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Full Length Research Paper

Constructional features of a 15-litre home-made bioreactor for fed-batch fermentations

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A 15-litre bench-top multipurpose bioreactor was designed and constructed. The vessel is a glass type with a stainless flat headplate incorporating 9 access ports allowing for a variety of interchangeable probes and actuators. The stirring speed ranges between 0 and 250 rpm, the aeration rate (0-2 l/m), the pH control loop uses HI 1131 probe, two 100 ml bottles of HCl and NaOH solutions and operates a close feedback system. The temperature control module is a close loop using a PT 100 RTD thermocouple and an auxiliary vessel containing a cooling solution. The aeration and feed flow rates are open loops. The system incorporates attributes of a good bioreactor design as discussed by Naraendranathan (1998). Sterility is achieved by autoclaving different units of the system. This machine has been tested on an array of local standard fermentation processes.

Key words: Fermentation, bioreactor, control, sensors, actuators.

INTRODUCTION

Bioreactors offer a possibility to provide an optimally controlled environment for microbial fermentation processes, a condition required for optimal yield (Williams, 2002). With this, one can specifically alter the metabolic fluxes and divert the cell's resources to more desirable pathways while inhibiting unwanted ones. For instance, by imposing a given temperature profile on the culture, one can selectively denature certain enzymes, thus prioritizing some metabolic routes over others within the cell (Gueguim -Kana et al., 2002). Process control through bioreactor in submerged cultures is based on the measurement of physical, chemical and biochemical properties of the broth, such as pH, dissolved oxygen, temperature, agitation rate and others, using dedicated probes followed by the manipulation of the physicochemical properties of the culture with suitable actuators (Lim and Lee, 1991). Some of the actuated parameters are: the agitation speed, the aeration rate, the heating intensity or cooling rate, and the nutrients feeding rate, acid or base valve. Precise environmental control is of considerable interest in fermentations since oscillations may lower the system efficiency (Yegneswaran et al., 1991), increase the plasmid instability (Namdev et al., 1993) and produce undesirable end products (Diaz et al*.,* 1996).

Attributes of a good bioreactor design as discussed by Naraendranathan (1998) include: an economical, robust and simple mechanical design, an easy operation under aseptic conditions, a flexibility with respect to various process requirements, an absence of dead zones, giving good control to bulk flow, and a good heat and mass transfer. In this work we have implemented the features above in a home-made bioreactor constructed from locally available materials.

MATERIALS AND METHODS

Bioreactor vessel

The bioreactor vessel is a Pyrex glass of size (ID 25 x HT 30 cm) with a stainless steel top plate of 20.5 cm diameter and 5 mm thickness. Various ports and standard nozzles (ID 10 mm) are provided on the stainless plate for actuators and probes. These include pH, thermocouple, and dissolved oxygen probes ports, defoaming, acid and base ports, inoculum port, pipe for sparging process air, agitator shaft and spare ports (Figure 1). A Teflon "O" ring secures the stainless top plate to the glass vessel; addition

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Figure 1. Functional Bioreactor. 1. Glass vessel; 2. DC motor; 3. Stainless top plate; 4. Water pump; 5. Cooling tank; 6. Power unit; 7. Basement; 8. Gear system; 9. Wires to control computer.

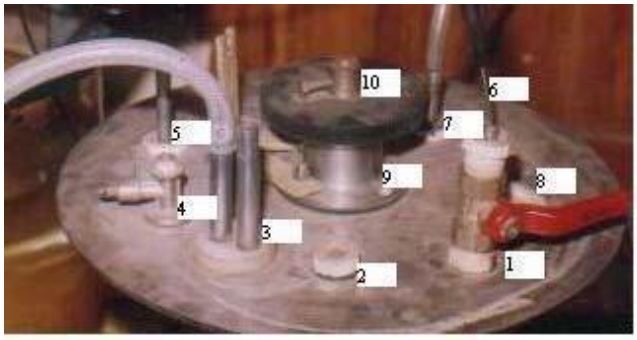

Figure 2. Stainless top plate. 1. Feed port; 2.Acid/alkali port; 3. Cooling water port; 4. Gas exhaust port; 5. Sampling port; 6. Temperature probe; 7. Aeration port; 8. pH probe port; 9. Support for agitation shaft; 10. Gear coupled to the shaft.

ports and nozzles are provided with union nuts. They were secured to the nozzles with Teflon cork.

Agitation system

The agitation system consists of two stage impellers with adjustable height on a vertical shaft. Impellers are six bladed Rushton type turbines. The upper end of the agitation shaft is coupled to a dynodrive through a gear system (Figure 2). The dynodrive is a 12 volts DC electric motor. The shaft is supported through the top plate by two ball bearings embedded in a cylindrical teflon rod held within a short stainless pipe screwed on the plate. This simple design yielded the dynamic stability required, and also narrows the possibility of process contamination through the shaft and the top plate interface. The disadvantage of this arrangement is that the shaft of the agitator has to cross a considerable distance in the vessel space in the reactor. A bottom driven bioreactor would have faced the problem of frequent leakage which annuls the advantage of having a shorter shaft length. The system is designed for a maximum agitation speed of 250 rpm. To prevent vortexing and ensure maximum turbulence during agitation, a detachable baffle unit consisting of four stainless blades of 2 cm width is placed radially to the internal wall of the vessel (Figure 1).

