Project no. **SES6-CT-2004-502824**

Project acronym: **CROPGEN**

Project title: **Renewable energy from crops and agrowastes**

Instrument: Specific Targeted Research Project

Thematic Priority: SUSTDEV: Sustainable Energy Systems

D7: Kinetic data from a modified BMP test with uncoupling of solids and liquid retention times

Due date of deliverable: Month 36
Actual submission date: Month 36

Start date of project: 01/03/2004 Duration: 39 months

Organisation name of lead contractor for this deliverable

University of Southampton (Soton)

Revision [0]

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)		
Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	PU
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

D7: KINETIC DATA FROM A MODIFIED BMP TEST WITH UNCOUPLED SOLIDS AND LIQUID RETENTION TIMES

1 Original rationale

Under the work package on reactor innovations (WP6) one of the aims was to look at the application of two phase digestion with the uncoupling of solids and liquids retention times. This approach has shown promise in the rapid treatment and solids destruction of certain waste fractions, for example municipal solid waste and abattoir wastes (Wang and Banks, 2000 and 2003). The original objective was to look at a rapid way of simulating this process on a small scale in order to give predictive data as to the probable methane yield and rate of production in a two phase system in which the solids and liquids retention times had been uncoupled.

2 Change of plan

The anticipated process advantages of using a two phase reactor system in which the solids and liquids retention time had been uncoupled was not apparent. A possible reason for this is that a whole crop plant material such as maize, which is rich in free sugars and starch, has a very rapid initial hydrolysis and acidification phase that is poorly buffered by the rest of the plant material. This leads to a rapid fall in pH and, even at the highest flush rates that are practically achievable, the acid fermentation products cannot be flushed from the system. The low pH leads to inhibition of the hydrolysis of the fibre material (cellulose and hemicellulose) which form most of the remaining volatile solids in the system. Hence the overall solids destruction in the first phase is low compared to better buffered substrates, and there appears to be little process advantage in adopting this approach compared to single phase digestion in the case of energy crops of this type. There may still be some advantages in phase separation without the uncoupling of solids and liquids retention time in a two stage digestion process where the hydraulic retention times in are independent of each other promoting differential acidification and methanogenesis in two different stages. There may also be some process advantage in retaining uncoupled solids and liquids retention time in a first phase reactor followed by two further separate phases, one of which further converts the acid liquid product to methane and another which further processes the fibre residue, in a conventional CSTR digester.

The results of work carried out in WP2 have indicated that the single phase Biochemical Methane Potential (BMP) test is a complex assay the results of which are influenced by many factors. Significant progress has been made on identifying some of these factors and working towards standard test protocols. While this work is incomplete, however, it was decided not to complicate the issue further with the development of a two or even three-stage procedure. The research presented in towards this deliverable has thus concentrated on measuring the product formation from the two approaches to the hydrolysis of crop material viz: in a solids liquid uncoupled phase; and in short retention time CSTR hydrolysis reactors without pH control. The research has gone further in then looking at using the products of these two types of reactor in a BMP testing strategy using CSTR digesters.

3 Measurement of hydrolysis and acidification in first stage reactors

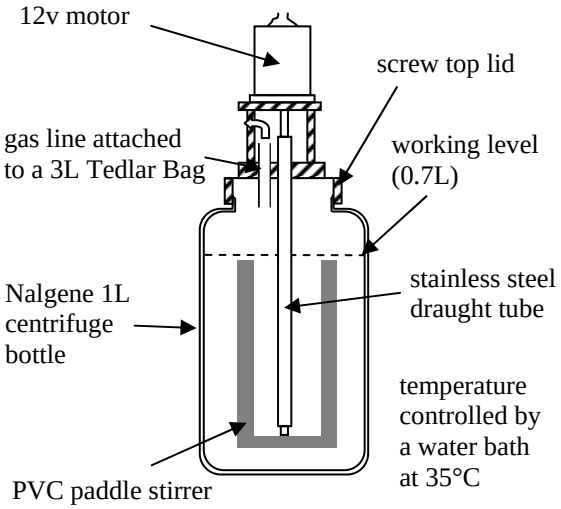
3.1 Uncoupled solids and liquid retention time (hydraulic flush reactors)

3.1.1 Method

Equipment design: The design of hydraulic flush reactor (HFR) used in this work is shown in Figure 1. A total of 8 of these reactors were used. These were operated in duplicate to give a total of eight operational conditions over two experimental runs.

The HFR were operated at a solids retention time (SRT) of 20 days and hydraulic retention times (HRT) of 4, 2.7 and 2 days, at two organic loading rates (OLR). In the first experiment the OLR was set at 2.01 gVS l^{-1} d^{-1}, and in the second at 4.03 gVS l^{-1} d^{-1}. In both experimental runs duplicate control reactors were run, which were maintained at a HRT and SRT of 114 days, i.e. there was no uncoupling of solids and liquids retention time. The SRT was set by the removal of a proportion of the mixed sludge content of each reactor, and the HRT was set by centrifuging the remaining reactor contents at 3000 rpm for 20 minutes and removing the appropriate volume of supernatant. Each HFR was then fed with ensiled maize to give the appropriate loading and tap water to maintain the desired flush volume and HRT. A summary of the conditions used in the two experiments is shown in table 1.

