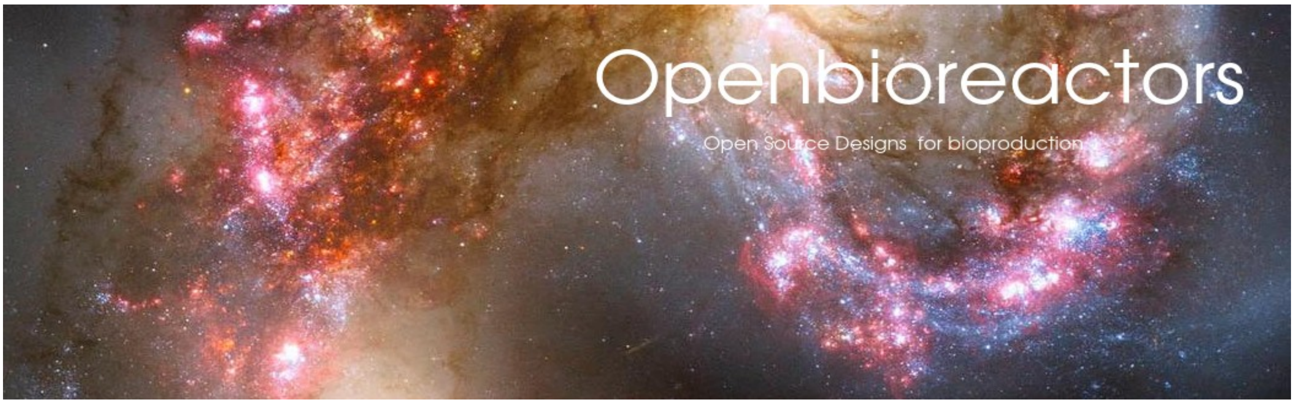


Technical Report

Propelled by SPACE GAMBIT The Hackerspace Space Program & Piloted by LA PAILLASSE

1-Introduction

Today, Bioreactor is used at a large industrial scale and thus is very complex and expensive.

Our objective was to make it cheaper and accessible for everyone through the development of an open-source, low-cost bioreactor bringing down the cost from at least 20 000 euros a piece to a thousand.
Beeing active but not everyday at the lab, we wanted to be able to monitor production from a distance.

Beeing one of the most important tool for a biohacker, we also needed sharing new applications and protocols of bioproduction thanks to a collaborative web platform wich would enable knowledge spreading with others communities.

1-a Définition

A bioreactor or fermenter, is a device wherein multiplying microorganisms (yeast , bacteria , fungi, algae , plant and animal cells) is sought for the production of biomass (ecology) , or for the production of a metabolite or bioconversion of a molecule of interest .
Unlike the simpler systems used for growing microorganisms , such as vials , the bioreactor seeks to control culture conditions (temperature , pH, aeration, etc. .) , And thus , it can raise information of higher reliability.
The laboratory models range from 0.1 to 15 liters . The models used to test for industrialization (known as "drivers ") go from 20 to 1000 liters, while those for industrial production may exceed 1,000 m3 (the case of ethanol) . Disposable bioreactors models available on the market since 1995 , mainly used for volumes ranging from a few hundred milliliters .
In tissue engineering , the term may designate a bioreactor system for tissue culture. The purpose here is not to produce metabolites but a complete tissue composed of cells and extracellular matrix.

1-b Specifications

A bioreactor comprising:
-A tub or glass enclosure (for laboratory models) or stainless steel
-A cap if necessary to not let the air of the indoor environment and that of the external environment
-A syringe with a catheter for injecting solution
-A stirring system comprising one or more turbines based on size
-Sensors for measuring the temperature (thermometer), the pH (pH meter), the dissolved oxygen concentration (oximetry sensor), the level ...
-A process control system managed by computer to record and control all parameters

