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Acid solubilization of lignin and bioconversion of treated newsprint to methane

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Abstract

Newsprint was pretreated with acetic-nitric acid reagent to solubilize and remove the lignin component and improve its conversion to methane in anaerobic digestion. Acetic acid itself cannot dissolve lignin even at a concentration as high as 80% at elevated temperature (in a boiling water bath). In order to effectively dissolve significant amounts of lignin, nitric acid must also be added. At an acetic acid concentration of 35% with 2% nitric acid, about 80% of lignin was removed from newsprint (resulting in a weight loss of about 40%). Hydrochloric acid may partially but not completely replace the nitric acid. The methane yield from treated newsprint bioconversion increased nearly three times over that of untreated newsprint in a 60-day test. Treated newsprint generated about 75% as much methane as office paper in the same 60-day test. Acid pretreatment produced newsprint with the same cellulose content (as a percentage of volatile solids) as office paper. Despite these promising results, however, the cellulose/lignin ratio of newsprint was increased from 1.6 to only 9.9 by acid pretreatment, compared to 22.9 for office paper. Since the lignin was not completely removed, the cellulose-lignin association is considered to be the major limiting factor on long-term anaerobic digestion of both untreated and treated newsprint. An additional limiting factor to development of a practical pretreatment method based on this approach, efficient recovery of acids following lignin solubilization, was not pursued in this research.

Introduction

Waste paper has been one of the most important categories of solid waste going to sanitary landfills. It is the major contributor to solid waste by either weight or volume. Currently, approximately 40 to 50% of landfill space is occupied by waste paper (Suflita et al. 1992). The buried waste paper is subject to microbial degradation under anaerobic conditions in landfills. Cellulose is the major biodegradable fraction of waste paper. However, its association with lignin, as in newsprint and magazines, forms a physical barrier which confers resistance to natural biodegradation (Tong et al. 1990). Pretreatment of newsprint may increase the rate and extent of anaerobic bioconversion, leading to application of anaerobic digestion as a practical alternative to landfilling of lignocellulosic wastes.

To date, much research has revealed that cellulose and lignocellulose can indeed be modified by various physical, chemical and biological manipulations. Physical subdivision by ball milling can thoroughly break the cellulose-lignin complex; irradiation with high energy electrons can significantly increase digestibility of lignocellulosic materials; base and ammonia soaking can swell and separate lignocellulosic structural elements; and selectively removing lignin with white rot fungi and other microorganisms may leave cellulose basically untouched. Although some of these treatments have been put aside because of economic or other limitations, others are still being investigated in a search for effective, low cost pretreatment alternatives. Details may be found in reviews by Millet et al. (1975, 1976), Chang et al. (1981), Puls & Dietrich (1980), Tsao (1984), Kirk & Farrell (1987), Schell et al. (1991), Walker & Wilson (1991), and

Kuhad & Singh (1993). Pretreatments may be divided into physical, chemical and biological methods. Combinations of different types of treatments are also under investigation.

Acids have been reported to be used to pretreat lignocellulosic materials to hydrolyze cellulose and hemicellulose. The sugars resulting from treatment are then subject to enzymatic or microbial conversion. Acid hydrolysis of cellulose is a well-known phenomenon and can be carried out with concentrated or dilute acid. The most extensively used acids are H_2SO_4 and HCl (Grethlein 1984; Han & Callihan 1974; Millett et al. 1976). Concentrated acid (72% H_2SO_4 or 42% HCl) usually gives 90% or more conversion of potential glucan in biomass to glucose, but to be a viable process the large amount of acid used per unit of glucose produced must be recovered (Grethlein 1984). By comparing the effectiveness of various acid treatments, Wayman (1986) claimed that the process based on 41% HCl has industrial potential, but the cost of recovery of the rather expensive acid is a deterrent to large scale implementation. To avoid the recovery problem, dilute acid (1% H_2SO_4) was used to simplify the process. However, in order to get reasonable reaction rates, the temperature must be raised to above 150 °C, and the glucose yield is reduced to 50 to 65% of the potential glucan with decomposed sugar compounds as byproducts (Grethlein 1984).

