Development of a dissolved carbon dioxide sensor with a HPTS-incorporated polymer membrane

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Abstract
In this study a dissolved carbon dioxide sensor is made by using the fluorescent dye, HPTS incorporated into a polymer matrix, polyHEMA-co-EGDA. The HPTS-incorporated polymer membrane soaking in sodium bicarbonate buffer solution is put into a well in a 24-well microtiter plate, and silicone is smeared above the polymer membrane. This dissolved carbon dioxide sensor is characterized. It has no cross-sensitivity to sodium ion in the range of 20-200 mM and to pH in the range of 3-10. It has also a long lifetime over 10 days. The sensor has been applied to monitor the concentrations of dissolved carbon dioxide in fermentation processes for Escherichia coli and Bacillus subtilis.

Key-Words: Carbon dioxide; Fermentation; Fluorescence; Polymer membrane; Sensor

1 Introduction
Dissolved carbon dioxide (dCO2) is one of the key parameters in a variety of biochemical processes, including wastewater treatment, fermentation monitoring and control. Therefore, the quantitative detection of dCO2 is of importance. Optical sensors (optodes) for the detection of dCO2 has been developed on the basis of the same principle as the Severinghaus electrode except that a pH-sentitive dye replaces the pH electrode to detect the change in pH caused by the diffusion of CO2. Most of the optical dCO2 sensor uses indicator dyes with pKa values between 7.4 and 10.0. Recently a few fluorescent dyes have been used for optical CO2 sensing, for example, tetraphenylporphyrin (TPP) with a pH-indicator-alpha-naphtholph-thalein or perfluorochemicals (PFC) with 4-[(p-N,N-dimethylamino)benzylidene]-2-phenyloxazole-5-one (DPO). However, the fluorescent pH indicator, 1-hydroxypyrene-3,6,8-trisulfonate (HPTS) has been one of the most frequently used indicator dyes in optical CO2 sensor. HPTS is an inexpensive, highly water-soluble pH indicator with a pKa of 7.3 in aqueous buffer. The pH indicator dye could be incorporated into either a buffered hydrophilic support material such as hydrogel or a moisture-rich sol-gel glass substrate located beneath a hydrophobic gas permeable membrane.

In this work the HPTS is incorporated into polymer, pHHEMA-co-EGDA. The HPTS-incorporated pHHEMA-co-EGDA is cut to a membrane of 14 mm diameter and 1 mm of thickness. The dCO2 sensing polymer membrane is soaked with bicarbonate buffer solution and put onto a well in 24-well microtiter plate. The planar dCO2 sensing membrane is embedded in hydrophobic polymer silicone in order to prevent any liquid entering or exiting the HPTS-incorporated polymer membrane. This planar dCO2 sensor for the detection of dissolved CO2 has been characterized and applied to fermentation processes.

2. Experimental
2.1. Materials
HPTS trisodium salt and silicone were obtained from Sigma Aldrich. HEMA (hydroxyethyl methacrylate), EGDA (ethylene glycol dimethacrylate) and AIBN (isobutyronitrile) were purchased from Aldrich. The HEMA and EGDA were purified by vacuum distillation. All other chemicals such as sodium phosphate, sodium carbonate, etc., used for the preparation of the phosphate buffered saline and the bicarbonate buffer were of analytical

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grade and used without further purification.

2.2. Preparation of a HPTS-incorporated polymer membrane

The HPTS-incorporated polymer membrane (pHEMA-co-EGDA) was prepared from HEMA and EGDA by heat-polymerization. A monomer mixture of HEMA and EGDA was mixed with heat-initiator AIBN to a weight ratio of 98.9:1:1.01. To 10 ml of this monomer mixture solution, 2 mg of HPTS was added and dissolved well in a 15 ml test tube. The HPTS-incorporated pHEMA-co-EGDA rod with 15 mm diameter and 100 mm length was made after polymerization at 65 °C for 24 hours. A polymer membrane with a diameter of 14 mm and a thickness of 1 mm was cut from the pHEMA-co-EGDA rod. The pHEMA-co-EGDA membrane was placed into a bicarbonate buffer (0.1 M, pH 8) for 24 hours. The swollen pHEMA-co-EGDA membrane is extracted from the bicarbonate buffer and put into one well in a 24-well microtiter plate. After that a hydrophobic polymer silicone was smeared on the bicarbonate-buffered pHEMA-co-EGDA membrane and dried at room temperature for 24 hours. This bicarbonate-buffered pHEMA-co-EGDA membrane with silicone is defined as a dCO2 sensor or a dCO2 sensing membrane.

2.3 Characterization of dCO2 sensing membrane

The dCO2 sensing membrane was characterized by measuring the fluorescence spectra changes. A multifunctional microtiter plate reader (Safire 2, Tecan Austria GmbH, Austria) was used to measure the fluorescence intensity. Excitation and emission wavelengths of 455 nm and 515 nm, respectively, were chosen for recognizing the change in the fluorescence intensity of the dCO2 sensing membrane.

