Design of Real-Time Detection System of Bacteria Concentration Changes in Biological Fermentation

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Abstract. In the process of bio-fermentation, there is a problem of low detection efficiency in the process of recording changes in the concentration of traditional bacterial cells. Therefore, a real-time detection system for the concentration of microbial cells in biological fermentation is designed. In the system hardware design process, the data of microbial concentration changes in the biological fermentation are analyzed to select the system measurement principle. An intermediate conversion circuit is designed based on the measurement principle to complete the system hardware design. The measurement principle is used to derive the software structure of the real-time detection system. Real-time data acquisition and detection are implemented in the software structure to realize system software design. According to the results of simulation experiments, the real-time detection system for the change of bacterial concentration in biological fermentation compares with the traditional detection method, the detection efficiency is improved by 11%, and the operation is stable.

Keywords: Biological fermentation · Cell concentration · Concentration detection · Real-time · Detection efficiency

1 Introduction

Biological fermentation is the foundation of bioengineering and modern biotechnology and its industrialization. With the progress of bioengineering technology and the expansion of the scale of fermentation industry, it is urgent to carry out advanced control and optimization of fermentation process. The existing fermentation measurement and control system lacks the intelligent detection unit of key biological parameters detection. The mechanism of biological fermentation is complex, and it is highly nonlinear and time-varying. It is difficult to realize real-time detection of these key biological parameters; The structure of these measurement and control systems are all unit structures, and their openness and reliability are poor, making it difficult to apply advanced optimization control algorithms and strategies to industrial applications and to meet the need for optimal control of fermentation processes. Therefore, it has important theoretical

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significance and application value to study the real-time detection system and its key technologies of the microbial concentration change in the process of biological fermentation [1]. Microbial fermentation process is an extremely complex biochemical reaction process, and many factors affect the fermentation, such as the composition of the fermentation broth, temperature, Ph value, dissolved oxygen, the type and concentration of viable bacteria. Among the above factors, the concentration of bacteria is the most important process parameter, but at present there is no real-time monitoring system that can meet the requirements, and it can accurately detect the bacterial concentration in the fermentation process in real time. The conventional measurement method of the concentration of bacteria is off-line. Off-line measurement brings two problems: On the one hand, the sampling process is easy to be contaminated; on the other hand, the automatic control of fermentation is difficult. Therefore, it is necessary to develop a real-time detection system for the concentration of bacteria, which is of great significance for understanding the fermentation information, mastering and controlling the biological fermentation process, and improving the quality of fermentation [2]. Therefore, a new method is proposed to detect the concentration of bacteria in the fermentation process in real time, and an automatic detection system for the concentration of bacteria is designed to realize the real-time detection of the concentration of bacteria in the fermentation process. It has good application value in biochemical pharmaceuticals, food fermentation, sewage treatment and other industrial fields.

2 Design of Real-Time Detection System of Bacteria Concentration Changes in Biological Fermentation

2.1 Real-Time Detection System Hardware Design of Change of Bacteria Concentration

Selected Measurement Principle

The presence of microorganisms affects the electrical properties of the fermentation broth, and the electrical properties can characterize the fermentation broth using its conductivity and permittivity. The permittivity (dielectric constant) of the fermentation broth is defined as the ability to store the charge. When the microbial strain is added to the medium, its permittivity will increase significantly; when the measurement frequency changes, the permittivity of the fermentation broth will also change. The phenomenon that the permittivity changes with the measurement frequency is called the permittivity distribution. Under normal circumstances, it is difficult for charged ions in the cell to cross the cell membrane to reach the outside of the cell, and it is difficult for extracellular charged ions to penetrate into the cell interior. If the fermentation broth is placed in an electric field, there will be an equal and opposite charge accumulation on the inner and outer surfaces of the cell membrane, each cell acting like a small capacitor. Under certain conditions, the amount of electric charge bound by living cells per unit volume is proportional to the number of live bacteria [3]. The greater the concentration of viable bacteria, the more the bound charge, the greater the permittivity of the fermentation broth. Therefore, the permittivity of the fermentation broth in the radio frequency range

