Copyright Warning & Restrictions

The copyright law of the United States (Title 17, United States Code) governs the making of photocopies or other reproductions of copyrighted material.

Under certain conditions specified in the law, libraries and archives are authorized to furnish a photocopy or other reproduction. One of these specified conditions is that the photocopy or reproduction is not to be "used for any purpose other than private study, scholarship, or research." If a, user makes a request for, or later uses, a photocopy or reproduction for purposes in excess of "fair use" that user may be liable for copyright infringement,

This institution reserves the right to refuse to accept a copying order if, in its judgment, fulfillment of the order would involve violation of copyright law.

Please Note: The author retains the copyright while the New Jersey Institute of Technology reserves the right to distribute this thesis or dissertation

Printing note: If you do not wish to print this page, then select "Pages from: first page # to: last page #" on the print dialog screen



The Van Houten library has removed some of the personal information and all signatures from the approval page and biographical sketches of theses and dissertations in order to protect the identity of NJIT graduates and faculty.

2) COMPUTER CONTROLLED OXYGEN BIOREACTOR

by Dinesh Sachdeva

Thesis submitted to the Faculty of the Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of Master of Science in Electrical Engineering 1989

APPROVAL SHEET

Title of Thesis: Computer Controlled Oxygen Bioreactor.

Name of Candidate: Dinesh Sachdeva Master of Science in Electrical Engineering, 1989

> Stanley Reisman, Professor Date Associate Chairman, Dept. of Elec. Eng.

Signatures of other members of the thesis committee.

> William Carr, Professor Date Sponsored Chair in Microelectronics

VITA

Name: Dinesh Sachdeva

Degree and Date to be conferred: M.S., 1989

Secondary education: Central School, Ahmedabad 1980

Collegiate institutions
attendedDatesDegreeDate of DegreeNew Jersey Institute
of Technology.
Newark, N.J.Sept 1986 to
Dec 1988M.S.May 1989L.D. College of
Engineering.
Ahmedabad, India.Sept 1980 to
Dec 1984B.E.Aug 1984

Major: Electrical Engineering

ABSTRACT

Title of Thesis: Computer Controlled Oxygen Bioreactor Dinesh Sachdeva, Master of Science in Electrical Engineering, 1989

Thesis Directed by: Sam S. Sofer, Professor

Sponsored Chair in Biotechnology

A computer controlled oxygen bioreactor has been designed to obtain more accurate values for oxygen bioreactor requirements and to optimize oxygen productivity with respect to oxygen concentration. This study solves a specific problem relating to oxygen concentration measurement and bioreactor control. The proposed reactor system is computer controlled to insure a desired oxygen concentration supply to an oxygen consuming reaction or organism. This thesis also explains and illustrates the bioreactor and its computer control system.

The computer controller incorporates oxygen sensing, oxygen measurement and temperature measurement. Electrical circuits have been designed and built for proper signal conditioning, data acquisition, data storage, real time recording and data printout. All processes, oxygen sensing, temperature control, data acquisition, and signal conditioning are integrated by a microcomputer system which supervises the process control. The computer controlled oxygen bioreactor shows that a computer control system is feasible and is recommended for application in biological processes.

 \bigcirc \langle

TABLE OF CONTENTS

Chapter	P	age
I.	INTRODUCTION	1
II.	MATERIALS AND METHODS	9
III.	INTRODUCTION TO MICROCOMPUTERS	14
IV.	INTERFACING SENSORS: ART/RC	23
ν.	INSTRUMENTATION: SYSTEM DESIGN AND HARDWARE	29
VI.	SOFTWARE DEVELOPMENT AND COMMUNICATION	. 35
VII.	INTELLIGENT CONTROL: PROCESS DESIGN AND CALCULATIONS	42
VIII.	RESULTS AND CONCLUSIONS	46
APPENDIX	A	57
APPENDIX	В	60
APPENDIX	с	63
APPENDIX	D	65
APPENDIX	Ε	69
APPENDIX	F	71
APPENDIX	G	73
BIBLIOGRA	АРНУ	75

LIST OF FIGURES

Figure

Page

1.1	Process Control of Detoxification Bioreactor3
1.2	Clark Oxygen Probe6
1.3	Experimental Setup of Microassay Reactor 8
2.1	Preparation of Catalase Beads11
2.2	Effect of Hydrogen peroxide on Catalase Beads13
3.1	K-9000 Microcomputer System16
3.2	Power Module Rack18
5.1	Block Diagram 8073 with 825532
8.1	Voltage vs.Oxygen Concentration Graph51
8.2	Oxygen Probe Calibration Graph(without shielding).52
8.3	Oxygen Probe Calibration Graph(without shielding).53
8.4	Oxygen Probe Calibration Graph(without shielding).54
8.5	Oxygen Probe Calibration Graph55
8.6	Oxygen Probe Calibration Graph56

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my supervising professor Sam S. Sofer, for his guidance, friendship and moral support throughout this research.

Special thanks are also due to Professor Stanley Reisman and Professor William Carr for being members on my Masters Committee.

Appreciation is also expressed to all members of the Biotech Lab for their timely help and suggestions in conducting this project.

Finally I would like to thank Emilia Rus, Fayaz Lakhwala, Carl Camp and Tim Roche for their help.

Chapter I

INTRODUCTION

This chapter deals with the research objectives, background and the previous work done [1,15,16]. Various methods of measuring oxygen concentration, oxygen sensors and polarographs are discussed in the background.

1.1 RESEARCH OBJECTIVES

The oxygen bioreactor produces oxygen with the help of immobilized catalase beads and hydrogen peroxide. Catalase is an enzyme that catalyzes the following reaction:

With the help of the microcomputer and electronic instrumentation, the bioreactor can be controlled intelligently, to supply the required amount of oxygen. The control aspects include sensing oxygen, signal conditioning and obtaining the amplified signal to read concentration of oxygen. These data are stored in an accessible storage medium such as floppy discs. This makes it easier to review the results and make important decisions for further control of the bioreactor.

1

The main research objectives are:

o Design a computer controller that measures oxygen concentration of a solution and supplies oxygen via a hydrogen peroxide and catalase reaction when required.

 Study the reliability of the system based on long duration runs.

o Make the controller easy to operate.

The block Diagram shown in Figure 1.1 describes the microcomputer system used to monitor oxygen in a detoxification bioreactor where the microorganisms consume oxygen. The figure shows the K-9000 microcomputer controlling the pumps, valves, temperature controller, etc., and also monitoring oxygen. The K-9000 microcomputer is connected to the 9020 (A/D Converter) and 9028 (Triac Controller) by a coaxial cable. If the oxygen concentration falls below a certain value where it endangers the microorganisms, the microcomputer system is programmed to sense this low concentration and react to it by opening a solenoid valve which releases oxygen from the bioreactor, or air, as the case may be, into the microorganism bioreactor. Once the oxygen concentration in the bioreactor reaches saturation level, the microcomputer system senses the upper limit of dissolved oxygen and shuts off the solenoid valve. Details are discussed in Chapter 7.



Figure 1.1 Process Control of Detoxification Bioreactor

1.2 BACKGROUND

1.2.1 Oxygen Measurement

An oxygen sensor is used for the detection of oxygen. It gives an output in the millivolt range, which is very sensitive to outer vibrations of pumps and motors and other factors like presence of magnetic fields. Therefore, the output signal needs to be conditioned and then amplified to a level such that it can be used as input to a recording device which can record the level of oxygen at different intervals of time.

The oxygen measurement system utilizes polarography[19], a widely used method. Polarographic oxygen sensors depend upon the chemical reduction of oxygen at the electrode's reactive surface; this reduction is caused by electrons imposed upon that surface as a result of an external voltage source. The reduction of dissolved oxygen to hydroxyl ion at or near the reactive surface of the sensing electrode produces an electric current[19] between reference electrode and oxygen electrode. Therefore, the polarographic electrode shows amperometrically the concentration of oxygen in a solution.