No reports of acetic acid as a reagent to pretreat lignocellulosic materials were found except for its use in certain analytical procedures. In his semimicro cellulose analysis method, Updegraff (1969) used a high concentration acetic-nitric acid reagent (80% acetic acid and 7.5% nitric acid) to dissolve lignin from lignocellulosic materials prior to analyzing the remaining cellulose. With this method, the removal of lignin was very effective and complete. The objective of this study was to determine whether lower concentrations and different combinations of acids could effectively remove less degradable lignin fractions from newsprint, potentially facilitating anaerobic digestion as an alternative use of waste paper. Batch anaerobic digestion experiments were conducted on treated and untreated newsprint samples to measure improvement in bioconversion to methane.

The practicality of using acid pretreatment to remove lignin and enhance digestion of lignocellulosic materials will depend on additional factors outside the scope of this study, such as whether reagent concentrations can be lowered enough to be economical, whether there are effective ways to separate the treatment liquid from the remaining cellulose, and whether dissolved lignin components can be separated from the treatment liquid efficiently without excessive loss of acetic acid, thus allowing for reuse of acid solutions.

Materials and methods

Newsprint

Unprinted newsprint was collected from the Stillwater (OK) NewsPress printing plant. The paper contained 20–25% recycled fiber and was supplied by Abitibi-Price.

Acid treatments

A group of experiments was initiated to find the lowest acetic acid concentration that could dissolve lignin to a significant extent. Different acetic acid concentrations were used to pretreat the newsprint, then the weight losses of paper due to the treatment and separation were determined. Weight loss was due to solubilization of non-cellulosic fractions of the newsprint. Cellulose and acid insoluble lignin (AIL) were determined in untreated and treated samples in order to estimate the weight loss attributable to lignin and to other components, such as hemicelluloses. Nitric acid is part of the reagent used to solubilize lignin in the cellulose analysis method of Updegraff (1969). In order to verify the function of the nitric acid as a catalyst or just an acidifier, the efficiencies of lignin removal at different nitric acid concentrations in the treatment solution were also investigated. Since nitric acid is more oxidative and expensive than other strong acids, its replacement with other strong acids would be preferred. Therefore, hydrochloric acid was tested to totally or partially replace nitric acid.

Possible methods for separating cellulose from the treatment liquid include washing and centrifuging. In weight loss tests, centrifugation was used to separate the treatment liquid from remaining solids. Ground newsprint (0.15–0.2 g) was weighed into 16 × 125 mm Kimble Kimax tubes. Treatment solution (10 ml) was added into each tube. The tubes were heated in a boiling water bath for 30 min. After being heated, the tubes were centrifuged at 2500 rpm for 5 min, then the supernatants were discarded. Water was added into the tubes, mixed with a vortex mixer and centrifuged again. This procedure was repeated three times. The tubes were then put into a 105 °C oven until dry. The

Table 1. Weight loss with acetic acid pretreatment

Acetic acid concentration, %	Weight loss, %
0	6.04
20	6.16
40	7.16
60	7.15
80	7.19

Note: averages of duplicate samples.

Table 2. Weight loss with acetic-nitric acid pretreatment

Acids concentration, %		Weight loss, %
Acetic	Nitric	
0	0	5.18
14.5	1.3	25.6
29	2.5	37.2
44	3.9	43.7
58	5.1	46.5

Note: averages of duplicate samples.

moisture content of the newsprint was determined at the same time. The weight loss of the acid treatment was calculated according to the weight loss in the tubes.

Bioconversion of acid pretreated sample

An optimized concentration of the treatment solution, which would be able to remove a significant amount of lignin from newsprint with relatively low acid concentration, was determined. This solution was used to treat newsprint prior to the following bioconversion test.

Wheaton '400' Brand serum bottles (160 ml) were used as batch anaerobic reactors. To each bottle, 100 ml total volume of solid sample, nutrient solution and inocula was fed, and the remaining 60 ml volume served as head space. The bottles were stoppered with black butyl rubber septum-type stoppers (GeoMicrobial Technology), which were air impermeable even after repeated sampling with syringes. Stoppers were secured with 20 mm aluminum crimp seals. Triplicate bottles were incubated at 35 °C.

Mixed inocula from a landfill, anaerobically digesting sewage sludge, and rumen fluid were described by Xiao (1996). A nutrient solution was supplied to each reactor. The formula was adapted from Owen et al. (1979) and reported by Xiao (1996).