is a function of the measurement frequency and the bacterial cell concentration. When the measurement frequency is fixed, the permittivity and the bacterial concentration of the fermentation broth are single-valued functional relationships. This means that the bacterial concentration can be detected by measuring the permittivity of the fermentation broth. Fermentation process mainly batch fermentation, batch fed fermentation and continuous feed fermentation in three forms. The fed batch fermentation process is between batch fermentation and continuous feed fermentation, both have the advantages, and overcome the disadvantages of both, it is a commonly used fermentation method, the study of different feeding strategies to increase the fermentation yield is one of the main directions for optimal control of fed batch fermentation processes. Therefore, this paper focuses on the soft-sensing system of fed batch fermentation process [4]. The fermentation process belongs to a complex and nonlinear dynamic process. The general material conservation equations for the feed fermentation process are as follows:

$$\frac{\mathrm{d}\xi}{\mathrm{dt}} = K_{\mathrm{m}}\mathrm{r}(\xi) + \mathrm{u} \tag{1}$$

State $\xi = [\xi_1, \xi_2, \ldots, \xi_{\mathrm{n}}]^T$ represents a state vector representing the concentration of a particular component in the fermenter. The first item $K_{\mathrm{m}}\mathrm{r}(\xi)$ in formula (1–2) represents the change of biochemical and biochemical reactions in the fermenter. Km represents a stochastic transformation matrix, $\mathrm{r}(\xi) = [\mathrm{r}_1(\xi), \mathrm{r}_2, \xi, \ldots, \mathrm{r}_{\mathrm{m}}(\xi)]^T$ represents the reaction rate vector for different components. The second item $\mathrm{u} = [\mathrm{u}_1, \mathrm{u}_2, \ldots, \mathrm{u}_{\mathrm{n}}]^T$ represents the control input vector of the fermenter, including the rate of various materials flowing into and out of the fermenter. Therefore, the dynamic characteristics of fed batch fermentation process can be represented by a set of ordinary differential equations, then, the state space is used to describe the real-time detection system of microbial concentration changes during fermentation, it can be expressed as:

$$\begin{cases} \frac{\mathrm{dx(t)}}{\mathrm{dt}} = \mathrm{f}(\mathrm{x}(t), u(t), w(t)) \\ y(t) = g(x(t), v(t)) \end{cases} \tag{2}$$

Among them, x is the state variable vector for the measurement output vector; x and y are Gaussian white noise vectors with a mean of 0; f(·) and g(·) represent a linear or nonlinear function of the state transition and measurement output, respectively.

From the above formula (2) we can see that, the similarity between a soft measurement hybrid system and a state space system is that, the system is composed of state differential equations and equations, it is the same as the dynamic system state space system, the state vector is an internal variable set that can completely characterize the time domain behavior of the system. Represents all the dynamic information of the system. But the difference is: soft measurement system, instead of the output equation, the conservation equation can be a measurement supplemental equation established using various artificial intelligence methods and prior knowledge [5]. In terms of expression, soft measurement systems include differential equations and algebraic equations, it can fully express the dynamic and static characteristics of the fermentation process, therefore, it is called the real-time detection system for the change of the

concentration of bacteria; the conservation equation contains the specific form of prior knowledge used in the soft measurement system, the dimension of the conservation equation can be seen as the amount of prior knowledge used by the system.

Intermediate Transform Circuit Design

The intermediate conversion circuit is mainly based on the bacteria concentration detection system, the system is mainly composed of a fermenter, a capacitance sensor—platinum electrode, an intermediate conversion circuit, an A/D board, a computer, a monitor, a printer, etc., as shown in Fig. 1:

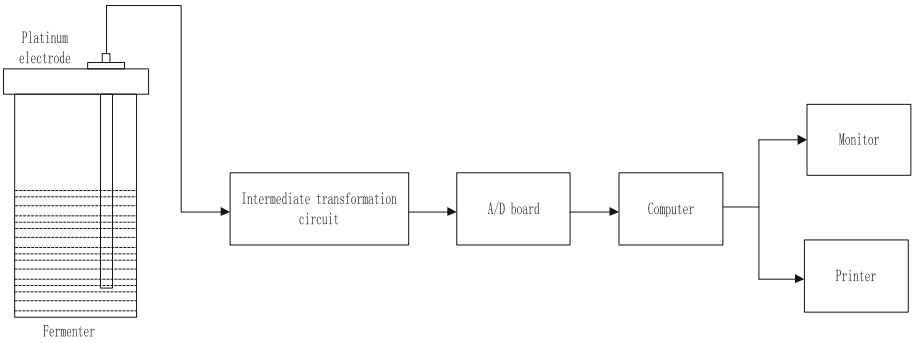

Fig. 1. Frame structure of real-time detection system of bacterial concentration change data

Based on the real-time detection system for the change of microbial concentration data, the intermediate conversion circuit converts the capacitance signal detected by the capacitance sensor into a voltage signal required by the A/D board, the intermediate conversion circuit is implemented using a lock-in amplifier [6]. Lock-in amplifiers are based on coherent detection technology, using the reference signal frequency is related to the input signal frequency, not related to noise frequency, this extracts useful signals from noise backgrounds. The intermediate conversion circuit is shown in Fig. 2:

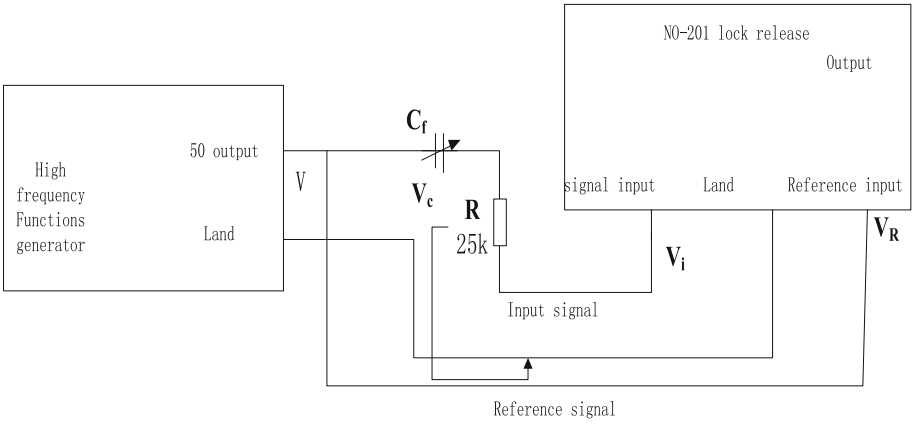

Fig. 2. Intermediate conversion circuit block diagram

In this circuit, when the capacitance of the capacitive sensor changes slightly, the amplitude and phase of the input signal voltage Vi of the lock-in amplifier changes accordingly. Because of the input signal $V_{\mathrm{i}} = \frac{R}{R + \frac{1}{\mathrm{j}w C_{\mathrm{f}}}} V_{\mathrm{s}}$ (ignoring the input impedance of the lock), where Vs is a high frequency sinusoidal signal. When the frequency of the input signal of the lock-in amplifier is locked at the frequency of the reference signal, that is w = wR, the phase difference between Vi and VR is equal to zero, the amplitude of the output voltage of the lock-in amplifier Vo = KVi. Where K is the magnification, after the parameters of the lock-in amplifier are selected, it is a constant [7]. Therefore, the output voltage has a monotonically increasing function relationship with the sensor capacitance. This circuit has a strong anti-interference, high sensitivity, a wide dynamic range, therefore, it is widely used as weak signal detection.

