



AMPTS II

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AMPTS II Light

Automatic Methane Potential Test System

Operation and Maintenance Manual



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1 PREFACE

BPC Instruments AB

BPC Instruments is a market leader in the area of low gas flow analytical instruments for biotechnology related applications. We invest in innovation and development of smart instruments that allow for more efficient, reliable, and higher quality research and analysis, leading to significant reductions in time and labour. We ensure the highest product quality throughout our portfolio and focus on being service minded and always meeting the needs of our customers.

The company's flagship product, the Automatic Methane Potential Test System (AMPTS), has become the preferred analytical instrument around the world for conducting various anaerobic batch fermentation tests. BPC's product portfolio offers academic and industrial actors working with biogas, animal feed, wastewater, and other fields exciting products for low gas flow measurements, substrate analyses and process simulations.

2 GENERAL INFORMATION

The design and construction of full-scale anaerobic digesters based on substrates from single or multiple sources require a good understanding of the physical and biochemical characteristics of each individual substrate. Biological or biochemical methane potential (BMP) tests, i.e., anaerobic biodegradability assays, are used to determine the anaerobic biodegradability and ultimate methane potential of wastes or biomass, as well as the biodegradation rate under laboratory conditions.

2.1 Conventional Biochemical Methane Potential (BMP) Assay

A conventional BMP test provides a preliminary indication of the biodegradability of a substrate and of its potential to produce methane via anaerobic digestion. Methods based on measuring the gaseous end product (bio-methane) have been developed and reported as standard protocols with a variety of experimental setups. The normal procedure to determine methanogenic activity involves inoculating a number of vials containing a small amount of the target media with anaerobic inoculum, incubating them at a controlled temperature, and periodically checking for the methane production by manual sampling and determination of the volume of gas released, and analyzing the gas composition using gas chromatography (GC). Unfortunately, the above-described procedures not only require expensive laboratory equipment (e.g., GC), but are also very time- and labor-consuming due to the large number of vials usually employed and fairly long incubation periods (e.g., over one month). Moreover, the biogas produced and its methane content can only be measured periodically, making it impossible to obtain satisfactory data to fully understand the degradation dynamics. Furthermore, it is difficult to perform the test in a satisfying way within the normal working routines of a laboratory. Therefore, there is a need for a highly automated methane potential test that allows for the collection of high quality data, that is less labor-intensive, and where no expensive laboratory equipment is needed for performing the test.

AMPTS II has been developed by BPC for on-line measurements of ultra low biogas and bio-methane flows produced from the anaerobic digestion of any biological degradable substrate (both solid and liquid form) at laboratory scale.

From the biogas producers' point of view, understanding the potential of a substrate to produce methane and its dynamic degradation profile has a significant impact on the choice of organic waste/material to digest when producing biogas, as well as provides a better understanding of the quality of the raw gas produced from a biogas production facility. The latter has in turn an impact on the total volume of upgraded bio-methane that can be produced from a commercial biogas site. Thus, understanding the methane potential of a substrate can have a direct bearing on the profitability of a commercial biogas producer, as well as the volume of bio-methane produced.

2.2 Advantages of AMPTS II over Conventional BMP Test

The AMPTS II provides the following advantages:

- Automated analytical procedure, reducing work load
- User friendly interface for experiment setup, real-time data display and analysis overview
- Real-time data logging of accumulated bio-methane volume and flow rate
- Real-time pressure and temperature compensation for gas volume and flow measurements
- Real-time gas flow and volume normalisation
- Algorithm to avoid an overestimation of gas volume and flow (which may be induced by the flush gas, such as nitrogen gas, during experimental setup)
- Possibility of multiplexing, allowing for simultaneous batch analysis at different startup times
- High quality data for extracting process kinetic information
- Standalone instrument with embedded data acquisition and web server for remote access
- Low and easy maintenance
- One of the most cost competitive solutions on the market today
- Supports all anaerobic biodegradability, biochemical methane potential (BMP) and specific methanogenic activity (SMA) test protocols, allowing for the standardisation of measurement procedures, data interpretation and reports.

2.3 Choosing the AMPTS II or the AMPTS II Light

There are mainly two things to consider when choosing between the AMPTS II and the AMPTS II Light: the need for statistics and the type of substrates that will be tested.

With 15 test vessels, the AMPTS II has the capacity for performing large batch tests with triplicates positive controls, and therefore is the recommended choice for research purposes.

The AMPTS II Light with 6 reactors (each 2000 ml) is suitable when mainly homogenous substrates are tested for input to operative decision-making in a plant environment.

3 DELIVERY CHECKS

At delivery, unpack and check that the contents match with what is listed below in the Section “Box Content”.

If the packaging or the equipment is broken at delivery, please:

- a) Document and take photos of the parts and packaging.
- b) Inform the transport company at the time of delivery.
- c) Make sure that the transport company documents the incident.
- d) Inform your local distributor or BPC of the incident.

BOX CONTENT

See also Chapter 7. Equipment Description (Design/Function) for pictures of the included parts.

AMPTS II – THERMOSTATIC WATER BATH (UNIT A)

15 glass bottles (500 ml) as reactors
15 plastic caps with agitators/motors
15 plastic screw caps with hole
15 helical couplings
14 short motor cables
1 long motor cable (from Motor Controller to first motor unit)
1 signal cable (from the Gas Volume Measuring Device to the Motor Controller)
15 plastic stoppers with 2 tubing ports and rotating shaft for mixing
1 thermostatic water bath (18 l)
1 plastic glass lid for the water bath, with 15 circular openings for reactors and 15 evaporation minimising rings
15 plastic tubing clamps

AMPTS II – CO₂-ABSORPTION TRAY (UNIT B)

1 bottle holder for 15 glass bottles
15 glass bottles, 100 ml
15 plastic screw caps with hole
15 plastic stoppers with 2 tubing ports

AMPTS II – GAS VOLUME MEASURING DEVICE (UNIT C)

1 water bath package (including water tank, flow cell holder, 15 injection mould flow cells containing magnetic metal pieces, base and protection plate)
1 plastic glass lid for the water tank (including straight connector)
1 manual plastic water pump
1 power adapter (input 100-240 V ~ 50/60 Hz, output 12 V DC / 5 A)

AMPTS II - OTHER COMPONENTS

- 1 shielded Ethernet cord
- 1 Motor Controller
- 2 boxes of 15 m flexible Tygon® tubing
- 1 number markers kit for tubes
- 30 plastic lids (blue)
- 1 Allen key (for helical couplings)
- 1 manual

AMPTS II - OPTIONAL EQUIPMENT

- 8 glass bottles, 1 l (bottles with one port designed for continuous pH monitoring during the batch fermentation) and 8 stirrers adjusted for 1 l bottles
- 6 glass bottles, 2 l (bottles with one port designed for continuous pH monitoring during the batch fermentation) and 6 stirrers adjusted for 2 l bottles
- 1 plastic glass lid for the water bath, with six or eight circular openings for reactors
- Gas sampling units

AMPTS II LIGHT - THERMOSTATIC WATER BATH (UNIT A)

- 6 glass bottles (2000 ml) as reactors
- 6 plastic caps with agitators/motors
- 5 short motor cables
- 6 plastic screw caps with hole
- 1 long motor cable (from Motor Controller to first motor unit)
- 1 signal cable (from the Gas Volume Measuring Device to the Motor Controller)
- 6 plastic stoppers with 2 tubing ports and rotating shaft for mixing
- 1 thermostatic water bath (the models can differ, therefore the picture does not necessary conform to the reality)
- 1 plastic glass lid for the water bath, with 6 circular openings for reactors
- 6 helical couplings
- 6 plastic tubing clamps

AMPTS II LIGHT - CO₂-ABSORPTION TRAY (UNIT B)

- 1 bottle holder for 6 glass bottles
- 6 glass bottles, 500 ml
- 6 plastic screw caps with hole
- 6 plastic stoppers with 2 tubing ports

AMPTS II LIGHT - GAS VOLUME MEASURING DEVICE (UNIT C)

- 1 measuring unit (including water tank, flow cell holder, 6 injection mould flow cells)
- 1 plastic glass lid for the water tank
- 1 manual plastic water pump

1 power adapter (input 100-240 V ~ 50/60 Hz, output 12 V DC / 5 A)

AMPTS II LIGHT - OTHER COMPONENTS

1 shielded Ethernet cord
1 Motor Controller
1 box of 15 m flexible Tygon® tubing
1 number markers kit for tubes
6 plastic lids (blue)
1 Allen key (for helical couplings)
1 manual

AMPTS II LIGHT - OPTIONAL EQUIPMENT

6 glass bottles, 1 l (bottles with one port designed for continuous pH monitoring during the batch fermentation) and 6 stirrers adjusted for 1 l bottles
6 glass bottles, 2 l (bottles with one port designed for continuous pH monitoring during the batch fermentation) and 6 stirrers adjusted for 2 l bottles
Gas sampling units
6 glass bottles, 500 ml for removing CO₂
6 plastic screw caps with hole
6 plastic stoppers with 2 tubing ports

4 PRE-COMMISSIONING

The following items are not included in the delivered AMPTS II, however they will / may be required to operate the AMPTS II:

- Thermometer for temperature indication in the thermostatic water bath
- N₂ or a mixture of N₂/CO₂ to obtain anaerobic conditions during the sample preparation phase (BPC recommends a mixture of N₂ (60%) and CO₂ (40%) to be used)
- NaOH (reagent grade 97%, pellets, e.g. Sigma-Aldrich 221465 or equivalent quality) for CO₂ absorption
- pH indicator: Thymolphthalein (2',2''-Dimethyl-5,5''-di-iso-propylphenolphthalein, C₂₈H₃₀O₄, CAS 125-20-2, ACS reagent, dye content 95%, e.g. Sigma-Aldrich 114553 or equivalent quality)
- Ethanol (ACS reagent 99.5%, e.g. Sigma-Aldrich 459844, or equivalent quality)
- Additional wall socket adapters (plugs/contacts) (the ones supplied are according to European, US or UK standards, depending on original purchase order)
- If the customer has a GC and is interested in off-line biogas composition analysis, gas-sampling units can be ordered separately from BPC.



Alternatively, reactors can be connected without / with NaOH bottles to obtain the total gas / methane production. In order to measure the total biogas, the flow rate of the produced gas should be higher than 2 l/day for minimising the impact of CO₂ solubility in the aqueous solution (e.g., in a continuous mode), otherwise the AMPTS II setup must be modified.

5 QUALITY RULES AND RECOMMENDATIONS

- The product guarantee provided corresponds to the guarantee stipulated on the confirmed product order form and shipping documentation. The removing of the bottom plate of the Gas Volume Measuring Device is considered as a breach of guarantee.
- For warranty claims relating to the thermostatic water bath, contact the manufacturer directly (see separate manual from Grant). Inform Bioprocess Control as well.
- BPC recommends using a mixed oxygen free gas (ideally 60% N₂ and 40% CO₂), to remove any remaining air in the headspace of the bottles to create anaerobic conditions.
- When 500 ml bottles are used as reactors, the recommended headspace in the bottles during the methane potential test analysis is between 200 and 300 ml.
- When 2000 ml bottles are used as reactors, the recommended headspace in the bottles during the methane potential test analysis is between 300 and 400 ml.
- For a high accuracy analysis, AMPTS II should not be exposed to mechanical vibrations and/or high frequency radio transmissions.
- Only the parts delivered with the product can be used in the system in order to guarantee the quality and performance of the product.
- The system contains batteries and need to be handled accordingly.
- BPC reserves the right to correct any possible text and image errors as well as changes to technical data in this manual.

BEFORE GETTING STARTED

- Read this manual and additional separate manuals for the individual instruments before installing and using the equipment.
- Keep the instruction manual for future reference and make sure it is easily available for people who regularly use the AMPTS II.
- Read the Bioreactor Agitation Systems instruction manual before using the equipment.

6 SAFETY CONSIDERATIONS

When handling NaOH, always use glasses, a lab coat and plastic gloves. Using the AMPTS II and the AMPTS II Light and the handling of NaOH should always be done in an environment with good ventilation. See the safety data sheet for your chemicals for further information. Always be cautious when handling electrical devices close to water.

The Agitation System contains rotating parts. Make sure to tie back any loose hanging objects like clothing or hair when using the instrument.

The power adapter for the multifunction brushless DC Agitation System must never be used in the AMPTS II or the AMPTS II Light.

7 EQUIPMENT DESCRIPTION (DESIGN/FUNCTION)

The AMPTS II follows the same measuring principles as conventional methane potential tests (see Appendix A), which make the analysis results fully comparable with standard methods. However, with the AMPTS II, the analysis and data recording are fully automatic during the long incubating period, which significantly reduces the time- and labor-demand for carrying out the analysis. Moreover, a high quality of data can be obtained, which can be used to extract kinetic information of the degradation process. This will in turn allow for a much better understanding of the degradation dynamic behavior of a specific biomass substrate, further leading to an improved process operation.

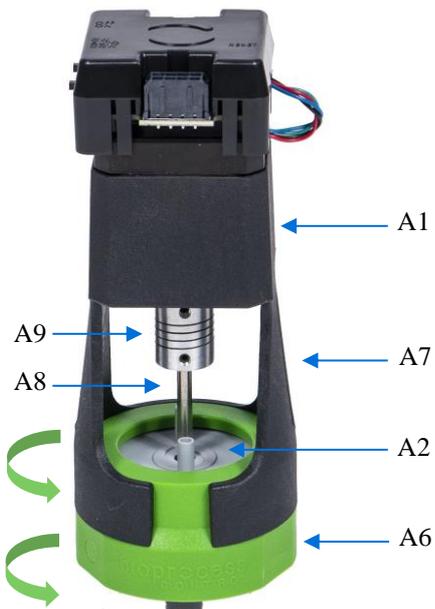
The instrument setup can be divided into three units: A, B, and C (Please refer to the included photos, which show the AMPTS II, but will be applicable to the AMPTS II Light as well. The only visible difference is that the number of test channels differs between 15 for the AMPTS II and 6 for the AMPTS II Light.)

In the *Sample Incubation Unit* (unit A), up to 6 or 15 vials containing small amounts of a sample with anaerobic inoculum are incubated at a desired temperature. The media in each vial is mixed by a slow rotating agitator. Biogas is then continuously produced, a parameter which is used to estimate the biomethanation activity inside each vial.

In the *CO₂-absorbing Unit* (unit B), the biogas produced in each vial passes through an individual vial containing an alkaline solution. Several acid gas fractions, such CO₂ and H₂S, are retained by chemical interaction with NaOH, only allowing CH₄ to pass through to the biomethane Gas Volume Measuring Device. A pH indicator is added into each vial for controlling the acid binding capacity of the solution.

In the *Gas Volume Measuring Device* (unit C), the volume of CH₄ gas released from unit B is measured using a wet gas flow measuring device with a multi-flow cell arrangement (6 or 15 cells). This measuring device works according to the principle of liquid displacement & buoyancy and can monitor ultra low gas flows; a digital pulse is generated when a defined volume of gas flows through the device. An integrated embedded data acquisition system is used to record, display and analyze the results.

7.1 Sample Incubation Unit



Unit A

A1 Multifunction Brushless DC motor

A2 Stopper with 2 tubing ports and rotating shaft for mixing

A3 Glass bottle 500 ml as reactor

A4 Plastic lid and evaporation minimising rings for thermostatic water bath¹

A5 Thermostatic Water Bath

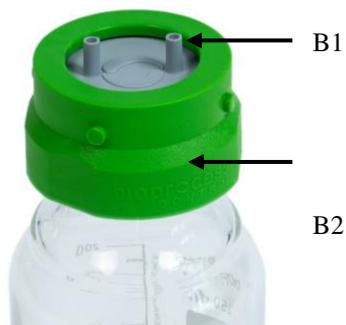
A6 Plastic screw cap with hole

A7 Motor holder

A8 Bent stir rod/rotating shaft

A9 Helical coupling

7.2 CO₂-Absorption Unit



Unit B

B1 Stopper

B2 Plastic screw cap with hole

B3 Glass bottle 100 ml for the AMPTS II (500 ml for the AMPTS II Light)

B4 Bottle holder

¹ Evaporation minimising rings only available for AMPTS II and not for AMPTS II Light.

7.3 Gas Volume Measuring Device





Unit C

- C1 Flow cell (15 on the AMPTS II and 6 on the AMPTS II Light)
- C2 Water bath package (including water tank, flow cell holder, protection plate, electronics)
- C3 Manual plastic water pump
- C4 Connection block (15 connections on the AMPTS II and 6 connections on the AMPTS II Light)
- C5 Motor signal connection socket for the Motor Controller: 12 V DC / 3 A
- C6 Power connection socket for the power adapter: 12 V DC / 5 A
- C7 Shielded Ethernet socket
- C8 Power adapter: 12 V DC / 5 A
- C9 Reset button for fixed IP
- C10 Reset button for DHCP
- C11 Indicator diodes
- C12 Serial number

7.4 Bioreactor Agitation System

The agitation system is a unique design of brushless DC stepper motors. For a full description of the system, please refer to the Bioreactor Agitation Systems manual. The system features:

- Reversal of motor direction (clockwise [CW]/counter-clockwise [CCW])
- Timer function to set time periods for the motor reversal
- Remote speed control on a broad range (i.e. 5-100%)

The complete system consists of the components shown in the picture below: Motor Controller, 24 V power adapter for the Motor Controller, brushless stepper DC motor units with plastic caps, helical couplings, short motor cables (0.2 m), long motor cable (1.5 m), signal cable and a hex tool.

