TOWARDS APPLICATION OF METABOLIC MODEL OF *SACCHAROMYCES CEREVISIAE* IN BIOPROCESS CONTROL ALGORITHM

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Abstract. Increasing bioprocess requirements for monitoring, control and identification of the process state require optimizing the performance and time to avoid disruption that may affect the success of the micro-organism cultivation process. Continuous estimation of the bioprocess parameters during fermentation of yeast *Saccharomyces cerevisiae* is the necessity to use models of cellular metabolism that enable to provide the essential information about the ongoing intracellular activities of biochemical bioprocesses and their impact on the quality of the desired product (ethanol) production process. It would bring significant benefits for experimenters and producers of biotechnological devices more objectively to determine the feedrate profile of substrates, nutrients and salts which must be supplied at a given time. A conceptual framework of the bioprocess algorithm has been developed on the basis of summarizing the current views in literature on system biology and process engineering linking capabilities. The developed approach gives a possibility early enough to send control commands for actuators to stabilize the bioprocess state and predict the future course of the procedure in order to ensure a high quality production process of ethanol.

Keywords: bioprocess control, metabolic model, bioethanol production.

Introduction

Nowadays with increasing development of biotechnology industry it is necessary to accurately monitor and control the biotechnological process to produce the target product of the highest quality and more profitable. Using less expensive raw materials, finding the ways for by-product minimization, tending to reach the theoretical maximal concentration of the desired product in fermentation, ensuring the optimal environment conditions (pH level, pO_2 (partial pressure of dissolved oxygen) temperature) for yeast *Saccharomyces cerevisiae* effective growing, these are only a few aspects of the biotechnologist aims to be resolved.

Reliable sensors to measure intercellular activities are rarely available, making the bioprocess states very difficult to characterise. Only the basic process variables (culture weight, temperature, pH, pO_2, CO_2 and O_2 off gas analysis, turbidity) can be directly measured online [1]. Accurate process models are rarely available due to the complexity of the biochemical processes. There is the lack of detail information in biochemical level, how the mutual response of metabolites ensure the vital functions as cell growing, regeneration, maintain their structures and rely on external influences.

Most described bioreactor control algorithms in the literature are PID control, model reference control, adaptive control, model predictive control, neural network control, fuzzy control and hybrid control [2-5]. In spite of the development of many advanced control algorithms, nearly 50 % of the controllers in the industrial field are using PID controllers [6]. This control is based on the feedback principle – the deviations of measurements are used as basis to perform quite accurate actions on deviations increase. The aim of PID control is to minimize deviations of the setpoint value. The main advantage of PID control is its simplicity, robustness and successful practical applications.

The aim of the paper is to discuss the opportunities to use a yeast metabolic model to improve the control of the bioreactor in the bioethanol production process. Therefore, with increasing availability of the new quantitative "omic" data and genome-scale metabolic models of yeast [7; 8] scientists search for solutions of effective application of these data of the bioreactor control algorithm.

Materials and methods

The last few years have seen substantial increase in fuel prices and various nations are trying to find alternatives to the fuel addiction. One of these alternatives is using biofuel "bioethanol". The technology to produce bioethanol from sugars is through the fermentation process of bioreactor whereby microorganisms such as yeast *S. cerevisiae* have the ability to convert sugar through their metabolism to the form of bioethanol. But industrial bioethanol producing is a complex process. It is essential to ensure optimal environment conditions (temperature, pH, pO_2), appropriate nutrients, proper yeast strains and optimal mass transfer rate of the bioreactor liquid choosing an appropriate

bioreactor construction (stirrer type, bioreactor vessel volume and design) [6]. Ethanol production by yeast *S. cerevisiae* is a well-studied application in biotechnology. Although near maximal concentration of ethanol is naturally produced by *S. cerevisiae*, this process is severely hampered by the stress caused by the high concentration of ethanol [9]. Currently the main strategies how to optimize the ethanol production process is trying to provide for all these affecting factors optimal conditions in action.

The described approach in this paper is that bioprocess control can be described in three levels (see Fig 1). The 1st level contains a control system for carrying out the parameters maintaining the given range and it is carried out by controlling devices (heater, peristaltic pumps, stirrer, O_2 and CO_2 flow controller). If the deviation of the parameter, for instance pO_2 value, is decreasing then the controller tries to run a control command to stabilize the state, change the stirrer rotation speed or start the oxygen enrichment process. PID controller is used for this control level most. One of the major difficulties for PID effective use is to set the appropriate PID controller P, I, D coefficients to be applied to a particular situation. Sometimes these parameters are necessary to change several times in microorganism fermentation because in the growth of the microorganism in different stages of the process dramatic changes are observed [10].

