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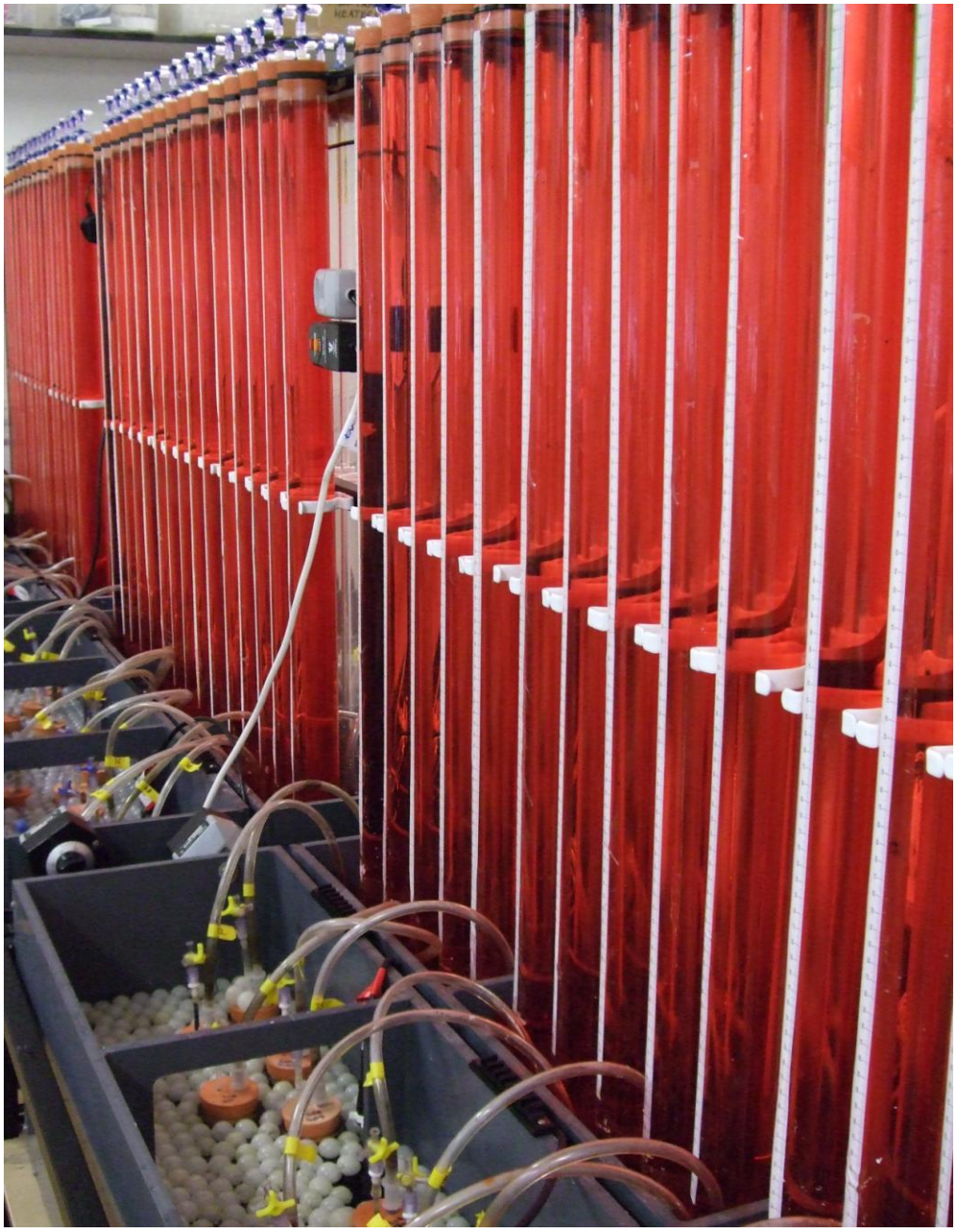
Final Report – 7th January 2010

Residual biogas potential test for digestates



Development and evaluation of a method for testing the residual biogas potential of digestates

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WRAP helps individuals, businesses and local authorities to reduce waste and recycle more, making better use of resources and helping to tackle climate change.

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Executive summary

This report concerns the development of a test procedure for determination of the residual biogas potential (RBP) of anaerobically digested materials, for inclusion in the PAS 110 specification which forms a key element in the UK Government's anaerobic digestion quality protocol (see http://www.environment-agency.gov.uk/business/topics/waste/39005.aspx).

The requirement to develop a procedure followed on from original screening work carried out by the Open University (OU) on behalf of the PAS 110 Advisory Committee, who subsequently identified the RBP test as an appropriate parameter for digestate stability and process performance.

The remit of the research was to develop the concept of the test further with the aim of producing a procedure that could be carried out in the minimum time period (preferably 28 days or less); that could be set up and operated by laboratories already involved in similar testing e.g. PAS 100, LATS, and soil analysis; and that would be suitable for analysing a range of materials from different process types, including wet and dry processes and separated fibre and liquid fractions (whole digestate and separated liquor) as might be applied to agricultural land. The remit also included that the outcome of the research should be interpreted in such a way as to give guidance to the PAS 110 Advisory Committee on the RBP limit values that should be set in PAS 110. The work tested a range of digestate samples as specified by WRAP and was undertaken by three separate organisations, the University of Southampton (UoS), Open University (OU) and the consultancy WRc Plc (WRc) in an inter-laboratory comparative study.

Before the inter-laboratory part of this project could take place it was necessary to carry out some test development work looking at factors that may influence a 28-day test. As a result of this development work and the subsequent inter-laboratory testing a detailed methodology of how the test should be set up and carried out is set out in this report, along with recommendations for RBP limit values based on the precautionary principle.

The main conclusions arising from the test development work were as follows:

Without the use of an inoculum the duration of an RBP test would be in excess of 50 days. The test duration could be reduced using inoculum derived from anaerobic digesters treating municipal wastewater biosolids. The use of inoculum is also essential where a digestate has been pasteurised. Although there was some difference in the test kinetics using inoculum from different sources, the final RBP values were consistent for the range of samples used. The suitability of the inoculum should, however, be assessed against certain quality control indicators which include testing a standard reference material as part of the procedure. Care must be exercised in selecting an appropriate inoculum to substrate ratio as insufficient inoculum can result in inhibition of gas production and an unreliable 28-day RBP value. Drying and grinding of samples is not recommended due to the loss of volatile components and an increased test duration.

As well as an RBP value it is recommended that the kinetics of gas production are reported as part of the test results to ensure that factors such as inoculum inhibition and negative net gas production can be noted and assessed against the quality control recommendations associated with the test. Detailed descriptions of the appropriate methods and the correction factors to be applied in gas measurement are given in the test procedure instructions. It is recommended that the test be carried out at a controlled temperature of 35 ^{o}C and that macro and micro nutrients are added to avoid any potential nutrient limitation. It is recommended that operators are given clear guidance about the point in the process at which samples are to be taken for testing.

The following main conclusions were drawn from the inter-laboratory comparative study:

Samples with high volatile fatty acid (VFA) concentration showed less reproducibility in a 28-day test period. It is recommended that inoculum to substrate ratio in the final test is increased to at least 4:1 and that samples are pre-screened for VFA content or soluble chemical oxygen demand (COD) to allow a stoichiometric prediction of the RBP from this component. This allows samples that will fail an RBP test due to their readily degradable soluble components to be identified at an early stage. If the suggested quality control measures relating to inoculum suitability and test procedure are followed, a repeatable and reproducible test can be achieved in a 28-day period.

The UoS, OU and WRc consortium proposed setting the RBP limit values for PAS 110 at levels comparable with RBP values for organic materials already commonly applied to agricultural land, in the context of applying a precautionary approach. Based on a study of the RBP values of a limited number of samples of such materials (cattle slurry, pig slurry and anaerobically digested municipal wastewater biosolids), a limit value of 0.25 l biogas / g VS was recommended. Determination of the impact of digestate on soils was not part of the development work. Further justification would be needed to set a RBP above these typical values and risk assessment work should be carried out to support any limits which are set. A further consideration in recommending a limit value of 0.25 l biogas / g VS was that some of the materials commonly applied to land are also regarded as suitable feedstocks for digestion because of their residual energy content. It follows that commercial digesters should be producing digestates which are more stable than these potential raw substrates.

Contents

Glossary

Abbreviations

BMP	Biochemical Methane Potential
COD	Chemical Oxygen Demand
IS	Insufficient Sample
NA	Not applicable
R	Inoculum to susbtrate ratio on a VS basis (g VS of inoculum per g VS substrate in the test)
RBP	Residual Biogas Potential
TS	Total Solids (also known as Dry Matter, DM)
VFA	Volatile Fatty Acids
VS	Volatile Solids (also known as Organic Dry Matter, ODM, and as Loss On Ignition, LOI)

Digestate Sample Descriptions

Five separate digestates were included in this study, other materials which were included as comparison materials are listed below

Sewage sludge 1 – wastewater treatment works, name and location confidential.
Sewage sludge 2 – wastewater treatment works, name and location confidential.
Sewage sludge 3 – sewage treatment works, name and location confidential.
Sewage sludge 4 – wastewater treatment works, name and location confidential.
Sewage sludge 5 – wastewater treatment works, name and location confidential.
Cattle Slurry 1 – farm name and location confidential.
Cattle Slurry 2 – farm name and location confidential.
Pig Slurry 1 – farm name and location confidential.
Pig Slurry 2 – farm name and location confidential.

Digestate Sample Nomenclature

D1	Digestate 1
D1W	Digestate 1 Whole
D1L	Digestate 1 Liquor
D1F	Digestate 1 Fibre
D1Raw	Digestate 1 Raw
D2	Digestate 2
D2W	Digestate 2 Whole
D2L	Digestate 2 Liquor
D2F	Digestate 2 Fibre
D2Raw	Digestate 2 Raw
D3	Digestate 3
D3W	Digestate 3 Whole
D3L	Digestate 3 Liquor
D3F	Digestate 3 Fibre
D4	Digestate 4
D4W	Digestate 4 Whole
D4L	Digestate 4 Liquor
D4F	Digestate 4 Fibre
D5	Digestate 5
D5W	Digestate 5 Whole
D5L	Digestate 5 Liquor
D5F	Digestate 5 Fibre
SS1	Sewage Sludge 1 Filtered
SS2 Cake	Sewage Sludge 2 Pressed Cake
SS1W	Sewage Sludge 1 Whole
SS2W	Sewage Sludge 2 Whole

SS3W	Sewage Sludge 3 Whole
SS4W	Sewage Sludge 4 Whole
SS5W	Sewage Sludge 5 Whole
CS1	Cattle Slurry 1
CS2	Cattle Slurry 2
PS1	Pig Slurry 1
PS2	Pig Slurry 2

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The authors would like to acknowledge the very useful advice offered by the PAS 110 Advisory Committee and the comments and recommendations made as a result of the user group feedback meeting held at WRAP's offices in Banbury on 16th July 2009. Thanks are also due to the organisations providing samples of digestate and other materials commonly applied to agricultural land that were tested in this work.

1.0 Introduction

This report concerns the development of a test procedure for determination of the residual biogas potential (RBP) of anaerobically digested materials complying with the UK Government's anaerobic digestion protocol (http://www.environment-agency.gov.uk/business/topics/waste/39005.aspx). Typical input materials are source segregated biowastes including domestic food wastes, supermarket wastes, food processing wastes, and agricultural wastes. The requirement to develop a testing procedure followed on from original screening work carried out by the Open University (OU) which is summarised in appendix 9.1, on behalf of the PAS 110 Advisory Committee who subsequently specified the RBP as a preferred parameter of digestate stability and process performance for inclusion in the PAS 110 specification. The specification is the British Standards Institution's 110th document of this type, a fast-track version of a British Standard. It's title is 'Publicly Available Specification for whole digestate, separated liquor and separated fibre derived from the anaerobic digestion of source-segregated biodegradable materials'. It sets a minimum baseline for digestate quality, requires its production using a quality management systems approach, and specifies information that must be supplied to digestate users.

The remit of the research was to develop the concept of the test further with the aim of producing a procedure that could be carried out in the minimum time period (preferably 28 days or less); that could be set up and operated by laboratories already involved in similar testing e.g. PAS 100, LATS, and soil analysis; and that would be suitable for analysing a range of materials from different process types, including wet and dry processes and separated fibre and liquid fractions (whole digestate and separated liquor) as might be applied to agricultural land. The test should be as reproducible as possible between different laboratories. The remit also included that the outcome of the research should be interpreted in such a way as to give guidance to the PAS 110 Advisory Committee on the limit values that should be set in PAS 110. WRAP's call for tenders specified that any procedure should be tested on a range of digestate samples as specified by WRAP and that this testing should be done by at least 3 separate laboratories in an inter-laboratory comparative study. For this reason a consortium of the University of Southampton (UoS), Open University (OU) and WRc Plc (WRc) was formed, all of which had the necessary facilities available for the intensive testing programme required to fulfil the above obligations within a very tight time frame.

The work consisted of two parts, the first of which was a development study to look at factors that may influence a 28-day test. This was carried out by UoS and OU. The second part was a comparative study with all three laboratories using the method as then defined. As a result of this development work and comprehensive testing a detailed methodology of how the test should be set up and carried out is reported.

2.0 Digestate Sources and Characterisation

The digestate samples used in this work were collected from full-scale anaerobic digestion facilities and were chosen to represent a broad range of digestate characteristics. The samples are referred as D1 to D5, and came from digesters treating the following materials (not in sample order)

- Food and Green waste
- Food waste only (2 digestate samples)
- Maize (fresh and ensiled) and cattle slurry
- Food waste and pig slurry

On receipt of the digestate samples a proportion of each 'wet', whole digestate was separated into a fibre and liquid fraction by passing the material through a 1 mm screen. D5 was received already separated into fractions by the facility operator.

The samples were characterised for chemical and physical properties relevant to biological stability and digestate quality. The characterisation was selectively duplicated in two laboratories (UoS and OU), and the same tests were carried out on inoculum sludges and materials for comparative assessment. The results are shown in Table 1 and Table 2.

Note that total solids (TS) and volatile solids (VS) are used throughout this report and are equivalent to dry matter (DM) and organic dry matter (ODM) respectively. The method used by UoS and OU to measure TS and VS was APHA method 2540G (APHA 2005) whereas WRc used the British Standards EN 14346:2006 and EN

15169:2007. These methods are functionally equivalent and should give the same results. This is supported by comparing the variation of TS and VS results shown in Table 1 and Table 2; the variation between the institutions using different methods is comparable to the variation between the two institutions using the same method. The determination of loss on ignition (LOI) as per BS EN 15169:2007, which is expressed per unit DM, can be converted to VS as a % of wet weight by a simple calculation which is described in section 4.3.

Table 1 Characterisation of samples in initial test development stage

UoS						
Sample	**Total Solids[1] (TS)**	**Volatile Solids[1] (VS)**	**TKN[2]**	**Ammonia[2]**	**Total VFA[3]**	**Total VFA**
	% wet	% wet	g N / kg (wet)	g N / kg (wet)	g VFA / l	g COD / l
D1W	7.13	5.23	8.53	7.06	28.7	44.78
D1 Raw	7.74	6.06	9.24	6.30	34.7	54.47
D1L	5.33	3.83	9.08	7.11	28.2	44.35
D1F	10.36	8.49	9.64	6.90	NA	NA
D2W	2.29	1.41	4.27	3.73	5.45	7.52
D2 Raw	3.03	2.19	3.77	2.93	12.4	17.82
D2L	2.04	1.25	4.30	3.73	5.68	7.89
D2F	8.24	6.16	5.04	3.78	NA	NA
CS1	14.25	8.60	3.08	0.77	0.84	1.15
PS1	3.91	3.24	4.23	0.68	0.58	0.74
PS2	2.19	1.22	4.12	3.72	10.8	13.98
SS1	4.02	2.52	2.97	1.72	0.58	0.88
SS2 Cake	28.34	16.44	13.60	3.40	NA	NA

OU							
Sample	**Total Solids[1] (TS)**	**Volatile Solids[1] (VS)**	**TKN[2]**	**TKN**	**Ammonia[2]**	**pH[4]**	**Conductivity[5]**
	% wet	% wet	% wet	% TS	mg / l in filtrate		mS / cm
D1W	7.7	6.0	0.89	11.6	8114	7.8	18.8
D1 Raw	6.9	5.0	0.87	12.6	4923	8.5	23.2
D1L	5.3	3.8	0.88	16.5	6545	8.4	26.0
D1F	10.4	8.2	0.91	8.8	1817	8.8	NA
D2W	2.5	1.8	0.39	15.4	3298	7.7	11.2
D2 Raw	2.0	1.2	0.44	22.0	2858	7.9	145.0
D2L	2.0	1.3	0.45	21.9	4927	7.7	16.7
D2F	8.4	6.4	IS	IS	NA	8.9	IS
CS1	13.1	7.9	0.30	2.3	673	7.0	NA
PS2	1.8	1.0	0.39	21.5	1329	7.6	17.8
SS1	3.9	2.4	0.25	6.5	1707	7.4	9.9
SS2 Cake	25.2	14.6	1.22	4.8	NA	NA	NA

Table 2 Characterisation of samples in the inter-laboratory testing stage

	UoS	UoS	UoS	UoS	UoS	WRc	WRc
Sample	**TS[1]**	**VS[1]**	**Ammonia[2]**	**Total VFA[3]**	**Total VFA**	**DM[6]**	**LOI[7]**
	% wet	% wet	g N / kg wet	g VFA / l	g COD / l	% wet	% wet
D1W	5.50	4.07	7.04	23.70	36.69	5.40	3.99
D1F	11.04	8.68	6.87	NA	NA	12.51	9.77
D1L	4.65	3.28	6.97	23.33	36.19	4.20	2.89
D3W	3.14	2.14	6.42	3.94	5.96	3.46	2.32
D3F	13.21	10.39	6.12	NA	NA	14.49	11.50
D3L	3.01	2.03	6.42	3.82	5.78	2.92	1.95
D4W	4.96	3.27	3.52	0.53	0.60	5.34	3.60
D4F	12.35	9.30	3.81	NA	NA	12.45	9.44
D4L	4.30	2.69	3.49	0.40	0.43	4.34	2.75
D5W	24.05	12.44	2.74	1.29	1.47	25.58	15.24
D5F	43.22	24.88	2.11	NA	NA	41.68	25.45
D5L	17.12	8.50	2.74	0.59	0.68	16.07	8.57
CS1	7.49	5.82	0.39	2.10	2.57		
CS2	13.28	7.49	0.57	2.52	3.19		
PS1	5.35	4.39	0.83	0.34	0.40		
SS1W	4.28	2.77	1.63	0.03	0.03		
SS2W	4.71	2.78	IS	IS	IS		
SS3W	3.25	1.99	1.48	0.02	0.02		
SS4W	3.55	2.40	1.47	0.04	0.05		
SS5W	4.21	2.66	1.04	0.03	0.03		
SS1	NA	NA	1.73	0.03	0.03		
Cellulose						94.59	94.35

[1] Standard Method 2540 G (APHA 2005)
[2] According to the manufacturer's procedure (Kjeltec, Foss, Warrington, UK)
[3] According to the manufacturer's procedure (Shimadzu, Milton Keynes, UK) see appendix 9.2.
[4] BS EN 12176
[5] BS EN 13370
[6] BS EN 14346:2006
[7] BS EN 15169:2007

3.0 Development of the RBP Test

3.1 Initial Test Development

The RBP test development was carried out by UoS and OU and looked at a number of parameters that could affect the robustness, reproducibility and duration of the test. The test equipment and procedures were based on methods developed and used by the OU and WRc for the Landfill Allowance Trading Scheme (LATS) testing (Environment Agency, 2005). The full OU method is presented in appendix 9.3. The current work aimed to develop this approach further in order to make it more suitable for the purposes of digestate stability assessment. Modifications were based on the preliminary work as carried out by the OU and incorporating experience gained at UoS in running Biochemical Methane Potential tests on a variety of substrates and feedstocks.

3.1.1 Modifications to the OU RBP Method

The following modifications to the earlier OU method were included in this project's development version of the test.

- **Use of inoculum –** Where an inoculum was needed, sludge from an anaerobic digester treating municipal wastewater biosolids was collected the day before the test began. The sludge was passed through a 1 mm screen to remove larger solids and was incubated at 35 ºC until use. The amount of inoculum and digestate used was based on the VS concentration of each materials. At a total test volume of 400 ml the quantities of inoculum and substrate are given by equations 1 and 2:

$$\text{Digestate added (g)} = \frac{400}{1+\left(\frac{R*VS_{digestate}}{VS_{inoculum}}\right)} \quad [1]$$

Where R is the number of parts of inoculum to one part substrate, on a VS basis (i.e. g VS of inoculum per g VS of digestate) also commonly referred to as the inoculum to substrate ratio, which in the development work was 2.

Inoculum added (g) = 400 – digestate added [2]

where VS of the digestate and inoculum are expressed as a proportion of wet weight.

- **Calculation of gas volume -** Except where specified the gas collection method is by liquid displacement. The method involves collection of gas under a liquid barrier solution consisting of tap water acidified to pH 2 with HCl and containing 270 g / l of common salt (e.g. table or cooking salt as sold in supermarkets). For the purposes of this work gas volumes were calculated by applying the appropriate correction factors, as follows:

The volume of gas in the gasometer was calculated each time a reading of the barrier solution level was taken. The following notation is used and is shown in Figure 1;

V	Volume (m^3)
p	Pressure (Pa)
A	X-section of gasometer (m^2)
T	Temperature (K)
ρ	Density (kg m^{-3})
h	Distances measured relating to the position of the barrier solution level.

stp, atm, H_2O, b, t, c
Respectively, the subscripts refer to standard temperature and pressure, atmospheric, water, barrier solution, trough and column.

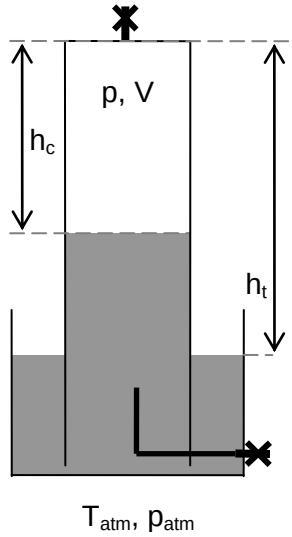

Figure 1 Liquid displacement gasometer notation

The volume of gas is calculated from equation 3:

$$V_{stp} = \frac{T_{stp}A}{T_{atm}p_{stp}}\left(\left(p_{atm} - p_{H_2O}(T_{atm}) - \rho_b g\left(h_t - h_c\right)\right)h_c\right) \quad [3]$$

Where $p_{H_2O}(T) = 101324.6 \times 10^z$ [4]

and

$$z = -7.90298\left(\frac{373.16}{T} - 1\right) + 5.02808\log_{10}\left(\frac{373.16}{T}\right)$$
$$-0.00000013816\left(10^{\left(11.34\left(1-\frac{T}{373.16}\right)\right)} - 1\right) + 0.00813289\left(10^{\left(-3.49149\left(\frac{373.16}{T}-1\right)\right)} - 1\right) \quad [5]$$

3.1.2 Experimental design

The test development programme considered the following factors that are likely to affect the progress of the test and ultimately the reported RBP value. Each trial was carried out in triplicate. The samples tested are shown in Table 3 and the basis for selection of each set of test parameters is summarised below.

