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A Microcomputer-Based Oxygen Measurement and Control System for Fermentation Processes

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ABSTRACT

The availability of oxygen in aerobic fermentation processes affects the growth and metabolism of microorganisms. Thus, measurement and control of oxygen in these processes is very essential. This paper presents a description of a computer-based oxygen measurement and control system. The rate of oxygen response to changes in the concentration of dissolved oxygen and the long-term stability of the system were evaluated. The system performed satisfactorily under simulated biological conditions and is currently being used in a real fermentation process for the production of single cell protein.

Index Entries: Oxygen; control; measurement; computer.

INTRODUCTION

Oxygen in the free state is of fundamental importance to the life and metabolism of a group of organisms called obligate aerobes. These microorganisms cannot grow or survive in the absence of oxygen. A limited supply of oxygen can inhibit the growth of such microorganisms. Thus, since the availability of oxygen affects the rate of growth and metabolisms

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of microorganisms, dissolved oxygen measurement and control in fermentation tanks is of paramount importance.

The interest in controlling the dissolved oxygen concentration lies in the changes noted in bacterial metabolism at different oxygen concentrations. Brookes (*1*) and Ghaly et al. (*2*) reported that, in conventional batch cultures, the oxygen demand increases as the microorganisms multiply and consequently the oxygen tension varies continuously. The most obvious effect of aeration level is on the total yield of microorganisms, since oxygen can be considered as a growth limiting nutrient. Hunt et al. (*3*) stated that there is a direct correlation between the yield of cells relative to substrate utilized, together with the total cell concentration, and the oxygenation efficiency of the vessel used in the growth experiments. Thus, in a biological reactor, the dissolved oxygen concentration will be depleted rapidly by the microbial metabolism unless sufficient oxygen is added to the system to meet the oxygen demand of the microorganisms.

Our work on single cell protein production (*4,5*) shows that oxygen is one of the major fermentation parameters that is difficult to monitor and control. Localized anaerobic conditions may exist in aerobic systems resulting in the production of undesirable end products and a decreased system efficiency. One of the requirements of an automatic oxygen control system is to account for the rate of response of the oxygen probe. This is particularly important because of the low oxygen capacity of the culture media compared to the potentially high rate of oxygen turnover.

Nonetheless, the development of an automatic measurement and control systems for dissolved oxygen in culture media has been considerably delayed because of the lack of a suitable oxygen probe. Many researchers (*5–8*) stressed the need for a probe that: (a) is stable over a long period of time without requiring frequent calibration, (b) must have a linear response to increasing oxygen concentration in the culture media, and (c) must be capable of *in situ* sterilization. It is the long-term stability that most electrodes to date have proven deficient.

OBJECTIVES

The objectives of this study were to develop a computer-based oxygen measurement and control system for fermentation processes requiring *in situ* oxygen measurement and control; and to evaluate the rate of the system response to changes in the concentration of dissolved oxygen in the fermentation media as well as its long-term stability.

KINETICS OF OXYGEN TRANSFER

Aeration is used to transfer oxygen to biological systems. Oxygen in the air is transferred from air bubbles through the liquid medium to the

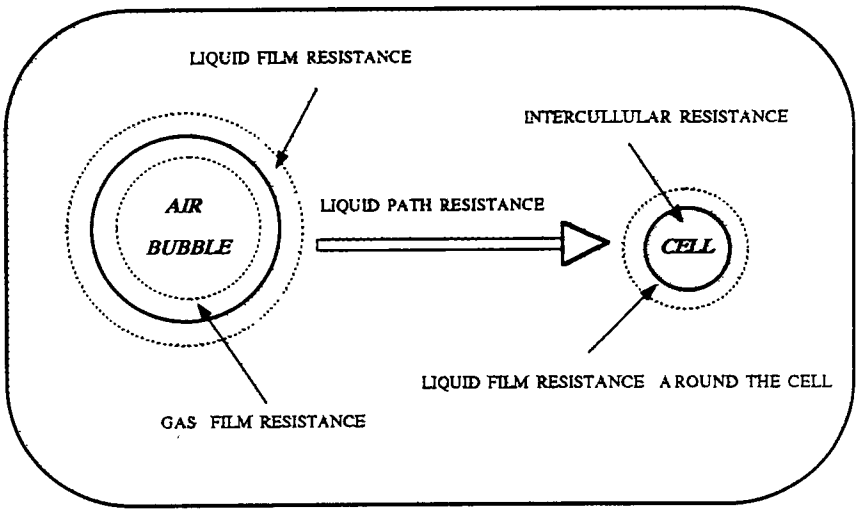

Fig. 1. Oxygen transfer from the air bubbles to the microbial cells.

microbial cells as shown in Fig. 1. Dissolved oxygen is utilized at a rate proportional to the rate of organic matter removal by the microorganisms. According to Loehr (*9*), the basic relationship used to estimate the rate at which oxygen must be supplied by an aeration system is as follows:

$$dC / dt = K_L a \, (C_s - C) \tag{1}$$

where:

- dC/dt is the rate of change of dissolved oxygen concentration with time (mg/L/s)
- $K_L a$ is the overall oxygen transfer coefficient (per s)
- C_s is the solubility of oxygen at given temperature and atmospheric pressure (mg/L)
- C is the actual oxygen concentration at time t (mg/L)

The overall oxygen transfer coefficient ($K_L a$) is a function of the gas-liquid interfacial area, the liquid turbulence, the diffusivity of oxygen in the liquid, the temperature of the liquid, and the concentration of the inorganic and organic materials. The solubility of oxygen (C_s) is affected by the temperature, the dissolved inorganic material and the air pressure at the depth where the air is introduced into the fermentation tank. Because standard conditions rarely exist in practice, some modifications to Eq. (1) are made to reflect the actual process condition. These modifications include factors to adjust for $K_L a$, C_s, temperature, pressure, and the oxygen utilization rate of microorganisms (R_r) as follows:

$$dC / dt = \alpha \, k_L a_{20} \, (\beta \, C_s - C) \, \Theta^{(T-20)} \, P / 101.325 - R_r \tag{2}$$

where:

K_La_{20} is K_La in water at 20°C (per s)
α is the ratio of K_La in the mixed liquor to K_La in water at 20°C (–)
β is the ratio of the oxygen solubility in mixed liquor at temperature T to the oxygen solubility in water at 20°C, standard barometric pressure and the absence of chlorine salts (–)
Θ is the temperature correction factor for the system (–)
T is the system operating temperature (°C)
P is the parametric pressure (Pa)
R_r is the microbial oxygen uptake rate (mg/L/s)

Temperature affects both the oxygen solubility and the overall oxygen transfer coefficient. As the temperature increases, C_s decreases and K_La increases. A temperature correction factor (Θ) value of 1.024 is commonly used for most aeration systems operating in the range of 15–35°C (*9*). The characteristics of the medium affect α and β directly and, thus, influence the oxygenation capacity of an aeration system to a greater degree than does temperature. According to Eckenfelder and O'Connor (*10*), the constituents of an aeration medium can affect K_La primarily by affecting the ability of oxygen to be transferred from the gaseous phase to the liquid phase. Values of α generally are less than 1.0 with most values in the range of 0.5–0.9. Beta (B) values are also usually less than 1.0 with most values in the range of 0.7–0.9 (*9*).

The oxygenation capacity (OC) of an aeration unit is a measure of the rate of input of oxygen (usually mg O_2/h) that can be achieved under given operating conditions. For a biological system operating at the steady state condition, the oxygenation capacity of the aeration system should equal or exceed the oxygen demand of microorganisms according to the following equation:

$$OC = R_r = \alpha K_La_{20} (\beta C_s - C) \Theta^{(T-20)} P / 101.325 \quad (3)$$

DETERMINATION OF AERATION PARAMETERS

Aeration equipment are usually evaluated in a water free of dissolved oxygen with the temperature being adjusted to 20°C. However, it is occasionally desirable to measure K_La in the presence of microbes. In practice, the unsteady state method with active microbes is used for the determination of K_La. This method provides information on K_La, C_s and R_r under actual process conditions according to the following equation:

$$dC/dt = (\alpha K_La \beta C_s - R_r) - (\alpha K_La C) \quad (4)$$

The procedure involves turning off the aeration equipment and noting the decrease in dissolved oxygen with time. The aeration equipment remains off until zero or low dissolved oxygen level is obtained. In most cases, the microorganisms will deplete the oxygen within a short time

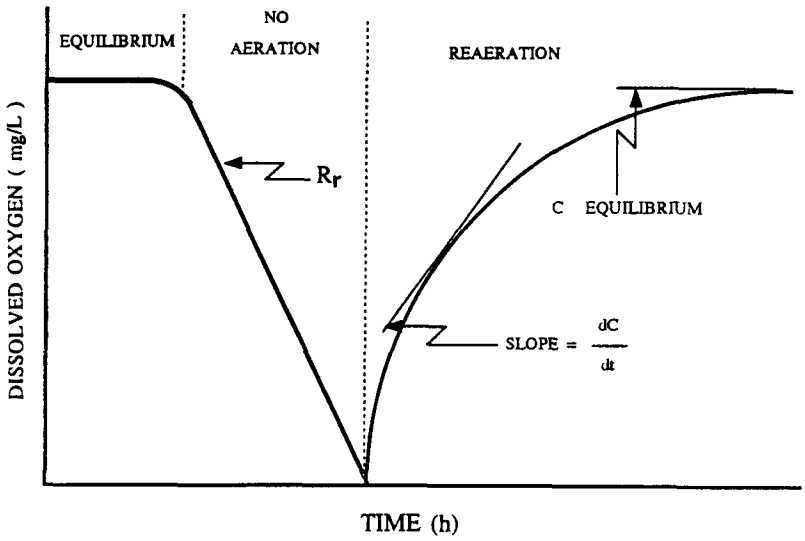

Fig. 2. Determination of R_r.

after the aerator is turned off. Since no oxygen is entering the aeration unit, the rate of dissolved oxygen decrease will be the microbial oxygen uptake rate (R_r). A plot of dissolved oxygen with time should result in a straight line throughout most of the range (Fig. 2). The slope of the straight line is R_r. Variation from straight line response may occur at the beginning of the experiment if the equilibrium conditions were not established and at the end of the experiment when the dissolved oxygen concentration becomes a limiting factor.

