REVIEW

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Bioprocess modelling for the design and optimization of lignocellulosic biomass fermentation

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Abstract

Lignocellulosic feedstocks, which are currently under-exploited, can be used for the production of biofuels, such as ethanol, and for biorefinery applications to produce a variety of value-added products. Although bioconversion of lignocellulose by microbial or yeast fermentation have been reported, efficient and economical lignocellulosic fermentation process is still a challenge due to multiple process parameters involved for bioprocess design, optimization and scale-up. Bioprocess modelling strategies have been proven effective for achieving high-production process[*] efficiency in yield, productivity or titer of desired product. Several types of bioprocess modelling for lignocellulosic application have been developed and successfully validated as a promising alternative for rapid design, optimization and scaling up of biomass-based process. This review aims to summarize the important development of bioprocess modelling for lignocellulosic bioprocess applications towards the success of biorefineries and bio-based economy. In particular, we discuss modelling relevant to lignocellulosic bioprocess including cell modelling based on kinetics, stoichiometry and integrative approaches and fermentation kinetic modelling for process performance assessment. An overview of these modelling approaches and their application for systematic design of efficient and economical lignocellulose-based bioprocesses are given.

Keywords: Lignocellulosic bioprocess, Systematic process optimization, Integrative cell modelling, Fermentation model, Process integration

Background

Low-priced, abundant and renewable lignocellulosic biomass has become an attractive alternative feedstock to significantly supplement corn and starch as a fermentation feedstock for bio-based production (FitzPatrick et al. 2010; Kircher 2012). These substrates can be obtained from agricultural, industrial and municipal solid wastes and forestry residues. The use of lignocellulose resources for the production of biochemicals and biofuels is considered as cost-effective and environmentally sustainable serving bio-based economy (Binod et al. 2010; Lopes 2015). The optimization of the technology and scale-up for lignocellulosic bioprocess is rapidly developing by

several biotech companies and pilot plants in Europe and the US. Bioconversion of lignocellulose to bioproducts requires lignocellulosic biomass to be hydrolysed in order to generate monomeric sugars for the fermentation step. Hydrolysis of lignocellulose is usually achieved by means of a thermal and/or chemical pretreatment followed by enzyme hydrolysis. Many studies have demonstrated the feasible production of bioproducts by both bacteria (e.g. *Zymomonas mobilis, Escherichia coli*) and yeasts (e.g. *Saccharomyces cerevisiae, Scheffersomyces stipitis*) using lignocellulosic feedstock (Geddes et al. 2015; Zhang and Lynd 2010; Van Zyl et al. 2007; Unrean and Nguyen 2012). However, several challenges remain for achieving the efficient hydrolysis and fermentation of lignocellulose. Studying enzymatic and chemical hydrolysis of lignocellulosic biomass based on experimental and modelling approaches has been extensively reviewed elsewhere (Van Dyk and Pletschke 2012; Bansal et al.

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2009; Meng and Ragauskas 2014; Hodge et al. 2009; Geddes et al. 2010; Sun and Cheng 2002). Hence, this review focuses on the fermentation step of lignocellulosic bioprocess based upon integrative cell and fermentation kinetic modelling framework.

