Notes on using the Bio-fermenter Control Unit

These notes describe how to set up the Bio-fermenter Control Unit and detail three experimental setups that the Bio-fermenter Control Unit is typically used in. At the end of the notes, there is an overview of how to use the ALBA software to set up and control your experiment.

Note that the Control Unit requires the ALBA Interface to have version 1.10 of the firmware. See the section *Using the Software* on how to check this.

Setting up the Bio-fermenter Control Unit

The Bio-fermenter Control Unit is only for use with the ALBA Interface and Logger and the **djb microtech** fermentation vessel.

Connect a 14V, 3A dc supply to the sockets at the back of the Bio-fermenter Control Unit.

> ***Caution****. Take care with the polarity. If you connect the supply the wrong way then an internal re-settable fuse will automatically remove the power and protect the Bio-fermenter Control Unit. Remove the power and wait a few seconds. The re-settable fuse may take up to 20 seconds to reset.*

The Bio-fermenter Control Unit can be used in many different and varied experimental setups to support biotechnology investigations. Three typical setups are outlined below and are described in more detail in following sections of the notes.

Setup 1: (1 output & 1 sensor)

- heater
- temperature sensor
- vent to prevent gas build up
- tube to enable samples to be taken

Setup 2: (2 outputs & 2 sensors)

- heater
- temperature sensor
- vent to prevent gas build up
- tube to enable samples to be taken
- tube for aeration of the broth
- pH sensor
- peristaltic pump

Setup 3: (2 outputs & 3 sensors)

- heater
- temperature sensor
- vent to prevent gas build up
- tube to enable samples to be taken
- tube to enable air to be added
- pH sensor
- peristaltic pump
- light sensor to indicate turbidity

Safety: Physical

The cartridge heater becomes hot in use and no attempt should be made to adjust its position when in use.

The peristaltic pump should be mounted so that it cannot topple.

The maximum control temperature allowed by the software is 44°C. It should be noted that when holding the temperature constant at this value the Bio-fermenter Control Unit will become warm.

Overheating results in automatic shut down. If this ever happens the power should be removed and the unit allowed to cool before continuing.

Safety: Biological

It is essential that users of this equipment follow their LEA's code of practice for working in Biotechnology. In addition, teachers must be trained to the appropriate level.

Teachers are referred to the following document, which has been issued by SSERC[1] and sent to all Scottish schools and colleges: "Biology /Biotechnology, Safety in Microbiology, A Code of Practice for Scottish Schools and Colleges".

Reference

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Setup 1 — (1 output & 1 sensor)

Let's suppose our experiment has the following requirements:

- a heater
- a temperature sensor
- a vent to prevent gas build up
- a tube to enable samples to be taken.

This setup requires a Type 1 lid for the fermentation vessel (catalogue number H3-1130.00). This lid has 1 large hole and 4 small holes. Since this experiment requires only three small holes, the fourth should be sealed with a solid rubber bung.

Refer to Figure 1 while following the steps below.

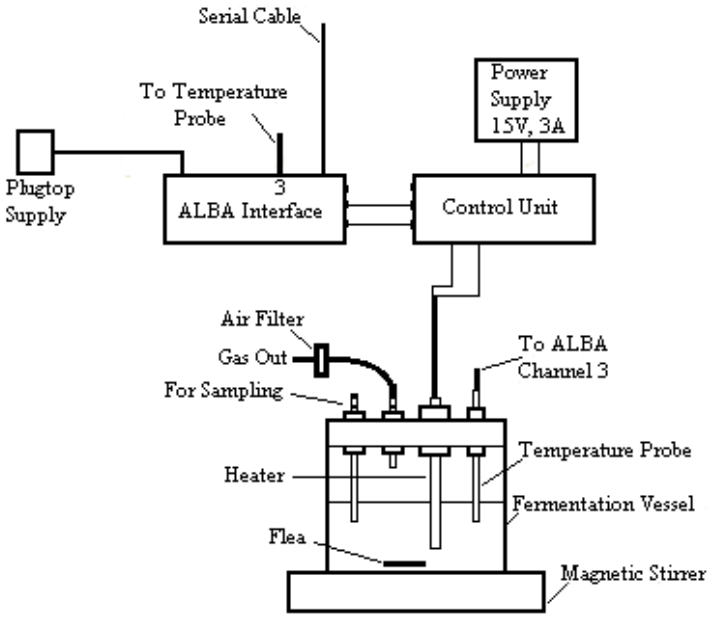


Figure 1

> ***Important.*** *The heater must be rated at 20W, 10V or less. The recommended heater is* ***djb microtech's*** *H3-1140.00.*

Step 1. Insert the heater through the 12.5mm diameter hole in a size 21 rubber bung and place it through the hole in the lid. When in use the top of the heater will get warm. In order to avoid the possibility of burning yourself, you can place another bung over the exposed section of the heater.