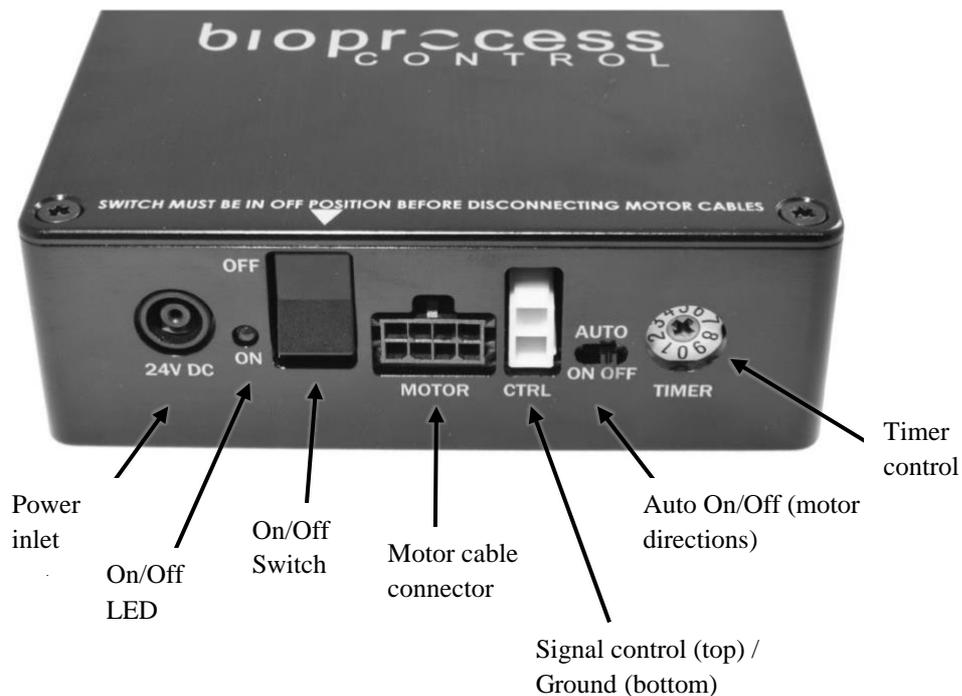


MOTOR CONTROLLER

The Motor Controller provides power to the motors via the motor cables, and **it is very important that the Motor Controller always is turned off and is unplugged from the power source when any cables are connected or disconnected from the motors.** Turning the switch off is not enough.

The Motor Controller is the hub of the agitation system. It interprets the speed signal sent from the Gas Volume Measuring Device of the AMPTS II system and controls the direction of the motors. All the mixers receive the same information from the Motor Controller.

The picture below shows the control panel on the front side of the Motor Controller.



The **ON/OFF switch** (shown in the picture above) turns the power of the Motor Controller on and off. When the switch is ON (in the upper position), the red LED will be lit up to indicate that the system is active. It is recommended to set the switch to OFF before connecting / disconnecting the Motor Controller to / from the power mains.

When the **AUTO switch** on Motor Controller is set to OFF, the mixers will be operated in continuous rotation mode, i.e. the mixers will always rotate in the same direction. Setting the switch to ON will activate the AUTO reversing mode, which will make the Motor Controller change the mixer directions at regular intervals.

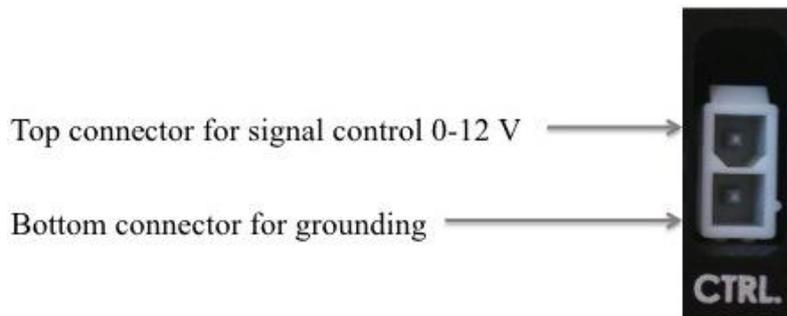
For the Auto reversing mode to work as intended, the DIRECTION switch on each motor needs to be in the AUTO (middle) position as well. If the switch is in either CW or CCW position, the mixer will start and stop but never change direction. See section Direction Switch for further explanation of the different combinations of settings on the Motor Controller and the motor units.

SIGNAL CABLE

The Motor Controller receives signals from the test system through an analogue signal cable (pictured below).



WARNING: The position of the signal and grounding terminals of the connectors are reversed on the Motor Controller compared to the AMPTS II system. On the Motor Controller, the ground is at the bottom terminal (see the picture below). Always make sure the proper cable is used and that it is inserted the correct way, or the system could be permanently damaged!



MOTOR CABLES

The signals from the Motor Controller are distributed to each motor unit through the motor cables (see picture below). They should be connected serially, and are fastened to the motor units with the help of latches. The 1.5 m cable is used only to connect the “MOTOR” output on the Motor Controller to the first motor.



MIXING INTENSITY

The motors can be operated at different speeds ranging from 10-200 RPM. The speed is adjusted linearly between 10 and 200 RPM, referred to as per cent (5 – 100%) in the AMPTS II software.

At a DC-signal of 0 (zero) V, the motor is out of function, and at 12 V it is rotating at top speed (200 RPM). An acceleration /deceleration ramp is built into the system to provide a smooth transition between different speeds.

TIMER TO CONTROL INTERVALS OF MOTOR REVERSAL

The rotary Timer switch can be used to set the time that should elapse before the direction is changed. 10 positions are available ranging between 5 s to 1 h.

Timer Switch Position	Time
0	5 s
1	15 s
2	30 s
3	45 s
4	60 s (1 min)
5	120 s (2 min)
6	300 s (5 min)
7	600 s (10 min)
8	1800 s (30 min)
9	3600 s (60 min)

FUSE

To protect the Motor Controller, the Printed Circuit Board (PCB) is fitted with a slow-blow 2 A fuse (522.520). If none of the mixers are working even though the power supply is connected, this fuse should be replaced. The fuse is located inside the Motor Controller, which can be opened by removing the four screws placed in each corner of the top cover. (See picture below).



MOTOR UNIT

Each motor unit has its own driver board, which controls the power distribution to the actual motor. The switches are explained below.



MOTOR UNIT ON/OFF SWITCH

The ON/OFF switch is located on the side of the motor and marked on the very top of the motor unit, just above where the actual switch is located. When a mixer is active and power is supplied, an LED next to the ON/OFF switch will be green. If not all mixers will be used, setting the ON/OFF switch to OFF can turn each motor off individually. The motor unit will make a sound to indicate when this is done. It is recommended to set the switch on each Motor Unit to the OFF position before connecting or disconnecting the Motor Controller or any of the motor cables.

When the system has been turned off, and then switched back on, it can take up to 8 seconds for it to start up again.

DIRECTION SWITCH

The DIRECTION switch is located on the side of the motor, and marked on the very top of the motor unit, just above where the actual switch is located. It is used to set the motor to rotate in a CW or a CCW direction. Setting the switch to AUTO gives the Motor Controller automatic control over the reversal of the direction. Below is a table that will show the output of the different combinations of switch settings on the Motor Controller and the motor unit.

Settings		Mixer output	
Motor Controller AUTO switch	Motor unit switch	Intervals	Rotation
ON	CW	Starting and stopping according to Motor Controller intervals	Constantly CW
ON	AUTO	Starting and stopping according to Motor Controller intervals	Reversing directions
ON	CCW	Starting and stopping according to Motor Controller intervals	Constantly CCW

OFF	CW	N/A	Constantly CW
OFF	AUTO	N/A	The latest direction used before the Motor Controller AUTO switch was set to off
OFF	CCW	N/A	Constantly CCW

RESET BUTTON

If an error is encountered somewhere on the driver board in the motor unit, a red LED will light up on the side of the motor unit, opposite to where the switches are located. A marking on the top of the motor unit shows where to find it (see picture below).

The problems could be e.g. high temperature, a disconnected motor cable, or power loss. If this happens, the mixer can be reset with a quick press on the RESET button. The RESET button is marked on the very top of the motor unit, just above the actual button. It can be reached by using a pen or other pointy object. If the mixer was reset successfully, the red LED will turn off.

POWER

The Motor Controller is powered by a 24 V / DC 2.71 A power supply adapter. The power supply is then distributed serially from the Motor Controller, through the 8-pin signal cables, to each motor unit.

WARNING: The power supply always has to be disconnected from the Motor Controller before removing or connecting any of the cables from the motors.



7.5 Technical Characteristics

GENERAL

CE certification: this product meets the requirements of applicable CE-directives. A copy of the corresponding Declaration of Conformity is available on request.

Usage: indoor in an environment free of corrosive agents and with normal humidity level
Power supply: 100 or 240 V ~ 50/60 Hz (Thermostatic Water Bath and power adapter)
Tubing material: Tygon® (ID 3.2 mm, OD 6.4 mm)
AMPTS II weight: approx. 30 kg
AMPTS II Light weight: approx. 30 kg

AMPTS II – SAMPLE INCUBATION UNIT

Maximum number of reactors per system: 15
Reactor material: glass
Standard reactor volume: 500 ml
Power consumption: 1300 W (maximum)
Dimension: 50×30×15 cm
Temperature control: up to 100 °C (precision 0.2 °C)
Mixing in the reactor: mechanical (adjustable interval and speed), maximum speed 140 rpm

AMPTS II – CO₂-ABSORPTION UNIT

CO₂ absorption bottles: 15
Volume of CO₂ absorption bottles: 100 ml
Dimension of unit: 44×30×6 cm
Absorption liquid: 3 M NaOH with pH indicator, 80 ml per bottle (not included)
Absorption efficiency: > 98%

AMPTS II – GAS VOLUME MEASURING DEVICE

Working principle: liquid displacement & buoyancy
Up to 15 cells running in parallel
Built-in pressure and temperature sensors
Integrated data acquisition (maximum capacity 2×10^4 flow cell openings)²
Web based software running on embedded server
Measuring resolution: 10 ml
Detection capacity: up to 13 l cumulative gas per channel for each batch test
Measuring range for instant gas flow rate: 10 to 120 ml per min³
Power consumption: 15 W (average), 28 W (maximum)
Dimension: 51×44×18 cm
Precision: 1% (CV)

AMPTS II LIGHT – SAMPLE INCUBATION UNIT

Maximum number of reactors per system: 6
Reactor material: glass
Standard reactor volume: 2000 ml

² See the “Data Reset” section in chapter 9 on how to proceed if this limitation is exceeded.

³ Unit calibration is not performed before delivery; the flow cell volumes represent the averages for 100 calibrated systems.

Power consumption: 1300 W (maximum)

Dimension: 53×33×24 cm

Temperature control: up to 95 °C (precision 0.2 °C)

Mixing in the reactor: mechanical (adjustable interval and speed), maximum speed 140 rpm

AMPTS II LIGHT – CO₂-ABSORPTION UNIT

CO₂ absorption bottles: 6

Volume of CO₂ absorption bottles: 500 ml

Dimension of unit: 37,7×28,3×5,6 cm

Absorption liquid: 3 M NaOH with pH indicator, 400 ml per bottle (not included)

Absorption efficiency: > 98%

AMPTS II LIGHT – GAS VOLUME MEASURING DEVICE

Working principle: liquid displacement & buoyancy

Up to 6 cells running in parallel

Built-in pressure and temperature sensors

Embedded data acquisition (maximum capacity 2×10⁴ flow cell openings)⁴

Web based software running on embedded server

Detection capacity: up to 33 l cumulative gas per channel for each batch test

Measuring range for instant gas flow rate: 10 to 120 ml per min

Measuring resolution: 10 ml

Power consumption: 15 W (average), 28 W (maximum)

Dimension: 55×37×31 cm

Housing: plastic

Precision: 1% (CV)

⁴ See the “Data Reset” section in chapter 9 on how to proceed if this limitation is exceeded.

8 OPERATION

In this section, references are given to pictures in the Chapter 7. Equipment Description (Design/Function) in the form of a letter and number within parenthesis e.g. (A1).

See also the user manual for the Thermostatic Water Bath before installation and use (Operating instructions, Bioprocess Control VWX).

8.1 Before Start up

CO₂-ABSORPTION UNIT

Preparation of NaOH solution for CO₂-absorption: Be careful and use the necessary protection. All work should be carried out inside a fume hood while wearing protective equipment (see Chapter 6. Safety Considerations).

For the AMPTS II, at least 1200 ml is needed of the NaOH solution with pH indicator in order to fill 15 bottles with 80 ml each.

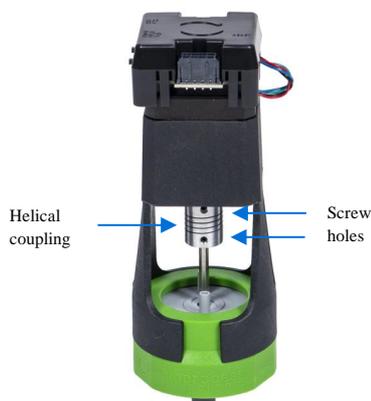
For the AMPTS II Light, at least 2400 ml is needed of the NaOH solution with pH indicator in order to fill 6 bottles with 400 ml each.

- a) Prepare a 3 M NaOH solution. Be careful and follow safety precautions, as this is a highly alkaline solution. After weighing the necessary amount of NaOH, mix it with approximately $\frac{3}{4}$ of the required total volume of distilled water (e.g. 120 g NaOH in $\frac{3}{4}$ of 1 l water). The heat generation following dissolution of NaOH in water is high, so that adding small amounts of supplementary water followed by mixing is recommended. When the NaOH is completely dissolved, add the whole amount of remaining water and mix well.
- b) Prepare a 0.4 % Thymolphthalein pH-indicator solution (40 mg in 9 ml ethanol 99.5% followed by addition of 1 ml water). Thymolphthalein is insoluble in water, but it is freely soluble in ethanol.
- c) Prepare the NaOH solution containing the pH indicator, by mixing 5 ml of the 0.4 % Thymolphthalein solution per 1 l 3M NaOH solution. It is recommended to prepare enough of the NaOH solution with pH indicator to fill up at least three replacement bottles for a quick change if the absorption solution becomes impaired (the color change from blue to colorless due to the pH-indicator Thymolphthalein).
- d) If grey plastic stoppers are used, make sure that there is no plastic ring attached to the rim of the bottle. (However, if blue rubber stoppers are used, the plastic ring should be kept on the bottle.) Add approximately 80 ml of the mixture containing NaOH solution and Thymolphthalein pH indicator into each of the 15 glass bottles (100 ml) for the AMPTS II. The 500 ml bottles for the AMPTS II Light should be filled with approximately 400 ml each (B3).

- e) Place a plastic stopper (with two tubes (B1)) in the opening of each bottle (B3), seal by placing the plastic lid (B2) on the top, and screw until the thread on the bottle is no longer visible and the lid is properly sealed.
- f) If the CO₂-absorption bottles will be stored before use, they must be protected from the atmosphere. Cut 6 or 15 pieces of Tygon® tubing to a length of 5 cm each. Bend them into U-shapes and put their ends over the tubes on the bottle tops.

REACTORS

- a) If grey plastic stoppers are used, make sure that there is no plastic attached to the rim of the reactor. (However, if blue rubber stoppers are used, the plastic ring should be kept on the reactor.)
 - For the AMTPS II: Add the sample (e.g. 400 g of mixture between substrate and inoculum), the blank (e.g. 400 g inoculums) and the positive control (e.g. 400 g of mixture between cellulose and inoculum) into the reactors. Use triplicates for statistical significance (see Appendix A for more details).
 - For the AMTPS II Light: Add the sample (e.g. 2000 g of mixture between substrate and inoculum), the blank (e.g. 2000 g inoculums) and the positive control (e.g. 2000 g of mixture between cellulose and inoculum) into the reactors. Use duplicates for statistical significance (see Appendix A for more details).



- Insert the stirrer into the bottle and press down on the motor holder and plastic stopper. Screw until the thread on the bottle is no longer visible. The lid must be properly sealed to ensure that the equipment does not risk being damaged. (See picture to the far right above for the position of the cap on the reactor bottle.)
- The bent stir rod can be fastened to the motor by attaching a helical coupling to it by using an Allen key to fasten the screws in the coupling. **Make sure the metal rod is not pushed too far down and drills a hole in the bottom of the stirrer.**
- Cut 6 or 15 pieces of Tygon® tubing sufficiently in length to connect one of the small plastic tubes on top of the lid of each reactor to one tube on the lid of the corresponding bottle for CO₂ absorption (B3).
- Cut 6 or 15 pieces of Tygon® tubing at lengths of about 10 cm and connect them to

each of the free small plastic tubes on top of the lids of the reactors. Close these tubing pieces with the help of plastic tubing clamps.

- Perform a simple leakage test for each reactor by creating some overpressure by blocking one of the plastic tubing ports and injecting air from the remaining tubing port, and then immerse the reactor in water to check if there is any air bubble escaping from the reactor. Make sure that all tubing is properly connected and there is no potential leakage anywhere.

THERMOSTATIC WATER BATH

Do not install or use the equipment before reading the separate instructions for the thermostatic water bath.