Table 3 Summary of initial test development

UoS	#	OU	#
Reproducibility check		**Reproducibility check**	
With inoculum		*With inoculum*	
SS1+D1W	3	SS1+D1W	3
SS1+D1F	3	SS1+D1F	3
SS1+D1L	3	SS1+D1L	3
SS1+D1W Pasteurised	3	SS1+D1W Pasteurised	3
SS1	3	SS1	3
Inoculum addition		**Effect of storage**	
No inoculum		*No inoculum*	
D1W	3	D1 Raw	3
D1F	3	D1W	3
D1L	3	D2 Raw	3
D1W Pasteurised	3	D2W	3
With digester inoculum		D1 Raw pasteurised	3
D1W	3	D1W pasteurised	3
D1F	3	*With inoculum*	
D1L	3	SS1+D1 Raw	3
D1W Pasteurised	3	SS1+D1W	3
		SS1+D2 Raw	3
Gas collection method		SS1+D2W	3
Impermeable gas storage bags			
SS1+D1W	3	**Effect of nutrient addition**	
SS1+D1F	3	*No nutrients, no inoculum*	
SS1+D1L	3	D1W	3
		D1F	3
		D1L	3
		No nutrients, with inoculum	
		SS1+D1W	3
		SS1+D1F	3
		SS1+D1L	3
		Effect of temperature	
		32ºC	
		SS1+D1W	3
		SS1+D1F	3
		SS1+D1L	3
		Digestate drying	
		Dry, with inoculum	
		SS1+D1W	3
		SS1+D1F	3
		SS1+Cellulose	3
		Inoculum from different sources	
		SS2	3
		SS3	3
		SS2+D1W	3
		SS3+D1W	3
		SS2+Cellulose	3
		SS3+Cellulose	3
sub-total	**48**	**sub-total**	**96**

- **Reproducibility check** – This was carried out using a single digestate taken from post-digestion storage and included whole digestate, digestate fibre, digestate liquor, and a pasteurised sample prepared by heating the whole digestate to 70 ºC for a period of 1 hour. The results provide a direct comparison of tests carried out in the two different laboratories of UoS and OU.

- **Inoculum addition** – There is some potential for debate on whether an inoculum is required for the test, as the samples themselves originate from an anaerobic digester rich in methanogenic organisms. However, it is possible for this methanogenic activity to be lost as a result of post digestion pasteurisation or cold storage for long periods of time. To ascertain the value of adding inoculum, a trial was run using whole digestate, digestate fibre, digestate liquor and pasteurised whole digestate (all from a single digestate taken from post digestion storage). The test was set up so that one set of laboratory-scale reactors had no inoculum, and a second set received an inoculum taken from the digester that produced the digestate subsequently used in this test The test was run in parallel with and using the same samples as the reproducibility check, allowing a further comparison with the use of sewage sludge as an inoculum.

- **Gas collection method** – Liquid displacement gas collection is known to be affected by gas solubility and diffusion through the barrier liquid (Soto et al, 1993), as well as depending on a number of critical height measurements for accurate determination of the final gas volume. Although the method adopted (see section 3.1.1) is designed to minimise these errors it is possible that they can be further reduced by the use of impermeable gas storage bags (e.g. Tedlar bags from SKC Ltd, Dorset, UK) which are attached directly to the reaction vessel. These are not subject to dissolution of gases in a barrier solution and over a long duration may provide a more accurate measurement. They are relatively low cost and could therefore increase the suitability of this method for use in commercial laboratories by simplifying the gas collection procedure. In theory the bag size could be selected to provide sufficient capacity for the whole test period, so that only one measurement of volume would be needed. The disadvantages of this approach are that it does not provide an easy method of measuring gas production during the test, and determination of the gas volume in the bag requires additional equipment. To compare these two approaches a set of reactors using whole digestate, digestate fibre, and digestate liquor was set up as for the reproducibility check but using gas collection bags. The volume of biogas in the bag at the end of the run was measured by evacuating it through a water displacement gasometer, with gas volume correction as in equations 3 to 5 above.

- **Digestate drying** – To solve the problem of lack of homogeneity within a sample, the standard LATS BM100 test method uses material that has been dried at 70 ^{o}C and ground. The conversion of the sample to a powder allows the testing of a wide range of waste materials with significant biodegradability and high solids content. However, with digestates, while samples can differ considerably from each other depending on process characteristics, within a single sample there should be a relatively high degree of homogeneity. It is therefore questionable whether drying and grinding is either necessary or advisable as it leads to potential loss of volatile materials, including volatile fatty acids (VFA) which are known to be present in high concentrations in some digestates. A trial was run using whole digestate and digestate fibre samples which were air dried (at 40 ^{o}C), ground to 4 mm and tested with inoculum added. The results were compared to those from the reproducibility check which used the same digestate samples without drying or grinding, and the same inoculum. To check the effectiveness of the inoculum added to a dry material, cellulose was also tested in triplicate as per the BM100 method. Inclusion of a standard substrate such as cellulose also provides a positive control against which to compare any other tests that use the same inoculum.

- **Nutrient addition** - Addition of nutrients may be unnecessary, especially where sewage sludge is used as an inoculum as this provides a rich source of macro and microelements. Addition of nutrients represents an extra cost and complication. To test nutrient influences, samples of whole digestate, digestate fibre, and digestate liquor (all from post-digestion storage) were run without nutrient addition, both with and without inoculum. These results were compared with the reproducibility check which used the same digestate samples with added inoculum and nutrients; and with the inoculum addition trial in which the digestate samples in one set of laboratory-scale reactors had nutrients added but no inoculum.

- **Digestate storage/maturation** - As the feedstock passes through the anaerobic digester its biogas and methane potential are depleted. This process will continue in the post digestion storage tank and result later in a final product with a lower RBP. As part of the test development procedure, digestate from different stages in the process was tested to ensure that the methodology can cover the range of RBP likely to be encountered in practice, i.e. from material stabilised after prolonged storage to very active material sampled immediately after leaving the main digester vessel (referred to as 'raw' digestate in this report). The trial used samples taken from two anaerobic digestion facilities that are known to have storage tanks of sufficient size for long retention of treated digestate. Samples from their storage tanks were tested against samples taken directly from their digesters: the test was carried out both with and without inoculum and with and without pasteurisation during the anaerobic digestion process.

- **Temperature** - Equipment for carrying out the LATS BM100 test is often set up to provide a controlled temperature of 32 ^{o}C, which is achievable with low-cost aquarium-type heaters. The accepted optimum for mesophilic anaerobic digestion is 35 to 37 ^{o}C, and this is the typical target operating temperature range of commercial digesters. The aim of this aspect of the test development phase was to see whether running the test at 32 ^{o}C would affect the progress of the test or the RBP result.

- **Inoculum from different sources** – The sludge from most anaerobic digesters provides an active methanogenic population and a wide variety of organisms capable of providing the intermediate products needed by the methanogens. An ideal inoculum is one that is fully acclimated to the incoming waste while also being depleted of residual primary and intermediate substrates. The trials described above tested the effect of using no inoculum, inoculum derived from the digester that produced the test sample, and treated sludge from a municipal wastewater biosolids digester as an 'external' inoculum source. Digestate from municipal wastewater plants is likely to be the most homogeneous and commonly available potential source of inoculum on a nationwide basis. So long as it is sourced correctly, used promptly (within 2 days), incubated at a suitable temperature and protected from excessive exposure to the air, its characteristics should remain reasonably uniform. In these trials anaerobically digested sewage sludge inoculum taken from two additional municipal wastewater treatment works was used in the RBP test in three ways; alone, as inocula to whole digestate 1 and as inocula to the cellulose control.

3.1.3 Results and discussion

General comments

- Due to the very limited budget for this project, the experimental programme did not contain sufficient replication for statistical analysis of the results and only qualitative observations on the empirical findings are presented.
- Volatile solids (VS) are obtained by multiplying Loss On Ignition (LOI) by dry matter and dividing the result by 100 %. All RBP and biogas production data presented as L / g refers to litres of biogas per g of VS and all VS data is expressed as a proportion of wet sample weight.

- Some of the UoS trials ran for only 19 days as a tank heating problem resulted in a temperature elevation which caused the tests in one water bath to fail. The OU trial ended at day 56 to observe the characteristics of the test beyond the planned operational period. The means of 3 replicates are presented throughout unless stated otherwise. OU digestate D1 is a mean of 2 replicates only.

Reproducibility check (UoS and OU)

A reproducibility check was carried out using D1W, D1F and D1L and D1W pasteurised with SS1 inoculum, by both UoS and OU. Biogas production from the inoculum was broadly similar in both laboratories over a 50-day period (Figure 2).

Figure 3 shows the results from the test samples, and although the UoS trial of D1W, D1F and D1L and D1W pasteurised failed after 19 days, biogas production up to this time was similar in both trials. For D1W pasteurised, the OU found that biogas production was comparable to that from the unpasteurised digestate, while at UoS biogas production up to 19 days was much lower than the value for unpasteurised digestate (Figure 3). OU biogas production expressed as litres / g VS was highest for the separated liquor > whole digestate > separated fibre. All OU samples were still producing biogas at 50 days although at a very low rate, indicating that most of the material degradable under these conditions had been converted to biogas.

The findings indicate that at least 50 days is needed to determine the RBP under the conditions used and that the originally suggested 28-day period may not be sufficient for a final value to be obtained.

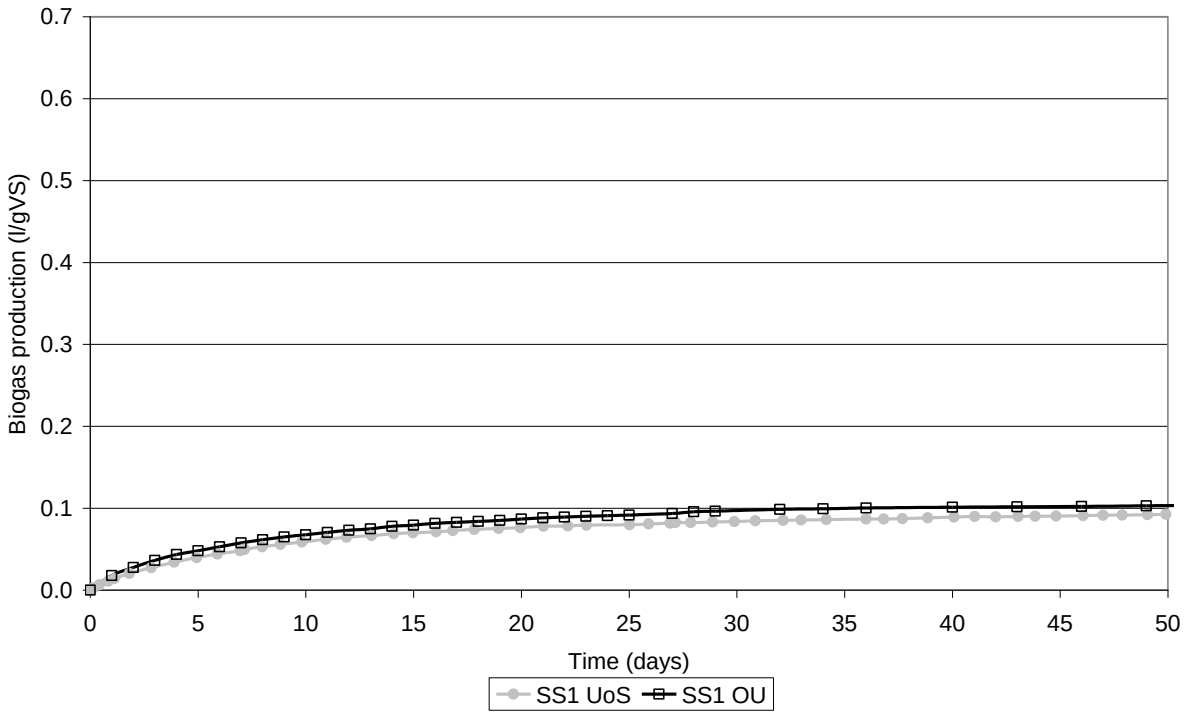

Figure 2 Reproducibility check SS1 (inoculum) biogas production for UoS and OU

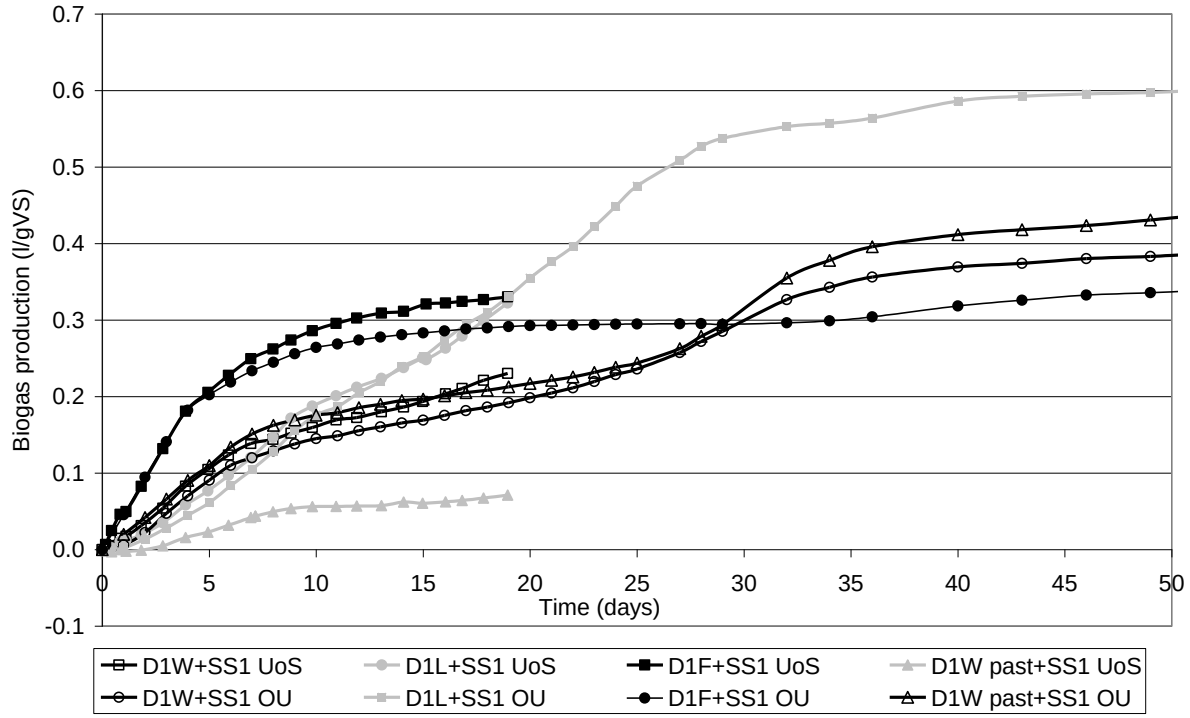

Figure 3 Reproducibility check RBP data for D1W, D1F and D1L inoculated with SS1 for UoS and OU

Effect of digestate storage

The test was carried out using D1 Raw and D1W both pasteurised and unpasteurised and with and without SS1 inoculum; and D2 Raw and D2W with and without inoculum. Figure 4 shows the RBP values for raw digestates with inoculum and shows that the test was reaching completion after 25 to 30 days. Gas production from the raw digestates was considerably higher than from digestates which had been further digested (D1) or stored (D2). Overall, the effect of storage or further digestion was to reduce RBP values by 50 % and 33 %, respectively.

Without an inoculum biogas production during the early stages of the test was very low, and only increased after day 20 to 25, as can be seen in Figure 5. Cumulative gas production from the raw digestates showed no sign of reaching a plateau within the 50-day period, and although gas production for the stored digestates had slowed down, the final totals were not as high as when inoculum was added.

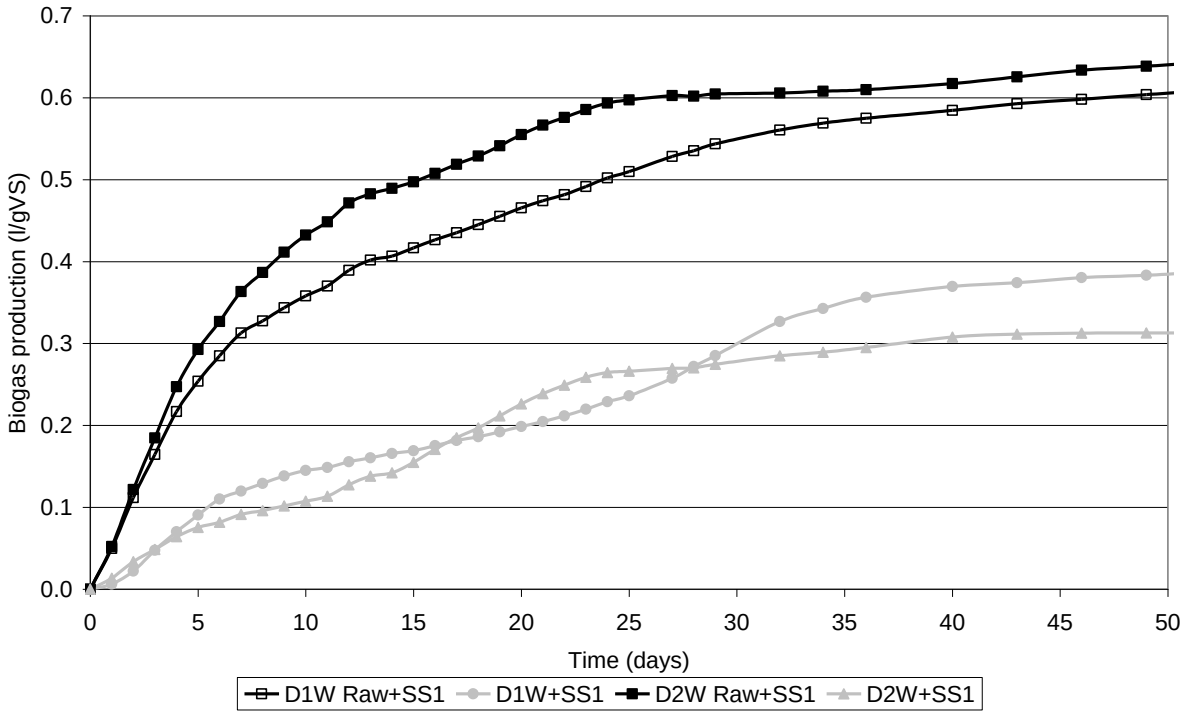

Figure 4 Storage - RBP data for digestates 1 and 2 (raw = direct from primary digestion tank) using standard method with inoculum

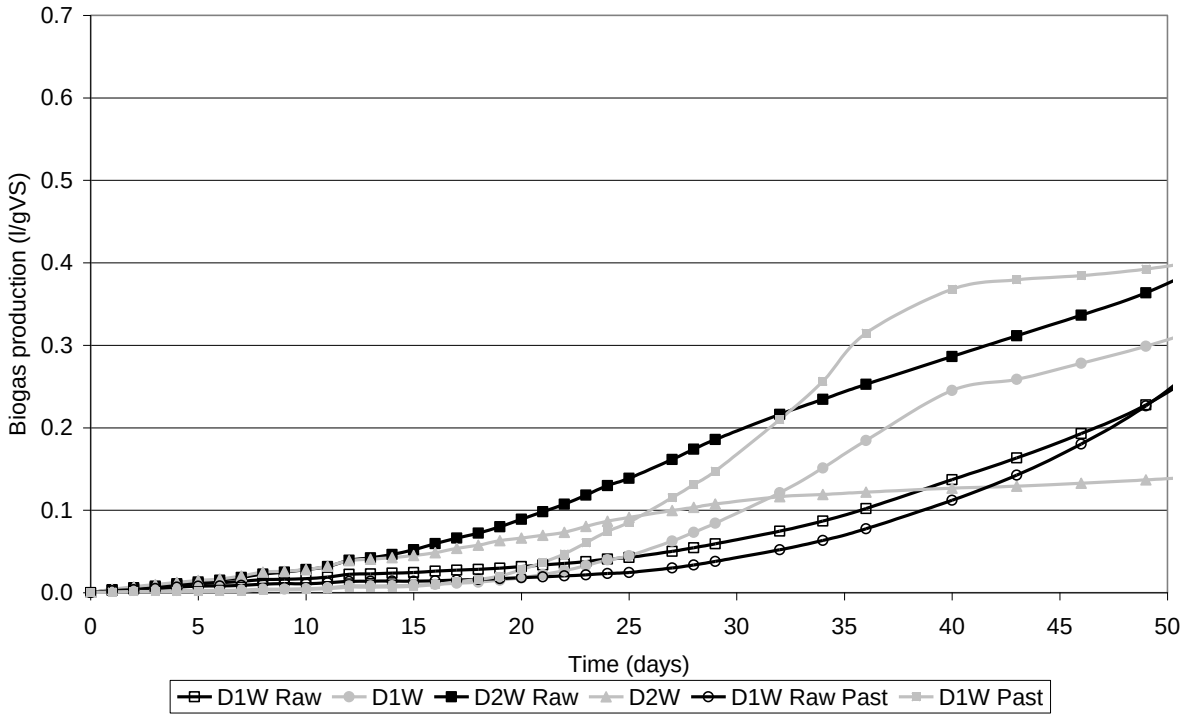

Figure 5 Storage - RBP data for digestates 1 and 2 (raw = direct from primary digestion tank) using standard method with no inoculum

Effect of nutrient addition

This trial was carried out on D1W, D1F and D1L with no nutrient additions and with and without SS1 inoculum. The results are shown in Figure 6 and can be compared with the reproducibility check in which the same digestates had both SS1 inoculum and added nutrients. There was no evidence that omitting the additional nutrients reduced biogas production by day 50. Omitting the inoculum considerably reduced the rate of biogas production up to day 50 for all fractions. The experiment suggested that it may be possible to omit nutrients, but consideration needs to be given to the risk of doing so compared with the perceived benefits. The experiment provided more evidence that adding an inoculum is necessary if the test is to be completed within a limited time period.

