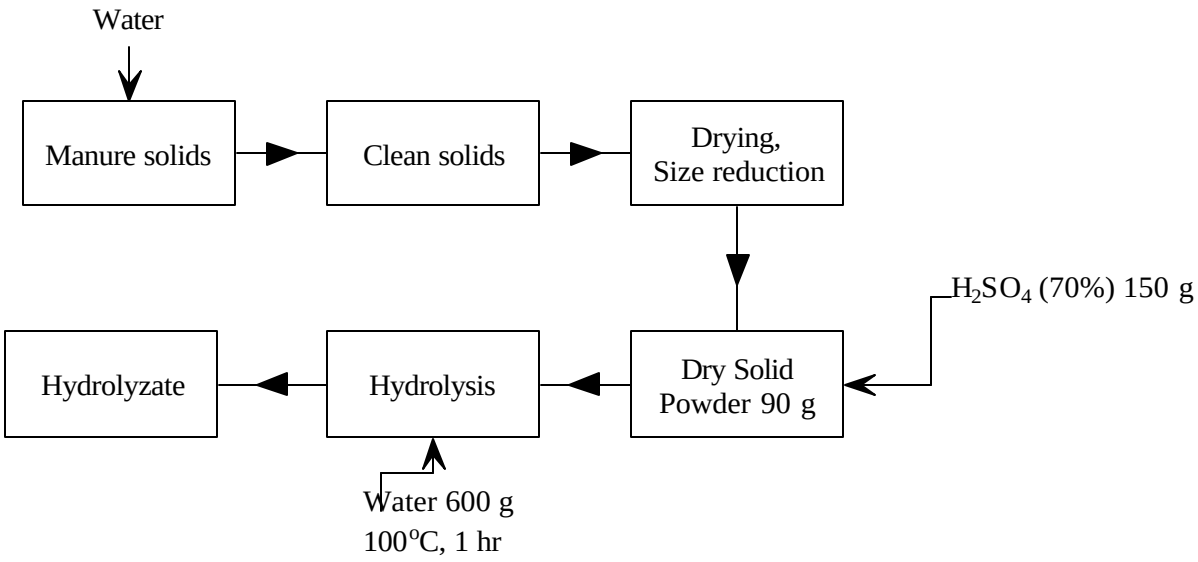


Figure 2.29. Preparation of manure hydrolysate.

The manure hydrolysate contained 47.25 g/L of reducing sugar, 9.71 g/L of water-soluble peptides, and a limited amount of byproducts from lignin degradation. Water-soluble peptides have been proven to be the major toxic component in manure hydrolysate, which can decrease the activity of catalysts dramatically. Accordingly, our work focused on the removal of peptides from the manure hydrolysate. The purification strategies applied here included adsorption (at acid or neutral conditions), strong basic ion exchange (at neutral condition), week basic ion exchange resin (at neutral condition), and ion exclusion.

Adsorption

In order to study the efficiency of adsorption on peptides removal from manure hydrolysate, a widely used adsorption resin, Duolite XAD 76, was applied to treat manure hydrolysate at acid and neutral conditions, respectively. The results are shown in Figure 2.30. One can easily find that peptides could not be efficiently removed by the adsorption resin at either acid or neutral conditions. A significant loss of sugar, our objective product, after

adsorption treatment was also revealed from the results presented in Figure 2.30. Therefore, adsorption is not suitable for the purification of manure hydrolysate.

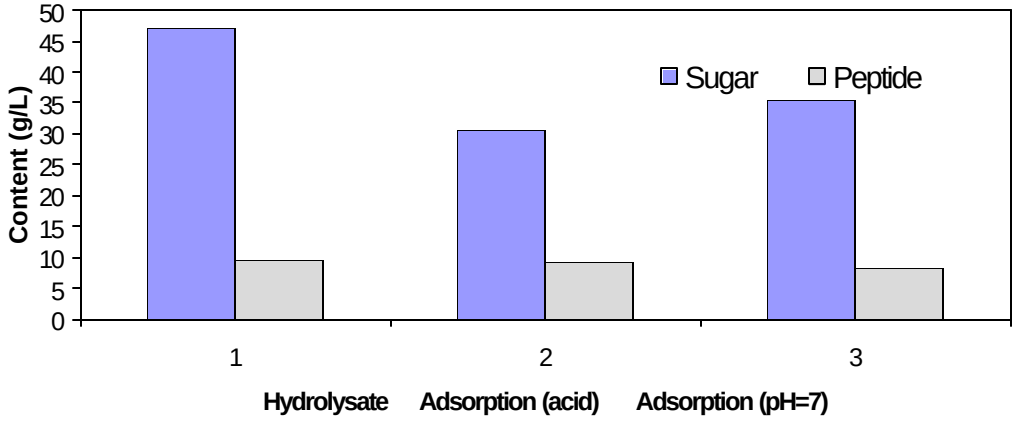


Figure 2.30. Adsorption treatment manure hydrolysate using Duolite XAD 76

Ion exchange

Considering that peptides exist in solution in the form of ions, the peptides in manure hydrolysate should be able to be removed by ion exchange treatment. Two ion exchange resins, strong basic ion exchange resin Amberlite IRA 958 and weak basic ion exchange resin Amberlite IRA 96, were applied. The effectiveness of these two resins is presented in Figure 2.31. Similar to adsorption treatment, ion exchange is not very effective for the removal of peptides. Significant loss of sugar were also observed.

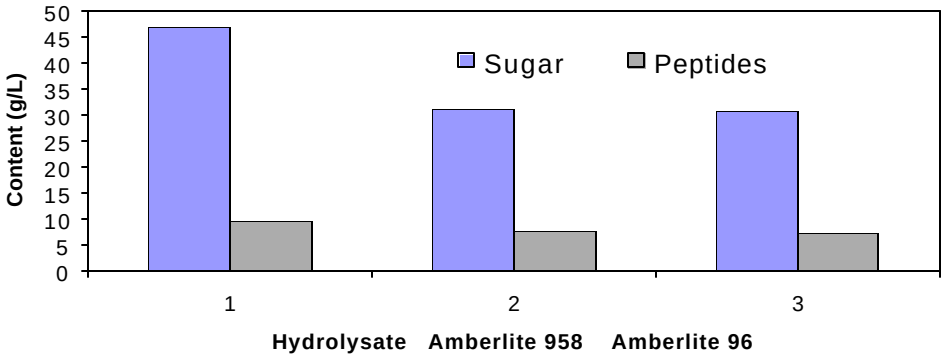


Figure 2.31. Ion exchange treatment of manure hydrolysate

Ion Exclusion

Ion exclusion (IE) has been proven to be an effective way to separate an acid/sugar mixture into its components. Separation by IE was favored because acid can be recovered and reused. Sulfuric acid is excluded from entering the porous resin because of ion repulsion due to a high chemical potential of acid groups inside the resin. Thus, acid will pass through a resin-packed bed faster than sugar, which penetrates the resin's microporous structure. When an acid/sugar mixture is passed through a resin bed, an acid-rich stream will elute first, then a sugar-rich stream. The composition of manure hydrolysate is much more complicated due to the existence of peptides and many other impurities. As peptides exist in hydrolysate as ions, we deduced that peptides would be excluded from entering the porous resin, and exist in the acid stream. A rather pure sugar stream should be obtained via IE. The results shown in Figures 2.32 and 2.33 confirm this assumption.

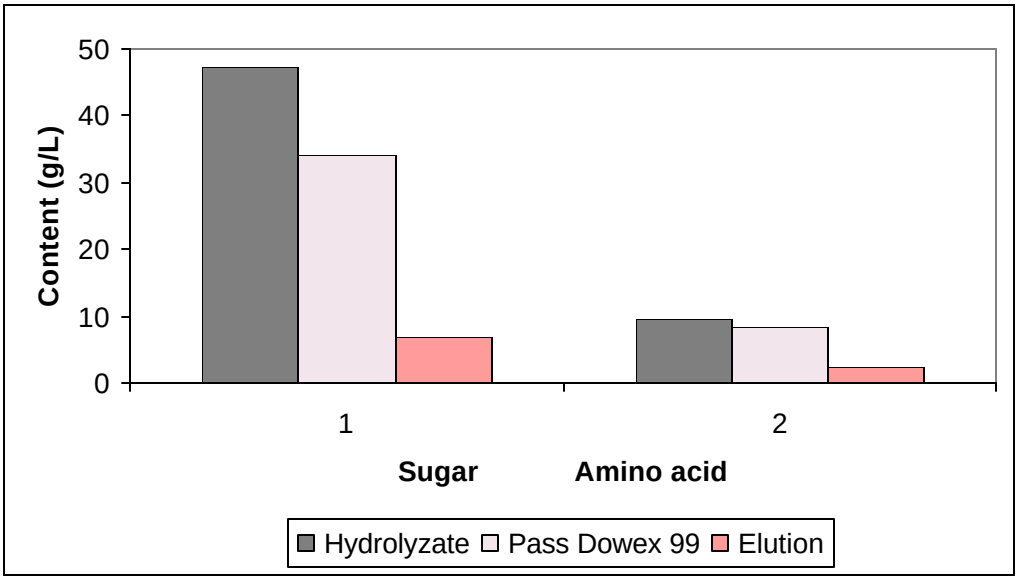


Figure 2.32. Ion exclusion treatment of manure hydrolysate using Dowex 99 H

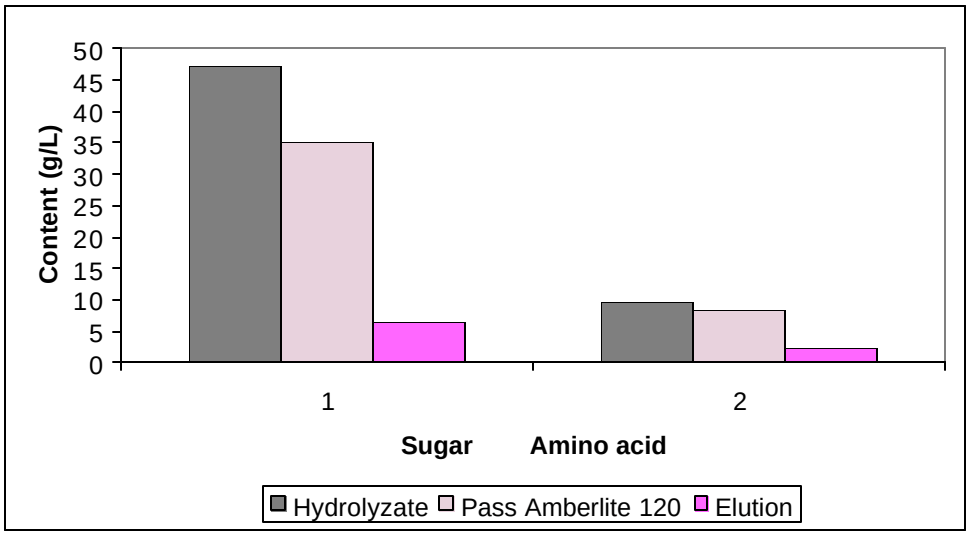


Figure 2.33. Ion exclusion treatment of manure hydrolysate using Amberlite 120

Production of cellulase by *Trichoderma reesei* from dairy manure

The aim of this part of study was to develop a cost-effective process for cellulase production and subsequent enzymatic hydrolysis of manure lignocellulose. Cellulolytic enzymes could be produced by a number of bacteria and fungi, which can use cellulose as a primary carbon source. Pure, crystalline cellulose, such as Solka Floc, Avicel, and cotton are good cellulase inducers, but are expensive. To keep costs down, other less expensive substrate can be considered. Many cellulosic materials, such as wood, wastepaper, bagasse, wheat straw, corncob, wheat bran, and fruit pomace, have been studied as potential substrates for the production of cellulase. However, few, if any, investigations have been conducted on cellulase production from manure cellulosics.

In this study, cellulase production by the fungi *Trichoderma reesei* was examined using dairy manure as a substrate. Two fungal strains, *Trichoderma reesei* RUT-C30 (ATCC 56765) and *Trichoderma reesei* QM 9414 (ATCC 26921), were used. The fungi were maintained in potato dextrose agar slant at 4°C. The subculture medium was a salt solution with 2 ml/L tween-80, 1 g/L peptone, and 10 g/L glucose added (Table 2.12). The initial pH of the medium was adjusted to 4.8 before being autoclaved at 121°C for 15 min. Fungal cells were sub-cultured

Table 2.12. Medium composition for subculture of the fungi *T. reesei*

Components	Unit	Concentration
Salt solution		
KH_2PO_4	g/L	2.0
$CaCl_2 \cdot 2H_2O$	g/L	0.4
$MgSO_4 \cdot 7H_2O$	g/L	0.3
$(NH_4)_2SO_4$	g/L	1.4
Urea	g/L	0.3
Trace elements		
$FeSO_4 \cdot 7H_2O$	mg/L	5.0
$MnSO_4 \cdot H_2O$	mg/L	1.6
$ZnSO_4 \cdot 7H_2O$	mg/L	1.4
$CoCl_2$	mg/L	2.0
Tween-80	mL/L	2.0
Peptone	g/L	1.0
Glucose	g/L	10

in an orbital shaker (175 rpm) at 30°C for 1~2 generations and then used for inoculum. Five milliliters of exponential cells was inoculated into 50 ml of medium containing manure as a substrate. The medium composition was the same as the subculture medium (Table 2.12), except that peptone was eliminated and glucose was replaced with manure.

The activities of total cellulase (filter paper activity, FPA), endo-β-1,4 glucanase (CMCase), and β-glucosidase were determined according to standard IUPAC procedures and expressed as an IU. One unit of FPA and CMCase activity was defined as the amount of enzymes that release 1 μmol of glucose equivalents from Whatman No. 1 filter paper and carboxymethyl cellulose (CMC) in 1 min, respectively. One unit of β-glucosidase activity was defined as the amount of enzyme converting 1 μmol of cellubiose to produce 2 μmol of glucose in 1 min. The glucose concentrations in the cellubiose hydrolysates were measured using the enzyme assay kit from Sigma (Product No. GAGO-20).

Data showed that *T. reesei* RUT-C30 had higher cellulase production than *T. reesei* QM 9414 and that a homogenized manure, treated by a blender to reduce fiber size, led to higher cellulase production. The cellulase production was further optimized by growing *T. reesei* RUT-C30 on homogenized manure. The effects of manure concentration, pH, and temperature on cellulase production were investigated with optimal parameter values determined to be 10 g/L

manure (dry basis), 25.5°C, and pH 5.7, respectively. Eliminating $CaCl_2$, $MgSO_4$, nitrogen sources (NH_4^+ and urea) and trace elements (Fe^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+}) from the original salt solution had no negative influence on the cellulase production, while phosphate elimination did reduce cellulase production. Based on above results, the final medium composition was simplified with manure additives being KH_2PO_4, Tween-80, and $CoCl_2$ only. Using this medium composition and a reaction time of 6~8 days, a maximum cellulose production activity of 1.74 IU/mL of filter paper activity (FPA), 12.22 IU/mL of CMCase activity, and 0.0978 IU/mL of β-glucosidase was obtained. This FPA is the highest ever reported in cellulase production from agricultural wastes.

Feasibility of using manure as a substrate for cellulase production

Manure has cellulosic components that can induce the production of cellulase when used as carbon sources for fungi growth. Of all the celluloytic fungi, *Trichoderma reesei* has been the most extensively studied, with the mutants *T. reesei* RUT-C30 and *T. reesei* QM 9414 having been identified as possessing improved FPA. In this work, the two mutants were respectively grown in medium containing manure as substrate. Both the untreated manure and homogenized manure were used. The manure concentration was equivalent to 6.7 g/L (dry basis).

It was found that both of the fungi could produce cellulase in medium containing untreated or homogenized manure (Figure 2.34). The un-treated manure resulted in a lower FPA than the homogenized manure, and the average length of fiber size was about 10 mm in untreated manure and less than 2 mm for homogenized manure. One possible explanation is that in the longer fiber, the accessibility of cellulose to fungi cells was lower due to reduced specific surface area, ultimately leading to lower cellulose production.

The time courses of cellulase production by *T reesei* in medium containing homogenized manure are shown in Figure 2.34b. The patterns of cellulase production were similar for the two mutants. Cellulase activity increased during the first 5 days, reached the maximum level between day 5 and day 8, and then decreased at the end of cultivation.

The results in Figure 2.34 also show that the mutant *T. reesei* RUT-C30 produced higher cellulase activity than *T. reesei* QM-9414, and in addition, the homogenized manure resulted in

higher cellulase activity as well. The fungi *T. reesei* RUT-C30 and homogenized manure were therefore used for optimizing fungi cellulase production.

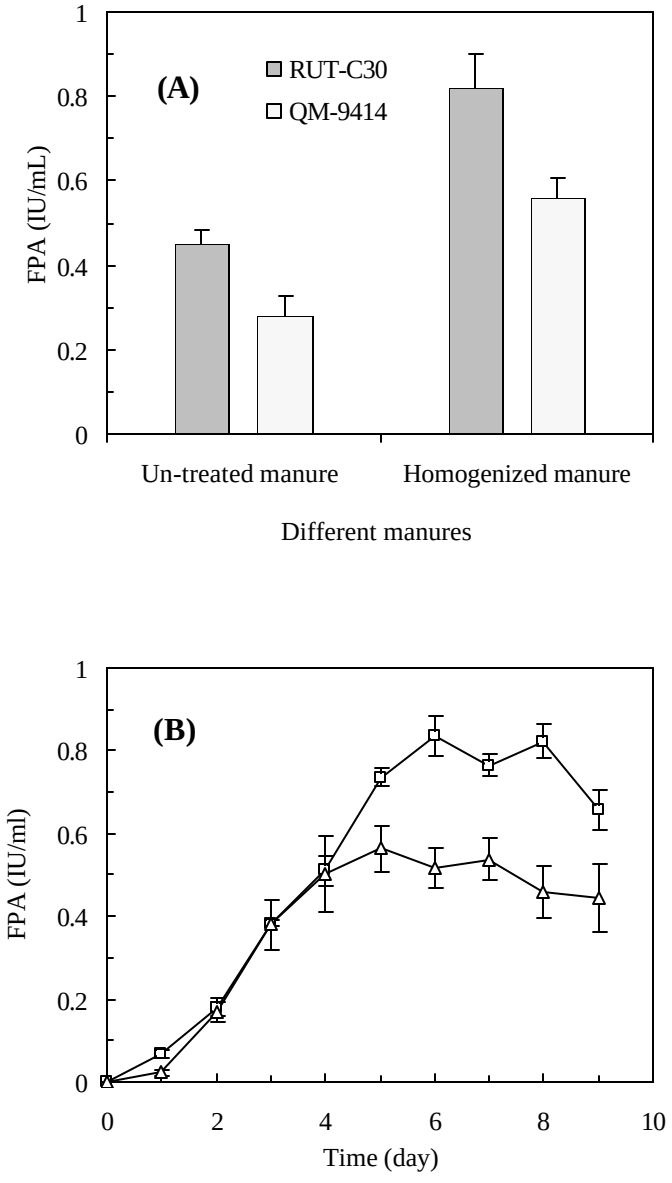


Figure 2.34. Cellulase production by two mutants of *T. reesei* using different dairy manures as a substrate. (A) Filter paper activity (FPA) after 6 days of cultivation in medium containing untreated manure and homogenized manure. (B) Time course of FPA in medium containing homogenized manure. □, *T. reesei* RUT-C30; △, *T. reesei* QM 9414. Data are means of three replicates and error bars show standard deviation.

Optimization of cellulase production by T. reesei RUT-C30

1.6. Effects of manure concentration. Cellulase production by *T. reesei* RUT-C30 investigated at different manure concentrations showed that FPA increased with manure concentration from 3.35 to 10 g/L (dry basis), with the highest FPA of 1.2 IU/mL being obtained for the range from 10 to 13.38 g/L of manure (Figure 2.35A). FPA decreased when the manure concentration was over 13.38 g/L. The variation of manure concentration resulted in different cellulose levels in the media; therefore, the cellulase yield based on added cellulose (FPA per gram of cellulose added) was presented. The highest cellulase yield, 708 IU/g cellulose, was obtained at the lowest manure level (Figure 2.35B). The yield monotonically decreased with increasing manure/cellulose concentration.

The effects of manure concentration on cellulase production did not solely depend on the amount of cellulose, but also perhaps on other nutrients or ions. For example, the lower FPA at high manure concentration (16.75 g/L, Figure 2.35A) can probably be attributed to the inhibitory effects caused by high mineral salts and lignin content. Ultimately, though, since 10 g/L (dry basis) was the optimal level for FPA, this manure level was used in optimizing temperature and pH and for eliminating of the addition of external mineral salts to the salt solution.

1.7. Effects of temperature and pH. The effects of temperature (T) and pH on cellulase production were investigated by a central composite design (Table 2.13). The cellulase activity of runs 1-8 were the means of two duplicates, while the central point was run in triplicate (runs 9-11), and its standard deviation was 0.03 IU/mL. The results showed a high reproducibility as the deviation of each run was less than 5%.

Filter paper activity in Table 2.13 was correlated as a function of T and pH (coded value) (Eq. 1) by the NCSS 2000 software. The resultant equation was:

$$FPA = 1.252 - 0.181 \cdot T + 0.474 \cdot pH - 0.401 \cdot T^2 - 0.277 \cdot pH^2 - 0.130 \cdot T \cdot pH \quad \textbf{(2)}$$

The correlation coefficient (R^2) of the above equation was 0.984. *F*-tests showed that the model had a significance of 99% ($P<0.01$). These data indicated that the model was reliable in reflecting the relationship between T and pH with cellulase production. According to Eq. (2), the

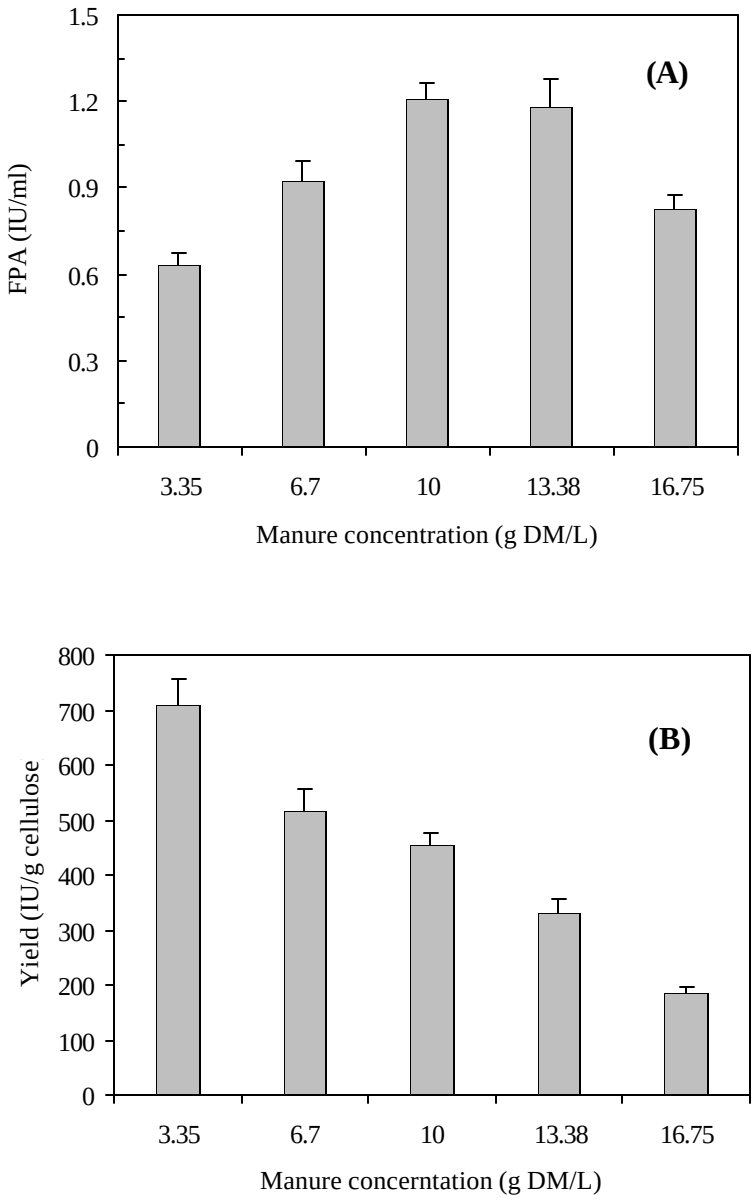


Figure 2.35. Effects of manure concentration on **(A)** cellulase production and **(B)** yield of cellulase based on cellulose in the medium. Data are means of three replicates and error bars show standard deviation.

exact optimal T and pH values (in coded unit) were obtained as –0.285 and 0.922, which corresponded to real values of 25.5°C and pH 5.7, respectively. Eq. (2) predicted the maximum FPA as 1.51 IU/mL, and was verified by the experimental data (1.59 IU/mL) obtained under the optimal conditions.

Table 2.13. Central composite design of temperature (T) and pH with the FPA as response

Run [a]	Variables				Response	
	Coded unit		Real value			
	T	pH	T	pH	FPA (IU/L)	percentage of deviation
1	-1	-1	22°C	3.8	0.083 ± 0.004	4.80%
2	-1	+1	22°C	5.8	1.387 ± 0.024	1.73%
3	+1	-1	32°C	3.8	0.085 ± 0.002	2.35%
4	+1	+1	32°C	5.8	0.869 ± 0.031	3.56%
5	-1.41	0	20°C	4.8	0.743 ± 0.019	2.56%
6	+1.41	0	34°C	4.8	0.087 ± 0.002	2.29%
7	0	-1.41	27°C	3.4	0.061 ± 0.001	1.69%
8	0	+1.41	27°C	6.2	1.263 ± 0.048	3.80%
9	0	0	27°C	4.8	1.239	[b]
10	0	0	27°C	4.8	1.271	[b]
11	0	0	27°C	4.8	1.234	[b]

[a] Runs 1-8 were performed duplicates, while run 9 (central point) was performed triplicates.
[b] The standard deviation of runs 9-11 is 0.02 IU/mL, with a percentage of 1.6%.

1.8. Effects of eliminating nutrients in salt solution. The medium used in this work is a salt solution with manure as substrate. As manure is a complex mixture consisting not only of cellulosic materials but also other elements, it was logical to test if the nutrients in the salt solution could be replaced by the corresponding manure nutrients.

As shown in Table 2.14, each nutrient (or nutrients group) contained in salt solution was respectively eliminated from the medium (runs 1 to 5). The medium containing full nutrients was the control (run 6). The cellulase production from each run was determined. It was found that *T. reesei* produced much lower cellulase in the KH_2PO_4-eliminated medium (run 1). However, the cellulase production in the other runs was almost the same (runs 4 and 5) or even better (runs 2 and 3) than that of control (Table 2.14).