Because of potential reactions that could arise from the reduction of metal ions in the solution near the reactive surface, one of the noble metals, usually gold or platinum, is used in polarographic oxygen sensors. For oxygen measurements, the negatively polarized sensing electrode is termed the cathode (-) while the reference electrode, positively polarized with respect to the sensor, is termed the anode (+).

The cathode is polarized negatively between -0.3 and -0.8 volts with respect to the silver/silver chloride reference electrode[19]. The resulting polarographic current is directly proportional to the rate of oxygen diffusing to the reactive surface of the sensing electrode.

1.2.2 Oxygen Sensors and Polarographs

The oxygen probe is selected depending upon various factors like application, response and accuracy. The oxygen probe used here has fast response, high accuracy and it overcomes many inherent problems encountered with conventional sensors such as Model 54ABP oxygen sensor, Model 9708 oxygen electrode and Model 51B oxygen sensor.

The Clark sensor (Figure 1.2) can be used in various applications where acid gases are present in large concentrations (such as carbon dioxide). When this oxygen sensor is operating in a gas mixture that varies in total pressure, or in highly mobile background gases, differential pressure will not be produced across the sensing membrane to degrade performance. In other electrochemical sensors where the sensor is sealed, a pressure differential can develop across the membrane, resulting in a change in the thickness of the



Figure 1.2 Clark Oxygen Probe Source: Yellow Spring Instrument Co. (Ohio) Catalog. electrolyte film maintained between the cathode and the membrane. This in turn, will cause a change in the output of the sensor by varying the diffusion path of the cathode.

The sensing of oxygen relies upon its diffusion through a membrane and since diffusion is a temperature dependent process, it is necessary to maintain the environment around the probe at constant temperature [20]. There are different kinds of polarographs and chart recorders which can be used to record oxygen signals from the sensor. The signal is conditioned and amplified before being input to the chart recorder, and if required, an offset voltage is also used, depending upon the application. The offset voltage is used to set the scale of measurement on the chart recorder. The polarograph used has three different modules: an amplifier unit, an offset module and the recording unit. Figure 1.3 shows the conventional method of oxygen measurement using a polarograph. A chart recorder as shown in the figure is the line recording medium which receives a signal from the amplifier. The signal obtained from the oxygen probe is amplified and recorded on the chart recorder. The temperature of the reactor is maintained at 37°C through a water bath. The magnetic stirrer stirs the beads and hydrogen peroxide continuously.



Figure 1.3 Experimental Setup of Microassay Reactor

Chapter II

MATERIALS AND METHODS

This chapter describes immobilization, its advantages and the experimental method of immobilizing. This chapter also includes preliminary experiments performed to see the effect of nitrogen and catalase beads on oxygen concentration.

2.1 IMMOBILIZATION AND ITS ADVANTAGES

Immobilization means localizing of biomass or enzymes by attachment on solid supports or entrapment within a semipermeable matrix.

Application of immobilized catalase for oxygen production has been a subject of intensive study in recent years. The technique of immobilization has a lot of advantages over the conventional methods[2]. Washout of catalase is one of the most common problems encountered in biological production of oxygen from chemicals. Furthermore, long residence times are required, so that the catalase can evolve greater amounts of oxygen and use the hydrogen peroxide completely. Many of these conditions can be significantly improved using immobilized catalase. The system facilitates easy separation and has a greater degree of operational flexibility as continuous processes become practical. Immobilized catalase can be much more

9

resistant to high concentrations of hydrogen peroxide and toxic chemicals[4]. In addition, the catalase density of immobilized catalase can be much higher than that of the free catalase, resulting in higher rates of production per unit volume of the reactor. Moreover immobilized catalase can also be dried and stored as a convenient source of reproducible catalase. Numerous methods have been developed for immobilized biocatalyst preparation [4]. There is no universal carrier nor immobilization method for all living cells or catalase, and each application should be separately tested and optimized. The support material should be able to withstand substrate, product and reaction conditions, and it should be suitable for continuous or repeated use in the scale desired.

Moreover, the method (Section 2.2) should be sufficiently gentle for the living cells and catalase. Suitable polymers for entrapment of cells include alginate, K-carageenan, polyacrylamide and polyvinyl alcohol.

Figure 2.1 describes the method of immobilization in the form of a flow diagram. Catalase is mixed with alginate gel and stirred until a homogeneous suspension is formed. This homogeneous suspension is extruded into a calcium chloride solution forming immobilized beads. These beads can be dried gently.

METHOD OF IMMOBILIZATION

Catalase + Alginate Gel MIX (Homogenous Suspension)



Gentle drying

DRY BEADS

Figure 2.1 Preparation of Catalase Beads

2.2 EXPERIMENTAL METHODS

Different experiments such as preparation of catalase beads, effect of nitrogen on solubility of oxygen, effect of hydrogen peroxide on oxygen concentration (Figure 2.2) are described in this section and in Appendix-A.1.

Different steps involved in preparation of catalase beads are discussed here.

The first step in preparation of catalase beads is the preparation of calcium alginate. Calcium alginate gel is made by reacting sodium alginate gel with calcium chloride. The method of preparing calcium alginate gel and calcium chloride is described in Appendix. Alginic acid is a naturally occurring polysaccharide[18]. Calcium alginate has been used in the past as an immobilizing matrix for both enzymes and microorganisms [17,18].



Figure 2.2: Effect of Hydrogen peroxide on Catalase beads

Chapter III

INTRODUCTION TO MICROCOMPUTERS

This chapter describes microcomputers and the system K-9000 used for control of the bioreactor experiments. This chapter also includes a discussion of the master-slave concept used for a distributed control system.

3.1 GENERAL INTRODUCTION

The invention of the microprocessor is revolutionizing process control. During the initial development of the microprocessor in the early 1970s, it was used primarily in electronic systems. Instruments such as digital voltmeters and high quality oscilloscopes began to include data manipulation and storage capabilities, along with the sophisticated automatic control functions that were previously unavailable. As the quality and performance of microprocessors improved, they acquired other applications. One of them was computers. At the present time, we have computers based on the microprocessors that are important to several different fields. A microcomputer is a low cost computer system that utilizes a single IC chip containing the entire CPU and perhaps some memory. Generally, a microcomputer is much less expensive than a comparable minicomputer. The word size of a microcomputer ranges from 4 to 32 bits.

14

The microprocessors were originally used more in control and instrumentation applications than in computer applications[6]. As microcomputer capability increased, computer applications became more common. When used as a basis for a computer, the microprocessor generally must provide a large external memory space. [5]

3.2 K-9000 MICROCOMPUTER

The K-9000 system can be referred to as an Input-Decision-Output cycle(Figure 3.1). Input is given to the K-9000 system by the temperature sensors or the oxygen sensors etc. and the output is given by the triac controller. The decisions are made by the cpu depending on the inputs.

The decisions handled by the 8073 'TinyBasic' microcomputer are communicated via the ART/RC device to two types of remotely located slave units. Communications with the slave A/D converter module entails reading data for twenty separate sources. Up to 128 Triac Controller modules can be addressed on a single coax by one K-9000.

The K-9000 has built-in bus allocation logic for both multiprocessing and DMA applications[5]. All the necessary control logic and timing signal generation is provided on-chip. In a multiprocessing configuration, daisy chaining automatically assigns the priorities of each processor, thus



eliminating the need for external priority generation logic. This feature is very useful in control applications. The K-9000 consists of a PMR-6 Power Module Rack which is daisy chained configured. It includes two RS-232 ports. The K-9000 utilizes the NSC 8073 chip as the cpu. This device features XMOS technology and can operate at high speeds. When operating the cpu at high speeds (16 MHz), there are certain restrictions, for example, the cpu at high clock speeds operates at higher temperature and limits operating temperature at 0 to 50° C. Also, at higher operating speeds all peripheral devices must have adequate access speed to accommodate the fast addressing and data transfer rate. Due to various propagation delays and parasitic capacitance, extreme care must be taken while connecting peripheral devices and the processor.

