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Enzyme Activity Monitoring In Industrial Solid-State Fermentation Processes Based On Colorimetric Loc Compatible With R2R Fabrication

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Extended Abstract

In this work we present a user-friendly, disposable lab-on-a-chip (LoC) for colorimetric enzyme activity monitoring in industrial solid-state fermentation (SSF) processes. The chip, compatible with roll-to-roll (R2R) extrusion coating fabrication, significantly reduces the cost per device improving the efficiency [1]. COC was chosen for the chip fabrication for its flexibility required in R2R manufacturing and its transparency necessary for colorimetric detection [2]. An adhesive tape is used to cover the structured microfluidic foil.

The chip works on the principle of capillary-driven flow microfluidics [3]. The user adds a drop in the inlet then the chip is self-filled by capillary action and the sample arrives to the reaction chamber. The flows continue until reaching the capillary pump, which removes the liquid excess and isolates the detection area where the enzymatic reaction takes place.

Amylase and cellulase were selected for this work due to its industrial importance in SSF processes. To be suitable on LoC, enzymatic assays should provide a robust signal output and easy handling for the final user. These requirements are fulfilled by one-step colorimetric assays, where a dye is released from a substrate by enzymatic action.

Two strategies have been studied for the integration of the enzymatic substrates in the chip. One the one hand, drop-casting of the reagents followed by freeze-drying. On the other hand, spotting of the reagents by piezoelectric deposition, with subsequently air-drying. Different storage conditions were tested to calculate the shelf-life of the LoC and provide optimal reagent stability.

To measure changes in enzymatic activity on-site in the SSF plant, a miniaturised colorimetric reader adapted to the LoC geometry and the specific enzymatic reactions has been developed. This device incorporates customized miniaturized

electronics design for electro-optical output and optic detection and data acquisition elements. Besides, an Android application to enable communication with the device.

First, a functional validation of the LoC was carried out with commercial enzymes. A calibration curve with increasing enzymatic concentrations was build up to calculate the limit of detection (LoD) of the system. Functional validation was then performed using enzymes extracted from real samples from a SSF plant. A simple and fast procedure was set up for the extraction of the enzymes from solid samples. Finally, the enzymatic activity of the samples obtained at different time-points of the fermentation process was measured in 5-10 minutes. The samples show increasing enzymatic activity indicating that as the fermentation process progresses, the amount of generated enzymes is higher.

In conclusion a LoC for one-step detection of amylase and cellulase was developed using R2R extrusion coating. Enzymatic activity was measured using a customized colorimetric reader, and the performance of the chip was validated and tested with commercial enzymes and real samples from a SSF plant. This technology enables quick analysis of enzymatic activity in enzyme-containing ferments.

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