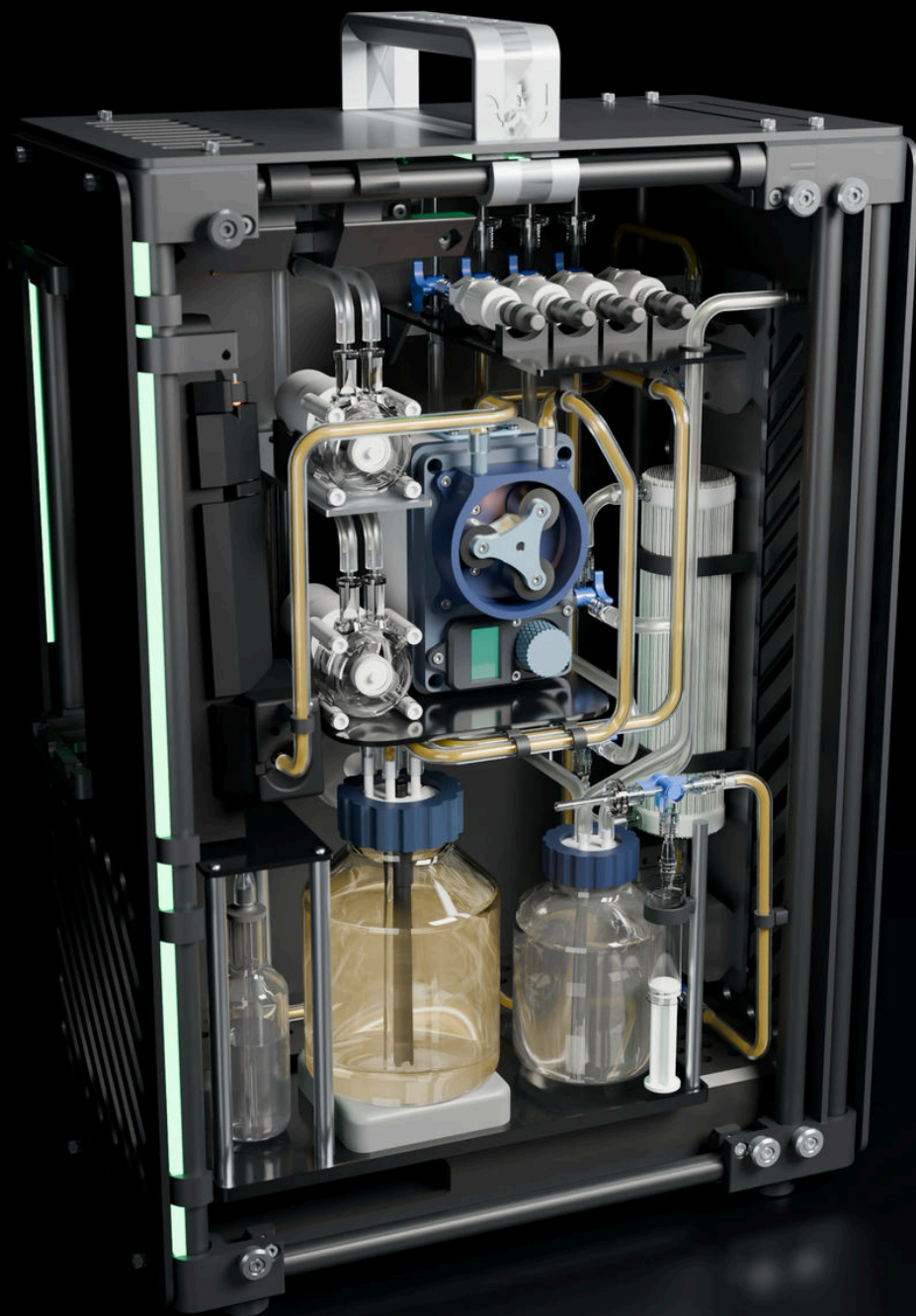




# ReLEAF Bioreactor

## User Manual

Perfusion hollow-fiber bioreactor for contained production of plant protectants

**Revision B**  
18 October 2026

**Software v6.2.2**  
Firmware v4.2.2

**GEMS-Taiwan**  
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*Laboratory research equipment for trained personnel at Biosafety Level 1. **Read Section 1 before use.***

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## About This Manual

This manual covers installation, commissioning, daily operation, maintenance, troubleshooting and disposal of the ReLEAF perfusion bioreactor. It applies to the integrative bioreactor, prototype 3, running controller software v6.2.2 with MCU firmware v4.2.2. The cover shows the commercial bioreactor, our design for the product, which has not been built yet; the procedures in this manual are for the lab system.

The bioreactor is intended for trained laboratory personnel working at Biosafety Level 1. Read Section 1 in full before the first run. Section 4.1 is a single-page checklist for a complete run; the sections after it give the detail behind each step.

| Symbol  | Meaning                                                               |
|---------|-----------------------------------------------------------------------|
| DANGER  | A step that can injure a person or destroy hardware if it is ignored. |
| CAUTION | A step that can lose a run or damage a part if it is ignored.         |
| NOTE    | A point worth knowing during normal use.                              |

**Conventions.** Controls are named in bold exactly as the controller displays them, for example **Zero calibrate**.

Pressures are given in bar, the controller's default unit; derived membrane indicators are given in kPa, as the controller displays them. 1 bar equals 100 kPa.

**Terms.** *Lumen side*: the inside of the fibers, where the culture circulates. *Shell side*: the space around the fibers, where the product collects. *Feed*, *retentate* and *permeate*: the pressures at the lumen inlet, the lumen outlet and the shell port. *TMP*: transmembrane pressure,  $(\text{Feed} + \text{Retentate}) \div 2 - \text{Permeate}$ . *PWM*: the 0 to 255 power setting sent to a pump.

Site-specific values that are set during installation, such as the base concentration and the network address, are recorded on the commissioning record at the back of this manual.

## 1 Safety

### 1.1 Intended use

The ReLEAF bioreactor is laboratory research equipment. It cultures engineered *Bacillus subtilis* in a closed perfusion loop and collects cell-free shell-side fluid for analysis.

- Operate the device only at Biosafety Level 1 (BSL-1), by personnel trained in aseptic technique and in the local biosafety rules.
- Do not apply shell-side fluid to soil, to a hydroponic line or to any plant.
- Do not use the device, or anything it produces, for medical, food or clinical purposes.
- The device carries no electrical, medical or biosafety certification (Section 9).

