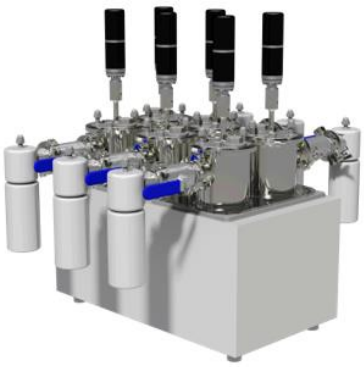
Anaero Technology

Anaerobic Digestion & Fermentation Equipment and Research

# OPERATING MANUAL

*for* Batch/ Fermenter Range

(Nautilus, Phoenix, Pegasus models)



Anaero Technology Ltd.
Landsite 2, Cowley road, Cambridge, CB4 0DL
Email: edgar.blanco@anaero.co.uk / rashmi.patil@anaero.co.uk
Phone: +44 (0) 7397555544

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# LIST OF ACRONYMS

| | | | |
|---|---|---|---|
| **BMP** | Biomethane Potential | **STP** | Standard conditions for Temperature and Pressure |
| **AD** | Anaerobic Digestion | **VAC** | Volts of Alternating Current |
| **CSTR** | Continuous Stirred-Tank Reactor | **VDC** | Volts of Direct Current |
| **HDPE** | High-Density Polyethylene | **VFA** | Volatile Fatty Acids |

## 1. INTRODUCTION

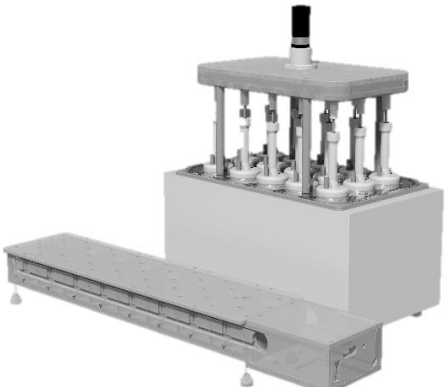


Figure 1. Standard BMP/RBP set *(water bath, reactors, miext, gas flow meter with temperature and pressure sensor*

The Biomethane Potential (BMP)[1] or Biogas Potential Test is a biological method to evaluate the potential methane/biogas volume generated from a material (substrate). The principle is simple and the test can be made using basic equipment, including drinks bottles and manually made gasometers, or more engineered equipment available commercially.

The test comprises two elements: a) Inoculum and b) sample. These are represented in terms of volatile solids (VS) and in some cases as COD. Test reactors are added a controlled amount of inoculum (seed) and a measured amount of sample at a selected inoculum to substrate ratio (ISR) measured on the basis of VS. Gas production is then measured and converted to standard temperature and pressure (STP), 273.15°K; 101.325 kPa.

The biogas produced by the inoculum is subtracted from total biogas produced in each test bottle by calculating how much biogas would the gVS of inoculum in each bottle would have produced and subtracting from total biogas. Since the gVS of sample is known, the gas produced by the sample fraction can be calculated. For this inoculum, only reactors are used to determine the baseline yield of inoculum on ml/g VS inoc., which improves the consistency of the test.

Through a gearbox, Anaero BMP equipment uses one single motor to mix 15x1-litre reactors, thus delivering exactly the same mixing[2] for all reactors. By immersion in a water bath with a tight water lid, temperature is maintained constant for all reactors and bath water evaporation minimised even when operating in thermophilic mode. The control of the mixing and temperature minimises operational differences between reactors, improving the consistency of the results. Moreover, Anaero BMP operates at low gas head pressure to reflect low pressure operating conditions in most Continuous Stirred-Tank Reactor (CSTR) digesters. The equipment has a built-in battery backup for the gas flow meter (up to 15h) to avoid data loss during power outage.

The Anaero Technology BMP sets optimise technical performance in the following areas:

- **Larger reactor volume.** 1-Litre reactors allow larger sample use, easier to handle and smaller errors (more gas generated in bigger reactors = less error from missed gas bubbles). However, it is also possible to operate with smaller test volumes in the bottle (from 450ml to 900ml).
- **Mixing consistency.** All reactors are mixed at exactly the same speed through stainless/silicone paddles driven by a gearbox (1 motor for 15 reactors). Consistent mixing even for high % Dry Solids (DS).
- **Real time gas flow measurement** with automatic conversion to standard temperature and pressure and protection of data with built-in power back-up for gas flow meter. Open source code for meter.

[1] In the UK, residual biogas potential (RBP) is an equivalent test. It is described in WRAP report OFW004-005 and it has become a certified method.

[2] Variations in mixing speed between reactors in a set can impact the kinetics of biogas production.

- **Ease of sample handling.** Wide mouth eases test preparation when using solid or sticky material that could otherwise be a challenge to dose through narrow openings impacting precision and effort.
- **Low bath water evaporation loss.** Standard water bath with tight-fitting cover that minimises bath water evaporation loss even when operating at thermophilic temperature.
- **Access to reactors during test.** Gas-tight access ports to interior of digesters without affecting gas monitoring (measure pH, redox, VFA, or supplement during test without opening reactors).
- **Ease of calibration**. Use a simple syringe to confirm the consistency of flow measurements.
- **Flexible addition of bespoke ports.** Large diameter cap allows fitting of additional ports as required by your protocol, as septum or larger probes (*small additional cost for extra ports).

# 3. Equipment Overview

Table 1.BMP equipment features.

| | |
|---|---|
| **Reactors per set** | 15 |
| **Reactor volume (L)** | 1 |
| **Materials** | High-density polyethylene[3] (HDPE) (reactors), 316 Stainless steel[4] (mixers), polycarbonate[5] (gas flow meter) and silicone (blades). Autoclave tolerance. |
| **Mixer motors per set** | 1 |
| **Power** | 110 or 220VAC for bath. 6vDC backup incl. for gas flow meter; 24 vDC mixer motor. |
| **Measurement resolution (ml)** | 7 – 10 |
| **Measurement method** | Volumetric displacement with real-time temperature and pressure sensors for STP. |
| **Software** | Python for Arduino Uno, open source by Anaero Technology |
| **Dimensions** | W570mm X D340mm x H 700mm (reactor set); W1000mm X D200mm x H200mm (gas flow meter). |
| **Country of origin** | United Kingdom. |

## 3.1 Reactor Module

1) 1-litre HDPE reactor bottle
2) Reactor heads
3) Water bath
4) Bath lid
5) Access port
6) Gas outlet
7) Reactor mixer paddle

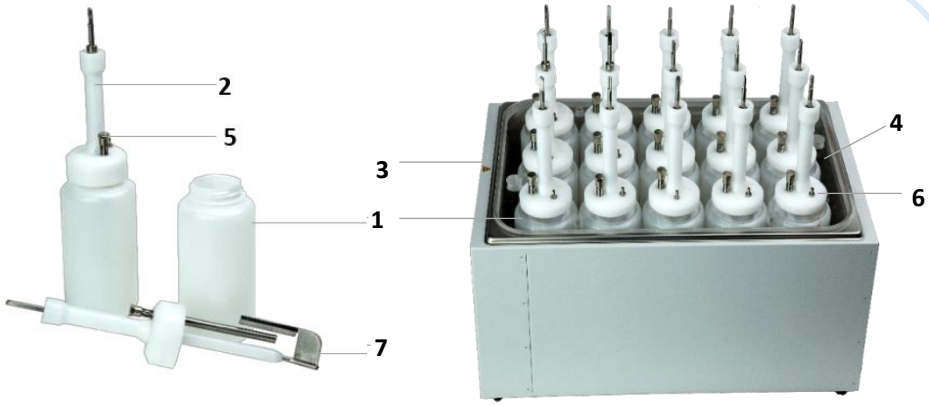



*Figure 2. Reactor module*

**Description:**

[3] Reference for material tolerance: British plastic federation (2017). 'Polyethylene (High Density) HDPE' http://www.bpf.co.uk/plastipedia/polymers/hdpe.aspx
[4] Reference for material tolerance: British Stainless Steel Association (2001). 'Structural Design of Stainless Steel'. http://www.bssa.org.uk/cms/File/SCI%20291%20Structural%20Design%20of%20Stainless%20Steel.pdf
[5] Reference for material tolerance: British plastic federation (2017). 'Polycarbonate PC' http://www.bpf.co.uk/plastipedia/polymers/polycarbonate.aspx

**- Bioreactors**: 1-litre wide-mouth HDPE bottles with easy handling, allow larger samples and inoculums to be processed easily, further reducing potential errors. Stainlees steel/silicone paddles deliver consitent and continuous mixing to all digesters and addapt well to the higher resistance when high solids samples/inoculum are tested. HPDE is biologicaly innert and remains stable at 100°C. Most commercially-available BMP sets do not guarantee equal mixing for all reactors in the set. Some rely on daily manual shaking, shaking surfaces, magnetic stirrers, etc., which is ok for absolute values of BMP but limits the consistency of kinetic data for rapid evaluation of inhibition or of the effect of feedstock composition on the dynamics of biogas production.

**- Water bath**: The reactors temperature of operation is controlled by immersion of reactors in a water bath. A lid that holds the reactors in place has been designed to minimise the loss of bath water by evaporation, facilitating tests at thermophilic temperatures without the common issues of water evaporation when operating at those conditions.

## 3.2 MIXING MODULE

Important note: Never switch on the water bath without water

8) Mixer drive box
9) Motor
10) Mixer module support posts

**Description:** All reactors are mixed at exactly the same speed and using stainless steel/silicone paddle systems that guarantee even mixing, even for high solids mixtures (e.g. 12%DS). At higher solids, it is needed to check if the motor is not over loaded and fit rods to avoid reactor spinning.

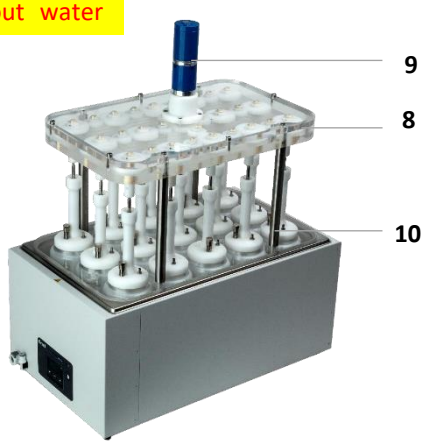
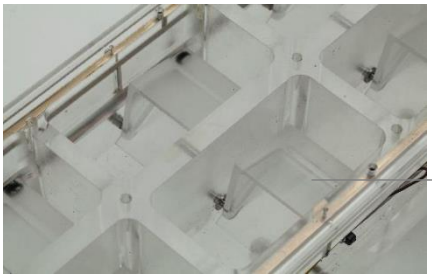



*Figure 3. Mixing module and reactor module.*

## 3.3 GAS FLOW METER MODULE

11) Gas flow meter block
12) Tumbler bucket
13) Temperature sensor



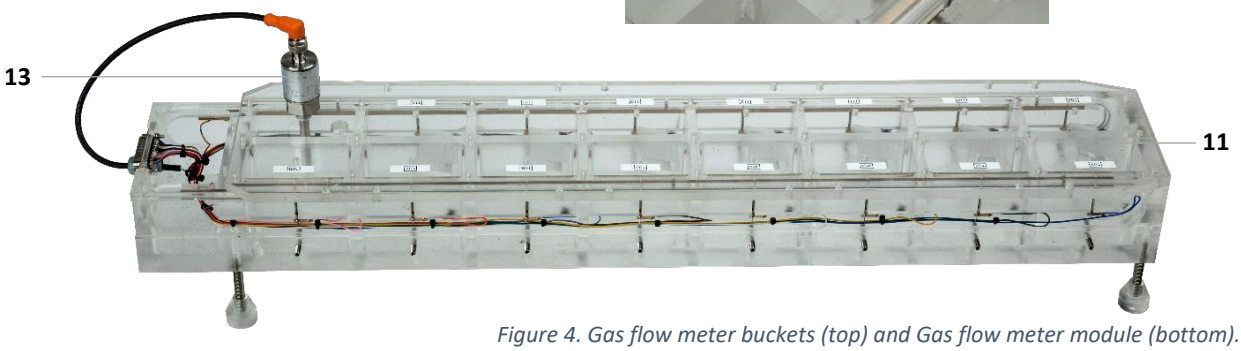
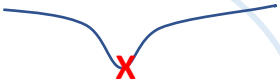




*Figure 4. Gas flow meter buckets (top) and Gas flow meter module (bottom).*

- **Gas flow meter**: Gas production is measured using a liquid displacement gas flow meter, consisting of 15 x 0.2l Perspex cells and Perspex with pivoting buckets of around 7ml gas volume. A spare cell (cell 16) is used for automatic monitoring of temperature. A barometer in the **Arduino logger** continuously monitors atmospheric pressure for STP correction. The liquid in all cells is interconnected to keep them at the same depth for equal gas head pressure in all reactors (liquid specifications: Table 2). Each cell has a pivoting (tumbling) bucket with active volume of around 7ml (easily calibrated by the user- See section 3.1). Smaller measuring volume allows for better recording of kinetics of tests, especially after the initial phase of high gas production. However, the smaller the higher the volume resolution, the larger the potential error from for an odd gas bubble escaping uncounted as a pivot takes place. To minimise potential error during gas measurement a minimum counting volume of 5ml has been designed and attention has been paid to the size of gas bubbles entering the measuring vessel. The diameter of the nozzle orifice where gas enters the measurement cell is machined to 1.5mm, reducing the size of gas bubbles and minimising the potential impact if an uncounted bubble bypasses the tumbling bucket.