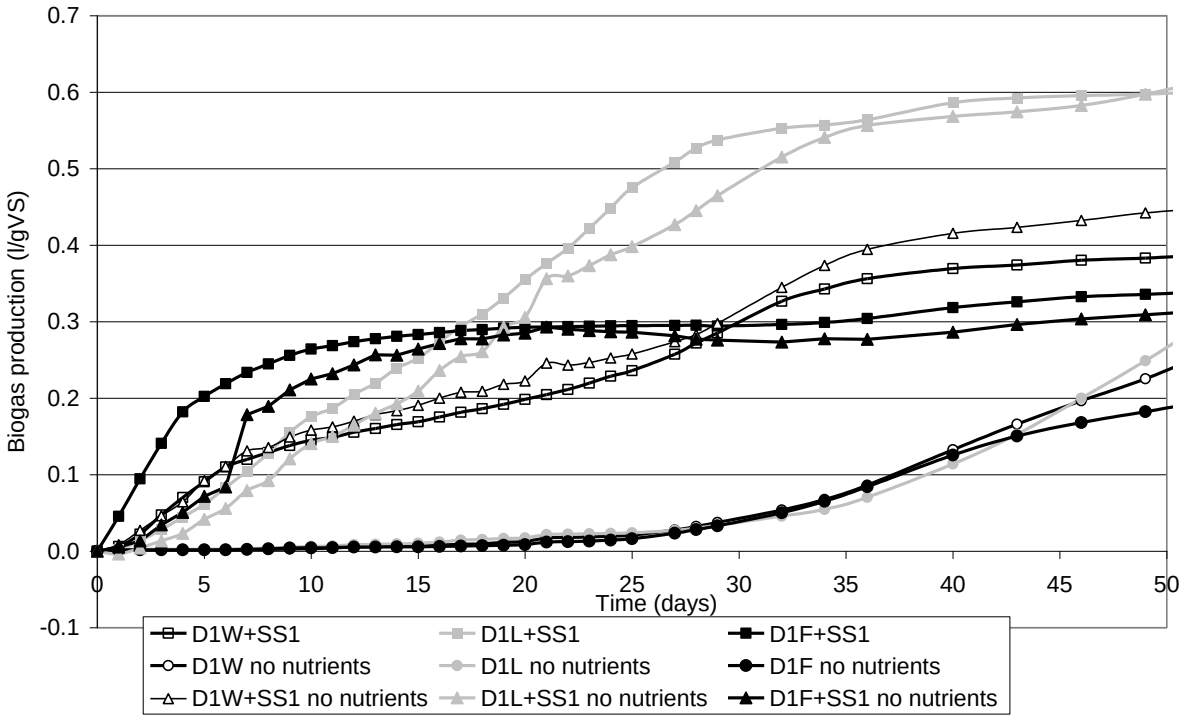

Figure 6 Nutrient addition - RBP data for D1W, D1L and D1F with and without nutrients and inoculum

Effect of temperature

In this trial D1W, D1F and D1L with SS1 inoculum were tested at 32 °C and the results compared with those from the reproducibility check. The results are shown in Figure 7. There was no clear pattern of difference in biogas production for the inoculum and fibre fractions when incubated at 32 °C or 35 °C. Incubating the separated liquor fraction at 35 °C increased biogas production compared with 32 °C, while the whole digestate produced less biogas when incubated at 35 °C compared with 32 °C.

Although there was no strong evidence that any advantage was gained either in terms of test characteristics or increased biogas production by incubating at 35 °C. The majority of evidence from the literature and the provisional recommendations of the IWA working group on anaerobic assays suggests that this is a preferable operating temperature. It is important that the test protocol allows completion in the shortest possible period and a stable, higher temperature of 35 °C is more likely to achieve this than a stable, lower temperature of 32 °C.

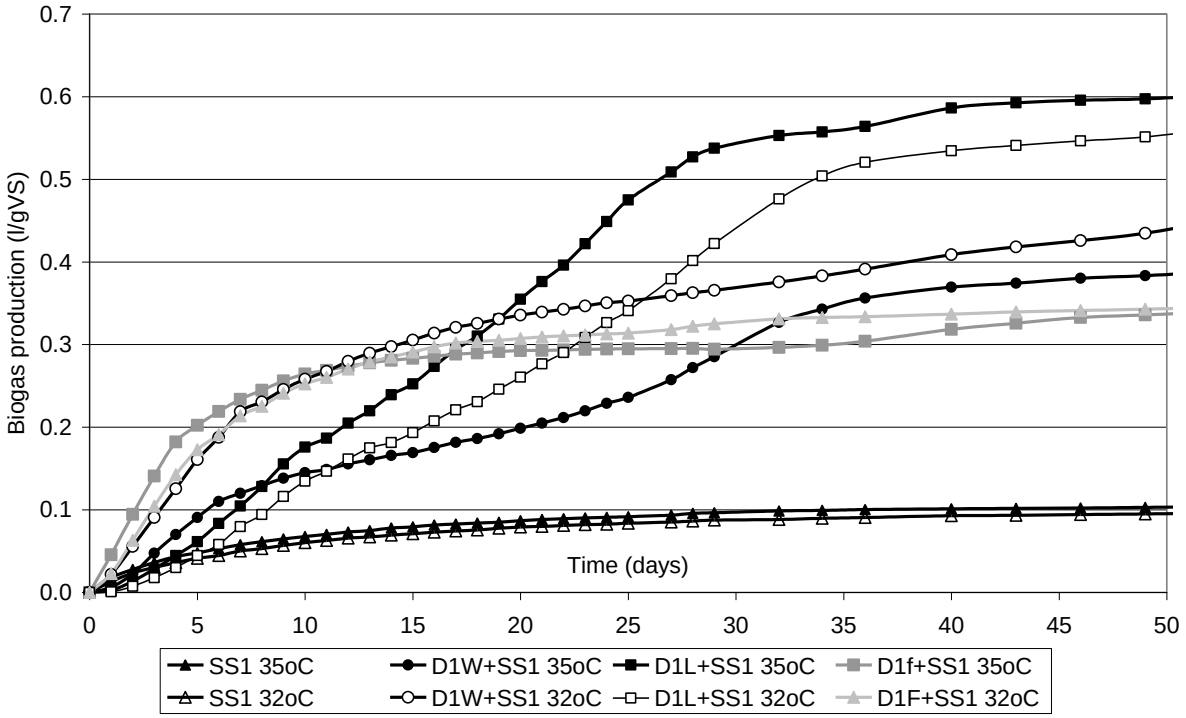

Figure 7 Temperature - RBP data for SS1, D1W, D1L and D1F operated at 35 °C as standard or at 32 °C

Digestate drying

This trial was carried out with D1W, D1F, D2W and a cellulose reference material using SS1 inoculum according to the BM100 method. The results are shown in Figure 8 and can be compared with those from the reproducibility check in Figure 3. It is clear that the RBP values for the dried samples after 50 days are relatively low in comparison with the fresh material. The OU's experience when using dried samples is of a significant lag period in biogas production, and Figure 8 shows biogas production to be increasing even at 50 days. It is likely however that the total biogas production will not match that seen in Figure 3 due to volatilisation of VFAs during sample preparation.

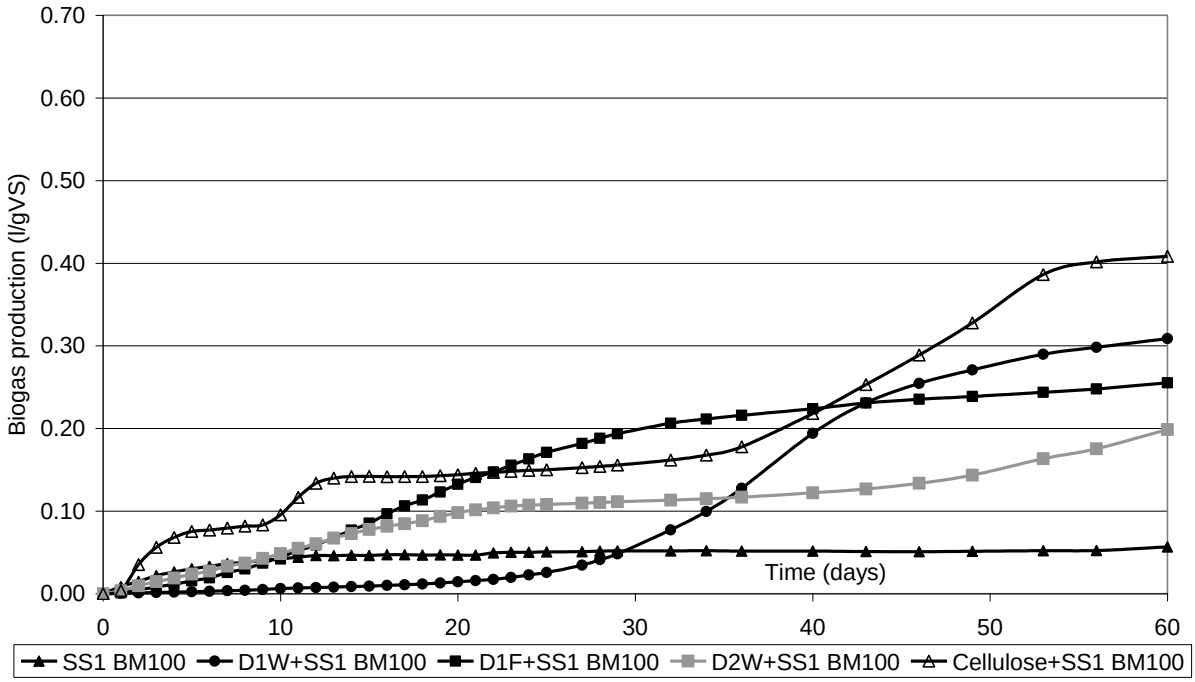

Figure 8 The effect of drying digestate samples on RBP in a BM100 style test

Inoculum addition

In this trial D1W, D1F, D1L and pasteurised D1W were tested with and without and inoculum. The results are shown in Figure 9 and Figure 10 and can be compared with those from the reproducibility check in Figure 3. Because of the failure of the UoS trial (see 3rd bullet point in 3.1.3's General sub-section), comparisons are made with OU data only. From Figure 9 it can be seen that the D1L sample without inoculum had a much reduced 50-day RBP in comparison with the use of a SS1 inoculum.

The pasteurised D1W sample without a sewage sludge inoculum produced zero biogas (Figure 9). In comparison, in Figure 3 it can be seen that the pasteurised D1W sample with SS1 inoculum had a RBP similar to the unpasteurised D1W sample which received a SS1 inoculum.

Figure 10 confirms that for all D1 samples tested, using the digestate taken from the primary digester as the inoculum was not as effective in converting residual substrate to biogas as using a well-prepared sewage sludge inoculum (as shown in Figure 3). RBP values for D1W, D1F and D1L at 50 days were 0.015, 0.16, 0.30 l / g VS respectively which are much lower than those obtained with SS1 inoculum (0.39, 0.34 and 0.60 l / g VS).

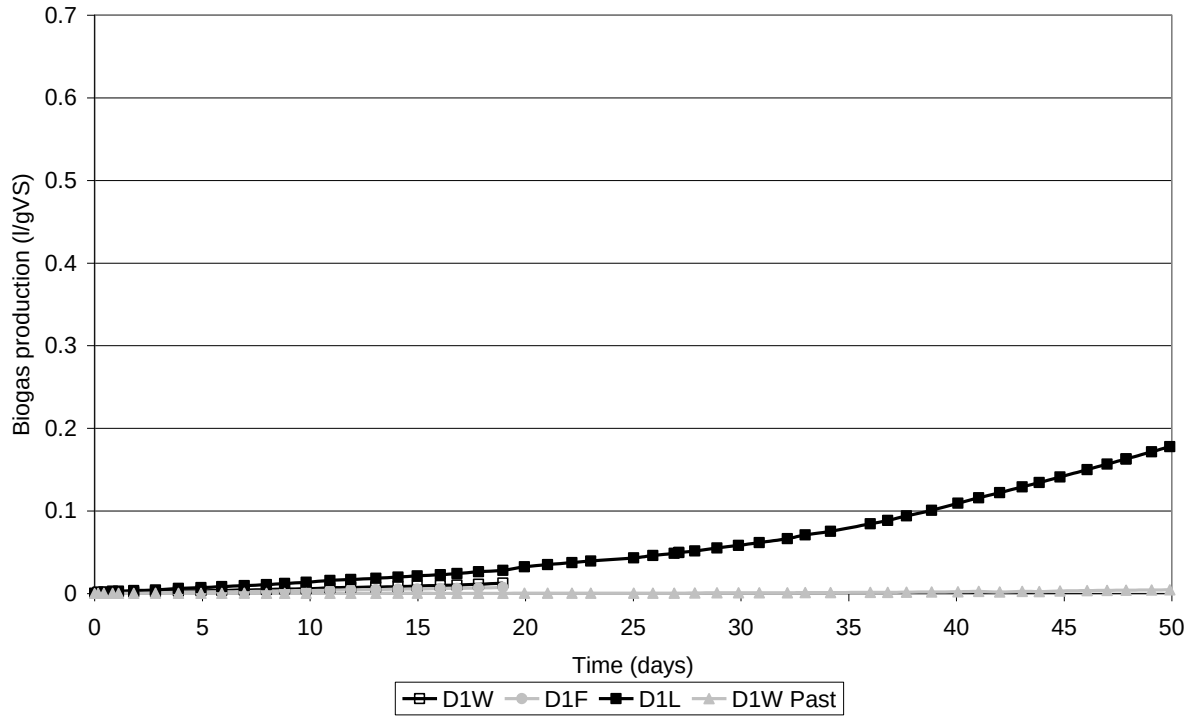

Figure 9 Inoculum addition - RBP data for D1W, D1L and D1F with no inoculum addition

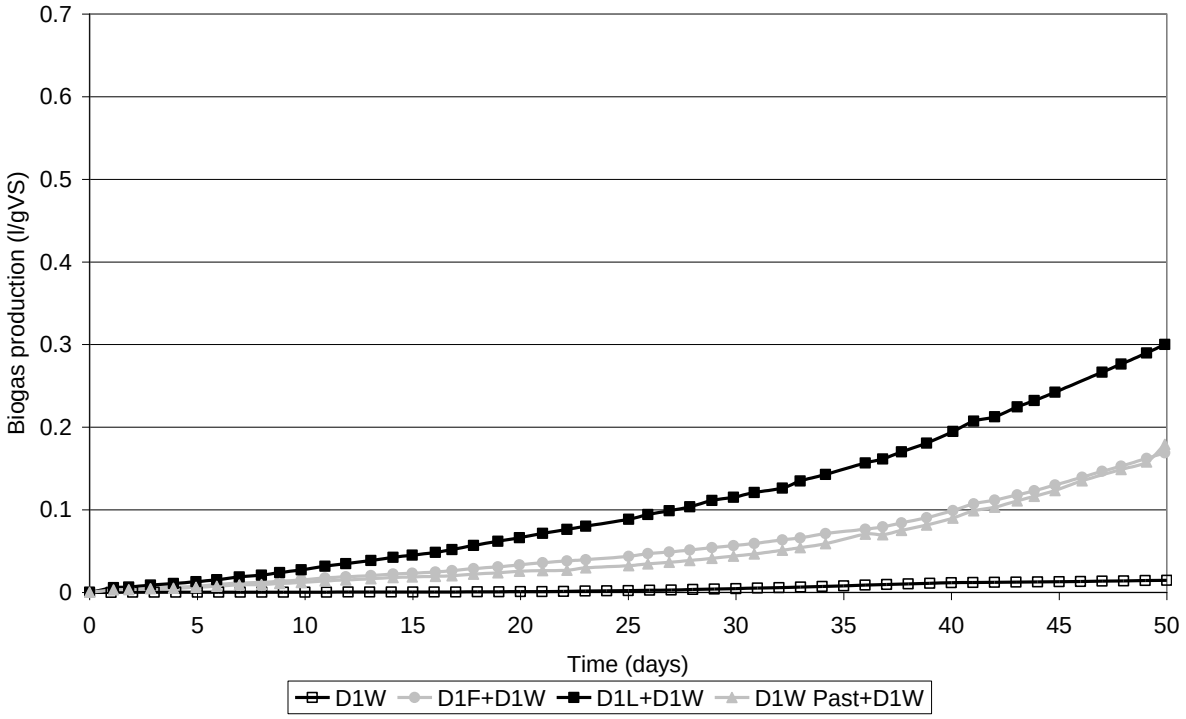

Figure 10 Inoculum addition - RBP data for D1W, D1F and D1L with D1W as inoculum

Note to Figure 10: OU data for the RBP of D1W is shown after day 19 and was also used to calculate the contribution of D1W as an inoculum since the D1W only test at UoS failed after 19 days.

Inoculum from different sources

The results from testing D1W and cellulose with different inoculum samples are shown in Figure 11. It can be seen that despite each inoculum having a different RBP (0.05 - 0.1 l / g VS), when used with D1W and cellulose the results showed good agreement for the RBP after 50 days. The test kinetics were different for each inoculum, however. This is particularly noticeable with the digestate samples, where a test period shorter than 28 days would lead to inconsistent results because of the occurrence of lag periods.

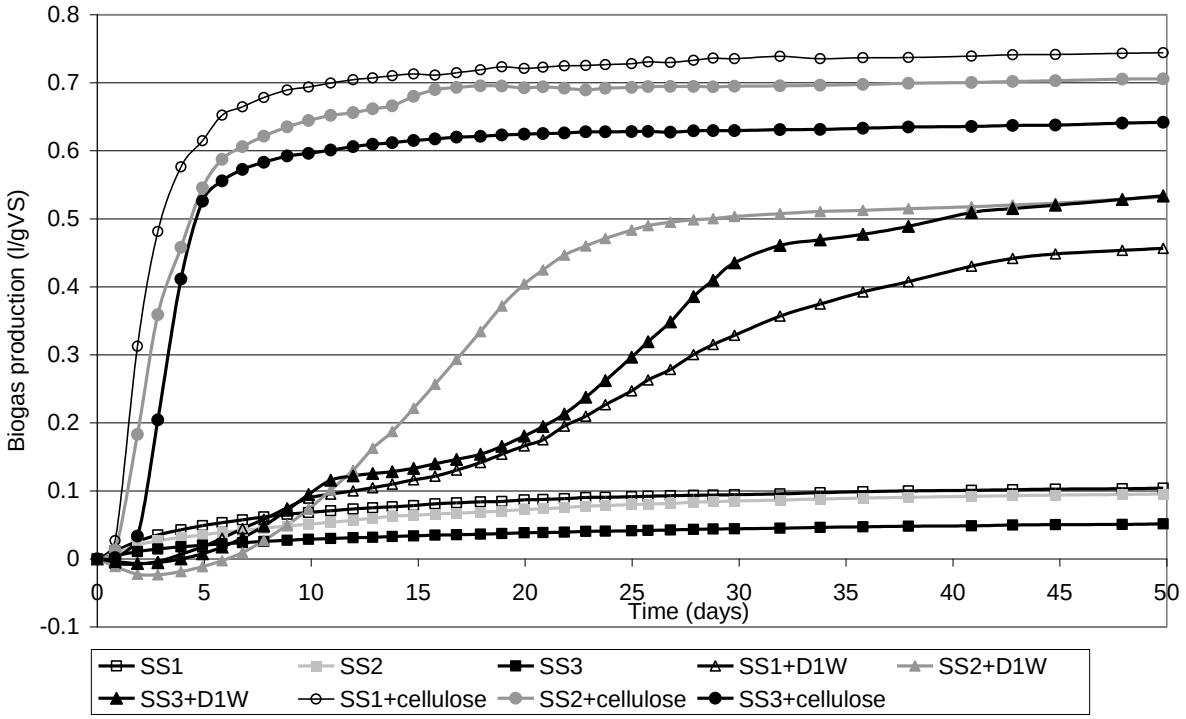

Figure 11 Different inoculum – RBP data for D1W with inoculum addition of SS1, SS2 and SS3

Gas collection method

This trial was carried out using D1W, D1F and D1L with SS1 inoculum. The results are presented in Table 4 and can be compared with those from the reproducibility check (gas collection in water displacement gasometers) in which the 28-day values for these materials were approximately 0.3, 0.3 and 0.55 l / g VS, respectively. While the D1L values for both gas collection techniques compare well, for the D1W and D1F fractions the impermeable gas storage bags appeared to collect more biogas than collection under a barrier solution. The advantage of using this type of gas storage bag is that the time spent monitoring the test is greatly reduced as a single reading of gas volume can be made at the end of the test period. The associated disadvantage of this method, however, is that it produces a single point value on the date chosen for reading the result and without the test kinetics it is difficult to tell if the test has reached completion. More frequent measurement is possible but negates the advantage of reduced effort and may lead to increased error in correction of gas volumes.

Table 4 RBP values D1 fractions measured with impermeable gas storage bags after 28 days

Test substrate	Substrate 28-day gas production l / g VS added	
	biogas	methane
D1W	0.38	0.29
D1F	0.34	0.26
D1L	0.57	0.45

3.1.4 Conclusions from the test development work

The main conclusions arising from the test development programme were as follows:

- **RBP test duration -** Findings from the test development phase using samples of D1 and D2 indicated that the test duration to reach completion would be over 50 days when inoculum is not used.
- **Use of an inoculum -** The results indicate that sludge from anaerobic digesters treating municipal wastewater biosolids is a suitable inoculum. The main function of the inoculum is to increase the rate of biogas production by as much as 10 times which may allow the RBP test to be competed in an acceptable time period. It is also an essential requirement where digestate has been pasteurised.
- **Use of inoculum from different sources -** Inoculum from different sources (different sewage works) showed different test kinetics but the final RBP was the same in all cases. In short duration tests, particularly those on liquid fraction samples, the lag phases were of different durations and intensities for the different inoculum sludges: this could lead to different reported RBP values in a short-duration test where the total RBP was not realised. The test using three inoculum sludges tests all gave similar values and kinetics when used with the cellulose standard substrate.
- **Sample drying -** Using dried samples for the RBP test was found to have major disadvantages when testing digestates: a longer lag phase before biogas production compared with testing digestates as received, and the strong probability that a significant amount of biodegradable carbon as VFAs may be volatilised during drying. It is unlikely that the consistency offered by sample drying outweighs the disadvantages outlined above.
- **Gas collection method -** The use of impermeable gas storage bags may reduce biogas losses during collection but these are minimised by the use of an acidified saline solution and the differences seen were therefore small. The advantage of collection under a barrier solution is that it allows the kinetics of gas production to be followed, and this outweighs the advantages associated with the use of impermeable gas storage bags for this test as evaluated in this project.
- **Temperature -** There appear to be no clear advantages in reducing the incubation temperature to 32 $^{\circ}$C compared with the accepted incubation temperature of 35 $^{\circ}$C, however a temperature of 35 $^{\circ}$C is most commonly recommended in scientific literature on this subject.
- **Nutrient addition -** When using an inoculum from anaerobically digested municipal wastewater sludge there appeared to be no benefit from nutrient addition. It is possible however that that certain combinations of digestate and inoculum could be nutrient limited leading to low biogas production and a false positive result

(pass). Given the small cost of the nutrient addition relative to the cost of the test, the consortium recommends that nutrients are added.

