Open-Source bioreactor controller for bacterial protein expression

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Abstract

Growing microorganisms for laboratory experiments or industrial biotechnological process is an activity which involves the use of bioreactors. Although there are many commercially available equipment, most of them lack the flexibility of an open-source solution. This work proposes a cost effective Arduino based bioreactor controller for growing suspended microbial cells. To exemplify its functionality, this study provides the parts list and schematics necessary to make a functional laboratory scale bench top stirred tank bioreactor. Using the built prototype, an *E. coli* culture is grown maintaining the preset parameters, protein expression is induced and culture is harvested at preset culture density. Automatically recorded process data shows stable environmental parameters and reliable bacterial growing curve.

Introduction

The bioreactor is the term used for to carry out any bioprocess in a closed system in which organisms that express a product of interest are grown (Eibl et al., 2010; IUPAC, 2018). Bioreactors offer best conditions for growing bacterial, yeast or eukaryotic cells under monitored and controlled environmental and operational conditions, like pH, temperature, CO_2/O_2 exchange and carbon source supply, nutrient level (Gueguim Kana et al., 2003; Pörtner et al., 2005; Doucha & Lívanský, 2006; Zhang et al., 2011). They differ by mode of operation (batch, fed-batch or continuous), the agent used (living cells or with pre-attached enzymes), the method of organisms growing (suspended, immobilized) and by the presence or absence of the oxygen and requirement of stirring (non stirred non aerated, non stirred aerated, stirred and aerated bioreactors) (Scott, 1987; Heath & Belfort, 1990; Edwards et al., 1999; Rani & Rao, 1999, Pörtner et al., 2005; Najafpour, 2006).

Stirred-tank and aerated bioreactors are the most frequently used for growing microbial cells on large scale and at a high cell density for industrial purposes. In traditionally shake flasks the culture is fed at the inoculation time and when this initial carbon source is consumed the culture stops growing. Moreover, the control of environment is limited. The acids from fermentation lowers pH of the broth which inhibits the microbial growth (Riesenberg & Guthke, 1999; Knoll et al., 2007; Willey, Sherwood & Woolverton, 2009; Obom, Magno & Cummings, 2013). Bioreactors control the pH by addition through a pump of 1-5 N NaOH or 25% ammonium hydroxide (w/v) for bacterial culture (Rault, Bouix & Béal, 2009; Obom, Magno & Cummings, 2013; Cheng et al., 2013) or 10 N KOH for yeast culture (Zelle et al., 2010). For eukaryotic cells culture, generally acid addition is required (1M HCl) (Johnstone & Alexandrov, 2014; Meghrous et al., 2015). Another key variable in the bioprocess is the temperature which must be tightly regulated either by heating or cooling (Farrell & Rose, 1967; Pearson, Glennon & Kieran, 2003). The rotation velocity during the stirring also facilitates distribution of temperature and increase uniformity of the fermentation broth (Zashkova, Penkova & Karamfilowa, 2006). Several temperature control systems have been designed and tested. In small size cultures, placing the

stirred-tank inside a thermal regulated bath ensures temperature control. When the bioreactor is scaled up the heat transfer becomes problematic and requires the tank jacketed with internal coils (Pérez-Correa, Fernández & Von, 2006; Palomares & Ramirez, 2009). Microorganism growth is supported by the nutrient composition from the growth medium, as well as the presence or absence of oxygen (VanMeter & Hubert, 2015). The control of aeration rate is critically for aerobic organisms and depend on dissolved oxygen, rate of diffusion into the fermentation broth, mixing and bioreactor capacity. In stirred tank bioreactor, the stirrer have a pronounced effect on mass transfer and on the aeration rate which may be assured by submerged (bubble oxygen supply) or surface aeration (Galaction et al., 2004; Garcia-Ochoa & Gomez, 2009; VanMeter & Hubert, 2015). It is also important to monitor the biomass, usually by means of different types of optical sensors such as turbidimetric, near-/mid-infrared (NIR, MIR) or fluorimetric (Kiviharju et al., 2008; Ude et al, 2014; van Noort, 2016). The small scale bioreactor is most similar to the flasks culture conditions but offers the advantage of better monitoring and feed-back optimization (Obom, Magno & Cummings, 2013). The sensors used for accurately quantification of environment and culture parameters (pH, culture density, temperature, aeration rate) are usually controlled by programming a micro-controller (Fisher & Gould, 2012; Einarsdóttir, 2014).

Arduino is one of the most popular open-source hardware platforms providing ready to use micro-controller printed circuit boards (PCB) with digital and analog input and output breakout pins. It is supported by a large community of enthusiasts who design and share hardware extensions named “shields”, software libraries and code examples. The programming language is a simplified C++ language, in a processing based Integrated Development Environment (IDE) (D’Ausilio, 2012). The programming code (*.ino) files called sketches are compiled and uploaded to the configured board. Because its simplicity, fast development, easy interfacing, great open-source software resources available, Arduino is already the platform of choice for many industrial applications.

The goal of this study was to design a simple, open source, cost effective bioreactor controller, easy to make from readily available components.

Materials and methods

Reagents

The reagents were purcheased form Sigma Aldrich unless otherwise stated. The Luria-Bertani (LB) culture media was supplemented with D-glucose (1%). Induction of protein expression was performed by addition of Isopropyl β-D-1-thiogalactopyranoside (IPTG).

Microbial cells

Escherichia coli bacterial cells, strain BL21(DE3*)pLysS-pET28a-hdNadV* with genotype *E. coli* B F–dcm ompT hsdS(rB– m B–) gal λ(DE3) [pLysS Cam^r] [pET28a-hdNadV Km^r] from our laboratory stock harboring pET28a-hdNadV plasmid (Addgene ID #83362) were used as bioreactor culture inoculum (Marinescu et al., 2018).

Bioreactor modular design overview

The components used in this study were chosen having availability and cost effectiveness as main targets. The bioreactor controller was designed, and a functional prototype was built. The components, suppliers and acquisition cost are listed in Table 1.

The bioreactor controller collects information from sensors about the culture density, pH and temperature. According to the preset process parameters, it commands the effectors circuits in order to maintain the growing conditions in the preset limits. Although the proposed design (Figure 1) shows as a functional prototype a bench top bioreactor, the controller module is independent of the effector circuits therefore it may control a bioreactor of virtually any scale. In our implementation, the O_2 supply is provided by M2 stirrer motor and /or by the Air Pump. The temperature is set and controlled on the PIDT1 module which commands the heater (HT1). OD Pump provides external culture flow through the sensors outside of the bioreactor (e.g. optical density sensor OD1). OH Pump is used for pumping concentrated base solution in order to maintain pH in the desired preset range. If the grown microorganism produces base, acid concentrated solution should be used instead. The protein expression is induced by commanding the IPTG Pump. At preset optical density, the Harvest Pump is commanded to automatically harvest the culture into the separatory funnel stored at 10ºC. Alternatively a tangential flow filtration system may be used instead.