A further analysis of the nutrients distribution in manure and in salt solution is presented in Table 2.15. It was found that the amounts of calcium, magnesium, iron, manganese, and zinc contained in the manure are much higher than those in salt solution. As *T. reesei* could utilize those nutrients from manure, the elimination of nitrogen, calcium, magnesium, and trace elements from salt solution had no negative influence on the cellulase production. It is noticeable that the

Table 2.14. Experimental design for eliminating various nutrients from salt solution in Table 2.12 and corresponding cellulase activity [a]

Run	Nutrients					FPA (IU/mL)
	KH_2PO_4	$CaCl_2$	$MgSO_4$	$(NH_4)_2SO_4$ & Urea	Trace-elements	
1	-	+	+	+	+	0.797 ± 0.085
2	+	-	+	+	+	1.74 ± 0.037
3	+	+	-	+	+	1.71 ± 0.057
4	+	+	+	-	+	1.61 ± 0.123
5	+	+	+	+	-	1.58 ± 0.109
6	+	+	+	+	+	1.59 ± 0.066

[a] the symbol - represents the corresponding nutrient is eliminated from salt solution while + represents the corresponding nutrient is included in salt solution.

Table 2.15. The distribution of nutrients in manure and salt solution [a]

Nutrient	From Manure	From salt solution	Total concentration in medium	Nutrient ratio of manure to salt solution
Calcium	0.241 g/L	0.11 g/L	0.350 g/L	2.19 : 1
Magnesium	0.097 g/L	0.029 g/L	0.126 g/L	3.34 : 1
Iron	13.4 mg/L	1.0 mg/L	14.4 mg/L	13.4 : 1
Manganese	1.5 mg/L	0.521 mg/L	2.021 mg/L	2.88 : 1
Zinc	1.3 mg/L	0.317 mg/L	1.617 mg/L	4.10 : 1
Cobalt	0.02 mg/L	0.908 mg/L	0.928 mg/L	0.02 : 1
Nitrogen	0.303 g/L	0.436 g/L	0.739 g/L	0.69 : 1
Potassium	0.124 g/L	0.573 g/L	0.697 g/L	0.22 : 1
Phosphorus	0.081 g/L	0.456 g/L	0.537 g/L	0.18 : 1

[a] The calculation was based on 10 g/L (DM) of manure and the composition of salt solution in Table 2.12.

cobalt level in manure is low, suggesting cobalt is probably not a crucial element for cellulase production. Although nitrogen from manure is lower than that from the salt solution, it may be sufficient for fungi culture; therefore, elimination of a nitrogen source also had no negative influence.

The contributions of potassium and phosphorus from the manure were much lower than those from the salt solution (Table 2.15). This may be the reason why cellulase production in the

KH_2PO_4-eliminated medium was much lower than the other cases; because potassium and phosphorus within the manure are insufficient to support the cellulase production by the fungi. Another reason may be that most of manure phosphorus is in the form of organic phosphate and polyphosphates, which makes the utilization more difficult.

A further experiment was conducted by growing the fungi in medium containing manure (10 g/L) added with KH_2PO_4 (2g/L), $CoCl_2$ (2mg/L), and tween-80 (2 g/L). Nitrogen, calcium, magnesium, and trace elements (except cobalt) were simultaneously eliminated from the salt solution. The medium containing manure added with full-nutrients (as in salt solution in Table 2.12) was taken as control. To give a detailed cellulase profile of the fungi under this condition, the time course of FPA, CMCase activity, and β-glucosidase activity were monitored, respectively. As shown in Figure 2.36, the three enzymes were in a similar pattern and increased in parallel with incubation time. The filter paper activity and β-glucosidase activity reached their highest levels at day 6, while the highest CMCase production occurred near day 8. It was also found that the activities of all the three enzymes were higher than the control, suggesting the

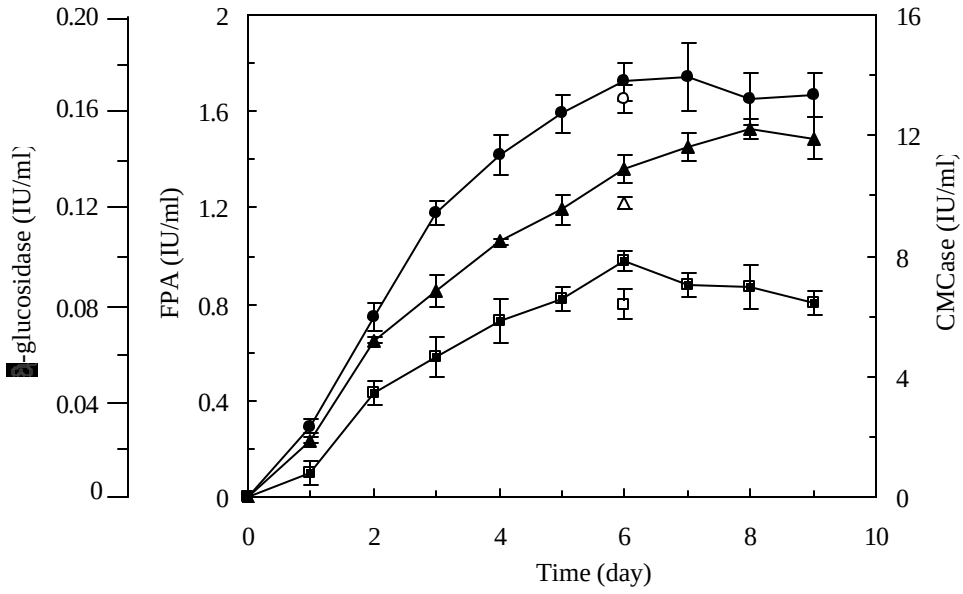


Figure 2.36. Time course of filter paper activity (FPA), CMCase activity, and β-glucosidase activity in medium containing manure added with KH_2PO_4, $CoCl_2$, and tween-80. Symbols: ►, FPA; ▲, CMCase activity; ■, β-glucosidase activity. The open symbols are the control medium containing manure added with full-nutrients (as in salt solution in Table 2.12). Data are means of three replicates and error bars show standard deviation.

medium with reduced nutrients could sufficiently support a high cellulase production by *T. reesei* (Figure 2.36).

Comparison of cellulase production

The highest cellulase production value (based on filter paper activity) achieved was 1.7 IU/mL and was obtained in medium that contained 10 g/L (dry basis) homogenized manure supplemented with 2 g/L KH_2PO_4, 2 mg/L $CoCl_2$, and 2 g/L tween-80 at pH 5.7 and 25.5°C. An overall comparison of cellulase production by different fungal species and substrates is presented in Table 2.16. It was found that pure substrate (cellulose or lactose) resulted in a higher cellulase activity than lignocellulosic substrate. Among different lignocellulosic residues, however, the cellulase production obtained in this work is much higher than previous reports, suggesting dairy manure is a good source for cellulase production.

Table 2.16. Comparison of cellulase production by different fungal species and substrates

Fungal species	Substrate	FPA (IU/mL)	Reference
	Lignocellulosic substrate		
Trichoderma reesei	Steam-treated willow	0.66	Reczey et al., 1996
Chaetomium globosum	Oil palm fruit fiber	0.95	Umikalsom et al., 1997
Neurospora crassa	Wheat straw	1.33	Romero et al., 1999
Trichoderma reesei	Wastepaper	0.30	Ju and Afolabi, 1999
Scytalidium thermophilum	Apple pomace	0.39	Ogel et al., 2001
Scytalidium thermophilum	Lentil bran	0.23	Ogel et al., 2001
Scytalidium thermophilum	Bagasse	0.21	Ogel et al., 2001
Trichoderma reesei	Steam-treated willow	1.55	Szengyel and Zacchi, 2000
Trichoderma reesei	Dairy manure	1.72	This work
	Pure cellulose or reducing sugar		
Trichoderma reesei	Acid-swollen cellulose	0.54	Gadgil et al., 1995
Trichoderma reesei	Solka floc (cellulose)	4.65	Velkovska et al., 1997
Trichoderma reesei	Solka floc (cellulose)	2.10	Domingues et al., 2000
Trichoderma reesei	Lactose	1.30	Domingues et al., 2000

Task 3.
Study the aqueous phase hydrogenation used to convert manure hydrolysates

Innovative catalytic processing approaches recently have been developed that convert dissolved sugars into a variety of valuable chemical products. These processes utilize heterogeneous catalysts in the aqueous phase to perform hydrogenation or hydrogenolysis reactions under mild thermal conditions of 100–200°C at elevated pressures. Present catalysts work well for converting clean C_5 and C_6 sugars to chemicals. The objective of this work was to develop basic data on the influence of manure components – inorganics and protein – on catalyst performance in the hydrogenation of manure hydrolysates. The research was focused to determine how much (or little) separation is required, and how catalyst performance could be impacted.

Hydrogenation is a general term applied to any chemical reaction involving the addition of hydrogen to a compound to form a new hydrogenated compound. While the hydrolysis reactions described in Task 2 are inherently water-mediated reactions, hydrogenation reactions may be accomplished in aqueous or non-aqueous mediating solvents. In carbohydrate chemistry, examples of hydrogenation include formation of the sugar alcohol, sorbitol, by addition of hydrogen to glucose, or formation of the 5-carbon sugar alcohol, xylitol, from the pentose sugar, xylose. These are typically heterogeneously or homogeneously catalyzed reactions at modest elevated temperature (~ 100 to 150°C), and with significant (e.g., ~ 6 to 10 MPa) hydrogen atmosphere pressure. There is little past practice for hydrogenation of mixed C_5 and C_6 sugars. Rather, established technology has been directed to C_5 <u>or</u> C_6 sugar hydrogenation -- but not both at the same time.

Glucose Hydrogenation: $C_6H_{12}O_6 + H_2 = C_6H_{14}O_6$ (catalyst, H_2 overpressure)
glucose sorbitol

Xylose Hydrogenation: $C_5H_{10}O_5 + H_2 = C_5H_{12}O_5$ (catalyst, H_2 overpressure)
xylose xylitol

Hydrogenation of glucose in water is the standard industrial method for producing the sugar alcohol, sorbitol – an alditol much valued as a dietetic sugar and as an intermediate for manufacture of certain fine organic chemicals. Another technology pathway, hydrogenolysis, is

under development and can lead to subsequent production of commodity chemicals, such as glycols, glycerol, and other polyols from the sugar alcohols.

Catalytic processing tests for hydrogenation were performed at PNNL in existing semi-batch and continuous-flow bench-scale reactor systems to verify product yields from the manure-derived carbohydrate feedstock. The testing was conducted using a ruthenium/titania catalyst produced for PNNL by Degussa based on a formula developed by PNNL (U.S. Patent # 6,235,797). This catalyst is a new-generation heterogeneous catalyst designed for stability in aqueous processing systems.

We evaluated the hydrogenation process using a semi-batch reactor system that was in place at PNNL. The unit was a 300-ml stirred batch reactor equipped with continuous hydrogen feed. The reactor also had a sample recovery system so that multiple liquid samples were withdrawn from the reactor throughout the test. A total of 121 semi-batch reactor tests were completed. We also evaluated long-term catalyst stability issues using a fixed-bed, micro-scale, tubular catalytic reactor system.

The major issue addressed by these tests was the effect of the noncarbohydrate components recovered from the manure on the aqueous-phase catalytic chemistry. Catalyst tests of two types were performed. First, carbohydrates-to-chemicals tests using model compound systems were evaluated using models of expected impurities. In this way, a controlled evaluation was made of the potential contaminants, and the effects of individual species, without interference from other species, were determined. In the second series of tests actual separated manure streams were catalytically processed to produce value-added chemical products. These tests evaluated reaction kinetics and the effect of impurities remaining after the different separation processes.

PNNL led the Task 3 catalytic conversion research. WSU was involved as appropriate to assist with the engineering and processing aspects of the work. The products of manure hydrolysis at WSU were processed by catalytic hydrogenation to sugar alcohols.

Methods

These experiments were designed to model the hydrogenation process being developed for use in converting biomass-derived sugars into sugar alcohol products. In this case, the sugars were relatively dilute. The manure was processed at different levels of severity to produce either

a mixed xylose and glucose solution or a primarily glucose solution, as derived from mainly the hemicellulose or the cellulose, respectively.

A 300-ml Parr bomb reactor was operated in a semi-batch mode wherein hydrogen pressure was maintained and multiple liquid samples were removed over the 6-h period of the test. The sugar-water solution feedstock and catalyst particles (3 wt% ruthenium metal on rutile titania) were stirred in the reactor, which was maintained at constant temperature (typically 100°C) and hydrogen over-pressure (8.3 MPa).

The micro-scale continuous-flow reactor system was reassembled from existing components in a new laboratory location as part of the project and was operated for 7 days on line. The 30 ml catalyst bed was fed with a continuous flow of hydrogen gas from a cylinder manifold and with a continuous flow of feedstock solution from a twin piston high-pressure, syringe metering pump. The reactor was heated with a hot oil jacket served by a controlled-temperature circulating bath.

The product liquids from these tests were analyzed by high-pressure liquid chromatography (HPLC) using a conventional carbohydrate column (Bio-Rad Aminex HPX-87H, 300 x 7.8 mm,) at 65°C with a 5-mM sulfuric acid mobile phase (isocratic), at a flow rate of 0.6 ml/min and a refractive index detector. Column calibration was maintained by continual analysis of standard compounds. Analysis of the products showed, in most cases, a very high selectivity to the sugar alcohol product. Traces of hydrogenolysis products (lower molecular weight polyols) were noted, as were trace yields of methane and carbon dioxide. Glucose (or xylose) conversion is typically reported here based on reduction of sugar concentration, with time zero at temperature being the point when heat-up of the reactor raised the aqueous temperature to 100°C.

The nonsugar components in the manure hydrolysates were determined to be a collection of metals, anions, and nitrogenous material. The inorganic elements (Ca, K, Mg, Na, S, P, Al, Si) were measured by inductively coupled plasma-optical emission spectrometer (ICP-OES). Dissolved ammonium was measured with an ion selective electrode.

The ICP was a Perkin-Elmer 3000DV with an AS90 Autosampler, which has an instrument detection limit of about 1 ppb (for most elements) with a linear calibration up to 100 ppm (for most elements). Solid samples were prepared via microwave digestion in concentrated nitric and hydrochloric acids, then diluted to volume. The ICP was calibrated and

verified with two independent certified standard sets. Spikes and dilutions were down for each batch of samples to check for and/or mitigate any matrix effects. The ICP process ran a constant pump rate of 1.5 ml/min for all samples and standards during analysis. A 3-ml/min rinse and initial sample flush was used to switch between each sample and standard. The plasma was run at 1450 W with argon flow. Trace metal grade (sub-ppb) acids and two independently NIST-certified calibration standard sets were used for calibration and method verification.

Anions, including chloride, were measured by ion chromatography (IC) using a Dionex DX 500 IC consisting of a GP40 Pump, EG40 Elluent Generator, ED40 Electrochemical Detector, with an AS3500 autosampler. An ASRS-Ultra 4-mm suppressor was used (at 100 mW) to minimize baseline drift. The chromatography was performed using an AG-11 guard column and an AS-11HC column running at 30°C with an hydroxide gradient from 0.5 mM to 41 mM and a flow rate of 1.2 ml/min. Certified standards were used to calibrate the IC.

Ultrafiltration and carbon treatment were applied to manure hydrolysates in an attempt to remove impurities that slow the sugar hydrogenation reaction. We tested the use of various ultrafiltration membranes to try to remove the protein contaminants from the manure hydrolysate. We suspected these contaminants poisoned the catalyst, preventing it from performing the desired hydrogenation reactions of the feedstock.

We have a significant amount of experience using a simple, pressurized, stirred cell filtration unit for the ultrafiltration. The stirred cell held up to 450 ml of feedstock, operated at up to 100 psi (limited by the nitrogen gas pressure regulator), and used a 3-inch diameter flat membrane. The stirred cell filter was very slow, and took from between 6 and 60 h to process 300 ml of feedstock.

Because of the very slow flux of the stirred cell filter, we tested two other filter designs: a recirculating plate filter and a recirculating spiral wound filter. The plate filter circulated the feed across a 4-inch diameter membrane and was operated between 50 and 200 psi. The spiral wound filter used an approximate 12 inch by 12 inch membrane sheet that was rolled into a 1.5-inch tube. The membrane was fitted into chamber where the feedstock was passed over the filter at a high velocity. The spiral wound filter was operated between 30 and 200 psi. Both of these filters performed much faster than the stirred cell filter, processing in minutes what took days to filter in the stirred cell.

Because of the dead volume inherent in the recirculating design of these two filters, more feedstock was required to generate the same amount of filtrate. The plate filter had approximately 150 ml of dead volume, while the spiral wound filter had approximately 500 ml. Thus, the minimum amount of material to generate 300 ml of filtrate was 450 ml for the plate filter, and 800 ml for the spiral wound filter.

We processed the hydrolysate through membranes with three different pore sizes.

GM: MWCO 8000 (MWCO = molecular weight cut off)

PT: MWCO 6000

GK: MWCO 3500

The PT membrane did not perform as would be expected based on its pore size and rating. In fact, in the stirred cell, no feedstock was able to be processed through the membrane. Using the other filters, the membrane performed better – that is to say, we were able to pass material through the membrane – but it filtered out essentially all the sugars. In other words, the membrane performed more like what would be expected from a 300 to 600 MWCO filter than a 6000 MWCO filter.

The GM and the GK membranes performed much better, allowing most of the sugars to pass through the filter.

In addition to the ultrafiltration techniques, carbon treatment was also tested for removing the hydrogenation-inhibiting contaminants. The carbon treatment was performed with a 100-cc column of Norit ROX 0.08 extruded activated carbon. The column was operated to process between 30 and 50 ml per minute. The column was pre-rinsed with 200 ml of deionized water before the initial pass of hydrolysate over the carbon. The carbon was rinsed with a minimum of 100 ml of deionized water following each treatment, and another 100 ml prior to the next treatment of feedstock.

Results and Discussion

Model compound sugar hydrogenation results

In our program of process development for bio-based chemical products from manures, we conducted extensive model compound testing to evaluate the effect of various manure contaminants on catalytic processing. Specifically, we evaluated catalytic hydrogenation of

sugars to sugar alcohols in the aqueous phase, using a supported ruthenium metal catalyst. The experiments involved reagent glucose and/or xylose sugar as the models of the manure hydrolysate products. To the sugar solutions, we added various chemicals to model the contaminants that will be derived from manures in actual process applications. The tests determined changes in the rate of reaction and mechanistic modifications caused by various contaminants. The sugar solution contaminant levels tested were based on the compositions of acid hydrolysis product from dairy manure solids.

The experimental results are presented in several groups. The tests in the first group were performed with contaminant-free sugar solution to provide a baseline for comparison with the tests involving contaminants. The subsequent groups are collections to evaluate certain contaminants perceived to be of greatest significance.

Sugar hydrogenation studies

The tests to quantify the reaction rate and its reproducibility used reagent sugars in deionized water as the feedstock. Figure 3.1 shows the sugar hydrogenation and sugar alcohol production over the time of the tests for comparison of glucose and xylose. Clearly, the xylose was hydrogenated more readily. These tests at higher concentrations of sugars showed a concentration effect in the glucose case, but not in the xylose case, as the reaction was so rapid that the concentration appears to have little impact. High selectivity (85% to 95%) to the sugar alcohol product was seen in all cases.

In another test with a mixed solution of 10% glucose and 10% xylose (20% total concentration), the interaction of the two sugars did not appear to occur. Figure 3.2 shows conversion curves for both glucose (signified by diamonds) and xylose (signified by squares). As seen, the xylose reaction was very fast at either 10% or 20% concentration and at essentially the same rate with 10% glucose added to 10% xylose. The glucose reaction rate was reduced slightly by the competition of the 10% xylose, but not nearly as much as with the addition of the extra 10% of glucose, which reacted more slowly.

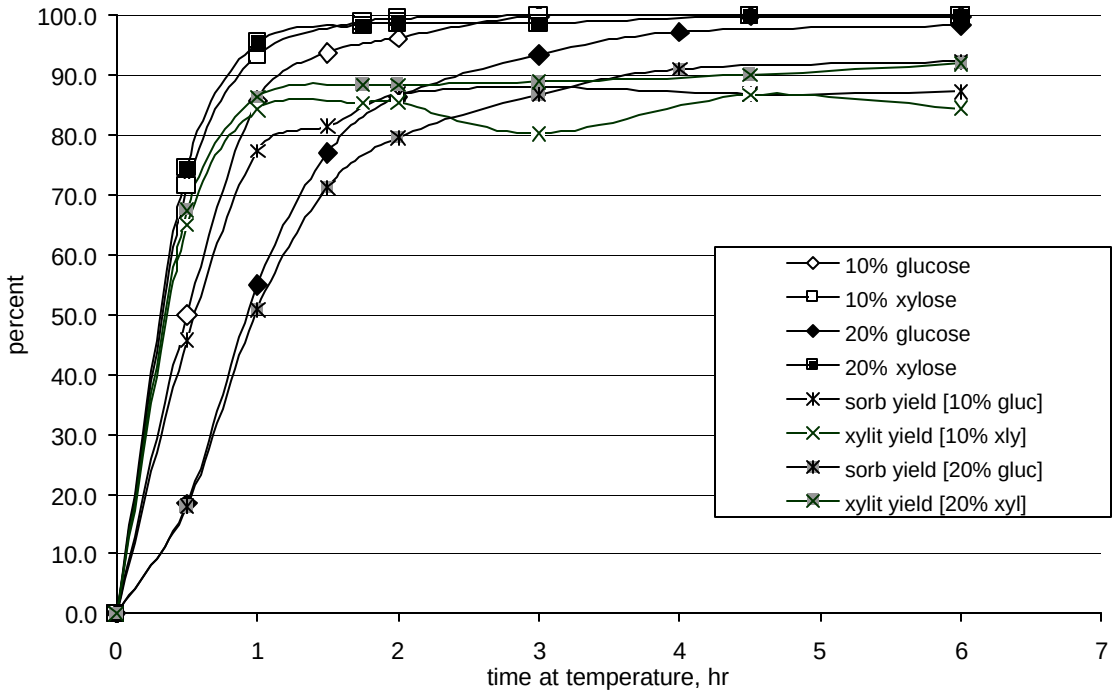


Figure 3.1. Sugar conversion to sugar alcohol at 100°C

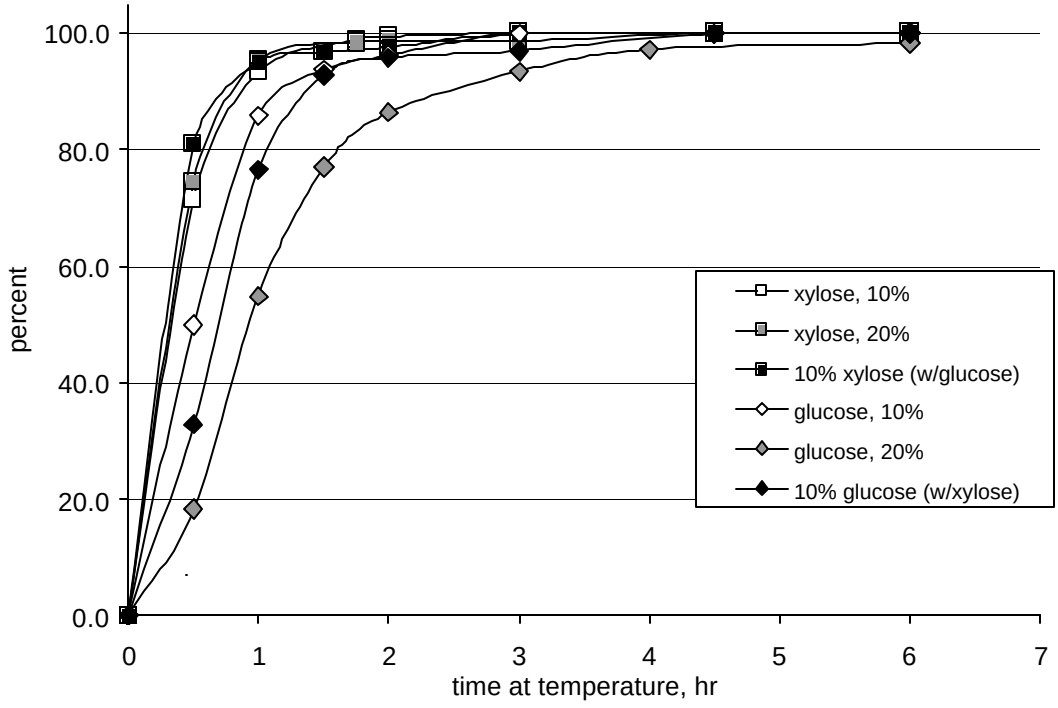


Figure 3.2. Glucose/xylose conversion

A subsequent series of glucose tests (10% concentration) were performed with added acids and bases to model expected anion and cation contaminants from manure hydrolysates (see Figure 3.3). The contaminants were added at 100 ppm. The ammonium (added as carbonate) showed a decided inhibition of the catalysis. Calcium (added as carbonate) had a mild effect and the nitric acid contaminant even less, nearer the range of experimental variation. The potassium (added as carbonate) effect was negligible (within experimental variation), as was the effect of the other acids.

Manure hydrolysate model contaminant tests

Additional tests with added acids and bases were also performed to more accurately model expected anion and cation contaminants from manure. The results are shown in Figure 3.4. The contaminants were added at the levels noted in the key, based on expected values in manure-derived feedstocks. The ammonium compounds showed a decided inhibition of the catalysis at this higher concentration, especially when added as carbonate but also to a lesser degree in the hydroxide form. Calcium (added as carbonate) had a mild effect. Neither the magnesium nor the potassium bases, nor the calcium sulfate, appeared to have an effect at these concentrations.

A shorter companion series of tests was performed with xylose as the feedstock. As shown in Figure 3.5, ammonium carbonate also inhibited the xylose hydrogenation. However, while the xylose hydrogenation was noticeably faster than the glucose hydrogenation, the effect of the ammonium carbonate was less in the xylose case as compared to the glucose. The calcium carbonate appeared to have almost no impact on the xylose hydrogenation at these concentrations.