- a) Remove the protection film from the plastic glass lid with 15 circular openings (A4) before using it. Place it on top of the water bath, in order to minimise the evaporation of water during the experiment. After inserting all the reactors into the thermostatic water bath (A5) fill it up with enough water to completely cover the equivalent height of the content in the reactors (ca 8 l). If normal tap water is used, calcareous deposits may appear in the bath and on the heating element. It is therefore recommended to use deionised or distilled water. (It is also possible to use silicone oil (e.g., LABOTHERMOL S from NeoLab) as heat exchanging fluid if water evaporation is expected to be an issue in the experiment.)
- b) Place the evaporation minimising rings over the reactor bottlenecks and press gently so they close the gap between the plastic lid and the reactors.

MOTOR CONNECTIONS

- a) Make sure that the power adapter for the Motor Controller is disconnected from the power supply.
- b) The motor units should all have the ON/OFF switch set to the OFF mode.
- c) Connect one short motor cable to each motor (excluding the last motor in the chain) and then connect the free end of the cable from motor 1 to the free port on motor 2 and so on until the last motor.
- d) The Motor Controller should have the System ON/OFF switch in the OFF mode.
- e) Connect the long motor cable from motor 1 to the Motor Controller.
- f) Connect the signal cable from the Motor Controller to the motor signal port on the Gas Volume Measuring Device.

GAS VOLUME MEASURING DEVICE

- a) Install the Gas Volume Measuring Device on a flat and stable surface. Use appropriate instrumentation (i.e., bubble level device) to verify the horizontality of the surface and to adjust the level of the system placed on the surface.

- b) Fill up the water bath (C2) with deionised or distilled water. The water level should be within the limits of the marking on the water bath.
- c) Cut pieces of Tygon® tubing sufficient in length to couple the second connection on the lid of the CO₂ absorption bottles (B3) to the corresponding tubing connector on the connection block (C4) located on the back of the Gas Volume Measuring Device. (If the AMPTS II is used, cut 15 pieces, and if the AMPTS II Light is used, cut 6 pieces.)
- d) Make sure to remove the protective film from the transparent lid of the water tank.

NETWORK CONNECTION

- a) Connect one end of the Ethernet cord to the Gas Volume Measuring device.
- b) Connect the other end of the Ethernet cord to a computer or to an internal network.
- c) Make sure the computer is not connected to a wireless network. If possible temporarily completely disable the wireless capability.
- d) Make sure that the network cable is connected to the network prior to connecting the power supply.

8.2 Start up

FLUSHING THE REACTORS

- a) Before flushing the system with gas to create anaerobic conditions, disconnect the Tygon® tube from the CO₂-absorption bottles or the Gas Volume Measuring Device.
- b) Connect the gas source to the Tygon® tubing with the red tube clamp.
- c) Open the red tube clamp.
- d) Flush the reactors with a low gas flow (i.e., 2.5 to 10 l/min) gently for 30 - 120 seconds.
- e) Stop the flush gas and close the red tube clamp.
- f) Disconnect the flush gas source.
- g) Re-connect the Tygon® tubing to the CO₂-absorption bottles or to the Gas Volume Measuring Device.

Repeat the procedure for all the reactors used in the test, as there is no way to flush them all at once.

Important! Before flushing the system, please, disconnect the Tygon® tube from the Gas Volume Measuring Device in order to eliminate the risk of damaging its built-in check valves by the high-pressure gas flow.

After flushing the system and before starting the data logging, please open manually all the flow cells in the gas measuring device in order to release any remaining flush gas trapped in the flow cell chambers.

THERMOSTATIC WATER BATH

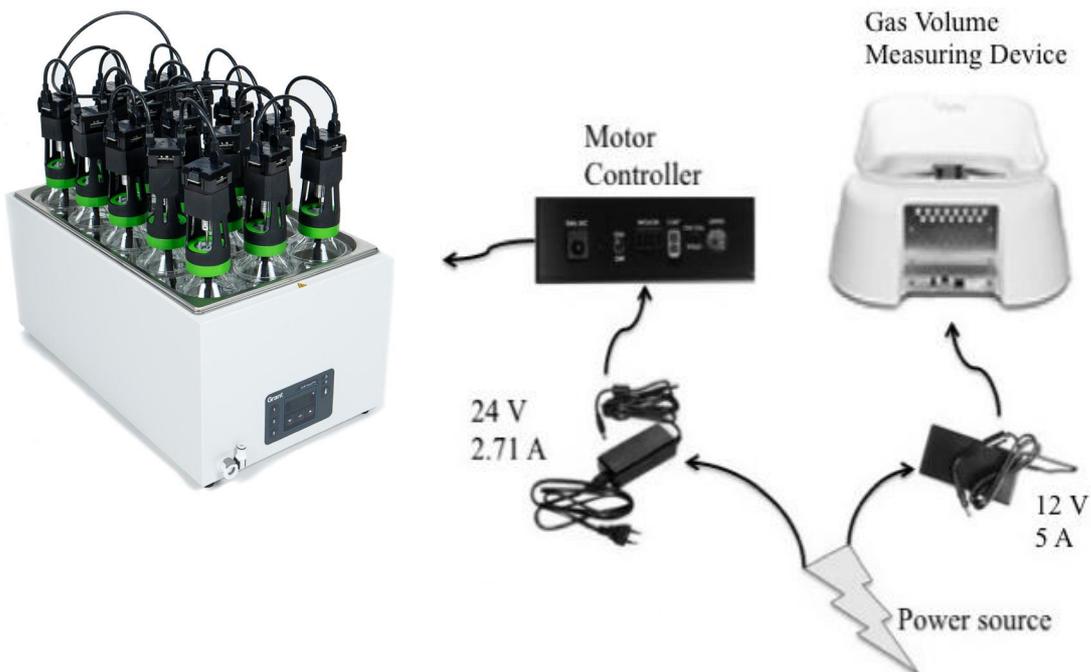
- a) Start up the Thermostatic Water Bath using the main switch. The green LED indicator is illuminated.
- b) Set the operating temperature if it is different from the value set before the last switch-off.

MOTOR AND POWER CABLES

- a) Check that all motor cables are connected to each other, and that the long motor cable is connected between the first motor and the Motor Controller.
- b) Check that the analogue signal cable is connected between the Motor Controller and the Gas Volume Measuring Device.
- c) Connect the **12 V** power adapter to the Gas Volume Measuring Device (C6), and to a 100-240 V 50/60 Hz standard power socket. After this, connect the **24 V** power adapter to the Motor Controller and to a 100-240 V 50/60 Hz standard power socket.

IMPORTANT: The power adapters for the Motor Controller and the Gas Volume Measuring Device of AMPTS II have different characteristics. Make sure the right ones are used, or the system could be permanently damaged!

- d) Set the System switch on the Motor Controller to ON.
- e) Set the ON/OFF switches on each motor unit to ON.



SOFTWARE

- a) Make sure that the shielded Ethernet cord is connected to the Gas Volume Measuring Device and a computer or a local internal network. Make sure that the network cable is connected prior to connecting the power supply.
- b) The AMPTS II software for embedded data acquisition and the built in web server will be loaded and started up automatically after powering up the Gas Volume Measuring Device.
- c) Follow the instructions in Chapter 9. AMPTS II Software step by step for accessing the software interface through an Internet browser, such as Google Chrome, Firefox or the latest version of Internet Explorer. Using Google Chrome is always recommended.
- d) When software and experiment configuration have been completed, start the data logging function of the AMPTS II software. In the Control menu, activate all the cells for data registration. Open each cell in the gas volume measuring device manually several times in numerical order (from 1-15 on AMPTS II and 1-6 on AMPTS II Light), and repeat in total 3 times. Follow the corresponding result of each opening on the plots in the Graph menu of the software to make sure that both the detection system and data acquisition system function properly.
- e) Check the files obtained when generating a report in the Download report menu. Download these files and open them using Microsoft Excel 2007 (or a later version). If prompted by the software to install Adobe Flash, please do so.
- f) Re-start the AMPTS II software by pressing the “End experiment” and “Start experiment” buttons for each individual cell.

8.3 Monitoring

REACTORS

- a) If possible, perform a GC analysis to determine the fraction of methane in the raw biogas samples or simply estimate the methane content based on any literature study or previous experimental result for a similar substrate sample.
- b) Periodically check that the hard tubing pieces that connect the bent stir rod to the motor are undamaged and the mixing works properly. Otherwise check the couplings and adjust them if needed by fastening the screws.
- c) Check twice a week that the water level in the Thermostatic Water Bath reaches up to the plastic lid, and fill it up if needed. If the water level is dropping too much, the temperature may change enough to effect the gas production in the reactors in unwanted ways. It is possible to eliminate the need for water level checks if silicone oil (e.g., LABOTHERMOL S from NeoLab) is used as heat exchanging fluid instead of water.
- d) Make sure that the Tygon[®] tubing is not sharply bent, interfering with the gas flow, and that it doesn't take damage by the rotating parts of the stirrer / motor.

THERMOSTATIC WATER BATH

- a) Periodically check the water level in the thermostatic water bath.
- b) Fill up with additional deionised or distilled water when necessary.

GAS VOLUME MEASURING DEVICE

- a) Check that the water level in the water bath for the gas flow and the Gas Volume Measuring Device is within the recommended range.
- b) Fill up with additional deionised or distilled water to the recommended water level when necessary.

CO₂-ABSORPTION UNIT

- a) The pH indicator Thymolphthalein will turn from blue to colourless when the CO₂ binding capacity of the NaOH solution decreases below optimal. At this point, replacement of the bottle with NaOH solution is recommended, for avoiding the CO₂ gas to pass to the Gas Volume Measuring Device. At 22 °C, about 2.9 l CO₂ can theoretically be captured in each bottle before it needs to be changed.
- b) To change a NaOH solution bottle, place the tubing clamps on the tubing on each side of the bottle and then disconnect the bottle by gently pulling the Tygon® tubings. Replace the old NaOH solution with a fresh one and open/take off the clamps.

SOFTWARE

See the Chapter 9. AMPTS II Software for information regarding software function and operation.

8.4 End of Operation

- a) Note the total running time if this is of interest. In the saved data files, the last data collected will be from the last opening of a cell.
- b) Generate a report in the Download report menu. Download the report and open it to make sure that the report has been generated properly and that no errors occurred while downloading the file.
- c) Stop the logging by pressing the pause button and then the stop button. Note: When pressing the stop button, the experiment associated with that particular cell can no longer be continued.
- d) Turn off the Thermostatic Water Bath.
- e) Set the ON/OFF switch on each motor unit to OFF.
- f) Set the System switch on the Motor Controller to OFF.
- g) Unplug the power adapters (for the Motor Controller and the Gas Volume Measuring Device) from the power source.
- h) Disconnect all tubing connections between the reactors and the NaOH bottles.

- i) Disconnect all tubing connections between the NaOH bottles and the Gas Volume Measuring Device.
- j) Empty the water from the water bath by using the manual plastic water pump only. Do not pour out water from water bath in any other way. Directly pouring out the water without using the water pump might allow water to flow into the space between the water tank and the stand, as well as flow into the stand. There is a potential risk of electronic damage even if only very limited amounts of water are accidentally in contact with the electronic circuit boards inside the stand.

DATA HANDLING

When a report is generated, save the report in a desired folder on the local computer used to visualize the software interface.

See also Appendix and Chapter 9. AMPTS II Software for details on the data handling.

The report files are of XML format (with the ending “.xml”), can be generated anytime during the experiment, and can be used for further data analysis and curve plotting in Microsoft Excel or other software capable of reading this file format. The name of data file is automatically generated, consisting of the actual date and time. For example, a file saved on 1st of April 2011 at 12:30 will get the name “report_2011-04-01_1230.xml” or “report_2011-04-01_1230.csv”. The user will have the possibility to generate a report with or without elimination of the headspace overestimation (that is a compensation for the volume of gas initially used for flushing the system).

The following data are stored in the files:

- a) Time (hours, days) since the start of the experiment
- b) The total gas volume in normalised liter (Nl) (total accumulated gas volume during the experiment)
- c) Average gas flow (Nml/h or Nml/day)
- d) Experimental parameters such as CO₂ concentration (%) of flush gas, process temperature (°C), mixer on/off times (s), mixer speed adjustment (%)
- e) Amount of substrate and inoculum VS (g), volume of the headspace (ml).

The generated file contains the volume of gas already normalised (1.0 standard atmospheric pressure, 0°C and zero moisture content). The calculations are carried out to compensate for pressure, temperature and saturated moisture content at the above given conditions (based on the values of pressure and temperature registered by the sensors from the Gas Volume Measuring Device).

9 AMPTS II SOFTWARE

9.1 First Time Connecting and Setting up the Network

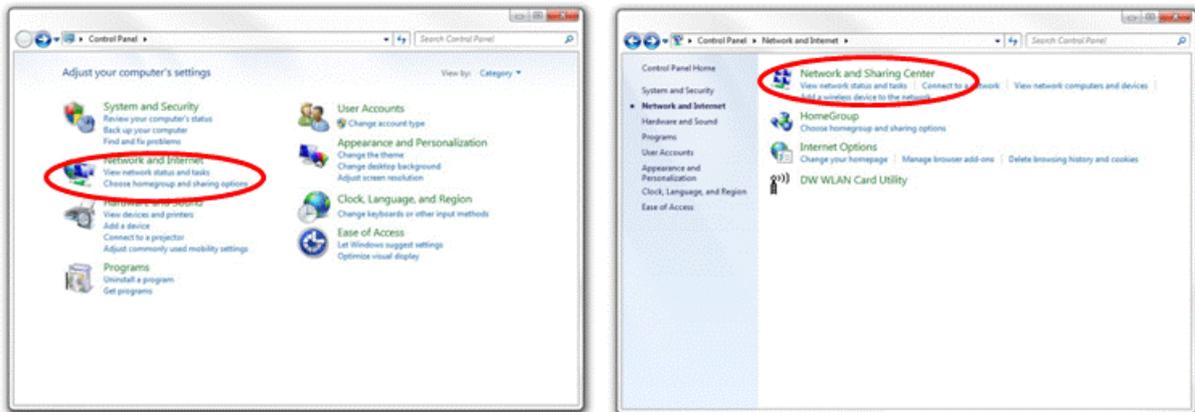
Important! Make sure the computer is not connected to a wireless network. If possible, temporarily completely disable the wireless capability.

- a) Connect the shielded Ethernet cable to the Gas Volume Measuring Device.
- b) Connect the shielded Ethernet cable to a computer.

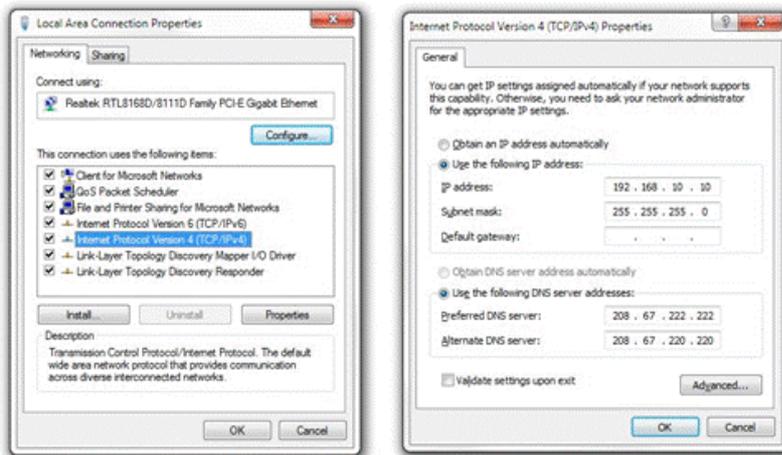
Important! Make sure that the network cable is connected prior to connecting the power.

- c) Connect the power supply (12 V DC) first to the motor module, and then to a standard 100-240 V ~ 50/60 Hz power socket.

WINDOWS 7

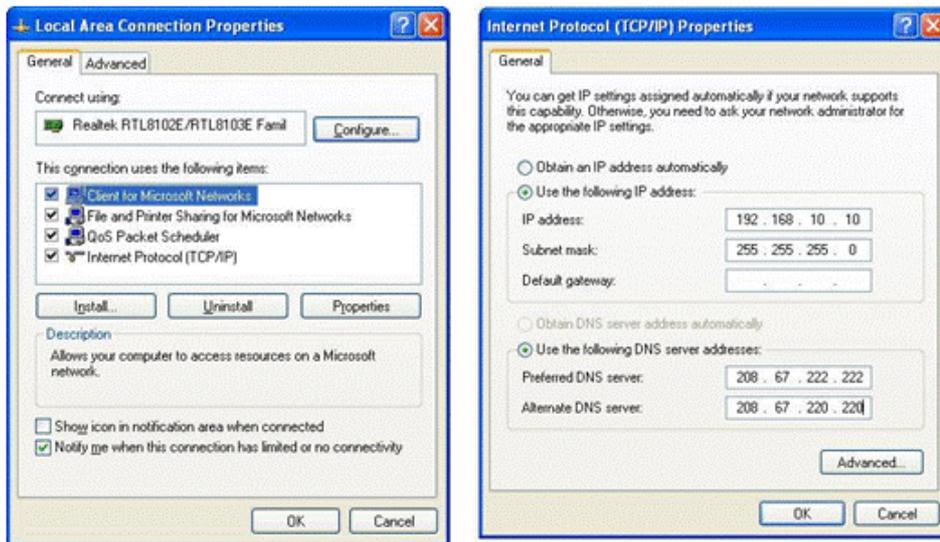


- a) In the Windows Start Menu, select “Control Panel”.
- b) In the Control Panel, select “Network and Internet”.
- c) Select “Network and Sharing Center”.