## Some recommendations for the use of the Gas flow meter:




- Avoid sagging in gas lines which can accumulate condensation and increase head pressure in reactors affected. Ensure that any condensation would flow to meter or to reactors
- Avoid connecting only a few gas bags (to collect gas samples) in the Gas flow meter. If it is possible, connect all cells to gas bags to avoid pressure differentials between cells.
- Ideally, the gas flow meter should be placed lower than reactors to minimise vacuum effect sucking liquid from flow meter into reactors if bath cools down rapidly in long power cuts
- Avoid kinks in gas lines that would restrict flow. Low kink gas line is supplied.
- Ensure liquid covers buckets in resting position. Lower liquid level=higher tumble volume.

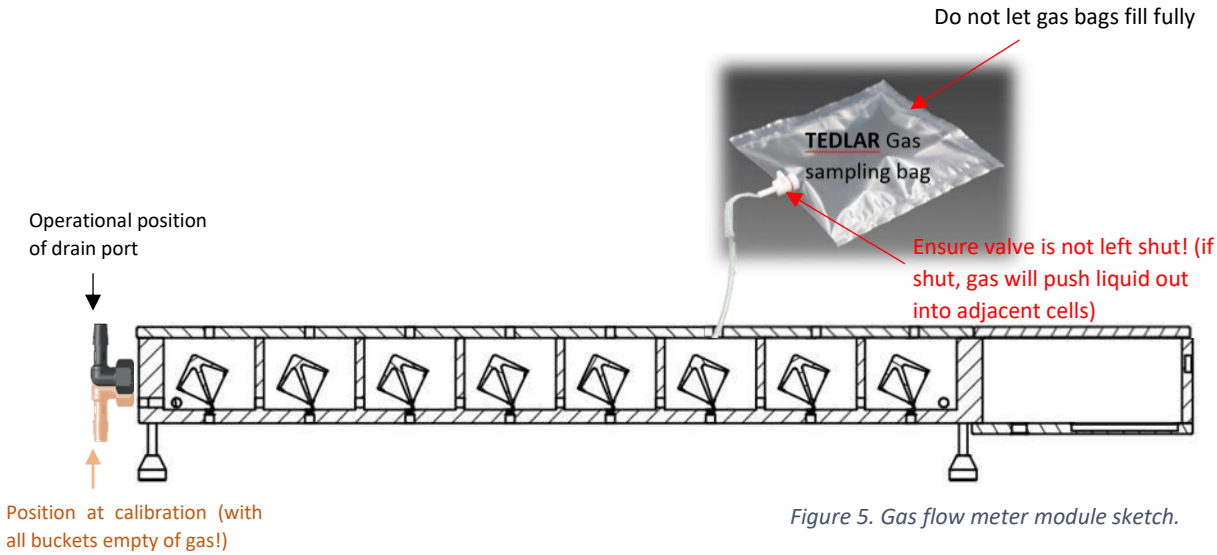



*Figure 5. Gas flow meter module sketch.*

The **liquid inside the Gas flow meter** can be an acidified or distilled water (for total biogas), or caustic solution (for $CH_4$-only). Different possibilities are detailed bellow.

*Table 2. Gas flow meter liquid.*

| Liquid in the Gas flow meter | pH or molar concentration | Parameter measured |
|---|---|---|
| **$H_2O$** | - | $CH_4$, $CO_2$, $H_2S$, etc. |
| **Na(OH) + $H_2O$** | 3 M NaOH | $CH_4$ only |
| **HCl + $H_2O$** | pH=2 [6] | $CH_4$. $CO_2$, $H_2S$ |

### 3.4 Arduino Datalogger

The Data logger consists of an Arduino UNO microcontroller which acts as the main controller. Connected to this is a specially designed 'latch shield' consisting of 15 separate Set/Reset latches. When one of the tumblers in the gas flow meter tips, a magnet attached to the tumbler causes a reed switch to close, and the latch associated with that channel locks to store the occurrence, time, temperature and pressure. Each tumbler event is captured in hardware by the latch shield, guarantying that all tumble events are registered even if these occur at the same time. The system has 6x1.5V AA rechargeable batteries for logger power back up for up to 15 hours. Users could add a solar power unit to make system totally black-out proof.

## 4. Operation principles: Setting up of a BMP test

### General steps to set up a BMP/RBP test

1- Collect samples and Inoculum, ideally from operational site using similar feedstock. Leaving them unfed for over 3 days would improve the consistency of results by reducing the biogas produced by the inoculum. However, if you want to focus on inhibition/toxicity potential, fresh samples may be useful although there may be larger standard deviations
2- Analyse DS and VS to determine loading of reactors depending on ISR selected
3- Prepare a table of calculations with target ISRs and mass of inoculum and sample for each reactor. If you can, print a table giving you target weights so it is at hand when you weigh
4- Check liquid levels and calibration of gas flow meter to ensure data quality. Check all cells record. It is very painful to find out a cell failed or suspect after you start test. This applies to all technologies. Check, check, check, it is your data, your time
5- Tare bottles one by one and weigh inoculum into each of them (scale to 5kg, d=0.1g)
6- Tare balance and dispense sample into reactors using balance (d=0.0001g)
7- Fit reactor caps and gently place in the water bath. Fit bath lid then connect the gas lines to reactors. If you flush with $N_2$, do it so that reactor and flowmeter headspaces are flushed (flush from reactors so complete gas line is cleared)
8- Fit mixer module
9- Load set up data on Arduino logger and get system to Start Run
10- Switch water bath and mixer on. Test has started

[6] Reference:Walker, M. *et al.* (2010). 'Residual biogas potential test'. Bioresource Technology. 100(24) 6339-6346. http://www.wrap.org.uk/sites/files/wrap/Residual%20Biogas%20Potential.pdf

11- We recommend you check the following day or some hours later to ensure your readings are being logged by doing a data download. Experience has taught us to double check to protect data. After this you can leave the machine to do its work and take downloads as required.

## 4.1 PREPARATION OF THE GAS FLOW METER

1. **Pass the hose provided through the holes** in the lid of the gas flow meter and connect firmly to the gas inlet at the bottom. Keep the gas hose close to body of the flow meter, but not tight. This avoids sagging which would increase reactor back pressure. Tight hose on body can obstruct gas flow.

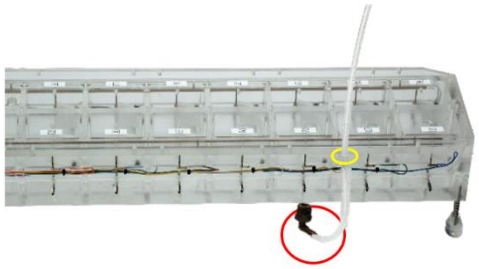


*Figure 6. Gas flow meter, hose position detailed.*

2. **Fill the gas flow meters** (see table 2) until liquid drops from turned down spill point. The measurement is essentially a buoyancy measurement so, higher water level results in more buoyancy and this in turn would give smaller volume to make tumbles. The most common cause of cell stuck in the middle, apart from excessive gas flow is liquid level too high.

*Figure 7. How to fill the gas flow meter.*

3. **Calibrate the gas flow meters**: You should regularly calibrate the gas flow meter to obtain accurate results. To control human error it is best is one operator does a complete calibration. For this, a calibration syringe is provided. Too fast calibration will give false high volumes, thus avoid rushing this step.

3.1 Level the gas flow meter.

3.2 Ensure that all buckets are empty of gas.

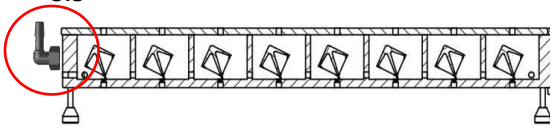
3.3 Change the position of drain port to up position.

3.4 Fit calibration syringe to gas line and gently introduce air to cause one tumble (dummy tumble) and do 3 tumbles each way (1 dummy tumble + 6 tumbles in total).

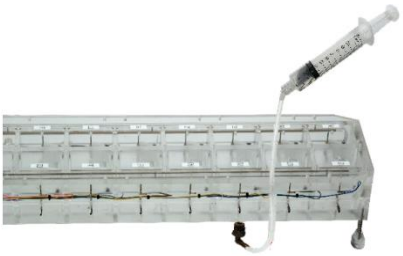
Avoid pressing the calibration syringe when the tumbler is tipping as you will cause bubbles to not be counted, increasing the error.

**3.1**

**3.3**



**3.4**



You can fill the buckets fast at the beginning and slow down just before tumbler starts tipping.

*Figure 8. (3.1) Gas flow meter levelled, (3.2) Operational position of the drain pipe, (3.4) Gas flow meter calibration.*

Gas flow meter calibration Date: 07/09/20

| Reactor Nº | Start point ml | End point ml | Nº of tumbles | ml/tumble |
|---|---|---|---|---|
| **1** | 56 | 17.5 | 6 | 6.41 |
| **2** | 57 | 17.5 | 6 | 6.58 |
| **3** | 57 | 18 | 6 | 6.50 |
| **4** | 56 | 17 | 6 | 6.50 |
| **5** | 56 | 17.5 | 6 | 6.41 |
| **6** | 57 | 17.8 | 6 | 6.53 |
| **7** | 57.5 | 17 | 6 | 6.66 |
| **8** | 56 | 17 | 6 | 6.50 |
| **9** | 57 | 17.5 | 6 | 6.53 |
| **10** | 57 | 18 | 6 | 6.50 |
| **11** | 57 | 17.5 | 6 | 6.58 |
| **12** | 56 | 17 | 6 | 6.50 |
| **13** | 57.5 | 17 | 6 | 6.66 |
| **14** | 57 | 18 | 6 | 6.50 |
| **15** | 56 | 17 | 6 | 6.50 |

*Figure 9. Gas flow meter calibration sample sheet.*

3.5 Syringe air into the bucket until the first tumble happens. The first tumble is a dummy tumble to take out any air present in the bucket. Once the dummy tumble is made take the reading on the syringe as your Start point and continue introducing air to make at least 6 tumbles (3 each way). Take note of the end reading on the syringe and calculate the average ml/tumble by dividing the distance travelled by the syringe to make the six tumbles by number of tumbles (6) as shown below

3.6 To calculate the millilitres per tumble use the formula showed below:

$$\frac{ml}{tumble} = \frac{(Start\ point - End\ point)}{Nº\ of\ tumbles}\ or\ \frac{(distance\ travelled\ by\ the\ syringe(ml))}{Nº\ of\ tumbles}$$

$$Example\ Reactor\ Nº1\ \frac{ml}{tumble} = \frac{(56 - 17.5)}{6} = 6.41\ \frac{ml}{tumble}$$

3.7 Once complete, the calibration values (ml/tumble) from the table can be entered in the set-up file of the Arduino software installed in your laptop (See section 3 to see the operational principles of the BMP and section 4.3 where Arduino set up is explained in detail).

## 4.2 PREPARATION OF THE WATER BATH

Before switching on the water bath, prepare all reactors (See section number 4) and place inside the bath. Then fill with tepid water, if possible, to cover up to the level of digestate.

a) Once the **samples** are **prepared**, fit the reactor heads and place all the reactor bottles in water bath.

*Figure 10. Water bath with reactors.*

b) Place the **bath lid**. Flush the reactor bottles and the gas flow meter with selected gas as required and ensure all the tumblers in the gas flow meter are in the down position.

