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# Utilization of Enzymatically Hydrolyzed Wood Hemicelluloses by Microorganisms for Production of Liquid Fuels

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Hemicellulose-derived sugars were obtained from a variety of pretreated wood substrates such as water-soluble fractions from steam-exploded aspen, solvent-extracted aspen, and commercial xylan. These fractions were enzymatically hydrolyzed by commercial enzyme preparations and by the culture filtrates of eight highly cellulolytic fungi. The sugars released were assayed by high-pressure liquid chromatography. Over 30% of the hemicellulose fractions, at a 10% substrate concentration, could be hydrolyzed to monosaccharides. These hemicellulose hydrolysates were used as the substrates for growth of *Clostridium acetobutylicum* and *Klebsiella pneumoniae*. Comparatively low butanol values were obtained with *C. acetobutylicum*, although over 50% of the hemicellulose fraction, at a 1% substrate concentration, could be converted to 2,3-butanediol, ethanol, and acetic acid by *K. pneumoniae*.

Fuel production from biomass, in the form of agricultural and forest residues, has been particularly appealing as a renewable energy source. Although progress has been made in the pretreatment processes and microbial conversion of such cellulosic wastes to liquid fuels, most research to date has dealt with the utilization of the cellulose portion of the lignocellulosic substrate. Little emphasis has been placed on the hemicellulose content, even though it can account for 20 to 40% of most woody residues (21). At present, the production of liquid fuels from cellulosic materials has not been shown to be economically feasible on an industrial scale. The utilization of hemicellulose in addition to cellulose should play a role in making this process more economically attractive (4).

Although most recent work (6, 9, 23) on the utilization of hemicellulose-derived sugars has primarily concentrated on xylose, hemicellulose is a relatively complex component of lignocellulosic materials and, depending on its origin, can be composed of a variety of different hexoses, pentoses, and uronic acids (22). For the hemicellulose component to be efficiently utilized, it must first be extracted from the lignocellulosic complex and then hydrolyzed to its component sugars, which must all be readily utilized by fermentative microorganisms. Although some workers (5, 23) have concentrated on the use of yeasts to convert pentose sugars to ethanol, we have used a variety of different aerobic and anaerobic bacteria capable of using all of the sugars normally found in hemicellulose hydrolysates and producing a variety of liquid fuels. In this paper we report on the solvents and acids produced by these organisms when they are grown on a variety of enzymatically hydrolyzed hemicellulose fractions.

## MATERIALS AND METHODS

**Microorganisms and media.** All of the fungi used were taken from the Forintek culture collection; *Trichoderma reesei* C30 was originally supplied by Rutgers University. A spore inoculum was used to initiate growth in shake flasks, using Vogel's medium (16). The fungi were grown in 100-ml quantities at 28°C on 2% Solka floc. Culture filtrates were obtained by filtration through a Whatman glass-fiber filter after 8 days of growth.

*Klebsiella pneumoniae (Aerobacter aerogenes)* NRRL B-199 (ATCC 8724) was obtained from the National Research Council of Canada culture collection (NRCC 3006). Culture conditions were as described previously (25), with cultures shaken (130 rpm) at 37°C under a finite air supply.

*Clostridium acetobutylicum* ATCC 824 was routinely maintained in 10 ml of reinforced clostridium medium (Oxoid) in 60-ml serum vials (15) under $N_2$-$CO_2$ (80:20) head space gas. Cultures were incubated as previously described (13) at 37°C under stationary conditions.

**Assays.** Soluble protein was determined by the

method of Lowry et al. (11), using bovine serum albumin (Sigma Chemical Co.) as a standard. Total sugars were estimated colorimetrically with dinitrosalicylic acid reagent (14). Pentosans were assayed by the method outlined in TAPPI (Technical Association of the Pulp and Paper Industry) standard T223-05-71, acid-insoluble lignin was assayed by the method outlined in TAPPI standard T222-05-75, and hexosans were measured by the anthrone (19) reaction. Monosaccharides were detected by high-pressure liquid chromatography with a Varian 5000 chromatograph, a Micropak $NH_2$-10 column, an acetonitrile-water (80:20) solvent system, a flow rate of 1.5 ml/min, and a refractive index detector. Solvents and acids were determined by gas-liquid chromatography following a modification of the Ackman method (1) and using a Chromosorb 101 column.

