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A Computer-Based pH Measurement and Control System for Fermentation Processes

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ABSTRACT

The measurement and control of pH is essential in most fermentation processes. The highly nonlinear response of pH to the addition of acid or base makes pH control by conventional means very difficult. This paper presents a description of the hardware and software of a computer-based pH measurement and control system. The system is applicable to any fermentation process requiring *in situ* pH measurement and control and is capable of controlling the pH within ±0.1 U with a very short response time. It has a proportional metering device capable of adding the required amount of acid (or alkaline) solution with high accuracy, thereby preventing overshooting.

Index Entries: pH; control; measurement; overshooting; computer.

INTRODUCTION

Recent advances in biochemistry and microbiology have resulted in a better understanding of fermentation processes, particularly with respect to substrate metabolism, microbial growth requirements, and enzyme

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regulation (*1*). However, the problems associated with the monitoring and control of the many factors and parameters, which are known to significantly influence the fermentation processes, have not been satisfactorily addressed. One such important parameter is pH.

pH control is essential in industrial fermentation processes where biological activities tend to substantially alter the pH of the system from the desirable optimum values. Denitrification, organic nitrogen breakdown, and sulfate reduction are examples of biological reactions that can cause an increase in pH. A decrease in pH may be caused by sulfate oxidation, nitrification, and organic carbon oxidation (*2*). However, the actual changes in pH are influenced and determined by the buffer capacity of the system and the amount of substrate utilized by the microorganisms.

Our work on single-cell protein production (*3–5*) shows that pH is one such major fermentation parameter that poses difficult problems of monitoring and control, especially as the most commonly used yeast species, *K. fragilis*, has a very narrow preferred pH range of 4.0–4.6. An increase in the pH resulted in the inhibition of the yeast growth and the promotion of the growth of undesirable microorganisms such as mold and bacteria. At pH above 5.5, the yeast ceased to grow and formed spores.

Usually, pH control is achieved by the addition of an acid or a base to maintain the pH within the optimum level. Unfortunately, the response of pH to the addition of an acid or a base is highly nonlinear. This makes pH control by conventional metering devices very difficult, particularly for strongly acidic or alkaline systems.

OBJECTIVES

The objectives of this study were to develop a computer-based pH measurement and control system for fermentation processes, and to evaluate the performance of the system under extreme pH conditions using strong acid and alkaline solutions to restore the pH to preselected optimum values.

DEVELOPMENT OF SYSTEM COMPONENTS

Figure 1 shows a flowchart illustrating the pH measurement and control system. The system consists of a microcomputer, a data acquisition unit, a solid state relay and power supply, pumps and solution tanks, a pH probe, and a signal conditioning circuit.

A digital microcomputer (TRS-80 model 100) was employed for the on-line measurement, control, and data analysis. The computer has 32K of random access memory (RAM). A Starbuck 8232 data acquisition and