- **Effect of storage -** The RBP values for the raw digestates from the primary digestion tanks were appreciably higher than those for digestates which had been stored or further digested. Overall, the effect of further digestion (D1) or storage (D2) for the digestates tested was to reduce RBP values by 50% and 33% respectively. It is recommended that operators are given clear guidance about the point in the process from which samples are to be taken for testing.

3.2 Inter-laboratory Testing

The second part of the work was an inter-laboratory comparison to assess the variability in results from different laboratories following the same test procedure with a broad range of digestate types. To reduce the effect of differences in sample collection and preparation this work was carried out by a single laboratory and samples for testing were then distributed to all partners. All laboratories used the same inoculum.

3.2.1 Methodology

Digestates D1, D3, D4 and D5 and their separated fractions were tested in triplicate in the laboratories of OU, UoS and WRc, as shown in Table 5. The method of test used took into account the results of the test development work although it was considered that the inter-laboratory study presented a further opportunity to refine the procedure. The main features of the inter-laboratory test were as follows:

- Test duration of 50 days
- Sewage sludge inoculum (SS1) added at a VS ratio of 2:1 (inoculum:digestate)
- Test carried out on samples taken from post-digestion storage (where applicable)
- Total weight of test material (sample plus inoculum) of 400 g
- Test temperature 35 °C
- Gas measurement in liquid displacement gasometers filled with acidified (pH 2) salt solution (270 g / l), corrected to STP (273.15 K, 10^5 Pa) and reported per gram VS of digestate added to the test
- Nutrients added to all test reactors (see appendix 9.3)

Samples were collected and processed into their fibre and liquor fractions by WRc and then distributed to each partner. The day before testing was due to start, a fresh inoculum of digested sewage sludge was collected from Millbrook wastewater treatment works, Southampton and sieved though a 1 mm mesh at UoS (SS1) before distribution to the other laboratories. All tests started on the same day and lasted 50 days.

Table 5 Summary of inter-laboratory testing

UoS	#	OU	#	WRc	#
SS1	6	SS1	3	SS1	3
SS1+Cellulose	3	SS1+Cellulose	3	SS1+Cellulose	3
SS1+D1W	6	SS1+D1W	3	SS1+D1W	3
SS1+D1L	3	SS1+D1L	3	SS1+D1L	3
SS1+D1F	3	SS1+D1F	3	SS1+D1F	3
SS1+D3W	3	SS1+D3W	3	SS1+D3W	3
SS1+D3L	3	SS1+D3L	3	SS1+D3L	3
SS1+D3F	3	SS1+D3F	3	SS1+D3F	3
SS1+D4W	3	SS1+D4W	3	SS1+D4W	3
SS1+D4L	3	SS1+D4L	3	SS1+D4L	3
SS1+D4F	3	SS1+D4F	3	SS1+D4F	3
SS1+D5W	3	SS1+D5W	3	SS1+D5W	3
SS1+D5L	3	SS1+D5L	3	SS1+D5L	3
SS1+D5F	3	SS1+D5F	3	SS1+D5F	3
Total	**58**	**Total**	**52**	**Total**	**52**

3.2.2 Results and discussion

Figures 12 and 13 show biogas production after 28 days and 50 days of biogas production from all of the samples for all three laboratories; error bars show the range of the triplicate test results. Examination of the data shows that in most cases the in-house repeatability of the test was good. In some cases the inter-laboratory reproducibility was poor, particularly at day 28 of the test and for samples with the highest biogas production (D1W, D1F, D1L, D3L). Inter-laboratory agreement on the RBP values at day 50 was better.

Some variability is always expected in the results of biological tests of this type, especially when the substances being tested are heterogeneous and relatively small quantities are required in the test. Effort was made by the consortium members to use representative samples for the RBP testing but the methods were limited to mixing (either by shaking or stirring) and the use of judgement to observe the nature of the subsamples and similarity with the bulk samples. Apart from this no other physical homogenisation (e.g. cutting, pulping, crushing) of each of the digestate samples was performed since it is doubted, given the wide range of physical characteristics of digestate samples, that a standard method could be developed for this process. The in-house repeatability of the test depends on an assumed homogeneity, and if this does not exist there is also the potential for a degree of human error in selection of a representative sample. This may particularly apply in the case of digestate that contains garden waste in which large diameter woody materials may be present.

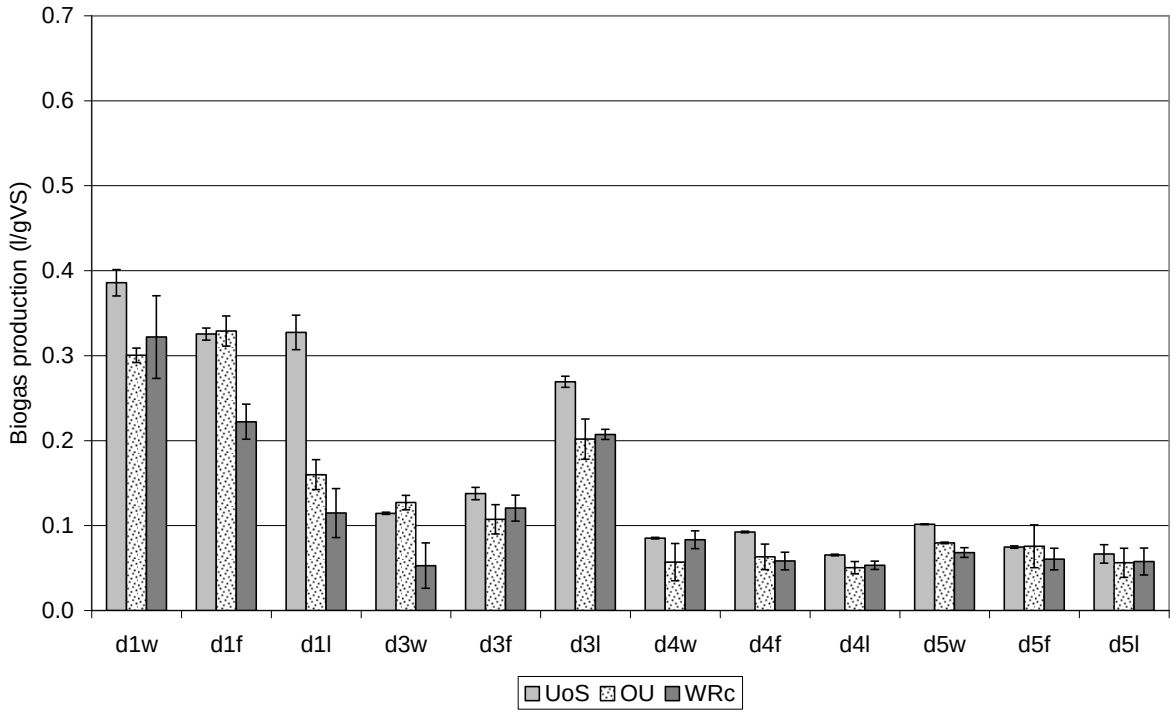

Figure 12 Summary of inter-laboratory testing results of digestates 1 to 5 after 28 days (Average of triplicate tests, error bars show range)

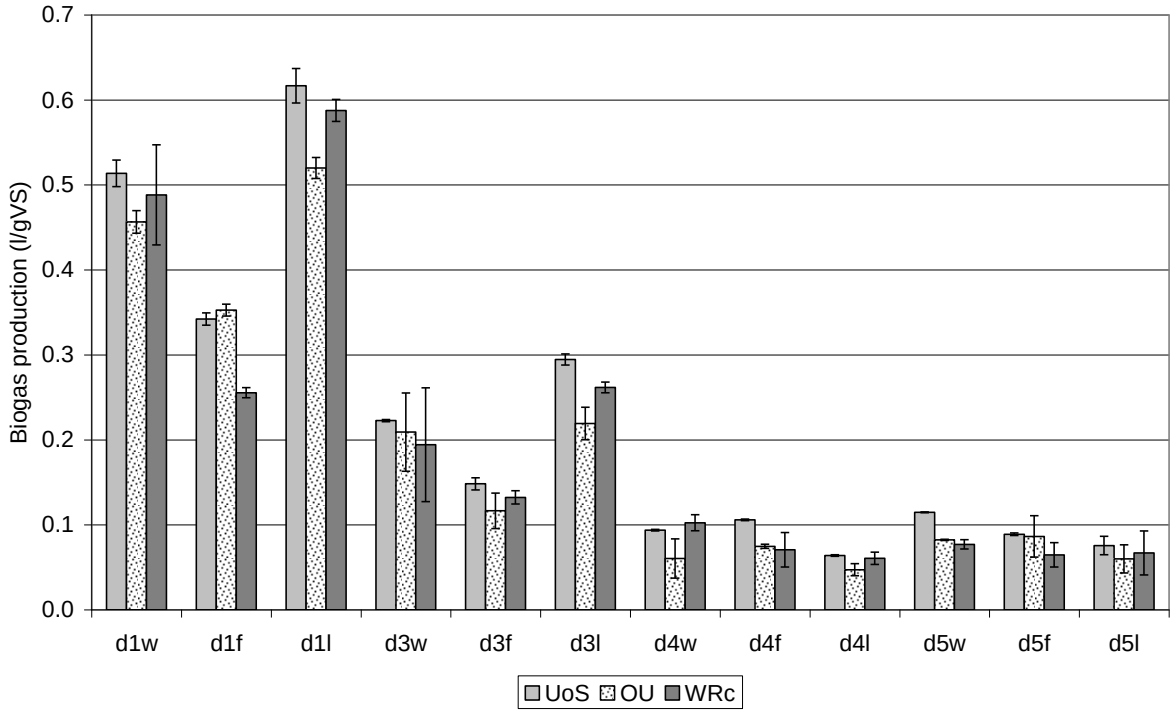

Figure 13 Summary of inter-laboratory testing results of digestates 1 to 5 after 50 days (Average of triplicate tests, error bars show range)

Where there is good sample homogeneity, as in the case of the inoculum (a smooth liquid) and the cellulose standard substance (powder) the tests show very good agreement both in-house and between the different laboratories as seen in Figure 14, which shows the average result from triplicate tests with error bars showing the range. The cellulose tests were operated at an inoculum to substrate ratio of 6:1 on a volatile solids basis, and showed little variation in kinetics between the laboratories.

In-house repeatability of the test was measured by UoS by testing sextuplicate samples of D1W(+SS1) and SS1 only. The results of this are shown in Figure 15 and Figure 16 for 28 and 50 days respectively. The coefficients of variation at 28 and 50 days were 5.1 % and 4.9 % for SS1 and 3.1 % and 4.8 % for D1W respectively which, given the relatively low homogeneity of the samples and the natural variation of biological materials, indicates that good repeatability was achieved.

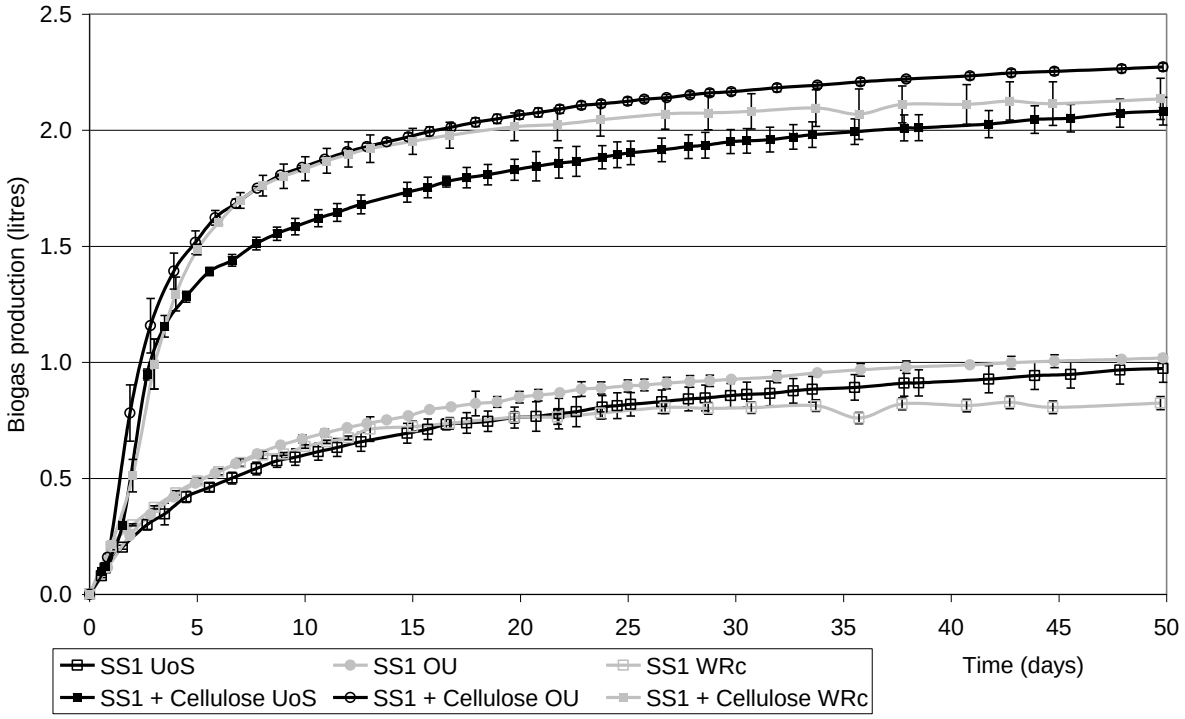

Figure 14 Inter-laboratory testing of the inoculum (SS1) and standard sample (cellulose) (Average of triplicate tests, error bars show range)

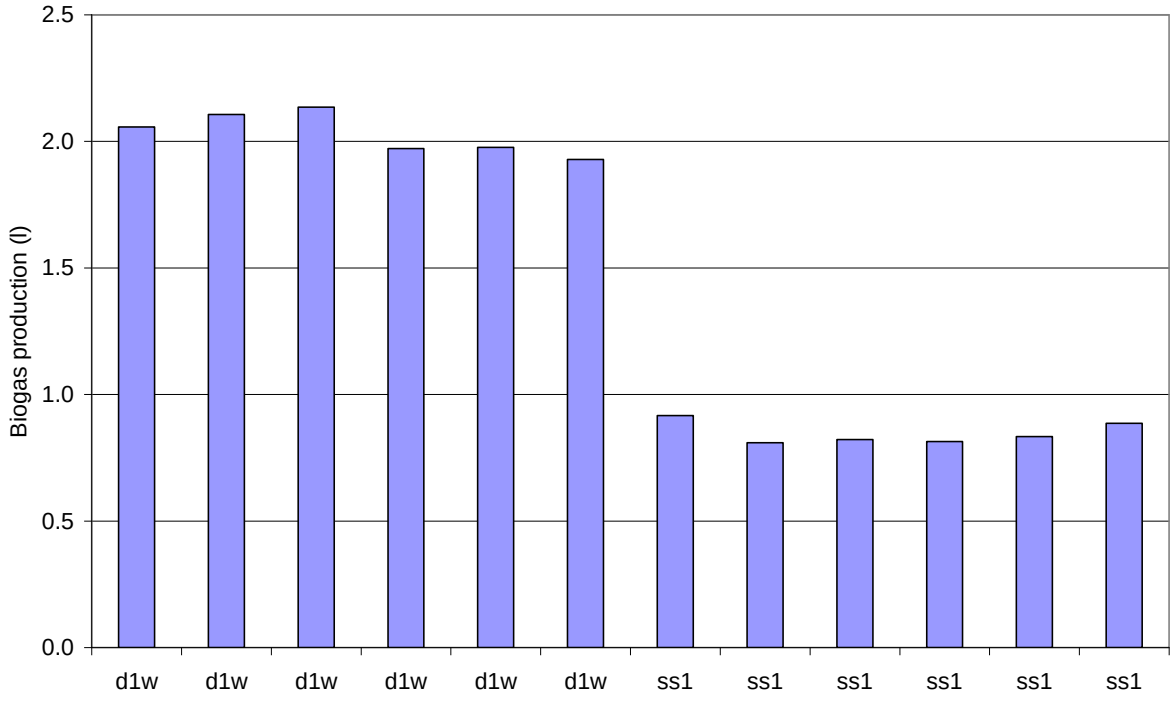

Figure 15 Repeatability of the 28-day RBP test based on sextuplicate testing performed by UoS

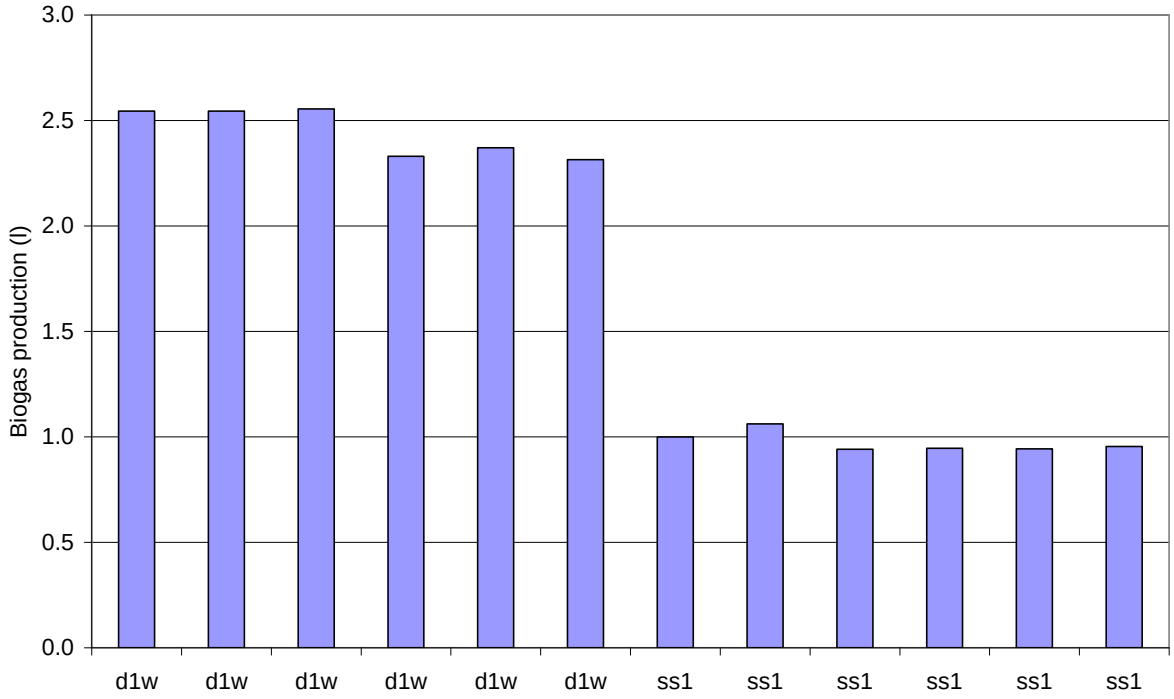

Figure 16 Repeatability of the 50-day RBP test based on sextuplicate testing performed by UoS

The biogas production curves for all samples are included in appendix 9.4 (Figure 24 to Figure 35 inclusive). Further analysis of the results and plotted curves indicated that the variation seen in the 28-day results is due to differences in test kinetics, and in most cases after 50 days the tests reached a similar ultimate RBP value. Ideally the RBP test should be designed to provide sufficient microorganisms and nutrients combined with the correct physical conditions, such that the limiting factor governing the kinetics of biogas production is the rate at which the microorganisms can degrade the substrate. Therefore the ideal gas production kinetic in a successful test shows a zero or short lag phase followed by a steady rate of gas production, which then decreases either gradually or rapidly as the substrate is depleted. The rate at which this occurs is very dependent on the biodegradability of the substrate. This pattern is shown as curve 1 in Figure 17. In practice, however, some of the results showed a long lag phase where, after deduction of the gas produced by the inoculum, the net biogas production from the tests is negative. This is shown in Figure 17 as curve 2. Negative net biogas production is generally associated with some inhibition of the inoculum by the substrate (digestate), either because of a component already present in the substrate or due to rapid fermentation of a readily degradable material producing acidic conditions. In this case the rate of net substrate biogas production can be increased by increasing the inoculum to substrate ratio. Given sufficient time, the final RBP value for a test with less inoculum addition is usually similar to a test with an optimal inoculum to substrate ratio. If a shorter test duration is required it is better to use a higher ratio to avoid problems with inhibition which may lead to incomplete degradation of the digestate in the test period and an apparently low RBP value.

The tests on D1W, D1L and to some extent D3W and D3L (Figure 24, Figure 25, Figure 27 and Figure 28 respectively) show a lag phase of variable length. This is thought to be caused by the high VFA concentrations found in these samples. High concentrations of VFA can exceed the initial methane conversion capacity of the inoculum. The test kinetics for the associated fibre fractions (D1F, D3F) do not show this characteristic. This occurs both because the VFAs are primarily dissolved in the liquid, which is removed when the fibre is separated; and because the fibre has a higher VS content which reduces the volume of sample added to the test. These results indicate that the inoculum to substrate ratio of 2:1 on a volatile solids basis, as used in the inter-laboratory study is not suitable for testing digestate samples with high VFA concentrations in a 28-day period, whereas a 50-day test period gave acceptable results. In order to reduce the effect of high VFA concentrations on the test a higher inoculum to substrate ratio should be used, and is recommended in the proposed full method described in section 4.0 of this report. This recommendation is further supported by the results from the standard cellulose sample (Figure 14) where a higher inoculum to substrate ratio gave a good agreement in test kinetics between laboratories.

The 28-day results in Figure 12 also show that in general the order of total biogas production between the institutions was UoS > OU > WRc. Possible explanations for this are the start-up and day-to-day management of

the test. For example although all attempts were made to ensure the careful handling of the inoculum, the time taken to deliver it from UoS to the other institutions (approximately 6 hours) could have been enough for a temperature variation to disturb the methanogenic population, making it less able to degrade the incoming substrate at the beginning of the test. In this period the inoculum used at UoS was maintained in a water bath at 35 °C and therefore was not subjected to this shock.

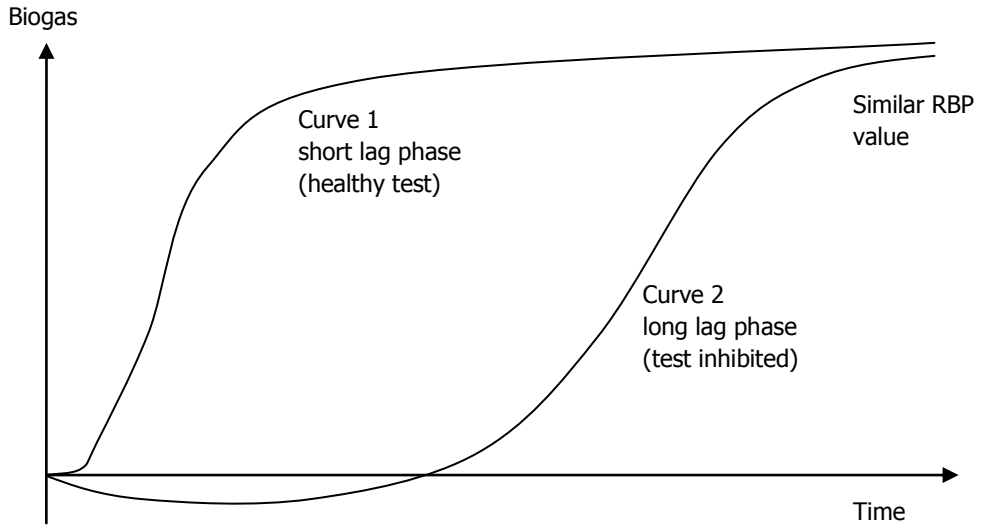

Figure 17 Idealised RBP test result showing similar ultimate biogas production but different test dynamics where the test is inhibited

3.3 Conclusions from the development work and inter-laboratory study

The following main conclusions were drawn from the inter-laboratory study:

- **Effect of VFA –** Samples with high VFA concentration caused a decrease in the inter-laboratory reproducibility of the method. This could be addressed by increasing the inoculum to substrate ratio.