The sampling duration of the serum bottles was determined by the biogas production rate. A 30 ml glass syringe was used to collect the biogas produced and to release the pressure built up inside the serum bottles. The volume of biogas produced during the sampling period was read directly from the scale of the glass syringe. The sample was taken at 35 °C. The temperature effect was corrected to standard conditions (0 °C and 1 atm). After the biogas volume was noted, a sleeve stopper was used to cap the front end of the syringe as soon as the needle was removed. The needle was then removed from the serum bottle stopper immediately. Samples were taken with a 250 μl Hamilton gas-tight syringe from the 30 ml glass syringe, which was always held vertically with its mouth downward. Under the weight of the plunger, there was a positive pressure inside the syringe and less equilibrium time for the injection syringe was needed. The gas samples were injected into a Gow-Mac model 350 gas chromatograph (GC) equipped with a thermal conductivity detector (TCD).

Analysis techniques

Gas chromatographic method described in Standard Methods (APHA et al. 1985), Section 511B, was followed for determination of methane and carbon dioxide contents of the biogas. Total solids (TS) and volatile solids (VS) were measured according to Standard Methods (APHA et al. 1985), Section 209 and 209 D. Cellulose was measured with the methods developed by Updegraff (1969). The method described in Analysis of Paper (Browning 1977) Chapter 7, Part IIA, was adapted to analyze acid insoluble lignin (AIL).

Results and discussion

Weight loss of acid treatment

When newsprint paper was treated with acetic acid alone in a series of different concentrations, weight loss of the newsprint was shown to be minimal. The results in Table 1 clearly show that up to 80% acetic acid by itself did not solubilize lignin or other non-cellulose materials appreciably. Therefore different concentrations of acetic-nitric acid solutions were used to treat the newsprint in the next series of experiments.

Table 2 shows that with a low concentration of nitric acid, the weight loss of the newsprint increased very significantly even at relatively low acetic acid concentrations. From Table 2, in order to achieve approxi-

Table 3. Weight loss by alternative acids pretreatment

Acids concentration, %			Weight loss, %
Acetic	Nitric	Hydrochloric	
35	3	0	43.4
35	2	0	39.7
35	1	0	30.5
35	0	3	28.1
35	0	2	26.2
35	0	1	23.4

Note: averages of duplicate samples.

Table 4. Weight loss of mixed acids pretreatment

Acids concentration, volume %			Weight loss, %
Acetic	Nitric	Hydrochloric	
35	2	0	39.7
35	1	1	38.3
35	0.5	1.5	36.6
35	0.25	1.75	35.5
35	0.125	1.875	30.8
35	0	2	26.1

Note: averages of duplicate samples.

mately 40% weight loss, about 35% strength acetic acid should be used in the presence of nitric acid. The target figure of 40% weight loss should represent removal of mostly lignin and some hemicellulose from this newsprint paper, containing approximately 50% cellulose, 30% acid insoluble lignin (therefore 20% other components, inferred to be mainly hemicellulose) by our analysis. Since nitric is a strong, oxidative acid, if it could be replaced by a non-oxidative, more economical acid such as hydrochloric the practicality of acid pretreatment would be enhanced. To find the optimum nitric acid concentration and to test the feasibility of replacement by hydrochloric acid, newsprint was treated with 35% strength acetic acid in combination with nitric or hydrochloric acid at three different levels.

The results listed in Table 3 clearly indicate that nitric acid significantly increases lignin solubilization, compared to HCl. This suggests that the nitric acid most probably acts as a catalyst instead of an acidifier because at the same strong acid concentrations the weight loss of the samples is significantly different. With 2% nitric acid in the treatment solution, weight loss of the sample was about 40%.

Although it is obvious that nitric acid cannot be replaced totally by hydrochloric acid, the next experiment was conducted to verify whether partial substitution of HCl would be feasible. With acetic acid concentration still fixed at 35%, the total concentration of strong acids was set at 2% and the ratio of nitric to hydrochloric acid was varied. The results are listed in Table 4.

It is interesting to note that when the nitric acid concentration was as low as 0.125% with total strong acid concentration of 2%, a higher weight loss was observed than with 3% hydrochloric acid only (Tables 3 and 4). At the same total strong acid concentration, half of the nitric acid could be replaced with hydrochloric acid, resulting in weight loss only slightly lower than with all nitric acid treatment.

