

Aseptic Monitoring & Control with the YSI 2700 SELECT Biochemistry Analyzer

I. Introduction

Fermentations and cell cultures require tight control of system variables in order to achieve consistent, desirable results. Measurement of these variables, such as oxygen, pH, and temperature, has been accepted for a long time. Recently, researchers have realized the importance of nutrient availability and byproduct reduction on the health and productivity of these processes. Off-line analysis generally does not allow for timely adjustment of process variables. On-line analysis can provide this capability. The YSI system was designed to do just that.

When installed on the YSI 2700 Biochemistry Analyzer, the YSI 2730 Monitoring & Control Accessory allows for:

- On-line aseptic monitoring of key nutrients and byproducts
- Regulation of key nutrients and byproducts
- Data logging on a PC through analog signals representative of analyte concentrations (RS232 also available)

II. Operating Principle

To achieve aseptic monitoring, the YSI system pulls sample from a process vessel at programmed intervals. After analysis, antiseptic solution is flushed through the sample lines, thus disinfecting the stream path and eliminating potential for microbiological growth to occur. The antiseptic solution remains in the sample lines until the next sample analysis. This cycle repeats through the course of the bioreaction, as often as every 2 minutes.

Monitoring can be done directly or through the use of an on-line filtration device. Such a device with 0.2 μ pores or smaller provides a barrier to environmentally born contaminants. If the process requires monitoring infrequently and the loss of a small amount of cells is not a concern, direct sample acquisition will normally suffice. However, if cell loss is a concern, an in-line filtration device will be needed. In either case, the YSI system maintains aseptic monitoring.

Regulation of a bioprocess is achieved through PID control on the 2700 SELECT. After entering the desired setpoint of the analyte(s) into the 2700 SELECT, the 2700 SELECT triggers a feed pump to replenish nutrients, broth, or media when necessary. In this way, nutrients can be replenished to a setpoint as they are consumed, or byproducts can be diluted to a setpoint as they are produced.

The 2730 Monitoring & Control Accessory includes a cable which carries these regulation signals. To achieve regulation, this cable needs to be connected to a user-supplied feed pump. If desired, analog signals that represent the analyte(s) concentration(s) are also available through this same cable. These signals can be sent to a computer's data acquisition software or to a process loop controller. A computer or process loop controller can perform the control function as an alternative to having the 2700 SELECT perform the regulation.

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III. Equipment

- **YSI 2700 SELECT Biochemistry Analyzer equipped with a YSI 2730 Monitoring & Control Accessory**

Install the 2730 Monitoring & Control Accessory on the 2700 SELECT using the 2730 manual as a guide. The YSI 2730 Monitoring & Control Accessory can be installed on any 2700 SELECT with software version 2.41 or higher. The installation of this accessory eliminates the turntable functionality.

- **Filtration Device**

Several filtration devices are available commercially. As was mentioned earlier, the use of a filtration device is optional. YSI has used the Applikon A-Sep™ in conjunction with the YSI System. It requires the use of filtrate flow rates no greater than 360 µL/min. Information on this device and others is provided at the end of this note.

- **Feed Pump(s)**

If regulation of nutrients or byproducts is desired, the YSI system can turn on and off an external feed pump (up to 2) using normal or inverted TTL signal output(s). Therefore, the pump chosen must have TTL on/off remote logic. When nutrient concentration falls below the programmed setpoint or byproduct concentration rises above the programmed setpoint, the 2700 SELECT will signal the operation of the external feed pump. This will initiate the replenishing of nutrients or the dilution of byproducts.

Masterflex™ and Watson-Marlow™ pumps have been used for this purpose. Information on these pumps is included at the end of this application.

- **Computer / Process Loop Controller**

A PC or PLC may be used to simply log concentration data. RS232 signals and analog signals that represent analyte(s) concentration(s) are available from the 2700. If RS232 signals will be used, refer to Section 9 of the 2700 SELECT manual for assistance in set up and operation. If analog signals will be used, the PC will need to be equipped with a data acquisition program and an A to D converter that accommodates +5V or +10V full scale.

The PC or PLC can act simply as a data acquisition tool. However, regulation may also be achieved with the PC or PLC, bypassing the regulation capability of the 2700 SELECT. If other parameters, such as oxygen and pH, are being regulated by the PC or PLC, centralizing all of the regulation on a PC or PLC may be more convenient.

IV. Set up and Operation

1. Install the 2730 Monitoring & Control Accessory onto the 2700 SELECT as instructed in the 2730 manual.
2. Connect tubing to the fermentation vessel directly or through a filtration device. Bulk silicone tubing (0.02" ID, 5.1 µL/in) is supplied with the 2730 accessory, however, other tubing may be substituted.