Step 2. Place the temperature sensor through a hole in a size 13 bung and position it in the lid.

Step 3. Insert a glass tube through the hole in a size 13 bung and position it in the lid – this tube is to allow any gas to escape.

Step 4. Place the lid on the fermentation vessel and clip it in place. The rubber seal on the lid keeps the fermentation vessel airtight.

Step 5. Connect the heater to Output X on the Bio-fermenter Control Unit.

Step 6. Connect a red wire from Output X on ALBA to Input X on the Bio-fermenter Control Unit and connect a black wire between the two units.

Step 7. Place the fermentation vessel on a magnetic stirrer and start the stirrer. If you find that the flea is rattling on the slightly raised centre part of the fermentation vessel then raise one side on the vessel.

The technique for withdrawing a sample is discussed later in the manual.

Setup 2 — (2 outputs & 2 sensors)

Let's suppose our experiment has the following requirements:

- a heater
- a temperature sensor
- a vent to prevent gas build up
- a tube to enable samples to be taken
- a tube for aeration of the broth
- a pH sensor
- a peristaltic pump.

For this setup a Type 2 lid (catalogue number H3-1130.05) is required. In order to aerate the broth an air pump is required. **djb microtech** supply a suitable unit H3-1150.00. The air should pass through a filter then through a glass tube in the lid.

Refer to Figure 2 while following the steps below.

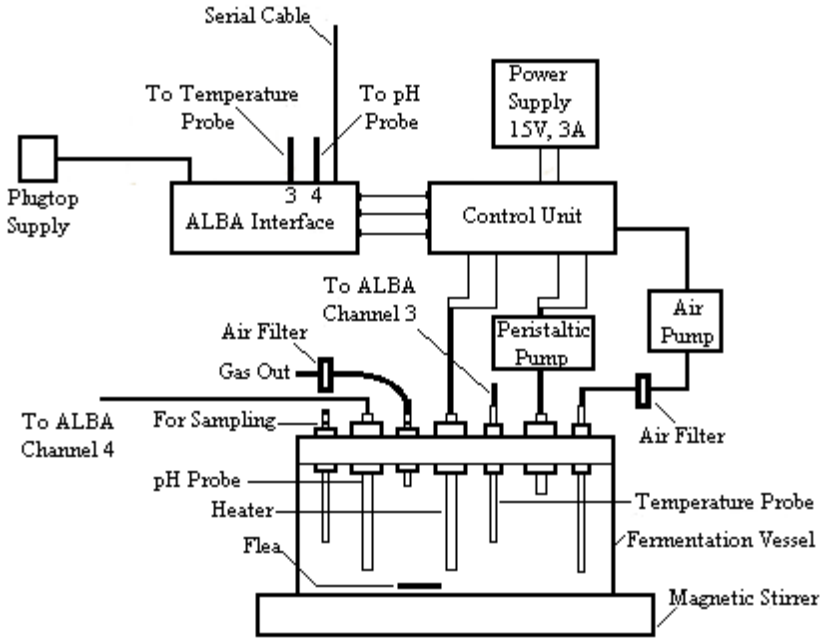


Figure 2

Step 8. Follow steps 1–4 of Setup 1.

Step 9. Place a pH sensor through the lid in a similar way to the temperature sensor. Ensure that the pH probe is below the surface of the broth but not so low that it could be hit by the magnetic flea.

Step 10. Set up the peristaltic pump (how to do this is described in a separate section, later in the notes). If your broth becomes more acidic as the experiment progresses then you can hold the pH constant by adding alkali using a peristaltic pump. The software will automatically do this for you once you have set the target pH.

Step 11. Make the following connections to the Bio-fermenter Control Unit

- three wires from the output of ALBA to the input sockets on the left hand side of the Control unit.
- the heater and the peristaltic pump to the terminals on the front.
- the air pump to the jack socket on the right hand side of the Control unit.
-

Step 12. Seal the unused holes in the lid of the fermentation vessel with solid rubber bungs.