Aeration

The aeration system has a perforated pipe sparger discharging directly under agitating impellers (Figure 3). A peristaltic air pump motor type Rena 301 supply air into the sparger via an air-filtering unit. Signal from the system control unit actuates a 6 volts relay directing the air pump. The aeration rate ranges from 0-2.0 l/min.

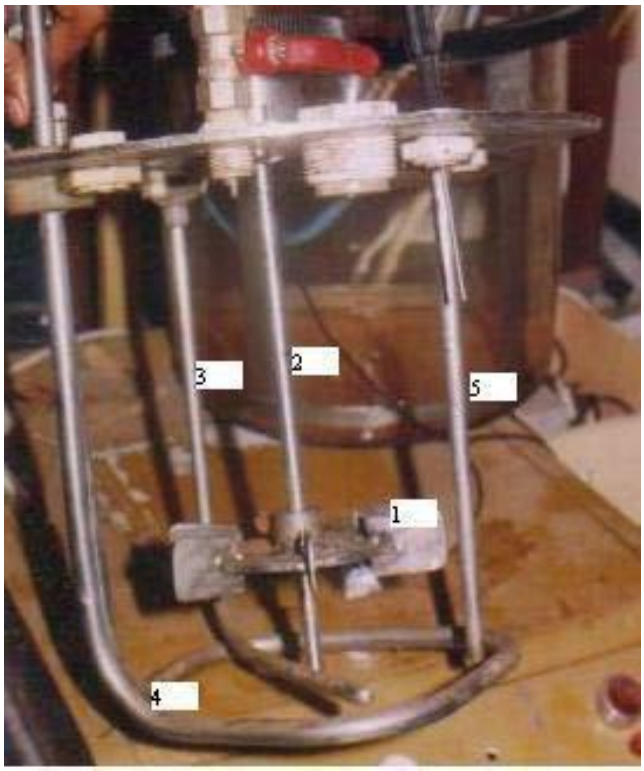

Figure 3. Some internal structures of the reactor. 1. Impeller; 2. Agitating shaft; 3. Air sparger; 4. Cooling coil; 5. Sampling pipe.

Temperature control Module

Fermentation heat is removed by re-circulation of cooling water at controlled temperature through an internal stainless coiled pipe within the vessel (Figure 3). The pipe has 10 mm diameter and an immersion length of 100 cm. The cooling water for bioreactor is stored in an auxiliary vessel having 10 litres capacity, although this could be substituted with larger capacity vessel while operating a highly exothermic fermentation process. Water is driven through the

