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Feedback control based on NADH fluorescence intensity for *Saccharomyces cerevisiae* cultivations

Supasuda Assawajaruwan*, Fiona Kuon, Matthias Funke and Bernd Hitzmann

Abstract

Background: A glucose concentration is an important factor for a fed-batch process of *Saccharomyces cerevisiae*. Therefore, it is necessary to be controlled under a critical concentration to avoid overflow metabolism and to gain high productivity of biomass. In the study, 2D fluorescence spectroscopy was applied for an online monitoring and controlling of the yeast cultivations to attain the pure oxidative metabolism.

Results: The characteristic of the NADH intensity can effectively identify the metabolic switch between oxidative and oxidoreductive states. Consequently, the feed rate was regulated using the single signal based on the fluorescence intensity of NADH. With this closed-loop control of the glucose concentration, a biomass yield was obtained at 0.5 $g_{biomass}/g_{glucose}$. In addition, ethanol production could be avoided during the controlled feeding phase.

Conclusions: The fluorescence sensor with a single signal of the NADH fluorescence intensity has potential to control a glucose concentration under the critical value in real time. Therefore, this achievement of the feedback control is promising to build up a compact and economical fluorescence sensor with the specific wavelength using light-emitting diodes and photodiodes. The sensor could be advantageous to the bioprocess monitoring because of a cost-effective and miniaturized device for routine analysis.

Keywords: Bioprocess monitoring, Fluorescence spectroscopy, Closed-loop control, *Saccharomyces cerevisiae*, NADH

Background

The fundamental purpose of a fed-batch process is to achieve a high production yield at the low cost. The objective of the fed-batch cultivation of baker's yeast is to obtain a high yield of biomass. The main factor, which is considered in the fed-batch cultivation, is a glucose concentration, because it plays a pivotal role in regulating yeast metabolism. When the glucose concentration in a yeast cultivation is above a critical value, it leads to oxidoreductive metabolism and ethanol is produced as an overflow metabolite under an aerobic condition (Walker 1998). Due to the ethanol production, the yeast cultivation gains a lower biomass yield (Pham et al. 1998). This phenomenon has been known as the Crabtree effect caused by a limited respiratory capacity (Sonnleitner and Käppeli 1986). A critical glucose concentration is in the range between 0.04 and 0.07 g/L depending on yeast strains, media components, and operation systems (Pham et al. 1998; van Hoek et al. 1998; Hantelmann et al. 2006). In industries, the oxidoreductive growth of yeasts is necessarily avoided to reach a high biomass yield. Consequently, the substrate feed rate is controlled under the critical glucose concentration to maintain oxidative metabolism. Literally, the yield coefficient ($Y_{X/G}$) of an oxidative growth of yeasts attains in the range of 0.47–0.50 $g_{biomass}/g_{glucose}$ (Sonnleitner and Käppeli 1986; Pham et al. 1998; Hantelmann et al. 2006). Many studies have investigated effective approaches, such as the feed rate control with models or online measurements during a fed-batch process (Hantelmann et al. 2006; Henes and Sonnleitner 2007; Klockow et al. 2008; Craven et al. 2014; Mears et al. 2017; Vann and Sheppard 2017).

*Correspondence: sassawaj@uni-hohenheim.de
Department of Process Analytics and Cereal Science, University of Hohenheim, Garbenstraße 23, 70599 Stuttgart, Germany

To manipulate the glucose concentration under the critical value, an online measurement device is required to detect overflow metabolism. Two-dimensional (2D) fluorescence spectroscopy is an effective tool for online monitoring of cultivation processes (Biechele et al. 2015; Faassen and Hitzmann 2015). It was developed for measuring fluorescent molecules in a wide range of excitation and emission wavelengths to detect non-identified overlapping peaks and quenching of different fluorophores (Marose et al. 1998). Besides, it is possible to perform a non-invasive measurement without interfering an inner system of cultivation processes. The fluorescence sensor has been applied in many studies for online bioprocess monitoring and the fluorescence data can be used for quantification of fluorescence substances, particularly proteins, and for estimation of cell mass, glucose and ethanol concentrations (Haack et al. 2004; Rhee and Kang 2007; Srivastava et al. 2008; Odman et al. 2009; Rossi et al. 2012; Almqvist et al. 2016; Assawajaruwan et al. 2017a). Furthermore, 2D fluorescence spectroscopy can recognize metabolic changes during yeast cultivations (Hantelmann et al. 2006; Assawajaruwan et al. 2017b).

