BPC INSTRUMENTS

OPERATION AND
MAINTENANCE MANUAL

Gas Endevour® III

Gas volume and flow measurement system for diverse applications

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Produced in Sweden.

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GENERAL INFORMATION

Before operating the Gas Endeavour III (hereafter referred to as “the instrument”, “the system” or “the equipment” interchangeably) from BPC Instruments AB (hereafter referred to as “BPC Instruments”, “BPC”, “Bioprocess Control” or “BPC Instruments AB” interchangeably), carefully read this operator manual for the instrument, any separate instructions for other equipment used together or in conjunction with the instrument, as well as the safety instructions for any and all chemicals used in the process of utilising the instrument.

Safety Information

When performing experiments with the instrument, always use protective eyewear, gloves, and lab coat. Always make sure there is adequate ventilation and take proper precautions when handling electrical devices near water or explosive gases. Make sure to tie back any hanging objects, such as hair and clothing, when working near rotating or other moving parts.

Do not modify the instrument without the prior consent of the manufacturer. BPC Instruments AB do not assume responsibility for any errors due to equipment modification.

Do not clean or service the instrument while it is running.

Do not expose the instrument to mechanical vibrations or high frequency radio transmissions.

Never operate the instrument in a way it was not intended.

Never operate the instrument or let anyone else operate it without proper training.

Never use the instrument outside or in environments with parameters outside of the instruments recommended range.

Never connect additional electrical equipment not supplied by BPC Instruments AB for the express purpose of using with the instrument. This is true even if the connections can mate.

Always back-up important data to an external device.

Always keep the instrument level and on a flat and stable surface. Failing to do so can, among other things, generate an erroneous gas reading.

Always make sure all safety guards are in place and working before operating the instrument.

Always make sure that all parts are functioning properly immediately after start-up.

Always keep the instrument clean.

Always make sure to have access to relevant chemicals before starting an experiment.

Always dispose of parts and chemicals according to applicable rules in the country of usage.

Periodic maintenance of the instrument and its various accessories is essential. Always make sure they are in working condition. If service or spare parts are required, please visit https://webshop.bpcinstruments.com or contact BPC Instruments AB directly or one of its representatives.

Always make sure to connect the power supply so that it is easy to remove from the mains power outlet and so it doesn’t risk becoming damaged.

Always make sure that the gas outlet of the Flow Cell Unit (FCU) is able to release pressure in the event of pressure build-up inside of the instrument. Do not obstruct or block it.

Always wait 60 seconds between powering the system on and off. This will allow for the operating system to shut down properly and for the capacitators to properly cycle.

Always use deionized water to minimise the risk of residue or rust forming on the inside of the FCU.

Limited Warranty

The product warranty provided with the instrument corresponds to the stipulations in Orgalime S 2012, unless otherwise agreed upon with BPC Instruments AB. BPC Instruments AB reserves the right to correct any possible errors, mistakes, changes, updates, technical data or otherwise relevant information in this manual or any other documents, where applicable by law.

Electrical Safety

Compliance is required with respect to voltage, frequency and current requirements indicated on relevant parts. Improper operation, damage to the equipment, fire or otherwise undesired effects might be caused by connecting to a different power source. There are no user-serviceable parts in the equipment, unless otherwise agreed upon with BPC Instruments AB.

Before Getting Started

Read this manual before installing and using the instrument. In addition, keep this instruction manual for future reference and make sure it is easily available for people who regularly use the system.

Contact Information

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Chapter 01: Overview

Gas Endeavour III (GE III) is an automatic analytical platform designed to accurately measure gas volume and flow for ***batch and continuous processes***. GE III offers a fully integrated and automated system for sampling, recording, and generating reports with remote access to real-time data. Available in different configurations, including 18 or 9 channels, GE III is versatile in investigating processes centered around both gas production and consumption. This flexibility positions GE III as a suitable analytical instrument for both research and industrial applications:

- In-vitro digestibility studies
- Assessing aerobic and anaerobic biodegradability
- Analysing biogas production
- Monitoring hydrogen production
- Facilitating ethanol fermentation processes
- Conducting wastewater analysis
- Evaluating microbial activity

GE III retains important features of its previous version, which include the following:

- Same configuration in two versions (GE III and GE III Light).
- Automated sampling, recording, data analysis and report generation.
- Multifunctional agitation system.
- Standalone instrument with embedded data acquisition and web server for remote access (no need for software installation).
- The measuring cell is pre-calibrated by the factory.
- Gas composition estimation.
- Specific configuration dedicated to Animal Nutrition.
- The software can be accessed locally or remotely from any device with a web browser (preferably Google Chrome).
- Option for real-time automatic compensation for atmospheric pressure, environmental temperature and moisture content changes to normalise the data to standard conditions.
- Function to remove gas overestimation and automatic calculation of the amount of substrate and inoculum needed per reactor in order to perform BMP tests.
- Reliable operation and easy maintenance where most of the components can be easily exchanged without special requirements.
- System log for operational diagnosis.

The next generation of this analytical platform presents multiple new features and improvements in comparison with its previous generation (**Table 1**).

Table 1. Overview of the new features of GE III.

Gas Endeavour III	
INCUBATION UNIT 	- 18 channels (GE III) and 9 channels (GE III Light) - GE III standard configuration with 1 L reactors (0.5 L reactors as an option) - GE III Light comes with 2 L reactors - New thermostatic water bath with capacity to place 18 (0.5 / 1 L) or 9 (2 L) reactors - Shaking thermostatic water bath with 18 channels for 250 mL reactors (GE III Animal Nutrition) - Configuration including 9 CSTR-2G reactors (GE III Max)
CO_2-ABSORPTION UNIT 	- Improved bottle holder design for CO_2 removal bottles - GE III comes with two bottle holder units, each for nine bottles, allowing more flexibility to arrange the instrument - Higher capacity for CO_2 removal with 250 mL sized bottles
BPC CORE UNIT AND SOFTWARE  	- Two measurement resolutions (2 and 9 mL) - New electronics for faster and more stable operation compared to the previous version - 150 times higher storage capacity compared to GE II - USB port for software upgrade - Addition of a power button - Factory reset function - OLED screen to easy access the status and IP address of the instrument. - Built-in accelerometer for leveling of the unit C - New software platform Aurora™ - It is possible to start and stop all channels with one click - Three types of normalisation - New set of settings to visualise data and generate reports
Accessories	- More robust and less gas permeable tubing - Tubing connectors to simplify and ensure a good and tight connection between the tubing and the inlet of the flow cell chamber and the outlet of the reactor - Push-in valves to simplify the flushing step of the headspace of the reactor in order to create an anaerobic environment - New tubing markers for easy identification of the different channels - New tool for removing lids and tubing

2.1 GE III Standard and GE III Standard Light

GE III Standard (**Figure 1**) and GE III Standard Light (**Figure 2**) are delivered with the components listed below:

Figure 1. GE III Standard Complete System.

Unit A – Incubation Unit

18 glass reactors (0.5 or 1 L)	1 motor controller signal cable	1 MCU power adapter
18 brushless DC motors 1 motor power splitter	18 axis couplings for brushless DC motors	1 thermostatic water bath 1 base tray
19 brushless DC motor cables 250 mm (15 units) 500 mm (3 units) 1500 mm (1 unit)	18 stirrers GL 45 (0.5 or 1 L) standard	1 thermostatic water bath lid for 18 reactors (0.5 or 1 L)
	1 motor controller unit (MCU)	18 push-in valves 6 mm

Unit B – CO_2-absorption Unit

2 bottle holders 9 x 250 mL	18 bottle nuts GL 45
18 glass bottles (250 mL)	18 lids GL 45

Unit C – BPC Core Unit

1 BPC Core AMPTS	36 check valves	1 main unit power adapter
36 flow cell units (FCUs) 9 mL FCUs (18 units) 2 mL FCUs (18 units)	1 plastic syringe	1 ethernet cable

Additional Components

1 Festo tubing 50 m	36 push-in connectors 6 mm	1 bottle/tube opening tool
1 funnel	12 soft binders 7/180 mm	FCU volume sheet
18 tubing stoppers	36 multi-coloured marker clamps	

Figure 2. GE III Standard Light Complete System.

Unit A – Incubation Unit

9 glass reactors (2 L)	1 motor controller signal cable	1 MCU power adapter
9 brushless DC motors 1 motor power splitter	9 axis couplings for brushless DC motors	1 thermostatic water bath
10 brushless DC motor cables 250 mm (6 units) 500 mm (3 units) 1500 mm (1 unit)	9 stirrers GL 45 (2 L) standard	1 thermostatic water bath lid for 9 reactors (2 L)
	1 motor controller unit (MCU)	9 push-in valves 6 mm

Unit B – CO_2-absorption Unit

1 bottle holders 9 x 250 mL	9 bottle nuts GL 45
9 glass bottles (250 mL)	9 lids GL 45

Unit C – BPC Core Light Unit

1 BPC Core AMPTS	18 check valves	1 main unit power adapter
18 flow cell units (FCUs) 9 mL FCUs (9 units) 2 mL FCUs (9 units)	1 plastic syringe	1 ethernet cable

Additional components

1 Festo tubing 50 m	9 push-in connectors 6 mm	1 bottle/tube opening tool
1 funnel	6 soft binders 7/180 mm	FCU volume sheet
9 tubing stoppers	18 multi-coloured marker clamps	

The following items are **NOT** provided in the package of both instruments:

- Flushing gas to obtain anaerobic conditions inside the reactors during the sample preparation phase.
- 3 mol/L sodium hydroxide solution, pH indicator (Thymolphthalein) and ethanol.
- Additional wall socket adapters (plugs/contacts). The ones supplied are according to European, US or UK standards, depending on the country where the instrument will be operated.
- Gas sampling units and gas bags for off-line gas composition analysis.

2.2 GE III DUO and GE III Light DUO

GE III DUO consists of two BPC Core Units, where users can measure the total gas (e.g., CH_4 + CO_2) and a gas component (e.g., CH_4) from 18 samples simultaneously (**Figure 3**).

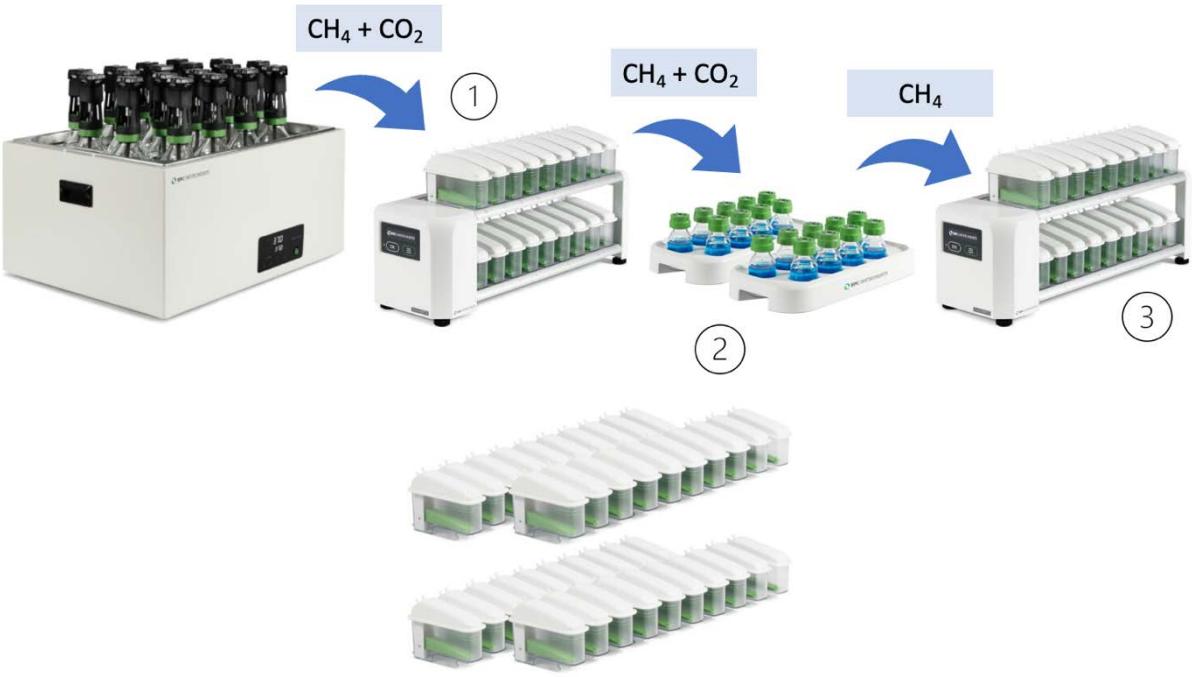


Figure 3. GE III DUO configuration.

