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HEAT TREATMENT OF ORGANICS
FOR
INCREASING ANAEROBIC BIODEGRADABILITY

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A. INTRODUCTION

The objective of this study is to evaluate thermochemical pretreatment as a method for increasing the anaerobic biodegradability of organic materials so that they can be more completely fermented to methane gas, a potential source of fuel. The current study has four specific phases: (1) biological conversion of lignocellulose to methane, (2) biodegradation of lignin and lignin fractions, (3) pretreatment of nitrogenous organics for increasing biodegradability, (4) biodegradation of lignin aromatic compounds, and (5) biochemical methane potential and toxicity testing.

Results are reported for phases one, two, and three. No new information is available for phases four and five at this time.

B. BIOLOGICAL CONVERSION OF LIGNOCELLULOSE TO METHANE

K. Baugh and P. L. McCarty

Background

Previous investigations at Stanford (Owen et al., 1978) have shown that staged heat treatment has the best potential for maximizing the solubilization of organics from wood products. The increased solubilization of organics by staged autohydrolysis offers a potential for increasing the bioconversion efficiency of lignocellulosic materials to methane in anaerobic digesters. Besides maximizing the yield of solubilized organic materials, staged heat treatment of wood products could also reduce the formation of toxic products present in all heat-treatment products attributable to soluble dehydration products (furan compounds) formed from heat treatment of polysaccharides.

Staged heat treatment was developed by Owen to increase the solubilization of the biodegradable fraction of wood products, the hemicellulose and cellulose. The first stage is a mild heat treatment for solubilization of the easily solubilized polysaccharides, notably hemicellulose, while minimizing condensation reactions. The two following stages require more severe conditions of temperature and heating duration to separate the more resistant polysaccharides, such as cellulose. Thus, a non-biodegradable lignocellulosic material is converted into two easily separable fractions: a soluble fraction resulting from carbohydrates, and a particulate residue consisting mainly of lignin condensation products. This report contains the results of Biochemical Methane Potential (BMP) tests of the products of a three-stage heat treatment.

The BMP results will show if increasing the solubilized organic concentration by staged autohydrolysis also increases the biodegradability of wood products.

Procedures and Results

A white fir slurry (61.5 g/l total solids) was hydrolyzed in staged, sequential reactions as reported previously (Owen et al., 1978). In Stage 1, 1 liter of white fir slurry was heated to 200°C and immediately cooled to room temperature. Stages 2 and 3 were both conducted for two hours at 225°C. After each stage, the product was assayed for total and soluble COD, and the particulate fraction was separated by filtration. The recovered particulate

solids were resuspended in 0.75 liters of deionized water and treated in the subsequent stage. All chemical analyses, solids and chemical oxygen demand (COD), were performed in accordance with Standard Methods (1976). A representative sample was taken from the products (total and soluble) of each heat treatment stage to determine biodegradability by the BMP test (Owen et al., 1979).

The results from the stage heat treatment of white fir are shown in Table 1. The COD values are similar to those reported previously (Owen et al., 1978). The particulate yield (32.4%) approximates 81 percent of the COD represented by the lignin fraction, and the soluble yield (48.8%) approximates 82 percent of the carbohydrate COD. These results confirm that a three-stage scheme is effective in recovering a significant portion of the initial solids either as soluble products or insoluble residue. The BMP and anaerobic bioconversion efficiencies for the autohydrolysis products of white fir are summarized in Table 2. The biodegradability of the solubilized products was quite high, with the first stage showing almost an eighty percent bioconversion efficiency while the last two stages were slightly under sixty percent. The overall bioconversion efficiency of the solubilized organics when referenced to the initial feed total COD was 32 percent. This compares reasonably to an estimated maximum overall bioconversion efficiency of 40 percent by Owen (1979). Some improvement in the overall bioconversion efficiency should be possible when difficulty in solids recovery between stages is minimized.

Summary

In summary, staged autohydrolysis pretreatment of a relatively nonbiodegradable forest product residue can be used to maximize the production of biodegradable materials through the solubilization of the carbohydrate fraction of the residue.

Future studies will further address the potential of staged treatment. The various soluble organic compounds formed during staged autocatalytic treatment will be determined to evaluate potential for recovery and influence on the overall process.