Figure 1 HFR Design



HF Reactor

Table 1. Conditions used in the two experimental runs

Experimental run	volumetric loading (g VS $l^{-1}d^{-1}$)	solids retention times(d)	hydraulic retention times (d)	run length (d)
1	2.01	20	4.0, 2.7,& 2.0	142
2	4.03	20	4.0, 2.7,& 2.0	92
control (run 1)	2.01	114	114	142
control (run 2)	4.03	114	114	92

Analytical Techniques: pH was read daily using a Jenway pH probe. TS, VS and SCOD were measured on 5-day composite samples using standard methods (APHA 1998 and

2005). Biogas production, when measured, was taken from water displacement in columns containing acidified tap water (pH 2). Gas composition was measured weekly using a Varian CP 3800 gas chromatograph with a gas sampling loop using argon as the carrier gas at a flow of 50 ml min^{-1}. The GC was fitted with a Haysep C column and a molecular sieve 13 x (80-100 mesh) operated at a temperature of 50 ^{o}C. The GC was calibrated using a standard gas containing 35% CO_2 and 65% CH_4. Volatile fatty acids were quantified in a Shimazdu 2010 gas chromatograph, using a flame ionization detector and a capillary column type SGE BP 21 with helium as the carrier gas. Three standard solutions containing 50, 250 and 500 mg l^{-1} of acetic, proprionic, iso-butyric, n-butyric, iso-valeric, valeric, hexanoic and heptanoic acids were used to calibrate the instrument.

3.1.2 Results

The two parameters of importance in assessment of the results are the volatile solids destruction and the total soluble COD produced. The first represents the disappearance of the substrate, the second the appearance of the product. Other monitoring parameters include the pH of the reactor, the VFA concentration in the supernatant and the level of suspended solids in the supernatant. The latter is important as these must be counted as solids lost from the system but not destroyed. The other ratio parameters shown in table 2 and 3 are calculated values which give an insight into the nature of the intermediate products: for example the ratios of VFA and of theoretical COD to measured COD indicate the proportion of substrate being converted to products other than VFA, which may not be converted to methane or may represent a less efficient conversion pathway. The ratio of VFA to theoretical COD provides an indication of the balance between lower and higher VFAs. The results indicate that in the reactors with hydraulic flush the majority of COD is produced in the form of VFA whereas in the controls the COD occurs in other forms including alcohols and other acids.

The results from the control reactors at both the loading of 2.01 and 4.03 gVS $l^{-1}d^{-1}$ showed that these operated at the lowest pH, the highest reactor TS, the lowest VS destruction, and the lowest daily production of soluble COD and VFA. It appears that that the acidic conditions within the reactors are preventing a high level of hydrolysis and further fermentation. Where the solids and liquid retention times have been uncoupled then as the hydraulic retention time is decreased the volatile solids destruction increases at both loading rates. At a loading rate of 4.03 and a HRT of 2.0 days the VS destruction is above 50% (table 3). There is a trend of increasing VS destruction with decreasing HRT which is more pronounced than the difference in %VS destruction between the two different loadings. The total amount of VS destroyed and the soluble COD produced increases with both decreasing HRT and increasing loading. The output COD can be expressed in terms of theoretical methane potential as shown in tables 2 and 3.

Table 2. Summary of results taken over 30 days of steady state conditions in run 1

Reactor		C1	C2	C3	C4	C5	C6	C7	C8
OLR	gVS/d	1.61	1.61	1.61	1.61	1.61	1.61	1.61	1.61
	gVS/l-d	2.01	2.01	2.01	2.01	2.01	2.01	2.01	2.01
RT	days	20	20	20	20	20	20	20	20
Flush	ml/d	7	7	200	200	300	300	400	400
Vol withdrawn	ml/d	47	47	240	240	340	340	440	440
HRT	days	114	114	4.0	4.0	2.7	2.7	2.0	2.0
pH		3.39	3.44	4.04	4.04	4.10	4.09	4.18	4.22
Supernatant solids	mg/l	343	343	323	336	369	343	332	337
Reactor TS	g/l	29.5	29.6	21.7	21.8	18.7	20.0	18.3	18.1
COD	mg/l	16813	15935	4634	4228	3918	2911	2475	2213
	mg/d	790	749	1112	1015	1332	990	1089	974
VS destruction	%	0.30	0.30	0.45	0.44	0.49	0.46	0.49	0.49
VFA	mg/d	226	242	407	400	439	446	458	410
thCOD	mg/d	286	320	873	863	865	869	879	801
	mg/l	6095	6816	3636	3597	2545	2556	1998	1821
ratio VFA:thCOD		0.79	0.75	0.47	0.46	0.51	0.51	0.52	0.51
ratio VFA:COD		0.29	0.32	0.37	0.39	0.33	0.45	0.42	0.42
ratio thCOD:COD		0.36	0.43	0.78	0.85	0.65	0.88	0.81	0.82
thCH4 (thCOD)	litres/gVSadded	0.06	0.07	0.19	0.19	0.19	0.19	0.19	0.17
	litres/gVSdest	0.21	0.23	0.43	0.43	0.38	0.41	0.39	0.35
thCH4 (COD)	litres/gVSadded	0.17	0.16	0.24	0.22	0.29	0.22	0.24	0.21
	litres/gVSdest	0.57	0.54	0.54	0.50	0.59	0.46	0.49	0.43

Table 3. Summary of results taken over 30 days of steady state conditions in run 2