Ammonium ion detailed studies

The ammonium carbonate inhibition was further studied with glucose to evaluate its scale of effect over a range of concentration. As seen in Figure 3.6, the inhibition of the glucose hydrogenation was a reproducible effect and was directly proportional to the concentration of the ammonium carbonate. Consequently, if the higher concentration of ammonium was present in the manure-derived feedstocks, there likely would have been a significant effect on the rate of

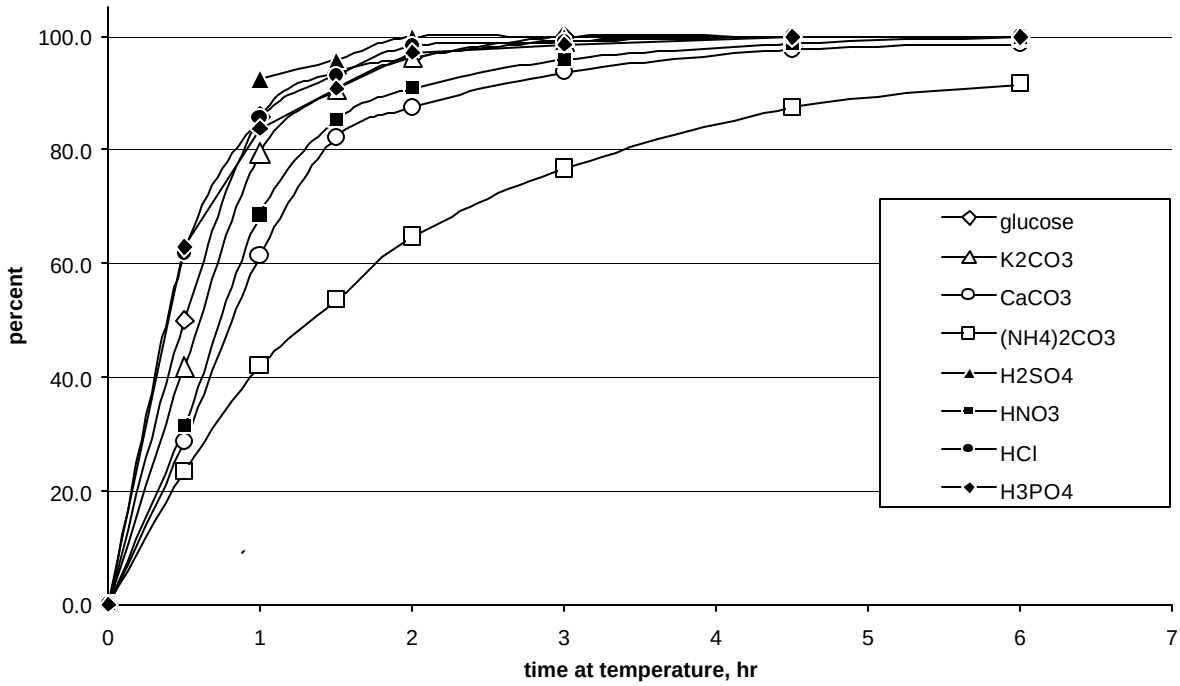


Figure 3.3. Glucose conversion in the presence of inorganic contaminants

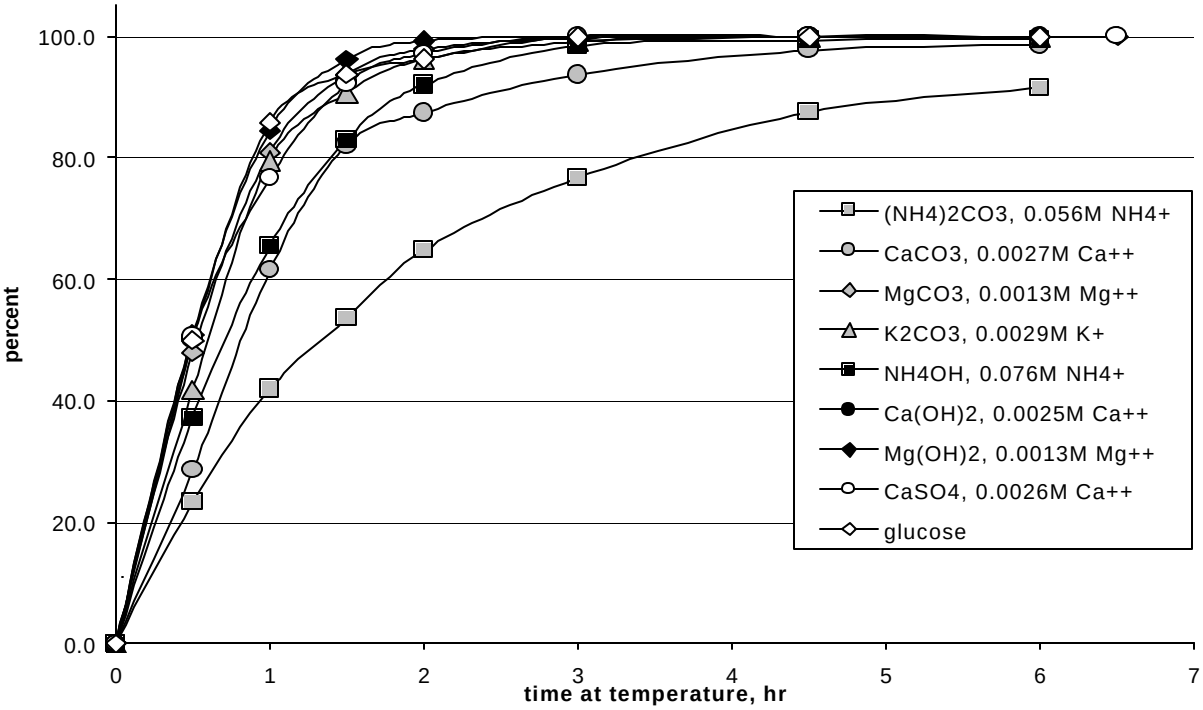


Figure 3.4. Glucose conversion inhibition by manure model contaminants

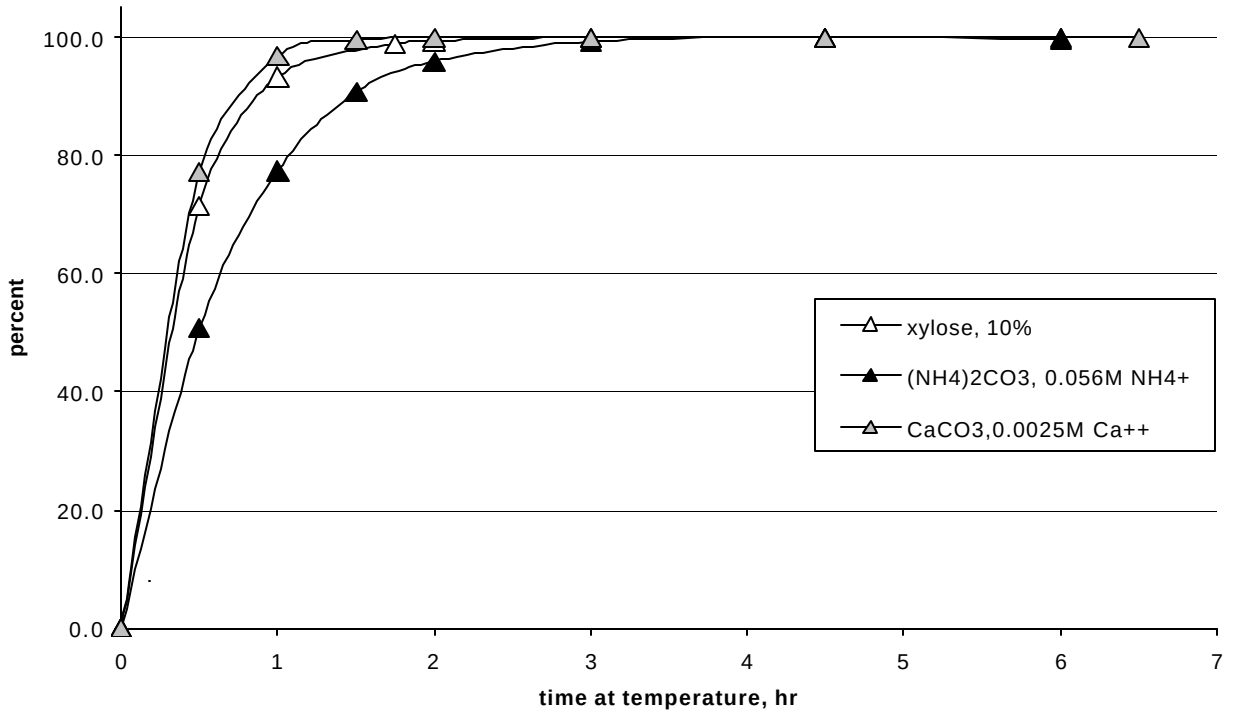


Figure 3.5. Xylose conversion with contaminants

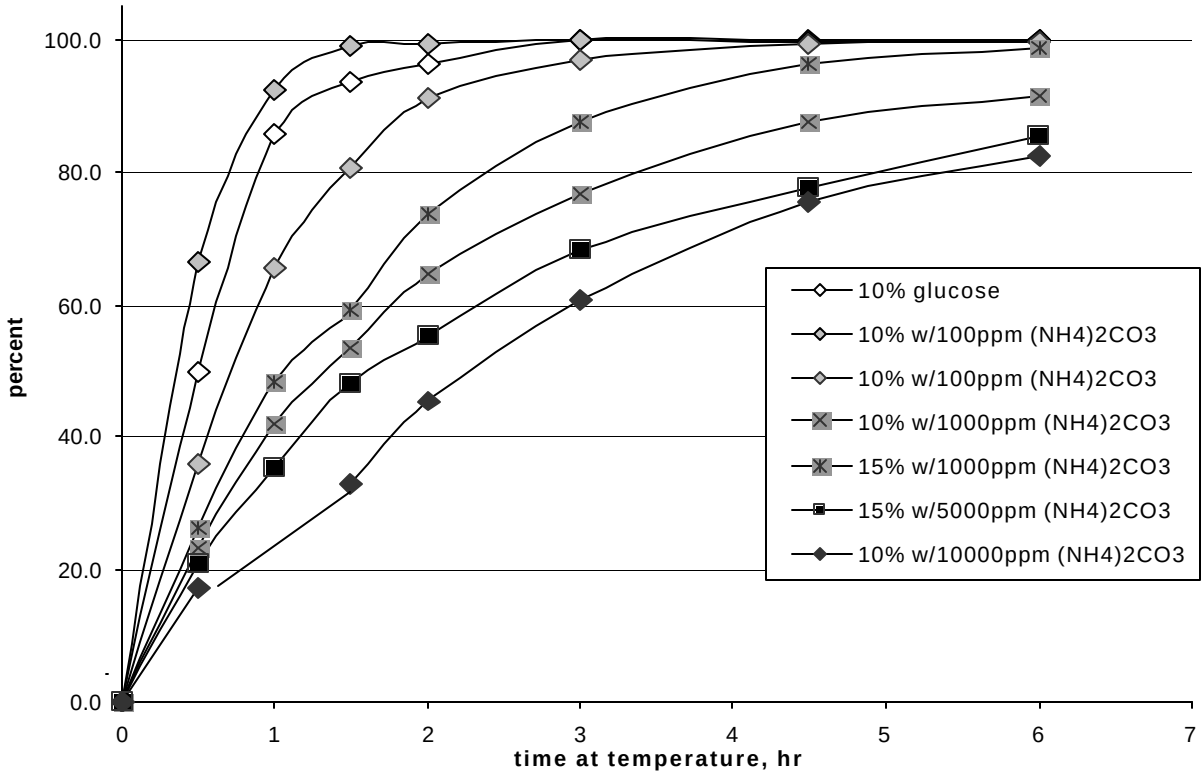


Figure 3.6. Glucose conversion with ammonium present

hydrogenation of the glucose. Also noted at the higher concentrations was the competitive reaction of isomerization of the glucose to form fructose. The fructose yield increased from less than detectible levels at 100 ppm ammonium carbonate to 6% at 1000 to almost 18% at 10,000 ppm. Correlating with this production of fructose was a reduction in the sorbitol product. As a result, the true inhibition of the glucose hydrogenation was even more severe than suggested by Figure 3.6.

Additional tests with ammonium compounds were performed to address the effect of ammonium ion (see Figure 3.7). It was clear that the catalyst inhibition was not based only on the presence of ammonium ion. Ammonium carbonate showed the largest inhibition of the glucose hydrogenation reaction, while chloride and hydroxide had lesser effects. The ammonium nitrate caused no apparent inhibition on glucose conversion. A similar lack of effect was shown with potassium nitrate. However, in the presence of the nitrates (and to a lesser degree with the ammonium carbonate), there was isomerization of the glucose to fructose. In the case of potassium nitrate, although there was a large amount of isomerization underway, the inhibition of hydrogenation was not large, as the overall yield of sorbitol (produced by hydrogenation of both glucose and fructose) was only slightly reduced. In the case of the ammonium nitrate, the sorbitol yield was reduced by about 20%, and numerous byproducts and overreaction products (lower molecular weight polyols) were evident.

Calcium ion detailed studies

The effect of calcium was not so straightforward. As seen in Figure 3.8, calcium carbonate concentration appeared to have little effect on the extent of inhibition of the glucose conversion, which was low, and was about the same in all cases, i.e., from 100 to 5300 ppm. Calcium hydroxide appeared to have no inhibitory effect.

Comparative catalyst tests

Contaminant poisoning of the catalyst is likely to be catalyst specific. To evaluate this parameter, comparative tests of the ruthenium catalyst were made with a more conventional nickel catalyst (50% Ni metal on high-surface area alumina). Figure 3.9 shows glucose conversion comparisons for two catalysts. The ruthenium and nickel catalysts exhibited similar

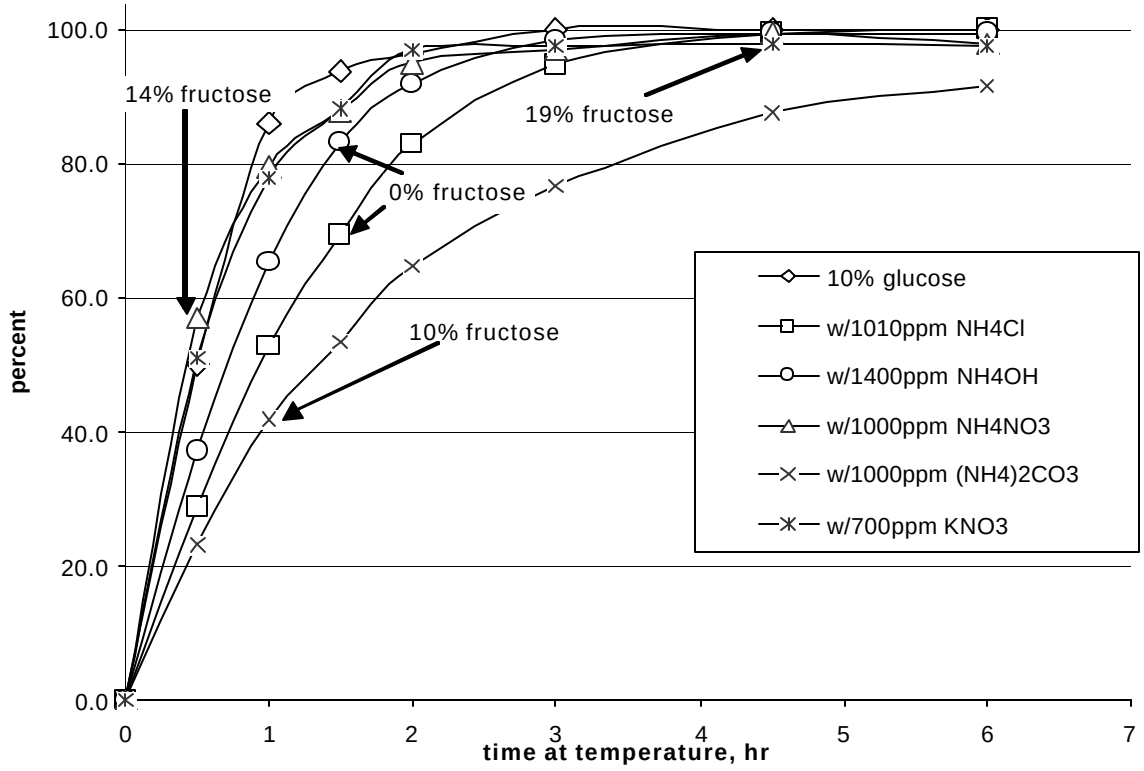


Figure 3.7. Glucose conversion with ammonium and nitrates

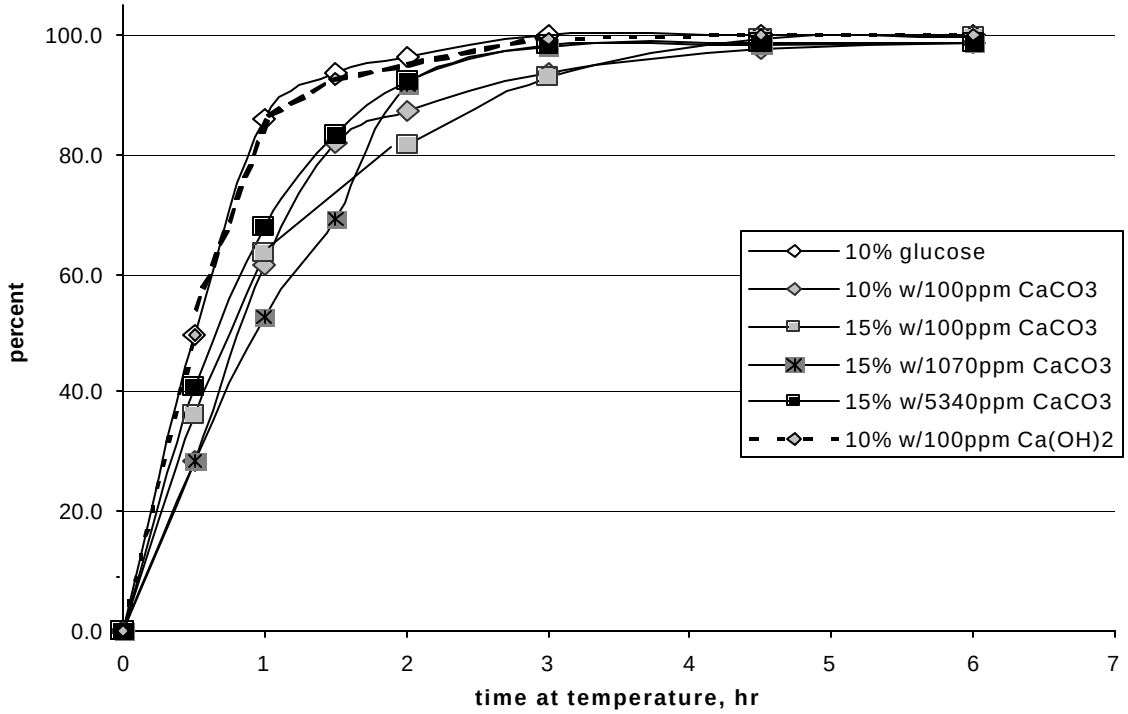


Figure 3.8. Glucose conversion with calcium present

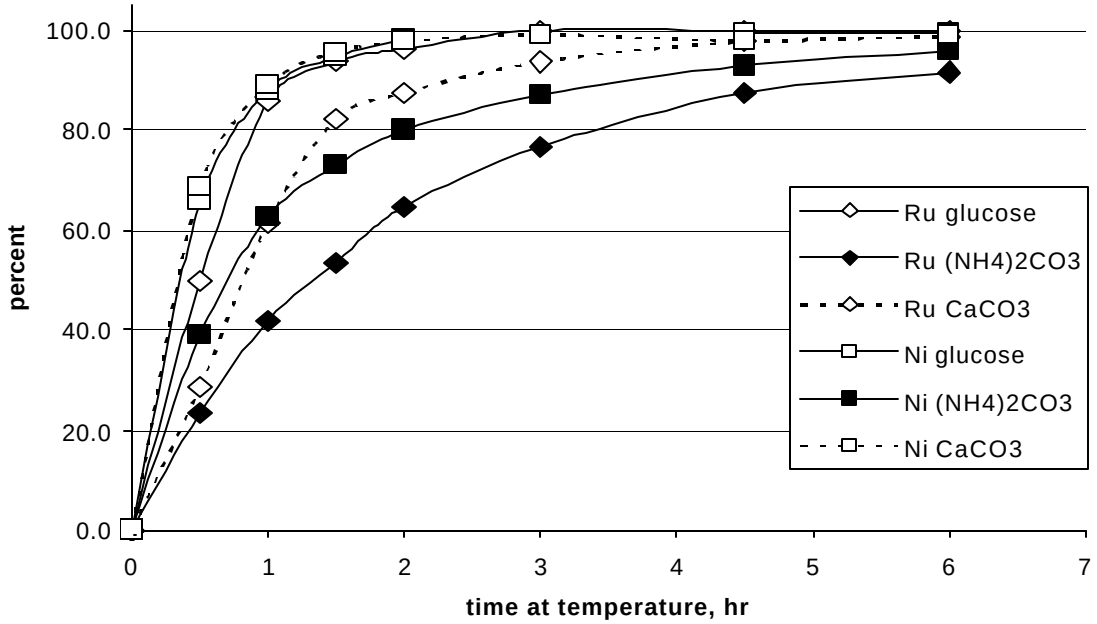


Figure 3.9. Glucose conversion with different catalysts

activity in this batch test. However, the reaction inhibition, seen with the ruthenium catalyst in the presence of the ammonium carbonate (0.056M NH_4^+), was much less severe when using the nickel catalyst in the presence of ammonium carbonate. The reaction inhibition seen with the ruthenium, with addition of calcium carbonate (0.0025M Ca^{++}), was not significant when using the nickel catalyst in the presence of the same amount of calcium carbonate.

A model manure solution was prepared based on 10% glucose (as a carbohydrate hydrolysate model) with the various mineral components. The model solution was processed with three different catalyst formulations for comparison. The two nickel catalysts, ruthenium-stabilized and copper-stabilized (U.S. Patent # 6,152,975), exhibited no effects from the contaminants, while the ruthenium showed reduced activity similar to that noted above.

Detailed sulfate and ammonium tests

Initial analyses showed that the manure hydrolysis feedstocks were relatively dilute (~1% sugars) and mainly consisted of xylose when hydrolyzed at low temperature (~100°C). The

sulfuric acid remainder from the hydrolysis could be significant. Hydrogenation tests with manure acid hydrolysates showed slow reaction rates in all cases, suggesting a large inhibition of the catalytic chemistry. Model tests were performed with 1% xylose in water with a range of sulfuric acid, from 0 to 6 wt%, to test the effect of the residual acid from the hydrolysis treatment. The results, depicted in Figure 3.10, showed that sulfuric acid has only a minor effect on the catalytic chemistry, noticeable only at the highest concentration tested and much too small to explain the results of the manure-derived feedstocks.

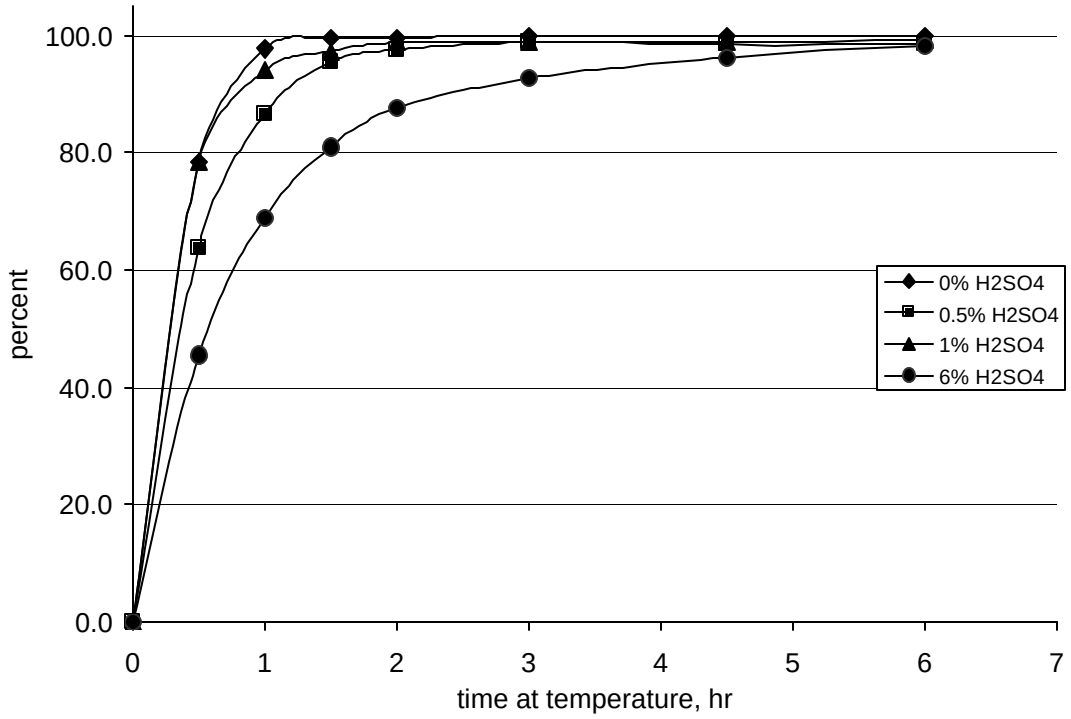


Figure 3.10. Xylose (1%) conversion with sulfuric acid.

Analysis of ammonia performed on the manure hydrolysate samples showed ammonia concentrations of about 1300 ppm in the low-temperature acid hydrolysates and about 400 ppm in the moderate-temperature (135°C) acid hydroysates. As reported above, model tests of ammonia at this concentration range appeared to show little or no effect. The sulfuric acid remaining in the samples from the hydrolysis ranged from 1 to 8 wt%. Again as reported above, tests of sulfuric acid in the range of the hydrolysates also appeared to have only a minor effect. A combination of the two (ammonia and sulfuric acid) were tested as an explanation of the

catalyst inhibition. Table 3.1 summarizes these results. No significant effect on xylose hydrogenation was apparent at any of these sulfate concentrations with a clean xylose feedstock.

The addition of ammonia with or without sulfate present also did not appear to affect xylose hydrogenation.