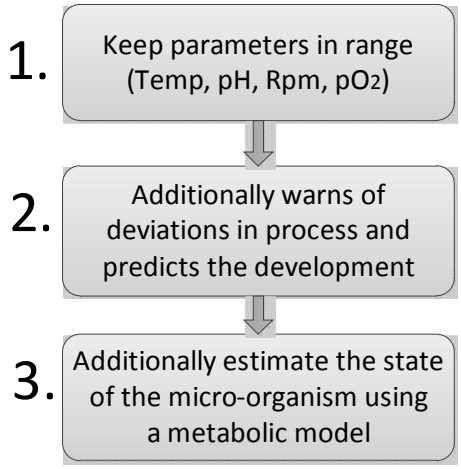

Fig. 1. **Levels of bioreactor control**

The 2nd level is more advanced that additionally warns of deviations in the process and predicts the development. Mostly the control algorithms of the current level are based on the model reference control, adaptive control, model predictive control and hybrid control. The quality of the model and above all its structure must correspond to the objective for which the model was built. The model could be used to detect deviations in the functioning of the process and could be used as a prediction tool to stabilize the fermentation process state early enough while the process is not left in an irreversible situation. The model predictive control has become a popular topic in the recent years [11-14]. Furthermore, the fact should be taken into account that more complex models require more information and it is more difficult to evaluate them and apply in automatic control systems for ethanol production-scale bioreactors.

The 3rd level additionally estimates the state of the micro-organism using a metabolic model. This allows controlling on a more detailed level (metabolome, fluxome) the process according to the intracellular changes of reactions fluxes, but there is a need to find a connection point between the real measured data of the sensors and metabolic model provided information. Only limited number variables of the metabolic model are observed in close correlation with the currently examined chemical, physical principles. With the aim to prevent this situation mathematical methods are used, which allow to detect the "hidden" information from the on-line data and thereby find a correlation with many opportunities for the off-line data [15].

Historically, bioprocess mathematical modelling was based on simple cell models where the biological system is inspected as a catalyst for the conversation of substrates into products without futher consideration about the intracellular processes. The intracellular processes of cells are viewed as "black-boxes" [16]. Mostly interactions between the bioreactor extracellular environment and

intracellular cells metabolites and enzymes as catalysers of reactions are generally ignored. Sometimes descriptive models are available using measurements of the optical density of the biomass, the substrate and product concentration changes. These models in most cases describe the microbial growth rate and product formation.

During fermentation it is important not only to follow the measured on-line or off-line parameter changes in the bioreactor, but also determine the actual physiological state of the micro-organism. The qualitative estimation of the micro-organism state [17] depends on the available measurements of pO_2, pH, temperature (T), off-gas (Fig. 2.).

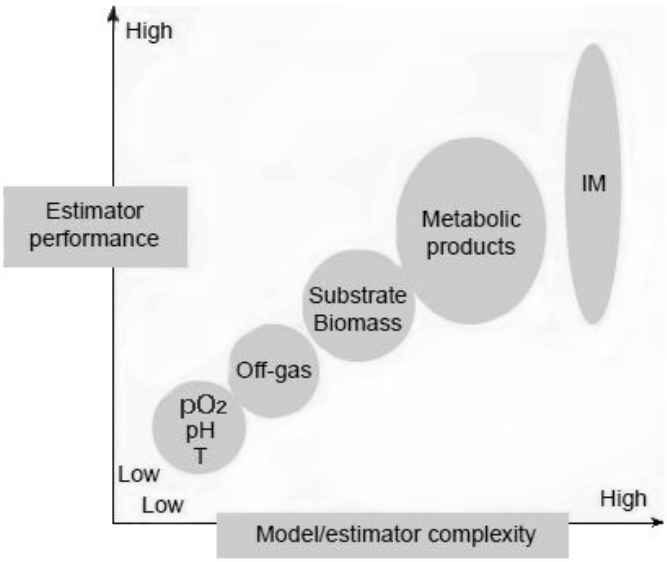

Fig. 2. **Estimatition performance of bioprocess** [17]

More detailed level information provides concentration measurements of products, substrates or intercellular metabolites (IM). Consequently, if a more detailed measurement level is supplied then the model/estimator complexity increases and the model performance more accurately describes the state of the micro-organism at a given time.

Results and discussion

The developed solution for more efficient link between the yeast metabolic model and the measurements of the bioreactor is applicable in literature more, and the flux balance analysis (FBA) approach is more described [18-21]. FBA is a modelling approach based on the constraints of a metabolic network. It is applicable method for the determination of the metabolic flux distribution in undetermined systems. The main source of constraints is the stoichiometric matrix which describes precisely which substrates at which quantity are necessary to be supplied to create the desired product. Fluxes are determined by the linear programming method where the selected objective criteria (target reaction flux) are optimized taking into account all cell physiological state constraints.

The next described solution for yeast fermentation optimization is using the elementary flux mode (EFM) approach [22-24]. EFM is a mathematical tool for metabolic pathway analysis. It decomposes a metabolic network into elementary modes which are the simplest paths able to operate in steady-state. The elementary mode includes the pathway from the substrate uptake to the desired product formation. Some authors have already presented applications of EFM [25-28] and showed experimental results. The problem is mentioned that the size of elementary modes dramatically increases with the size of the model [29].