Reactor		C1	C2	C3	C4	C5	C6	C7	C8
OLR	gVS/d	3.22	3.22	3.22	3.22	3.22	3.22	3.22	3.22
	gVS/l-d	4.03	4.03	4.03	4.03	4.03	4.03	4.03	4.03
RT	days	20	20	20	20	20	20	20	20
Flush	ml/d	7	7	200	200	300	300	400	400
Vol withdrawn	ml/d	47	47	240	240	340	340	440	440
HRT	days	114	114	4.0	4.0	2.7	2.7	2.0	2.0
pH		3.60	3.61	3.95	3.95	3.93	5.12	3.92	3.91
Supernatant solids	mg/l	609	609	616	575	608	597	624	643
Reactor TS	g/l	65.3	66.4	42.0	42.1	39.6	39.3	34.6	36.0
COD	mg/l	20179	21679	8577	8257	5773	5479	5358	5613
	mg/d	948	1019	2058	1982	1963	1863	2358	2470
VS destruction	%	0.30	0.27	0.49	0.49	0.50	0.49	0.52	0.51
VFA	mg/d	183	181	742	661	756	730	814	824
thCOD	mg/d	236	256	1576	1450	1614	1544	1702	1689
	mg/l	5020	5444	6568	6040	4747	4541	3867	3838
ratio VFA:thCOD		0.78	0.71	0.47	0.46	0.47	0.47	0.48	0.49
ratio VFA:COD		0.19	0.18	0.36	0.33	0.39	0.39	0.35	0.33
ratio thCOD:COD		0.25	0.25	0.77	0.73	0.82	0.83	0.72	0.68
thCH4 (thCOD)	litres/gVSadded	0.03	0.03	0.17	0.16	0.18	0.17	0.18	0.18
	litres/gVSdest	0.08	0.10	0.35	0.32	0.35	0.34	0.36	0.36
thCH4 (COD)	litres/gVSadded	0.10	0.11	0.22	0.22	0.21	0.20	0.26	0.27
	litres/gVSdest	0.34	0.40	0.46	0.44	0.43	0.41	0.49	0.53

3.2 Short retention time hydrolysis/acidification reactors

3.2.1 Method

Ten CSTR design digesters (Plate 1) with a working volume of 1.5 litres were used to assess the hydrolysis and acidification potential at short (less than 10 days) HRT. The digesters were maintained at 35^{o}C±2.0 ^{o}C, and fed on a semi-continuous basis by the daily addition of ensiled maize. Biogas generated was collected and volume and gas composition determined.

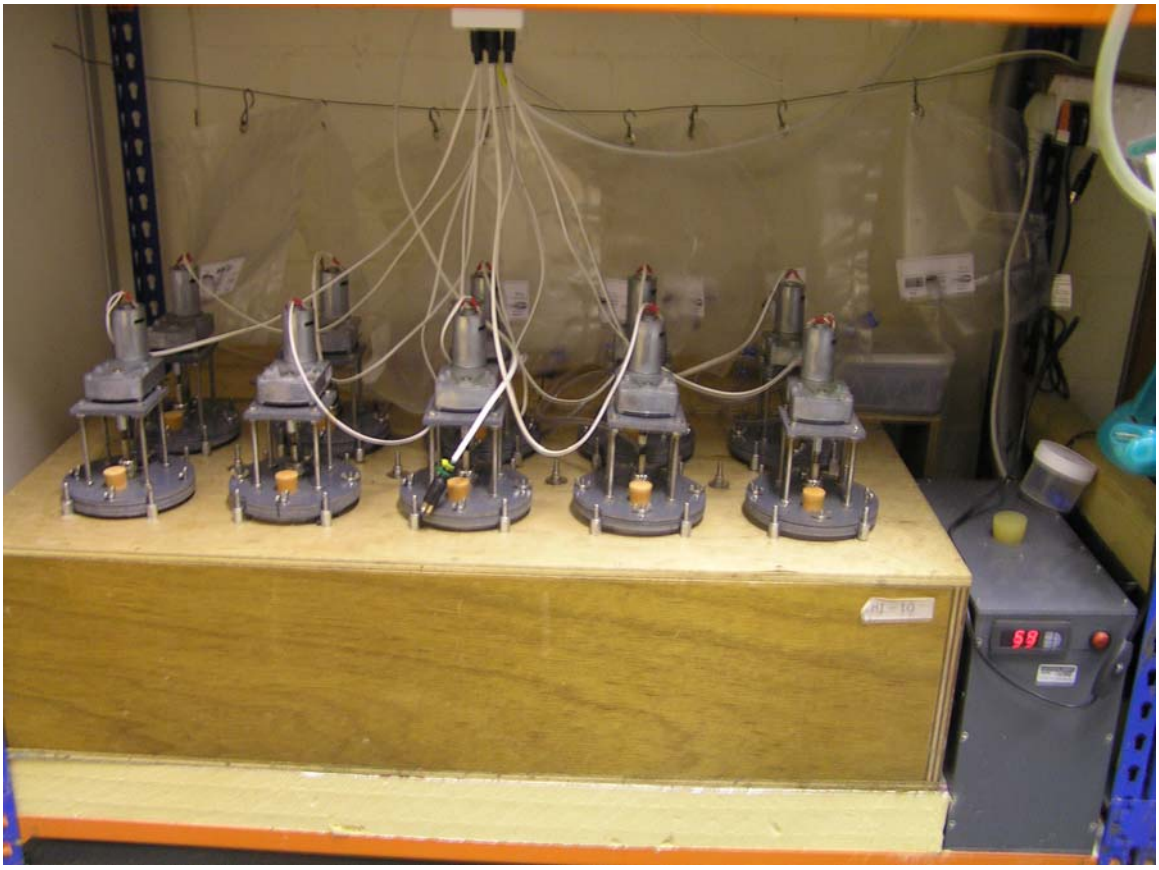


Plate 1. 1.5 litre working volume CSTR design digesters with gas sampling bags for biogas collection

The digesters were operated with retention times of 2, 4, 6, 8 and 12 days by daily removal of the appropriate volume of digestate and replacing this with maize diluted with water containing a trace element supplement (see appendix 2). After an initial period of acclimatisation, in which the HRT of the digesters were dropped systematically to their proposed operating conditions, the digesters were run for at least three retention times (table 4)

Sampling frequencies and analytical methods. Total solids (TS) and volatile solids (VS) in the material removed from each reactor were measured three times a week in accordance with standard methods. pH was measured daily using a Jenway 3010 pH-meter and the temperature was measured regularly check the stability of the thermo-circulator. Chemical oxygen demand (COD) of the digestate supernatant was measured twice a week using the closed tube reflux method. The VFA concentration was measured at least three times a week and analysed by GC as described earlier. Total VFA concentrations are expressed as acetic acid equivalents and converted to theoretical COD. Gas composition was determined by GC as described earlier.

