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Automated rapid blood culture sensor system based on diode laser wavelength-modulation spectroscopy for microbial growth analysis

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ABSTRACT

An automated rapid blood culture sensor system was developed to detect CO_2 concentration levels in blood culture bottles and thereby monitoring microbial growth. The sensor system is based on diode laser wavelength-modulation spectroscopy (DLWMS), which offers low cost, high sensitivity, fast response as well as label-free and non-intrusive detection. The optical gas sensing module was based on a careful design to realize automated and rapid CO_2 detection with the assistance of control and storage elements. A 2-month field test was carried out at the Jinhua Guangfu Hospital, Jinhua, China for the new DLWMS based sensor system, which exhibits similar performance to a commercial BacT/ALERT 3D automated blood culture system in terms of accuracy but with significantly reduced detection time.

1. Introduction

Blood cultures in which microbial organisms reproduce in predetermined culture media under controlled laboratory conditions were pioneered in the early 20th century [1,2]. It is one of the primary diagnostic methods of microbiology and used as a tool to determine the cause of infectious diseases. When a patient shows signs or symptoms of a systemic infection, the result from a blood culture can verify that an infection is present and identify the type (or types) of microorganism that is responsible for the infection. For example, blood tests can identify causative organisms in severe pneumonia, puerperal fever, pelvic inflammatory disease, neonatal epiglottitis, sepsis, and fever of unknown origin (FUO). The mortality rate of infectious complications in critically ill patients is 25% and can be as high as 55.2% when infectious complications progress to the development of sepsis or severe sepsis, which has been reported in the United States, France, and Germany [3–6]. Therefore, rapid identification of the microbial growth is of the highest priority as this allows for early adoption of an antibiotic treatment.

At present, the detection of microbial growth is mainly divided into four classes: 1. dry weight measurement [7], 2. volume measurement [8], 3. turbidimetry [9] and 4. physiological index method [10]. Conventional techniques for the microorganism growth detection include the first three which are widely used in experiments due to their universality and easy operation. Nevertheless, the conventional methods require a long operation time and involve a large amount of manual operations. These disadvantages make the currently available techniques impractical for automated measurements. Furthermore, invasive operation can cause miscellaneous bacterial interference and the loss of the content medium, leading to a low accuracy and efficiency. Thus, conventional methods are unable to achieve a rapid and accurate analysis of microbial growth.

Hence, there is an urgent need for rapid and reliable diagnostic methods allowing for the detection of the growth of microorganisms in a fully automated manner. As the growth of microorganism is always accompanied by a series of changes of physiological index parallel to the growth quantity, a physiological index method has been used in microbial growth measurements in recent years. The biological indicators available for determination include deoxyribonucleic acid (DNA), ribonucleic acid (RNA), adenosine triphosphate (ATP), natural actomyosin (NAM), carbon dioxide (CO_2) production, oxygen (O_2) consumption, viscosity, transparency as well as heat production [11,12]. The amount of CO_2 produced in metabolic processes varies rapidly with time and reflects the state of bacterial growth. Techniques for detection of the presence of CO_2 based on colorimetric analysis have been applied to the study of microbial growth. For example, a commercially available BacT/ALERT 3D automated blood culture system was used in a clinical setting for blood culture monitoring [13–16]. The

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presence of CO_2 will make CO_2-sensitive receptors at the bottom of a culture bottle change from green to yellow, which can be detected by reflectance spectrometry. This method can be fully automated. However, the CO_2 receptors are easily contaminated by bacteria production, since the acquisition of a data point can take up to half an hour. Another drawback of this method is that the label-requirement not only increases sources of error in detection but also adds to detection cost and causes environmental pollution. An alternative realization is to utilize chromatography, which also requires a long response time and which is difficult to measure accurately in long term tests.

Diode laser wavelength-modulation spectroscopy (DLWMS) is a well-established technique that features high sensitivity for gas sensing and has been used for numerous applications such as environmental monitoring and combustion analysis [17–25]. This method was first applied to the measurement of the growth curve of microorganisms successfully by Shao et al. in 2016 [26]. Since CO_2 is one of the main metabolites of bacteria growth, its content can reflect the growth state of the bacteria. In this work, we developed an automated blood culture sensor system based on DLWMS and demonstrated a fast and accurate determination of the CO_2 concentration level that is produced during the microbial metabolic process. In contrast to the commercial BacT/ALERT 3D automated blood culture system based on colorimetric analysis, wavelength-modulation spectroscopy based on direct concentration measurement of CO_2 produced during bacterial growth is a non-intrusive, label-free and real-time monitoring technique

2. DLWMS for microbial growth analysis

2.1. Selection of target line for CO_2 detection

The most common non-extractive technique for online CO_2 detection is based on tunable diode laser absorption spectrometry (TDLAS) combined with wavelength modulation spectrometry (WMS) for noise reduction. Near-infrared distributed feedback (DFB) lasers are used to target the CO_2 overtone bands at 1.57 μm. However, the near-infrared CO_2 detection sensitivity is not sufficient for the CO_2 detection in the blood cultures because of the short optical path length. This technique can offer a ppb-level CO_2 detection sensitivity at 4.25 μm by means of mid-infrared quantum cascade lasers (QCLs) and interband cascade lasers (ICLs), where the absorption line strength is 3–4 orders larger than those in the near-infrared overtone bands. However the cost of mid-infrared laser sources and blood culture bottles made of mid-infrared material do not have any competitive advantages with respect to current commercial equipment based on colorimetric analysis. Therefore it is of interest to detect CO_2 concentration levels using the 20012-00001 overtone bands in the 2.0 μm wavelength region, which provides a compromise between detection sensitivity and cost.

