0273-2289/99/77–79/0455/$14.50

Bioconversion of Mixed Solids Waste to Ethanol

Quang A. Nguyen,*[,1] Fred A. Keller,[1] Melvin P. Tucker,[1] Charles K. Lombard,[2] Bryan M. Jenkins,[3] David E. Yomogida,[3] and Valentino M. Tiangco[4]

[1]National Renewable Energy Laboratory, 1617 Cole Blvd., Golden, CO 80401, E-mail: quang_nguyen@nrel.gov; [2]Waste Energy Integrated Systems, Palo Alto, CA; [3]University of California, Biological and Agricultural Engineering, Davis, CA; and [4]California Energy Commission, Sacramento, CA

Abstract

A mixed solids waste (MSW) feedstock, comprising construction lumber waste (35% oven-dry basis), almond tree prunings (20%), wheat straw (20%), office waste paper (12.5%), and newsprint (12.5%), was converted to ethanol via dilute-acid pretreatment followed by enzymatic hydrolysis and yeast fermentation. The MSW was pretreated with dilute sulfuric acid (0.4% w/w) at 210°C for 3 min in a 4-L steam explosion reactor, then washed with water to recover the solubilized hemicellulose. The digestibility of water-washed, pretreated MSW was 90% in batch enzymatic hydrolysis at 66 FPU/g cellulose. Using an enzyme-recycle bioreactor system, greater than 90% cellulose hydrolysis was achieved at a net enzyme loading of about 10 FPU/g cellulose. Enzyme recycling using membrane filtration and a fed-batch fermentation technique is a promising option for significantly reducing the cost of enzyme in cellulose hydrolysis. The hexose sugars were readily fermentable using a *Saccharomyces cerevisiae* yeast strain that was adapted to the hydrolysate. Solid residue after enzyme digestion was subjected to various furnace experiments designed to assess the fouling and slagging characteristics. Results of these analyses suggest the residue to be of a low to moderate slagging and fouling type if burned by itself.

Index Entries: Biomass; ethanol; pretreatment; MSW; bioconversion; hydrolysis; enzyme recycle.

Introduction

California produces abundant lignocellulosic biomass, more than 32 million bone-dry tons (BDT)/year, from agricultural and forestry residues,

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

urban wood wastes, yard wastes, food-processing wastes, chaparral, and lumber-mill wastes. The state's biomass power industry has provided some avenues for productive use of these resources as boiler fuel, about 4 million BDT/year. The future of the biomass power industry is uncertain at present because of a myriad of disposal problems and the uncertainties of electricity deregulation. The vast quantities of biomass residues might be available for conversion to ethanol and alleviate part of the waste disposal problem at the same time. San Joaquin County generates about 350,000 tons (dry weight basis) of biomass residues annually. The California Energy Commission (CEC) and San Joaquin County are actively seeking viable technologies (including bioethanol production) to use these wastes to reduce landfill requirements and provide economic opportunities in the county.

Waste Energy Integrated System (WEIS; Palo Alto, CA), the CEC, and the National Renewable Energy Laboratory (NREL) formed a partnership via the Sustainable Technology Energy Partnerships Program to conduct a feasibility study on the conversion of biomass residues to ethanol. This paper focuses on two key issues: potential ethanol yield and combustion characteristics of the ligneous residue. Specifically, the study examines the potential of lowering the cost of enzymatic hydrolysis through enzyme recycle.

Enzymatic hydrolysis has been recognized as one of the most promising processes for converting cellulosic biomass to fermentable sugar. The enzyme-based conversion of lignocellulosic biomass into ethanol consists of four major steps:

1. Pretreat biomass feedstock to hydrolyze the hemicellulose and to make the residual cellulose accessible to enzyme.
2. Enzymatically hydrolyze the cellulose to glucose.
3. Ferment sugars released in steps (1) and (2) to ethanol. Steps (2) and (3) can be combined in a single bioreactor, an operation referred to as simultaneous saccharification and fermentation (SSF).
4. Recover the ethanol.

Dilute-acid pretreatment has been recognized as one of the most effective pretreatment methods, as it generally leads to high hemicellulose recovery and high enzyme digestibility. The main focus of this study is to generate pretreated substrate that is highly digestible for testing the enzyme recycle bioreactor system. Optimization of hemicellulose recovery will be examined in future studies.

Capital and operating costs associated with enzyme production and enzymatic hydrolysis contribute to a significant portion of the total ethanol-production cost *(1–3)*. One method of lowering this cost is to keep the enzyme loading (usually expressed as FPU/g cellulose) low. However, this strategy has two major disadvantages. The low enzyme loadings make cellulose hydrolysis the rate-limiting step, which tends to increase the size and costs of the hydrolysis reactors. Also, at low enzyme concentrations,

Table 1
Gross Composition of Mixed Solids Waste (MSW)

Biomass	Weight % (dry basis)	Preparation
Fir	35.0	Chipped from 2 × 2 fir strips using a small chipper and knife-milled through 3/8-in screen
Almond tree prunings	20.0	Hammer-milled through a 1-in screen then knife-milled through a 3/8-in screen
Wheat straw	20.0	Hammer-milled through a 1-in screen then knife-milled through a 3/8-in screen
Office waste paper	12.5	Shredded then knife-milled through a 3/8-in screen
Newsprint	12.5	Shredded then knife-milled through a 3/8-in screen

cellulase activity is further reduced by the inhibitory hydrolysis products, cellobiose and glucose. One method of minimizing enzyme cost is to achieve more effective use through enzyme recycle *(4–12)*. This work examines the possibility of retaining the hydrolyzing enzyme in the hydrolysis bioreactor, effectively using it over and over on freshly fed biomass, while removing the inhibitory products. Because its use is extended over potentially large quantities of biomass, the effective enzyme loading is greatly reduced. Progress has been made during the past several years reducing both the capital and operating costs of using cross-flow ultra filtration (UF). We assembled an enzyme-recycle bioreactor system that employs membrane filters for separating liquid hydrolysate from insoluble solids and recovering enzyme from the liquid fraction.

