A Noval Microbial Optical Density Detection Method Based on a Logarithmic Amplifier

Zhigang Xu, Na Li

Abstract— **Microbial detection plays an important role in food safety, environmental monitoring, and drug safety. The optical density (OD) detection method is the most commonly used method in the field of microbial concentration detection. In OD detection, aerobic bacteria require a large volume of oxygenated culture, which can lead to a higher OD of the bacterial solution. Therefore, how to expand the detection range is a key issue. To address the limitations of using operational amplifiers and lock-in amplifiers with limited dynamic range, a microbial OD detection circuit based on a logarithmic amplifier is proposed. The circuit uses the logarithmic amplifier AD8304 to meet the high dynamic range characteristics of OD detection. The theoretical calculation proves that this method can directly convert OD and output into a linear relationship, simplifying complex logarithmic calculations. To verify the feasibility of the scheme, an experimental device was built, and the linearity and stability of the circuit were verified by detecting the OD of standard OD tablets. In addition, the OD of three different concentrations of lactobacillus was detected simultaneously with a spectrophotometer, proving that the detection circuit can accurately detect the OD of microorganisms.**

Index Terms— **Dynamic Range; Log Amplifier; Microorganisms; Optical Density**

I. INTRODUCTION

Methods for measuring microbial concentration mainly include spectrophotometry, colony counting, turbidity measurement, flow cytometry, fluorescent quantitative PCR, microscopic counting, and biosensors. Spectrophotometry is a method used to quantitatively determine substance concentration by detecting the optical density of a substance at specific wavelengths. It has diverse analysis methods, reliable results, and wide applications in the fields of chemistry, materials, and biology. Specific applications include enzyme kinetics, organic compounds in water, plasma components, optical density of sugars and peptides, protein concentration, inorganic anions, fiber components, and others[1-9].

In aerobic bacteria growth detection, the large volume of aerobic culture leads to higher optical density values in the bacterial suspension. Therefore, improving the measurement range of optical density detection becomes a key issue. The optical signal detection module is an important component of the optical density detection system, mainly including the photoelectric conversion module, signal amplification module, and signal acquisition module. The design performance of these modules is a critical factor affecting the measurement range, resolution, and accuracy of optical density detection. According to the Lambert-Beer law, optical density is logarithmically related to light intensity, resulting in a significant range of light intensity variations in microbial optical density detection. The input signal dynamic range of the signal amplification module is large, but the signal-to-noise ratio of the system will decrease under high optical density. Therefore, common weak light signal detection techniques for acquiring weak light signals include gain amplification, light enhancement technology, and ultra-high sensitivity detectors. Li et al. used an APD photodetector and designed a corresponding processing circuit using operational amplifiers, achieving a dynamic range of up to 60 dB. Qi et al. implemented a weak light signal detection circuit using DSP and LabVIEW for lock-in amplifiers[10]. Operational amplifiers have the characteristics of high gain and low noise, but their dynamic range is limited. Lock-in amplifiers are sensitive to noise and have a relatively narrow dynamic range. The above solutions cannot meet the requirements of a high measurement range in optical density detection.

A logarithmic amplifier has an input-output relationship that is logarithmic, and the output signal generated by the logarithmic amplifier is directly proportional to the logarithm of its input[11]. Due to the compression characteristics of the function, a logarithmic amplifier can be used to process wide dynamic range signals, such as signals with amplitude changes exceeding 100 dB. Some aerobic bacteria in microorganisms require large-volume aerobic culture, and the optical density often exceeds 5. According to the Lambert-Beer law, optical density is directly proportional to the logarithm of the input light intensity. Using a logarithmic amplifier, the optical density can be directly converted into a linear relationship with the output signal, simplifying the logarithmic calculation.

Therefore, this paper adopts a light density detection circuit based on the logarithmic amplifier AD8304 and establishes an experimental platform to validate the circuit for detecting optical density in culture dishes.