The aeration unit is, then, returned to normal and the dissolved oxygen concentration is determined at frequent intervals until the steady state condition is achieved. The dissolved oxygen concentration (C) is plotted with time. Tangents to this curve at various values of C are used to establish the values of dC/dt (Fig. 2). These values are then plotted against C (Fig. 3) to determine K_La of the system under actual process conditions. If a straight line is obtained from the plot of dC/dt vs C, the system is stable and the parameters R_r and K_La are constants. If the system is unstable, a curve will result when dC/dt is plotted against C, which indicates that R_r and K_La are variables. Several factors contribute to the instability of aeration systems; the most important of which being the nonuniformity of dissolved oxygen concentration in all parts of the medium. Varying concentrations of dissolved oxygen at different points in the system can create instability throughout the entire system, even though sufficient dissolved oxygen may be present at specific parts of the system.

The values of α and β of a specific medium can be determined by running a small scale laboratory experiment using sterilized medium and water. The sterilized medium and water are aerated separately under standard conditions of temperature and pressure and similar aeration

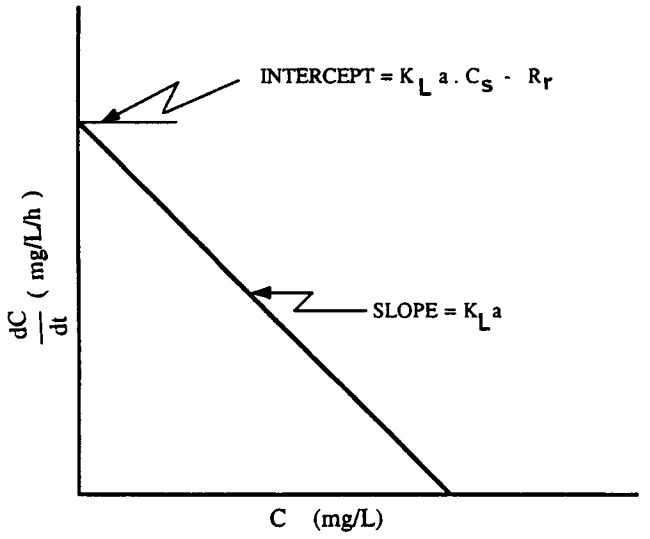

Fig. 3. Determination of $K_L a$.

parameters (such as mixing speed, air flow rate, aerator shape, and dimension) until the oxygen concentrations reach constant values ($C_{s\ \text{medium}}$ and $C_{s\ \text{water}}$). The value of β is determined by dividing $C_{s\ \text{medium}}$ by $C_{s\ \text{water}}$. The values of $K_L a$ can be determined using Eq. (1). By plotting $(C_s - C)$ for both the medium and water against time on a semilogarithmic paper, straight lines will be obtained, the slope of which are $K_L a_{\ \text{medium}}$ and $K_L a_{\ \text{water}}$, respectively. The value of α is obtained by dividing $K_L a_{\ \text{medium}}$ by $K_L a_{\ \text{water}}$.

SYSTEM DEVELOPMENT

The developed oxygen measurement and control system (Fig. 4) consisted of a portable computer, data acquisition unit, an oxygen probe, a signal conditioning circuit, an air compressor pump, an air tank, a cut-off switch, a pressure regulator, an air filter, an air flow meter, an air distributor, a mixing system, a stepper motor with power supply and coil switches, and an electronic pot.

A Radio Shack portable computer (TRS-80 Model 100) was employed for the on-line measurement and control. The computer has 32K of random access memory (RAM). A Starbuck 8232, multi-channel data acquisition and control unit served as an interface between the computer and the oxygen measurement and control system. The starbuck unit has eight digital inputs, eight analog inputs, and eight digital outputs. As the unit can only read analog voltage between 0 and 5 V, sensors that provide very small output voltage cannot be read accurately without voltage amplification.

The oxygen was monitored with an Orion Oxygen Electrode (Cole-Parmer cat. no. J-5717-00) made for use with a pH meter for direct ppm