The ligneous residue obtained after enzymatic hydrolysis can be used as fuel for power and steam production via thermal conversion including combustion and gasification. The behaviors of the fuel and transformations among inorganic constituents at elevated temperatures are of particular importance for this type of residue utilization. This study examines composition, ash fusibility, and fouling and slagging characteristics.

Materials and Methods

Feedstock Preparation

A synthetic mixed solids waste (MSW) feedstock was prepared using five different biomass materials chosen to simulate the lignocellulosic component of municipal solid waste stream in San Joaquin County, CA. The percentage of individual components in the mixed feedstock is shown in Table 1.

Each feedstock component was milled separately. The 2 × 2 fir (supplier was unable to furnish species information, possibly Douglas fir) furring strips were obtained at a local lumber yard and chipped using a small garden chipper/shredder (Amerind-MacKissic, model Mighty Mac, Parker Ford, PA) equipped with a 1-in (25-mm) rejection screen. The wheat straw was obtained at a local agricultural feed store and was shredded using the garden chipper/shredder with a 1-in rejection screen. The office waste paper and newsprint were obtained locally and shredded using an office paper shredder (Fellowes, model Powershred 1216N, Sanford, NC). The almond tree prunings were obtained from an orchard in California and shredded with the garden chipper/shredder equipped with a 1-in rejection screen. Subsequently, all five of the chipped or shredded biomass feedstocks were then individually processed through a Mitts and Merrill rotary knife mill (now Reduction Technology, Inc., model 10 × 12, Leeds, AL) equipped with a 3/8-in (9.5-mm) rejection screen. The knife mill was thoroughly cleaned before processing the next feedstock.

A 100-kg batch of mixed feedstock was prepared from the stocks of individual biomass feedstocks prepared, by mixing in the proper proportions (dry basis) the various feedstocks, followed by coning/quartering blending a total of four times. The blended feedstocks were stored sealed in polyethylene lined 55-gal drums at –20°C.

Pretreatment

Acid Impregnation

Acid impregnation was carried out by soaking approximately 10 kg of the MSW in 110 L of 0.4% H_2SO_4 at 60°C for 3 h. The excess acid in the wetted feedstock was allowed to drain overnight at room temperature. The acid-soaked MSW was air-dried to approximately 55% solids, then weighed into sealed polyethylene bags before pretreatment.

Steam Pretreatment

All pretreatment experiments were performed using the 4-L NREL steam explosion reactor. The reactor is equipped with a steam jacket, external electrical heaters, a 4-in (10-cm) top and 2-in (5-cm) bottom ball valve, two direct steam-injection ports near the top and bottom, and K-type thermocouples inserted near the top and bottom of the reactor used for temperature measurements. The reactor was made of Hastelloy C-22™ to resist corrosion. The reaction temperature was controlled at or near the desired value by using a pressure control valve to control the steam-supply pressure. Before each pretreatment experiment, the reactor was preheated to near the desired operating temperature by admitting steam into the jacket and cycling steam repeatedly through the reactor. A batch of preweighed acid-soaked MSW (approximately 500–750 g) was loaded into the reactor, and saturated steam was then admitted (defined as time zero). After a predetermined cooking time, the steam was shut off, then the contents of the reactor (pretreated solids, condensate, and steam) were dis-

charged into a flash tank where some of the condensate flashed off into a condenser and the pretreated material collected in the bottom of the tank. The pretreated MSW was blended, then stored in polyethylene containers at 4°C. Samples of the pretreated material were processed into liquor samples (obtained by pressing the liquid from the wet samples) and water-insoluble samples (obtained by extensively washing the solids in the samples) for chemical analyses.

Batch Enzymatic Hydrolysis

A large batch (15.1 kg @ 25.8% total solids) of pretreated MSW was extensively washed with hot water (until the pH of the filtrate was 4.5), then enzymatically hydrolyzed to generate ligneous solid residue for combustion characterization. Pretreated MSW was slurried in 60°C water to approximately 6 wt% consistency. After letting the slurry settle for several hours in a 55-gal plastic drum, the mixture was pumped into a 24-in (61-cm) vacuum Buchner filter fitted with a muslin cloth liner to collect the washed solids. The cake was re-slurried in hot water then filtered. This process was repeated five times until the pH measured higher than 5.0. After the final washing, the washed solids (10.8 kg @ 25.1% total solids) were transferred to a 190-L fermentor. Citrate buffer (pH 4.8) was added to a final volume of 100 L. The temperature of the vessel and slurry (approximately 2.7% total solids) was raised to 121°C for 1 h, then cooled to 50°C. Approximately 1.16 L of filter-sterilized (filtered through a 120-mm × 0.45-µm Millipore filter) cellulase enzyme (Iogen, Ottawa, ON, Canada) was added to obtain approximately 66 FPU/g cellulose. To control bacterial contamination, a solution containing 1.5 g tetracycline (Sigma Chemicals, St. Louis, MO) in 100 mL of 70% ethanol was added through a sterilized port using a 0.2-µm sterile filter and syringe. The vessel was stirred at 150 rpm and the temperature controlled between 49° and 50°C for the 7-d digestion. Samples were taken aseptically during the digestion. At the end of the hydrolysis, the digested solids were pumped into an 18-in (45.7-cm) vacuum Buchner filter fitted with a muslin liner, and the solid residue was washed five times with hot water and then air-dried before thermal characterization.