## 1.2 Biological safety

- The culture loop contains engineered *B. subtilis*. Wear gloves, a lab coat, long trousers and closed-toe shoes whenever the device is running or being serviced.
- The 0.2 µm hollow-fiber membrane is the containment barrier. Treat shell-side fluid as live culture until a plate taken from that run has grown nothing.
- The culture loop has three openings that you control: the Luer sampling valve, the feed line and the permeate collection. The pump head and the tubing are part of the containment boundary as well. Section 4.8 gives the rules for each.
- Open Luer ports with aseptic technique. Wipe each port with 70% ethanol before and after use, and cap it when it is not in use. Ethanol does not kill spores; inactivation is by bleach or autoclave (Section 8).
- Never discharge culture or shell-side fluid to a drain, to soil or to a hydroponic line. Inactivate every liquid as described in Section 8.

## 1.3 Electrical safety

### **DANGER · External voltage destroys the controller board**

Follow the power-up and shutdown order in Section 3.2 every time.

Switch off everything, the 12 V supply, the power distribution board and the controller board, before any rewiring. Never connect or disconnect a wire while the system is powered, and remove the old wire before fitting the new one.

The controller board's 3.3 V, 5 V and VIN pins supply small loads outward only, such as the thermometer and its pull-up resistor. Never connect them to the power distribution board, to the motor driver or to any external supply.

- The signal pins use 3.3 V logic and are not 5 V tolerant. Every line entering the board must be at or below 3.3 V. Measure each line with a multimeter before connecting a replacement board.
- Every signal line carries a series resistor, 1 kΩ or 4.7 kΩ, which limits the current if a higher voltage reaches the line. Do not bypass them.
- The motor and servo rail runs at 12 V, the limit of the motor driver's on-board regulator. Do not exceed it.
- Keep liquid and dried salt residue away from the breadboard, the motor driver and the wiring. The wet section of the device sits inside an incubator beside open medium.
- Position the device so that the 12 V supply and the controller board's USB-C cable can be reached and disconnected immediately.

## 1.4 Chemical and mechanical safety

- Automatic pH control doses sodium hydroxide into the culture loop. Sodium hydroxide is corrosive: wear eye protection when preparing, filling or handling the base bottle, and follow the safety data sheet for the concentration in use.
- The loop operates under pressure. Pressure protection stops both pumps when any transmitter exceeds the trip limit. Never raise that limit to work around a blockage; find and clear the blockage.
- The pinch valve and the four servo-driven Luer valves move without warning when a command is sent or an interlock trips. Keep fingers clear of the servo horns while the controller is running.
- The peristaltic pump head closes on the tubing with force. Stop the pump before loading or removing tubing.

## 1.5 In an emergency

| Situation                                                   | Action                                                                                                                                                                |
|-------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Liquid leaking onto the bench or the incubator floor        | Press <b>Emergency Stop</b> in the main pump panel, then switch off the 12 V supply. Contain the spill with the spill kit and disinfect with 10% bleach.              |
| Red pressure-trip banner on the interface                   | Do not clear it immediately. Inspect every line for a closed valve, a kinked tube or a clogged filter, then follow Section 4.6.                                       |
| Burning smell, smoke or a hot board                         | Switch off the 12 V supply first, then disconnect the controller board's USB-C cable. Do not touch the board until it has cooled.                                     |
| Culture on skin or in the eyes                              | Wash skin with soap and water. Flush eyes at the eyewash station for at least 15 minutes, holding the eyelids open. Report the incident to the laboratory supervisor. |
| Suspected membrane breach: shell side turbid, TMP collapses | Stop the main pump, close the shell sampling valve, and plate shell-side medium before any further use. Do not use the shell-side fluid.                              |

## 2 System Overview

### 2.1 How the device works

The ReLEAF bioreactor is a closed perfusion loop built around a hollow-fiber membrane module. Culture circulates on the lumen side of the fibers and is retained there. Soluble product crosses the fiber wall to the shell side and is drawn off to the permeate reservoir. The two fluid paths meet only at the fiber wall, so the harvested stream leaves the culture behind.

The wet section is designed to run on a shelf inside a 37 °C incubator. The controller board, the motor driver and the wiring sit on a shelf above it, outside the wet zone.

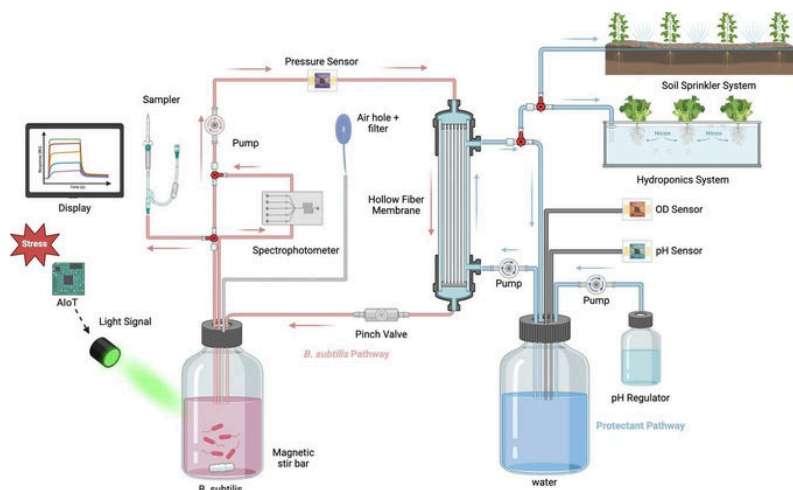


**Figure 1.** System diagram. The lumen path (red) carries culture from the vessel through the pump, the in-line photometer and the fiber lumens, and back. The shell path (blue) carries permeate from the module to the permeate reservoir. Delivery lines and harvest-side sensing shown beyond the reservoir are not part of the delivered system.

**Lumen path:** medium reservoir → peristaltic pump → in-line photometer → fiber lumens → pinch valve → back to the reservoir. The loop stays closed for the whole run, and its only access is the dual Luer sampling valve. **Shell path:** the space around the fibers fills across the fiber wall, is drawn off through the shell ports by the harvest pump, and collects in the permeate reservoir.