II. MEASUREMENT PRINCIPLE

A. Signal Acquisition of Optical Density

According to the Lambert-Beer law, optical density is defined as the logarithm of the ratio of transmitted light intensity to incident light intensity, as shown in Equation 1, where $I_{0(\lambda)}$ represents the transmitted light intensity and $I_{(\lambda)}$ represents the incident light intensity. Mainstream optical signal acquisition systems utilize operational amplifiers for amplifying the photocurrent signals. Operational amplifiers operate effectively within their linear range, beyond which they are unable to detect the signal. On the other hand, logarithmic amplifiers exhibit an output-input relationship in logarithmic form and possess a wide dynamic range, making them suitable for detecting microbial optical density.

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Therefore, in this study, a logarithmic amplifier is employed for signal acquisition. In summary, the signal acquisition process is based on the principles of the Lambert-Beer law. Optical density is defined by the logarithm of the ratio between transmitted and incident light intensities. While operational amplifiers are commonly used for signal amplification, logarithmic amplifiers are preferred due to their wide dynamic range, which meets the requirements for microbial optical density detection.

$$OD = \alpha_\lambda CL = \lg\frac{I_{0(\lambda)}}{I_{(\lambda)}} \#(1)$$

The logarithmic amplifier AD8304 is employed in this study, which is optimized for low-frequency signal power measurement in optical fiber systems. It utilizes advanced transconductance linearization technology, providing a versatile and user-friendly ultra-wide dynamic range (160 dB) to improve the signal-to-noise ratio[12]. It features a dedicated optoelectronic diode interface and incorporates temperature compensation circuitry to enhance conversion accuracy. The AD8304 consists of two main components: a logarithmic ratio converter and a linear operational amplifier.

The output voltage of the logarithmic amplifier AD8304 exhibits a linear relationship with the measured optical density value (OD). According to the data sheet of the AD8304 logarithmic amplifier[13], we can derive the following equation:

$$V_{out} = G \cdot V_y \cdot \lg\frac{\rho \cdot P_{opt}}{I_z} \#(2)$$

In this equation, G represents the gain coefficient of the amplifier's post-stage and varies with the design of the resistance values in the peripheral circuit. V_y denotes the slope voltage of the amplifier, which characterizes the voltage change corresponding to a tenfold increase in the logarithmic amplifier's input. I_z represents the intercept current of the amplifier, indicating the minimum detectable input current. ρ is the responsivity of the photodiode, and P_{opt} is the optical power generated by the photodiode.

Based on the light intensity attenuation law during light propagation, laser power attenuates as laser propagation distance increases. This can be expressed using the following equation:

$$P_2 = \frac{P_1}{d^2} \#(3)$$

In this equation, P_1 represents the initial power output, i.e., the power output when the laser is first emitted. P_2 denotes the power output at a propagation distance of d, and d represents the propagation distance. In the detection device, since the laser is precisely aligned, the propagation distance for each hole position is the same. Therefore, the optical density (OD) value can be expressed as follows:

$$OD = -\lg\frac{I_{trans}}{I_{inc}} \#(4)$$

In this equation, I_{trans} represents the transmitted light intensity, and I_{inc} denotes the incident light intensity. Light intensity is expressed as the optical power per unit area, which is defined as follows:

$$I = \frac{P}{S} \#(5)$$

The formula for light intensity can be expressed as follows when the area reaching the photodetector in the detection system is completely the same:

$$OD = -\lg\frac{P_{trans}}{P_{inc}} = \lg P_{inc} - \lg P_{trans} = \frac{V_{inc} - V_{trans}}{G \cdot V_y} \#(6)$$

The output voltage (V) of AD8304 corresponds to the photodiode-generated photocurrent and varies based on the design of the external resistor network. The specific formula is as follows:

$$V_{out} = G\left(V_y \cdot \frac{R_z}{R_z + R_{LOG}} \cdot \log_{10}\frac{I_{pd}}{I_z} + V_{REF} \cdot \frac{R_{LOG}}{R_{LOG} + R_z}\right) \#(7)$$

In this equation, $G = 1 + \frac{R_a}{R_b}$

Since the incident power of the laser diode is constant, the value of V_{inc} remains fixed. Therefore, the formula can be transformed as follows:

$$OD = K \cdot V_{trans} + C \#(8)$$

K and C are constants determined by the external resistor network, as illustrated in Figure 1.