3.2.1 PMR-6 Power Module Rack

The PMR-6 Power Module Rack houses the MB-9006, a six slotted mother board (Figure 3.2) J-1 through J-6. It has two RS-232 ports, a 32 pin I/O connector, reset circuitry for multiprocessing and a 3 amp 5 volt switching power supply. When using one processor (priority 1) the K-9000 card is put in slot J-1; for the RS-232 interface, connector J-8 is used. When working on multiprocessing, the second processor or priority 2 must use the J-9 connector for its RS-232 communications.



Figure 3.2 Power Module Rack Source: Transwave (Vanderbilt) Manual

3.2.2 M-9048 Memory Card

The M-9048 Memory card is capable of being used with RAM devices of 6264 (8k) type or EPROM either the 2764 (8k) or the 27128 (16k) EPROM a memory[8]. The M-9048 card has a hardwired socket so that EPROMs can be programmed. A Zero Insertion Force socket on the M-9048 is where the EPROM is programmed, using the COPY command in the Utility Firmware. To program the EPROM, an external supply voltage of 21 Volts must be supplied to the M-9048 via the J-2 connector. M-9048 provides battery backup to the Real Time clock and the RAM memory.

3.2.3 C-9004 Communication Card

The communication card supports a Real Time clock, 24 line parallel I/O and ART/RC. In the K-9000 series the ART/RC is software operated in a master mode which gives the C-9004 the ability to serially communicate over long distances with up to 128 remote slave stations via a simple co-axial cable. The MM58174 is an accurate crystal controlled CMOS device that functions as a real time clock and calendar. The RTC can be software programmed to periodically interrupt the processor at specified time intervals.

3.2.4 D-9020 A/D Card

The 9020 functions as a 16 channel 8 bit A/D converter. It operates in a multiplex mode. By the use of the 8-bit latch

any analog channel may be selected to read directly to the ADC804 A/D[10] residing on the bus. The analog switches are sequenced by successive approximation logic to match the analog difference input voltage Vin (+) - Vin (-) to a corresponding tap on the R network. After eight comparisons a digital 8-bit binary code (1111 1111 = full scale) is transferred to an input latch and then an interrupt is asserted.

3.2.5 D-9028L Triac Controller Card

The D-9028 forms a complete local control system. The D-9028 provides a direct method of controlling relays, contactors, valves, etc. The Triac Drivers control the ON/OFF operation of the relays. The D-9028 circuit has 3-position toggle switches for manual over-ride of the load. The 3-position switches give manual, off and automatic control of each load in the circuit. Eight load LED's provide visual monitoring of circuit conditions.(see Appendix-A.3). The D-9028 is designed for low voltage AC control with loads normally around 20 watts per channel. The current limiting resistors for the load LED indicators are 1.0 K ohm and designed for 24 VAC operation. If voltages higher than 24 VAC are used then these resistors must be increased in value to prevent damage to the LED load indicators. The program listed in Appendix-C.2 is a test program. It will first shut off all the channels and then turn on each channel one at a time until all have been turned on. Before it turns the next channel on it will delay the maximum amount of time and then continue.

3.3 MASTER SLAVE CONCEPT

The essential requirement of a distributed computer control system is a two way transfer of data between master and slave microprocessor units (MPU)[12]. Data may be in the form of blocks of data which are called by the slave and transferred to the master for processing; or it may be a set data point which is passed by the master to the slave so that the slave can hold a process parameter (temperature, pressure, thickness etc.) at a referred value. Hence, the slave MPU must be connected to the data and control bus of the master MPU.

The interconnection of a master computer with various slave MPUs must be done with care to ensure valid data transfer and to avoid a situation where a slave MPU loads the data bus of the master MPU. For this reason an interface circuit is required between the master and each slave MPU. The PPI in its mode2 operation provides the required interface. The necessary requirements of this interface circuit can be summarized as: o A two way or bidirectional data flow must be allowed. o Because the transfer of data to and from the master may occur at any time, handshaking signals are required to ensure an orderly data flow between master and slave. o Since the data buses of the master and slave MPU must be interfaced, tri-state buffers are required between the master MPU and the PPI (to ensure that the slave MPU does not load the master MPU data bus) and between the PPI and the slave MPU (to ensure that the data lines of the master do not load the slave MPU data bus). The first three-state buffer on the slave side of the PPI, is enabled by the PPI chip select line. The second three state buffer on the slave side of the PPI is enabled by the PPI chip select line. The second three state buffer on the slave side of the PPI is enabled by a handshaking signal from the slave MPU when it is ready to accept data from the master.

o Data that are to be transferred from master to slave MPU must be latched by the interface circuit and held until the slave MPU is ready to accept the data.

The PPI has been configured with port A used for data output (and therefore to transmit data to the slave) and port B used for data input (and therefore to receive data from the slave MPU).

Chapter IV

INTERFACING SENSORS: ART/RC

This chapter describes the characteristics of oxygen and temperature sensors. Interfacing sensors with the computer and communication through the ART/RC is also discussed in this chapter.

4.1 OXYGEN PROBE

The probe is a complete system in itself, which is relatively unaffected by, and does not affect, its external environment. For this reason, the electrode may also be used to measure oxygen in non-conducting liquids or gases. Further, the probe is bathed in a known medium and protected from contamination by the membrane. Thus, the probe will measure oxygen in solutions contaminated by ionic reducing agents and reducing (in consuming) organic matter. The probe is subject to interference only from low molecular weight reducing gases such as the halogens and halogen sulfides.

4.1.1 Sensor Characteristics

The different sensor probe characteristics such as voltage plateau, oxygen depletion, membrane coefficient are discussed here.

1. Voltage Plateau: The current oxygen pressure is essentially independent of polarizing voltage within a certain voltage range. Specifically the output signal shall change less than 3 when the polarizing voltage is lowered from 0.8 to 0.65 volts.

2. Oxygen Depletion: Reading error in liquids is due to oxygen depletion in the vicinity of the membrane, which will occur unless adequate stirring is provided. Oxygen usage is 8*11⁻¹¹ grams oxygen sec per sensor current of 1 microampere.

3. Membrane coefficient: The approximate 4% per degree C membrane coefficient necessitates good temperature control. Temperature equilibrium time must be considered when making changes in the setup.

4.2 AMPLIFICATION

The signal obtained from the sensor is amplified.

The signal conditioning unit requires 0.8 volts DC. This voltage can be obtained from a battery. If a line operated supply is used, the DC must be well filtered and regulated. The sensor load resistor restricts the voltage across the load resistor to less than 0.10 volts (10 to 25 mV preferred). The YSI 4004 Sensor will develop about 2.0 microamp in air at 30°C and (10.0microamp in oxygen at 30°C.

Amplifier input requirement: The input impedance of the amplifier should be large compared to the sensor load resistor; so as not to shunt the sensor load resistor. But in a situation where the amplifier input impedance is purely resistive and has a value of 100,000 ohms, the sensor load may be increased so that the resultant paralleled resistors are of desired value. The 10 turn potentiometer (Bourns) gives better resolution. A switch is installed to permit easy change from air to oxygen. There is a reference zero switch which has three positions: float position, ground position and zero switch position. In float position the reference electrode is grounded to internal circuit of the microsensor, in the zero switch position the current is prevented from flowing through the electrode; and in ground position the reference electrode is grounded to earth ground (Appendix-D.4).

