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Real time phase detection based online monitoring of batch fermentation processes

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ABSTRACT

Industrial fermentations conducted in a batch or semi-batch mode demonstrate significant batch-to-batch variability. Current batch process monitoring strategies involve manual interpretation of highly informative but low frequency offline measurements such as concentrations of products, biomass and substrates. Fermentors are also fitted with computer interfaced instrumentation, enabling high frequency online measurements of several variables and automated techniques which can utilize this data would be desirable. Evolution of a batch fermentation, which typically uses complex medium, can be conceptualized as a sequence of several distinct metabolic phases. Monitoring of batch processes can then be achieved by detecting the phase change events, also termed as singular points (SP). In this work, we propose a novel moving window based real-time monitoring strategy for SP detection based only on online measurements. The key hypothesis of the strategy is that the statistical properties of the online data undergo a significant change around an SP. The strategy is easily implementable and does not require past data or prior knowledge of the number or time of occurrence of SPs. The efficacy of the proposed approach has been demonstrated to be superior compared to that of reported techniques for industrially relevant model organisms. The proposed approach can be used to decide offline sampling timings in real time.

1. Introduction

Fermentation processes are widely used in food, pharmaceutical, agrochemical and chemical industries. The production units range from small scale for biopharmaceuticals to large scale for bulk chemicals. A majority of the processes are operated in a batch or semi-batch mode. Intense competition and regulatory requirements pose severe demands on consistency of these batches in terms of the end of batch productivity and product quality [1]. However, fermentation processes are subject to intrinsic batch-to-batch variability due to variability in raw material quality, state of the seed culture and operator skills. It is therefore desirable to automate monitoring, fault detection and diagnosis and control of fermentation processes. This can lead to improved process reliability, product quality and productivity as well as reduced development time, manpower inputs and cost of production [2]. Typically, during operation, the product quality and batch performance are monitored via off-line measurements of concentrations of the product, byproducts, biomass and substrates. These measurements are expensive, labor intensive and time consuming, are obtained at low frequencies (e.g., every few hours) at pre-defined intervals and hence, may not always lead to timely information about the status of the batch. Further, in some processes, the product formation begins only towards the later parts of the batch and this leads to additional difficulty in adequately monitoring the process using these offline measurements [3]. Fermentors are typically equipped with several on-line sensors such as pH, temperature, concentrations of dissolved oxygen (DO) and carbon dioxide and partial pressure of oxygen and carbon dioxide in the exhaust gas. These measurements are inexpensive, usually available at high frequencies (e.g., every few seconds) and are obtained in an automated fashion. Hence, there is enormous potential to use these measurements to effectively monitor batch fermentation processes.

In the general process systems engineering literature, several different techniques have been reported for process monitoring and fault diagnosis [4]. These can be broadly classified as process model based, knowledge based and historical data based. The success of any model based strategy depends critically on the

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adequacy of the underlying model. Industrial fermentation processes typically employ complex media with multiple substitutable carbon and nitrogen substrates, which leads to difficulties in developing adequate process models. Further, several aspects of fermentation processes such as the dynamic evolution of pH and concentration of dissolved oxygen, are not well understood in general and this may lead to additional difficulties in developing reliable process models. Hence, model based strategies may not be suitable for monitoring of majority of industrial fermentation processes. Knowledge based monitoring techniques such as those based on fuzzy logic require expert knowledge of the system and therefore are system specific [4,5]. Such expert knowledge may not be available for the system of interest. Further, even for cases where such knowledge exists in terms of the manpower knowledgeable about the system, it is not straightforward to translate such knowledge to a form that can be readily utilized by automated monitoring systems. Historical data based methods rely on large amount of past data to capture the underlying relationships between the process variables [4,6,7]. However, due to batch-to-batch variability intrinsic to fermentation processes, it is difficult for these techniques to delineate between normal and abnormal variations.

Another set of methods, based on ideas from statistical control literature, have been proposed that rely only on data available from the current batch [8–10]. Fermentation processes typically utilize complex organic substrates such as yeast extract in addition to defined components such as glucose and ammonia. This provides a substitutable multisubstrate milieu, which may result in sequential and/or simultaneous utilization of the substrates. The cellular metabolism may be different in each such substrate uptake phase [11]. Evolution of a batch fermentation process can then be conceptualized as a sequence of such phases, each with its own duration and dynamics. It is expected that batch-to-batch variability would therefore, among other things, translate to variations in switching times between the phases [12]. Hence, effective monitoring can be achieved by detecting the time of occurrence of these various phases. The reported technique based on this philosophy detects the phase change time by identifying qualitative changes in trajectories of the test statistic T^2 and principal component score plots [9]. Being qualitative in nature, this technique is difficult to automate. While other statistical process monitoring techniques such as Shewhart Charts, Cumulative sum (CUSUM) and Exponentially weighted moving average (EWMA) [8,10,13] have been applied for monitoring batch processes in general, they have not been specifically applied for monitoring fermentation processes characterized by multiple phases since it is typically assumed in these techniques that the entire batch data is characterized by single set of statistical properties (such as mean and covariance).

In this article, we present a real time phase detection based process monitoring scheme that does not require process model or historical data. The scheme is inspired from statistical control literature, is multivariate in nature, relies only on online measurements and can be easily automated to work with industrial processes. The basic premise in our approach is that statistical properties of online measured data are different in different phases. Hence, the problem of phase change detection is treated to be equivalent to that of detection of changes in statistical properties of the data. To be consistent with earlier work [9,12], we refer to a point where phase change is detected as a singular point (SP).