- **Repeatability –** Given the non-homogeneity of the digestate samples and the natural variation in biological materials the in-house repeatability of the test was considered to be good. Care needs to be taken at all times in the handling and preparation of the digestate samples and inoculum to achieve this.

- **Reproducibility –** The inter-laboratory reproducibility of the test is good for samples with relatively low residual biodegradability but some errors occur when testing samples of high VFA concentration. The reproducibility with these samples is better with a longer test. Reproducibility with different inoculum materials was similarly better in a longer test.

- **Test length -** The 28-day test is suitable for substances that contain low concentrations of VFA whereas at high concentrations the test kinetics are variable. This means that at any time before completion some tests will be closer to reaching the final value, leading to variation between results in a shorter test. This effect was minimised in the 50-day results since most of the tests had reached completion in this period.

- **Inoculum sensitivity –** Some of the variation in kinetics of the 28-day result may have been caused by different treatment of the inoculum (temperature shock). The inoculum should be used promptly after collection and kept at 35 °C until required.

3.3.1 Suitability of the test

The test was able to provide information on the RBP of the digestate samples. Samples containing a high VFA concentration had a large RBP which primarily derived from their liquid content. None of the digested fibre samples tested had a large RBP but it can be seen from the response of the test to the standard cellulose sample that it was suitable for materials with high residual biodegradability in their solids content. Some variation between RBP test results in-house and between laboratories is inevitable given the heterogeneity of the samples but test kinetics showed a larger variation where high VFA concentrations were present. Since the initial brief was to create a relatively short test (28 days) it can be said that the test method used in the development work was unsuitable for some of the samples which contained high concentration of VFAs. This is discussed further in section 6.0.

4.0 Full Description of the RBP Test

This section contains a detailed method which, based on the development work and inter-laboratory testing presented in section 3.0, the consortium believes will provide a satisfactory version of the RBP test in terms of robustness, repeatability and inter-laboratory reproducibility. While the concept of the test is simple, the nature of biological testing methods means that care must be taken at all times in order to ensure that valid results are obtained. A detailed method description has therefore been given.

Care should be taken to follow all instructions and advice given in this method to avoid the risk of problems with the test and consequent errors in the results.

4.1 Principle

The residual biogas potential test is designed to measure the stability of digestate samples under anaerobic conditions. Stability is assessed by the measurement of the total quantity of biogas produced by the digestate sample during a specified period of time, which is an indicator of its residual biodegradability.

The test involves placing the digestate, along with excess nutrients and microorganisms, in laboratory reactors and measuring the biogas production for a period of 28 days at a constant temperature of 35°C. Excess microorganisms are provided by mixing the digestate with an inoculum of sewage sludge digestate at the beginning of the test.

Biogas production from the digestate sample mixed with inoculum, as well as the inoculum alone, is measured which allows the biogas production of the digestate sample to be calculated. The biogas production of a control substance of cellulose mixed with inoculum is also measured for quality control purposes.

4.2 Materials and Equipment

The test is carried out in a reactor held in a temperature controlled environment and connected to a liquid displacement gasometer. The simplest type of reactor is a bottle sealed with a bung and the simplest type of gasometer is a cylinder inverted in a trough as shown in Figure 18.

Reactors should be made of glass and each have a volume of between 550 and 650 ml to accommodate the 400 g sample size, leaving sufficient headspace for their samples to expand in size (when biogas is produced) and to allow shaking by hand without the contents entering the gas connection tube.

Reactors should be maintained at a constant temperature of 35 ^{o}C. If a **water bath** is used it should be sufficiently deep that the sample within the bottle is completely submerged (below the water level in the bath).

Bungs if used should have a single hole allowing a rigid tube (3 mm OD) to be inserted, to which flexible tubing can be connected.

Tubing should be flexible and made from PVC or other low-permeability material. All connections between the bungs, tubing and valves should be gas tight.

Nutrient solutions should comprise a mixture of the nutrient types and quantities shown in Table 6, and be stored in a fridge.

Reference material is alpha cellulose as supplied by Sigma-Aldrich (Dorset, UK), product number C8002.

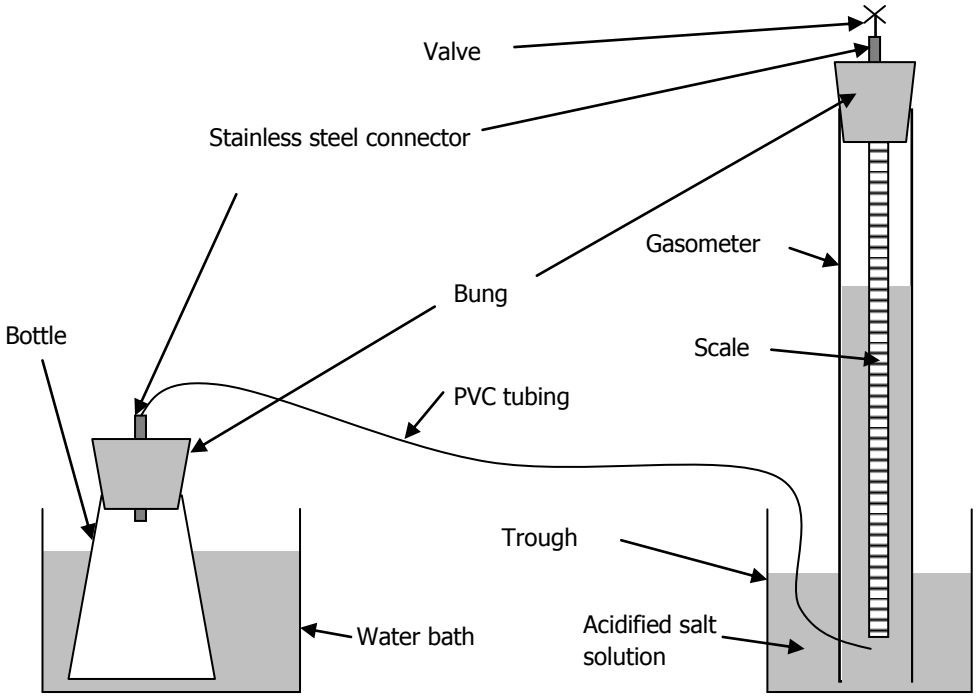

Figure 18 Equipment setup

Table 6 Nutrient solutions (based on Environment Agency (2005))

Major elements (use 10 ml / sample replicate)	**g / L**
KH_2PO_4	13.2
NH_4Cl	13.5
$CaCl_2.2H_2O$	1.88
$MgCl_2.6H_2O$	2.5
Trace elements (use 1 ml / sample replicate)	**mg / L**
$FeCl_2.4H_2O$	50
H2BO3	1.25
$ZnCl_2$	12
$CuCl_2.2H_2O$	1.7
$MnCl_2.4H_2O$	160
$(NH_4)_6Mo_7O_{24}.4H_2O$	2.5
$AlCl_3.6H_2O$	2.5
$CoCl_2.6H_2O$	5.0

4.2.1 Liquid Displacement Gasometers

The use of liquid displacement gasometers allows daily monitoring of the gas production from the RBP tests. This is advantageous as outlined in section 3.1.4. Incorrect biogas volumes can be measured when using liquid displacement gasometers due to:

- diffusion of biogas through the barrier solution into the atmosphere
- diffusion of air through the barrier solution into the gasometers
- no correction for pressure forces within the headspace leading to incorrect calculation of the quantity of biogas present in the gasometers

The method presented is designed to minimise errors in gas volume determination by the use of these liquid displacement gasometers and is based on Walker et al. (2009).

The gasometers are filled with a solution of common table or cooking salt at a concentration of 270 g / l (approx 75 % saturated). The salt should be fully dissolved before the solution is used. Hydrochloric or sulphuric acid should be added dropwise to the solution until the pH is 2. The density of this solution is needed for correct calculation of the gas volumes and should be measured. This can be done simply by weighing a known volume of the solution (e.g. in a volumetric flask).

Density = Weight / Volume

Gasometers should be made from glass or acrylic tube with an inner diameter of 40 to 50 mm and should have a stopper and valve in one end. The cross sectional area of the gasometer (internal) should be calculated as it is needed for gas volume calculation. The tubes should be supported upright in a trough. Measurements that need to be taken from this device are:

- distance from the base of the bung to the column liquid level (hc)
- distance from the base of the bung to the trough liquid level (ht)

This is shown in Figure 1 with the nomenclature as presented in section 3.1.1. Gasometers should have a marked scale against which readings of the meniscus level can be accurately taken: the simplest way is to have a scale that starts where the bung ends (at the top of the gasometer) and runs down the length of the tube into the trough containing the barrier solution. This allows both measurements (hc and ht) to be made from the same scale.

Each time a gas measurement is taken atmospheric temperature (±1 °C or K) and pressure (± 100 Pa) should be recorded. The volume calculation is made using the following equations to give gas volume corrected to standard temperature and pressure (STP) of 273.15 K and 10^5 Pa (1 bar). These standard values should be quoted when reporting the results.

$$V_{stp} = \frac{T_{stp} A}{T_{atm} p_{stp}} \left(\left(p_{atm} - p_{H_2O}(T_{atm}) - \rho_b g \left(h_t - h_c \right) \right) h_c \right) \quad [3]$$

Where $p_{H_2O}(T) = 101324.6 \times 10^z$ [4]

$$z = -7.90298\left(\frac{373.16}{T} - 1\right) + 5.02808 \log_{10}\left(\frac{373.16}{T}\right) - 0.00000013816\left(10^{\left(11.34\left(1-\frac{T}{373.16}\right)\right)} - 1\right) + 0.00813289\left(10^{\left(-3.49149\left(\frac{373.16}{T}-1\right)\right)} - 1\right) \quad [5]$$

Other methods for collecting gas may be substituted but in choosing a method attention should be given to ensure that appropriate arrangements are made for correcting to STP either by calculation or in the equipment itself.

4.3 Digestate Sample, Inoculum and Reference Material Preparation

Samples supplied by the digester operator should be appropriately labelled with the date and time of sampling and the location and point within the process from which the sample was taken.

Testing should begin no later than one week after sample collection.

The digestate sample supplied should be representative of the material produced at that point in the process. As a guide, the sample size supplied to the laboratory should be not less than 1 kg, but to obtain a representative and homogeneous sample it may be necessary to derive this from a larger sample taken on site.

Fresh inoculum should be collected from a mesophilic anaerobic digester treating municipal wastewater biosolids which is reported to be operating satisfactorily. As soon as possible after collection, the inoculum should be kept at the test temperature (35 °C). The inoculum should be passed through a 1 mm screen before use to remove larger particles.

The reference material of cellulose should be used as supplied (in powder form) from Sigma-Aldrich (Dorset, UK).

Testing should begin within 48 hours of inoculum collection.

Total Solids and Volatile Solids are reported as a proportion of the wet weight of the sample material (digestate, inoculum and reference material). Before measurement of these parameters, the sample is thoroughly mixed to ensure good homogeneity.

Total Solids (TS) in g / kg is obtained by multiplying the percentage dry matter (w_{dm}) by 1000 g / kg and dividing by 100 %, where w_{dm} is measured in accordance with BS EN 14346:2006.

$$\text{TS (g / kg)} = \frac{w_{dm}\ (\%) \times 1000\ (\text{g / kg})}{100\ \%}$$

Volatile Solids (VS) in g / kg is obtained by multiplying the TS by the loss on ignition (W_{LOI}) and dividing by 100 %, where W_{LOI} is measured in accordance with BS EN 15169:2007.

$$\text{VS (g / kg)} = \frac{\text{TS (g / kg)} \times W_{LOI}\ (\%)}{100\ \%}$$

TS and VS are measured in triplicate and the average value is reported and used in all calculations.

4.4 Test Method

Good laboratory practice should be followed at all times. Maintain clean working spaces and make sure that all equipment is thoroughly cleaned before use.

All tests should be performed in triplicate (3 x digestate sample with inoculum, 3 x reference material with inoculum, and 3 x inoculum control). Note that if more than one digestate sample is being tested, common tests for reference material and inoculum can be used (3 x digestate sample 1 with inoculum, 3 x digestate sample 2 with inoculum,….. 3 x reference material with inoculum, and 3 x inoculum control)

1. Use the VS content (g / kg of wet weight) of the digestate and inoculum to calculate the weights for use in each digestate sample test using the following formula:

$$\text{Digestate added (g)} = \frac{400}{1+\left(\frac{R * VS_{digestate}}{VS_{inoculum}}\right)}$$, where the inoculum to substrate ratio, R = 4. [1]

Inoculum added (g) = 400 – digestate added. [2]

For each reference material test use the following formula to calculate the amount of cellulose to add;

$$\text{Cellulose added (g)} = \frac{400}{1+\left(\frac{R*VS_{cellulose}}{VS_{inoculum}}\right)}$$, where the inoculum to substrate ratio, R = 6. [6]

Inoculum added (g) = 400 – cellulose added. [7]

2. Ensure the digestate sample is well mixed and add the required amount to the reactor. Representative sampling is more important than achieving the exact weight required. Record the weight of sample used for each test, accurate to ± 0.1 g. Use the measured weight rather than the calculated weight in all further calculations.

   a. If the test is for inoculum only (the control) no digestate is added.
   b. If the test is for the reference material the weight of cellulose added should be accurate to ± 0.01 g.

3. Ensure that the inoculum is well mixed and add the required amount to the test bottle. Record the weight of inoculum added, accurate to ± 1 g. Use the measured weight rather than the calculated weight in all further calculations.

   a. If the test is an inoculum control, 400 g of inoculum should be added, accurate to ± 1 g.
   b. If the test is of reference material add the amount of inoculum as calculated in equation 7, accurate to ± 1 g.

4. Add 10 ml of the major elements solution and 1 ml of the trace elements solution.

5. Place the reactor in the temperature controlled environment (e.g. water bath) at the test temperature; flush the headspace of the bottle with nitrogen before attaching to the gasometer. Swirl the contents of the bottle to ensure they are mixed.

6. Wait for 15 minutes to allow the temperature of the headspace gas to equilibrate.

7. Use a vacuum pump to draw the barrier solution to the top of the gasometer, then close the valve.

8. Record the time, date, water bath temperature, atmospheric temperature and pressure, hc and ht.

9. Each day at approximately the same time;

   a. Shake the bottle carefully to mix the contents and allow any trapped gas to escape, ensuring that digestate does not enter the tube as a blockage could cause the bung to be ejected from the bottle leading to test failure.
   b. Record the time, date, thermostatically controlled temperature, atmospheric temperature and pressure, hc and ht.

10. The test is complete 28 days after its start date.

4.5 Result Calculation and Reporting

Table 7 shows the nomenclature used in the method for calculating the RBP for each of the sample types (digestate, reference and inoculum). The text and equations below set out the method, including calculated results for use when assessing the quality control of the test (see section 4.6). An example of the RBP calculation for a digestate, reference and inoculum sample is available as an Excel Worksheet titled Example_RBP_calculation.xls. This can be obtained from the WRAP website or by contacting the corresponding author (mark.walker@soton.ac.uk).

The RBP of each digestate, inoculum and reference sample can be calculated on any day of the test where biogas measurements are taken (for some of the quality control checks in section 4.6), however the final result of the RBP test uses the biogas production from the 28th day after its start date (for digestate sample RBP evaluation and for further quality control checks on the reference material and inoculum used in the tests).

The RBP of each digestate sample (RBPD) tested in each of the triplicate reactors should be calculated separately using the following formulae;

$$RBPDa\ (l / g\ VS) = \frac{(DGa\ (l) - AIDa\ (l)) \times 1000}{Da\ (g) \times DVS\ (g / kg)} \quad [8a]$$

$$RBPDb\ (l / g\ VS) = \frac{(DGb\ (l) - AIDb\ (l)) \times 1000}{Db\ (g) \times DVS\ (g / kg)} \quad [8b]$$

$$RBPDc\ (l / g\ VS) = \frac{(DGc\ (l) - AIDc\ (l)) \times 1000}{Dc\ (g) \times DVS\ (g / kg)} \quad [8c]$$

Where;

$$AIDa\ (l) = ASIG\ (l / g) \times IDa\ (g) \quad [9a]$$

$$AIDb\ (l) = ASIG\ (l / g) \times IDb\ (g) \quad [9b]$$

$$AIDc\ (l) = ASIG\ (l / g) \times IDc\ (g) \quad [9c]$$

The RBP of each reference sample (RBPR) tested in each of the triplicate reactors should be calculated separately using the following formulae;

$$RBPRa\ (l / g\ VS) = \frac{(RGa\ (l) - AIRa\ (l)) \times 1000}{Ra\ (g) \times RVS\ (g / kg)} \quad [10a]$$

$$RBPRb\ (l / g\ VS) = \frac{(RGb\ (l) - AIRb\ (l)) \times 1000}{Rb\ (g) \times RVS\ (g / kg)} \quad [10b]$$

$$RBPRc\ (l / g\ VS) = \frac{(RGc\ (l) - AIRc\ (l)) \times 1000}{Rc\ (g) \times RVS\ (g / kg)} \quad [10c]$$

Where

$$AIRa\ (l) = ASIG\ (l / g) \times IRa\ (g) \quad [11a]$$

$$AIRb\ (l) = ASIG\ (l / g) \times IRb\ (g) \quad [11b]$$

$$AIRc\ (l) = ASIG\ (l / g) \times IRc\ (g) \quad [11c]$$

Calculation of the RBP of each inoculum control (RBPI) should be done using the following formulae;

$$RBPIa\ (l / g\ VS) = \frac{IGa\ (l) \times 1000}{Ia\ (g) \times IVS\ (g / kg)} \quad [12a]$$

$$RBPIb\ (l / g\ VS) = \frac{IGb\ (l) \times 1000}{Ib\ (g) \times IVS\ (g / kg)} \quad [12b]$$

$$RBPIc\ (l / g\ VS) = \frac{IGc\ (l) \times 1000}{Ic\ (g) \times IVS\ (g / kg)} \quad [12c]$$

Calculation of the specific inoculum gas production (SIG), the average of which (ASIG) is used in equations 9 and 11 should be done using the following formulae;

$$SIGa\ (l/g) = \frac{IGa\ (l)}{Ia\ (g)} \quad [13a]$$

$$SIGb\ (l/g) = \frac{IGb\ (l)}{Ib\ (g)} \quad [13b]$$

$$SIGc\ (l/g) = \frac{IGc\ (l)}{Ic\ (g)} \quad [13c]$$

$$ASIG\ (l/g) = \frac{SIGa\ (l/g) + SIGb\ (l/g) + SIGc\ (l/g)}{3} \quad [13d]$$

The results are reported as follows:

1. A plot of digestate RBP (RBPD), based on the average of the triplicate values (RBPDa, b, c ~ digestate with inoculum) for each day the biogas was measured.
2. On the same graph as for item 1, a plot of the reference sample RBP (RBPR), based on the average of the triplicate values (RBPRa, b, c ~ cellulose with inoculum) for each day the biogas was measured.
3. On the same graph as for item 1, a plot of the inoculum RBP (RBPI), based on the average of the triplicate values (RBPRa, b, c ~ inoculum only) for each day the biogas was measured.
4. The triplicate TS and VS values of each material (digestate, reference and inoculum before use in the RBP tests), and the calculated averages of each material's TS and VS values.
5. The triplicate values of the 28-day inoculum control RBP (RBPIa,b,c ~ inoculum only), and the calculated average of those values.
6. The triplicate values of the 28-day reference sample RBP (RBPRa,b,c ~ cellulose with inoculum), and the calculated average of those values.
7. The triplicate values of the 28-day digestate sample RBP (RBPDa,b,c ~ digestate with inoculum), and the calculated average of those values.

The report should include quality control information and evaluation, see section 4.6.

Table 7 Nomenclature for the RBP calculation

Example calculation for a digestate sample (a, b and c refer to triplicate tests)				
Quantity	**Source**	**Nomenclature**		
VS digestate (g / kg)	tested as per BS EN 15169:2007 then calculated as instructed in report section 4.3, average of triplicate values	DVS		
weight of digestate added (g)	calculated in Eq. 1 then measured out	Da	Db	Dc
weight of inoculum added (g)	calculated in Eq. 2 then measured out	IDa	IDb	IDc
digestate test gas production (l)	measured in RBP test	DGa	DGb	DGc
average inoculum contribution (l)	calculated using Eq. 9	AIDa	AIDb	AIDc
RBP test sample (l / g VS)	calculated using Eq. 8	RBPDa	RBPDb	RBPDc
average RBP test sample (l / g VS)	average (mean) of RBPDa, RBPDb and RBPDc	RBPD		
Example calculation for a reference sample (a, b and c refer to triplicate tests)				
Quantity	**Source**	**Nomenclature**		
VS reference (g / kg)	tested as per BS EN 15169:2007 then calculated as instructed in report section 4.3, average of triplicate values	RVS		
weight of reference material added (g)	calculated in Eq. 6 then measured out	Ra	Rb	Rc
weight of inoculum added (g)	Calculated in Eq. 7 then measured out	IRa	IRb	IRc
reference test gas production (l)	measured in RBP test	RGa	RGb	RGc
average inoculum contribution (l)	calculated using Eq. 11	AIRa	AIRb	AIRc
RBP reference sample (l / g VS)	calculated using Eq. 10	RBPRa	RBPRb	RBPRc
average RBP reference sample (l / g VS)	average (mean) of RBPRa, RBPRb and RBPRc	RBPR		
Example calculation for an inoculum control sample (a, b and c refer to triplicate tests)				
Quantity	**Source**	**Nomenclature**		
VS inoculum (g / kg)	tested as per BS EN 15169:2007 then calculated as instructed in report section 4.3, average of triplicate values	IVS		
weight of inoculum added (g)	given as 400 g per reactor vessel in report section 4.4 then measured out	Ia	Ib	Ic
inoculum test gas production (l)	measured in RBP test	IGa	IGb	IGc
RBP of inoculum (l / g VS)	calculated using Eq. 12	RBPIa	RBPIb	RBPIc
average RBP inoculum (l / g VS)	average (mean) of RBPIa, RBPIb and RBPIc	RBPD		
specific inoculum gas production (l / g)	calculated using Eq. 13	SIGa	SIGb	SIGc
average inoculum gas production (l / g)	average (mean) of SIGa, SIGb and SIGc	ASIG		

4.6 Quality Control

A number of conditions must be met for the test results to be valid; these can be split into three groups of quality control information. The RBP of each digestate, inoculum and reference sample should be calculated for each day on which biogas measurements are taken during the test. The final results of the inoculum RBP test and reference material RBP test use the biogas production from the 28th day after their start date.