There is currently no commercial device, which can measure a glucose concentration at the low level of the critical point in real time. For this reason, a 2D fluorescence spectroscopy was applied in the study to investigate a signal, which can determine a metabolic switch between oxidative and oxidoreductive states. The biogenic fluorophores, such as NADH, tryptophan, flavins, and pyridoxine, were taken into consideration of the investigation because they are significantly related to the growth characteristics of yeasts (Marose et al. 1998; Hantelmann et al. 2006; Assawajaruwan et al. 2017b). The fluorescence intensity, which can greatly indicate the metabolic switch, was chosen and applied as a metabolic signal to control the glucose feed rate. In addition, it is possible to see from the study if it is promising to build up a specific-wavelength fluorescence sensor equipped with light-emitting diodes and photodiodes for yeast cultivations. The sensor will be a cost-effective and miniaturized device for routine analysis (O'Toole and Diamond 2008).

Methods

Yeast strain and cultivation conditions

The fed-batch cultivations were operated in a 2.5-L stainless steel tank bioreactor (Minifors, Inifors HT, Bottmingen, Switzerland) with an initial volume of 1.35 L. The amount of 2.5-g dry baker's yeast or *S. cerevisiae* (SAF Instant Red, S.I.Lesaffre, Marcq, France) was precultivated in 100 mL Schatzmann medium, which consists of 0.34 g/L $MgSO_4{\cdot}7H_2O$, 0.42 g/L $CaCl_2{\cdot}2H_2O$, 4.5 g/L $(NH_4)2SO_4$, 1.9 g/L $(NH_4)2HPO_4$, and 0.9 g/L KCl (Schatzmann 1975). The pre-culture was shaken for 30 min at 180 rpm and was then pumped into the bioreactor. The medium for the yeast cultivations was the same as for the pre-culture, but with glucose, 1 mL/L trace elements solution (0.015 g/L $FeCl_3{\cdot}6H_2O$, 9 mg/L $ZnSO_4{\cdot}7H_2O$, 10.5 mg/L $MnSO_4{\cdot}2H_2O$, and 2.4 mg/L $CuSO_4{\cdot}5H_2O$), 1 mL/L vitamin solution (0.06 g/L myo-inositol, 0.03 g/L Ca-pantothenate, 6 mg/L thiamine HCl, 1.5 mg/L pyridoxine HCl, and 0.03 mg/L biotin), and 200 µL/L antifoam agent. The fed-batch cultivations to test relevant fluorescence signals of the metabolic switch were performed with 1.5 g/L initial glucose concentration. For the fed-batch cultivations with the feedback control, the initial glucose concentration was reduced to 1.0 g/L for minimizing time of the batch phase in the beginning. The yeast cultivations were conducted in triplicate. The glucose concentration of the feed solution was 15 g/L in Schatzmann medium with trace elements and vitamin solutions. All fed-batch cultivations were performed with a maintained temperature and pH at 30 °C and 5, respectively. The aeration and agitation rates were kept constant at 4 L/min and 450 rpm, respectively. Iris software (Inifors HT, Bottmingen, Switzerland) was applied as a process control system for the bioreactor.

Bioprocess setup and control

Manual control

A peristaltic pump (Ismatec MCP Process, Cole-Parmer GmbH, Wertheim, Germany) was connected to the bioreactor and computer. For the investigation of significant fluorescence signals, the feed rate was calculated based on Eq. 1, which is referred from a mathematical model of a fed-batch cultivation process (Rode Kristensen 2003; Henes and Sonnleitner 2007):

$$F_0 = \frac{\mu}{Y_{x/s}(S_f - S_0)} V_0 X_0 e^{\mu t_0} \tag{1}$$

$$t_p = \frac{1}{\mu_p} < \frac{1}{\mu_{\max \text{ on glucose}}} \tag{2}$$

$$F(t) = F_0 e^{\frac{t}{t_p}}, \tag{3}$$

where F_0 and $F(t)$ are the feed rate at the beginning and at time t, respectively. μ is the maximum specific growth rate of yeasts on glucose. V_0 is the volume of the culture broth at the start of feeding. X_0 is the biomass concentration at the start of feeding. $Y_{x/s}$ is the yield coefficient for biomass with respect to glucose. S_f and S_0 are the glucose concentration of the feed solution and the cultivation at the start of feeding, respectively. t_0 is the time at the start of feeding. t_p is a time constant converted from the specific growth rate (h^{-1}), which is considered to be

higher than the maximum specific growth rate on glucose ($\mu_p > \mu_{max\ on\ glucose}$) to drive the metabolism from a state of pure oxidative glucose consumption to an oxidoreductive mode.

After the initial glucose in the batch phase was depleted, the glucose feed solution was pumped with the starting feed rate (F_0) at 0.87 mL/min to maintain an oxidative consumption of glucose. When the steady state of an oxidative phase was reached, the feed solution was exponentially pumped into the bioreactor based on Eq. 3. The feed rate was manually controlled via MATLAB (R2015b).