Filter paper activity was determined by the method of Mandels et al. (12). Xylanase activity was determined by incubating 1 ml of an appropriately diluted enzyme solution with 10 mg of larchwood xylan (Sigma) in 1 ml of 0.05 M citrate buffer (pH 4.8) at 50°C for 30 min. The reaction was terminated by the addition of 3 ml of dinitrosalicylic acid reagent. The tubes were placed in a boiling-water bath for 5 min and then cooled to room temperature; the absorbance was read at 575 nm. Endoglucanase and β-glucosidase activities were assayed as described previously (17).

**Substrates. (i) Commercial substrates.** Xylan from larchwood (molecular weight, 20,000) was obtained from Sigma.

**(ii) Solvent-extracted aspen (SEA).** Aspen powder (pass 20 mesh) was extracted with an ethanol-benzene mixture (1:2), using the method outlined in TAPPI standard T6 M-50. This material was dried, chlorited three times at 70°C with $NaClO_2$ solution at pH 4 by the method of Wise et al. (24), and then extracted with 24% KOH under $N_2$ for 2 h at room temperature, and the hemicellulose in solution was precipitated with an ethanol-acetic acid mixture (10:1).

**(iii)** Aspen wood chips were steam exploded, using a high-pressure gun with a 250-ml capacity (17).

**(a) Steam-exploded wood, water-soluble fractions (SEW-WS1).** Aspen wafers which had been exposed to saturated steam at 560 lb/in$^2$ (250°C) for 40 s were extracted at room temperature for 2 h with water.

**(b) SEW-WS2.** Aspen wafers which had been exposed to saturated steam at 560 lb/in$^2$ (250°C) for 15 s were extracted at room temperature for 2 h with water.

**(c) SEW-WS2-0.1%A.** SEW-WS2-0.1%A are water-soluble fractions from aspen wafers soaked in 0.1% $H_2SO_4$ (0.2% $H_2SO_4$ based on wood) before exposure to saturated steam and water extraction as in SEW-WS2.

**(d) SEW-WS2-0.2%A.** SEW-WS2-0.2%A are water-soluble fractions from aspen wafers soaked in 0.2% $H_2SO_4$ (0.4% $H_2SO_4$ based on wood) before exposure to saturated steam and water extraction as in SEW-WS2.

**Substrate hydrolysis. (i) Method A.** Substrates were suspended at a fixed concentration in 0.05 M citrate buffer, pH 4.8. A 1-ml portion of appropriately diluted culture filtrate was preheated to 50°C before adding it to 1 ml of the preheated substrate and incubating at 50°C. To terminate the incubation, the tubes were placed in a boiling-water bath for approximately 5 min. The contents of each tube were then filtered through a Reeve-Angel glass-fiber filter paper, and the filtrate was used to assay for sugars.

**(ii) Method B.** Substrates were suspended at a concentration of 100 mg/ml in 20 ml of 0.05 M citrate buffer, pH 4.8. Culture filtrates of *Trichoderma* sp. E58 were concentrated to a protein concentration of 8 mg/ml on a rotary evaporator and again assayed for cellulase activity. A 4-ml amount of the concentrated enzyme was preheated to 50°C before its addition to 20 ml of the preheated substrate and incubation for 24 h at 50°C. The same procedure as in method A was followed to terminate the reaction and assay for products.

## RESULTS

A variety of different methods for obtaining hemicellulose from aspen wood samples were initially attempted, and the pentosan, hexosan, and lignin contents were compared with those from commercially available xylan (Table 1). High pentosan values were obtained from the commercial xylan; however, 4% of this material was also detected as hexosans. The hemicellulose from the SEA had a pentosan content 6% greater than that of the commercial xylan, whereas the pentosan content of the water-soluble fractions from the steam-exploded samples were all in the range of 51 to 62% of the total material.