3.2.2 Results

All of the reactors rapidly reached a pH lower than 4 and showed negligible methane production with relatively high levels of VFA in the digestate. The percentage VS destruction was similar in the reactors with HRTS less than 10 days at around 40% but there was quite a degree of daily variability in this figure even after a period of steady state operation. This can probably be attributed to the difficulty of obtaining

representative samples from this type of digester due to the non-homogeneity of the contents. In terms translation of this small-scale experimental data to a larger-scale operational plant it appears that the optimal HRT is likely to be around 4 days: although the daily yield of acids is higher in the 2-day HRT reactor, the concentration is lower and the volume to be processed is higher. The theoretical methane yield based on COD production is low and confirms that a considerable quantity of material remains unfermented. The ratio of VFA to COD indicates that the longer the retention time the more likely it is that the hydrolysed substrates are transformed to VFA.

Table 4. Operational conditions and average results for hydrolysis/acidification reactors at 5 different HRT

Parameter	unit	R1&2	R3&4	R5&6	R7&8	R9&10
Acclimation period	days	28	24	18	8	12
Stable operation	days	37	77	77	37	55/77
OLR	gVS/l-d	4.03	4.03	4.03	4.03	4.03
Volume withdrawn	ml/d	750	375	250	188	125
HRT	days	2	4	6	8	12
pH		3.90	3.80	3.76	3.57	3.71
VS destruction	%	40.3%	47.4%	36.4%	40.7%	12.8%
COD	mg/l	6502	9870	11793	7441	8234
	mg/gVS-d	807	612	488	231	170
VFA	mg/l	2400	4299	3790	3841	6225
	mg/gVS-d	298	267	157	119	129
ratio VFA:COD		0.37	0.44	0.32	0.52	0.76
thCH4 (COD)	litres/gVSadded	0.28	0.21	0.17	0.08	0.06
	litres/gVSdest	0.70	0.45	0.47	0.20	0.47

4 Quantification of the methane potential of products from hydrolysis/ acidification reactors

4.1 Measurement of Biochemical Methane Potential of liquor from the hydraulic flush reactor (HFR)

The BMP of the liquor taken from one of the hydraulic flush experiments was measured using the method adopted for the project by the Bioenergy Research Group at the University of Southampton (see Appendix 1 of this report).

4.1.1 Experimental set up

The inoculum for the BMP test was digester sludge taken for the municipal sewage sludge digester at Millbrook WWTP, Southampton UK. The reactors used were 500 ml CSTRs with slow (30 rpm) bar mixers, maintained at 37 ^{o}C in a water bath. The gas collection vessels for each reactor were instrumented to measure the height of the water column automatically and log this to computer. Four controls were run which contained inoculum sludge only and duplicates of each HFR liquor were tested along with liquor from the control reactor without a hydraulic flush. The COD of the mixed reactor contents was measured at the end of the test. The test was run over a period of 11 days which was sufficient for gas production to stop when compared to the control.

4.1.2 Results

The results of the BMP test are shown in table 4. The COD destroyed in the experiment is presented both as a weight and a percentage. From this is calculated the proportion of COD destroyed that was derived from the substrate added. The volume of methane from the test reactors was determined (CH_4 litres) by taking the volume of biogas accumulated in the gas collectors and measuring the methane content by GC analysis. Figures are corrected to standard temperature and pressure (0^oC, 101.325 kPa). The theoretical methane potential of the COD destroyed ($thCH_4$) was calculated by multiplying the COD destroyed by the factor of 0.35, which is volume of methane (litres) produced from 1 gram of COD. The percentage of methane actually collected compared with that theoretically possible was then calculated along with the actual methane yield (m^3 CH_4 g^{-1} COD destroyed). It is usual to express the BMP result in terms of CH_4 produced per gram of VS added. In this case it was appropriate for interpretation of the results to express this in terms of CH_4 produced per gram of VS destroyed, although the former can be calculated from the data given in table 4 and is shown in Figures 2a-d.

The biogas, and hence methane, generation rate was logged automatically and is plotted as methane volume against time for each of the tests and the control reactors. A first order rate equation was then fitted to the data to give the rate constant k for each of the test conditions.

The COD destroyed in all the test flasks was roughly equal between 85-88%. The liquor from the reactor with no flush and from the 4-day HRT reactor showed a value for COD destroyed more or less equal to the theoretical yield. The liquors taken from the HFRs with shorter HRT (2.7. and 2.0 days) showed a lower conversion. The reasons for this are not proven but it is possible that that the shorter HRT liquors, which had a lower suspended solids content, were more rapidly degradable and other non methane producing reactions may have been competing for this substrate in the test reducing the overall efficiency of the methanogenic pathway. The difference in the nature of the liquors is also apparent from the differences in k values for each of the tests. The high k values associated with the short HRT flush liquors indicate that the substrate is rapidly converted to methane and this is consistent with the observation that it is primarily soluble material. The lower k values for the longer HRT flush liquors and the liquor from the reactor with no flush indicated that the substrate may have been more colloidal in nature. The apparent over-production of methane (106.4 and 109.2%) compared to the theoretical estimation for the liquor from the 4 day HRT reactors could be as a result of under-estimation of the initial COD as some of this colloidal material may not have been oxidised under the COD test conditions.