Closed-loop control

For the fed-batch cultivations with the feedback control, the feed rate was also calculated based on Eqs. 1–3, but it was regulated with the online measured fluorescence spectral data from the fluorescence spectrometer (BioView, DELTA Lights & Optics, Hørsholm, Denmark). The computer connecting to BioView collected the measured spectra and sent the spectra to another computer, which was connected to the pump, as illustrated in Fig. 1a.

The feed rate was manipulated based on the NADH intensity (ex330/em450) according to its intensity change referred to the metabolic switch from oxidative to oxidoreductive states. The signal of the metabolic change to an oxidoreductive state is detected as a noise signal in the oxidative metabolism. For this reason, the Savitzky–Golay filter for derivative of signal curves was applied to pronounce the peak of the metabolic change. The NADH spectra were first smoothed with the three-point median filter to reduce regular noises occurring from the turbidity of the culture and the device itself, not from the metabolic change. Then the median-smoothed NADH spectra were processed with the Savitzky–Golay filter for first derivative (quadratic, five-point size) as in Eq. 4 (Gorry 2002). These derivative values were named as a metabolic signal. The metabolic signal was used to indicate the metabolic switch between oxidative and oxidoreductive states:

$$N_j^{'} = (-2 \times N_{j-2} - 1 \times N_{j-1} + 0 \times N_j + 1 \times N_{j+1} + 2 \times N_{j+2}) \times \frac{1}{10\Delta t}, \quad (4)$$

where N_j and $N_j^{'}$ are the median-smoothed NADH intensities and its derivative at the central point of each subset, respectively. j is an index presenting the data point of the NADH measurement and Δt is the time interval between each measurement of the NADH intensity, which is 1.5 min.

The glucose feed solution was being pumped with the starting feed rate (F_0) into the fermenter when the initial glucose in the batch phase ran out. Then, the feed solution was exponentially pumped into the bioreactor as the function shown in Eq. 3 after reaching the steady state of an oxidative phase. The feed rate was manipulated under the program control by MATLAB (R2015b) as shown in Fig. 1b. As illustrated in the scheme, the current feed rate at that time point will be 10% reduced when the derivative value is more than or equal to the upper threshold signal ($N^{'} \geq 1.3$ rel. unit/min). Then, the 10%—reduced

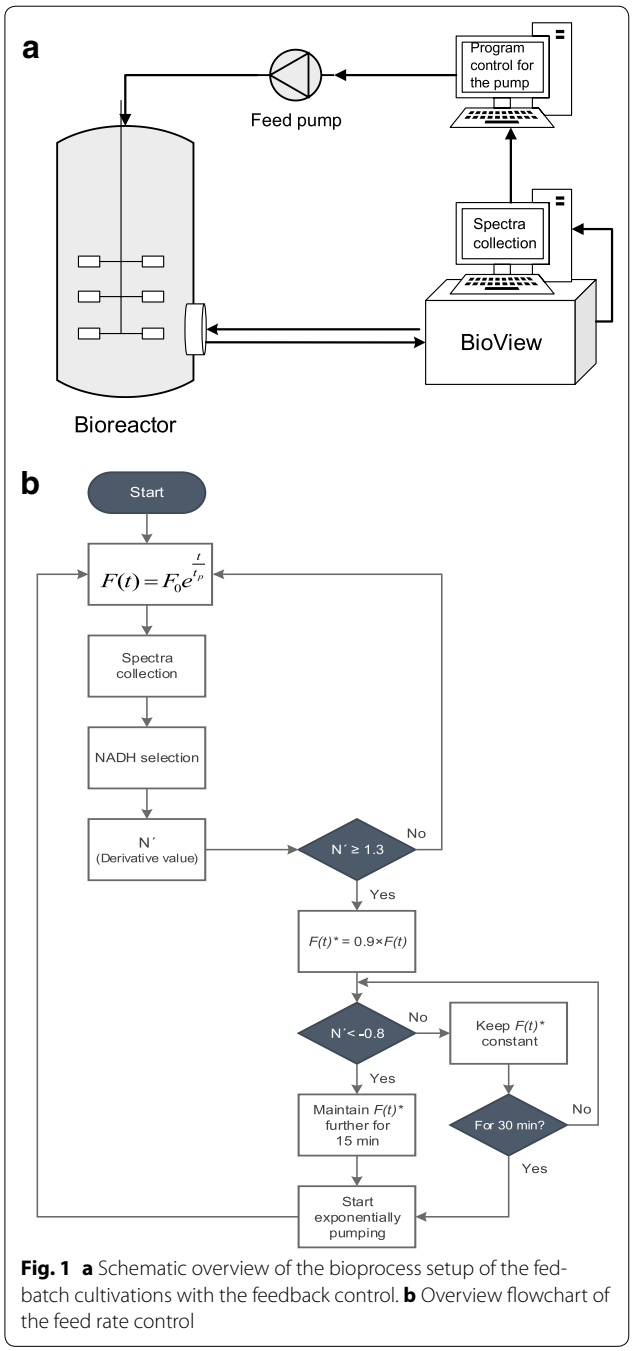


Fig. 1 **a** Schematic overview of the bioprocess setup of the fed-batch cultivations with the feedback control. **b** Overview flowchart of the feed rate control

