Regulating Glucose and pH, and Monitoring Oxygen in a Bioreactor

Glucose and oxygen concentrations are monitored, and glucose concentration and pH are adjusted as needed.

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Figure 1 is a simplified schematic diagram of a system that automatically regulates the concentration of glucose or pH in a liquid culture medium that is circulated through a rotating-wall perfused bioreactor. Another system, shown in Figure 2, monitors the concentration of oxygen in the culture medium.

The glucose-regulating system includes an electrochemical sensor that measures the concentration of glucose in the medium flowing out of the bioreactor, a reservoir containing a concentrated glucose stock solution, and a peristaltic pump. The sensor reading is digitized and monitored by a computer, which generates commands to open and close valves, as described below, to adjust the concentration of glucose. This allows the system to maintain an optimal concentration of glucose without using large volumes of cell culture medium. The glucose control system operates in one of the following three modes (see Figure 1): perfusion, where the medium is simply circulated without adding or removing any liquid; infusion, where fresh cell medium is introduced into circulation, replacing the spent medium in the bioreactor; injection, where a small amount of concentrated glucose solution is added to the bioreactor when the glucose concentration falls below 75 mg/dL.

The electrochemical glucose sensor includes a membrane that is covered with a thin (10 to 200 mm thick) layer of immobilized glucose oxidase enzyme and is coupled to a three-electrode amperometric probe and a flow cell. Glucose diffuses through the membrane and, in the presence of the glucose oxidase, is converted to hydrogen peroxide and gluconic acid. The rate of generation of hydrogen peroxide is measured amperometrically on a platinum working electrode at a potential of 0.7 V with respect to an Ag/AgCl reference electrode. The sensor reading is correlated with the concentration of glucose by the Michaelis-Menten equation,

$$I = I_{max} S/(K + S),$$

where I is the sensor current, I_{max} is the maximum sensor current for the amperometric reaction, K is a constant, and S is the concentration of glucose. The constants I_{max} and K are determined by means of a two-point calibration, using a commercial glucose analyzer to determine the glucose concentrations. This sensor system has been tested in cell culture experiments for over 50 days. The control of glucose in this manner reduces the amount of culture medium required to optimally grow cells.

The pH sensor, described in "Optoelectronic Instrument Monitors pH in a Culture Medium" (MSC-23107), *NASA Tech Briefs*, Vol. 28, No. 9 (September 2004), page 4a, was coupled to the above control system containing a small volume of a mixture of sodium and potassium hydroxide and bicarbonate solution to form a pH control system. When the sensor pH output fell below a preset level, a small amount of buffer was injected to the bioreactor to bring the pH to the desired level. This system was tested in a 120-day cell run, where the pH was controlled at 7.1±0.1 pH unit. The volume of buffer used was negligible compared to the volume of cell culture medium required for the same level of pH control. By controlling the culture pH in this manner, the cells produced less biofilm.

The oxygen-monitoring system is designed to satisfy special requirements for noninvasiveness, sterilizability, compactness, light weight, and nontoxicity to the cells nourished by the solution. The oxygen sensor exploits dynamic quenching of fluorescence by oxygen molecules. The sensor includes a capillary tube of inside diameter 2 mm, lined with a 0.1-mm-thick sensing layer of oxygen-sensitive fluorescent dye, and titanium oxide encapsulated in a gas-permeable, ion-impermeable silicone rubber. The sensing layer is overlaid with a black shielding layer of carbon black encapsulated in silicone rubber. The solution of interest flows through the tube, and oxygen from the solution permeates the silicone-rubber layers. The degree of permeation