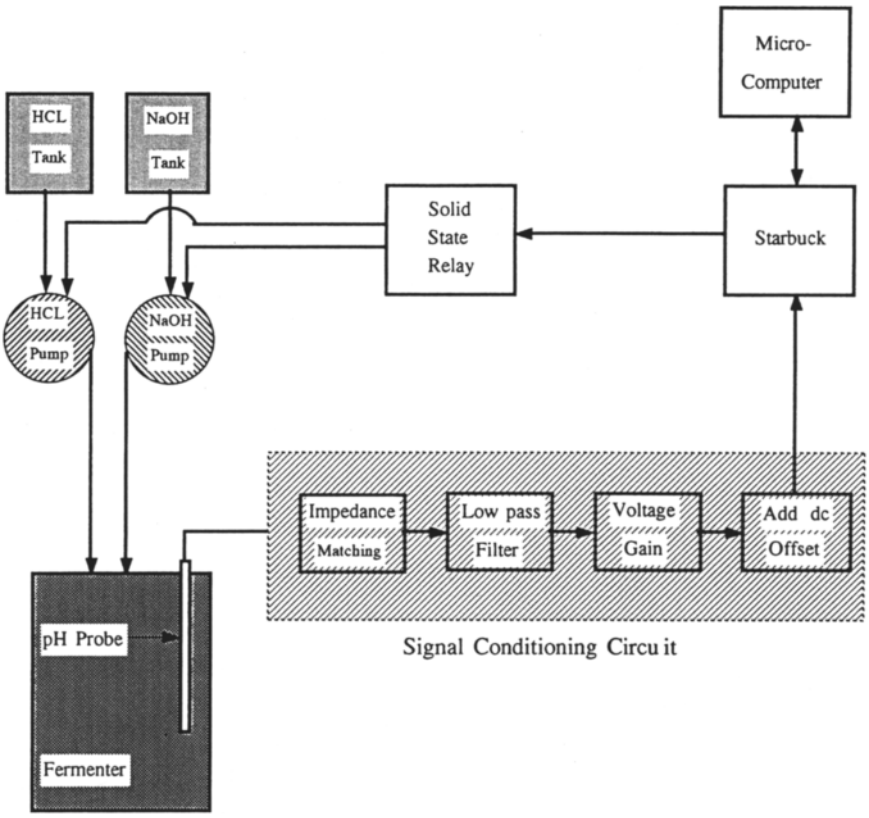

Fig. 1. A flowchart illustrating the pH measurement and control system.

control unit served as an interface between the computer and the pH measurement and control system. The Starbuck is a flexible, multichannel unit. It has eight digital inputs, eight analog inputs, and eight digital outputs. The Starbuck has an analog input voltage range of 0–5 V. Sensors that provide very small output voltage cannot, therefore, be read accurately by the unit without voltage amplification.

The pH was monitored with a long, thin-neck, combination pH electrode (Cole-Parmer cat. no. J-5990-40). The electrode was calibrated in different buffer solutions. The pH of the solutions used varied from 4–10. A pH/mV calibrator (Cole-Parmer cat. no. 05657-10) was used to determine the voltage output of the pH solutions. The mV output of the pH electrode ranged from −180 to +180 mV, depending on the pH value of the solution used.

Since the Starbuck 8232 data acquisition unit accepts voltage only in the range of 0–5 V, a signal conditioning circuit was built to convert the voltage obtained from the pH electrode from −180 to +180 mV to 0–5 V. Figure 2 presents the details of the electronic components circuitry and