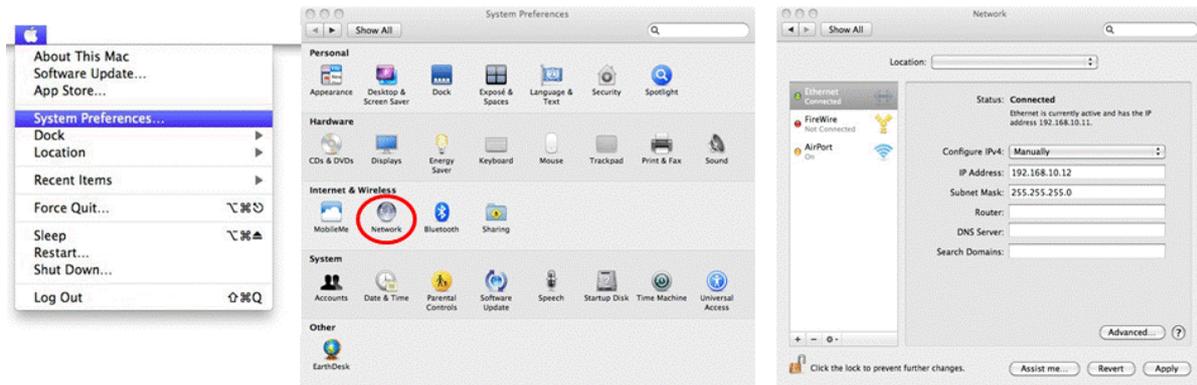
- d) On the left side of the window, select “Change adapter settings”.
- e) Right click on the appropriate network adapter and select “Properties”. Usually this is named “Local Area Connection”, possibly followed by a number. It is important that the adapter corresponding to the connected Ethernet cable is selected.
- f) Select “Internet Protocol Version 4 (TCP/IPv4)” and click on the button marked “Properties”.
- g) Write down the initial settings (e.g., IP address, subnet mask).
- h) Select “Use the following IP address” and enter the following values in the fields:
 - IP address: 192.168.10.10
 - Subnet mask: 255.255.255.0
- i) Select “OK”.
- j) Click “Close” in the remaining window.

WINDOWS XP



- a) In the Windows Start Menu, select “Control Panel”.
- b) Select “Network Connections”.
- c) Right click on the appropriate network adapter and select “Properties”. Usually this is named “Local Area Connection”, possibly followed by a number. It is important that the adapter corresponding to the connected Ethernet cable is selected.
- d) Select “Internet Protocol” and click on the button marked “Properties”.
- e) Write down the initial settings (e.g., IP address, subnet mask).
- f) Select “Use the following IP address” and enter the following values in the fields:
 - IP address: 192.168.10.10
 - Subnet mask: 255.255.255.0
- g) Select “OK”.
- h) Select “OK” in the remaining window.

MAC OS X



Select the Apple Menu button and select “System Preferences”.

- a) Select “Network”.
- b) Write down the initial settings (e.g., IP address, subnet mask).
- c) Select “Manually” from the “configure IPv4” dropdown menu and enter the following values in the fields:
 - IP Address: 192.168.10.10
 - Subnet mask: 255.255.255.0
- d) Select “Apply”.

ALL SYSTEMS

To access the instrument, open a web browser (Google Chrome is recommended as it provides the best and most compatible feature set for the AMPTS II).

In the address field enter, <http://192.168.10.11>

The address can be saved in the bookmarks of the web browser. If multiple AMPTS II units are installed, log the IP address for each individual system.

Bellow is located a quick guide to make sure that all settings have been made correctly.

AMPTS II

IP Address	192.168.10.11
Subnet mask	255.255.255.0

Computer

IP Address	192.168.10.10
Subnet mask	255.255.255.0

Please note that the IP address for the computer and the IP address for the AMPTS II are different. This is a design requirement of the IP protocol. Care needs to be taken so that the same address is not used in both locations, as it will render the system inaccessible from the designated computer.

Connect the system to a network

If the system is to be connected to an internal network (and/or the Internet), please follow the steps below:

- a) Log in to the AMPTS II by entering the user name (user) and the password (bpc).
- b) In the web browser window, showing the AMPTS II web application, click on the tab marked “System”.
- c) If using a Dynamic Host Configuration Protocol (DHCP) server located on the internal network to assign an IP address to the system, click on the checkbox marked “Obtain an IP address and DNS information automatically”, select “Apply network settings” and click “Yes” in the confirmation dialogue.

If the system is not supposed to use a DHCP server, manually enter the required network information followed by selecting “Apply network settings” and clicking “Yes” in the confirmation dialogue.

Important! Changing the network settings might make your system unreachable if you enter incorrect information. Contact the IT department to make sure that the correct settings are applied. If, for any reason, the wrong settings are applied, the AMPTS II can be reset to the factory default settings by pressing the reset button located at the back of the Gas Volume Measuring Device. This will reset the unit to its default state with the default network settings.

- d) Disconnect the provided shielded Ethernet cable from the computer (not the AMPTS II) and connect it to the internal network.
- e) Change the network settings on the computer to reflect the state they were in prior to starting the AMPTS II configuration and, if needed, reconnect it to the internal network.

9.2 Finding the IP of AMPTS II

If you configure the AMPTS II system to use a DHCP server, the unit will request an IP address from a local server on the network. In order to access the system, this IP address needs to be known. Due to the complexities and different setups that can be present in a network, there is no easy way to describe how this IP address can be found. The recommended procedure is to get this information from the local IT department.

9.3 Function and Operation

The description of the software refers to AMPTS II, but it is also valid for the AMPTS II Light. The only difference is the number of cells (15 for AMPTS II and 6 for AMPTS II Light).

LOG IN

The logo for bioprocess CONTROL, with 'bioprocess' in a green and black font and 'CONTROL' in a smaller black font below it.

Log in to the AMPTS II instrument

The page is used to log in to the AMPTS II.

- a) Press “Log in to the AMPTS II instrument”
- b) Write in the user name: user
- c) Write in the password: bpc (default password)



The web interface is organized into a number of pages, all reachable from the start page named “Home”, which is the first page the user enters. The pages are ordered as an experiment would be setup, executed, monitored and finally documented.

The following pages are available:

Home

The first page seen upon logging in.

Experiment

The user prepares an experiment by calculating and setting up individual data for each reactor/cell.

Control

The user can start, pause and end an experiment. The user can also control the speed of the motors driving the reactor stirrers.

Graphs

Two graphs showing the accumulated gas volume and flow rate vs. time are displayed.

Download Report

The user can generate a report from the page. The report contains experimental data for each reactor/cell.

System

This page contains the configuration of IP addresses and cell volume settings, the system log and set date and time. Performing a system reboot is also possible from this page.

EXPERIMENT

Home Experiment Control Graphs Download report System

Experiment settings

Choose experiment bottle to edit

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Bottle #1

Name	<input type="text"/>
Total sample amount [g] ⓘ	<input type="text"/>
Inoculum concentration [% w/w]	<input type="text"/>
Substrate concentration [% w/w]	<input type="text"/>
I/S ratio	<input type="text"/>
Total volume of reactor [ml]	<input type="text"/>
Assumed CH ₄ content [%]	<input type="text"/>
Type of unit [VS/COD] ⓘ	<input type="text" value="VS"/>

Experiment guidelines

Calculated value for setting up the experiment bottle

Inoculum amount [g]	<input type="text" value="0"/>
Substrate amount [g]	<input type="text" value="0"/>
Inoculum VS or COD amount [g]	<input type="text" value="0"/>
Substrate VS or COD amount [g]	<input type="text" value="0"/>
Headspace volume [ml] ⓘ	<input type="text" value="0"/>

Guideline matrix (Show ↓)

Experiment common settings

Eliminate overestimation

Activated Deactivated

CO₂ in flush gas [%]

Process temperature

Assumed temperature [Celsius]

This page is used to setup an experiment and to give guidelines to the user regarding the setup of the experimental reactors. The user starts by selecting which flow cell reactor that shall be used for the experiment by selecting one of the radio buttons.

Experiment settings

The user enters relevant values for the total amount of sample, volatile solids (VS) content of inoculum and substrate (relative to the total amount of wet sample, i.e. VS%), inoculum to substrate VS ratio (e.g. 2; 1.5 or 0 if no substrate is added), total reactor volume (for 500 mL bottles, this volume was estimated to 600 mL, also including the volume of the connection tubing) and the expected CH₄ content of the produced biogas (which can usually vary between 50 and 70% or it should be inserted as 100% if the total volume of raw biogas has to be measured). When the user has filled in the values, the data is saved by pressing the button: “Store settings”. The software then automatically calculates and presents the values for the amount of substrate and inoculum which need to be added in each reactor.

The user has the possibility to see simultaneously the calculated values for all cells by activating the hidden Guideline matrix function (Show!).

Guideline matrix ([Hide ↑](#))

	Bottle #1 Inoculum 1 (Blank)	Bottle #2 Inoculum 2 (Blank)	Bottle #3 Inoculum 3 (Blank)	Bottle #4 Sample A1	Bottle #5 Sample A2	Bottle #6 Sample A3	Bottle #7 Sample B1	Bottle #8 Sample B2	Bottle #9 Sample B3	Bottle #10 Sample C1	Bottle #11 Sample C2	Bottle #12 Sample C3	Bottle #13 Cellulose 1 (Control)	Bottle #14 Cellulose 2 (Control)	Bottle #15 Cellulose 3 (Control)
Row 1:	400	400	400	347.54	347.54	347.54	388.57	388.57	388.57	391.06	391.06	391.06	396.66	396.66	396.66
Row 2:	0	0	0	52.46	52.46	52.46	11.43	11.43	11.43	8.94	8.94	8.94	3.34	3.34	3.34

Row 1 is Inoculum amount [g]
Row 2 is Substrate amount [g]

Experiment common settings

Some settings are common for all cells, for example the process temperature, the content of CO₂ in the flush gas and the overestimation compensation.

The overestimation calculations are necessary since the produced biogas and the flush gas (N₂ is commonly used) do not have the same properties. The inert part of the flush gas (part that is not dissolved in CO₂-absorption unit) will pass through the system the same way as methane. This means that the biogas produced at the beginning of the experiment will push away the flush gas and it will be registered as methane. Therefore, the overestimation compensation corrects for the volume of gas used to flush the system prior to initiating the experiment by considering the headspace of the bioreactors and the CO₂ concentration of the flush gas.

To save the settings press the button “Store settings”.

Important! If nitrogen is using for flushing the system, then the CO₂ concentration in the flush gas should be inserted as “0”. No empty space will be left here.

CONTROL

bioprocess
CONTROL

Home Experiment **Control** Graphs Download report System

Control

Motor control

Speed adjustment [%]
Mixers on time [min]
Mixers off time [min]
 Off On

Cell control

Cell id	Control	Status
Cell 1:		Stopped
Cell 2:		Stopped
Cell 3:		Stopped
Cell 4:		Stopped
Cell 5:		Stopped
Cell 6:		Stopped
Cell 7:		Stopped
Cell 8:		Stopped
Cell 9:		Stopped
Cell 10:		Stopped
Cell 11:		Stopped
Cell 12:		Stopped
Cell 13:		Stopped
Cell 14:		Stopped
Cell 15:		Stopped

This section handles the Control pages. The page shows different controls that can be changed by the user.

Motor Control

Through this function the user can control the speed and the mixer on/off time. The motor status is either on or off. To activate the settings, the button “Apply” has to be pressed. The settings are the same for all motors.

Cell id Control Status
Cell 1: Ended

Cell id Control Status
Cell 1: Running

Cell id Control Status
Cell 1: Paused

Cell control

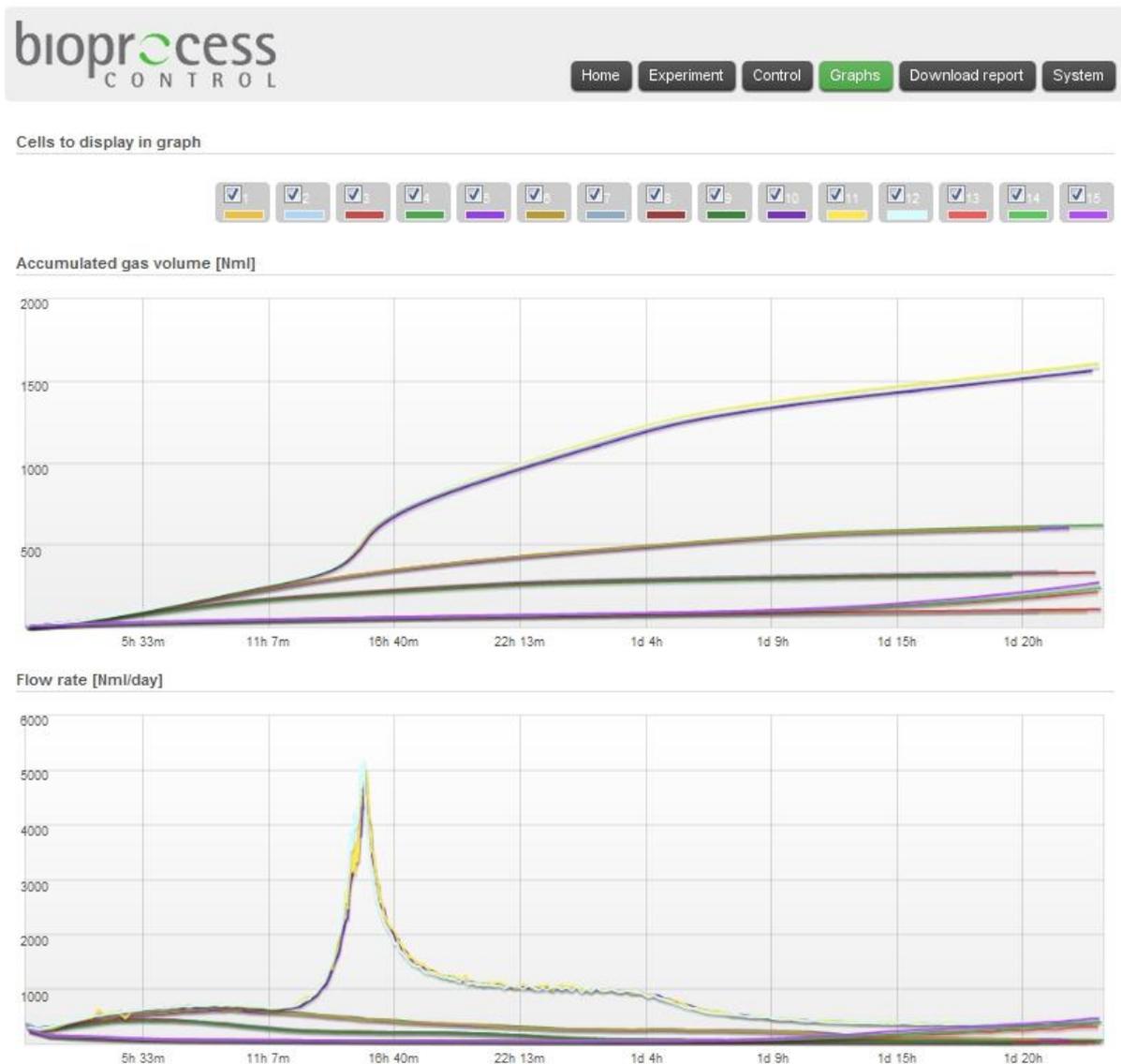
Each cell has a set of buttons that control the experiment. The first is the start experiment button. When hovering over it with the mouse the text “Start experiment” is displayed.

Important! When pressing the “Start experiment” button a new session is started and all data from the previous session will be deleted.

An experiment may be paused at any time. This is done by pressing the corresponding “Pause” button. During pause no data is recorded, but the time is still registered. By pressing the “Pause” button again the experiment will continue normally.

When the pause button has been pressed, the “End experiment” button becomes available. By pressing the “End experiment” button neither data nor time are further recorded. The collected data is available until the next time the Start experiment is pressed.

GRAPH



This section shows what the Graphs page may look like. In some browsers the data might initially not be displayed and to reload the page might be necessary to press “Reload”.

Cell status

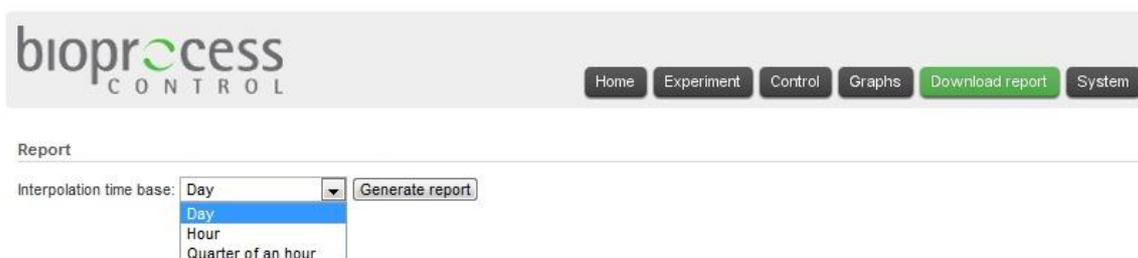
All 6 or 15 cells have a checkbox. Active cells have a colour assigned to them. If a cell is not active, the assigned colour is grey. When hovering over an active cell, the latest opening cell time is displayed. Checking/unchecking a box will add / remove the curve from the graph.