The configuration operates based on three different stages:

- **Stage** ①: The accumulated gas volume and flow rate generated by the anaerobic digestion process (CH_4 + CO_2) is continuously measured by the first BPC Core Unit.
- **Stage** ②: Carbon dioxide is removed using a 3 mol/L NaOH solution (selective absorption). In this case, only a single gas component will be measured by the second detection unit.
- **Stage** ③: CH_4 is collected and measured by the second BPC Core Unit.

The average gas composition can be backcalculated and plotted at any time during the test.

Additionally, different measurement resolutions (2 and 9 mL) can be combined in this configuration depending on the application and requirements of the test. For instance, the first detection unit can have 9 mL FCUs and the second one 2 mL FCUs.

Unit A – Incubation Unit

18 glass reactors (1 L)	1 motor controller signal cable	1 MCU power adapter
18 brushless DC motors 1 motor power splitter	18 axis couplings for brushless DC motors	1 thermostatic water bath 1 base tray
19 brushless DC motor cables 250 mm (15 units) 500 mm (3 units) 1500 mm (1 unit)	18 stirrers GL 45 (1 L) standard	1 thermostatic water bath lid for 18 reactors (1 L)
	1 motor controller unit (MCU)	18 push-in valves 6 mm

Unit B – CO_2-absorption Unit

2 bottle holders 9 x 250 mL	18 bottle nuts GL 45
18 glass bottles (250 mL)	18 lids GL 45

Unit C – BPC Core Unit

2 BPC Core	72 check valves	2 main unit power adapters
72 flow cell units (FCUs) 9 mL FCUs (36 units) 2 mL FCUs (36 units)	1 plastic syringe	2 ethernet cables

Additional Components

1 Festo tubing 50 m	72 push-in connectors 6 mm	1 bottle/tube opening tool
1 funnel	12 soft binders 7/180 mm	FCU volume sheet
18 tubing stoppers	36 multi-coloured marker clamps	

For GE III Light DUO, the package comes with one BPC Core Unit with 18 active channels for both total and single gas measurements (**Figure 4**).

Figure 4. GE III Light DUO complete system.

Unit A – Incubation Unit

9 glass reactors (2 L)	1 motor controller signal cable	1 MCU power adapter
9 brushless DC motors 1 motor power splitter	9 axis couplings for brushless DC motors	1 thermostatic water bath 1 base tray

10 brushless DC motor cables 250 mm (6 units) 500 mm (3 units) 1500 mm (1 unit)	9 stirrers GL 45 (2 L) standard	1 thermostatic water bath lid for 9 reactors (2 L)
	1 motor controller unit (MCU)	9 push-in valves 6 mm

Unit B – CO_2-absorption Unit

1 bottle holders 9 x 250 mL	9 bottle nuts GL 45
9 glass bottles (250 mL)	9 lids GL 45

Unit C – BPC Core Light Unit

1 BPC Core	36 check valves	1 main unit power adapter
36 flow cell units (FCUs) 9 mL FCUs (18 units) 2 mL FCUs (18 units)	1 plastic syringe	1 ethernet cable

Additional components

1 Festo tubing 50 m	18 push-in connectors 6 mm	1 bottle/tube opening tool
1 funnel	6 soft binders 7/180 mm	FCU volume sheet
9 tubing stoppers	18 multi-coloured marker clamps	

2.3 GE III Animal Nutrition

In the realm of rumen nutrition, *in vitro* rumen incubation analyses have long been employed for feed evaluation, originally using manual pressure-based gas measurement methods, which are both time- and labor-intensive. In the 1970s, the correlation between fermentation gas accumulation and the feed's metabolizable energy content was established. Subsequently, *in vitro* gas production techniques have evolved, but many early studies still relied on manual methods.

For non-ruminant animals, the link between *in vitro* gas production and feed nutritive qualities is gradually emerging. Automated systems based on volumetric gas measurements offer advantages such as minimizing human error, reducing workload demand, enhancing precision and accuracy, and standardizing data processing. This automated approach facilitates continuous monitoring of gas production in high-throughput *in vitro* digestibility tests.

Applications include:

- Continuous monitoring for process kinetic information extraction
- Determination of feed digestibility and metabolizable energy content
- Optimization of feed composition for livestock
- Exploration of feed additives or supplements to enhance fermentation

- Study of functional components with potential effects on methane emissions and organic matter digestibility *in vivo*
- Screening numerous feeds or additives before *in vivo* testing
- Comparison of different pre-treatments of feed compounds

The GE III Animal Nutrition configuration is equipped with the following items (**Figure 5**):

Figure 5. GE III Animal Nutrition System.

Unit A – Incubation Unit

18 glass reactors (250 mL)	Shaking thermostatic water bath	18 push-in valves 6 mm

Unit B – CO_2-absorption Unit

2 bottle holders 9 x 250 mL	18 bottle nuts GL 45
18 glass bottles (250 mL)	18 lids GL 45

Unit C – BPC Core Unit

1 BPC Core AMPTS	36 check valves	1 main unit power adapter
36 flow cell units (FCUs) 9 mL FCUs (18 units) 2 mL FCUs (18 units)	1 plastic syringe	1 ethernet cable

Additional Components

1 Festo tubing 50 m	36 push-in connectors 6 mm	1 bottle/tube opening tool
1 funnel	12 soft binders 7/180 mm	FCU volume sheet
18 tubing stoppers	36 multi-coloured marker clamps	

2.4 GE III Max

Gas Endeavour MAX offers a complete set of essential equipment and accessories necessary for conducting experiments under both anaerobic and aerobic conditions, whether in batch or continuous mode (**Figure 6**).

Figure 6. GE III Max Complete System.

Unit A – Incubation Unit

18 glass reactors (1 L) 9 CSTR-2G reactors (2 L)	1 motor controller signal cable	1 MCU power adapter 1 motor controller unit (MCU)
18 brushless DC motors 1 motor power splitter	18 axis couplings for brushless DC motors	1 thermostatic water bath 1 base tray
19 brushless DC motor cables 250 mm (15 units) 500 mm (3 units) 1500 mm (1 unit)	18 stirrers GL 45 (1 L) GAA 18 stirrers GL 45 (1 L) Standard 9 stirrers GL 45 (2 L) 18 push-in valves 6 mm	1 thermostatic water bath lid for 18 reactors (1 L) 1 thermostatic water bath lid for 9 reactors (2 L)

Unit B – CO_2-absorption Unit

18 Gas Absorption attachment (GAA) units	4 funnels for GAA
2 bottle holders 9 x 250 mL	18 bottle nuts GL 45
18 glass bottles (250 mL)	18 lids GL 45

Unit C – BPC Core Unit

2 BPC Core	72 check valves	2 main unit power adapters
72 flow cell units (FCUs) 18 flow cell units (9 mL) 18 flow cell units (2 mL)	1 plastic syringe	2 ethernet cables

Additional components

2 Festo tubing 50 m	72 push-in connectors 6 mm	1 bottle/tube opening tool
1 funnel	12 soft binders 7/180 mm	3 gasbags
18 tubing stoppers	36 multi-coloured marker clamps	FCU volume sheet
1 Gas distribution Manifold 3 Mk 2	3 Gas distribution Manifolds 6 Mk 2	

GE III configurations consist mainly of three parts: Incubation unit, CO_2-absorption unit and detection unit (also called gas volume measuring device).

1) **<u>Incubation Unit (Unit A)</u>**: Here is where the fermentation process takes place (**Figure 7**). 18 (0.5 or 1 L) or 9 (2 L) reactors are placed inside a thermostatic water bath (68 x 56 x 33 cm), which is used for temperature control (up to 60° C, precision of 0.2° C). The thermostatic water bath is provided with a lid for 18 or 9 reactors to minimize water loss and ensure that the temperature set point is rapidly reached.

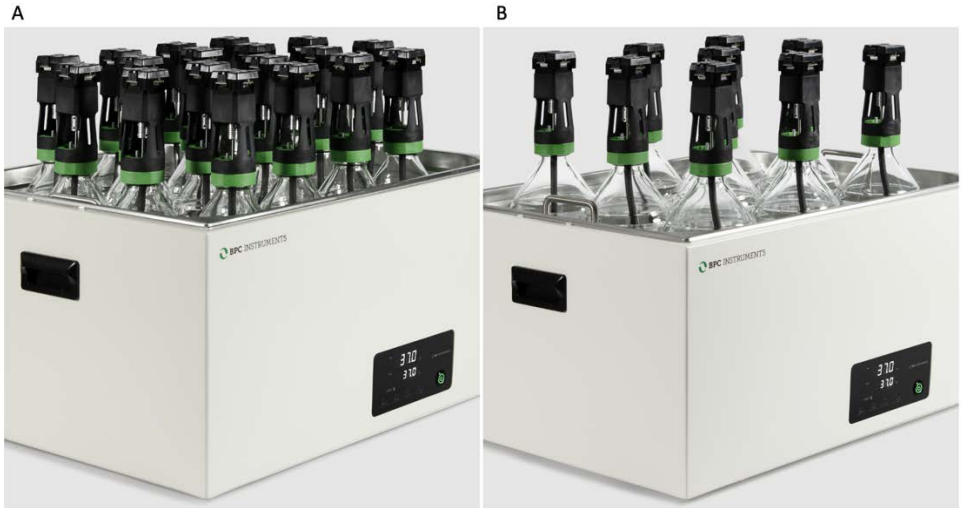


Figure 7. (A) Incubation unit for GE III standard and (B) GE III Standard Light.

Each reactor is connected to a brushless DC motor that ensures a well-mixed content through a strong, reliable, and multifunctional agitation system (**Figure 8**). A motor controller provides the power via motor cables, where all the motors receive the same information. **The MCU needs to be turned off and unplugged from the power source when any cables are connected or disconnected from the motors.**

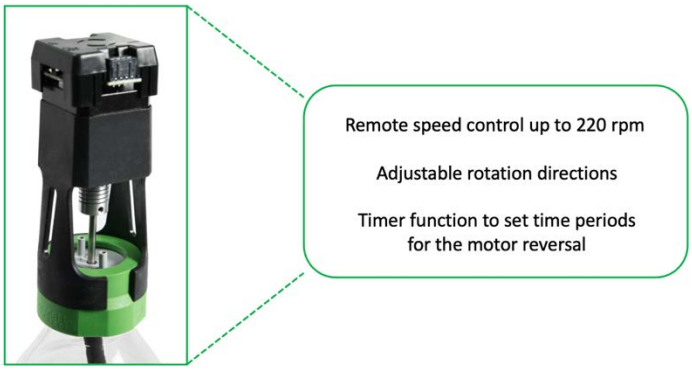


Figure 8. Main features of the agitation system.