TABLE 1
SUMMARY OF 3-STAGE AUTOHYDROLYSIS OF WHITE FIR

Stage	Reaction Conditions ^a		Initial Feed COD (g/l)	Product COD (g/l)			Percent of Initial Feed COD ^b	
	Temp. (°C)	Time (hr)		Total	Soluble	Particulate	Particulate	Soluble
1	200	0.0	77.4	62.9	17.1	45.8	59.2	22.1
2	225	2.0	50.6	50.6	13.7	36.9	43.2	16.0
3	225	2.0	20.9	20.9	5.2	15.7	32.4	10.7
Reaction Sum							32.4	48.8

^aFirst stage employed 1-liter liquid volume; for stages two and three, a portion of the recovered particulates from the preceding stage was resuspended in 0.75 liter liquid volume.

^bRespective fraction referenced to the initial feed COD of stage one, and based on percent particulate recovery of preceding stage as referenced to feed.

TABLE 2

BIOCHEMICAL METHANE POTENTIAL OF 3-STAGE AUTOHYDROLYZED WHITE FIR

Stage	Reaction Conditions ^a		Referenced to Product				Overall Bioconversion Efficiency of Soluble COD Referenced to Initial Feed COD (%)
			BMP (1 CH ₄ /g COD)		Bioconversion Efficiency ^b (%)		
	Temp. (°C)	Time (hr)	Total	Soluble	Total	Soluble	
1	200	0.0	0.094	0.27	26.7	77.1	17.0
2	225	2.0	0.089	0.19	25.4	54.3	8.7
3	225	2.0	0.048	0.20	13.7	57.1	6.1
Reaction Sum							31.8
Column No.	1	2	3	4	5	6	7

^aReaction conditions as per Table 1, note (a).

^bBased on theoretical BMP of 0.35 l CH₄/g COD at STP.

^cOverall bioconversion efficiency = (Column 6, Table 2) x (Column 8, Table 1) x 100%.

C. BIODEGRADATION OF LIGNIN AND LIGNIN FRACTIONS

P. J. Colberg and L. Y. Young

Previous studies in our laboratories at Stanford have related enhanced biodegradability of lignocellulosics to the solubilization of lignin during alkaline heat treatment (Healy et al., 1977). Chemically altered lignin fractions separated according to molecular weight were examined for their biodegradability and toxicity.

Preparation of Peat Lignin for Biodegradation Studies

Preparation of peat lignin and its separation by gel filtration chromatography has been described in previous reports (Healy et al., 1978). For purposes of identification, the largest peak of MW ~ 1400⁺ is designated Fraction 1 in this report; a smaller peak of MW ~ 600 is Fraction 2; and the smallest peak of MW ~ 200 is designated Fraction 3.

Individual fractions were added to pre-reduced, defined media as the sole source of carbon according to the Biochemical Methane Potential (BMP) and Anaerobic Toxicity Assay (ATA) protocols developed by Owen et al. (1979). Since the quantities of Fractions 2 and 3 eluted from the column with each passing were small, repeated fractionations were required in order to obtain sufficient quantities of substrate for biodegradation experiments. Consequently, the serum-bottle size was also reduced to a 14-ml capacity. The total liquid volume was 12 ml (medium + substrate = 10 ml; seed = 2 ml), with a void space of 2 ml. The Total Organic Carbon (TOC) concentration (mg carbon/l) tested for each fraction in the BMP and ATA tests were as follows: 100, 300, 500, 750, 1000, 1200, and 1500. The seed organisms were obtained from a laboratory digester fed waste-activated sludge. Gas volumes were measured periodically during the 30-day incubation period and gas composition was determined by gas partitioning analysis.

Results and Discussion

A comparison of the percent total carbon converted to CO₂ and CH₄ for each of the three peat fractions tested in the BMP test resulted in the following observations (see Table 3).

1. For Fraction 1 samples, the lowest concentration (100 mg/l) had the most carbon conversion to gas. Among the other concentrations, which were all significantly lower, there were no major differences.

TABLE 3
CONVERSION OF LIGNIN FRACTIONS TO GAS

	Percent TOC Converted to CO ₂ and CH ₄						
	100 mgC/l	300 mgC/l	500 mgC/l	750c mgC/l	1000 mgC/l	1200 mgC/l	1500 mgC/l
Fraction 1 (MW ~ 1400)	10.8 ± 2.9	4.1 ± 4.7	2.3 ± 1.5	3.6 ± 2.4	3.7 ± 0.5	3.5 ± 0.1	4.9 ± 0.1
Fraction 2 (MW ~ 600)	5.6 ± 1.2	3.3 ± 1.2	2.8 ± 3.8	4.2 ± 3.0	4.2 ± 7.8	6.1 ± 1.6	5.9 ± 2.8
Fraction 3 (MW ~ 200)	8.1 ± 1.6	5.8 ± 2.0	1.5 ± 0.4	4.6 ± 0.5	5.4 ± 1.7	4.4 ± 1.4	5.3 ± 0.6
Values reported as mean ± standard deviation.							

