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Detection of phase shifts in batch fermentation via statistical analysis of the online measurements: A case study with rifamycin B fermentation

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Abstract

Industrial production of antibiotics, biopharmaceuticals and enzymes is typically carried out via a batch or fed-batch fermentation process. These processes go through various phases based on sequential substrate uptake, growth and product formation, which require monitoring due to the potential batch-to-batch variability. The phase shifts can be identified directly by measuring the concentrations of substrates and products or by morphological examinations under microscope. However, such measurements are cumbersome to obtain. We present a method to identify phase transitions in batch fermentation using readily available online measurements. Our approach is based on *Dynamic Principal Component Analysis* (DPCA), a multivariate statistical approach that can model the dynamics of non-stationary processes. Phase-transitions in fermentation produce distinct patterns in the DPCA scores, which can be identified as singular points. We illustrate the application of the method to detect transitions such as the onset of exponential growth phase, substrate exhaustion and substrate switching for rifamycin B fermentation batches. Further, we analyze the loading vectors of DPCA model to illustrate the mechanism by which the statistical model accounts for process dynamics. The approach can be readily applied to other industrially important processes and may have implications in online monitoring of fermentation batches in a production facility.

Keywords: *Amycolatopsis mediterranei*; Complex medium; Multivariate statistical analysis

1. Introduction

Fermentation processes have innumerable applications in food, agrochemical and pharmaceutical industries. For safety and health reasons, fermentation products are subjected to stringent regulatory standard (de Noronha Pissarra, 2004). Further, the cost-competitive nature of such products demands an optimal operation of the process (Nielsen, 1998; Nissen et al., 2000; Olsson et al., 1998; Vara et al., 2002). Therefore, fermentation process supervision is of particular importance to ensure consistent operation and thereby achieve high quality products. Industrial fermentation is typically carried out in batch or fed-batch mode to overcome the limitations of carbon and nitrogen catabolite repression (Bapat et al., 2006b). The key challenges in the monitoring of fermentation processes are batch-to-batch variation and complex dynamics. The batch-to-batch variation may result from the variation in the raw material quality or the variations in the seed culture. The variables that are desired to be monitored and controlled may include the biomass or product concentration(s). These variables are typically available only via offline measurements. Online measurements that are readily available include pH, temperature, agitation speed, dissolved oxygen, and exhaust CO_2, and O_2. However, these measurements do not give direct information on the state of the process (Vaidyanathan et al., 1999).

Abbreviations: Glc, glucose; DSF, defatted soybean flour; CSL, corn steep liquor; AMS, ammonium sulfate; DPCA, dynamic principal component analysis; SP, singular point; PC, principal component; PLS, partial least square

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Industrial fermentations typically use a multi-substrate complex medium, which may result in sequential and/or simultaneous utilization of the available substrates (Bapat et al., 2006a; Bapat and Wangikar, 2004). The metabolism in each phase is different and hence deserves its own consideration in terms of modeling, and supervisory control strategy (Konstantinov and Yoshida, 1989; Muthuswamy and Srinivasan, 2003). In addition, it is desirable to minimize offline sampling and the concomitant risk of contamination, yet obtain sufficient information on nutrient uptake and product formation in real time. As a result, online identification of phases and phase shifts in complex media is of critical importance.

The recently published methods for the identification of phase shift via online measurements have required the quantitative evaluation of key components such as the biomass concentration. Consequently these methods require advanced sensors such as infrared/mass spectrometers (Feng and Glassey, 2000; Grube et al., 2002), electronic nose (Bachinger and Mandenius, 2001; Pinheiro et al., 2002), or calorimetric sensors (Voisard et al., 2002). In addition, these methods suffer from the disadvantage that extensive time and experience are often required to implement them. Further, the low signal-to-noise ratio (Schugerl, 2001) and specific requirements of aseptic conditions (Clementschitsch and Bayer, 2006) of such sensors has limited their application in large-scale industrial processes. Another class of methods has focused on utilizing the routinely available online data for qualitatively identifying fermentation phases. Qualitative trend analysis and expert system are the two most common methods belonging to this class. A formal framework for deducing process trends from the online process variables was developed (Cheung and Stephanopoulos, 1990) and applied to fermentation data (Stephanopoulos et al., 1997). Alternatively, (Srinivasan et al., 2004) proposed a clustering approach using similarity factor derived from dynamic principal component analysis for process state identification. The approach relied on identifying the steady states to locate and subsequently segment historical data into different process phases. Consequently, it is not readily applicable in batch fermentation processes, where steady states do not normally exist. An expert system uses process knowledge gathered from experts such as biochemical engineers, biochemists, and microbiologists and coded in forms of "if–then" rules. These rules may be crisp or based on fuzzy logic (Kamimura et al., 1996). However, the limitation of expert system technique is that it is system-specific and difficult to customize for different fermentation processes (Venkatasubramanian et al., 2003).

