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Anaerobic biodegradability of cellulose and hemicellulose in excavated refuse samples using a biochemical methane potential assay

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SUMMARY

Improved techniques are needed to predict potential methane generation from refuse buried in landfills. The Biochemical Methane Potential (BMP) test was used to measure the methane potential of ten refuse samples excavated from a Berkeley, CA, landfill. The test was conducted in 125-ml serum bottles containing phosphate-buffered medium and inoculated with anaerobically digested sewage sludge. Comparison of the measured BMP to the theoretical BMP calculated from measured cellulose and hemicellulose concentrations indicated that cellulose plus hemicellulose is not well correlated with the measured BMP. The average of the measured to theoretical BMP was 19.1% (range 0–53%, s.d. = 16.9%). Measured sulfate concentrations showed that sulfate was an insignificant electron sink in the samples tested. Once methane production from the refuse was complete, 0.072 g of Whatman no. 1 filter paper was added to two of the four serum bottles incubated for each sample. An average of 84.9% (s.d. = 2.5%) of the added filter paper was recovered as methane, suggesting that some cellulose and hemicellulose present in refuse is recalcitrant or otherwise not bioavailable.

INTRODUCTION

Approximately 196 million tons of municipal solid waste were generated in the US in 1990 and 65% of this material was disposed of by burial in landfills [25]. In addition to the 6600 existing landfills, numerous facilities were closed prior to 1986 [26]. Municipal solid waste is composed of 40–50% cellulose, 12% hemicellulose and 10–15% lignin on a dry weight basis and the cellulose plus hemicellulose fraction makes up over 90% of its methane potential [2,6]. A complex series of chemical and microbiological reactions is initiated with the burial of refuse in a sanitary landfill. The oxygen entrained in the refuse at burial is rapidly depleted, leading to the development of an anaerobic ecosystem. In the absence of nitrate and sulfate, or once these electron acceptors are depleted, the terminal products of refuse decomposition are carbon dioxide and methane.

Methane is recovered in commercial quantities at about 110 landfills in the US [23]. However, many potential methane recovery projects are rejected due to uncertainty in the volume of methane available for recovery and its rate of production. When not recovered or otherwise controlled, methane may cause an explosion hazard by lateral subsurface migration and accumulation in basements. In addition, refuse settles as it decomposes. Thus, techniques are needed to estimate the potential for: (1) future settling, (2) subsurface methane migration and (3) recovery of commercial volumes of methane. Finally, methane is a greenhouse gas and better estimates of the contribution of landfill methane to atmospheric methane accumulation are needed [17].

In this paper, we demonstrate the use of the Biochemical Methane Potential (BMP) test [5,16,21] to measure the methane potential of decomposed refuse excavated from a Berkeley, CA, USA landfill. We compare the methane potential measured by the BMP test with the theoretical methane potential calculated from measured cellulose and hemicellulose concentrations. In addition, we document that some of the cellulose present in excavated refuse samples is not available for anaerobic biodegradation.

MATERIALS AND METHODS

Refuse

Refuse was excavated in 1990 from the North Waterfront Park Landfill in Berkeley, CA, USA with a bucket auger. The landfill received refuse between 1969 and 1983. Four holes were drilled at strategic locations in the landfill and samples were collected at approximately 3-m intervals to a depth of 9–12 m in each hole. No attempt was made to date the refuse during sampling by recording dates on old newspapers. During excavation, grab samples weighing 3–5 kg were removed from the pile of excavated refuse associated with each interval. Samples were dried at 70 °C, ground to

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a particle size of 5–8 mm and shipped to our laboratory. Once in the laboratory, samples were ground in a wiley mill to a particle size of 1 mm, redried at 65 °C and stored in mason jars at 4 °C prior to testing.