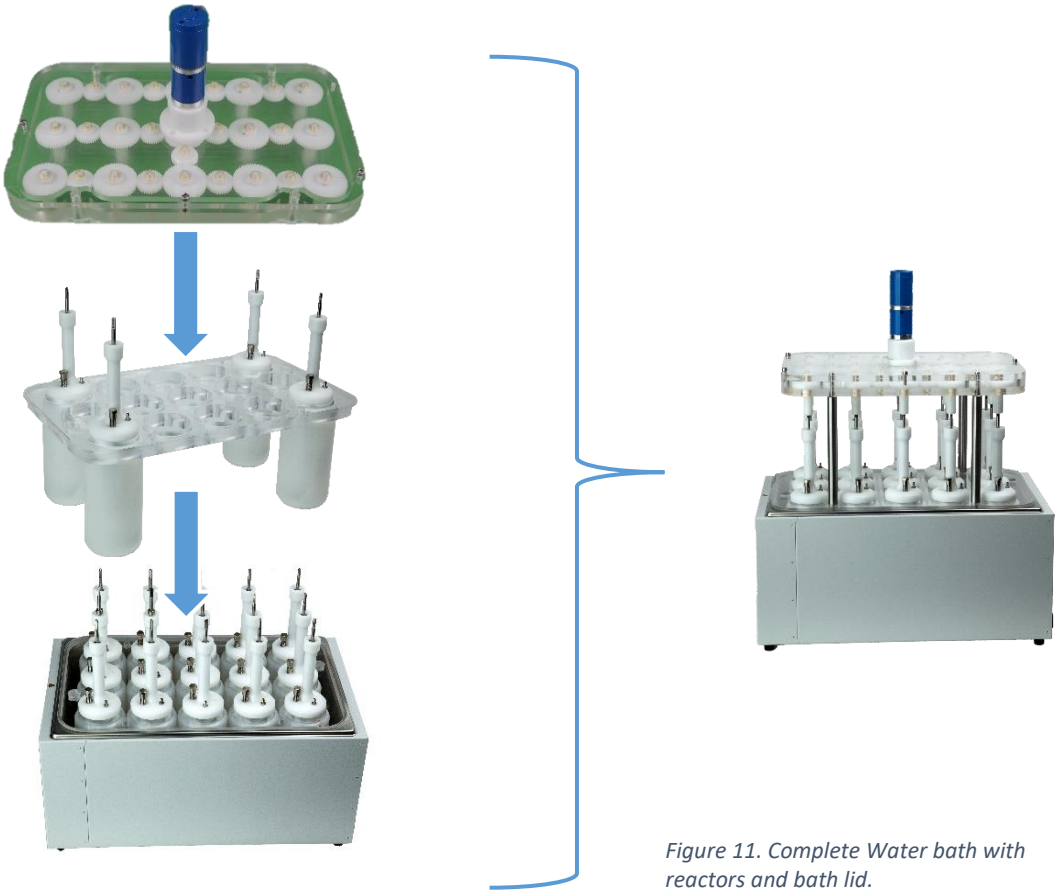


*Figure 11. Complete Water bath with reactors and bath lid.*

c) **Fill water** in the water bath and set it to the required temperature (maximum temperature 95º).

d) **Connect all the reactor bottles** to the other end of the hose coming out the gas flow meter.

e) **Fit the mixing gear box** ensuring all mixer shafts fit gears and move up and down.

f) **Connect the mixer motor** to the power supply, check gas flow meter and switch water bath.

# 5. How to start your test and prepare your samples

## 5.1 Pre-analysis

*Figure 12. Crucibles with samples before and after Total solids and volatile solid analysis.*

- **Dry Solids (DS):**

Dry Solids is the dry matter of a sample after moisture has been completely removed.

To measure DS, crucibles can be used to test multiple samples (see picture 3). Weigh the empty crucibles (C) g using a weighing scale, weigh the crucibles again with fresh feedstock samples (C+S1)g **.** Dry the samples in an oven at 105ºC for 24 hours. After 24 hours take the crucibles out, cool and then weigh them again (C+S2) to determine the dry solids. The same crucibles after weighing for dry solids can be used to determine the volatile solids (VS).

- **Volatile Solids (VS):**

Volatile Solid represents the organic dry matter of a sample. To carry out the analysis of VS, place the crucibles with the dried samples obtained previously in an oven at 550ºC for >6 hours, ideally overnight. After this, weigh the ash left in the crucibles (C+S3) and subtracting this from the DS would give the gVS of the sample.

## 5.1 Detailed process of BMP analysis and Calculations

Measurement of DS and VS is required to know determine g sample in each reactor.

1. First, complete a spreadsheet (tab: TS VS) with the measurements done for DS and VS.
    - **C**: Weight empty crucibles.
    - **C+S1**: Crucibles with the samples.
    - **C+S2**: Weight of the dry crucible and dry solids after oven.
    - **C+S3:** Weight of the crucible and dry solids after furnace.

2. Calculate the weight of the samples without the crucible weight:

**Wet sample**

S1=(C+S1)-C

**Dry solids**

S2=(C+S2)-C

**Ash**

S3=(C+S3)-C

3. Calculations of: volatile solids (dried solids – ash), dry solids % of wet weight, volatile solid % of wet weight, volatile solids % of dry solids (wet weight).

| | A | I | J | K | L |
|---|---|---|---|---|---|
| 1 | | | | | |
| 2 | **Sample** | **S2-S3 (volatile solids)** | **DS %wet weight** | **VS%wet weight** | **VS %DS wet weight** |
| 3 | Inoculum | 0.4031 | 9.54 | 6.09 | 63.87 |
| 4 | | 0.2627 | 9.41 | 5.96 | 63.36 |
| 5 | | 0.2465 | 9.36 | 5.94 | 63.43 |
| 6 | Parsnip | 2.5778 | 16.06 | 14.54 | 90.53 |
| 7 | | 1.1598 | 16.08 | 14.49 | 90.11 |
| 8 | | 0.9172 | 16.38 | 14.74 | 89.97 |
| 9 | Pulp | 2.6286 | 16.33 | 14.25 | 87.25 |
| 10 | | 0.9965 | 16.78 | 14.74 | 87.85 |
| 11 | | 0.6935 | 16.80 | 14.77 | 87.92 |
| 12 | S.Beet | 1.6164 | 14.81 | 10.51 | 70.99 |
| 13 | | 0.7186 | 12.00 | 9.09 | 75.77 |
| 14 | | 0.4081 | 12.39 | 9.36 | 75.57 |
| 15 | Paper | 2.7951 | 94.33 | 27.95 | 29.63 |

TS VS | Set-up calcs

**Volatile solids**

VS=S2-S3

**DS % wet weight**

$$DS\%\frac{w}{w}=\frac{S2}{S1}X100$$

**VS % wet weight**

$$VS\%\frac{w}{w}=\frac{S2-S3}{S1}X100$$

**VS % DS wet weight**

$$VS\%DS\frac{w}{w}=\frac{S2-S3}{S2}X100$$

4. Calculations of: DS % wet weight average; VS % wet weight average, VS % DS wet weight average.

| | A | M | N | O |
|---|---|---|---|---|
| 1 | | | **Average** | |
| 2 | **Sample** | **DS % wet weight** | **VS % wet weight** | **VS% DS wet weight** |
| 3 | Inoculum | | | |
| 4 | | | | |
| 5 | | 9.44 | 6.00 | 63.56 |
| 6 | Parsnip | | | |
| 7 | | | | |
| 8 | | 16.17 | 14.59 | 90.20 |
| 9 | Pulp | | | |
| 10 | | | | |
| 11 | | 16.64 | 14.59 | 87.67 |
| 12 | S.Beet | | | |
| 13 | | | | |
| 14 | | 13.07 | 9.66 | 74.11 |
| 15 | Paper | | | |
| 16 | | | | |
| 17 | | 94.64 | 27.97 | 29.55 |
| 18 | Straw | | | |
| 19 | | | | |
| 20 | | 91.45 | 86.90 | 95.02 |
| 21 | Chicken Litter | | | |
| 22 | | | | |

TS VS | Set-up calcs

**EXAMPLES:**

**Average DS % wet weight**

$$DS\%\frac{w}{w}=\frac{J3+J4+J5}{3}$$

**Average VS % wet weight**

$$VS\%\frac{w}{w}=\frac{K3+K4+K5}{3}$$

**Average VS % DS wet weight**

$$VS\%DS\frac{w}{w}=\frac{L3+L4+L5}{3}$$

5. Open the second tab (Set-up calcs). The Inoculum VS (%w/w) value will appear at the top of the tab.
6. Give to each reactor a sample description in the spreadsheet, in order to easily recognise which samples are in the different reactors. To differentiate them while you are conducting the BMP analysis, it is necessary to allocate numbers to each reactor (See figure12).

7. **Weigh the empty 1-litre HDPE bottles** using a weighing scale and allocate numbers to each reactor.

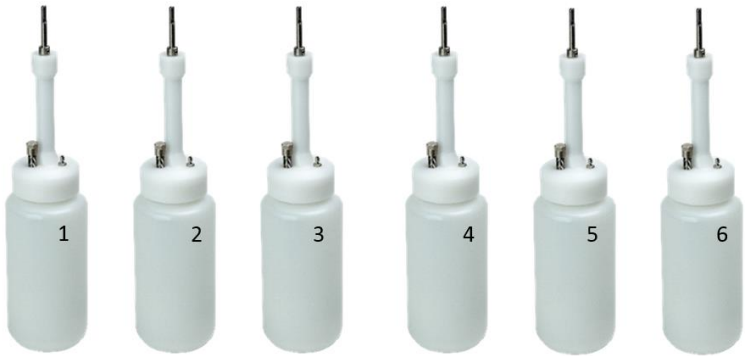



*Figure 13. Reactors number allocation.*

8. **Fill all the 15 1-litre bottles** with 'X' grams of inoculum (Target weigh of inoculum = 650g) and weigh again (actual weight). Put the value of the actual weight value in the Set-up calcs tab (e.g 650.3g). The spreadsheet will automatically calculate the grams of VS Inoculum mass.

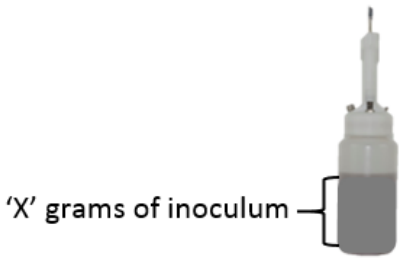



*Figure 14. Inoculum disposed in HDPE bottles.*

9. **Set up a ratio**[7] of Substrate: Inoculum (e.g. 1:4). Do not exceed **1g Sample:10g Inoculum** (See reference 1 to understand how set up a ratio).

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Inoculum VS (%w/w) | 6.00 | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | Sample description | Reactor number | Target weight of inoculum (g) | Actual weight of inoculum (g) added to reactors | Inoculum mass VS (g) | Substrate:inoculum (1:x) |
| 5 | Inoc1 | 1 | 650 | 650.3 | 39.00 | |
| 6 | Inoc2 | 2 | 650 | 650.4 | 39.01 | |
| 7 | Inoc3 | 3 | 650 | 650.4 | 39.01 | |
| 8 | Parsnip | 4 | 650 | 650.7 | 39.03 | 4 |
| 9 | Pulp | 5 | 650 | 650 | 38.98 | 4 |
| 10 | S.Beet | 6 | 650 | 650.7 | 39.03 | 4 |
| 11 | Paper | 7 | 650 | 650.1 | 38.99 | 4 |

TS VS | Set-up calcs

**EXAMPLES:**

**Inoculum mass VS:**

$$\text{Inoc. mass VS} = \text{Inoculum VS}\left(\%\frac{\text{w}}{\text{w}}\right) x \frac{\text{Actual Weight of inoc.(g)}}{100} = 6.00\ x\ \frac{650.3}{100} = 39\ \text{g}$$

**Ratio Substrate:Inoculum → 1:4**

[7] Black and white values are automatically calculated by the spreadsheet. Red ones have to be manually written.

