

Through Use of an On-Line Fibre Detector to Determine the Biomass of a Filamentous Biocatalyst

Abstract

In bioprocess engineering, it's critical to keep an eye on all the important process parameters. On-line temperature, pH, stirring control, and data logging are just a few of the parameters for which sensors have been developed in the past. However, further development is required for biomass monitoring. The ability to measure the biomass of animal and insect cells is a limitation of every current non-invasive technology, including Near Infra-Red. Still, sample removal from the vessel is required for biomass monitoring of industrial bioprocesses containing filamentous microorganisms, which could compromise sterility. The application of a non-invasive optical sensor in the on-line monitoring of the biomass of the filamentous microorganism *Streptomyces coelicolor* A3 has been the primary focus of this research (2). The results successfully demonstrate that the optical sensor is sensitive in identifying various levels of biomass when compared to data from sensors measuring dissolved oxygen levels and off gas evolution. The raw output data from the biomass monitor were then directly compared to that data. As a result, the straightforward output data can be used to provide real-time information on the biomass levels of filamentous microorganisms, making it an extremely useful tool in bioprocess engineering.

Keywords: *Streptomyces coelicolor* • Non-invasive • Optical sensor • Filamentous • Biomass monitoring

Introduction

Bioprocess engineering focuses primarily on real-time monitoring and precise control of all critical process parameters (CPPs). High reproducibility, robustness, and productivity will all be guaranteed by this. Sensors for CPPs, such as temperature, pH, and stirring, are well-established; On the other hand, significant progress is needed for online monitoring of crucial process variables like biomass [1]. Analyse concentration profiles, such as glucose, are commonly used in near infra-red spectroscopy (NIRS) for growth monitoring, but its application is limited to dispersed cell growth.

Complex systems like vigorous stirring, high aeration, high cell densities, and complex media components like yeast extract and malt extract are used in industrial fermentations of filamentous microorganisms. The removal of a culture sample for off-line analysis is required by the majority of current methods for measuring biomass, which compromises sterility [2].

In the filamentous fermentation of *Streptomyces fradiae*, NIRS technology has previously been used to measure analytes like methyl oleate, glucose, glutamate, and ammonium but not biomass concentrations.

Discussion

In this study we zeroed in on the utilization of another harmless optical sensor for the constant

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Received: 01-Nov- 2022, Manuscript No. FMPB-22-81266; **Editor assigned:** 03-Nov-2022, PreQC No. FMPB-22-81266 (PQ); **Reviewed:** 17-Nov-2022, QC No. FMPB-22-81266; **Revised:** 22-Nov-2022, Manuscript No. FMPB-22-81266 (R); **Published:** 30-Nov-2022, DOI: 10.37532/2048-9145.2022.10(6).109-111

observing of biomass of the filamentous microorganism *Streptomyces coelicolor* (2). The genome of *S. coelicolor*, the typical actinomycete, was sequenced in 2002.

The Backlight stain (Cell Viability Kit;) and fluorescence microscopy were used to examine the viability of the culture. Molecular Probes, Eugene, OR, USA), which stains all population cells green and membrane-deficient cells red [3]. Bioprocess In accordance with the manufacturer's instructions, two identical autoclavable glass bioreactors (Applikon Biotechnology, Tewkesbury, UK) containing 1.8 L of culture media were assembled and sterilized for 30 minutes at 393.15 K. A Rushton impeller with 45 mm outside diameters was installed on both stirred tank vessels. *S. coelicolor* A3 (2) was inoculated into the single batch vessels, resulting in a final spore concentration of 1×10^6 spores mL⁻¹. Controlled conditions for the fermentation included 700 rpm, 30 °C, and 1.0 v/v/min aeration [4].

2CPPs CPPs, such as temperature, stirring, pH, dissolved oxygen, and evolution of carbon dioxide and oxygen, were continuously monitored and recorded [5, 6]. The instrument was connected to the fermenter and the optical sensor was mounted on the vessel's glass (Applikon Biotechnology, Gloucestershire, UK, EZ controller). Part of it is a monitor and an optical sensor that doesn't hurt. A group of 850 nm vertical cavity surface emitting lasers and detectors designed to detect light reflected from the vessel's cells at multiple laser-detector distances make up the sensor. The lasers are controlled, the signals from the detectors are read, and the signals from each laser-detector pair are analyzed by the monitor. The user is then shown the result. BugFree software is used to display the BugLab units in real time and calibrate them into dry weight in accordance with the manufacturer's instructions. Analysis of the Sensor [7, 8]. The sensor's performance was compared to the data from the dissolved oxygen analysis and gas evolution to see if there were any differences. At this point, growth slowed down (19–21 h), the culture morphology became pelleted, and there was evidence of a second phase of biomass accumulation (21–30 h). Following a period of cell death lasting up to 46 hours, biomass and CO₂ concentrations reached their highest levels after 30 hours of fermentation. The BugLab monitor also showed signs of cell lysis and a decrease in biomass [9].

Plots from one of the two batches represent the

results of the two bioprocesses, which behaved very similarly. Differences in the values collected from the gas evolution and dissolved oxygen levels were immediately linked to any changes in the raw output of the biomass screen in both cultures under the same stringent conditions. Unicellular microbial cultures, such as *Escherichia coli* and the budding yeast *Saccharomyces cerevisiae*, have previously been used to test the technology. This is, to our knowledge, the first report of a non-invasive optical sensor being used to monitor a filamentous fermentation [10].

Results

Because *Streptomyces* species are prolific antibiotic producers and account for approximately 70% of antibiotics on the market today, biomass measurements of these filamentous microorganisms are crucial to bioprocess engineering. There is a lot of evidence that the amount of biomass produced is directly related to antibiotic production. In order to achieve the highest antibiotic productivity, it will be beneficial to ensure maximum growth and precise documentation. A filamentous fermentation that is prone to pelleting can be tracked through the use of an optical device, as demonstrated in the study. The BugLab has proven to be highly reliable, sensitive to low cell concentrations, and reliable in our hands. At this point, growth slowed down (19–21 h), the culture morphology became pelleted, and there was evidence of a second phase of biomass accumulation (21–30 h). Following a period of cell death lasting up to 46 hours, biomass and CO₂ concentrations reached their highest levels after 30 hours of fermentation. The BugLab monitor also showed signs of cell lysis and a decrease in biomass. Plots from one of the two batches represent the results of the two bioprocesses, which behaved very similarly. Differences in the values collected from the gas evolution and dissolved oxygen levels were immediately linked to any changes in the raw output of the biomass screen in both cultures under the same stringent conditions. Unicellular microbial cultures, such as *Escherichia coli* and the budding yeast *Saccharomyces cerevisiae*, have previously been used to test the technology. This is, to our knowledge, the first report of a non-invasive optical sensor being used to monitor a filamentous fermentation.

Acknowledgement

None

Conflict of Interest

None

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