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Investigation of metabolic changes due to recombinant protein production in *E. coli* using 2D fluorescence spectroscopy and principal component analysis

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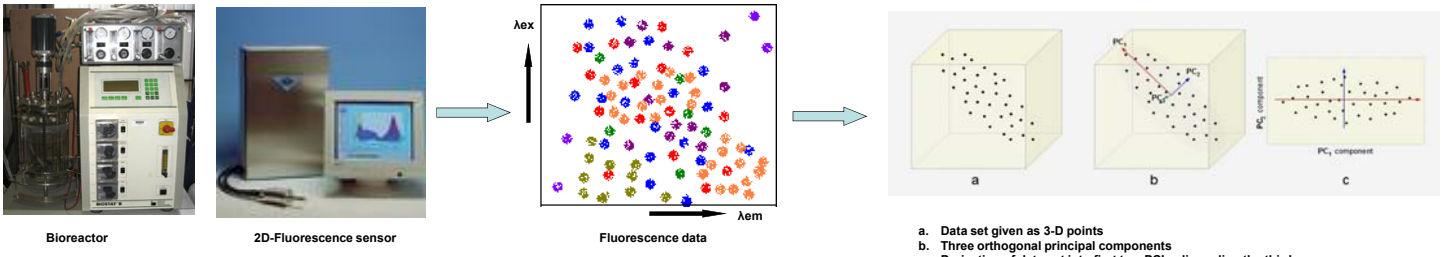
INTRODUCTION: 2-Dimensional fluorescence spectroscopy can be used as an on-line tool for continuous monitoring of bioprocesses. It monitors the metabolic state of cells on-line and gives useful information about various key molecules, e.g. FAD/FMN, NADH, pyridoxine, tryptophan, tyrosine and phenylalanine. This study gives a comparison in the metabolic state of *Escherichia coli* [BL21(DE3)+pET-29c-hbFGF] with and without recombinant protein (human basic fibroblast growth factor) production in batch cultivations using principal component analysis (PCA).



Bioreactor | 2D-Fluorescence sensor | Fluorescence data

a. Data set given as 3-D points
b. Three orthogonal principal components
c. Projection of data set into first two PC's, discarding the third one

Figure 1: Showing an illustration of principal component analysis

Principal component analysis of induced and uninduced cultures:

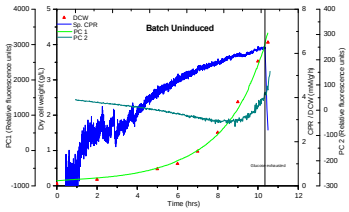


Figure 2

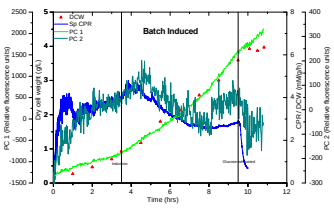


Figure 3

Figs. 2 and 3: Showing dry cell weight (g/L) (DCW), specific carbon dioxide production rate (mM/g/h) (Sp CPR), principal component 1 (PC 1) and principal component 2 (PC 2) values for uninduced and induced batch cultivations, respectively.

OBSERVATIONS AND CONCLUSIONS :

- PC 1 component correlates very well with biomass formation.
- PC 2 correlates with the specific carbon dioxide production rate in the batch cultivation with recombinant protein production. This shows that PC 2 contains information about major metabolic changes after induction.

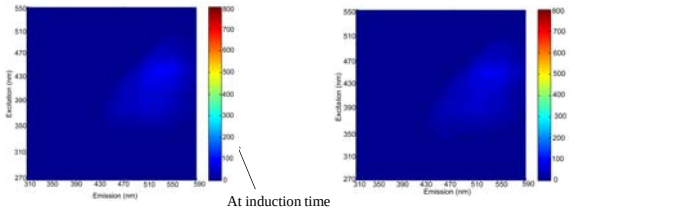


Figure 4 Induced batch

Figure 5 Uninduced batch

Fig. 4 and 5: Showing surface plots of fluorescence spectrum (at points with same biomass) at the time of induction (DCW = 0.8 g/L) and spectrum at the end of cultivations (DCW = 3.0 g/L) in induced and uninduced batch cultivations, respectively.

OBSERVATIONS AND CONCLUSIONS :

- At the end of the cultivation, the amount of FMN/FAD and NADH is relatively higher in batch cultivation with induction as compared to batch cultivation without induction.
- Amount of FMN/FAD and NADH are affected due to changes in the respiratory activity upon induction.
- This is indicated by the correlation of PC 2 with the specific carbon dioxide production rate as shown in Fig. 3.

SUMMARY: 2-D fluorescence spectroscopy can give useful insights in metabolic changes in *E. coli* in response to recombinant protein production. PC 2 data alignment with sp. CPR (only in induced batches) indicates that PCA contains information related to changes in the respiratory activity. These changes are mainly observed in the FMN/FAD and NADH region of the spectrum.

FUTURE PLANS:

- **To make partial least square-regression models for prediction of various off-line measurements e.g. biomass, glucose, acetate.**
- **To extend this analysis for fed-batch cultivations.**

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