10. Based on the ratio of grams of VS of inoculum to grams to VS of substrate used, it is **calculated the amount of feedstock to be added** in the 15 1-litre reactor bottles (for example, for a ratio of 4g of VS of inoculum to 1 g of VS of substrate in digestate analysis you will need **Inoculum mass VS ÷4** g VS as sample).

| | A | F | G | H | I | J |
|---|---|---|---|---|---|---|
| 1 | Inoculum VS (%w/w) | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | Sample description | Substrate:inoculum (1:x) | Target sample VS (g) to achieve the ratio in Column F | Sample VS (%wet weight) | Target weight of sample (g) to achieve the ratio in column F | Actual sample weight (g) added to the reactors |
| 5 | Inoc1 | | - | - | - | - |
| 6 | Inoc2 | | - | - | - | - |
| 7 | Inoc3 | | - | - | - | - |
| 8 | Parsnip | 4 | 9.76 | 14.59 | 66.87 | 66.89 |
| 9 | Pulp | 4 | 9.75 | 14.59 | 66.80 | 66.79 |
| 10 | S.Beet | 4 | 9.76 | 9.66 | 101.00 | 101.3 |
| 11 | Paper | 4 | 9.75 | 27.97 | 34.85 | 34.9 |

TS VS | Set-up calcs

**EXAMPLES:**

**Target sample VS (g) to achieve the ratio fixed:**

$$\text{Target sample VS (g)} = \frac{\text{Inoculum mass (g)}}{\text{Subsdtrate: Inoculum}} = \frac{39}{4} = 9.76$$

**Sample VS (%w/w): Calculated in the previous tab (TS VS).**

**Target weight of sample (g) to achieve the ratio fixed:**

$$\text{Target sample VS (g)} = \frac{\text{Target smaple VS (g)x100}}{\text{Sample VS (\%}\frac{w}{w})} = \frac{9.76 * 100}{14.59} = 66.87$$

11. Pour into reactors the calculated grams of sample ('Target weight of sample (g) to achieve the ratio fixed') in the spreadsheet, weight them and write the value in 'Actual sample weight (g) added to the reactors' column.

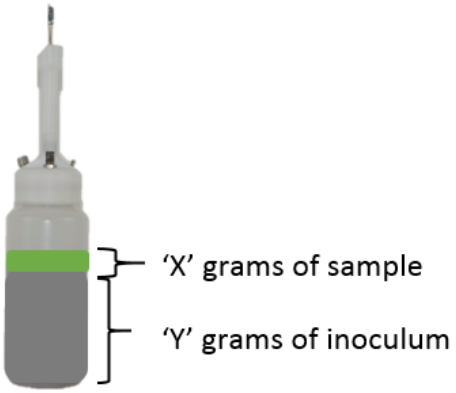



*Figure 15. Inoculum and sample disposed in HDPE*

12. The spreadsheet will automatically calculate the amount of sample mass in terms of VS.

| | A | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|
| 1 | Inoculum VS (%w/w) | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | Sample description | Substrate:inoculum (1:x) | Target sample VS (g) to achieve the ratio in Column F | Sample VS (%wet weight) | Target weight of sample (g) to achieve the ratio in column F | Actual sample weight (g) added to the reactors | Sample mass VS (g) |
| 5 | Inoc1 | | - | - | - | - | - |
| 6 | Inoc2 | | - | - | - | - | - |
| 7 | Inoc3 | | - | - | - | - | - |
| 8 | Parsnip | 4 | 9.76 | 14.59 | 66.87 | 66.89 | 9.759 |
| 9 | Pulp | 4 | 9.75 | 14.59 | 66.80 | 66.79 | 9.745 |
| 10 | S.Beet | 4 | 9.76 | 9.66 | 101.00 | 101.3 | 9.786 |
| 11 | Paper | 4 | 9.75 | 27.97 | 34.85 | 34.9 | 9.762 |

TS VS | Set-up calcs

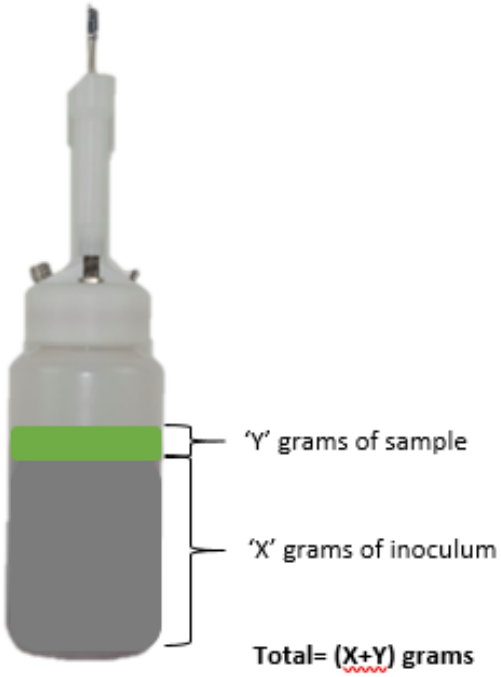
**EXAMPLES:**

**Sample mass VS:**

$$\text{Sample mass VS (g)} = \frac{\text{Inoculum mass (g)}}{100} = \frac{66.89 \text{ x } 14.59}{100} = 9.759$$

13. Finally fit the reactor heads and place all reactor bottles in water bath (follow the instructions given in the Section 3.3).

The total amount of biogas produced by the material will be calculated by the gas flow meter. The gas produced by the material can be calculated as below. If a "negative" biogas yield is produced what this indicates is that the sample is causing the reactor to produce less gas than it would have produced without any sample, indicating inhibition or toxicity that could be chronic or acute:




**Total Gas produced by (X+Y) = a**

**Total Gas produced by X = b**

**Total Gas produced by Y (Sample) = a-b**

*Example 2:*

Total Gas produced by (X+Y) = 250 L Kg $VS^{-1}$

Total Gas produced by X = 200 L Kg $VS^{-1}$

Total Gas produced by Y (Sample) = 250 – 200= 50 L Kg $VS^{-1}$

*Example 3:*

Total Gas produced by (X+Y) = 200 L Kg $VS^{-1}$

Total Gas produced by X = 250 L Kg $VS^{-1}$

Total Gas produced by Y (Sample) = 200 – 250= - 50 L Kg V $S^{-1}$ → **NEGATIVE VALUE** (See section 5)

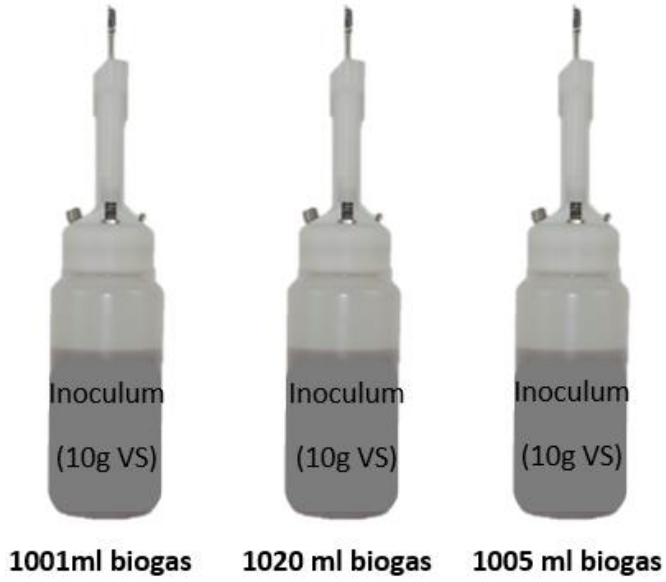
*Example 4:*

**SAMPLE:** As an example, suppose you need to measure the biomethane potential of a food waste. Assume a 20g sample of food waste with 10%VS of wet weight. Therefore, you have 2g of volatile solids (VS) as sample assumed to be mainly organic degradable material.


Since we want to evaluate how much biogas would a digester produce when fed this material, we use anaerobic digestate as inoculum (seed) for the test.

It has been established that in order to not shock the inoculum with too much sample and to be able to make use of the kinetics of biogas production, a volatile solids (VS) ratio Inoculum: Sample between 4:1 and <10:1 is recommended to minimise potential organic shock to the inoculum as the test is set. In this example we will use a 5:1 ratio. Note to maintain more relevance to real life we avoid pH buffering (not used in real life)

**INOCULUM:** Assuming the inoculum is 2.5% VS of wet weight (means that for one kilo of liquid inoculum we have 25g of volatiles solids, assumed to be mainly anaerobic bacteria). We have 400g of inoculum at 2.5%VS of wet weight. This means that we would have total 10g VS as anaerobic inoculum.




From the above results would have the following yields for the inoculum:

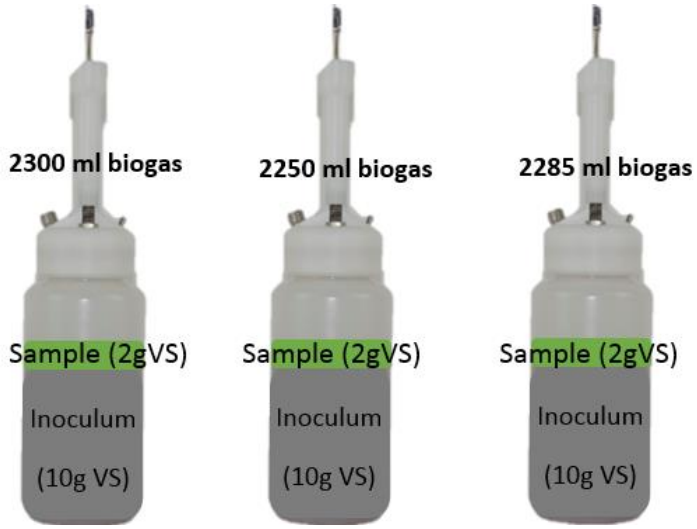
1001ml/10gVS
1020ml/10gVS ➔ 100.1, 102, 100.5
1005ml/10gVS

**Average inoculum yield =**
(100.1 + 102 +100.5)/3 = **100.86 ml biogas/ g VS inoculum**
(or $m^3$/ton VS)

**MEASURING THE POTENTIAL BIOGAS YIELD OF A SAMPLE:** To carry out the test, first we need to measure how much biogas is produced by the inoculum alone (litre of biogas per kg of VS). This is done preparing three digesters containing inoculum alone and measuring the biogas produce over a period of 30 days, or as long as the test lasts.

We subtract the biogas produced by the inoculum in the test from the "inoculum plus sample reactors". This subtraction gives us the biogas potential of the sample, we then convert it to litres of biogas per kg of VS.

Ideally, measure each sample, or inoculum, in **triplicate to avoid outliers impacting test consistency**. This is because this is a biological test, **susceptible to unpredicted variability**, and samples can be blends of different compounds with potential for bias, for example if lumps of sample are present. **Never do a test using only one control reactor**




Since we know the average biogas yield of the inoculum per gram VS is 100.86ml/gVS inoculum, and we have 10g VS of inoculum in each of the samples, **each of the reactors above containing sample would have 1008.6ml of biogas corresponding to the inoculum** with which the sample was seeded (area in grey).

Therefore, the sample present in each of the reactors above would have produced:

2300ml gas total - 1008.6ml biogas from inoculum = 1291.4 ml biogas/2 g sample: 638.2ml/gVS

2250ml gas total - 1008.6 ml biogas from inoculum = 1241.1 ml biogas/2 g sample: 620.5ml/gVS

2285ml gas total - 1008.6 ml biogas from inoculum = 1276.4 ml biogas/2 g sample: 638.2ml/gVS

Thus, the average Biogas potential for the sample is: (638.2+620.5+638.2)/3 = **632.3ml/g VS**

(This calculation is automatically made on a daily basis and results in the cumulative graphs, a sample will normally produce more biogas per gram).

If the sample used produces biogas, there will be a positive value when the gas from the inoculum is subtracted. This will be reflected on an upward yield graph.

However, when inhibition or toxicity occurs, subtracting the biogas produced by the inoculum from the sample reactor would give a negative value reflected on a downward trend in the cumulative biogas yield (See section 5). Users can interpret the significance of results rather than mathematically smoothing them

## 5.2 Setting up Arduino Datalogger

A BMP280 barometric pressure, a microSD card reader and a thermocouple amplifier are connected to the Arduino. All tumbler events are written to a log file on the microSD card including the channel number, the time at which the event occurred, the air pressure at that time and the temperature as recorded by the thermocouple. For ease of use, other log files (e.g. a summary of what has happened in the last 24 hours) which are calculated from this event data, are also stored on the memory card. A suite of Python utilities has also been developed to simplify set up and operation of the data logger.