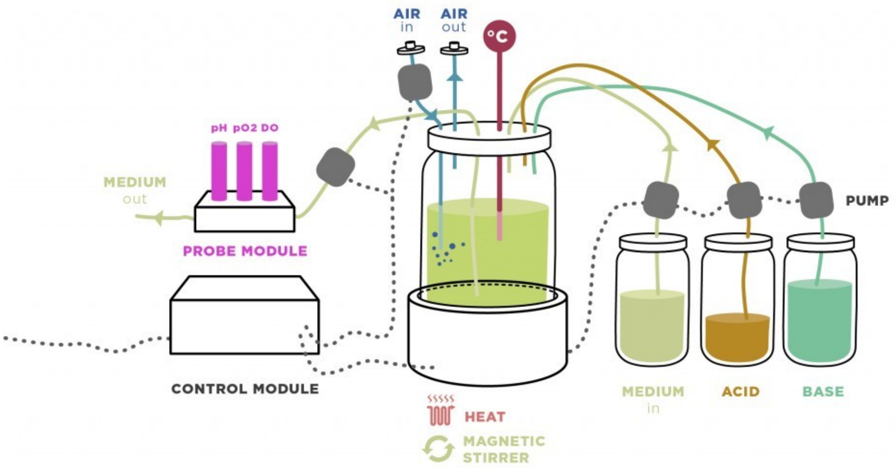
The bioreactor we develop builds on concepts that make the success of open source engineering. The bioreactor is meant easy to use simple design, and modify open source, scalable, fault tolerant, with sub parts counter, easily replaceable, cheap, usable network, well documented and independently.
All these sometimes contradictory imperatives require the choice of technical method and special equipment.

1-c State of the art

The objective of developing the software and hardware needed to operate the bioreactor was achieved.
These steps were performed successfully using two different physical design under ongoing improvement.
Issues considering sterility, and an additional cost reduction will require time for investigation and more development work .
With the number of Paillase growing around the globe (6 including Paris), and sharing the job done with other communities we met trough thoses first steps, we will be more efficient by dividing the optimisation phase in submodules using the web platform.

2-Hardware

2-a Global hardware design



We tested two diffferent designs based on the same idea of probe externalisation

Raspberry Pi
Hardware: Arduino Uno, Adafruit Motorshield / arduino 2560
Sensors: pH, O2, Temperature, Density (not implemented)
Engine: 4 motors 12V DC current
Supply via arduino wiring

Connection serial cables
Raspberry Pi
Hardware: Arduino 2560, 2 relays
Sensors: pH, O2, Temperature, Density (not implemented)
Engine: 4 motors 24V DC current
external power

For optimum culture, the control of temperature, pH, pO2, turbidity, air inlet flow and require the addition of pumps, also involving the use of numerous sensors for monitoring of these variables are generally very sensitive.

Except for the turbidity sensor (referred to but not implemented), the majority of probes are not openhardware but were chosen for the calibration hardware and software, keeping in mind that to meet the specifications a real job must be made on these probes, to lower the manufacturing cost (acquisition :))
(it takes a hundred euros per autoclavable probes available on the market) and make openhardware / opensource (firmware).

2-b Communication protocols

We temporarily opted for a serial connection, even if the wireless connection remains the ultimate goal.
We chose several (nrf24), and Zigbee as network architecture modules .
The zigbee is compatible with the wifi network and the nrf24L has a limited cost used as an arduino module.
The architecture is a star network, further investigation is still necessary for the implementation of the communication process at software level, and for secure communication (to prevent piracy of motor control for example).

ref
http://jeromeabel.net/fr/ressources/xbee-arduino

2-c Hardware Details (more specs on website)

-Board :

We chosed arduino boards (arduino uno and 2580) for their reliability, good documentation and because its fairly easy easily programmation.
There are many tools that facilitate the development around this board (Arduino IDE tool to compile python code etc ...) as well as the large number of shield supports.
In addition, this card can be programmed in python (Python is an open source programming language, easy access for beginners which can be programmed with the Arduino board).
We can even consider programming the arduino card in full python.

-Motor Control:

Several card interfaced with arduino motor control were tested : dualstep motorshield, L298N and vellman kit. However, the small LN 298N caught our attention because of its price .

The Adafruit motorshield allows you to use 4 motors powered by 12v via arduino, with a limited number of connection (no need to board prototyping) because the motors connect directly to the card, power is supplied by the arduino, of there is more possibility of stacker cards and thus be able to control up to 96 motors! While keeping the number of pins required for connection of sensors.
Schedule a library is needed for motor control by arduino but the programming of those is very simple.