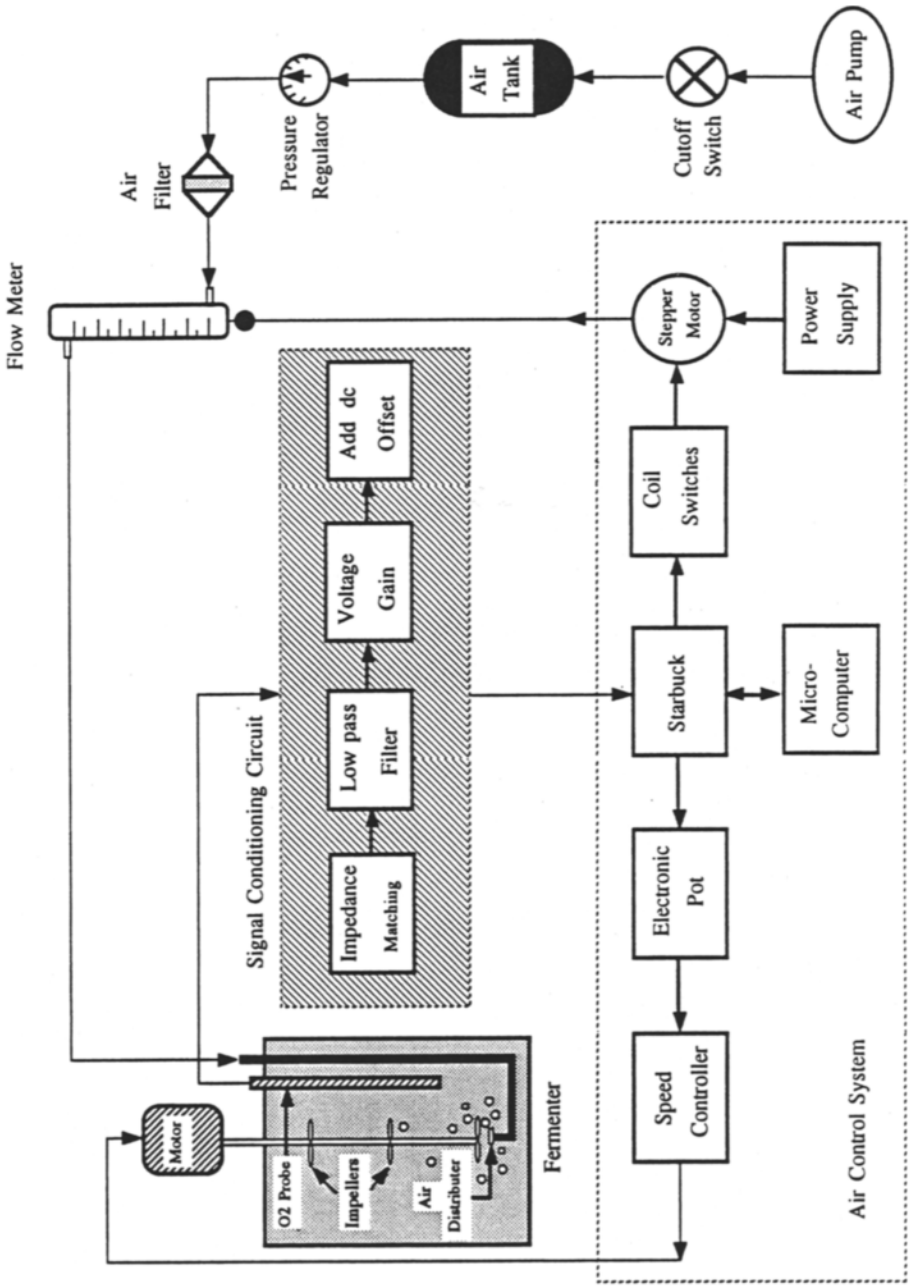

Fig. 4. A schematic diagram showing the oxygen measurement and control system.

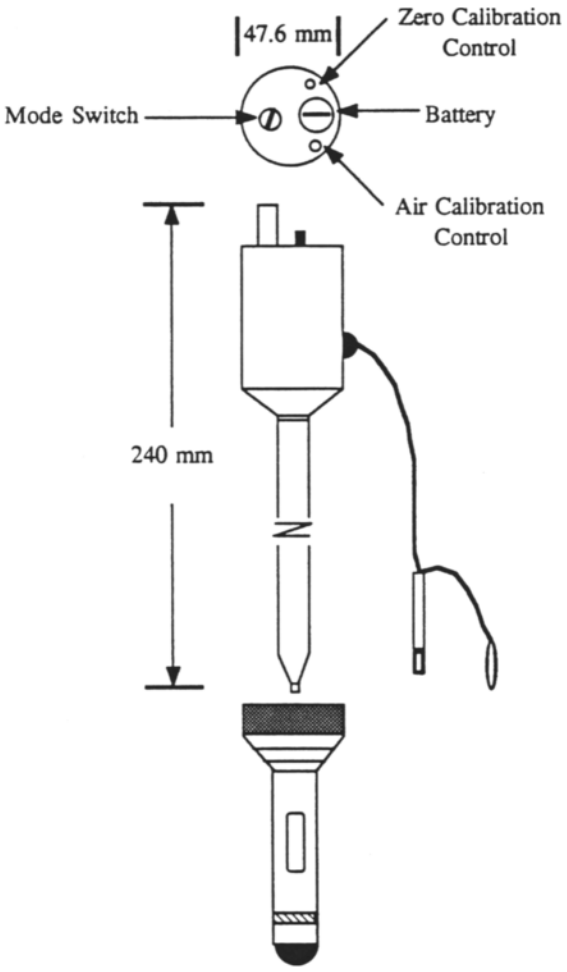

Fig. 5. The orion oxygen probe.

readout of dissolved oxygen. The Orion Oxygen Electrode (Fig. 5) houses an oxygen sensor, electronic circuits, and operating controls. On top of the unit are switches for battery check, air calibration, zero calibration, and water sensing modes. The oxygen electrode has an input range of 0–14 ppm, which corresponds to an output range of +414 to −414 mV. The oxygen electrode is very stable and does not require any calibration as the sensing portion of the electrode can be replaced easily.