Here, we present a method for the detection of phase shifts in batch fermentation via dynamic principal component analysis (DPCA) of the online measurements. We illustrate the application of the method for rifamycin B fermentation. Rifamycin B is a polyketide antibiotic from ansamycin family with a pronounced anti-mycobacterial activity and is extensively used in clinical treatment of tuberculosis, leprosy and AIDS-related mycobacterial infections (Sepkowitz et al., 1995). Further, we analyze the DPCA model in terms of the loading vectors in an attempt to understand the mechanism by which the DPCA model uses the process history.

2. Materials and methods

2.1. Experimental methods

2.1.1. Strain and fermentation medium

Prof. Heinz Floss (Washington University, USA) kindly donated the rifamycin B overproducing strain of *Amycolatopsis mediterranei* S699 that does not require barbital (Yu et al., 2001). The preculture was propagated as described by (Kim et al., 1996). One hundred and fifty milliliters of pre culture (10%, v/v) was used to inoculate the bioreactor. The media contained (per liter of distilled water) glucose, 80 g; potassium phosphate, 1 g; magnesium sulphate, 1 g; ferrous sulfate, 1 g; zinc sulfate, 0.010 g; cobalt chloride, 0.0030 g. In addition, the medium contained one or more of the following: ammonium sulfate, 4 g; potassium nitrate, 5.1 g; defatted soybean flour (DSF), 8 g; corn steep liquor solids (CSL), 8 g. After adjusting the pH to 7.0 with 1 N sodium hydroxide, the fermentor was sterilized by autoclaving at 121 °C for 15 min.

2.1.2. Bioreactor and cultivation conditions

Batch cultivations were conducted in 6.5-l BIOSTAT® B (BBI; B. Braun Biotech International, Schwarzenberger, Germany) bioreactor at working volume of 1.501 at 28 °C. The pH and the dissolved oxygen (pO_2) were recorded by using autoclavable pH-electrode and polarographic pO_2-electrode (INGOLD, USA), respectively. Agitator speed was used as a control variable to maintain dissolved oxygen at 40% via cascade control. Mass flow controller (BBI, Germany) was used to supply a constant airflow of 1.0 vvm (volume of air per minute per volume of media). The concentration of O_2 and CO_2 in the exit gas stream from the bioreactor were measured by infrared spectroscopy and paramagnetic analysis, respectively (Analyzer BINOS1002M® with sample conditioning unit, Rosemount analytical, Germany).

2.1.3. Analytical techniques

Samples were drawn from the fermentation medium at regular intervals to analyze the dry cell weight and the concentrations of glucose, ammonium sulfate, free amino acids and rifamycin B as described previously (Bapat et al., 2006a). Glucose was analyzed via RI detector on HPLC (Hitachi, Merck KgaA, Darmstadt, Germany) using HP-Aminex-87-H column (Biorad, Hercules, CA, USA) at 60 °C. The concentration of free amino acids was estimated via the ninhydrin method (Moore, 1968). The concentrations of the ammonium and nitrate ions were determined by the respective ion specific electrodes (EA940 Ion analyzer, Thermo Orion, USA). Rifamycin B was detected on spectrophotometer (V-540, Jasco, Tokyo, Japan) at a wavelength of 425 nm.

2.2. Data analysis methods

2.2.1. Principal component analysis

Principal component analysis (PCA) is a linear dimensionality reduction technique, which is optimal in capturing the variance in the data. It determines a set of orthogonal vectors,