-Probes

The task is more complicated for O2 and pH sensors , it does not exist on the market probes operating in aqueous phase that is autoclavable at affordable cost : they are often over a hundred euros. Autoclavable probes in aqueous phase remains expensive .

After using probes Atlas Scientific, cheap and relatively reliable but not open hardware , we made a draft investigation of alternative methods of sterilization (cold sterilization) . The problem is less serious for temperature probes (autoclavable cheap models) , and DO probes that can be encapsulated and therefore do not need to be sterilized .

The important point is that the current equipment is not expensive and costs openhardware and proposed solutions are not very practical.

We are currently looking to replicate probes open hardware at the lowest cost . Commercial probes acquired will enable us to verify the calibration.

- Pumps

We found not all pump to be equal in quality. While Adafruit 's tubes stand the autoclave, we recommand 12V ones for their great robustness .Most og the time changing the inner tube is a good option for increasing durability

-Misc

The heat controler tested (heating belt) is impractical (slippery) but does not seem to pose any particular problem, the peltier module was heating a very small portion of the bioreactor and we wish to experiment a solution based on a platter IR diodes.

A test OD probe will be performed by coupling a photoreceptor with a laser, without cost should be very low, but the implementation will require a very fine calibration, which require real work on the downstream software (influence external light, influence of

turbidity own media, etc ....).
Similarly, the agitator has been succesfully design and mounted, it will be integrated into a demo version of the bioreactor version, it can be controlled with a potentiometer for the moment, but will be remotely controllable in the future using a rotative encoder.

2-d Sterilisation

We could, thanks to the dotation buy a lab grade autoclave

Autoclaving :

Sterilization technique of medical waste (Waste from care activities potentially infectious) using saturated steam under pressure or superheated water. The heat associated with the moisture causing the destruction of germs. For a material to be considered as sterile, the theoretical probability of isolating a seed should be less than 1%. This is the level of sterility assurance (NAS) regulated by EN 556.

Using a pressure cooker can be an option when food production is not the objective

Regarding the cost , we had several lectures on low-temperature sterilisation and will try some of them considering their efficiency in the specific bioreactor's case
Sterilization with ethylene oxide
Plasma sterilization
The equipment to be sterilized is subjected to the electrons, photons and ions from a plasma.
Electron beam sterilization
Sterilization by gamma irradiation
Radiation sterilization of medical and surgical equipment by gamma irradiation is performed with a dose of about 25 kGy and can be done on the material already placed in its final packaging.

2-e Next steps

-Experimenting the open hardware DO probe found
-Pursue the investigation on Open Ph probe
Pursue the noisebridge investigations on Do with an interested team of engineers
https://www.noisebridge.net/wiki/BioBoard/Documentation/Oxygen
-Testing liquid level monitoring with a glass melted solution
-Experimenting on autoclavable electronics with our partners
-Experiment vaporisation of medium and sampling with an electronic cigarette and gas Co2 probe
-Testing IR heating (specially its influence in culture growth)
-Pushing wireless communication making it more stable, and securing communication (to prevent piracy of motor control for example)

3-Software

The API monitoring and remote control of the bioreactor are useful for the establishment of self-station production.
All code is available on the (as it may change, it is better to access to it via the « fork me on github » ribbon on the www.openbioreactors.org website

3-a Software

Actual Linux installation procedure :

modules to install :
python27 :
python-PyQT :
python-Tornado :
python-Numpy
python-Pyserial
javascript – jquery (compris dans LeeLaopenbioreactor)

included in arduino IDE :
Arduino C
Adafruit motoshielV2 (a télécharger et à ajouter aux dossier des librairies arduino)

Download « LeeLa openbioreacteur » from the github repository (via website)

-Monitoring :

Arduino board returns (Similic code), the data collected by sensors to a raspberry (star center) port, these data are processed by the listener (listener openbioreactor python code) sent to a database and tornado server (python openbioreactor-server), this one displays the data thanks to a coded jquery (javascript) graphical interface, the web interface is modular and can be easily structured through an XML file (tutorial coming soon) . Addition of a new page for a new bioreactor prints probes data of several bioreactors on a same graph, data overlay temperature on the same graph, or a different graph
The server is listenable on localhost: 8888/BIOREACTOR

-Control :

The motors are controlled by a GUI coded in pyqt, python (openbioreactor-gui) and by commands sent on the serial port of Arduino. The GUI can also monitor the sensor data.