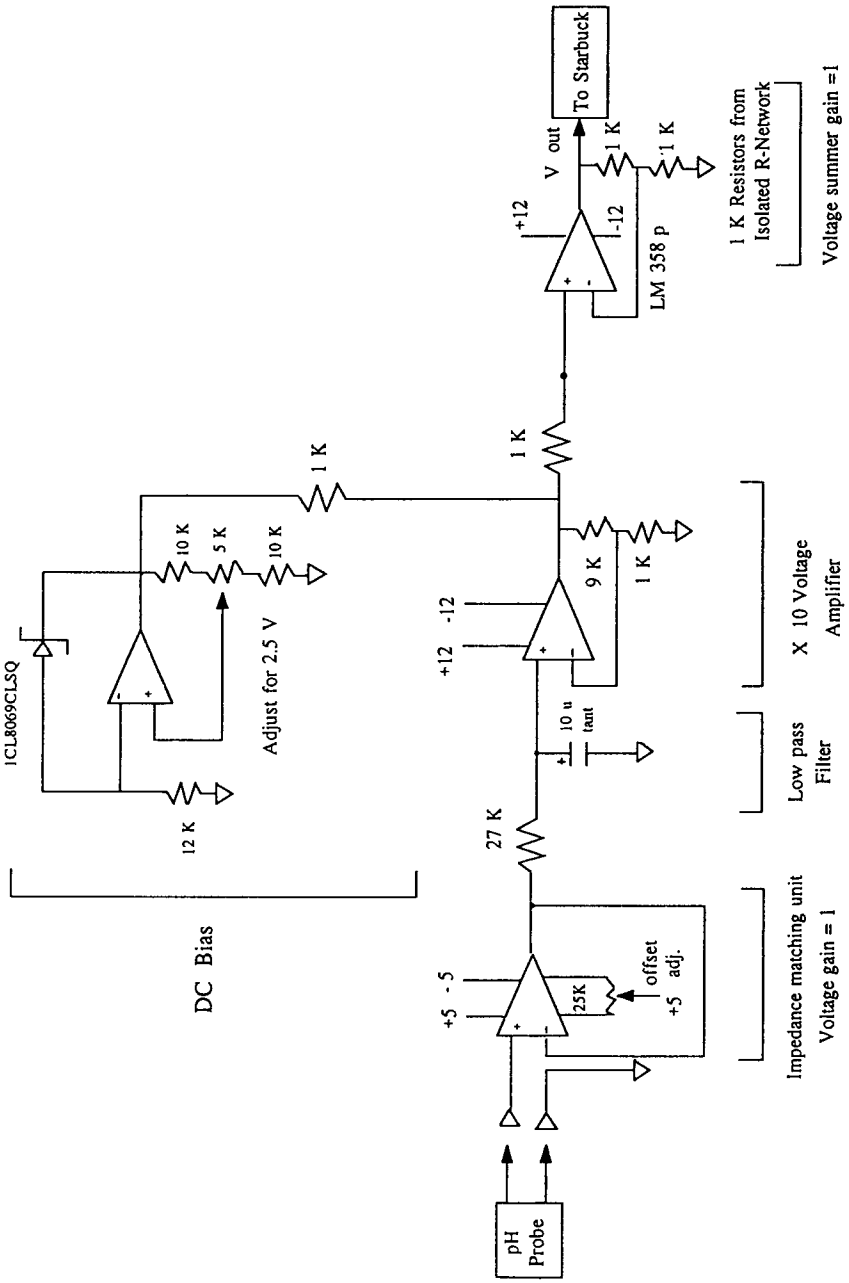

Fig. 2. The component of the signal conditioning circuit.

Table 1
Summary of the Voltage Output at Different pH Values

pH Value	Probe voltage output (mV)	Amplified voltage (V)	Summed voltage output (V)
4	+180	+1.8	+4.3
7	0.0	0.0	+2.5
10	−180	−1.8	+0.7

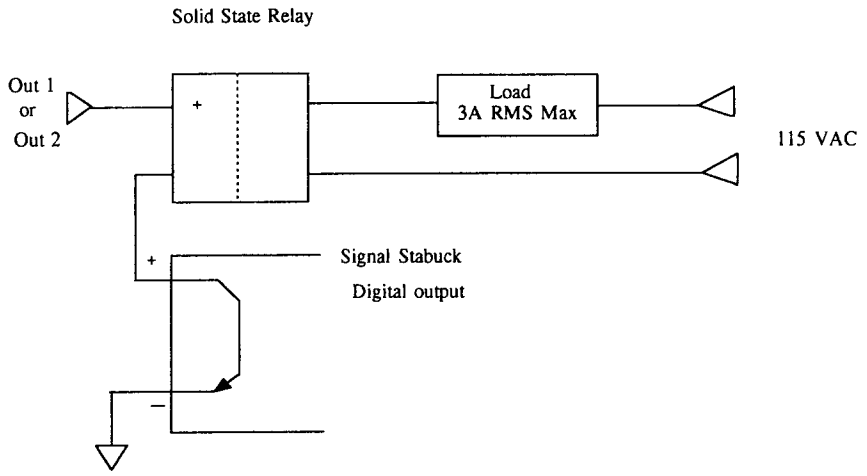

Fig. 3. The solid state relay.

the signal conditioning circuit. In this circuit, the pH probe is connected to the input of a complementary metal-oxide semiconductor (CMOS) operational amplifier having an input impedance of about 10^{12} ohms. The operational amplifier has a temperature dependent input impedance. However, at about a temperature of 25–35°C, the output of the buffer closely matched the expected values. There was 60 hertz interference found in the output of the buffer, probably as a result of the long cable and high impedance of the pH probe. This interference was removed with a simple low-pass RC network. The pH signal was amplified from ±0.18 to ±1.8 V. The amplified pH signal is then summed with the dc bias of 2.5 V. Table 1 shows the summary of the voltage input and output.

Two Perstalic Pumps (Cole-Parmer model no. 7543-02) were used for metering the acid and the base into the fermentation vessel. These pumps require 110 VAC and draw large amounts of current. Because of the current voltage limitation of the Starbuck 8232's digital outputs, a solid-state relay (Fig. 3), commonly used for the control of heavy power loads with standard digital signal was used to operate these pumps. The relay module comes equipped with opto-isolation between the digital control signal and the power output loads, to protect the digital circuits from electrically