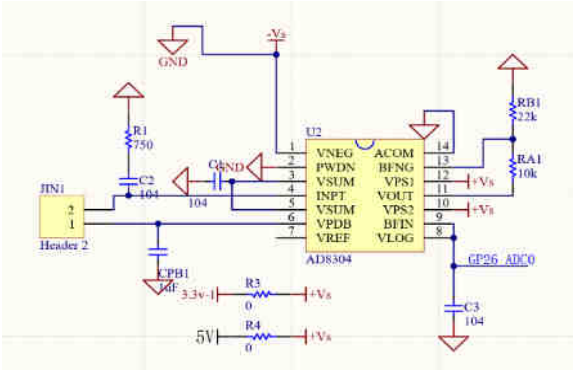

Figure 1. Circuit Diagram of Optical Density Detection Periphery

B. Selection of Photodetectors

The chosen photodetector is a silicon photodiode, which is a P-N junction photovoltaic device that can convert light energy into electrical energy without requiring bias voltage. According to their applications, silicon photodiodes are classified into two categories: solar cells and measurement photodiodes. The main function of a measurement photodiode is to serve as a photodetector that converts light signals into electrical signals without any bias. The requirements for a measurement photodiode include a wide linear range, high sensitivity, suitable spectral response, good stability, and long lifespan. They are widely used in photometry, colorimetry, optical precision measurement, and testing equipment. Silicon photodiodes have the characteristics of a large photosensitive surface, a large junction capacitance, and low frequency response. The actual

photodiode selected is the 2DU6 silicon photodiode, which is a semiconductor light receiver designed specifically for various optical instruments such as spectrophotometers and colorimeters.

III. Experimental Validation and Results Analysis

a) Building an Experimental Platform for Optical Density Measurement

The experimental setup of the optical density detection system is shown in Figure 2, which consists of five main components: a 650nm laser diode, sample dishes, a photodetector, an optical density detection circuit, and a shell. The laser diode and photodetector were mounted on a custom-designed bracket made of PLA material using 3D printing technology. The position of the laser diode and photodetector was adjusted to ensure that the laser beam was perpendicular to the sample dishes, and the transmitted light through the sample dishes was vertically incident on the photodetector. The microcontroller unit (MCU) of the detection circuit was selected as STM32C8T6, and the analog-to-digital converter module was selected as ADS1220 with a resolution of 24 bits. The data transmission between the MCU and PC was carried out through UART. During the actual measurement process, the apparatus was covered with a shell to avoid interference from background light.

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Figure 2. Photograph of the Internal Structure of the Optical Density Detection Device

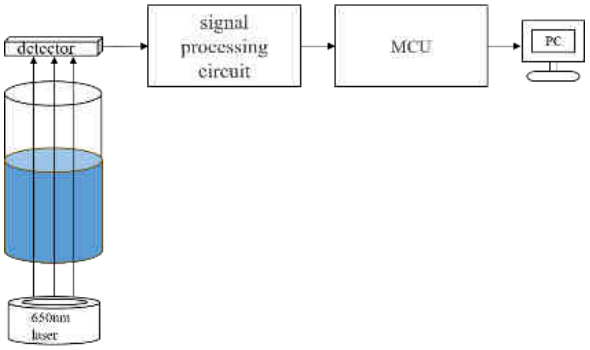

Figure 3. Architectural Diagram of the Optical Density Detection Experimental Setup

b) Linearity Testing of Logarithmic Amplification Circuit

To evaluate the linearity of the signal acquisition circuit for detecting optical density and verify Equation (8), a 35mW 650nm laser diode and eight fixed standard optical density filters were used. The laser diode emitted laser light, which was vertically transmitted through the standard optical density filters and illuminated the detector. The voltage value obtained by the amplified electrical signal of the light signal passing through the standard optical density filter was collected and linearly fitted with the actual optical density value. The results are shown in Figure 4 and Table 1, with a goodness of fit R-squared value of 0.98317. The results indicate that there is good linearity between the voltage value obtained by the logarithmic amplification circuit and the optical density value.