feed rate ($F(t)^{*} = 0.9F(t)$) will be kept pumping for 30 min or till the lower threshold signal is achieved, which is −0.8 rel. unit/min. In the latter case, the 10%—reduced feed rate will be constantly pumped further for 15 min. After 15 min, it will start pumping the feed solution with the exponential rate from the 10%—reduced feed rate as demonstrated in Fig. 1b.

Online monitoring/analysis

Relative fluorescence intensity of relevant fluorophores, such as NADH, tryptophan, pyridoxine, and flavins, was online monitored during the yeast cultivations by the BioView fluorescence spectrometer (Marose et al. 1998; Haack et al. 2004; Hantelmann et al. 2006; Faassen and Hitzmann 2015). The device is equipped with 15 different filters for excitation and emission wavelengths. The multi-wavelength fluorescence in range of 270–550 nm excitation (ex) and 310–590 nm emission (em) with increment of 20 nm were measured during the cultivations. The BioView fluorescence sensor has a xenon flash lamp as a light source for exciting molecules. The excitation light goes via the fiber optic as a guide light into the bioreactor, and the fluorescent light, which is emitted in a 180° angle, is monitored after passing the emission filters. Then, the fluorescent light is detected by a photomultiplier. The process runs continuously until a complete rotation of excitation and emission filters. The fluorescence sensor measured the yeast culture through a quartz window in 25-mm standard port as a non-invasive monitoring. The measurement for a single scan of the spectrum was achieved within 1.5 min. The spectrum of a scanning contains the combinations of excitation and emission wavelengths.

Dissolved oxygen was monitored continuously with OxyFerm DO sensor (Hamilton Bonaduz AG, Bonaduz, Switzerland). The online measurements of the dissolved oxygen were observed during the cultivations with the Iris Software (Inifors HT, Bottmingen, Switzerland).

Offline analysis

Offline samples were regularly taken from the bioreactor and put into preweighed and predried microcentrifuge tubes for analyzing biomass, glucose, and ethanol concentrations in triplicate. Dry cell mass was determined by centrifugation (Mega Star 600R, VWR International BVBA, Haasrode, Belgium) of a sample with 1.5 mL (two times) at 14,000 rpm for 10 min at 4 °C. The wet cells were put in a drying oven at 100 °C for 24 h. Subsequently, they were cooled down for 30 min before weighing. The supernatant of the samples after the centrifugation was examined by HPLC (ProStar, Variant, Walnut Creek, CA, USA) to determine glucose and ethanol concentrations. The supernatant was firstly filtrated with pore size filter, 0.2 µm, polypropylene membrane (VWR, Darmstadt, Germany). Then, the filtrate was injected 20 µL into a Rezex ROA-organic acid H+ (8%) column (Phenomenex, Aschaffenburg, Germany) and operated at 70 °C with 5 mM H_2SO_4 as an eluent at 0.6 mL/min flow rate. The concentrations of glucose and ethanol were calculated by Software GalaxieTM Chromatography (Varian, Walnut Creek, CA, USA).