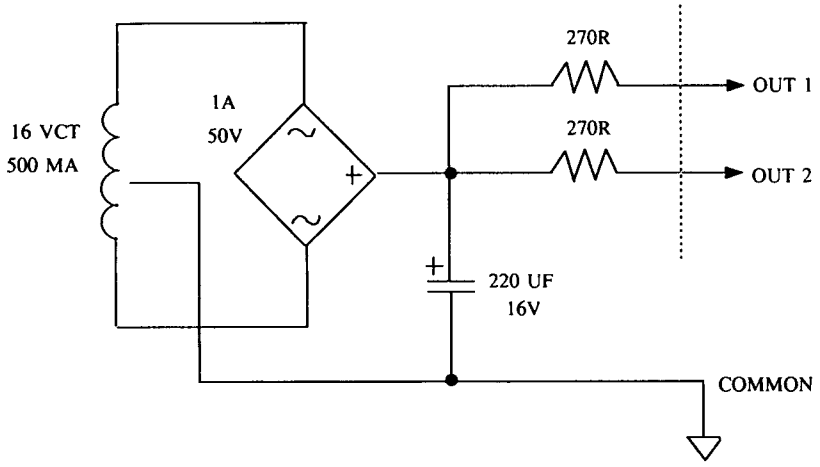

Fig. 4. The input power supply.

noisy environments. A power supply (Fig. 4) was used in the control system. The Starbuck 8232's digital outputs served as a switcher to turn the power on or off. When the digital output is not activated (i.e., no current flows through it), the power supply is off. When the digital output is activated (i.e., the current flows through it), the power supply is on.

DEVELOPMENT OF TITRATION CURVES

Titration experiments were carried out to obtain data on which to base the development of the computer program. Solutions of known initial pH (2.5 and 11.3) were used in these experiments. Aliquots of 1*N* NaOH (or 1*N* HCl) were added to change the pH.

Addition of NaOH

To 1 L of an acidic solution of known initial pH (2.5), 0.5 mL aliquots of 1*N* NaOH solution were added at intervals timed to maintain isothermal conditions. The solution was stirred continuously and maintained at a total volume of 1 L by removing excess liquid prior to each addition. The pH was recorded after each addition.

A plot of the cumulative volume of NaOH solution added vs the corresponding pH values yielded a highly nonlinear curve (solid line) as shown in Fig. 5. The peculiar shape of the curve suggested the use of the piecewise regression technique to analyze the pH response to the volumes of base added, using the following model (*6*).

$$E(y) = B_0 + B_i x + B_2 (x - k_1) C_1 + B_3 (x - k_2) C_2 \quad (1)$$

where:

$$k_1, k_2 = \text{knot values of } x;\ k_1 < k_2$$

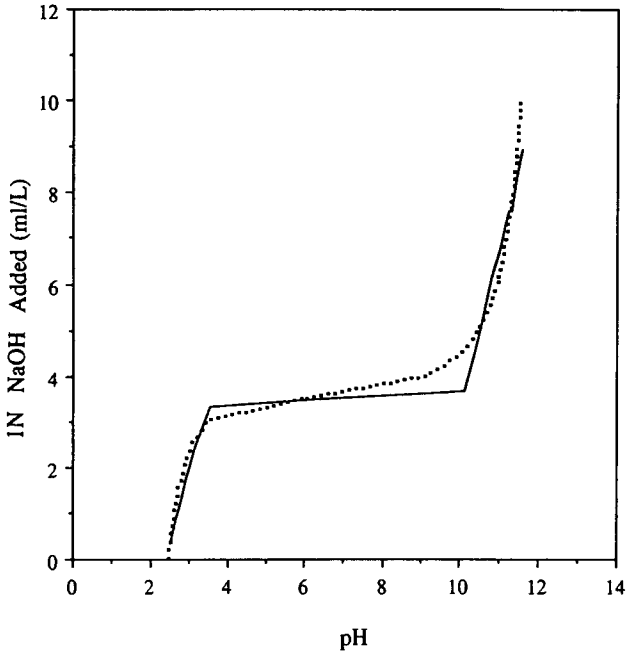

Fig. 5. Change in pH with addition of 1*N* NaOH solution. ····· Actual; —— Predicted.

Table 2
Analysis of Variance of the NaOH-pH Data

Source	DF	SS	MS	F	P>F
Total	20	192.5000			
Model	3	187.3735	62.4578	207.119	0.0001
Error	17	5.1265	0.3015		

R^2=0.973

$$C_1 = 1 \text{ if } x > k_1$$
$$0 \text{ if } x \leq k_1$$

$$C_2 = 1 \text{ if } x > k_2$$
$$0 \text{ if } x \leq k_2$$

In this study, pH is the independent variable and the values of k_1 and k_2 were obtained from Fig. 5 as follows:

$$k_1 = 3.4$$

$$k_2 = 10.2$$

An analysis of variance was carried out on the data as shown in Table 2. The coefficient of correlation (R^2) was over 0.97. A *t*-test was also carried out on the regression coefficients. The values of the B coefficients and the corresponding *t*-values are shown in Table 3.