2.2 Real-Time Detection System Software Design for the Change of Bacterial Concentration

Real-Time Detection System Software Structure

The real-time detection system software design of the concentration change data of bacteria, using a networked and modular integrated approach, taking full account of the system's openness and reliability principles, designed real-time detection system software, Fig. 3 shows the integrated architecture of the intelligent measurement and control system for the fermentation process:

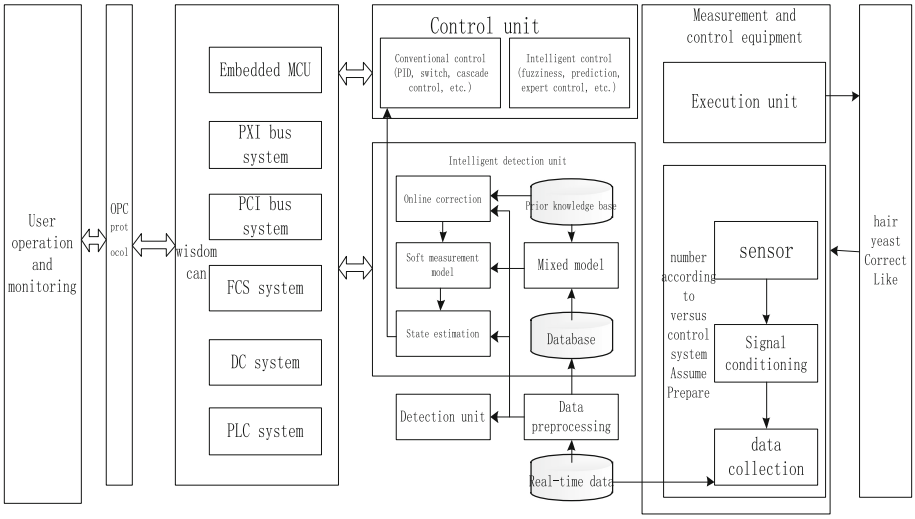

Fig. 3. Real-time detection system software structure

Collecting and controlling the state information of fermentation process objects through real-time detection system software structure is the basic function realized by the

existing fermentation process measurement and control system, the intelligent measurement and control system of the fermentation process is based on the analysis of the objects of the fermentation process, extract useful prior knowledge, and analyze and systematically prior knowledge, through the smart detection processing unit, using the latest artificial intelligence technology, real-time estimation of unpredictable key parameters in bio-fermentation process, therefore, it is possible to effectively achieve optimal control of biological parameters that are difficult to measure [8]. The functions of each functional module unit in the intelligent measurement and control system of the fermentation process are as follows:

Measurement and control equipment: It is mainly composed of data acquisition unit and execution unit. The data acquisition unit collects the signals of the hardware sensors. Through signal conditioning and data acquisition modules, convert the measurement information of the fermented object from analog to digital, as the real-time measurement data of the fermentation process measurement and control system; the execution unit sends a control command, control various switches and valves, etc., to achieve optimal control of the fermentation process;

Detection unit: difference and intelligent detection unit, refers to a normally measurable inspection unit, realize the routine measurable fermentation process (such as pH, temperature, dissolved oxygen concentration) detection;

Intelligent detection unit: The biggest difference between the intelligent measurement and control system of the fermentation process and the existing measurement and control system is that the intelligent measurement and control system has an intelligent detection unit. In information processing, the intelligent detection processing module uses artificial intelligence technology to analyze and deal with unpredictable key quality parameters, and according to the detection results of the intelligent detection unit, the biological parameters of the fermentation process are feedback controlled, to increase the yield and efficiency of fermentation;

Control unit: The control unit includes two modules, conventional control and intelligent control. Conventional control has PID, switch, cascade control and so on. Intelligent control has fuzzy, forecast, expert control and so on. The control algorithm is written in a programming language and packaged into standard modules, then directly called by the application, only need to design the interface type of control process, input parameters (pass or call), return value, can realize the control function of the control module;

Intelligent processing unit: The intelligent processing unit realizes the comprehensive processing of the information of the fermentation process measurement and control system through an intelligent processor. The intelligent detection unit and control unit are components of the intelligent processing unit, and the data flow and information flow are represented in the architecture [9]. The intelligent processing unit is usually composed of a PCI bus system, a PXI bus system, an embedded 1 VICU. PLC system, a DCS system or an FCS system;