Table 5. Comparison of cellulose-lignin ratio and 60 day methane yield

Parameter	Acid pretreated newsprint	Untreated newsprint	Office paper
Cellulose, %VS	83.4 (5.8)	49.8 (1.7)	82.4 (1.4)
AIL, %VS	8.4 (0.3)	30.3 (0.5)	3.6 (0.5)
Cellulose/lignin ratio	9.9	1.6	22.9
Methane, ml gVS^{-1}	271 (10)	97.0 (12.0)	364 (27)

Note: data in parentheses are standard deviations of parameter estimates.

Bioconversion of treated sample

The newsprint sample to be used in the following bioconversion test was treated with 35% acetic acid and 2% nitric acid. The solid residue from pretreatment was rinsed in a No. 40 sieve with tap water to neutral pH, then homogenized in a Waring blender. Cellulose and AIL in the treated sample were determined, and the results are listed in Table 5. The TS and VS were also determined to quantify the cellulose and AIL components as percentage of VS. For comparison, the cellulose and AIL in the untreated newsprint and office paper are also listed in Table 5.

Table 5 shows that about 80% of the AIL was removed by the acid treatment while the cellulose percentage increased from 49.8 to 83.4% VS. Compared with office paper, AIL concentration in the treated newsprint sample is much higher while cellulose content is equivalent. This suggests that under the conditions adopted for the above pretreatment, lignin removal is not complete and a certain part of the weight loss must be due to newsprint components other than cellulose and AIL, such as hemicelluloses.

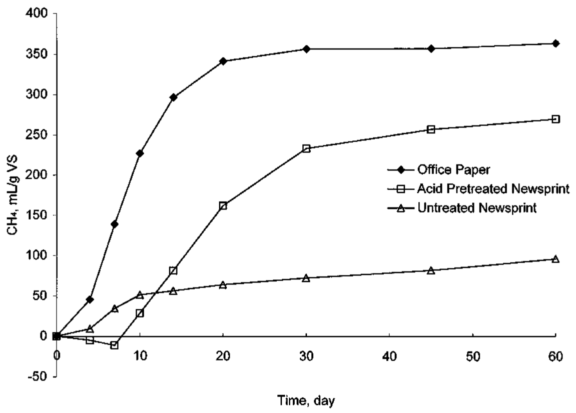

Figure 1. Methane yield comparison of acid pretreated newsprint with untreated newsprint and office paper.

The pretreated newsprint sample was used as substrate for anaerobic conversion. The results and a comparison of the acid pretreated sample with unprinted newsprint and office paper are shown in Figure 1 and Table 5, which clearly show that acid pretreatment significantly improved bioconversion of the newsprint. The methane yield of the treated sample was increased by close to 3 times on a volatile solids basis during the 60 day incubation period. Cellulose to lignin ratio was shown to exert a great effect on bioconversion. Even though the cellulose content of the acid pretreated sample was in the same range as office paper, the conversion rate and extent were appreciably lower because of its higher lignin content.

Figure 1 also shows that methane production from acid pretreated newsprint was subject to a lag phase of approximately 7 days, possibly due to incomplete washing of acid solution from the paper. Apparent negative cumulative methane production of the treated sample in Figure 1 is an experimental artifact due to the effects of the lag phase compared to the unprinted newsprint control. At this point, methane conversion rate of the pretreated sample was significantly greater than the untreated newsprint sample, but not as fast as that of office paper. After about 30 days, the cumulative methane production curves of both treated and untreated newsprint samples appear roughly linear and parallel, indicating that most of the cellulose exposed to enzymatic attack by the acid pretreatment had been converted to biogas by this time.

Possible application of acid pretreatment

Experimental results indicate that acetic-nitric-acid can effectively remove a significant portion of lignin from newsprint with much lower acid concentrations than previously known. However, the acid usage for achieving substantial lignin removal is still too high from a practical or economic point of view, unless the treatment liquid can be utilized repeatedly.

Due to its absorbent nature, the amount of paper that can be treated with a certain volume of acid solution in each batch is limited. However, during chemical treatment, the volume of solids is greatly reduced. Thus, centrifugation was used to separate the treatment liquid from the solids for reuse in the next batch treatment. It was found in these limited experiments that the recovered liquid was as effective as new acid solution in lignin solubilization for at least three uses (data not shown). Presumably, the buildup of a certain amount of solubilized lignin components may limit the effectiveness of the acid solution, but this was not observed in these experiments. Nor were any tests performed to identify or measure lignin solubilization products. Separation of these components is likely to be expensive and technically challenging, so disposal of spent acid solutions would have to be addressed at some point in implementing this process.