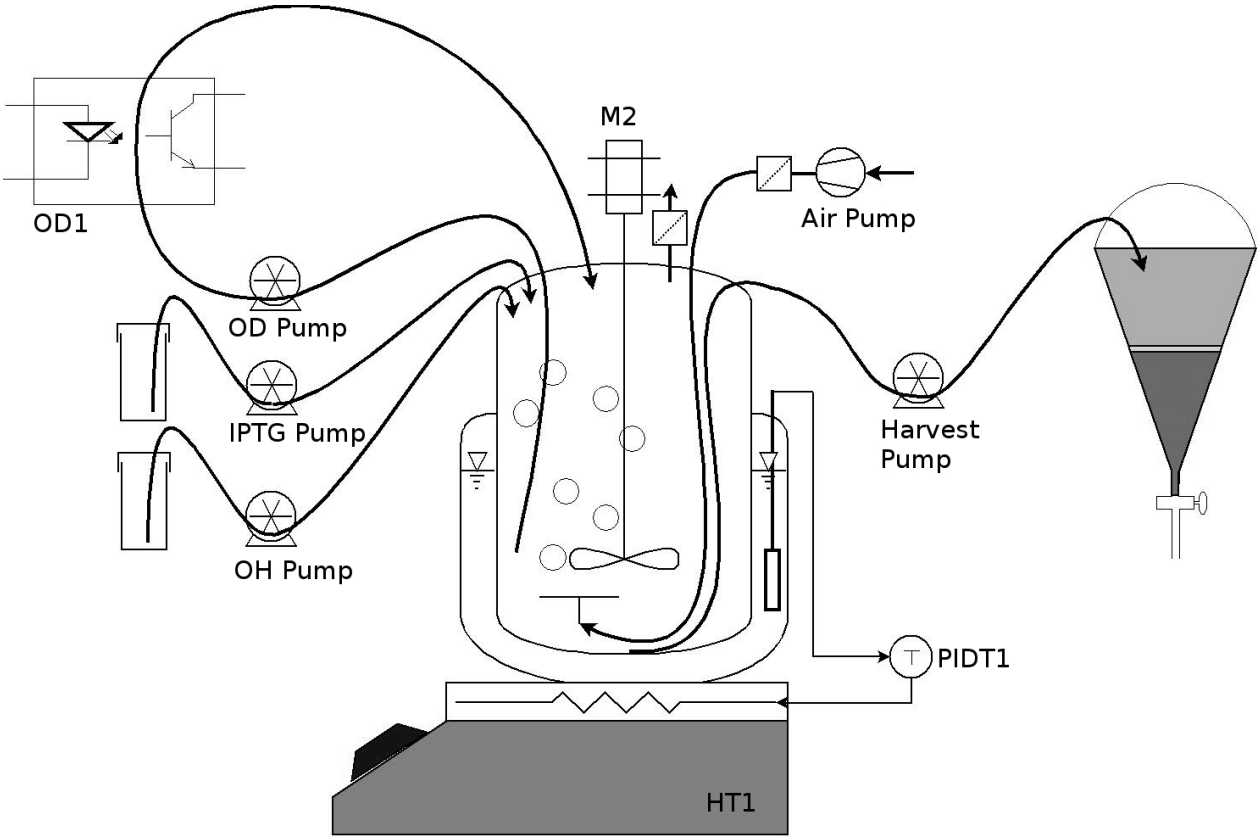

Figure 1: Bioreactor block diagram.

Arduino Bioreactor Controller

The main board, Arduino Mega 2560 R3 (AM2560) was connected to the PC using the USB port. The serial debug interface was used for debug and process log. The controller and low voltage modules were powered by a standard 500W ATX computer power supply which provides +5V and +12V DC. Arduino Ethernet Shield 2 (ETH1) was staked on top of AM2560 in order to implement network remote monitoring of the process, micro SD card data logging and micro SD card process parameters loading and saving. The start process button (B0) is connected between pin 28 and GND. Real time information about the bioprocess is shown on the I2C display Modtronix LCD2S-204BIW (DS1) connected to AM2560 i2c serial data bus (PIN 20 – SDA, PIN 21 – SCL). The display initialization and control software was written according to the manufacturer manual. Jumpers 1 and 2 were set to OFF position. The I2C address was this way set to 0x28 as specified by the LCD manufacturer. The peripherals (effectors) like pumps, motors, heaters are controlled by AM2560 through the Relay Shield (RM8), each relay supporting up to 10A current. Detailed wiring diagram is presented in Figure 2. Arduino boards images were generated with Fritzing 0.9.3b software available at http://fritzing.org.

Network access

The Arduino Ethernet2 version 1.0.3 library was used. Dynamic Host Configuration Protocol (DHCP) client was configured to automatically obtain an IP address. If no IP address was provided by any local DHCP server accessible in the local area network, the default IP 10.5.5.171/24 is used with gateway 10.5.5.1. Because time is an important factor in the microbial growing process (Willey, Sherwood & Woolverton, 2009) and accurate process data records depend on accurate time settings, after power up, the bioreactor controller connects to a public accessible internet NTP time server in order to set its internal clock. As the proposed bioreactor controller provides real time process data as a web service, it can be accessed from the network opening the IP address of the bioreactor in a browser (http://10.5.5.171).

Time library

Arduino time library DS1307RTC written by Michael Margolis, version 1.4.0 and real time clock module DS1307(RTC1) from Spark Fun Electronics was connected on the I2C serial data bus of AM2560 (PIN 20 – SDA, PIN 21 – SCL) as shown in Figure 2. When the controller starts up, if the network time server is available, the exact time is set on the internal clock and RTC1. If the time server is unavailable, the internal time is updated from RTC1.

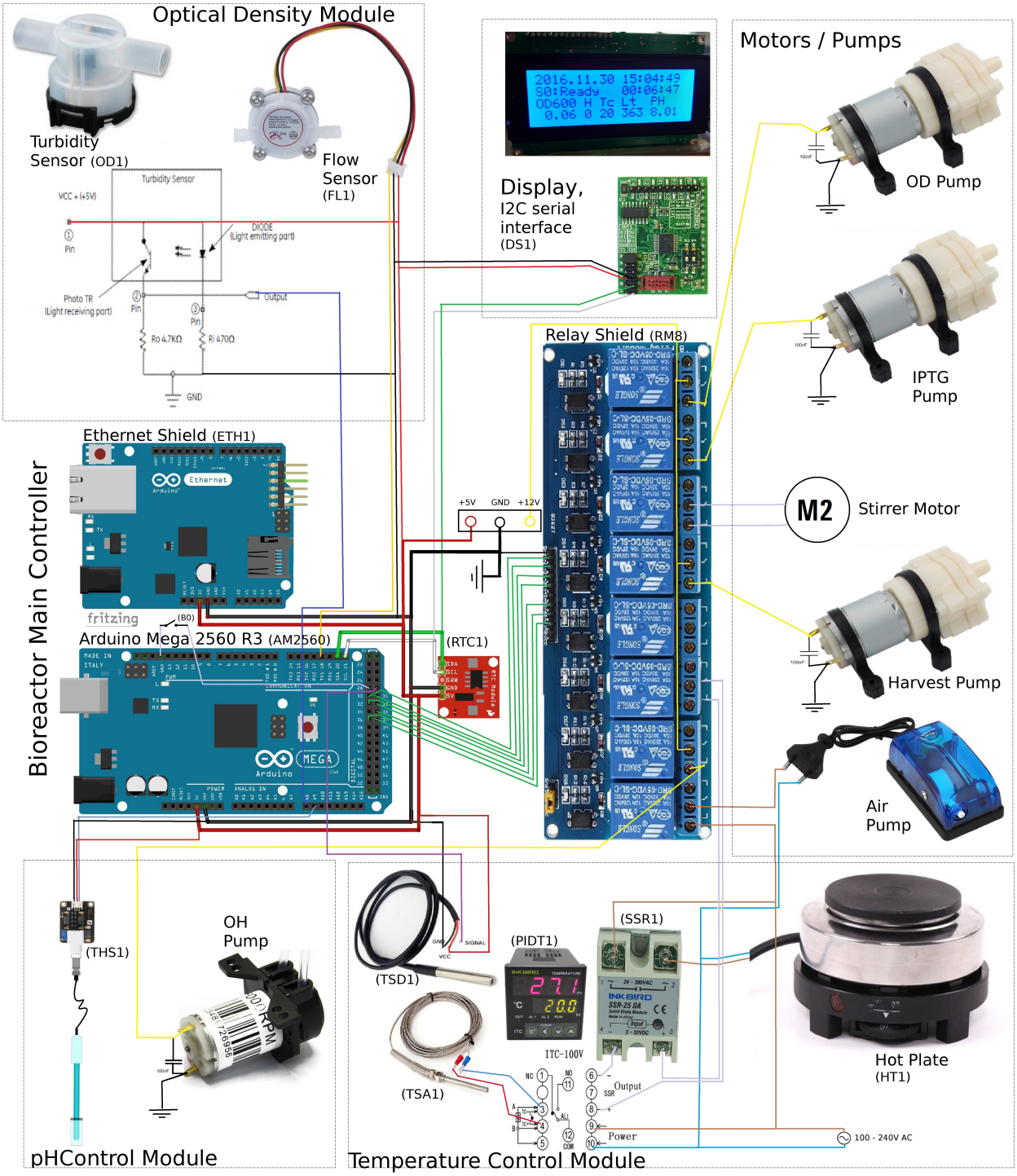

Figure 2: Bioreactor components and wiring schematics.