Step 13. Place the lid on the fermentation vessel and clip in place.

Step 14. Place the fermentation vessel on a magnetic stirrer.

Setup 3 — (2 outputs & 3 sensors)

Let's suppose our experiment has the following requirements:

- a heater
- a temperature sensor
- a vent to prevent gas build up
- a tube to enable samples to be taken
- a tube to enable air to be added
- a pH sensor
- a peristaltic pump
- a light sensor to indicate turbidity.

For this setup a Type 2 lid is required.

This is the same setup as Setup 2, with the addition of a light sensor.

Examination of the above requirements shows that three sensors are required. In order to connect three sensors you will require the Input Extension Unit G1-1000.50. This unit enables self-identifying sensors to be connected to inputs 1 and 2 of the ALBA Interface. The software enables you to assign sensors and calibrations to each channel.

Refer to Figure 3 while following the steps below.

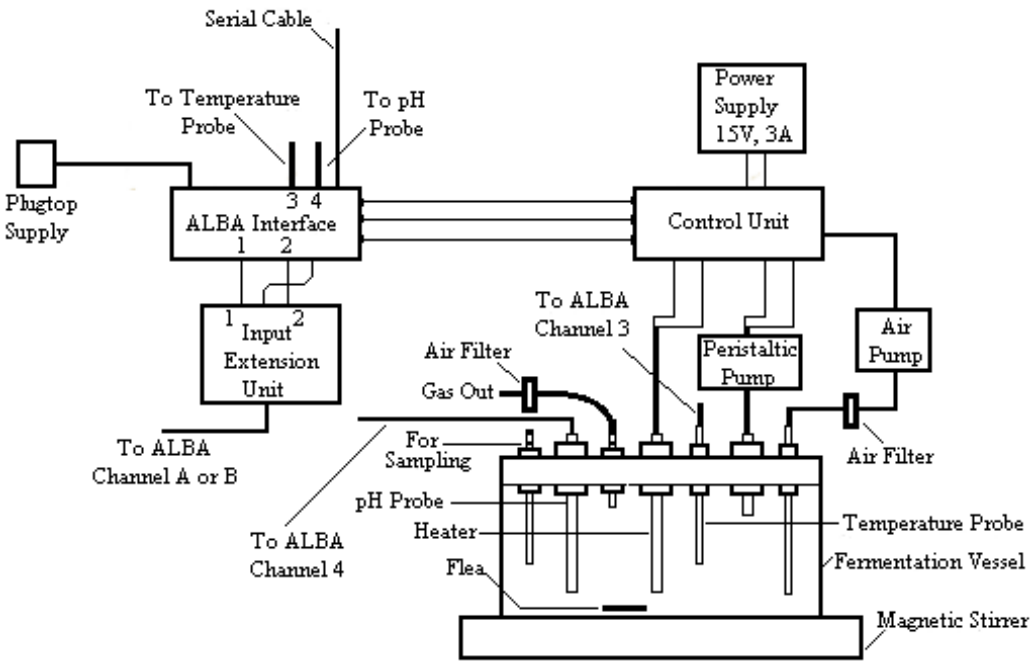


Figure 3

Step 15. Complete the setup for Setup 2.

Step 16. Connect 3 wires from the Input Extension Unit to the corresponding inputs on the ALBA Interface.

Step 17. Position the dissolved oxygen probe through the lid in the same way as the heater and pH probe.

Step 18. The holes in Types 1 and 2 lids are positioned so that it is possible to position a light sensor and source at one end of the fermentation vessel. A 12V dc lamp is recommended with its separate supply. Alternatively you could try a 2.2V lens end bulb.

Step 19. Place the lid on the fermentation vessel and clip in place.

Step 20. Place the fermentation vessel on a magnetic stirrer.

Step 21.

Setting up the Peristaltic Pump

For illustrative purposes we will assume that during an experiment the broth becomes acidic and we wish to maintain a constant pH by adding an alkali.

It is necessary to prime the pump before use and this should be done in advance of setting up your fermentation vessel.

