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Degradation of Furfural (2-Furaldehyde) to Methane and Carbon Dioxide by an Anaerobic Consortium

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ABSTRACT

Furfural, a byproduct formed during the thermal/chemical pretreatment of hemicellulosic biomass, was degraded to methane and carbon dioxide under anaerobic conditions. The consortium of anaerobic microbes responsible for the degradation was enriched using small continuously stirred tank reactor (CSTR) systems with daily batch feeding of biomass pretreatment liquor and continuous addition of furfural. Although the continuous infusion of furfural was initially inhibitory to the anaerobic CSTR system, adaptation of the consortium occurred rapidly with high rates of furfural addition. Addition rates of 7.35 mg furfural/700-mL reactor/d resulted in biogas productions of 375%, of that produced in control CSTR systems, fed the biomass pretreatment liquor only. The anaerobic CSTR system fed high levels of furfural was stable, with a sludge pH of 7.1 and methane gas composition of 69%, compared to the control CSTR, which had a pH of 7.2 and 77% methane. CSTR systems in which furfural was continuously added resulted in 80% of the theoretically expected biogas. Intermediates in the anaerobic biodegradation of furfural were determined by spike additions in serum-bottle assays using the enriched consortium from the CSTR systems. Furfural was converted to several intermediates, including furfuryl alcohol, furoic acid, and acetic acid, before final conversion to methane and carbon dioxide.

Index Entries: Anaerobic digestion; CSTR; furfural; furfuryl alcohol; furoic acid; methane production.

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INTRODUCTION

Furfural (2-furaldehyde) is reported to be the most widely distributed simple furan in nature (*1*). It is a byproduct from the production and storage of fruit juices (*2–5*), wines (*6,7*), and medical solutions (*8–10*). Furfural is also a degradation byproduct in the thermal/chemical treatment of hemicellulosic feedstocks (biomass) (*11–19*) and refuse (*20*) for chemical and fuel production. During thermal/chemical treatment, the hemicellulosic portion of biomass is solubilized; this serves to increase the effectiveness of further treatment with cellulose-hydrolyzing enzymes that break down the cellulose portion of the cellulose–lignin residue. The kinetics of the degradation of aldopentoses to furfural has been determined (*15,16*), and furfural concentrations may reach levels of 1% or more, depending on treatment conditions. Furfural is also a major contaminant of evaporation condensate from sulfite-pulping processes used in the pulp and paper industry (*21*).

Furfural has been shown to inhibit the fermentation of glucose to ethanol by important yeast strains (*22,23*). Although little is known about microbial degradation of the furan ring, some yeasts convert furfural to furfuryl alcohol (*24*), and many bacteria, including species of *Pseudomonas, Clostridium,* and *Bacillus,* have been shown to metabolize furoic acid (*25–27*). Strains of *Pseudomonas* convert furoic acid to 2-oxoglutaric acid under aerobic conditions (*28*). Additionally, furfural has been degraded to methane by acclimatized anaerobic sludge cultures (*18,20*). The anaerobic conversion of sulfite evaporator condensate containing high levels of furfural revealed a coculture of a sulfate-reducing bacterium converting furfural to acetate and a methanogenic bacteria converting the acetate to methane and carbon dioxide (*29*). The sulfate-reducing bacterium was characterized as a species of *Desulfovibrio* (*30,31*).

Previously, we have documented the anaerobic biodegradation of furfural-containing liquors derived from the thermal/dilute phosphoric acid pretreatment of biomass to methane and carbon dioxide, using upflow anaerobic fixed-film reactors (*18*). In this study, CSTRs were used to examine the anaerobic degradation of furfural and related compounds. Additionally, serum-bottle assays were used to determine major intermediates in the mixed-anaerobic-culture degradation using spike loadings.

MATERIALS AND METHODS

Anaerobic Digesters

One-liter Applikon fermenters (700 mL working volume, Applikon BV, Schiedam, Holland) equipped with motor-speed controller, pH control (model 704, Horizon Ecology, Chicago, IL), and temperature control at 37°C by a temperature-controlled circulating water bath (Model 8000,