Table 3
t-test for the B Coefficients of the NaOH-pH Regression Equation

Coefficient	DF	Value	sT	P>T
B_0	1	−7.9869	1.8950	0.0006
B_1	1	3.3289	0.6555	0.0001
B_2	1	−3.2750	0.7157	0.0003
B_3	1	3.6850	0.3882	0.0001

To use the model, the optimum pH is defined as pH_o whereas the current pH is designated as pH_c. From Eq. (1), the volume of the base needed to change the pH from pH_c to pH_o is determined as follows:

$$V_{pHo} = B_0 + B_1 pH_o + B_2 (pH_o - k_1) C_1 + B_3 (pH_o - k_2) C_2 \quad (2)$$

$$V_{pHo} = B_0 + B_1 pH_c + B_2 (pH_c - k_1) C_1 + B_3 (pH_c - k_2) C_2 \quad (3)$$

$$\Delta V_B = V_{pHo} - V_{pHc} \quad (4)$$

where:

ΔV_B is the volume of base to be added per L.

Figure 5 presents the actual values of NaOH solution and those predicted using the model. There is a good agreement between the model and the observed data.

Addition of HCl

To 1 L of an alkaline solution of known initial pH (11.3), 0.5 mL aliquots of 1*N* HCl solution were added at intervals timed to maintain isothermal conditions. The solution was stirred continuously and maintained at a total volume of 1 L by removing excess liquid prior to each addition. The pH was recorded after each addition of acid. A plot of the cumulative volume of HCl solution added vs the corresponding pH values is shown in Fig. 6. The C coefficients were as follows:

$$C_1 = \begin{array}{l} 1 \text{ if } x < k_2 \\ 0 \text{ if } x \leq k_2 \end{array}$$

$$C_2 = \begin{array}{l} 1 \text{ if } x < k_1 \\ 0 \text{ if } x \geq k_1 \end{array}$$

The *k* values were then obtained from Fig. 6 as follows:

$$k_1 = 3.4$$

$$k_2 = 10.2$$

In fact, Fig. 6 is virtually a mirror image of Fig. 5 and the *K* values for the alkaline solution were same as for the acidic solution. An analysis of variance was carried out on the data as shown in Table 4. The coefficients

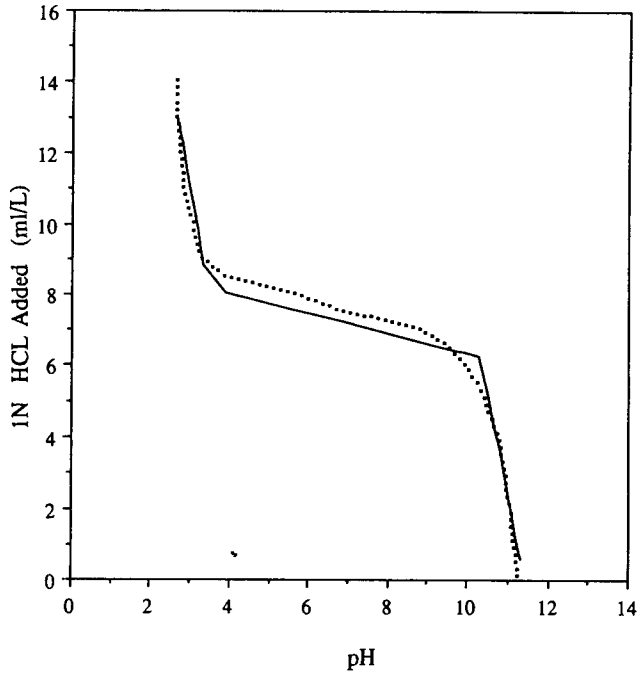

Fig. 6. Change in pH with addition of 1*N* HCl solution. ····· Actual; —— Predicted.

Table 4
Analysis of Variance of the HCl-pH Data

Source	DF	SS	MS	F	P>F
Total	28	507.5027			
Model	3	502.3674	167.4549	815.2080	0.0001
Error	25	5.1353	0.2044		

$R^2 = 0.99$

of correlation (R^2) was 0.99. A *t*-test was performed on the regression coefficients as shown in Table 5.

