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Maximizing biomass concentration in baker's yeast process by using a decoupled geometric controller for substrate and dissolved oxygen

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HIGHLIGHTS

- Biomass maximization depends on the cumulative effect of control action.
- Smoother control action achieved by decoupling interactions between state variables.
- Implementation of simultaneous control of substrate and dissolved oxygen.
- A single set of tuning parameters shown to be valid over a wide metabolic range.
- Higher biomass concentration achieved (23% vs. PID and 18% vs. PID-ℓ controller).

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ABSTRACT

Biomass production by baker's yeast in a fed-batch reactor depends on the metabolic regime determined by the concentration of glucose and dissolved oxygen in the reactor. Achieving high biomass concentration in turn is dependent on the dynamic interaction between the glucose and dissolved oxygen concentration. Taking this into account, we present in this paper the implementation of a decoupled input–output linearizing controller (DIOLC) for maximizing biomass in a fed-batch yeast process. The decoupling is based on the inversion of 2×2 input–output matrix resulting from global linearization. The DIOLC was implemented online using a platform created in LabVIEW employing a TCP/IP protocol via the reactor's built-in electronic system. An improvement in biomass yield by 23% was obtained compared to that using a PID controller. The results demonstrate superior capability of the DIOLC and that the cumulative effect of smoother control action contributes to biomass maximization.

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1. Introduction

The ever-increasing global energy requirement has led to a gradual depletion of natural energy resources such as fossil fuels, coal and natural gas. The looming energy crisis has a potential to severely affect industrial production and profitability. Already, the exploitation of natural resources has adversely impacted the eco-system (Lin and Tanaka, 2006; Dellomonaco et al., 2010), highlighting the urgent need to develop affordable, sustainable, and eco-friendly renewable energy resources. Using biological processes for generating clean energy economically is a possible solution to this conundrum. Over the years, bioethanol and biomass have emerged as alternatives to the limited natural resources (Mussatto et al., 2010; Munasinghe and Khanal, 2010; Pereira et al., 2010; Binod et al., 2010). The approximate worldwide production of industrial ethanol has crossed four million tons annually, 80% of which comes from biological fermentation (Gupta and Prakash, 2015). Likewise, biomass is a valuable product used in the food and baking industry. Yeast is the preferred organism for the production of industrial-scale ethanol, alcoholic beverages, biomass and a range of metabolic products (Cardona and Sánchez, 2007; Costa and De Morais, 2011). In addition, yeast is a well-accepted expression system for the production of many recombinant proteins and vaccines. Hence, maximizing biomass production is of significant interest to the biotech industry.

Although the use of yeast is as old as human civilization, exploiting its full potential for meeting process objectives remains a daunting challenge because of its multifaceted metabolism. Through its Crabtree or overflow metabolism, yeast cells can switch from a purely oxidative metabolism to a respiro-fermentative metabolism even under fully aerobic conditions as soon as glucose exceeds the critical concentration of about 0.1 g/L (Kasperski and Miśkiewicz, 2008). The value of critical

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glucose concentration varies depending on the strain, media and operating conditions. After crossing this stage, the central carbon flux is directed towards the production of ethanol and acetic acid, resulting in a decrease in the biomass production yield. Thus, biomass and ethanol are products formed in the extremes of metabolic regimes from yeast (Atasoy et al., 2013). Irrespective of the product at the end, the yeast fermentation process needs stringent regulation of critical process parameters to follow a particular metabolic regime. Moreover, there are chances of a substantial variation in feed material used in yeast fermentation processes at larger scales. Therefore, to enable the achievement of desired objectives, implementation of adequate process control is essential. However, control of such dynamic, time-variant and non-linear bioprocesses is a major challenge (Alford, 2006).

Most industrial fermentation processes rely on fed-batch operation due to the resulting economic benefits and the abundant literature that exists on the real time control of fed-batch yeast fermentation (Vovsík et al., 2013). Despite this, the current industrial practices are based on manual controls based on off-line measurements or the use of proportional-integral-derivative (PID) control. Manual interventions are, however, subjective and error prone (Cosenza and Galluzzo, 2012). PID controllers require accurate, online measurement of the variable to be controlled which may not be possible due to the lack of instrumentation. Often these variables are estimated indirectly from other measurements or obtained off-line. Both situations can result in unacceptable perturbation to the process. The performance of a PID controller may also degrade because the control parameters tuned for one set of conditions may not be suitable for another. Therefore, to exploit the full potential of the microorganism and maximize the economic benefit, there is a need to develop an advanced yet simple control strategy.