Refer to Figure 4 while carrying out the following steps:

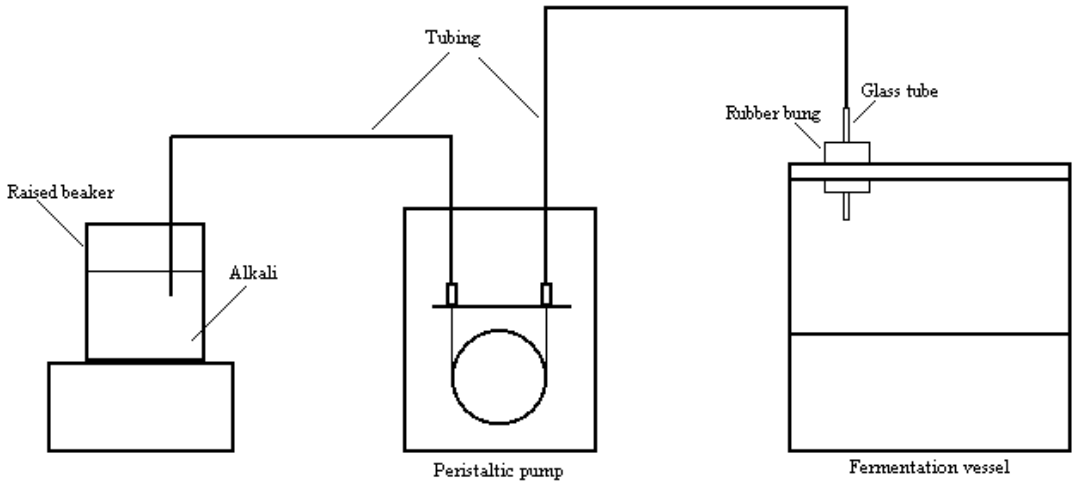


Figure 4

Step 22. Connect the tubing to the pump.

Step 23. Place the tubing on the left hand side of the pump in the beaker containing the alkali. This beaker should be slightly higher than pump.

Step 24. Connect the pump to a 5V dc supply and switch on. Take care with the polarity. The pump turns anti-clockwise (looking at the front) when correctly wired. The other end of this lead has a jack connection which should be connected to the socket on the back of the pump. Note that the switch on the back of the pump should be in the down position for 'on'.

Step 25. The tube slowly fills with alkali. When both sides are full, switch off the pump. The pump is now primed.

Step 26. When you are ready to start your experiment the tubing on the right hand side should be connected to the fermentation vessel. To avoid getting alkali on your hands wear disposable gloves when connecting the tubing to the glass rod. For the best control use adjacent holes in the lid for the pH probe and the alkali.

Step 27. Remove the lead from the 5V supply and connect it to the Control Unit.

Note that during the experiment it may be necessary to top up the beaker containing the alkali. There must also be sufficient room in the fermentation vessel for the addition of alkali.

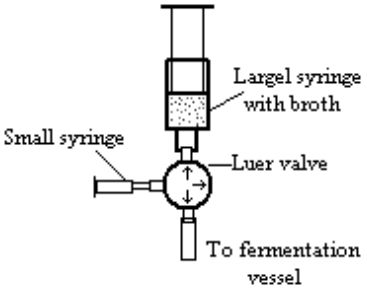
Restriction on Sensors

Dissolved oxygen, pH, and conductivity sensors should not be used together in the same fermentation vessel – only one of these sensors should be used in a fermentation vessel at a time. The principle of operation of these sensors will cause erroneous readings if placed in the same container as each other.

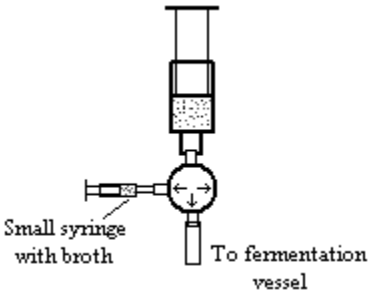
Taking a Sample

The three way Luer valve (catalogue number H3-1180.00) can be used in conjunction with a large and small syringe to obtain a sample. The following steps should be taken:

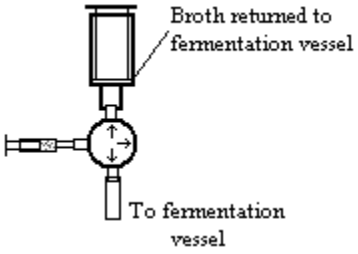
Step 28. Position the valve so that the larger syringe can remove liquid from the fermentation vessel. This ensures that any liquid that had been lying in the tubing is removed.



Step 29. Change the valve position so that the smaller syringe can take a sample.