2. Experimental methods

In this study, experimental data has been collected for two different strains, *Amycolatopsis balhimycina* DSM5908 *and Bacillus pumilus* ATCC 21951 while the data for *Amycolatopsis mediterranei* S699 was taken from Doan et al. [9]. For *A. balhimycina* and *B. pumilus*, the fermentation experiments were performed in a 2.5 l fermentor equipped with various sensors and data acquisition system (Model: Biostat B, B. Braun, Germany). The fermentor was aerated at a constant flow rate of 1.0 vvm (volume of air per unit volume of medium per minute) using a mass flow controller. Dissolved oxygen (DO) concentration in the fermentor was maintained at 40% of saturation value by controlling the stirrer speed in cascade mode with DO. The concentrations of oxygen and carbon dioxide in the exhaust gas were measured by infrared spectroscopy and paramagnetic analysis, respectively (Analyser BINOS1002 M, Rosemount Analytical, Germany). The online measurements were stored at 5 min intervals.

The *Amycolatopsis balhimycina* strain was a gift from Prof Anna Eliasson Lantz of Denmark's Technical University, Denmark, and was stored on Bennett agar plates at 4 °C. Seed culture was grown in 100 ml medium in a 500 ml capacity Erlenmeyer flask with single baffle and incubated at 30 °C and 150 rpm. The seed medium contained per liter of distilled water: glucose: 15 g, glycerol: 15 g, soya peptone: 15 g, NaCl: 5 g and yeast extract: 3 g. Upon reaching an optical density of ~12 at 600 nm, 25 ml of the seed culture was transferred to a fermentor containing 1 l of production medium. The production medium contained, per liter of distilled water, glucose: 54–100 g, glycerol: 0–16 g, ammonium sulfate: 3–6.6 g, yeast extract: 0.75–1.5 g, defatted soybean flour: 0.25–1.0 g, $ZnSO_4$: 0.02 g, $FeSO_4$: 0.02 g, trisodium citrate: 0.025 g, $MgSO_4$: 1.5 g, $MnSO_4$: 0.01 g, NaCl: 1 g, MES: 1.045 g and KH_2PO_4: 0.2 g. In addition, the following vitamins were added: biotin: 0.00005 g, calcium-pantothenate: 0.001 g, nicotinic acid: 0.001 g, myo-inositol: 0.025 g, thiamin HCL: 0.001 g, pyridoxine HCL: 0.001 g and para-aminobenzoic acid: 0.0002 g. Temperature was maintained at 30 °C and pH was maintained at 7.0 by adding 1.5N NaOH solution by using a pH controller. The online measurements included NaOH flow rate, pH, agitator speed and DO.

A transketolase (*tkt*) deficient strain of *Bacillus pumilus* ATCC 21951 was procured from Institute for fermentation, Osaka, Japan. The strain was maintained on Luria Bertani agar slant and was stored at 4 °C. The preparation of pre-seed and seed cultures and the culture transfer criteria were as described earlier [14]. The production medium contained per liter of distilled water: glucose: 200 g, cas amino acids: 15 g or corn steep liquor: 12 g, ammonium sulfate: 5 g, $CaCO_3$: 16 g, $MnSO_4$: 0.5 g, leucine: 0.5 g and tryptophan: 0.05 g. The temperature was maintained at 37 °C. The online measurements available for *Bacillus pumilus* were: pH, dissolved oxygen, agitator speed and CO_2 and O_2 concentration in exhaust gas.

For both the strains, samples were drawn from the fermentation medium at regular intervals to obtain the time profiles of concentrations of dry cell weight (DCW), product(s) and substrate(s). Glucose, glycerol, D-ribose, acetate, acetoin and 2,3-butanediol were analyzed via RI detector on HPLC (Hitachi, Merck KgaA, Darmstadt, Germany) using HP-Aminex-87-H column (Biorad, Hercules, CA, USA) with column temperature maintained at 60 °C. A mobile phase of 5 mM sulfuric acid with flow rate of 0.6 ml/min was used. The concentration of free amino acids was estimated via the ninhydrin method. The details are described in earlier works [11,14,15]. Ammonia was measured using Nessler's reagent [16]. For A. balhimycina, DCW was measured by filtering 10 ml of the fermentation broth using pre weighted filter papers (Whatman, Brentford, Middlesex, UK) as reported elsewhere[11]. Micrococcus luteus was used as a test organism to measure antimicrobial activity of balhimycin [17]. For this purpose, agar test plates with Micrococcus luteus growth medium were prepared. Holes were punched in the agar medium and filled with fermentor samples. Then the plates were incubated for two days at 30 °C. The growth inhibition diameter around the holes was measured and concentration of balhimycin was determined using pre-computed calibration curve.

The data for *Amycolatopsis mediterranei* S699 was taken from literature [9] and consisted of the following online measurements: pH, dissolved oxygen, agitator speed and CO_2 and O_2 concentration in exhaust gas.

3. Phase detection technique

3.1. Algorithm

In this work, the problem of monitoring of fermentation process has been posed as that of detection of singular points (SPs). We assume that the underlying characteristic dynamics and in turn the statistical properties of the online data vary from one phase to another. Thus, we propose that an SP can be detected by appropriately detecting the change in the statistical properties of the available online data as described below (Fig. 1). For the current phase ϕ_i and a new data point x_k ($x_k = [x_{1k}\ x_{2k}\ x_{3k}\ldots x_{pk}]$ where p is the number of variables being measured), the following hypothesis is checked:

$$\begin{aligned} &\textit{Null hypothesis} : H_0 : \mathrm{x_k} \in \phi_i \\ &\textit{Alternative hypothesis} : H_1 : \mathrm{x_k} \notin \phi_i \end{aligned} \quad (1)$$