### 2.2 What the system contains

| Item                                 | Description                                                                                                                                                                                                           | Qty    |
|--------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|
| <b>Controller board</b>              | Single-board computer with an on-board microcontroller. Serves the web interface on port 7000 and holds the safety interlocks. Includes a 7-inch 1024×600 touch screen.                                               | 1      |
| <b>Motor driver</b>                  | Dual-channel driver on the 12 V rail: channel 1 main pump, channel 2 feed pump.                                                                                                                                       | 1      |
| <b>Main pump</b>                     | Peristaltic pump for crossflow (ZP4000-N79H, up to 1 L/min).                                                                                                                                                          | 1      |
| <b>Feed pump</b>                     | Single-direction peristaltic pump for base dosing and external feed.                                                                                                                                                  | 1      |
| <b>Harvest pump</b>                  | Draws permeate from the shell line to the permeate reservoir.                                                                                                                                                         | 1      |
| <b>Servo valves</b>                  | Five STS3215 12 V bus servos: one pinch valve on the return line and four automated Luer valves on the membrane's lines, among them the shell sampling valve and the feed valve. Each Luer valve has three positions. | 5      |
| <b>Pressure transmitters</b>         | 0 to 1 MPa, 0 to 5 V output, one at each membrane port, each behind its own filter.                                                                                                                                   | 4      |
| <b>Flow meter</b>                    | Pulse-output meter on the lumen line.                                                                                                                                                                                 | 1      |
| <b>pH probe and thermometer</b>      | Probe in the reservoir cap with a waterproof temperature probe for compensation.                                                                                                                                      | 1 each |
| <b>In-line photometer</b>            | Measures OD600 through a 2 mm flow cuvette with sample and reference sensors, without drawing a sample.                                                                                                               | 1      |
| <b>Membrane module</b>               | mPES hollow fiber (Cobetter MiniLab), 0.2 µm pore, 200 cm <sup>2</sup> over 11 fibers.                                                                                                                                | 1      |
| <b>Reservoirs</b>                    | 1 L medium reservoir with GL45 cap; base bottle; permeate reservoir with collection bottle.                                                                                                                           | 3      |
| <b>Magnetic stirrer and stir bar</b> | Keeps the reservoir homogeneous during a run.                                                                                                                                                                         | 1 each |

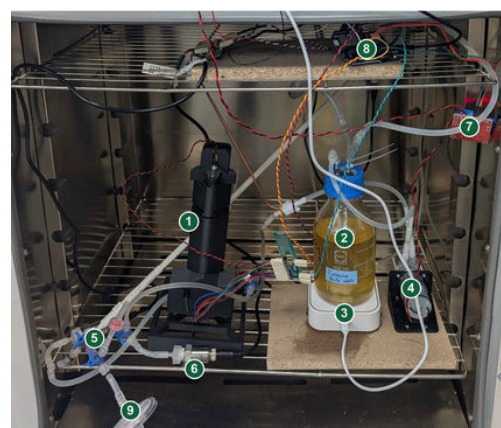


**Figure 2.** The device installed in an incubator. 1 membrane module; 2 culture vessel; 3 stirrer; 4 pump head; 5 Luer manifold and sampling port; 6 pressure transmitter; 7 sensor breakouts; 8 controller board; 9 vent filter.

| Item                              | Description                                                                                                                                                                                                                                        | Qty   |
|-----------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| <b>Filters, valves and tubing</b> | 0.45 µm hydrophobic PTFE vent filters for the sampling line and the cap air hole; one filter per transmitter line; Luer valves (three-way stopcocks) including the dual sampling valve; silicone pump and loop tubing, 4 × 6 mm and 5 × 7 mm, 50A. | set   |
| <b>Illumination strip</b>         | Green strip at approximately 535 nm fitted around the culture vessel, driven by the AIoT controller.                                                                                                                                               | 1     |
| <b>Power</b>                      | 12 V DC supply, power distribution board, and a USB-C supply for the controller board.                                                                                                                                                             | 1 set |

Check the delivered system against this list before installation. The membrane module, the tubing, the filters and the pump tubing are consumable items (Section 5.4).

## 2.3 What crosses the membrane



**Figure 3.** Containment check. Lumen-side medium (left) and shell-side medium (right) on LB agar after 24 h at 37 °C. The lumen plate grew colonies; the shell plate grew none.

### CAUTION · The membrane separates by size, not by molecule

Everything smaller than the 0.2 µm pore crosses to the shell side: the product, medium components, vesicles and cell debris. Whole cells are retained. Plate the shell side on the first run after every membrane change, and treat the harvested fluid as live culture until that plate has grown nothing.

## 3 Installation and Commissioning

Work through this section in order on a new installation, and again after new tubing is fitted, after the membrane module is changed, or after the controller board is replaced. Record the site-specific values on the commissioning record at the back of this manual as you go.

### 3.1 Siting the device

- Place the wet section on a shelf inside a 37 °C incubator, with the membrane module upright in its clamp.
- Place the controller board, the motor driver and the wiring on the shelf above, outside the wet zone, where condensation and salt residue cannot reach them.
- Leave the 12 V supply and the controller board's USB-C cable within reach so that either can be disconnected immediately.
- Allow room beneath the device to inspect the bench and incubator floor for leaks at every check.

### 3.2 Powering up and shutting down

#### DANGER · Power-up and shutdown order

**Power up:** connect the controller board first and wait until the serial monitor reaches its idle line. Then switch on the 12 V supply and the power distribution board.

**Shut down in reverse:** switch off the 12 V supply and the distribution board first, then disconnect the controller board.

Switching external power on first back-feeds through the signal pins. The board will not boot, and repeated back-feeding destroys it.

The pinch valve moves to open on every boot. If the board does not start, or the console reports that a method is unavailable with no boot lines, disconnect the USB-C cable for at least 10 seconds, reconnect it, and repeat the order above.

### 3.3 Connecting to the controller

The device is operated entirely from one web interface served by the controller board. The browser displays values and sends commands; the safety interlocks run on the microcontroller and remain in force even if the browser or the board's Linux side stops responding.

1. Open the interface at the address recorded on the commissioning record, or use the touch screen on the board. Do not open the page through a development file preview: the buttons will not work and the page reports that the interface is not defined.

2. Check the status line. It must show **Connection: Connected**, **MCU: OK** and matching interface and backend versions. If the firmware and backend versions differ, they are a mismatched pair: replace both files together and reflash.
3. Choose a layout at the top right. **Desktop** gives three columns: OD600 and pH, pressure and derived analysis, and pumps, valves and recording. **Local 1024×600** suits the touch screen, with five tabs: **Monitor**, **pH / Temp**, **Pumps / Valves**, **Record** and **Settings**.

The interface opens in English; the switch beside the layout control changes it to Traditional Chinese. Messages generated by the backend remain in English in either language. Any value marked with an information symbol opens an explanation of its meaning and normal range.

## 3.4 Fitting the modules

### 3.4.1 Membrane module

1. Mount the module upright in its clamp. The height difference between its two ends sets the liquid-column pressure that the controller subtracts, so the mounting angle matters.
2. Connect the lumen inlet at the bottom and the lumen outlet at the top, each through its pressure transmitter tee and that tee's filter.
3. Connect the shell port to the permeate line through its own transmitter tee and filter.
4. Run the gravity-offset self-test (Section 3.5.2) after every re-mounting.

