

# Open and Modular Photobioreactor Projects.

September 18, 2017

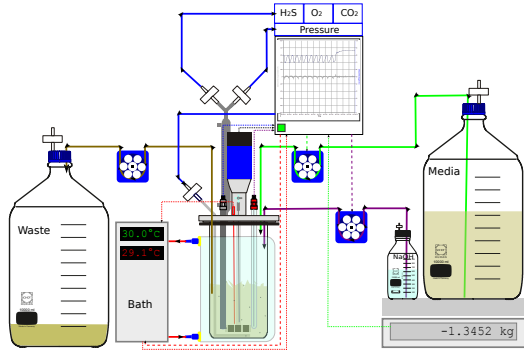
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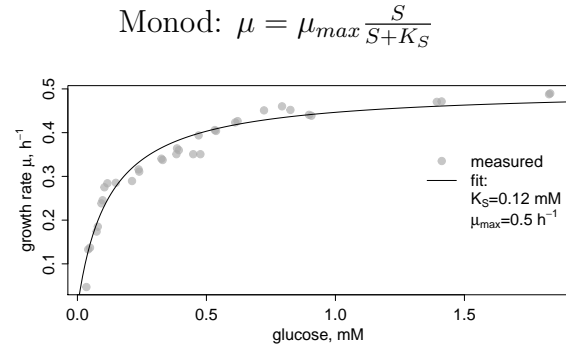
# 1 Resources - Materials, Designs and Ideas

Please see the Hack'a'thing Wiki for link lists of DIY bioreactors, DIY lab ware, 3D printer designs, general DIY websites, and electronics supply shops.

All code is at the github project.



(a) Dougie's Reactor



(b) Snoep *et al.* 2009

Figure 1: Bioreactors

$$\begin{aligned}
 \frac{dX}{dt} &= (\mu_{ab} - \phi)X \\
 \frac{dS}{dt} &= \phi(S_{in} - S) - (\mu_{ab} + \mu_{cd})X \\
 \frac{datp}{dt} &= (n_{cd}\mu_{cd} - n_{ab}\mu_{ab} - \mu_m)\frac{C_c}{V_c} - \mu_{ab}atp \\
 \frac{dG}{dt} &= k_L a(G_{in}^* - G) - n_g\mu_{cd}X \\
 adp &= a_{tot} - atp
 \end{aligned}
 \quad
 \begin{aligned}
 \mu_{ab} &\equiv f(S, atp) \\
 \mu_{cd} &\equiv f(S, G, adp) \\
 \mu_m &\equiv f(S, ROS)n_{cd}, \quad n_{ab} \equiv f(S)
 \end{aligned}$$

Figure 2: Growth in Continuous Culture: liquid and gas flux equations

## 2 PBR Modules

### 2.1 Gas Flux: Gas‘o’meter

**Project:** Run co2meter’s O<sub>2</sub> and CO<sub>2</sub> sensors via Sainsmart’s Arduino Mega+Touch screen); add a digital mass flow meter Aalborg XFM; write calibration routines for all sensors; build water trap and tubing to connect to the PSI or our DIY reactor and increase gas transfer (smaller bubbles) and decrease overall gas flow so that we can measure cellular activity. Perhaps add valve control to measure multiple reactors in series.

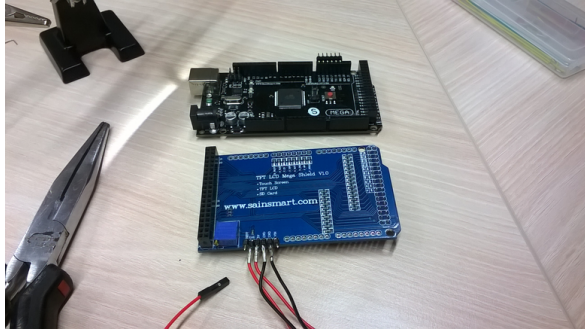
1. **build**: COZIR and UV Flux probes to Arduino Mega’s Tx/Rx pins, solder 5V/3.3V and Gnd connections to touchscreen shield; **code**: display of measurement values on screen, and optional “record data” mode to store data on the SD card of the touchscreen
2. **build** water trap, tubing path from reactor, and casing for sensors and Arduino; **build** improved gassing system (glas blowers!) to allow lower flow
3. **code** sensor calibration routines via touch-screen (use PSI gas mixing system)
4. **build** & **code** interface to Aalborg XFM digital mass flow meter: connect the Aalborg’s RS 485 interface to Arduino hardware serial Tx3/Rx3, and Ground
5. **build** & **code** valve control to measure several reactors; connect via Arduino software serial connections; perhaps attach to PSI Multicultivator