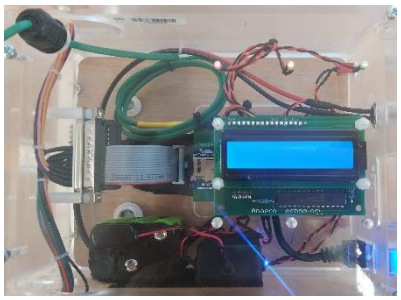


*Figure 16. Arduino datalogger.*

### Data logger use

a) A master folder of Arduino software will be provided by Anaero with two versions of software (software that runs on a 32 bit operating system and software suitable for 64 bit operating system). Check the properties of your computer's processor (also called a CPU) and see if it is 32-bit or 64-bit[8].

b) Depending on your computer properties copy the right version of Arduino software (64 bit folder or the 32 bit) from the Master software folder provided and paste it on your desktop. Rename the folder copied : right click on the folder, select copy, right click on the desktop and select paste then right click on the new folder, select Rename and rename the folder to relevant name (e.g.: Gas_Flow_1 or Gas_Flow_2).

c) **Open the folder**[9] you have just created (it should contain the four Python utilities (**filegrab, fastgrab, monitor and startrun**) in the form of .exe files, along with a template for the setup.csv file.

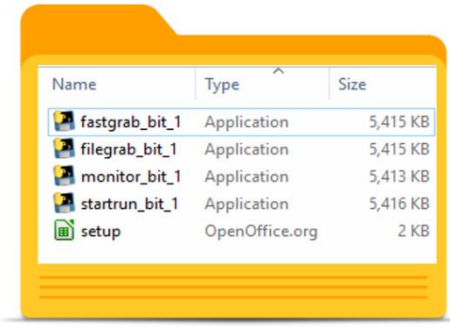



*Figure 17. Contents of Arduino folder.*

d) Open the **setup file** in Excel format (normally the computer will open the folder via Excel by default). The setup file will be displayed in the style of a spreadsheet. The **setup file** allows a sample name to be associated with each of the 15 channels, **specifies which channels are being**

[8] Open the main menu of your computer, press 'your computer', click right on 'C:', go to properties and check type of system (32 or 64-bit).

[9] If you are running more than one system you will need a different folder for each system, each of which should contain the relevant setup.csv and the three .exe files.

**used and named them**, which channels are 'inoculum only', the mass of inoculum volatile solids for each channel and the mass of sample volatile solids for each channel (inoculum only channels will, by definition have a zero entry for their mass of sample volatile solids). The final column contains the calibrated tumbler volume for each channel.

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Sample description | In service | Inoculum only | Inoculum mass VS (g) | Sample mass VS (g) | Tumbler volume (ml) |
| 2 | Inoculum 1 | 1 | 1 | 15.46 | 0 | 6.41 |
| 3 | Inoculum 2 | 1 | 1 | 15.48 | 0 | 6.58 |
| 4 | Inoculum 3 | 1 | 1 | 15.5 | 0 | 6.5 |
| 5 | Cellu 10 | 1 | 0 | 14.27 | 2.39 | 6.5 |
| 6 | Cellu 11 | 1 | 0 | 14.28 | 2.42 | 6.41 |
| 7 | Cellu 12 | 1 | 0 | 14.27 | 2.38 | 6.53 |
| 8 | 1623 Mar | 1 | 0 | 15.31 | 3.92 | 6.66 |
| 9 | 1623 Mar | 1 | 0 | 15.32 | 3.87 | 6.5 |
| 10 | 1623 Mar | 1 | 0 | 15.3 | 3.83 | 6.53 |
| 11 | 1624 Agri | 1 | 0 | 11.85 | 2.97 | 6.5 |
| 12 | 1624 Agri | 1 | 0 | 11.8 | 2.98 | 6.58 |
| 13 | 1624 Agri | 1 | 0 | 11.8 | 2.98 | 6.5 |
| 14 | 54 Bryn 1 | 1 | 0 | 11.92 | 3.1 | 6.66 |
| 15 | 54 Bryn 2 | 1 | 0 | 11.9 | 2.95 | 6.5 |
| 16 | 54 Bryn 3 | 1 | 0 | 11.88 | 2.96 | 6.5 |

*Figure 18. Data CSV format setup spreadsheet file.*

e) **Calibrate the gas flow meter and keep the values ready to be entered into the set-up file** (See section 2.3 where the calibration of the gas flow meter is explained).

f) **Edit the contents** of the spreadsheet to match your system/experiment: **Insert the ml/ tumble values (**Section 2.3) obtained during the gas flow meter calibration (Column Figure 18). Insert the Inoculum VS mass (g) and sample VS mass (g) , already calculated in the calculation sheet provided (Section 4.1 and 4.2 – Coloumn E and Coloumn K in sample spreadsheet highlighted in yellow).

g) **Save the Excel spreadsheet**, clicking on File -> Save. This brings up a prompt to check that you wish to save the file in .csv format. Click on "Use TEXT CSV Format". **Close the Excel spreadsheet**.

h) Once the setup.csv file has been generated by editing the template provided, it can be transferred to the Arduino[10] and the run started using the '**startrun**' utility. Make sure the **gas flow system is powered using the power supply provided (Figure 19 → 1 to 2) and the gas flow meter is connected to the laptop** using the USB cable (3 to 4) then double click on the startrun icon in the folder created at Step 1.

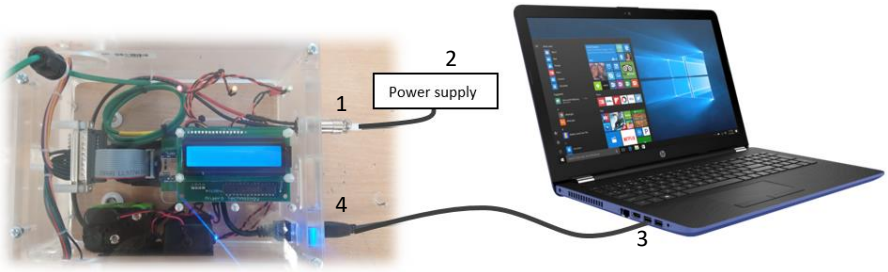



*Figure 19. Arduino connexions. The numbers show the connection procedure order to be followed.*

[10] When connecting a computer to the Arduino, always disconnect and reconnect the computer from the Arduino end (i.e. the square shaped 'type B' USB plug), not by unplugging or connecting the USB lead from computer using the rectangular 'type A' plug. If you unplug or reconnect at the computer end, there is a risk that a spike over the USB connection as you make the connection can cause the Arduino to reset – this is much less likely if the connection is made/broken using the USB 'type B' plug/socket at the Arduino end.

g) Make sure that the ON/OFF Arduino button is in ON mode (See Figure 20).

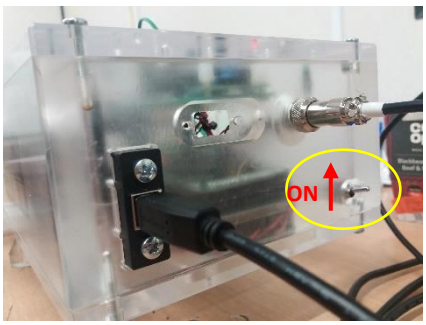


*Figure 20. Arduino connections details.*

h) Startrun utility initialises the logging system and transfers the data from the setup.csv file over to the system. The system will ask which port of the computer you will use (called COM + a number). In the case of the example Figure 21, the name of the port is COM10 (the system will tell you which port are you using), simply **write COM**10 **and press enter**[11].

```
>>> ********************************************************************************
>>> anaero technology - www.anaero.co.uk - gas flow monitor startup utility
>>>
>>> serial ports being used on this machine are:

    ('COM10', 'Arduino Mega 2560 (COM10)', 'USB VID:PID=2A03:0042 SNR=953363336353513041C2')

>>> enter the name of the COM port the Arduino Mega 2560 is connected to
>>> e.g. COM1 or COM2 or COM3 or COM4 or similar, and then press enter
```

*Figure 21. Arduino startrun.*

i) Arduino will ask if you want to copy or deleted old data. Directly press 'D' and 'enter' to delete you data or copy it and then delete it (See figure 22).

j) Once the files have been copied or deleted, the system will complete its **self-test routines** and prepare to transfer the data in the setup.csv file.

```
>>> ********************************************************************************
>>> anaero technology - www.anaero.co.uk - gas flow monitor startup utility
>>>
>>> serial ports being used on this machine are:

    ('COM10', 'Arduino Mega 2560 (COM10)', 'USB VID:PID=2A03:0042 SNR=953363336353513041C2')

>>> enter the name of the COM port the Arduino Mega 2560 is connected to
>>> e.g. COM1 or COM2 or COM3 or COM4 or similar, and then press enter

>>> COM10
>>> opening serial port connection to arduino

 2017-12-01 11:26:27

Arduino has been reset

Arduino running - starting self test

Running Bittern software build

Internal Temp = 24.06
Thermocouple reading is 23.25C
Barometer OK
Air pressure is: 1022.45 hPa

Checking SD card...SD card present

Card type: SDHC

Files found on the card (name and size in bytes):

Opening SD card for file read/write

SD card opened OK

*** This program loads a new setup file and starts a complete new run ***
*** if you continue, existing log files on the SD card will be deleted ***

*** Do you want to save existing log files to your computer before continuing? ***

To save the existing log files from SD card on your computer before new run starts press Enter now...

OR

To delete existing log files on SD card and start new run, press d followed by the Enter key...
```

*Figure 22. Arduino startrun 2.*

[11] If there is any problem with Arduino COM connection to the laptop, try to change the wire to another computer port.