Inoculum test quality control

1. The inoculum control should produce a measurable volume of biogas over the 28 day period. If no biogas production is observed the inoculum is unsuitable (RBPI). Report this item, with a statement of whether the test is valid.
2. The plots of the inoculum RBP (one plot line of RPBI results for each of the sample's triplicates) should be smooth with no obvious spikes or inconsistencies that suggest faulty equipment (temperature, leaks etc.) or incorrect calculation methods. Include in the report a statement based on qualitative evaluation of the plots.

Reference material test quality control

1. The reference material RBP (RBPR) is allowed to be negative only during the first 5 days of the test. If the reference material RBP (RBPR) is negative beyond the first 5 days of the test the inoculum is unsuitable. Report this item, with a statement of whether the test is valid.
2. The 28-day RBP of the reference material (RBPR) should exceed 0.5 litre of biogas per gram volatile solids (l / g VS). Report this item, with a statement of whether the test is valid.
3. The plots of the reference RBP (one plot line of RBPR results for each of the sample's triplicates) should be smooth with no obvious spikes or inconsistencies that suggest faulty equipment (temperature, leaks etc.) or incorrect calculation methods. Include in the report a statement based on qualitative evaluation of the plots.

Digestate test quality control

1. The digestate RBP (RBPD) is allowed to be negative only during the first 5 days of the test. If the digestate RBP (RBPD) is negative beyond the first 5 days of the test, the test is invalid as the inoculum is being inhibited. Report this item, with a statement of whether the test is valid.
2. The plots of the digestate RBP (one plot line of RBPD results for each of the sample's triplicates) should be smooth with no obvious spikes or inconsistencies that suggest faulty equipment (temperature, leaks etc.) or incorrect calculation methods. Include in the report a statement based on qualitative evaluation of the plots.

5.0 RBP Testing of 'Other Materials' Commonly Applied to Agricultural Land

There is a lack of evidence on which to base an RBP value that represents product stability and process efficiency. Consequently it was decided to run parallel tests on a number of other materials commonly applied to agricultural land. Two cattle slurries, two pig slurries and five sewage sludge samples were tested as summarised in Table 8.

Table 8 Summary of other materials tested

Gas collection method	Sample	Test length (days)	#
Liquid displacement	CS1	50	3
	CS2	50	3
	PS1	50	3
	SS1W	50	3
	SS2W	50	3
	SS3W	50	3
	SS4W	50	3
	SS5W	50	3
Impermeable gas storage bags	PS2	28	3
	SS2 Cake	28	3
		Total	**30**

The 28-day and 50-day RBP values for these materials are shown in Figure 19 and Figure 20. The biogas production curves for materials where gas collection was by liquid displacement is shown in Figure 21. The specific biogas production after 28 days ranged from 0.067 to 0.260 l / g VS and all showed a long, gradual biogas production. It should be noted that although the cattle and pig slurries are applied to agricultural land, they are also used by anaerobic digestion operators as a feed material. Their residual energy potential is thus greater than that shown by the samples derived from sewage sludge digesters which have already been through a digestion process.

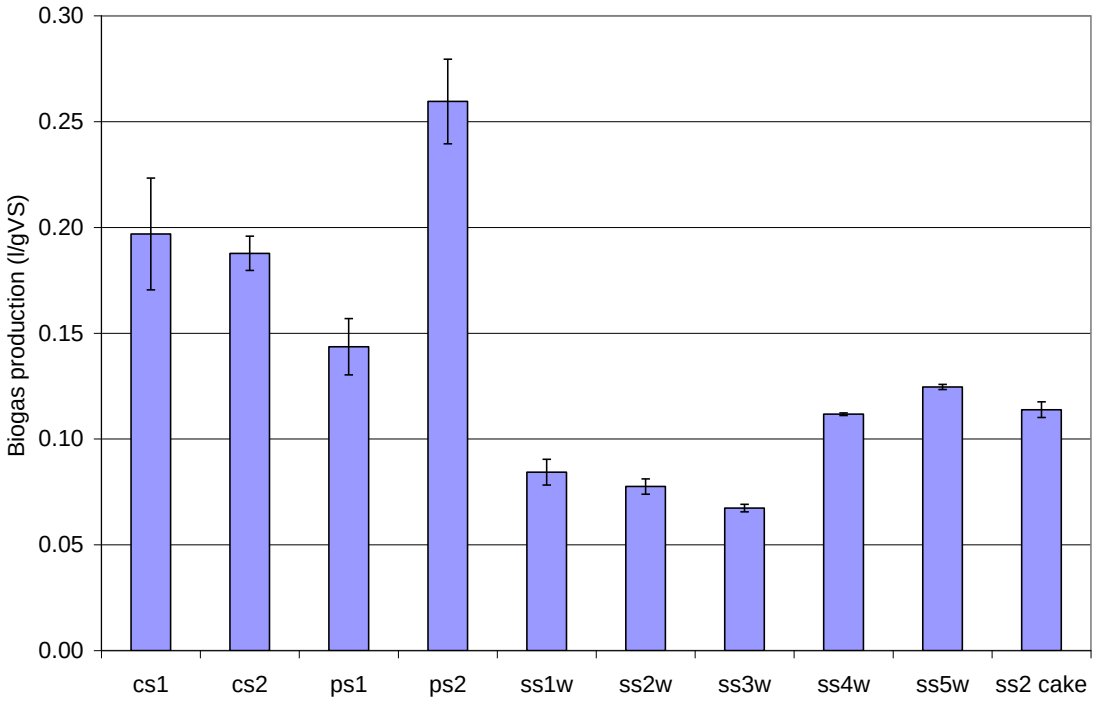

Figure 19 Biogas production of 'other materials' after 28 days (Average of triplicate tests, error bars show range)

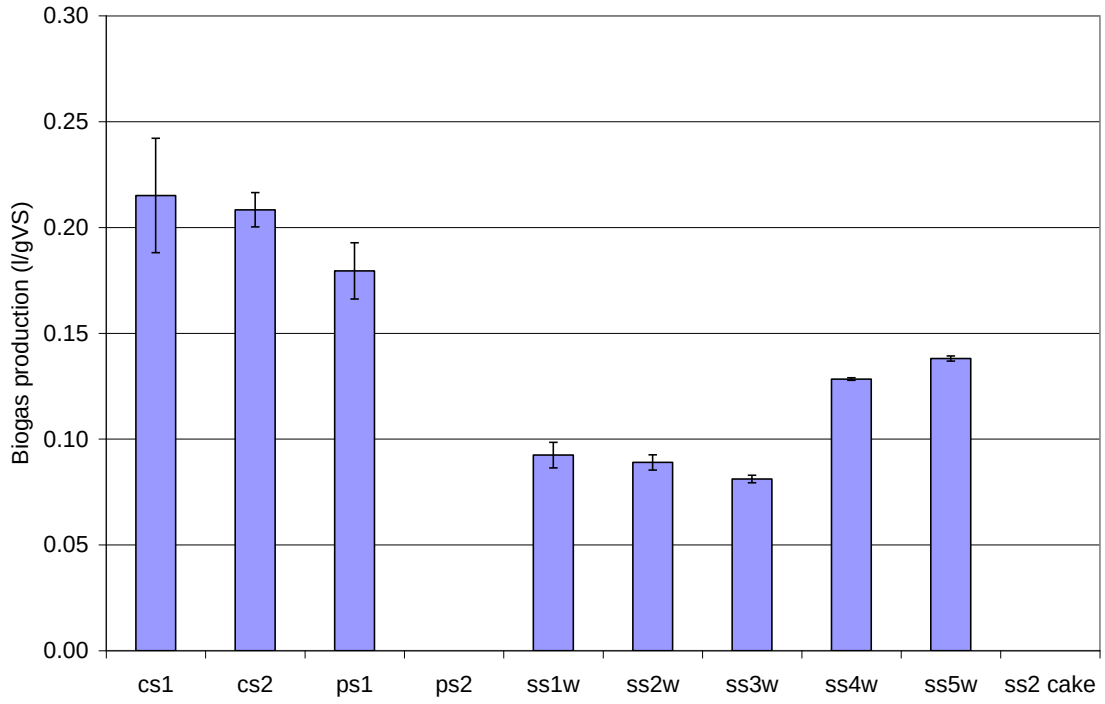

Figure 20 Biogas production of 'other materials' after 50 days (Average of triplicate tests, error bars show range)

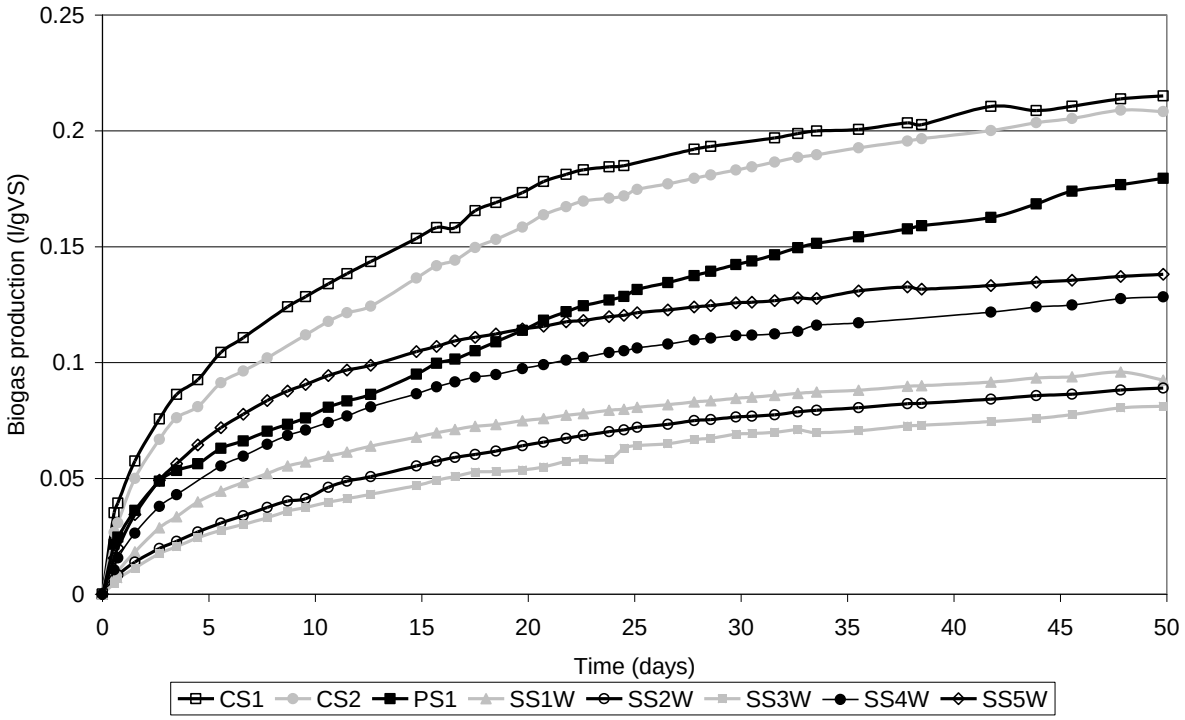

Figure 21 Biogas production curves for 'other materials' (Average of triplicate tests)

6.0 Suggested Limits and Recommendations for PAS 110

Precautionary principle

The recommendations in this report are made in the context of the precautionary principle. Determination of digestate impact on soils was not part of the development work, and it may be that at RBP similar to that of raw slurry there is a detrimental impact; or it may be that higher levels can be tolerated. Further justification would be needed, however, to set a RBP above these typical values, and risk assessment work should be carried out to support any limits which are set.

6.1 Recommended RBP Limit

The consortium recommends an RBP limit value of 0.25 l / g VS based on the RBP values of other materials which are commonly applied to land. This assumes a 28-day test which has passed the quality control requirements as described in section 4.6.

The conclusions of the development work suggest that a 28-day test may be unsuitable for materials with a high VFA concentration which may produce inhibition in the RBP test. Therefore the second recommendation is that digestate samples be pre-screened based on their dissolved chemical oxygen demand (COD), on the basis that failure in the pre-screening test guarantees failure of the RBP test.

6.2 Pre-screening test

The VFA concentration was considered as a method of assessing stability and quality of digestate samples for PAS 110. While a high VFA concentration indicates high potential biodegradability, the absence of VFA does not necessarily indicate the opposite. For example the VFA content of fresh food waste is very low but does not mean this is a stable product in the context of land application. Figure 22 shows the correlation between VFA concentration and RBP for all the materials tested in this work and while there is some relationship, the correlation is weak and therefore VFA concentration alone cannot be related to stability.

The second complication with the use of VFA concentration is that a wide range of methods are in use which do not always produce identical results, as shown in previous work carried out by OU which is summarised in 9.1.

Despite these difficulties, it can be said that if a sample has a VFA concentration with a stoichiometric equivalent biogas value greater than the RBP limit, it will fail the RBP test since these VFAs are the precursors to biogas production in the digestion process. Pre-screening of samples for VFA would therefore allow rejection of those whose biogas potential exceeds the RBP limit value recommended for PAS 110. This is advantageous as it reduces the time and cost of testing and eliminates samples that will fail the recommended RBP test limit of 0.25 l / g VS.

Given the variability in VFA quantification methods noted above, the consortium recommends the use of a soluble COD test as a pre-screening test, on the following basis. In anaerobic digestion the soluble COD is closely related to the VFA concentration and can be stoichiometrically converted to a theoretical methane volume (2.86 g COD / litre methane). Measurement of soluble COD is relatively simple and is a standard method (Standard Methods 5520 C. and 5520 D. (APHA 2005)). Where VFAs can be quantified by chromatographic measurement using a recognised method (e.g. gas chromatography with FID detection; ion exclusion chromatography), then the results of VFA expressed as COD equivalent should be accepted for the purposes of the test. As an example of a method for the determination of VFA by gas chromatography see appendix 9.2 where the method recommended by Shimadzu (Milton Keynes, UK) is given. This is the method that was used to determine the VFA concentrations in this work.

The recommended pre-screening test limit value of 0.43 g COD / g VS is based on the COD of the methane in the recommended RBP test limit of 0.25 l / g VS. This should be calculated by the following method.

$$\text{g COD / g VS} = \frac{\text{soluble COD of the digestate (mg / l)}}{\text{1000*VS of digestate (g / l)}} \quad [14]$$

$$\text{Where VS of digestate (g / l)} = \text{10*VS content as a percentage of wet weight (\%)} \quad [15]$$

The conversion between biogas and COD is based on the following logic;

- The COD equivalent of 0.25 litres of biogas can be calculated from the methane content.
- In order to estimate the methane content of biogas produced from VFA, the Buswell equation (Buswell and Mueller, 1952) can be used to calculate the theoretical value from each VFA species as shown in Table 9. For the current purpose, a value of 60 % methane in biogas can be taken as a conservative but realistic choice.
- At 60 % methane, biogas has a COD equivalent of 1.71 g COD / litre biogas.
- 0.25 litres of biogas is therefore equivalent to 0.43 g COD and 0.25 l / g VS is equivalent to 0.43 g COD / g VS.

If VFAs are quantified these can be converted to COD by the formula in equation [16], where each VFA is expressed in grams per litre (g / l)

VFA as COD = (1.066*Acetic acid) + (1.512*Propionic acid) + (1.816*Butyric acid) + (2.037*Valeric acid) + (2.235*Hexanoic acid) + (2.306*Heptanoic acid) [16]

In a sample with no VS, equation [15] could not be applied, but the COD could still be directly converted into biogas equivalent using the above assumptions.

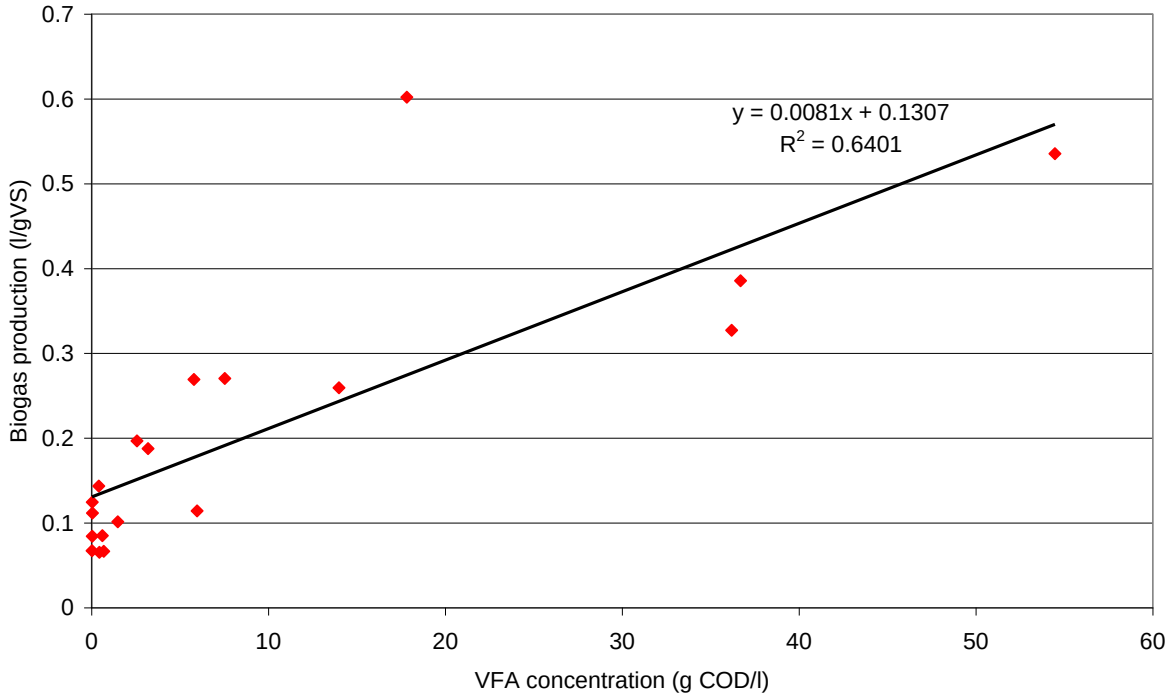

Figure 22 Correlation of VFA with 28-day RBP for all samples tested

Table 9 Theoretical methane content of biogas from VFA degradation

VFA	Theoretical methane composition in biogas (%)
Acetic acid	50.0
Propionic acid	58.3
Butyric acid	62.5
Valeric acid	65.0
Hexanoic acid	66.7
Heptanoic acid	67.9

6.3 RBP and pre-screening test results

Table 10 shows how the results from the trials carried out relate to the recommended limits as proposed in the previous section. It can be seen that the proposed pre-screening test produces no false negative RBP results but would eliminate the need for some RPB tests that would show a 'fail' result.

Table 10 Summary of VFA pre-screening test results

Sample	VS (%)	VFA (as g COD / l)	Pre-test result (g COD / g VS)	RBP result (l / g VS) (UoS data)	Pre-test outcome	RBP outcome
D1W	4.07	36.69	0.901	0.38	Fail	Fail
D1F	8.68	N/A	N/A	0.33	N/A	Fail
D1L	3.28	36.19	1.102	0.33	Fail	Fail
D2W	1.41	7.52	0.533	0.27	Fail	Fail
D3W	2.14	5.96	0.278	0.11	Pass	Pass
D3F	10.39	N/A	N/A	0.14	N/A	Pass
D3L	2.03	5.78	0.285	0.27	Pass	Fail
D4W	3.27	0.60	0.018	0.09	Pass	Pass
D4F	9.30	N/A	N/A	0.09	N/A	Pass
D4L	2.69	0.43	0.016	0.07	Pass	Pass
D5W	12.44	1.47	0.012	0.10	Pass	Pass
D5F	24.88	N/A	N/A	0.07	N/A	Pass
D5L	8.50	0.68	0.008	0.07	Pass	Pass

7.0 Conclusions

The test development and inter-laboratory comparison were carried out in a very limited time frame and under the constraints of a limited number of samples and replicates. The results are therefore not statistically validated. It is likely that as more data become available a better understanding of the range of test values and kinetics will be developed, which may allow further refinement of the PAS 110 stability requirements and a greater awareness of the relationship between RBP and anaerobic digestion process efficiency.

It is possible to have a 28-day RBP test but in order to achieve this certain quality control measures must be followed relating to the inoculum suitability and test procedure. Where test kinetics are suspect due to poor inoculum performance or substrate inhibition, the test result is invalidated. To minimise the occurrence of this a pre-screening test is recommended based on soluble COD or VFA concentrations. To confirm the suitability of the inoculum and demonstrate the efficacy of the test procedures, the RBP of a standard reference material (e.g. cellulose) with inoculum added should also be determined, when each batch of tests is carried out on digestate with inoculum added.

The recommendations on the RBP limit value for PAS 110 have been made based only on equivalent values for materials commonly applied to agricultural land. They do not take into account any soil-related factors such as impact on soil oxygen demand, phyto-toxicity, zoo-toxicity, odour, nutrient imbalance or complexing, effect on soil moisture content etc. It is therefore strongly recommended that further research is carried out on these factors by suitably qualified organisations.

8.0 References

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9.0 Appendices

9.1 OU Scoping Work Assessing Methods of Quantifying Digestate Stability

The Open University undertook a preliminary scoping study in autumn 2008, aimed at assessing the merits of specific test methods for measuring the degree of digestion undergone by digestates (i.e. also known as levels of stability and biodegradability). The aim of this was to enhance understanding of the suitability of particular test methods and to provide background data on the typical levels of stability found for whole digestates and separated fractions. Two main test methods or approaches to measuring stability for digestates were investigated. These were the German RAL-GZ 256 approach which seeks to determine the level of volatile fatty acids (VFAs) present in digestate and the Biochemical Methane Potential (BMP) test which aims to determine the volume of biogas generated by digestates under controlled anaerobic conditions. Also investigated was the use of respiration tests to determine the stability of separated digestate fibre under aerobic conditions, which is taken to reflect the behaviour of digestate fibre in composting environments.

Study objectives

To evaluate three "field" methods for determining volatile fatty acid (VFA) content, involving distillation / titration techniques and one analytical method based on gas chromatography, and to report VFA results.

To subject whole digestate samples and separated liquor and fibre fractions to a programme of Biochemical Methane Testing (BMP) for 21 days to help assess digestate biodegradability.

To subject digestate fibre samples to two aerobic respirometry tests (the CO_2 evolution method specified in the PAS 100:2005 specification for composted materials and DR4 (dynamic aerobic respiration over 4 days at 37°C)) to help assess digestate fibre biodegradability.

