

Enzyme-Free Electrochemical Sensing for Real-Time In-Process Monitoring

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Summary:

Real-time monitoring is essential for scalable bioprocessing, yet key metabolites are still measured off-line. We present an enzyme-free electrochemical sensor for continuous in-line and on-line operation under industrial conditions. The platform enables accurate, stable, and sterilizable monitoring in complex media. Performance is demonstrated during fermentation with high linearity, low detection limits, and long-term stability.

Keywords: process analytical technology, electrochemical sensing, glucose monitoring, bioprocessing, enzyme-free sensor

Background, Motivation and Objective

Process analytical technology (PAT) is a key enabler for the transition from empirical bioprocessing toward data-driven and autonomous operation [1]. However, most industrial and laboratory-scale processes still rely on off-line measurements for critical metabolites such as glucose. This limits temporal resolution and process insight.

Biosensors are very attractive due to their operational simplicity, low cost, and rapid analysis. They have been used effectively for real-time monitoring platform in a yeast fed-batch fermentation [2]. However, most electrochemical biosensors rely on enzymes, such as glucose oxidase, and this creates several practical limitations. The enzyme can lose activity over time, which leads to limited stability and short operational lifetime. These systems also often depend on oxygen, which can be a problem in some bioprocess conditions. In addition, they are usually sensitive to sterilization and may require frequent calibration or replacement.

Description of the New Method or System

We present an enzyme-free electrochemical sensing platform for monitoring of key

metabolites such as glucose, lactate and ammonium. The system is intended for both in-line use inside the bioreactor and on-line operation using an external flow-through setup. The overall idea is to enable continuous measurements under realistic bioprocess conditions, where traditional biosensors often have limitations.

The sensing principle is based on a solid-state electrode structure with synthetic sensing layers, so no biological components are used. The electrodes are fabricated as 3D pyrolytic carbon microstructures in a three-electrode chip configuration. This is done using photolithography followed by pyrolysis of SU-8 photoresist, which forms stable carbon structures [3].

The sensing itself is based on electrochemical reactions occurring at the electrode surface. The electrode is coated with selective materials that interact with the target molecules, and this leads to a measurable current signal. On top of this, a membrane layer is used to control diffusion of molecules to the surface and to reduce interference from larger compounds or particles in the medium. Additional coating layers are used to improve stability and reduce fouling during operation. The combination of coating and membrane is important to get a stable signal over time

and to make the sensor usable in complex fermentation media

For the in-line configuration, the sensor is integrated into a probe. The design helps to have more controlled mass transport at the sensor surface and reduces effects from bubbles and mixing in the reactor. For the on-line configuration, a flow-through cell is used, where the liquid is circulated outside the reactor and passes over the sensor. This makes it easier to handle and test the system. The platform is operated with a compact in-house potentiostat, which is used to control the measurement and record the signal.

Results

The glucose, lactate and ammonium sensors demonstrate a dynamic range of 0.1–150 mM, linearity above $R^2 > 0.996$, and detection limits below 0.05 mM. Accuracy and precision were below 5% and 7%, respectively, with response times of 1–3 minutes. The sensor maintains a stable and linear response after gamma sterilization (50 kGy) (Fig. 1). While a slight decrease in absolute signal is observed after irradiation, the linear relationship between current and glucose concentration is preserved across the full measurement range. This indicates that the sensing mechanism remains intact, and calibration can be maintained post-sterilization.

On-line glucose monitoring was performed using a flow-through setup during yeast fermentation (Fig. 2). The sensor shows stable operation over time and follows the general trend of glucose consumption throughout the 8 hours. The measurements are in reasonable agreement with HPLC data, which were collected every hour, following sampling and off-line analysis.

At a later stage, a glucose spike was intentionally introduced to check if the sensor is still responsive. This appears as a sharp increase in the signal. After this, the signal goes back to the expected level. This confirms that the sensor is still active, responds quickly to changes, and does not lose functionality during operation.

Conclusion

The presented enzyme-free electrochemical platform enables robust in-line and flexible on-line monitoring of multiple key metabolites. By combining sterilizability, long-term stability, and multi-analyte capability, this work addresses key limitations of existing biosensors and provides a practical route toward scalable PAT and autonomous bioprocessing.

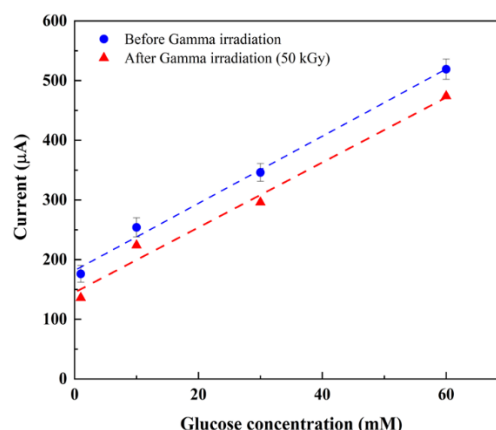


Fig. 1. Stable and linear glucose response is maintained after gamma sterilization, confirming sensor robustness.

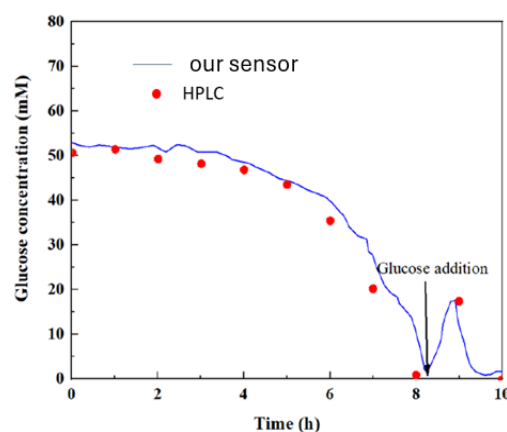


Fig. 2. On-line Glucose Monitoring During a yeast fermentation

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