One of the challenges of lignocellulose fermentation is the presence of sugar mixture (mainly glucose and xylose) released during the pretreatment and enzyme hydrolysis of lignocellulosic materials. From an economic point of view, these sugars must be efficiently fermented by organisms into desired product (Bera et al. 2010; Konishi et al. 2015; Unrean and Srienc 2010). The fluctuation of sugar composition, 30–50 % and 10–25 % of dry weight for glucose and xylose content, respectively, in different biomass feedstock strongly affects fermentation performance since an organism may not be able to optimally adjust its fermentation capacity to match with the change in sugar composition resulting in long fermentation time. A culture system that is able to handle the variation of sugar composition and efficiently ferment the sugar mixture is therefore required in order to meet the technical and economic requirements of industrial lignocellulose-based process. Another challenge for lignocellulosic fermentation is the presence of inhibitory substances (such as acetic acid and furans) generated during the pretreatment strongly inhibiting growth and fermentation performance of fermenting organism (Almeida et al. 2007; Allen et al. 2010; Klinke et al. 2004). These inhibitors are significant hurdles for the implementation of large-scale lignocellulose-based bioprocess. Removal of the inhibitors by physical and chemical means significantly adds to the overall process cost and causes loss of sugars (Liu and Blaschek 2010). Therefore, the use of inhibitor-tolerant microorganisms in the fermentation or the use of optimized process configuration to minimize inhibitory effects is required to improve process efficiency. The development of inhibitor-tolerant cell factory is previously reviewed by Liu (2006, 2011) describing the mechanisms of action of known inhibitors as well as metabolic and evolutionary engineering strategies for tolerant strain development. Thus, this review focuses on fermentation process configuration to overcome inhibition issue caused by the inhibitors and fermentative end products. Moreover, problems with viscosity and partial insolubility of lignocellulosic biomass can cause poor mixing and limited mass and heat transfer especially at high solid operation of fermentation process. Design of fed-batch process configuration with sufficient mixing is required to improve process efficiency (Nguyen et al. 2015; Geddes et al. 2010; Unrean et al. 2015).

Hence, this review article discusses the development of cell and bioprocess modelling to provide a comprehensive update of the model-based approach for the design,

optimization and scale-up of biomass-based processes. Specific modelling strategies for optimizing fermentation control in lignocellulosic bioprocess based on integrative cell modelling and fermentation kinetics are discussed.

Cell modelling for growth and fermentation of lignocellulosic hydrolysate

Different type of cell modelling that is relevant to cell growth and fermentation of lignocellulosic bioprocesses is explored as follows:

Monod cell growth kinetics

An unsegregated and unstructured model based on Monod kinetic has been the most commonly used model to describe the overall cell growth and fermentation in batch, fed-batch or continuous lignocellulosic biomass processes. The Monod's cell modelling which considers cell growth as one, single reaction is typically composed of the kinetics of (1) cell growth determined by limiting substrate (i.e. glucose or xylose present in biomass feedstock), and (2) cell death due to the endogenous metabolism as well as toxicity caused by end product or inhibitors (e.g. furfural, HMF or acetic acid) present in hydrolysates. A generalized Monod's cell growth equation with competitive and non-competitive inhibition of inhibitors and cell growth inhibition of end product is

Specific cell growth rate:

$$\mu_{S_i} = \mu_{\max,S_i} \left(\frac{C_{S_i}}{K_{\mathrm{mu},S_i}\left(1 + I_i/K_i\right) + C_{S_i}} \right) \prod_j \left(\frac{1}{1 + I_j/K_j} \right) \left(1 - \frac{C_P}{C_{P,\max}} \right)^n. \tag{1}$$

In addition, cell death kinetics is typically applied to prevent an over-prediction of cell viability in lignocellulosic process (Zhang et al. 2009a, b). Both cell death rate caused by end product and cultivation temperature can be described by Arrhenius-type kinetics (Mutturi and Lidén 2014). Furthermore, two distinct population of cells: one is active population able to replicate, Type I cell, and another is stalled population unable to replicate due to toxicity of inhibitors, Type II cell, can also be included in the cell growth model (Wang et al. 2014). The predicted cell death rates due to endogenous metabolism, temperature, end product and toxicity of inhibitors present in hydrolysate are given as follows:

Specific cell death rate:

$$K_D = \sum_{S_i} \left(m_{S_i} Y_{X,S_i}^{\max} \right). \tag{2}$$

Temperature-dependent cell death rate:

$$K_{DT} = A \exp(-E/RT). \tag{3}$$

Product-dependent cell death rate:

$$K_{\text{De}} = a \exp(bC_{\text{P}}). \tag{4}$$

Transformation rate from type I to type II cell:

$$K_{\text{trf}} = k_{\text{T}} + \frac{v_{\text{T}}^{\max} I_k^{\text{H}}}{k_{\text{T}}^{\text{H}} + I_k^{\text{H}}}. \tag{5}$$

The generalized equation describing cell growth can then be generated by combining Eqs. (1, 2, 3, 4 and 5):

$$\frac{dX}{dt} = \left(\sum_{S_i} \mu_{S_i} - K_{\text{D}} - K_{\text{DT}} - K_{\text{De}} - K_{\text{trf}} \right) X - DX. \tag{6}$$

This equation is commonly applied for predicting cell growth during the fermentation of lignocellulosic hydrolysate.