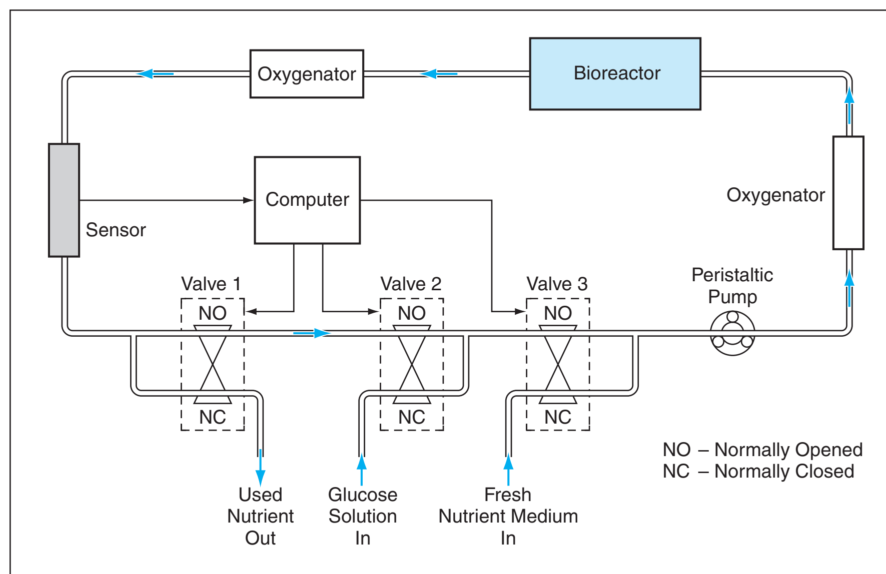

Figure 1. **Valves Are Momentarily Switched** between open and closed positions, to inject concentrated glucose solution and drain used nutrient medium, when the measured concentration of glucose in the circulating nutrient medium falls to a preset minimum level.

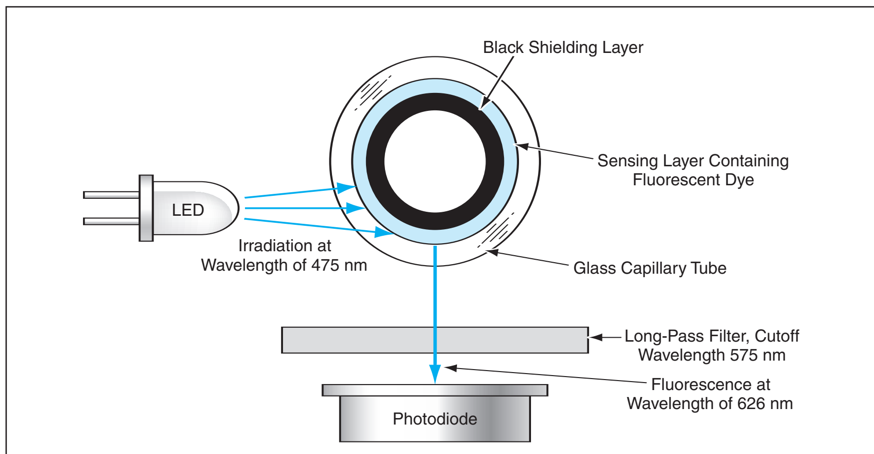

Figure 2. **Blue Light From the LED** excites red fluorescence in the dye encapsulated in the silicone rubber. The intensity of the fluorescence, measured by use of the photodiode, varies with the concentration of oxygen dissolved in the solution flowing through the tube.

(and, hence, the rate of quenching of fluorescence) is reversible; that is, it varies along with the concentration of dissolved oxygen.

The dye is tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(ll)chloride [$Ru(dpp)_3Cl_2$], which exhibits a high quantum yield (30 percent) of red (wavelength 626 nm) fluorescence with a lifetime of 5.34 ms after irradiation with blue light. A pulsed light-emitting diode (LED) of wavelength 465 nm is used as the radiation source. Operation in pulse mode minimizes the dye-bleaching effect that could occur if the LED were to irradiate the dye continuously during long-term monitoring. A long-wavelength-pass filter is used to remove the blue LED light reflected and scattered by the irradiated capillary tube and its contents. The remaining light — predominantly the red fluorescence — is detected by a photodiode.

The pulsed output of the photodiode is digitized and processed to determine the concentration of dissolved oxygen in terms of the partial pressure of oxygen (pO_2). This determination is made by use of the Stern-Volmer equation for the intensity and fluorescence lifetime of oxygen-quenched fluorescence of a luminescent dye:

$$I_0/I = \tau_0/\tau = 1 + K_{sv}(pO_2),$$

where I_0 and I are the intensities of fluorescence in the absence and presence of oxygen, respectively; τ_0 and τ are the fluorescence-decay lifetimes in the absence and presence of oxygen, respectively; and K_{sv} (the Stern-Volmer constant) depends on details of the sensor design and construction. The sensor is calibrated by use of a phosphate-buffered saline solution containing a known elevated concentration of oxygen. The oxygen sensor was tested for over 180 days in a perfused rotating-wall bioreactor, using a single, initial calibration for the entire experiment.

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This invention is owned by NASA, and a patent application has been filed. Inquiries concerning nonexclusive or exclusive license for its commercial development should be addressed to the Patent Counsel, Johnson Space Center, (281) 483-0837. Refer to MSC-23473/504/13/54.