Biochemical Methane Potential assay

The BMP procedure was modified from previously developed procedures [5,21]. Tests were conducted in 125-ml serum bottles (Wheaton, Millville, NJ, USA) sealed with black butyl rubber stoppers (Bellco Biotechnology, Vineland, NJ, USA) and aluminum crimps. Medium composition is presented in Table 1. The N_2/CO_2 (80/20) gas mixture was passed over a hot copper column to remove traces of oxygen.

The carbon source in the BMP test was wiley-milled refuse obtained as described above. Sufficient refuse was used in each BMP test so that the theoretical methane potential, based on complete cellulose and hemicellulose conversion to methane, was 50 ml. Theoretical methane potential was calculated using the stoichiometry presented in Eqn 1 [7]. Using Eqn 1, the calculated methane potential of cellulose ($C_6H_{10}O_5$) and hemicellulose ($C_5H_8O_4$) is 415 and 424 ml CH_4 at STP per dry g of cellulose and hemicellulose, respectively.

$$C_nH_aO_b + [n-(a/4)-(b/2)]H_2O \rightarrow [(n/2)-(a/8)+(b/4)]CO_2 + [(n/2)+(a/8)-(b/4)]CH_4 \quad (1)$$

BMP tests were inoculated with 5 ml of anaerobically digested sludge obtained just before use from the Orange County Wastewater Treatment plant in Carrboro, NC, USA. Immediately after inoculation, bottles were adjusted to atmospheric pressure using a 5-ml wetted glass syringe filled with the N_2/CO_2 gas mixture. Tests were conducted in quadruplicate and incubated at 37 °C. Background methane production associated with the inoculum was measured in a set of five controls.

To measure gas production, a 60-ml plastic syringe was used to measure the majority of the overpressure. A 5-ml glass syringe was then used to measure the remaining overpressure and to adjust bottles to atmospheric pressure. Gas volumes were corrected to dry gas at STP. Gas production was measured after 28 days and again after 43 days. The absence of additional methane production on day 43, after correction for background, suggested that biodegradation of the refuse samples was essentially complete.

Where average methane production in a set of four test bottles was significantly greater than that in the controls on day 43 ($P = 0.05$), methane production due to refuse was calculated by subtracting methane production in the controls from that in the test bottles. Where methane production was not significantly greater than that in the controls, the BMP is reported as 0. Background methane production was 3.4 ml (s.d. = 1 ml) on day 43. Dissolved methane was assumed to be comparable in the control and test bottles and no additional correction for dissolved methane was made.

Cellulose spike

As presented in the Results, the measured BMP was well below the BMP calculated from Eqn 1. To explore whether carbon availability or toxicity limited methane production, a cellulose spike was added on day 43 to two of the four bottles used to test each sample; 0.072 g of Whatman no.1 filter paper (99% cellulose) was added to two randomly selected bottles for each sample. The filter paper was ground in a wiley mill to pass a 1-mm screen prior to use. The mass added had a theoretical methane potential of 29.57 ml. To add the filter paper, a serum bottle was opened under a stream of N_2/CO_2, 0.072 g of preweighed filter paper were added and, after about 10 min, the stopper and aluminum crimp were replaced. This procedure served to maintain anaerobic conditions and remove all methane from the bottles. It was applied equally to all serum bottles and controls, whether or not they received a filter paper addition. Methane production was measured 41 days after the filter paper addition. Net methane production from filter paper was calculated by correction for: (1) methane produced from the original controls initiated to measure methane production from the inoculum and (2) additional methane produced from the refuse samples after day 43 in the two bottles for each sample which did not receive filter paper. The total correction was an average of 1.2 ml (s.d. = 1.2 ml), or about 4.6% of the total methane measured in the cellulose spike experiment. All of the correction was attributed to background methane production from the inoculum controls.