Step 30. Position the valve so that you can return the liquid in the larger syringe to the fermentation vessel.



Sterilization

The following items may be autoclaved:

- the rubber bungs - note that they become hard after three sessions in the autoclave and would eventually have to be disposed of.
- the flexible tubing type H3-1195.00
- glass tubing
- the air filter type H3-1190.00
- Luer valve

All other items would require to be chemically disinfected.

Note

- As of June 2013 the fermentation vessel and its lid should no longer be autoclaved and must be chemically disinfected. The original containers are no longer manufactured.

Using the Software

The installation of the software on your PC places the Bio-fermenter Application in the Biology Applications folder.

The Control Application requires version V1.10 of the ALBA firmware in the ALBA Interface. To check your version number select *Help* then click *About*. The version number is displayed in the middle of the window. Users requiring an upgrade please see the website www.djb.co.uk for details or contact djb microtech directly.

Before running the software check that the power lead and the serial lead are connected to ALBA. To run this Application click on the ALBA icon on your desktop and select the *Load Application* option.

Click on the Biology radio button. Select *Bio-fermenter* then click the *Load Application* button – Figure 5.

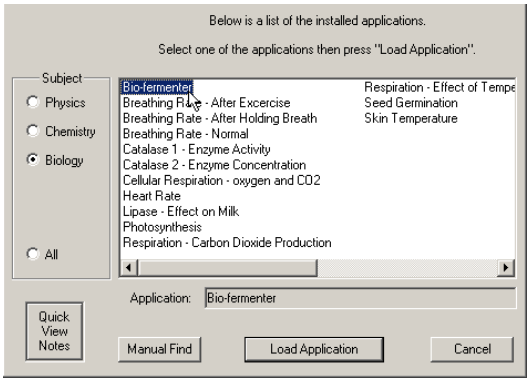


Figure 5

Click OK to close the splash screen. It should be noted that the Bio-fermenter Control program is a general purpose tool that enables the user to set up and control their own specific experiment.

Select the *Experiment Notes* icon which is the second from the left on the Toolbar. The notes are similar to the text in this manual.

Password: The Teacher's Notes are accessed from the Experiment menu. Note that the password is **JohnLogieBaird** (no spaces and case does not matter).

Once you have set up your experiment and are ready to start, click the *GO* icon.

For this tutorial, a temperature and pH sensor have been connected to ALBA inputs 3 and 4 respectively. The temperature has to be kept constant at 28°C and the pH at 6.8.

Figure 6 shows the Bio-fermenter setup screen.

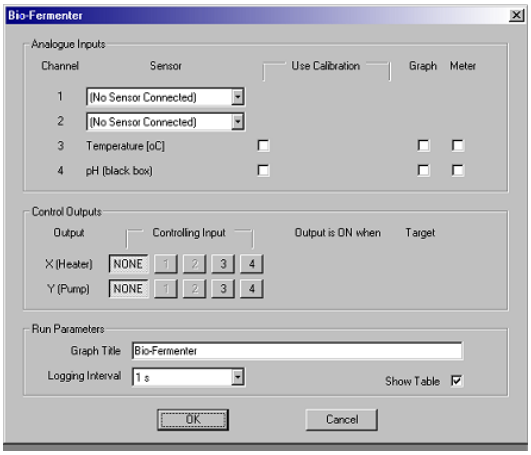


Figure 6

First consider the Analogue Inputs.

Notice that the sensors have been identified by the software and that no sensors are connected on channels 1 & 2 – Figure 7.

Connecting sensors to channels 1 & 2 will be discussed later.

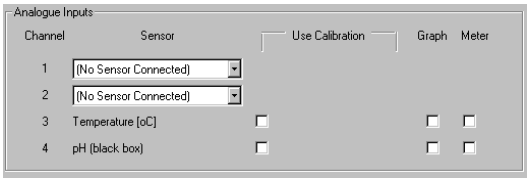


Figure 7

The *Use Calibration* section in the middle of the screen lets you assign a calibration to a sensor. Click on the check box and then select the required calibration – Figure 8. If you are unfamiliar with the calibration procedure then select *Help*, *How to* and then *Calibrate a sensor*. Note that the calibration of a sensor should be carried out before setting up the bio-fermenter. If the check box is left unchecked then the default calibration for the sensor will be used. On the right hand side of the window you can select how to display your readings. You will always have a Table (choice of maximized or minimized – see below) but in addition you can have a graph and/or a meter.