Data logging

Beside the maintenance of the culture environment in optimal limits, another important function of the bioreactor controller is data recording (Einarsdóttir, 2014). Arduino Ethernet2

shield provides the hardware interface for a Micro SD card. Ethernet2 library version 1.0.3 was used to implement both micro SD card data logging and network communication. Every time the controller starts up, it creates a standard comma separated values (CSV) text file with the following data, as shown in the file header: timestamp, stage, Lt, OD600, pH, Heater, Tc, OHuL, l_hour. The file name is unique and derived from the current date and time at the controller start up. The significance of each field is: current time as number of seconds that have elapsed since January 1, 1970 (midnight UTC/GMT) largely used and known as epoch time or Unix time, culture stage (1 = Ready; 2 = Growing; 3= IPTG ON, the protein expression was induced; 4 = Harvest), light intensity received by the phototransistor, calculated OD600, culture pH, Heater status (0 = OFF; 1 = ON), Culture temperature in Celsius Degrees, total volume of base (µL NaOH) pumped for pH adjustments, culture flow through the external sensors tubing. A line of values is recorded in the file at a fixed time interval defined by the variable SD_LOG_FREQ_SEC initialized by default to 900 seconds. Complete log file example is provided as supplementary information in Supplement S1.

Additionally there is a serial log assigned to the default Arduino serial console port where more verbose output related to the bioreactor and process status is provided. Relevant sections of the serial log is shown in the Supplement S2.

Controlling the microbial growing process.

Temperature

The temperature is one of the most critical parameters of the microbial culture (Ratkowsky et al., 1982). In the proposed design, it is set on the stand alone temperature controller INKBIRD ITC-100V (PIDT1) which uses power wave modulation algorithm to command the heater element (Hot Plate HT1 in our implementation) through the solid state relay INKBIRD SSR-25 DA (SSR1). A directly connected analogical Stainless Steel Probe K type Sensor (TSA1) is immersed in the water bath of the bioreactor. ON/OFF command to start or stop the temperature adjusting process is provided by AM2560 on PIN 35 which energizes relay 6 (normal open) of the Relay Shield (RM8) and closes the SSR1 input circuit (Figure 2). The temperature is also measured by AM2560 module using a one wire bus digital Thermal Sensor DS18B20, data pin was connected to PIN29, the sensor was powered from the +5VDC output of PSU1. The Arduino library for Dallas Temperature ICs version 3.7.5 and One Wire version 2.3.1 were used for interfacing the digital temperature sensor.

pH

The pH value is continuously monitored in order to keep the environmental in the optimal range for the growing microorganism (Griswold, 2004). For pH reading, Analog pH Sensor Pro Kit For Arduino SKU:SEN0169 (THS1) was connected to analog PIN A9 of AM2560. The analog readings were translated to pH values using a calibrated Thermo Orion 310 laboratory pH meter and sample solutions with pH range from 1 to 14. A linear calibration curve with correlation coefficient of 0.998 was obtained (Figure 5F). The calibration constants were

initialized in the controller software in variables PH_A=0.0335768807 and PH_B=-5.9885198198. The current pH value was calculated as ph_instant = PH_A * ph_adc +PH_B, where ph_adc was the voltage reading on the analog pin 9 of AM2560. In our bacterial growing experiments optimal pH interval was set between PH_MIN=7.0 and PH_MAX=7.4, this range was previously reported as optimal for growth of neutrophilic microorganisms (Jain & Sinha, 2009). While growing, bacteria produces acids (Willey, Sherwood & Woolverton, 2009), therefore adjusting pH involves automatic titration of base, 5N NaOH in our case. OH Pump was connected to relay 7 of RM8, controlled by PIN 36 of AM2560. Once the pH goes below PH_MIN, the feedback algorithm starts compensation by dropping a quantity of NaOH defined by the variable OH_DROP_VOLUME (µL). There is a waiting period defined by the variable OH_ON_OFF_MILISEC necessary for diffusion and pH equilibration in medium. After 10 consecutive drops, if pH is still below limit, the OH droplet doubles in size. This process continues until the desired pH is reached. Although there is no need for a precise initially estimate of the necessary OH flow or quantity, as it is automatically managed by the described adaptive feedback loop, for an accurate total OH record shown in the logs (OHuL field), the OH pump constant in µL/s must be set in the variable OH_FLOW (default, for our pump was 438 µL/s). The desired initial OH drop volume was set in variable OH_DROP_VOLUME, in our example it was set to 20 µL.

Culture density

Traditionally, the culture density is determined by in optical density (OD) units measured using a wave length of 600 nm (OD600), which is according to Lambert-Beer law the logarithm of the ratio of intensity of transmitted light to the intensity of the incident light passing through the sample (Swinehart, 1962; VanMeter & Hubert, 2015). In fact, it is not light absorption but light scattering or turbidity that is measured. The light from the emitting diode passes through a fixed length of microbial culture and is then received by the phototransistor. There are two methods to pass the cell culture between light emitting and light receiving components of the sensor: either pumping the culture out of the bioreactor or immersing the sensor into the culture inside the bioreactor (Cox et al., 1989). Our controller works with both types of optical density sensors. For external approach, OD1 pump was connected to Relay 1 of the RM8 controlled by PIN30 of AM250. The media was pumped from the bioreactor through 1/8'' autoclavable silicone tube to the flow sensor (FL1) and Turbidity Sensor (OD1). FL1 output was connected to PIN18 of AM2560 and was used to monitor the functioning of OD1 Pump, the output of OD1 was connected to AM2560 on PIN A8 pulled to ground through a 4.7 kOhm resistor (R1). The input light emitting diode of OD1 was replaced with a known 600 nm led (D1) connected to +5V DC and to ground through a 470 Ohms resistor (R2). For the immersed version of OD600 sensor, the two elements of OD1 (LED and Phototransistor) were removed from the plastic case, the connecting wires were extended and inserted each into a 16x125 mm glass tube and mounted into a 3D printed plastic part, as shown in Figure 4. The 3D CAD file is provided as

Supplement S3. The two elements of the sensor must be positioned inside the tubes facing each other maximizing the light intensity from the LED arriving on the phototransistor. Black color heat-shrink tube was used to minimize unwanted light reflections into the glass tubes. The fine positioning of the two OD600 sensor elements was done by obtaining a maximum possible Light Intensity (Lt) value shown on the LC Display (DS1) or in the serial port log. The OD600 value is calculated in real time with the formula: od = - log(lt/l0) where lt is the light intensity reading through the actual microbial culture and l0 is the light intensity reference reading through the medium only. The proposed implementation initializes l0 when the bioreactor goes from stage 0 (Ready) to 1 (Growing) as a consequence of switching B0 from OFF to ON. It worth mentioning here that the ambient light influences the light sensor reading, therefore it must be protected from any external light source.