Analytical methods

The concentrations of methane and carbon dioxide were measured on a Gow Mac gas chromatograph equipped with a thermal conductivity detector and an Alltech Haysep Q

TABLE 1

Medium composition

Component	per liter
PO_4 solution[a]	100 ml
M3 solution[b]	100 ml
Mineral solution[c]	10 ml
Vitamin solution[d]	10 ml
Resazurin (0.1%)	2 ml
Distilled water	768 ml
Refuse	50 ml CH_4 potential (see text)
$NaHCO_3$[e]	3.5 g
Cysteine hydrochloride[e] (5%)	10 ml

[a]The phosphate solution contained 16.1 g KH_2PO_4 and 31.89 g $Na_2HPO_4 \cdot 7H_2O$ per liter. It was prepared in carbonate-free water and stored under N_2 at 4 °C.

[b]As given in reference 3.

[c]As given in reference 13 with the addition of 0.033 g $Na_2WO_4 \cdot 2H_2O$ per liter.

[d]As given in reference 28.

[e]Added after adjustment of the media to pH 7.2 and boiling under an 80/20 mixture of N_2/CO_2.

80/100 column. Sulfates were measured either by ion chromatography (IC) or inductively coupled plasma emission spectroscopy (ICP). Sulfate concentration prior to initiation of the BMP were measured by IC using a Dionex ioscratic pump and conductivity detector and a Dionex AS4A column. A sodium carbonate/bicarbonate buffer served as the mobile phase. Sulfate concentrations at termination of the BMP test were measured as total sulfur by ICP. Sulfides were removed from samples prior to analysis by acidification to pH 2 and sparging with nitrogen to drive off any hydrogen sulfide.

Acid-insoluble lignin was measured by the sulfuric acid digestion technique [10]. Cellulose and hemicellulose concentrations were measured at the Forest Products Laboratory in Madison, WI using liquid chromatography [18]. Briefly, samples were digested in 72% sulfuric acid to convert cellulose and hemicellulose to their respective monomers. The monomers were then separated on an anion exchange column and measured using a pulsed ampiometric detector. We define degradable carbohydrates as the sum of cellulose plus hemicellulose.

RESULTS

The excavation depth of each sample and its cellulose, hemicellulose and lignin concentrations are presented in Table 2. Cover soil is typically applied to refuse on a daily basis. Thus, excavated refuse samples often consist of a mixture of soil and refuse which dilutes the chemical composition of the refuse. For this reason, others have suggested analysis of the cellulose to lignin ratio (hemicellulose was not measured) and showed that the ratio decreased with age in excavated refuse samples [6]. The cellulose and hemicellulose concentrations measured here would be expected to decrease with sample depth as depth should roughly correspond to refuse age and level of decomposition. Here, we look at the ratio of the degradable carbohydrates (cellulose plus hemicellulose) to lignin (Car/L) as a function of sample depth. In borehole B this ratio followed the expected trend while in boreholes A, C and D it did not. When all ratios are plotted together (Fig. 1), there is no discernable decrease with sample depth. The relatively high concentrations of lignin reported here (68–86%) suggest that sample dilution due to cover soil was minimal. These data also reflect the recalcitrance of lignin under anaerobic conditions [8]. As refuse carbohydrates are degraded, the lignin concentration increases.

The measured BMP (BMP_m) of each sample, the theoretical BMP based on Eqn 1 and the sulfate concentrations at the beginning and end of the BMP test are presented in Table 3. Eight of the ten samples tested had a BMP_m greater than zero.

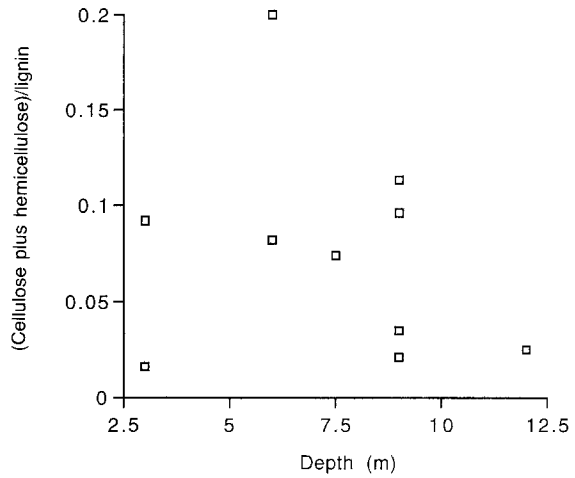

Fig. 1. Relationship between the depth of a sample and the ratio of carbohydrate content to lignin content in excavated refuse.

