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Differences in Xylan Degradation by Various Noncellulolytic Thermophilic Anaerobes and *Clostridium thermocellum*

JÜRGEN WIEGEL,[1]* CHERYLE P. MOTHERSHED,[1] AND JÜRGEN PULS[2]

Department of Microbiology and Center for Biological Resource Recovery, University of Georgia, Athens, Georgia 30602,[1] and Institute of Wood Chemistry and Chemical Technology of Wood, BFA-Hamburg, D-2050 Hamburg 80, Federal Republic of Germany[2]

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Hemicellulose fractions with a predetermined distribution of xylose, xylooligomers, and xylan fractions were obtained through steam explosion of wood by the steam explosion-extraction process of BFA-Hamburg, Hamburg, Federal Republic of Germany. A differential utilization of various molecular-weight fractions by several thermophilic anaerobic bacteria was determined during their growth on the hemicellulose preparations. *Clostridium thermocellum* (60°C) first utilized the high-molecular-weight fractions (polymerization degree of 15 to 40 xylose units). Xylose and xylooligomers of $n = 2$ to 5 accumulated while *C. thermocellum* was not growing, as evident from the fermentation products formed. Whereas the xylan was hydrolyzed and the small oligoxylans were utilized after more than 100 h of incubation, xylose was not significantly utilized. In contrast to this, *C. thermohydrosulfuricum* (70°C) and *Thermoanaerobium brockii* (70°C) utilized xylose first and then xylooligomers of $n = 2$ to 5, but xylooligomers of n greater than 6 were only slowly utilized. *Thermoanaerobacter ethanolicus* (70°C), *Thermobacteroides acetoethylicus* (70°C), and *C. thermosaccharolyticum* (60°C) utilized xylose preferentially. Xylooligomers of $n = 2$ to 5 and $n = 6$ and greater were apparently concomitantly utilized without significant differences. In contrast to *C. thermocellum*, the non-cellulolytic organisms grew during xylan hydrolysis, producing ethanol, lactate, acetate, CO_2, and H_2.

Cellulose and hemicellulose are regarded as the two most abundant and, thus, most important biologically renewable resources for bioconversion into gaseous and liquid fuels as well as feedstock chemicals (11). Several processes have been developed to separate the carbohydrate portions of a lignocellulosic biomass into its three main components: cellulose, a glucose polymer with β-1,4-glycosidic bonds; hemicellulose, mainly a pentose polymer of various structures and, in some instances, including hexose polymers, such as mannan; and lignin, a complex polyaromatic polymer.

Hemicellulose fractions are conveniently obtained through steam explosion-extraction processes. The BFA-Hamburg process, as described previously (2, 10), yields relatively pure hemicellulose fractions. In the case of hardwoods, a mixture of xylans of up to 40 units is obtained. The ratio of xylose to xylooligomers and xylopolymers depends on the source, the incubation temperature, and the incubation time.

The utilization of hemicellulose by thermophilic and extreme thermophilic anaerobic bacteria has not been extensively studied, although they are regarded as very promising for biotechnological conversion processes (3; J. Wiegel and L. G. Ljungdahl, Crit. Rev. Biotechnol., in press). Thermophiles offer many advantages to yeasts and most mesophilic anaerobes (4, 7, 12).

Weimar et al. (6) recently described some new hemicellulolytic anaerobic thermophiles which had been specifically isolated on hemicellulose. It has recently been reported that *Thermoanaerobacter ethanolicus* and some other thermophiles can utilize hemicellulose but not cellulose (9). Depending on growth conditions, the main product is ethanol or acetate. The ratios of the products ethanol, acetate, lactate, H_2, and CO_2 depend on the substrate and its concentration (9).