The incubation unit for the GE III Animal Nutrition configuration is simplified, with 18 (250 mL) reactors placed inside a shaking thermostatic water bath, eliminating the need for motors. (**Figure 9**).

Figure 9. Incubation unit for GE III Animal Nutrition.

To conduct continuous fermentation tests with the GE III Max configuration, nine CSTR-2G units are used (**Figure 10**).

Figure 10. Incubation unit for GE III Max designed for conducting continuous fermentation experiments.

2) **<u>CO_2-absorption Unit (Unit B)</u>**: Different amounts of biogas is generated during the AD process based on the content of the reactors (blank, positive control, negative control, and samples). Biogas is mainly made of methane (CH_4) and carbon dioxide (CO_2). The unit B is where the CO_2 and traces of hydrogen sulphide are removed through a chemical reaction with a 3 mol/L NaOH solution (**Figure 11**). ***The absorption efficiency is higher than 98% even at high flow rates.***

Figure 11. Bottler holder designed for 9 glass bottles (250 mL). AMPTS III is equipped with two bottle holders.

Carbon dioxide reacts with sodium hydroxide in an acid-base reaction to generate sodium carbonate and sodium bicarbonate:

$$2\ NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$
$$Na_2CO_3 + CO_2 + H_2O \rightarrow 2NaHCO_3$$

In this case, only methane will reach the detection unit (single gas measurements). If the test aims to measure biogas, the Unit B is not needed, where the setup should present the following configuration (**Figure 12**).

Figure 12. Setup for total gas (e.g., biogas) measurements.

A pH indicator, Thymolphthalein, is used to keep track of the capacity of the alkaline solution to retain CO_2. Since a high concentration of a strong base is used, the initial pH is 14. When CO_2 is added, NaOH is consumed and the pH decreases. This pH indicator changes from blue to colorless around pH 9 (**Figure 13**). In this pH, the capacity of NaOH to remove the CO_2 is reduced, indicating that the solution needs to be changed.

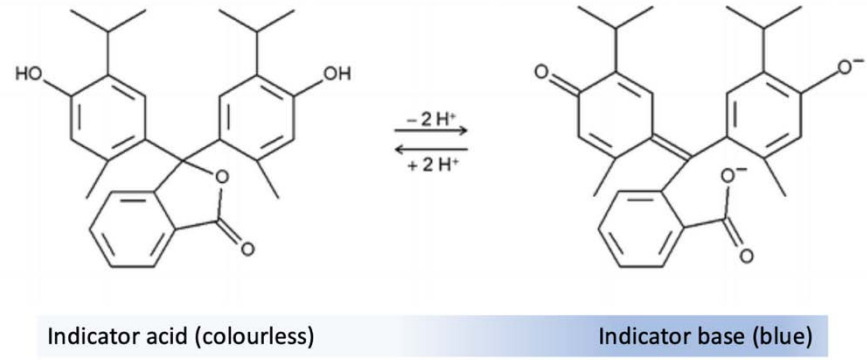


Figure 13. Structure of Thymolphthalein depending on the pH.

NOTE: For most applications it is not necessary to submerge the tubing inside the alkaline solution to remove CO_2.

For tests conducted under aerobic conditions, the *in-situ* setup is utilized (**Figure 14**). In this configuration, the Gas Absorption Attachment (GAA) unit, which is attached to the reactor, contains the alkaline solution. The absorption of CO_2 by the sodium hydroxide solution generates negative pressure within the reactor, resulting in an inflow of oxygen from the gas bags towards the reactor.

Figure 14. Setup for tests perform under aerobic conditions.

3) **<u>BPC Core Unit (Unit C)</u>**: Here the gas is recorded and processed. The BPC Core Unit consists of 18 or 9 flow cell units for simultaneous gas volume detection from 18 or 9 independently operating reactors (**Figure 15**). Unit C comes with an OLED screen which will display various information, including IP address of the instrument, version of the software, current environmental parameters (temperature and pressure), and alignment indicator. The information can be accessed through two physical buttons located below the screen. The detection unit is equipped with an USB port for software upgrades and possible new applications and a power button.

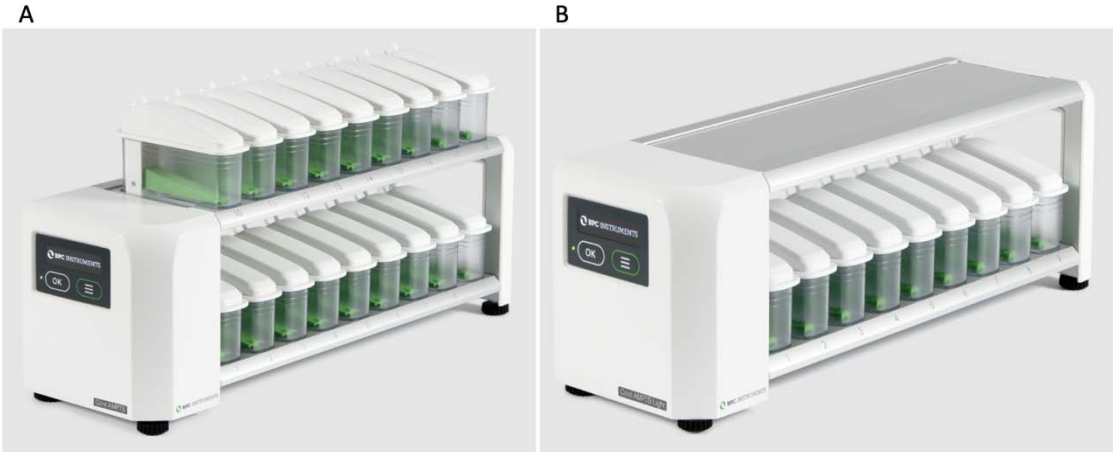


Figure 15. (A) Gas volume measuring device for GE III and (B) GE III Light.

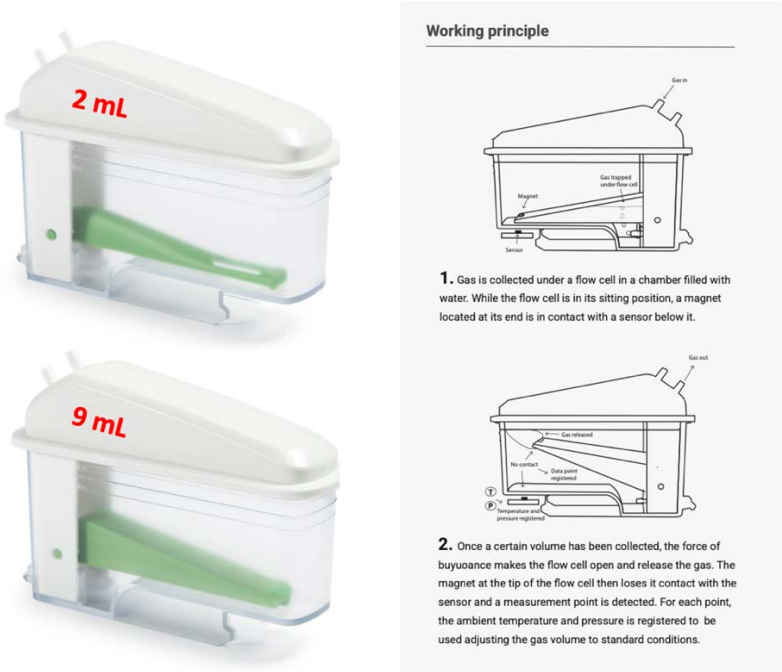


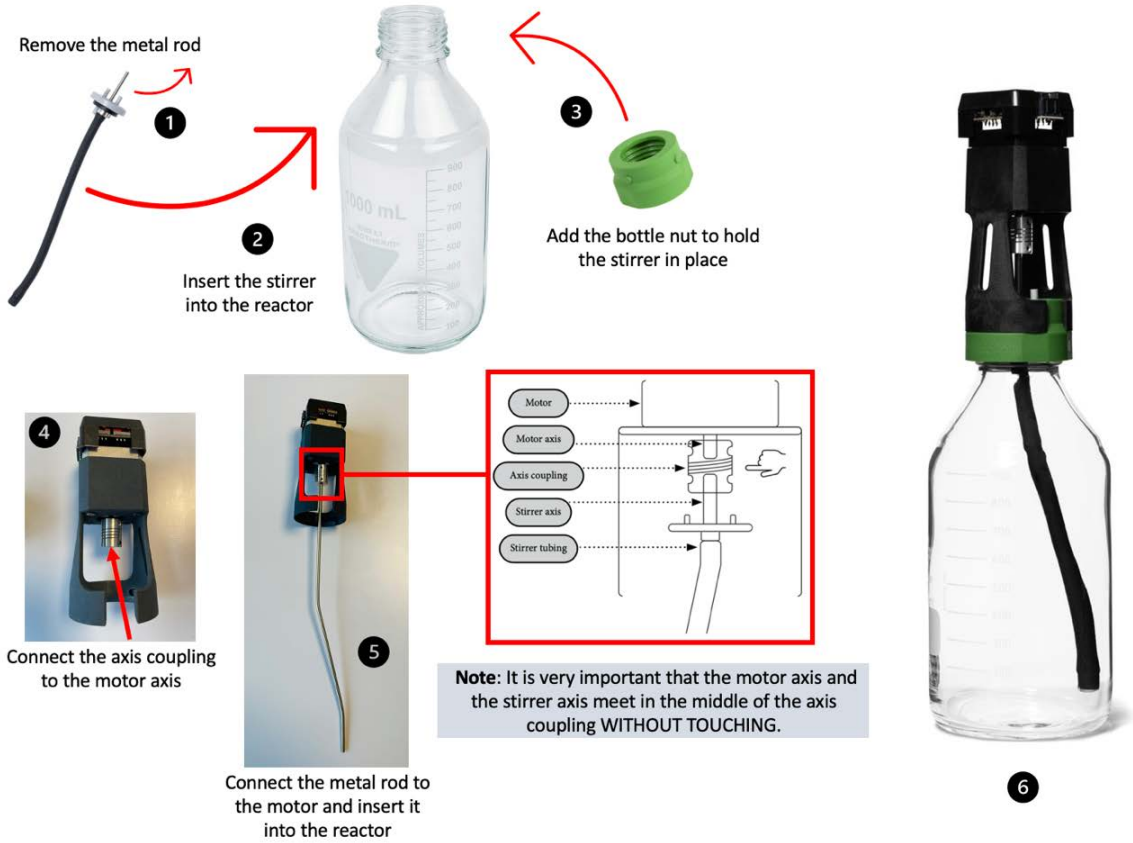
Figure 16. 2 and 9 mL FCUs and working principle.

The FCU has 2 possible resolutions, 9 mL and 2 mL for low and ultra-low gas measurements. ***The operational principle is based on liquid displacement and buoyance***. When a certain gas volume enters the flow cell unit, the force of buoyancy leads the flow cell to open and releases the entrapped gas (**Figure 16**). The system counts each flow cell opening and registers the temperature and pressure for automatic compensation to normalise the flow rate and volume to standard conditions.