4.3 SENSOR INPUT AND SIGNAL CONDITIONING

4.3.1 Temperature Sensor

The ADC804 has the ability to be adjusted over various input ranges. A typical case is the ST-273 which has a Nationals LM-335 temperature sensor I.C (Appendix B.1). Like all the new integrated temperature sensors, the output voltage per degree is in the millivolt range. Therefore, a 100 degree change in temperature provides an overall voltage change of one volt which is often offset above ground. If the A/D converter operated input voltage is normally of 0-5 volts, the 8-bit word resolution would be undesirable. However, if the 1.0 volt range could be spanned to represent the full 8-bit word of 255 then the resolution would be acceptable. See Appendix-B.1 for circuit diagram.

Transwave's ST-273 temperature probe is referenced to absolute zero degrees kelvin. Its linear output is 10 mV/degree Celsius. Therefore, at 0°C the output voltage of the sensor is 2.73 volts and at +100°C the voltage output is 3.73 volts. See Appendix-D.4 for circuit diagram.

4.3.2 Oxygen Sensor

For oxygen sensor YSI 4004 Clark, the voltage span can be adjusted over the entire 0 - 5 Volts range. Some adjustments are necessary in this application. It is necessary to add a resistance value of approximately 1 megaohm in the position provided as R-13 and wire jumper W5 between B and C. This will allow a finer adjustment of the regulator voltage output in the lower range. (Circuit Diagram Appendix-B.2).

4.3.3 Light sensor

The Light energy sensor as shown in Appendix-B.3 is an interactive non-amplified passive device that allows the necessary offset voltage adjustment for direct matching to the 8020 and 9020 A/D units. The circuit also provides a linear output voltage span over the desired 8-bit word.
4.4 ASYNCHRONOUS RECEIVER TRANSMITTER / REMOTE CONTROLLER

The ART/RC is National MM54240 Asynchronous Receiver Transmitter/Remote Controller [10], an easy-to-use device for duplex serial data transmission applications to and from remotely located positions. The Transwave's K-8073 Microcomputer is used as the information handling center, and up to 128 remote information control stations are all connected over a single wire. ART/RC can be used in many applications, such as gathering data from temperature sensors, humidity, light energy devices, etc. Another application is to provide subsequent control for operating valves, motors, fans, etc. To satisfy both of these requirements for peripheral use, 16 A to D and 4 on/off input channels are in the 8020 Card, an 8channel TRIAC controller.

The simplest route between the ART/RC and A/D at a distance is by means of a twisted pair or coaxial cable. The single line I/O circuit of all ART/RC devices is an open drain, driver output. Because this line is floating, the ART/RC on the 8073 Card has the I/O communication line pulled up to via a 1 K ohm resistor. This pull up provides excellent data transmission over a distance of a few thousand feet using standard coaxial cable such as RG-58. Care should be taken to reduce capacitance and resistance and maintain good ground continuity between the master and the slave units. The 8020 and 8028 can be operated from different power supplies, provided there is a good ground. This ground can be provided by the coaxial cable.

By the use of pulse width modulation techniques, frequency tolerance between the master and slave circuits is broadened. The frequency between each ART/RC can vary as much as 50% before performance would be affected.

Chapter V

INSTRUMENTATION: SYSTEM DESIGN AND HARDWARE

This chapter describes the hardware design aspects of the K-9000 system. System Design is described in Appendix-F.1. A brief introduction of how to interface the microcomputer with an 8255 PPI is given in section 5.3.

5.1 ANALOG AND DIGITAL CIRCUIT DESIGN

Sample and hold: Before digitizing an analog signal (for collecting data or for any other purpose), it is strobed at precise time intervals and then sequentially digitized by the A to D converter. A sample and hold (S/H) circuit holds or freezes a changing analog voltage [11]. Usually the voltage thus frozen is converted into another form, either by a voltage controlled oscillator, A/D, or, as in this case, it is done by 54240 remote controller. Normally one sample and hold is used for each A/D convertor with any multiplexing between input channels done. However, for a large number of channels this leads to errors due to the different conversion times of the various channels. Static and dynamic errors exist while using a sample and hold circuit. The static errors are offset error, hold error and gain error [10]. The dynamic errors involve change in gain during the sample mode as a function of frequency, hold step as a function of Vout, dVout/dt or fre quency (aperture - shift error). Considering an ideal sample and hold circuit, the amplifiers are assumed to be ideal with infinite input impedances and bandwidths, zero output impedances and unity gains. The electronic switch is also considered ideal with infinite speed, zero impedance in the sample position and infinite impedance in the hold position. Also, the sampling capacitor C is assumed to have no leakage or dielectric absorption. The aperture time is defined as if the analog input signal to an ADC alters its value during the conversion process.

5.2 MICROCOMPUTER INTERFACING WITH THE 8255 PPI

The PPI is a 40 pin large scale integrated (LSI) chip that is used as an interface between the microcomputer data bus and external input/output devices [3].

o PPI is designed for parallel data transfer.

o USART is designed for serial data transfer.

The PPI is connected to the microcomputer via the databus and it connects the microcomputer to the outside world via 24 I/O lines. These are generally divided into three 8 bit bytes, which are labelled PAO-PA7, PBO-PB7, and PCO-PC7.

There are two major advantages in using the PPI in a

microcomputer system:

1. The PPI concentrates parallel I/O operations into one integrated circuit, unless more than 24 I/O lines are required. Since all I/O logic is on one IC, both the interfacing complexity and chip count are reduced, with a resulting reduction in cost.

2. The PPI brings tremendous flexibility to microcomputer I/O interfacing. The flexibility is obtained by making the PPI software configurable-hence its name, PPI. The configuration of its 24 I/O lines for input, output, or perhaps bidirectional I/O, is then under software rather than hardware control. This makes the allocation of I/O lines and any subsequent alterations much easier.

The block diagram (Figure 5.1) of the PPI can be divided into three main units, i.e., the interface circuitry to the 8073 cpu, a peripheral interface unit, and an internal control logic unit shown in Figure 5.1.

When programming the operating mode for the PPI, you are not restricted to committing all of the PPI I/O lines to one particular operating role. The mode control word, which is used to define the operating mode for each of the PPI ports, is set up so each port can be assigned a different operating mode. If, for example, port A is programmed for Mode 2 operation, the remaining eight lines of port B and three of port C can be configured for either Mode 0 or Mode 1 operation.





Internal Control Logic The operating modes of ports A through C as well as the bit-set/reset control byte are under software control. The destination of this control word is the control register (within the read write control logic block) whose code is AO=1, A1=1. The internal logic of the chip then manages the transfer of data and control information of the internal data bus. The mode control byte is transferred to two group port controllers, which are designated GROUP A and GROUP B control.

The GROUP A control module controls the mode definition of (and data transfer to and from) port A and the most significant four bits of port C. Similarly, the GROUP B control module supervises port C.

5.3 INTERFACE BETWEEN MASTER AND SLAVE MICROCOMPUTERS

This section discusses the interface between master and slave microcomputer. This was tried using the 8255 PPI. In distributed computer control system a number of small computers-increasingly microcomputers-are dedicated to control small portions of the overall process.

In a chemical process, for example, a microcomputer can be used to control small portions of the process such as the pressure or temperature in a distillation column. With a microcomputer used in this way, a larger computer with increased speed, memory and disc storage is then used to supervise the operation of the overall process by monitoring and altering, by command as necessary, the operation of the dedicated microcomputer controllers. In the example given, the supervising computer was at a higher level than the dedicated microcomputers. Because of this, the supervising computer is often referred to as a master while the lower level, dedicated microcomputers are called slaves.