Table 4. COD removed and methane generated in the BMP test on liquor taken from 3 pairs of hydraulic flush reactors and corresponding control without flushing.

	COD start	COD end	COD destroyed		minus control	CH4	thCH4	CH4:thCH4	CH4 yield	1st order k
	g	g	g	%	g	litres	litres		m3 CH4/g COD destroyed	day-1
Blank1	0.245	0.138	0.107	43.7%	0.001					
Blank2	0.245	0.141	0.104	42.4%	-0.001					
No flush	1.443	0.204	1.239	85.9%	1.134	0.419	0.434	96.7%	0.338	0.46
4-day HRT	1.561	0.186	1.375	88.1%	1.270	0.512	0.481	106.4%	0.372	0.54
4-day HRT	1.561	0.188	1.374	88.0%	1.268	0.525	0.481	109.2%	0.382	0.55
2.7-day HRT	1.299	0.165	1.134	87.3%	1.029	0.365	0.397	91.9%	0.321	0.79
2.7-day HRT	1.299	0.176	1.124	86.5%	1.018	0.390	0.393	99.2%	0.347	0.77
2-day HRT	1.096	0.158	0.939	85.6%	0.833	0.289	0.328	88.1%	0.308	0.89
2-day HRT	1.096	0.162	0.934	85.2%	0.829	0.289	0.327	88.4%	0.309	0.94

Figures 2a -d show the actual methane volume generated per gram of COD added to each of the flasks. The data has been fitted to the first order rate equation and the model line is also shown in each of the figures.

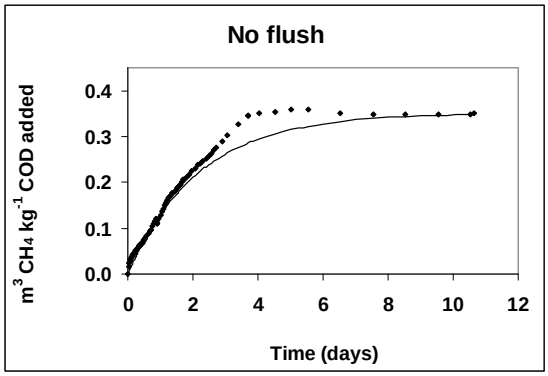


Fig. 2a. Methane production (m^3 kg^{-1} COD added) for liquor from a reactor with no flush

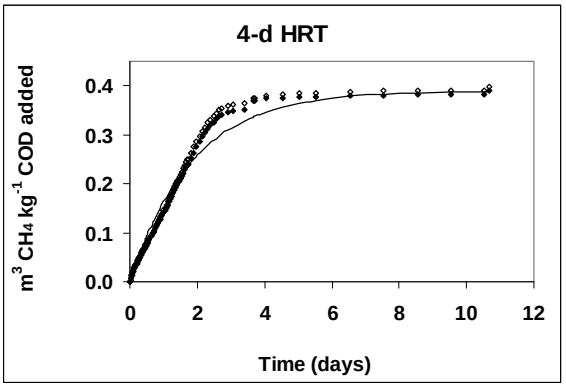


Fig. 2b. Methane production (m^3 kg^{-1} COD added) for liquor from a reactor with a 4 day HRT

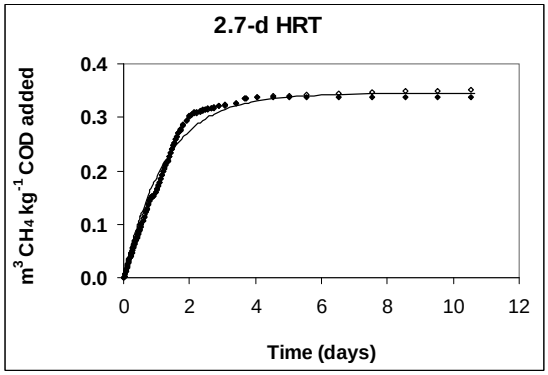


Fig. 2c. Methane production (m^3 kg^{-1} COD added) for liquor from a reactor with a 2.7 day HRT

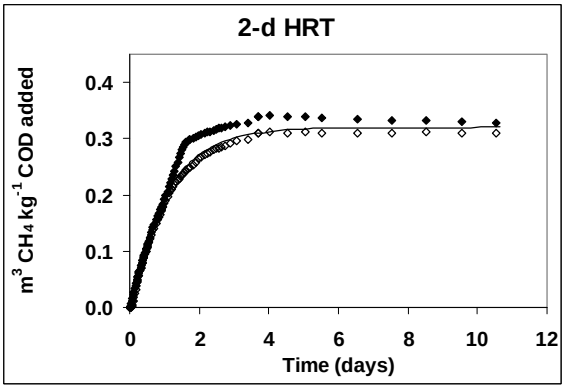


Fig. 2d. Methane production (m^3 kg^{-1} COD added) for liquor from a reactor with a 2.0 day HRT

It is clear that there are differences in the nature of the flush liquors taken from reactors with different HRT when measured using the BMP assay. The differences in flush liquor were also noted earlier in terms of the volatile fatty acid composition as a proportion of the COD. It is clear that where the COD is predominantly present as VFA then all of this COD is not being converted directly into methane: this aspect is just noted for the present but needs further investigation.