Results and discussion

Investigation of fluorescence signals corresponding to the metabolic change

The biogenic fluorophores, which were examined during the fed-batch process, are the peak intensity in NADH (ex330/em450), tryptophan (ex290/em350), flavins (ex450/em530), and pyridoxine (ex330/em390) regions. These fluorophores were regularly mentioned in several studies that they are related to some important metabolic pathways of yeast cells, e.g., glycolysis and TCA cycle (Marose et al. 1998; Hantelmann et al. 2006; Assawajaruwan et al. 2017b). According to an increase of the dissolved oxygen at approximately 3 h, the glucose feed solution was being pumped into the bioreactor as illustrated in Fig. 2a, b. The slight increase of the dissolved oxygen at around 3 h was assumed that glucose and ethanol substrates in the batch phase were depleted, which can be seen in Fig. 2a. After pumping the glucose feed solution, the dissolved oxygen was immediately decreasing. The feed solution was constantly pumped at the minimum rate for around 2.5 h. The dissolved oxygen dramatically increased at about 5 h and then kept slightly increasing as demonstrated in Fig. 2b. From this evolution of the dissolved oxygen between roughly 3 and 5 h, it was presumed that the cells were starving and tried to adapt themselves with the feed condition. Due to the slight increase of the dissolved oxygen after 5 h, it was assumed that the yeasts slowly maintained the steady state of oxidative metabolism. In addition, it was also assumed that there was no produced ethanol after the increment of the dissolved oxygen. If there is ethanol in the system, the dissolved oxygen would be lower, because yeasts consume more oxygen during the metabolization of ethanol (Henes and Sonnleitner 2007). It means that the metabolism after about 5 h was in a complete oxidative mode due to no production of ethanol, which can be seen in Fig. 2a. Subsequently, the feed solution was exponentially pumped until the dissolved oxygen was decreasing almost to zero, as shown in Fig. 2b. Then, the feed rate was manually set back to the minimum rate and kept constant till the dissolved oxygen increased and reached the steady state again (Fig. 2a, b). Afterwards, the feed rate was exponentially pumped again until the depletion of the dissolved oxygen. In Fig. 2a, the ethanol

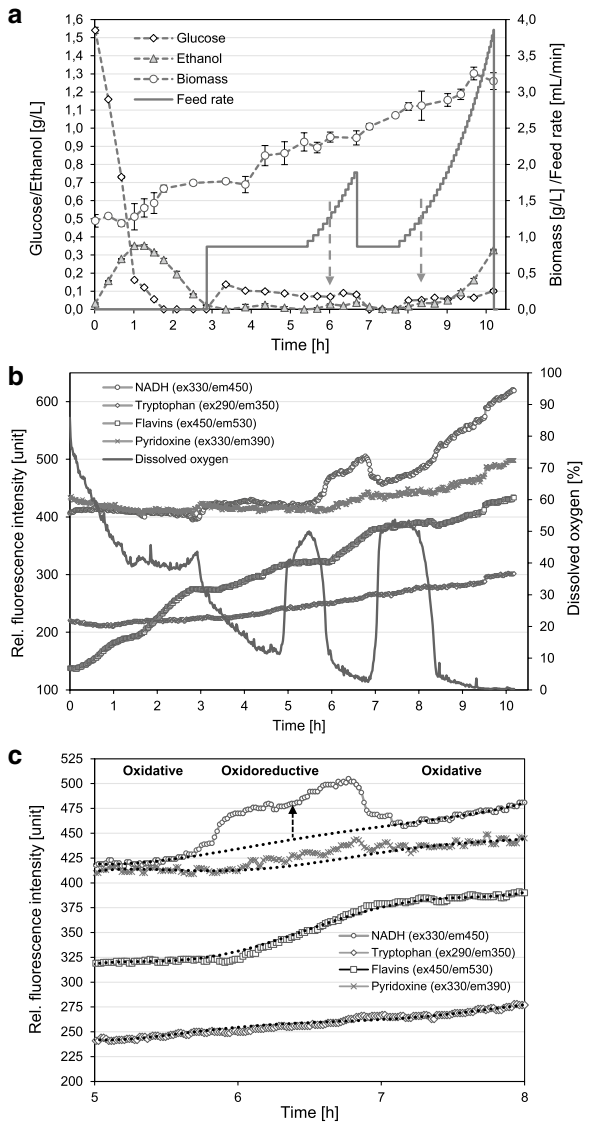


Fig. 2 **a** Growth characteristic of yeasts and the feed rate profile during the fed-batch operation without a closed-loop control. The arrows point out the produced ethanol due to overflow metabolism. **b** Evolution of the dissolved oxygen and the intensity of NADH, tryptophan, flavins, and pyridoxine during the fed-batch operation without a closed-loop control. **c** Characteristics of the fluorophores in different metabolic states of oxidative and oxidoreductive metabolism. The dotted lines are plotted from the polynomial function based on the state of oxidative metabolism to see the intensity change between these two metabolic states. The small arrow shows the different intensity between the oxidative and oxidoreductive states