Protein expression

Some of the most used techniques in biotechnology involves protein expression induction by pumping an inductor (e.g. Isopropyl β-D-1-thiogalactopyranoside – IPTG) or lactose (Lin & Hsu, 1997; Tian et al., 2011; Faust, Stand & Weuster-Botz, 2015). For maximum yield of product of interest, the induction is optimized to take place when the culture has reached a specified density (Glazyrina et al., 2010). For our process, we have determined by successive experiments that OD600 = 0.7 is the optimum induction time. The optical density which triggers the protein expression induction was initialized in the variable OD_IPTG. The IPTG induction solution was prepared to get a final concentration in the fermentation broth of 1 mM. The pumping time was calculated based on the pump flow and set into the variable IPTG_PUMPING_SECONDS. IPTG Pump was connected to the second relay of RM8 which is controlled by PIN 31 of AM2560.

Culture harvest

Once the culture reached the OD600 preset in variable OD_MAX, the harvest pump connected to relay 4 of RM8 controlled by pin 33 of AM2560 were pumping out the culture into a separatory funnel cooled at 10ºC. There was no fuel level sensor installed in the bioreactor vessel therefore the harvest pump running time was defined in the variable HARVEST_MINUTES as the calculated report between the total known culture volume divided by known pump flow rate (Clincke et al., 2013; Jaekel et al., 2015).

Bioreactor controller software

Arduino development studio version 1.6.5 was installed on a computer running MS Windows 7 operating system. The necessary Arduino libraries (Dhcp, Ethernet2, EthernetClient, EthernetServer, Wire, DS1307RTC, Time, SPI, EthernetUdp2, SD, OneWire, DallasTemperature, DallasTemperature) and usage examples were downloaded from www.arduino.cc unless otherwise stated. The Arduino sketch file starts with the above listed libraries inclusion part, followed by the hardware dependent constants that never change in the proposed hardware configuration. These constants like pin definitions were assigned using the #define clause

inherited from C programming language, which instructs the compiler to replace the text with assigned value, this way these constants do not use any programming memory, as following:
#define OD600 8 // OD600 analog sensor connected to A8.
The variables not related to the hardware, but to the process were defined as following:
unsigned int I0 = 427; // light intensity reading through 1 cm path of LB medium (blank, reference optical density reading).

In order to make possible bioprocess changes without recompiling and uploading the software, the above values may be overwritten. To do this, a text file named “setup.txt” containing the variable names and values must be placed in the root folder of the micro SD card. The “setup.txt” file format is: [VARIABLE1=value1]

Any text not included between brackets is ignored. For example, in order to keep the pH of the next culture above 6 and harvest the culture at OD600 = 2, “setup.txt” file should contain:
[PH_MIN=6.0] // min ph value
[OD_MAX=2] // at this OD the harvest pump will start

A complete example of “setup.txt” file is provided as Supplement S4. The same principle and file formatting was used to store the initial, reference optical density light reading value (I0). When button B0 is switched from OFF to ON, a text file named “nvmemory.txt” (Supplement S5) with the text: [I0=value] is written on the micro SD card. In case of controller reset (caused by power failure for example), the stored variable is loaded from “nvmemory.txt” file overriding the default written in the variable declarations section. The implementation of saving and loading micro SD card variables was adapted from the elegant code written by Alex Shu available online at: http://overskill.alexshu.com/saving-loading-settings-on-sd-card-with-arduino/

The initialization variables of IP network, time server, micro SD card, flow meter are followed by functions definitions. Time synchronization functions implement a User Datagram Protocol (UDP) client for the widely used Network Time Protocol (NTP). The function refreshDisplay() fills in the variable LCD_string with 80 characters which are then written on the LCD using the I2C protocol. Because this screen is slow, the writing is done line by line, 20 characters at a time. The actual display photo is presented in Figure 3.

The hardware peripherals are initialized in setup() function. Arduino is a flexible platform, the same hardware pin for example can function as a digital input, or digital output (I/O), or as a pin for a serial data bus. The setup() function is the place where all the hardware is initialized and runs only once after micro-controller reset. Then the control is taken by the never ending loop() function which has an execution time in our implementation of about 750 ms, which means that every instruction in the loop() function runs once at every 750 ms. The sensors values are evaluated every cycle, but for important decisions like inducing protein expression, 10 consecutive values must be over the defined OD level. The button B0 position OFF triggers the stage 0 (Ready) status of the bioreactor. In this stage, the sensor readings are displayed and logged onto SD card and on the serial port, but all pumps, stirrer, heater are off. Switching B0 to

ON determines the stage shift to 1 (Growing) which starts the OD pump, stirrer and heater. Once OD reaches the defined protein induction level, the stage rises to 2 (IPTG ON) which makes the IPTG Pump run for the defined period of time. Once OD reaches the defined harvest value, the stage goes to 3 (Harvest) which runs the harvest pump for the time defined by HARVEST_MINUTES variable and stops all the other pumps, heater and stirrer. Once HARVEST_MINUTES expires, the stage goes to 4 (Done) which stops the harvest pump. The bioreactor is ready to be cleaned up, sterilized and prepared for the next culture. The LCD refresh function is called form the loop() function if the current second is different from the last display refresh second. Because all the actions are called from loop() function, the processes must be implemented in non blocking way. If for example the pH adjustment requires base (OH) pumping time longer than the measured loop() time, the pump is started in a cycle, the start time is retained in a variable and the pump is turned off in another cycle, when the calculated pumping time passed. The last part of the loop() function is a simple web server implementation which listens for client connections and replies with a simple auto refresh web page showing current process variables and sensors readings. The complete source code is provided in Supplement S6.

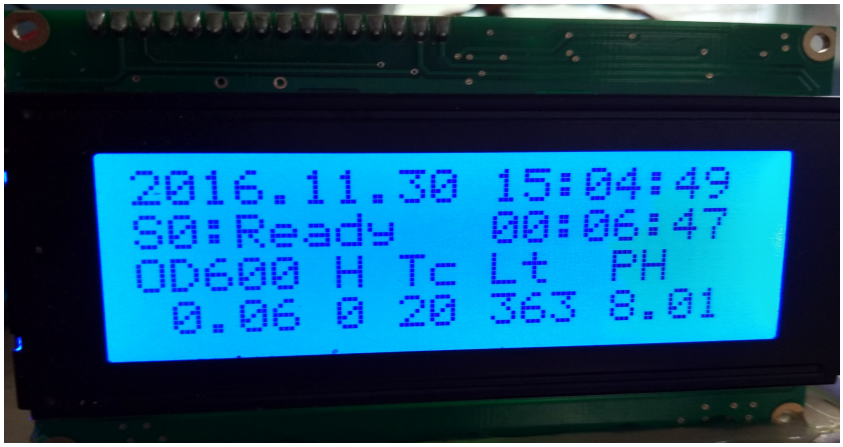

Figure 3: Real time process LC Display.

Operation of the proposed controller for growing bacteria in a Benchtop bioreactor.

A 0.5 L bioreactor tank vessel containing the growing medium were sterilized in autoclave for 20 min at 120ºC. Once cooled down below 60ºC, in order to prevent the plasmids loss, sterile filtered antibiotics were added to a final concentrations of 50 µg/mL kanamycin and 25 µg/mL chloramphenicol, respectively. The temperature was set to 37ºC on the temperature controller PIDT1. The probes and connecting tubes were chemically sterilized with 70% ethanol. The pH measuring shield (THS1) was calibrated at initial hardware setup. The calibration is needed only when this shield is replaced while the pH probe replacement produced the same calibration

curve as the old one. Once powered ON, the bioreactor controller was started in “Ready” stage. To begin the process, button B0 was switched ON which triggers the stage change to “Growing”. The optical density sensor (our preferred was the immersed version) initial reading was automatically saved and used to continuously calculate the culture density. The constant temperature for fermentation broth was maintained by placing the bioreactor tank in a 10 L water bath on top of hotplate HT1. The pH was preset to 7.0-7.2 and the controller pumped 5N NaOH as necessary to compensate for the acids produced by bacteria. The optical density was monitored by measuring the turbidity of the fermentation broth at 600 nm using an immersed OD600 sensor (Figure 4). When the OD600 value of the culture reached 0.7, the stage changed to “IPTG ON”, IPTG was automatic pumped into the fermentation broth to a final concentration of 1 mM to induce NAMPT protein expression coding by the nadV gene from the pET28a-hdNadV plasmid (Addgene ID #83362). Once the culture density reached the preset harvest optical density value (OD600 = 2.0 in our case), the culture stage changed to “HARVEST” and the culture was pumped by the Harvest Pump into a separatory funnel stored at 10ºC for further processing. During the entire process the controller operated independently, writing on micro SD card the measured process parameters in a standard coma separated values (CSV) format file as presented in Supplement S1. The real-time measured parameters were also updated every second on the LCD as shown in Figure 3.