#### CAUTION · Keep a service log for every module

Record the running hours of each membrane module. Plate the shell side on the first run after a module change, and after any run in which the pressure trend changes. Retire a module that has been breached or whose fouling indicators reach the critical threshold, and dispose of it as described in Section 8.

### 3.4.2 Pumps

1. Stop the pump before loading tubing.
2. Load the tubing into the peristaltic head and close the head over it. The head acts on the outside of the tubing, so the fluid path stays closed.
3. Check that the tube is seated along its whole arc, with no twist at either clamp.
4. Set a low PWM, press **Apply**, and confirm that the flow meter registers pulses within a few seconds. If it does not, see Section 6.

Pump tubing is the component that wears first. Inspect the tube at the head for flattening or scoring before every long run, and replace it at the interval in Section 5.4.

### 3.4.3 Pinch valve

1. Fit the return line into the pinch valve jaw with the servo released.
2. In the valve panel, use **Move** to bring the servo to the point where the tube is just closed, then press **Save current as pinched**. Move the servo to the fully open position and save it the same way.
3. Confirm the saved TMP limit, lumen-in limit and maximum pinch time before the first pinch of the run.

### 3.4.4 Luer and servo valves

The dual Luer sampling valve at the reservoir outlet is the only opening in the culture loop. The four automated Luer valves on the membrane's lines are servo-driven; the positions of the shell sampling valve and the feed valve are listed in Section 4.3.

1. With the servos unpowered, set each valve by hand to its closed position and check that the handle clears its neighbours.
2. Power up, step each valve through its three positions from the panel, and confirm the angle reached. Correct any offset with **Save current position as**.
3. Cap the sampling port when it is not in use.

#### CAUTION · Test the valves before fitting tubing

On a new installation, step every servo-driven Luer valve through its three positions before any tubing is fitted. A valve mounted a few degrees out of position will close on a tube that should be open, which appears as a pressure trip in the first minutes of a run.

Each servo is supplied with the same bus address. Set a new servo's address before reconnecting the others, one servo at a time, under **Valve setup** → **Change servo ID**. Two servos sharing an address will collide on the bus and move together.

### 3.4.5 Stirrer and reservoirs

1. Place the stir bar in the medium reservoir, seat the reservoir squarely on the stirrer plate, and set 200 rpm. Stirring is not optional: without it, cells settle, the reservoir stratifies, and a sample drawn from the reservoir outlet does not represent the culture.
2. Fill the medium reservoir with growth medium to the working volume.

3. Fit the cap carrying the loop inlet, the loop outlet, the pH probe, the sampling line, and the air hole with its 0.45 µm vent filter. The air hole prevents a vacuum forming when a sample is drawn.
4. Prepare the base solution, wearing eye protection, and connect the base bottle to the feed valve's **Base** port. Record the concentration on the commissioning record.
5. Place the permeate reservoir below the shell outlet and connect the harvest pump.

### 3.4.6 Illumination strip

Fit the green strip around the culture vessel so that the light reaches the medium but not the photometer's flow cuvette. The strip is commanded by the AIoT controller, not from the bioreactor interface.

#### NOTE · Keep stray light off the photometer

The photometer measures 600 nm light through the flow cuvette. Keep the illumination strip, the incubator lamp and room light off the cuvette and its sensors during a run, and take the blank under the same lighting conditions as the run itself.

## 3.5 Calibration

Run this sequence in order after installation, after new tubing, after a membrane change and after a board change. When it is complete, export the result as a backup from **Calibration Values** → **Export calibration**. The file holds the blanks, the pressure zero offsets, the path-length factor, the pH points and the module settings. Calibration values are stored on the board and survive a reboot.

### 3.5.1 Pressure transmitter zeroing

1. Vent the system so that every line is open to atmosphere, with no pump running.
2. Press **Zero calibrate** in the pressure panel. The controller averages three seconds of raw readings and stores an offset for each channel.
3. Confirm that all three channels read close to zero.

The offsets are also pushed to the microcontroller, because the safety interlocks compare against absolute pressure. Read pressure as a trend: the transmitters are 1 MPa parts working at a few tens of kPa, so a single absolute value sits within sensor error.

### 3.5.2 Gravity offset self-test

1. Stop the pump, PWM 0, and press **Start self-test**. The controller waits 10 seconds for the flow to decay, then records for 30 seconds.
2. The reading must be a plateau, drifting no more than 0.15 kPa per minute. If it drifts, a line is still flowing or a valve is leaking.

Repeat this test after every re-mounting of the module. It measures the liquid column between the module's two ends, which the controller subtracts from every derived pressure.

### 3.5.3 Clean-membrane pressure-flow sweep

1. Fit a clean membrane module and open the pinch valve.
2. Enter between two and ten PWM points and press **Start sweep**, highest PWM first so that the pump does not have to start from rest at a low setting. Each point settles for 20 seconds and records for 30 seconds.
3. Review the fit. Take the linear fit when the quadratic term is negative or when there are fewer than four points.
4. Press **Apply**. Nothing is written until then, and the original PWM is restored afterwards.

This fit is the clean-membrane baseline against which the fouling indicators in Section 4.5 are judged, so it must be repeated after every membrane change.

### 3.5.4 pH calibration

1. Rinse the probe with distilled water and blot it dry. Place the probe and the thermometer together in pH 7.00 buffer, wait for the voltage to settle, and record the point; the controller averages the last five seconds.
2. Rinse, then repeat in pH 4.01 buffer. The buffer temperature at this point becomes the calibration temperature for the slope.
3. Check that the reading is correct in each buffer before returning the probe to the reservoir cap. Points can also be entered by hand under **Calibration Values**.

### 3.5.5 Photometer blank

1. Fill the loop with the growth medium that will be used in the run, with no cells, and circulate until the cuvette is free of bubbles.
2. In engineering mode, take a new blank. The blank is stored separately for each of the eight estimators.
3. Confirm that the sample-to-reference ratio is stable and the bubble rejection is low before inoculating.

**CAUTION · Blank on medium, not on water**

Blank the photometer on growth medium, and blank any reference spectrophotometer the same way. Blanking the two instruments differently is the most common cause of disagreement between them.

Changing the sensor settings, the estimator or the path-length factor invalidates the blank. Take a new one; the controller flags an old blank with a notice rather than refusing it.

No calibration curve against a reference spectrophotometer is applied. At high cell density the source and sensor are not linear, so check the top of the range with a dilution series.

## 4 Operation

### 4.1 Run checklist

The complete sequence for one run. Each step names the section that gives the detail. A skipped step is the most common cause of a lost run.