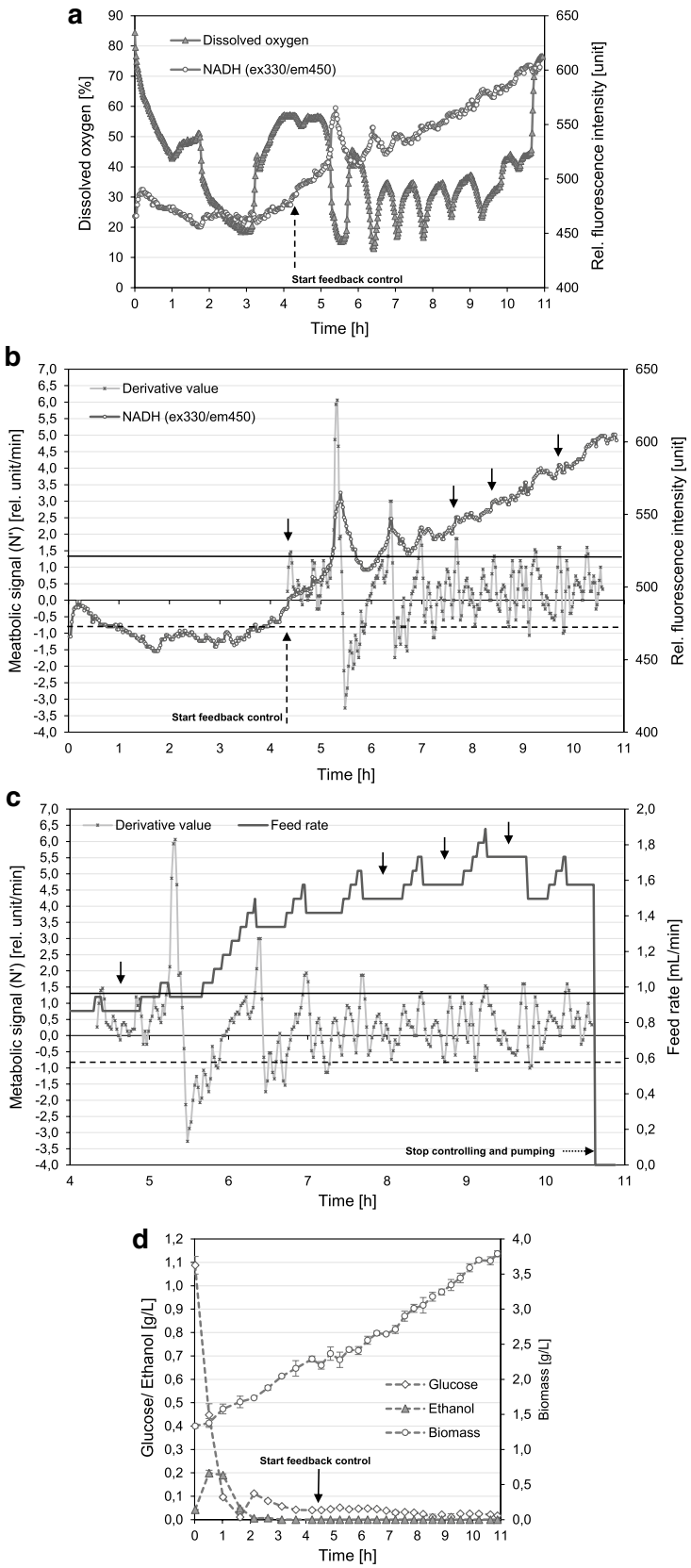
was slightly produced after the feed rate was exponentially increasing (see arrows), which is corresponding to the decrease of the dissolved oxygen. Besides, the ethanol was rapidly produced, since about 9 h, because there was the lack of oxygen in the cultivation (Fig. 2a, b).

The characteristics of NADH, tryptophan, flavins, and pyridoxine intensities during the fed-batch operation were presented in Fig. 2b, c. During the exponential feeding of glucose, the NADH intensity instantly and distinctly increased comparing to other fluorophores. When the feed rate was reduced to the minimum rate and kept constant, the NADH intensity was later dropping to the oxidative state as demonstrated in Fig. 2b, c. The oxidative state can be proved from the completely consumed ethanol at about 7 h in Fig. 2a. The phenomenon of the NADH intensity has a reverse correlation with the evolution of the dissolved oxygen. The change of their fluorescence intensities between the oxidative and oxidoreductive metabolism is quantified using the polynomial function based on the oxidative state as presented in Fig. 2c. It is clear to see the increased intensity of NADH from the oxidative state, which is around 40 rel. fluorescence units in average. For other three fluorophores, the difference of their intensities from their polynomial functions is less than the half of the increased intensity of NADH, as illustrated in Fig. 2c. Regarding the results, the metabolic switch between oxidative and oxidoreductive states is recognizable from the NADH intensity, but it is not obvious to see from tryptophan, flavins, and pyridoxine. Although the intensity of flavins was slowly increasing after 6 h, it responded to the overflow metabolism slower than NADH did. Furthermore, it cannot be recognized when the oxidative mode returns.