Chapter VI

SOFTWARE DEVELOPMENT AND COMMUNICATION

This chapter describes the role of the ART/RC in software development. It also discusses how the ART/RC can be programmed to read and write various channels. The actual programs developed are discussed in chapter 7, but the basis and fundamentals of the software are discussed in this chapter. The chapter includes a brief overview of communication software used.

6.1 SOFTWARE MONITORING

The ART/RC has been discussed in Chapter 4. The ART/RC helps in remote measuring and control; therefore it plays an important part in software monitoring. The system K-9000 and K-8073 use the ART/RC. Software monitoring of the operational status (STATUS) of the ART/RC permits broader, more guaranteed control of the ART/RC network. The 'RDART' routine contained in the firmware contains a utility which automatically obtains the operating status of the ART/RC and stores the code under the variable S. The status may be read by printing the value of 'S', or used within a program with an IF statement

Reset the ART/RC's Status Code: The small subroutine given in Appendix 3.7 consisting of a LINK statement to address #9DAB.

35

6.1.1 Programming the ART/RC to Write

The ART/RC is relatively slow. It requires approximately 4 msec to address a slave and transmit the data. To read the slave requires twice this time plus an additional 1.2 msec for the slave to get its act together. Thus the total time required to address a particular slave and acquire its data is approximately 9 msec. Since clock frequencies may vary somewhat it would be best to allow 5 msec to read data from a slave and 10 msec for reading slave data. On the C-9004 Communication card, the ART/RC lives at address #E900 to #E97F (One memory address for each of the 128 slave stations). The slave stations are address selectable with an onboard DIP switch. The program given in Appendix-C.1 is an example of an output routine that transmits sequentially to all eight channels of a remote ART/RC configured in the 'write only' mode. Channel ON/OFF Routine :

The CHON/CHOFF utilities makes the task of writing to a TRIAC or RELAY controller much simpler. The eight channels of a Triac or Relay controller card correspond to the eight bits of a data byte in one's complement form. CHON/CHOFF takes the burden of calculating the value to write to a Triac or Relay Control card off to the programmer. Both CHON/CHOFF expect to find the controller address in the variable A and channel in C. Neither A nor C is destroyed, but since the specified address is read before any writing is done, the variable D will be returned before any channel is toggled. The CHON/CHOFF routine is given in Appendix-C.2.

6.1.2 Programming the ART/RC to read

Reading data from a remote ART/RC requires a different technique. This results from the manner in which the ART/RC interfaces to the 8073 mpu - using Tiny Basic. To do a read requires a 'LINK' to a special machine language program that properly handles the entire program and returns the slave data already assigned to a variable. This machine language routine RDART uses three variables namely A,D, and S.

Part of the same Appendix-C.2 shows a program used to read D-9020 A/D. There are 16 analog channels on the card which consists of 1 bank of 16 channels with one ART/RC controlling this bank. A second ART/RC is used for channel selection through a multiplexer which selects one sensor input for the A/D conversion and passes that data on to the respective ART/RC. The remaining four channels on the ART/RC provide digital(ON/OFF) input.

6.2 COMMUNICATION

The K-9000 Series of microcomputer has access to the Real Time Clock on board the C-9004 Communications card that is extremely useful when operating with process control devices. The C-9004 Communications Card utilizes National Semiconductor's MM58174 [10] microprocessor bus oriented clock that provides tenths of seconds through months. The clock is crystal controlled and includes provision for a very low drain battery backup. The real time clock is automatically switched to the 160Mah Lithium Battery. This battery controlled circuit prevents glitches, thus assuring data is held in the clock counters. The real time clock requires three tasks to be useful in a program:

o Setting the clock time and date.

o Reading the clock.

o Extracting data from the clock string.

A program for setting the clock is given in the Appendix-C.3 Reading the clock requires a speed faster than that possible from Tiny Basic. The clock read routine is written in machine language. It is in the Firmware [5] and it requires a LINK, command from Tiny Basic program to the GETIME machine language routine. The Program is given in the Appendix-C.3.

6.3 INTRODUCTION TO COMMUNICATION SOFTWARE

There is an ever increasing need to move information from one computer to another. Information can be exchanged using magnetic media - tapes or disks - or over a network, a network is expensive. Microcomputer users have no access to tapes. Tapes and disks must to be physically moved - the effort and delay could be significant if the systems are widely separated. The telecommunication line provides an alternative to networks and magnetic media. Asynchronous telecommunication is the method used by most terminals to connect to most terminals. The computer comes equipped with a serial port, connector configuration (DB-25 or DB-9), transmission signals EIA RS-232, with a communication transmission speeds (baud rate) and a convention for encoding character in storage and during transmission (AS-CII). These standards provide the physical medium and the data format, but they do not specify a process for exchanging data [14].

6.3.1 Kermit

Kermit is a file transfer protocol. It is specifically designed for transfer of sequential files over ordinary serial telecommunication lines. Kermit transfers data by encapsulating it in packets of control information. It includes a synchronization marker, a packet sequence number to allow detection of lost packets, a length indicator, and a 'block check' to allow verification of the data.

Commands used are briefly described here:

The MARK (usually an ASCII Control-A character) appears at the beginning of the packet. The next character is a length

39

field (LEN), specifying how long the rest of the packet is. The sequence number (SEQ) is used to detect lost or duplicated packets; retransmission is requested for lost packets and duplicate packets are discarded. The TYPE field specifies whether the packet contains data or control information.

The (check) field contains a quantity obtained by combining all the other characters in the packet together in one of the several ways; the sender computes this value and sends it. The packet receiver also computes the value and checks it against the value sent; if they agree, the packet is accepted; if they disagree, the packet has been corrupted and retransmission is requested.

The (Data) field contains up to 90 characters of data. All fields except the mark are encoded as printable ASCII characters, to prevent host or network interference.

The main kermit commands used are: Send, Receive, Get: for exchanging files. Connect, set line, set parity, set duplex, set speed etc. : for connecting to remote host. Set block-check, Set debug, Set delay, Set file, Set parity: setting nonstandard transmission and file parameters. Define: for defining macros.

Control-X, Control-Z,

Control-C, Control-C: for interrupting transmission.

Close: for closing log files.

Exit, Quit: leaving the program.

Chapter VII

INTELLIGENT CONTROL: PROCESS DESIGN AND CALCULATIONS

7.1 INTELLIGENT CONTROL

The oxygen bioreactor produces oxygen with the help of catalase beads and hydrogen peroxide. But as discussed in the previous chapters, with the help of the microcomputer and instrumentation, the bioreactor can be controlled intelligently, to supply the required amount of oxygen. The control aspects include sensing oxygen concentration, signal conditioning and obtaining the amplified signal to read concentration of oxygen. Further using the data acquisition system, data are stored in a accessible storage medium. This makes it easier to review the results and make important decisions for further control of the bioreactor.

In a specific example, the microcomputer system is used to monitor in a detoxification bioreactor where the bacteria consume oxygen. With increase in biodegradation, the concentration of dissolved oxygen in the bioreactor decreases.

If the oxygen falls below a certain value where it endangers the microorganisms, the microcomputer system is programmed to sense this low concentration and react to it by opening a solenoid value which releases oxygen from the bioreactor, or air, as the case may be, into the microorganism bioreactor. Once the oxygen concentration in the bioreactor reaches saturation level, the microcomputer system senses the upper limit of dissolved oxygen and shuts off the solenoid valve.

The micro-computer system is also programmed (Appendix-D.1) to periodically monitor and calculate the rate of consumption of dissolved oxygen. This provides an effective method to follow the reaction which takes place in the bioreactor and thereby control it. In an extreme case, if the above control system fails to energize the solenoid valve, the microorganisms in the reactor will die due to lack of oxygen. In such a case, there is a subroutine in the program which de-energizes an emergency valve (normally open) which exposes the microorganism bioreactor to the atmosphere. In case there is a power failure, or the microcomputer system shuts down, the emergency valve gets de-energized and saves microorganisms from dying.

