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Journal of Biotechnology 121 (2006) 544–554
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Determination of cell mass and polymyxin using multi-wavelength fluorescence

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Received 18 March 2005; received in revised form 8 July 2005; accepted 4 August 2005

Abstract

Multi-wavelength fluorescence was applied for on-line monitoring of cell mass and the antibiotic polymyxin B in *Bacillus polymyxa* cultivations. By varying the phosphate and nitrogen content of the medium different polymyxin–cell mass ratios could be obtained. Using this strategy, it was possible to investigate if multi-wavelength fluorescence is able to give independent prediction of the two parameters.

Partial least square (PLS) regression was applied to establish mathematical relationships between off-line determined cell mass and polymyxin concentrations and on-line collected fluorescence data. For polymyxin one universal PLS model, with a correlation of 0.95 and a root mean square error of cross validation (RMSECV) of 35 mg l^{-1}, could be constructed. However, correlation between fluorescence and cell mass dry weight could not be established including data from all three types of cultivations. For data from each type of cultivation, separate models with high correlation and low RMSECV values were built. A large variation in cellular composition as a result of the different levels of nitrogen and phosphorus in the cultivations was the probable reason to the necessity of building three models.

The results of the present investigation indicate that production of polymyxin is concomitantly regulated by phosphate and nitrogen as the highest polymyxin yield on cell mass, 0.17 ± 0.01 g g^{-1}, was reached in the cultivations where both nitrogen and phosphate concentrations were kept low.

Keywords: On-line monitoring; Chemometrics; Multi-wavelength fluorescence; Cell mass; Polymyxin; *Bacillus polymyxa*

1. Introduction

Control and monitoring of submerged bioprocesses today is mainly based on few physical and chemi-

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doi:10.1016/j.jbiotec.2005.08.007

cal variables (Olsson et al., 1998; Sonnleitner, 2000). However, the current industrial development is towards analyzing more variables and in particular there is a large interest in analysis of biological variables. The quality of products and also the possibility to optimize production in submerged cultivations would be greatly enhanced if more on-line/real-time information about biochemical or biological variables were at hand.

Several promising spectroscopic methods for on-line monitoring of submerged microbial bioprocesses have emerged. Advantageous is that these methods are non-invasive, have no need for sampling and give real-time measurements (Marose et al., 1999; Ulber et al., 2003). Together with recent development within fiber optic techniques this has opened up the field of reactor monitoring and control.

One of these techniques, multi-wavelength fluorescence, is a relatively new technique for monitoring of bioprocesses and it has a high potential due to that it covers a broad range in the UV–vis area. The possibility to concurrently monitor several biogenic fluorophores, such as vitamins, coenzymes and proteins, gives a much better picture of the cellular activities than obtained by the earlier fluorescence sensors, which were limited to the range of NAD(P)H fluorescence (Li et al., 1991; Stärk et al., 2002). Furthermore, the broad wavelength range may also give a possibility to distinguish between signals from different fluorophores and reduce the problems with overlapping peaks and quenching.

The potential of multi-wavelength fluorescence has been evaluated by monitoring cultivations of different micro-organisms (Boehl et al., 2003; Haack et al., 2004; Hagedorn et al., 2004; Marose et al., 1998; Mukherjee et al., 1999; Skibsted et al., 2001; Solle et al., 2003; Sonnleitner, 2000; Tartakovsky et al., 1996a,b). In these studies information about substrates, cell mass, products and changes in metabolism could be extracted and the technique seems promising for monitoring of cultivation processes. Yet there are only few reports dealing with quantitative analysis of submerged cultivations using multi-wavelength fluorescence and chemometrics in combination.

The objective of the present work was to investigate if it is possible, with multi-wavelength fluorescence, to determine the two central, biological variables in polymyxin B production, namely polymyxin and cell mass. Polymyxin B is a cyclic, branched decapeptide synthesized by *Bacillus polymyxa*. The antibiotic is typically produced in batch cultivations where only standard variables such as temperature and pH are monitored. Temperature is the only variable that is controlled, a start pH is set, but no control is applied. Polymyxin B is analysed off-line by HPLC.

Furthermore, in the present study our aim was to investigate whether polymyxin and cell mass could be determined *independently* of each other. The production medium is a complex medium based on starch as carbon source and oatmeal as nitrogen source and in this medium production of polymyxin occurs parallel to the formation of cell mass. To achieve well-controlled conditions for evaluation of the sensor a defined medium containing soluble starch and ammonium was designed. By varying the phosphate and nitrogen content in the medium different polymyxin–cell mass ratios could be achieved and thereby uncoupling of the variables was possible. In addition to investigation of the usage of multi-wavelength fluorescence for independent detection of polymyxin and cell mass, the aim was also to establish quantitative on-line methods for monitoring. Partial least square regression models were applied in order to establish the mathematical relationships between on-line and off-line data.

2. Materials and methods

2.1. Cultivations

2.1.1. Strain and propagation

The strain used in this study, *B. polymyxa* POL4-2 (Alpharma ApS), was stored at −80 °C. The strain is derived from the wild type strain *B. polymyxa* NRLL 1551 through repeated steps of random mutagenesis and screening by Alpharma ApS. For propagation 200 μl frozen stock was transferred to baffled shake flasks (250 ml) containing 30 ml TSB-YE medium (40 g l^{-1} trypticase soy broth, 7 g l^{-1} yeast extract, pH 6.5) and incubated at 30 °C and 200 rpm for 7 h in an orbital incubator (GFL Gesellschaft für Labortechnik mbH, Burgwedel, Germany). Precultures, 400 ml medium (glucose 20 g l^{-1}, starch 20 g l^{-1}, $(NH_4)_2SO_4$ 20 g l^{-1}, yeast extract 10 g l^{-1}, K_2HPO_4 2.6 g l^{-1}, $FeSO_4 \cdot 7H_2O$ 0.1 g l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.5 g l^{-1}, NaCl