Table 3.1. Model Compound Xylose Hydrogenation Results

			Batch Feed		6 h	
	Ammonium, ppm	Sulfate, %	Xylose	Sulfate (area)	Xylose Conversion	Xylitol Yield
No additives	0	0	0.471	0	98%	78%
Low NH_4OH	445	0	0.471	0	98%	92%
Low $(NH_4)_2SO_4$	488	0.1	0.471	202999	100%	78%
Low $(NH_4)_2SO_4$ + H_2SO_4	398	4.6	0.471	7583905	98%	89%
High NH_4OH	1258	0	3.94	0	99.3%	79%
High $(NH_4)_2SO_4$	1370	0.4	3.94	617589	99.8%	92%
High $(NH_4)_2SO_4$ + low H_2SO_4	1524	1.5	3.94	1815351	99.8%	99%
High $(NH_4)_2SO_4$ + H_2SO_4	1258	7.1	3.94	12429875	99.7%	82%

Tests with peptone contaminant

Another impurity in the manure hydrolysates was the protein-derived material, and peptone was added to the glucose solution to model this material. Figure 3.11 shows a peptone-concentration-dependent effect on the rate of glucose hydrogenation. As the peptone addition was increased from 0.1 g to 1 g and to 5 g, glucose hydrogenation was slowed to the point that only a partial conversion was achieved after the full 6-h test, compared to nearly complete conversion achieved in 2 h with reagent glucose alone. In addition, the reduction in rate of glucose hydrogenation was in the same range as that found in processing of the actual manure hydrolysates. The amount of peptone added resulted in nitrogen contents similar to that found in the manure hydrolysates.

Low-temperature hydrolysate hydrogenation results

Tests completed using hydrolysate solutions from the two-stage low-temperature acid hydrolysis work at WSU, showed that the hydrogenation proceeded slowly in all cases, suggesting a large inhibition of the catalytic chemistry. Comparable tests made with a nickel catalyst, instead of the standard ruthenium catalyst, were largely inhibited as well.

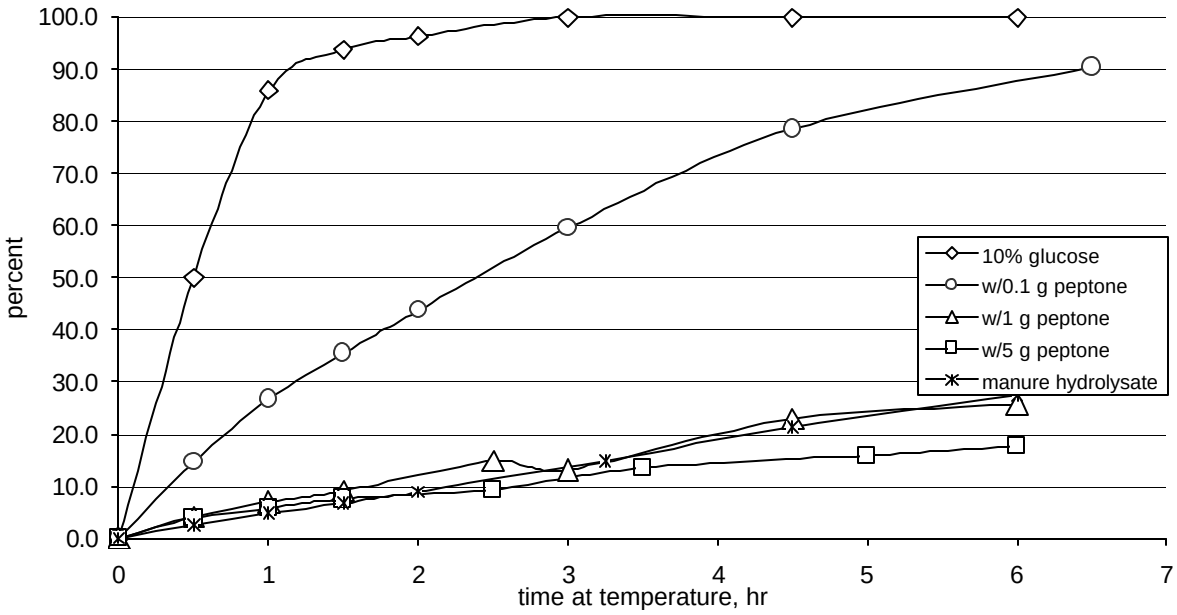


Figure 3.11. Glucose conversion with addition of peptone.

The manure-derived feedstocks were pretreated with a lab-scale, dead-end membrane ultrafiltration test rig. Membranes rated at 8000 and 10000 MWCO were used to filter two of the hydrolyzed manure feedstocks. These ultra-filtered feedstocks were then tested in the catalytic hydrogenation and seemed to show some improvement. The finer filtration medium resulted in a higher activity feedstock. However, the rates of reaction were still significantly reduced compared to reagent sugar feedstocks.

The ammonia in the hydrolysate samples was analyzed to evaluate the correlation of reactivity with ammonia concentration. The ammonia concentrations were found to be about 1300 ppm in the 1st stage hydrolysates and about 400 ppm in the 2nd stage hydroysates. Earlier model tests of ammonia at this concentration range appeared to show little or no effect. The sulfuric acid remained in the samples from the hydrolysis at from 1 to 8 wt%. However, tests of sulfuric acid in the range of the hydrolysates also appeared to show only a minor effect.

Table 3.2 summarizes these results. Increasing acid in the hydrolysis process yielded a higher level of monomer sugars, and oligomers trended upward and then decreased with the formation of additional monomers. The 1st stage samples with a higher level of ammonia generally seemed to show more inhibition of hydrogenation. An increasing level of sulfuric acid had little effect at this higher level of ammonia. For the 2nd stage samples with a lower

concentration of ammonia, there appeared to be a noticeable trend of inhibition with increasing sulfuric acid concentration.

Table 3.2. Hydrolysate hydrogenation processing results

	NH4	acid	batch feed (except 1st Stg, #4, which is at time=0)					expt	6 hr yield		
	ppm	%	glucose	xylose	arabinose	H2SO4	oligomers	number	sorbitol	xylitol	arabinitol
1st Stg #1	1160	0.5	0	0	0	1433267	56945	no test	---	---	---
1st Stg #2	1230	1	0	0.008	0.059	2623725	115641	68	0	incl in arab	83%
1st Stg #3	1540	2	0.045	0.194	0.165	4488203	237613	spilled	---	---	---
1st Stg #4	1310	3	0.120	0.403	0.183	5817857	168322	56	12%	12%	incl in xyl
1st Stg #5	1310	4	0.153	0.506	0.162	7706570	152206	57	9%	18%	incl in xyl
1st Stg #6	1320	5	0.167	0.564	0.153	9604494	78037	58	12%	14%	incl in xyl
2nd Stg #1	454	1	0.137	0.555	0.135	2354546	122264	59	32%	40%	incl in xyl
2nd Stg #2	432	2	0.147	0.594	0.083	4427699	146449	60	11%	19%	incl in xyl
2nd Stg #3	403	4	0.172	0.380	0.035	8272323	85402	65 (UF)	9%	20%	incl in xyl
2nd Stg #4	416	6	0.228	0.275	0.029	12872007	63558	66 (UF)	22%	51%	incl in xyl
2nd Stg #5	358	8	0.268	0.196	0.022	17094523	43879	67	0.4%	1.4%	??
2nd Stg #6	19?	10	0.320	0.142	0.016	21380481	37021	no test	---	---	---
reagent xylose	~0 ppm	0-6%	0	1.000	0		0	61-64	---	94-84%	

Second-stage high-temperature hydrolysate hydrogenation processing results

Additional hydrolysate samples were received from WSU representing the two-stage hydrolysis, including a 2nd stage at high temperature (170°C with 3% acid). One sample was an "as produced" hydrolysate – "Solution A," and the second was a concentrated (by evaporation after calcium carbonate neutralization) sample – "Solution B." These two samples were both hydrogenated. The results shown in Table 3.3 (similar to those seen with earlier hydrolysate samples) suggested strong inhibition of the glucose hydrogenation to sorbitol.

Table 3.3. Second-stage Hydrolysate hydrogenation processing results

	NH4	acid	batch feed				expt	3.5 hr yield	
	ppm	%	glucose	xylose	sulfate	oligomers	number	sorbitol	xylitol
2nd Stg #2	432	2	0.147	0.594	4427699	146449	60	11%	19%
Solution A	NA	3	0.608	0.049	7531832	81518	69	3.5%	8.7%
Solution B	NA	3	2.92	0.172*	1099211	77357	70	0.3%	3.3%*

* appears to be fructose, rather than xylose or xylitol

Two-stage hydrolysis of manure solids at PNNL

We completed a two-stage hydrolysis in our 2-gallon autoclave at PNNL, as well. Based on the conditions identified in the WSU optimization work, we processed manure solids from WSU through a low-temperature acid hydrolysis and filtration, followed by a second acid hydrolysis of the undissolved solids at a higher temperature. This method generated several liters of hydrolysate solutions (1st and 2nd stage solutions) for subsequent testing and processing. The hydrolysis processing conditions and results are given in Table 3.4.

Table 3.4. Two-stage hydrolysis of manure solids

	1st stage	**2nd stage**
temperature	110°C	170°C
time at temperature	60 min	30 min
solids concentration	9.9%	13.3%
acid concentration	2.8%	1.0%
dissolution of solids	34.6%	20.0%
analysis of product solutions		
glucose	0.20%	0.51%
xylose	1.20%	0.10%
arabinose	0.16%	0.0%
sulfate	3.1%	2.4%
calcium	600 ppm	235 ppm
potassium	439 ppm	173 ppm
magnesium	165 ppm	65 ppm
chloride	150 ppm	55 ppm
sodium	126 ppm	51 ppm
phosphorus (phosphate)	104 (141) ppm	52 (116) ppm
silicon	61 ppm	126 ppm
manganese	27 ppm	105 ppm
iron	1365 ppm	3080 ppm
chromium	263 ppm	836 ppm
nickel	101 ppm	505 ppm

Study of the two hydrolysis solutions showed important differences. The 1st stage product contained C5 sugars representing about 13.7% of the dry solids put into the hydrolysis. This number was similar to the expected amount of hemicellulose in the manure and agreed well with the numbers generated at WSU. There was a substantial amount of other material also dissolved in the hydrolysis. Some glucose was present as well as typical manure mineral

elements. There was also a noticeable amount of material derived from the stainless steel vessel (iron, chromium and nickel). In the 2nd stage, higher temperature hydrolysate, these components were present at higher levels, suggesting even more attack on the processing vessel. The other manure minerals were present at lower levels in the 2nd stage, suggesting that they were readily removed at the lower temperature. Glucose was the most prevalent sugar in the 2nd stage hydrolysate, yet it represented only a fraction (approximately 1/10th) of the cellulose target. Formation of furfural and hydroxymethylfurfural, over-reaction products of the carbohydrate hydrolysis, was noted. The 2nd stage was apparently at temperature for longer than the optimum time.

Two-stage hydrolysate hydrogenation results

Comparison of the hydrogenation results with the hydrolysis solutions from WSU and PNNL (shown in Table 3.5) showed some important similarities and some differences. The catalysis of the hydrogenations worked better in the 1st stage solutions but was still significantly inhibited. With the 2nd stage solutions, almost no hydrogenation was achieved. There were significant amounts of oligomers in the hydrolysates such that, in the presence of the residual acid, additional glucose was generated. In experiment #80, the resolution of the xylose and sorbitol peaks was insufficient to accurately determine their respective amounts.

Table 3.5 Hydrolysis solution hydrogenation results

	NH_4^+	sulfate	batch feed		expt	6 hr			
	ppm	%	glucose	xylose	number	xylose conversion	xylitol yield	glucose conversion	sorbitol yield
1st Stage #6, WSU	1320	4.8	0.17	0.56	58	9%	14%	increased	12.4%
1st Stage, PNNL	NA	3.1	0.20	1.2	80	44%?	44%	16.9%	136%?
2nd Stage #5, WSU	358	7.6	0.27	0.2	67	11%	1.4%	increased	0.4%
2nd Stage, PNNL	NA	2.4	0.51	0.1	79	16%	decreased	2.2%	0%

Cleanup of Two-stage hydrolysates for hydrogenation

Ultrafiltration and carbon treatment were applied to these hydrolysates in an attempt to remove impurities that slow the sugar hydrogenation reaction. The ultrafiltration was involved the dead-end filtration cell using a membrane material with a nominal 8000 MWCO. Several

hours of filtration at 105 psig pressure were required to recover sufficient filtered solution for testing in the catalytic hydrogenation. The ultrafiltered solution was carbon-treated in a down-flow packed-bed column of particulate carbon (Norit ROX 0.8).

Low-temperature hydrolysate hydrogenation results

Comparing the reactivity of the two hydrolysates, the catalysis of the hydrogenation reaction worked better in the 1st stage (MH1) low-temperature hydrolysis solution, but was still significantly inhibited, as shown by comparison with reagent xylose in Figure 3.12. The application of ultra-filtration (8000 MWCO) improved the xylose conversion rate. Subsequent carbon treatment of the ultrafiltered hydrolysate (MH1 UF/CT) improved the reactivity further, almost to the level of the reagent xylose. A second low-temperature hydrolysate (MH3) was ultrafiltered with a smaller pore membrane (3500 MWCO). There was improved activity in the catalytic hydrogenation after this filtration, but the carbon treatment was still required to achieve a nearly full activity level.

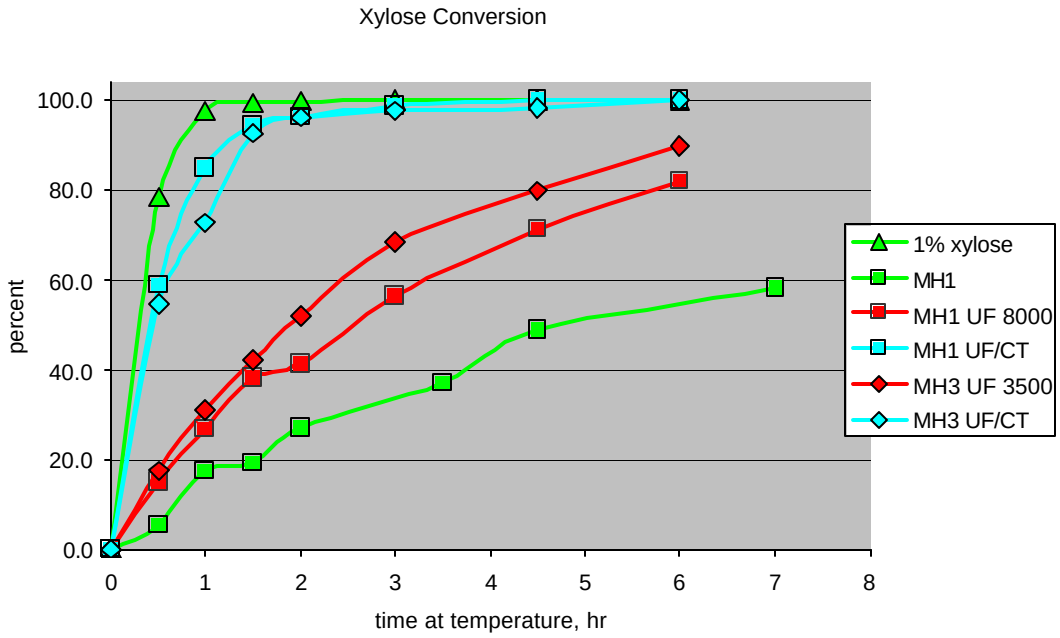


Figure 3.12. Low-temperature hydrolysate hydrogenations
UF = ultrafiltration; CT = carbon treatment

Additional hydrogenation tests at higher temperatures resulted in high activity for the hydrolysates. As shown in Figure 3.13, both ultrafiltered hydrolysates had reaction rates when processed at 150°C at the level of reagent xylose processed at 100°C. The carbon-treated ultra-filtrate was processed at higher temperature (185°C) and showed activity similar to that of pure xylose when processed at 150°C. Higher-temperature processing may be a relatively simple fix, considering that the ruthenium hydrogenation catalyst allowed us to perform our hydrogenations at lower temperatures than conventional nickel catalyst, which typically process at 120 to 140°C.

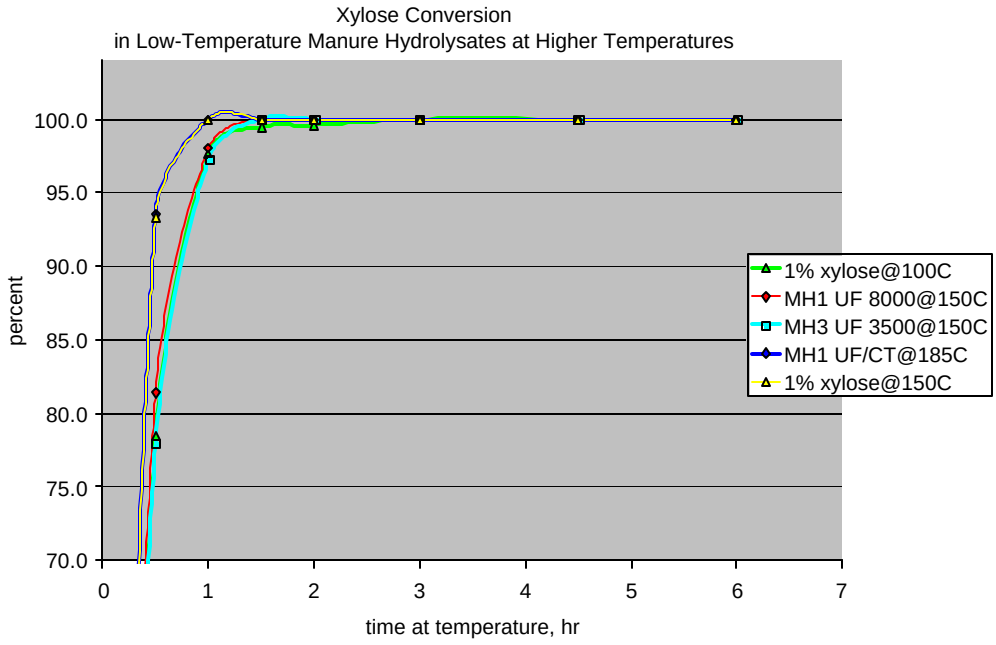


Figure 3.13. Comparison of hydrogenation results at higher temperatures

High-temperature hydrolysate hydrogenation results

With the 2nd stage, high-temperature hydrolysis solution (MH2) there was almost no hydrogenation achieved, as seen in Figure 3.14. Ultrafiltration of the hydrolysate (MH2 UF) had little positive effect. Even after the carbon treatment of the ultrafiltered hydrolysate (MH2 UF/CT), the activity was low with potential for some activity after an incubation period. However, use of the smaller pore membrane (3500 MWCO) for filtration of a second batch of high-temperature hydrolysate (MH4) was more effective in recovering the catalytic effect.

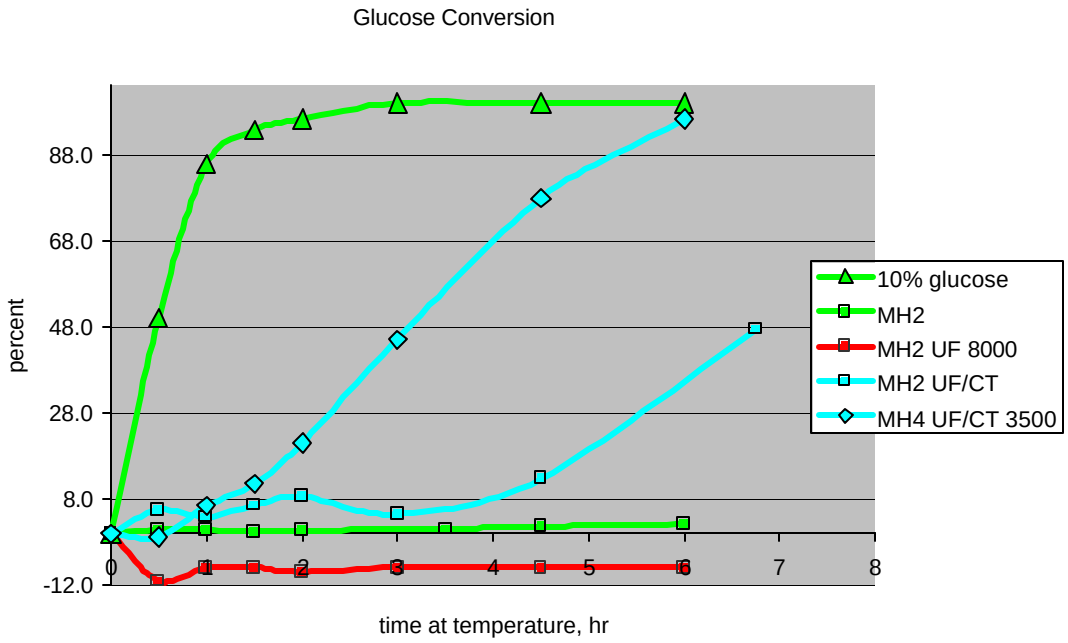


Figure 3.14. High-temperature hydrolysate hydrogenations

Cleanup of peptone-contaminated feedstock

Earlier tests with peptone suggested that it modeled the catalyst inhibition seen with manure hydrolysates quite well. The cleanup steps of ultrafiltration and carbon bed treatment, which have been found to be quite effective with the manure hydrolysates, were also applied to peptone to see if the model applied to the cleanup steps, as well. The results of a series of batch tests at 100°C with peptone are shown in Figure 3.15. As seen before, the peptone addition greatly reduces the catalytic activity for glucose hydrogenation. There are two peptone addition tests shown: the one at 1 g peptone was conducted at 100°C and shows activity reduced to the level of a manure hydrolysate; the second with 1.5 g peptone addition was conducted at 118°C and shows the increased activity at higher temperature but still a dramatic reduction of activity relative to the reagent glucose. Treatment by ultrafiltration recovers some of the lost activity and subsequent treatment with a carbon bed returns the activity nearly to that of the reagent glucose. These tests further point to the hydrolyzed protein as the component of interest when concerned about catalyst activity. These treatment methods appear to be useful for restoring catalyst activity.

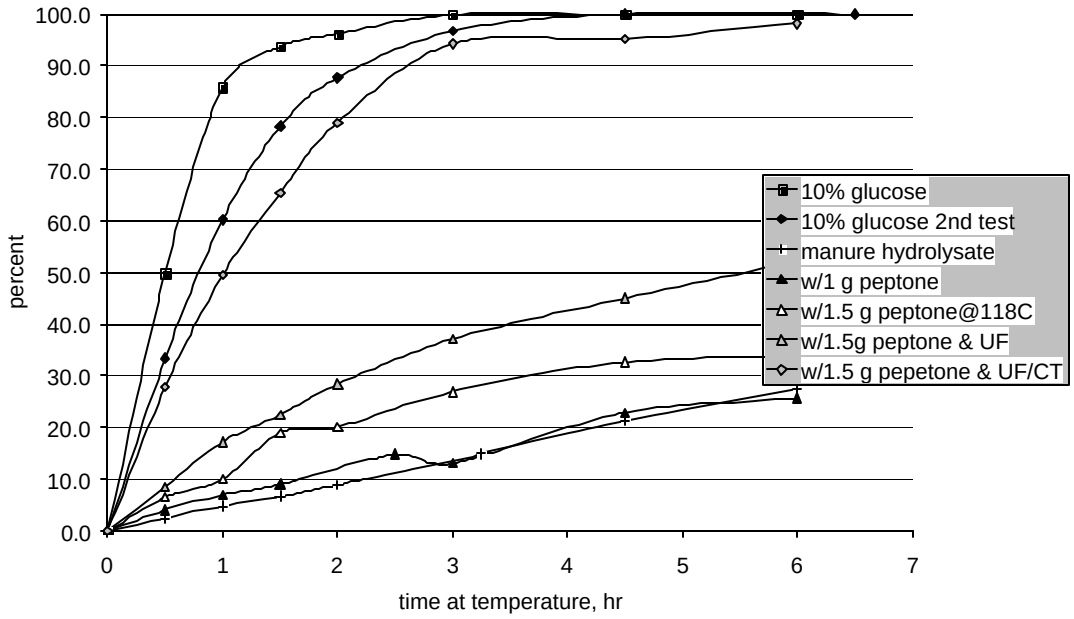


Figure 3.15. Glucose conversion with addition of peptone and after cleaning

Hydrogenation of enzymatic hydrolyzed manure solids

A sample of manure hydrolysate from WSU was produced by a process that involved a low-temperature acid hydrolysis followed by an enzymatic hydrolysis with cellulases. The product solution was concentrated and treated in an ion exchange (IX) column. This product solution, containing about 2 wt% glucose, was hydrogenated in three separate tests: as received, following UF, and following carbon bed (CB) treatment. The results given in Figure 3.16 show that the as-received solution was readily hydrogenated, but with some inhibition. Activity slightly improved after the UF and CB treatments, but was still not equivalent to reagent glucose.

Hydrogenation of two-stage hydrolysates with decrystallization

As described in Task 2, acid hydrolysis with decrystallization is the most effective method for manure hydrolysis. Therefore, the manure hydrolysate used in this part of the study was prepared using this method. Clean dairy manure solids obtained by washing the manure with water were treated by 70% H_2SO_4 for decrystallization. The mixture was then diluted with water, and hydrolyzed at 100^{o}C for 1 hour. The manure hydrolyzate contained 47 g/L of reducing

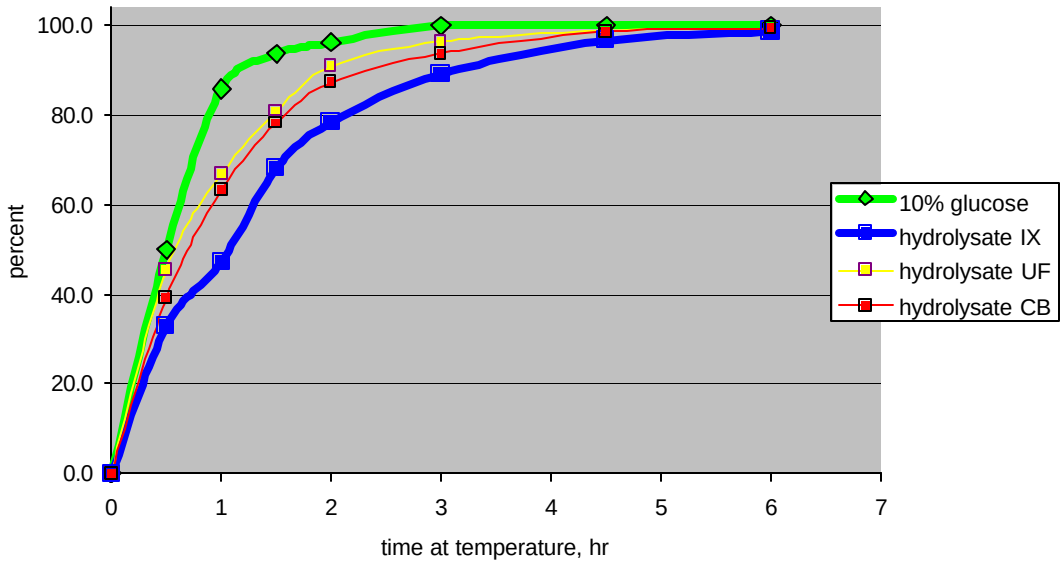


Figure 3.16. Comparison of catalyst activity of enzyme hydrolysate after treatments

sugar, 9.7 g/L of water-soluble peptides, and a limited amount of byproducts from lignin degradation. Two purification strategies were applied prior to hydrogenation, adsorption (at neutral condition) and ion exclusion on two different resins, as described in Task 2. The results of hydrogenation of the raw hydrolysate and following cleanup steps are presented in Table 3.6 for experiments at 100°C and 1200 psig with hydrogen overpressure for a total of 6 h at temperature. The adsorption exhibits greatly improved hydrogenation, although it is still significantly inhibited compared to reagent glucose and xylose. The ion exclusion process produces a similarly improved product for catalytic hydrogenation. The Amberlite resin gives somewhat better results with less loss of sugar onto the adsorption column. There is little difference between the passing hydrolysate and the eluent fraction, except that the eluent is more dilute and as a result is hydrogenated more quickly.