Stoichiometric metabolic model

Cell growth can also been simulated based on a steady-state flux balance model which solves the stoichiometric mass balance of metabolic reaction network within cell. The model allows for quantification of carbon flux occurring within the cell by coupling extracellular fluxes for cell growth, substrate uptake and product secretion with the intracellular flux distribution in matrix form:

Stoichiometric flux balance:

$$\underline{\underline{S}} \cdot \underline{q} = \underline{J}. \tag{7}$$

The most common mathematical tool used for solving these balance equations is flux balance analysis (FBA). The reader is referred to Maarleveld et al. (2013) for the thorough review of concept and application of this computation tool. Briefly, FBA yields a single flux solution that satisfies specified objective and constraints based on linear program (LP) optimization. The commonly used objectives are as follows:

Objective : $\max \mu, q_P$ or q_{ATP}
Subject to : $q_{\min} \le q \le q_{\max}$
$\qquad\qquad J_i = J_i, i \in E$
$\qquad\qquad J_i = 0, i \in N$
$\qquad\qquad J_{i,\min} \le J_i \le J_{i,\max}, i \in M$

The stoichiometry metabolic model has been utilized to study the response of cell metabolism to different environmental and genetic perturbations or different stresses caused by inhibitors during lignocellulosic fermentation process (Heer et al. 2009; Hanly and Henson 2014). By constraining fluxes associated with corresponding genes, the stoichiometric model can be applied to guide genetic engineering for increasing production of biorefinery products such as ethanol, malic acid and succinic acid (Pizarro et al. 2007; Oberhardt et al. 2009) as well as

to aid process development, optimization and scale-up (Baart et al. 2007). Integration of stoichiometric metabolic model with dynamic model, regulatory and signalling network in the future could significantly increase the usefulness of the model for guiding cell engineering and optimizing lignocellulosic bioprocesses.

Fermentation kinetic model

Kinetic model to describe fermentation profile of lignocellulosic hydrolysate can be developed by taking into account growth-limiting factor such as sugar and/or nitrogen content, product titer and temperature influenced fermentation process. The proposed lignocellulosic fermentation kinetic model typically comprises (1) the sugar uptake equation and (2) the fermentation equation of secreting products. Sugar uptake model following Michaelis–Menten kinetics considers the uptake rate of hexose and pentose sugars (e.g. glucose or xylose) for cell growth, product synthesis and for maintenance process, the competitive inhibition between hexoses for each transporter (Pizarro et al. 2007) and the non-competitive inhibition between hexoses and pentoses (Zhang et al. 2009a, b). The non-competitive inhibition of sugar transport caused by increasing concentration of end product and by the presence of inhibitors (e.g. acetic acid, furfural or HMF) are also commonly included in the model to capture the adverse effects of these compounds on sugar fermentation (Hanly and Henson 2014). A generalized kinetics of sugar uptake is

Specific sugar uptake rate:

$$q_{S_i} = \left(\frac{V_{\max,S_i} C_{S_i}}{K_{m,S_i} + C_{S_i}} \right) \prod_{S_j} \left(\frac{1}{1 + C_{S_j} / K_{S_j}} \right) \prod_j \left(\frac{1}{1 + I_j / K_j} \right). \tag{8}$$

The balance equation of sugar during lignocellulosic hydrolysate is as follows:

Balance equation of sugar:

$$\frac{dC_{S_i}}{dt} = -q_{S_i} X - m_{S_i} X + D \left(C_{S_i,\text{feed}} - C_{S_i} \right). \tag{9}$$