The volume of the acid needed to restore the pH to the optimum value is computed as follows:

$$V_{pHo} = B_0 + B_1 pH_o + B_2 (k_2 - pH_o) C_1 + B_3 (k_1 - pH_o) C_2 \quad (5)$$

$$V_{pHo} = B_0 + B_1 pH_c + B_2 (k_2 - pH_c) C_1 + B_3 (k_1 - pH_c) C_2 \quad (6)$$

$$\Delta V_A = V_{pHo} - V_{pHc} \quad (7)$$

where:

V_A is the volume of the acid to be added per L.

The actual and predicted values of HCl solution are shown in Fig. 6. There is a good agreement between the model and the observed data.

Table 5
t-Test for the Coefficients of the HCl-pH Regression Equation

Coefficient	DF	Value	ST	P>T
B_0	1	64.0586	3.5598	0.0001
B_1	1	−5.6125	0.3289	0.0001
B_2	1	−5.3294	0.3629	0.0001
B_3	1	6.2559	0.5484	0.0001

DEVELOPMENT OF THE COMPUTER PROGRAM

The software for pH measurement and control was developed according to the flowchart given in Fig. 7. The major parts of the program include 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 the optimum pH value.
2. The pH measurement subroutine. Here, the voltage output (V) of the pH probe is read and converted to a pH value using the following regression equation, based on the data presented in Table 1:

$$pH = 11.17 - 1.67V \quad (8)$$

3. The pH control subroutine. At this point, the current pH measured as in (2) above is compared with the optimum pH. If the current pH of the fermenter is less than the set optimum pH range (i.e., $pH_c < pH_o$), then the volume of NaOH solution needed to raise the pH to the optimum value is computed using Eqs. (2) to (4). Then, the data acquisition system is activated to turn on the NaOH pump to add the computed volume of the base. If $pH_c > pH_o$, a similar computation for the needed volume of the acid solution is made using Eqs. (5) to (7) and the HCl pump is turned on to add the computed volume of the acid. But, if pH_c is within the set optimum range, the computer pauses for a specified period of time before returning to read the (new) pH_c.

EVALUATION OF THE SYSTEM

A 1-L capacity fermentation vessel, with an overflow port to maintain its working volume at 1L, was used to evaluate the performance of the control system. An alkaline solution of 11.31 pH and an acidic solution of