Fed-Batch Enzymatic Hydrolysis with Enzyme Recycle

Figure 1 shows a simplified block flow diagram of the bioconversion of MSW to ethanol. The enzymatic hydrolysis system consists a Braun-UD 50-L bioreactor (B-Braun Biotech International, Melsungen, Germany) used as the feed vessel, a Braun-UD 20-L hydrolysis bioreactor, and a Niro UF/RO Pilot Plant (Niro Hudson, Inc., Hudson, WI). The Niro UF/RO Pilot Plant is equipped with a feed pump and a recirculation pump for use as either micro-filtration (MF) or UF or reverse osmosis (RO), but not with more than one filter at the same time. The system was modified to allow two types of filters (MF and UF) to be used simultaneously. A surge tank, placed on load cells to facilitate mass-balance calculations, was added to collect the perme-

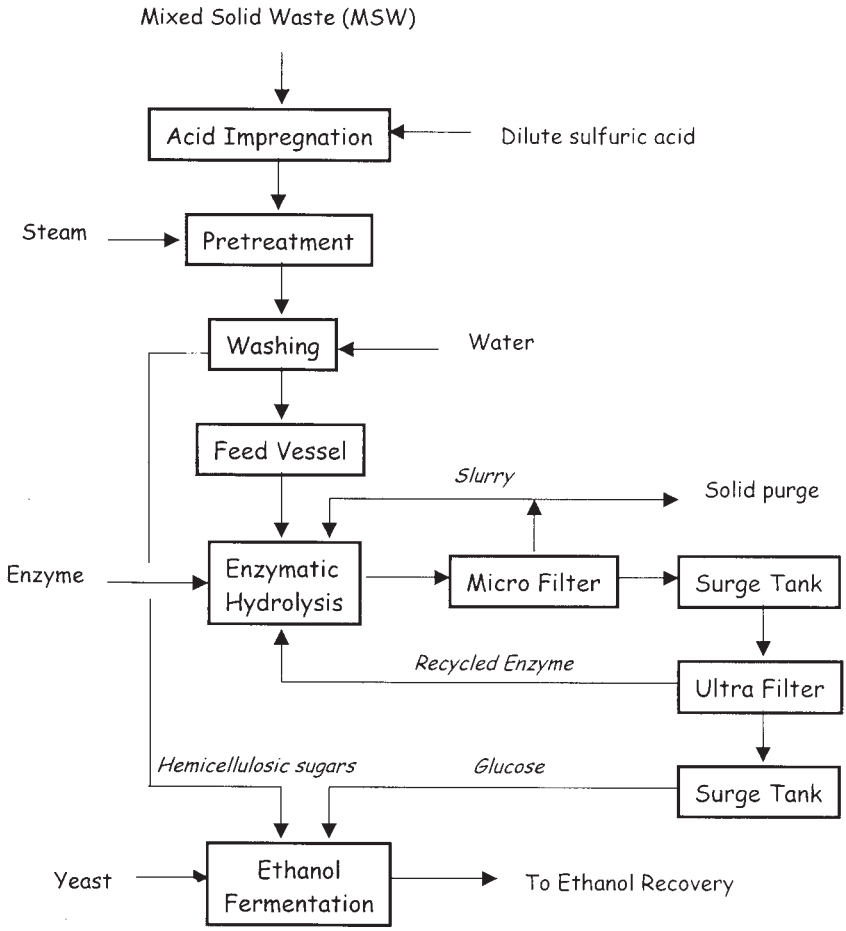

Fig. 1. Block flow diagram of ethanol production from MSW.

ate (which contains both enzyme and glucose) from the MF unit. The MF permeate was pushed through the UF membrane using air pressure. The MF membrane is a single U.S. Filter #P19-40E85A, 0.2-μm ceramic membrane microfilter (U.S. Filter, Warrendale, PA). It has a surface area of 0.2 m^2. The UF membrane is a spiral wound, Desal UF#PW3838C (Desalination Systems, Inc., Escondido, CA), 48-mil spacing polyether sulfone element with MW cut off of 10,000–30,000, and a surface area of 4.9 m^2. The area of MF membrane proved to be undersized for this setup. The UF membrane was selected to retain the cellulase enzymes, which have molecular weights of about 56,000. An Allen-Bradley PLC (Allen-Bradley Co./Rockwell Automation, Milwaukee, WI) displayed flow, pressure, and temperature data on a monitor. The Rockwell WINtelligent LINX software (Rockwell Software, West Allis, WI) was used to record these data. Before pretreated MSW was added to the bioreactors, the system was steam-sterilized. The pumps, filters, and associated piping of the Niro pilot plant were sterilized chemically.

As a precaution to prevent blockage of the narrow channels in the MF cartridge, the water-washed, pretreated MSW was blended, using a 1-gallon commercial blender (Hamilton Beach/Proctor-Silex, Inc., model 990, Washington, NC) and sieved through a 1.6-mm mesh stainless screen to remove oversized particles. The blended material was then slurried (10 wt% solids) with 0.05 *M* sodium citrate buffer, pH adjusted to 4.5 with phosphoric acid, and sterilized in the feed vessel. The hydrolysis vessel was filled with the pH-adjusted slurry, steam-sterilized, then diluted to about 4 wt%, before being circulated through the membrane system. The lower solid concentration was used to maintain adequate flux through the MF membrane. During the course of the 12-d hydrolysis, the slurry was periodically pumped from the feed vessel, via a recirculating loop, into the hydrolysis vessel to maintain a constant working level. The total biomass (dry weight basis) fed into the hydrolysis vessel was 6.7 times the starting amount. Cellulase enzyme (Iogen) was added into the hydrolysis bioreactor at the beginning of the experiment to achieve an initial enzyme loading of 55 FPU/g cellulose. No fresh enzyme was added afterward. The temperature of the feed vessel and the hydrolysis bioreactor was maintained at 45°C. Slurry from the hydrolysis bioreactor was fed to the Niro pilot plant via a recirculation loop to maintain uniform temperature and solids concentration in the feed. The hydrolysis slurry was first fed to the MF unit, with back-pulse, semicontinuously, generating a clear MF permeate, which was collected in the first surge tank. The retentate, which contains the suspended solids, was returned to the hydrolysis vessel. At least once each day, the Niro system was started up, recirculating the hydrolysis slurry. Over a few hours, 10 kg of MF permeate was collected. Air pressure was used to push the MF permeate through the UF membrane, returning the enzyme-containing retentate to the hydrolysis vessel, and generating UF permeate as glucose syrup. The syrup product was collected in a sterile surge tank. At night, recirculation through the MF unit was controlled at a reduced differential pressure (3–4 psi) to maintain the temperature in the whole system but not to generate any significant amount of permeate. This continuous recirculation also minimized fouling of the MF membrane.