The intelligent detection unit is the most critical part of the intelligent measurement and control system architecture of the fermentation process, soft measurement technology is a key technology for intelligent detection units. The intelligent detection unit utilizes the measurable information of the hardware sensor and priori information of the process object, the soft measurement system enables real-time detection of key

biological parameters that are difficult to measure in real time. As shown in the smart detection unit block diagram in Fig. 2, by integrating prior knowledge and historical database data, by integrating prior knowledge and historical database data, after system identification, construct a corresponding soft measurement system; the hardware sensor collects measurement data through the data acquisition device, pre-processing measurement data, combining soft helium J system and real-time state estimation method, realize accurate estimates of key unpredictable biological parameters. At the same time, the real-time correction module in the intelligent detection unit, using state estimation and control unit output and system real-time measurement data, through the correction data provided by the prior knowledge base, real-time correction of soft measurement systems, improve system detection accuracy.

Realizing Data Acquisition and Detection in Real Time

The real-time data acquisition and sorting of the capacitive sensor for detecting the concentration of bacteria is mainly made of PTFE rods, rubber seals, leads, two platinum electrodes. The sensor lead is a coaxial shielded cable, the electrode has a coaxial cylindrical structure, the inner and outer electrodes are covered with a layer of PTFE film, for internal and external electrode insulation. The electrodes are resistant to high temperatures and are non-toxic, meeting the special requirements of bio-fermentation sensors [10]. The diameter of the external electrode is 16 mm, the diameter of the internal electrode is 10 mm, and some small holes are drilled on the external electrode, makes live cells evenly distributed in the fermentation broth. There is the following relationship between the electrode capacitance Cf and the permittivity ef of the fermentation broth: $e_f = \frac{\ln\frac{R}{r}}{2\pi e_0 l} C_f$ or $e_f = kC_f (k = \frac{\ln\frac{R}{r}}{2\pi e_0 l}$ Is a constant). Therefore, the permittivity of the fermentation broth can be determined by measuring the electrode capacitance.

The voltage signal output from the lock-in amplifier needs to be sent to the computer for data filtering after A/D conversion, get the data you need. The article uses the 9012 modular interface board as the data acquisition interface board of the system, it includes a 12-bit A/D converter, 16 multiplexers, sample/hold, bus interface control logic, etc. the board is equipped with an address switch to set the I/O address arbitrarily. Data processing is done in software, the filtering method used is the median averaging filter. In our detection system, the data acquisition system has been able to automatically collect and process data through the hardware and software design.

3 Experimental Analysis

3.1 Experimental Procedure

To verify the effectiveness of the real-time detection system for the change of bacterial concentration in biological fermentation, the following comparison experiments were designed. Seven different fermentation strains were selected for testing, including Streptococcus, Lactococcus, Leuconostoc, Lactobacillus, Propionic Acid Bacteria, Brevibacterium, and Enterococcus. Experiments were performed on each of the fermenting bacteria. The experimental medium is yellow pulp water, one liter fermenter, in order to make the cells in the fermenter uniform, the fermentation liquid should be

continuously stirred, the stirring speed is 100 rpm/min, the fermentation temperature is basically controlled at about 25 °C, and the pH is controlled at About 5.0, the measurement frequency is 50 kHz, and the experimental results are observed under a high-power microscope environment.

The bacterial organisms in the same biological fermentation were used as experimental objects, divided into two groups, the real-time detection system for the change of bacterial concentration in the biological fermentation is the experimental group, the traditional data measurement method is used as a control group, under the premise of controlling a single variable, record the accuracy rate of data records in the two groups of cell concentration changes, the change of cell concentration data records real-time performance. Set corresponding conditions for two sets of experimental data, in order to ensure the fairness of the experiment, the parameters of the experimental group and the control group are always the same. In order to verify the differences between the SME management systems based on clustering algorithms and traditional SME management, the parameters of the experimental group and the control group are always the same. In order to verify the differences between the SME management systems based on clustering algorithms and traditional SME management, the experimental group will use the real-time detection system for the change of the concentration of bacterial cells in the biological fermentation according to the requirements. The traditional data detection mainly adopts manual processing.