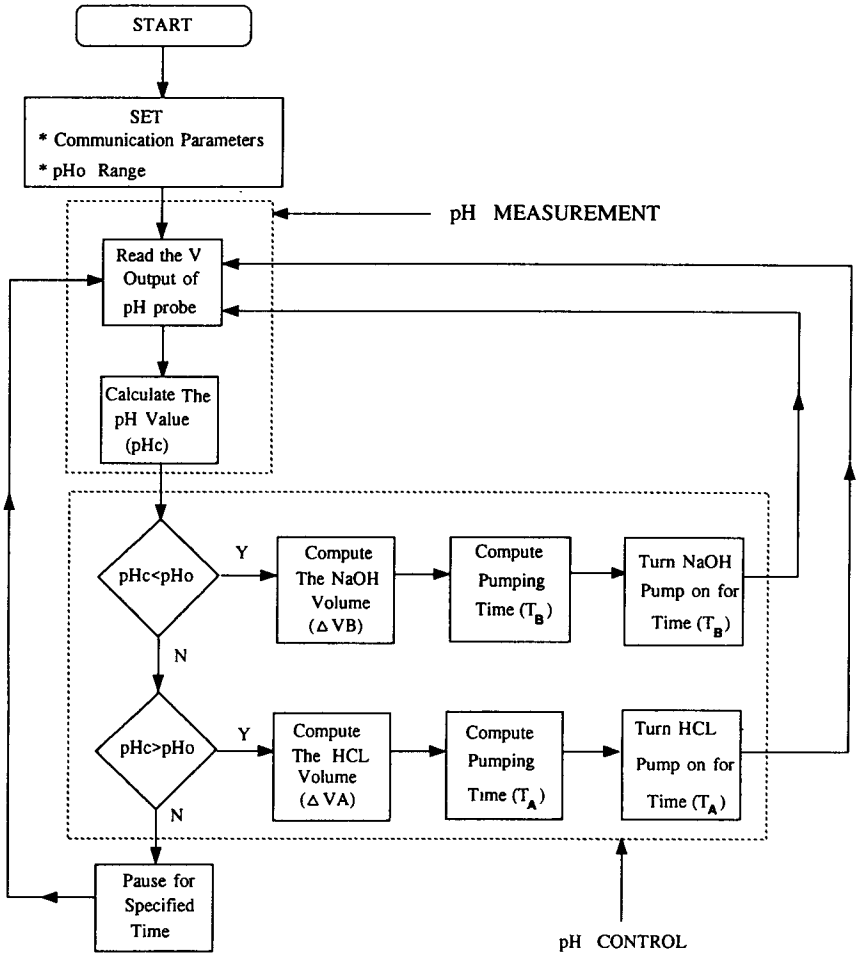

Fig. 7. Logic flow chart of the pH measurement and control program.

2.50 pH were used to test the response of the system to extreme pH conditions. The optimum pH was set at 4.0 ± 0.25 and the system was allowed to run. The volume of a base (or an acid) required to change the pH of the medium from one value to another was determined exactly according to Eqs. (2) to (4) or Eqs. (5) to (7). It must be noted that if the volume used is different, say N L, then Eqs. (4) and (7) have to be multiplied by N to determine the needed volume of base or acid.

In the case of alkaline solution (pH = 11.31), the pH of the medium was automatically measured, the amount of acid required to bring the pH to the optimum range was calculated, and the HCl pump was immediately turned on and run for some time to deliver the required amount of acid and stopped. Then, the computer measured the new pH value of the medium. Soon after, the computer indicated that it had gone into pause,

as programmed. The pH of the medium was at once measured manually and found to be 3.85, which was within the set optimum pH range and very close to the pH value of 3.84712 indicated on the computer printout.

In the case of acid solution (pH=2.5), the pH of the medium was automatically measured, the amount of alkali required to bring the pH to the optimum range was calculated, and the NaOH pump was immediately turned on and run for some time to deliver the required amount of alkali and stopped. The pH of the medium was at once measured manually and found to be 4.15, which was within the optimum range and very close to the pH value of 4.14738 indicated by the computer printout.

To study the effect of both the amount and kind of alkakility (or acidity) on the system response, three different buffers having three different pH values were used. These were: (a) 0.05*M* Potassium Biphthalate Buffer with a pH value of 4.00 (Fisher Scientific cat. no. 101 B-500), (b) 0.05*M* Potassium Hydroxide Buffer with a pH value of 7.00 (Fisher Scientific cat. no. 513107-500) and (c) 0.05*M* Potassium Carbonate-Potassium Borate-Potassium Hydroxide Buffer with a pH value of 10.00 (Fisher Scientific cat. no. 50B-115). Five optimum pH ranges were tried in this set of experiments. These were 3.5 ± 0.1, 4.5 ± 0.1, 5.5 ± 0.1, 6.5 ± 0.1, and 7.5 ± 0.1. In all cases the system was capable of calculating the required amount of acid (or alkali) required and restored the pH of the medium to the optimum pH range.

For a number of times, the pH of the medium was altered by the addition of an acid or a base, with the resultant pH being measured manually each time. The computer-based pH measurement and control system restored the pH to the set optimum range. These experiments were repeated while the optimum pH was set at a given value ±0.1 and the pH was altered manually each time. The system was capable of restoring the pH to the set optimum range.

The system was then used in a 2.5-L fermentation system for the production of single cell protein from cheese whey using the yeast *K. fragilis.* The system always kept the pH of the medium at 4.3 ± 0.1. It was capable of calculating the exact amount of acid (or alkali) required to restore the pH without any overshooting. It should be noted that the computer always paused following the addition of the base or acid solution. This indicates the effectiveness of the system that performs without cycling (overshooting).

It should be noted that the design of the interface and the development of the titration curves were carried out for a selected pH range of 2.5–11.3. Thus, the empirical models used in the computer program are only valid within this pH range. Conditions that may change the environment within this pH range will not have any effect on the pH measurement and control. It should also be emphasized that the interface and the titration curves as well as the empirical models could be developed for a wider range of pH. However, this would be of no practical value as most industrial fermentation processes requiring pH control operate within the pH range of 2.5–11.3.

CONCLUSION

A computer-based pH measurement and control system was developed and tested. The system performed satisfactorily in a biologically inert, acidic and alkaline solutions. The fact that it performed without cycling (overshooting) is a particularly useful feature of the system. The system is being applied to the fermentation process of our single-cell protein production system. Its performance in a biologically active system is satisfactory.

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