Cleanup of catalysts following hydrogenation of hydrolysates

Another important issue is the permanence of the catalyst inhibition or poisoning. The permanence of catalyst inhibition depends on the mechanism of the chemical interaction between the poison and the catalyst. The catalyst inhibition and the resulting reaction rate reduction

Table 3.6. Hydrogenation of decrystallized hydrolysates without and with cleanup.

feed	sugar conversion	polyol yield	glucose conversion	glucose conversion at 3 hr	g/L sugars (HPLC)	g/L reducing sugars	g/L amino acids
hydrolysate	62.1%	75.1%	31.8%	17.4%	30	47	9.7
adsorption	88.4%	140%	74.2%	57.3%	22	35	8.5
IE1 passing	84.0%	99.5%	65.5%	40.0%	21	29	7.9
IE1 elution	91.2%	105%	80.8%	70.4%	6.4	7.0	2.4
IE2 passing	85.0%	91.2%	79.4%	60.9%	34	44	11
IE2 elution	93.8%	95.7%	90.1%	86.6%	7.4	6.5	2.4

IE1 = ion exclusion with Dowex 99H
IE2 = ion exclusion with Amberlite 120

could result from competition between the poison and the preferred reactant at the catalytic site, either because of a high affinity of the poison for the catalyst site or because of its slow reaction once on the catalyst site. If the affinity is too high, as when the poison actually reacts with, and is covalently bound to, the catalyst, the catalyst is permanently poisoned. If the inhibition is only related to a slow rate of reaction, it may be possible to remove the poison from the catalyst surface and restore catalyst activity.

Catalyst-washing tests were performed to determine if catalyst activity could be recovered (see Figure 3.17). Catalysts used in hydrogenation tests with manure hydrolysates

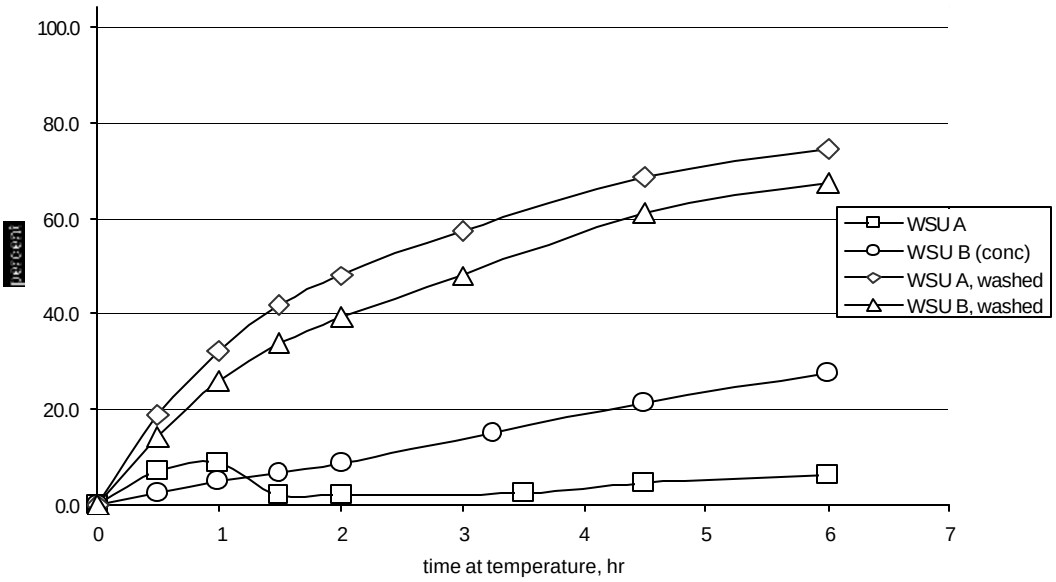


Figure 3.17. Comparison of catalyst activity with raw hydrolysates and after washing

were washed with water and reused to hydrogenate reagent glucose. The two high-temperature hydrolysates, designated solution A and solution B, gave low rates of conversion of glucose when hydrogenated directly as produced. The catalysts from those two tests, after washing, showed much improved reaction rates, approaching that of unused catalyst.

This reversibility of the poisoning is an important parameter. Two of the catalysts exhibiting inhibited activity due to ammonium were also tested after a water wash to determine the permanence of the deactivation. In the test results shown in Figure 3.18, it was apparent that the catalyst deactivation noted in an initial test could be reversed by the water wash, and the catalyst activity could be returned to a level at or near that of unused catalyst. The effect was demonstrated for both ammonium carbonate and ammonium hydroxide.

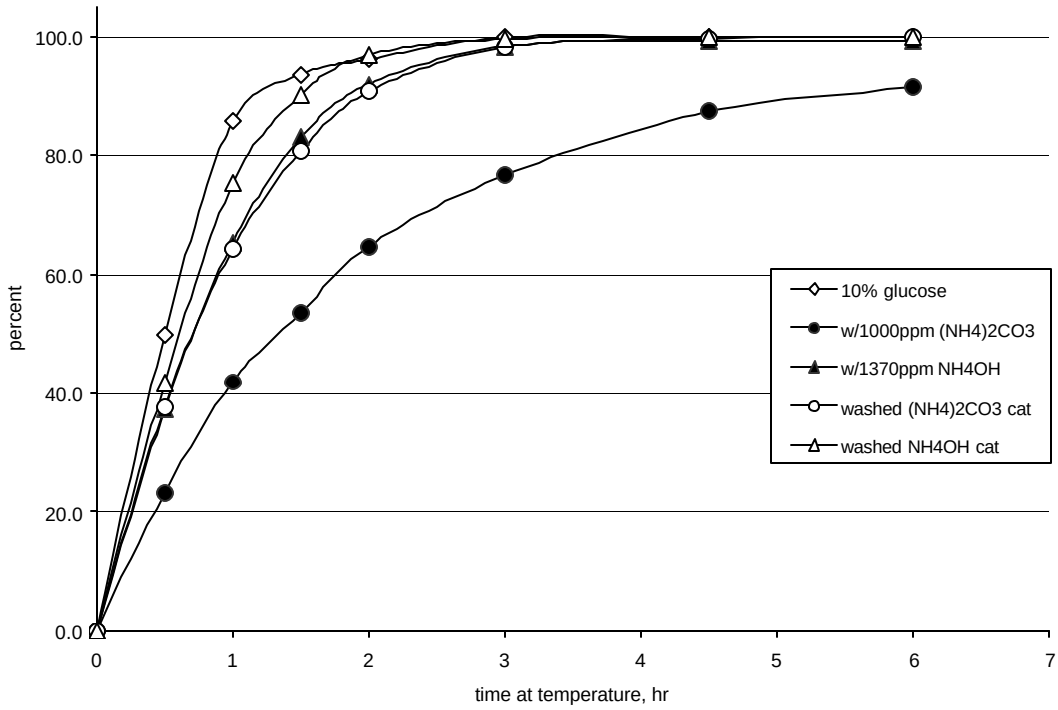


Figure 3.18. Glucose conversion with reused catalyst

Additional washing tests with the peptone-poisoned catalysts showed a similar relationship. As seen in Figure 3.19, the washed catalysts showed greatly improved activity compared to the result initially with the peptone-contaminated environment. It appeared that the

water washing of the catalyst improved the activity equivalent to an order of magnitude reduction in the peptone contamination. However, in this case, there was not a total recovery of catalyst activity to the pre-contaminated state. This recovery of activity indicated that a significant portion of the catalyst deactivation resulted from a competition of the peptone with the glucose for the catalytic site. In addition, also illustrated in these results, was that a second wash did not improve the activity any further. This result appeared to verify a two-pronged catalyst deactivation effect with competition for the catalytic site as well as a more permanent poisoning of the catalytic site by the peptone.

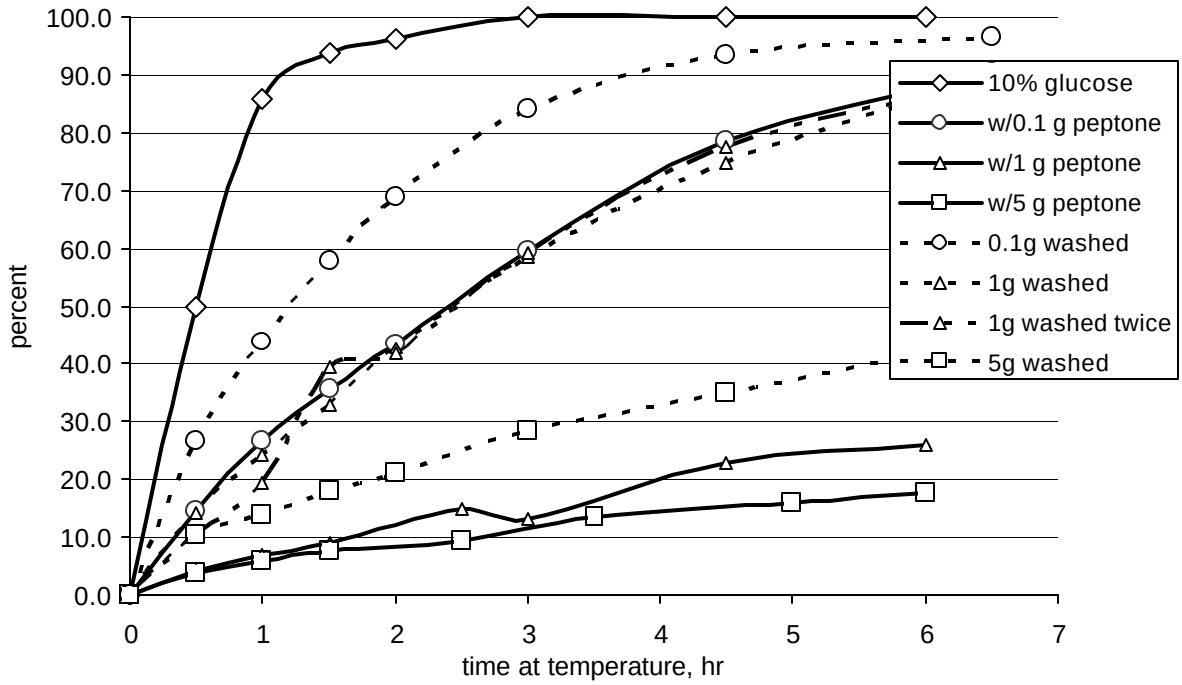


Figure 3.19. Effect of washing peptone-contaminated catalysts.

Other used catalysts from several previous tests were cleaned for reuse to evaluate the ability to regenerate deactivated catalysts by several relatively simple cleaning steps. These experiments extended the water-wash study to the use of an ultrasonic bath or the use of caustic for the wash step. Figure 3.20 shows glucose conversion plots for low-temperature hydrolysates and with several used catalysts.

For comparison purposes, the model test with reagent glucose is given, as well as the actual hydrolysate processing test, identified here as MH1. The use of an ultrafiltration cleanup step for the hydrolysate (MH1 UF 8000 or MH3 UF 3500) showed improved reactivity but still significantly inhibited relative to the reagent glucose. Both a deionized water wash of the MH1 UF catalyst and the caustic wash of the MH3 UF showed a significant recovery of the catalyst activity (when retested with reagent glucose) but not a complete recovery of activity. The combination of ultra-filtration and carbon bed treatment (MH1 UF/CT or MH3 UF/CT) greatly improved the processing rate for the hydrolysate. The caustic wash of the used catalyst in the MH1 case showed an essentially complete recovery of catalyst activity, while the use of the ultrasonic bath wash with deionized water actually had little positive value.

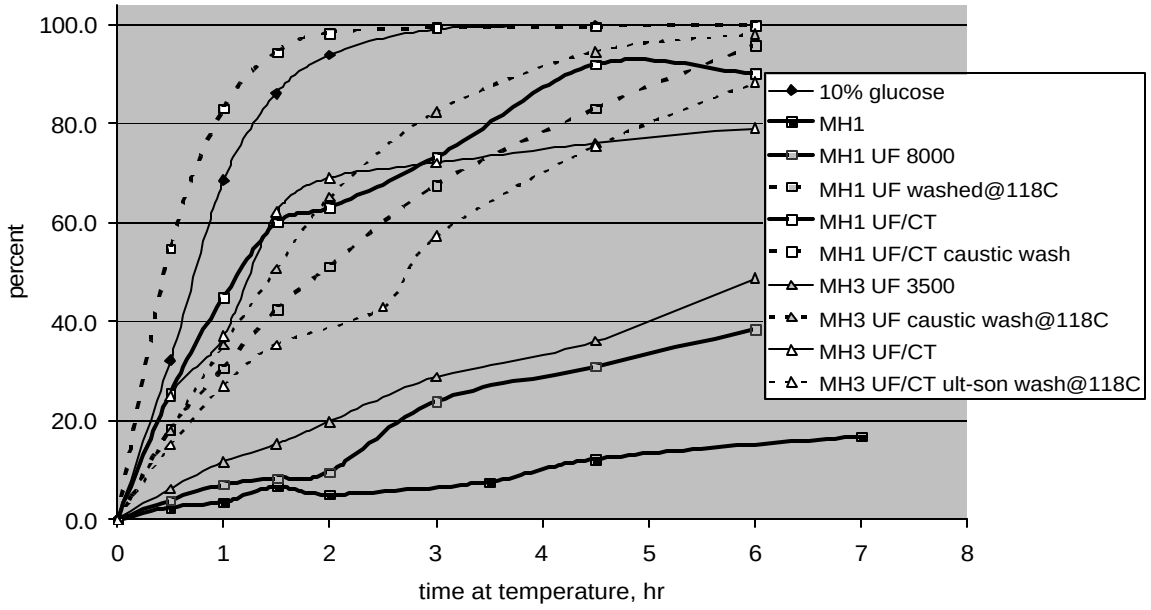


Figure 3.20. Glucose Conversion in Low-Temperature Manure Hydrolysates

Similarly in Figure 3.21, glucose conversion results are shown for high-temperature (second stage) hydrolysates and with cleaned catalysts. For comparison, a test result showing high conversion of reagent glucose is given, as well as the nearly-zero activity result from processing the as-produced hydrolysate solution (MH2). Ultrafiltration alone (MH2 UF 8000) did not produce a positive result, but following a caustic wash of the used catalyst, its activity

was essentially restored to that of a fresh catalyst. The combination of ultrafiltration and carbon bed treatment (MH2 UF8000/CT) showed some improvement in the glucose conversion, and in this case the ultrasonic deionized water bath showed some additional improvement. However, the catalyst activity remained significantly below that of fresh catalyst.

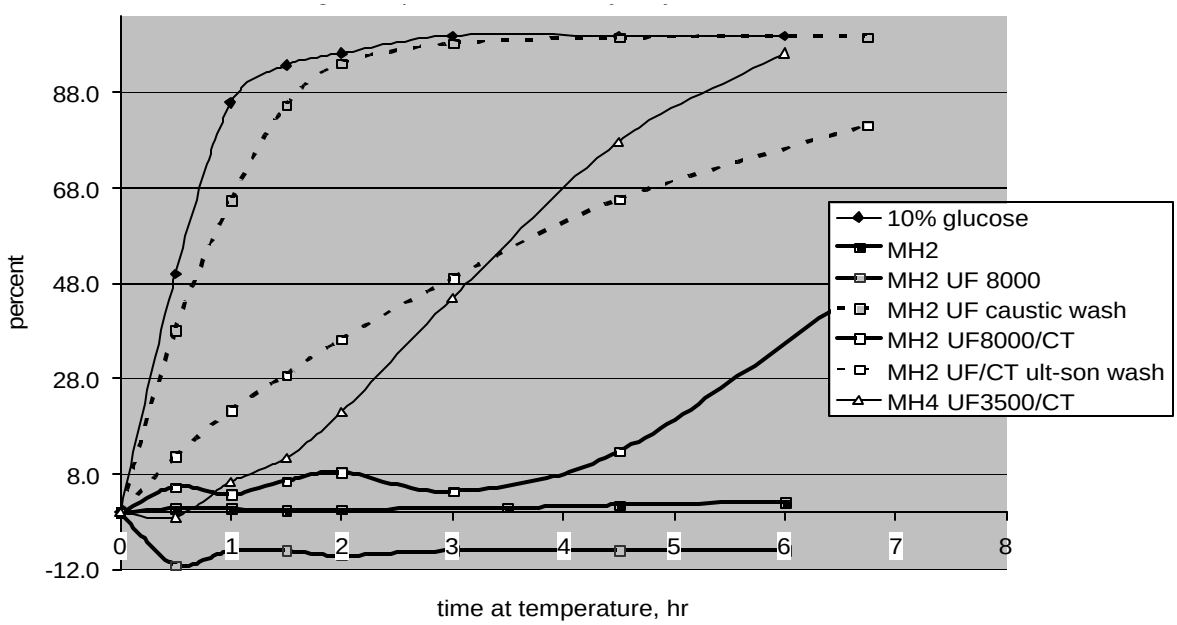


Figure 3.21. Glucose Conversion in High-Temperature Manure Hydrolysates

Catalyst lifetime testing in a continuous-flow micro-reactor

A micro-scale (30-ml bed) catalyst reactor was reassembled at our laboratory. The unit had an online data acquisition and process control system, which allowed continuous 24-h operation for catalyst lifetime testing. The unit was fed by a dual-piston, high-pressure metering pump, which also provided for continuous feed of solution. The reactor tube was heated with a circulating oil bath. Hydrogen was fed continuously from a high-pressure manifold with pressure controlled by a dome-loaded, back-pressure regulator. The feedstock used was produced by a two-step batch hydrolysis of manure solids from the WSU dairy; first at 110°C and followed at 170°C for the undissolved solids from the first step. These hydrolysis solutions were processed through ultra-filtration and an activated carbon bed prior to the catalytic hydrogenation. There is some question as to whether the carbon bed became saturated and failed to clean the feedstock after the first day.

The results of the 7-day test, given in Table 3.6, showed a strong conversion of the sugars to sugar alcohols initially. Over the period of several days the activity fell away. The processing equipment functioned mostly as expected. The operating temperature was very stable at 102°C in the catalyst bed. The pressure control was more erratic, having been set at 1200 psig but fluctuating from 700 to 1350 psig. The processing rate was steady at a liquid hourly space velocity (LHSV) of 2 liters of feed per liter of catalyst bed per hour. These results suggested that the feedstock was still contaminated enough to poison the catalyst.

Table 3.6. Long-term continuous flow test of hydrolysate hydrogenation

date-time	time on stream, h	glucose fed, wt %	xylose fed, wt %	sugar conversion, %	sugar alcohol yield, %	carbon gasification, %
8/27-18:00	0	0.47	0.82	--	--	--
8/28-11:35	17.58	0.47	0.82	98.5	93.8	5.1
8/29-11:45	41.75	0.50	0.87	92.8	94.4	4.1
8/30-10:53	64.88	0.50	0.87	70.6	73.0	6.8
8/31-18:45	92.97	0.50	0.87	22.5	25.1	0.0
9/2-14:42	135.84	0.50	0.87	17.6	18.9	--

For comparison, results in an internally funded research program showed catalytic stability over several weeks of operation in similar tests with reagent sugars and corn wet mill glucose. In addition, the processing rate at a much higher concentration of sugars was significantly higher than that achieved with manure hydrolysate solutions. Results in the other tests have shown that a LHSV of 2 could process 40 wt% glucose to a 99.9% conversion level and that 20% glucose could be processed to 99.95% conversion at as high as 6 LHSV. Carbon gas yields were only 0.1 to 0.2% in those tests, also.

Analysis of the liquid products showed that over the first 3 days of operation the amounts of calcium, magnesium, phosphorus, and sulfur were much reduced compared to the amount fed to the reactor, suggesting significant amounts were deposited in the catalyst bed. We suspect insoluble alkaline earth phosphate deposits and sulfur reaction with the ruthenium catalyst. After the first 3 days, the amounts of these elements returned to the feed level suggesting a saturation of the catalyst bed. In contrast, the amounts of iron, chromium, nickel, molybdenum, as well as cobalt, copper manganese, and titanium, were significantly increased in the products, suggesting

corrosion of the reactor system construction materials by the residual acid. A green tint seen in the products after hydrogenation also suggests this mechanism. The highly soluble sodium and potassium, as well as silicon and zinc, were essentially constant in and out of the reactor.

Analysis of the used catalyst provided some confirmation of these findings. Elemental analysis by ICP showed metal contaminants, Cu, Cr, Co, Fe, and Ni, had migrated into the catalyst bed. Sulfur and calcium were also present in low amounts. Magnesium, zinc, and manganese were also present in trace amounts. There was a significant level of sodium in the used catalyst, which does not correlate with the steady level of sodium in the feed and the product. At the same time, there was no potassium found in the catalyst, as expected.

Task 4.
Life-Cycle Analysis and Economic Analyses

Availability of manures and potential to sequester carbon or produce carbon savings

A major source of methane emissions, and hence greenhouse gases, is livestock. Collecting cow manure for use in making industrial chemicals would help in reducing these emissions, as well as eliminating or reducing the need for petroleum to make the chemicals. The methane contained in cattle manure escapes into the atmosphere over time and is oxidized into carbon dioxide, which contributes to global warming. The amount of greenhouse gas the entire U.S. cattle population releases is actually quite significant. In the United States, the third largest source of methane emission to the atmosphere is livestock, which produce about six million tons, or 19% of all methane emissions [a]. Two studies were conducted to determine the amount of carbon savings resulting from collection and use of cow manure. Limited data are available on carbon content of manures, and, therefore, the final value, 0.06 kg carbon in gases saved/kg manure collected, is considered only a reasonable estimate.

Methodology

As a baseline comparison, it is assumed here that instead of leaving the manure out on the field, during which 100% of the carbon would be released directly into the atmosphere, the wet manure would be collected immediately and all of its carbon would be converted to bioproducts.

The carbon content and amount of cattle manure produced per animal depends on the type and size of cow, what and how much it eats, its productivity level (when producing milk), and even the climate [b]. However, the most profound discrepancy of manure composition occurs between beef and dairy cattle. Dairy cattle in the U.S. produce many more emissions than beef cattle, especially as their milk production increases. As a group, dairy cattle release 653×10^9 kg of methane per year in their manure. Beef cattle, although many more in number (61.05 million versus 12.95 million dairy [c]), only produce 161×10^9 kg of methane per year [d]. Because of this lower production and the fact that dairy cattle stay within a more limited area where manure collection is easier (beef cattle usually are raised on open range or feedlots), cattle manure collection for this study will be limited to only dairy [e].

Two sets of data were used for this analysis: tests conducted by a group of scientists at Washington State University (WSU) in Puyallup, Washington, and an article received from the same group on a study at the University of Jordan in Amman, Jordan.

Results

The WSU analysis of dairy manures shows a 47.22% dry mass carbon content [f]. Wet manure has a moisture content of approximately 85-88% [g]. Therefore, assuming 100% conversion of carbon to gases (methane and carbon dioxide), the result from dairy manure is 0.06375 kg carbon in gases per kg manure, according to the calculation below.

$$\frac{.4722 \text{ kg C}}{1 \text{ kg dry mass}} * \frac{.135 \text{ kg dry mass}}{1 \text{ kg manure}} = 0.06375 \text{ kg carbon / kg manure}$$

The study of cattle in Jordan reports a similar carbon content. Scientists were trying to extract biogas from manure using a digester. Using 40 kg manure per day, an average of 2.098 m^3 of biogas (approximately 60% methane and 40% carbon dioxide) per day was extracted, at an average temperature of 18.5°C [h]. Using a biogas density of 1.1515 kg/m^3 [i], results in 0.0266 kg carbon in gases/kg manure, according to the equation below.

$$\frac{2.098 \text{ m}^3 \text{ biogas}}{\text{day}} * \frac{1.1515 \text{ kg biogas}}{\text{m}^3 \text{ biogas}} * \frac{\text{day}}{40 \text{ kg manure}} * \frac{12 \text{ kg carbon/kgmole}}{27.2 \text{ kg biogas/kgmole}} = 0.0266 \text{ kg carbon / kg manure}$$

Discussion

Because the study in Jordan was extracting biogas (methane and carbon dioxide) from manure by an inefficient biological process, it is safe to assume that a smaller amount of carbon gases was produced with significant levels of unconverted carbon residual requiring additional disposal. Also, it was not specified which type of cow was used in this study. Most likely they were dairy cows, but Jordan and U.S. dairy cattle may differ, since they live in different climates and cultures, where farming methods and feed most likely differ.

In conclusion, while the data from Jordan remains to be good supporting evidence, it should not be used as a reliable number on which to base future calculations. However, it is not good practice to use a single data point (the WSU case) to base calculations on either, even if the data is known to be good. Therefore the significant digits should be reduced, resulting in an average carbon amount of 0.06 kg/kg manure. This is the amount of carbon that would be saved from being released into the atmosphere if dairy cow manure was collected wet from the field to be used as bioproducts instead of being left out to dry and decompose releasing carbon gases.

Conclusion

Collecting manure in order to use its carbon would be extremely beneficial in emissions reduction, odor reduction, and natural resource savings, especially in the case of dairy cattle. The advantage of using cattle manure for biomass conversion is in taking the carbon excreted from the cow and directly transferring it to useful carbon in industrial chemicals. This not only prevents the carbon in manure from escaping into the atmosphere, but also eliminates the need for the petroleum typically used to develop the chemical. By using manure to displace petroleum, emissions and energy are being saved from two separate sources.

The energy required to process the manure for conversion to industrial chemicals is still a cost. With proper process development, the energy saved should still exceed the energy used. Either way, there will be a positive savings of carbon emissions.