In blood culture bottles, the CO_2 concentration gradually increases during bacteria metabolic processes, resulting in an increasing total pressure between 1 to 1.5 atmospheres. The water content almost reaches saturation at an operating temperature of 37 °C. The main spectral interference is from H_2O vapor. The simulated spectra of 380 ppm CO_2 and 4.5% H_2O between 4975 cm^{-1} and 5000 cm^{-1} at a temperature of 37 °C and a pressure of one atmosphere are shown in Fig. 1, based on the HITRAN database [27]. A light path length of 3 cm was used. From Fig. 1, it can be seen that the R16 absorption line located at 4991.26 cm^{-1} with a line strength of 1.29×10^{-21} cm/mol is free from H_2O spectroscopic interference, which improves the detection sensitivity and selectivity for a quantitative CO_2 concentration assessments. The following experiments employ the R16 line as the optimum CO_2 target line.

2.2. Experimental setup of an optical sensing system based on DLWMS

The experimental setup of the optical sensing system is shown in Fig. 2. A 2004 nm DFB diode laser with an output power of 10 mW was

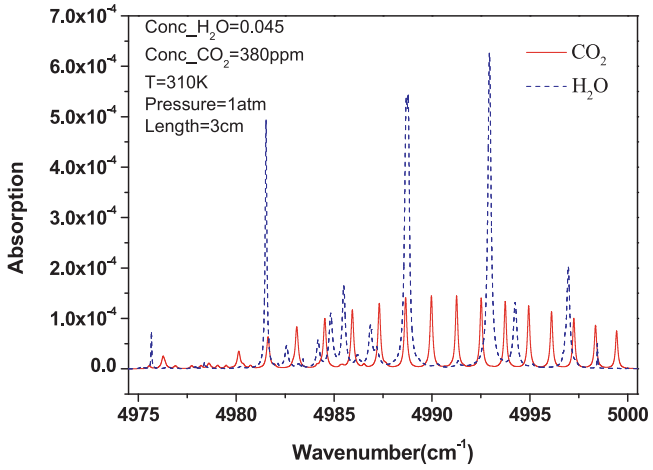

Fig. 1. Simulated HITRAN spectra between 4975 and 5000 cm^{-1} at one atmosphere and 37 °C for a gas compositions consisting of 380 ppm CO and 4.5% H_2O.

used as the excitation source. The center wavelength of the diode laser was set to 4991.26 cm^{-1} by a current driver and a temperature controller. The fiber-coupled diode laser was connected to a fiber attenuator (VOA50PM-APC, THORLABS). The exiting beam from the fiber attenuator was collimated with an antireflection coated lens (F260APC-1550, THORLABS) and then passed through the culture bottle. The culture bottle has a path length of 3 cm and a volume of 100 mL. The transmitted laser beam was focused onto a photodiode detector (DET100 M, THORLABS). The wavelength of the DFB diode laser was modulated sinusoidally at 33.1 kHz and demodulated by two custom-made digital lock-in amplifiers. The diode laser current was scanned by a 50 Hz triangular voltage wave from a custom made function generator. The signal from the photodiode detector was received simultaneously by the two lock-in amplifiers operating at 1*f* and 2*f* modes, respectively with a 10-ms time constant and 24-dB/oct digital filtering, which corresponds to an 8 Hz detection bandwidth. The 1*f* and 2*f* signals were acquired using a 16-bit DAQ card (USB-6351, National Instruments) that was connected to an industrial personal computer (IPC) with a program written in LabVIEW.

The fundamental of WMS can be found in References [20,26]. The acquired spectra were processed using a three-step algorithm. In the first step, a theoretical calculation of 1*f* and 2*f* waveforms, which can be written as Eq. (1) in the case of a small absorbance, was fitted to the 1*f* and 2*f* raw signals using a Levenberg-Marquardt least-squares fit procedure [28–30].