Chapter 04: Setting up the Instrument

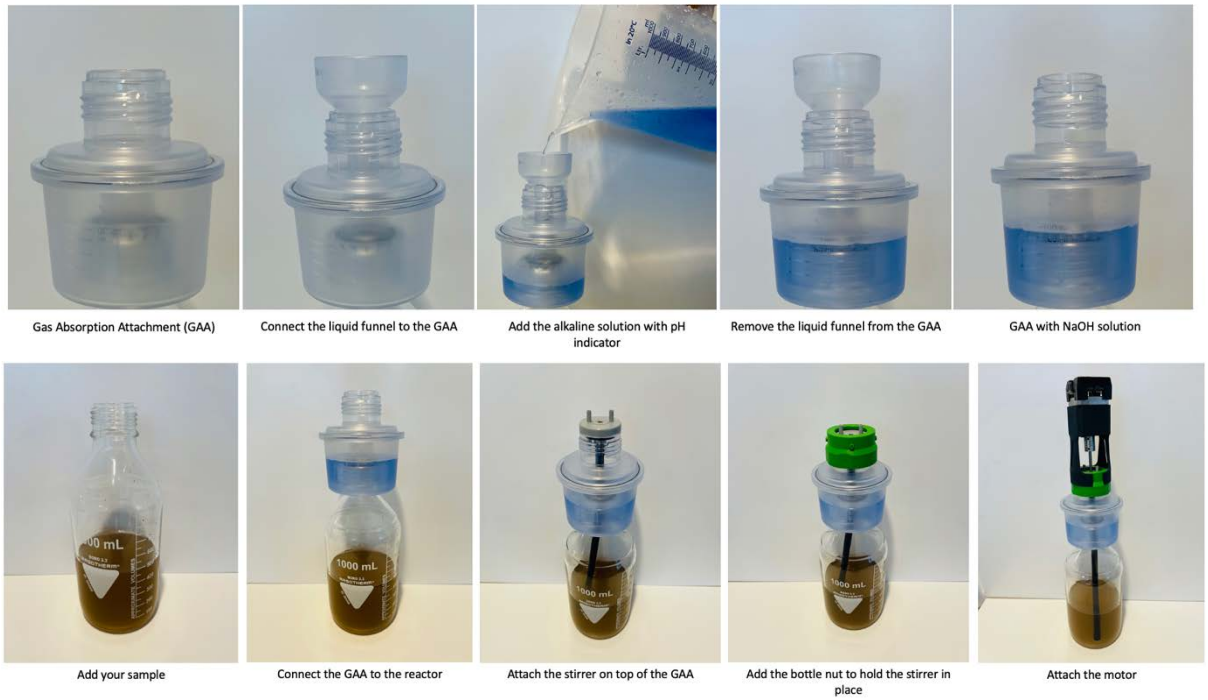
In order to install the system properly, the steps described below need to be followed:

Step 1: Unpack the instrument and place the thermostatic water bath on a flat and stable surface.
Step 2: Remove the blue plastic protection on the thermostatic water bath lid, mount the legs and handles and place the lid on top of the thermostatic water bath.
Step 3: Fix the reactors after adding the samples by following the instructions below:



For applications under aerobic conditions:

Before fixing each reactor according to the illustration above, attach the Gas Absorption Attachment (GAA) to the reactor and add the sodium hydroxide solution with pH indicator as described below*:

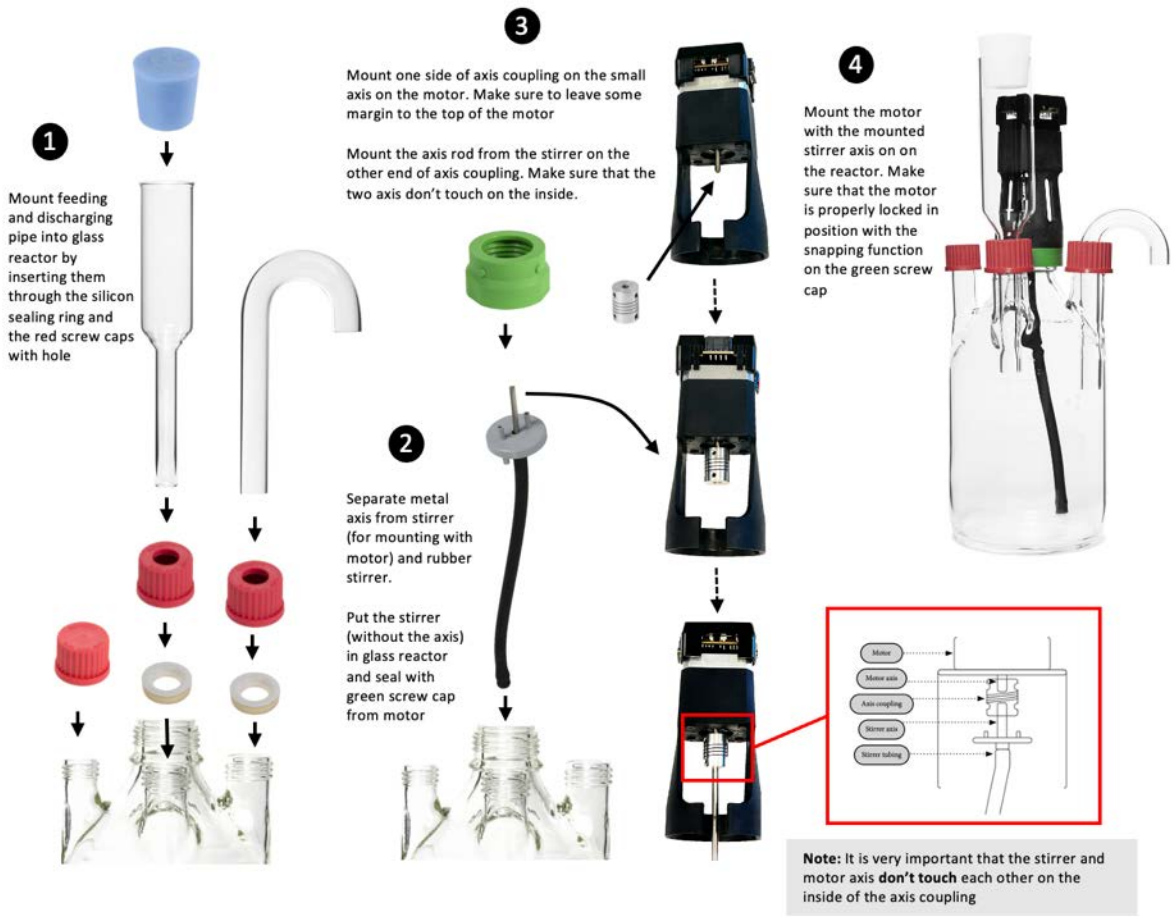


* Illustration using the previous GAA unit. Same procedure for the new configuration.

NOTE: Make sure that the GAA is very well attached to the reactor in order to avoid gas leakage.

Next, fill up the gas bags using an oxygen cylinder equipped with a pressure regulator:

For applications using the CSTR-2G:



Step 4: Place the reactors inside the thermostatic water bath. Add a piece of tubing to the push-in valve and attach it to one of the 2 ports on the lid (the other one is the gas outlet).

A funnel is provided with the instrument to facilitate the introduction of solid samples inside the reactor. Once all reactors are in place, distilled/deionised water needs to be added into

the thermostatic water bath until the recommended level by using the designated hole on the thermostatic water bath lid.

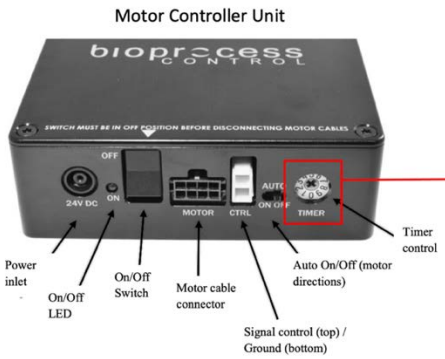
In the thermostatic water bath, the temperature is controlled by a simple and intuitive digital interface. The instrument contains 2 LED screens: the first one displaying the current temperature (PV) and the second one showing the desired temperature (SV). Press the button ***SET*** to determine SV. The desired temperature can be easily selected by using the shift digit, increase and decrease buttons. When the button ***SET*** is pressed, the last digit of the value displayed in SV blinks, indicating that the respective digit can be changed by pressing the buttons increase (∧) and decrease (∨). If there is a considerable difference between the current and desired temperature in SV, the user can use the shift digit button (R/S) to select the first digit and quickly go from 30 to 60°C, for example. Once the desired temperature is selected, press the button ***SET*** again to save the value. Once the desired temperature is established, the instrument will heat the water until PV and SV reach the same values. When the light ***HEAT*** is on, it is an indication that the instrument is on heating mode. When the temperature in PV is close to reach the SV, 2° C from SV, the light ***HEAT*** will start blinking until the desired temperature is achieved.

NOTE: Check periodically the water level inside the thermostic water bath.

Step 5: Fixing the agitation system for GE III. Connect the short motor cables (250 mm) in series by attaching one short motor cable to each motor (excluding the last motor in the chain), and then connecting the free end of the cable from motor 1 to the free port on motor 2 and so on until motor 9. Repeat the same operation for the remaining nine motors, creating two groups of motors connected to each other through short cables. After that, connect the 500 mm motor cable from the last motor in the chain of each group to the motor power splitter. Finally, use the 1500 mm motor cable to connect the power splitter to the MCU. The last step is to connect the signal cable from the motor controller to the detection unit.

NOTE: Make sure that the power adapter for the motor controller is disconnected from the power supply when inserting or removing the motor cables.

From the motor it is only possible to control the direction of the mixing, clockwise (CW), AUTO and counterclockwise (CCW). If users select AUTO, the motor controller will dictate the change of direction at certain intervals using the timer, if the motor controller's AUTO is ON. Since the signal cable connects the motor controller to the unit C, the phase (single or double) and speed can be controlled using the software (see page 33).



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

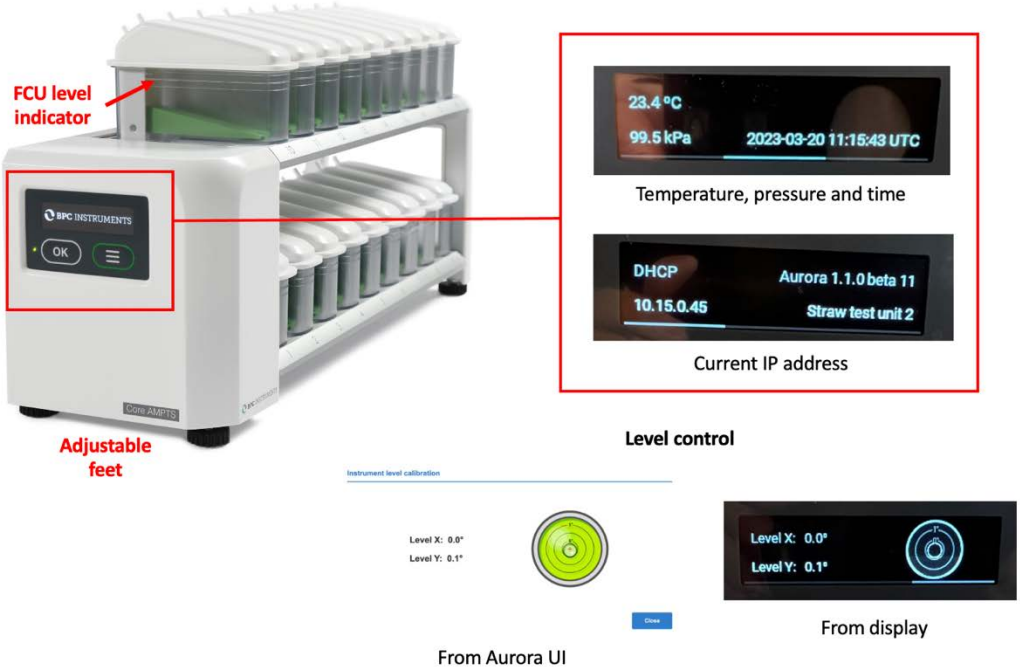
Step 6: Prepare 3 mol/L NaOH solution. The 250 mL bottles should be filled with 200 mL of the alkaline solution which makes it necessary to add 3.6 L and 1.8 L of 3 mol/L NaOH for 18 and 9 glass bottles, respectively. After weighing 432 g (for 18 bottles) or 216 g (for 9 bottles) of NaOH pellets, mix it with approximately 3⁄4 of the required total volume of distilled water. The heat generation following dissolution of NaOH in water is high, it is therefore recommended to add small amounts of supplementary water followed by mixing. When the NaOH is completely dissolved, add the remaining amount of water until the total volume (3.6 or 1.8 L) is reached. Prepare 0.4% Thymolphthalein by dissolving 40 mg in 9 mL ethanol followed by 1 mL of water. Mix it with 3 mol/L NaOH solution. After adding 200 mL of 3 mol/L NaOH solution with pH indicator in each bottle, place them on the bottle holder.