#### Before the run

1. Inspect the tubing at the pump head, every Luer connection and all filters. Filters, bench and incubator floor must be dry (Section 5.2).
2. Power up in order: controller board, wait for the idle line, then the 12 V supply and the distribution board (Section 3.2).
3. Open the interface and check the status line: connected, microcontroller healthy, versions matched (Section 3.3).
4. Check the free disk space on the board; the interface warns below 2 GB (Section 4.9).
5. Zero the pressure transmitters with every line vented and both pumps stopped (Section 3.5.1).
6. If the module was re-mounted, run the gravity self-test. After a membrane change, also repeat the clean-membrane sweep (Sections 3.5.2 and 3.5.3).
7. Fill the loop with the run's medium, circulate until the cuvette is bubble-free, and take the blank under the run's lighting (Section 3.5.5).
8. Confirm that the pH calibration is current, or recalibrate on pH 7.00 then 4.01 (Section 3.5.4).
9. Confirm the saved pinch positions and limits (Section 3.4.3). Set the stirrer to 200 rpm. Fill the base bottle (Section 3.4.5).

#### Starting the run

10. Set the main pump PWM and press **Apply**. Confirm that the flow card shows pulses and that the pressures sit within their normal ranges (Sections 4.3 and 7).
11. Inoculate through the sampling valve using aseptic technique (Section 4.8). Declare the inoculation in the OD600 panel so that the growth-rate window restarts.
12. Start recording (Section 4.9).
13. For a pH-controlled run, enable automatic base dosing (Section 4.4).

#### During the run

14. Once a day: check the flow rate against the set PWM, the TMP trend, the pH, the permeate bottle level, and that there is no liquid on the bench or the incubator floor (Section 5.2).
15. Pinch only for a collection, with the pump running and the flow stable. Release when the collection is finished (Section 4.3).
16. Empty the permeate bottle before it fills (Section 4.8). Refill medium through the feed path and declare the dilution (Section 5.1).
17. Act on every fault, trip or alarm as described in Section 4.6. Never raise a limit to clear a trip.

#### Ending the run

18. Follow Section 4.10: release the pinch valve, stop both pumps, stop recording, export the calibration file, download the recording, drain and flush the loop, then shut down in order.

### 4.2 Readings

Every reading is logged to the recording file once per sampling window. Pressure is displayed in bar by default; psi and MPa are available from the unit selector in the pressure panel toolbar. Flow for a window is the total pulse count divided by the total time, which removes the quantisation error of a one-second gate count, so the window figure is the one to use.

#### Optical density (OD600)

Optical density at 600 nm is read in line through the flow cuvette, so a growth curve requires no sample to be drawn.

- **Current OD600** is the value after gating. The raw estimate is shown beneath it.

- **Specific growth rate  $\mu$**  is the least-squares slope of  $\ln(\text{OD}_{600})$  over the last 60 minutes, in  $\text{h}^{-1}$ , with its  $R^2$ , the number of points in the fit and the doubling time. The window restarts after a declared dilution or inoculation, so a dilution step is not read as negative growth.
- **Gate decision** shows whether a reading was accepted. The gate rejects a reading whose dispersion, or whose jump from the last accepted value, is too large, and reports the median of the last three accepted values.
- **Bubble / outlier reject** is the share of readings in the burst that fell more than 5% below the upper envelope. Above 50% the measurement is discarded and retaken at once.

## pH

pH is read once a second and displayed with the probe voltage and the compensation temperature. A manually entered temperature is used only when the thermometer cannot be read. A jump of more than 0.5 pH is accepted only after three consecutive readings, and the accepted value is smoothed. A reading outside the valid probe range is flagged and is not written to the recording.

## 4.3 Controls

### Main pump

The main peristaltic pump sets the crossflow. Its panel holds a PWM slider and input box, a live flow rate and pulse frequency, a direction control and **Emergency Stop**.

1. Set the PWM value. The slider and the box are a preview; nothing is sent until **Apply** is pressed.
2. Press **Apply**. The pump starts at the kick PWM for 500 ms, then falls back to the target value. The kick is what starts the pump turning at low PWM.
3. Read the flow rate card to confirm that the pump is moving liquid. Click the card to open the live flow chart.

#### CAUTION · Changing direction or stopping sets the PWM to zero

Reversing the direction or pressing **Emergency Stop** sets the PWM to zero first, and **Emergency Stop** also releases the pinch valve. After either action the pump stays stopped until a value is set and **Apply** is pressed again.

### Pinch valve

The pinch valve closes on the return line to raise transmembrane pressure and drive permeate across the fiber wall. Its panel holds **Pinch** and **Release**, the pinched time, the live TMP, a fault indicator with **Clear**, and an event log of every pinch.

1. Confirm that the main pump is running and the flow rate is stable. Press **Pinch**; the servo moves to the saved pinched position and the TMP begins to rise.
2. Watch the live TMP. The valve releases itself and latches a fault if any interlock in Section 4.6 trips.
3. Press **Release** when the collection is finished. Do not leave the valve pinched beyond the maximum pinch time.

### Servo valves

| Valve                | Positions                                                     | State after boot                                           |
|----------------------|---------------------------------------------------------------|------------------------------------------------------------|
| Shell sampling valve | Closed · To sensor · Fully open, sensor and collection bottle | To sensor, or the state last selected                      |
| Feed valve           | Closed · Base · External feed                                 | Closed. The Base position is never restored automatically. |

If a valve's mounting is out of position, use **Torque off (turn by hand)** or **Move** to bring it to the true position, then press **Save position** for that state.

### Feed pump

- The feed pump has its own PWM slider, **Apply** and **Stop**. It runs in one direction only; if liquid moves the wrong way, exchange the two motor wires at the driver output.
- The pump will not start while the feed valve is fully closed, and automatic pH control pauses while the pump is run by hand.
- Manual dosing works only with the feed valve set to **Base** and the feed pump stopped.

## 4.4 Automatic pH control

Automatic control adds base only. There is no acid or carbon dioxide dosing, so a high pH raises an alarm and nothing more. Enable it from **pH meter calibration & adjustment** → **Enable automatic base dosing**, which asks for confirmation. The control loop runs once a second:

1. The pH falls below the target minus the deadband.
2. The feed valve moves to **Base** and the pump doses for the pulse length.
3. The controller waits at least 60 seconds, and until the pH slope over the steady window falls below 0.02 pH per minute, up to the maximum wait.
4. If the pH has reached the target, the feed valve returns to its previous position. Otherwise the loop doses again, up to the hourly dose limit.

If three consecutive pulses raise the pH by less than the minimum response, the controller latches a fault, returns the feed valve and stops dosing: the base bottle is empty or the line is blocked. Refill or clear the line, then press **Clear FAULT**. Every dose is written to the dosing event file.