To undertake VFA, BMP and respiration tests using two types of digestate (digestate type A and type B).

To undertake appropriate physico-chemical determinations, relevant to characterising digestate properties.

Study findings

For accurate determination of VFA concentrations in digestates within an analytical laboratory context, it is recommended that gas chromatography (GC) should be the preferred method. However, in terms of the "field" methods tested, the German RAL method (distillation and titration) produced total VFA results (Digestate A 2540 mg / l; Digestate B 240 mg / l) which were the closest to the GC results (Digestate A 2597 mg / l; Digestate B 130 mg / l). The double titration and wastewater methods both gave much higher VFA values compared with the GC method and the German RAL "field" method. The German RAL-GZ 256 standard approach is to limit VFAs in digestate to minimise odour / phytotoxicity problems when applied to land. The RAL limit is reported to be 4,000 mg / l of organic acids (VFAs) as acetic acid.

The BMP tests were not complete after 21 days and were continued to completion (110 days). The levels of biogas production from Digestate A and Digestate B (whole digestates, fibre and liquor fractions) appeared to be reasonably low after 21 days and at completion. BMP levels for the whole digestates, fibre and liquor fractions were broadly in the range expected for composted and digested materials which had undergone significant biological treatment, indicating relatively low levels of biodegradability. For example, BMP values for Digestate A and Digestate B whole digestate samples were found to be 65 and 26 l (biogas) / kg LoI respectively after 21 days. These may be compared with values after 21 days found for treated materials in other studies such as digested sewage sludge (80 l (biogas) / kg LoI), fully composted green and kitchen waste (74 l (biogas) / kg LoI) and partially composted and screened green waste (165 l (biogas) / kg LoI). The BMP test and modifications used during this study appeared to be "fit for purpose" for testing all three digestate fractions (whole, fibre and liquor).

From the respirometry testing, the laboratory separated fibre from both digestate samples appeared to be well stabilised (i.e. low biodegradability) if the method of test specified in PAS 100 is adopted which records respiration rates as mg (CO_2) / g (LOI) / day for days 4 to 7 only. The PAS 100 limit for compost is 16 mg (CO_2) / g (LOI) / day. The Digestate A fresh fibre and the Digestate A very mature fibre sample (stored for about 12 months) recorded respiration values for both test methods below this limit. The PAS 100 test recorded a value of

20.8 mg (CO_2) / g (LOI) / day for the Digestate B separated fibre, which exceeded the limit, while the comparable DR4 test recorded a value of 12.5 mg (CO_2) / g (LOI) / day for days 4 to 7 for the same test material.

Study conclusions and recommendations:

- It is recommended that the most suitable analytical method for determining VFAs in digestate is gas chromatography although the Method 1 "field test" gave comparable results and was straightforward to undertake. The digestates tested were reported to have been stored for either 3 or 6 months and VFA levels for both were within the German RAL-GZ 256 standard limit for application to land (4,000 mg / l of organic acids (VFAs) as acetic acid).
- The biochemical methane potential (BMP) tests were not complete after 21 days and a longer test duration is recommended. The BMP test and modifications used during this study appeared to be "fit for purpose" for testing all three digestate fractions (whole, fibre and liquor) but further refinements are recommended. The levels of biogas production for the whole digestates, fibre and liquor fractions were broadly in the range expected for composted and digested materials which had undergone significant biological treatment. This appears to be consistent with the long storage times reported for the digestates and the relatively low levels of VFAs detected.
- Under aerobic conditions, the laboratory separated fibre from both digestate type A and B samples appeared to be well stabilised (i.e. low biodegradability).
- It is recommended that further research is undertaken to refine the BMP test method and to determine its suitability for a wide range of digestate types.

9.2 Quantification of VFA by gas chromatography

Volatile fatty acids (VFA) 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 at a flow of 190.8 ml / min, with a split ratio of 100 giving a flow rate of 1.86 ml / min in the column and a 3.0 ml / min purge. The GC oven temperature was programmed to increase from 60 to 210 ^{o}C in 15 min, with a final hold time of 3 min. The temperatures of injector and detector were 200 and 250 ^{o}C, respectively.

Preparation of samples involved centrifuging at approximately 38,000 g for 10 minutes, then dilution to required concentration and preservation in formic acid (10 % concentration v / v). Three standard solutions containing 50, 250 and 500 mg / l of acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric, hexanoic and heptanoic acids were used for calibration of the instrument. The VFA retention characteristics of the instrument are shown in Table 11. The VFA concentration of the diluted samples was calculated on a proportional area basis of the chromatograph when compared with the calibration standards.

Table 11 Retention characteristics of VFA in a Shimazdu 2010 gas chromatograph

Retention time (minutes)	Acetic	6.33
	Formic	6.92
	Propionic	7.32
	iso-Butyric	7.67
	n-Butyric	8.39
	iso-Valeric	8.86
	n-Valeric	9.64
	Hexanoic	10.81
	Heptanoic	11.94

9.3 OU BMP / RBP Method

Prepared by Open University
30.01.09

This test method determines the residual biogas potential of digestates by measuring the production of biogas under anaerobic conditions. The version given here has been amended for whole digestates and separated "liquor" fraction, and may be used for samples with relatively high solid content such as separated solid "fibre" fractions using additional water. It is based on the BM100 method described in "Guidance on monitoring MBT and other pre-treatment processes for the landfill allowances schemes (England and Wales) published by the Environment Agency, August 2005. These procedures and apparatus are derived from the Blue Book method on suitability of sewage sludge for anaerobic digestion (SCA 1978, Amenability of sewage sludge to anaerobic digestion 1977, ISBN 0117512508).

Principles

Under anaerobic methanogenic conditions the decomposition of organic carbon proceeds by producing biogas (mostly CH_4 + CO_2). The test is set up in a small vessel containing the test substrate and a mineral aqueous medium. An inoculum of methanogenic bacteria, taken from an active anaerobic digester, may be added if the original sample has been pasteurised. The test is monitored by collecting the biogas produced and recording its volume, adjusted to standard temperature and pressure. The duration of the test when using digestates as samples may be 21 to 50 days or further to completion.

Reagents

Nutrient media: Mix the following in distilled H_2O, adjust pH to 7.5 with NaOH

Major elements (use 10 ml / sample rep)	*g / l*	*Alternative*
KH_2PO_4	13.2	
NH_4Cl	13.5	
$CaCl_2.2H_2O$	1.88	$CaCl_2.6H_2O$ 2.63 g / l
$MgCl_2.6H_2O$	2.5	
Trace elements (use 1ml/sample rep)	*mg / l*	*alternative*
$FeCl_2.4H_2O$	50	
H2BO3	1.25	
$ZnCl_2$	12	$ZnSO_4.7H_2O$ 25 mg / l
$CuCl_2.2H_2O$	1.7	$CuSO_4.5H_2O$ 2.5 mg / l
$MnCl_2.4H_2O$	160	$MnSO_4.H_2O$ 125 ml / l
$(NH_4)_6Mo_7O_{24}.4H_2O$	2.5	
$AlCl_3.6H_2O$	2.5	
$CoCl_2.6H_2O$	5.0	
Additional trace nutrients if required		
NaCl	250	
$NiSO_2.6H_2O$	7.5	
Na_2SeO_3	5.0	
$Na_2WO_4.2H_2O$	5.0	

Apparatus

The experimental set up is shown in Figure 23.

- Vessel – 500 ml bottle with rubber bung (or similar) and flexible plastic tubing.
- Incubator or water bath set at 35 $^{\circ}$C for test vessels
- Biogas collection cylinder calibrated in volume with valve at top of cylinder for connection to suction pump (e.g. cylinder I.D. 50 mm, height 1100 mm and capacity approximately 1500 ml).
- Water bath of acidified water used for filling biogas collection cylinder. Preferably water should be acidified to pH <2 (pH 4 is acceptable) with HCl to reduce adsorption of CO_2 and contain a pH indicator dye.
- Air pump to fill collection vessel with acidified water by suction.
- Delivery system for nitrogen gas.

- Weighing balances.
- pH meter properly calibrated.
- Temperature measurement

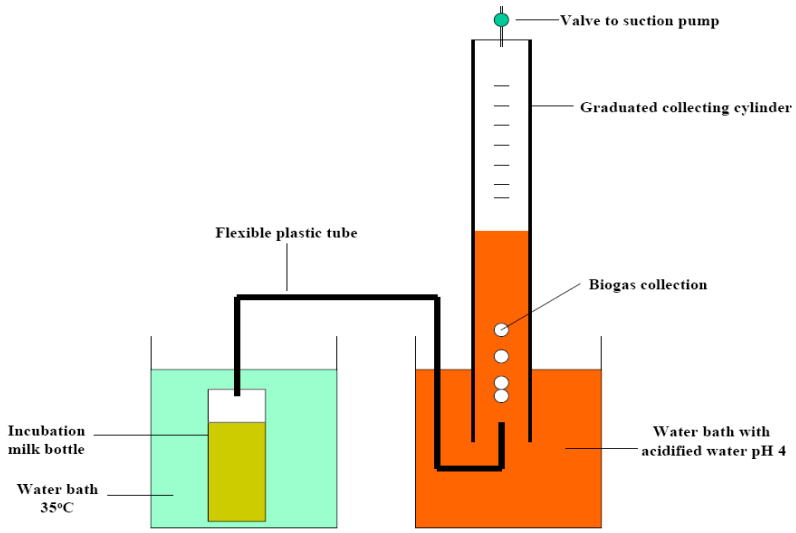

Figure 23 Experimental setup of OU method

Procedure

1. Sample preparation

Determine dry matter content (103 ºC) and loss on ignition (LoI) (550 ºC). Whole digestates and separated liquor from whole digestates may be used without amendment, though a reliable sub-sampling technique must be used. Samples with high solids content such as separated fibre are likely to require additional water.

2. Preparation of seed sludge (if used)

Microbial seed/inoculum:- 3 g DM of well digested anaerobic sludge added in 50 ml, i.e. a 6 % sludge by DM.
The microbial seed may be stored at 0 to 5 ºC for several days before use.
The seed sludge is incubated for at least 48 hours at 35 ºC in order to ensure the microbial seed is acclimated to the test temperature and is active.
Note: active anaerobic sludge samples generally will not need inoculum. In the case of a pasteurised / sterilised sample, the best inoculum is a small amount of the same sludge before pasteurisation / sterilisation.

3. Filling the test vessels

To each test vessel add:
- sample equivalent to 20 g loss on ignition.
  For liquid samples with low LoI content this may not be possible as the sample volume may exceed the volume of the vessel; in this case use either a larger vessel or reduced sample. Some headspace is required to allow mixing of contents by swirling; in practice about 400ml volume is maximum for a 500 ml flask. Replicable sub-sampling technique is essential.
- 10 ml major element nutrient solution and 1 ml of trace element solution.
- Seed sludge if used (up to 10 % of sample loss on ignition).
- Record weights of sample and seed sludge used.
- Check pH and add Na_2CO_3 to bring the pH to 7.5 if required.
- Sparge with N_2 and mix well by swirling the bottle.

4. Gas collection system initiation

Remove gas from the gas collection system by suction using the air pump from the top of the cylinder setting the level of water in the gas collection vessel to zero. This is the start of the incubation period (zero time).

5. Test monitoring

Record biogas daily at first, reducing monitoring frequency as appropriate.

- Mix the contents of the reaction vessels daily by swirling.
- Record the level of biogas collected in the gas collection system.
- When the water level in the collection cylinder has reduced by over 500 mm reset the level of water in the cylinder to zero and record this event. If analysing CH_4 content, take samples before resetting.
- The test is monitored for a defined number of days or until there is no or minimal gas production which indicates completion of the test.

6. Quality Assurance

Each waste to be tested in triplicate.
Blank: Sludge seed only (if used, triplicate)
Control: Cellulose standard (α-cellulose from Sigma), 5g LoI per chamber (triplicate)

7. Calculation and expression of results

Report biogas production as L(biogas) per kg (sample LoI). A plot of the cumulative curve clarifies progress of the test. The biogas volume in the collection cylinder must be corrected to standard temperature and pressure, including the effect of the hanging water column in the gas collection tubes.

9.4 Supplementary Data

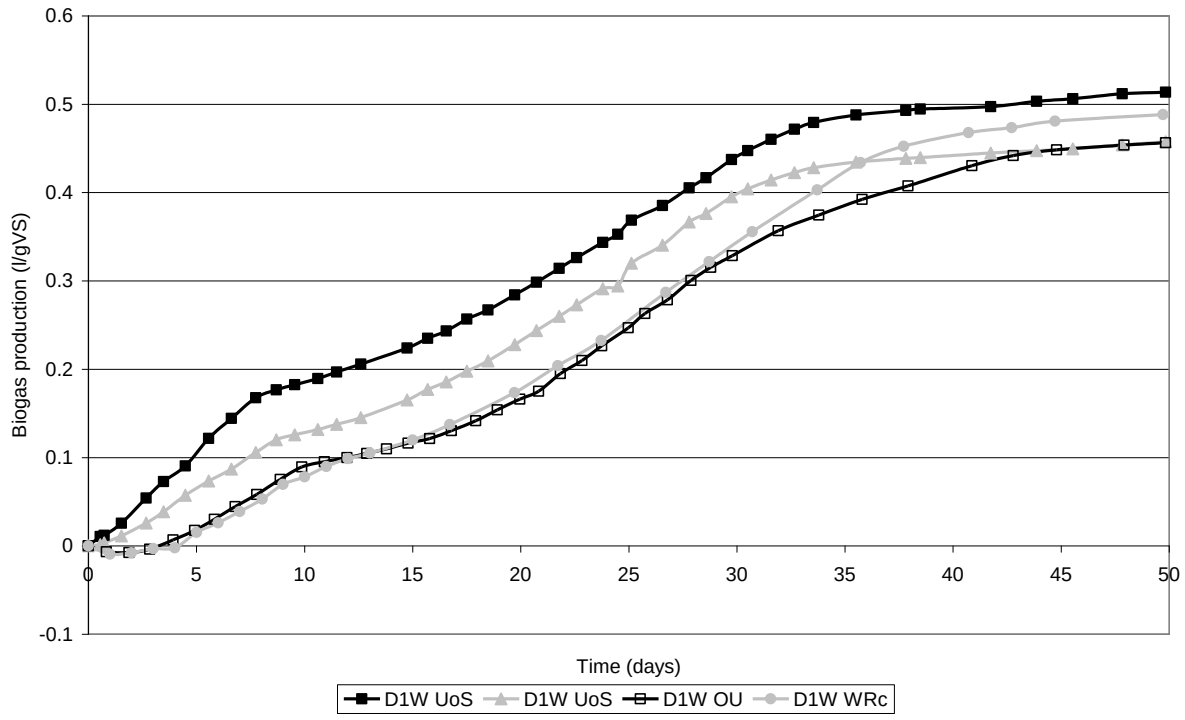

Figure 24 Inter-laboratory determination of RBP for D1W (Average of triplicate tests)

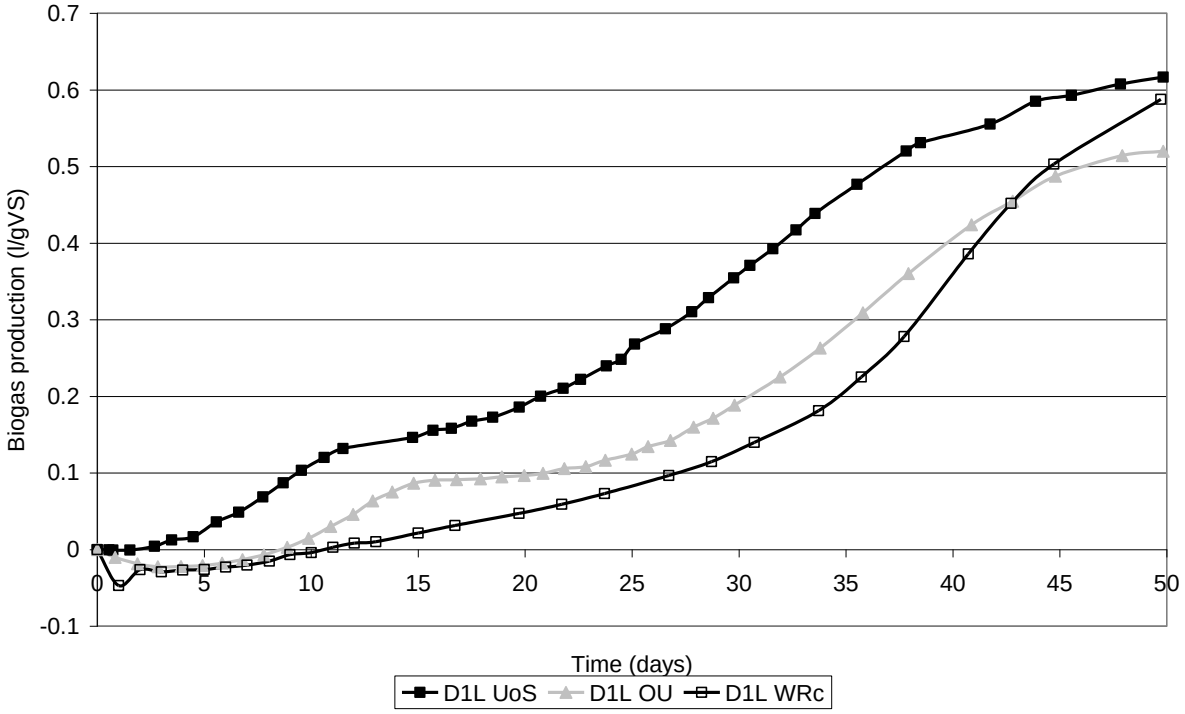

Figure 25 Inter-laboratory determination of RBP for D1L (Average of triplicate tests)

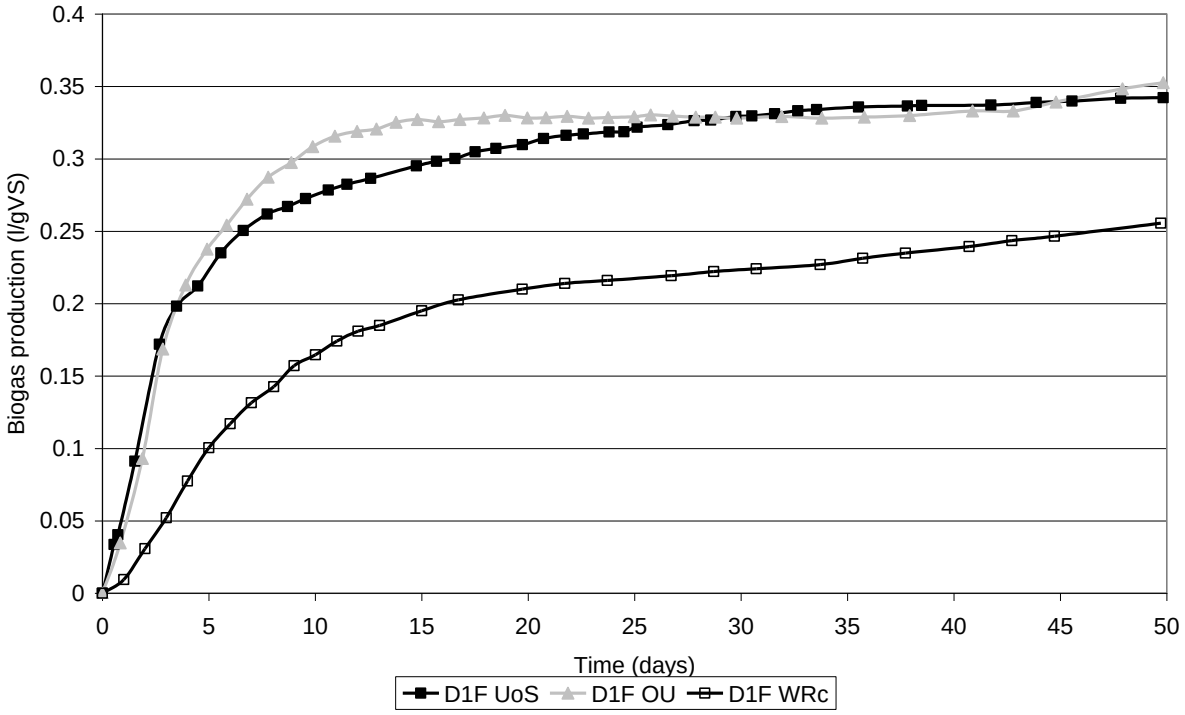

Figure 26 Inter-laboratory determination of RBP for D1F (Average of triplicate tests)

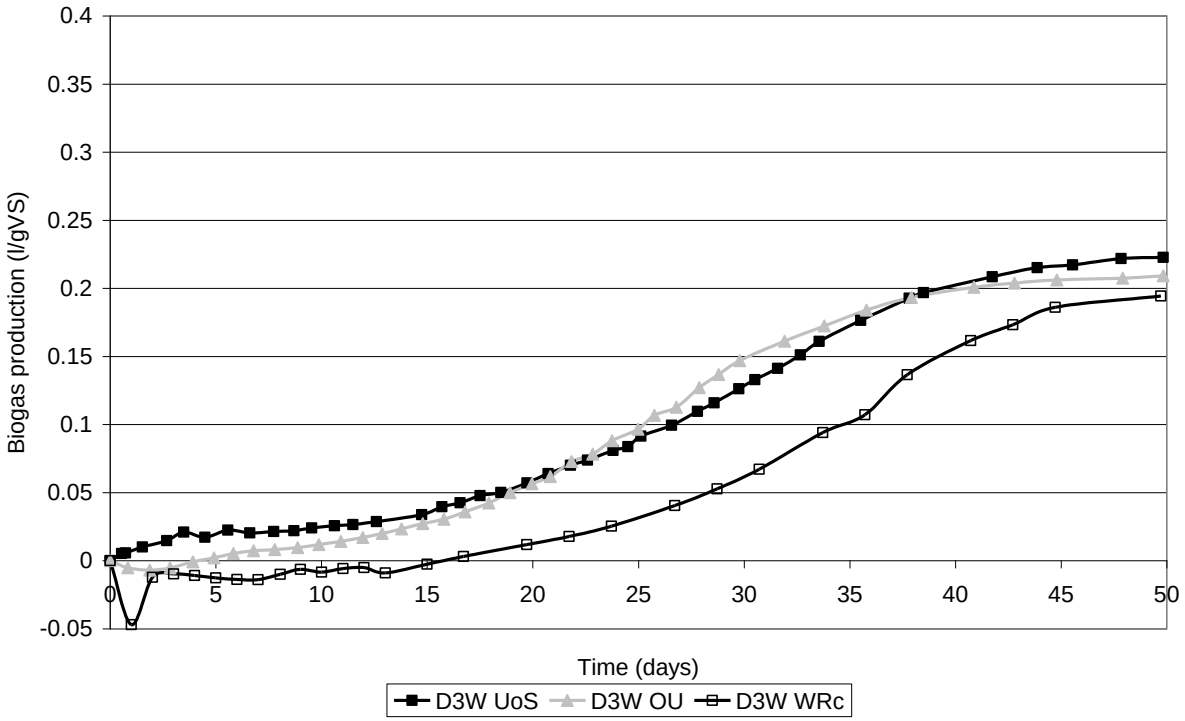

Figure 27 Inter-laboratory determination of RBP for D3W (Average of triplicate tests)