If a cell is not showing any activity, it is a good idea to find out if the problem is a lack of gas flow or if the Gas Volume Measuring Device is not working. This can be done by opening the flow cell manually, to see if the graph is registering an opening. If it registers the opening, the mechanics of the system is intact and you need to figure out if there is a gas leakage or a biological issue (e.g. very low activity of inoculum, substrate inhibition, etc).

Accumulated gas volume and flow rate

The two graphs show the accumulated gas volume and the current flow rate for the selected cells. The values displayed in the graphs are adjusted for “Overestimation” (if selected) and the gas volumes are normalized to 1.0 atmospheric pressure, 0°C and zero moisture content.

DOWNLOAD REPORT



On the Download report page, it is possible to select the unit for the interpolation time (e.g. one minute, 5 minutes, quarter of hour, hour or day), as well as to generate the report.

Before downloading the first report of an experiment, please go to the system tab and make sure that the values in the Flow Cell Volumes (at the bottom left section) have changed, from the defaulted value of 10 ml in each cell, to any other values.

Press the Generate report button, and the report will be available for download as seen in the next picture. Click on the green arrows to download them.

Note: The system registers the data for each bioreactor / flow cell until the last opening of the cell. This means that if a reactor is not producing gas any more, its corresponding cell will have empty cells showing up in the downloaded spreadsheet.

SYSTEM

The screenshot shows the 'System' page of the bioprocess CONTROL interface. At the top, there is a navigation bar with buttons for Home, Experiment, Control, Graphs, Download report, and System. The main content area is divided into several sections:

- System version ampts_2.1 (v1.2948)**
- Network**: Shows the Ethernet MAC address (04:79:b7:ae:61:80) and an option to obtain IP and DNS information automatically. A highlighted box provides the following network configuration:

IP address	192.168.10.11
Network mask	255.255.255.0
Gateway address	0.0.0.0
DNS server addresses	208.67.222.222 208.67.220.220

An 'Apply network settings' button is located below.
- Time settings**: Shows the instrument time (Wed Jun 17 09:49:46 2020 GMT+1) and a dropdown menu for selecting a timezone (currently set to GMT +1:00 hour Brussels, Copenhagen, Madrid, Paris, Stockholm). There is also an option to synchronize time automatically over Internet and a highlighted box showing the local browser time (2020-06-17 10:49 GMT+1) with a 'Use this time for instrument' button.
- System power**: Includes a 'Restart the system: Restart' button.
- Change system password**: Includes fields for 'New password' and 'Confirm password', and a 'Change password' button.
- Flow cell volumes [ml]**: A grid of 15 cells with their respective volumes:

Cell 1	Cell 2	Cell 3	Cell 4	Cell 5
9.07	8.97	9.04	9.04	9.07
Cell 6	Cell 7	Cell 8	Cell 9	Cell 10
9.04	9.16	9.15	9.03	9.12
Cell 11	Cell 12	Cell 13	Cell 14	Cell 15
9.11	9.09	9.12	9.09	9.13

Buttons for 'Store cell volume settings' and 'Reset to factory default' are at the bottom.
- Temperature and pressure sensor calibration**: A table showing current and sensor values for temperature and pressure, with input fields for 'Actual value'.

	Temperature (°C)	Pressure (kPA)
Current offset	0	0.1
Sensor value	60.9	100.3
Actual value	<input type="text"/>	<input type="text"/>
- System log**: Includes a 'Show system log ↓' button.

This section shows what the System page looks like. The page contains settings that are at the installation of the system and do not need to be modified afterwards. The page also gives the user the possibility to log the status/latest actions of the system.

Network

The system can be set up to obtain an IP address automatically; if this is not possible (e.g. no DHCP server available), an IP address can be entered manually.

The system has a default IP address. If this address is changed, a system reset will change it back to its default value.

Flow cell volumes

Each cell has a unique volume. When the cells are manufactured, the volumes are calibrated and pre-saved in this file of the system.

Time settings

The system time can be set either by synchronising towards the connected browser or an NTP server. Using an NTP server requires that the system has a working connection to the Internet.

System power

This menu allows a system restart. This does not affect the ongoing experiments. They will continue as soon as the system is up and running. Be aware that during the reboot, no values will be registered.

Temperature and pressure sensor calibration

This area lists the current sensor values and offset values for the temperature and pressure sensors. The two bottom fields are used to change the offset values.

System log



The screenshot shows a window titled "System log" with a "Hide system log" button. The log content is as follows:

```
2011-04-12 20:39:00 :: [sampler] sensor:13 no:36 time:30454 temp:20.8 pressure:100.9
2011-04-12 20:39:58 :: [motor] Running motor with dutycycle=114 for 60 sec.
2011-04-12 20:40:58 :: [motor] Stopping motor for 60 sec.
2011-04-12 20:41:23 :: [sampler] sensor:12 no:36 time:30603 temp:20.8 pressure:100.9
2011-04-12 20:41:58 :: [motor] Running motor with dutycycle=114 for 60 sec.
2011-04-12 20:42:26 :: [sampler] sensor:2 no:34 time:30698 temp:20.8 pressure:100.9
2011-04-12 20:42:58 :: [motor] Stopping motor for 60 sec.
2011-04-12 20:43:49 :: [sampler] sensor:7 no:33 time:30765 temp:20.8 pressure:100.9
2011-04-12 20:43:58 :: [motor] Running motor with dutycycle=114 for 60 sec.
2011-04-12 20:44:58 :: [motor] Stopping motor for 60 sec.
2011-04-12 20:45:19 :: [sampler] sensor:6 no:33 time:30857 temp:20.8 pressure:100.9
2011-04-12 20:45:58 :: [motor] Running motor with dutycycle=114 for 60 sec.
2011-04-12 20:46:54 :: [sampler] sensor:11 no:35 time:30936 temp:20.8 pressure:100.9
2011-04-12 20:46:58 :: [motor] Stopping motor for 60 sec.
2011-04-12 20:47:58 :: [motor] Running motor with dutycycle=114 for 60 sec.
2011-04-12 20:48:16 :: [sampler] sensor:5 no:33 time:31038 temp:20.8 pressure:100.9
2011-04-12 20:48:32 :: [sampler] sensor:9 no:32 time:31042 temp:20.8 pressure:100.9
2011-04-12 20:48:58 :: [motor] Stopping motor for 60 sec.
2011-04-12 20:49:58 :: [motor] Running motor with dutycycle=114 for 60 sec.
```

The system log provides the user with an updated view of what the AMPTS II logging software has registered. This includes both general events, such as flow cell openings (together with time, temperature and pressure) and time for motors starting and stopping. It also includes errors experienced by the various subsystems of the AMPTS II, such as unexpected power loss.

The AMPTS II has an embedded computer that runs inside of it, collecting data points independently. Restarting the standalone computer will not affect the data collecting.

When contacting BPC with support questions, it is good to have an up to date copy of the system log and to be able to provide this upon request.

DATA

The screenshot shows the 'bioprocess CONTROL' web interface. At the top, there is a navigation bar with buttons for 'Home', 'Experiments', 'Control', 'Graphs', 'Download report', and 'System'. Below the navigation bar, the page title is 'View cell data'. On the left side, there is a vertical list of radio buttons numbered 1 through 15. Radio button 1 is selected. To the right of the radio buttons, the text 'Cell 1' is displayed. Below 'Cell 1', there is a large rectangular area containing a table of data. The table has a header 'Time, Temp., Pres.' and five rows of data.

Time	Temp.	Pres.
11	27	120.5
93	27	120.4
99	27	120.4
914	27.2	120.4
961	27.2	120.4
4268	27.2	120.3

This section shows what the Data page looks like. After entering the IP address to the page followed by /app.html#data (e.g., <http://192.168.10.11/app.html#data>), the raw logged cell data in table format is displayed. In the current state, the cell data is just an image of the file that contains the data. The file that is displayed depends on the selected radio button. Only data for one cell at a time can be displayed.

This view is meant as an overview of the raw data and should not be interpreted as the final result of the running experiments. To make full use of the AMPTS II capabilities, a full report using the built-in features of the system should be generated.

DATA RESET



Reset instrument data

WARNING: This will erase **ALL** experimental data currently collected by the instrument. It is recommended that you generate any necessary reports before continuing!

Erase all data

[Back to the instrument main page](#)

This section shows what the Data Reset page looks like. When the AMPTS II is instructed to collect more data than the system is designed for, it might become unresponsive and difficult to access. In order to restore the system to a clean state, this page can be used. To access it, enter the IP address of the system followed by /datareset.html. For example, with the default settings enter http://192.168.10.11/datareset.html in the browser address bar.

WARNING: Please note that this function should only be used as a last resort. It will remove ALL collected data from the system. After using this function, no report will be possible to generate without conducting a new experiment. It is therefore highly recommended not to rely on this usage as a step in a normal workflow.

CONNECTIVITY

In order for the system to be able to function during an experiment, there is no requirement that a computer is connected to the system or that the system is connected to a network.

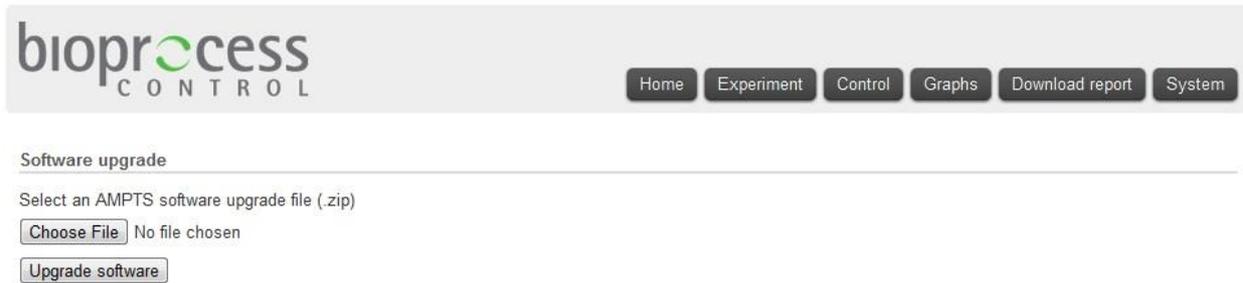
The only time there is a demand for a direct connection between the system and a computer through an Ethernet cable is during the initial setup of the system.

The only time there is a demand for either a direct connection between the system and a computer or through a network is during the setup of an experiment and during the generation of a report. There is no effect on the data collection capabilities of the system by disconnecting the Ethernet cable or turning off the connected computer.

If the AMPTS II system loses power during an experiment, the data previously stored in the system is again made available after the system is powered up. While the system is unpowered, no gas generated by the reactors will be registered but once power is restored, all active experiments will start to register the gas again.

As with all computer equipment, care should be taken to assert that the system functions after an unscheduled reboot. Make sure that the experiment is running correctly and that data is being collected in an appropriate manner. Also make sure that any other connected equipment, such as the thermal water bath, has restarted safely and with the correct settings.

SOFTWARE UPGRADE



Software upgrade

Select an AMPTS software upgrade file (.zip)

No file chosen

Care should always be taken to keep the built-in software of the AMPTS II up to date. BPC will, from time to time, issue software updates that can be installed on the system through an easy procedure. These updates can contain both new features and bug fixes so it is imperative that, when an update is received, the system is updated at an appropriate time.

In order to install an update issued by BPC, append “upgrade” after the # to the standard AMPTS II url visible in the address bar of the web browser. Example for a standard configured unit would be <http://192.168.10.11/app.html#upgrade>. This will display the software upgrade interface.

Click on the “Choose File” button and select the .zip file sent by BPC. Note: Do not unzip this file and never make any changes to the files contained inside of it. Also, make sure that the filename does not include any parentheses. Some operating systems, such as Microsoft Windows, will sometimes append parentheses to filenames when the same file is downloaded more than once. If this is the case, remove the parentheses from the filenames before using it to upgrade the system.

When the file has been properly selected, click on the “Upgrade software” button. During the upgrade process, make sure that the system is not restarted or otherwise loses power. This might result in a failed upgrade. If this happens, redo the entire upgrade process from the start.

It is recommended to leave the AMPTS II to finish its upgrade for five minutes to ensure that everything has been installed appropriately. When the upgrade is completed, the system will restart, with all previous data and settings intact and ready for use. To verify that the upgrade has taken place, go to the system tab of the system and, in the top left corner, compare the stated software version number to the version number of the file issued by BPC.

9.4 Troubleshooting

This section deals with problems that might arise during the operation of the AMPTS II. The instrument is highly advanced and needs to be handled as such. This means that, from time to time, unintended events might occur or difficulties in handling the equipment might be experienced. It is the intention of this chapter to help minimizing the probability for such events to occur and provide advice on how to solve some of the most common problems which can come up during system operation.

FINDING THE AMPTS II ON THE INTERNAL NETWORK

If there is a problem of locating the assigned IP address of the AMPTS II after connecting it to the Internal network using automatic IP settings (NOTE: This does not apply if you have setup the instrument to be used via a direct the shielded Ethernet cable connection from a computer to the AMPTS II instrument), there are few ways of manually detecting the unit. Due to the different ways networks can be setup, there is no way to guarantee that these steps will work. They should thus not be seen as replacements for contacting the local IT department (which is the recommended way of obtaining the IP address of the instrument), but assist if, for some reason, this is not feasible. These instructions are meant for the Microsoft Windows operating systems.

USING AN EXTERNAL TOOL (IP TOOLS)

Start by entering the following address: <http://www.softpedia.com/get/Network-Tools/Protocol-Analyzers-Sniffers/IP-Sniffer.shtml> into the web browser to download the IP Sniffer tool.

After downloading the file, unzip it to a suitable location and enter the directory created. There, a file named iptools.exe should be available. Start the program and in the main window by selecting Tools -> ARP -> ARP Scan / MAC to IP on the top toolbar.

A new window will appear. In the subnet field, there will be a pre-defined value. Usually, this will be the appropriate value for the network the AMPTS II is connected to. If the value here should be changed to reflect the internal network address assigned to new systems, do so.

The program will now display a list of IPv4 addresses with associated Ethernet MAC hardware addresses. Check if any of these addresses match the “Ethernet MAC hardware address” associated with the AMPTS II instrument (printed on the back of the instrument). If a match is found, enter this address into a web browser to access the AMPTS unit.

USING THE COMMAND PROMPT IN WINDOWS

Start the windows command prompt via the Windows start menu. This is usually located in All Programs / Accessories / Command Prompt. At the command prompt, write “arp -a” (without the quotation marks) and press enter. This will generate a list of the IP addresses currently in the arp cache. Check if any of the values located under “Physical Address” match the “Ethernet MAC hardware address” associated to the new AMPTS II Instrument. If a match is found, enter this address into a web browser to access the AMPTS unit.

If none of these addresses match the “Ethernet MAC hardware address” of the unit, the next step is to update the arp cache by issuing a broadcast ping to your local network. First, find out what the IP address of the currently used computer is by writing “ipconfig” (without the quotation marks) in the command prompt and pressing enter.

Find the section that starting with “IPv4 Address”. Next to it, a four block number should be visible, similar in style to 192.168.0.1. If there are several IPv4 sections, this means there is more than one network card active in the computer and care needs to be taken to choose the one representing the internal network that the computer and the AMPTS II are connected to.

After finding the mentioned IPv4 field, the subnet mask of the network needs to be determined. This should be located below the IPv4 field and be similar in style to 255.255.255.0. To determine the broadcast IP of the internal network, combine the IPv4 address and the subnet mask. Where the subnet mask indicates 255, retain the original field in the IPv4 address and where the subnet mask indicates something else, substitute that field in the IPv4 address for 255. In the previous example, that gives 192.168.0.1 and 255.255.255.0 and thus the broadcast address becomes 192.168.0.255.

Next, in the command prompt, write “ping 192.168.0.255” (without the quotation marks and substituting the broadcast address for the one relevant to the network, calculated as described in the previous step).

Wait for the program to run its course (this should take less than a few minutes). Write in the command prompt “arp -a” (without the quotation marks) and check the output. Try to match the AMPTS II Ethernet MAC hardware address to one of the fields and check what IP it is associated with.

If a match is found, enter this address into a web browser to access the AMPTS unit.

Depending on the internal network setup, the correct Ethernet MAC hardware address will not be reported. Compare the results from the last “arp -a” to the first “arp -a” actions performed and check if any new IP addresses have shown up. If such addresses are identified, manually introduce them one by one into a web browser to verify if any of them can be connected to the AMPTS II unit.

UNRESPONSIVE SYSTEM

If the AMPTS II is used to collect more data than is advisable, the system might become slow and unresponsive. It might become difficult to generate reports and change settings. It is thus highly recommended not to exceed the recommended gas production as, in some cases, it can rendere the collected data completely inaccessible.

During this time, the page for the system might still appear to load but no changes will take effect. In order to determine if the system is properly loaded or if the system is unresponsive, go to the systems tab and look at the “flow cell volumes” fields. While the system is in a non-responsive state, these values will be displayed as “10”. Once the system is ready, these values will be updated to the values calibrated into the system by BPC.

If Google Chrome is being used, it might, from time to time, show a dialog box asking if it should keep trying or if it should stop the page. This is due to Google Chrome assuming that the delay is due to a faulty executed program. This is not the case. The delay is because of the very large amount of data accumulated. If using other web browsers, a dialog choice might not be presented and the web browser will simply stop executing the program, thus making it impossible to properly access the system. BPC always recommends using Google Chrome with the AMPTS II.