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e. http://www.westbioenergy.org/cattle/intro.htm
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g. http://muextension.missouri.edu/explore/envqual/eq0385.htm
h. Hammad, M., Badarneh, D., Tahboub, K. "Evaluating variable organic waste to produce methane," *Energy Conversion and Management* 40, p 1463-75, 1999.
i. Rose, J. W. and Cooper, J. R. eds. *Technical Data on Fuel* 7th edition, p 267, John Wiley & Sons, New York, 1977.

Flowsheet Cost Estimation

A techno-economic assessment of the feasibility of converting waste manures to valuable products was developed using laboratory results and standard chemical engineering plant design and costing concepts.

Several flowsheets were developed that potentially could convert manure into valuable products. In the first flowsheet, the residual undigested protein products are extracted from the solid portion of the manure and the remaining carbohydratesare converted to sorbitol via a two-stage acid hydrolysis and hydrotreatment. In the second, all manure solids are hydrolyzed directly without protein extraction. In the third, the manure solids are pre-treated using a concentrated sulfuric acid "decrystallization" process (Farone and Cuzens, 1998) prior to hydrolysis.

For the first flowsheet (BASELINE), the estimated total capital cost to build a plant capable of treating the waste from a 2000-head dairy is estimated at $8.85M. Annual operating costs are estimated at $2.66M. The value of the products produced by the plant is estimated at $1.13M for an annual return on investment (ROI) of approximately -17%.

A second flowsheet (SIMPLE) converted all carbohydrates to sorbitol was developed to examine the relative costs of the protein extraction front-end processing. If the manure solids are directly hydrolyzed and hydrogenated, a substantial capital cost for extraction equipment can be avoided. Total capital cost for this simple flowsheet for a 2000-head dairy is estimated to be approximately $4.5M with annual operating costs around $2.0M. The value of the sorbitol product is only estimated at about $0.79M annually, for an ROI of -26 %.

For the third flowsheet (DECRYSTAL), the estimated capital costs are $8.2M. Annual operating costs are $2.9M. The value of the products is $1.2M for an ROI of -21%.

A sensitivity analysis was performed to determine the effect of herd size, manure cost, and product value on the rate of return. Clearly, plant size appears to have the greatest effect on ROI. Increasing the herd size to 3000 animals, raises the ROI about 6%, from -21 to -13%. Breakeven occurs at just over 5000 animals. Varying the cost of manure by +/- $10/ton changes the ROI only a few percent either way. Product value has a marginal effect on profitability. Each 10% increase in product value produces approximately a 1% improvement in ROI.

Concept

The overall process concept generally involves the extraction of the undigested and novel proteins from manures followed by hydrolysis and conversion of the remaining lignocellulosic materials to sorbitol. The preliminary laboratory testing for the extractions and conceptual flowsheet, as shown in Figure 4.1, was developed by WSU. This BASELINE flowsheet results in an alkali-soluble protein, an alcohol-soluble protein, and a carbohydrate material for hydrolysis and further conversion to polyols. For our economic analysis, we assumed that all of this carbohydrate (as glucose) is catalytically hydrogenated to sorbitol, a valuable C_6 polyol used in a variety of food and consumer products, which easily can be converted to a variety of commodity chemicals.

We divided the flowsheet model into two discrete sections: a protein separation followed by the hydrolysis and hydrogenation.

Modeling Strategy

The strategy for modeling the manure process involved the development of a large Excel spreadsheet. The model begins with a design basis and flowsheets, calculates specific stream data, summarizes the baseline unit operations equipment list, calculates the baseline equipment costs, calculates the total capital investment, summarizes the annual operating costs, and conducts a sensitivity analysis.

Cost estimating for chemical plants is typically carried out in several steps, as outlined in Figure 4.2. These methods are time-tested by decades of chemical plant engineers and are well documented in Peters and Timmerhaus (1968).

First, purchased capital equipment costs are developed from an equipment list generated from the flowsheet. Secondly, a series of direct costs, such as equipment installation, piping, instrumentation, etc., are scaled from the purchased equipment costs to fold into total direct costs (TDC), which is about 70% of the total fixed capital investment (FCI). Finally, a number of indirect costs such as engineering, construction expenses, and contingency are scaled from TDC and summed to total indirect costs (TIC). The FCI is the sum of the direct and indirect costs of construction. The total capital investment (TCI) typically includes a 10-20% adder for estimated working capital.

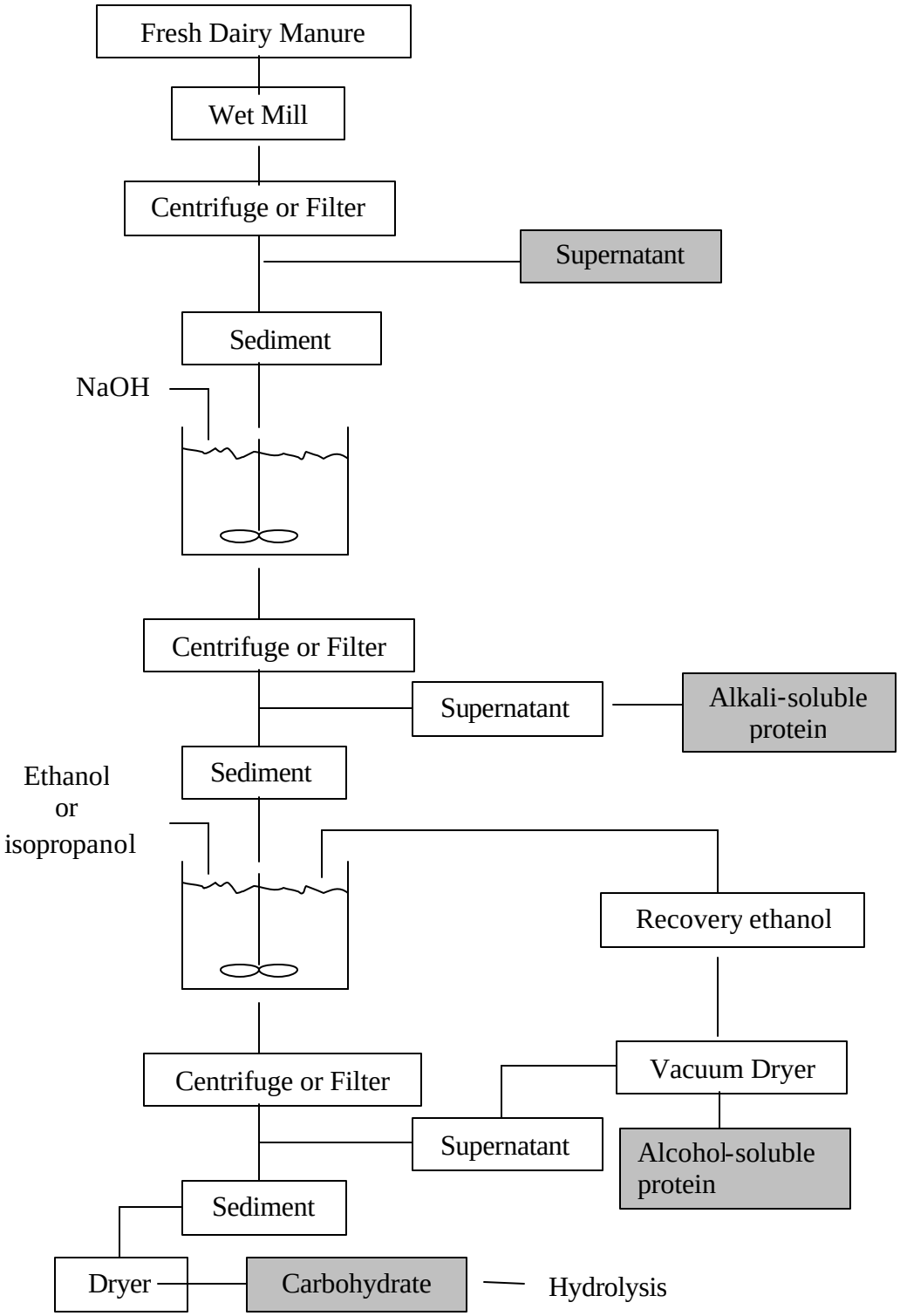

Figure 4.1 Conceptual flow schematic

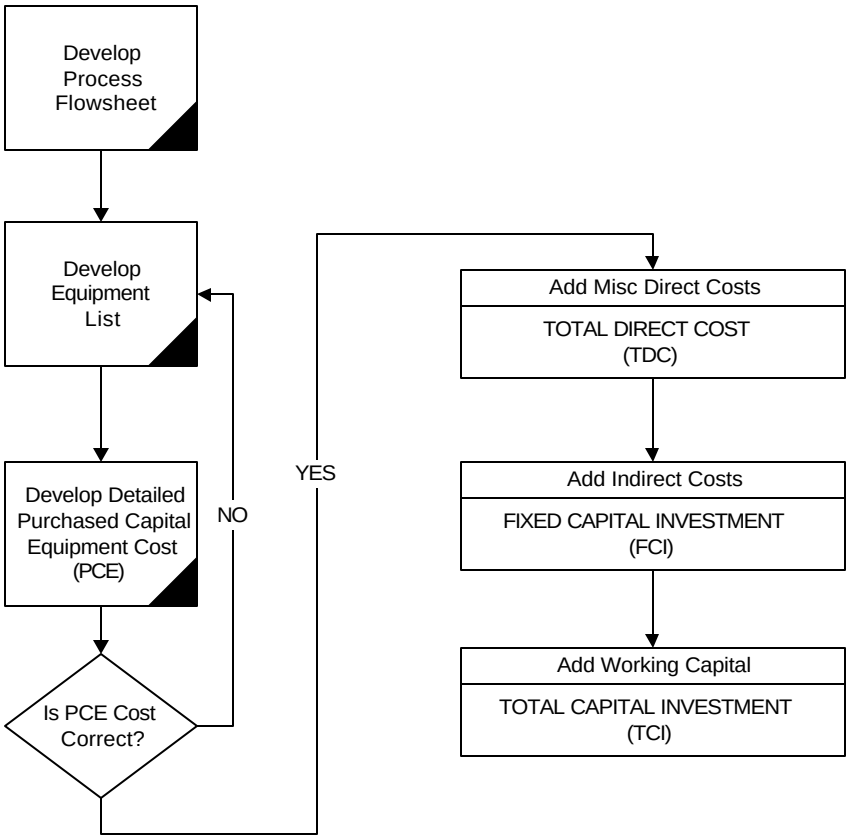

Figure 4.2. Cost estimating process steps

Plant design basis

The plant design basis page is a summary of the assumptions used in the model. It is a compilation of manure properties, baseline flowrates, separation efficiencies, conversions, and other chemical requirements. All calculations that follow in the model can be traced back to the design basis page. By changing assumptions on this page, the effects on stream properties, costs, and profitability can be seen.

The design basis starts with a typical 2000-head dairy. There are many smaller dairies, and some larger, but a typical large commercial dairy is between 1500 and 2000 animals. Each animal produces about 105 lb of manure per day (www.maeap.org/mwps_manure_charts.pdf) at an average of 12% volatile solids. Other manure properties are summarized in Table 4.1.

Table 4.1. Dry Manure Properties

Water+caustic solubles	40.82%
Alcohol solubles	5.27%
Insolubles	53.91%
Total	100.00%

Elemental Analysis	
Carbon	49.12%
Hydrogen	
Nitrogen	2.70%
Oxygen (by difference)	

The plant produces two protein fractions and a sorbitol product.

Protein separation section

The protein separation section is composed of an initial solids separation followed by a caustic extraction and an alcohol extraction. The overall process flowsheet is shown in Figure 4.3. The laboratory analysis results demonstrate that the bulk of the carbon and nitrogen is contained in the manure solids, and very little (a few %) is lost in the green supernatant. Therefore, the initial filtration is accomplished on a belt filter, and the filtrate is sent to the existing settling ponds and spray field.

The initial solids are washed with 0.02 M sodium hydroxide and centrifuged. The centrate is neutralized by 5% sulfuric acid to precipitate the caustic-soluble protein, which is then spray dried.

The remaining solids from the caustic wash are then washed with 80% ethanol to extract additional proteins and centrifuged. The centrate is vacuum dried to leave the alcohol-soluble proteins. The centrifuged solids are also dried to recover the ethanol prior to hydrotreatment. The ethanol vapor removed in the drying processes is condensed and recycled to the alcohol wash tank. The remaining solids from the alcohol wash are sent to the hydrolysis step.

In the second SIMPLE flowsheet scenario, the initial separation step was proposed to be only a belt filter to separate all manure solids for conversion to sugars. This was proposed for a variety of reasons:

- The protein separation section has high capital costs.

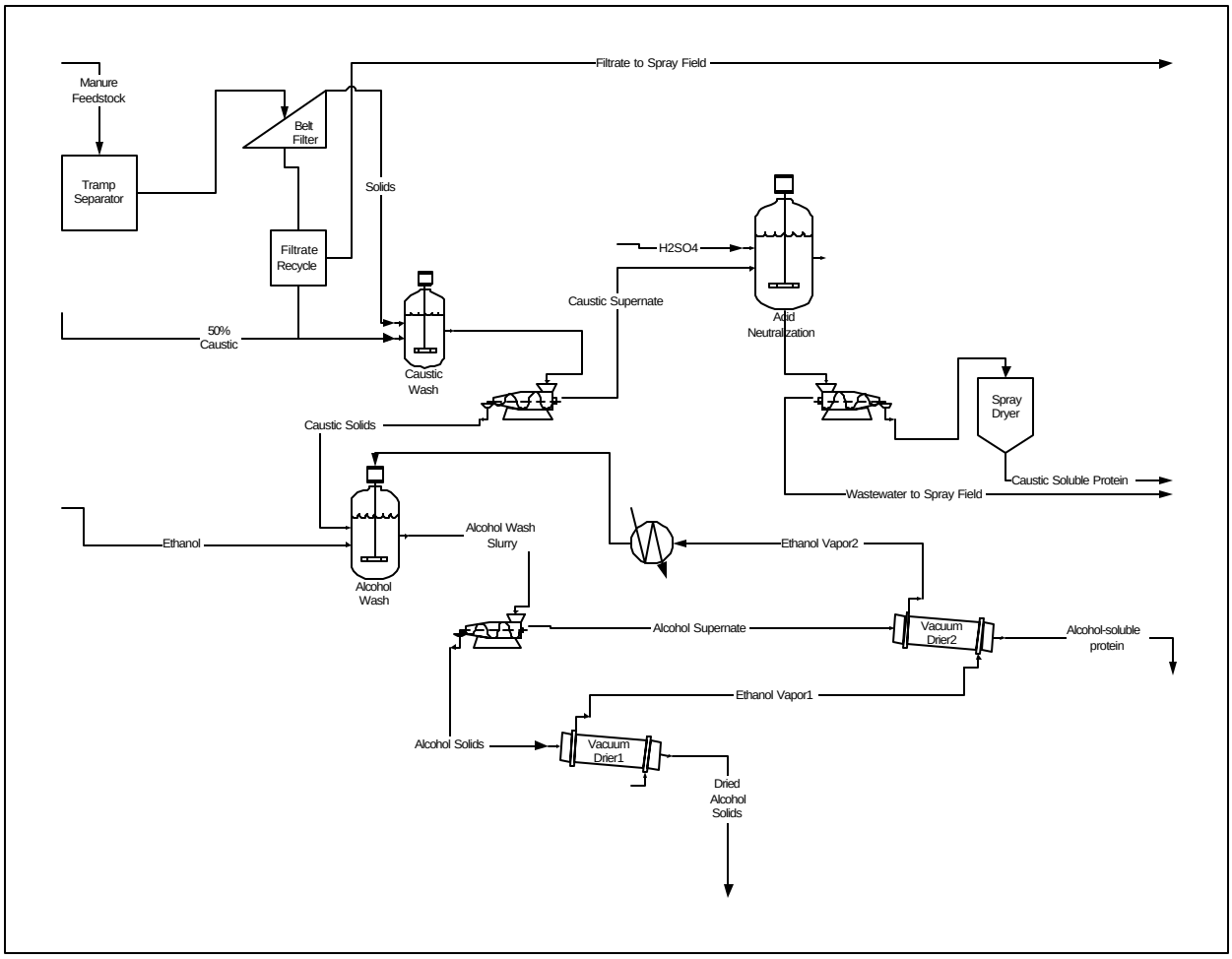

Figure 4.3. Manure processing including protein separation

- High annual operating cost contribution by the ethanol solvent (due to losses) was evident.
- No high-value market for these proteins has been identified; therefore, no large price incentive exists to separate them.

The third flowsheet scenario (DECRYSTAL) also used the same strategy of converting as much of the available solids directly to sugars.

Hydrolysis and hydrotreatment section

The hydrolysis and hydrotreating section converts the remaining carbohydrates to sugars via a two-stage acid hydrolysis and then to the final product, sorbitol, via catalytic hydrogenation. The schematic for first baseline hydrolysis (employed in both BASELINE and SIMPLE flowsheets) is shown in Figure 4.4.

The remaining sediment from the protein separation section is introduced to the first stage of the hydrolysis with 5% sulfuric acid. The reaction conditions are approximately 105°C and 0.5 Bar of pressure (1.5 Bar absolute). Laboratory tests suggest that approximately 60% of the available carbohydrates are converted to sugars in the overall hydrolysis process, about 40% in this first step. Sugar conversions are calculated on a basis of grams of sugars per gram of bone dry manure solids. Experimental data from WSU suggest that about 12 grams of sugars are produced per 100 grams of manure solids in this first stage. On centrifugation, the sugary liquid centrate is sent to the hydrotreater and the solids are added to the second hydrolysis step.

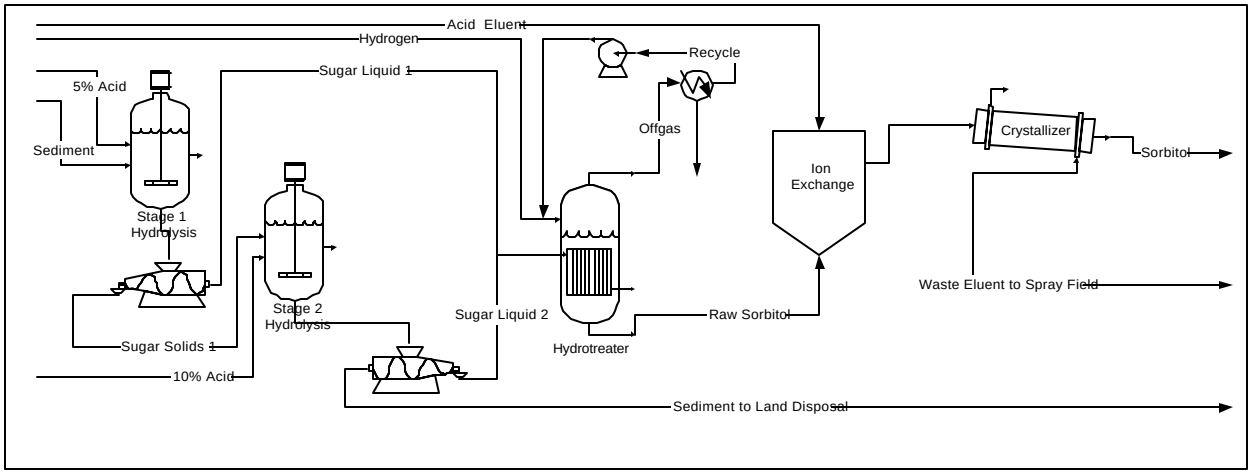

Figure 4.4. BASELINE two-stage hydrolysis/hydrotreatment flowsheet

Much of the sugars produced in this first-stage hydrolysis are xylose. However, the model treats all sugars as glucose. This may be unfortunate, because the hydrogenation product of xylose (xylitol) is worth considerably more than sorbitol. The price of xylitol is about $3 per kg (USDA) vs. about $1.50 for sorbitol. Clearly the market for xylitol is much smaller (about

$28M), and significant production would saturate the market quickly. Xylose is somewhat difficult to separate, and hangs up on an activated carbon bed.

The second hydrolysis step is conducted at 130°C and 0.5 Bar (1.5 Bar absolute) using 10% sulfuric acid. Approximately 20% of the original carbohydrates are supposedly converted in this second step. However, experimental data show only about 3 grams of sugar per 100 grams of manure solids are produced in this step. On centrifugation, the sugary centrate is also sent to the hydrotreater. The remaining manure solids are sent for landfarming.

In the DECRYSTAL flowsheet (Figure 4.5), the dried manure solids are subjected to a cold acid soak in 70% sulfuric acid followed by dilution to 20% acid and further hydrolysis at 100°C. Following the hydrolysis section is a continuous ion exclusion chromatography as offered by Ameridia (2003) and others for sugar recovery. The 20% sweet acid solutions are circulated through the sequencing beds to recover the sugars from the acid. Pure water is used as the eluent, generating a pure water-sugar stream. The lean 20% acid is then concentrated back to 70% acid for recycle to the cold acid soak.

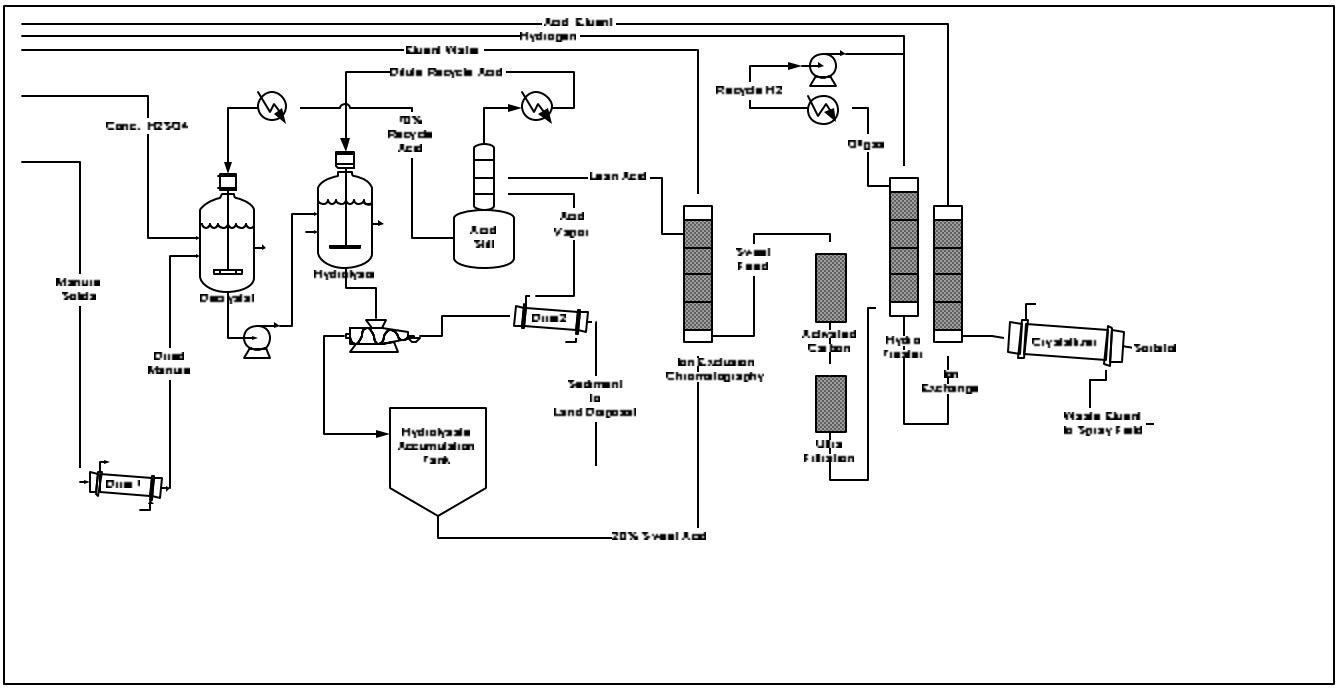

Figure 4.5. Manure Processing with decrystallization, hydrolysis, hydrotreatment, and cleanup

An initial drying step is required of the manure solids to allow the decrystallization reaction to take place at a low enough temperature (50°C). Wet solids cause the acid solution reaction to heat appreciably. A gas-fired rotary dryer utilizing chains, balls, or other mechanical methods of grinding the material is desired. Manure is quite corrosive, and the dryers chosen should be capable of handling this severe service.

The initial decrystallization reaction is done at a ratio of 5:4 acid to solids, which creates an almost paste material that is not easily mixed. One alternative for performing this reaction is a "Holoflite"-type screw reactor shown in Figure 4.6. These reactors are capable of circulating heating or cooling fluids within the screw for temperature control and can produce reliable residence times and some reasonable mixing.

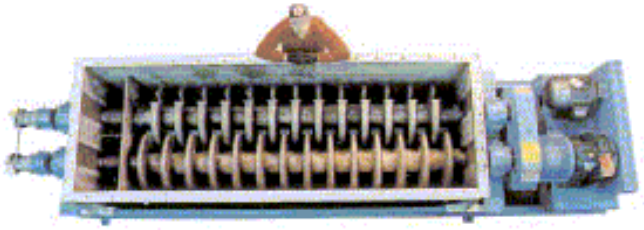

Figure 4.6. Rotary ThermalProcessor, courtesy of Komline-Sanderson, Inc.

Following this decrystallization, the high acid paste is slurried with dilute recycle acid to yield a 20% acid mixture, which undergoes the actual hydrolysis at 135°C. The remaining solids are removed via centrifuge, and the sweet acid hydrolysate is subjected to ion exclusion chromatography to capture and elute the sugars. In the baseline case, the acidic sludge is dried for disposal using an indirectly fired rotary kiln dryer. Alternatives to be considered would be the direct land farming of this material in sulfur-poor cropland or direct neutralization. An acid distillation column concentrates the acid vapors generated from this dryer and the 20% lean acid stream to produce a 70% concentrated acid for recycle to the decrystallization step. Prior to hydrotreatment, the acidic sugar solution is ultrafiltered and passed over activated carbon to remove any remaining impurities.

For all flowsheets, the hydrotreating section converts sugars to alcohols through hydrogenation over a catalyst under high pressure. Reaction conditions are typically 100°C and 80 Bar. The reactor vessel is expected to be a 2500-L (500-gal) ASME-stamped pressure vessel

made of high-alloy stainless steel operated with a fixed bed of catalyst. Typical catalysts used in these reactions include the supported metals (Ni or Ru). Obtaining an active catalyst that can stand up to the challenge of manure residues over long periods of time may be difficult. For this analysis, we have assumed that a single 3300-kg charge of the catalyst at $50/kg will be replaced once per year.

Product Revenues

The three specific products that are produced in the BASELINE flowsheet include a caustic-soluble protein fraction, an alcohol-soluble protein fraction, and sorbitol. For the BASELINE case, the product revenue streams are listed in Table 4.2.