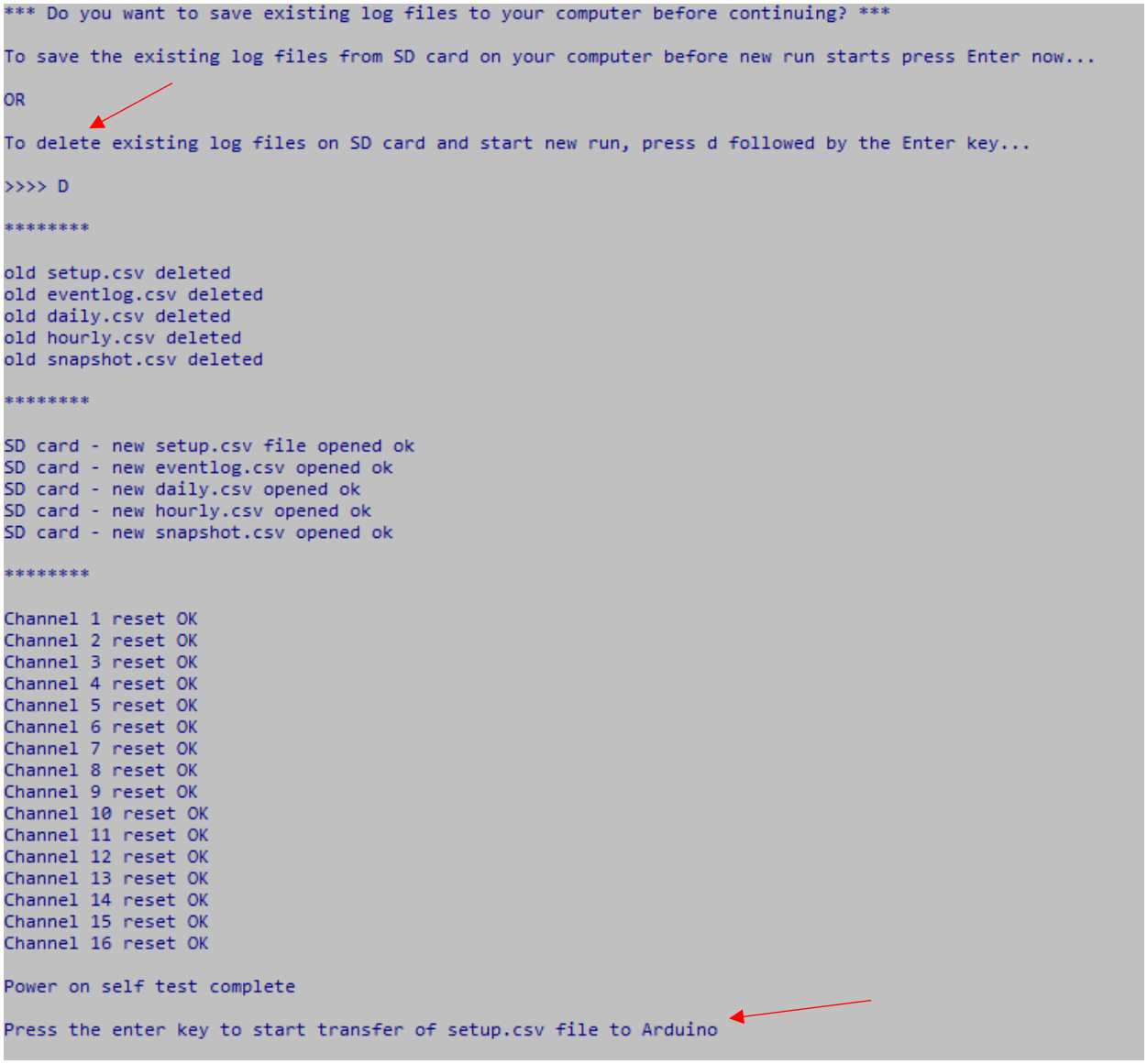



*Figure 23. Arduino startrun 3.*

k) **Pressing 'enter' again to starts the transfer process** (once this has completed, the data which has been stored by the logging system is written back to the console and also to the setup.csv file). The data logger does perform range checks on the data received and will display warnings if unexpected or out of range entries are found. It is important to check that the data written back match what was send. This can be done by examining the information displayed on the console or by opening the setup.csv file in Libre Office. You will see that the data written back from the data logger are appended to the file.

```
Press the enter key to start transfer of setup.csv file to Arduino

>>>>

Inoculum 1,1,1,15.46,0,5
Inoculum 2,1,1,15.48,0,5.2
Inoculum 3,1,1,15.5,0,5.17
Cellu 10,1,0,14.27,2.39,5.17
Cellu 11,1,0,14.28,2.42,5.2
Cellu 12,1,0,14.27,2.38,5.2
1623 Mar,1,0,15.31,3.92,5.17
1623 Mar,1,0,15.32,3.87,5
1623 Mar,1,0,15.3,3.83,5
1624 Agri,1,0,11.85,2.97,5
1624 Agri,1,0,11.8,2.98,5.17
1624 Agri,1,0,11.8,2.98,5.17
54 Bryn 1,1,0,11.92,3.1,5.17
54 Bryn 2,1,0,11.9,2.95,5.17
54 Bryn 3,1,0,11.88,2.96,5.17
End of data,,,,,

Data tables loaded

Setup file written to SD card

starting setup.csv writeback

Inoculum 1,1,1,15.46,0.00,5.00
Inoculum 2,1,1,15.48,0.00,5.20
Inoculum 3,1,1,15.50,0.00,5.17
Cellu 10,1,0,14.27,2.39,5.17
Cellu 11,1,0,14.28,2.42,5.20
Cellu 12,1,0,14.27,2.38,5.20
1623 Mar,1,0,15.31,3.92,5.17
1623 Mar,1,0,15.32,3.87,5.00
1623 Mar,1,0,15.30,3.83,5.00
1624 Agri,1,0,11.85,2.97,5.00
1624 Agri,1,0,11.80,2.98,5.17
1624 Agri,1,0,11.80,2.98,5.17
54 Bryn 1,1,0,11.92,3.10,5.17
54 Bryn 2,1,0,11.90,2.95,5.17
54 Bryn 3,1,0,11.88,2.96,5.17

writeback completed - setup.csv closed

Press the enter key to start data logging
```

```
Data tables loaded

Setup file written to SD card

starting setup.csv writeback

Inoculum 1,1,1,15.46,0.00,5.00
Inoculum 2,1,1,15.48,0.00,5.20
Inoculum 3,1,1,15.50,0.00,5.17
Cellu 10,1,0,14.27,2.39,5.17
Cellu 11,1,0,14.28,2.42,5.20
Cellu 12,1,0,14.27,2.38,5.20
1623 Mar,1,0,15.31,3.92,5.17
1623 Mar,1,0,15.32,3.87,5.00
1623 Mar,1,0,15.30,3.83,5.00
1624 Agri,1,0,11.85,2.97,5.00
1624 Agri,1,0,11.80,2.98,5.17
1624 Agri,1,0,11.80,2.98,5.17
54 Bryn 1,1,0,11.92,3.10,5.17
54 Bryn 2,1,0,11.90,2.95,5.17
54 Bryn 3,1,0,11.88,2.96,5.17

writeback completed - setup.csv closed

Press the enter key to start data logging

>>>>

--- data log started ---

-----------------------------------
```

*Figure 24. Arduino transfer process.*

l) Make sure that laptop-Arduino and Arduino-Power supply are connected. Also, at this stage all the reactors are placed in the waterbath and all the gas hoses are connected from the reactors to the gas flow meter. To start the **gas flow log**, press 'enter' – the system is now live and

recording all tumbler tips. When a tumbler tip is detected an update is sent to the console. In between tumbler events, the time stamp on the **console is updated every 10 seconds**. To leave the startrun program press any key on the keyboard. It can take the utility a short while to tidy up and close down.

m) Arduino will collect the gas production, which could be downloaded in your laptop.

## Biogas production curve

n) **Plotting Pivot chart:** Copy daily.csv to the desktop so the original remains in the test directory and then double click on the daily.csv file that is on the desktop to open it.

o) Delete the first row ('file uploaded 2017-11-28…'), and the last one (that says 'writeback completed 2017-11-28…').

| | A | B | C | D | E | F | G | H | I | J | K |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Channel nu | Name | Timestamp | Days | Hours | Mins | In service | Tips this da | Vol this da | Net vol this day (ml/g) | |
| 2 | 1 | inoculum | 86400028 | 1 | 0 | 0 | 1 | 9 | 41.96 | 0 | |
| 3 | 2 | inoculum | 86400028 | 1 | 0 | 0 | 1 | 9 | 42.78 | 0 | |

*Figure 25. Excel file with the values downloaded from Arduino.*

p) Now highlight all the data you wish to graph (whole table) and select Pivot Chart and click on OK.

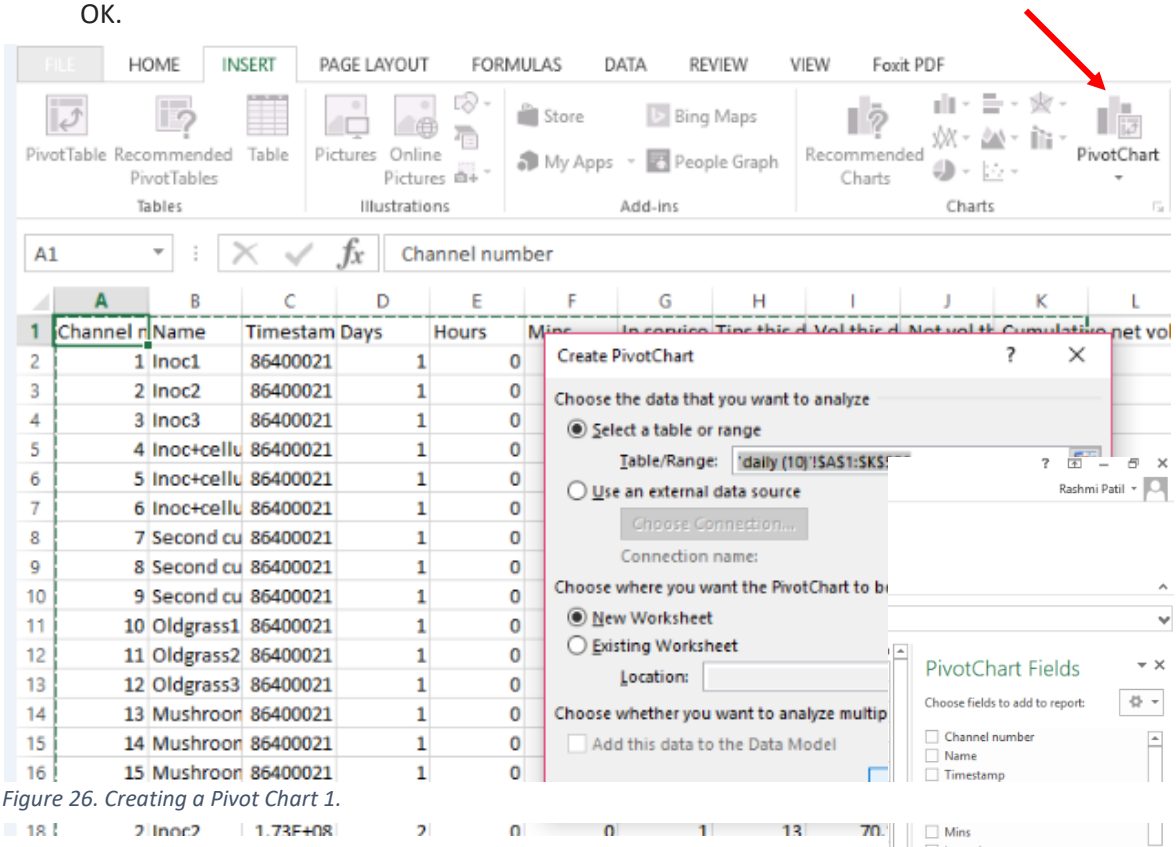



*Figure 26. Creating a Pivot Chart 1.*

q) A new tab will be created with the Pivot Chart, as it is showed the figure 27.

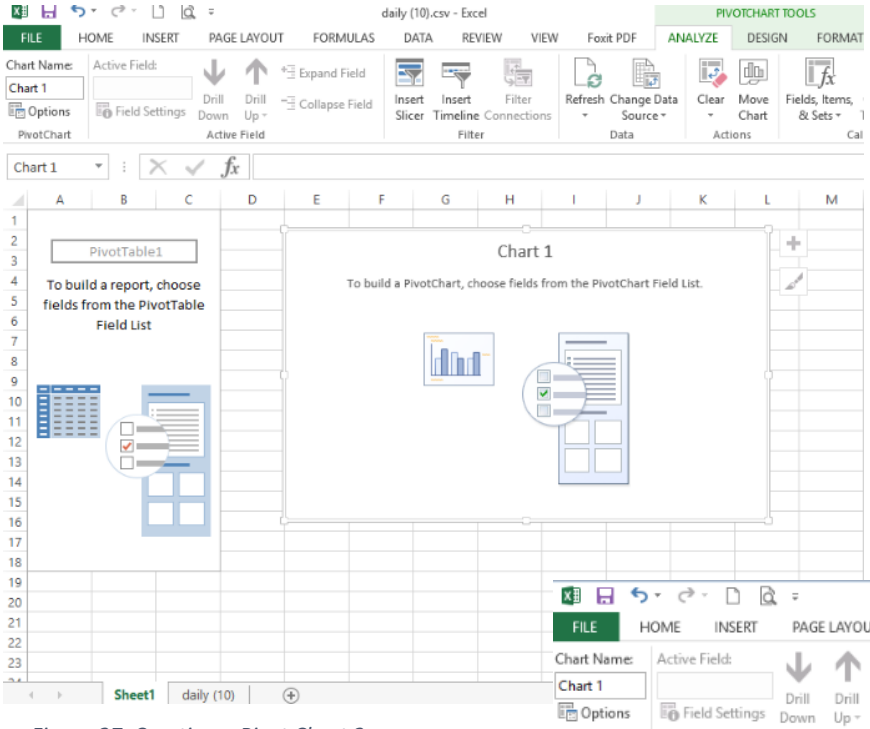



*Figure 27. Creating a Pivot Chart 2.*

r) Select the Pivot Chart Fields and drag that fields to the areas below (see figure 28).

| Areas | Pivot Chart Fields |
|---|---|
| • LEGEND: | Channel number |
| • AXIS: | Days |
| • VALUES: | Sums of Cumulative net volume. |

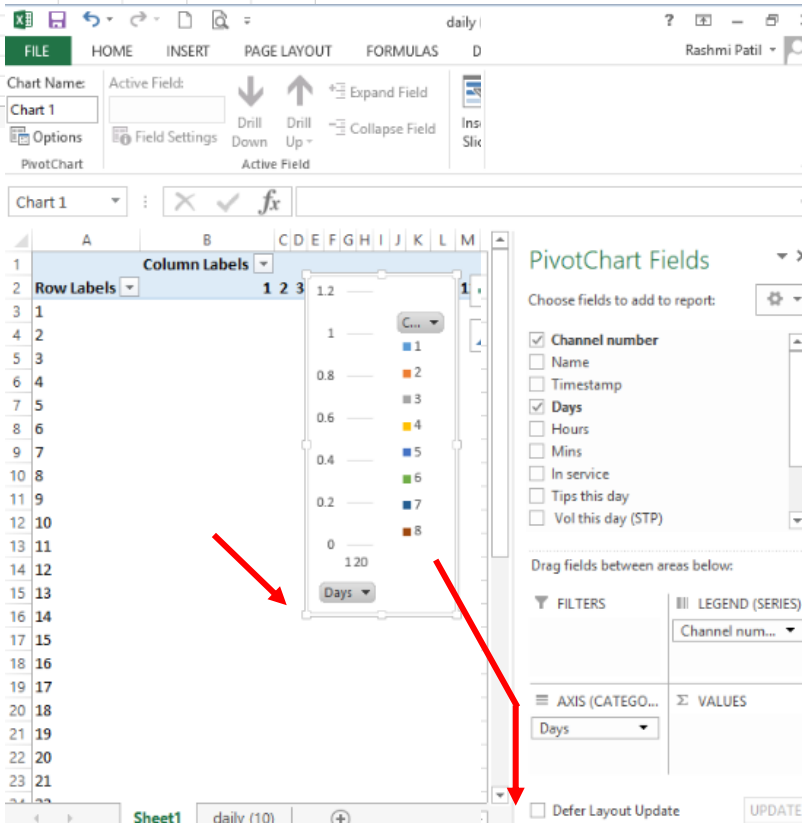



*Figure 28. Creating a Pivot Chart 3.*

s) Move the Chart to a new tab (see figure 29) and change the chart type to: Line Chart (see figure 30).