Table 1 Measurement Results of Voltage Values in a Detection Circuit for Standard Optical Density Slides

OD	0.1	0.15	0.2	0.5	1	2	3	4
Voltage/V	1.237	1.231	1.227	1.196	1.135	1.008	0.838	0.608

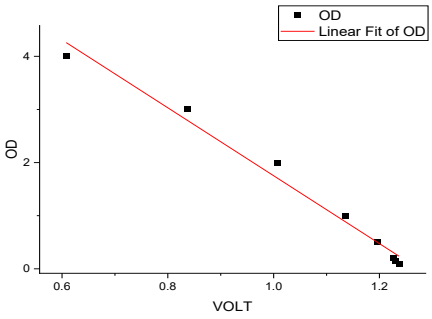

Figure 4. Linear Fitting Results of Optical Density and Measured Voltage Values

c) System Stability Testing

In order to test the stability of the signal acquisition in the logarithmic amplification circuit, we conducted ten repeated measurements of the light signals under the standard optical density filter. Each measurement was taken with a time interval of 1 second, and the standard deviation was calculated for the data collected at different optical density levels. The results, shown in Table 2 and Figure 5, reveal that the standard deviation obtained from the measurements at each optical density level was consistently below 0.001. These results highlight the high stability of the system.

Table 2 Measurement Results of System Stability

OD	0.1	0.15	0.2	0.5	1	2	3	4
Voltage1	1.237774	1.231455	1.228028	1.195431	1.134409	1.008421	0.837333	0.60777
Voltage2	1.237754	1.23009	1.227597	1.196803	1.1352	1.008848	0.837468	0.608562
Voltage3	1.238173	1.231535	1.226659	1.196331	1.135191	1.008809	0.837418	0.60766
Voltage4	1.237394	1.23205	1.227339	1.195658	1.135334	1.008316	0.837856	0.607588
Voltage5	1.237365	1.231665	1.22714	1.195689	1.134722	1.009006	0.837838	0.607745
Voltage6	1.237124	1.231481	1.225937	1.196514	1.134848	1.008374	0.838309	0.607789
Voltage7	1.236672	1.232062	1.226854	1.196445	1.134119	1.007826	0.838547	0.607533
Voltage8	1.238226	1.231657	1.226475	1.195658	1.135095	1.008683	0.83819	0.608237
Voltage9	1.236138	1.230107	1.227604	1.196704	1.134748	1.008578	0.838309	0.607668
Voltage10	1.237605	1.230226	1.227175	1.196168	1.134818	1.0076	0.837443	0.608274
STD	0.000648	0.000782	0.000616	0.000494	0.000378	0.000448	0.000445	0.000347

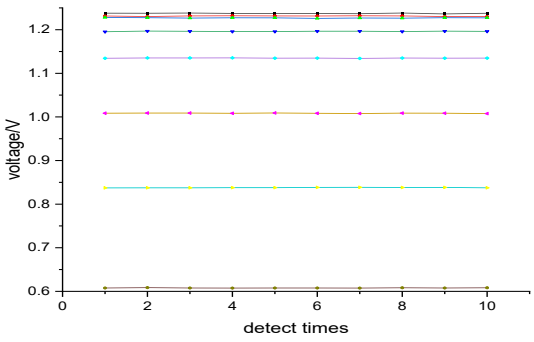

Figure 5. Measurement Results Figure for System Stability

d) Lactobacillus Optical Density Detection

To assess the performance of the experimental platform, a comparative experiment was conducted using both the experimental platform and a spectrophotometer to measure the optical density. The experiment involved the detection of optical density for four different concentrations of three strains of Lactobacillus.