2. There were no apparent differences among the samples in Fraction 2; whereas, in Fraction 3, the lowest concentration (100 mg/l) tended to provide more carbon conversion than the higher concentrations.
3. When examining the conversions between the different fractions, Fraction 1 at 100 mg/l displayed greater conversion to gas than Fraction 2.
3. Although the overall conversions were small, the larger values at the lowest concentration suggests some toxicity occurred in the other samples.

If the BMP results for each of the three fractions are totalled for substrate concentrations corresponding to those reported by Owen et al. (1979) in an equivalent alkaline heat-treated peat, the results concur. Owen et al. (1979) conducted BMP tests on whole (unfractionated) peat and observed a 20 percent conversion of substrate carbon to CH_4 . If the appropriate data are totaled from each of the three fractions in this study, a conversion of 17.3 percent of substrate carbon to CH_4 is realized.

It is evident from the data that all fractions tested produced CO_2 and CH_4 , although the overall conversions of substrate carbon to gas were small. There does not appear to be any trend regarding molecular-weight fractions and biodegradability. However, a number of considerations in addition to molecular size complicate the interpretation of available data.

The most obvious complication may be that the seed organisms did not acclimate to the substrate in the 30-day test period. Upon longer incubation and repeated feedings, it may be possible to selectively enrich for a more active population of microorganisms.

Because of the similar percentage conversions of substrate carbon to gas observed between fractions, it might be surmised that the same types of compounds are undergoing degradation within each fraction regardless of fraction size. However, since the fractions are physically separated via gel filtration chromatography, these suspected compounds would have to be unaffected by molecular sieving and be eluted indiscriminately with each fraction. Large MW carbohydrates such as cellulose might meet this criterion, yet available evidence indicates that cellulose is solubilized during alkaline heat treatment by an end-wise degradation scheme with the production of isosaccharinic acid (MW 192), which, in turn, should be eluted with Fraction 3 based on its molecular size. If cellulose solubilization were incomplete following

alkaline pretreatment, the remaining cellulose fragments might have different molecular weights and would elute with the different fractions.

Based on the observed data, it might be suspected that fractions of peat lignin exert toxic effects regardless of molecular size. ATA data for these fractions were not conclusive, since controls in this study yielded only 50 percent of expected gas production from the acetate-propionate spike. Consequently, ATA tests on peat fractions are currently being retested.

The observed degradation of peat fractions may be due, at least in part, to attack on functional groups. Although the number of available functional groups is probably greater in the larger MW fractions, steric effects may retard attack, accounting for the insignificant differences in degradation between fractions. Or it is possible that degradation of the two large fractions is limited to functional groups only, while organic acids of carbohydrate origin (Gossett, 1977) are perhaps being degraded in Fraction 3.

Future work

In addition to developing experiments to test some of the questions posed in this study, current work is focusing on development of reverse-phase High Pressure Liquid Chromatography (HPLC) for classification and identification of potential degradation products, preparation of ^{14}C -(lignin)-lignocellulose (Crawford and Crawford, 1976) for tracing the degradation of lignin-derived structures and compounds, and gas chromatographic analyses of peat BMP cultures for volatile fatty acid content (Healy, 1979).

D. PRETREATMENT OF NITROGENOUS ORGANICS

D. Stuckey and P. L. McCarty

The effect of thermochemical pretreatment on the degradability and toxicity of pure nitrogen compounds and sugars is currently being investigated.

Biodegradability of Amino Acids, Sugars and Bases

In order to more fully understand the behavior of complex nitrogenous organics under thermochemical pretreatment, the individual components were studied separately.

The percentage of each of these components converted to methane was assessed using the Biochemical Methane Potential (BMP) bioassay. Destruction of each component was assessed both before and after thermochemical pretreatment at 200°C for 1 hour and in the presence of 300 meq/l NaOH.. Data after 35 and 78 days are set out in Table 4. Also, in order to obtain some idea of the effect of pretreatment on the chemical structure of the pure components, chemical analyses were carried out for NH_3 and organic nitrogen. These data are included in Table 4 and indicate NH_3 as a percentage of the total nitrogen measured (organic N + NH_2), and the percentage of total nitrogen measured compared to the theoretical value.