TABLE 2

Chemical composition of excavated refuse

Sample	Location[a]	Excavation depth (m)	Cellulose %	Hemicellulose %	Lignin %	Degradable carbohydrate[b]/ Lignin
1	A	3	0.9	0.5	85.6	0.016
2	A	6	4.5	1.5	73.2	0.082
3	A	7.5	3.8	1.9	76.9	0.074
4	A	9	1.9	0.8	77.2	0.035
5	B	6	11.7	2.5	70.9	0.20
6	B	9	5.4	1.8	75.3	0.096
7	B	12	1.5	0.6	84.5	0.025
8	C	3	1.0	0.4	85.2	0.016
9	C	9	5.7	2.5	72.1	0.113
10	D	3	4.6	1.7	68.2	0.092
11	D	9	1.0	0.5	72.0	0.21

[a]The letter differentiates boreholes.

[b]The ratio of cellulose plus hemicellulose to lignin.

TABLE 3

Biochemical methane potential of excavated refuse samples

Sample	Refuse mass used in BMP test, (g)	Measured BMP[a] (s.d.)	Theoretical BMP[b]	Initial sulfate[c] mg L^{-1} (s.d.)	Final sulfate[d] mg L^{-1} (s.d.)	Corrected theoretical BMP[e]	$\frac{BMP_m}{BMP_{th}}$[f]
1	8.48	1.0 (0.12)	5.9	35.2 (2.8)	16.7 (4.0)	5.8	0.17
2	2.02	9.1 (2.1)	24.8	17.8 (2.5)	14.5 (0.8)	24.7	0.37
3	2.1	3.2 (0.3)	23.8	110.7 (8.1)	17.6 (1.1)	22.8	0.14
4	4.46	2.5 (0.4)	11.2	29.3 (3.7)	14.6 (1.1)	11.1	0.23
5	0.84	30.7 (0.8)	59.3	92.4 (29.6)	42.6 (5.8)	57.9	0.53
6	1.66	9.1 (0.4)	30.2	31.8 (14.0)	18.0 (3.4)	30.1	0.30
7	5.91	1.0 (0.29)	8.5	26.4 (3.6)	12.1 (1.5)	8.3	0.12
8	8.62	0	5.8	32.0 (7.8)	7.5 (0.2)	5.7	0
9	1.46	0	34.3	10.5 (2.0)	11.2 (1.1)	34.3	0
10	1.9	1.6 (1.09)	26.4	48.4 (11.7)	15.7 (1.3)	26.2	0.06

[a]Biochemical methane potential in ml CH_4 corrected to dry gas at STP per g of refuse. Data are the average of four replicates followed by the standard deviation.

[b]Theoretical BMP calculated using Eqn 1 and the cellulose and hemicellulose concentrations reported in Table 2.

[c]Measured sulfate concentration in test bottle after addition of refuse and media but prior to inoculation. Data are the average of four replicates followed by the standard deviation.

[d]The concentration of sulfate in each bottle at the completion of the BMP and cellulose spike tests (Day 84). Data are the average of the four test bottles followed by the standard deviation.

[e]The theoretical BMP assuming that the difference between the sulfate present initially and at the completion of a test served as the terminal electron acceptor for the conversion of cellulose to carbon dioxide as given in Eqn 2. Sulfate consumed was calculated as the difference between the average initial sulfate mass for each set of four bottles and the final sulfate mass in each individual bottle. Only the average final sulfate concentration is presented. The initial volume in each bottle was 100 ml while the final volume, which included the inoculum, was 105 ml.