Bioethanol Re-circulation Bioreactor

Biomass utilization in many countries offers a significant opportunity for efficient energy conversion. Ethanol can be produced from starchy raw materials like corn, potato, wheat grains, etc. Ethanol production from grains requires pretreatment of grains to convert the starch to fermentable sugar (glucose). In this experiment, cornmeal is used as a source of glucose. Corn contains 78% starch. In a hydrolysis reaction, approximately 90% of the starch can be converted to glucose using the enzymes Takatherm and Diazyme at $60 - 90^{\circ}$ C temperature. The glucose water obtained from the hydrolysis of corn starch can be used for fermentation.

Fermentation of glucose takes place by immobilized yeast in a recirculatory bioreactor. This reactor consists of a reservoir with capacity of 3 liters, that is usually filled with 1.8 liter sugar water which is recirculated by a peristaltic pump through an immobilized yeast bioreactor. Immobilized yeast catalyzes the conversion of glucose to ethanol in a steady state fashion. Ethanol water can be distilled for separation of alcohol from water. Yeast cells grow by consuming glucose as a nutrient. Due to cell growth, they get bigger in size. This cell growth can increase the density of beads and decrease the flow rate of glucose water through the bioreactor. The continuous pumping can reduce the flowrate due to clustering of beads in the reactor, thus causing clogging at the top of the reactor. This prevents regular flow rate of glucose water and affects the production rate.

By switching the main pump ON and OFF, the process is made unsteady state. The computer is used to vary the switching time and finds out the switching time that gives maximum activity. The program is given in Appendix-D.2. It is observed that the rate of carbon dioxide increases in the unsteady state process.

7.2 CALCULATIONS

Hydrogen peroxide in the presence of catalase gives pure oxygen and water. Calcium alginate immobilized catalase was used to convert hydrogen peroxide to pure oxygen and water. The oxygen released dissolves in water, which is used in biological reactions. As the concentration of dissolved oxygen in water decreases, more hydrogen peroxide needs to be added. The calculations (Appendix-G.1) show determination of required moles of hydrogen peroxide.

Chapter VIII

RESULTS AND CONCLUSIONS

8.1 Catalase Bioreactor and Production of Oxygen

Oxygen is produced using hydrogen peroxide and catalase. The catalase is in the form of immobilized beads so that oxygen can be produced for a longer duration. Initially oxygen was measured using a Clark type oxygen probe and recorded using a chart recorder. The graphs in Chapter 2 have been drawn using a chart recorder. To find out rate of oxyen consumption from graphs recorded on a chart recorder is time consuming and moreover it is difficult to find out the instantaneous rate. On the other hand after conversion from A/D using a computer the rate of oxygen consumption and various other factors can be found and the results can be easily stored and reproduced at any time. The system is capable of monitoring on line data and can also control the oxygen concentration in the bioreactor automatically using the software.

8.2 Calibration of Oxygen Probe

The experimental set up for the recirculation reactor adopted for this calibration is shown in Figure 1.1. It consists of a reservoir which is saturated with oxygen by bubbling air. The pump is used to circulate the medium from

46

the reservoir through the reactor and back to the reservoir. The oxygen probe on line reads concentration of dissolved oxygen.

a) Using a Pump: As seen in Figures 8.2, 8.3 and 8.5 line AB is the region when the medium in the reservoir is saturated with oxygen. At point 'B' the pump is turned OFF and it is observed that the probe indicates a drop in concentration of dissolved oxygen (BC). This is because no liquid is flowing across the probe. At point 'C' the pump is again turned ON and as seen from line CD, the probe reads increasing concentration of dissolved oxygen and levels along line AB, which is the starting concentration. Figure 8.3 shows the results obtained on a similar run and it clearly shows the regions AB and BC which have already been explained.

b) Using Nitrogen: Using the same experimental setup as in Figure 1.1, the probe is now calibrated to read changes in oxygen concentration while the pump is still ON. The result obtained is shown in Figure 8.6. Line AB shows that the system is saturated with oxygen by bubbling air. At point 'B' air supply is cut off and nitrogen is bubbled in the reservoir. This time the probe reads actual decrease in concentration of dissolved oxygen as indicated by the line BC. The concentration reaches a minimum level, where it remains constant. At point 'C' nitrogen supply is cut off and air is introduced in the reservoir, and the concentration of oxygen starts increasing. This is shown by line CD. Line DE shows that system has reached a saturation level which is the same as the initial value.

8.3 A/D Conversion of Oxygen Concentration Data

Oxygen concentration data collected over the duration of an experimental run used a Clark type oxygen sensor to measure the oxygen concentration. The Clark type oxygen sensor delivers an analog signal of 0.1 to 1.0 millivolts. This range of signals is overly sensitive and also under powered. To fix the sensitivity and signal range problem the output is stabilized and then amplified to a range of 0-5 volts. This is an analog voltage, but the computer needs a digital voltage; necessitating an Analog to Digital [A/D] converter. Conversion was routed through the A/D card of a K-9000 system. The card accepts an analog voltage of 0-2.73 volts, but was modified to accept 0-5 volts. The digitized voltage signal ranged from 0 to 1111 1111 binary (0 to 255 decimal); 0 corresponds to 0 volts and 1111 1111 binary corresponds to 5 volts. The relationship between analog voltage and digitized voltage is linear. In addition, oxygen concentration co-relation is straight forward; oxygen concentration (nmoles/ml) is related to the digitized signal by a straight line: 0 percent maximum oxygen solubility is equivalent to 0 voltage from the A/D card, likewise 100 percent maximum oxygen solubility is equivalent to 1111 1111 binary from the A/D card.

Figures 8.1 to 8.6 show the graph of the oxygen probe calibration with the same experimental setup as figure 1.1. A twisted pair cable was used initially for communication between different modules and the resulting graphs are shown in Figures 8.2 and 8.3. A thick noisy band was obtained which does not give exact oxygen concentration. This band of oxygen concentration was made smooth to some extent by using a coaxial cable (Figure 8.4, 8.5). Finally, using different software techniques and averaging 50 readings the noise was essentially eliminated. The averaging was done by collecting 50 consecutive readings over a period of time and representing them as a single reading by taking their time average (Figure 8.6).

8.4 CONCLUSIONS

Oxygen concentration was read in terms of analog voltage and then converted into binary digits. This facilitates data acquisition, real time recording, data storage and data printout. Using signal conditioning a stable output signal was obtained from which instantaneous rates of oxygen consumption could be made available. The features and advantages discussed in the above sections makes a microcomputer system feasible and highly recommendable for use in biological processes.



Figure 8.1 Voltage vs Oxygen Concentration Grapa



Figure 8.2 Oxygen Probe Calibration Graph (without shielding)



Figure 8.3 Oxygen Probe Calibration Graph (without shielding)



Figure 8.4 Oxygen Probe Calibration Graph (without shielding)



Figure 8.5 Oxygen Probe Calibration Graph



Figure 8.6 Oxygen Probe Calibration Graph (averaging 50 readings)

APPENDIX-A.1

Preparation of calcium alginate gel:

Two grams of alginic acid (Sigma Co. practical Grade IV) is slowly added into 100 ml of NaCl solution (1.87 grams sodium chloride per 100ml pH 7.4 buffer). The mixture is heated while it is constantly stirred until all alginic acid is dissolved into a gel mixture. The use of a blender is also suggested for obtaining gel concentrations more than 2%. The alginate gel should then be sterilized in an autoclave for 30 min at 121°C and 15 psi.

Preparation of .1M calcium chloride solution

From chemistry, the reaction between sodium alginate and calcium chloride gives calcium alginate gel. Hence a solution of calcium chloride is made to have calcium ions in the solution for the reaction. After calculations, 147 grams of anhydrous calcium chloride is dissolved in 1000 ml to make 1M calcium chloride solution. We need to add 14.7 grams in 1000 ml to make 0.1 molar calcium chloride solution.