$NADH/NAD^+$ as redox carriers are prerequisite for catabolic and anabolic reactions, particularly, for providing cells with energy in the form of ATP. Due to the overflow metabolism, NADH is accumulated as shown in Fig. 2c and yeast cells need to maintain their cellular redox balance or metabolic homeostasis of $NADH/NAD^+$ ratio. Therefore, the accumulation of NADH due to high glycolytic fluxes leads to the formation of byproducts, such as ethanol and glycerol (Vemuri et al. 2007; Chen et al. 2014). Then, these fermentation products, e.g., ethanol and glycerol, are further oxidized through the TCA cycle and the oxidative phosphorylation for generating ATP to reach the requirement of growth (Brauer et al. 2005).

Online controlling of the feed rate using the metabolic signal

The NADH intensity was applied as a single signal for the feed control in real time. The evolution of the NADH intensity during the fed-batch process is illustrated in Fig. 3a. The feedback control started functioning at the same time as starting exponential feed rates (see an arrow in Fig. 3a). After approximately 5 h, the NADH intensity significantly increased, whereas the dissolved oxygen declined. As mentioned above that the NADH intensity


a
Dissolved oxygen
NADH (ex330/em450)
Dissolved oxygen [%]
Rel. fluorescence intensity [unit]
Start feedback control
Time [h]
b
Derivative value
NADH (ex330/em450)
Meatbolic signal (N') [rel. unit/min]
Rel. fluorescence intensity [unit]
Start feedback control
Time [h]
c
Derivative value
Feed rate
Metabolic signal (N') [rel. unit/min]
Feed rate [mL/min]
Stop controlling and pumping
Time [h]
d
Glucose
Ethanol
Biomass
Glucose/ Ethanol [g/L]
Biomass [g/L]
Start feedback control
Time [h]

(See figure on previous page)
Fig. 3 **a** Evolution of the dissolved oxygen and the NADH intensity during the fed-batch operation with the closed-loop control. **b** Evolution of the NADH intensity and its derivative as a metabolic signal during the fed-batch operation with the closed-loop control. The arrows show the points that the upper threshold was reached, but it might be because of the noise of the NADH intensity. **c** Metabolic signals and the feed rate profile during the controlled feeding phase. The arrows refer to the time that the lower threshold signal was not reached. **d** Glucose, ethanol, and biomass concentrations during the fed-batch operation with the closed-loop control

drops back to the same gradient of the linear rate, which indicates a state of oxidative metabolism, when the feed rate was reduced and kept constant. Besides, no matter how high the incline of the NADH peak is, the intensity will decrease to the same state of an oxidative phase (Fig. 3a). This phenomenon is observed empirically during the fed-batch cultivations. Due to the reduction of the dissolved oxygen, it can be presumed that more substrate was applied as shown in Figs. 2b and 3a. However, it is not clear to see which level of the dissolved oxygen indicates the overflow metabolism.

The NADH intensity obtained in real time during the fed-batch operation was computationally converted to the metabolic signal. The metabolic signals or derivative values during the controlled feeding phase are demonstrated in Fig. 3b, c. The upper and lower threshold signals were illustrated with the solid and dashed lines, respectively (Fig. 3b, c). The level of the threshold signals was determined from experiments by trial and error. However, they were realized based on outside noise area, which was between these two lines. For the upper threshold ($N' \geq 1.3$ rel. unit/min), the level was not set too high from the noise area to deter reaching overflow metabolism in time. The lower threshold ($N' < -0.8$ rel. unit/min) was also not set too low from the noises, because, if the lower threshold is not reached, it will take too long to maintain the constant reduced feed rate. Then, the glucose substrate might be too low for the yeast cells to reach an optimum growth. Although the lower threshold is achieved, the reduced feed rate is still maintained constant for a short while to make sure that the metabolism turns back to an oxidative state (Fig. 3b, c). There were a few times that the lower threshold was not reached after the upper threshold arrived (see arrows in Fig. 3c). In this case, the reduced feed rate will be maintained constant for 30 min before exponential feeding (Fig. 1b). The pause time to maintain the constant reduced feed rate was determined based on experiments. In some cases of reaching the upper threshold, it might be due to the noise intensity of NADH, as shown in Fig. 3b, with the small arrows. It could be also the reason why the lower threshold was sometimes not reached. In Fig. 3c, the feed rate at about 10 h was more reduced after the pause time, because the upper threshold was reached right away after 30 min. However, as shown in Fig. 3b, it might be because of the noise of the NADH intensity (see the arrow between 9 and 10 h). According to the results, the feed rate was decently regulated with the metabolic signals based on the NADH intensity.