Table 4.2. Product values from BASELINE case

Product/Byproduct Stream	Production Rate, kg per day	Annual Production, kg (365 days per year at 90% operability	Unit Value of Products, $/kg	Value of Products, $K per year
Caustic-soluble Proteins	4,023	1,321,530	$ 0.50	661
Alcohol-soluble Proteins	520	170,955	$ 0.50	85
Sorbitol	725	238,241	$ 1.60	381
Total Value of Products				**1,127**

Extracted protein valuations are largely unknown. While a specific protein of interest might be worth several dollars per gram, the bulk extraction material is generally not easily marketable. We based our initial economics on the worst-case scenario, where the value would be equivalent to the cost of protein from raw alfalfa hay. At $100/ton @ 20% protein, this translates to about $0.50 per kg. No specific wholesale marketing opportunities were identified during our research.

The latest sorbitol values were derived from the trade publication Chemical Marketing Reporter, which has weekly (http://www.chemicalmarketreporter.com/home/frameset.htm)

prices for a whole host of commodity chemicals. The value of $1.60/kg was calculated from the February 2003 numbers for crystalline sorbitol in drums, FOB plant.

In the SIMPLE flowsheet, neither of the soluble proteins are extracted and recovered. The sorbitol production is increased to about 1500 kg per day due to the increased conversion of carbohydrates to sugars during the hydrolysis step. In the DECRYSTAL flowsheet, this conversion is further enhanced by the advanced hydrolysis, and the sorbitol production is approximately 2300 kg per day.

Neither of these steps was sufficient to increase the production of valuable chemicals above the breakeven point. However, it may be worthwhile to examine the potential economic viability of segregating the xylose from the first stage hydrolysis and hydrogenating it separately to xylitol, which is worth considerably more (>$6/kg) than sorbitol. Unfortunately, this may add substantial complexity to the separations issues.

Equipment Summary

The purchased equipment list was developed by selecting specific unit operations equipment based on the flowsheet needs. Each of the three flowsheets is highly developmental in nature, changing frequently, and would clearly need to undergo further pilot testing and scale-up to confirm these laboratory values and the suitability of specific process equipment. For this level of effort, the equipment selection and costs are probably a rough order of magnitude (ROM) estimate. No effort was made to get into specific design information and only rough sizing calculations were made based on the baseline 2000-head dairy.

Costs for individual pieces of capital equipment may be derived from several sources, including previous cost quotes, telephone estimates, estimates scaled from actual equipment costs, and equipment cost reference materials. The most accurate estimates are derived directly from vendor quotes, and thus most of the equipment costs were obtained by direct interaction with several vendors.

Table 4.3 presents the purchase cost for the major equipment and systems in the manure conversion plant in 2002 dollars. These costs are subdivided by process operation (i.e., Feed Preparation, Protein Extraction, Hydrolysis, etc.). As indicated in Table 4.3, equipment costs for

Table 4.3. Baseline flowsheet equipment list

System/Equipment	Purchase Cost ($K) *	Subtotal ($K)	Source for Cost Estimate
Feedstock Preparation		**141 (K$) SUBTOTAL**	
Tramp separator (10x10x4 concrete settling pit)	6		Richardson (1980)
Belt Filter	60		"starts at under $60K" @ http://www.alrickpress.com/products.html#Belt Feeder
Front-end Loader	75		Used - Cat966 or equal @ http://www.clevelandbrothers.com/used/eqpres1.cfm?prod_type=W
Caustic Washing		**398 (K$) SUBTOTAL**	
Caustic Wash Tank w/ agitator (7500 gal - SS or FRP	54		$15K (1967$) per Peters & Timmerhaus
Caustic Sludge Centrifuge - 10gpm, 25hp, 12"diameter Bird solid bowl, stainless	108		$30K (1967$) per Peters & Timmerhaus
Acid Neutralization Tank w/ agitator 3000 gal - SS or FRP	36		$10K (1967$) per Peters & Timmerhaus
Acid Neutralization Centrifuge 5 gpm, 15hp, 6" dia Bird solid bowl, stainless	90		$25K (1967$) per Peters & Timmerhaus
Spray Drier for Caustic Soluble Protein - 400 kg/hr evap – SS	100		$3K (1967$) per Peters & Timmerhaus for 10' diameter spray dryer. These costs could be radically higher if NIRO or other packaged unit is used.
Pumps for Caustic Washing and Neutralization	10		
Alcohol Washing		**398 (K$) SUBTOTAL**	
Alcohol Wash Tank w/ agitator 12,000 gal SS	72		$20K (1967$) per Peters & Timmerhaus
Alcohol Sludge Centrifuge - 10gpm, 25hp, 12"diameter Bird solid bowl, stainless	108		$30K (1967$) per Peters & Timmerhaus
Vacuum Drier - 1000 kg/hr EtOH evap - 15-20 sq m, SS	100		$4K (1967$) per Peters & Timmerhaus for 10' diameter spray dryer. These costs could be radically higher if NIRO or other packaged unit is used.
Vacuum Drier2 for solids - 1000 kg/hr EtOH evap - 15-20 sq m, SS	100		probably rotary needed here to handle solids.
Ethanol recovery condenser (1000kg/hr EtOH condensed) 500 sq ft CS U-tube	18		$5K (1967$) per Peters & Timmerhaus
Hydrolysis & Hydrotreating		**382 (K$) SUBTOTAL**	
Stage 1 Hydrolysis Tank w/ agitator (5000 gal - SS)	54		$15K (1967$) per Peters & Timmerhaus
Stage 1 Solids Centrifuge 5gpm 6" Bird SS solid bowl	90		$25K (1967$) per Peters & Timmerhaus
Stage 2 Hydrolysis Tank w/ agitator (5000 gal - SS)	54		$15K (1967$) per Peters & Timmerhaus
Stage 2 Solids Centrifuge 5gpm 6" Bird SS solid bowl	90		$25K (1967$) per Peters & Timmerhaus
Hydrotreater High Pressure	90		$25K (1967$) per Peters & Timmerhaus

Vessel (2167 L -500gal), 80 Atm, High Alloy		
High Pressure Feed Pump (10gpm SS, rotary, 80 Atm)	4	$500 (1967$) per Peters & Timmerhaus -may be higher due to harsh conditions
Offgas Condenser	10	
Hydrogen Recycle Compressor (200cfm, 3 stage, 3000 psi outlet pressure)	65	$18K (1967$) per Peters & Timmerhaus
Separation/Purification of Sorbitol		**220 (K$) SUBTOTAL**
UF Cartridge Filter System to Remove High MW Impurities (2-5gpm, SS)	20	SWAG
Ion Exchange Purification skid (2-5gpm flow, SS, with pumps and eluent controls)	100	SWAG
Crystallizer (Swenson-Walker type, SS, 50 ft length)	90	$25K (1967$) per Peters & Timmerhaus
Pumps for separation/purification	10	SWAG
Product Handling Systems		**87 (K$) SUBTOTAL**
Pneumatic Conveyance System (cooler, blowers, valves, hoppers)	20	Scaled to Inulin project estimate
Product Silos (3 at 300 cu ft), SS	32	$3K ea (1967$) per Peters & Timmerhaus
Super-sacking System (1000 lb/hr capacity)	25	Scaled to Inulin project estimate
Forklift	10	Used, market price
Total Purchased Capital Equipment Costs		**1626(K$)**

* Costs are estimated costs X 3.59 cost index multiplier (1967=109, 2002=394)

this evaluation were based on vendor budgetary quotes, cost data from Peters and Timmerhaus (1968), and from scaling costs from similar operations. Cost information from sources prior to 2002 were escalated to 2002 dollars using the Chemical Engineering Plant Cost Index (*Chemical Engineering* 2003). For the BASELINE flowsheet, the total purchased cost for the major equipment in the plant is estimated at $1.63 million.

For the SIMPLE flowsheet, the entire protein extraction area is removed, saving a substantial amount of washing and solvent recovery equipment. The total equipment costs for this option are less than half of the BASELINE flowsheet and are listed in Table 4.4.

Table 4.4. Simple flowsheet equipment list

System/Equipment	Purchase Cost ($K) *	Subtotal ($K)	Source for Cost Estimate
Feedstock Preparation		**141 (K$) SUBTOTAL**	
Tramp separator (10x10x4 concrete settling pit)	6		Richardson (1980)
Belt Filter	60		"starts at under $60K" @ http://www.alrickpress.com/products.html#Belt Feeder
Front-end Loader	75		Used - Cat966 or equal @ http://www.clevelandbrothers.com/used/eqpres1.cfm?prod_type=W
Hydrolysis & Hydrotreating		**382 (K$) SUBTOTAL**	
Stage 1 Hydrolysis Tank w/ agitator (5000 gal - SS)	54		$15K (1967$) per Peters & Timmerhaus
Stage 1 Solids Centrifuge 5gpm 6" Bird SS solid bowl	90		$25K (1967$) per Peters & Timmerhaus
Stage 2 Hydrolysis Tank w/ agitator (5000 gal - SS)	54		$15K (1967$) per Peters & Timmerhaus
Stage 2 Solids Centrifuge 5gpm 6" Bird SS solid bowl	90		$25K (1967$) per Peters & Timmerhaus
Hydrotreater High Pressure Vessel (2167 L -500gal), 80 Atm, High Alloy	90		$25K (1967$) per Peters & Timmerhaus
High Pressure Feed Pump (10gpm SS, rotary, 80 Atm)	4		$500 (1967$) per Peters & Timmerhaus-may be higher due to harsh conditions
Offgas Condenser	10		
Hydrogen Recycle Compressor (200cfm, 3 stage, 3000 psi outlet pressure)	65		$18K (1967$) per Peters & Timmerhaus
Separation/Purification of Sorbitol		**220 (K$) SUBTOTAL**	
UF Cartridge Filter System to Remove High MW Impurities (2-5gpm, SS)	20		SWAG
Ion Exchange Purification skid (2-5gpm flow, SS, with pumps and eluent controls)	100		SWAG
Crystallizer (Swenson-Walker type, SS, 50 ft length)	90		$25K (1967$) per Peters & Timmerhaus
Pumps for separation/purification	10		SWAG
Product Handling Systems		**87 (K$) SUBTOTAL**	
Pneumatic Conveyance System (cooler, blowers, valves, hoppers)	20		Scaled to Inulin project estimate
Product Silos (3 at 300 cu ft), SS	32		$3K ea (1967$) per Peters & Timmerhaus
Super-sacking System (1000 lb/hr capacity)	25		Scaled to Inulin project estimate
Forklift	10		Used, market price
Total Purchased Capital Equipment Costs		**830(K$)**	

* Costs are estimated costs X 3.59 cost index multiplier (1967=109, 2002=394)

In the DECRYSTAL flowsheet, the protein extraction section is also removed, but there is a substantial increase in capital equipment costs over the SIMPLE flowsheet due to the added drying capability, increasing complexity of the acid recycle system, and the ion exclusion chromatography system used to recover the sugars from the hydrolysis stream. The capital equipment costs for this flowsheet are summarized in Table 4.5.

Table 4.5. Decrystal Flowsheet Equipment List

System/Equipment	Purchase Cost ($K) *	Subtotal ($K)	Source for Cost Estimate
Feedstock Preparation		**501**	**(K$) SUBTOTAL**
Tramp separator (10x10x4 concrete settling pit)	6		Richardson (1980)
Belt Filter	60		"starts at under $60K" @ http://www.alrickpress.com/products.html#Belt Feeder
Manure Drier 1 - 6'dia x 40' long, 5MMBtu/hr, rotary kiln, w/ balls, 304SS, direct-fired	360		SEVERE SERVICE -THIS SEEMS LOW http://www.matche.com/EquipCost/Dryer.htm
Front-end Loader	75		Used - Cat966 or equal @ http://www.clevelandbrothers.com/used/eqpres1.cfm?prod_type=W
Hydrolysis & Hydrotreating		**1054**	**(K$) SUBTOTAL**
DECRYSTAL REACTOR "Holoflite Thermal Processor" 24"x 24' Severe service stainless	100		Used - Bepex TJ-24 or equal http://www.equipnetdirect.com/marketplace/browse/asset_view.asp?listid=10442&acro=MKT&custid=17
Stage 2 Hydrolysis Tank w/ agitator (5000 gal - SS)	54		$15K (1967$) per Peters & Timmerhaus
Stage 2 Solids Centrifuge 5gpm 6" Bird SS solid bowl	90		$25K (1967$) per Peters & Timmerhaus
Hydrolysate Accumulator Tank, 10,000 gal SS	54		$15K (1967$) per Peters & Timmerhaus
Acid Recovery Column,SS, packed column, with reboiler (50 gpm)	100		SWAG-this is a very large column - 25MMBtu/hr heat duty
Sediment Drier 2 - indirect fired rotary kiln	360		SEVERE SERVICE -THIS SEEMS LOW http://www.matche.com/EquipCost/Dryer.htm
Dilute Acid Accumulation Tank, 5000 gal SS	36		$10K (1967$) per Peters & Timmerhaus
Conc Acid Accumulation Tank, 5000 gal SS	36		$10K (1967$) per Peters & Timmerhaus
Simulated Moving Bed 2-4gpm	200		$1.2M for 40 gpm (inulin). Using 0.6 power rule, 2 gpm gives about $200K

Chromatography Separation Skid			
Ultra-filtration Skid, 1-2 gpm	20		SWAG
Activated Carbon Polishing Filter Skid,2-55gal in parallel, 1-2 gpm	10		SWAG
Hydrotreater High Pressure Vessel (2167 L -500gal), 80 Atm, High Alloy	90		$25K (1967$) per Peters & Timmerhaus
High Pressure Feed Pump (10gpm SS, rotary, 80 Atm)	4		$500 (1967$) per Peters & Timmerhaus -may be higher due to harsh conditions
Offgas Condenser	10		
Hydrogen Recycle Compressor (200cfm, 3 stage, 3000 psi outlet pressure)	65		$18K (1967$) per Peters & Timmerhaus
Separation/Purification of Sorbitol		**220**	**(K$) SUBTOTAL**
UF Cartridge Filter System to Remove High MW Impurities (2-5gpm, SS)	20		SWAG
Ion Exchange Purification skid (2-5gpm flow, SS, with pumps and eluent controls)	100		SWAG
Crystallizer (Swenson-Walker type, SS, 50 ft length)	90		$25K (1967$) per Peters & Timmerhaus
Pumps for separation/purification	10		SWAG
Product Handling Systems		**87**	**(K$) SUBTOTAL**
Pneumatic Conveyance System (cooler, blowers, valves, hoppers)	20		Scaled to Inulin project estimate
Product Silos (3 at 300 cu ft), SS	32		$3K ea (1967$) per Peters & Timmerhaus
Super-sacking System (1000 lb/hr capacity)	25		Scaled to Inulin project estimate
Forklift	10		Used, market price
Total Purchased Capital Equipment Costs		**1862**	**(K$)**

* Costs are estimated costs X 3.59 cost index multiplier (1967=109, 2002=394)

Capital costs

Total direct costs (TDC) are developed by scaling from the total purchased equipment costs. Other direct costs also include instrumentation and controls, installed piping, installed electrical, the cost of buildings, site preparation, and the cost of land. Table 4.6 lists the TDC for the manure conversion plant BASELINE flowsheet. Peters and Timmerhaus (1968) summarize typical values for both direct and indirect costs of solid processing plants, of solid-fluid processing plants, and of fluid processing plants. Since this processing plant is a solid-fluid plant, these percentage costs were developed by interpolating closely around the solid-fluid costs. The ratios for both the direct and indirect costs are based on standard estimates for a grassroots plant at an undeveloped site. Since such a plant would presumably be sited on an existing dairy, with substantial rural land, some allowances can be made for land costs.

Table 4.6. Total Direct Costs (TDC) for BASELINE flow

Item	% of Purchased Equipment Cost (Typical Ranges)	% of Purchased Equipment Costs for Manure Process	Cost, $K
Direct Costs			
Purchased Equipment (delivered)	100	100	1626
Equipment Installation	25 to 55	45	732
Insulation (installed)	0 to 9	5	81
Instrumentation and Controls (installed)	6 to 30	13	211
Piping (installed)	16 to 66	30	488
Electrical (installed)	10 to 11	9	146
Buildings (including services)	45 to 68	47	764
Site Preparation, Yard Improvements	10 to 13	13	211
Services/Support Facilities (utilities and distributions systems, waste management, communications)	30 to 80	55	894
Land Costs	4 to 8	4	65
Total Direct Costs (TDC)		**321**	**5219**

TDC are $5.2M for the BASELINE flowsheet, $2.66M for the SIMPLE flowsheet, and $5.97M for the DECRYSTAL flowsheet.

Indirect costs typically include the cost of engineering and construction expenses that cannot be directly tied to specific pieces of equipment or bricks and mortar. Again, they are estimated as a percentage of the total direct costs as outlined in Peters and Timmerhaus (1968) for solid-liquid processing plants. Estimates of these percentages were compiled from a study of fixed capital investment (FCI) from over 100 chemical process projects, many at the 95% confidence level.

Indirect costs are $0.94M for the BASELINE flowsheet (Table 4.7.), $0.48M for the SIMPLE flowsheet, and $1.08M for the DECRYSTAL flowsheet.

Table 4.7. Indirect costs in BASELINE flow

Indirect Costs (IC)	Basis Used for Analysis	Percentage of Purchased Equip	$K
Engineering	8% of TDC	26%	418
Construction Expenses	10% of TDC	32%	522
Total Indircet Costs (TIC)			**940**

Although technically considered part of the indirect costs, the contractors fee, contingency, and startup expenses are calculated after the direct and indirect plant costs are totaled (Table 4.8). Typical contractor fees vary widely, depending on the complexity of the job. A mid-range contractor fee of 5% was assumed. Approximately $600K of the FCI has been allocated as contingency funds for unpredicted occurrences, errors in estimates, and design changes. Also, an additional $600K has been added to the FCI to account for startup expenses (i.e., staff training, initial reduced capacity throughput, process modifications, and optimization). The manure conversion plant will be the first of its kind in the U.S., and ample allocations for both contingency and startup are warranted.

The Total Direct and Indirect Costs (TDC+TIC) is $6.16M for the BASELINE flowsheet (Table 4.8), $3.14M for the SIMPLE flowsheet, and $7.05M for the DECRYSTAL flowsheet.

The FCI for the BASELINE flowsheet was determined by summing the direct costs, indirect costs, contractor fee, contingency, and startup expenses as percentages of the total purchase cost for the major equipment (Table 4.8). The TDCs include equipment, equipment installation, insulation, instrumentation, controls, piping, electrical, site preparation, buildings,

service/support facilities, and land. Indirect costs include engineering, supervision, and construction expenses for building the plant.

Table 4.8. Other costs for BASELINE flow

Total Direct and Indirect Costs (TDC + TIC)	from Table 4.6 and 4.7	**379%**	**6159**
Contractors Fee (typical range is 2% to 8% of TDC + TIC)	5% of TDC + TIC	19%	308
Contingency (typical range is 5% to 15% of TDC + TIC)	10% of TDC + TIC	38%	616
Startup Expenses (typical range is 8% to 10% of FCI)	10% of TDC + TIC	38%	616
Total (FCI)			**7699**

Table 4.6 provides the various elements of the fixed costs, including information on the ranges of typical ratios for new plants on undeveloped sites. The ratios selected for the manure conversion plant fall into the mid-range of the typical ratio ranges. In general, the selected values line up with the typical ratios used for a solid-fluid processing plant, as compared to the ratios for a solid-processing or a fluid-processing plant (Peters and Timmerhaus 1968). Use of the ratios in Table 4.6 results in a total FCI of $7.7 million, which is 473% of the purchased cost of the equipment for the plant.

The most significant items contributing to the FCI are the direct purchase equipment costs, equipment installation, buildings, service and support facilities, contingency, and startup expenses. Included within the combined factor ($900K) for buildings, and service/support facilities are the costs of all building, including plumbing, heating, lighting and ventilation. Service and support facilities include the facilities and services for steam generation and distribution, water and wastewater treatment and distribution, electrical substations and distribution, natural gas supply and distribution, waste disposal, communications systems, manure storage, product storage, fire protection systems, and safety installations.

The TCI in Table 4.9 includes the FCI plus the working capital. Working capital is the money invested in raw materials, product in inventory, accounts receivable, cash on hand for operating expenses (i.e., salaries, supplies), accounts payable, and taxes. For plants that operate year-round, the working capital is typically equivalent to the production costs for 1 month of

operations. Peters and Timmerhaus (1968) state the initial working capital required for most chemical plants ranges from 10% to 20% of the TCI. Therefore, for this analysis, it has been assumed that required working capital will be about 15% of the TCI.

Table 4.9. Fixed Capital and Total Capital Investment for the BASELINE flow

Fixed Capital Investment (FCI)		**473%**	**7699**
Working Capital (typical range is 10% to 20% of TCI)	15% of FCI	71%	1155
Total Capital Investment (TCI)		**544%**	**8854**

The total capital investment for the BASELINE flowsheet plant is $8.85M, for the SIMPLE flowsheet plant is $4.52M, and for the DECRYSTAL flowsheet plant is $10.14M

Operating costs

Operating costs for the manure protein recovery plant include the cost of the raw material (manure) and other required chemicals, utilities (steam, gas, electricity, fuel, and water), operating labor, supervision and support labor, special maintenance/replacement items, general maintenance and supplies, taxes, and plant/equipment depreciation. The annual operating costs for producing proteins and chemicals from manures are estimated to be $2.67 million for the baseline case, a 24-hour-per-day, 365 day-per-year operating schedule, with an assumed operating efficiency of 90%. Table 4.10 lists the components that comprise the operating costs.

Table 4.10. Operating costs for BASELINE flow

Raw Materials	602
Utilities	51
Operating Labor	560
Special Maintenance Items	10
Other Overheads & Directs	1736
Annual Operating Costs (AOC)	**2959**

Raw materials cost is a significant fraction of the annual operating costs of the plant. The primary raw materials are manure and hydrogen. Some ethanol, caustic, and sulfuric acid are used during the extraction process, but their costs are negligible.

At a dairy size of 2000 cows and the operating efficiency of 90%, approximately 96 MT of manure slurry will be processed per day. The baseline assumption is that manure costs will be zero. While it is unlikely that the dairyman will be "paid" a tipping fee to dispose of the manure, tax credits tied to quantities of manure processed may make this cost slightly negative.

Hydrogen costs are a fairly significant item in the annual operating costs. This analysis assumes that the plant will be sited within pipeline distance of a source of relatively inexpensive hydrogen, such as an oil refinery with excess hydrogen, a methanol plant, an ammonia fertilizer plant, or any other natural gas reforming plant. Per Chemical Marketing Reporter's Feb. 24, 2003, "Highlight Report on Hydrogen," pipeline hydrogen costs $0.18-0.80 per 100 SCF, while cryogenic liquid hydrogen costs $1.15-1.80 per 100 SCF. These costs calculate to $ 0.06-0.28 per SCM by pipeline or $0.50-0.78 per SCM for cryogenic liquid hydrogen. This plant would not be cost effective if required to operate on cryogenic liquid hydrogen.

The plant utility requirements were estimated based on vendor projections and energy balance calculations. The "per-unit" utility costs were based on local (Benton and Franklin counties) utility rates for industrial customers. Annual utility costs were estimated to be just $50-75K per year (~2% of the total operating cost) because of the low heat requirements and the probable heat recovery.

For determining the operating labor requirements, the number of operations staff (operators and supervisors) required during production was estimated by examining the staffing needed per shift for each major piece of equipment. It is estimated that one working supervisor and three operators will be required to run the plant. For the baseline operating case of 24-hour operation, it was assumed that the plant would be staffed using four rotating shifts. Unburdened labor rates for operators ($15/h) and supervisors ($25/h) were based on review of "1998 Area Wage Survey for Kennewick, Richland, and Pasco, WA" (provided by the Tri-City Industrial Development Council). Annual labor costs (unburdened), associated with production, were estimated to be $560K per year (19% of total operating costs).

Along with the staff associated with production, additional management, clerical, maintenance, and other facilities support staff are required. The costs associated with these administrative and support staff are estimated to be 15% ($360K per year) of the operating labor (Peters and Timmerhaus 1968).

Items within the flowsheet were called out as requiring routine replacement. The primary maintenance item will be periodic replacement of the catalyst. For this review, replacement of 1000 kg of catalyst per year at $50/kg is estimated. Depending on the specific catalyst used, this may be low.

In the process industries, plant maintenance is typically assumed to be about 6% of the FCI (Peters and Timmerhaus 1968). This calculates to $460K.

The plant overhead costs ($442K) account for about 16% of the annual operating costs. The plant overhead costs are closely tied to the labor costs, and include employee benefits and general plant services.

To write off the FCI as a production expense, the equipment, buildings and inventory are typically depreciated. For the operating cost analysis, the FCI was depreciated at 7% per year, which corresponds to a 15-year straight-line depreciation. At $539K, depreciation amounts to about 20% of the annual operating costs. There are other methods for analyzing capital recovery and interest expenses. The technique used, along with the interest rate/depreciation rate selected, will impact the overall economic analysis. For example, if an analysis were to be performed with a 10% interest rate, and interest payments were to be made on the TCI (FCI + working capital), the annual cost for associated with this item would be $885K (0.1 x $8.85 million).

The DECRYSTAL flowsheet has a slightly different operating cost due to the increased drying capacity, which was reflected in the higher labor and overheads. The operating costs for this flowsheet are $3.3M.

Baseline case economics

Using the BASELINE flowsheet and the initial product projections, the baseline case economic analysis appears to be poor. Operating costs exceeded revenues by nearly $1.5M for a dismal ROI of -17%. Table 4.11 summarizes the results of the BASELINE case analysis.

Because the anticipated performance of this flowsheet was poor, the SIMPLE flowsheet, which eliminated the protein extraction steps and focused solely on the production of sorbitol, was developed. The SIMPLE flowsheet economic summary is shown in Table 4.12. Despite having decreased the capital and operating costs, the loss of the extracted protein revenue was sufficient to decrease the ROI to an even more dismal –26%. Clearly, another strategy was in order to

- increase the amount and value of products recovered,
- increase the conversion of sugars in the hydrolysis, or
- decrease the annual operating costs.

The final DECRYSTAL flowsheet was developed primarily on WSU's experimental data that showed additional sugars could be produced during the hydrolysis step. The second stage hydrolysis reportedly went from 1-2% conversion to nearly 25%. This apparently is due to better breakdown of the cellulose structure of the manure fibers. Results are shown in Table 4.13.