[f]Measured BMP divided by theoretical BMP corrected for diversion of electrons to sulfate reduction as described in note e.

Sulfate data were used to evaluate the significance of sulfate as an electron sink in our refuse samples. Previous work [11] showed that sulfate may be an important electron acceptor in landfills. Sulfate-reducing bacteria are reported to out-compete methanogens for hydrogen, although some methane production will occur in the presence of sulfate [20,27]. Thus, sulfate reduction could be responsible for reduced methane production in our samples. Using the initial and final sulfate concentrations in each bottle, the maximum amount of cellulose which could have been oxidized with sulfate as the terminal electron acceptor was calculated using Eqn 2.

$$C_6H_{10}O_5 + 3SO_4^{-2} + 3H^+ \rightarrow 6CO_2 + 3HS^- + 5H_2O \quad (2)$$

The theoretical BMP of each sample was corrected for sulfate reduction assuming that all sulfate consumption was used to divert electrons from CO_2 to SO_4^{-2}. The sulfate-corrected theoretical BMP (BMP_{th}) for each sample is reported in Table 3 and is at least 96% of the uncorrected data. Thus, sulfate was not a significant electron sink in the refuse tested here.

BMP_m data are presented as a function of the degradable carbohydrate concentration and Car/L in Figs 2(A) and 2(B), respectively. The BMP_m generally increased as a function of both the sum of the cellulose and hemicellulose and Car/L. The correlation coefficients are 0.68 and 0.67 in Figs 2(A) and 2(B), respectively. However, there are anomalous data points in both figures and samples with

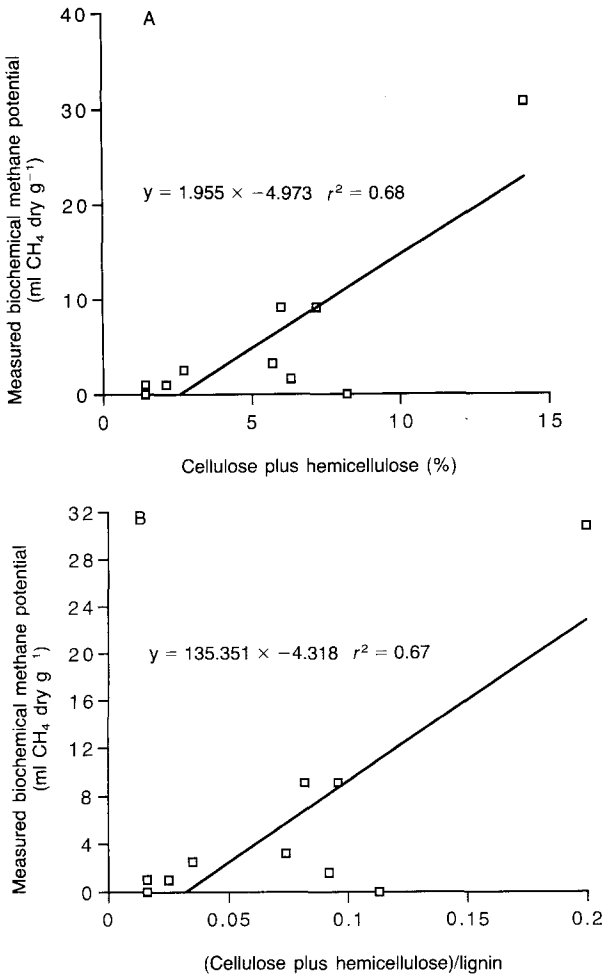

Fig. 2. Relationship between biochemical methane potential and (A) total carbohydrates (%) and (B) the total carbohydrate to lignin ratio in excavated refuse.

similar total carbohydrate concentrations exhibited large differences in BMP_m. When samples with a BMP_m of zero are deleted from the data set, the correlation coefficients for Figs 2(A) and 2(B) increase to 0.85 and 0.84, respectively. Even so, neither total carbohydrates, nor Car/L are completely reliable indicators of BMP_m. The fraction of the BMP_{th} actually measured (BMP_m/BMP_{th}) was also evaluated as a function of both degradable carbohydrates (r^2 = 0.36) and Car/L (r^2 = 0.35) (figure not shown). However, these relationships are weaker than those presented in Fig. 2.