After the system has loaded properly, the report should be possible to generate. As soon as this is done, the running experiments containing large sets of data should be cleared and a new experiment should be started. This does not, however, mean that the system cannot be used to analyse large data sets. The procedure to do so is to run the experiment up until the recommended limit of the system, generate a report and save it, clear the cells and continue. This procedure is then repeated until the full experiment has been finished. After this, the individual reports need to be combined and care taken to assure that the data is combined in an appropriate way.

If the system becomes completely unresponsive and it is not possible to access the data the regular way, two options remain. If the data can be considered forfeitable, i.e. it is only for a short time period and the loss is not considered to have too large of an effect on the end results, the data can be completely cleared by using the built in function referred to under the head line “Data Reset” in chapter 9 (only available for AMPTS II software version 1.04 and above). This will make it possible to use the system again without affecting any of the network settings. Please note that the reset function on the back of the unit should NOT be used for this, as it only resets the network settings and leaves the data intact.

If the data is considered high value, it is possible for BPC to access the raw data of the system. Raw data is defined as the time when a flow cell opened as well as the associated temperature and pressure. If internet access is given (to BPC) to the system IP (port 80 and port 22), this raw data can be extracted and the system can also be reset. Please note that this should not be viewed as a solution taken lightly. The raw data, while in itself useful, needs to later on be analysed by the customer. The raw data does not take into account such features built into the AMPTS II report generation function such normalization, calibration of the flow cells, flush gas calculations and so forth.

10 MAINTENANCE

10.1 Clean the Equipment

REACTORS

Use a bottlebrush and washing-up liquid (detergent) to clean the inside and outside of the reactors. Rinse well with water.

Autoclaving the grey plastic stoppers and their stirrers works fine, but only after disconnecting the motors. **The motor and its holder must not be autoclaved. The same goes for the blue rubber stoppers with metal tubes and their stirrers, which** contain plastic and rubber parts, which are characterised by uncertain heat stability and are therefore not suitable for autoclaving.

THERMOSTATIC WATER BATH

If normal tap water is used, calcareous deposits may appear on the reactors, in the water bath and on the heating element. Periodical cleaning of the inside of the tank and the heating element with a solution of 10 to 20% hydrochloric acid or a solution of water and vinegar (acetic acid) is advised. Rinse with clean water afterwards.

For cleaning of the different parts, use the following products: (i) alcohol (for cleaning of stainless steel) or alcohol with cotton duster (for cleaning of plastic).

GAS VOLUME MEASURING DEVICE

Rinse with water after use. A soft brush can be used very gently if needed.

Make sure that all the flow cells are in the correct position. The position can be checked by lifting the flow cell and making sure that the software registers the opening of the flow cell.

CO₂-ABSORPTION UNIT

The used NaOH must **NOT** be poured into the sink. It should be saved in dedicated vessels and disposed as hazardous waste. See the safety data sheet for the chemicals for further information.

APPENDIX A

BMP TEST USING AMPTS II – METHOD DESCRIPTION

This method description is based on the method used in the BPC-laboratory. It can be used as it is, used as base for developing a new method or it can be replaced entirely by the customer's own method.

Determination of Total (TS) and Volatile Solids (VS)

Before starting any BMP test, the biomass should be characterized with regard to total (TS) and volatiles solids (VS).

The dry matter, i.e. all inorganic and organic compounds is often expressed as TS and can be measured according to a standard protocol. For a given biomass sample, it is necessary to heat the sample up to 105 °C in order to remove all water content.

VS is represented by the organic compounds in the sample. After finishing the TS measurement, heating the sample up to 550 °C for 2 hours should be continued for burning up the organic matter. The weight difference between the sample after heating at 105 °C and 550 °C reflects the VS content of the biomass.

The next three steps are usually followed to determine the TS and VS in a target sample:

1). Preparation

- a) Heat a dish to 550 °C for 1 h.
- b) Place the dish in a desiccator for cooling.

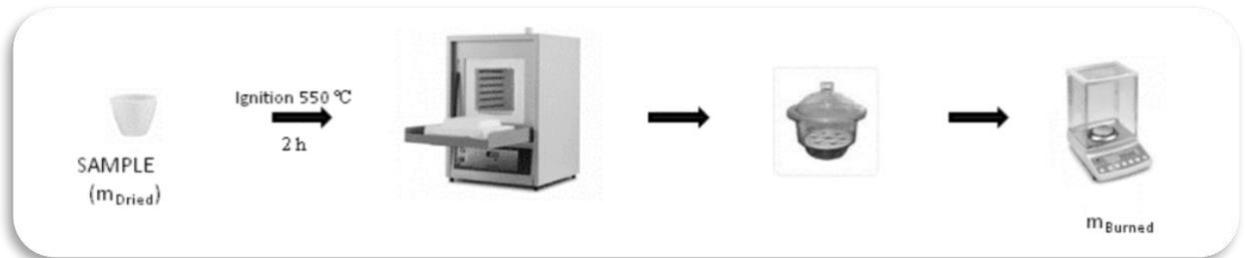
2). TS determination

- a) Weigh the dish and record this value.
- b) Add 2-3 ml of a representative sample into the dish.
- c) Place the dish with the sample in an oven preheated to 105 °C and allow the volatiles to evaporate for 20 h.



3). VS determination

- a) Take the dish out of the oven and allow it to cool to RT in a desiccator.
- b) Weigh the dish and record this value.
- c) Transfer this dish into a furnace pre-heated to 550 °C (ignition).
- d) After 2 h, take the dish out of the furnace and cool it to RT in a desiccator.
- e) Weigh the dish and record this value.



TS is calculated as the ratio between the amount of dried sample (m_{Dried}) and the initial amount of wet sample (m_{Wet}). VS content provides an estimation of the organic material in the sample and it is expressed either as the percent of the TS or as the percent of the wet sample, the second case being applied in the BPC protocol, when VS is calculated as the ratio between the difference in the amount of sample after drying and burning (m_{Burned}) and the initial amount of sample.

$$TS (\%) = \frac{m_{Dried}}{m_{Wet}}$$

$$VS(\%) = \frac{m_{Dried} - m_{Burned}}{m_{Wet}}$$

A template for automatic calculation of the two parameters can be provided by BPC. Please contact BPC in order to get more information.

Preparation⁵

To be able to start an experiment, at least one *substrate* and an *inoculum* are needed. The inoculum is typically the sludge from a full-scale biogas plant. It is recommended to use an inoculum, which is adapted to the current substrate. If the substrate to test is already used in an existing plant, the inoculum from that plant might be suitable. Be aware that the time between sampling the inoculum and experiment start up should be as short as possible; moreover, the substrate should be as fresh as possible. If this is not achievable, they should be stored

⁵ The text below describes a case where the AMPTS II bottles were used. If the AMPTS II Light sized bottles are used, the volumes need to be adjusted accordingly.

refrigerated or frozen. Take into consideration the time necessary to measure TS and VS before starting the experiment.

PREPARATION OF THE INOCULUM (OPTIONAL)

In order to minimize the amount of biogas that is produced by the inoculum itself, pre-incubate the inoculum in anaerobic conditions (apply e.g. N₂) in order to deplete the residual biodegradable organic material present. This is typically carried out by storing the sludge for 2 to 5 days at a temperature corresponding to the process temperature from where the sludge was taken from and releasing the gas that is produced. When no significant gas production is observed, the sludge is ready to be used. After the pre-incubation step, check the pH and measure the TS and VS of the inoculum.

If this step is not performed, subtracting the amount of biogas produced by the inoculum itself from the one produced by the mixture between inoculum and substrate is necessary for calculating the methane potential of that certain substrate.

PREPARATION OF THE SUBSTRATE

Check the pH of the substrate to make sure that it is not acid, which may cause an inhibition of the biodegradation process. Measure TS and VS. Keep the substrate refrigerated or frozen until the experiment starts. It is recommended to have as short time as possible between sampling of the substrate and start of the experiment.

PREPARATION OF NAOH-BOTTLES FOR CO₂ ABSORPTION

- a) Prepare a 3 M NaOH-solution by mixing 240 g pure NaOH with distilled water up to 2 l.
- b) Prepare a 0.4 % Thymolphthalein pH-indicator solution (40 mg in 9 ml ethanol 99.5% and 1 ml water).
- c) Add 10 ml pH-indicator solution to the prepared 2 l NaOH solution.
- d) Prepare bottles (100 ml) containing about 80 ml NaOH solution (3M) and Thymolphthalein pH-indicator.
- e) Place the plastic lid (with two pipes) into the bottle and screw cap.

PREPARATION OF THE THERMOSTATIC WATER BATH

- a) Fill up the Thermostatic Water Bath with enough water to cover the reactors (approximately 8 l). It is recommended to use distilled water.
- b) Make sure that the correct temperature is set.

PREPARATION OF THE GAS VOLUME MEASURING DEVICE

- a) Fill up the water bath with enough distilled water to cover the cells in open position. The water level should be according to the water level marking on the water bath.
- b) To avoid trouble when starting the experiment, it is recommended to start the program and try to open each cell several times to make sure that the detection step is functioning properly.

PREPARATION OF REACTORS

- a) It is recommended to use triplicates of each combination inoculum-substrate. For one sample, use:
 - *3 reactors as blanks* (containing only inoculum and (optional) distilled water for having the same total volume in all reactors) – used to compensate for the amount of biogas produced by the inoculums itself;
 - *3 reactors with inoculum + substrate* (samples)
 - *3 reactors as positive controls* (containing cellulose or other standard substrate) – to test the quality of the inoculum.
- b) When preparing the experiment, a certain inoculum to substrate ratio needs to be chosen. The appropriate ratio depends on the method chosen or the properties of the substrate. At the BPC laboratory, inoculum to substrate ratios of 3:2 - 2:1 (based on VS) are normally used. However, if it is known that the substrate contains inhibitory substances it is recommended to test higher ratios (i.e., less substrate).
- c) For high accuracy data the experiment should be set-up correctly: high amount of inoculum-substrate mixture (e.g. 400 g in 500 mL bottles), low headspace volume, low inoculum to substrate ratio (in the range 2 to 4) in order to generate a high volume of gas at high flow rate during an AD process.
- d) When this ratio has been decided, the amounts of inoculum (m_{IS}) and substrate (m_{SS}) in the sample need to be calculated. A total amount of maximum 400 g liquid per reactor (for 500 ml bottle) is normally used in the BPC-laboratory to avoid problems if foaming occurs. The amount of inoculum can be either constant in all the bottles or it can vary from one sample bottle to another one.
- e) If several substrates are to be tested with the same inoculum, calculate the amount of inoculum in accordance with the substrate with the lowest VS value (see formulas below) if the amount of inoculum has to be kept constant in all the bottles. This amount of inoculum is then subsequently used for the other substrates, and the amount of substrate is then adjusted so that the desired VS ratio is achieved. In this way, only one set of blanks (corresponding to the amount of inoculum added in each reactor) can be used. However, some of the reactors will then contain a total volume smaller than 400 g. If the same amount is desired in all bottles, these reactors may be filled up to 400 g using distilled water.

$$\left\{ \begin{array}{l} \text{ratio 2:1} \quad \frac{m_{IS} \times VS_I}{m_{SS} \times VS_S} = 2 \\ \\ \text{or} \\ \\ \text{ratio 3:2} \quad \frac{m_{IS} \times VS_I}{m_{SS} \times VS_S} = 1.5 \\ \\ \text{and} \\ \\ m_{SS} + m_{IS} = m_{tot} \end{array} \right.$$

PREPARATION OF THE REACTORS (EXAMPLE)

If a ratio of 2:1 and a total amount of 400 g are chosen:

$$\frac{m_{IS} \times VS_I}{m_{SS} \times VS_S} = 2$$

$$m_{SS} + m_{IS} = 400$$

The amount of inoculum needed can be calculated according to the following equation:

$$m_{IS} = \frac{800 \cdot VS_S}{(VS_I + 2 \cdot VS_S)}$$

The amount of substrate will then be:

$$m_{SS} = 400 - m_{IS}$$

If another substrate will be used with the same inoculum, the amount of new substrate is calculated according to the equation below:

$$m_{SS} = \frac{m_{IS} \cdot VS_I}{2 \cdot VS_S}$$

Important! All calculations to determine the necessary amounts of substrate and inoculum for a certain VS ratio are automatically carried out by the AMPTS II Software (see Section “Experiment” in the Chapter 9).

Start Up Procedure

Two different methods may be applied depending on the presentation of the substrate.

START UP ALTERNATIVE 1

All bottles are filled with the same amount of inoculum (prepare one extra mix of each sample for measuring pH, TS and VS). Leave 3 bottles as blanks (add distilled water up to 400 g if desired). For each substrate, add the calculated amount (depending on the inoculum to substrate ratio desired) to each one of the three replicate reactors. For control, add cellulose to three of the reactors (fill up to 400 g with distilled water if desired). This method is especially suitable for solid substrates.

START UP ALTERNATIVE 2

Mix the inoculum and substrate for all three reactors with the same substrate/cellulose in one batch and then distribute 400 g of this mix into each bottle. This alternative method is recommended only for liquid and homogeneous substrates.

GENERAL START UP PROCEDURE

- a) Mark all reactors and their lids.
- b) Fill up the reactors with inoculum and substrate.
- c) Close the reactor with a plastic stopper and tighten the plastic screw cap carefully.
- d) Fasten the stirring stick to the motor.
- e) If not already done, mount a short piece of Tygon® tubing on one of the tubes of the lid and place a tube stopper on it.
- f) Place all reactors in the water bath and connect to the CO₂-absorption bottles and to the flow cells. Fill up with more water if necessary.
- g) Connect the contacts for the stirring to the individual motors and, lastly, to the gas volume measuring device. Connect the Ethernet cable to the internal network (or computer if the system is not used within a network) and to the gas volume measuring device. Lastly, connect the power supply (input 100-240 V ~ 50/60 Hz, output 12 V DC/3 A) to the wall outlet and to the gas volume measuring device.
- h) Flush all reactors with N₂ for approximately 1 minute, using the extra inlet in the lid, to achieve anaerobic conditions (see the instructions in the AMPTS II manual).
- i) Empty each flow cell.
- j) Start the data logging program, see instructions in the AMPTS II Software Chapter.
- k) Measure pH, TS and VS in the extra bottles/mixtures prepared.

During the Experiment

- a) Check the water level in the thermostatic water bath and the water bath periodically and fill up with distilled water when necessary.
- b) If the system has been equipped with the extra gas sampling valves (can be ordered at BPC), GC analysis can be performed to measure the gas composition any time during the experiment.

End of Experiment

The test period often spans between 30-60 days. End the test when CH₄ production is negligible (<5 ml/day⁶).

- a) Turn off the Thermostatic Water Bath and disconnect the contacts for the stirring.
- b) Disconnect the reactors one by one from the CO₂-absorption bottles and measure the pH in each reactor.
- c) Measure TS and VS in each reactor in order to check for VS reduction.
- d) Disconnect the CO₂-absorption bottles.
- e) Wash reactors and other equipment.
- f) Empty the Thermostatic Water Bath after it has cooled down to ambient temperature.
- g) Process the data.

Data Processing

- a) If the biochemical methane potential (BMP) of a substrate is evaluated, the most interesting parameter to evaluate is the amount of gas produced per gram of VS added. Use a mean value of the three blanks when withdrawing the gas production from the inoculum.

$$BMP = \frac{V_S - V_B \frac{m_{IS}}{m_{IB}}}{m_{VS,SS}}$$

- *BMP* - is the normalised volume of methane produced per gram VS of substrate added (Nl/gVS)

⁶ A. Lehtomäki, S. Huttunen, S. Rintala, (2007). *Resources Conservation and Recycling* (2007), 51 (3), 591-609.

- V_S - is the accumulated volume of methane produced from the reactor with sample (i.e., inoculum and substrate)
- V_B - is the mean value of the accumulated volume of methane produced by the three blanks (i.e., inoculum)
- m_{IS} - is the total amount of inoculum in the sample
- m_{IB} - is the total amount of inoculum in the blank
- $m_{VS,SS}$ - is the amount of organic material (i.e., volatile solids) of substrate contained in the sample bottle.

The degradation profile vs. time can also be plotted. If only one substrate is used, the accumulated volume can be plotted directly. If several substrates with different inoculum to substrate ratios should be compared, calculate the methane potential at every time step and compare the volume produced per gram of VS instead.

APPENDIX B

BMP CALCULATION FROM DATA GENERATED BY AMPTS II

In this section some issues important for the definition of the ultimate anaerobic biodegradation or biochemical methane potential (BMP) of organic substrates determined in batch assays and an example for the calculation of BMP of a target substrate using the data generated from AMPTS-II are presented.

Biochemical Methane Potential (BMP)

BMP test is a simple batch assay developed to determine the methane production of a given organic substrate during its anaerobic decomposition. The conventional assay involves incubating a substrate inoculated with anaerobic bacteria for a period of 30 to 60 days commonly at 37°C and monitoring the production of biogas together with its composition throughout the test. The presented gas volume or gas flow rate should be normalized based on dry gas (water content is removed) at 1 atm and 0°C.

The experiment is normally carried out with tests in triplicates for statistical significance: 3 reactors as *blanks* (reactors containing only inoculum), 3 reactors as *reference control* (e.g., cellulose) and the rest of reactors contain *sample(s)* (i.e., inoculum and substrate).