## 4.5 Membrane monitoring

The derived-parameter table turns the three pressures and the flow into indicators of how the membrane is behaving. Collapsed, its header shows the permeability, the inlet TMP, the shear ratio and the number of warning and critical items. Expanded, it lists every indicator with abnormal rows highlighted.

| Indicator         | What it means                                         | Alarm threshold                                                             |
|-------------------|-------------------------------------------------------|-----------------------------------------------------------------------------|
| TMP_out           | Retentate minus permeate pressure                     | At or below 0: backflow risk. Check the sampling valve and collection line. |
| TMP_mean          | Mean transmembrane pressure across the module         | Above 50 kPa                                                                |
| ΔP ratio          | Measured friction loss against the clean-membrane fit | Above 1.3: the module is more restrictive than when clean                   |
| y_ratio           | Shear from pressure against shear from flow           | Above 1.3: effective fiber count dropping, clogging warning                 |
| L_p               | Permeability, flux divided by mean TMP                | Below 70% of the value captured at steady state                             |
| P_s               | Shell-side pressure against the self-test value       | More than 2 kPa above: collection-side drainage may be blocked              |
| TMP peak / trough | Extremes within the pump pulsation                    | Peak above 50 kPa; trough below -2 kPa                                      |

Clogging alarms are suspended while the pinch valve is pinched and for 60 seconds after it releases, because a pinched valve raises the pressures for a reason unrelated to fouling. Ratio parameters are not computed below 10 mL/min or when the flow reading is stale. Log the running hours of every module and record the trend.

## 4.6 Faults and trips

### Pinch valve fault

These interlocks run on the microcontroller and stay in force if the board's Linux side or the browser stops responding. While a fault is latched, pinching is refused.

| Trigger, while pinched                                              | Action                                                       |
|---------------------------------------------------------------------|--------------------------------------------------------------|
| Lumen-in pressure above the feed limit for five consecutive samples | Release and latch a fault                                    |
| TMP above the TMP limit for five consecutive samples                | Release and latch a fault                                    |
| Pinched longer than the maximum pinch time                          | Release and latch a fault                                    |
| Servo temperature at or above its cut-out                           | Release and latch a fault                                    |
| Servo fails to answer three times in a row                          | Latch a fault; the valve opens when the servo responds again |
| Pump emergency stop, or controller boot                             | Release, without latching                                    |

Remove the cause, then press **Clear**. If the valve releases repeatedly on lumen-in pressure, the peak is catching the pump's own pulsation: switch the basis from **Instantaneous peak** to **Mean** in the valve settings, or raise the limit moderately.

### Pressure trip

Pressure protection is independent of the pinch valve interlock and applies whether the valve is open or closed. Any one of the feed, retentate or permeate pressures above the trip limit for five consecutive samples stops the main pump and the feed pump, releases the pinch valve and latches the trip.

- While the trip is latched, the controller refuses any pump command above zero and the pump panel states the reason. **Clear pressure trip** succeeds only once the pressure has fallen back below the limit.
- After clearing, the main pump stays stopped until a PWM is set and **Apply** is pressed.
- Rebooting the controller clears the microcontroller's latch but not the Linux side's, so the pump is never restarted without the operator.

## 4.7 Leak detection

A sudden or abnormal change in transmembrane pressure indicates a leak, a breach or a blockage, and the controller raises an alarm on the thresholds in Section 4.5.

### CAUTION · Inspect for leaks visually

Pressure monitoring does not replace inspection. Check the bench and the incubator floor beneath the device at every routine check, and again before leaving a run unattended.

## 4.8 Sampling and harvest

- Sample only through the dual Luer sampling valve at the reservoir outlet, using aseptic technique. Wipe the port with 70% ethanol before and after, and cap it afterwards. The vent filter and the cap air hole prevent a vacuum forming while drawing.
- A sample represents the culture only while the stirrer is running.
- Inoculate through the same valve.
- Treat shell-side fluid as live culture until a plate from that run has grown nothing (Section 1.2).
- Set the shell sampling valve to **To sensor** for monitoring, or to **Fully open** to collect into the bottle. Leave it **Closed** otherwise.
- Empty the permeate collection bottle by hand before it fills; there is no level sensing. Wear gloves and eye protection, and inactivate the fluid as described in Section 8 unless it is being kept for analysis.
- Connect the external feed only for a refill (Section 5.1), with the feed valve set to **External feed**. Return the valve to **Closed** or **Base** afterwards.

## 4.9 Recording and data

- **Recording file.** Every reading in Section 4.2, written once per sampling window. Start and stop recording from the recording panel; the status line shows the start time and the point count.
- **Raw pressure.** High-rate pressure batches are written to the raw data folder. The interface warns below 2 GB free and stops raw logging below 500 MB, while continuing to write the aggregate record.
- **Event files.** Every automatic base dose, and every pinch with its duration, mean and peak TMP and release reason.
- **Calibration file.** Exported from **Calibration Values** → **Export calibration**.
- After every run, download the recording and its raw data folder and export the calibration file. The storage location and download route are on the commissioning record.

## 4.10 Ending a run

1. Press **Release** on the pinch valve, then stop both pumps and stop recording.
2. Export the calibration file and download the recording and its raw data folder (Section 4.9).
3. Drain the culture into a container of disinfectant.
4. Flush the lumen and shell paths with disinfectant, then with water, running the pump for several loop volumes each time.
5. Only then break any connection or remove any sensor.
6. Inspect the membrane module and log its hours (Section 3.4.1).
7. Shut down in order: 12 V supply and distribution board first, then the controller board (Section 3.2).

# 5 Maintenance

## 5.1 Refilling medium

The culture loop stays closed for the run. Refilling means adding fresh medium through the feed path, not opening the reservoir.

1. Set the feed valve to **External feed** and connect the fresh-medium line.
2. Run the feed pump at a low PWM and watch the reservoir level. There is no level sensor, so the level must be watched by eye.
3. Press **Declare operation (dilution)** in the OD600 panel before or immediately after the addition, so that the growth-rate window restarts and the dilution is not read as negative growth.
4. Return the feed valve to **Closed**, or to **Base** if automatic pH control is to continue.

### NOTE · Sealed refill cartridges are a plan

The commercial bioreactor is designed to take two sealed, single-use cartridges, ready-to-use sterile medium and a *B. subtilis* spore stock, in one keyed dock, so that nothing is poured by hand. They are a concept and not part of this system: refill through the feed path as described above.