Preparation of Catalase beads

0.034 grams of catalase is added to 80 grams alginate gel under continuous stirring to form a homogeneous paste. With the help of a syringe pump, the catalase gel is then extruded as discrete droplets in a slowly stirred solution of 0.1 calcium chloride. The droplets harden to form beads. The catalase beads are cured in calcium chloride solution for about 24 hours.

Effect of Nitrogen on Oxygen Concentration in buffer solution:

Nitrogen is added to buffer solution to decrease the amount of oxygen concentration, it produces the same effect as the oxygen is being consumed.

The oxygen probe is set up in the bioreactor. The probe is filled with probe solution (potassium chloride). Using a membrane as shown in Figure 1.2, cover the probe end and fix the membrane using an 'O' ring. Put the probe in cleaned reactor. Take a calculated amount of buffer solution (pH 7.4) and put it in the reactor (amount = 1.8 ml) Keep the stirrer on and temperature maintained at 37°C. Bubble air through the buffer solution under constant stirring, and when the polarograph shows the oxygen saturation, change the air bubbling by nitrogen. The content of oxygen shown by the polarograph starts reducing drastically, but after some time the oxygen content and the reduction rate become less. Then it remains at a minimum constant and the curve again starts becoming straight. This amount of oxygen left in the buffer solution is shown by the graph in Figure which is 30% or even less. This experiment proves the reduction in oxygen content

and also helps in showing the effect of introduction of nitrogen to the fully saturated oxygen. Further it is used in calculating the amount of Oxygen consumption and how much oxygen should be added (chapter 7).

Effect of Hydrogen peroxide on catalase beads:

In the previous method, when nitrogen is added (Figure 2.2 B to C) and the amount of oxygen in the buffer solution reaches a minimum constant value and stays there for some time(Figure 2.2 C to D), hydrogen peroxide is added(D to E), the chart-recorder shoots to 100% and oxygen concentration is beyond that (Figure 2.2). This experiment shows that Oxygen is produced by adding Hydrogen peroxide. Temperature Application Uncalibrated Probe







61




Subroutine: RESET THE ART/RC STATUS CODE

40 A=0 : LINK#9DAB : IF S<>∩ GOSUB 100
.
.
.
90 STOP ... (END OF PROGRAM)
100 IF@#E980 <> 7 THEN X=@#E900 : GOTO 100
110 RETURN

Program: Output routine that transmits sequentially to ART/RC

```
100 REM PROGRAM TO WRITE TO CHANNEL TRIAC OR RELAY
CONTROL CARD
110 C=1 : I=3 : REM CHANNEL AND ADDRESS SELECT
120 DO : REM START WRITING TO CONTROLLER
130 Y=Y+1 : REM NUMBER THE CHANNELS
140 @(#E900 + I) = 255-C : REM WRITE TO SLAVE ART/RC
150 PRINT "SLAVE" , I , "CHANNEL" , Y ;
160 PRINT "--- CHANNEL SELECT NUMBER" , C , "IS ON"
170 DELAY O : DELAY O :REM LEAVE CHANNEL ON AWHILE
180 C=C+C : REM SELECT COMPLEMENTED VALUE FOR NEXT CHANNEL
190 UNTIL C>128: REM STOP AFTER LAST CHANNEL
200 STOP
```

63

CHON/CHOFF ROUTINE

10	A=2 :REM CHON ADDRESS OF THE TRIAC OR RELAY CARD
20	FOR C=0 TO 7
30	LINK #9COO : REM WAIT AND TURN CHANNEL C ON
40	DELAY O : NEXT C : REM WAIT AND DO THE NEXT CHANNEL
10	A=2 : REM CHOFE ADDRESS OF THE TRIAC OR RELAY CARD
-	
20	FOR $C=0$ TO 7
30	LINK #9C10 : REM TURN CHANNEL C OFF

Program: To READ D-9020R, 16 CHANNEL A/D CARD

100	FOR C=0	TO 7 : REM SELECT CHANNEL
110	@∦E900=2	55-C : REM START A/D CONVERSION
120	A=1 :	REM ART/RC SLAVE NUMBER 1
130	LINK∦9DA	B
140	PRINT "S	LAVE" , A , "CHANNEL" , C , "IS" , D: REM
	DISPLAY	DATA
150	IF S <> 5	THEN GOSUB 200
160	NEXT C	
200	IF S=0	THEN PRINT "MASTER ERROR": STOP
210	IF S=1	THEN GOTO 130 : REM INVALID DATA - READ AGAIN
220	IF S=3	THEN PRINT "NO RESPONSE FROM SLAVE" , A : STOP
230	IF S=6	THEN PRINT "FIRMWARE ERROR" : STOP

Program: To find out RATE OF OXYGEN CONSUMPTION and indicate

WARNINGS

10 PRINT "INITIAL OXYGEN CONCENTRATION" : INPUT A 20 PRINT "STARTING TIME (IN MIN), T1=" : INPUT T1 30 PRINT "FINAL OXYGEN CONCENTRATION " : INPUT B 40 PRINT "TIME (IN MIN) AT FINAL OXYGEN CONC.,T2=" INPUT T2 50 C=A-B: PRINT "OXYGEN CONSUMED, C=": PRINT C 60 PRINT "100% REPRESENTS HOW MANY MOLES PER ML" : INPUT D 70 PRINT "TOTAL REACTOR VOLUME : V=" : INPUT V 80 P=V*D : F=P*C/100 90 IF (P/F) 1.9 THEN PRINT "WARNING" 100 IF (P/F) 1.75 THEN PRINT "BEEP, WARNING , BEEP" 110 PRINT "RATE=": R=F/(T2-T1) : PRINT R 120 PRINT P,F : END

APPENDIX D-2

Program for Ethanol Bioreactor Control

- 10 FOR I=1 TO 10
- 20 @#ED00 = 2
- 30 FOR J = 1 TO 100
- 40 DELAY 100
- 50 PRINT "PUMP IS ON"
- 60 NEXT J
- 70 NEXT I
- 80 FOR I = 1 TO 50
- 100 @#ED00 =255
- 110 DELAY 100
- 120 PRINT "PUMP IS OFF"
- 130 NEXT I
- 140 GO TO 10

Program: To READ A/D Channel for LONG DURATION RUNS

```
5 K=3000
10 I=1
20 FOR Q=1 TO 4
30 FOR J=1 TO 250
40 @#ECOO=1 : B=@#ECOO : REM READ CHANNEL 1
50 T=TOP : LINK#9A2E : REM READ CLOCK
60 C=B
70 PRINT I, (Q-1)*250+J, $T, B
80 NEXT J
90 @(K+Q) = C
100 NEXT Q
110 K = K+Q
120 I =I+1
130 GOTO 20
140 K=#3000 : REM MEMORY ADDRESS ON RAM
150 FOR L=1 to 2000
160 PRINT L, @(K+L)
180 NEXT L
200 STOP
```



APPENDIX-E.1

10 PRINT S, or 10 IF S= n goto nnn.

S=0: A 'Master Error or Undefined data' error has occurred. This simply means that for some reason the ART/RC is unable to process the request. For example, an attempt is made to write to a slave station with a number greater than 127 or place a number greater than 255 into a particular address.

S=1 : An invalid data error has occurred. This is generally caused by a spike or noise on the DPW and is corrected by rereading the slave.

S=3 : A master receiving (No Answer) error means that although the Master ART/RC is working properly, it is not receiving an answer from the slave, a break in the DPW or no slave at that address.

S=5 : 'Valid Data Receiving' means that everything is working properly.