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3. Cut the tubing to an appropriate length and connect it to the adapter of the 2730 pump.
4. If aseptic operation is desired, clamp the tubing at the base of the 2730 external sample well, disconnect from the external sample well, and sterilize. Reassemble and remove clamps.
5. Install antiseptic solution at the antiseptic tubing inlet as instructed in the 2730 manual. Sodium hydroxide (1%) or hypochlorite solution (0.25%) may be used as an antiseptic. Other reagents compatible with silicone tubing may also be used.
6. Run the 2730 waste tubing into an appropriate collection vessel.
7. Connect the cable to the AUXILIARY connector on the back of the 2700 SELECT.
8. When using an in-line filter, a separate filtrate pump is usually not necessary. However, if using an external filtrate pump, it will need to turn on and off at the same time as the 2730 pump. This can be achieved using a signal from the AUXILIARY cable. Choose the "Filtrate Pump Control" color-coded wire described in the 2730 manual, Section 4, and connect this (and a ground wire) to the filtrate pump. Program the 2730 pump rate at a rate compatible with the filter's flux characteristics.
9. For regulation of analytes, refer to Section 3 of the 2730 manual, and configure the switch to the output polarity compatible with the feed pump associated with the analyte measured on the black probe (Black Pump), the feed pump associated with the analyte measured on the white probe (White Pump), and the external filtrate pump (Filtrate Pump), if one will be used. The position of this switch determines the state of the signal for on and off control (normal or inverted) and is dependent upon the type of external pump used.
10. Refer to the table in Section 4 of the 2730 manual describing the color-coded wire assignments. If the regulated analyte is on the black pump, choose the "Black Pump Control" wire. Likewise, if the regulated analyte is on the white probe, choose the "White Pump Control" wire. If two analyte concentrations are to be controlled, both signals will be used simultaneously. Connect this wire and a ground wire to a feed pump with TTL on/off remote logic.
11. Before connecting feed solution to the feed pump and connecting the fluid line to the bioreactor, check that proper installation has been achieved. Select Service, then Analog I/O on the menu of the 2700 SELECT. Select Discrete I/O. At this point, selecting Black Control, White Control, or Fil (filtrate) should drive the operation of any pumps installed. If operation does not occur, check the board switch positions and wire assignments to confirm that those have been properly configured.
12. Once proper operation has been verified, prime the feed fluid lines, and connect to the bioreactor using sterile techniques.
13. If data logging to a PC, connect the appropriate "Black Analog Output," "White Analog Output," and "Ground" wires described in Section 4 of the 2730 manual to the PC via an A to D converter. Confirm proper connection by selecting Service, then Analog I/O, then Analog Outputs on the 2700 SELECT menu. Drive the signal to full scale and to zero and confirm that communication is occurring.

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14. Several parameters will need to be programmed into the 2700 SELECT. Please refer to the 2700 SELECT manual and 2730 manual for assistance. Enter choices for each of the following parameters:

Sample size - in μL ; usually 25 μL

Sample station - station where discrete samples can be run while monitoring; Station #2, for example

Chemistry measured - black and white probes; glucose, lactate, etc.

Units of measure - black and white probes; g/L, for example

Calibrator concentration - black and white probes

Measurement endpoint - black and white probes; usually 30 seconds

Calibration station - black and white probes; usually Station #1

Calibration frequency - in minutes or by sample; every 5 samples or 15 minutes, for example

Monitoring station - for 2730, this is sample station #5

Sampling interval - in minutes, minimum of 2

Precalibration time - time before sample that instrument will calibrate. If set to 0, Calibration frequency parameters will determine calibration protocol.

Purge time - in seconds, time required to purge fresh sample through tubing. Refer to 2730 manual for calculation. If set to 0, monitoring will be disabled.

Flow rate - in $\mu\text{L}/\text{min.}$, of 2730 pump. This can be adjusted to accommodate flow of different filtration devices. If no filtration device is used, set to 2500 $\mu\text{L}/\text{min.}$

Antiseptic - on or off. If not using antiseptic solution, tube end must be clear and unobstructed.

Full scale output of analog signals - for both black and white probes, either 1x, 2x, 3x, or 4x the calibrator concentration. For example, if calibrating at 2.5 g/L glucose and bioreactor will be operated at 7 g/L, choose 3x.

Time per unit error (TPU) - for both black and white probes. This calculated feed parameter indicates to the 2700 the magnitude of the response (pump on time) per unit of deviation from the setpoint. Refer to 2730 manual for calculation. If set to 0, regulation will not occur.

15. Install membranes and instrument fluids on the 2700 SELECT as instructed in the 2700 SELECT manual.
16. Purge the fluid lines by selecting Service, then Monitor on the 2700 SELECT menu.
17. Enter RUN on the 2700 SELECT. After calibration, the instrument will pull a sample from the bioreactor at the programmed interval. Additional feed will be added automatically as necessary.

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V. Notes

1. While the 2700 SELECT is monitoring, discrete samples can be run. While in RUN mode, select sample station #2 or #3, and press SAMPLE on the keypad. The results from the discrete sample will not initiate regulation and will not affect analog signal outputs.
2. For optimum regulation, the concentration of the regulated analyte at startup should be within 10% of the setpoint.

VI. Supplementary Equipment

Filtration Devices

Applikon Dependable Instruments, Inc.

(A-Sep™ filtration device)

USA

Phone 650 578-1396

Fax 650 578-8836

The Netherlands

Phone (0)10-462 18 55

Fax (0)10-437 96 48

Telex 26302

Eppendorf

USA

Phone 800 421-9988

608 231-1188

Fax 608 231-1339

Germany

Phone (0 40) 5 38 01-0

Fax (0 40) 5 38 01-556

Sartorius Corp.

USA

Phone 800 635-2906

Fax 516 254-4261

Feed Pumps

Cole-Parmer Instrument Co. (Masterflex™ pumps)

USA

Phone 800 323-4340

708 647-7600

Fax 708 647-9660

Watson-Marlow Inc.

USA

Phone 800 282-8823

508 658-6168

Fax 508 658-0041

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