In previous studies we had noted that commercially available cellobiase (Novo) had a specific activity for xylanase which was even higher than its β-glucosidase activity: endoglucanase, 2.1 IU/mg; β-glucosidase, 10 IU/mg; filter paper activity, 0.2 IU/mg; and xylanase, 11.1 IU/mg. This enzyme preparation was diluted to a protein concentration of 1 mg/ml after first precipitating it with ethanol to remove the chemical inhibitors which were present. Increasing concentrations of xylan were incubated with this partially purified enzyme preparation, and the amount of reducing sugars liberated during the time of incubation was measured (Fig. 1). A total of 100% of the 1-mg/ml xylan sample was detected as reducing sugars after 60 min, whereas over

TABLE 1. Lignin, pentosan, and hexosan content of various lignocellulosic materials used as substrates[a]

| Substrate[b] | % of total | | |
|---|---|---|---|
| | Lignin | Pentosans | Hexosans |
| Xylan (Sigma) | <0.1 | 79.5 | 4.1 |
| SEW-WS1 | 11.5 | 60.4 | 15.2 |
| SEW-WS2 | 12.2 | 61.5 | 16.8 |
| SEW-WS2-0.1%A | 17.5 | 56.3 | 15.3 |
| SEW-WS2-0.2%A | 13.1 | 51.0 | 16.1 |
| SEA | 0.5 | 85.5 | 3.8 |

[a] Values are not additive to 100% because values for ash content, uronic acids, etc., were omitted.

[b] See text for full description of substrates.

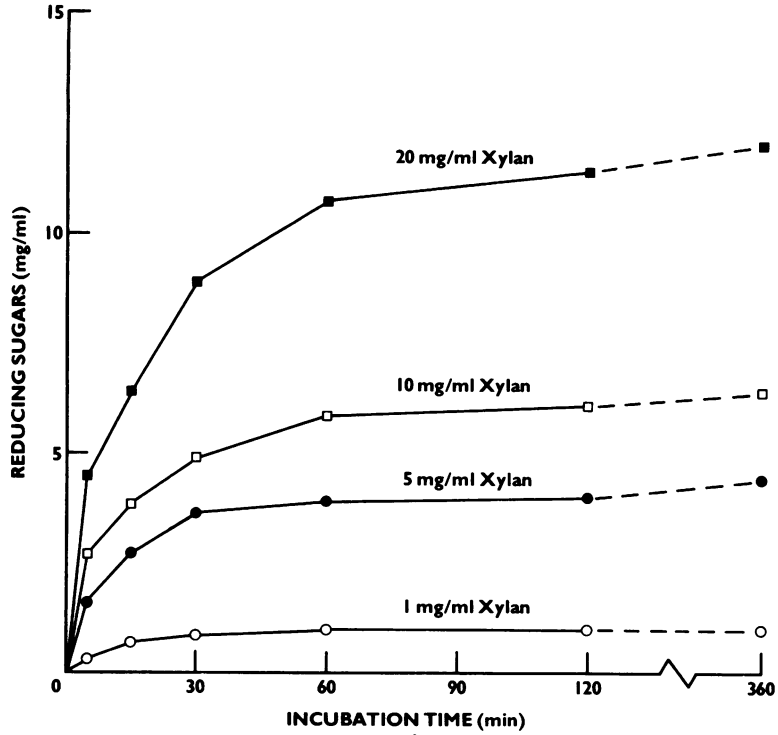


FIG. 1. Hydrolysis of increasing concentrations of xylan by Novo cellobiase.

50% of all of the other xylan concentrations were also hydrolyzed after this time. The greatest amounts of reducing sugars detected were after 6-h incubation with the 20-mg/ml xylan substrate, where 12.5 mg of reducing sugars per ml was obtained. However, this was equivalent to hydrolysis of only 62.5% of the initial substrate.

The same enzyme preparation derived from the ethanol-precipitated Novo cellobiase was incubated with various pretreated aspen wood substrates (Table 2), and the amounts of reducing sugars and monosaccharides liberated were measured. Fifty to 60% of each of these samples was hydrolyzed to reducing sugars after 3 h of incubation, with the commercial xylan being the most readily hydrolyzed, whereas hemicellulose fractions from the pretreated wood were all similarly hydrolyzed. The monosaccharides liberated by the hydrolysis of these samples, however, were all equally low, falling within the range of 1.3 to 1.6 mg/ml and amounting to less than 15% of the reducing sugars detected.