Growth characteristics of yeasts under the feedback control

The growth characteristics of yeasts during the fed-batch process with the feedback control are demonstrated in Table 1 and Fig. 3d. The batch phase took almost 2 h until the concentration of glucose and ethanol was depleted. The yield coefficient ($Y_{X/G}$) during the batch phase is 0.31 $g_{biomass}/g_{glucose}$, which shows a characteristic of an oxidoreductive growth (Woehrer and Roehr 1981; Hantelmann et al. 2006). After all substrates in the batch phase ran out, the glucose feed solution was pumped into the cultivations, as can be seen at around 2 h in Fig. 3d. During 3–4 h, the fed-batch process became steady and the glucose concentrations were approximately 0.04–0.05 g/L, which are in the range of the critical value (Pham et al. 1998; van Hoek et al. 1998; Hantelmann et al. 2006). Thus, the feed solution was pumped with the exponential rate and the feedback control also started functioning. During the controlled feeding phase, the feed rate was continuously regulated in real time to keep the glucose concentrations in the range or under the range of the critical value, as shown in Fig. 3d. Furthermore, there was no production of ethanol during the controlled feeding phase, which indicates the state of oxidative metabolism. The yield coefficient during the controlled feeding phase reached 0.49 $g_{biomass}/g_{glucose}$, which shows the sign of a pure oxidative growth of yeasts (Sonnleitner and Käppeli 1986; Pham et al. 1998; Hantelmann et al. 2006). For the entire cultivation, the yield coefficient is reduced due to the oxidoreductive growth during the batch phase, as presented in Table 1.

Table 1 Yield coefficients during the fed-batch process of the yeast cultivations in triplicate

Cultivation phase	$Y_{X/G}$ ($g_{biomass}/g_{glucose}$)
Batch phase	0.31 ± 0.01
Controlled feeding phase	0.49 ± 0.01
Entire cultivation	0.46 ± 0.02

The values in the table are mean value ± standard deviation

The starting time of the controlled feeding phase is shown in Fig. 3d

According to the achievement of the typical high yield coefficient during the controlled phase, the 2D fluorescence spectrometer using the single signal of the NADH intensity has great potential to detect the metabolic change within 1.5 min and delivers instantaneous results to control the feed rate in real time. Although a conventional approach for the fed-batch control by off-gas analysis as respiratory quotient (RQ) is robust and widely used in industries, there is still the problem regarding a certain time delay comparing with the control by the NADH signal (Claes and van Impe 2000; Jobé et al. 2003). Besides, the cost of the fluorescence sensor with the specific wavelength equipped with light-emitting diodes and photodiodes will be more effective in comparison with off-gas analyzers (O'Toole and Diamond 2008; Yang et al. 2009). Another concern is the comparison between the potential of a direct and indirect measurement of the critical glucose concentration. Although it seems more reliable to measure directly the glucose concentration from the broth, there is a concern in a time delay and certain errors at the low critical concentration of glucose (Shimizu et al. 1988; Arndt and Hitzmann 2004).

Conclusions

In this contribution, we proposed a control of the feed rate based on the fluorescence intensity of NADH, which was selected over tryptophan, flavins, and pyridoxine. The signal of the NADH intensity showed the best performance to determine the metabolic switch between oxidative and oxidoreductive states. Under the feedback control, the glucose concentration was capably maintained under the range of the critical value. Accordingly, the glucose was oxidatively metabolized by the cells during the controlled feeding phase. From the results, the fluorescence sensor shows great potential not only for the applications in process monitoring, but also in the process control. However, there is still a challenging task for the scale-up fermentation process using the fluorescence sensor based on the NADH signal. There are many technical issues to be considered concerning transferring the bench scale to the larger scale cultivations, i.e., pilot and production scales (Formenti et al. 2014). The critical issues of the scale-up process can be basically categorized into biological, chemical, and physical impacts. The production strains should be fundamentally robust enough to withstand changing environmental conditions in the large scale, such as new media components, substrate, pH, temperature, and oxygen inhomogeneities (Takors 2012). These changing conditions make microorganisms stressed during the cultivations and affect metabolic activities in cells, particularly, the product yield and productivity. A scale-down tool, which is principally used as a lab test simulation of large-scale conditions, is based on the monitoring of metabolic responses (Takors 2012). Without doubt, the fluorescence sensor is the one of the powerful tools to detect the metabolic change in real time. Particularly, the NADH-based measurement can provide significant information about metabolic states of the yeast cells.

Authors' contributions

SA designed research and experiments, analyzed data, and wrote the article under the guidance of BH. MF carried out the experiments for the part of "Investigation of fluorescence signals corresponding to the metabolic change". FK performed the experiments of the feedback control of the yeast cultivations. SA reviewed the results and edited the manuscript under the guidance of BH. All authors read and approved the final manuscript.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data sets are presented in the main paper.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The research does not contain any studies with human participants or animals performed by any of the authors.

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