Conclusions

Acetic acid pretreatments have significant effects on newsprint bioconversion. Efforts to establish an acid pretreatment protocol based on analytical methods for lignin determination (80% acetic acid, with 7.5% nitric acid in a boiling water bath for 30 min) did not result in a practical pretreatment alternative. Without adding nitric acid, acetic acid by itself could not solubilize lignin, even under elevated temperature conditions. The nitric acid, which could not be completely replaced by another strong acid like hydrochloric acid, must also be added to effect lignin solubilization. About 80% of the lignin was removed from newsprint by treatment with a combination of 35% acetic and 2% nitric acids. A portion of the nitric acid may be replaced by hydrochloric acid; however, a longer reaction time may be required for treatments with lower nitric acid concentrations.

The bioconversion rate of the treated sample was significantly higher than that of the untreated sample. The methane production from treated sample volatile solids also increased by close to three times over untreated newsprint in 60 days incubation. However, in the long run, bioconversion of the treated sample will sooner or later encounter a physical barrier due to the complex association of cellulose and lignin in the remaining treated residue.

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References

APHA, AWWA & WPCF (1985) Standard Methods for the Examination of Water and Wastewater, 16th edition. American Public Health Association, Washington, DC

Browning BL (1977) Analysis of Paper, 2nd edition. Marcel Dekker, Inc., New York

Chang MM, Chou TYC & Tsao GT (1981) Structure, pretreatment, and hydrolysis of cellulose. Adv. Biochem. Engr. 20: 15–42

Grethlein HE (1984) Acid hydrolysis review. In: Ferrero GL, Ferranti MP & Naveau PH (Eds) Anaerobic Digestion and Carbohydrate Hydrolysis of Waste (pp 14–31). Elsevier Applied Science Publishers

Han YW & Callihan CC (1974) Cellulose fermentation: effect of substrate pretreatment on microbial growth. Appl. Microbiol. 27: 159–165

Kirk TK & Farrell RL (1987) Enzymatic 'combustion': the microbial degradation of lignin. Ann. Rev. Microbiol. 41: 465–505

Kuhad RC & Singh A (1993) Lignocellulose biotechnology: current and future prospects. Crit. Rev. Biotechnol. 13: 151–172

Millett MA, Baker AJ & Satter LD (1975) Pretreatments to enhance chemical, enzymatic, and microbiological attack of cellulosic materials. Biotechnol. Bioeng. Symp. 5: 193–220

Millett MA, Baker AJ & Satter LD (1976) Physical and chemical pretreatments for enhancing cellulose saccharification. Biotechnol. Bioeng. Symp. 6: 125–153

Puls J & Dietrich HH (1980) Separation of lignocellulosics into highly accessible fiber materials and hemicellulose fraction by the steaming-extraction process. In: Energy from Biomass, Proc. Int. Conf. Biomass (pp. 348–353). Brighton, UK

Schell DJ, Torget R, Power A, Walter PJ, Grohmann K & Hinman ND (1991) A technical and economic analysis of acid-catalyzed steam explosion and dilute sulfuric acid pretreatments using wheat straw or aspen wood chips. Appl. Biochem. Biotechnol. 28/29: 87–97

Suflita JM, Gerba CP, Ham RK, Palmisano AC, Rathje WL & Robinson JA (1992) The world's largest landfill: a multidisciplinary investigation. Environ. Sci. Technol. 26: 1486–1494

Tong M, Smith LH & McCarty PL (1990) Methane fermentation of selected lignocellulosic materials. Biomass. 21: 239–255

Tsao GT (1984) Bacterial hydrolysis: a review. In: Ferrero GL, Ferranti MP & Naveau PH (Eds) Anaerobic Digestion and Carbohydrate Hydrolysis of Waste (pp 83–99). Elsevier Applied Science Publishers

Updegraff DM (1969) Semimicro determination of cellulose in biological materials. Anal. Biochem. 32: 420–424

Walker L & Wilson DB (1991) Enzymatic hydrolysis of cellulose: an overview. Biores. Technol. 36: 3–14

Wayman M (1986) Comparative effectiveness of various acids for hydrolysis of cellulosics. In: Young RA & Rowell RM (Eds) Cellulose Structure, Modification and Hydrolysis (pp 265–280). John Wiley and Sons

Xiao W (1996) Anaerobic bioconversion of waste paper and newsprint (Ph.D. thesis), Oklahoma State University