Fisher Scientific, Denver, CO) were used to conduct anaerobic fermentations with continuous addition of furfural or furfuryl alcohol. The anaerobic consortium was derived from the original up-flow fixed-film reactors used to anaerobically degrade the thermal/dilute phosphoric acid pretreatment liquor (*18*). This consortium was also developed in a 3.5-L CSTR fed biomass pretreatment liquor. The anaerobic reactor systems was operated as previously described (*32,33*), including daily batch feeding of a liquid biomass pretreatment liquor (*34*). Briefly, the pretreatment liquor was the supernatant derived from the hydrolysis of the hemicellulosic portion of wheat straw after processing for 2 h at 121°C with 0.2% phosphoric acid. The pH of the pretreatment liquor was adjusted to 7.5 using NaOH. The analysis and composition of the pretreatment liquor was previously described (*34*). Test compounds, including furfural and furfuryl alcohol, were administered to CSTR systems on a continuous basis using a multiple-position syringe pump (Harvard Apparatus, South Natick, MA). All chemical components were reagent grade and obtained from national laboratory supply houses. Effluent was removed on a daily basis and stored at 4°C until analyses were performed. Spike-addition studies were conducted in 155-mL serum bottles at 37°C and mixed using an orbital shaker as previously described (*33*).

CSTR-Effluent Analysis

Reactor-effluent samples were prepared and analyzed for volatile organic acids (C2–C5, iso- and normal acids) by gas–liquid chromatography (GLC) as previously described (*32*), using a model 5840A gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector and a 60/80 Carbopack C/0.3% Carbowax 20*M*/0.1% H_3PO_4 packed column (183 cm×2 mm; Supelco, Bellefonte, PA).

Analysis of nonvolatile organic acids in digester sludge effluent was accomplished by high-performance liquid chromatography (HPLC). Experimental samples were clarified by centrifugation (13,800*g* for 10 min) and filtration through 0.2-μm Acrodisc disposable syringe filters (Gelman Sciences, Ann Arbor, MI). The samples were diluted with 0.01*N* sulfuric acid and analyzed using a BioRad HPX-87H HPLC column (BioRad, Richmond, CA) controlled at 45°C. Analysis by HPLC identified both nonvolatile and volatile organic acids and thus served as a back-up to the GLC analysis for volatile fatty acids.

The presence of furfural, furfuryl alcohol, and furoic acid were determined as described above for nonvolatile organic acids using HPLC analysis (*18*). Sulfate, sulfite, nitrate, and nitrite ions present in the biomass pretreatment liquor feed or the reactor-effluent samples were determined by HPLC as previously described (*33*).

Gas Analysis

Production of biogas from anaerobic reactors was monitored on a daily basis using calibrated water-displacement reservoirs. Biogas produced in

studies conducted using 155-mL serum bottles was measured using a pressure transducer equipped with a 22-gage needle for penetration into, and subsequent release of excess pressure from, the serum bottle. The composition of biogas produced was analyzed for methane and carbon dioxide composition by GLC using a Gow-Mac Instruments (Bridgewater, NJ) model 550 gas chromatograph equipped with a thermal-conductivity detector and a Porapack Q column (183 cm × 6.4 mm; Supelco).

Enumeration of Digester Microflora

Anaerobic digester effluents were analyzed for viable cell numbers of several important groups of microorganisms. Digester effluent (10 mL) was removed 24 h after batch feeding and was subjected to 10-fold dilutions in 9-mL anaerobic dilution blanks. Anaerobic dilution blanks consisted of basal medium under an oxygen-free N_2/CO_2 (80%/20%) gas phase. Anaerobic basal medium was prepared in serum tubes using the methods of Hungate (*35*) as described by Balch et al. (*36*). Basal medium composition was as follows: trace vitamin solution (*37*), 10 mL; trace mineral solution (*37*), 10 mL; NH_4Cl, 1.0 g; K_2HPO_4, 2.0 g; $NaHCO_3$, 5.0 g; resazurin, 0.001 g; cysteine HCl, 0.5 g; and clarified digester effluent (nonamended pretreatment liquor, batch fed), 10 mL; distilled water to 1000 mL. One milliliter of each dilution was injected into 25 mL of tempered agar medium (basal medium, additions, and 2% Noble agar [Difco, Detroit, MI]) contained in 155-mL serum bottles. Inoculated bottles were then rolled in a level pan containing cool water until the agar solidified. The roll bottles were incubated at 37°C, inverted, with either a N_2/CO_2 or a H_2/CO_2 (both 80%/20%) gas phase. Colonies were examined and counted with an Olympus stereo microscope after 1, 2, and 3 wk of incubation.