The third described approach is the dynamic flux balance analysis [30; 31]. The dynamic flux balance analysis (DFBA) provides modelling of detailed metabolic models in the absence of enzyme kinetics and substantial information about intracellular regulatory processes. The method is based on the reasonable assumption that metabolite concentrations rapidly equilibrate in response to extracellular perturbations. A flux balance description of intracellular metabolism combined with dynamic mass balances on extracellular substrates and products allows the prediction of the cellular

behaviour as the extracellular environment changes with time [31]. DFBA may be used to generate dynamic predictions of extracellular metabolite profiles.

Examining the literature sources and analysing the possibility to efficiently provide optimal external environment conditions in the yeast fermentation process, a conceptual framework is developed (Fig. 3) that includes a dynamic metabolic model of yeast in the bioreactor control algorithm. This concept includes the bioreactor monitoring of the measured on-line and off-line data. The obtained sensor data are used in the mathematical model in the form of differential equations. A dynamic metabolic model of yeast has been used in addition, that has much more detailed level information for ensuring more efficient environment conditions on the microorganism state at a given time.

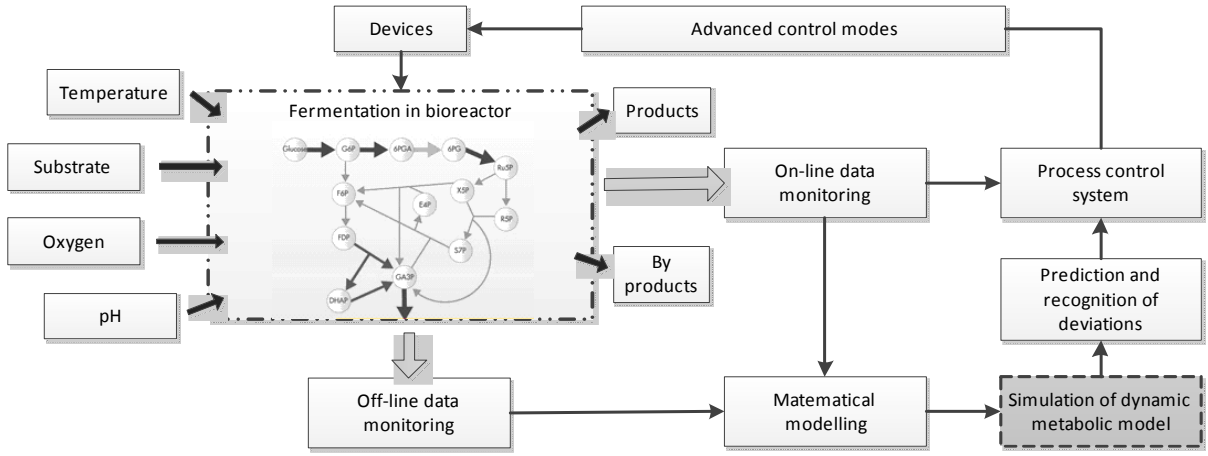

Fig. 3. **Application of metabolic model in bioprocess control algorithm (adapted from [15])**

The simulation of the dynamic metabolic model gives more advanced information (fluxome level) about deviation from the reference model at the current time. Using the outlined above FBA, EFM or DFBA approach into "simulation of the dynamic metabolic model" part of conceptual framework could be opportunities to dynamically generate the necessary feeding profile and would be a possibility to directly supply the yeast with actual nutrients, salts as calculated predictions with the metabolic model. Also it is needed be taken into account that simultaneously must be ensured the maximum product (ethanol) formation rate, minimized by-products (acetaldehyde) formation and the yeast would have high osmotic stress resistance to high ethanol concentrations. This approach provides prediction of the fermentation process and gives chances to eliminate the possible risks of deviations early enough. The obtained data are used for the process control system that automatically chooses advanced control modes for different fermentation process states. The advanced control modes involve specific control actions of devices (heater, peristaltic pumps, stirrer, O_2 and CO_2 flow controller) depending on the given process conditions.

Conclusions

The main aim of the work was making research in methods and possible solutions for the bioreactor optimal control algorithm that gives an opportunity to link the yeast *Saccharomyces cerevisiae* metabolic model with the measurable data in the bioreactor. Many methodologies have been published for genome-scale models (reconstructions) data analysis to obtain more detailed information on the cells intracellular level. But only some of them (FBA, EFM, DFBA) have showed good opportunities for implementation in the bioprocess control algorithm. It would allow on a more detailed level (metabolome, fluxome) to control the process according to the intracellular changes of reaction fluxes, generate dynamic substrate feedrate profiles, estimate the physiological state of the yeast and early enough change the extracellular environment conditions according to the metabolic model predictions using control devices (heater, peristaltic pumps, stirrer, O_2 and CO_2 flow controller).

A conceptual framework has been developed on the basis of summarizing the current views in literature on system biology and process engineering linking capabilities. It is planned to develop this approach on a more detailed level and demonstrate the results on real practical experiments of yeast fermentation using genome-scale models as basis for advanced control and make the framework

validation. Future development of the bioreactor control algorithm will depend on further progress in system biology and process engineering industries.

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