S=6 : The 'Master Transmitting Write' status code is a brief condition of the status between the execution of a LINK command and the return to Tiny Basic. It will notify a problem in the Firmware. Address:= @#E900 = Slave 0 Address:= @#E901 = Slave 1 Address:= @#E902 = Slave 2 . = . = . . = . = . Address:= @#E97F = Slave 127

Suppose that on the DPW line there is a D-9028 whose address is 5, we can write to Slave 5 as follows:

```
10 \ @\#E905 = 255
```

255 is the data in complementary binary 05 is the Slave address. Now if the Triac Relay channel # 1 is to be operated the instruction would be :

10 @ #E905 = 255 - 1.

Further if Triac or Relay channel 4 is to be turned on the program would be:

 $10 \quad @\#E905 = 255 - 8.$

SYSTEM DESIGN

Hardware Design and Modification:

For maximum flexibility, the ADC 804 has been designed to accommodate a 5 Vdc, 2.5 Vdc, or an adjusted voltage reference. The reference voltage for the ADC is either 1/2 of the voltage which is applied to the Vcc supply pin, or is equal to the voltage which is externally forced at the Vref/2 pin. This allows for a ratiometric voltage reference using the Vcc supply. A 5V reference voltage can be used for the Vcc supply or a voltage less than 2.5 Vdc can be applied to the Vref/2 input for increased application flexibility. The internal gain of the Vref/2 input allows this factor of 2 reduction in the Vref/2 voltage. An example of an adjusted reference voltage is the accommodation of a reduced span or dynamic voltage range of analog input voltage. If the analog voltage were to range from 0.5 Vdc to 3.5Vdc, instead of 0 to 5Vdc, the span would be 3V. With 0.5VDC applied to the Vin (-) pin to absorb the offset, the reference voltage can be made equal to 1/2 of the 3 V span from 0.5 V to 3.5 V with 0.5V input corresponding to zero and the 3.5 Vdc input corresponding to the full-scale. The full 8 bits of resolution are therefore applied over this reduced analog input voltage range providing greater resolution.

The converter can be operated in a ratiometric mode or an absolute mode. In ratiometric converter applications, the magnitude of the reference voltage is a factor in both the output of the source transducer and the output of the A/D convertor, and therefore cancels out in the final digit code. In absolute conversion applications, both the initial value and the temperature stability of the reference voltage are important factors for accuracy in the operation of the A/D convertor.

8020 A/D Card:

The A/D Card features 4 LED's for monitoring the circuit performance. Three of the LED's (1,2 and 4) represent the binary channel or position that the multiplexer is reading. The fourth LED indicates serial data signals present on the datapathway line between the master and slave. The variable resistors are adjusted to 2800 ohms because this value supplies 1.0 ma of current to the sensors.

Wire jumpers:

The jumpers W-1, W-2 (Figure 3.2) are wired from A to B and their function is input voltage from the A/D convertor. The jumpers W-3 are all wired from A to C. Their function is analog input voltage trimming, the same as for all the jumpers W-4. The W-5 jumper is wired from B to A, and its function is A/D span regulator Vref/2.

72

The determination of required moles of hydrogen peroxide. Using solubility in water at different temperatures [21] we can calculate the amount of pure oxygen dis solved in water at 37°C (experiment temperature). The solubility of oxygen in water at 37°C is 2.4ml oxygen/100ml water.

> 1 mole = 22.4 ltrs x mole = 2.4 ml

imol
2.4ml * ----- = 1071 nmoles
22.4 l

Therefore, 2.4ml is equivalent to 1071 nmoles 02.

For 100ml of reactor volume, total moles of oxygen is:

100ml * 1071 nmoles $O_2/ml = 107100$ nmoles = 107 mmoles

This is the total moles present initially. If suppose 50% oxygen is consumed by microorganism in 30 minutes we can calculate the amount consumed in micromoles

is 50 * 107/100 = 53.5 micromoles

Therefore, after 30 minutes 53.5 micromoles O_2 is needed. From reaction: mol.wt. of $H_2O_2 = 34$ grams mol.wt. of $H_2O_2 = 18$ grams mol.wt. of O = 16 grams H_2O_2 required to obtain 107 mmoles of O_2 at start is given by,

```
catalase

H_2O_2 -----> 1/2O_2 + H_2O

(1 mole) (1/2 mole) (1 mole)

(214 mmoles) (107 mmoles)
```

Therefore, requirement for 214 mmoles H_2O_2

```
moles = ------
mol. wt
```

1.0 Mole = 32 grams O_2 / 1000ml 1 milli mole = 32 * 10^{-3} grams O_2 / 1000ml 1 micro mole = 32 * 10^{-6} grams O_2 / 1000ml

```
1 micro mole = 32 * 10^{-6} grams/ 1000ml

107 micro mole = ?

= 107 micromole * 32 * 10^{-6} / 1 micro mole

= 3.424 * 10^{-3} grams / 1000 ml
```

In this case it is necessary to use 7.276 * 10^{-3} grams H_2O_2 34 * 3.424 * 10^{-3} /16 = 7.276 * 10^{-3} grams H_2O_2 - 100.

BIBLIOGRAPHY

[1] Camp, C.E. "Preliminary System and Process Design of a Microsomal Bioreactor". M.S. Thesis, Univ. of Okla. 1985.
[2] Kennedy, J.F. and Cabral, J.M.S. "Immobilized Living Cells and their Applications". Appl. Biochem. Bioeng., 1983
[3] Hall, Douglas V., "Microprocessors and Interfacing, Programming and Hardware". McGraw Hill Book Co., 1986 [4] Dwyer, D.F., Krume, M.L., Boyd, S.A., and Tiedje, J.M., "Kinetics of Phenol Degradation by an Immobilized Methanogenic Consortium". Appl. Environ. Microbiol. 1986.

[5] K-9000 Series Microcomputer, Vanderbilt Inc.

[6] Leventhal, L.A., "Fundamentals of Microprocessors" ,1984

[7] Comer, D.J., "Microprocessor-Based System Design". Holt, Rinehart and Winston. 1986

[8] M O S Memory Data Book., National Semiconductor Corp. 1984[9] INTEL Component Data Cat. Intel Corp. 1983

[10] Linear Databook, National Semiconductor Corp. 1982

[11] Intersil's Component Data Cat. Excellence in Integrated Signal Processing ICs. 1987

[12] Goldsborough, Paul F., "Microcomputer Interfacing with 8255 PPI Chip", Howard W. Sams Co., 1980

[13] Morrison, D.R., "Space Bioreactor Requirements Document"
First Draft, NASA/JSC , Aug. 1985.

[14] Kermit User's Manual.

75

[15] Camp C.E., and Sofer S.S., 'Calcium Alginate-immobilized hepatic microsomes: Effect of NADPH cofactor on oxidation rates' Enzyme Microb. Technol., 1987, vol.9

[16] Lakhwala F., Lodaya M., Yang K., Sofer S.S., and Lewandowski G., 'Viability Studies of Micro-organisms Entrapped in Alginic Gel' Presentation at International Conference of Physiochemical and Biological Detox of Hazardous Wastes' 1988.

[17] Chien N.K., and Sofer S.S., 'Flowrate and Beadsize as Critical Parameters for Immobilized Yeast Reactors.' Enzyme Microb. Technol., 1985

[18] Westmeier F., and Rehm H.,J., 'Biodegradation of 4chlorophenol by entrapped Alcaligenes' Appl. Microb. Biotech., 1985

[19] Skoog D.A., 'Principles of Instrumental Analysis' 3rd edition Saunders College Pub., 1985

[20] Trebal, Robert E., 'Mass Transfer Operations' McGraw Hill Pub., 1980

[21] Perry Robert H., Green Don., 'Chemical Engineers Handbook' McGraw Hill Pub., 1986