4.2 Measurement of the BMP of digestate from short retention time hydrolysis acidification reactors

4.2.1 Method

The BMP of the material from the 4-day retention time hydrolysis/acidification reactor was determined using the procedure described in appendix 1. The inoculum was digester sludge taken for the municipal sewage sludge digester at Millbrook WWTP, Southampton UK; this was used at a ratio of 2 parts VS to 1 part substrate VS in the test. The reactors were 500 ml CSTRs with slow (30 rpm) bar mixers, maintained at 37^{o}C in a water bath.

Nine reactors were used in the test: 3 of these were controls containing only the sludge inoculum; 3 contained unseparated digestate, and 3 contained the solids that had been separated by sieving from the digestate. In all cases 120 ml of inoculum was added and the remaining volume was made up to 300 ml with feed and water (table 5) The gas collection vessels for each reactor were instrumented to measure the height of the water column automatically and log this to computer.

Table 5. Make-up of the BMP test reactors

BMP test reactor	Type of feed	feed added (g)	VS added (g l^{-1})	inoculum sludge (ml)	water added (ml)
1 + 2 + 3	none	0	8 (inoculum)	120	180
4 + 5 + 6	separated solids	13.8g	4 (feed)	120	166.2
7 + 8 + 9	unseparated digestate	144.6 ml	4 (feed)	120	35.4

4.2.2 Results

Figures 3a and b show the methane production per gVS added for mixed digestate and the separated solid fraction. Figure 3c shows the difference which can be attributed to the liquid fraction. Overall the methane productivity is close to the expected value for whole crop material indicating that no significant losses have occurred as a result of the separate hydrolysis and acidification. The methane potential of the solids fraction was 0.18 litres CH4 gVS^{-1} added, accounting for approximately 44% of the total methane yield. The rate of degradation of the material, as indicated by the rate of product formation, was low.

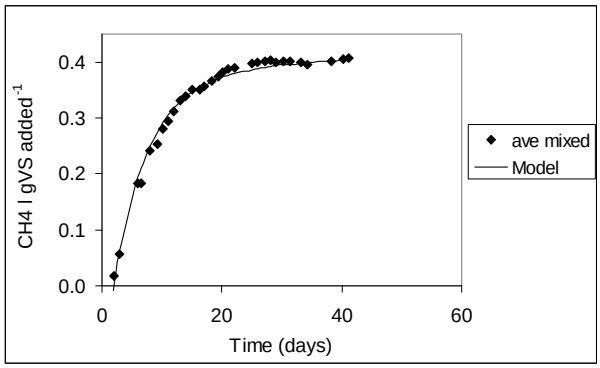


Fig. 3a Methane production (l gVS^{-1} added) for mixed reactor contents from 4-day HRT

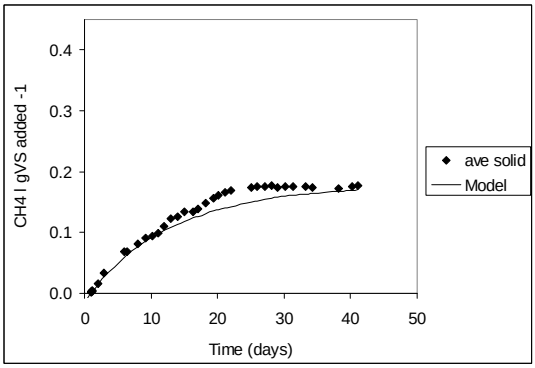


Fig. 3b Methane production (l gVS^{-1} added) for separated solids from 4-day

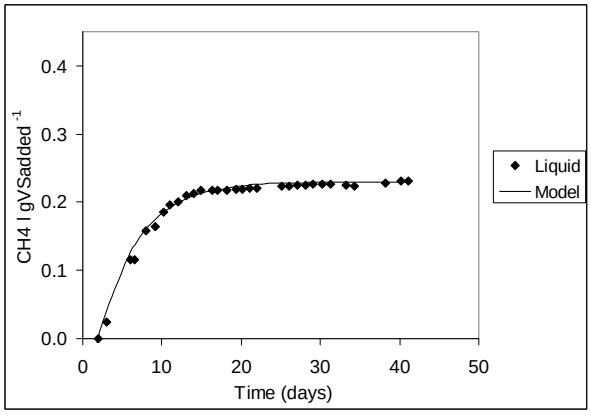


Fig. 3b Assumed methane production (l gVS^{-1} added) for liquid fraction from 4-day HRT

Table 6 gives rate constants for the solid, mixed and liquid fractions based on a pseudo-parallel first order kinetic model.

$$Y = Y_m (1 - Ae^{-k_1 t} - (1-A) e^{-k_2 t}) \qquad [2]$$

where:
Y is the cumulative methane yield at time t
Y_m is the ultimate methane yield
k_1 is the first order rate constant for the proportion of readily degradable material
k_2 is the first order rate constant for the proportion of less readily degradable material
A is the proportion of readily degradable material

The values suggest that the liquid fraction can be modelled by a typical single-component first-order equation. The solid fraction can be modelled as consisting of a proportion of more rapidly degradable material with the majority of material more resistant to degradation. The mixed material can then be modelled as a combination of the two. In this set of experimental results the need for a pseudo-parallel equation is less marked but the approach has proved effective in interpretation of energy crop data elsewhere in the current research programme.