Step 7: Add distilled water inside the FCU and place them on the detection unit.

Each FCU has an inlet and outlet for the gas. Use the syringe to introduce water through the inlet of the FCU

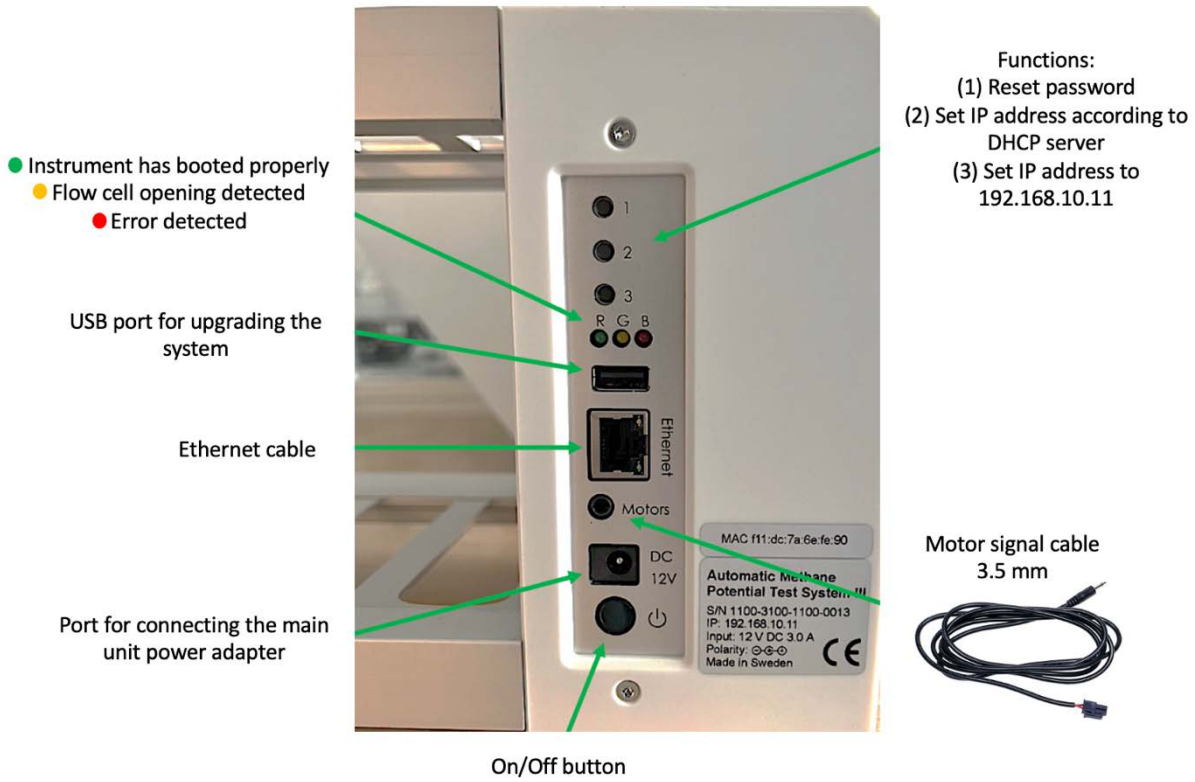
The water should be added until the recommended level

Make sure that each FCU is perfectly seated (horizontally levelled) on the BPC Core Unit. It can be easily observed by checking the **FCU level indicator** and the level control from the software or the OLED screen as shown below. **Adjustable feet** are used to level the instrument.



The BPC Core Unit is equipped with an OLED screen that provides information regarding the status of the instrument (environmental conditions, time, current IP address used by the instrument and alignment indicator). This can be accessed through two physical buttons located below the screen.

Backside of the BPC Core Unit:



Step 8: Cut 36 (GE III Standard) or 18 (GE III Standard Light) pieces of festo tubing sufficient in length to connect the gas outlet of each reactor to the corresponding alkaline solution bottle (Unit B) and then a second piece to couple the glass bottle with NaOH solution to the FCU. In order to simplify this procedure, use push-in connectors (two per channel) provided with the instrument. To avoid water flowing back from Unit C to Unit B, check valves can be inserted close to the inlet of each FCU in order to the target substance flow in one direction only.

NOTE: If the user wants to flush the reactors to create the anaerobic environment, disconnect the tubing from the CO_2-absorption unit before to eliminate the risk of damaging the external check valves (if used) by the high-pressure gas flow.

Step 9: Use the softer binder to group tubing of channels that are related to each other. If the test is conducted in triplicate, it is possible to put together six groups with three channels (tubing) each based on the content of the reactors. In order to easily identify each channel, add the multi-coloured marker clamps (two per channel) provided in different colors. Mark each channel by writing the proper description using a ballpoint pen, water-based pen, marker, etc. After the experiment, users can wipe the label off with a wet tissue and reuse them.

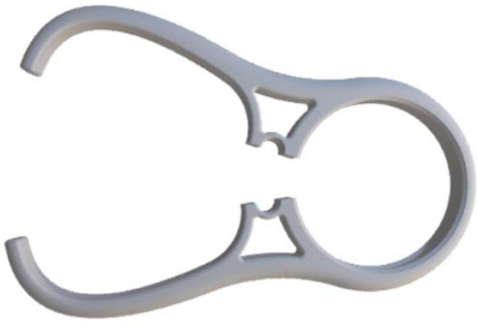
Soft binder 7/180 mm

Multi-coloured marker clamps

Step 10: In order to start the test, access the software following the instructions in Chapter 5 of this manual. Add the parameters required for the test and make sure that the status of each channel is classified as **ready** before starting the gas measurements. When using the instrument for the first time, the cell volume of each channel needs to be entered in the control page. A calibration sheet is delivered with the equipment, where the serial number of the flow cell unit is listed together with its calibrated volume. It is important to know where each flow cell unit will be placed on the system, so that the calibrated values can be entered in the right position.

NOTE: Testing the detection unit and software. Before starting an experiment, simulate a flow cell opening by manually removing each FCU and put it back in numerical order from 1 to 18 (repeat this procedure three times). Follow the corresponding result of each opening on the plots in the graph page of the software to make sure that both the detection unit and data acquisition system function properly.

Step 11: Once the experiment is finished, generate and download a report in the report menu of the software. Turn off the thermostatic water bath and the motors, including the motor controller. Next, unplug the power adapters (for the Motor Controller and the Gas Volume Measuring Device) from the power source. Disconnect the tubing and remove the lids from the reactors and CO_2 removal bottles after the tests. These components are often difficult to remove after the experiments, since they sit very tightly after usage, specially the tubing to the different connectors. GE III is equipped with a bottle/tube opening tool to easily remove lids and tubing without the risk of damaging them and the connectors.

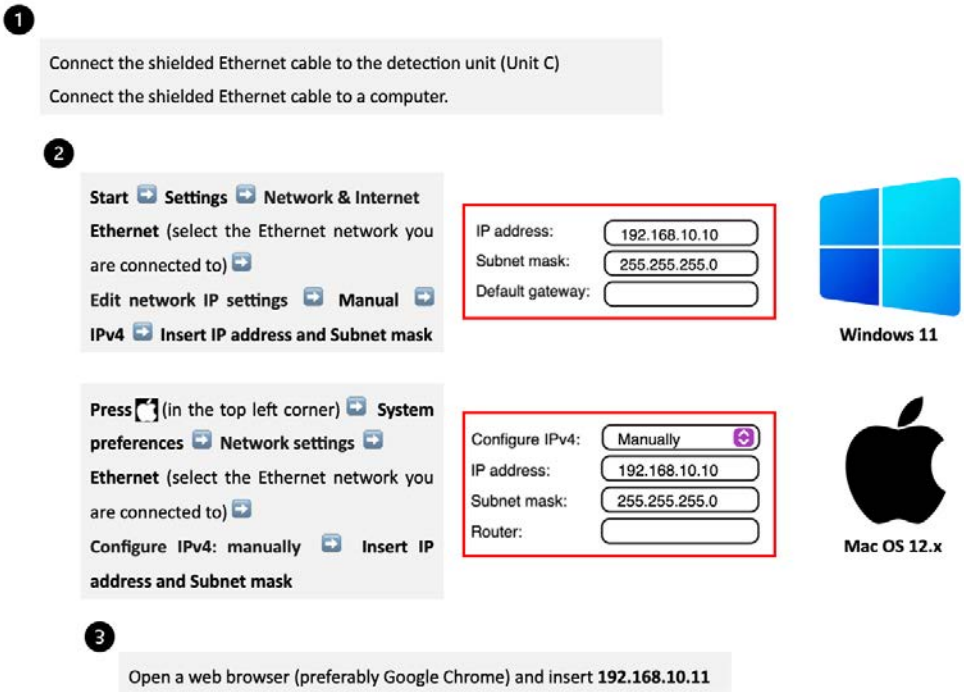


Bottle/tube opening tool

Chapter 05: GE III Web-based Software

5.1 Computer Network Configuration

In order to have access to the web-based software Aurora™, the following steps need to be followed (this procedure is described considering the most common operating system setups – **Windows 11** and **Mac Os 12.x**):



5.2 Network Quick Guide

	Instrument	Computer
IP address	192.168.10.11	192.168.10.10
Subnet mask	255.255.255.0	255.255.255.0
Default gateway / Router	Leave empty	Leave empty

NOTE: IP address for the computer and the IP address for the GE III 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. When using multiple BPC Core units, make sure that each unit has its own IP address. This can be achieved by changing the last digit of the IP address.

The OLED screen in the detection unit will display whether the instrument uses a manually assigned IP or an automatic IP assigned by a DHCP server as well as what IP address is currently in use and the version of the instrument's Aurora software. In this way, it is simple and easy to connect the instrument to a computer or network.

5.3 Web-based Software Aurora™

After inserting the default IP address of the instrument, users will have access to the **Log in** page (**Figure 17**). In the Log in page, the default password **bpc** needs to be added.

