Ocean Insight

Noninvasive, Real-Time Monitoring of Oxygen and pH During E. coli Fermentation

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Application Note

KEYWORDS

- Biofermentation
- E. coli
- Oxygen
- pH

TECHNIQUES

- Oxygen sensing
- pH measurement
- Fluorescence
- Colorimetry

APPLICATIONS

- Bioprocess monitoring
- Biotechnology
- Pharmaceuticals
- Life sciences

Advances in sensor materials and optoelectronics have enabled novel optical sensors for applications in life sciences, pharmaceuticals, biotechnology and more. Compared with electrochemical sensing techniques such as galvanic sensors, optical sensors can be made in small and customizable form factors, allow non-intrusive measurements and do not consume the sample.

The principle of operation is to trap an oxygen-sensitive fluorophore or pH indicator dye in a sol-gel host matrix that is applied to the tip of a fiber, an adhesive membrane such as a patch or a flat substrate such as a microtiter plate. The indicator materials change optical properties in response to specific analytes and electronics measure the response. For oxygen, a phase fluorometer measures the partial pressure of dissolved or gaseous oxygen; for pH, a miniature spectrometer measures the colorimetric (absorbance) response of the pH dye.

Introduction

To demonstrate the viability of optical oxygen and pH sensors for monitoring biological parameters in bioreactor environments, we placed oxygen- and pH-sensitive adhesive patches inside a bioflask to monitor conditions during an E. coli fermentation reaction. Bioreactors are closed-environment systems where the cells are cultured under specific conditions to synthesize the final product. Such systems require constant monitoring of DO and pH to optimize the bioprocesses. Optical sensors were used to provide oxygen and pH measurements in the liquid phase of the bioreactor.

Monitoring of E. coli Fermentation Processes

E. coli fermentation for the production of recombinant proteins and other products is a complex, multistep process that requires rigorous control of culture conditions. For optimal fermentation to take place in the bioreactor, oxygen level and pH must be maintained within a narrow range. The bacterial cells consume oxygen as they replicate, so the culture must be sparged with oxygen as necessary. Also, the bacterial cells release metabolic by-products during the growth process, requiring periodic rebalancing of pH levels. Maintaining optimal conditions to ensure high yields requires frequent monitoring of oxygen and pH in real time during the reaction.

Various technologies exist for oxygen and pH measurements, including galvanic sensors, paramagnetic sensors, fuel-cell sensors and even paper. Yet all these techniques are invasive and require sampling the culture or placing a probe or sensor into the reactor vessel, which can introduce contamination or alter growth conditions.

Optical sensors provide non-contact alternatives to more invasive monitoring methods. Fluorescence can be used to monitor oxygen in the liquid and headspace phases of the bioreactor. Partial pressure of dissolved or gaseous oxygen is monitored using a phase fluorometer to measure the phase shift between the excitation signal of a blue LED and the emission signal of the fluorescence.

Figure 1: Optical pH sensor includes an electroformed gold mesh applied atop the sensor layer (inset), which both admits the sample and reflects the probe signal.

Non-invasive monitoring of pH in the liquid phase can be accomplished by measuring the colorimetric response of a pH dye using a miniature spectrometer. This method is ratiometric and relatively unaffected by drift. Colorimetric pH sensors also can be implemented as reflective sensors immune to the effects of solution color, sediment or turbidity.

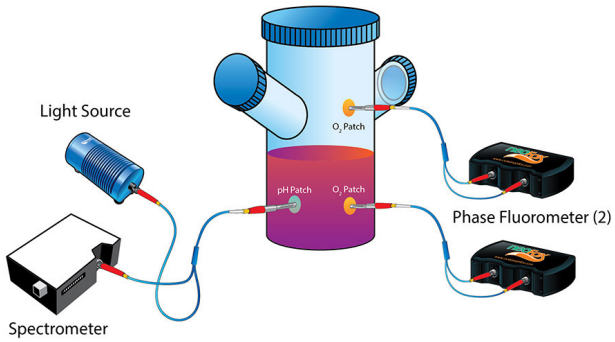

Figure 2: Oxygen and pH are monitored in a bioflask during an E. coli fermentation reaction.

Typically, reflective pH and oxygen sensors comprise patches consisting of an active sensor layer formed by doping a thin sheet of sol gel host matrix with target-sensitive fluorophores for oxygen sensing or indicator dyes for pH sensing. In some pH sensors a gold mesh can improve reflectivity and be customized for transmission levels, pore sizes, thicknesses and other parameters. As shown in **Figure 1**, the pH sensor layer is sandwiched between this mesh layer and a layer of adhesive that allows the patch to be affixed to the vessel wall. Oxygen sensor patches can be left bare or coated with a silicone overcoat to make them more robust for harsh environments.

Real-Time Noninvasive Monitoring of Oxygen and pH

To monitor oxygen and pH in the liquid phase during E. coli fermentation, oxygen- and pH-sensitive adhesive patches were placed inside a bioflask. The setup used for these measurements is shown in **Figure 2**.