To see whether we could alleviate this problem, a variety of different cellulolytic fungi were screened and their culture filtrates were assayed for high specific xylanase activity (Table 3). All of the *Trichoderma* spp. cultures assayed exhibited high xylanase activity as well as high cellulase activity. *Trichoderma* sp. E58 was used as

TABLE 2. Sugars released from different pretreated wood substrates after incubation with 1 mg of ethanol-precipitated Novo cell cellobiase per ml[a]

| Substrate (20 mg/ml)[b] | Pentosan originally present as % of total | Reducing sugars (mg/ml) | Monosaccharides (mg/ml) | | | |
|---|---|---|---|---|---|---|
| | | | Xylose | Galactose | Glucose | Total |
| Xylan (Sigma) | 79.5 | 11.7 | 1.5 | 0.1 | 0.1 | 1.7 |
| SEA | 85.5 | 10.8 | 1.4 | 0.2 | 0.1 | 1.7 |
| SEW-WS2 | 61.5 | 9.5 | 1.2 | 0.1 | 0.1 | 1.4 |
| SEW-WS2-0.1%A | 56.3 | 9.7 | 1.3 | 0.1 | 0.1 | 1.5 |
| SEW-WS2-0.2%A | 51.0 | 9.9 | 1.3 | 0.1 | 0.1 | 1.5 |

[a] Samples were incubated for 3 h at 50°C, and the reaction was terminated by placing in a boiling-water bath for 10 min.

[b] See text for full description of substrates.

TABLE 3. Specific cellulase and xylanase activities of culture filtrates from cellulolytic fungi

| Enzyme source | Filter paper activity (IU/mg) | Xylanase activity (IU/mg) | Original protein concn in culture supernatant (mg/ml) |
|---|---|---|---|
| *Trichoderma* sp. E58 | 2.3 | 132.6 | 1.6 |
| *Trichoderma reesei* C30 | 2.6 | 130.4 | 2.2 |
| *T. viride* D39 | 1.0 | 59.0 | 1.0 |
| *T. viride* D59 | 2.7 | 119.1 | 0.9 |
| *Phialophora americana* | 0.5 | 11.6 | 0.5 |
| *Scytalidium lignicola* | 1.1 | 20.1 | 0.3 |
| *Libertella* spp. | 0.1 | 2.0 | 0.1 |
| *Myrothecium verrucaria* | 1.6 | 50.5 | 1.5 |
| Novo cellobiase | 0.1 | 5.9 | NA[a] |
| Novo celluclast | 1.7 | 8.5 | NA |

[a] NA, Not applicable.

the source of xylanase activity for subsequent work because of the high xylanase activity and its relatively high protein production. When the culture supernatant of *Trichoderma* sp. E58 diluted to a protein concentration of 1 mg/ml was incubated with increasing concentrations of xylan (Fig. 2), a marked increase in hydrolysis was obtained compared with that obtained when the Novo cellobiase preparation was used. Approximately 75% of the 20- and 10-mg/ml xylan preparations were hydrolyzed to reducing sugars after 2.5 h of incubation, whereas 42 and 31% of the 50- and 100-mg/ml xylan solution, respectively, were hydrolyzed to reducing sugars after this time. When an ethanol-precipitated enzyme preparation from *Trichoderma* sp. E58 was used to hydrolyze the pretreated wood substrates (Table 4), more than 50% of the reducing sugars detected were present as monosaccharides. Again, the hemicellulose fraction obtained by solvent extraction (SEA) gave values similar to those obtained with the commercial xylan, whereas the samples which had been slightly acidified before steam explosion were more easily hydrolyzed to reducing sugars and monosaccharides than the nonacidified sample.

Two different microorganisms were used as possible vectors for converting the sugars liberated from the hydrolyzed hemicellulose fractions to liquid fuels. In the first of these approaches, *C. acetobutylicum* was initially grown on sugars which were reported as being present (22) in wood hemicellulose hydrolysates (Table 5). The highest acetone, butanol, and ethanol values were obtained after growth on glucose and cellobiose, whereas those obtained after growth on xylose and galactose were the lowest.