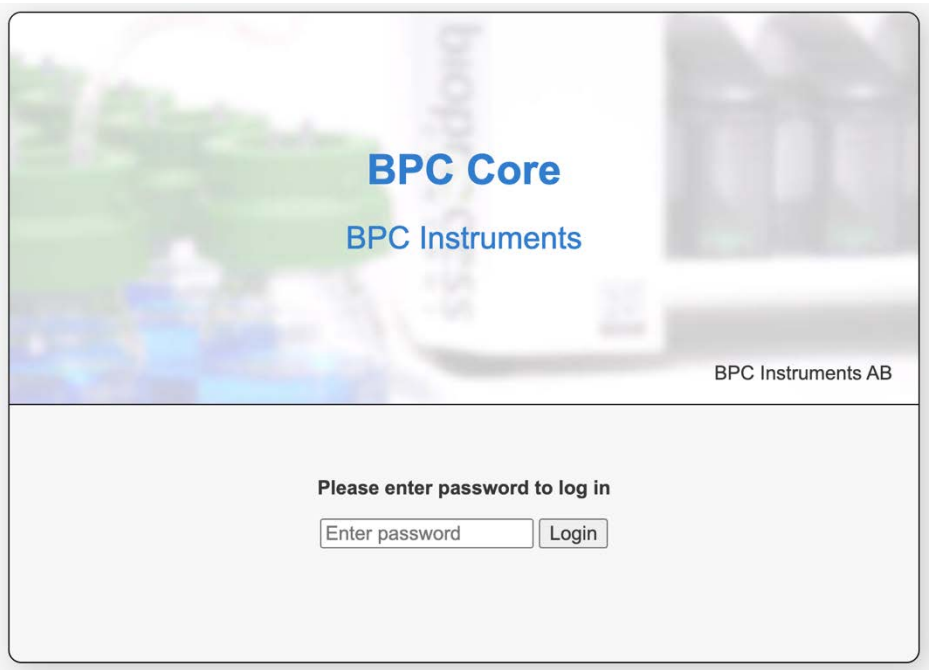


Figure 17. Log in page for GE III System.

On the **Home** page, users get an overview of the features of the software, where the topics are ordered according to experimental setup, execution, monitoring and finally documented. In addition, three useful links are provided together with the Log out function (**Figure 18**).

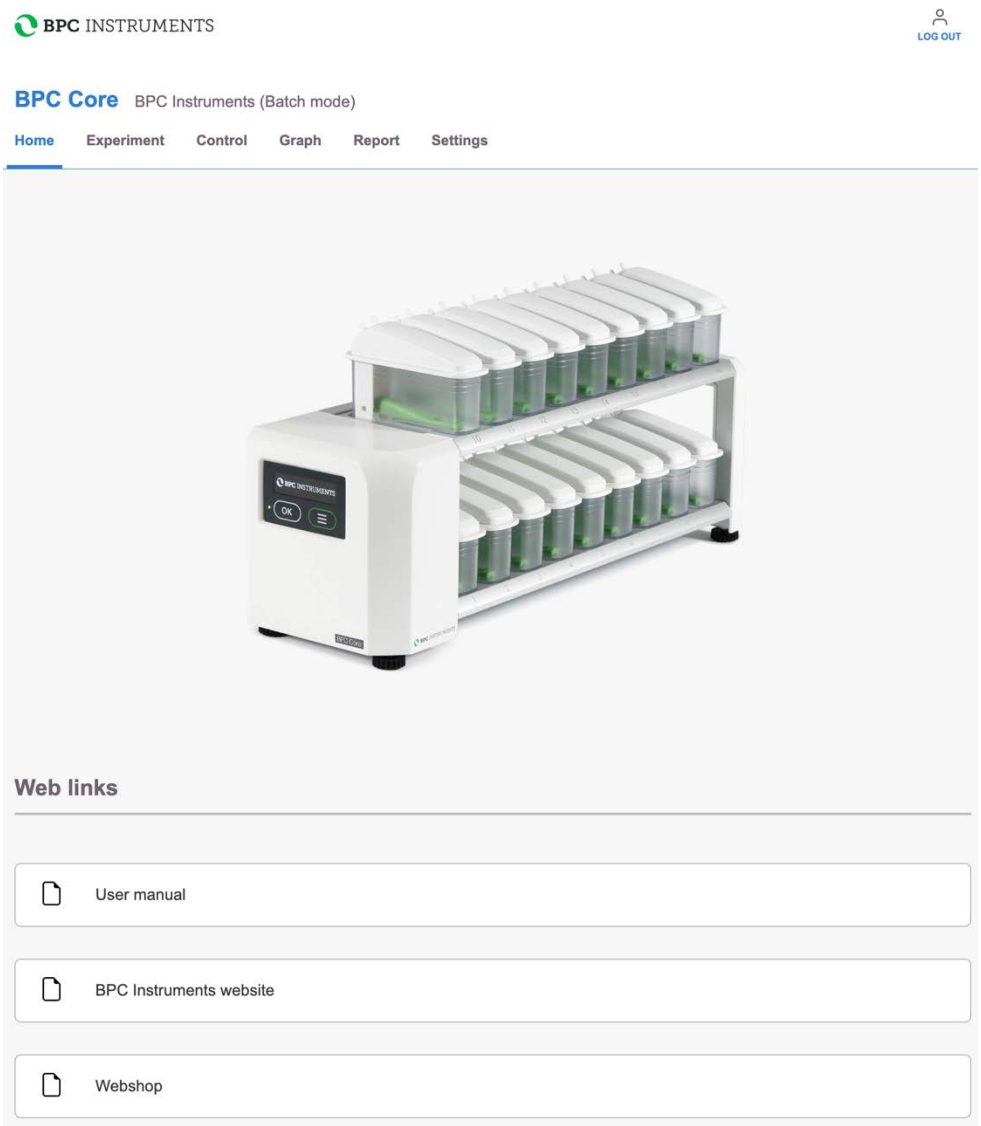


Figure 18. Home page for GE III.

The loading time of this new software is significantly shorter than the previous version. Additionally, several new features were implemented to offer a better user experience.

GE III offers two operating modes: batch and continuous. The transition between these modes is flexible, allowing for changes at any point, even during the experiment's runtime. This adjustment can be made within the System Settings tab under the Experiment type session (**Figure 19**).

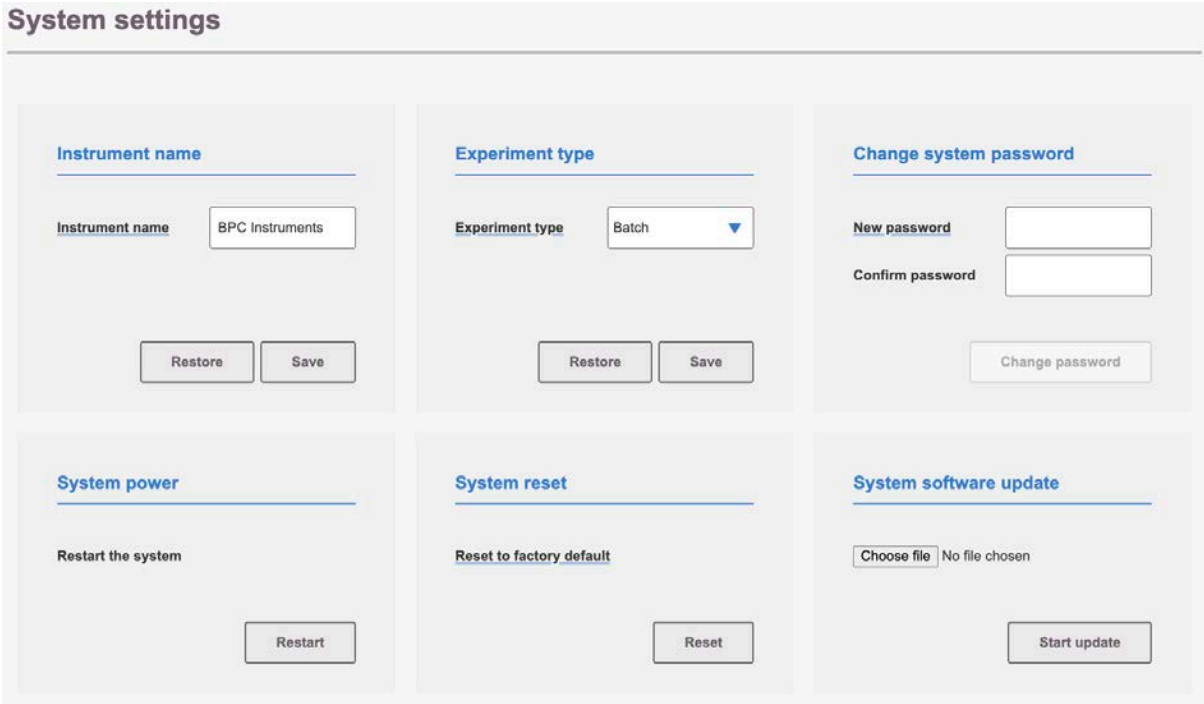


Figure 19. Switching between Continuous and Batch modes within the System Settings tab.

5.3.1 Batch Mode

On the **Experiment** page for GE III (the same description holds true for GE III Light, including 9 channels), 18 lines are related to the 18 reactors where each one of them should be properly labelled based on its content. Within this page, utilizing the "Print Experiment Guidelines" function allows users to directly print or save the page in PDF format, containing crucial information about the established parameters for the test. The experiment setup can be configured in two modes: calculated or manual (**Figure 20**).

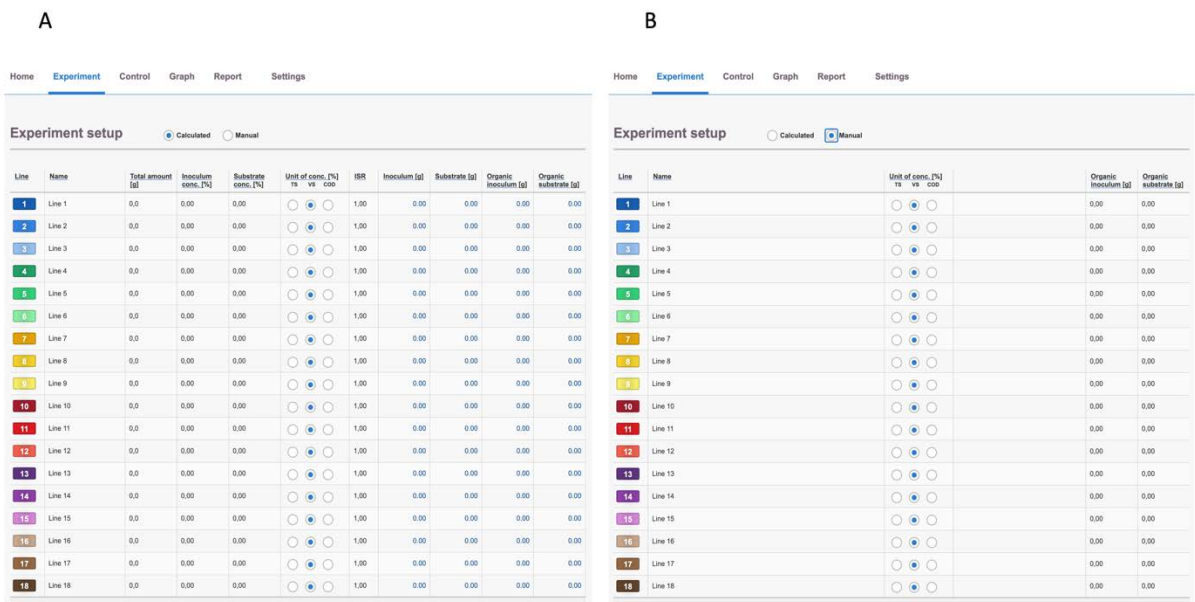
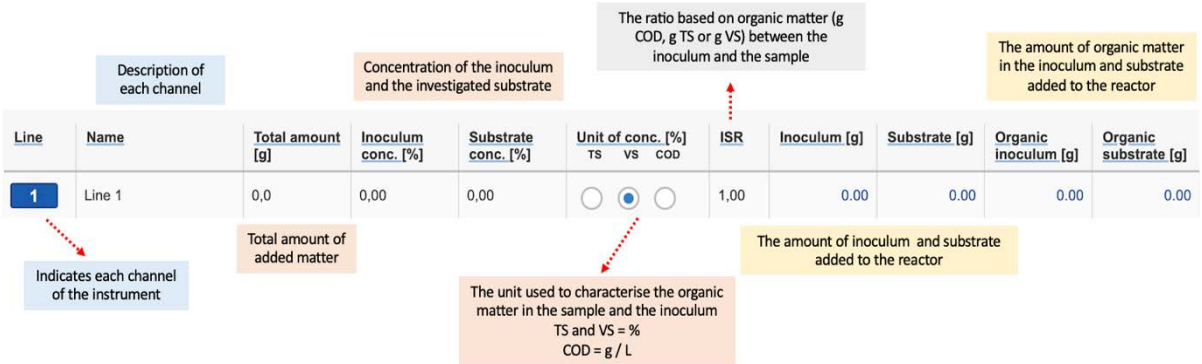


Figure 20. Experiment setup (A) Calculated and (B) Manual for GE III.