$$H_k(\bar{\nu}, \nu_a) = -2PXSLf \int_{-1/2f}^{1/2f} \phi[\bar{\nu} + \nu_a \cos(2\pi ft)]\cos(2\pi kft)dt \quad (1)$$

where $\bar{\nu}$ is the laser center frequency, ν_a is the modulation amplitude, f is the modulation frequency, $\phi(\nu)$ is the area normalized line shape function, P is the total pressure, X is the mole fraction of the absorbing species, S is the line strength of the target line and L is the path length. $H_k(\bar{\nu}, \nu_a)$ is referred to as the *kth*-order Fourier coefficient. When the value of k is equal to 1 or 2, $H_k(\bar{\nu}, \nu_a)$ corresponds to the 1*f* or 2*f* signals, respectively. However, the 2*f* signal cannot determine gas concentration level directly in harsh environments, due to the optical transmission losses from scattering, beam steering, mechanical misalignments, soot, and window contamination. Therefore it requires the transmission corrections. The 1*f* signal is an effective normalization signal. By normalization of the 2*f* signal magnitude with the 1*f* signal magnitude, common terms such as laser output intensity, optical- electrical gain, and laser transmission variations are eliminated [31–34]. Fig. 3 shows an example of the 2*f* and 1*f* signals of the CO_2 R16 absorption line located at 4991.26 cm^{-1} in the absence of bacteria in the culture bottle. The local minimum around the relative wavenumber of 0.5 cm^{-1} in

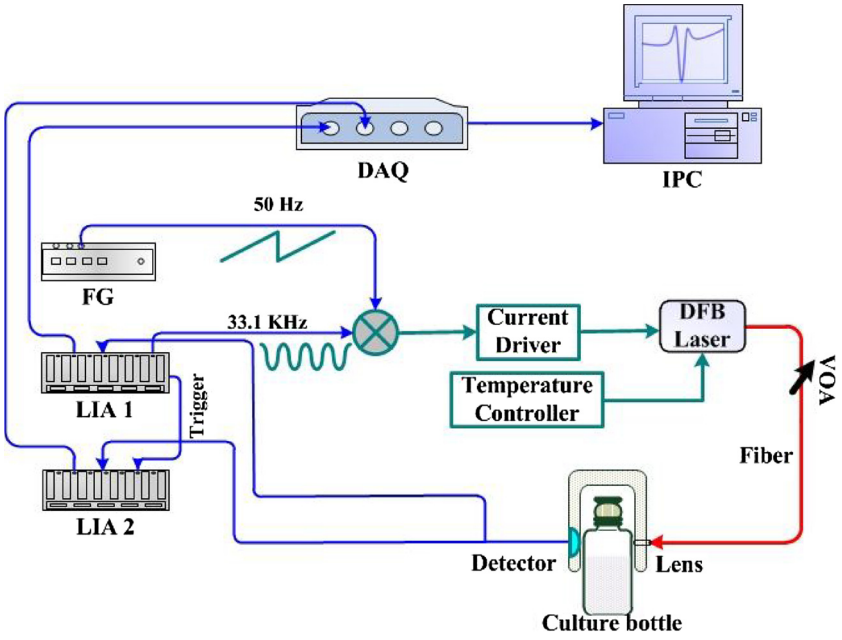

Fig. 2. Schematic diagram of optical sensing module based on DLWMS for CO_2 concentration levels. FG: function generator; LIA: lock-in amplifier; VOA: variable optical attenuator; DAQ: data acquisition card; IPC: industrial personal computer.