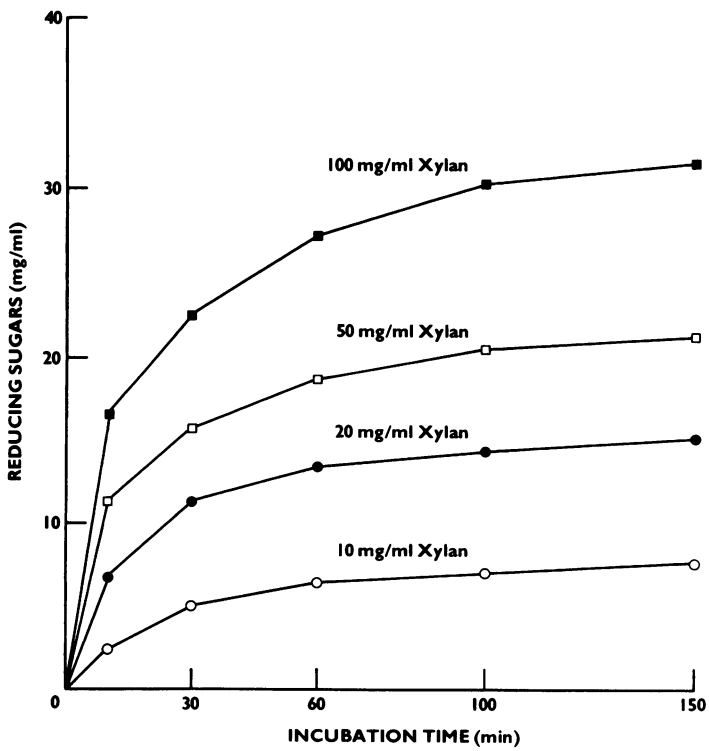


FIG. 2. Hydrolysis of increasing concentrations of xylan by *Trichoderma* sp. E58 culture filtrate.

TABLE 4. Sugars released from different pretreated wood substrates after incubation with concentrated culture supernatants from *Trichoderma* sp. E58[a]

| Substrate (100 mg/ml)[b] | Pentosan originally present as % of total | Reducing sugars (mg/ml) | Monosaccharides (mg/ml) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Xylose | Galactose | Glucose | Mannose | Arabinose | Total |
| Xylan (Sigma) | 79.5 | 58.6 | 22.8 | 0.5 | 4.2 | 1.6 | 4.8 | 33.9 |
| SEA | 85.5 | 56.9 | 26.3 | 5.0 | <0.1 | 2.6 | 0.1 | 34.0 |
| SEW-WS2 | 61.5 | 33.2 | 23.6 | 2.7 | <0.1 | <0.1 | <0.1 | 26.3 |
| SEW-WS2-0.1%A | 56.3 | 34.9 | 28.5 | 1.6 | <0.1 | 1.5 | <0.1 | 31.6 |
| SEW-WS2-0.2%A | 51.0 | 42.9 | 23.2 | 7.9 | 5.7 | <0.1 | <0.1 | 36.8 |

[a] A 20-ml amount of each of the substrates at a concentration of 100 mg/ml was incubated for 24 h at 50°C with 4 ml of *Trichoderma* sp. E58 at a protein concentration of 8 mg/ml.
[b] See text for full description of substrates.

Attempts were made to use enzymatically hydrolyzed, pretreated wood substrates for growth of *C. acetobutylicum* (Table 6). Although most of those substrates were apparently utilized, the solvent values obtained were generally low.

The best butanol yield was obtained from cultures grown on SEW (water-soluble fractions), with 0.26 mol of butanol being produced per mol of sugars utilized (about 48% of the theoretical yield) (10).

In the second approach, *K. pneumoniae* was first grown on those sugars normally found in hemicellulose hydrolysates (Table 7). All of the sugars were completely utilized within 24 h, with the highest butanediol and ethanol values obtained when xylose was used as the carbon substrate. Almost 50% of the xylose substrate was converted to solvents, whereas even the poorest values obtained from galactose utilization showed a 30% conversion to solvents. *K. pneumoniae* was then grown on enzymatically hydrolyzed, pretreated wood substrates (Table 8). The highest butanediol values were detected when hydrolyzed xylan was used as the substrate even though all of the available sugar was not utilized and only 60% of the initial substrate was hydrolyzed to reducing sugars. Comparatively low diol values were obtained when the water-soluble fraction from SEW was used as the substrate, with most of the products being detected as acetic acid. High acetic acid values were also obtained with the hemicellulose fraction obtained by solvent extraction (SEA), although relatively high butanediol values were obtained compared with the amount of reducing sugars that were available for utilization.