Yeast Fermentation

A *Saccharomyces cerevisiae* strain, which was adapted to Douglas fir prehydrolysate over a few months, was adapted to MSW prehydrolysate over only a couple of weeks. The prehydrolysate liquor was prepared by adding deionized water to pretreated MSW to make up 23 wt% slurry, then filtered through a Buchner filter. Fermentation tests on the filtrate were carried out in DeLong flasks (Bellco, Vineland, NJ) with stainless steel Morton closures. The flasks were placed in an orbital shaker set at 30°C and 150 rpm. The pH of the liquor was adjusted to pH 5.0 before adding 10–20% yeast inoculum and nutrients. Samples were withdrawn from the flasks at predetermined time intervals and analyzed for sugar and ethanol content.

Chemical Analyses

Analysis of Feedstock and Pretreated Solids

Dry weights (by oven-drying at 105°C to constant weight) *(13)* and Klason acid-soluble and acid-insoluble lignin were determined by standard methods *(14)*. Anhydrosugars in the whole wood and pretreated solids were determined by a procedure slightly modified from that developed at the U.S. Forest Products Laboratory *(15,16)*. Ash in the wood and pretreated solid residues were analyzed by standard gravimetric methods *(17)*.

Analysis of Liquor

Organic acids, glycerol, hydroxymethyl furfural (HMF), and furfural in the filtrate and rinsate fractions were determined by high-performance liquid chromatography (HPLC) using Bio-Rad Aminex HPX-87H columns (Bio-Rad Laboratories, Hercules, CA) *(15,16,18)*. Monomeric sugars were determined by HPLC using Bio-Rad Aminex HPX-87P columns *(15,16,18)*. Subsequent to the HPLC analysis, the oligomeric sugars in the liquors and rinsate fractions were converted to monomers using 4% H_2SO_4 hydrolysis at 121°C for 1 h, and the monomeric sugars analyzed using the Bio-Rad HPX-87P column and corrected for sugar losses using sugar recovery standards *(16,18)*.

Thermal Characterization of Ligneous Solid Residue

Composition, Density, and Heating Value

The enzyme-digested residue was analyzed for composition, including moisture, ash, volatile matter, fixed carbon, and concentrations of major elements. Bulk density and particle-size distribution were also determined. Moisture content was determined via air oven method (ASTM E871) on previously air-dried material. Ash content was determined by igniting the sample in air while heating to 575°C in a muffle furnace, and holding for 2 h (ASTM D1102). Volatile matter, which is indicative of the fraction weight loss during pyrolysis, was determined by heating the sample at 950°C for 7 min under a nonoxidizing atmosphere in accordance with ASTM E872. Fixed carbon was determined by the difference on dry matter and the sum of ash and volatile matter.

Bulk density was determined using a modified drop test (based on ASTM E873) using a 300 mL sample volume. As-received material, which was in the form of a slurry, was air-dried and carefully broken up by mortar and pestle to separate individual particles, then placed in the sample beaker. Initial density was measured, and the sample repeatedly dropped from a height of 10 cm onto a cushioned surface. Additional material was added as needed to maintain original volume. The test was continued to constant density. Particle-size distribution was determined on separated material by sieve analysis (ASAE S319).

Constant volume higher heating value was determined via adiabatic oxygen bomb calorimeter, following ASTM E711/D2015. Analyses of ulti-

mate composition, elemental ash, and water-soluble alkali were conducted by Hazen Research Incorporated, Golden, CO. The elemental ash analysis was conducted after ashing at 600°C.

Ash Volatility

Ashing tests were conducted to determine the fractional amounts of ash volatilized at temperatures of 750°, 900°, and 970°C (maximum furnace temperature for this test) relative to ash at 575°C. The fraction of volatile ash is partially indicative of the fouling potential of the residue; that is, the greater the fractional ash volatilization, the greater the perceived fouling potential at the specified temperature because of the presence of volatile inorganic species available for deposition on heat-transfer equipment. Dry fuel samples were placed in an air atmosphere muffle furnace and slowly heated at a rate of approximately 15°C/min to 575°C, held for 2 h, cooled to ambient, and weighed to determine ash fraction. The samples were then heated repeatedly using the same procedure but at temperatures of 750°, then 900°, and finally 970°C, recording the fraction of ash remaining at each temperature.