#### Resources:

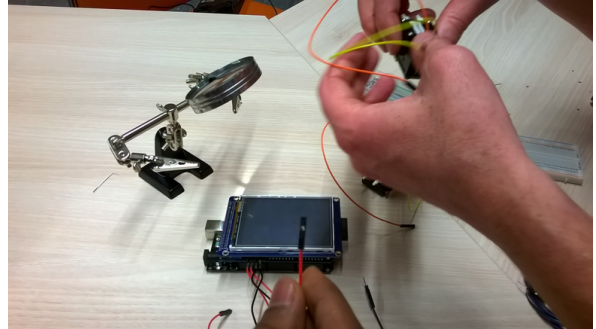
- Sensor manuals in `manuals/offgas/` at the PBR git:  
`Manual-CM-0201-UV-Flux-Oxygen.pdf`, `Manual-GSS-Sensors.pdf`, and `A_XFM_Manual_TD0701M[...].pdf`
- Code in `code/gas/gasometer/` at the PBR git

#### Materials:

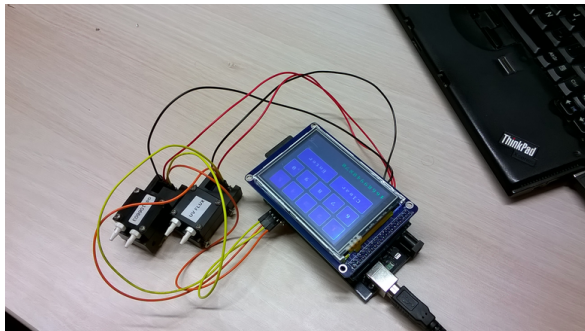
- Sainsmart’s Arduino Mega R3 + 3.2’ Touchscreen
- co2meter’s CO<sub>2</sub> and O<sub>2</sub> sensors, with cap adapter for flow: COZIR and UV Flux
- Aalborg XFM, with RS 485 interface + Arduino TTL-to-RS485 converter
- Temperature & Humidity sensor: AM2302 via elecrow
- Valve system for gas tubing, controllable *via* serial interface



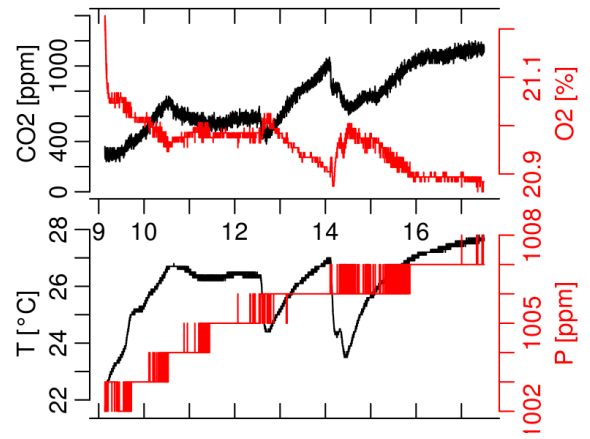
(a) The Gas'o'meter: making of



(b) The Gas'o'meter: making of



(c) The Gas'o'meter



(d) Room 03.30, 2016-01-13

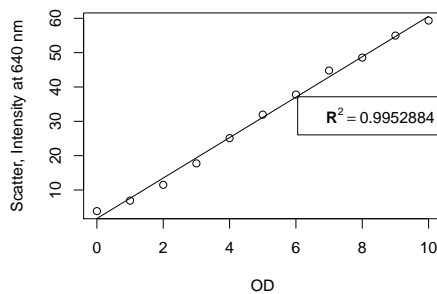
Figure 3: Gas Measurement Module.

## 2.2 Light Flux: Light Scatter for Biomass

**Background.** This technique is known as nephelometry. Light scatter can be used as an estimate of cell number and biomass, and gives a monotonous signal for  $OD > 1$  without dilution [Hancher et al., 1974]. This would allow use as an online measurement tool, either in a flow-cell, where culture is pumped through [Hancher et al., 1974]. Backscatter, at  $180^\circ$ , can be used to measure from the outside of a transparent reactor vessel [Ude et al., 2014, Bruder et al., 2016]. Measurement of scatter at  $90^\circ$  works better for low to intermediate cell densities [Ude et al., 2014].