## 5.2 Routine checks

| Interval           | Check                                                                                                                                       |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Before every run   | Tubing at the pump head; every Luer connection; filters dry; blank taken; pressure zeroed; self-test plateau; free disk space on the board. |
| Daily during a run | Flow rate against the set PWM; TMP trend; pH within range; permeate bottle level; no liquid on the bench or the incubator floor.            |

| Interval                | Check                                                                                                                     |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------|
| After every run         | Flush the loop; inspect the membrane module; export the calibration file; download the recording and its raw data folder. |
| After a membrane change | Re-run the gravity self-test and the clean-membrane sweep; plate shell-side medium on the first run.                      |

### 5.3 Cleaning and storage

- Flush the lumen and shell paths with disinfectant and then with water at the end of every run, before any connection is broken.
- Wipe the exterior, the stirrer plate and the shelf with 70% ethanol. Keep liquid away from the controller board, the motor driver and the wiring.
- Store the device dry. Leave the pinch valve released and the pump heads open so that the tubing is not held compressed between runs.
- Store the pH probe wet, in the storage solution recommended by its supplier. A probe that has dried out will not calibrate.

### 5.4 Consumables and replacement

| Item                              | Replace                                                                                                                                                |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Pump tubing</b>                | At the first sign of flattening or scoring at the head, and as a precaution before any long run.                                                       |
| <b>Membrane module</b>            | When the fouling indicators in Section 4.5 reach the critical threshold, when a breach is suspected, or when cleaning no longer restores permeability. |
| <b>0.45 µm vent filters</b>       | Whenever a filter has been wetted, and at every membrane change.                                                                                       |
| <b>Transmitter line filters</b>   | At every membrane change, or if a transmitter reading becomes erratic.                                                                                 |
| <b>pH probe</b>                   | When two-point calibration no longer holds, or the response becomes slow.                                                                              |
| <b>Luer valves and connectors</b> | At any sign of leakage, cracking or a loose fit.                                                                                                       |

Treat every used consumable that has held culture as biological waste (Section 8).

## 6 Troubleshooting

If a fault is not listed here, or persists after the action given, stop the run, shut down in the order in Section 3.2, and consult the project record (Section 10).

| Symptom                                       | Likely cause                                                    | What to do                                                                                                                        |
|-----------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Pump does not turn at a low setting           | Static friction in the head                                     | Kick-start is on by default. Raise the PWM. Confirm the head is not over-tightened on the tube.                                   |
| Pump will not start; the panel gives a reason | Pressure trip is latched                                        | Clear the cause, then <b>Clear pressure trip</b> , then set the PWM and press <b>Apply</b> (Section 4.6).                         |
| Feed pump moves liquid the wrong way          | Motor wiring                                                    | Exchange the two motor wires at the driver output. The pump runs in one direction only.                                           |
| Feed pump refuses to start                    | Feed valve fully closed                                         | Set the feed valve to <b>Base</b> or <b>External feed</b> first.                                                                  |
| Flow reads zero while the pump turns          | No pulses reaching the controller, or the line is air-locked    | Check the flow meter signal line and its divider. Prime the loop.                                                                 |
| Flow reading dimmed                           | The value is stale                                              | Unread values are shown empty rather than zero. Check the sensor connection.                                                      |
| Pinch valve releases itself repeatedly        | Pump pulsation peaks above the lumen-in limit                   | Switch the limit basis from instantaneous peak to mean, or raise the limit moderately (Section 4.6).                              |
| A servo cannot be found by the scan           | Bus wiring, jumper or power                                     | Check the adapter jumper and that the two bus lines are not crossed. Use the full scan when more than one servo is on the bus.    |
| Two valves move together                      | Two servos share a bus address                                  | Set the addresses one servo at a time with the others disconnected (Section 3.4.4).                                               |
| OD600 freezes or falls to zero                | A light sensor has left measurement mode                        | The controller resets the sensor bus by itself. If it repeats, check the sensor supply and shorten the sensor wiring.             |
| OD600 jumps and the gate rejects readings     | Bubbles in the flow cuvette                                     | Check the reservoir level and the air filter. Look for a loose Luer connection drawing air.                                       |
| Growth rate shows nothing                     | Not enough accepted readings yet                                | The fit needs six accepted points spanning 15 minutes, above the minimum density.                                                 |
| pH reads out of range                         | Probe dry, unplugged or out of calibration                      | Check that the probe is immersed, reseal the connector, and recalibrate on pH 7.00 then 4.01 (Section 3.5.4).                     |
| Thermometer not found                         | The temperature line is held low by a wiring fault              | Check the pull-up resistor from the data line to 3.3 V, and the probe colour code.                                                |
| pH control latches a fault                    | Three doses with no response: base bottle empty or line blocked | Refill or clear the line, then press <b>Clear FAULT</b> (Section 4.4).                                                            |
| TMP falls to zero or goes negative            | Backflow, or the collection line is open                        | Check the shell sampling valve position and the collection line. Do not pinch until it is resolved.                               |
| Clogging warnings rise over hours             | The membrane is fouling                                         | Record the trend (Section 4.5) and plan a module change (Section 5.4).                                                            |
| Pressure readings out by about 1%             | Divider resistors differ from the values in firmware            | Measure the actual resistances and enter them.                                                                                    |
| Buttons do nothing, data still updating       | The command channel to the board has failed                     | Recording continues. Stop and restart the application; the pump stops and the pinch valve opens.                                  |
| Interface reports a firmware version mismatch | Firmware and backend versions do not match                      | Replace both files together and reflash (Section 3.3).                                                                            |
| Board does not boot                           | External power was switched on first, or back-feeding           | Disconnect USB-C for at least 10 seconds. Follow the power-up order (Section 3.2). Measure every signal line before reconnecting. |

## 7 Specifications

### 7.1 Process

| Parameter                     | Value                                                    |
|-------------------------------|----------------------------------------------------------|
| Membrane                      | mPES hollow fiber, 0.2 µm pore rating                    |
| Membrane area                 | 200 cm <sup>2</sup> over 11 fibers                       |
| Culture working volume        | 1 L in the standard configuration                        |
| Culture temperature           | 37 °C in an incubator                                    |
| Stirring                      | 200 rpm, magnetic                                        |
| Feed pressure at working flow | 0.10 to 0.25 bar                                         |
| Retentate pressure            | Below feed; 0.02 to 0.20 bar                             |
| Permeate pressure             | Lowest of the three; 0.02 to 0.10 bar                    |
| Transmembrane pressure        | A few kPa in normal operation; read as a trend           |
| Pressure trip limit           | 0.70 bar default, adjustable 0.1 to 5.0 bar              |
| Pinch TMP and lumen-in limits | 0.5 bar default, on instantaneous peak or mean           |
| Maximum pinch time            | 900 s default                                            |
| pH target and deadband        | 7.00 and 0.05 default; control starts below 6.95         |
| Valid pH probe range          | 2 to 12                                                  |
| Base dosing                   | Base only; pulse 5 s default, up to twelve doses an hour |