Ash Fusibility

Ash fusibility tests were conducted at temperatures ranging from 800°–1600°C in increments of 50°C to describe the fusibility characteristics of the ash. The test used *(19)* has been developed recently in preference to the ASTM pyrometric cone test (ASTM D1857/E953), which uses calcined ash rather than dry fuel as starting material. Approximately 0.7–1 g of air-dried residue was pelletized, weighed, and placed on an alumina support. The pellet and support were placed in a Kanthal EPD High Temperature Melting Furnace (Kanthal Corporation, Bethel, CT) preheated to a specified temperature between 800° and 1600°C in an air atmosphere. After 20 min, the support and residue were removed from the furnace and cooled to ambient temperature. The condition of the pellet was then rated and recorded. Loose samples of residue (rather then pelletized) were heated at temperatures of 1400°–1600°C to improve possibly the detection of light-particle sintering. Results of ash fusibility and compositional analyses were compared against predictions of ash melting from phase diagrams of the alkali-alumina-silicate system, which comprises the bulk of the inorganic fraction of the residue.

Results and Discussion

Pretreatment

Feedstock Composition

The synthetic MSW feedstock was prepared by blending five different biomass materials, as shown in Table 1. The chemical composition of the blended mixture is shown in Table 2. Based on this composition and assuming that all hexose and pentose sugars are fermented, the theoretical ethanol yield would be approximately 95 gal per oven-dry ton of feedstock.

Table 2
Chemical Composition of Mixed Solids Waste (in wt% Oven-Dry Basis)

Material	G	X	GA	A	M	Lignin	Ash	Unidentified
MSW	41.7	13.3	0.8	1.8	5.3	24.2	4.8	8.1

G, glucan; X, xylan; GA, galactan; A, arabinan; M, mannan.
Note: Lignin = acid insoluble lignin + acid soluble lignin.

Table 3
Chemical Analysis of Liquid Fraction of Pretreated MSW (g/L)

Sample	CEL	G	X	GA	A	M	AC	HMF	FL
As is	1.4	19.7	16.5	5.0	3.5	10.5	5.7	3.6	3.3
After 4% acid hydrolysis	0.0	23.4	17.5	6.0	3.4	10.1	N/M	N/M	N/M

CEL, cellobiose; G, glucose; X, xylose; GA, galactose; A, arabinose; M, mannose; AC, acetic acid; HMF, hydroxymethyl furfural; FL, furfural; N/M, not measured.

Dilute-Acid Pretreatment

The MSW was pretreated at 210°C for 185 s using the 4-L NREL steam-explosion reactor. The pretreated material was analyzed for chemical composition, and evaluated for enzyme digestibility and fermentability. The compositions of liquor and water-washed solids of the pretreated material are shown in Tables 3 and 4. More than 80% of the sugars in the prehydrolysate are in monomeric form and essentially all the hemicellulose was solubilized. However, recoveries in the prehydrolysate were low: 12.7% for glucose, 54.3% for mannose, 52.8% for arabinose, and 34.6% for xylose. No attempt was made to optimize the hemicellulosic sugar recovery.

Dilute-acid pretreatment of MSW solubilizes essentially all the hemicellulose; however, it is difficult to optimize single-stage pretreatment of feedstock such as MSW that contains a mixture of various biomass components. Effective pretreatment of softwood hemicellulosic components require conditions that are severe compared to conditions for effective solubilization of the xylan in wheat straw. As a result, approximately 10% of the xylan was converted to furfural. High concentrations of HMF also indicate that a considerable amount of mannose was destroyed at the pretreatment conditions used in this study.

Batch Enzymatic Hydrolysis

A batch of pretreated material (composition shown in Tables 3 and 4) was extensively washed with 40–50°C water until the pH of the filtrate measured approximately 5.0. The water-insoluble solids were digested with enzyme (using a loading of 66 FPU/g cellulose) in a 190-L fermentor at 50°C for 7 d. Glucose analysis of samples taken during the hydrolysis indicated that hydrolysis of cellulose was essentially completed after 5 d,

Table 4
Composition of Pretreated MSW After Water Wash (% Oven-Dry Weight)

G	X	GA	A	M	Lignin	Ash	Unidentified
54.5	0.4	0	0.1	0.5	37.9	3.8	2.8

G, glucan; X, xylan; GA, galactan; A, arabinan; M, mannan.
Note: Lignin = Klason acid insoluble lignin + acid soluble lignin.

Table 5
Composition of Enzyme Digested Solids (% Oven-Dry Weight)

G	X	GA	A	M	Lignin	Ash	Unidentified
15.6	0.8	0.3	0	1.4	72.9	4.8	4.2

G, glucan; X, xylan; GA, galactan; A, arabinan; M, mannan.
Note: Lignin = Klason acid insoluble lignin + acid soluble lignin.

and the cellulose digestibility based on glucose released was approximately 90% of theoretical. The final glucose concentration was 15.7 g/L. The digested mixture was washed with water and the composition of the residual solids was analyzed (Table 5). This ligneous residue was further analyzed for thermal characteristics.

Fed-Batch Enzymatic Hydrolysis with Enzyme Recycle

Because of the narrow MF channels, pretreated MSW was bridged at the entrance of the membrane cartridge. As a result, the permeate flux through the membrane dropped from 30 to as low as 5 kg/h.m^2, as shown in Fig. 2. Back pulsing only helped minimize blockage of the membrane and not the feed-distribution manifold at the inlet of the membrane housing. After about 150 h, the MF distribution manifold was manually cleaned, and the flux was restored to 20 kg/h.m^2. During the 300-h operation of the hydrolysis system, 6.7 times the biomass that was charged initially to the hydrolysis vessel was fed. The average purge rate (including sampling) was 500 g of slurry/d at 3–5 wt% solid concentration. The enzyme entrained in the purge stream was not recovered. The cumulative total solids in the hydrolysis vessel was 19 wt%. This fed-batch mode reduced the effective enzyme loading from 55.1 to 12.7 FPU/g cellulose at 130 h, and to 8.2 FPU/g cellulose after all the prepared biomass was fed into the hydrolysis reactor at 240 h. The high initial enzyme loading resulted in excellent glucose hydrolysis yield of 98–100% at 60 h after the initial hydrolysis began (Fig. 3). And again, the hydrolysis yield of glucose was more than 90% theoretical at 108 h. The high glucose yield and hydrolysis rate would probably have been repeated if it were not for contamination, which developed after losing temperature control at about 170 h. The temperature of the feed vessel, lines, and double diaphragm pump dropped to 30°C, probably pulling in nonsterile air. The contamination was cured by raising the temperature of