A problem is that scatter can not be blanked to empty medium, and the value will depend on the light intensity and path, etc. A solution would be to express the measured value as a fraction of a standard scattering solution. Possible standards are discussed by Buffone [1976]. The commercial solution Ludox has been used by Maron and Lou [1954] and may be standardized enough for such a purpose, e.g. Sigma Aldrich, CAS Number 7631-86-9, LUDOX<sup>®</sup> TM-40 colloidal silica.

**Preliminary Results.** We found a linear relation of  $90^\circ$  scatter for OD 1–12 for *E. coli*, at ca. 640 nm the peak of a red light LED.



**Plan.**

**Ressources:**

- DIY Arduino-based Modules:
  - <https://github.com/ebgoldstein/OpenOBS> is a simple backscatter sensor with LED display,
  - <https://hackaday.io/project/12125-pulse-profiling-underwater-light-sensor> is a more involved underwater light- and backscatter sensors
- Commercial Products:

- Ude et al. [2014] test a shake flask system for PreSens
- Bruder et al. [2016] test a shake flask system for Aquila Biolabs

## 2.3 Light Flux: High-light Panel

## 2.4 Light Flux: Spectrometer

**Project:** Simple spectrometric measuring tool based on AvaSpec-Mini2048l-V25

1. Basic: Connect to Raspberry Pi, using drivers provides by Avantes; **code** simple interface with display and/or recording functions
2. Advanced: use LED for absorbance, reflectance, or fluorescence measurements; **build** light paths and perhaps a reactor probe for online recording

### Resources:

- AvaSpec-Mini data sheet in `manuals/light/` at the PBR git
- The AS5216 microprocessor board - lib for Rasp. Pi 1 B+ at `code/light/`
- Tsuda *et al.* PLoS ONE 2015: 3D printed casing for OD measurement

### Materials:

- AvaSpec-Mini2048l-V25, Minispectrometer:  
Mini spectrometer, 2048 Large pixels, grating-MN0600-0.50 (350-885nm), OSC, 25 $\mu$ m slit, USB2 interface, AvaSoft-Basic
- Fiber optic cables, VIS/NIR: 1 m, 200  $\mu$ m VIS/NIR and 1m, 600  $\mu$ m SMA terminations, metal protection sleeves
- Raspberry Pi Version 1 Model B+ or Version 2 (libs for both available)
- LED system: use PSI LEDs or **obtain**
- Reactor probe: **build**; 3D print and/or fine mechanics and glas blowers



(a) AvaSpec-Mini 2048

setup sketch here  
(b)

Figure 4: **Spectrometer Module.**



## 2.5 Liquid Flux: the Liq‘o’meter

For running cultures in chemostat (continuous culture) or turbidostat modes.

**Project:** build a module consisting of media and waste bottles, a reactor vessel, peristaltic pump(s), and a scale; pump and scale are controlled *via* serial interfaces from an Arduino+Touchscreen. The flow rate is controlled *via* the pump motor speed and recorded *via* the scale; the flow rate is recorded or can be set after a setup-specific (tubing) calibration routine

1. **build** a simple reactor vessel (Schott bottles) with liquid media flow, from media bottles through reactor vessel and out to waste bottle
2. **code**: calibration routine for the weight sensor module
3. **code**: analog control of peristaltic motor speed and recording of weight loss and/or gain to record mass flow (g/min)
4. **code**: routine to calibrate pump speed to weight loss/gain for a specific setup; store calibration on SD card, which allows to also set pump speed in g/min, or if provided with a culture volume, as culture dilution rate ( $\text{h}^{-1}$ )
5. **build** & **code**: combine with 2.4 to make turbidostatic control
6. **build**: add gassing system of project 2.1 to make a first simple bioreactor

### Resources:

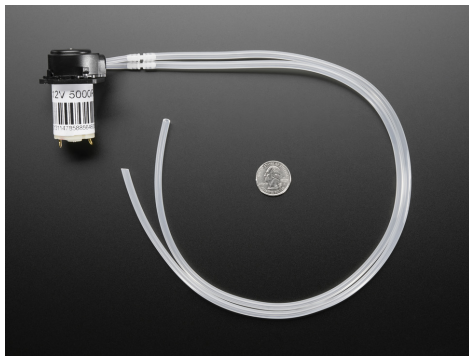
- Arduino library for Elecrow weight sensor kit based on older version
- HX711 24-bit analog-to-digital converter (ADC) for load cells