Figure 4: Optical Density immersed sensor.

Results and discussion

Arduino Bioreactor Controller

There are a few open source bioreactors builds available online, the most notable found were the IGEM 10 mL scale "Do-It-Yourself chemostat" (http://2015.igem.org/Team:Aachen/Lab/Bioreactor), which is able to control many small volume cultures in the same time and the Aalto University project for rotary cell culture system bioreactor (https://wiki.aalto.fi/display/MechP/Bioreactor+0.1) However, we were unable to find any functional implementation simple and ready to be used for a common *E. coli* protein expression culture (Figure 1). Most of other open-source projects are in beta/incomplete stage and not maintained for years. Our proposed modular design shown in Figure 2. was built around Arduino Mega 2560 which provides 16 analogical and 54 digital input/output connections. Our implementation uses 7 digital outputs to control the pumps and heater, 2 digital inputs for temperature and flow sensor and one digital input for ON/OFF button. There are 2 digital pins used for the serial I2C data bus where the real time clock (RTC1) and LC Display (DS1) are connected. Only two of the available analog inputs are used by pH (THS1) and turbidity (OD1) sensors. Thus the board offers plenty of available analog and digital input/output pins for future improvements like additional sensors or effectors. Any such implementation should be simple, starting from the provided functional examples. The total cost of the controller components including the sensors was 477 USD (Table 1). Total assembly time for the electronic components was 6 hours.

Data logging

Ethernet shield provides support for micro-sd card which is used for process data recording and also for reading initial settings from the micro-SD card. The recommended setting for parameter "SD_LOG_FREQ_SEC" which sets the SD card parameters record frequency, The default 600 seconds results in over 100 data points for each recorded parameter for our *E. coli* culture harvested at OD600=2. (Suplement S1). The recorded data is in standard CSV format is readily accesible for a spreadsheet software like Microsoft Excell or OpenOffice Calc. Figure 5 shows consistent data records on the micro-SD card.

Controlling the microbial growing process

Temperature

Usually, each microorganism has an optimum growing temperature (Ratkowsky et al., 1982), so the temperature should be as close as possible to a defined constant value. In order to keep enough available processing resource on the main controller board (AM2560), we choose a separate power wave modulation temperature controller (PIDT1). As our bacterial culture allways grows at 37 ºC (Faust, Stand & Weuster-Botz, 2015), PIDT1 was set to this temperature. It reads the temeprature through a directly attached temperature sensor (TSA1) and its internal

algorythm directly controlls the solid state relay (SSR1) which sends power wave modulation to the hot plate (HT1). The heating element can be as well an internal electrical heating element of a large scale bioreactor. This design permits changing the heating element without any bioreactor controller modification, as the main controller only decides if the temperature controll is ON or OFF by opening or closing the circuit between PIDT1 and SSR1 input. TSD1 which is a digital sensor connected to AM2560 provides the actual culture temeparture shown on the LCD and recorded in the log files.

pH

The THS1 Arduino pH sensor shield reads the pH value from the pH probe and serves an analogic value on A9 analog pin of AM2560. We have also observed that THS1 does some kind of error filtering and mediation of the readings. Therefore it was not necessary to implement in the Arduino code any error detection or mediation on the pH data readings. In order to convert the analog voltage reading to the pH value, as we were unable to get any calibration curve from the manufacturer, we calibrated it using the reference a known calibrated pH meter from our laboratory, and sample solutions covering the pH range from 1 to 13. The calibration curve (Figure 5F) shows a surpising precise curve (R^2 = 0.9987). The coeficients were set as defaults in the source code (float PH_A = 0.0335768807; float PH_B = -5.9885198198;), but they can be overridden by placing corresponding values in the "setup.txt" file on the micro-SD card as explained in *Bioreactor controller software* section. It worth mentioning that replacing the pH probe did not affect the calibration, our callibration attempt produced the same curve. Though, we did not try to replace the THS1 shiled which probably requires a new calibration. The pH control in involves pumping concentrated base in order to compensate for the acids produced by the growing microorganisms (Willey, Sherwood & Woolverton, 2009; Obom, Magno & Cummings, 2013) which in our implementation was done by pumping NaOH solution using OH Pump. As the necessary NaOH quantity is variable, the implemented algorithm is adaptive. If pH is below the preset value, once every minute a NaOH quantity is pumped. If the pump was triggered 10 times in a row (that is no minute pumping pause), as feedback response to this high OH demand, the pumped quantity is doubled for the next consecutive events. As expected, the total NaOH pumped recorded in the micro-SD card log shows an exponential rise in the last stage of the culture (Figure 5C). Though the NaOH pumped volume spiked with the rise of culture density, the described addaptive algorithm succesfuly maintained pH over the preset value of 7 (Figures 5C and D). The pH is successfuly maintained in a narrow optimal range for *E. coli*, between 7.0 and 7.3 during the entire growing process (Figure 5D).

Culture Density

The traditional method for culture density measuring involves measuring 600 nm absorbance of the culture relative to the medium (Myers et al., 2013). This is named the optical

density of the culture (OD600). The light is not absorbed but is scattered and the relative turbidity of the culture is in fact measured. Though a turbidity sensor is a device which emits light, passes it whrough the culture and reads the light intensity received (Kiviharju et al., 2008). Optical density sensors are external (which involves pumping the culture through it) or internal (which involves immersing the sensor into the bioreactor) (Cox et al., 1989). We used the OD1 sensor (Table 1, Figure 2) which was previously used with Arduino. But the wavelength of the light emitting diode component of OD1 was unknown and no information about this was available from the manufacturer, we have replaced the LED with a known 600 nm one. For the external OD600 sensor version, it was necessary to add OD Pump and also FL1 to monitor the flow through it. This design is classical for external culture density sensors (Cox et al., 1989). Regarding the immersed sensor version, the active components are the same 600 nm LED and phototransistor. They were inserted facing each other in two separated laboratory tubes as shown in Figure 4. To hold the two glass tubes at a fixed distance of 10 mm, a 3D printed part was designed (Supplement S3). In order to obtain a stable reading bubbles must be kept away from the sensor. The sensor provided consistent light intensity reading which was recorded on the micro-SD card during our 20 hours bacterial culture (Figure 5B). OD600=-log(Lt/L0) was also plotted (Figures 5A and E) showing a normal bacterial growing curve with Lag phase, Log (exponential growing) phase, and Stationary phase. Microorganism growth is supported by the nutrient composition from the growth medium, as well as the presence or absence of oxygen (VanMeter & Hubert, 2015). The stirrer and air pump oxygen supply assured mass transfer and aeration rate of the fermentation broth (Galaction et al., 2004; Garcia-Ochoa & Gomez, 2009). The rate of medium acidification is related to the growth rate (Brown, 1975; Willey , Sherwood & Woolverton, 2009). The cell density during the Lag and Log growing phases is related to the total NaOH pumped (Figures 5C and E). This suggests that recorded total NaOH value may be used alone as a cell density estimation. However, once the culture enters stationary phase, the total NaOH rises sharply and is not anymore proportional to OD600.