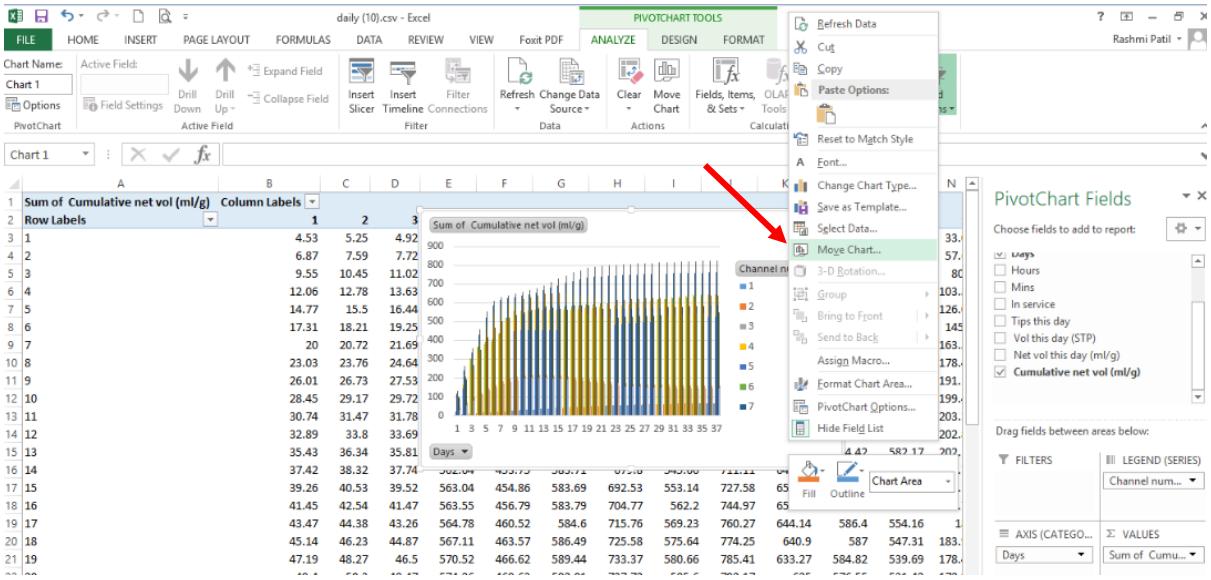



*Figure 29. Creating a Pivot Chart 4.*

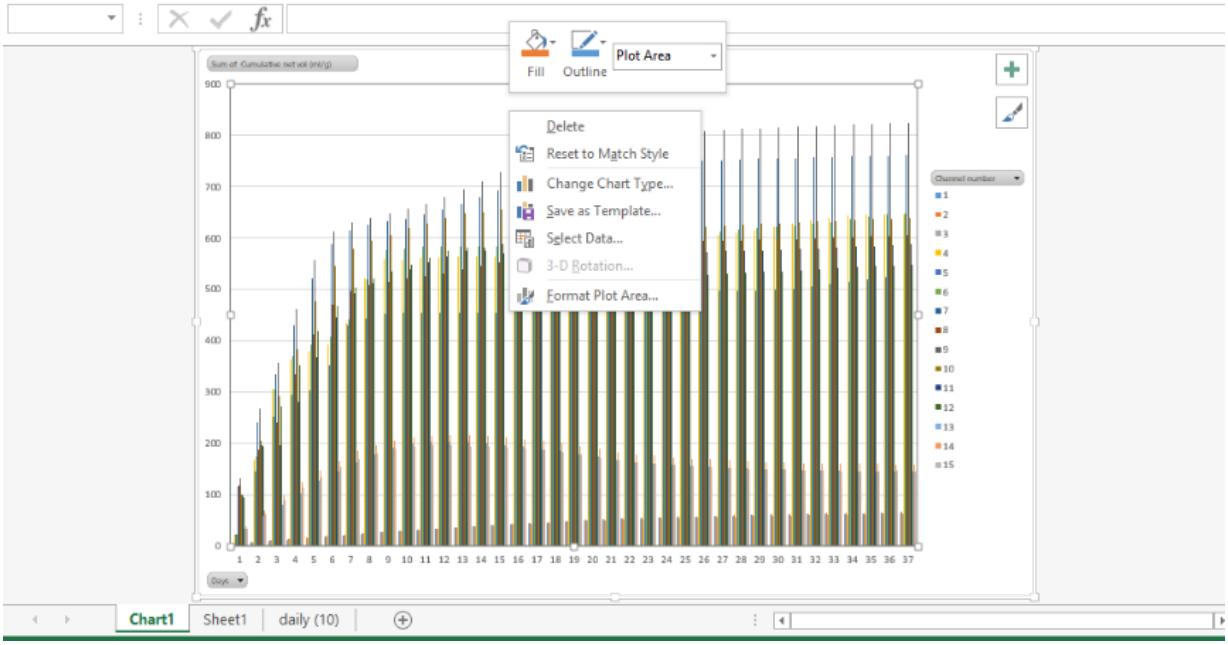



*Figure 30. Creating a Pivot Chart 5.*

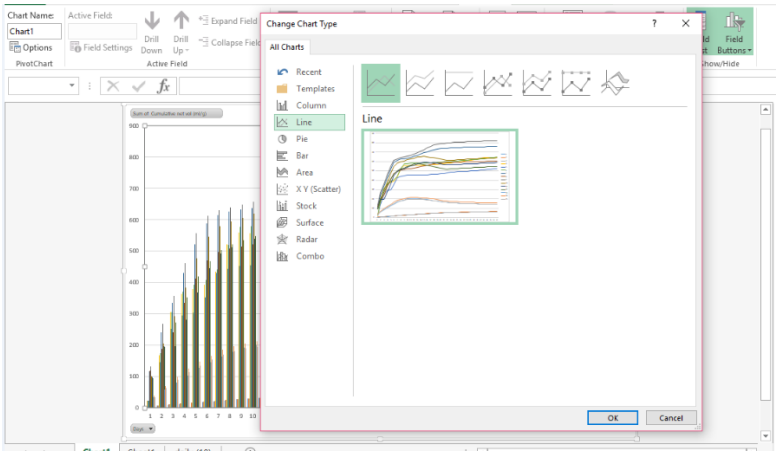



*Figure 32. Creating a Pivot Chart 6 (Change to Line Chart).*

*Figure 311. Creating a Pivot Chart 6.*

t) Finally you obtain a graphic showing the cumulative biogas production curve of each sample, produced during the BMP test.

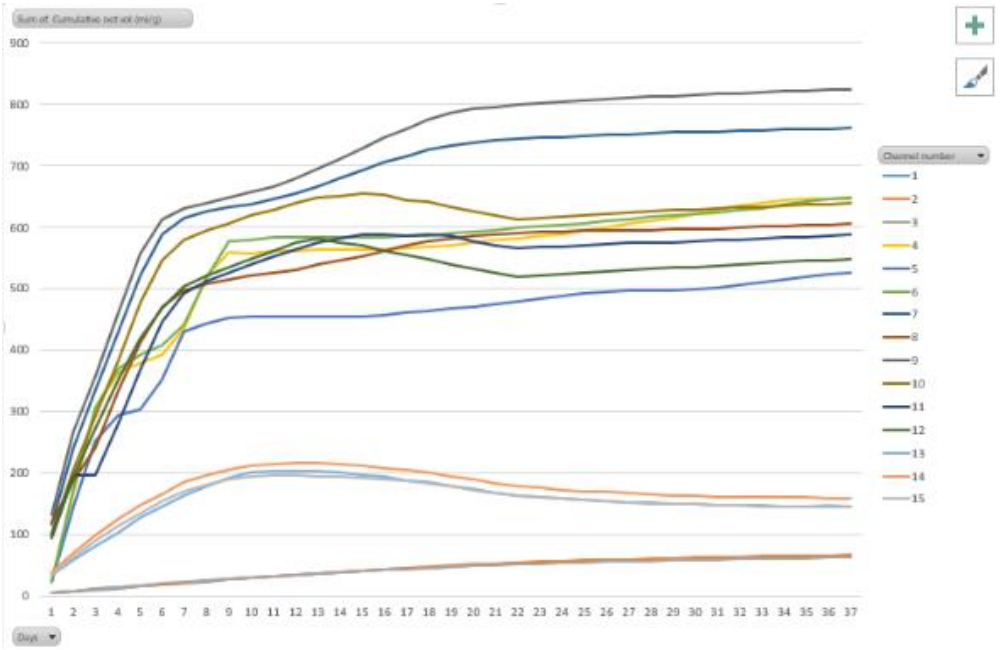



*Figure 33. Final biogas production chart.*

## Other utilities

- **Filegrab**: implements transfer of ALL data recorded by the logging system to the laptop. This comprises the event log, a cumulative and incremental hourly report, a cumulative and incremental daily report and a cumulative four hourly snapshot. After a run of several days several thousand lined of data are produced so it can take a while for all data to be transferred. The utility closes itself a short time after all data have been transferred. The name of each file written on the laptop includes a time and date so is unique; as such the utility can be run as many times as required without having to remove any files from the folder
- **Fastgrab**: performs the same function as filegrab but only transfers the event log and the daily summary so takes much less time to complete. The utility closes itself a short time after all data have been transferred. As with filegrab, the file names include a time and date so previous transfers are not over-written.
- **Monitor:** allows live observation of the system but doesn't transfer any data.

Note that when connecting to a 'live' gas flow meter mid-run, in order to minimise the possibility of a short circuit or spike disrupting things, always **power up the laptop unattached**, then plug the USB lead into the laptop and then connect the USB lead from the laptop to the flying lead coming off the gas flow meter. If the USB lead is connected in the reverse order there is a risk that as the lead is plugged into the laptop a short or power spike is generated which could affect the data logger.

**Reference: Labatut, 2012. Anaerobic Biodegradability of complex substrates: Performance and Stability at Mesophilic and Thermophilic Conditions. *Cornell University*, 25- 28.**

As well as an indicator of biogas potential, the test can provide information about potential sample inhibition, or inoculum strength. The kinetics of biogas generation obtained through BMP/Biogas Potential tests can provide data on potential sample inhibition, toxicity or inoculum strength. Biodegradability characteristics of substrates and production of inhibitory intermediate products will mainly control the kinetics of the different steps of anaerobic digestion and define the shape of the biogas production curve. Therefore, you should consider what is the purpose of the test you are carrying out? If it is to just estimate the maximum biogas potential of a sample inoculum type, ISR, or dilution should not impact much when long term biogas production is measured. Equally, if you just want to measure $CH_4$ it does not matter whether or not you strip $CO_2$ from the gas. However, if in addition you want to gain some information about potential inhibition, some idea of behaviour in a plant, and the potential issues from $H_2S$ levels in biogas, or dissolved sulphides (residual odour potential), you might choose to source inoculum as close to operational digestate. Fresher inoculum is better to focus on inhibition at a specific site but can have higher standard deviation in triplicates. Still the kinetic picture should be clear if inhibition is present. No commercial AD plant uses dilution water as it would massively increase operational costs; use of dilution in BMP tests can make the kinetics of a test even less representative of that of a real plant. We use no dilution in our own tests. Be cautious when interpreting kinetics of tests to understand not only the inoculum acclimatisation but any possible dilution, buffering or supplementation.