The effect of pH and temperature on the dCO2 sensor was investigated in the pH range from 3 to 10 and also from 30 °C to 40 °C. 1N NaOH or 1N HCl was used to adjust the pH values of 0.1 M PBS (Phosphate buffered saline) solution. 1 mL of PBS solution at pH 10 were added to a well containing the dCO2 sensing membrane in a 24-well microtiter plate. The microtiter plate was then inserted into the measurement chamber of the microplate reader to measure the fluorescence intensity. After performing the measurement at pH 10, the dCO2 sensing membrane was washed with distilled water several times, and then 0.1 M PBS solution at pH 9 was added to the well and its fluorescence intensity was measured. These steps were repeated for PBS solutions of lower pH. To test the effect of temperature, 1 mL of bicarbonate buffer solution at 128 mg/L dCO2 concentration was added to the well holding the dCO2 sensing membrane. The solution was incubated in the measurement chamber of the microplate reader for 10 minutes at a given temperature from 30 to 40 °C and its fluorescence intensity was measured.

Different sodium concentrations (0, 20, 50, 100, 200 mM) were also tested at a fixed dCO2 concentration of 128 mg/L.

The stability of the sensing membrane was determined from the change in the fluorescence intensity of the dCO2 sensing membrane at dCO2 concentrations of 128 mg/L after repeating the measurements at least three times. After the measurements, the sensing membranes were stored in 0.1M phosphate buffer (pH 7.0) under dark conditions at 4 °C.

2.4. Fermentation

The fermentation experiment was carried out using E.coli DH5α and Bacillus subtilis. The seed culture consisted of 1% inoculums in 20 mL LB medium incubated at 37 ºC with shaking at 300 rpm for 15 hours. The fermentation was carried out at 37 ºC in a 24-well microtiter plate, of which each well contained 1.5 mL of LB medium with 150 uL of the seed culture. The microtiter plate was placed on a home-made microtiter plate based bioreactor with online optical monitoring system (MABOOMS ) at 280 rpm. The 24-well microplate equipped with the dCO2 sensing membrane was placed in the UV-chamber for 24 hours (at overnight).

3. Results and discussion

3.1. Optical properties of the dCO2 polymer membrane

The fluorescence spectrum change of the dCO2 sensing membrane when excited at 455 nm under various dCO2 concentrations is also shown in Fig. 1. The fluorescence intensity decreased with increasing the concentrations of dCO2. The plot for the fluorescence intensity against dCO2 concentrations shows a linear response in the dCO2 concentrations range of 0-426 mg/L. The limit of detection (LOD) is about 0.5 mg/L dCO2, calculated as three times the
3.2. Characterization of the dCO2 polymer membrane

The response and recovery characteristics of the dCO2 sensing membrane are studied. The change in the fluorescence intensity due to the addition of different concentrations of dCO2 was measured. First, 1 mL of nitrogen-saturated 0.45 M NaCl is added to the well containing the dCO2 sensor and the fluorescence intensity is measured. After this measurement 0.9 ml of 0.45 M NaCl solution was removed from the well. As second measurement 0.9 ml of 20 mg/L of dCO2 is added to the well and the fluorescence intensity is measured. With this method the dCO2 concentrations in the well is varied randomly and the fluorescence intensity is measured. From the Fig the signal changes is reversible and measurement hysteresis is not observed. The time when 90 % equilibrium was reached (t\text{90}) is also recorded as response time. The response times are between 10 min and 20 min. This long response time is due to the presence of the additional silicone membrane. It can also arise because most of the CO2 crossing the dCO2 sensing membrane is consumed for the protonation of carbonate, bicarbonate, H2O and unprotonated dye, before equilibrium between CO2 across the dCO2 sensing membrane is established. The long response time can be improved by using thinner dCO2 sensing membrane as well as the silicone polymer.

The effects of pH and temperature on the dCO2 sensor are investigated. As shown in Fig. 2, the temperature and pH had only little effects on the dCO2 sensing membrane. That is, in the range of pH from 3 to 10, the fluorescence intensity of the dCO2 sensing membrane does not change at concentration of 128 mg/L dCO2 significantly. From 30 °C to 40 °C the dCO2 sensor has no significant change in the fluorescence intensity in the dCO2 concentration range from 21 mg/L to 426 mg/L.
3.3 Application of the dCO2 sensing membrane to fermentation processes.

The dCO2 sensing membrane is tested in a batch fermentation of E.coli and B.subtilis. Fig. 4 shows the dCO2 concentration in the media recorded online and the optical density of the media, which is used as a measure of the biomass production. From the figure the fermentation process undergo three different stages: lag phase, exponential growth phase and stationary phase. During the lag phase, the dCO2 concentration remained relatively stable. As the cells entered the exponential growth phase, a sharp increase in optical density was observed, followed by a corresponding increase in dCO2 concentration due to active respiration.

As the cells entered the stationary phase, the cells stopped growing and the dCO2 concentration begins to decline. This ability to track dCO2 simply should be great utility in fermentation and cell culture application.

4. Conclusion

An optical dissolved carbon dioxide sensor has been developed based on the HPTS-incorporated polymer membrane. The sensor has linear response to dCO2 concentrations in the range of 0-426 mg/L. It is not greatly affected by pH, temperature and sodium ions. The sensor has been applied for the monitoring of dCO2 concentrations during fermentation processes of E.coli and B.subtilis.

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