As the Starbuck 8232 data acquisition unit accepts voltages only in the range of 0–5 V, a signal conditioning circuit was built to convert the voltage obtained from the oxygen electrode from −414 to +414 mV to 0–5 V. Details of the signal conditioning circuit are shown in Fig. 6. In this circuit, the oxygen probe is connected to the input of a complementary metal-oxide semi-conductor (CMOS) operational amplifier, which has an input

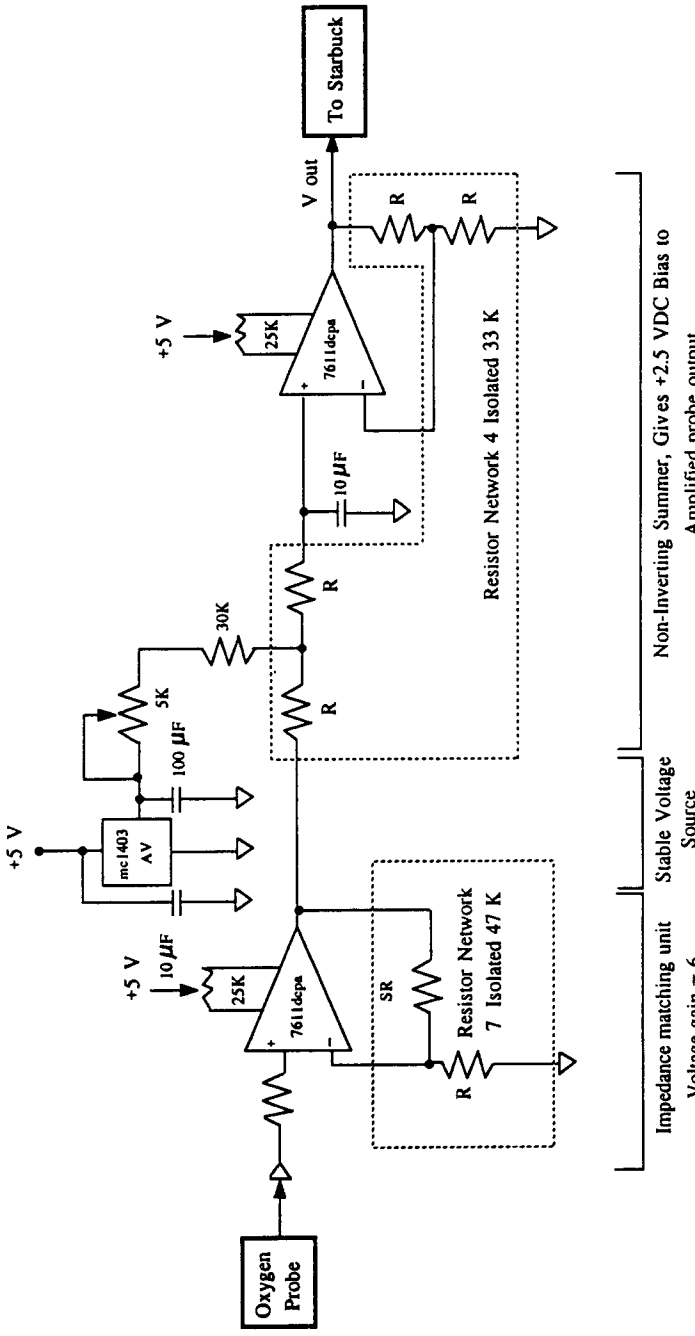

Fig. 6. The signal conditioning circuit.

impedance of about 10^{12} ohms. The buffer output is amplified by 6.0 (from ±414 mV to ±2.484 V). The amplified signal is then given a +2.5 V bias in order to have an output range from 0.016–4.984 V.

The mixing system (Cole-Parmer model no. HS240 MDMT) included the mixing motor, impeller, and speed controller. The speed control unit has a highly accurate electronic control system that is easily adapted to computer interface. The controller provides filtered DC power for the motor, and changes the voltage as necessary to keep the motor speed constant. The system can also be used with chart recorder for recording the motor speed if necessary and has the ability of measuring the torque. The latter is a highly desirable feature in our fermentation vessel as it can be related to microbial density.

An air compressor pump (Cole-Parmer model no. LGH 210. HO2) with an air tank, a pressure gage, an automatic cutoff switch, an air filter, and a flowmeter was used to supply air to the fermentation system. A 12 V stepper motor (301-SM-AIRPAX 4SH-12A56S) was used for controlling the valve of the flowmeter to increase or decrease the amount of air coming from the air compressor pump and going to the fermentation tank.