## DISCUSSION

It is increasingly apparent that for the production of liquid fuels from lignocellulosics to become more economically attractive the hemicellulose as well as the cellulose components of woody substrates must be utilized. (4; D. I. C. Wang and H.-Y. Fang, Proc. Am. Chem. Soc. Meet., 1980, p. 639–649). However, most workers who have advocated the use of sugars derived from hemicellulose have focused their attention on the utilization of xylose alone (6, 9, 23; Wang and Fang, Proc. Am. Chem. Meet., 1980). It can be seen from this work and that of others (3) that the composition of hemicelluloses depends not only on the source of the material, but also on the method of extraction. Only 80% of commercial xylan could be detected as pentosans, which corresponds to its reported structure (2) of an unbranched chain of xylopyranose residues with every fifth or sixth residue substituted at C-2 with a 4-*O*-methyl-D-glucuronic acid

TABLE 5. Substrate utilization and solvent production of *C. acetobutylicum* grown on sugars found in wood hemicellulose hydrolysates[a]

| Substrate | Substrate utilized (%) | Solvents produced (g/100 g of carbon utilized) | | | | |
|---|---|---|---|---|---|---|
| | | Ethanol | Acetone | Acetic acid | Butanol | Butyric acid |
| Glucose | 97.5 | 1.6 | 6.2 | 4.4 | 20.9 | 2.5 |
| Cellobiose | 93.5 | 2.1 | 6.0 | 3.8 | 22.5 | 2.2 |
| Mannose | 84.5 | 1.7 | 5.3 | 7.7 | 18.3 | 6.4 |
| Arabinose | 54.0 | 1.2 | 4.9 | 16.8 | 9.1 | 13.2 |
| Xylose | 61.0 | 1.3 | 2.3 | 10.0 | 7.1 | 17.6 |
| Galactose | 58.0 | 1.4 | 1.3 | 9.3 | 2.2 | 19.7 |

[a] Work was performed in 60-ml serum vials containing 10 ml of media and 2% substrate. Incubation was at 37°C under stationary conditions for 2 days.

TABLE 6. Substrate utilization and solvent production of *C. acetobutylicum* grown[a] on enzymatically hydrolyzed wood hemicellulose fractions[b]

| Substrate[c] | % of monosaccharides utilized | Solvents produced (g/100 g of carbon utilized) | | | | |
|---|---|---|---|---|---|---|
| | | Ethanol | Acetone | Acetic acid | Butanol | Butyric acid |
| Xylan (Sigma) | 87 | ND[d] | 1.5 | 6.3 | 4.0 | 7.9 |
| SEW-WS1 | 91 | ND | 1.2 | 28.4 | 13.5 | 14.8 |
| SEA | 73 | ND | 1.2 | 44.7 | 4.4 | 5.6 |

[a] Growth of *C. acetobutylicum* was carried out under conditions described in Table 5, footnote *a*.
[b] These fractions were hydrolyzed by *Trichoderma* sp. E58 enzyme activity under conditions described in Table 4, footnote *a*, before diluting to a 1% substrate concentration with culture medium.
[c] See text for full description of substrate.
[d] ND, Not detected.

unit and a small number of xylopyranose residues substituted at C-3 with arabofuranose.

We were primarily interested in utilizing the hemicellulose fraction obtained as water-soluble fractions after aspen wood had been pretreated by steam explosion, as this material can be obtained as a by-product in the pretreatment of wood to obtain cellulose for ethanol production (17). Initial results with commercial enzymes indicated that the hemicellulose fractions obtained after steam explosion could be as readily broken down to reducing sugars as the commercial xylan. Although about half of a 2% solution of the different hemicellulose fractions could be hydrolyzed to reducing sugars, very few monosaccharides were obtained with the commercial enzyme preparation. An initial screening of some known highly cellulolytic fungi indicated that some of the strains which were high in cellulase activity also had a high xylanase activity. This was of interest as the process of enzymatic hydrolysis of the hemicellulose and cellulose would be greatly enhanced if this could be carried out by the one enzyme mixture. Further enzymatic hydrolysis was carried out with the culture filtrate from *Trichoderma* sp. E58 as this strain not only had a high xylanase activity, but also had been shown to have a high β-glucosidase activity which enhanced the conversion of cellulose to glucose (16a). This enzyme preparation hydrolyzed greater amounts of the hemicellulose substrates to reducing sugars, with approximately 60% of these reducing sugars detected as monosaccharides. It is probable that the enzymatic hydrolysis of hemicellulose is dependent on the synergistic action of several enzymes, as has been demonstrated with cellulose hydrolysis, and that the amount of monosaccharides liberated will be dependent on the efficiency of this synergism as well as on the activity of the individual enzymes.

