

Brief Report

The Application of an On-Line Optical Sensor to Measure Biomass of a Filamentous Bioprocess

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Abstract: Monitoring of all critical process parameters in bioprocess engineering is essential. Sensors have been previously developed for specific parameters such as on-line temperature, pH or stirring control and data logging. However, biomass monitoring needs further development. All current non-invasive technology, such as Near Infra-Red, is limited on biomass measurement of animal and insect cells. Biomass monitoring of industrial bioprocesses of filamentous microorganisms still requires sample removal from the vessel, which could potentially compromise sterility. This study has focused on the application of a non-invasive optical sensor in the on-line monitoring of the biomass of the filamentous microorganism *Streptomyces coelicolor* A3 (2). Raw output data from the biomass monitor were directly compared to data from the sensors measuring dissolved oxygen levels and off gas evolution and the results successfully demonstrate that the optical sensor is sensitive in identifying different levels of biomass. Therefore, it is possible to use the simple output data to provide real time information on biomass levels of filamentous microorganisms, a very powerful tool in bioprocess engineering.

Keywords: non-invasive; optical sensor; *Streptomyces coelicolor* A3 (2); filamentous; on-line; biomass monitoring

1. Introduction

Real time monitoring and precise control of all critical process parameters (CPPs) belong to the main focuses of bioprocess engineering [1]. This will ensure high productivity, robustness and reproducibility. The use of sensors for CPPs, such as stirring, temperature and pH are well established; however considerable progress is required for on-line monitoring of important process variables such as biomass [1,2]. Near infra-red spectroscopy (NIRS) is commonly practiced for growth monitoring through analyte concentration profiles, such as glucose, but its application is limited to dispersed cell growth [3].

Industrial fermentations of filamentous microorganisms are complex systems that employ vigorous stirring, high aeration, high cell densities and complex media components such as yeast extract and malt extract. Most current methods of biomass measurements require the withdrawal of a culture sample for off line analysis, which compromises sterility [4,5].

NIRS technology has been previously employed to measure analytes in the filamentous fermentation of *Streptomyces fradiae*, such as methyl oleate, glucose, glutamate and ammonium, but not biomass concentrations [6].

In this study we focused on the use of a new non-invasive optical sensor (BugEye® 100) for the real time monitoring of biomass of the filamentous microorganism *Streptomyces coelicolor* A3 (2). *S. coelicolor* is the model actinomycete and its genome was sequenced in 2002 [7].

2. Experimental Section

2.1. Organism

S. coelicolor A3 (2) was employed for this study. A pure culture of *S. coelicolor* A3 (2) was stored in a bacterial preservation kit (Technical Service Consultants Ltd., Lancashire, UK) at 193 K.

2.2. Culture Media

The culture media used in this study contained 4 g L⁻¹ yeast extract (Oxoid, Basingstoke, UK), 10 g L⁻¹ malt extract (Sigma, Gillingham, UK) and 4 g L⁻¹ D-(+)-glucose (Sigma) dissolved in H₂O.

2.3. Culture Viability

Culture viability was monitored using fluorescence microscopy and the BacLight™ stain (Cell Viability Kit; Molecular Probes, Eugene, OR, USA), which stains all cells within the population green and those with compromised membranes red.

2.4. Bioprocess

Two identical autoclavable glass bioreactors (Applikon Biotechnology, Tewkesbury, UK) containing 1.8 L of culture media were assembled and sterilised at 393.15 K for 30 min according to manufacturer's instructions. Both stirred tank vessels were equipped with a Rushton impeller with outside diameters 45 mm. The single batch vessels were inoculated with *S. coelicolor* A3 (2) to give a final spore concentration of 1×10^6 spores mL⁻¹. The fermentation was controlled at 700 rpm, 30 °C and 1.0 v/v/min aeration.

2.5. CPPs

CPPs, including, temperature, stirring, pH, dissolved oxygen and carbon dioxide/oxygen evolution were monitored and data logged in real-time. The optical sensor was mounted on the glass of the vessel and the instrument was linked to the fermenter (Applikon Biotechnology, Gloucestershire, UK, EZ controller).

2.6. BugEye® 100

A non-invasive optical sensor and a monitor are part of the BugEye® 100.

The sensor contains a group of lasers (vertical cavity surface emitting lasers) emitting at 850 nm and detectors manufactured to detect the light reflected from the cells in the vessel at multiple laser-detector distances [8].

The monitor controls the lasers, reads the signals from the detectors, and analyses the signals generated by each of the laser-detector pairs. The result is then presented to the user. Real-time display and calibration of the BugLab units into dry weight is completed through BugFree software following the manufacturer's instructions.

2.7. Sensor Comparison Analysis

The performance of the sensor was compared to data from the dissolved oxygen analysis and gas evolution in order to identify any possible discrepancies.