Protein expression and culture harvest time

Tight control of pH, the induction time of protein expression and temperature contribute to a much higher biomass and protein yield in biotechnological process (Gueguim et al., 2003). The precise control of the protein expression induction is critical for maximizing the useful product yield (Tian et al., 2011). Inducing a culture too early, may prevent its growth, while inducing it too late may not have enough nutrients left in the fermentation broth to support the biosynthesis of the desired product. The proposed bioreactor controller provides precise control on the protein expression induction and culture harvest time as a function of cell culture density (OD600). Its precision and accurate data logging facilitates pinpoint protein expression and culture harvest optimization.

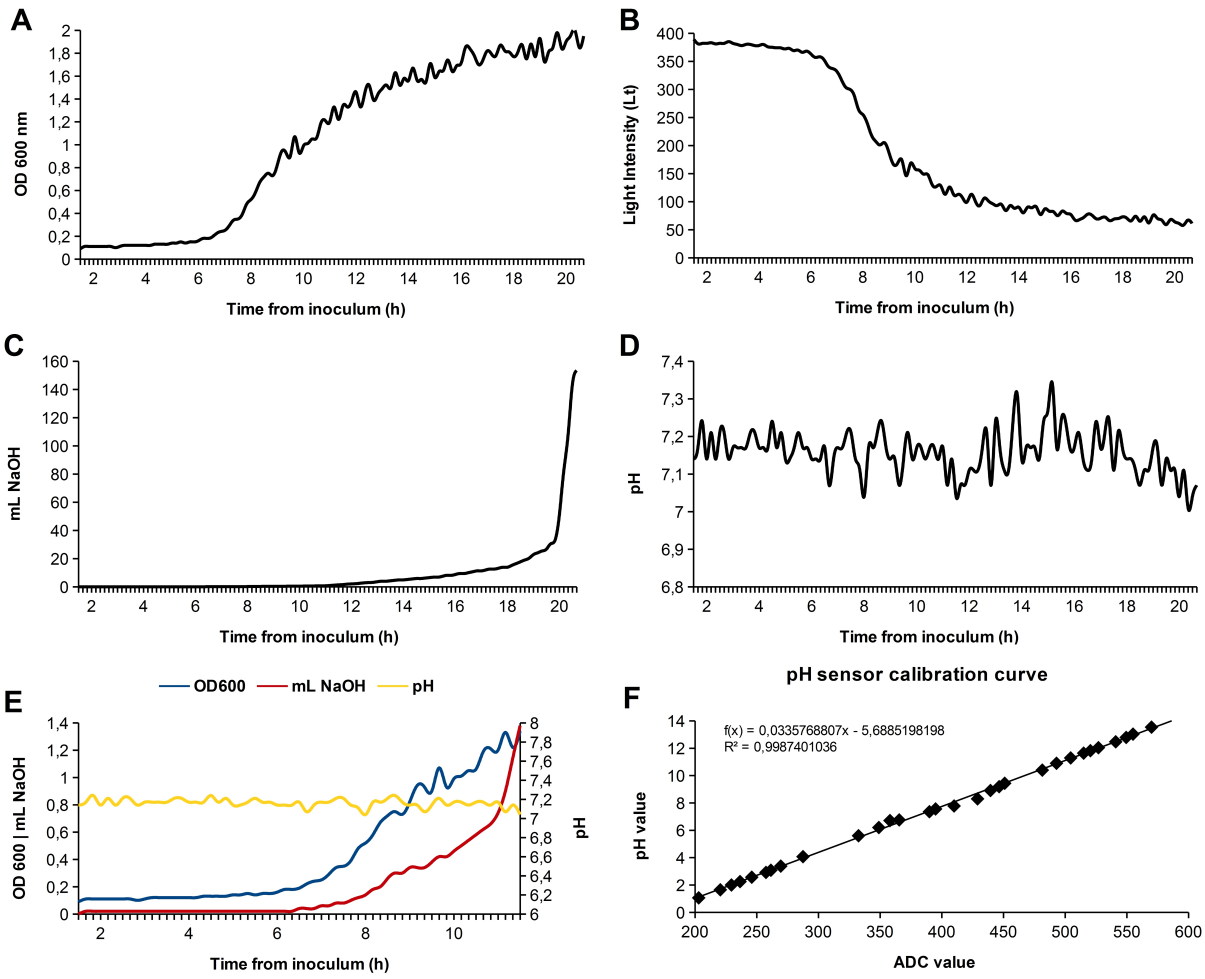

Figure 5: Bioprocess data records. **(A)** Growth curve, optical density at 600 nm (OD600). **(B)** The values of light Intensity (Lt) measured during the growth process of *E. coli* BL21(DE3*)pLysS-pET28a-hdNadV* strain. **(C)** Total volume of 5N NaOH pumped during the fermentation process. **(D)** Maintained pH range during the fermentation process. **(E)** OD600 values versus total volume of 5N NaOH pumped during the Lag and Log growing phases. (**F**) Arduino pH shield calibration curve.

Bioreactor Controller Software

Although there are planty of open source code examples available and Arduino is a well documented platform, the controller software writing and testing was a time consumming process. The current functional version totals 891 code lines includding comments and adnotations. It is based on the Arduino usage examples and contributions of other authors, some of them listed, some of them are unknown. Complete source file (current stable version 2.5) is available in Supplement S6. Anybody may use it as is, modify and redistribute freely. A sample serial output (debug) log is presented in Supplement S2.

Conclusions

In this study, we designed an open source bioreactor controller for microbial growing in suspension, using Arduino open electronics platform. The functional prototype proved itself

effective in controlling the bacterial culture environmental factors, recording culture parameters and growing curve. It autonomously induced protein expression and harvested the culture according to programmed settings. The presented controller is an easy to build and more flexible, cost effective alternative to the existing commercially available solutions. It is an efficient laboratory tool and may be effortlessly adapted for any scale microbial culture. However, the environmental sensors may need improvements and better mechanical protection to ensure long term reliability in industrial environments.

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Author contributions

G.C.M. conceived and designed the study. G.C.M. and R.G.P. performed all experiments, analysed and interpreted the data and drafted the manuscript.

Conflict of interests

The authors declared that they have no competing interests.

References

Brown DE. The Effect of Acid pH on the Growth Kinetics of Trichoderma viride. 1975;XVII:1199-1210.

Cheng LK, Wang J, Xu QY, et al. Strategy for pH control and pH feedback-controlled substrate feeding for high-level production of l-tryptophan by Escherichia coli. *World J Microbiol Biotechnol*. 2013;29(5):883-890. doi:10.1007/s11274-012-1243-7.

Clincke MF, Mölleryd C, Samani PK, et al. Very high density of Chinese hamster ovary cells in perfusion by alternating tangential flow or tangential flow filtration in WAVE bioreactor™-part II: Applications for antibody production and cryopreservation. *Biotechnol Prog*. 2013;29(3):768-777. doi:10.1002/btpr.1703.

Cox RP, Miller M, Bang Nielsen J, Nielsen M, Kirk Thomsen J. Continuous turbidometric measurements of microbial cell density in bioreactors using a light-emitting diode and a photodiode. *J Microbiol Methods*. 1989;10(1):25-31. doi:10.1016/0167-7012(89)90043-2.

D'Ausilio A. Arduino: A low-cost multipurpose lab equipment. *Behav Res Methods*. 2012;44(2):305-313. doi:10.3758/s13428-011-0163-z.

Doucha J, Lívanský K. Productivity, CO2/O2 exchange and hydraulics in outdoor open high density microalgal (Chlorella sp.) photobioreactors operated in a Middle and Southern European climate. *J Appl Phycol*. 2006;18(6):811-826. doi:10.1007/s10811-006-9100-4.

Edwards W, Bownes R, Leukes WD, et al. A capillary membrane bioreactor using immobilized polyphenol oxidase for the removal of phenols from industrial effluents. *Enzyme Microb Technol*. 1999;24(3-4):209-217. doi:10.1016/S0141-0229(98)00110-0.