With the untreated amino acids, after 35 days, approximately one-third were highly degradable, one-third were moderately degradable, and the remainder were quite refractory. This behavior seems to be related in a general sense to structure. If the functional group of the amino acid is either branched, or contains a ring compound, the acid appears to be harder to degrade.

After 78 days of degradation the degradability of only three acids had increased significantly: valine, leucine and tryptophan. Since these compounds are toxic at the concentrations tested (see Table 5), it is possible that this increase could be due to acclimation to the toxic effect.

Of the nucleic acid bases, four of them were moderately degradable, while thymine was virtually inert. After 78 days thymine was 26 percent degradable, while there was not significant increase in the other bases. Since thymine is identical to the other pyrimidines except for a methyl group on the fifth carbon, it is apparent that small structural changes can have profound effects on degradability.

TABLE 4
BMPs OF AMINO ACIDS, SUGARS AND BASES

Component	Destruction of Pure Component		Destruction of Component after Pretreatment ^a		Effect of HT on N	
	35 days	78 days	35 days	78 days	% NH ₃ total N	% Theoretical N Accounted For
Alanine	91	91	87	83	0.3	79
Valine	20	90	16	74	2	87
Leucine	18	70	16	30	1	96
Isoleucine	86	86	93	95	1	90
Proline	90	88	87	87	0.5	66
Phenylalanine	73	74	37	91	21	7
Tryptophan	59	75	59	83	3	93
Methionine	82	90	68	69	8	82
Glycine	94	92	92	91	2	88
Serine	99	98	67	69	14	111
Threonine	97	97	35	35	5	86
Cysteine	94	96	12	12	83	77
Tyrosine	72	76	45	52	1	95
Asparagine	83	91	84	80	67	86
Aspartic acid	79	73	88	82	50	103
Glutamic acid	39	37	120	118	2	95
Lysine	77	78	70	74	4	44
Arginine	-	-	-	-	49	63
Histidine	58	57	60	58	4	61
Cystine	64	71	6	15	74	89
Adenine	73	61	89	78	8	97
Guanine	71	50	65	69	14	79
Uracil	77	77	62	69	17	91
Thymine	0	26	1	36	6	88
Cytosine	61	64	24	26	75	53
Glucose	92	95	36	39	-	-
Ribose	97	99	35	38	-	-
Deoxyribose	91	93	4	6	-	-
Glucose/leucine (20/20)	68	66	38	41		
Uracil/ribose (17.1/22.9)	95	95	52	53		

^a200°C for 1 hour and with 300 meq/l NaOH.

TABLE 5

ATAs OF AMINO ACIDS, SUGARS AND BASES EXPRESSED AS 7-DAY MMRR^a
(All stock solutions 40 g/l, mesophilic condition used)

Chemical		Non-Heat Treated Controls (1/2 Dilu.)	Heat-Treated Samples, 200°C NaOH (300 meq/l)		
			1/30 Dilu.	1/5 Dilu.	1/2 Dilu.
Alanine	non-polar R groups	0.43	1.10	0.62	0.22
Valine		0.42	1.10	0.62	0.20
Leucine		0.42	1.17	0.56	0.39
Isoleucine		0.41	0.87	0.51	0.11
Proline		0.72	0.65	0.38	0.22
Phenylalanine		0.14	0.89	0.57	0.04
Tryptophan		0.02	0.71	0.04	0.01
Methionine		0.39	0.96	0.56	0.06
Glycine	unchanged polar R groups	0.20	0.52	0.26	0.15
Serine		1.09	1.13	0.85	0.18
Threonine		0.22	0.97	0.25	0.10
Cysteine		0.11	0.35	0.01	0.01
Tyrosine		0.58	0.79	0.63	0.25
Asparagine		0.61	1.11	0.76	0.39
Aspartic acid	negative at pH 6	0.61	0.92	0.58	0.16
Glutamic acid		1.10	1.17	1.03	0.58
Lysine	positive at pH 6	0.28	1.29	0.85	0.03
Arginine		0.14	1.20	0.79	0.17
Histidine		0.61	0.89	0.65	0.22
Cystine		0.16	0.48	0.0	0.0
Adenine	purines	0.39	0.59	0.16	0.15
Guanine		0.17	0.93	0.68	0.43
Uracil	pyrimidines	0.68	1.12	0.73	0.08
Thymine		0.0	1.08	0.60	0.49
Cytosine		0.37	1.10	0.49	0.05
Glucose	sugars	0.06	1.00	0.01	0.01
Ribose		0.06	0.98	0.20	0.0
Deoxyribose		0.03	0.78	0.53	0.0
Uracil/ribose (17.1/22.9)		0.06	0.96	0.42	0.03
Leucine/glucose (20/20)		0.0	0.87	0.0	0.0

^a Maximum methane rate ratio.