### Materials:

- Sainsmart’s Arduino Mega R3 + 3.2’ Touchscreen + Arduino Motor Shield R3
- Scale:  
Elecrow Weight Sensor kit 3kg for Arduino via elecrow
- Peristaltic pumps, options:  
12V DC motor (5000 rpm): at ebay  
12V/24V stepper motor and metal tube holder (pump head) via aliexpress  
12V Nema17 stepper motor with 3D-printed pump head



(a) Elecrow Scale Module, 3 kg



(b) Adafruit Peristaltic Pump

Figure 5: **Continuous Culture Module.** The elecrow scale module is based on the HX711 load-cell amplifier (24-bit analog- to-digital converter) and connected to 5V, Gnd and two analog pins of the Arduino and comes with an Arduino library. An Adafruit peristaltic pump (12V; a “geared down DC motor”) is run via the Arduino motor shield or alternatively via a a Toshiba TA7291P Bridge Driver (0-20V 1A; 2A peak) (instructable [here](#)).

## 2.6 The Server

**Project:** a master software running on a (detachable) linux desktop that synchronizes and speaks via a common interface to all Arduino and Raspberry Pi modules; the modules themselves can interpret get, set and act impulses (use arguments only when absolutely necessary).

During an initialization the server may inquire what an attached module provides (*via* data IDs and SI units, meaningful time resolution) and handle it automatically.

Variable higher order control or processing logics can be built using defined data and control IDs. Ultimately, a direct integration with mathematical models may be desirable. For example, measured O<sub>2</sub> and CO<sub>2</sub> levels may be used to estimate metabolic activities, such as catabolic ATP/ADP turnover; required data and equations can be loaded and interpreted *via* SBML encoded models.

1. **build** combine of gas (2.1), liquid (2.5) and light (2.4) modules into a bioreactor
2. **code**: master program to synchronize and record data from the three modules
3. **code**: combine e.g. 2.4 & 2.5 to implement turbidostat control
4. **code**: higher order data evaluation logics, *eg.*, estimate metabolic rates from gas exchange measurements

### Materials:

- `setTime(time_t t)`: sets the current master time to all modules
- `get(..., time_t t)`: get all values, currently available (with a time stamp), or from a previous time  $t$
- `act(..., time_t t)`: act (switch on and off, set to a specific value), now or at future time  $t$

## 2.7 Heat Flux: Water Bath Thermostat

**Project:** build a water bath for growth vessels, control T, read-out energy required for maintaining constant T and estimate the amount of heat withdrawn or administered

1.

### Materials:

- Jacketed reactor vessel: **build** or **obtain**
- Julabo water bath, e.g. F25-ME
- Arduino and/or Raspberry Pi

## 2.8 The Kaiten Eppi: Automated Sampling Device

**Projects:** build sterile and automated sampling device; using a controllable syringe pump, sampling into the Kaiten Eppi (automated: pump sample into tubes, potentially pre-filled with chemicals, vortex, and transport them into liquid N<sub>2</sub> or other storage containers)

### Materials:

- Sainsmart's Arduino Mega & Touchscreen
- Sterile sampling device by HHU glas blowers
- 12V Nema17 Stepper Motor 45oz,0.4A,34mm for 3D printer CE (as in ultimaker2):  
via act motor
- Plastic syringe pump + 3D-printed holder for pump and motor
- Kaiten Eppi: **build** a circular tube-holder, run by a stepper motor (same as above for pump)

## 2.9 Single Cell Biology: Microfluidic Device

**Project:** Basic microfluidics and live-cell imaging device; scratch growth chambers and liquid flow channels into microscope slide; attach 2–3 pumps; and control *via* arduino/screen

### Resources:

- Tsuda *et al.* PLoS ONE 2015: 3D Printed 'Plug and Play' Millifluidic

### Materials:

- Ilka's lab microscope: **available**
- Microscopy slides: **available**
- 2–3 peristaltic pumps for microfluidics: **obtain**
- Sainsmart's Arduino Mega + Touchscreen: **obtain**

## References

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