Early efforts in implementing process control for fed-batch baker's yeast fermentation were based on feedback PID controllers. The objective usually was to regulate respiratory quotient (RQ), specific growth rate and ethanol concentration by manipulating the substrate feed rate. Currently, many industrial protocols use RQ, the ratio of the carbon dioxide evolution rate (CER) to the oxygen uptake rate (OUR), to regulate the switching of metabolic regimes in the baker's yeast process. However, measurement of the RQ alone is not sufficient to capture the dynamic variations in the pathways of baker's yeast fermentation. In general, $RQ > 1.0$ indicates ethanol formation and for optimal growth, the range $0.9 < RQ < 1$ is required. At lower RQ values, the carbon source is utilized more rapidly until depletion begins to occur. Further, RQ based control cannot be applied to all strains as shown by Nagamori et al. (2013). Also, RQ-based control is an indirect control since the glucose concentration in the culture is not tightly regulated as demonstrated by Kiran and Jana (2009). Hence, controllers that directly influence measured variables such as substrate and dissolved oxygen concentration simultaneously, the major drivers of the yeast metabolism, are more desirable.

With the advancement in monitoring techniques, researchers have explored the application of dielectric spectroscopy and calorimetry (Schuler and Marison, 2012; Biener et al., 2012) to control the feed rate of the substrate in small-scale bioreactors. However, these tools thus far remain untested in an industrial environment. The exponentially increasing substrate feed rate, the difference in the characteristic time constants between substrate and oxygen utilization and ethanol production create hindrances in control design. In a general industrial application, the substrate is fed according to a precalculated feeding profile with PID regulation of the feed rate. Several advanced developments have been reported concerning the control of fed-batch fermentation processes in the literature. Recently, for example, various control strategies including those based on neural networks, adaptive models and fuzzy logic have been validated on fed-batch yeast fermentation processes. Considering the limitations of robust on-line monitoring tools, two neural soft sensor estimators have been developed by Karakuzu et al. (2006) to compute the specific growth rate and biomass concentration, respectively, and thereby facilitate the implementation of a fuzzy control scheme. Their results showed that the proposed controller gave satisfactory performance in both small and large scale bioreactors. Valentinotti et al. (2003) had proposed a control strategy for growth of *Saccharomyces cerevisiae* in fed-batch culture in which ethanol and biomass formation was decoupled for optimal control of the substrate feed rate slightly above the critical value. This concept of decoupling was further tested by Cannizzaro et al. (2004) at the laboratory scale to control fed-batch baker's yeast fermentation in order to maximize biomass production throughout the fermentation. Hocalar and Türker (2010) regulated the ethanol concentration around a fixed set point or a predetermined time varying profile by manipulating the substrate feed rate and employing control of the minimal overflow metabolite as suggested by Valentinotti et al. (2003) and Cannizzaro et al. (2004).

Most of the control strategies reported in the literature employ manipulation of either the substrate feed rate or the air flow rate separately to achieve process targets. However, these variables are interactive and affect process outputs significantly. Hence, to achieve the possibly conflicting goals of maximization of biomass while keeping ethanol at a minimal level requires accurate regulation of both substrate and air feed rate simultaneously, and hence their interaction must be decoupled. In this paper, we have shown how the biomass production can be maximized by implementing a decoupled input–output linearizing controller (DIOLC) to control the baker's yeast fed-batch fermentation. The interaction between the glucose and dissolved oxygen concentration was decoupled using a simple matrix inversion principle. Further, in accordance with Process Analytical Technology (PAT) guidelines, an initiative driven by the European and North American regulatory authorities, the control scheme was based on real time measurements and responded quickly to trajectory deviation (Gomes et al., 2014). The baker's yeast fermentation process was chosen for the DIOLC implementation because it offers sufficient metabolic complexity for testing. The DIOLC was implemented online and its performance in baker's yeast fed batch fermentation was benchmarked against the PID and linearly scheduled PID (PID-ℓ) controllers in a laboratory scale reactor. While executing this strategy, real-time measurements of dissolved oxygen (DO) and of residual glucose concentration were performed to control the process on-line while biomass and ethanol concentrations were measured offline. It has been demonstrated that biomass production using the proposed DIOLC results in higher yields compared to a PID or a PID-ℓ controller.

2. Methods

2.1. Microorganism culture and maintenance

Yeast strain *S. cerevisiae* NRRL-Y-11857 was gifted by Agricultural Research Service (ARS), Washington DC, USA. The yeast strain was maintained on agar slants and stored at 4 °C with periodic sub-culturing once a month. The composition of the medium used for preparation of the seed culture was 2% glucose, 1% yeast extract and 2% peptone. The medium was sterilized by autoclaving; the glucose solution was autoclaved separately to prevent Maillard reaction and then mixed under aseptic conditions. For preparing the seed culture, a slant was used to inoculate a 250 ml Erlenmeyer flask containing 50 ml of culture medium and the culture was incubated at 30 °C for 8–10 h at 200 rpm in an

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