DETERMINATION OF THE SYSTEM PARAMETERS UNDER SIMULATED BIOLOGICAL CONDITIONS

The unsteady state aeration procedure, proposed by Gaden (*11*) and adopted by many authors (*12–16*) as the standard, has been used in this study. The test involves chemical removal of dissolved oxygen from water by the addition of sodium sulfite ($Na_2 SO_3$) with cobalt chloride ($CoCl_2$) added as a catalyst. The subsequent increase in oxygen concentration is measured during the aeration using an oxygen probe and $K_L a$ is calculated according to Eq. (1). The amount of sodium sulfite that should be added is calculated from the stoichiometric relationship as follows:

$$Na_2 SO_3 + 0.05\, O_2 \xrightarrow{Co\, Cl_2} Na_2 SO_4 \qquad (5)$$

Theoretically, 7.9 mg/L sodium sulfite is needed for each mg/L of dissolved oxygen. However, because it is necessary to mix the sodium sulfite throughout the aeration tank before the test starts, oxidation of some sulfite may occur because of mixing. Therefore, the addition of two times of the theoretical quantity of sodium sulfite has been suggested by Stukenberg and Wahbeh (*13*) and Ghaly and Kok (*14*). Although, Kalinske (*12*) showed that a cobalt concentration as low as 0.05 mg/L can be effective in catalyzing the reaction between the oxygen and sodium sulfite, Ghaly and Kok (*16*) recommended the use of 0.2 mg/L of cobalt chloride ($Co\, Cl_2 \cdot 6H_20$).

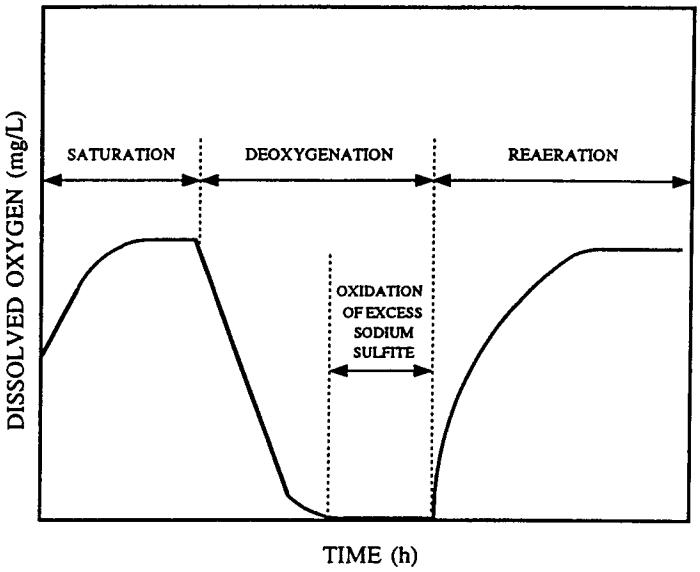

Fig. 7. A typical recorded curve of the saturation-deoxygenation-reaeration process.

Determination of K_La

A number of experiments were performed to determine the values of K_La in distilled-deionized water under standard conditions of pressure and temperature at three mixing speeds (200, 300, and 400 rpm) and three airflow rates (10, 20, and 30 cm^3/min) in a 1-L reactor. During each experimental run, the reactor contents were aerated, deoxygenated, and reaerated. Before each experimental run, the reactor was flushed several times with distilled deionized water. One liter of distilled-deionized water was then added to the reactor. The air supply, mixer, and oxygen measurement and recording system were started simultaneously. When the saturation concentration (C_s) had been reached, the air supply was switched off and the mixer speed was reduced. Then 1.15 mL of 1*M* Na_2SO_3 (144 mg of sodium sulfite) and 0.75 mL of 0.01*M* Co $Cl_2 \cdot 6H_2O$ solution (1.8 mg of cobalt chloride) were added. When the water was completely deoxygenated, both the airflow rate and the mixer speed were brought to the required levels and the oxygen concentration increased with time. During a typical experiment, C remained at zero until the excess sodium sulfite had been oxidized, rose rapidly at the beginning, and then slowly approached its saturation value. A typical recorded curve of the entire process of saturation-deoxygenation-reaeration is shown in Fig. 7. The values of K_La at various mixing speeds and airflow rates are presented in Table 1. A multiple regression analysis was performed on the data and the following relationship was obtained.