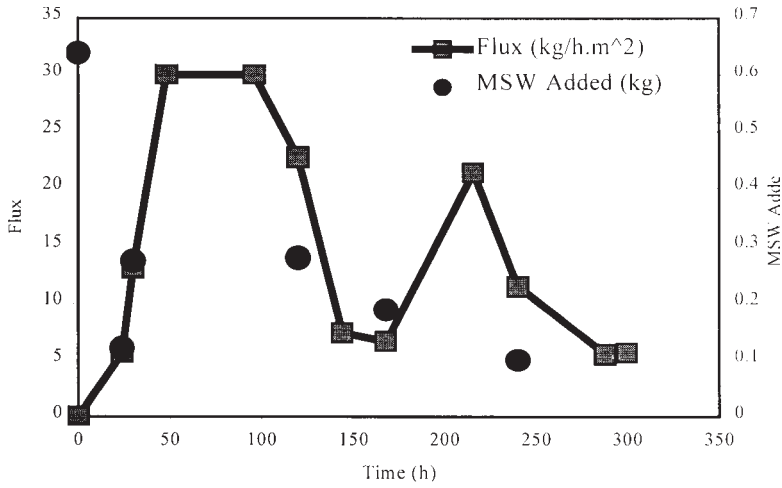

Fig. 2. Flux through Micro Filter.

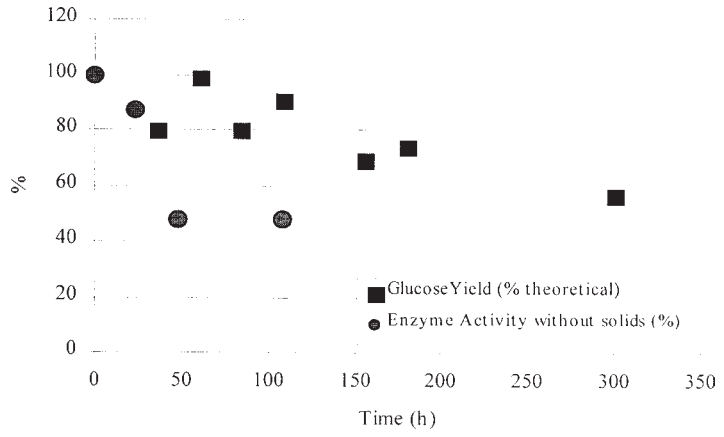

Fig. 3. Glucose yield from pretreated MSW in fed-batch system.

the hydrolysis bioreactor from 45° to 55°C. During this period, the thermostat leaked in air, which resulted in a temperature excursion up to 62°C over 2.5 h, before it could be brought under control. The enzyme half-life at 62°C is most probably very short *(20)*. Coupled with the shear and air-liquid interfacial energy, the enzyme probably denatured as noted by a significant drop in cellulase activity (to about 0.05 FPU/mL or about 5% of the initial activity) in the MF permeate and reduction in glucose yield to 74% shortly afterward.

An experiment was carried out to determine the effect of shear and air-liquid interfacial energy on the cellulase activity at hydrolysis conditions but without the protective biomass. Fresh enzyme in pH 5.0 buffer solution

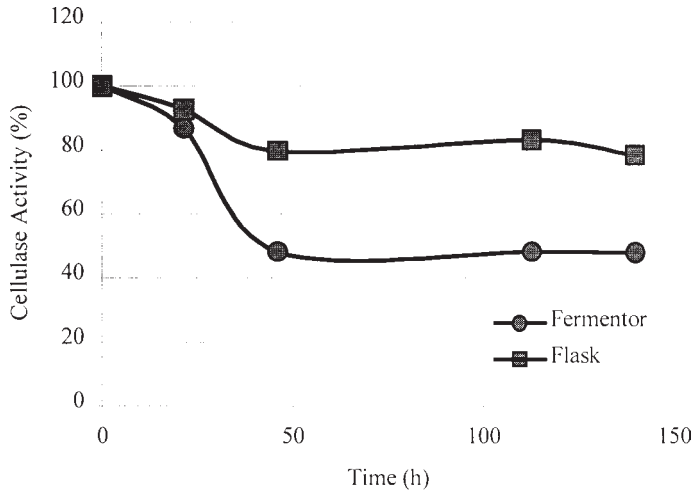

Fig. 4. Enzyme deactivation in shake flask and enzyme recycle fermentor. (Note: Agitation speed: 150 rpm for shake flask and 400 rpm for fermentor).

was placed in the 20-L fermentor (set at 400 rpm and 55°C) and circulated through the MF membrane. Filter-paper assays showed the enzyme activity dropped to 50% of the original activity in 48 h and appeared to level off afterward. A control, baffled shake flask at 150 rpm and 56°C lost 10% activity in the first 20 h, then maintained 80% activity through 134 h.

A similar baffled flask at 60°C, lost 82% of its initial activity in the first 20 h. The shake-flask results show reasonable shear and air-liquid interfacial area stability, but considerable temperature sensitivity above 55°C. The large drop in enzyme activity without biomass in the membrane bioreactor system is probably owing to both shear and air-liquid interface energy deactivation *(21–23)*. The large difference in enzyme deactivation between the enzyme recycle fermentor system and shake flask (Fig. 4) indicates the need for improvement in the design and operation of the bioreactor and enzyme-recycle system to minimize deactivation of enzyme. One design *(24)* employs intermittent mixing of the substrate slurry in a plug-flow reactor to reduce residence time and overall mixing shear that the enzyme is subjected to.