3.2 Accuracy of Data Accumulation of Changes in Microbial Concentration

The experimental group and the control group simultaneously recorded the change data of the same bacteria concentration, compared with its record accuracy, after recording 2, 4, 6, 8, and 10 h respectively, differences between the records of bacterial concentration changes and actual data. To avoid the interference caused by unexpected events on the experimental results, the treatment parameters of the experimental group and the control group are the same, the specific results are as follows (Fig. 4):

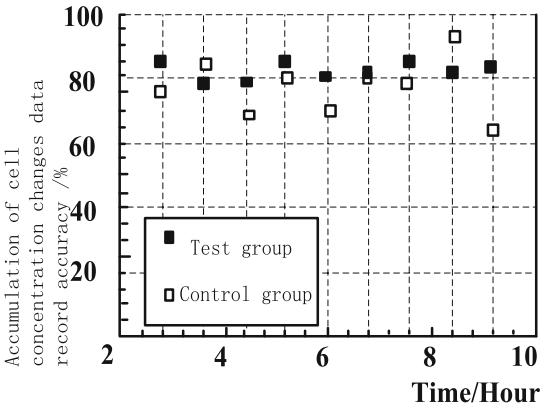

Fig. 4. Accuracy rate of data change of cell concentration

The analysis of the above figure shows that the accuracy of the data recording of bacterial cell concentration changes is compared. With the increase of time, the accuracy of data recording of the cumulative concentration of cell concentration in the experimental group is about 94%, and the data points are similar in value, and the changes are not Big. In the control group, the cumulative data change accuracy of the cell concentration was about 83%, but the values of the data points differed greatly. Comparing the experimental results, the recording accuracy of the proposed method is improved by 11% compared with the traditional method, and the accuracy of the proposed method is higher, which can prove the effectiveness of the real-time detection system.

3.3 Comparison of Bacterial Cell Concentration Changes in Real-Time Data Records

The experimental group and the control group processed the same data at the same time, after recording 200, 400, 600, 800, 1000 sets of data, respectively, the change of cell concentration data records real-time performance. To avoid the interference caused by unexpected events on the experimental results, the treatment parameters of the experimental group and the control group are the same. The specific results are as follows (Fig. 5):

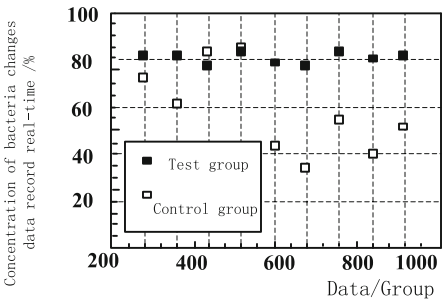

Fig. 5. The real-time recording of the data of bacterial concentration changes

Compared with the above figure, we can see that, in the process of recording the changes in the concentration of bacterial cells, as the demand for processing increases, the real-time performance of the real-time detection system for the change of bacterial concentration in biological fermentation is high, stay around 92%. The control group continues to increase with the demand for processing, the processing efficiency shows a declining trend, record real-time performance is about 79%. Therefore, it can prove the real-time detection system for the application of microbial concentration change in biological fermentation, it can effectively improve the real-time performance of the data of bacterial concentration changes.

4 Conclusion

Biofermentation is the foundation of bioengineering and modern biotechnology and its industrialization, with the advancement of bioengineering technology and the continuous expansion of the production scale of the fermentation industry, there is an urgent need for advanced control and optimization of the fermentation process. The existing fermentation monitoring and control system lacks a key biological parameter detection unit, the mechanism of biological fermentation is complex, with a high degree of nonlinearity and time-variation, it is difficult to achieve real-time detection of these key biological parameters; and these measurement and control systems are all unit structures, poor openness and reliability, making advanced optimization control algorithms and strategies difficult for industrial applications, the need for optimal control of the fermentation process cannot be met. Therefore, the system for real-time detection of microbial concentration changes in biological fermentation, can promote the optimization of the control of the fermentation process engineering application level.

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