Information necessary for the BMP determination

To be able to determine the BMP the following information is necessary:

Parameter	Unit	Symbol
Accumulated biomethane volume from sample bottle(s)	Nml	V _S
Accumulated biomethane volume from blank bottle(s)	Nml	V _B
VS amount of substrate in sample bottle(s)*	g	m _{VS,SS}
VS amount of inoculum in sample bottle(s)*	g	m _{VS,IS}
VS amount of inoculum in blank bottle(s)*	g	m _{VS,IB}

*For wastewater samples, COD (chemical oxygen demand) is often used instead of VS, where VS stands for the volatile solids.

All this information is available in the report generated by the AMPTS-II equipment. Of course this requires that the user has entered the correct values for total sample amounts and concentrations of substrate and inoculum in the “Experiment” window and also added the amounts of substrate and inoculum calculated by the software in the experimental bottles.

In order to determine the true (ultimate) BMP of an organic material it is important to continuously monitor the biomethane gas production through anaerobic degradation of substrate until the gas production is nearly ceased. Preferably, this is confirmed by generating a report and controlling the two flow rates.

Formula for calculation of BMP

BMP is defined as the volume of methane produced per amount of organic substrate material added to the reactor. Consequently, the accumulated volume from an experiment has to be divided by the amount of organic substrate material added into the reactor. However, in the experimental sample bottle, there will also be organic residues present in the added inoculum which will produce certain amount of biogas in the anaerobic process and its volume fraction of biomethane has to be subtracted from the total accumulated biomethanes volume in order to get the true production from the substrate. Thus, BMP can be expressed according to equation (1).

$$BMP = \frac{V_S - V_I}{m_{VS,SS}} \quad (1)$$

In equation (1), V_S is the accumulated volume of biomethane from the reactor containing the sample (i.e., substrate and inoculum), V_I is the volume of biomethane coming from the inoculum present in the sample bottle and $m_{VS,SS}$ is the amount of organic material of substrate contained in the sample bottle. In case of wastewater samples, chemical oxygen demand (COD) is often used instead of VS.

The blank samples will generate only the amount of biomethane originating from the inoculum (V_B), and can further on be normalized to the biomethane production per unit weight of dry organic material in the inoculum. The obtained fraction ($V_B/m_{VS,IB}$) has to be multiplied with the amount of dry organic material (i.e., gram VS or COD) from the inoculum in each sample bottle ($m_{VS,IS}$) in order to calculate how much biomethane is coming from the inoculum in the sample reactor, as following:

$$BMP = \frac{V_S - V_I}{m_{VS,SS}} = \frac{V_S - V_B \frac{m_{VS,IS}}{m_{VS,IB}}}{m_{VS,SS}} = \frac{V_S - V_B \frac{m_{IS}}{m_{IB}}}{m_{VS,SS}} \quad (2)$$

As seen from the equation (2), the ratio between the amount of organic material from the inoculum in the sample vs. the one in the blank is equal to the ratio between the total amount of the inoculum in the sample (m_{IS}) and the one in the blank (m_{IB}).

Example of a BMP calculation

In this example, the BMP will be determined for three different substrates (s1, s2 and s3) and a control (c). Data is collected from an AMPTS-II system that has been running for 30 days. All

the samples (S), the control (C) and blank (B) have been tested in triplicates (3 identical setups for each).

In Figure 1, a screenshot of a generated report from this test can be seen. In the screenshot, the information necessary for the determination of the BMP for sample 1 (S1) is clearly marked out. Three values are given for each parameter since triplicates are used. BMP can then be calculated according to equation (2). For substrate 1 this becomes:

$$BMP = \frac{2565.4 - 1077.3 * \frac{11.69}{16.08}}{5.84} = 305.2 \text{ Nml/gVS} \quad (3)$$

For this particular example, BMP can be calculated in Excel with equation 4:

$$BMP = (AVERAGE(H47:J47) - AVERAGE(B47:D47) * AVERAGE(H11:J11) / AVERAGE(B11:D11)) / AVERAGE(H10:J10) \quad (4)$$

In equation (4), the function “AVERAGE” is used to calculate the mean value of the replicates. The replicates are either in columns H:J (cells 7:9) for the substrate 1 or in columns B:D (cells 1:3) for the blank.

Gas production (V_B , V_S)

The accumulated gas volumes are specified in the row number 47. However, this number is variable and depends on how long the experiment has been run and which time basis is used. As a general rule, the row number of the last obtained values in the accumulated volume columns should be used.

The column letters will be variable, depending on which cells are used and the number of replicates. In this example, columns H:J are used for the substrate since substrate s1 use flow cells 7:9 (Figure 1) and columns B:D since the blank use flow cells 1:3.

Organic material (m_{VS})

Row number 10 is always used to get the amount of organic material in the substrate and row number 11 is always used to get the amount of organic material in the inoculum in an AMPTS II generated report.

Calculation of BMP for the other substrates

The BMP of the other substrates can be calculated in a similar way. The only necessary thing is to replace the column letters for the substrate data. For example, if the BMP of substrate 3 is to be determined, H:J should be replaced with N:P since this substrate is evaluated in flow cells 13:15 (Figure 1).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	CO2 concentration [%] of flush gas	38														
2	Process temperature [Celsius]	37														
3	Mixer on time [sec]	60														
4	Mixer off time [sec]	60														
5	Mixer speed adjustment [%]	49														
6	Eliminate overestimation	No														
7																
8	Flow Cell nr.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
9	Name	B	B	B	C	C	C	C	S1	S1	S2	S2	S3	S3	S3	S3
10	Substrate VS/COD amount [g]	0	0	0	7.87	7.87	7.87	5.84	5.84	5.84	6.93	6.93	7.31	7.31	7.31	7.31
11	Inoculum VS/COD amount [g]	16.08	16.08	16.08	15.75	15.75	15.75	11.69	11.69	11.69	13.87	13.87	14.62	14.62	14.62	14.62
12	Type of unit [VS/COD]	V5	V5	V5	V5	V5	V5	V5	V5	V5	V5	V5	V5	V5	V5	V5
13	Headspace volume [ml]	240	240	240	240	240	240	240	240	240	240	240	240	240	240	240
14	Assumed CH4 content [%]	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60
15																
16	Day															
17		0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18		1	107,1	112,5	111,8	842,7	852,1	828,5	560,1	511,3	542,3	1129,3	1143,4	1129,3	1831,1	1824,9
19		2	204,0	213,8	212,6	1479,1	1489,8	1457,0	984,5	909,6	957,0	1944,0	1960,6	1944,0	2698,2	2698,2
20		3	291,7	305,0	303,4	1962,3	1970,1	1936,4	1307,9	1221,1	1275,9	2535,0	2548,4	2535,0	3130,6	3105,7
21		4	371,1	387,1	385,3	2331,5	2334,3	2304,3	1556,2	1466,2	1522,7	2966,8	2974,2	2966,8	3361,3	3370,1
22		5	442,9	461,1	459,0	2815,7	2812,8	2588,6	1748,2	1660,0	1715,0	3284,9	3285,7	3284,9	3509,7	3465,3
23		6	507,9	527,6	525,5	2826,3	2827,6	2810,0	1898,0	1814,3	1866,1	3521,6	3515,9	3521,6	3590,1	3602,0
24		7	566,6	587,5	585,4	3008,9	2995,0	2903,8	2016,0	1937,9	1985,7	3699,5	3688,1	3699,5	3658,4	3622,8
25		8	619,8	641,5	639,4	3145,4	3126,8	3121,6	2109,8	2037,6	2081,4	3835,1	3818,7	3835,1	3713,7	3727,8
26		9	668,0	690,1	688,1	3254,3	3231,7	3231,8	2185,3	2118,7	2198,5	3939,8	3919,4	3939,8	3760,6	3775,0
27		10	711,5	733,8	731,9	3342,3	3316,3	3320,9	2246,5	2185,2	2221,3	4021,8	3998,1	4021,8	3801,6	3816,1
28		11	750,9	773,2	771,4	3414,0	3385,2	3393,6	2296,8	2240,1	2273,0	4087,0	4050,8	4087,0	3838,0	3852,6
29		12	786,6	808,6	807,1	3473,1	3442,1	3453,6	2338,5	2285,7	2315,9	4139,7	4111,4	4139,7	3870,7	3885,2
30		13	818,8	840,5	839,2	3522,3	3489,5	3503,6	2373,4	2324,0	2351,8	4182,9	4153,0	4182,9	3899,9	3914,5
31		14	848,0	869,3	868,1	3563,7	3529,5	3545,6	2403,0	2356,4	2382,2	4218,8	4187,7	4218,8	3926,3	3940,9
32		15	874,4	895,1	894,2	3598,9	3563,5	3581,2	2428,0	2383,9	2408,1	4249,0	4217,0	4249,0	3950,1	3964,7
33		16	898,3	918,4	917,6	3629,0	3592,7	3611,7	2450,0	2407,5	2430,4	4274,0	4242,1	4274,0	3971,5	3986,1
34		17	920,0	939,4	938,8	3654,9	3617,9	3638,0	2468,8	2427,9	2449,6	4296,9	4263,7	4296,9	3990,8	4005,5
35		18	939,5	958,3	957,9	3677,5	3640,0	3660,8	2485,2	2445,5	2466,4	4316,1	4282,6	4316,1	4008,3	4022,9
36		19	957,2	975,3	975,1	3697,2	3659,2	3680,2	2499,6	2460,9	2481,1	4332,9	4299,9	4332,9	4024,0	4038,6
37		20	973,2	990,6	990,6	3714,5	3676,2	3698,2	2512,2	2474,3	2494,0	4347,7	4313,7	4347,7	4038,2	4052,8
38		21	987,8	1004,4	1004,6	3729,8	3691,3	3713,6	2523,5	2486,1	2505,3	4360,8	4326,6	4360,8	4051,0	4065,6
39		22	1000,9	1016,8	1017,2	3743,3	3704,6	3727,3	2533,4	2496,6	2515,4	4372,5	4338,1	4372,5	4062,5	4077,2
40		23	1012,7	1027,9	1028,5	3755,4	3716,5	3739,4	2542,2	2505,8	2524,4	4382,8	4348,4	4382,8	4073,0	4087,6
41		24	1023,5	1038,0	1038,7	3766,1	3727,1	3750,1	2550,2	2514,0	2532,4	4392,1	4357,6	4392,1	4082,4	4097,0
42		25	1033,2	1047,0	1048,0	3775,6	3736,5	3759,7	2557,2	2521,3	2539,5	4400,3	4365,8	4400,3	4090,8	4105,5
43		26	1042,0	1055,2	1056,3	3784,2	3745,0	3768,3	2563,6	2527,8	2545,9	4407,8	4373,2	4407,8	4098,5	4113,1
44		27	1050,0	1062,5	1063,7	3791,9	3752,7	3776,0	2569,2	2533,7	2551,6	4414,4	4379,8	4414,4	4105,4	4120,0
45		28	1057,2	1069,1	1070,5	3798,7	3759,5	3782,9	2574,3	2538,9	2556,7	4420,4	4385,8	4420,4	4111,6	4126,2
46		29	1063,7	1075,1	1076,6	3804,9	3765,6	3789,1	2578,9	2543,5	2561,3	4425,8	4391,2	4425,8	4117,2	4131,9
47		30	1069,6	1080,4	1082,0	3810,5	3771,2	3794,7	2583,0	2547,7	2565,4	4430,6	4396,0	4430,6	4122,3	4136,9
48																
49																
50																

Figure 1. Screenshot of a report generated from AMPTS-II.

Graphs from the generated data

The data presented in Figure 1 may further be used for plotting the methane production profiles over time. Figures 2, 3 and 4 show typical examples of how such graphs are presented. Figure 2 shows triplicate curves obtained for the target samples (including inoculum), for blanks containing only inoculum and for control containing cellulose as a reference substrate and inoculum. The specific methane production is reported as accumulated volume of CH₄ per gram VS after correction for the methane produced by the inoculum and this graph is represented in Figure 3.

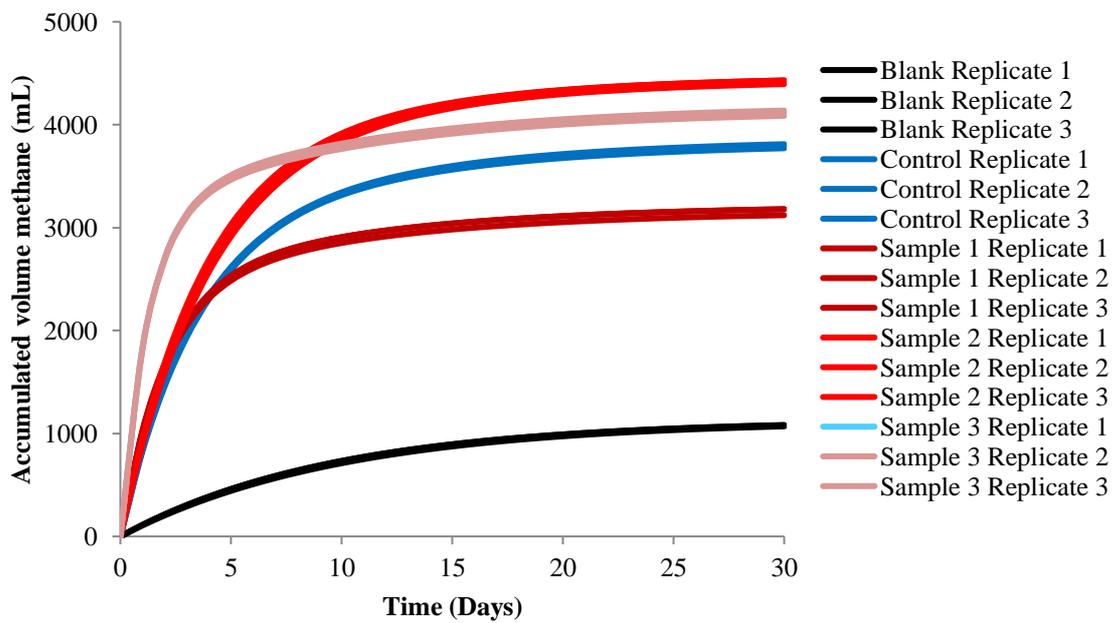


Figure 2. Methane production for triplicate samples of substrate 1, 2 and 3 (including inoculum), for blank containing only inoculums and for control samples.

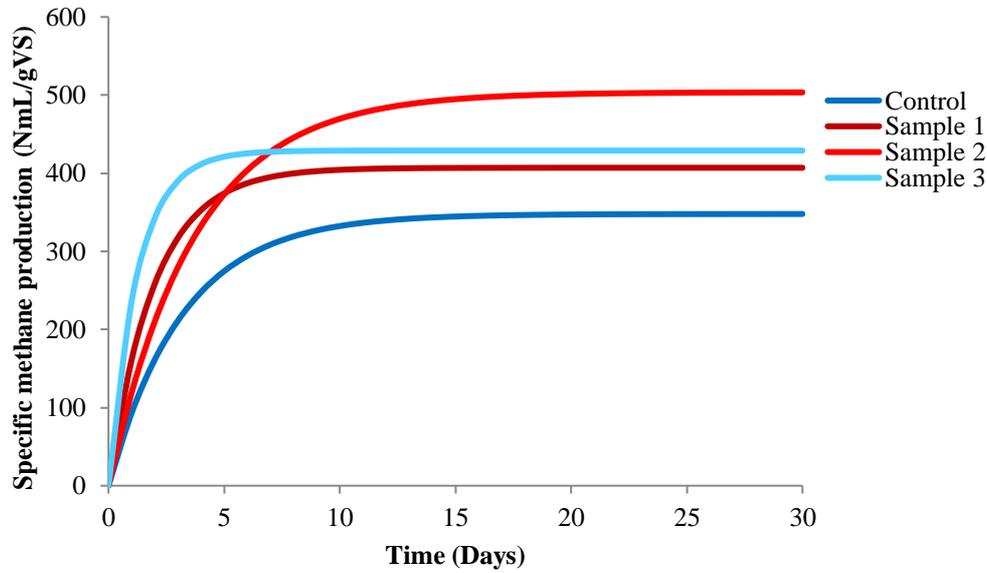


Figure 3. Specific methane production for the samples and control as average from triplicates (the gas from the inoculums has been subtracted and the data has been expressed as volume of CH₄ per gram VS added).

The fraction of bio-methane released daily as a ratio from the total accumulated methane production over 30 days is presented in Figure 4. As seen, in the studied case, more than 90% of the biogas/biomethane was produced within the first 10 days.

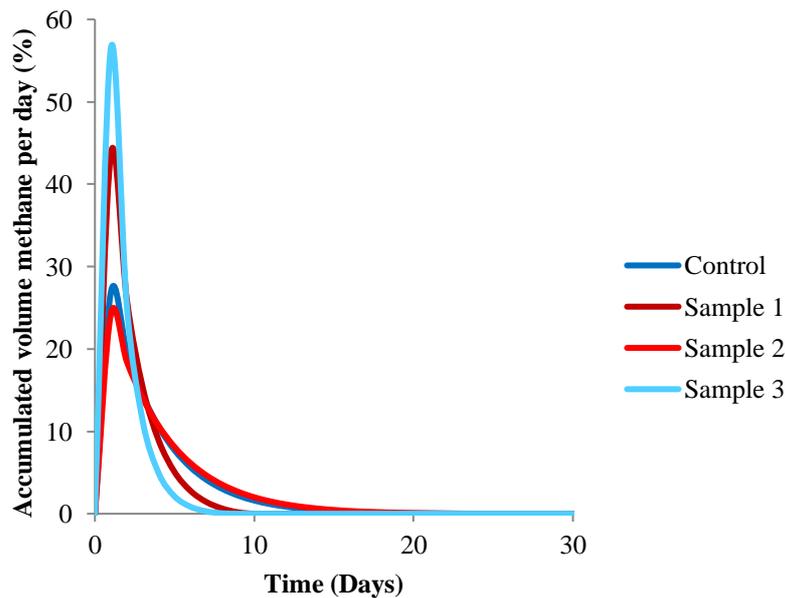


Figure 4. Daily methane production as % of the total accumulated volume (the gas from the inoculum has been subtracted from the data).