Eibl R, Kaiser S, Lombriser R, Eibl D. Disposable bioreactors: The current state-of-the-art and recommended applications in biotechnology. *Appl Microbiol Biotechnol*. 2010;86(1):41-49. doi:10.1007/s00253-009-2422-9.

Einarsdóttir G. Development of a bioreactor system and programming of an Arduino control unit. B.Sc. thesis, Faculty of Computer Science, University of Iceland; 2014.

Farrell J, Rose A. Temperature Effects on Microorganism. *Annu. Rev. Microbiol.* 1967;21:101-120.

Faust G, Stand A, Weuster-Botz D. IPTG can replace lactose in auto-induction media to enhance protein expression in batch-cultured Escherichia coli. *Eng Life Sci*. 2015;15(8):824-829. doi:10.1002/elsc.201500011.

Fisher DK, Gould PJ. Open-Source Hardware Is a Low-Cost Alternative for Scientific Instrumentation and Research. *Mod Instrum*. 2012;1(April):8-20. doi:10.4236/mi.2012.12002.

Galaction AI, Cascaval D, Oniscu C, Turnea M. Prediction of oxygen mass transfer coefficients in stirred bioreactors for bacteria, yeasts and fungus broths. *Biochem Eng J*. 2004;20(1):85-94. doi:10.1016/j.bej.2004.02.005.

Garcia-Ochoa F, Gomez E. Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. *Biotechnol Adv*. 2009;27(2):153-176. doi:10.1016/j.biotechadv.2008.10.006.

Glazyrina J, Materne E-M, Dreher T, et al. High cell density cultivation and recombinant protein production with Escherichia coli in a rocking-motion-type bioreactor. *Microb Cell Fact*. 2010;9:42. doi:10.1186/1475-2859-9-42.

Griswold AA. pH Control in a Miniaturized Bioreactor. B.Sc. thesis in Mechanical Engineering at Massachusetts Institute of Technology; 2004.

Gueguim Kana EB, Oloke JK, Lateef A, Zebaze Kana MG. Constructional features of a 15-litre home-made bioreactor for fed-batch fermentations. *African J Biotechnol*. 2003;2(8):245-249. http://www.scopus.com/inward/record.url?eid=2-s2.0-3042831712&partnerID=tZOtx3y1.

Heath C, Belfort G. Membranes and Bioreactors. *Minireview. Int. J. Biochem.* 1990;22(8), 823-835.

IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). Available at: https://goldbook.iupac.org/html/B/B00662.html. Accessed February 26, 2018.

Jaekel U, Zedelius J, Wilkes H, Musat F. Anaerobic degradation of cyclohexane by sulfate-reducing bacteria from hydrocarbon-contaminated marine sediments. *Front Microbiol*. 2015;6(FEB):1-11. doi:10.3389/fmicb.2015.00116.

Jain P, Sinha S. Neutrophiles: Acid Challenge and Comparison with Acidophiles. *Internet J Microbiol*. 2009;7(1):1-9. doi:10.5580/1ee7.

Johnstone WA, Alexandrov K. Production of eukaryotic cell-free lysate from Leishmania tarentolae. *Methods Mol Biol.* 2014;2014(1118):1-15. doi: 10.1007/978-1-62703-782-2_1.

Kiviharju K, Salonen K, Moilanen U, Eerikäinen T. Biomass measurement online: The performance of in situ measurements and software sensors. *J Ind Microbiol Biotechnol*. 2008;35(7):657-665. doi:10.1007/s10295-008-0346-5.

Knoll A, Bartsch S, Husemann B, et al. High cell density cultivation of recombinant yeasts and bacteria under non-pressurized and pressurized conditions in stirred tank bioreactors. *J Biotechnol*. 2007;132(2):167-179. doi:10.1016/j.jbiotec.2007.06.010.

Lin LL, Hsu WH. Lactose-induced expression of Bacillus sp. TS-23 amylase gene in Escherichia coli regulated by a T7 promoter. *Lett Appl Microbiol*. 1997;24(5):365-368.

http://www.ncbi.nlm.nih.gov/pubmed/9172444.

Marinescu GC, Popescu RG, Stoian G, Dinischiotu A. β-nicotinamide mononucleotide (NMN) production in Escherichia coli. *Sci Rep.* 2018;8(2018): 12278. doi:10.1038/s41598-018-30792-0.

Meghrous J, Khramtsov N, Buckland BC, Cox MMJ, Palomares LA, Srivastava IK. Dissolved carbon dioxide determines the productivity of a recombinant hemagglutinin component of an influenza vaccine produced by insect cells. *Biotechnol Bioeng*. 2015;112(11):2267-2275. doi:10.1002/bit.25634.

Myers JA, Curtis BS, Curtis WR, et al. Improving accuracy of cell and chromophore concentration measurements using optical density. *BMC Biophys*. 2013;6(1):4. doi:10.1186/2046-1682-6-4.

Najafpour GD. Chapter 6: Bioreactor Design.In: Najafpour GD. Biochemical Engineering and Biotechnology. Elsevier Science, 2006:142–169.

Obom KM, Magno A, Cummings PJ. Operation of a benchtop bioreactor. *J Vis Exp*. 2013; (September):e50582. Doi:10.3791/50582.

Palomares LA, Ramirez OT. Bioreactor scale-up. *Encycl Ind Biotechnol Bioprocess, Biosep Cell Technol*. 2009:1-17. doi:10.1002/9780470054581.eib143.

Pearson AP, Glennon B, Kieran PM. Comparison of morphological characteristics of Streptomyces natalensis by image analysis and focused beam reflectance measurement. *Biotechnol Prog*. 2003;19(4):1342-1347. doi:10.1021/bp025734p.

Pérez-Correa J, Fernández M, Von O. 28 Application of Automatic Control Strategies to SSF Bioreactors. *Bioreactors*. 2006. http://books.google.com/books?hl=en&lr=&id=7bYP3lq9bewC&oi=fnd&pg=PA387&dq=28+Application+of+Automatic+Control+Strategies+to+SSF+Bioreactors&ots=zanJWKpMqN&sig=7u81e7O7aWNZbKpNIdSP_0CpZWQ.

Pörtner R, Nagel-Heyer S, Goepfert C, Adamietz P, Meenen NM. Bioreactor design for tissue engineering. *J Biosci Bioeng*. 2005;100(3):235-245. doi:10.1263/jbb.100.235.

Rani KY, Rao VSR. Control of fermenters - A review. *Bioprocess Eng*. 1999;21(1):77-88. doi:10.1007/s004490050644.

Ratkowsky DA, Olley J, McMeekin TA, Ball A. Relationship between temperature and growth rate of bacterial cultures. *J Bacteriol*. 1982;149(1):1-5.

Rault A, Bouix M, Béal C. Fermentation pH influences the physiological-state dynamics of Lactobacillus bulgaricus CFL1 during pH-controlled culture. *Appl Environ Microbiol*. 2009;75(13):4374-4381. doi:10.1128/AEM.02725-08.

Riesenberg D, Guthke R. High-cell-density cultivation of microorganisms. *Appl Microbiol Biotechnol*. 1999;51(4):422-430. doi:10.1007/s002530051412.

Scott CD. Immobilized cells: a review of recent literature. *Enzyme Microb Technol*. 1987;9(2):66-72. doi:10.1016/0141-0229(87)90145-1.

Swinehart DF. The Beer-Lambert Law. *J Chem Educ*. 1962;39(7):333. doi:10.1021/ed039p333.

Tian H, Tang L, Wang Y, et al. Lactose induction increases production of recombinant keratinocyte growth factor-2 in escherichia coli. *Int J Pept Res Ther*. 2011;17(2):123-129. doi:10.1007/s10989-011-9249-9.