Table 1
Measured and Predicted K_La Values at Various Air Flowrates and Mixing Speeds

Air flow rate (cm^3/min)	Speed (rpm)	K_La (s^{-1})	
		Measured	Predicted
10	200	0.0980	0.0860
	300	0.1000	0.1075
	400	0.1130	0.1291
20	200	0.1350	0.1290
	300	0.1670	0.1506
	400	0.1900	0.1781
30	200	0.1630	0.1720
	300	0.1880	0.1936
	400	0.2100	0.2150

$R^2 = 0.9936$
$cv = 8.24\%$

$$K_La = 0.0043\,F + 0.000215\,S \tag{6}$$

where:

F is the air flow rate ($cm^3\ min^{-1}$)
S is the mixing speed (rpm)

Determination of C_s

A number of experiments were performed to determine the value of C_s in distilled-deionized water under standard conditions of pressure and temperature at three mixing speeds (200, 300, and 400 rpm), three air flow rates (10, 20, and 30 cm^3/min), and three simulated microbial-oxygen-uptake rates (2.4, 4.8, and 7.2 mg $O_2/cm^3/min$) in a 1-L reactor. Before each experimental run, the reactor was flushed several times with distilled-deionized water. One liter of distilled-deionized water was then added to the reactor. The air supply, cobalt chloride supply, sodium sulfite supply, mixer, and oxygen measurement and recording systems were started simultaneously. Continuous recording of C continued until a constant value was obtained.

The value of C at various air flow rates, mixing speeds, and simulated microbial-oxygen-uptake rates are presented in Table 2. A multiple regression analysis was performed on the data and the following relationship was obtained:

$$C = 8.81 - 21.56\,R_r + 0.0014\,F + 0.002\,S \tag{7}$$

Table 2
Measured and Predicted Values
of C at Various Simulated Oxygen Uptake Rates, Flow Rates and Mixing Speeds

Oxygen uptake rate ($mg/cm^3/min$)	Air flow rate (cm^3/min)	Speed (rpm)	C (mg/L)	
			Measured	Predicted
2.4	20	200	6.7	7.0
		300	6.9	7.2
		400	7.3	7.4
	30	200	6.8	7.0
		300	7.0	7.2
		400	7.4	7.4
	40	200	6.9	7.0
		300	7.2	7.2
		400	7.5	7.4
4.8	20	200	3.2	4.8
		300	4.4	5.0
		400	5.1	5.2
	30	200	4.5	4.8
		300	4.6	5.0
		400	5.5	5.3
	40	200	4.8	4.8
		300	4.9	5.0
		400	5.7	5.3
7.2	20	200	2.3	2.7
		300	2.7	2.9
		400	3.8	3.1
	30	200	2.7	2.7
		300	2.9	2.9
		400	3.6	3.1
	40	200	3.1	2.7
		300	3.3	2.9
		400	4.0	3.1

$R^2 = 0.96$
$cv = 7.6\%$

where:

C is the oxygen concentration at steady state (mg/L)
R_r is the simulated oxygen uptake (mg/s)

The constant 8.81 is the C_s value and Eq. (7) can be rewritten as follows:

$$C = C_s - 21.5600\,R_r - 0.0014\,F + 0.0020\,S \qquad (8)$$

SYSTEM EVALUATION

The system was evaluated under standard conditions of temperature and pressure using a continuous operation with distilled-deionized water. A retention time of 4 h was used and the microbial oxygen uptake rate was simulated with the continuous addition of sodium sulfite and cobalt chloride. Under these conditions, Eq. (2) can be rewritten as follows:

$$dC / dt = K_L a\,(C_s - C) - R_r \tag{9}$$

By integrating Eq. (9), the following equation was obtained:

$$K_L a\,(C_s - C) = R_r + (K_L a\, C_s - R_r)\, e^{K_L a\, t} \tag{10}$$

At the steady state condition, $dc/dt = 0$ and Eq. (10) can be rewritten as follows:

$$C_s - C = R_r / K_L a = \lambda \tag{11}$$

where:

λ is a known constant and can be obtained from $(C_s - C)$

At a given set of conditions (air flow rate and mixing speed) any change in R_r value will result in a change in C value. Therefore, in order to keep C at the desired value, $K_L a$ must be changed such that λ is maintained constant.

For a given value of C_s, R_r can be calculated from the following Eq. (8) as follows:

$$R_r = (C_s - C - 0.0014\,F + 0.0020\,S) / 21.56 \tag{12}$$

Then, the $K_L a$ value that is required to keep λ constant can be calculated from the following equation:

$$K_L a = \lambda / R_r \tag{13}$$

By using Eq. (6), it was possible to arrive at suitable values of F and S such that the desired $K_L a$ was achieved. The control system was then tried in a fermentation system for the production of single cell protein from cheese whey. Its performance was found satisfactory.

DEVELOPMENT OF THE COMPUTER PROGRAM

The software for oxygen measurement and control system was developed according to the flowchart given in Fig. 8. The major parts of the program included the following subroutines:

1. Initialization subroutine: This sets the communication parameters between the microcomputer and the data acquisition system, and sets the desired range of optimum C_s value.