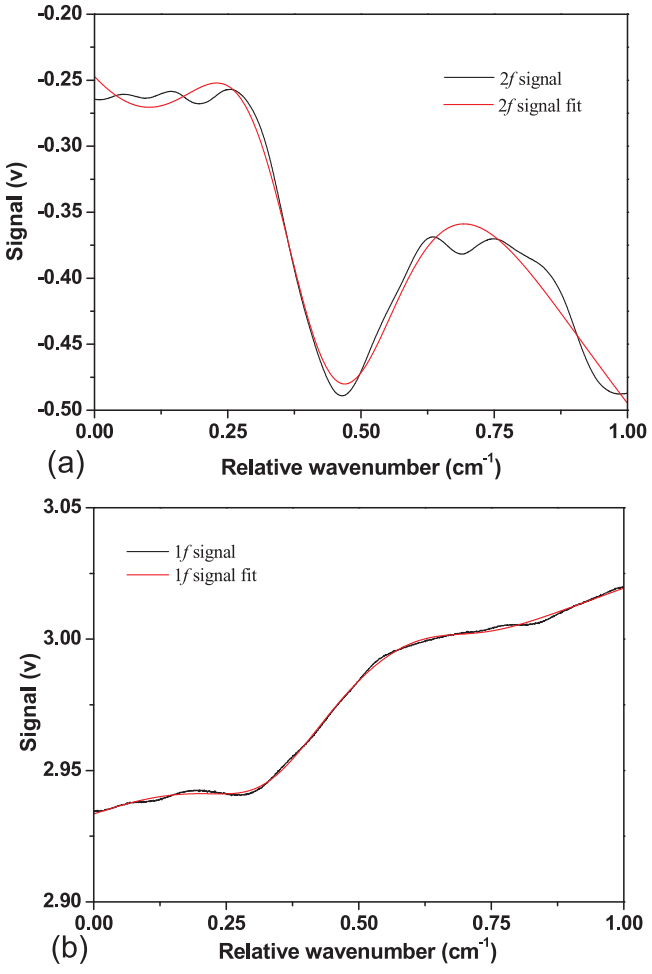

Fig. 3. Acquired 2*f* (a) and 1*f* (b) spectra (black lines) from culture bottle without bacteria, and their fitted spectra (red lines) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Fig. 3(a) corresponds to the peak of the absorption line. The horizontal axis of Fig. 3 (b) has the same wavenumber range from 0–1 cm^{-1} as Fig. 3(a). Therefore the peak of the absorption line corresponds to a positive offset value. The fitted lines are plotted in Fig. 3. A calibration-free measurement methodology is subsequently used to normalize the 2*f* signal magnitude with respect to the 1*f* signal magnitude on resonance in order to eliminate the influence of laser intensity and environmental factors. In the third step, the ratio of $H_2(\nu_0, \nu_a)$ and $H_1(\nu_0, \nu_a)$ was recorded.

3. Design of an automated rapid blood culture sensor system based on DLWMS

The automated blood culture sensor system consists of an optical sensing unit that has been described in Section 2.2 as well as a storage and control units. A 3D CAD model of the automated blood culture sensor system and a prototype photo are shown in Fig. 4(a) and (b), respectively.

All culture bottles were placed in a storage unit, which is a sample turntable installed in a drawer as shown in Fig. 5. The drawer was fixed by a tow chain and a slide rail, which can be taken out as a whole either automatically or manually. The sample turntable was divided into three storage circles A, B, C with 30, 20, 10 storage locations, respectively. In order to facilitate optical sensing, one fourth of the culture bottle body was outside the sample turntable. The automated sample stream switching between different bottles was performed by means of sample turntable rotation and only one storage location was detected by the optical sensing unit at the same time.

The control unit realized three functions of position control, temperature control and oscillation control. The position control determines the detection position of the optical sensing unit and the sample turntable removal. Pressure sensors were installed under each storage position. When the culture bottle was mounted, the position information and the mounting time were sent to the IPC. An electric sliding table with a positioning accuracy of ± 0.05 mm transported the optical sensing unit to the storage positions of the culture bottles according to the record of the IPC. Every half hour, a sensing cycle was completed from the circle A to the circle C and the sample turntable and the position sensing unit were reset. A temperature control module,

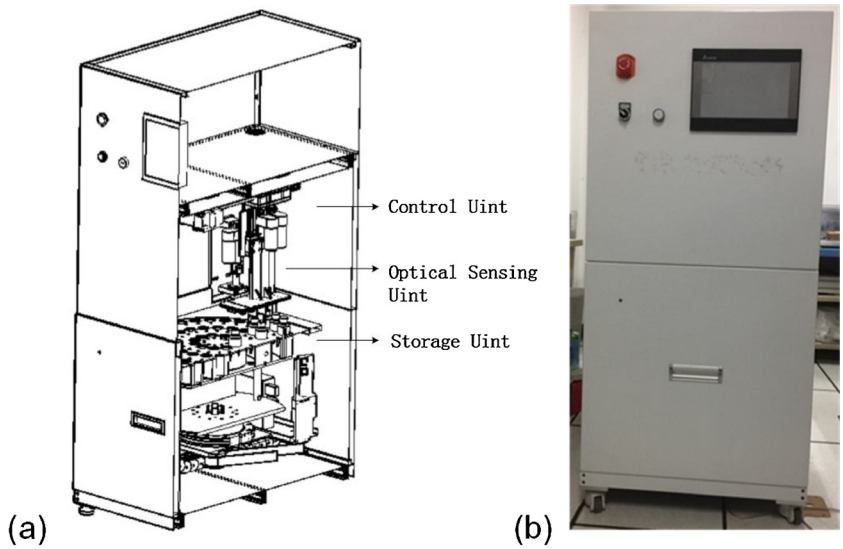

Fig. 4. (a) Cross-sectional view of a 3D CAD model of the automated blood culture sensor system; (b) prototype photo of the automated blood culture sensor system.

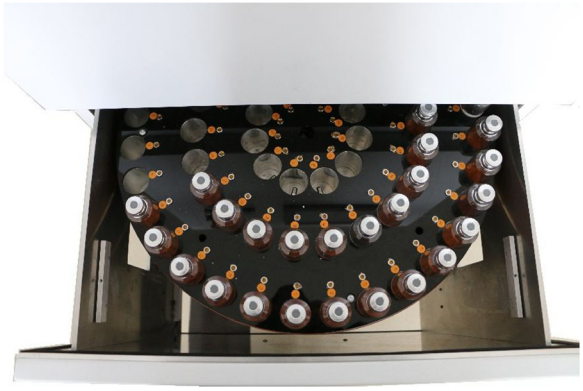

Fig. 5. Photo of sample turntable.

including heating sheets and thermocouple sensors, maintained the temperature of every culture bottle at a constant temperature. The cup-shaped heating sheets were installed in every storage position to make the culture bottle heating more evenly. The thermocouple sensor was used to detect the temperature of every culture bottle. The temperature control module can control the bottle temperature within ± 1°C. The oscillation control module is used for mixing of the medium and the blood samples in the culture bottle to promote the rapid growth of bacteria, which is realized by a servo drive and servo motor controller. In the culture process, the sample turntable was moved forward and backward every half hour in order to achieve uniform mixing of the fluid in the culture bottles. When a specific culture bottle is detected, the IPC will identify the bottle position and make a liquid crystal display (LCD) indicate its position and activate an alarm.