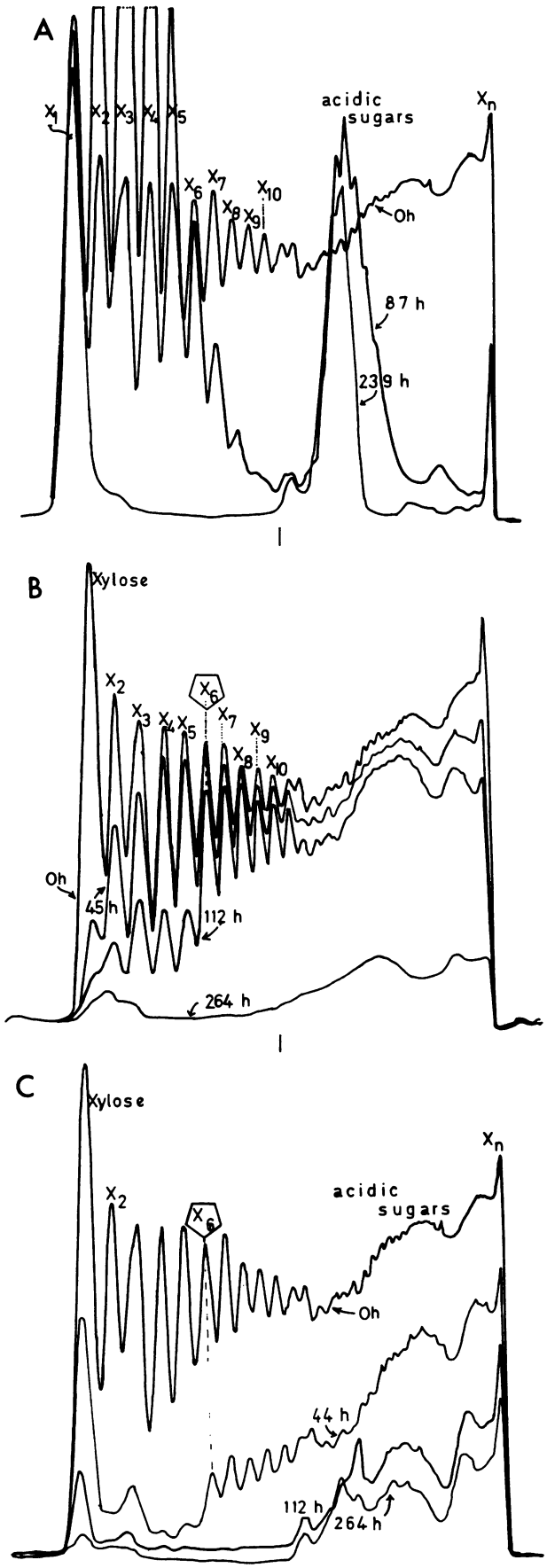
* Corresponding author.

In this publication, we describe differences in the kinetics of utilization of hemicellulose from birch wood by various validly published thermophilic and extreme thermophilic anaerobic bacteria which are being considered for use in industrial biomass conversion.

MATERIALS AND METHODS

Organisms and growth conditions. *T. ethanolicus* JW200 (ATCC 31550; DSM 2246) and its mutants described by Carreira et al. (1), *Clostridium thermohydrosulfuricum* JW102 (DSM 2247), *Thermoanaerobium brockii* HTD4 (DSM 1457), and *Thermobacteroides acetoethylicus* (ATCC 33265) were grown at 70°C. *C. thermosaccharolyticum* (ATCC 7956) and *C. thermocellum* JW20 (ATCC 31549) were grown at 60°C. The mineral media used were described previously (9), except that 5 mg of $FeSO_4$ per liter was added. The xylan degradation experiments were performed as described by Wiegel et al. (9).

Hemicellulose preparations. Hemicellulose preparations were obtained by the steam explosion-extraction process of BFA-Hamburg, Hamburg, Federal Republic of Germany, as described by Dietrichs et al. (2) and Körner et al. (H.-U. Körner, D. Gottschalk, J. Wiegel, and J. Puls, Anal. Biochem., in press). For a comparison of the breakdown patterns, the hemicellulose fractions obtained by treatment for 10 min at 190°C (9) were chosen, as most of the molecular fractions were present at similar concentrations. The reported fermentation time of ca. 160 h was caused by a low inoculum of 0.5% (vol/vol), which was chosen to allow better monitoring of the breakdown patterns. The hemicellulose preparation used had the following sugar composition (percent [weight/volume]): xylose, 83.6; glucose, 5.4; mannose, 5.4; and rhamnose, 2.4; 4-*O*-methylglucuronic acids were present but were not quantified. The various carbohydrates were analyzed with a high-pressure liquid chromatography-based sugar analyzer with an integrated after-column



reaction system for carbohydrate detection, as described by Körner et al. (in press).

Analyses of fermentation products. Fermentation products were analyzed as described previously (9) by high-pressure liquid chromatography and gas-liquid chromatography as well as by total-carbon analysis.

RESULTS AND DISCUSSION

In addition to *T. ethanolicus* the extreme thermophiles *T. brockii* (optimum temperature [T_{opt}], 68°C; maximum temperature [T_{max}], 78°C), *T. acetoethylicus* (T_{opt}, 68°C; T_{max}, 78°C), and *C. thermohydrosulfuricum* (T_{opt}, 68 to 69°C; T_{max}, 78°C), thermophilic *C. thermosaccharolyticum* (T_{opt}, 58 to 59°C; T_{max}, 69°C), and cellulolytic *C. thermocellum* (T_{opt}, 58 to 60°C; T_{max}, 69°C) and *Clostridium* sp. (T_{opt}, 65°C; T_{max}, 75°C) all grew on xylans, producing ethanol, lactate, acetate, CO_2, and H_2 and, in the case of *C. thermosaccharolyticum*, butyrate and traces of butanol. However, the patterns of the breakdown and utilization of the various fractions differed markedly among the different organisms (Fig. 1 and Table 1). Three characteristic patterns for the breakdown of hemicellulose from birch wood are shown in Fig. 1. *C. thermocellum* (Fig. 1A) first degraded the higher oligomeric xylan fractions with a polymerization grade of $n = 6$ or higher. Whether *C. thermocellum* JW20 contains a special xylanase or whether the reactions were a result of the nonspecificity of its cellulase complex is unknown. The xylose$_n$ of $n = 2$ to 5 accumulated at first, but after 240 h they were utilized. The monomer xylose and the acidic sugars [4-*O*-methylglucurono-(xylose)$_n$] containing less than three xylose units were not utilized even after 300 h.