Some yeast cells such as *S. cerevisiae* have ability to convert inhibitors (e.g. furfural or HMF) present in lignocellulosic hydrolysate into less toxic compounds. Thus, kinetics of inhibitor conversion should also be included when describing cell growth and fermentation. The conversion kinetics of inhibitors can be defined similarly to that of sugar uptake. A model developed by Hanly and Henson (2014) has described the detoxification of furfural and HMF from hydrolysate media by *S. cerevisiae*. The kinetics of fermentation describing growth-associated production based on Monod kinetics

and non-growth-associated production for fermentation products can be written in a general form as follows:

Secreting products:

$$\frac{dC_{P_i}}{dt} = q_{P_i}X - DC_{P_i} \tag{10}$$

$$q_{P_i} = \left(\frac{V_{max,\,P_i}C_{S_i}}{K_{P_i} + C_{S_i}}\right) = Y_{P_i,\,S_i}(q_{S_i} + m_{S_i}). \tag{11}$$

Combining cell growth and fermentation kinetic model then permits the prediction of time profiles for the production of bioproducts, such as ethanol, during lignocellulosic hydrolysate fermentation.
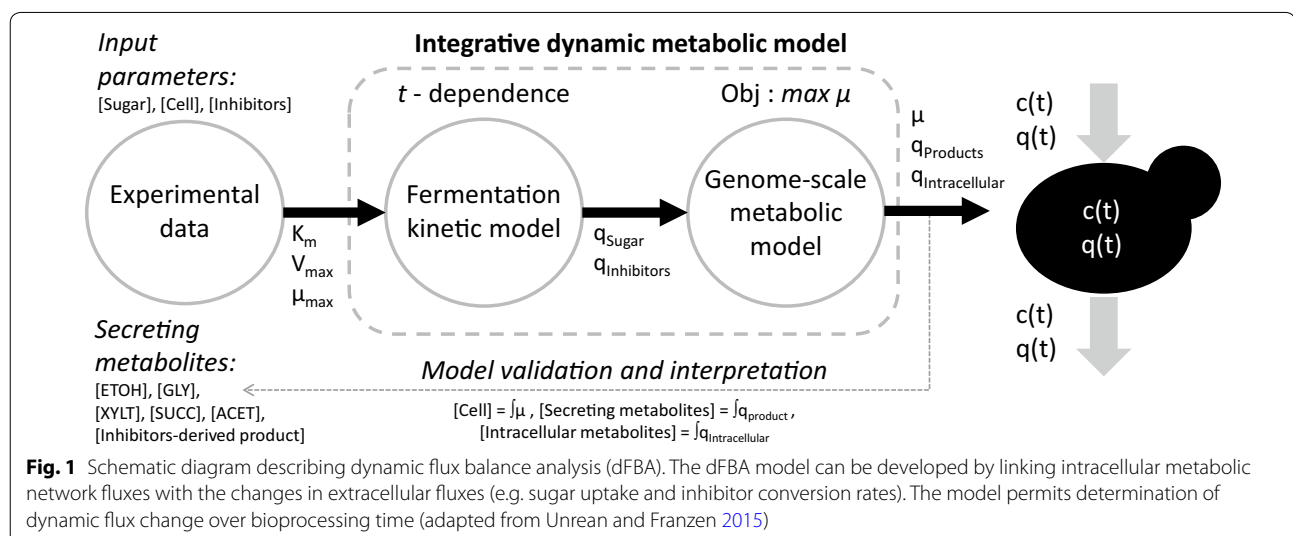
Integrative dynamic model for cell growth and fermentation

Most modelling frameworks describing cell growth and fermentation are based on a simple unstructured Monod kinetic model or a steady-state stoichiometric flux balance model. Integrative dynamic model framework has recently been proposed through incorporation of kinetic model and stoichiometric metabolic model for the prediction of dynamic whole-cell metabolism as the culture environment dynamically changes with time. Integrative dynamic model allows the thorough studies of a dynamic interaction of cell metabolism occurring during culture environment changes or genetic alternation by predicting optimal metabolic flux distribution at each instant time throughout the process. Such model may enable an expanded platform to design process or genetic modification candidates that may enhance the efficiency in batch or fed-batch of lignocellulosic bioprocesses. Two types of integrative dynamic model capable of simulating dynamics of cell growth and fermentation in batch or fed-batch

fermentation of lignocellulosic biomass have been developed: (1) dynamic flux balance model and (2) cybernetic model.