The three selected strains of Lactobacillus were as follows: 1) S-7 Lactobacillus iatae, 2) Plant Lactobacillus 6595, and 3) Cheese Lactobacillus. The MRS medium was used as the culture medium. For each strain, the initial bacterial solution was diluted 2-fold, 5-fold, and 10-fold, and the optical density of the bacterial solution was measured at the four different concentrations.

The results obtained from the experimental platform are shown in Figure 6. The key difference is that the experimental platform used a wavelength of 650nm as the light source, while the spectrophotometer used a wavelength of 600nm. Additionally, the experimental platform had a system volume of 2mL, whereas the spectrophotometer had a system volume of 200μL.

As the optical density is directly proportional to the path length for the same solution, a conversion is required for the optical density measured by the experimental platform, as described by Equation (9).

$$OD_{exp} = OD_{spec} \cdot \frac{l_{spec}}{l_{exp}} \#(9)$$

According to the experimental results, as shown in Figure 6 (c) after normalization calibration based on path length, it can be observed that the absorbance of the experimental setup is very close to that of the spectrophotometer. From the figure, it can be seen that the spectrophotometer cannot measure optical density greater than 2.5. Within the common range, the experimental platform exhibits similar linearity to the spectrophotometer. However, for solutions with high optical density beyond the range of the spectrophotometer, the linearity of the measured optical density by the experimental platform decreases. This is consistent with the applicable conditions of the Beer-Lambert law. The attenuation law for measuring optical density follows the Beer-Lambert law, but there are applicable conditions for the Beer-Lambert law. For systems with high optical density, precipitation and interferences such as reflection and scattering may occur during the cultivation process, which do not satisfy the applicable conditions of the Beer-Lambert law.

The results show that the log amplifier circuit-based optical density acquisition system can achieve accurate detection of optical density.

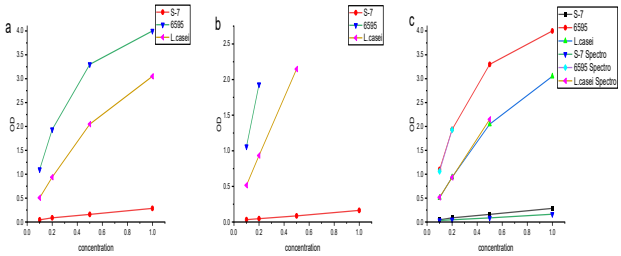

Figure 6. Comparison of Experimental Setup and Spectrophotometer for Measuring Optical Density Values (a) Optical density values of three strains of lactobacillus at four different concentrations measured by the experimental setup (b) Optical density values of three strains of lactobacillus at four different concentrations measured by the spectrophotometer (c) Comparison of normalized optical density values obtained from both methods

IV. Conclusion

This study addresses the issue of high optical density in aerobic bacterial cultures, which occurs due to the aerobic cultivation of large volumes of bacteria. A microbial optical

density detection method based on a log amplifier is proposed. The main conclusions of this work are as follows:

To overcome the limited dynamic range of operational amplifiers and lock-in amplifiers, a logarithmic amplifier is employed to compress the input signal range, enabling the detection of a wide range of microbial optical densities. The corresponding detection circuit is designed, and a linear transfer function between optical density and the output voltage of the log amplifier is derived through theoretical calculations.

An experimental setup for optical density detection is constructed, and the linearity of the detection circuit is validated using standard optical density filters. The goodness of fit (R^2) reaches 0.98317, demonstrating the linearity of the detection system. Multiple measurements are conducted, and the standard deviation of the detected values is consistently below 0.001, confirming the stability of the system.

Three types of lactobacillus with four different concentrations are simultaneously measured using the experimental system and a spectrophotometer. After normalizing the path length, the optical densities measured by the experimental system are compared to those obtained by the spectrophotometer. The results show good agreement between the two methods, validating the accuracy of the experimental system in detecting optical density. Additionally, it is observed that the relationship between optical density and concentration is non-linear under high optical density conditions, consistent with the applicable conditions of the Beer-Lambert law.

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