4. Performance assessment of automated blood culture sensor system based on DLWMS

4.1. Conventional colorimetric analysis

The developed DLWMS based blood culture sensor system was placed in the Jinhua Guangfu Hospital, Jinhua, China for a 2-month test period. For the purpose of a performance comparison, a commercial BacT/ALERT 3D automated blood culture system (BioMerieux) based on colorimetric analysis was employed. The bottom of each culture bottle used in the BacT/ALERT 3D system contains a CO_2 sensor, which is impregnated with water vapor and is separated from the broth medium by a semipermeable membrane. The membrane is impermeable to most ions, including hydrogen ions, and to components of media and degraded blood. It is nearly impermeable to water but is freely permeable to CO_2. When microbial organisms grow in culture medium, the released CO_2 reacts with H_2O to generate hydrogen ions (H+) as follows

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^- \quad (2)$$

which changes the pH value of the culture medium. The color of the CO_2 sensor varies from gray to yellow. The commercial system can identify positive blood culture bottles by observing its color variance. The specific detection method is illustrated in Fig. 6. A LED light beam illuminated the CO_2 sensor at the bottom of the culture bottle. The color can be detected by reflectance spectrometry using a photodiode.

In 1990, Thorpe et al. [35] studied the detection limit and response

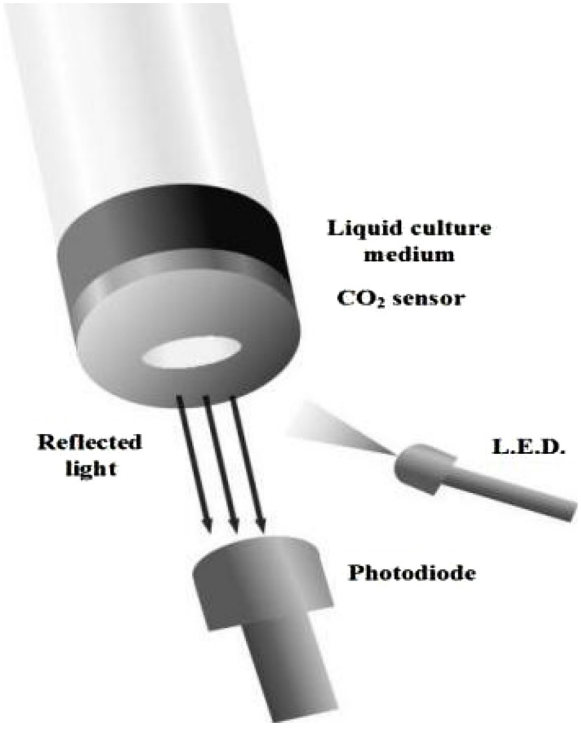

Fig. 6. Principle of a BacT/ALERT 3D automated blood culture system.