Ude C, Schmidt-Hager J, Findeis M, John GT, Scheper T, Beutel S. Application of an online-biomass sensor in an optical multisensory platform prototype for growth monitoring of biotechnical relevant microorganism and cell lines in single-use shake flasks. *Sensors (Basel)*. 2014;14(9):17390-17405. doi:10.3390/s140917390.

van Noort D. Chapter 3. Bioreactors on a Chip.In:Mandenius CF. Bioreactors - Design, Operation and Novel Applications. Wiley., 2016:90. https://doi.org/10.1002/9783527683369

VanMeter KC, Hubert RJ. Chapter 4. Bacteria and Archaea.In: VanMeter KC, Hubert RJ. Microbiology for the Healthcare Professional. 2nd edition. Elsevier - Health Sciences, 2015:88-101.

Willey JM, Sherwood LM, Woolverton CJ. Microbial growth. *Prescott's Princ Microbiol*. 2009:126-152. doi:10.2307/1935404.

Zashkova L, Penkova N, Karamfilowa R. Heat Transfer Processes in a Biogas-Reactor. *TASK Q*. 2006;9(4):427-438.

Zelle RM, De Hulster E, Kloezen W, Pronk JT, Van Maris AJA. Key process conditions for production of C4 dicarboxylic acids in bioreactor Batch cultures of an engineered saccharomyces cerevisiae strain. *Appl Environ Microbiol*. 2010;76(3):744-750. doi:10.1128/AEM.02396-09.

Zhang Y, Arends JBA, Van de Wiele T, Boon N. Bioreactor technology in marine microbiology: From design to future application. *Biotechnol Adv*. 2011;29(3):312-321. doi:10.1016/j.biotechadv.2011.01.004.

Table 1. Components

Designator	Hardware	Quantity	Price (USD)	Purchased from	Comment
		Bioreactor main controller			
AM2560	Arduino MEGA 2560 R3 Board Atmega2560 with USB cable	1	46	https://www.adafruit.com	Main board
ETH1	Arduino Ethernet2 shield with micro SD card and TCP/IP Embedded Ethernet Controller	1	59.95	https://www.sparkfun.com/	
RM8	5V Electronic Relay Module 8-Channel Shield	1	5	http://www.ebay.com	Controls up to 8 peripherals (pumps, motors, heaters, etc)
SW1	On/Off switch COM-11138	1	0.5	https://www.sparkfun.com/	
DS1	Modtronix LCD2S i2c display LCD2S-204BIW	1	30	http://modtronix.com	Main display module
RTC1	Real time clock DS1307 BOB-12708 Temperature Control Module	1	15	https://www.sparkfun.com/	
		Temperature control module			
PIDT1	ITC-100VH PID Temperature Controller Thermostat 110-240V;	1	38	Inkbird Tech. Co., Ltd	
TSA1	K Sensor (Stainless Steel Probe K type Sensor)	1			
SSR1	25A Solid State Relay	1			
TSD1	Waterproof Digital Thermal Sensor DS18B20 SKU:DFR0198	1	6.9	https://www.dfrobot.com	
HT1	Bench top 500 - 1000W electric hot	1	18	https://www.aliexpress.com/	Any banchtop hot plate for laboratory or cooking.

	plate				It is necessary for small size bioreactors whithout internal heating system.
CP1	10L stainless steel all puropse cooking pot	1	30	local supermarket	Used as water bath for bioreactor vessel
			pH Control Module		
PHS1	Analog pH Sensor / Meter Pro Kit For Arduino SKU:SEN0169	1	57	https://www.dfrobot.com	
OH PUMP	Peristaltic Liquid Pump with Silicone Tubing, PRODUCT ID: 1150	1	25	http://www.adafruit.com	
C1	Vishay Multilayer Ceramic Capacitors MLCC - Leaded 0.1uF 50volts 10% X7R 2.5mm LS part number K104K15X7RF53L2	1	0.01	http://eu.mouser.com	For filtering electromagnetic noise produced by the motor
			Optical Density Module		
D1	5mm Orange LED through hole thru 2 pin 600 nm clear lens part number 859-LTL-4273	1	0.1	http://eu.mouser.com	600nm light source
OD1	Amphenol Advanced Sensors TST-10 TST-10, Digi-Key Part Number 235-1367-ND	1	8	http://www.digikey.com/	Turbidity Sensor Phototransistor Output
OD PUMP	Mini Micro Self Prime Very Quiet Membrane Diaphragm Pump 12 VDC 31 GPH MM1512	1	29	http://www.ebay.com	Only needed if external optical density sensor is used
FL1	Arduino flow sensor 1/8'' SKU:SEN0216	1	9	http://www.ebay.com	Must be used if external OD sensor is used.
	1/8'' silicone tube 1m	1	16	https://www.dfrobot.com	Must be used if external OD sensor is used.

	16x125 mm glass tubes	2	1	http://www.ebay.com	Only needed if internal optical density sensor is used
	3D printed plastic spacer and support for 2 glass tubes	1	5	Designed and printed in house Supplement S3	Only needed if internal optical density sensor is used
C2	Vishay Multilayer Ceramic Capacitors MLCC - Leaded 0.1uF 50volts 10% X7R 2.5mm LS part number K104K15X7RF53L2	1	0.01	http://eu.mouser.com	For filtering electromagnetic noise produced by the motor
R1	4.7 Kohms resistor Mouser Part No: 603-MFR-25FBF52-4K7	1	0.1	http://eu.mouser.com	
R2	470 Ohms resistor Mouser Part No: 603-MFR50SFTE52-470R	1	0.1	http://eu.mouser.com	
	Protein Expression Induction Pump				
IPTGPUMP	Peristaltic Liquid Pump with Silicone Tubing, PRODUCT ID: 1150	1	25	http://www.adafruit.com	Only neeed if protein expression is induced with an inductor (eg: IPTG)
C3	Vishay Multilayer Ceramic Capacitors MLCC - Leaded 0.1uF 50volts 10% X7R 2.5mm LS part number K104K15X7RF53L2	1	0.01	http://eu.mouser.com	For filtering electromagnetic noise produced by the motor
	Air Pump				
AIRPUMP	Classica SuperX aquarium air pump 3W	1	23	Local aquarium accessories shop	
	Harvest Pump				
HPUMP	12v DC Diaphragm Pump Circulation Pump Micropump Flow: 2-3L/M	1	4	http://www.ebay.com	Cell culture harvest pump
C4	Vishay Multilayer Ceramic Capacitors MLCC - Leaded 0.1uF	1	0.01	http://eu.mouser.com	For filtering electromagnetic noise produced by the motor

	50volts 10% X7R 2.5mm LS part number K104K15X7RF53L2				
			Power Supply		
PSU1	Standard ATX computer switching power supply 500W		25	local computer parts supplier	
			Bioreactor Vessels		
	BELLCO Glass 500mL & 250mL Internal Overhead Bearing Spinner Flasks	1	100	http://www.ebay.com	Any of the vessels may be used
	BELLCO Glass 8000mL 8L Micro Carrier Spinner Flask Bioreactor GL-45 Sidearms	1	200	http://www.ebay.com	
	Bellco Digital Overhead Stirrer Drive 7774-10000 150 RPM	1	80	http://www.ebay.com	Only needed when the 8L vessel is used

Supplementary information

Supplement 1 S1 82154818.CSV. Example log file as recorded on micro SD card.
Supplement 2 S2 sample_serial_log.txt. Example of relevant sections from the serial port log
Supplement 3 S3 immersed_od_sensor_3dpart.stl. Computer Assisted Design file for 3D printing of the plastic support for the immersed version of OD600 sensor.
Supplement 4 S4 setup.txt. The process specific variables setup file.
Supplement 5 S5 nvmemory.txt. Example of micro SD card file storing non volatile memory variable (e.g. reference light intensity (I0) analog value) automatically saved at process startup.
Supplement 6 S6 bioreactor_2.5.ino. The complete Arduino source code for the proposed bioreactor controller.