The fermentation reaction was done using E. coli AG1 Competent Cells from Agilent Technologies (www.agilent.com) grown overnight at room temperature in LB broth growth medium. E. coli K12 growth medium supplemented with glucose, magnesium and thiamine was used as the medium for the fermentation reaction. Exponentially growing E. coli cells were added to the bioflask containing the K12 growth medium and nutrients to initiate the fermentation reaction.

Oxygen patches and pH patches were applied to the inside wall of the bioflask. The oxygen patches were attached to the container to monitor oxygen in the liquid phase of the bioflask (patches also could be placed in the headspace if desired). The pH patches were placed near the bottom of the bioflask to minimize the volume of buffer needed for calibration.

Oxygen was measured with a phase fluorometer equipped with a bifurcated optical fiber for the excitation and detection of fluorescence from the patches. Prior to adding the growth medium to the bioflask, the patches were calibrated at 0% and 20.9%. The fibers were situated normal to the outside surface of the flask pointing directly at the patch on the inside of the flask.

The pH was measured using a miniature spectrometer with tungsten halogen light source and a bifurcated optical fiber. One leg of the fiber transmitted light to the patch inside the container and the other leg read the response from the reflective patch inside the solution. Standard pH buffers were used for calibration. Absorbance curves were observed over time.

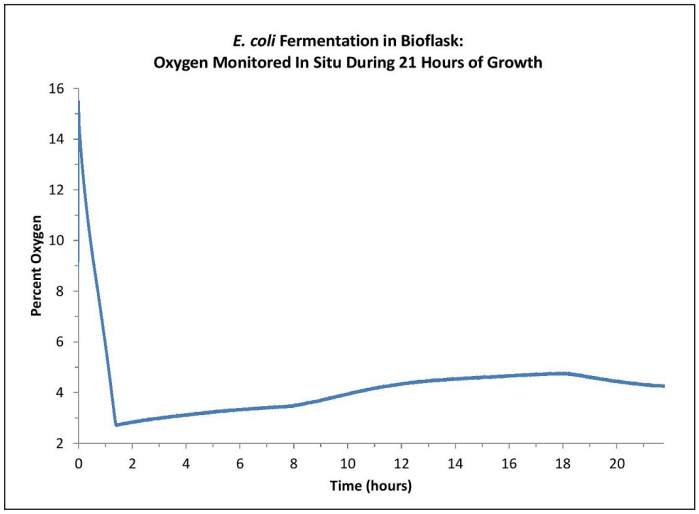

Figure 3: Oxygen concentration changes dramatically over 21 hours of E. coli fermentation.

Results

Oxygen consumption was fastest during the first two hours of the measurements as cell growth increased after the cells were added to fresh culture medium with added nutrients. Oxygen consumption tailed off and began to stabilize through the next six hours of the fermentation process **(Figure 3)**. Since the fermentation reaction was not run in a fully controlled environment, changes observed during the overnight period of the process (hours 8-18) may have been caused by fluctuations in the ambient environment.

As shown in **Figure 4**, over the 21-hour course of the experiment the pH dropped as cell growth increased, stimulated by exposure to fresh medium and nutrients. The pH dropped because the increased cell growth resulted in a buildup of metabolic by-products like lactic acid.

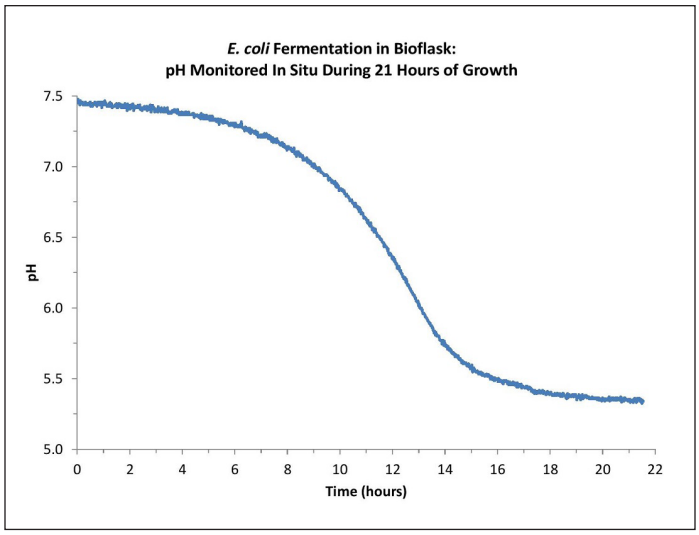

Figure 4: Over 21 hours of E. coli fermentation, pH levels drop as cell growth increases.

A more significant drop in pH observed between the eighth and fourteenth hours of the measurement may have been a function of ambient temperature increase, which increased cell growth as the temperature approached the optimal growth conditions for the cells. The tailing off in pH change observed at the end of the fermentation process was likely due to cell toxicity resulting from the buildup of lactic acid in the culture and depletion of critical culture medium components resulting in slowed cell growth.

Conclusion

The limitations of electrochemical-based oxygen and pH sensing are overcome by Ocean Insight optical oxygen and pH patches. Such patches can be integrated easily within a small-scale biosystem such as a bioflask and provide continuous, non-intrusive monitoring of key system parameters. The ability to monitor DO and pH in real time without perturbing a sealed environment can lead to an improved understanding of the processes in the bioreactor and, ultimately, help to facilitate the development of new biological products and processes.

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