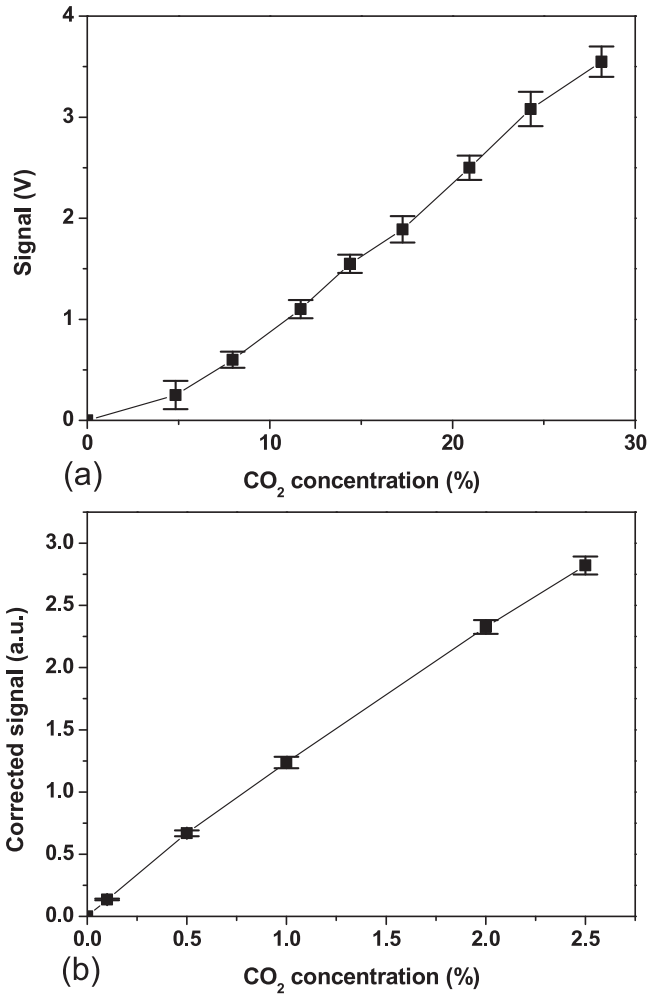

Fig. 7. (a) Detection responses of a BacT/ALERT 3D automated blood culture system to increasing amounts of CO_2 added to culture bottle [35]; (b) Corrected signal from the DLWMS system as a function of the CO_2 concentration levels. Standard deviations are indicated.

curve of a BacT/ALERT 3D automated blood culture system. An 80 mL bottle with a 30 mL sterile medium inside was employed to implement the experiment. The temperature of the bottle was set to 37 °C with an initial pressure of 1 atm. The different concentration levels of CO_2 were added to the bottle and allowed to equilibrate. The voltage signal from the photodiode was measured. For comparison purpose, the voltage signals in the CO_2 concentration range of 4.8–28% in the 50 mL gas space inside the bottle are shown in Fig. 7(a) [35]. It can be seen that an addition of 4.8% CO_2 can be detected by the conventional colorimetric analysis. The corresponding signal amplitude is 0.25 V with a standard deviation of 0.14 V, which results in a signal-to-noise ratio of ~1.8. Therefore, a minimum detection limit of 2.7% is achieved. The larger standard deviation limits the achievement of a higher detection sensitivity.

The same experiment was also carried out in the DLWMS system. The corrected signals based on the algorithm mentioned in Section 2 are shown in Fig. 7(b), which verifies the output signals from the DLWMS system are proportional to the concentration of CO_2. The corrected signals have a relative standard deviation of < ± 3%, which allows the DLWMS system to reach a lower detection limit. Based on the signal-to-noise ratio in Fig. 3, a minimum detection limit of 80 ppm was estimated, due to the fact that for Fig. 3, no bacteria existed in the culture bottle and hence a 380 ppm CO_2 background in ambient air was measured. The detection sensitivity is higher by three orders of magnitude than that of the commercial system. Due to clinical importance, each hour of earlier detection is crucial. Therefore, a rapider identification of the microbial growth is anticipated with the DLWMS system. A field test was implemented in the following.

4.2. Sample preparation and threshold determination

Samples of blood from patients with suspected infection in the Jinhua Guangfu Hospital, Jinhua, China were collected from May to June 2015. Each blood sample is equally divided into two parts for the DLWMS based sensor system and the commercial system. A total 173 blood culture samples for each system were tested in the reported study. The blood was collected using an aseptic technique, which requires that both the top of the culture bottles and the venipuncture site of the patient are cleaned prior to collection by swabbing with 70% isopropyl alcohol. Aseptic technology can effectively prevent bacterial interference from the skin.

It is often essential to isolate a pure culture of micro-organisms. The commercial system used a conventional method of bacterial culture, in which the desired bacteria are suspended in a nutrient medium. These cultures are ideal for the preparation of an antimicrobial assay. The experimenter can an inoculate liquid with bacteria and foster rapid growth. In order to make the results comparable with those from the commercial system, the DLWMS based sensor system was also used with a liquid sample.

Blood culture bottles were divided into two types according to the reproductive characteristics of the bacteria *i.e.* aerobic and anaerobic culture bottles. The aerobic culture bottles contain an 8 mL activated carbon suspension and a 22 mL culture media as well as an air environment containing CO_2 and oxygen above the medium. The anaerobic culture bottles contain both an 8 mL activated carbon suspension and a 32 mL culture media. However, the space above the medium was filled with nitrogen. All the blood culture bottles employed were of the same type from BioMerieux Inc. Therefore the effect of the culture bottle on the results can be ignored. After inoculating the culture bottles, the bottles were placed into two blood culture systems simultaneously and were operated at 37°C.

A change of CO_2 concentration levels in a culture bottle is mainly caused by bacterial metabolism. However, the growth of red blood cells in the bottle also produces a small dose of CO_2. It is critical to set a threshold to identify the CO_2 source and thereby identify the presence of bacteria. The ability of red blood cells to produce CO_2 varies from person to person due to individual differences. Nevertheless, from a statistical point of view, CO_2 produced by the red blood cell metabolism always fluctuates around a certain value. A pervious study showed that the CO_2 content increases monotonically with the growth of bacteria [26,36]. Thus when the obtained signal is larger than the threshold value, the bottle is identified as a positive result, otherwise the bottle is identified as a negative result.