Ethanol Fermentation

An *S. cerevisiae* strain, which was adapted to Douglas fir prehydrolysate over a few months, was adapted to MSW prehydrolysate over a period of 2 wk. Because of the short adaptation period, a fairly high level of nutrients (1% yeast extract and 1.5% corn-steep liquor) was used to obtain an ethanol yield of 80% theoretical and 0.8 g/L-h productivity. It is expected that with further adaptation of the yeast, higher ethanol yield and lower nutrient requirement can be achieved.

Table 6
Properties of Residue

Parameter	Average value	Number of samples for average	Range		Standard deviation
			High	Low	
Moisture[a] (%)	6.10	12	6.21	5.60	0.06
Ash (% db)	4.93	12	5.57	4.80	0.21
Volatile matter (% db)	66.00	4	66.31	65.80	0.26
Fixed carbon (% db)	29.00	NA[b]	NA[b]	NA[b]	NA[b]
Bulk density (kg m^{-3})	370.00	1	NA	NA	NA
HHV (MJ/kg) (db)	24.40	2	24.46	24.30	0.13

[a]Residue air-dried.
[b]NA, not applicable. Value was calculated from average values of volatile matter and ash.

Thermal Characterization

Properties of Residue

Table 6 gives the proximate analysis and heating values for the enzyme-digested residue. Table 7 gives the ultimate analysis, elemental composition of ash, and alkali index. Approximately 5% of residue was determined to be ash, and nearly 56% of the ash was comprised of silica, which tends to have a relatively high melting point (the melting point of pure silica is above 1700°C). Heating values have been correlated with ash content *(25,26)* and tend to follow an inverse trend with respect to ash content. The heating value, 24.4 MJ/kg (10,500 Btu/lb), is high compared to wood (typically 20 MJ/kg) or straw (15–16 MJ/kg) *(27)*. For each 1% increase in ash, biomass heating value has been observed to decline on average by approximately 0.2 MJ/kg from 20 MJ/kg *(25)*. However, lignin has a heating value (26.7 MJ/kg) that is greater than that of cellulose (17.3 MJ/kg). With a composition of 72.9% lignin, 4.9% ash, and 22.2% carbohydrate, the projected heating value is 23.3 MJ/kg (based on composition alone), which compares reasonably well with the experimental value of 24.4 MJ/kg.

Residue bulk density increased from its initial loose density of 310 kg/m^3 to 370 kg/m^3 after final settling (160 drops). Table 8 lists the particle-size distribution of residue. About 24% of the residue passed 200 mesh, which consists of a fine powder that is easily entrained and may lead to fugitive dust emissions in handling.

Ash Volatilization

Table 9 lists the ash-volatilization results for the residue. Samples show a decline in ash content with increasing temperature, suggesting a volatilization loss of alkali and other species (possibly including carbonates and water of hydration). Approximately 4.4% of the ash was volatilized when ash was heated to 970°C under oxidizing conditions. Although the trend is

Table 7
Elemental Analysis of Ash,
and Alkali Index of Residue

Ultimate analysis of residue (% dry matter)	
C	61.06
H	5.49
N	1.00
S	0.14
Cl[a]	0.02
Ash	5.02
O (by difference)	27.29
Elemental analysis of ash (%)[b]	
SiO_2	56.10
Al_2O_3	23.76
TiO_2	1.20
Fe_2O_3	2.01
CaO	3.62
MgO	1.75
Na_2O	2.93
K_2O	1.39
P_2O_5	0.23
SO_3	1.36
Cl	0.05
CO_2	0.32
Total	94.72
Water-soluble alkali (% dry matter)	
Na_2O	0.075
K_2O	0.004
Alkali index (kg/GJ)	0.090

[a]Cl usually not reported in ultimate analysis.
[b]Ashed at 600°C prior to analysis.

Table 8
Particle-Size Distribution of Residue

Particle size retained	Mass fraction (%)
>10 mesh	0.0
20 mesh	24.3
40 mesh	21.5
60 mesh	12.5
100 mesh	8.8
200 mesh	8.6
<200 mesh	24.3

a nearly linear increase between 575° and 970°C, there are no statistically significant differences between the ash contents determined at 575°, 750°, 900°, and 970°C at a confidence level of 95%. The low volatility is associated

Table 9
Ash Volatilization Results[a]

Furnace temp. (°C)	Ash (% db)[b]	Relative ash (%)	Color of ash	Ash fusibility	No. of samples	Ash range (% db)		Standard deviation
						High	Low	
575	4.93	100.0	Off-white	No sintering or slagging	12	5.57	4.80	0.21
750	4.88	98.7	Off-white	No sintering or slagging	9	5.56	4.67	0.26
900	4.84	96.8	Off-white	No sintering or slagging	6	5.43	4.67	0.30
970	4.68	95.6	Off-white	No sintering or slagging	3	4.79	4.57	0.11

[a]Fuel was hand crushed and pelletized. Continuous ashing for 2 h/temperature stage.
[b]*t*-test with a confidence level of 95% that the average ash amounts at furnace temperatures from 575° to 970°C are not significantly different.

with lower fouling caused by fewer alkali and other reactive species in the combustion gas at boiler superheaters and other heat-exchange surfaces.