Even though the value of the products increased markedly, because of the increased capital costs associated with drying, acid recycle, and chromatography, the ROI still did not break even at this low plant capacity. A plant size over 5000 head is required to break even.

As a final thought, we also evaluated the potential differences by simply buying glucose directly from the open market (or contracts) and converting this raw material directly to sorbitol. Glucose costs are relatively inexpensive in today's market -- typically $0.10-0.12/lb ($220-264/MT). For a stand-alone plant similar in size to a 2000-head manure plant, costs to purchase 2500 MT of glucose annually would be approximately $600K per year. This would increase annual operating costs to about $2.7M on revenues of $4.0M. Assuming that the low cost capital expenditure ($4.5M) is similar, an ROI of about 28% could be expected. A breakeven sorbitol price is calculated at approximately $1.20/kg.

Table 4.11. BASELINE case economic assessment

Total Direct Costs (TDC)		5219
Purchased Equipment	1626	
Equipment Installation	732	
Insulation	81	
Instrumentation & Controls	211	
Piping (installed)	488	
Electrical (installed)	146	
Buildings (including services)	764	
Site Preparation	211	
Improvements	894	
Land Costs	65	
Total Indirect Costs (TIC) - Engineering and Construction		940
Subtotal Direct and Indirect Costs (TDC + TIC)		**6159**
Contractor's Fee		308
Contingency		616
Startup Expenses		616
Fixed Capital Investment (FCI)		**7699**
Working Capital		1155
Total Capital Investment (TCI)		**8854**
Raw Materials		57
Utilities		50
Operating Labor		560
Special Maintenance Items		165
Other Overheads & Directs		1,827
Annual Operating Costs (AOC)		**2659**
Product Revenues (PV)		1127
Annual Rate of Return (PV-AOC)/TCI		**-17%**

Table 4.12. SIMPLE case economic assessment

		2664
Purchased Equipment	830	
Equipment Installation	374	
Insulation	42	
Instrumentation & Controls	108	
Piping (installed)	249	
Electrical (installed)	75	
Buildings (including services)	390	
Site Preparation	108	
Improvements	457	
Land Costs	33	
		480
Subtotal Direct and Indirect Costs (TDC + TIC)		**3144**
Contractor's Fee		157
Contingency		314
Startup Expenses		314
Fixed Capital Investment (FCI)		**3930**
Working Capital		589
Total Capital Investment (TCI)		**4519**
Raw Materials		106
Utilities		48
Operating Labor		560
Special Maintenance Items		166
Other Overheads & Directs		1,100
Annual Operating Costs (AOC)		**1980**
Product Revenues (PV)		787
Annual Rate of Return (PV-AOC)/TCI		**-26%**

Table 4.13. DECRYSTAL case economic assessment

Total Direct Costs (TDC)		**5977**
Purchased Equipment	1862	
Equipment Installation	838	
Insulation	93	
Instrumentation & Controls	242	
Piping (installed)	559	
Electrical (installed)	168	
Buildings (including services)	875	
Site Preparation	242	
Improvements	1024	
Land Costs	74	
Total Indirect Costs (TIC) - Engineering and Construction		**1076**
Engineering		478
Construction Expenses		598
Subtotal Direct and Indirect Costs (TDC + TIC)		**7053**
Contractor's Fee		353
Contingency		705
Startup Expenses		705
Fixed Capital Investment (FCI)		**8816**
Working Capital		1322
Total Capital Investment (TCI)		**10139**
Raw Materials		117
Utilities		408
Operating Labor		560
Special Maintenance Items		170
Other Overheads & Directs		2,043
Annual Operating Costs (AOC)		**3299**
Product Revenues (PV)		1,243
Annual Rate of Return (PV-AOC)/TCI		**-20%**

Sensitivity Analysis

A sensitivity analysis was conducted to evaluate the effect of plant size, manure cost, and product value on the profitability of a DECRYSTAL plant. A summary (Table 4.14) of the base-case economics for a 2000-head dairy shows that a DECRYSTAL plant for conversion to value-

added polyols cannot be expected to generate a positive ROI. If an allowance is made for the cost of manure disposal (Table 4.15), increased product values (Table 4.16), or a large plant size Tables 4.17-4.20), higher returns are possible; however, very few scenarios (essentially only at the largest dairy size analyzed) under the current flowsheet and product slates can be expected to generate a positive ROI. Clearly, a size some where between 5000 and 10,000 head is required for the economics of the processing concept to be interesting. At the 2000-head size, even a manure disposal credit of $20/ton would not make the process economical, as the cost to produce sorbitol would still be $3.43 (Table 4.17) versus the market price of $1.20. The cost of production drops under $1.20 only in the case of the 10,000 head dairy and a zero cost manure, see Table 4.20 and Figure 4.7 (a tipping fee for manure removal would lower the cost still further). Figure 4.8 similarly shows the sensitivity of sorbitol production costs relative to plant size with three different manure feedstock costs applied.

Table 4.14. Effect of Dairy Size on Rate of Return

	Dairy Size (Number of Head)				
Parameter	**1000**	**2000**	**3000**	**5000**	**10000**
Manure Slurry, metric tons per yr	15,798	31,596	47,394	118,484	592,420
Sorbitol Product (MT/yr)	388	777	1,165	1,942	3,885
Operating Efficiency, %	90	90	90	90	90
Production Campaign, days	365	365	365	365	365
Fixed Capital Investment (FCI), $K	5,816	8,816	11,244	15,277	23,156
Total Capital Investment (TCI), $K (FCI + working capital)	6,689	10,139	12,931	17,569	26,629
Annual Operating Costs (AOC), $K	2,872	3,299	3,578	3,963	4,552
Annual Product Value (PV) $K	622	1,243	1,865	3,108	6,216
Sorbitol Production Cost ($/kg)	$ 7.39	$ 4.25	$ 3.07	$ 2.04	$ 1.17
Annual Rate of Return on TCI, (PV-AOC/TCI)	-34%	-20%	-13%	-5%	6%

Table 4.15. Effect of Cost of Manure on Rate of Return[(a)]

			Baseline				
Cost of Manure Slurry, $/ton	10.00	5.00	-	(5.00)	(10.00)	(15.00)	(20.00)
Fixed Capital Investment (FCI), $K	8816	8816	8816	8816	8816	8816	8816
Total Capital Investment (TCI), $K (FCI + working capital)	10139	10139	10139	10139	10139	10139	10139
Annual Operating Costs (AOC), $K	3615	3457	3299	3141	2983	2825	2667
Annual Product Value (PV) $M	1,243	1,243	1,243	1,243	1,243	1,243	1,243
Annual Rate of Return on TCI, (PV-AOC/TCI)	-23.39%	-21.84%	-20.28%	-18.72%	-17.16%	-15.60%	-14.04%

(a) 2000 cow dairy, 90% onstream, 365-day operation, baseline product prices

Table 4.16. Value of Products $/kg [(a)]

	Baseline	10%	20%	30%	40%	50%
Caustic-soluble proteins ($/kg)	$ -	$ -	$ -	$ -	$ -	$ -
Alcohol-soluble proteins ($/kg)	$ -	$ -	$ -	$ -	$ -	$ -
Sorbitol ($/kg)	$ 1.60	$ 1.76	$ 1.92	$ 2.08	$ 2.24	$ 2.40
Total Capital Investment (TCI), $K (FCI + working capital)	10139	10139	10139	10139	10139	10139
Annual Operating Costs (AOC), $K	3299	3299	3299	3299	3299	3299
Annual Product Value (PV) $K	1,243	1368	1492	1616	1740	1865
Annual Rate of Return on TCI, (PV-AOC/TCI)	-20.28%	-19.05%	-17.82%	-16.60%	-15.37%	-14.15%

[a] 2000 cow dairy, 90% onstream, 365-day operation, baseline case

Table 4.17. Cost to Produce Sorbitol vs Manure Cost (2000 head)

			Baseline				
Cost of Manure Slurry, $/ton	$10.00	$ 5.00	$ -	$(5.00)	$(10.00)	$(15.00)	$ (20.00)
Fixed Capital Investment (FCI), $K	8816	8816	8816	8816	8816	8816	8816
Total Capital Investment (TCI), $K (FCI + working capital)	10139	10139	10139	10139	10139	10139	10139
Annual Operating Costs (AOC), $K	3615	3457	3299	3141	2983	2825	2667
Annual Product Value (PV) $M	1,243	1,243	1,243	1,243	1,243	1,243	1,243
Cost to Produce Sorbitol ($/kg)	$ 4.65	$ 4.45	$ 4.25	$ 4.04	$ 3.84	$ 3.64	$ 3.43

(a) 2000 cow dairy, 90% onstream, 365-day operation, baseline product prices

Table 4.18. Cost to Produce Sorbitol vs Manure Cost (3000 head)

			Baseline				
Cost of Manure Slurry, $/ton	$10.00	$ 5.00	$ -	$(5.00)	$(10.00)	$(15.00)	$(20.00)
Fixed Capital Investment (FCI), $K	11244	11244	11244	11244	11244	11244	11244
Total Capital Investment (TCI), $K (FCI + working capital)	12931	12931	12931	12931	12931	12931	12931
Annual Operating Costs (AOC), $K	4052	3815	3578	3341	3104	2867	2630
Annual Product Value (PV) $M	1,865	1,865	1,865	1,865	1,865	1,865	1,865
Cost to Produce Sorbitol ($/kg)	$ 3.48	$ 3.27	$ 3.07	$ 2.87	$ 2.66	$ 2.46	$ 2.26

(b) 3000 cow dairy, 90% onstream, 365-day operation, baseline product prices

Table 4.19. Cost to Produce Sorbitol vs Manure Cost (5000 head)

			Baseline				
Cost of Manure Slurry, $/ton	$10.00	$ 5.00	$ -	$ (5.00)	$(10.00)	$(15.00)	$(20.00)
Fixed Capital Investment (FCI), $K	15277	15277	15277	15277	15277	15277	15277
Total Capital Investment (TCI), $K (FCI + working capital)	17569	17569	17569	17569	17569	17569	17569
Annual Operating Costs (AOC), $K	4436	4199	3963	3726	3489	3252	3015
Annual Product Value (PV) $M	3,108	3,108	3,108	3,108	3,108	3,108	3,108
Cost to Produce Sorbitol ($/kg)	$ 2.28	$ 2.16	$ 2.04	$ 1.92	$ 1.80	$ 1.67	$ 1.55

(b) 3000 cow dairy, 90% onstream, 365-day operation, baseline product prices

Table 4.20. Cost to Produce Sorbitol vs Manure Cost (10,000 head)

			Baseline				
Cost of Manure Slurry, $/ton	$ 10.00	$ 5.00	$ -	$ (5.00)	$(10.00)	$(15.00)	$(20.00)
Fixed Capital Investment (FCI), $K	23156	23156	23156	23156	23156	23156	23156
Total Capital Investment (TCI), $K (FCI + working capital)	26629	26629	26629	26629	26629	26629	26629
Annual Operating Costs (AOC), $K	5026	4789	4552	4315	4078	3841	3604
Annual Product Value (PV) $M	6,216	6,216	6,216	6,216	6,216	6,216	6,216
Cost to Produce Sorbitol ($/kg)	$ 1.29	$ 1.23	$ 1.17	$ 1.11	$ 1.05	$ 0.99	$ 0.93

(b) 10,000 cow dairy, 90% onstream, 365-day operation, baseline product prices

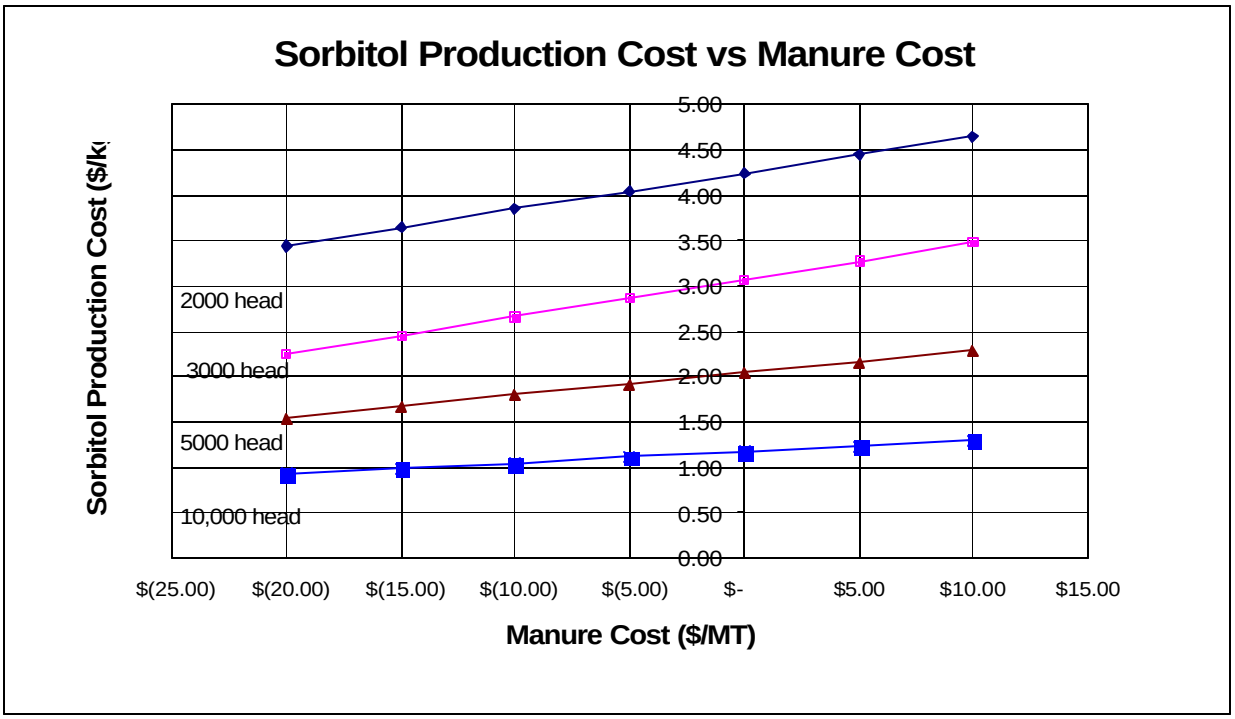

Figure 4.7. Sorbitol cost sensitivity relative to manure cost

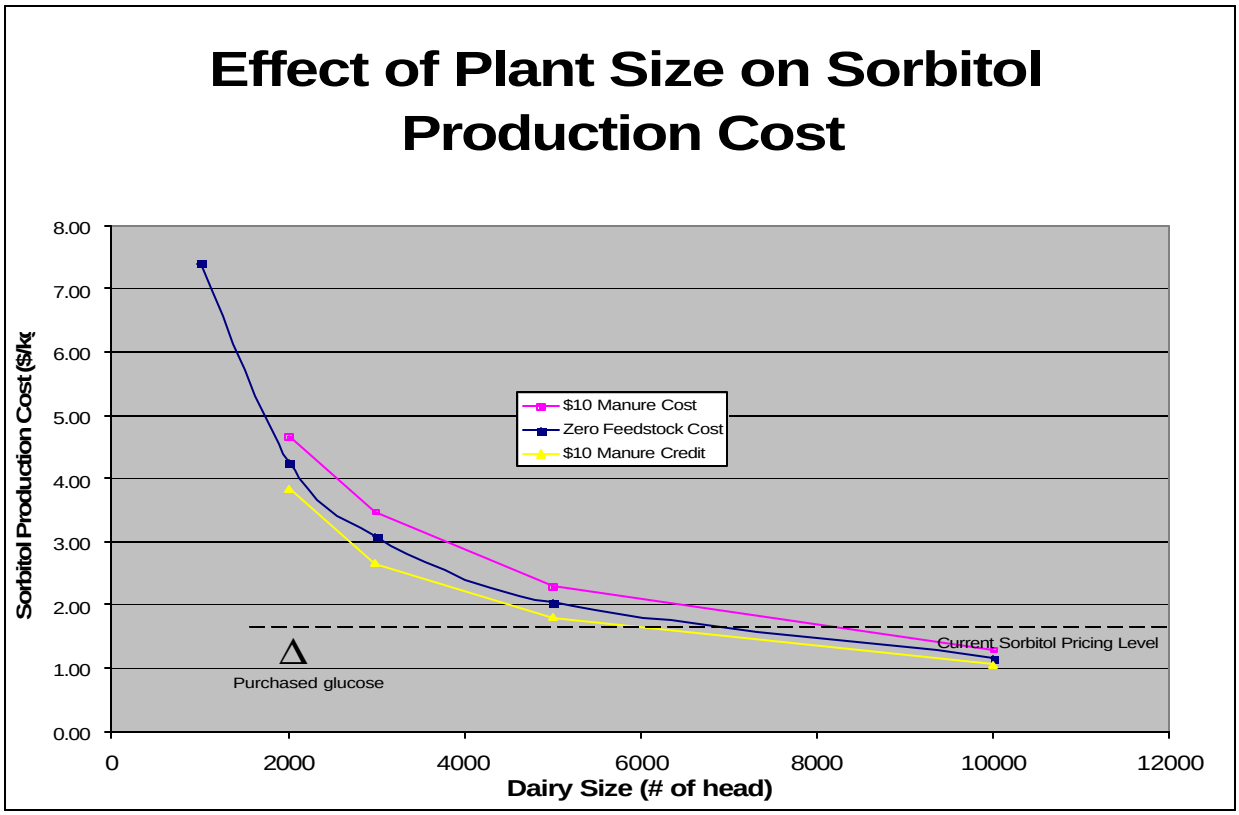

Figure 4.8. Production cost sensitivity versus plant size

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Conclusions

Collecting manure in order to use its carbon would be extremely beneficial in emissions reduction, odor reduction, and natural resource savings, especially in the case of dairy cattle. The advantage of using cattle manure for biomass conversion is in taking the carbon excreted from the cow and directly transferring it to useful carbon in industrial chemicals. This not only prevents the carbon in manure from escaping into the atmosphere, but also eliminates the need for the petroleum typically used to develop the chemical. By using manure to displace petroleum, emissions and energy are being saved from two separate sources.

The energy required to process the manure for conversion to industrial chemicals is still a cost. With proper process development, the energy saved should still exceed the energy used. Either way, there will be a positive savings of carbon emissions.

We have found that the protein contents of poultry manures were the highest among the manure types analyzed, accounting for 1/3 of the dry matter. Swine manures had about 1/4 of dry matter as protein. While protein contributed to no more than 1/5 of dry matter in cattle manures. On the contrary, the fiber (including hemicellulose, cellulose, and lignin) content in cattle manures was the highest, accounting for more than 50% of the dry matter. Total fiber in swine and poultry manures was less than 40% of dry matter. Because of the high content of fiber and low concentration of protein, cattle manure is most suitable for monosugars production. However, the utilization of animal manures is more difficult than the utilization of other lignocellulotic biomass such as wood and straw, because of the complicated composition and high protein content.

The results from the study of different hydrolysis of manure indicate that acid hydrolysis with decrystallization is the best one among all of procedures studied in the project. Decrystallization with 75% acid concentration, 3:5 sample to acid ratio, and 30 minutes of reaction time, followed by dilute acid hydrolysis with 12.5% acid and 10% sample at 130°C for 10 minutes were the optimal conditions producing 26 g/L glucose at a conversion rate of 84% and 11 g/L xylose at a conversion rate of 80%. Compared to acid hydrolysis of woods, manure has much higher nitrogen content, which makes some side-reactions such as browning reaction, and dehydration, happen much more easily. Thus, pretreatment of manure is a critical step in order to obtain higher sugar yield. In this particular case, acid hydrolysis with decrystallization

of pretreated dairy manure had a sugar yield (84% of theoretical) very close to the hydrolysis of clean wood chips (85% of theoretical).

For enzymatic hydrolysis, some treatments are necessary to improve sugar production. The highest sugar yield was from enzymatic hydrolysis of manure fiber without hemicellulose and lignin. The optimal conditions were 650 FPU/L cellulase, 250 IU/L β-glucosidase, and 50 g/L substrate at pH 4.8 and 46°C. The glucose yield under the optimal conditions reached 52%, which was more than half of the yield of acid hydrolysis with decrystallization. Although the yield of enzymatic hydrolysis was not as high as acid hydrolysis with decrystallization, it is still a promising method for producing sugars from lignocellulosic materials, since enzymatic hydrolysis is performed at moderate conditions and is environmentally friendly. Moreover, the comparison also indicates that decrystallization is the critical step in trying to improve glucose yield. This suggests that finding a non-acid decrystallization method may help enzymatic hydrolysis in effectively increasing sugar yield.

The present work showed that dairy manure was a suitable substrate for cellulase production by *T. reesei.* The optimal culture conditions were determined as follows: solid fraction of pre-treated manure as substrate medium containing 10 g/L manure (dry basis), 2 g/L KH_2PO_4, 2mg/L $CoCl_2$, and 2 g/L tween-80; and initial medium pH of 5.7 and temperature of 25.5 ^oC. The filter paper activity under these conditions achieved 1.72 IU/mL, which is much higher than results obtained using other lignocellulosics residues. However, β-glucosidase level produced by the fungi is still very low.

Model compound testing was conducted in batch a reactor to evaluate the effects of trace contaminant components on catalytic hydrogenation of sugars. Trace components are potential catalyst poisons when processing biomass feedstocks to value-added chemical products. Trace components included inorganic elements such as alkali metals and alkaline earths, phosphorus, sulfur, aluminum, silicon, chloride, or transition metals. Protein components in manure feedstocks can lead to formation of peptide fractions (from hydrolysis) or ammonium ions (from more severe breakdown), both of which might interfere with catalysis. The batch reactor tests with model compounds were performed in a 300-mL stirred autoclave, with multiple liquid samples withdrawn over the period of the experiment. Evaluation of these test results suggests that most of the catalyst inhibition is related to nitrogen-containing components, primarily the peptides. Additional tests with manure hydrolysates confirmed these results. Attempts to clean

the hydrolysate feedstocks with ultrafiltration and carbon bed adsorption have shown value in these methods, though they could not completely eliminate the inhibition of the catalytic process. A longer term test o f the catalytic hydrogenation in a continuous flow reactor was terminated after only a few days due to loss of catalytic activity. Both mineral deposits and sulfiding of the catalyst were found.

Three specific flowsheets have been developed to convert dairy manure to valuable proteins and chemicals. In the BASELINE flowsheet, soluble proteins are extracted from the manure solids prior to dilute acid hydrolysis and conversion of the glucose to sorbitol. In the SIMPLE flowsheet, all manure solids are hydrolyzed directly in dilute acid to glucose and then converted to sorbitol. Finally, in the DECRYSTAL flowsheet, the second hydrolysis step is carried out by concentrated sulfuric acid to enhance the glucose yield prior to sorbitol production.

These flowsheets were subjected to standard chemical engineering design practice cost estimation techniques to develop a rough economic feasibility of this process.

In all three flowsheets, the Total Capital Investments (TCI) range from $4.52M to $10.1M. Annual operating costs vary only slightly, from $2M to $3.3M, primarily labor and plant overheads. The value of the products varies widely, and depends highly on the conversion and separations efficiencies, and the assumptions made for protein value.

In all cases that we examined, no case could be found that resulted in a positive return on investment, except those based on the largest dairy herd size of 10,000 head. The probable reasons for this may be

- poor conversion of manure solids to glucose
- complicated separation processes
- high labor costs relative to products produced

If manure conversion is to be profitable, large collections of manure must be available for processing at low cost. Otherwise, specific high-value products must be identified, or an artificially high negative cost (tipping fee) must be placed on the manure, or both.

Suggestions for Future Studies

Future effort should be focusedon the development of a complete process for manure utilization, including solid-liquid separation, continuous decrystallization, enzyme hydrolysis of the decrystallized manure fiber and hydrogenation. As presented in this report, fibers from animal manure (primarily diary manure) can be separated first, then fed into a reactive extruder for continuous radical reaction so that the fiber will be decrystallized. The treated fiber can be used in enzyme hydrolysis to convert the fiber into simple sugars, which will be further processed through a hydrogenation process to obtain the final products such as glycols, polyols and diols etc.

The system should be tested at a bench-scale to provide design parameters for further pilot testing and commercialization to produce high value products. Specific project objectives should include:

(1) Developing a solid-liquid separation process to remove all non-fiber substances in animal manure,
(2) Determining the best radical reagents for fiber decrystallization,
(3) Developing a bench scale continuous decrystallization and hydrolysis process,
(4) Refining the process of sugar separation for hydrogenation,
(5) Improving the catalyst stability for the hydrogenation process, and
(6) Performing an economic analysis of the entire process.

Publications and Presentations from the Project

Journal papers

Elliott, D.C.; Peterson, K.L.; Muzatko, D.S.; Alderson, E.V.; Hart, T.R.; Neuenschwander, G.G.. "Effects of Trace Contaminants on Catalytic Processing of Biomass-Derived Sugar Feedstocks." *Applied Biochemistry and Biotechnology*, in press.

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Liao, W.; Liu, Y.; Liu, C.; Chen, S. "Optimizing Dilute Acid Hydrolysis of Hemicellulose in a Nitrogen-Rich Cellulosic Material – Dairy Manure." *Bioresource Technology*, submitted.

Chen, S., W. Liao, C. Liu, R. L., Kinkaid and J. H., Harrison. Use of animal manure as feedstock for bioproducts. in Proceedings of the 9th International Symposium on Animal, Agricultural, and Food Processing Wastes. pp 44-49. ASAE, St. Joseph, MI.

Presentations

Elliott, D.C.; Peterson, K.L.; Muzatko, D.S.; Alderson, E.V.; Hart, T.R.; Neuenschwander, G.G 2003. "Effects of Trace Contaminants on Catalytic Processing of Biomass-Derived Sugar Feedstocks." presented at the **25th Biotechnology for Fuels and Chemicals Symposium**, Breckinridge, CO, May 19, 2003.

Liao, W.; Chen, S.; Kincaid, R.L.; Harrison, J.H. "Value-Added Chemicals from Animal Manure." **The Tenth Biennial Bioenergy Conference, Bioenergy 2002**, Boise, Idaho, September 22-26, 2002.

Liao, W.; Wen, Z.; Liu, C.; Chen, S. "Comparison of Hydrolysis Processes for Dairy Manure Conversion." **ASAE 2003**. Las Vegas, Nevada, July 27-30, 2003.

Liu, C.; Kincaid, R.; Harrison, J.; Liao, W.; Wen, Z.; Chen, S. "Characterization of Animal Manures for Biorefinery," **ASAE 2003**, Las Vegas, Nevada, July 27-30, 2003.

Inventions

Elliott, D.C.; Peterson, K.L. "Catalytic Isomerization of Sugars." PNNL File # 14029-E