3. Results

Following a lag phase of approximately 10 h during which germination of the spores occurred (Figure 1), biomass levels increased rapidly over the period 10–19 h. This was accompanied by the decrease of dissolved oxygen and oxygen off gas evolution as well as the increase of biomass concentration and CO₂ production (Figures 2 and 3).

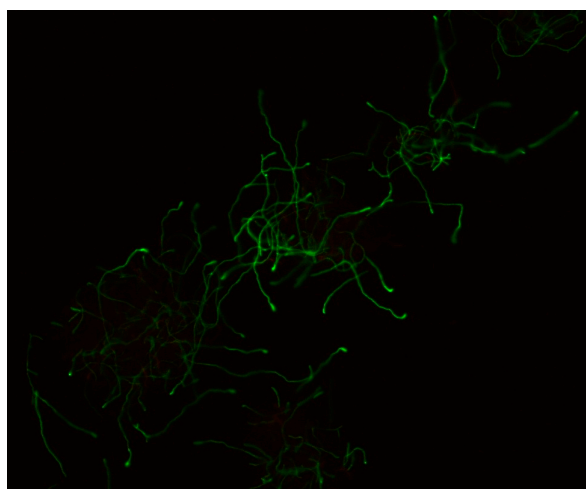


Figure 1. Fluorescent microscopy picture of *S. coelicolor*. The mycelia were stained with the fluorescent dye BacLight™, which stains all cells within the population green, and those with compromised membranes red.

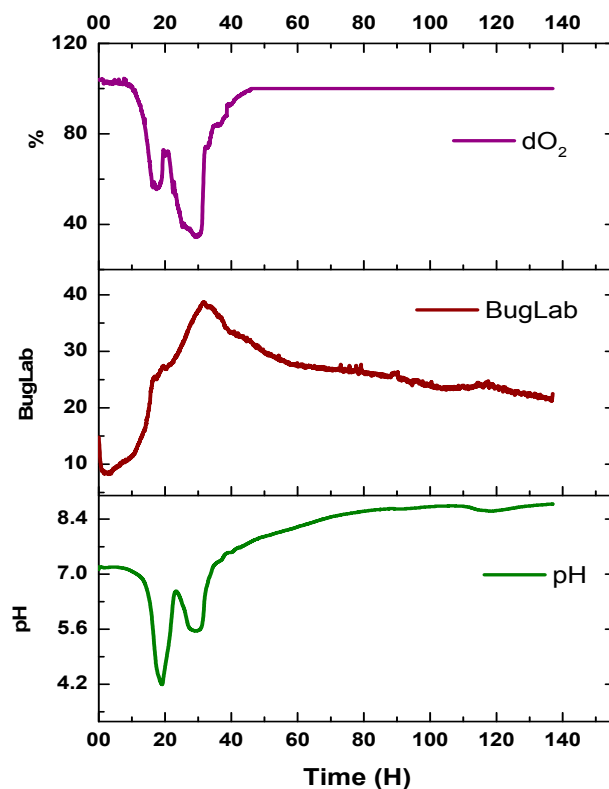


Figure 2. Fermentation profile of *S. coelicolor*. This includes changes in pH, dissolved oxygen and buglab biomass measurements during growth.

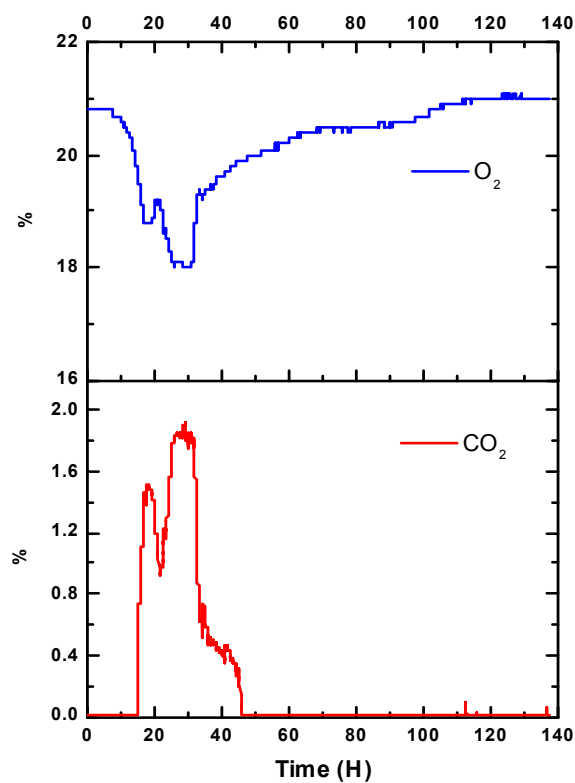


Figure 3. Off-gas analysis of the *S. coelicolor* fermentation. This includes oxygen evolution (consumption) and CO₂ production during growth.