All the sugars, as expected, were highly degradable. Glucose together with leucine seemed to increase leucine degradability. Since leucine was only half as concentrated as in the pure component test, it may be that toxicity was limiting leucine degradation, and that decreasing its concentration increased its degradability. The uracil/ribose mixture was highly biodegradable.

With thermochemical pretreatment of the amino acids the overall effect was either to decrease their degradability, or to affect it very little. In only three cases did it increase degradability, and only with glutamic acid was this increase substantial. It was postulated that any significant change in chemical structure during pretreatment might be reflected in the amount of free ammonia released, and the percentage of the measurable nitrogen to the theoretical amount that should be present. A value of the latter less than 100 percent may reflect formation of complex nitrogen forms not amenable to acid hydrolysis, and hence possibly to biodegradation.

In correlating these parameters to degradability it appears generally that when the ammonia released was low, and a high percentage of nitrogen was accounted for, the degradability was either unaffected or increased. The antithesis is that when a lot of ammonia was released, the degradability tended to decrease since the structure appears to have been altered considerably.

The effect of time on amino acid degradability was in general insignificant. However, with valine, leucine, phenylalanine and tryptophan there was a considerable increase with time.

With the purine and pyrimidine bases, thermochemical treatment only increased the degradability of adenine, while thymine was unaffected. The rest had decreased degradabilities. With time only thymine increased in degradability.

Finally, as expected with the sugars, pretreatment caused caramelization and a substantial decrease in COD destruction. Again the subtle difference between ribose and deoxyribose resulted in very different behavior under treatment. Time did not appear to have any effect in increasing the degradability of the sugars.

Toxicity of Amino Acids, Sugars and Bases

The toxicity of the individual components from the first phase were also assayed for toxicity using the anaerobic toxicity assay (ATA). These data are set out in Table 5.

In previous work toxicity was assessed by taking the ratio of total gas produced over a certain time in a sample bottle, to that of a spiked control. This ratio was denoted the maximum rate ratio (MRR). However, due to high rates of CO₂ production from certain samples this figure can be misleading, and can indicate no inhibition when in fact there is significant inhibition. To overcome this problem an alternative ratio was formulated, the maximum methane rate ratio (MMRR). To calculate this ratio, gas composition and production were both monitored, and the rate of methane production over a given time period was calculated. The MMRR was then calculated as the ratio of sample methane production rate to that of the control.

From Table 5 it is apparent that at a concentration of 20 g/l all of the amino acids except serine and glutamic acid were toxic. It is outside the scope of this report to examine all the structural aspects of this toxicity; however, a few points are worth mentioning. Alanine is considerably less toxic than glycine even though the R groups are similar, with alanine having a methyl group while glycine only hydrogen. Valine and leucine have identical end groups, and behave very similarly with regard to toxicity; however, in a similar situation with aspartic and glutamic acids the behavior is not similar.

All the bases were toxic, with thymine extremely so, again the methyl substitution on thymine compared with uracil seems to make it more toxic as well as less degradable.

While the data indicate that the three sugars were toxic, problems were experienced here in maintaining the pH above 6.4 and it is felt that the data do not give a true picture. It is known from the literature that these compounds are not toxic at these levels.

The effect of thermochemical pretreatment was to increase the toxicity in all cases except with adenine and tyrosine. Even at high dilution (1/30 = 1.33 g/l), amino acids such as cysteine, cystine and glycine were still extremely toxic.

Conclusions

1. With nitrogen-containing organics very small differences in structure can lead to substantial differences in degradability.
2. Thermochemical pretreatment tends to either decrease degradability, or affect it very little.
3. The toxicity of nitrogenous organics is similar to degradability inasmuch as small changes in structure can have profound changes on toxicity.
4. Thermochemical pretreatment in almost all cases lead to increased toxicity.

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