C. thermohydrosulfuricum (Fig. 1B) degraded xylose first and then the xylose$_n$ of $n = 2$ to 5. The degradation of xylose$_n$ of $n = 6$ to 40 was significant only after the smaller xylose oligomers had been utilized. After 264 h, 64% of the hemicellulose (0.8% [wt/vol]), including most of the acidic sugars, had been utilized. A similar pattern was obtained with *T. brockii*, in which over 71% of the hemicellulose was utilized after 264 h (Table 1). *T. ethanolicus* (Fig. 1C) utilized the lower xylose oligomers of n up to 3 at about the same rate as xylose or glucose; it utilized xylose$_n$ of $n = 4$ or 5 at a slightly slower rate. This was also shown by a comparison of the fermentation rates (doubling time for growth) of xylose and the isolated xylooligomers of n up to 7 (8, 9). Xylooligomers [tested up to xylose$_7$] are degraded by a stepwise removal of xylose units (kinetics are not shown). There were no indications that xylobiose was the main hydrolysis product. The acidic xylooligomers were utilized more slowly than the xylans containing only xylose. *T. acetoethylicus* and *C. thermosaccharolyticum* exhibited kinetic patterns similar to those of *T. ethanolicus*. Fermentation analyses for various thermophiles are shown in Table 1. The utilization of hemicellulose was calculated from a total sugar analysis, and all sugars were expressed as xylose units. As evident from the sugar analysis (see above), the xylan preparation contained some hexoses; thus, the ob-

FIG. 1. Degradation of birch wood hemicellulose prepared by the BFA-Hamburg process by various thermophilic and extreme thermophilic anaerobic bacteria. (A) *C. thermocellum*; (B) *C. thermohydrosulfuricum*; and (C) *T. ethanolicus*. Fermentation conditions were as described in the text. A detailed description of the carbohydrate analysis is provided by Körner et al. (in press). X_n, Xylose oligomers consisting out of n xylose units.

TABLE 1. Birch wood hemicellulose degradation by various thermophilic anaerobic bacteria

Organism (temp [°C] grown at)	Hemicellulose utilized[a]			Products produced (μmol/ml)			
	% (wt/vol)	By (h):	Xylose equivalents (μmol/ml)	Ethanol	Acetate	Lactate	Butyrate
C. thermocellum JW20 (60)	5.5	87	0.3	0.1	8.0[b]	0.1	—[c]
	68.5	240	27.6	10.1	23.9	15.1	—
C. thermohydrosulfuricum (70)	34.4	112	13.5	10.6	17.7	1.6	—
	62.4	264	24.5	19.0	29.2	3.4	—
T. ethanolicus (70)	33.7	40	14.0	13.3	9.4	1.2	—
	67.3	112	24.5	25.5	16.9	2.1	—
	71.8	264	29.5	32.3	18	2.4	—
T. brockii (70)	65.2	112					
	71.5	264	25.1	26.3	15.3	2.9	—
T. acetoethylicus (70)	63.6	112	25.4	26.4	15.7	2.1	—
C. thermosaccharolyticum (60)	17.5	18.5	7.1	7.0	9.0	0.0	—
	52	28	21.0	15.0	21.8	0.2	+
	88.5	264	35.9	ND[d]	26.0	2.5	++

[a] Derived by steam explosion-extraction process of BFA-Hamburg; 10 min, 190°C.
[b] CO_2 and H_2 were also produced; for an explanation of more than a 100% carbon recovery, see the text.
[c] —, Not detectable, i.e., below 0.05 mM; +, detectable in low amounts, below 1 mM; ++, compound present above 1 mM.
[d] ND, Not determined in this run. Based on a previous fermentation, ca. 25 μmol/ml should have been formed. In addition, 150 μmol of H_2 per ml of culture was produced.

tained carbon recovery in Table 1 was slightly higher than 100%. Most of the hexoses (glucose) were utilized early in the fermentation (data not shown). In addition, some of the acetic acid was not formed through fermentation but originated from acetylated xylose units. As the percentage formed as hydrolysis products could not be accurately determined, the total amount of acetate formed during incubation (corrected for initial acetate concentration) is given. In contrast to cellulolytic *C. thermocellum* JW20, the non-cellulolytic thermophiles immediately grew on the hydrolysis products of hemicellulose, as identified by the increase in biomass (data not shown) and fermentation products.