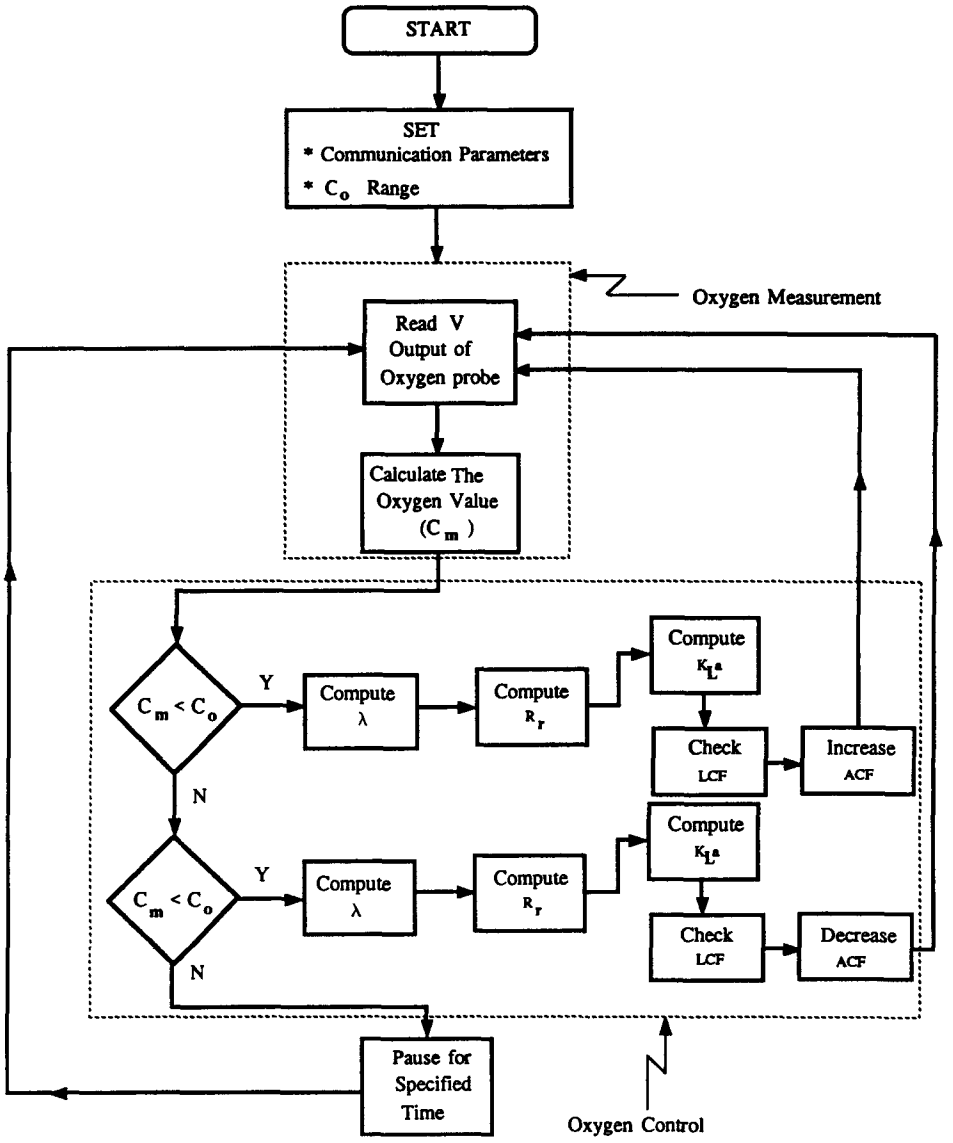

Fig. 8. A logic flowchart for oxygen measurement and control computer program.

2. The oxygen measurement subroutine: This reads the voltage output (V) of the oxygen probe and converts it to mg/L using the following regression equation in Table 1.

$$O_2 = -2.80969\,V + 14.0379 \qquad (14)$$

3. The oxygen control subroutine: The current O_2 as in (2) above is compared with the optimum O_2. If the current O_2 is less than the set optimum O_2 (i.e., $C_{measured} < C_{optimum}$), the new K_La value

needed to raise $C_{measured}$ to $C_{optimum}$ is computed using Eqs. (11–13). Then, the mixer speed and airflow rate will be recorded. As both the mixer speed and airflow rate will be kept at their minimum values (those which will provide O_2 equivalent to the initial oxygen uptake rate $R_{r\ initial}$), the decision is made to increase the mixer speed and airflow rate in turns, starting with the mixer speed. When $C_{measured}$ becomes less then $C_{optimum}$ for the first time the mixer speed that will produce K_La value that is required to produce $C_{optimum}$ will be computed. Then, the data acquisition system is activated to increase the mixer speed to the computed value. When $C_{measured}$ becomes less than $C_{optimum}$ once again the airflow value that will produce K_La value that is required to produce $C_{optimum}$ will be computed. The data acquisition will change the airflow rate to a new value. The change in speed and airflow rate will alternate, i.e., sequential control. If the $C_{measured}$ is within the set optimum ranges ($C_{optimum}$), the computer pauses for a specific period of time before returning to read the new $C_{measured}$.

CONCLUSIONS

A computer-based oxygen measurement and control system was developed and tested. The system performed satisfactorily under simulated biological conditions using steady state method with sodium sulfite. The system is being used in the fermentation process of our single cell protein production system for over a year. Its performance and long-term stability in biologically active medium have been very satisfactory. Detailed reports on its performance in biological systems can be found elsewhere.

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