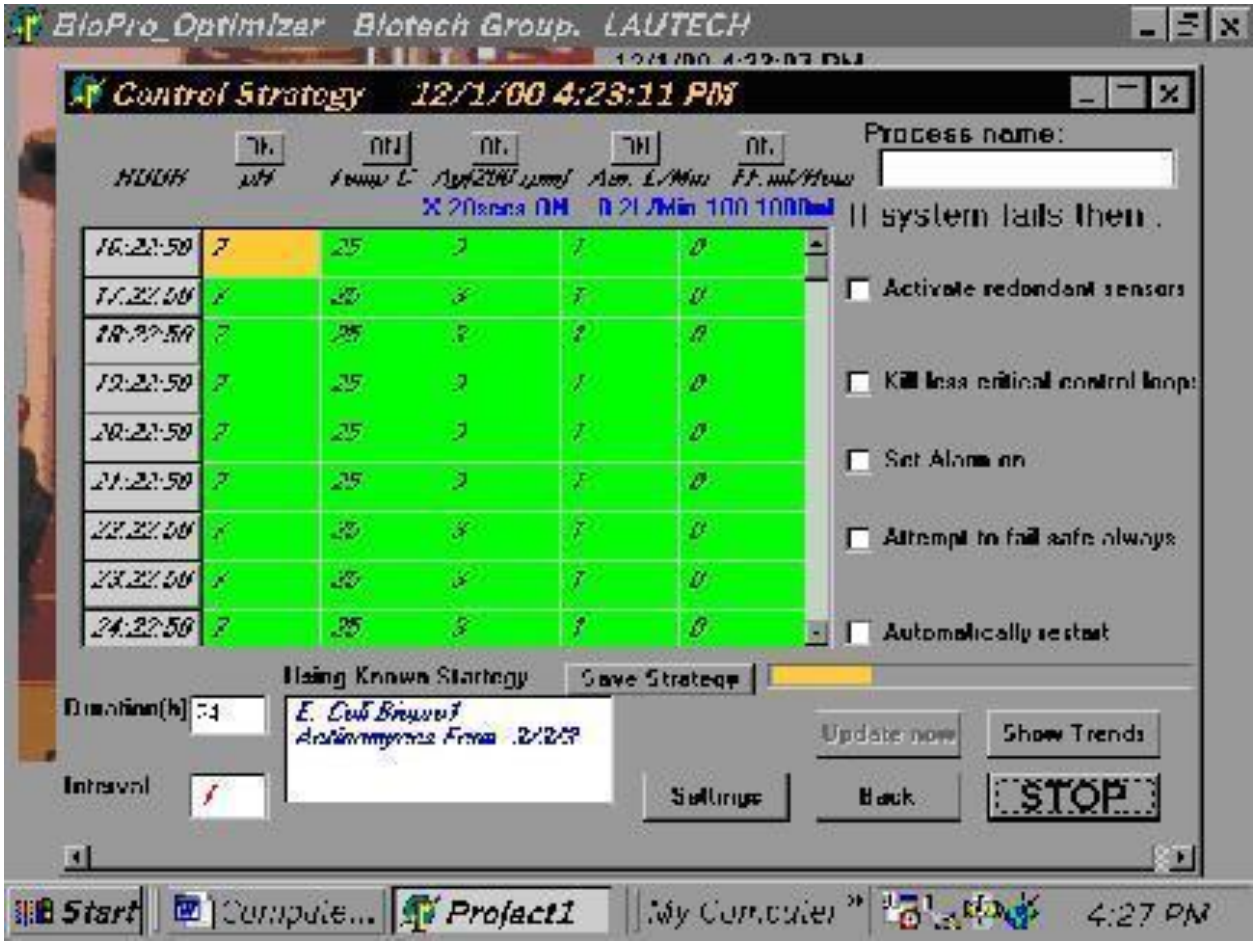

Figure 4. Control panel.

cooling coil by a water pump motor of 0.50 HP, type Pedrolla powered at 220 volts. Temperature sensor is a thermocouple of type PT 100 RTD having an immersion length of 150 mm. The overall length of sensor is 200 mm. It is located at an angle of 90^0C on the top plate. A standard twisted wire is used for sending output to the A/D converter of the controlling unit.

pH control Module

The pH electrode HI 1131 by Hanna Instruments tracks the pH change in the broth during the fermentations. Analog signals from the electrode are amplified and fed into the control unit on the computer which in turn sends millivolt signals to two relays actuating the base and acid pumps. Two auxiliary bottles of 250 ml of 3N NaOH/HCl are used. An amount of HCl or NaOH is added to the reactor when the pH sensed value deviates from the set range.

Substrate feeding control

The feeding range is between 0 to 2000 ml/h. The substrate is pumped into the reactor intermittently. Different feeding strategies can be operated on the system. Thus, a constant, an exponentially increasing or a decreasing feeding rate can be maintained. Signals from the control module direct the 6 volts relay of the feed flow peristaltic pump.

Sterility considerations

All the materials used are amenable to repeat cycles of steam sterilization (121^0C, 30 min.) with zero breakage. Other sterility considerations are smooth and crevice free welded joints of the baffle unit. The impeller's blade is polished to 180-200 grit finishes to avoid accumulation of pockets of contaminants during operation. All the pipelines are short, straight, and have slopes at appropriate places. Teflon is used as a material for sealing all threaded joints.

Control unit

Millivolts signal from the sensors are fed into AD channels of the data acquisition card PCL 818 obtained from Advantech (USA). This is interfaced to a computer. Monitoring and controlling software has been developed to direct the bioreactor. From the control sub-panel on the software, optimal set points of each control parameter are keyed in. These values can also be loaded from the archive folder of previous control policies. The time interval for each set of optimal values is 1 h, the optimal values of pH, temperature, aeration, agitation and feed rate for the 1st, 2nd, 3rd up to 300th h

of the fermentation process can be entered on the main window of this panel (Figure 4). Each of the control loops can be disabled using the ON/OFF toggle buttons located above the control window. Details of the computer interfacing and implementation of the supervisory software will be discussed elsewhere. The software controls the machine according to a programmed strategy.

RESULTS AND DISCUSSION

A number of low cost but functionally effective units have been implemented in the constructed bioreactor. The pH and Temperature control use the feedback close loop in an on-off frequency modulation, which is a variation of the on-off control system. For example, if the pH is very far from the set point, the valve will be opened for a long period of time and closed for a shorter period of time. The reversal happens when the pH is in the vicinity of the set point value. The frequency modulated on-off controller offers more accurate control. The Feed flow rate and aeration rate are open loop control schemes in which feed and air are added according to historical or predicted data. Process contamination through the agitation shaft and top lid junction is a critical problem in bioreactor design (Whitaker, 1994). Deshpande (1998) has proposed the use of a double mechanical seal with a sterile lubricating fluid. In our previous design (Gueguim-Kana et al., 2003) a single ball bearing encasted into a thick steel lid was tried. On the present system we have introduced the shaft through a double ball bearing embedded into cylindrical Teflon. With this design, an array of tests with standard processes has been successful (result not shown). The minimal cost of construction of this machine makes it very useful for laboratories and industries in developing nations.

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