Ash Fusibility

Below 1000°C, there was no apparent change in the fusion state of the ash from an initial unsintered condition. In the ash-fusibility analysis, the residue achieved a stage 2 fusibility rating (weak sintering of particles and pellet free of refractory support) at 1000°C in air; stage 3 fusibility at 1100°C (pellet contracted to spherical shape with rough surface texture, particles strongly sintered, low porosity surface, slagged to refractory support); stage 4 fusibility at 1200°C (pellet contracted to smooth, closed spherical shape, slagged to refractory support); stage 5 fusibility at 1400°C (ash fully molten with flat shape and thickness less than approximately 2 mm); and stage 6 fusibility at 1500°C (ash vaporized or absorbed by refractory support with no measurable thickness).

The low volatility, coupled with the moderately high melting point of the ash, suggest that the residue by itself should be a low to moderate fouling type. There is some potential that fuel blending would lead to worse rates of fouling because of interactions between residue silica and alkali metals in co-fired fuel, but the high alumina concentration of the residue ash might tend to mitigate such effects.

Conclusions

MSW can be readily converted to ethanol via dilute-acid pretreatment and enzyme hydrolysis followed by fermentation. Glucose yield in the range of 80–90% of theoretical was obtained. Yeast fermentation resulted in 80% ethanol yield from hexose. Enzyme recycle, together with the fed-batch technique, provides a promising option for obtaining higher hydrolysis rates and lower ethanol production cost by lowering the enzyme requirement. Residue from the enzymatic process was determined to be of low to moderate fouling type for direct-combustion energy conversion.

Acknowledgments

The authors wish to acknowledge technical assistance of D. Beaty, F.P. Eddy, and K. Connors in carrying out various experiments. Compositions of feedstock and solid residue were analyzed by Hauser Laboratories (Boulder, CO).

Funding for this project was provided by the National Renewable Energy Laboratory (NREL) Sustainable Technology Energy Partnerships initiative, Second Round (STEP-2) and by the California Energy Commission.

References

1. Wright, J. D., Wyman, C. E., and Grohman, K. (1988), *Appl. Biochem. Biotechnol.* **17/18,** 75–90.
2. Nguyen, Q. A. and Saddler, J. N. (1991), *Biores. Technol.* **35,** 275–282.

3. Hinman, N. D., Schell, D. J., Riley, C. J., Bergeron P. W., and Walter, P. J. (1992), *Appl. Biochem. Biotechnol.* **34/35,** 639–649.
4. Henley, R. G., Yang, R. Y. K., and Greenfield, P. F. (1980), *Enzyme Microb. Technol.* **2,** 206–208.
5. Tan, U. L., Yu, E. K. C., Mayers, P., and Saddler, J. N. (1987), *Appl. Microbiol. Biotechnol.* **26,** 21–27.
6. Vallander, L. and Ericksson, K. E. (1987), *Enzyme Microb. Technol.* **9,** 714–720.
7. Mes-Hartree, M., Hogan, C. M., and Saddler, J. N. (1987), *Biotechnol. Bioeng.* **30,** 558–564.
8. Lee, J. M. and Wolf, J. H. (1988), *Appl. Biochem. Biotechnol.* **17/18,** 203–215.
9. Tanaka, M., Fukui, M., and Matsuno, R. (1988), *Biotechnol. Bioeng.* **32,** 897–902.
10. Eklund, R., Galbe, M., and Zacchi, G. (1992), *Appl. Biochem. Biotechnol.* **34/35,** 105–113.
11. Girard, D. J. and Converse, A. O. (1993), *Appl. Biochem. Biotechnol.* **39/40,** 521–533.
12. Gregg, D. J. and Saddler, J. N. (1996), *Biotechnol. Bioeng.* **51,** 375–383.
13. TAPPI Test Methods (1991), T210 cm-86, *Weighing, Sampling and Testing Pulp for Moisture*, TAPPI, Atlanta, GA.
14. TAPPI Test Methods (1994–1995), T222 om-88, *Acid-Insoluble Lignin in Wood and Pulp*, TAPPI, Atlanta, GA.
15. Grohmann, K., Himmel, M., Rivard, C., Tucker, M., Baker, J., Torget, R., and Graboski, M. (1984), *Biotech. Bioeng. Symp.* **14,** 137.
16. Moore, W. E. and Johnson, D. B. (1967), *Procedures for the Chemical Analysis of Wood and Wood Products*, Forest Products Laboratory, U. S. Department of Agriculture, Madison, WI.
17. TAPPI Test Methods (1991), T211 om-85, *Ash in Wood and Pulp*, TAPPI, Atlanta, GA.
18. Ehrman, C. I. and Himmel, M. E. (1994), *Biotechnol. Tech.* **87(2),** 99–104.
19. Jenkins, B. M., Bakker, R. R., and Wei, J. B. (1996), *Biomass Bioenergy* **10,** 177–200.
20. Iogen Cellulase Technical Data Sheet (1995), *Effect of temperature on storage stability*, Iogen, Ottawa, Ontario, Canada.
21. Reese, E. T. and Mandels, M. (1980), *Biotechnol. Bioeng.* **22,** 323–335.
22. Reese, E. T. and Ryu, D. Y. (1980), *Enzyme Microb. Technol.* **2,** 239–240.
23. Mukataka, S., Tada, M., and Takahashi, J. (1983), *Ferment. Technol.* **61,** 615–621.
24. Nguyen, Q. A. (1998), U.S. Patent No. 5,733,758.
25. Jenkins, B. M. and Ebling, J. M. (1985), *Correlation of chemical and physical properties of terrestrial biomass with conversion*, Energy from Biomass and Wastes IX, Institute of Gas Technology, Chicago, IL.
26. Jenkins, B. M. (1989), in: *Biomass Handbook*, Kitani, O. and Hall, C. W., eds., Gordon and Breach Science Publishers, New York.
27. Jenkins, B. M. (1993), in: *Biomass Energy Fundamentals: Vol. 2: Appendices, Final Report, Electric Power Research Institute*, Wiltsee, G., ed., TR-102107, Research Project 3295-01.