Although xylose accounted for the majority of the sugars detected in each of the hemicellulose fractions, other hexoses and pentoses were also detected. To ensure that most of the sugars present in most hemicellulose fractions would be utilized for liquid fuel production, two microorganisms which were reported to utilize these sugars were initially grown on the individual sugars to see what levels of solvents and acids could be obtained. Product yields obtained for butanol and acetone production of *C. acetobutylicum* grown on the individual sugars were as high as those obtained by other workers (7, 10, 20); however, when the hemicellulose hydrolysates were used as substrate much lower yields were obtained. These low values were partly due to the poor utilization of xylose by *C. acetobutylicum*, and as this monosaccharide was probably the major substrate available, the

TABLE 7. Substrate utilization and solvent production of *K. pneumoniae* grown on various carbon sources[a]

| Substrate | Solvents produced (g/100 g of carbon utilized) | | | |
|---|---|---|---|---|
| | Ethanol | Acetic acid | 2,3-Butanediol | Total |
| Glucose | 7.4 | 9.6 | 21.4 | 38.4 |
| Cellobiose | 8.6 | 10.3 | 18.7 | 37.6 |
| Mannose | 9.8 | 15.1 | 24.8 | 49.7 |
| Arabinose | 8.4 | 12.4 | 22.6 | 43.4 |
| Xylose | 10.3 | 10.5 | 26.9 | 47.7 |
| Galactose | 6.5 | 9.3 | 14.4 | 30.3 |

[a] Cultures were grown at 37°C for 24 h (see text). All sugars (at 1% concentration) were completely utilized within 24 h.

TABLE 8. Substrate utilization and solvent production of *K. pneumoniae* grown[a] on enzymatically hydrolyzed wood hemicellulose fractions[b]

| Substrate | % of monosaccharides utilized | Solvents (g/100 g of carbon utilized) | | | |
|---|---|---|---|---|---|
| | | Ethanol | Acetic acid | 2,3-Butanediol | Total |
| Xylan (Sigma) | 92 | 7.2 | 11.4 | 35.2 | 53.8 |
| SEW-WS1 | 100 | 5.4 | 39.5 | 1.9 | 46.8 |
| SEA | 100 | 14.2 | 56.4 | 22.2 | 92.8 |

[a] Growth of *K. pneumoniae* was carried out under conditions described in Table 7, footnote *a*.
[b] Substrates (described in text) were hydrolyzed under conditions as described in Table 4, footnote *a*, before diluting to a 1% substrate concentration with culture medium.

low butanol and acetone values obtained were within the expected range. It is interesting that the highest butanol yields were obtained after growth on the water-soluble fractions of SEW, since water extraction is normally the procedure used to remove inhibitors from the insoluble cellulose fraction in the utilization of cellulose for liquid fuel production (8). It is possible that the organism may be resistant to the level of inhibitors present. The relatively high production of butanol may also be a result of low-molecular-weight oligomers such as cellobiose being present in the same fraction. It has been shown previously that *C. acetobutylicum* can efficiently utilize this sugar for solvent production (13).

Contrary to the results obtained with *C. acetobutylicum*, *K. pneumoniae* produced the highest amounts of ethanol and diol when xylose was used as the substrate, and relatively high values were obtained with most of the other sugars. High butanediol values were obtained when this microorganism was grown on the hydrolysates of commercial xylan and the hemicellulose derived by solvent extraction. The best values were obtained when the hemicellulose from SEA was used as the substrate, as over 50% of this substrate was converted to solvents and acid. Although monosaccharides present in the water-soluble fraction of SEW were completely utilized by *K. pneumoniae*, acetic acid was the major product detected, with a low level of diol being produced. The basis of such a shift in the metabolic pathway of this organism is at present undefined, but it appears likely that inhibitors present in the fraction, such as furfural and phenolics, may interfere with the enzymatic production of butanediol. This can be through a direct repression on the synthesis of enzymes responsible for diol production, viz., pH 6 acetolactate-forming enzyme, acetolactate decarboxylase, and diacetyl (acetoin) reductase, or by inhibition of the activities of the enzymes formed. Further work is presently being carried out to study the effects of furfural and other inhibitors commonly found in wood hydrolysate. Results obtained from diol production from enzymatically hydrolyzed xylan and SEA indicate that the use of *K. pneumoniae* grown on hemicellulose hydrolysates has potential as a means of producing "power solvents" from hemicellulose as well as in significantly affecting the economics of producing liquid fuels from lignocellulosics.

We are presently comparing the efficiencies of enzymatic and chemical hydrolysis of the different hemicellulose fractions as well as trying to increase the butanediol production of *K. pneumoniae* grown on the hemicellulose fraction obtained as a by-product during steam explosion.

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