Hydrogen-metabolizing methanogenic bacteria were cultivated in basal medium under a H_2/CO_2 gas phase. These methanogens were enumerated by counting colonies that autofluoresced when illuminated with 420-nm light (*38*).

Acetate-metabolizing methanogenic bacteria were enumerated by counting colonies that developed in basal medium containing 4 g/L sodium acetate under a N_2/CO_2 gas phase and that autofloresced when illuminated with 420-nm light.

Sulfate-reducing bacteria (SRB), which oxidize hydrogen or furfural, were enumerated using basal medium supplemented with sodium sulfate (1.0 g/L). Enumeration of hydrogen-oxidizing SRB utilized basal medium with a H_2/CO_2 gas phase, and enumeration of furfural-oxidizing SRB included 5 m*M* furfural in the agar medium. Prior to inoculation, 0.25 mL of a sterile $FeSO_4 \cdot 7H_2O$ solution (0.5 g/L final concentration) was added. Colonies that produced a black FeS precipitate, resulting from the sulfate reduction, were counted.

Table 1
Composition of Supernatant Feed
from the Thermal/Dilute Phosphoric Acid
Pretreatment of Wheat Straw Biomass

% Solids	2.08
Volatile Solids (g/L)	14.5
Total Sugars (Anthrone, g/L)	7.95
Volatile Fatty Acids (Acetate, %)	0.05
Furfural (2-furaldehyde, %)	0.01
Phosphate (%)	0.22
Nitrogen	
Free Ammonia (Phenate, mg/L)	0.44
Total (Kjeldahl, mg/L)	139
COD (mg/L)	12250
C:N Ratio	88

RESULTS

The characterization of hydrolysate from the thermal/dilute phosphoric acid pretreatment of wheat straw biomass is shown in Table 1. In part because of the high levels of furfural, which has been shown to inhibit yeast fermentation of sugars to ethanol (*22,23*), the pretreatment liquor was anaerobically digested to produce the gaseous fuel methane. During initial studies of the anaerobic fermentation of biomass pretreatment liquors, using up-flow anaerobic fixed-film reactor systems, complete conversion of furfural was determined (*18*). The consortia from this fixed-film reactor system was transferred to a CSTR system to further develop the furfural-degrading populations and provide adequate quantities of inoculum for in-depth spike-addition studies to determine intermediates in the anaerobic degradation.

Studies conducted with multiple replicate experimental samples in serum-bottle assays and using anaerobic digester sludge acclimated to furfural are shown in Figure 1 for spike additions of furfural (A), furfuryl alcohol (B), and furoic acid (C). Degradation intermediates identified for furfural spike additions include furfuryl alcohol and furoic acid (Fig. 1A) before conversion to acetate (acetate data not shown) and biogas (methane and carbon dioxide). Spike additions of furfuryl alcohol (Fig. 1B) did not result in detectable accumulations of furfural; rather, direct production of furoic acid was detected. Finally, the spike addition of furoic acid (Fig. 1C) did not result in detectable levels of either furfural or furfuryl alcohol, but in direct conversion to biogas.

An analysis of anaerobic bioconversion efficiency for added furfural and furfuryl alcohol in standard CSTR systems is shown in Table 2. Anaerobic process parameters were stable for all digester systems, as demonstrated by stable sludge pH and low volatile fatty acid levels. The anaerobic