The results from BMP tests can be used to obtain further information on the investigated substrates, such as the hydrolysis rate if the hydrolysis is limiting step of the anaerobic digestion process for a given non-easy biodegradable substrate. Therefore, using the linear part of the experimental curve build for the determination of the accumulated methane production of a target substrate, as plotted in Figure 2, it is possible to calculate the first order hydrolysis rate coefficient as the slope of the linear curve obtained. This value is characteristic to each substrate and gives information about the time required to generate a certain ratio of the ultimate methane potential.

BMP of control substrates

Compounds such as starch, cellulose, or gelatine can be used as positive control substrates. According to the literature, the average methane yields for these compounds at an inoculum to substrate ratio of 2 are 350 ± 33 , 350 ± 29 and respectively 380 ± 42 mL CH₄/g VS added (Raposo et al., 2011).

References

Raposo F, et al. (2011). Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international laboratory study. *J Chem Technol Biotechnol*, 86, 1088-1098.

APPENDIX C

GAS NORMALISATION CALCULATION IN AMPTS II

For comparison with other studies, the values given in the reports generated from AMPTS II are normalized to standard conditions, i.e., converted to 0°C, 1 atm and no humidity (i.e., removing the water content and considering the dry gas). This is carried out applying the following equation (1):

$$V_0 = f_{T,P} \times f_w \times V_{cell} \quad (1)$$

where V_0 is the normalised volume, $f_{T,P}$ is a factor considering the temperature and pressure of the gas, f_w is a factor considering the water content of the gas and V_{cell} is the registered volume for a cell opening.

Temperature and pressure

The factor considering the temperature and pressure ($f_{T,P}$) is calculated using the ideal gas law assumed to describe the physical characteristics of the gas within the temperature and pressure ranges used in an anaerobic digestion (AD) related test. $f_{T,P}$ is calculated according to equation (2):

$$f_{T,P} = \frac{T_0}{T_{gas}} \times \frac{p_{gas}}{p_0} = \frac{273.15}{t_{gas}+273.15} \times \frac{(p_{gas}+0.6)}{101.32} \quad (2)$$

where T_0 and p_0 are the standard temperature (273.15 K or 0 °C) and the standard pressure (101.32 kPa or 1 atm) respectively, T_{gas} , t_{gas} and p_{gas} represent the temperature (in K or °C) and pressure of the gas generated in the reactor at ambient conditions. These values are obtained from the internal temperature and pressure sensors of AMPTS II and are updated for each cell opening. The pressure of the gas is the sum of the measured pressure (expressed in kPa) and a constant factor equal to 0.6 (pressure exerted by approx 6 cm water column from the Gas Volume Measuring Device).

Water vapour

The factor considering the water content of the gas (f_w) is calculated using the Antoine equation which is assumed to provide a good approximate of the vapour pressure around atmospheric pressure and in the temperature interval from 0 to 100°C. f_w is calculated according to equation (3):

$$f_w = 1 - \frac{p_{vap}}{p_{gas}} = 1 - \frac{10^{8.1962 - \frac{1730.63}{233.426 + t_{gas}}}}{10 \times (p_{gas} + 0.6)} \quad (3)$$

In the equation above the vapour pressure of the gas is assumed to be proportional with the gas volume (according to the ideal gas law). The volumetric fraction of the water is then equivalent to

the pressure fraction obtained by dividing the water vapour pressure with the total pressure. The fraction of dry gas can then be calculated by removing the fraction of water from the wet gas. The values for the Antoine constants may be obtained from the Dortmund data bank (<http://www.ddbst.com>). Please note that the vapour pressure (i.e., p_{vap}) is calculated in mbar units and the digester gas pressure (i.e., p_{gas}) is expressed in kPa, also considering the pressure exerted by the water column (approx. 6 cm) of the Gas Volume Measuring Device.

In conclusion, the gas production is continuously registered and normalised by AMPTS II with real-time temperature and pressure compensation, according to equation (4) (derived from the equations (1)-(3)):

$$V_{0,i} = \frac{273.15}{t_{gas,i}+273.15} \times \frac{(p_{gas,i}+0.6)}{101.32} \times \left(1 - \frac{10^{8.1962 - \frac{1730.63}{233.426 + t_{gas,i}}}}{10 \times (p_{gas,i} + 0.6)} \right) \times V_{cell} \quad (4)$$

The accumulated volume reported by AMPTS II is calculated by summing up the cell volume for each cell opening. Each added volume is adjusted for pressure, temperature, and water vapour according to equation (5):

$$V_{acc,i} = V_{acc,i-1} + \frac{273.15}{t_{gas,i}+273.15} \times \frac{(p_{gas,i}+0.6)}{101.32} \times \left(1 - \frac{10^{8.1962 - \frac{1730.63}{233.426 + t_{gas,i}}}}{10 \times (p_{gas,i} + 0.6)} \right) \times V_{cell} \quad (5)$$

Flush gas characteristics

Another factor that impacts the biomethane quantification is the flush gas characteristics for the situation when the carbon dioxide is removed prior to the measurement.

When a reactor is flushed (deaerated) with an inert gas (e.g., nitrogen), an overestimation of the gas production can occur. As the biogas is produced, it will mix with the flush gas and push away the mixture of the two gases. When the gas mixture reaches the carbon dioxide absorption unit, only the fraction of carbon dioxide will be absorbed, whereas the inert part of the flush gas will continue to the measurement unit and be registered. Thus, the presence of less carbon dioxide in the flush gas as compared to the produced biogas may lead to an overestimation.

The under- or overestimated volume at every measurement point, in this case corresponding to every cell opening, is assumed to be the amount of inert part of the flush gas (usually N₂ or CH₄ which do not react with alkaline solution) replaced by carbon dioxide in the system, and can be expressed by equation 6:

$$V_{OE,i} = \Delta X_{N_2^{FG}} \times V_{HS} \times \Delta X_{CO_2} = (X_{N_2^{FG},i-1} - X_{N_2^{FG},i}) \times V_{HS} \times (X_{CO_2^B} - X_{CO_2^{FG}}) \quad (6)$$

where $V_{OE,i}$ is the overestimated volume at the specific measurement point i ($i \geq 1$), $X_{N_2^{FG},i}$ is the flush gas fraction in the system at the specific measurement point, V_{HS} is the headspace volume, whereas $X_{CO_2^B}$ and $X_{CO_2^{FG}}$ are the carbon dioxide fractions in the produced biogas and flush gas,

respectively. If the gas mixture inside the reactor is assumed to be homogeneously mixed, then $X_{N_2^{FG},i}$ can be calculated according to equation 7:

$$X_{N_2^{FG},i} = X_{N_2^{FG},i-1} - \frac{V_{cell}}{V_{HS}} \times X_{N_2^{FG},i-1} = X_{N_2^{FG},i-1} \times \left(1 - \frac{V_{cell}}{V_{HS}}\right) = X_{N_2^{FG},0} \times \left(1 - \frac{V_{cell}}{V_{HS}}\right)^i \quad (7)$$

where V_{cell} is the volume of the flow cell and $X_{N_2^{FG},0}$ is the initial flush gas concentration with regard to the inert part. By combining the equations 5 and 6, the overestimated volume can be expressed by equation 8:

$$V_{OE,i} = V_{HS} \times (X_{CO_2^B} - X_{CO_2^{FG}}) \times \left(\left(1 - \frac{V_{cell}}{V_{HS}}\right)^{i-1} - \left(1 - \frac{V_{cell}}{V_{HS}}\right)^i \right) \quad (8)$$

If the overestimation compensation is also considered for correcting the volume of gas used to flush the system prior initiating the experiment by taking into account the headspace of the bioreactor and the CO_2 concentration of the flush gas, then the accumulated volume will be calculated according to equation 9:

$$V_{acc,i} = V_{acc,i-1} + \frac{273.15}{t_{gas,i} + 273.15} \times \frac{(p_{gas,i} + 0.6)}{101.32} \times \left(1 - \frac{10^{8.1962 - \frac{1730.63}{233.426 + t_{gas,i}}}}{10 \times (p_{gas,i} + 0.6)} \right) \times (V_{cell} - V_{OE,i}) \quad (9)$$

APPENDIX D

FREQUENTLY ASKED QUESTIONS (FAQ) RELATED TO AMPTS II

1. Is there any reason for inconsistent gas production, which leads to a graph with multiple steady states during a BMP test?

When cycles of high gas production followed by plateau levels are periodically observed it is recommended to check the level of water in the Thermostatic Water Bath. When the amount of water decreases below the threshold level, there is a drop in temperature in the reactor, which leads to no more gas production and therefore the graph appears as multiple steady-states. The user needs to check at least twice per week the level of water in the water and add more water whenever it is necessary. The level of water should be up to the plastic glass lid of the Thermostatic Water Bath.

2. Which substrates can be used as positive controls in a BMP test?

The control experiments are usually performed for testing the quality and activity of inoculum using a standard substrate (e.g., cellulose, starch, gelatin). Cellulose is used as reference substrate for energy crops or waste from agriculture, starch for waste based on potatoes and cereal grains, whereas gelatin is used for household waste rich in meat products. The average methane yields for cellulose; starch and gelatin are reported in the literature as 350 ± 29 , 350 ± 33 , and 380 ± 42 NmL CH₄/g VS added at ISR of 2 (Raposo et al 2011). However, according with the latest publications the methane yield for cellulose and starch should be within 85-90% of the theoretical value (i.e. 415 Nml/gVS) which means above 350 NmL/gVS (Holliger et al 2016).

3. What is the impact of VS (volatile solids) calculation (i.e VS% or VS_{TS}%) on the experimental conditions in setting up a BMP test?

VS parameter provides an estimation of the organic material content in the sample and it is expressed either i) relative to the total amount of wet sample (VS%) or ii) relative to the total solids (VS_{TS}%). The first definition should be applied when a BMP test is set up with the help of AMPTS II software. This means that the entering data for the inoculum and substrate concentration is based on the initial amount of sample (i.e. fresh weight).

The user of the AMPTS II has to enter the VS values (VS%) for the inoculum and substrate together with inoculum to substrate ratio (ISR) and the total amount of mixture in the Experiment page of the AMPTS II software. The software will automatically calculate and present the values of the amount of substrate and inoculum which need to be added in each reactor.

4. We have noted water accumulation (e.g. blocks up to 3-4 mm in length) in the biogas collection tubes running in particular from the AMPTS reactor to the CO₂ scrubbers? What you would suggest to overcome this?

The biogas produced during an AD process contains water vapor and the fractional volume of water vapor is a function of AD process temperature. In AMPTS, the biogas produced in each bioreactor passes through individual CO₂-absorption units containing alkaline solutions before reaching the Gas Volume Measuring Device. A part of water vapor condensates inside the Tygon® tubing which connects the bioreactors to the CO₂-absorption unit. This phenomenon of water vapor condensation cannot be avoided since biogas which is produced at high temperature (e.g. 37 or 50°C) is coming in contact with a surface kept at lower temperature (i.e., RT).

The “block of liquid” accumulated in the Tygon® tubing (either from evaporation or condensation process) is slowly transferred into the CO₂- absorption unit once more biogas is produced inside the bioreactor. The level of liquid in the bottles with alkaline solution can increase from 80 to 100 mL during an AMPTS test, depending on the process temperature and volume of biogas produced during a BMP test. This “block of liquid” is not affecting the efficiency of CO₂- absorption unit or the accuracy of the measurements.

5. Do you recommend another fluid with a low evaporation rate, which can be used in the Thermostatic Water Bath?

To eliminate the necessity for periodical checks of the water level in the Thermostatic Water Bath during a BMP test and to avoid the addition of water due to its evaporation, some of the laboratories are using silicone oil (e.g., LABOTHERMOL S from NeoLab) as heat exchanging fluid. The price of such a silicone oil is approx. 60 €/L. We recommend to our customers to use such silicone oil, even if the price is not very low and it is not easy to clean the items which come in contact with such a silicone oil.

6. Why there is no gas registration in one channel of AMPTS II?

Have you made sure that the unit is receiving gas to that flow cell? You can check if the flow cell or the Gas Volume Measuring Device is the problem, by manually opening the flow cell in question with your hand and see if it registers or not. If it registers this opening, the mechanics of the system is intact and you need to figure out if there is a gas leakage or a biological issue (e.g. very low activity of inoculum, substrate inhibition, etc).

7. Can AMPTS II be used for monitoring the accumulation of biogas?

AMPTS II was developed for monitoring the accumulated volume of biomethane and not biogas (CH₄ and CO₂). Solubility of CO₂ is much higher than the solubility of CH₄ in water (0.74 vs. 0.03 l/l) and it will be always a problem to measure the total volume of biogas by a volumetric method working according to the water displacement principle. Even if the literature reports that the acidified water (pH 2), mineral oils, saturated NaCl or acidified saturated NaCl can decrease the solubility of CO₂ in water, this is usually done by a factor of max 3. Therefore, the acidified water will not be a suitable solution for measuring the volume of biogas.

Since the gas solubility and the gas transfer from the flow cell and liquid in the water bath are dependent on the flow rate at which the biogas is produced, a mathematical model which corrects the volume of CO₂/biogas solubilized in the water bath at a specific flow rate was implemented in the AMPTS II Light.

8. Which are the recommended experiment conditions for achieving a high accuracy data?

For high accuracy data the experiment should be set-up correctly: high amount of inoculum-substrate mixture (e.g. 400 g in 500 mL bottles), low headspace volume, low inoculum to substrate ratio (in the range 2 to 4) in order to generate a high volume of gas at high flow rate during an AD process.

9. Is it possible to shut down the computer when the AMPTS is running and the data has to be registered?

If you are using an AMPTS II then the system is collecting data points independent of the computer. The AMPTS II has an embedded computer that runs inside of it. Restarting the standalone computer will not affect the data collecting.

10. Do you have any recommendation for grinding/homogenization unit for preparation of samples such as silage, corn, grass and other large size particles?

In order to increase the substrate accessibility surface area of the investigated substrates to the bacteria you can use different types grinding/homogenization units. The simplest one is a mixer for home use.

There are also laboratory instruments, such as grinding mill (Willy type made from iron suitable to cut the solid substrates to fine mesh with adjustment of the blades. See the following link as an example.

<http://www.indiamart.com/esteemindustriesincbaddi/laboratory-instruments.html>

According to the literature, samples such as corn silage might be grounded through Wiley mill followed by grinding with Udy mill. A brief description of the working principle of the Udy cyclone sample mill is presented at the following address:

http://www.seedburo.com/productDetail.asp_Q_catID_E_517_A_subCatID_E_2525_A_productID_E_3163_A_Cyclone_Sample_Mill_E_Cyclone_Sample_Mill

11. How can we connect the anaerobic gas tube to the AMPTS unit? Do we have to flush each bottle separately or is there any way to do all at the same time?

For flushing the system prior BMP test each bottle has to be connected separately to the gas tube. There is no way to flush all of them at the same time.

The gas tube should contain an oxygen free gas (e.g. N₂ or a standard gas based on 60% CH₄ and 40% CO₂ or 60% N₂ and 40% CO₂). In most of the cases, the gas tube should be equipped with a double stage pressure regulator, e.g: i) First stage 0-200 bar; ii) Second stage: 0-30, 0-5 or 0-1.5 bar.

Before flushing the system with gas to create anaerobic conditions, the Tygon® tubing from the CO₂- absorption unit must be disconnected.

The gas source has to be connected to the Tygon® tubing with the red clamp by using a plastic

tubing connector/reduction adapter. The red clamp on the reactors should be opened and the reactors can be flushed with a gas flow gently for 0.5 – 1 minute. In order to control the gas flow rate from the gas source, the Tygon® tubing which was disconnected from the CO₂- absorption unit might be inserted into a beaker with water.

At the end of the flushing step, stop the flush gas, close the red tube clamp, take the Tygon® tubing from the water and connect it to the CO₂- absorption unit. Disconnect the flush gas source and repeat the procedure for all the reactors.

12. How does the AMPTS II use the temperature sensor? The user inputs the process/reactor temperature in the AMPTS II interface. Does the software calculate a loss of water vapour between the reactor and the Gas Volume Measuring Device if there is a drop in temperature between them?

The AMPTS II doesn't use the temperature that the user feeds in manually. That is more of a 'note' that is stored for future reference by the user. What the temperature probe does is that a measurement from it is taken at the same time as each flow cell opening occurs and then this is used to calculate the normalized gas volume (at 1 atm, 0 degrees C, no water vapour) that is then stored in the report.

13. How can I exchange a flow cell for a new one?

The instructions to replace a flow cell are as follows. If you look at the black 'fish bone' that holds the flow cells in place, there will be two screws that are attaching it from the top. There are two black circular plastic pieces that are hiding these two screws but they should be easily visible, like a small coin. Just remove the plastic plugs (no tool needed) and you will see the screws. Unscrew the two screws and lift the top half of the black 'fish bone' upwards. This will expose the flow cells and will let you remove the faulty one and replace it with a new one. Then put the 'fish bone' back from where you removed it and screw it back in place and put back the black plugs.

APPENDIX E

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