Dynamic flux balance model

Concept of dynamic flux balance analysis (dFBA) is relied on a flux balance stoichiometric network in combination with kinetic model describing cell growth and fermentation as illustrated in Fig. 1. First, the dynamic model utilizes kinetic equations to predict the substrate uptakes and additional flux constraints which are then used as inputs for the stoichiometric model analysis. The outputs of the flux balance model are the predicted specific rate of substrate and product (biomass and end product). The computed consumption and production rates based on flux balance are fed into the dynamic mass balance model, which are differential balance equations describing the concentration of the extracellular metabolites considered in the model. The dynamic mass balance is solved numerically to calculate time profiles of substrate and product in the fermentation process. The dFBA model has been used to predict cell growth and fermentation profiles in response to nitrogen source, culture temperature, inhibitory compounds (e.g. furfural, HMF) and ethanol toxicity in batch and fed-batch fermentation of lignocellulosic hydrolysates (Sainz et al. 2003; Pizarro et al. 2007; Hjersted and Henson 2006; Hanly and Henson 2014; Unrean and Franzen 2015; Unrean et al. 2015). In addition, the dFBA model can accurately predict the dynamic effects of genetic alternations and regulatory processes on the production performance (Pizarro et al. 2007; Lee et al. 2008). Thus, dFBA model proves useful for evaluation of the dynamic interactions between the cell metabolism and its changing environment in batch

Fig. 1 Schematic diagram describing dynamic flux balance analysis (dFBA). The dFBA model can be developed by linking intracellular metabolic network fluxes with the changes in extracellular fluxes (e.g. sugar uptake and inhibitor conversion rates). The model permits determination of dynamic flux change over bioprocessing time (adapted from Unrean and Franzen 2015)

and fed-batch fermentation which could lead to a better design of cell and fermentation conditions in lignocellulosic process.

Cybernatic model

Cybernetic modeling framework is based on the incorporation of internal dynamics of simplified regulated metabolic network of cell and the effects of external environment (Murthy et al. 2012). Similar to dFBA model, the cybernetic model can be divided into two distinct but interlinked models. The first model determines reaction rates of cybernetic metabolic network model consisting of simplified catabolic and anabolic pathways that produce energy, catabolic and anabolic precursors necessary for cell growth and fermentation. These pathways are optimally utilized by cell for maximizing cell growth. The second model determines kinetics for cell growth, substrate uptake and product secretion based on the metabolite balance equations. The cybernetic model is typically described by set of equations for reaction rates following Monod kinetics by assuming to vary directly with the relative enzyme concentration and to exhibit saturation dependence on all substrates.

Reaction rate expression:

$$r_i = k_i \varepsilon_i \prod_j \left(\frac{1}{1 + K_{m_j} / C_{m_j}} \right). \tag{12}$$

The enzyme balance equation is given by
Enzyme balance:

$$\frac{\mathrm{d}e_k}{\mathrm{d}t} = r_{e_k} - (\beta_k + \mu) \, e_k. \tag{13}$$

The balance equations for cell growth, substrate, product and intracellular metabolite can be described as follows:

Cell growth:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \left(\sum_i r_i v_i \right) X - DX. \tag{14}$$

Extracellular metabolite:

$$\frac{\mathrm{d}C_{mexj}}{\mathrm{d}t} = \left(\sum_i r_i v_i \right) X - D \left(C_{mexj,feed} - C_{mexj} \right). \tag{15}$$

Intracellular metabolite:

$$\frac{\mathrm{d}C_{m_j}}{\mathrm{d}t} = \sum_i r_i v_i - \mu_{S_i} C_{m_j}. \tag{16}$$

The cybernetic modelling approach has been used to accurately simulate yeast cell growth, ethanol fermentation and energy consumption in batch, fed-batch and continuous fermentation of lignocellulosic biomass (Straight and Ramakrishna 1994; Ko et al. 2010; Murthy et al. 2012).