In order to determine the threshold value, the signals of 20 groups of empty bottles (recorded as Si), and 20 groups of blood culture bottles with blood from healthy people (recorded as S2), for both aerobic and anaerobic cultures were measured. A standard deviation (STD) was obtained for a calculation of Si. According to the industrial standard, the threshold was set to $S2_{MAX}$ (the maximum of S2) +3STD. In this study the threshold values were set to 0.15 arbitrary units for an aerobic culture bottle and 0.055 arbitrary units for an anaerobic culture bottle, respectively.

4.3. Results and discussion

The total culture time was seven days. In order to compare the results obtained from the commercial and the DLWMS based sensor systems, the bottles in the DLWMS based sensor system were not removed until their counterparts in the commercial system were identified to possess a positive result or 7 days were reached.

The results recorded by the DLWMS based sensor system are shown

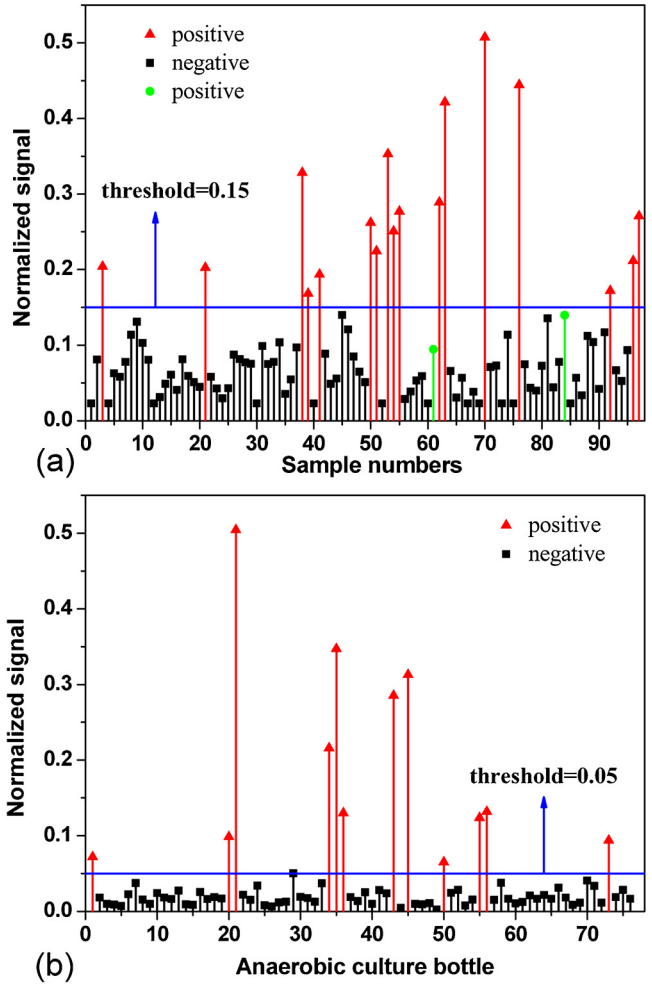

Fig. 8. Results of aerobic culture bottles (a) and anaerobic culture bottles (b) monitoring at the Jinhua Guangfu Hospital, Jinhua, China.

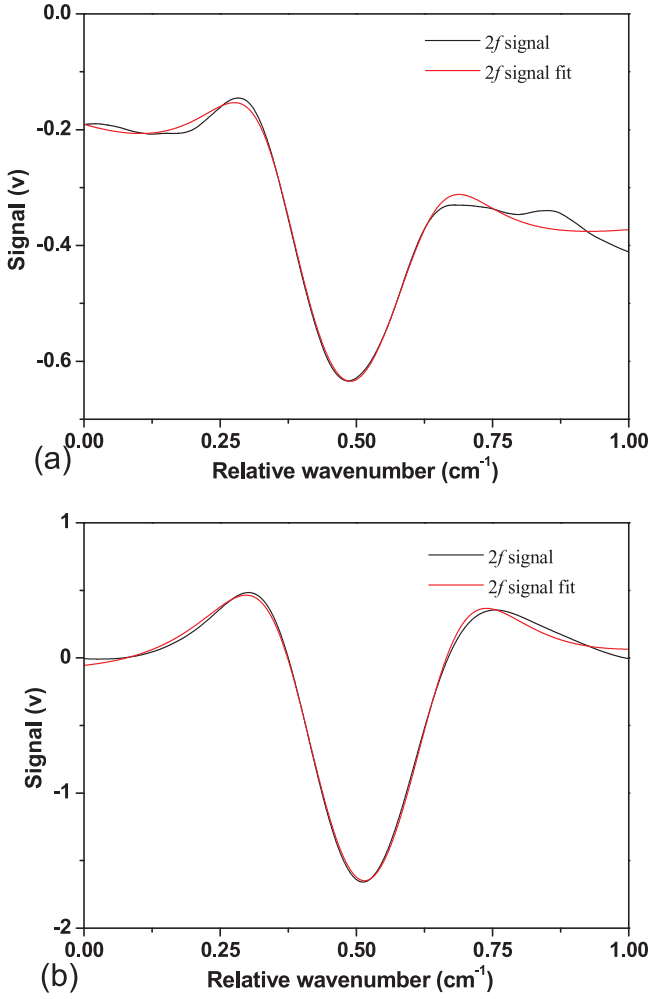

Fig. 9. 2*f* signals of #70 aerobic culture bottle from the DLWMS system when DLWMS based (a) and commercial (b) blood culture systems give positive alarms, respectively.