### 7.2 Measurement and control

| Parameter               | Value                                                                               |
|-------------------------|-------------------------------------------------------------------------------------|
| Optical density         | In-line at 600 nm, 2 mm flow cuvette, sample and reference sensors                  |
| OD600 measurement       | Four dark readings, source on 60 ms, sixteen lit readings per window                |
| Growth rate             | Least-squares slope of ln(OD600) over 60 minutes                                    |
| pH sampling             | Once a second, with temperature compensation                                        |
| Pressure transmitters   | 0 to 1 MPa, 0 to 5 V output, divided to 3.3 V logic                                 |
| Default sampling window | 30 s                                                                                |
| Raw pressure logging    | High rate; stops below 500 MB free, warning below 2 GB                              |
| Interface               | Web interface on port 7000, English or Traditional Chinese                          |
| Local display           | 7-inch, 1024×600                                                                    |
| Safety interlocks       | On the microcontroller; remain active if the browser or Linux side stops responding |

### 7.3 Electrical and environmental

| Parameter               | Value                                                                                                                                                  |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| Controller board supply | 5 V over USB-C, board only                                                                                                                             |
| Motor and servo rail    | 12 V DC; do not exceed                                                                                                                                 |
| Signal logic            | 3.3 V; not 5 V tolerant                                                                                                                                |
| Series resistors        | 1 kΩ on the servo bus and motor driver lines; 4.7 kΩ on the flow, pressure and pH lines                                                                |
| Electronics environment | Room temperature, outside the wet zone. Condensation on the board is a fault condition.                                                                |
| Servo thermal cut-out   | 60 °C                                                                                                                                                  |
| Dimensions              | Lab bioreactor: 600 × 900 mm wire-grid panel on two L-bracket feet. Commercial cabinet (designed, not yet built): about 400 × 240 × 620 mm (W × D × H) |
| Weight                  | Not yet measured                                                                                                                                       |

## 8 Disposal

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Everything that has held culture is biological waste. Everything electrical is electronic waste. Separate the two before disposal, and follow the local rules of your institution in addition to the instructions below.

### 8.1 Biological waste

- Autoclave solid waste, including culture plates, used tubing, filters and the spent membrane module, at 121 °C for 15 to 30 minutes before disposal.
- Treat liquid waste, including culture, shell-side fluid and flush water, with 10% bleach or an equivalent disinfectant before disposal.
- Never discharge culture or shell-side fluid to a drain, to soil or to a hydroponic line.

#### **CAUTION · Decommissioning a membrane module**

A spent module holds liquid inside the fibers that will not drain. Flush the lumen and the shell with disinfectant, leave the module filled, then autoclave the whole module before disposal.

### 8.2 Electrical and electronic waste

- The controller board, motor driver, servos, pumps, sensors and illumination strip are electronic waste. Dispose of them through your institution's electronic waste route, not in general waste.
- Decontaminate any sensor that has contacted culture, such as the pH probe, the flow meter and the photometer cuvette, before it leaves the laboratory.
- Separate the 12 V supply and its cabling as electrical waste.

## 9 Notices

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### 9.1 Certification and intended use

The ReLEAF bioreactor is laboratory research equipment built by a student team for the iGEM 2026 competition. It is supplied as research apparatus for use by trained personnel at Biosafety Level 1.

The device carries no electrical, medical or biosafety certification, and it has not been assessed against any product safety standard. It is not a medical device, not a food-production device and not an agricultural product. Nothing it produces may be used for medical, food, clinical or agricultural purposes, and shell-side fluid must not be applied to soil, hydroponic lines or plants.

### 9.2 Limitations

- Containment depends on the integrity of the membrane module and of the tubing, connectors and pump head around it. Plate the shell side after every module change and treat harvested fluid as live culture until that plate has grown nothing.
- No cleaning protocol for the membrane module is specified; a fouled module is replaced rather than cleaned.
- The device has no level sensing and no closed-loop feeding. The reservoir level and the permeate bottle must be watched by the operator.
- The optical density reading is uncalibrated raw output. It is linear across the working range but is not traceable to a reference instrument.

### 9.3 Responsibility of the operator

The operating institution is responsible for ensuring that personnel are trained, that local biosafety and electrical rules are met, that waste is handled as described in Section 8, and that the device is used only within the conditions given in this manual. The supplier accepts no liability for damage or injury arising from use outside those conditions.

## 10 Commissioning Record

Complete this record at installation and keep it with the device. These values are specific to your installation and are referenced from the sections listed.

| Item                                                  | Value | Section    |
|-------------------------------------------------------|-------|------------|
| Installation date and location                        |       | 3.1        |
| Serial or build identifier                            |       | —          |
| Controller software and firmware versions             |       | 3.3        |
| Interface address and local display                   |       | 3.3        |
| Procedure to stop and restart the application         |       | 3.3, 6     |
| Recording, raw data and event file locations          |       | 4.9        |
| Base solution: reagent, concentration, supplier       |       | 1.4, 3.4.5 |
| 12 V supply rating                                    |       | 7.3        |
| Pressure transmitter model and rated temperature      |       | 7.3        |
| Flow meter model and calibration factor               |       | 7.2        |
| Pump tubing type and size                             |       | 3.4.2      |
| Which transmitters the controller reads               |       | 2.2        |
| Harvest pump control arrangement                      |       | 2.2        |
| Clean-membrane sweep liquid                           |       | 3.5.3      |
| Shell sampling valve state after boot                 |       | 4.3        |
| pH control: maximum wait and minimum response         |       | 4.4        |
| Membrane module identifier and hours at commissioning |       | 3.4.1      |
| Local electronic waste route                          |       | 8.2        |
| Commissioned by, signature                            |       | —          |

### 10.1 Membrane module log

| Module ID | Installed | Hours at removal | Reason for removal |
|-----------|-----------|------------------|--------------------|
|           |           |                  |                    |
|           |           |                  |                    |
|           |           |                  |                    |
|           |           |                  |                    |
|           |           |                  |                    |

### 10.2 Project record

The design files, build history and test records for this device are published on the GEMS-Taiwan iGEM 2026 wiki and in the team's GitLab repository. Consult them for the engineering background behind the procedures in this manual.

*Document: ReLEAF Bioreactor User Manual, revision B, 18 October 2026. Applies to the integrative bioreactor, prototype 3, with controller software v6.2.2 and MCU firmware v4.2.2. Issued by GEMS-Taiwan, iGEM 2026.*