The relatively low BMP_m/BMP_{th} (Table 3) led to the question of whether toxicity or bioavailability was limiting cellulose conversion to methane. To address this, a cellulose spike was added to two of the four bottles used for measurement of the BMP in each sample. Spike recoveries ranged from 81% to 89% of the methane volume expected from filter paper addition (avg = 84.9%, s.d. = 2.5%). These data suggest that there was not an environmental condition within the BMP test which limited refuse conversion to methane, but rather the availability of degradable carbohydrates limited methane production.

DISCUSSION

The absence of the expected trend in Car/L value (Fig. 1) may be due to the extremely low concentrations of cellulose and hemicellulose and the low Car/L measured here (Table 2) relative to other studies [6,22]. Because of the likelihood that buried refuse is mixed with cover soil when excavated, it is more appropriate to compare Car/L ratios rather than direct comparison of cellulose concentrations. Cellulose/lignin (C/L) values of 0.16–0.24 have been reported for refuse buried between 1948 and 1957 in Wisconsin [6]. The lowest C/L value in refuse excavated from Fresh Kills landfill in New York, which began operation in 1948, was 0.74 [22]. Here, Car/L never exceeded 0.21 and eight of the eleven samples were less than 0.1 despite inclusion of hemicellulose in the ratio.

The data in Table 3 and Fig. 2 suggest that the methanogenic biodegradability of the refuse samples cannot be predicted by the degradable carbohydrate concentrations, at least not over the range of concentrations tested here. Alternative explanations for the low BMP_m/BMP_{th} are: (1) there was an accumulation of acidic endproducts and degradation was not complete and (2) organic matter in the refuse served as an electron acceptor. However, these explanations are not consistent with the high recoveries of methane in the cellulose spike experiment. The results of the spike experiment indicate that where substrate was available, the most significant endproduct which accumulates is methane as opposed to other reduced organics or carboxylic acids. Thus, we conclude that an absence of bioavailable cellulose and hemicellulose limited methane production in each refuse sample. This result is particularly noteworthy for sample 9, which had a relatively high cellulose plus hemicellulose concentration (8.2%), a BMP_m of 0 and a spike recovery of 80.5%. Several samples with lower carbohydrate concentrations had a BMP_m greater than 0.

While bioavailability limited anaerobic biodegradation, the degree to which carbohydrates are bioavailable could not be predicted from measured cellulose and hemicellulose concentrations. If refuse were degraded down to a constant cellulose concentration, then there would be some basis for using stoichiometry to estimate methane yields. In order to evaluate this possibility, a theoretical final cellulose concentration was calculated based on the assumption that all sulfate reduction and methane production resulted from cellulose degradation. The range of final calculated cellulose concentrations was 0.6% to 5.7% (avg = 2.7%, s.d. = 1.7%). Thus, no statements regarding a lower level of cellulose degradation can be made here.