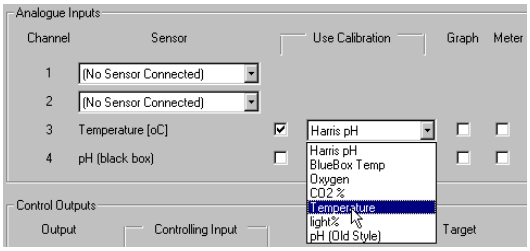


Figure 8

Controlling the Outputs

The heater on Output X can be controlled by the buttons that are not greyed out i.e. 3 and 4 – Figure 9. Since the temperature sensor is connected to channel 3 click button 3.

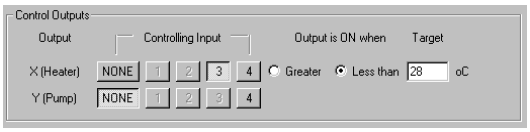


Figure 9

You can now enter the conditions for the heater being switched on i.e. if the temperature is less than 28°C then Output X will be on.

In a similar way we can control the pH so that alkali will be added if the pH is ever less than 6.8 – Figure 10.

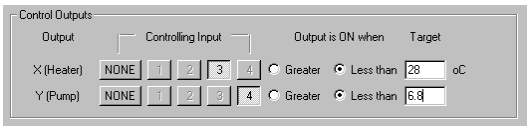


Figure 10

Note that selecting the NONE button resets the controlling input button – that output will not be controlled.

Run Parameters

Give your experiment a suitable title.

Select a suitable logging interval from the drop-down menu. If uncertain then select 10s as a starting point. If the *Show Table* check box is ticked then the results will be displayed in a Table along with any Meters and Graphs that may have been selected above. If this box is not checked then the Table will be minimized.

Click OK to start your experiment.

Normal graphing is against time. However if you wish to see a graph of pH/Temperature while the experiment is running, click on the Temperature and pH columns to select them, then click the *Quick Graph* icon.

Note that if you minimize the Application while it is running then you can use your computer for other work – provided of course that the serial port is not required. The results will continue to be logged and placed in your Table.

Using analogue Channels 1 & 2

Connect the Input Extension Unit to ALBA – see Figure 3. Assume that a temperature sensor is connected to channel 1 and a pH probe to channel 2.

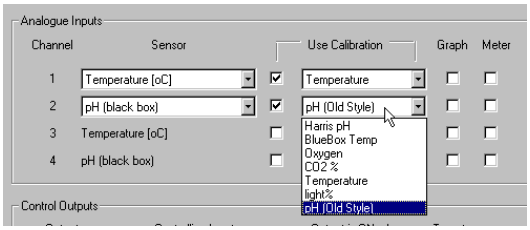


Figure 11

When you use channels 1 and 2 the software has no knowledge of the sensor type that is connected so you must give this information to the software. Click on the Sensor drop-down menu and select the sensor. As before, you now have the choice of using the default calibration or assigning your own calibration to the sensor. For the temperature probe check the *Use Calibration* box and then select the appropriate calibration from the drop-down menu. Similarly set up the pH probe – Figure 11. It is anticipated that channels 1 & 2 would normally be used for light and dissolved oxygen sensors and channels 3 & 4 for temperature and pH. However the software has been designed to handle any sensor on any input.

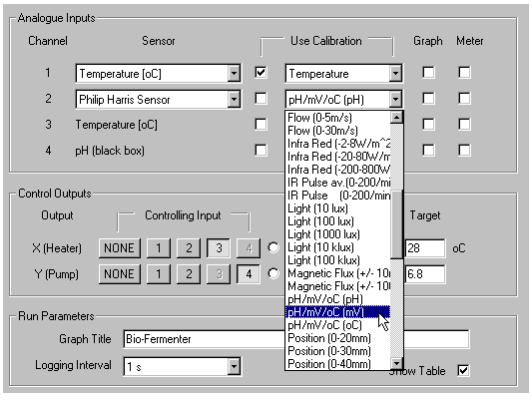


Figure 12

Note that other sensors may be connected to your fermentation vessel e.g. oxygen gas, carbon dioxide gas and pressure.

The Philip Harris SensorMeter range of sensors may connected to any of the analogue inputs – Figure 12. The ALBA software holds a default calibration for each of the SensorMeters.