Table 6. Kinetic parameters for BMP results with pseudo-parallel first order model

Parameter values				
Parameter		**Solid**	**Mixed**	**Liquid**
Ym		0.180	0.410	0.230
P		0.1	0.75	1
k1		0.2	0.2	0.2
k2		0.07	0.07	0
R2		0.9944	0.9955	0.9954

5 Conclusions

The first phase digesters with uncoupled solids and liquid retention times did not perform as well as expected and showed only a moderate solids destruction and conversion potential to methanogenic pre-cursors. This was also true for the short retention first stage hydrolysis/acidification reactor where a substantial proportion (46%) of the methane potential was still present in the solids after a 4-day hydraulic retention time.

Reducing the hydraulic retention time by means of a hydraulic flush or reducing the overall retention time in a single pass reactor without uncoupling the solids and liquid retention times both had the effect of increasing the yield of soluble COD. Both of these techniques resulted in some washout of the acid product, helping to reduce the accumulation that leads to inhibition. This process however was not fully effective, however, as it is clear from the results that inhibition still occurred and the flushing effect was not as effective as methanogenesis in removal of organic acids, as demonstrated by the much better performance obtained in conventional single pass reactors in VS destruction and overall methane production.

The use of the BMP test to asses the methane potential of the hydrolysates and methane precursors proved to be quite effective showing good agreement between theoretical

COD potential of the added substrates and the actual values obtained. It also potentially indicated that the hydrolysis products do not always have a uniform composition and amenability to conversion to methane as indicated by the kinetics associated with different fractions. The results of the work presented indicate that a two-phase test based on a mass balance approach between the phases is feasible but that the results will be highly dependent on the method adopted for hydrolysis and acidification. It is therefore possible to simulate a particular process but not to give a specific BMP value for a feedstock or substrate in a two phase system as this is process dependent.

References

APHA (2005). Standard Methods for the Examination of Water and Wastewater, American Public Health Association, American Water Works Association, Water Environment Federation.

Wang, Z., and C. J. Banks. (2000). Accelerated hydrolysis and acidification of municipal solid waste (MSW) in a flushing anaerobic bio-reactor using treated leachate recirculation. Waste Management & Research **18**(3), 215-223.

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APPENDIX 1

Biochemical Methane Potential Determination

Background

The biochemical methane potential assay is a procedure to determine the methane yield of an organic material during its anaerobic decomposition by a mixed microbial flora in a defined medium. This assay provides a simple means to monitor relative biodegradability of substrates. Various procedures have been developed for carrying out the test, dating back to methods using the anaerobic Warburg apparatus and serum-bottle techniques developed by Owen et al. (1979). There is also an outline procedure described in ASTM (1992) and examples of results from this method can be found in Owens and Chynoweth (1992).

The various protocols have been designed to assure that the degradation of the compound is not limited by nutrients, inoculum, substrate toxicity, pH, oxygen toxicity or substrate overloading. A stock solution of micronutrients may be added to ensure that appropriate trace metals are available in the final media.
In all tests a sample of substrate is anaerobically incubated with an inoculum for a period of time, which can either be pre-determined or until a defined point e.g. where gas production has stopped or the difference in gas production between the test flask and the inoculum control is the same. This can take at up to 30 days for simple substrates (e.g. sugars and starches) and up to 120 days for recalcitrant lignocellulosic substrates (e.g. paper and wood). The ASTM E 1196 method suggests an incubation period of 56 days or longer if gas is still being produced. To account for biogas production from residual degradable matter in the inoculum, triplicate sludge controls (sludge blanks) containing only media and inoculum are incubated and the gas is sampled and analysed simultaneously to allow subtraction of gas not attributed to the substrate. In some cases positive controls containing a standard material such as cellulose are also incubated and sampled simultaneously to ensure that inoculum, media, and sampling procedures are not affecting the results.

Biochemical methane potential (BMP) analyses carried out in our laboratory follow a standard procedure as described below.

Materials and Equipment

- Sample for analysis
- drying oven
- ashing oven
- balance (+/- 0.01 g)
- temperature controlled digestion vessels
- over water gas collectors
- gas chromatograph with TC detector
- gas standards
- suitable anaerobic inoculum
- various chemicals for preparation of trace element solution

Experimental Procedures

1) Where possible the test is set up in triplicate for each substrate.
2) There should be at least 3 controls of inoculum without added substrate.
3) A suitable digester sludge is chosen as inoculum. This may be from a municipal wastewater treatment plant anaerobic digester or a laboratory seed digester. The inoculum is sieved through a 1mm mesh to remove particles and grit
4) The total solids (TS), and volatile solids (VS) of the inoculum are determined in accordance with Standard Methods
5) Each reactor is weighed and a fixed mass (1.5 kg for 2litre BMP reactors or 0.5 kg for 0.8liter BMP reactors) of inoculum is added to each. The inoculum is kept homogeneous by constant mixing while sub-samples are taken for loading the digesters.
6) Throughout the filling procedure care should be taken to avoid undue agitation of the inoculum so as to minimize any oxygen transfer and to maintain anaerobicity.
7) The quantity of volatile solids added to each digester is calculated and noted for each digester

Total $VS_{(inoculum)}$ = weight of inoculum added (g) x $VS_{(inoculum)}$ (g/g)

8) If the test substrate is not homogeneous it is made so by pre-processing e.g. blender, grinder, cutting mill.
9) A preliminary determination of the total solids (TS) and volatile solids (VS) of the substrate is made to allow calculation of the quantity of substrate to be added. For liquid substrates the COD of the substrate is determined
10) The amount of substrate to be added to each test digester is calculated based on an inoculum to substrate volatile solids ratio of at least 2:1 (the ratio may be higher but NEVER below this value)

Weight of substrate added (g) x $VS_{(substrate)}$ (g/g) = total $VS_{(inoculum)}$ / 2

In the case of liquid substrates the loading of COD to inoculum VS should not exceed 3g COD gVS^{-1}

11) The calculated amount of 'wet' substrate + about 50 g additional material are placed in a beaker. The contents of the beaker are thoroughly mixed. From the beaker the required amount of substrate is added to the digester, which is placed on a balance to record the increase in weight. A sub-sample of 30-50g of the remaining material in the beaker is placed into a pre-weighed crucible for determination of TS and VS. In this way the quantity of TS and VS of the inoculum, the substrate and the ratio between them is known precisely.
12) 1.5 ml of trace element solution is added to each reactor
13) The tops are placed on the digesters using new gaskets and the digesters are connected to the gas collectors. The headspace is flushed with nitrogen as a precaution against any aerobic decomposition of the substrate in the very early stages of the test.

