Biomass estimation using 2-D fluorescence measurement in recombinant *E. coli* cultivation

Takagi, M[1]; Jan, G[2]; John, J; Ran, C[2]; Rinas, U[3] and Hitzmann, B[2].
1)Butantan Institute – Biotechnology Center – Bioprocess Laboratory – Brazil; 2) Institute of Technical Chemistry – Hannover University – Hannover - Deutschland; 3)Helmholtz Centre for Infection Research – Braunschweig – Deutschland

Introduction

The method of 2D fluorescence spectroscopy covers a wide range of excitation and emission wavelengths and offers variety information about the intracellular compound and its products.

Bioview sensor is a multichannel fluorescence detection system composed by two Independent filter wheels: excitation (270-550 nm) and emission (310 -590 nm).

Fluorophors compounds like vitamins, tryptophan, phenylalanine, NADH can be detected simultaneously (Figure 1).

Fluorophors	Excitation/emission (nm)
Tryptophan	287/348
Tyrosin	280/300
NAD(P)H	340/450
FAD	365/520
FMN	450/530

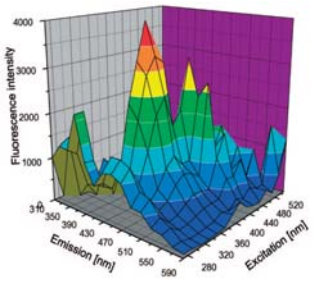

Figure 1. Fluorophors compounds and 3-dimensional representation of a fluorescence spectrum

For industrial applications, 2D fluorescence, is attractive because it is non-invasive which avoid problem of contamination, and considered as robust method. It has been used for prediction and monitoring the bioprocesses

The purpose of this work is to estimate the Biomass formation using 2D fluorescence technique during the cultivation of recombinant *Escherichia coli* BL21 DE3 expressing hFGF-2

Material and Methods

The experiment was performed in Batch cultivation using B.Braun Bioreactor with nominal capacity of 3 liters containing 1750 ml of mineral medium, pH controlled at 6.9 with ammonium hydroxide, DO controlled at 30% of air saturation, 1 VVM and 800 RPM as show figure 2. Induction was done with IPTG (1 mM) when OD_{600} achieved 15. Samples were collected each 1 hour for dry cell mass (DCW), OD_{600}, glucose and organic acids measurements and online data aquisited by RISP software.

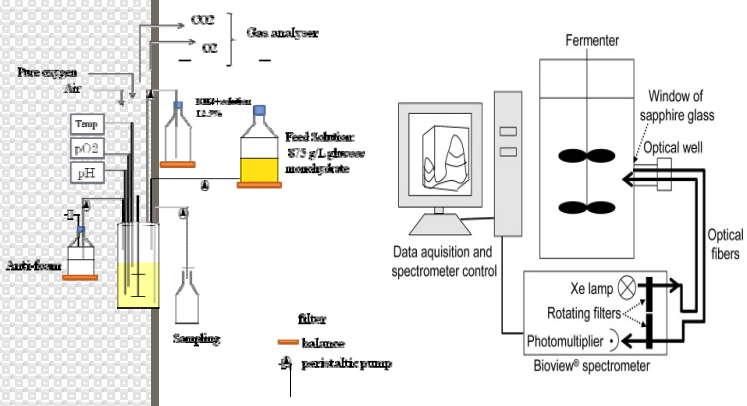

Figure 2. Set-up of the 2D fluorescence Bioview sensor and connection to bioreactor

Experiment

1) Batch culture – Non Induced– Model
2) Batch culture – Non Induced Prediction
3) Batch culture – Induced - Prediction

The fluorescence measurements were performed with the BioView (DELTA,Light & Optics, Lyngby, Denmark) spectrofluorometer, which is specially designed for on-line measurements. Figure 3 show a schematic picture of the BioView and how it was connected to the fermenter. Control of the spectrofluorometer and acquisition of measurement data was managed by CAMO software..

Results

In order to do multivariate data analysis, spectral data is exported to and processed in the software The Unscrambler.

The PLS model is based on only 3 PC's (principle components) and has a correlation of r=0,975 (250 elements), describing the correlation between fluorescence data and Biomass concentration (DCW).

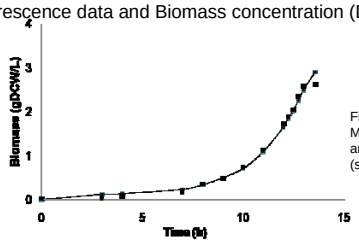

Figure 3. Batch culture without induction used as Model in multivariate data analysis . Estimated (line) and measured Biomass Concentration – DCW (scatter)

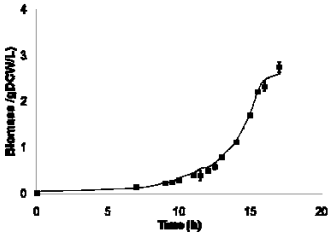

Figure 4. Batch culture without induction containing estimated (line) and measured Biomass data (scatter)

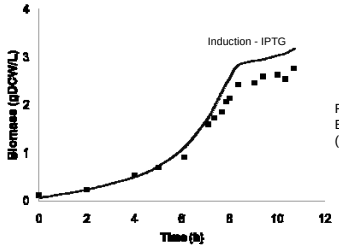

Figure 5. Batch culture with induction . Estimated (line) and measured Biomass data (scatter)

Data was analysed by PLS (Partial Least Squares) regression with the unfolded, but otherwise not modified, fluorescence data as x-variables and the Biomass concentration (DCW) as y-variables. This resulted in a model describing the correlation between fluorescence spectra and Biomass concentration (Figure 3). Fig. 4 shows the calculated values for Biomass concentration together with the reference values measured. The estimated Biomass concentration fit very well with the measured ones. The deviation is about 5 %. On the other hand after induction, the estimated and the measured values are different because the physiology of the bacterium changes with induction.

In conclusion, the estimated and measured values fit very well together and this kind of model might be applied to CO2 formation and glucose consumption, and for further experiments off-line sampling will not be necessary.

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