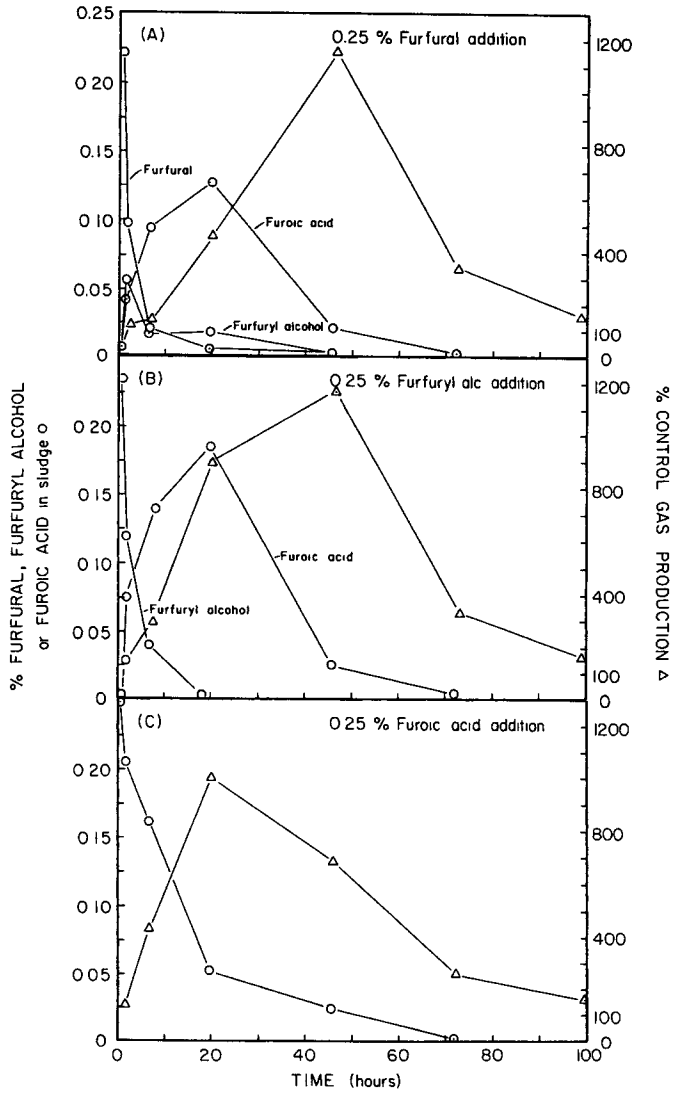

Fig. 1. Fate of spike additions of (A) furfural, (B) furfuryl alcohol, and (C) furoic acid in acclimated anaerobic digester sludge. Experiments were conducted in triplicate in serum bottle assays as described in the Materials and Methods section.

conversion efficiency for furfural and furfuryl alcohol to biogas end products were substantially complete with 80 and 94% of the furfural and furfuryl alcohol converted, respectively.

The relevance of sulfate-reducing bacteria to the degradation of furfural in the anaerobic system was examined through enumeration of viable colony-forming units in agar media. As shown in Table 3, viable numbers of sulfate-reducing bacteria were below the level of detection using classical media-detection methods. The relative number of methanogens increased with the addition of furfural and furfuryl alcohol above the control di-

Table 2
Analysis of Anaerobic Fermentation Performance
and Conversion Efficiency for Furfural and Related Compounds

Solution Added*	Water	Furfuryl Alcohol	Furfural	Furfural
Batch feed**	+	+	+	-
Total Biogas Production (mL biogas/reactor/day)	39 ± 8.0	197 ± 27	174 ± 26	137 ± 28
Gas Composition (% methane)	71.1	70.4	66.0	66.3
pH	7.1	7.0	7.0	6.9
Volatile Fatty Acids (mg/ 100 mL)				
Acetate	2.0	3.8	9.0	3.2
Propionate	Trace	Trace	1.4	Trace
Stock Solution Pump rate (mL/reactor/day)	----------	11.5 ± 1.6	----------	
Expected Biogas Production (mL biogas/reactor/day)		169	172	172
Actual Corrected Biogas Production (Total minus control biogas prod.)		158	135	137
Conversion Efficiency (% of Expected Biogas Production)		93.5	78.5	79.7

* Stock solutions contained 12 g/L test compound in 0.14 M phosphate buffer at pH 7.2.
** The batch biomass pretreatment liquor feed rate was 10 mL/reactor/day.

Table 3
Microbial Enumerations for Anaerobic Fermentation Systems
Acclimated to the Continuous Addition of Various Compounds

Solution Added*	Water	Furfuryl Alcohol	Furfural	Furfural
Batch feed**	+	+	+	-
Sulfate Reducers	N.D.	N.D.	N.D.	N.D.
Methanogens				
Hydrogen	1.2×10^6	1.4×10^7	1.3×10^7	1.1×10^7
Acetate	2.0×10^2	4.0×10^3	3.2×10^3	2.9×10^3

* Stock solutions contained 12 g/L test compound in 0.14 M phosphate buffer at pH 7.2.
** The batch biomass pretreatment liquor feed rate was 10 mL/reactor/day.
N.D. - Not detectable in roll tubes with either hydrogen or furfural.

gester. Increases in acetate- and hydrogen-utilizing methanogens were similar. The level of soluble oxidants in the biomass pretreatment liquor, used as the sole source of feed and nutrient for the anaerobic digestion consortia, was below the limit of detection for ion analysis (0.07, 0.79, and 0.12 m*M* sulfate, sulfite, and nitrate, respectively).