The use of *T. ethanolicus* and its mutants for biotechnological processes has been patented (L. G. Ljungdahl and L. H. Carreira, U.S. patent 4,385,117, May, 1983; L. H. Ljungdahl and J. Wiegel, U.S. patents 4,292,407 and 4,292,406, September, 1981), and its ability to utilize directly hemicellulose could be of importance for industrial applications. As long as the hemicellulose concentration is not higher than 1% (wt/vol, on the basis of xylose equivalents), the main product is ethanol. At higher substrate concentrations (above 2% [wt/vol] hemicellulose), acetate can become the main product.

As all the tested thermophilic and extreme thermophilic anaerobes were able to hydrolyze the hemicellulose fractions, it is concluded that in nature these organisms are involved in the degradation of hemicellulose biomass. The ability to hydrolyze hemicellulose seems to be more widespread among this group than the ability to hydrolyze cellulose. As cellulolytic *C. thermocellum* and *Clostridium* sp. grew so poorly on hemicellulose, as compared with their growth on cellulose or cellobiose (xylobiose has not been tested), these organisms are probably not directly involved in hemicellulose degradation. However, based on the above results and the known properties of *C. thermocellum* (3), it is assumed that the cellulose-hemicellulose-containing biomass, in which both compounds are closely associated and yield a complex structure (5), can be utilized through cooperative actions of cellulolytic and hemicellulolytic anaerobic thermophiles.

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LITERATURE CITED

1. **Carreira, L. H., J. Wiegel, and L. G. Ljungdahl.** 1983. Produtión of ethanol from biopolymers by anaerobic thermophilic and extreme thermophilic bacteria. I. Regulation of carbohydrate utilization in mutants of *Thermoanaerobacter ethanolicus*. Biotechnol. Bioeng. Symp. **13**:183–191.
2. **Dietrichs, H. H., M. Sinner, and J. Puls.** 1978. Potential of steaming hardwoods and straw for food and feed production. Holzforschung **32**:193–199.
3. **Duong, T.-V. C., E. A. Johnson, and A. L. Demain.** 1983. Thermophilic, anaerobic and cellulolytic bacteria. Top. Enzyme Ferment. Biotechnol. **7**:156–195.
4. **Sonnleitner, B., and A. Fiechter.** 1983. Advantages of using thermophiles in biotechnological processes: expectations and reality. Trends Biotechnol. **1**:74–80.
5. **Thomas, R. J.** 1983. Wood anatomy and permeability, pp. 1–32. *In* J. Soltes (ed.), Wood and agricultural residues. Academic Press, Inc., New York.
6. **Weimar, P. J., L. W. Wagner, S. Knowlton, and T. K. Ng.** 1984. Thermophilic anaerobic bacteria which ferment hemicellulose: characterization of organisms and identification of plasmids. Arch. Microbiol. **138**:31–36.
7. **Wiegel, J.** 1980. Formation of ethanol by bacteria. A pledge for the use of extreme thermophilic anaerobic bacteria in industrial ethanol fermentation processes. Experientia (Basel) **36**: 1434–1446.
8. **Wiegel, J., L. H. Carreira, L. G. Ljungdahl, and J. Puls.** 1984. Formation of ethanol and acetate from biomass using thermophilic and extreme thermophilic anaerobic bacteria. *In* Proceedings of the 7th International FPRS Industrial Wood Energy Forum 83, Nashville, Tenn. Forest Product Research Society, Madison, Wis.

9. **Wiegel, J. L. H. Carreira, C. P. Mothershed, and J. Puls** 1983. Production of ethanol from biopolymers by anaerobic thermophilic and extreme thermophilic bacteria. II. *Thermoanaerobacter ethanolicus* JW200 and its mutants in batch cultures and resting cell experiments. Biotechnol. Bioeng. Symp. **13**:193–205.
10. **Wiegel, J., and J. Puls.** 1983. Production of ethanol from hemicelluloses of hardwoods and annual plants, pp. 863–867. *In* A. Strub, P. Chartier, and G. Schleser (ed.), Energy from biomass: 2nd EC Conference. Applied Science Publishers, Inc., New York.
11. **Wise, D. L. (ed.).** 1984. CRC series in biotechnology. Vol. 1, Fuel gas development. Vol. 2. Fuel gas system. Vol. 3. Bioconversion system. Vol. 4. Liquid fuel system. Vol. 5. Liquid fuel developments. CRC Press, Inc., Boca Raton, Fla.
12. **Zeikus, J. G.** 1980. Chemical and fuel production by anaerobic bacteria. Annu. Rev. Microbiol. **314**:423–464.