Interpretation of BMP results can be of significant economic importance when evaluating the potential economic viability of a project, or improving performance of existing ones. Care should be taken not to assume that results of BMP assay, a laboratory scale batch test, can be directly extrapolated to predict the performance of semi- or continuous-flow, commercial size anaerobic digesters. As well as the physical differences in the fluid- and thermo-dynamic characteristics due to the scale and geometry of lab reactor compared with full-scale ones, full-scale digesters differ from BMP tests in their mode of operation (regular feeding). The way a reactor is fed has a fundamental impact on the thermodynamic equilibrium of the anaerobic process – and thus, on the food-biomass interactions. Semi- and continuous-flow digesters are characterised by dynamic changes in their chemistry (generation of vfas, etc), microbiology (biomass selection, wash out, etc), physical condition (gas dynamics in and out of solution) due to periodic substrate feeding and product removal. On the other hand, in a batch reactor,

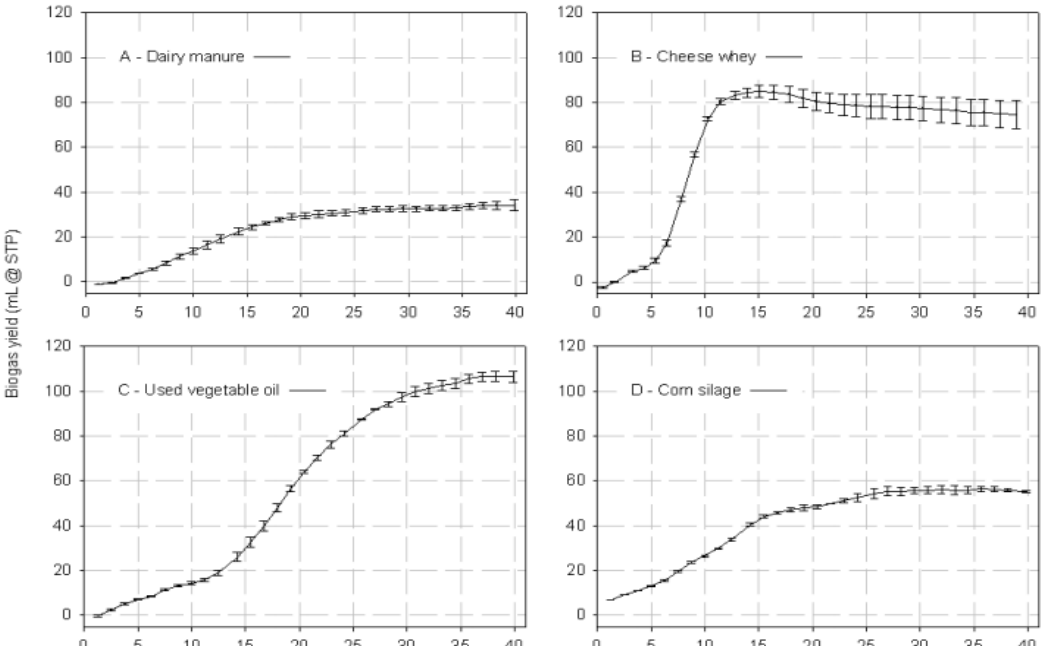



*Figure 34. BMP assay curves for a 40-day run showing four distinctive biogas production patterns (mL @ STP) from four different substrates; A: dairy manure, B: cheese whey, C: used vegetable oil, D: corn silage; error bars represent the standard*

substrates, microorganisms, enzymes, intermediate products, and (sometimes) final products are accumulated within the system, unless they are metabolised via biologically-mediated processes. When the concentration of an intermediate product (particularly, volatile fatty acids and hydrogen) reaches the homeostatic threshold of a certain organism, or group of organisms, the thermodynamic balance is altered, and one or several metabolic reactions may be inhibited, causing further product accumulation and delay of substrate degradation. In most cases, product inhibition is reversible, and as soon as thermodynamic conditions become favourable, reactions resume, this is more evident in semi-continuous tests and a reason to avoid direct extrapolation between batch and continuous evaluations.

The currently used BMP test method has been designed to provide ideal anaerobic digestion conditions (temperature, mixing, trace element supplementation), and prevent any form of biochemical inhibition (dilution and controlled ISR). How these are applied will influence how results should be interpreted. Three important conditions should be met throughout the BMP assay: 1) appropriate microbial community, enzyme pool, and nutrients are present in the inoculum; 2) environmental conditions are optimal (temperature, mixing, pH); and 3) substrate and intermediate product concentrations are well below inhibitory/toxic levels (often controlled through dilution of samples or ISR). Inhibition can be visualised in some BMP assays as it affects reaction kinetics. However, in most cases provided enough digestion time is allowed the final yield should not affected. However, a more difficult problem to foresee, which directly affects the biomethane potential, could be trace element deficiency, more relevant if a plant is single substrate and not regularly supplemented. This can occur during long-term anaerobic digestion of certain substrates lacking an essential element, such as cobalt in thin stillage (Agler et al., 2008). BMP results should be limited to a relative interpretation of the substrate's biomethane potential, and not for an absolute estimation of daily biomethane yields or the overall performance and stability of large scale digesters.

## FREQUENTLY ASKED QUESTIONS

### 1) What is the recommended minimum volume of digestate in 1L bioreactors?

We recommend using > 650-700 ml of inoculum + sample in the 1 litre reactor bottles. With this volume, any access port is below the liquid level and you can syringe out samples for analysis without having to lose biogas. However, if you want to use smaller sample volumes you can do so using the same 1 litre bottles although by producing less gas the potential error may increase.

### 2) Are there ports are available for sampling/dosing, can I measure pH?

There is a port for an optional access test tube on the reactor heads which goes below the liquid level. This allows you to syringe out sample for analysis and also to measure pH without losing biogas. You can use a hand held pH meter with 6 mm diameter pH probe to measure pH (https://www.coleparmer.co.uk/i/cole-parmer-ph-electrode-extra-long-220x6mm/0599045?searchterm=wz0599045). As well as the access and a biogas port, we have two additional free ports that can be fitted with flushing facilities or septum at user's facilities.

### 3) How can gas samples be taken for subsequent GC analysis?

You can analyse biogas composition either fitting a septum directly on the reactor caps, or using Tedlar gas bags fitted to the outlet discharge of the gas flow meters as illustrated in figure 5.

Our gas flow meters have individual chambers for each reactor. The gas from the bioreactor enters a gas flow meter cell from the bottom and bubbles through the water to fill one side of the rocking vessel. As the vessel fills, it floats then tumbles and this triggers the logging of the occurrence and the storage of the temperature and pressure at each occurrence. After the tipping, the gas is evacuated to the headspace of the gas flow meters. Each headspace has a hose tail fitting where a Tedlar gas bag of 5 litres or 10 litres can be fitted to collect the biogas after measuring gas flow. The biogas composition collected in the gasbags can be analysed using GC or a portable gas analyser.

To measure $CH_4$ only you can either use standard caustic soda bottle to remove $CO_2$ and $H_2S$ from the gas, or fill the gas flow meter directly with caustic solution instead of water. The caustic solution scrubs out the carbon dioxide as the gas enters the gas flow meter from the bottom and you will be measuring methane flow instead of biogas flow. We recommend using the Tedlar bag method as it gives flexibility to measure the $CH_4$, $CO_2$, $H_2S$ and $N_2$ content.

### 5) How are the reactors mixed?

All reactors are mixed at exactly the same speed through stainless/silicone paddles driven by a gearbox. One motor for 15 reactors. Stainless steel/silicone paddles deliver consistent mixing to all digesters in each set. Most commercially-available BMP sets do not guarantee equal mixing for all reactors in the set. Some rely on daily manual shaking, shaking surfaces, magnetic stirrers, etc., which is ok for absolute values of BMP but limits the use of kinetic data for rapid evaluation of inhibition or of the effect of feedstock composition on dynamics of biogas production

If needed, the mixing can be controlled by a commercial timer to come on and off at the set timings.

### 6) How is the temperature controlled?

The temperature of operation is controlled by immersion of reactors in a Grants © water bath up to 95ºC with 0.5º C setting resolution. A lid that holds the reactors in place has been designed to minimise the loss of bath water by evaporation, facilitating tests at thermophilic temperatures without the common issues of water evaporation when operating water baths at thermophilic temperature.

### 7) How is the data recorded?

The main data logging software is stored on the Arduino monitor which is integrated at the end of the gas flow meter. *To ensure no reading is lost when tumbles happen at the same time, the Arduino system has a latch shield that locks every occurrence and logs it once other readings clear the screen*.

There are three utilities which run on a laptop that is connected to the Arduino over USB. These utilities are 'startrun' - which is used to transfer basic experimental data to the Arduino at commencement of a run, 'filegrab' - which is used to transfer the log files built up by the Arduino back to the laptop and 'monitor' - which provides a simple monitoring facility. Results are written to a number of .csv log files which can be opened with Excel or similar. These files are held on a microSD card which is part of the Arduino system and can be uploaded to the laptop at any time and as often as required (eg daily) using the filegrab utility.

The gas flow meter automatically logs the final gas yield results in ml/day or ml/g VS and compensates to standard temperature and pressure (STP) automatically in real time. The results can be obtained using the 'Filegrab' utility on the laptop.

### 8) What is the minimum computer configuration to support the Arduino software?

A computer or laptop with Windows 10 with a spare USB port – can be a 32 bit machine or a 64 bit machine – software supports both.

### 9) How is the cleaning of the gas flow meter?

Some users dose algaecide into the gas flow meters. Prepare a solution of bleach and fill the gas flow meter with it leaving it soaking overnight. Remove the lid and brush each cell, blow air into each cell to clean gas inlet then wash with clean water. Then, shake the gas flow meter gently and flush connecting a water hose at a low flow for a few minutes until block looks clear. This will not leave the gas flow meter sparking clean, but it will be clearer. The ultimate wash would be to remove the tumbler buckets and give the machine a thorough clean. For the new model, remove reed switch, open cell and place in a dish washer or wash manually using soap and water. We recommend covering flow meter with dark cloth to stop light

## 7. ROUTINE CHECKS

a. Check the water level in the water bath.
b. Check the water level in the gas flow meter and top up if the water is below the spill point.
c. Check the tumbler buckets are not stuck in the vertical position (it could happen if gas flow exceeds the tumbler capacity).

## 8. TROUBLE SHOOTING

- **No Gas Counting**. Please check for monitoring factors including: ensure the Arduino logger has gone through Set up and Start run. Check that the logger is powered on. Check that the liquid level in the gas flow meter is covering the tumbler buckets. Ensure the buckets rock when you syringe air. If not, they could be too tight or have come off in which case you may need to remover the flow meter lid and reset the bucket. Make one tumble happen and check if it is registered on the screen. If not, check the reed switches passing a magnet over it. If still no recording, use a small wire to contact both sides of the reed switch and check for recording. If a recording happens the switch is broken and needs replacing. If not counting, check that the 25D connector inside the box is not lose. If you get a recording manually manipulating the flow meter, then the possible cause would be a leak in the gas areas of the equipment, such as reactor headspace (check that caps are tight and cap seals are not worn, check cap for leak), check that the liquid level in the reactors is not below the level of the shaft mixer tube as level too low would eliminate the liquid gas seal. Check that none of the fittings on the reactor caps is

leaking by preparing a foam solution, using a syringe pump air into the reactor head and look for signs of leak in the foam applied. Check that there is no blockage in the gas inlet to the gas flow meter by taking off the hose from the reactor and pushing some air, vigorous syringing can clear most such blockages. Be careful in case reactor is pressurised and you could get vigorous spill on removing the hose, cover the reactor with a cloth or pad before you undo the gas connection to contain any possible spill before it happens. **If you are using gas bags**: ensure the valves are open. A shut valve would cause gas to push water out of the cell and counts not to happen.

- **Gas Bags not filling**. Please ensure the valves are open (a shut valve would cause cell water to be emptied by pressure; this would alter calibration values for other cells until rebalanced. Check that gas bags are not leaky. Check that the lid of the flow meter is gas-tight, if necessary apply silicone. Check in the system if the sample has produced gas.
- **Atypical gas flow**. Are you using gas bags? Check all valves are open. Is the inoculum too fresh? Check the VS of sample and inoculum and check values entered in the set up file. Could water bath have been switched off by accident or a power cut affected it? Has the mixer been switched off by accident?
- **Noisy gearbox**. Remove from system; remove motor housing removing the 4 holding screws that fix motor to gearbox. Open and apply a thin veneer of Locktite Super Lube grease, if possible, to rotating areas of the white gears in contact with bushes (on both sides). Assemble again not overtightening screws. Check that solids or fibre in the reactor are not too high causing overload on the motor.

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