DISCUSSION

Furfural represents a major byproduct from pretreatment processes for lignocellulosic biomass either for energy production or pulp and paper

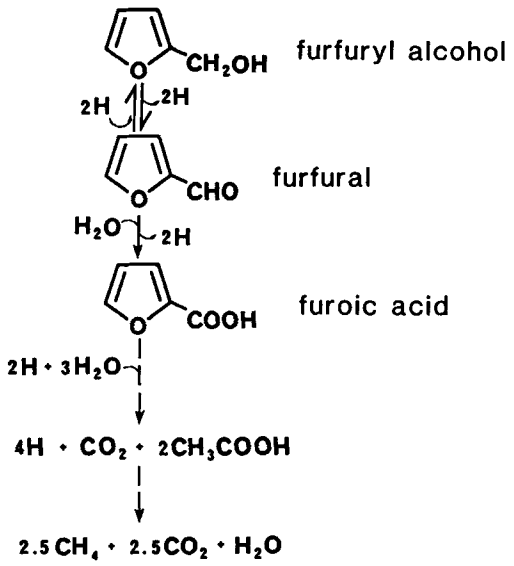

Fig. 2. Proposed disproportional anaerobic conversion of furfural to methane and carbon dioxide based on the stoichiometry given by Evans and Fuchs (*39*).

manufacture. Furfural has previously been shown to inhibit many microbial systems. Furfural also has been shown to be degraded under anaerobic conditions by acclimated microbial consortia. In the only well-defined furfural-degradation study, using sulfite evaporator condensate, the responsible microorganism was identified as a sulfate-reducing bacterium that metabolized furfural to acetate. In coculture with an acetate-utilizing methanogen, the furfural was converted to methane and carbon dioxide.

However, in this study a thermal/dilute phosphoric acid process was used for biomass pretreatment, which resulted in solubilization of the hemicellulosic portion of the biomass with no detectable sulfate present in the liquor used as feedstock. In this acclimated anaerobic system, furfural was effectively converted to methane and carbon dioxide. The efficiency in conversion of furfural and furfuryl alcohol to methane and carbon dioxide, based on carbon balance, was estimated to be 80% for furfural-added systems and 94% for furfuryl alcohol. The remainder of the carbon was presumably converted into microbial biomass. Intermediates in the degradation of furfural in this anaerobic system (apparently non-sulfate-influenced) were furfuryl alcohol, furoic acid, and acetate, as determined from spike-addition experiments. The proposed pathway for furfural conversion to methane and carbon dioxide is depicted in Fig. 2, from stoichiometry based on Evans and Fuchs (*39*), and is in agreement with the disproportionate amount of furfural to furfuryl alcohol and furoic acid proposed by Folkerts et al. (*31*). However, two anomalies with the data fitted to this pathway were identified in the current study.

In spike additions of furfuryl alcohol to acclimated digester sludge, furfural was not detected in the resulting conversion to methane and carbon dioxide. It is postulated that the lack of detection of furfural may be a result of the high binding efficiency of an alcohol dehydrogenase (*40*). Additional metabolic studies of the responsible microorganisms are indicated to elucidate the pathway.

Additionally, the biogas production resulting from degradation of furfural was projected to be 1/1 methane and carbon dioxide. Evaluation of the actual biogas identified a ratio significantly higher, approaching 1.8/1. However, the lower pH of the furfural-augmented digester effluent indicates elevated levels of dissolved carbon dioxide that may account for the elevated methane/carbon dioxide ratios.

In the only previous well-documented study of anaerobic furfural degradation (*31*), sulfate reducing bacteria were responsible for metabolic conversion of furfural to acetate. However, the extremely low levels of sulfate (below detection limits) in the biomass pretreatment liquor used as feedstock for the anaerobic consortium in this study resulted in selection against the development of detectable levels of sulfate reducing bacteria and thus their involvement in the anaerobic conversion of furfural to methane and carbon dioxide. Preliminary attempts to isolate the microbial population(s) responsible for initial degradation of furfural have resulted in coculture formation with acetate-utilizing *Methanosarcina*. Further isolations and characterization of the furfural-degrading strain is continuing. The degradation of added furfural or furfuryl alcohol resulted in higher levels of both hydrogen- and acetate-utilizing methanogenic bacteria over a control reactor system fed the pretreatment liquor only and was as expected because of increased levels of acetate, and presumably hydrogen, from the conversion of added furfural or furfuryl alcohol.

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