Growth at this point then slowed (19–21 h), culture morphology became pelleted (Figure 4) and a second biomass accumulation phase was evident (21–30 h). Biomass and CO₂ concentrations reached its maximum after 30 h of fermentation following a phase of cell death up to 46 h, where dissolved oxygen reached 100% and the BugLab monitor indicated a decrease of biomass with signs of cell lysis (Figure 5).

Both bioprocesses were behaving very similarly and the results presented are the plots from one of the two batches. In both cultures, under the same strict conditions, any changes to the raw output of the biomass screen were immediately linked to differences in values collected from the gas evolution and dissolve oxygen levels. The technology has been previously tested against unicellular microbial cultures, such as *Escherichia coli* and the budding yeast *Saccharomyces cerevisiae* [8]. To our knowledge this is the first report of the application of this non-invasive optical sensor for the monitoring of a filamentous fermentation.

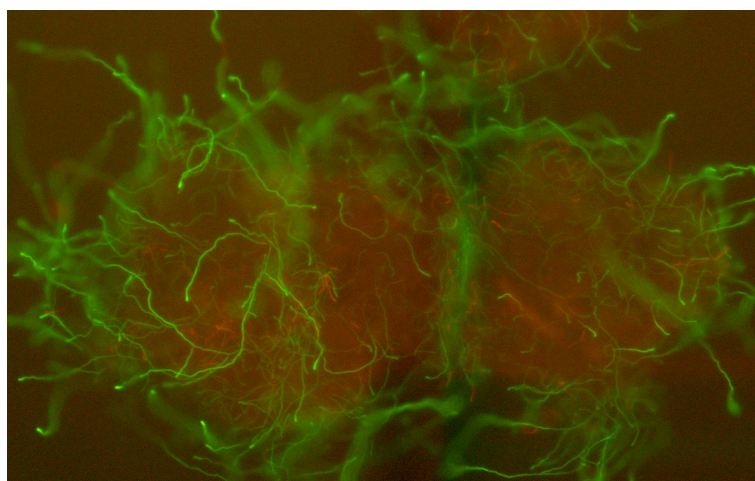


Figure 4. *S. coelicolor* pellets. The cells were stained with the fluorescent dye BacLight™. It is evident that the center of the pellets was dying due to oxygen and nutrient limitation.

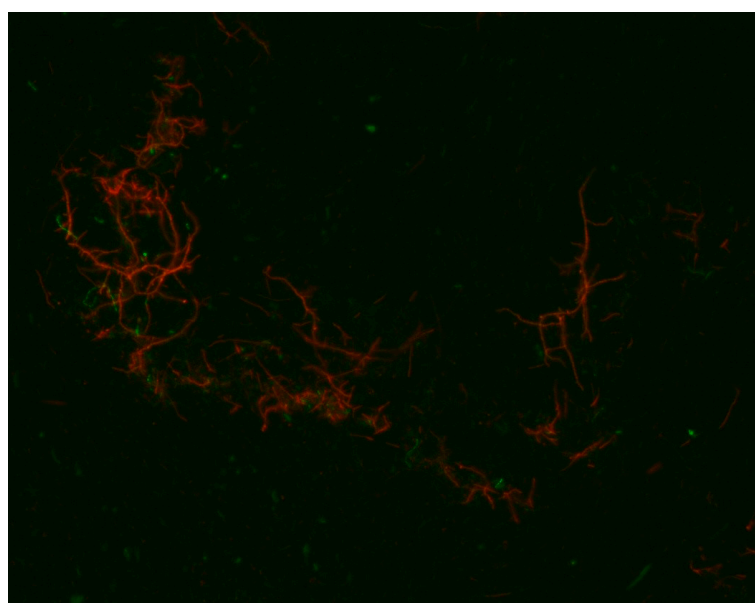


Figure 5. The *S. coelicolor* cells were stained red (BacLight™) due to lysis.

4. Discussion

Measuring biomass levels of *Streptomyces* species is very important in bioprocess engineering as the filamentous microorganisms are prolific antibiotic producers and account for some 70% of antibiotics currently on the market [9]. There is significant evidence that antibiotic production is directly linked to biomass levels [10,11]. Ensuring maximum growth and exact documentation will be beneficial in order to achieve the highest antibiotic productivity.

5. Conclusions

The study shows that an optical device can be used to monitor the progress of a filamentous fermentation that is prone to pelleting. In our hands the use of the BugLab has proved to be reliable, sensitive to low concentrations of cells and highly reliable.

Author Contributions

Both authors had equal input to both the research and the writing of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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