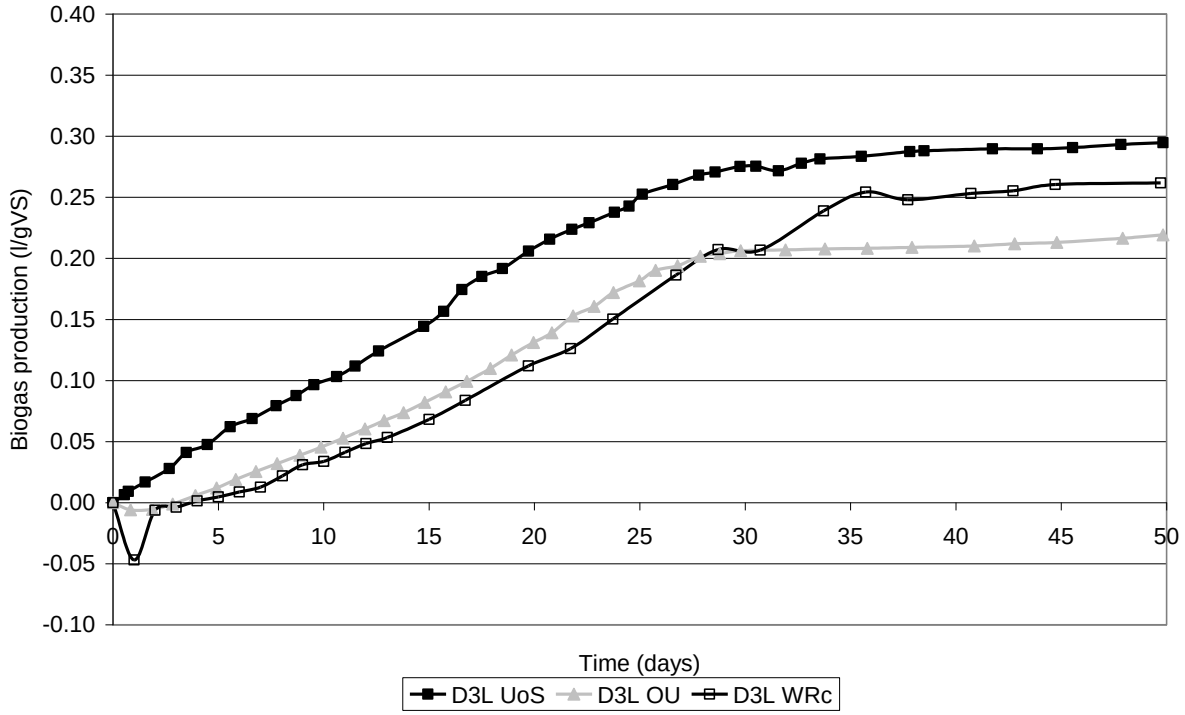

Figure 28 Inter-laboratory determination of RBP for D3L (Average of triplicate tests)

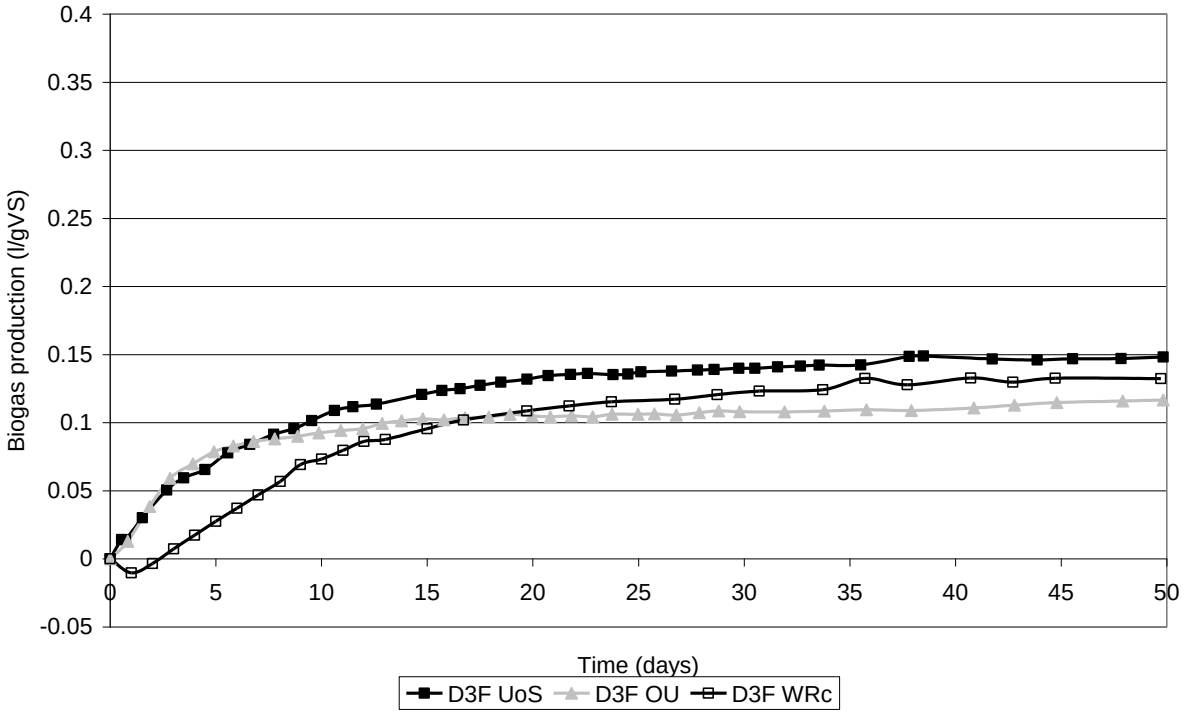

Figure 29 Inter-laboratory determination of RBP for D3F (Average of triplicate tests)

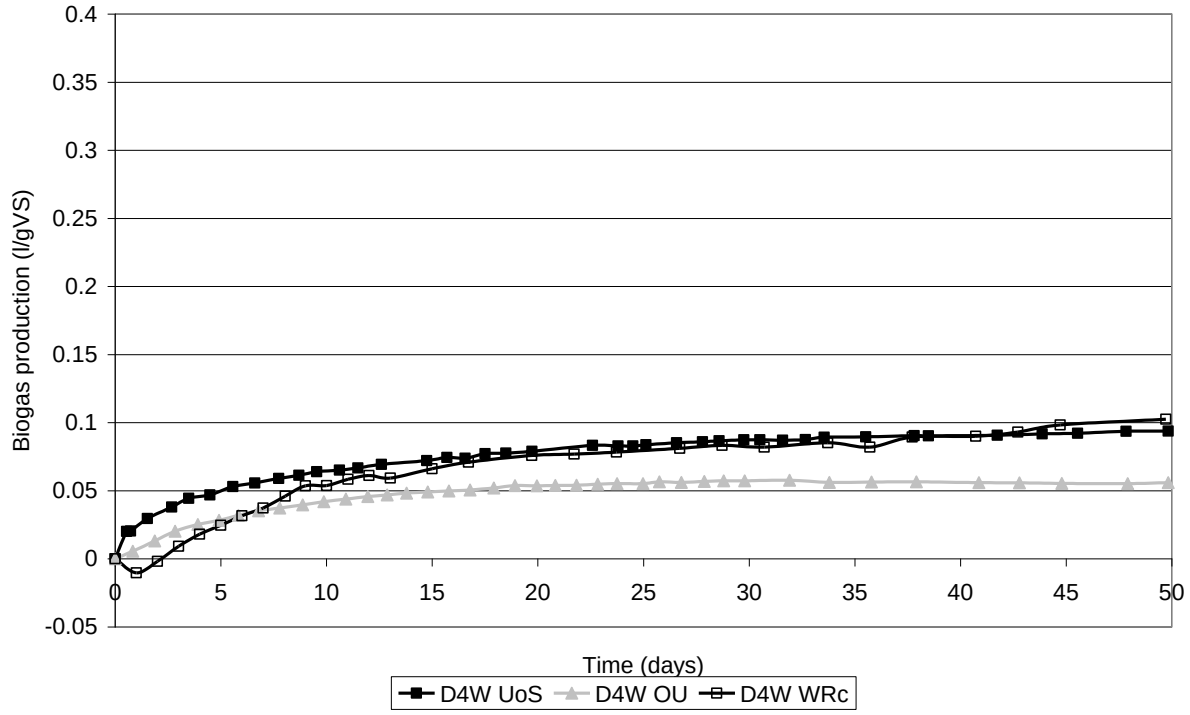

Figure 30 Inter-laboratory determination of RBP for D4W (Average of triplicate tests)

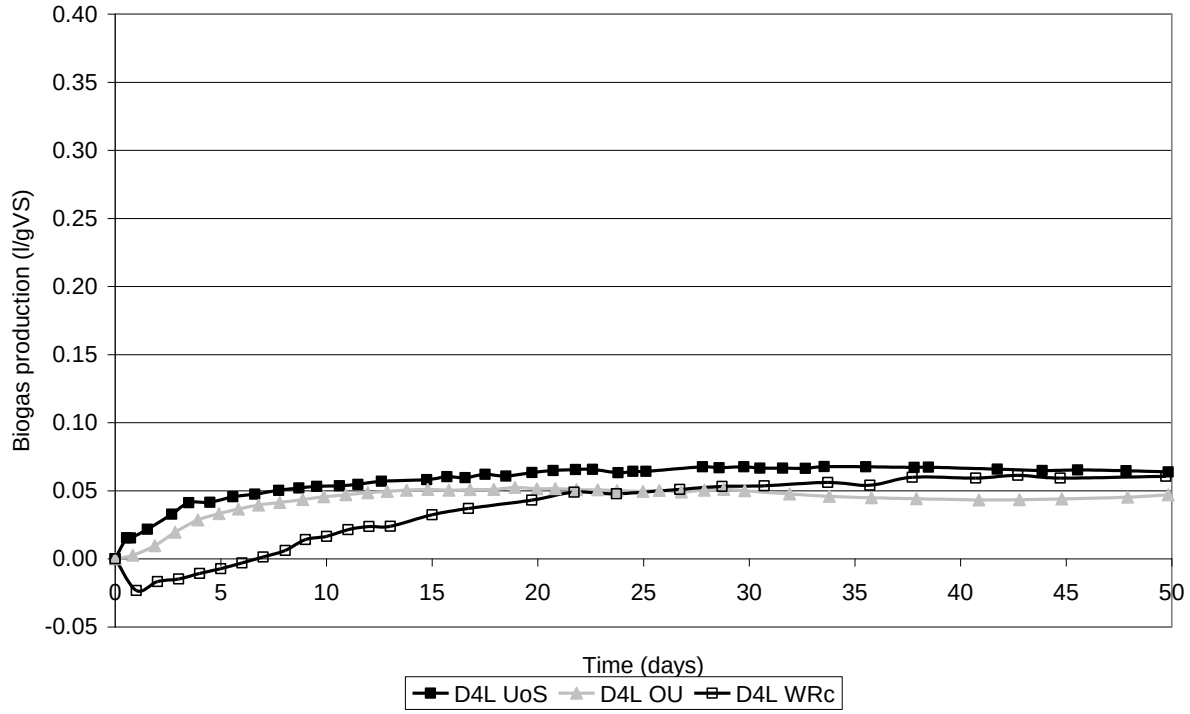

Figure 31 Inter-laboratory determination of RBP for D4L (Average of triplicate tests)

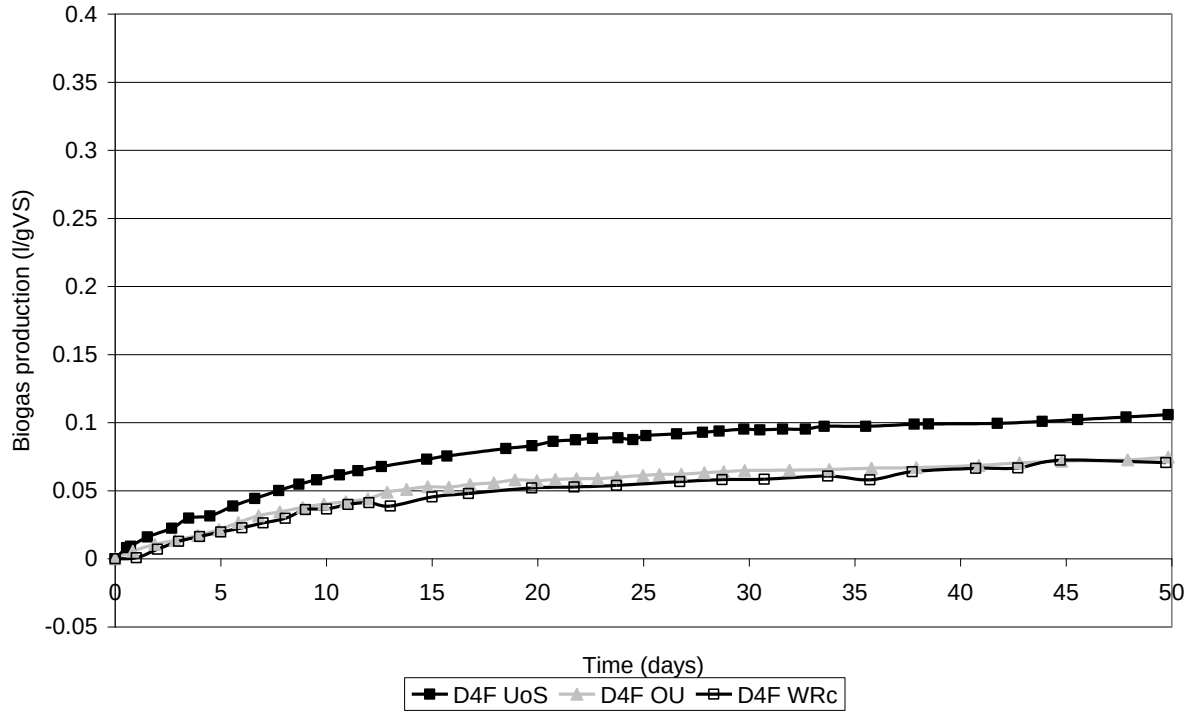

Figure 32 Inter-laboratory determination of RBP for D4F (Average of triplicate tests)

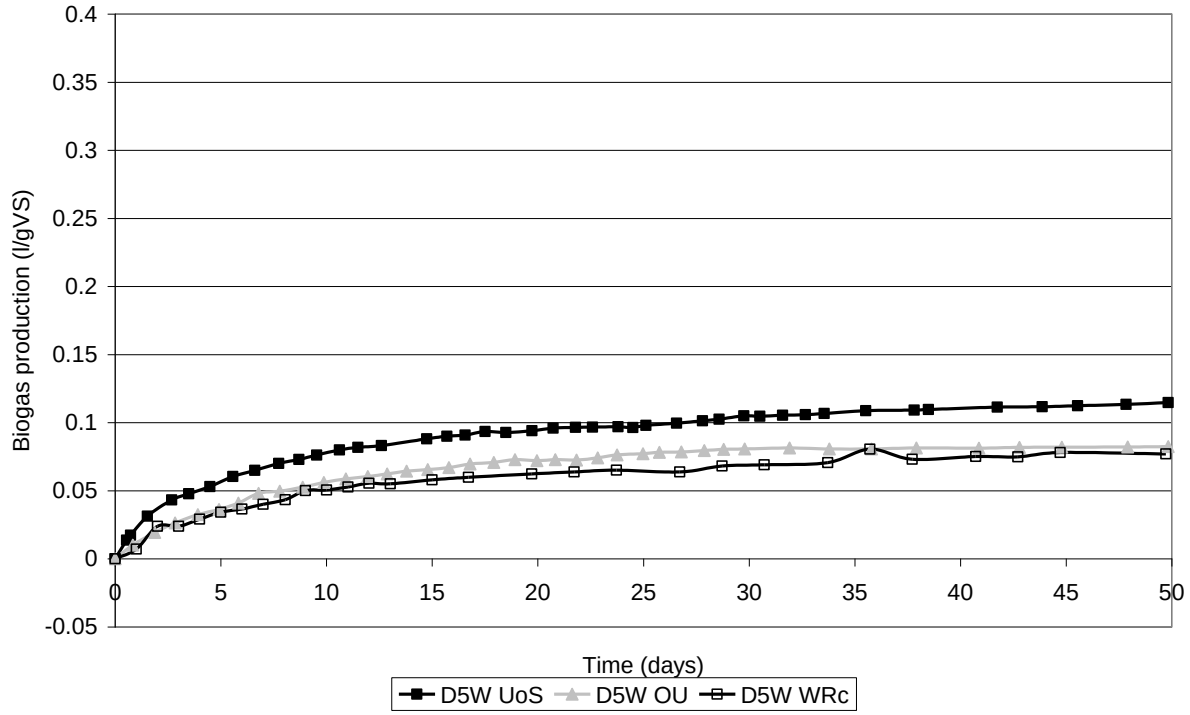

Figure 33 Inter-laboratory determination of RBP for D5W (Average of triplicate tests)

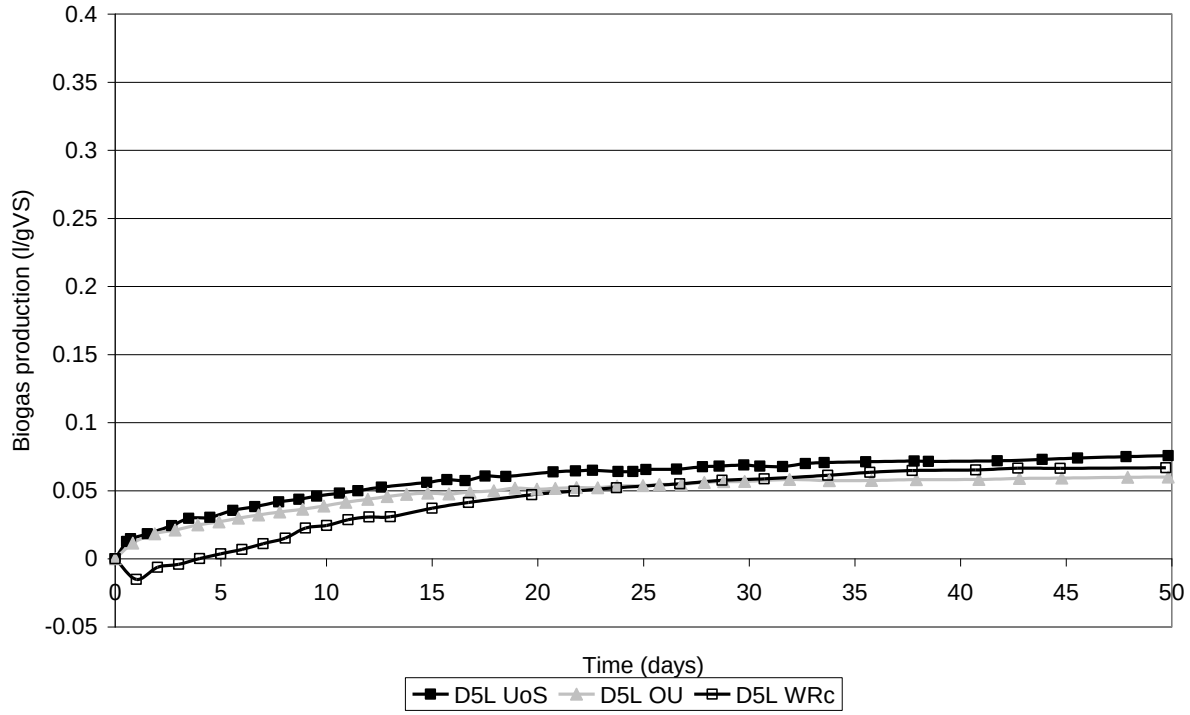

Figure 34 Inter-laboratory determination of RBP for D5L (Average of triplicate tests)

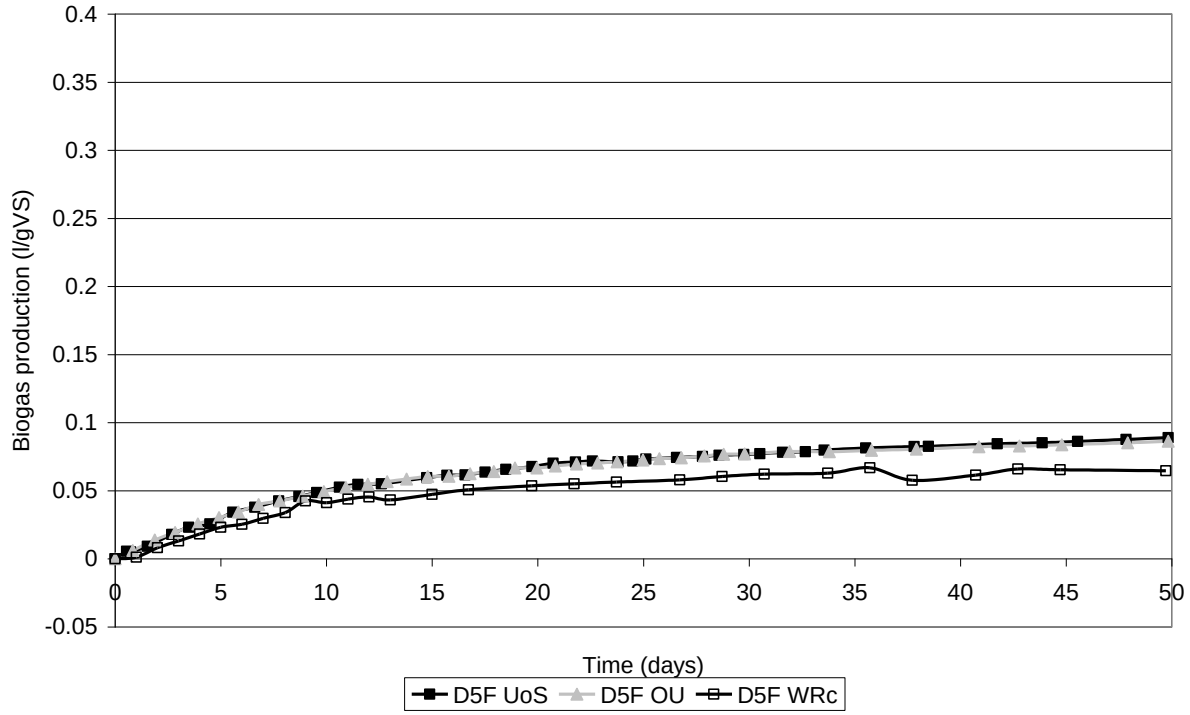

Figure 35 Inter-laboratory determination of RBP for D5F (Average of triplicate tests)

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