in Fig. 8 for both the aerobic culture bottles and the anaerobic culture bottles. The positive results were marked with a red triangle, while the negative results were marked with black square icon bars. The inconsistent results between the commercial and DLWMS based sensor systems were marked with circular icon bars. The red line heights of the positive results indicate the signal amplitudes from DLWMS sensor system when the commercial system identified a positive result. The black line heights of the negative results represent the signal amplitudes from DLWMS sensor system when the commercial system identified the negative result after 7 days were reached.

Based on Fig. 8(a) and (b), it was found that when the commercial blood culture system identified the culture bottle to be positive, the normalized signal values from the DLWMS based sensor system were greater than its set threshold. As a comparison, both the 2*f* signals of the #70 aerobic culture bottle from the DLWMS system at different times are depicted in Fig. 9(a) and (b), respectively. The 2*f* signal in Fig. 9(a) was acquired at the time when the DLWMS system gave a positive alarm; while the 2*f* signal in Fig. 9(b) was obtained when the commercial system gave a positive alarm. The amplitude of 2*f* signal in Fig. 9(b) is 4 times larger than that in Fig. 9(a), which implies that the DLWMS based sensor system can give a positive alarm at a lower CO_2 concentration level. In other words, the DLWMS based blood culture sensor system has a 3–12 h shorter positive alarm time than a culture measured with a commercial blood culture system. The large time variability of 3–12 h is due to that fact that the chemical reaction speed related to Eq. (2) in the BacT/ALERT 3D system depends on the pressure and temperature; moreover, the color discrimination of the commercial system is not very sensitive.

Fig. 8(a) shows the results of the aerobic culture bottles from the DLWMS based sensor system. It can be seen that the negative results obtained by the new DLWMS based sensor system are all below the threshold, which present a high degree of consistency with the results obtained with the commercial system. Moreover, the positive results also show consistency except for two bottles, # 61 and # 84, respectively, marked with circular icon bars. The culture bottles were identified to be a negative result by the DLWMS based sensor system, but a positive result by the commercial system. The #61 blood culture bottle came from a 52 years old man and was diagnosed to have *Listeria monocytogenes* in the blood. Further analysis of the hospital test revealed that the result from the commercial system was a false positive, since the real result was negative which was consistent with that obtained with the DLWMS based sensor system. The #84 blood culture bottle belonged to a 58 years old man. The culture was identified as a positive result by the commercial blood culture system and was found positive. In order to verify the reason for this behavior, the bacterial species were investigated and found to be fungi. Two reasons lead to this result: one reason was that the commercial system recognized the CO_2-sensitive receptors by their color but also analyzed them in terms of the bacteria growth rate by means of the color variation rate. When the color variation rate is faster than a setting threshold, the commercial system will directly give a positive result even if the color has not turned yellow yet. The DLWMS based sensor system only detected the

CO_2 concentration level. Thus, when the commercial system identified the bottle as a positive result after 2 days and 14 h, the CO_2 concentration in the bottle was below threshold. The other reason concerned the fungi growth rate. Differing from bacteria, fungi have an extremely rapid growth rate based on a logarithmic phase. After an extended culture period, our sensor system identified the sample as positive result after 2 days and 19 h, which is < a 7 days maximum culture time. This situation can be proved by analyzing the CO_2 concentration rate as measured by wavelength-modulated absorption spectroscopy.

Fig. 8(b) shows the results of the anaerobic culture bottles from the DLWMS based sensor system. The bottles identified by positive results were marked with triangular icon bars and the negative results were marked with square icon bars. There are no false positives and false negatives. The results from the DLWMS based sensor system are in good agreement with those from the commercial system.

5. Conclusions

An automated rapid blood culture sensor system based on DLWMS was developed and employed to detect CO_2 concentration level in blood culture bottles and thereby monitoring microbial growth. The performance of the designed blood culture sensor system was compared with a commercial automated blood culture system in the Jinhua Guangfu Hospital, Jinhua, China for a 2 months' period. The results from the DLWMS based sensor system were consistent with those obtained with a commercial system except for two samples, but with a 3–12 hours shorter positive alarm time. As a result, the new DLWMS based blood culture sensor system can contribute to the rescue time of patients. The developed DLWMS based blood culture sensor system can be readily adopted for rapid and reliable microbial growth analysis by hospitals. In a future development, an analysis of the change rate of the CO_2 concentration level will be added to accelerate the alarm time for fungi. Further improvements of the performance can be achieved by designing a multilayered structure for the storage unit to accommodate more culture bottles.

Acknowledgements

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