3-b Développement

The constant development of API and tools for the bioreactor should not prevent to have a permanently available stable code .
This is why certain practices of continuous integration can be considered (mainly facilitated after compilation deployment check-compilation environment in test etc ....).
The code should be updated regularly, can be loaded on central servers almost instantaneously, without errors.

Users must be able to load the firmware on Arduinos cards on many bioreactors, possibly thousands, without interrupting production.
This is the reason why some tools used in software development should not be seen as luxury, but must be integrated into development processes openbioreactor project.

-Git (gestionnaire de version), synchronisation of the repository with arduino card
-Compilation API in command lines
-Deployment server

3-c Calibration

An essential step requiring further automation is that of calibration, this phase (the automation) will be implemented as soon as the prototype is fully operational.
It will be initially to calibrate the probe measures the arrival media volumes, but also the inertia action.
In a second step, finally, control growth of microorganisms such as euglena gracilis may be considered.

3-d Next Steps

-It is intended to facilitate the work of novices by including all the necessary environment for steering bioreacteur a bootable drive, a single boot on the key allowing to control the bioreactor without complicated installation procedure modules.
-An extension to control the behavior of the engine and the thermostat (temperature) is in progress, in fact we should be able to program the environment easily with an exchangeable specifications .
This file should be loaded into a bioreactor remote production, depending on the period, or the type of microorganism to produce.
-Comment the code more explicitely

4-Web Portal www.openbioreactors.org

OPENBIOREACTORS
BIOPRODUCTION BYYOURSELF & FORYOURSELF

Considering the Reprap community and its enormous contribution to open source 3D printing, we wanted since the very beginning of the project to have a tool to share our work with others around the globe.

The combo Website + wiki+ forum + IRC prooved to be greatlly fulfilling this job but we wanted fresher and simpler tools than a mediawiki and a clasic php forum.

All the documentation around the bioreactor project is under continous developement on this website .

4 projects demonstrated their will to join the portal and several DIY BIO community actors are waiting the end of the beta phase to contribute to this great adventure.

4-a Wiki

Home wiki is stable when in place but nothing beats a hackpad to kickstart a documentation.

It can even be directly html embed when finished. We wanted to starting a hackpad and telling us when done in the forum so it can be smoothly added to the site wiki : Best of both worlds !

4-b Forums

While the actual version is standard we are seeing it as a first step to gather user's feedbacks, considering Node BB as a mid term developement solution and are actively looking at Discourse WP integration when final version will be released .

The last option brings a new level of responsive technologies massive asynchronous messages , thematic ...

4-c IRC

The ideal and most direct tool to quickly chat /meet / be helped in a bioreactor's developement and keep in touch with the community

users can join

-Using their own IRC client ,

-Via freenode interface

-Directly via the Freenode module integrated to the website

5-General conclusion

Although the phases of development hardware and software have not proved insurmountable difficulties, some gaps remain to be taken.
It appears that the development of openhardware probes, inexpensive, preferably autoclavable remains a goal that many resources should be concentrated at considering the risk of having to implement complex designs, or limit the scope of the bioreactor.
We will continue to realease open documentation on the www.openbioreactors.org website , considering this project as a flagship toward massivel distributed bioproduction and keep SpaceGambit informed of any further evolution.

We would like to deeply and sincerely thanks SpaceGambit for beeing behind this project since the beginning.
This has definitely been one more argument motivating the Paris city council to help us, making it possible to have a new place for the lab, 7 times bigger that the one we are in !

Considering this and the opening this year of (at least) 6 new “Paillasse” around the world this will definitly bring more and more energy around the openbioreactor projet.

THANKS !