Model-based process design and optimization
Cell consortium model for optimizing co-culture fermentation

The process using cell consortia holds promise for a better exploitation of individual species capabilities leading to an efficient fermentation of pentose and hexose sugars that compose lignocellulosic biomass. A mixture of multiple substrate-selective microbial or yeast strains is expected to act in concert to simultaneously uptake pentose and hexose sugars and efficiently convert to value-added bioproducts (Suriyachai et al. 2013; Henson and Hanly 2014). Several studies have developed cell consortium model based on cell growth, fermentation kinetic model and dynamic flux balance model to study the capability of co-culture system and to optimize cell growth and mixed sugar fermentation performance by co-culture (Unrean and Srienc 2010; Unrean and Khajeeram 2015; Hanly and Henson 2013). Using the co-culture of multiple strains enhances ethanol titer, production rate, shorten fermentation time, and reduce process costs making the co-culture process a promising technology for industrial applications (Chen 2011; Wan et al. 2012; Yadav et al. 2011; Li et al. 2011; Hickert et al. 2013). The dynamic co-culture model has been applied to optimize the inoculum cell concentration and aeration level that maximized fermentation process efficiency (Unrean and Srienc 2010; Hanly and Henson 2013). Co-culture model has also been used to predict the optimal relative cell ratio of each strain that yields simultaneous consumption of different sugar mixture with minimal fermentation time enabling improved productivity and less production cost (Hanly et al. 2012; Unrean and Khajeeram 2015). The co-culture model also demonstrates the flexibility of the cell consortia for optimally handling any sugar mixture available in different biomass feedstock. Additionally, Hanly and Henson (2013) applied the cell consortium modelling strategy for predicting targeted gene manipulation in the xylose-fermenting yeast cell in order to further improve ethanol fermentation by co-culture. The cell consortium modelling framework could, therefore, provide strategies for rapid process optimization of the multiple-strain culture by optimally adjusting each strain distribution based on the model prediction to match with varying sugar composition in lignocellulosic biomass feedstock for efficient and sustainable production of bioproducts.
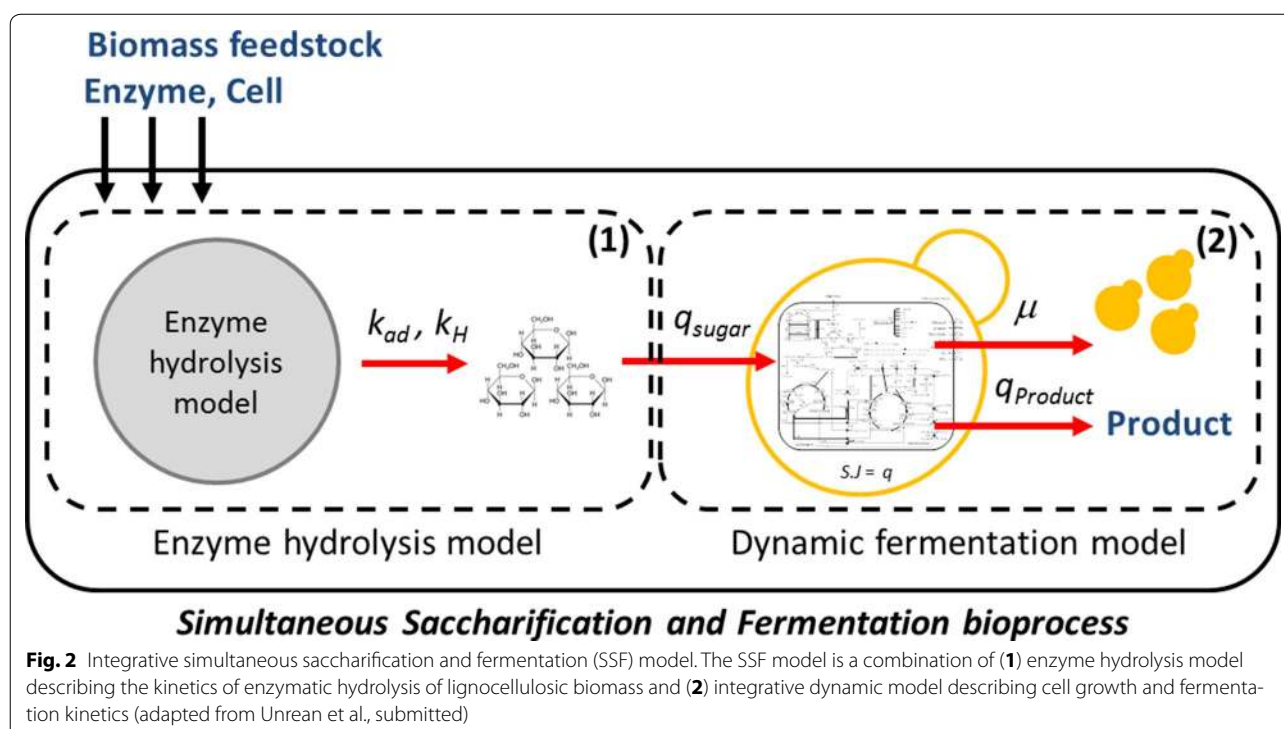
Fed-batch lignocellulosic bioprocess optimization