If the *calculated function* is activated, the following parameters must be considered for each line:



The *manual function* allows more set-up flexibility - information regarding the amount of organic matter in the inoculum and substrate is directly entered.

The **Control** tab is where direct interaction with the channels and motors are conducted (**Figure 21**). Using the Aurora^{TM} software, it is possible to start and stop all or just selected channels.

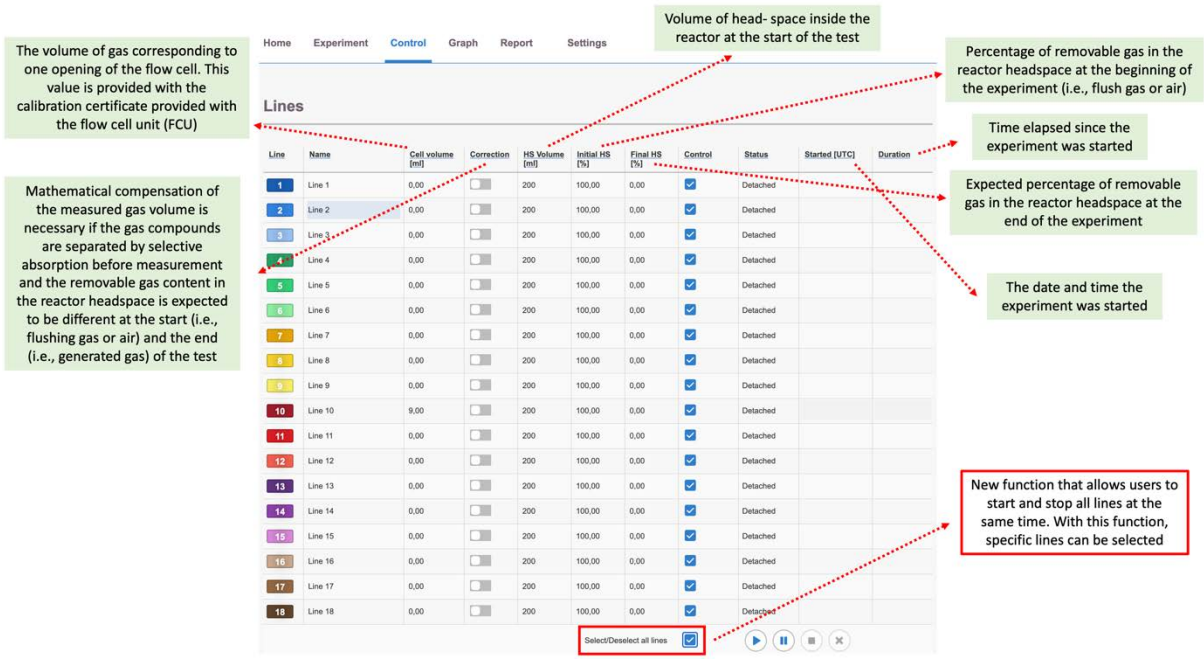
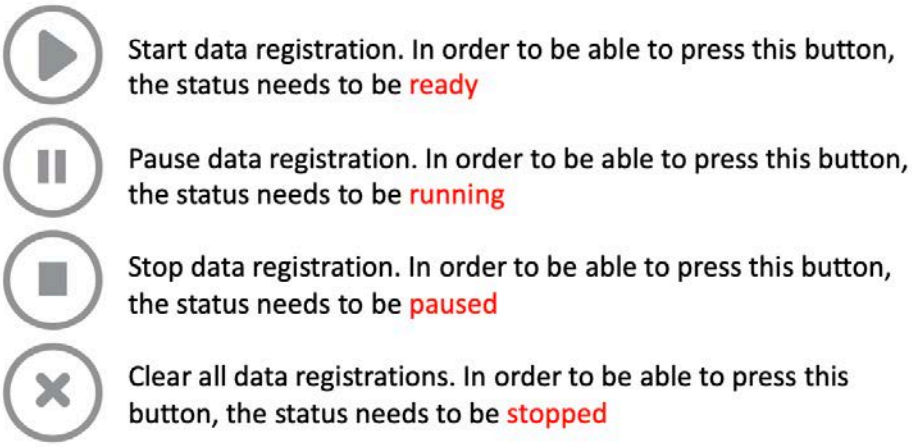


Figure 21. Full description of each line of the Control page.

Located at the bottom right of the **lines section** is the control panel for the flow cell units. These work as follows:



Regarding the agitation system, the motors can be controlled in *continuous (single) or two-phase (double)* modes. With the two-phase mode, the motors can run intermittently at two different speeds or as ON and OFF (**Figure 22**).

Figure 22. Controlling the agitation system through the software.

The software provides two graphs, ***accumulated gas volume as a function of time*** and ***flow rate as function of time***. The gas volume can be presented in three different ways: without normalization, with normalization without considering the moisture content (dry), and with normalization considering the moisture content (wet). There are three possible units for the gas flow: NmL / min, NmL / h, and NmL / day.

Once the gas registration starts, the box/line changes color from grayish to the corresponding color of a specific line, see line 10 in **Figure 23**. In this part, it is also possible to select all lines at once.

Figure 23. Gas flow segment in the Graph page.

In this new software, users have the option to zoom in both graphs in order to get a better visualisation of the real-time data.

NOTE: The graph requires at least two data points (i.e., flow cell openings) in order to display information. To simulate an opening during testing, briefly remove the FCU and place it right back; wait a few seconds and then repeat the action one more time. If everything is working properly, a line should appear in each graph.

Users can download and generate the report at any time from the **Report** tab. With Aurora Software, the raw data can be downloaded in a csv format either for each line separately or gathered in one file. Different settings can be applied to generate the report (**Figure 24**).

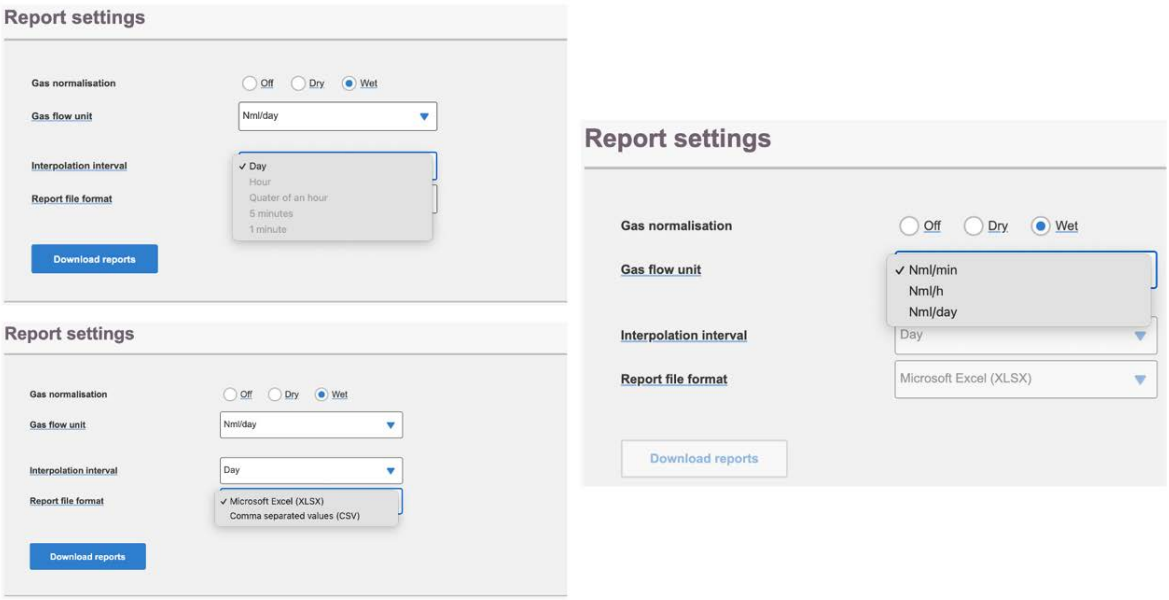


Figure 24. Report settings available in the report page.

The **Settings** page is home to a host of settings for the instrument. It also contains valuable information regarding versions, licenses, a logfile and so forth. Sometimes, a blue triangle with a white exclamation mark will appear next to the settings tab in the Aurora™ software. This symbol indicates that there is information in the logfile that needs to be reviewed or that one of the settings needs to be adjusted. If it is a setting that is in question, that particular setting will also display the same symbol; if it is the logfile, pressing the clear log button (after reviewing and potentially saving the information) will remove the warning.

Two settings are generally described below:

1. **Network settings**: Displays IP and Mac address for the instrument. Also allows for configuring the built-in network adapter using either DHCP or manual configuration.
2. **System warning log**: Contains a list of events registered by the instrument which might be useful when trying to resolve an issue. Please have this information ready when contacting support.

5.3.2 Continuous Mode

The **Feeding** tab is segmented into two sections: the upper section provides an overview of each line's settings, while the bottom section comprises a list of individual feedings recorded (**Figure 25**). It's important to clarify that the feedings in the software serve solely for data recording purposes. This functionality enables users to track gas production in conjunction with operational data within the software. It's crucial to note that the instrument itself does not have the capability to control actual feeding; this responsibility lies with the user, who can perform it either manually or through their custom automatic setup.