14) The gas collectors are filled with a 75% saturated solution of sodium chloride and acidified to pH2 using hydrochloric acid. This will minimize the absorption of CO_2 from the biogas being collected in the gas collector.
15) The level in the gas collectors is checked and a reading taken at least twice per day. At the same time the temperature and pressure are noted so that gas volumes can be corrected to standard temperature and pressure (STP).
16) Where the gas collectors are connected to pressure measuring devices to record the height of liquid continuously, these should be calibrated in accordance with the instructions provided. In no circumstances does the use of automatic recording equipment remove the need to check take manual readings at least daily and to check these against recorded values.
17) When the gas collector is full, or after 5 days (whichever is the shorter), the gas collector level is raised to its zero using a vacuum pump. At the same time a gas sample is taken from the gas collector (not the reactor headspace), and the gas composition analysed by gas chromatography (see method sheet on determination of biogas composition by GC).
18) Regular checks need to be made to ensure that the digester water bath is topped up with water and is maintaining a constant temperature of 35^{o}C; that the stirrers in the digesters are all turning; and that there are no apparent gas or liquid leaks in the system.
19) The digesters are allowed to run until there is no significant difference between the gas production of the control and test reactors. In some cases the assay may be terminated before this time if a reasonable estimate of the BMP can be made by approximation using a first order type model.
20) From the gas collector readings and the gas composition as measured in the gas collectors the methane volumes are determined for each time interval.

Data Analysis

After each sampling, the value of the measured volume of methane produced by the digesters is converted to dry gas at 1 atm and 0^{o}C (STP) and added to the previous measurements. The total cumulative methane volumes are corrected for methane production attributed to the medium and inoculum by subtracting the averaged blank control volumes from each bottle's total cumulative methane volume. Finally, the corrected cumulative methane yield is calculated by dividing the corrected volume by the weight of sample VS added to each bottle.

The degradation of each sample is often assumed to follow a first order rate of decay. Thus, the production of methane may follow:

$$Y = Y_m (1 - e^{-kt})$$

where:

Y - is the cumulative methane yield at time t
Y_m - is the ultimate methane yield
k - is the first order rate constant

The parameters Y_m and k may be estimated using a nonlinear regression fit to the yield data of a triplicate set. The regression can be performed on a computer using the Marquardt-Levenberg algorithm available in SigmaPlot or other appropriate software.

References

ASTM, E1196-92 Standard Test Method for Determining the Anaerobic Biodegradation Potential of Organic Chemicals, 1992 American Society for Testing and Materials, West Conshohocken, PA.

Owen, W. F., Stuckey, D. C., Healy, J. B., Jr., Young, L. Y. and McCarty, P. L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Research, 13, pp. 485-492.

Owens, J.M. and D.P. Chynoweth, "Biochemical Methane Potential of Municipal Solid Waste (MSW) Components", Water Science and Technology, Vol. 27, No. 2, pp. 1-14, 1993.

Shelton, D. R. and Tiedje, J. M. (1984). General method for determining anaerobic biodegradation potential. Applied and Environmental Microbiology, 47, No. 4, pp. 850-857.

Appendix 2 Trace element solution

Trace Elements Solution	
Compounds	Concentration (mg l^{-1})
$FeCl_2$	2000
$CoCl_2$	2000
$MnCl_2 . 4H_2O$	500
$AlCl_3 . 6H_2O$	90
H_3BO_3	50
$ZnCl_2$	50
$(NH_4)_6Mo_7O_{24} . 4H_2O$	50
$CuCl_2 . 2H_2O$	38
$NiCl_2 . 6H_2O$	50
$Na_2SeO_3 . 5H_2O$	194
EDTA	1000
Resazurine	200

Taken from:
Gonzalez-Gil G., Seghezzo L., Lettinga G. and Kleerebezem R. (2001).
Kinetics and Mass-Transfer Phenomena in Anaerobic Granular Sludge.
Biotechnology and Bioengineering 73 (2) 125-134.

The requirement for trace elements has been studied using a wide variety of substrates and sludge (Paulo et al, 2002). It is known that metal supplementation can significantly improve anaerobic reactors performance (Gonzalez-Gil et al, 2001) and cobalt has been shown to be the most important trace metal for methanogens (Florencio et al., 1994). As the requirement for cobalt is higher in methanogenic microorganisms, the medium shown above was chosen as it contains high concentration of this trace metal as well as others.

Other references:
Paulo PL, Jiang B, Cysneiros D, Stams AJM, Lettinga G. (2004). Effect of cobalt on the anaerobic thermophilic conversion of methanol. Biotechnology and Bioengineering 85 (4): 434-441.
Florencio L, Field JA, Lettinga G. (1994). Importance of cobalt for individual trophic groups in an anaerobic methanol-degrading consortium. Applied and Environmental Microbiology 60 (1): 227-234.