Refuse is composed of many individual components which are degradable to varying extents including grass, leaves, branches, food waste and several types of paper. The lignin concentration of these substrates varies from office paper, which is largely delignified, to branches and newspaper, which are highly lignified. On the whole, refuse must be considered as a lignocellulosic substrate. The presence of lignin is important because it is both a physical and chemical barrier to microbial attack [8,9,19,24]. Several studies have

shown that lignin is the major impediment to digestion of forage [1,4,9,12] and other types of biomass [24]. The lignin concentration has been related to both the rate and extent of cellulose plus hemicellulose degradation. However, the degree to which this relationship can be modeled varies between studies. Dehority and Johnston reported that cellulose digestibility was not directly correlated to the lignin concentration [9]. In their study, legume cellulose was digested to a lesser extent than the cellulose in four types of grass, although the grasses and legumes had similar lignin concentrations. Similarly, the methanogenic conversion of white fir and wood grass, which have similar lignin concentrations, was 9 and 66%, respectively [24]. However, a curvilinear relationship was shown to relate increasing lignin concentrations to a decrease in the digestibility of cellulose in eight species of grass [12].

Research on the methanogenic conversion of lignocellulosic components of municipal solid waste suggests that the presence of lignin reduces methanogenic degradability. About 40% of the cellulose in newsprint was degraded, while 90 and 97% of the cellulose in computer paper and Whatman filter paper was converted, respectively [14]. Both computer paper and filter paper are nearly completely delignified, while newsprint is heavily lignified. The methane yields of grass and office paper (low lignin) were two to three times higher than the comparable yields of newsprint and branches (high lignin) [15]. Removal of a portion of the lignin by alkaline treatment increased methane yields from fresh refuse [19].

The relationship between lignin concentration and both BMP_m and BMP_m/BMP_{th} is shown in Fig. 3. As illustrated in Fig. 3 and reflected in the correlation coefficients, samples with similar lignin concentrations exhibited large differences in both BMP_m(r^2 = 0.19) and BMP_m/BMP_{th} (r^2 = 0.11). Exclusion of data with a BMP of zero increased r^2 only marginally to 0.23 and 0.12, respectively. In a similar plot reported previously, the correlation coefficient was 0.59 [24].

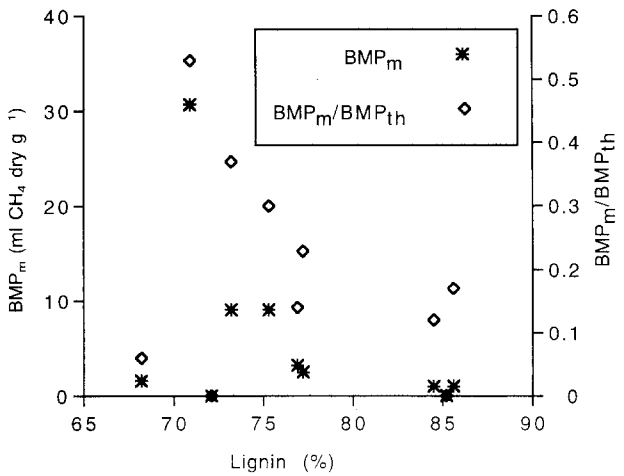

Fig. 3. Relationship between the amount of methane produced (BMP_m) and the ratio of the measured to theoretical methane as a function of the lignin concentration in excavated refuse samples.

Our results highlight the difficulty in predicting methane yields from well decomposed refuse and suggest that use of carbohydrate concentrations and Eqn 1 will produce misleading results. The BMP of a refuse sample can provide an upper bound on the amount of methane which may be produced if the refuse is allowed to continue to decompose in the landfill. Of course, environmental conditions in the BMP test are optimal for methanogenesis while conditions in a landfill will likely be suboptimal. In addition, representative sampling of landfills is difficult and numerous samples are required to gain an estimate of the state of refuse decomposition. The BMP will provide a relative measure of remaining methane potential and represents a more accurate assessment of the potential for future methane than degradable carbohydrate concentrations.

Our data also show that refuse does not decompose to a constant cellulose concentration or to a constant fraction of the theoretical yield. Clearly, other factors influence biodegradation. The results of the cellulose spike experiment suggest that toxicity was not a limiting factor. However, each sample may have contained remnants of different components of refuse which would lead to differing degrees of degradability.

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