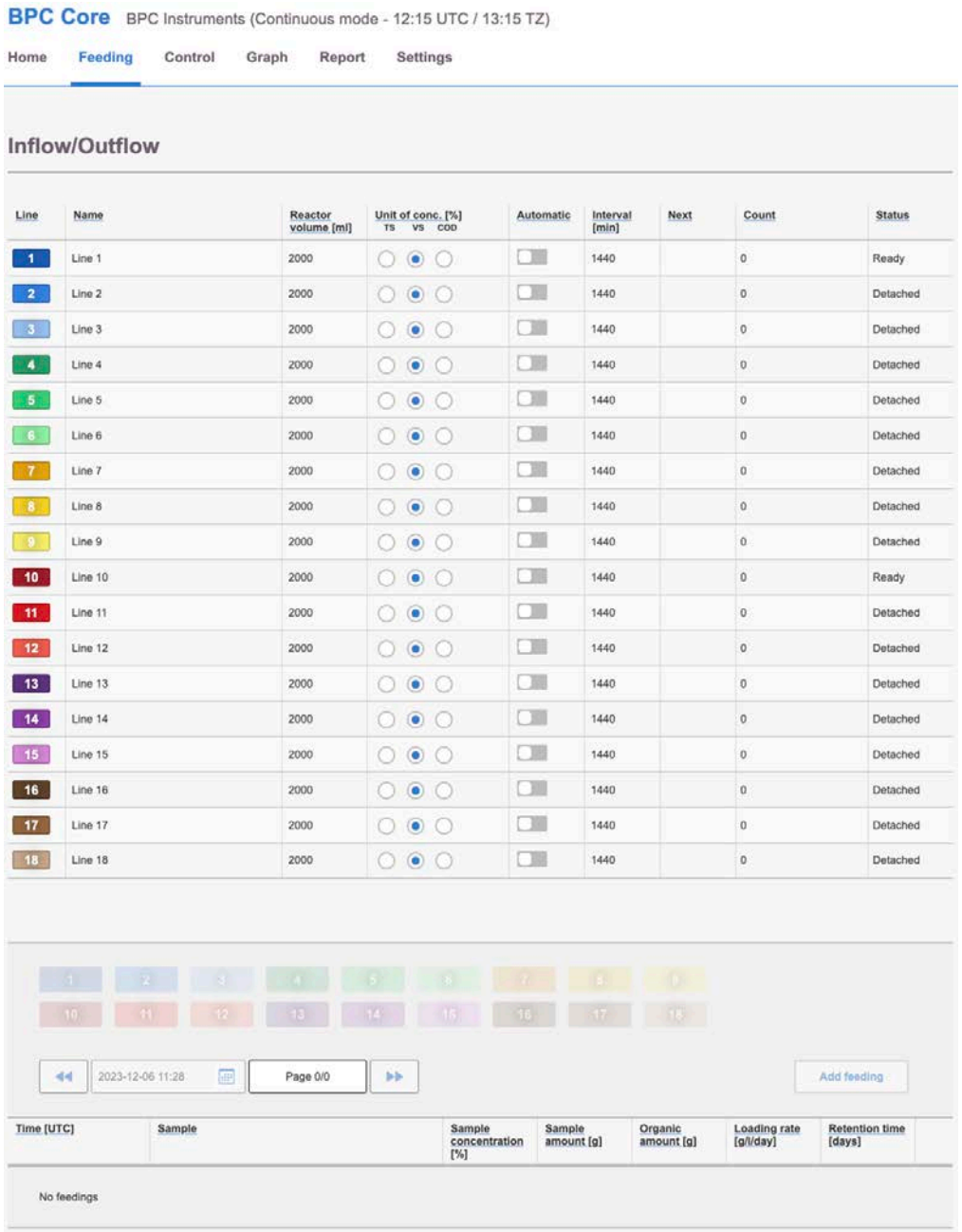
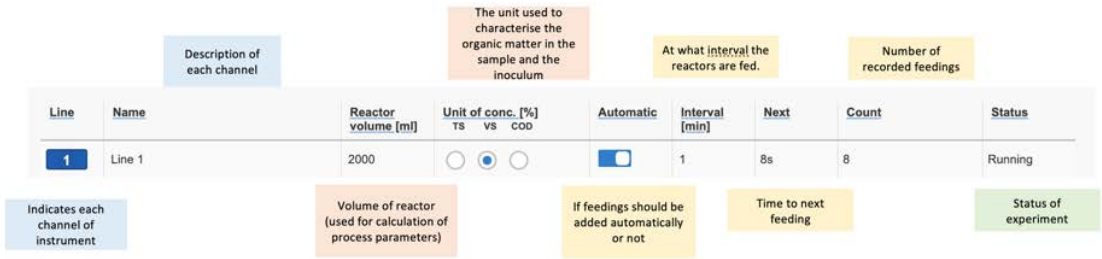
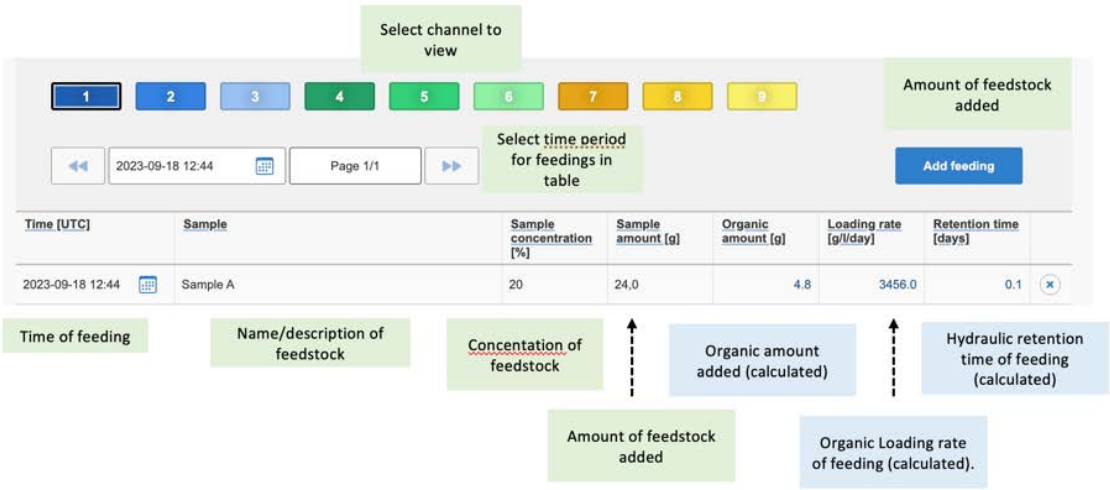


Figure 25. Report settings available in the report page.

The different parameters of the feeding settings are explained below:



Each channel features a list of recorded feedings, accessible in the table at the bottom of the feeding page. Within this table, users can add, remove, and modify already recorded feedings.



The **Control** page follows the same structure presented for batch mode (see pages 32 and 33).

The **Graph** tab presents two graphs, gas flow rate as a function of time and gas flow loading rate (g/l/day) as well as Retention time (days) as a function of time. As for the batch mode, the gas volume can be presented in three different ways: without normalization, with normalization without considering the moisture content (dry), and with normalization considering the moisture content (wet). There are three possible units for the gas flow: Nml /min, NmL /h, and Nml/day (**Figure 26**).

The **Report** and **Settings** pages for continuous mode share the same features found in batch mode.

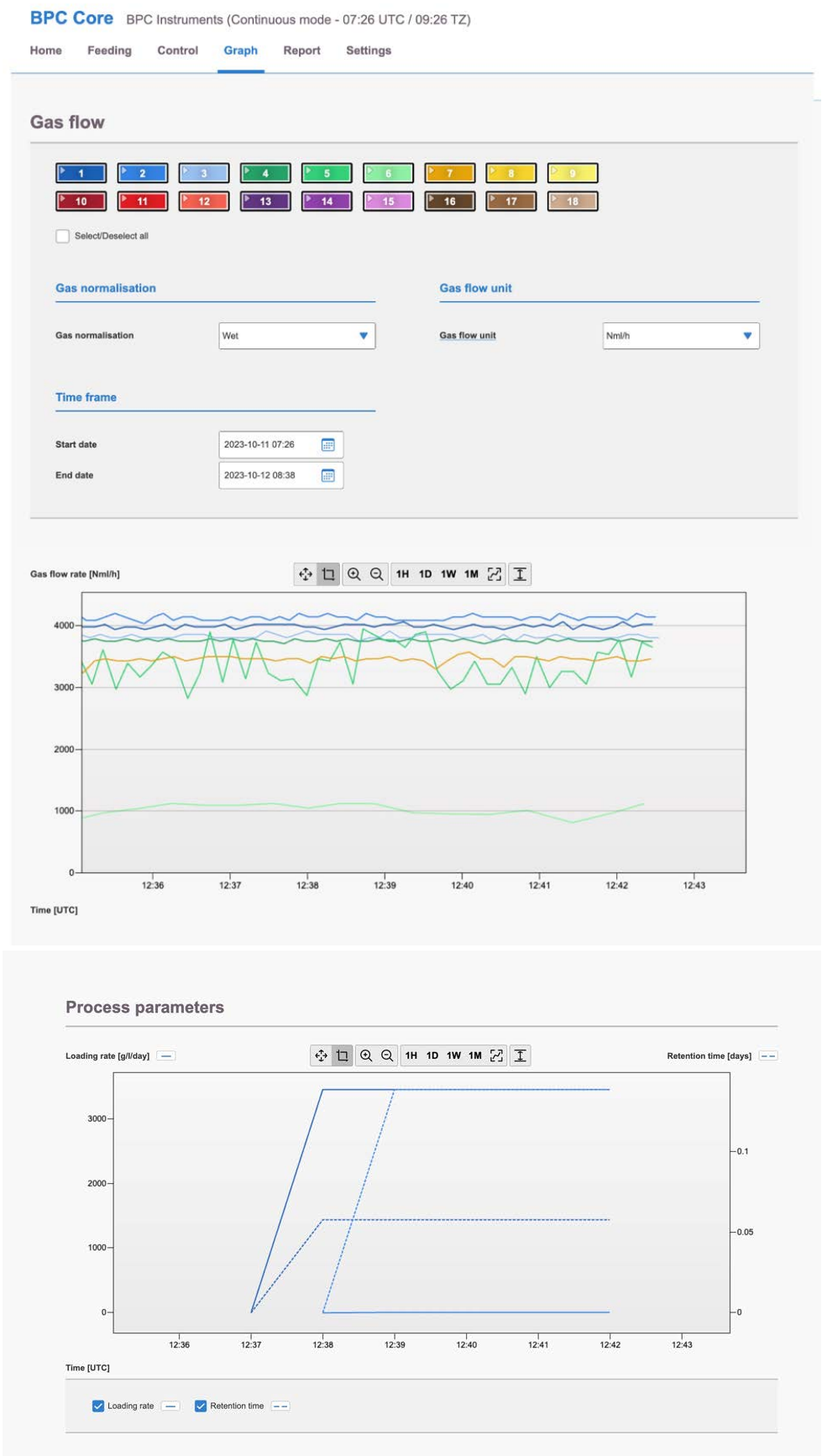


Figure 26. Graph page for continuous mode.

Chapter 06: Maintenance and Spare Parts

In order to ensure that the instrument and its constituent parts will operate properly for a long period of time, it is crucial to follow the instructions described on this manual. The lifespan of the instrument and its components will depend heavily on how the equipment is used and well maintained. Always make sure to use the instrument according to the following guidelines:

- The instrument must be kept in a dry and clean environment.
- Avoid applying tap water as bath liquid in the thermostatic water bath, since the minerals included in this type of water might negatively impact the material of the bath chamber (calcification or corrosion of stainless steel).
- The flow cell units cannot be opened and are considered consumables.
- Reactors and stirrers are consumables that can be autoclaved.
- To clean the detection unit, wipe it with a damp piece of cloth and, if required, a gentle form of detergent. Water must not get into the machine, since it can harm electrical components.
- The used NaOH solution must **NOT** be poured into the sink. It should be saved in dedicated vessels and disposed as hazardous waste.

All consumables are easily replaceable in the instrument. The spare parts can be ordered from BPC Instruments. For further information, please visit our webshop (link below):
https://webshop.bpcinstruments.com

BPC Instruments also provides a *Maintenance Package* where users can get all consumables that are recommended to be changed at regular intervals.