Fed-batch cultivation strategy by controlling the substrate feeding can be applied (1) to overcome inhibitory

effects by maintaining the inhibitors at low concentrations, (2) to avoid accumulation of undesired byproducts caused by overflow metabolism and (3) to ensure a balanced feeding of mixed hexose and pentose sugar available in biomass feedstock for achieving high yield, titer and productivity of the desired product (Abdel-Rahman et al. 2015; Rudolf et al. 2007; Petersson and Lidén 2007; Johnsson et al. 2013). A kinetic model based upon a system of linear differential equations can be formulated to design and optimize various process configurations such as batch and fed-batch process for efficient fermentation of biomass-derived sugars. The model-based process optimization was demonstrated in designing feed strategy with optimal specific cell growth rate of fed-batch for efficient mixed glucose–xylose fermentation (Unrean and Nguyen 2012). The optimized batch with cell recycle or with in situ ethanol removal was also simulated based on the kinetic model (Slininger et al. 2014). Besides application of the integrative dynamic model to study whole-cell metabolism during batch and fed-batch processes, the modeling approach can be used for in silico determination of the optimal operating conditions, such as feed rate or feed medium composition, for fed-batch fermentation of lignocellulosic hydrolysate (Unrean and Franzen 2015).

Coupling cell and fermentation kinetic model with enzyme hydrolysis model permits the prediction of dynamic cell growth and fermentation during simultaneous saccharification and fermentation (SSF) process.

The fed-batch SSF offers several advantages including less water consumption, lower production cost through the reduced number and size of required equipment and utility as well as minimized negative effects of inhibitors present in lignocellulosic hydrolysate (Olofsson et al. 2008; Mohagheghi and Schell 2010; Koppram et al. 2014). An SSF modelling approach is a useful guiding tool for rational design of the optimal feed profiles of solid substrate, enzyme and yeast cell in fed-batch SSF to avoid poor mass and heat transfer caused by high viscosity and to maximize process efficiency, thereby meeting the technical and economic requirement of the lignocellulosic biomass process (Zhang et al. 2010; Zhao et al. 2013; Huang et al. 2014). Several mechanistic models for SSF have been previously developed which describe kinetics of enzyme hydrolysis and yeast cell fermentation (Van Zyl et al. 2011; Morales-Rodriguez et al. 2011; Mutturi and Lidén 2014; Wang et al. 2014). The SSF model comprises two interlinked models, the enzyme hydrolysis model providing the quantitative analysis of the enzyme kinetics and the fermentation kinetic model describing kinetics of cell growth and sugar fermentation by organisms. The integrative SSF model has also been developed which integrates the enzyme hydrolysis model with the dynamic cell metabolic model to quantitatively capture the dynamic responses of enzyme and cell metabolism with changing culture environment (e.g. substrates, inhibitors and end products) during SSF. Figure 2 represents

Fig. 2 Integrative simultaneous saccharification and fermentation (SSF) model. The SSF model is a combination of (**1**) enzyme hydrolysis model describing the kinetics of enzymatic hydrolysis of lignocellulosic biomass and (**2**) integrative dynamic model describing cell growth and fermentation kinetics (adapted from Unrean et al., submitted)

schematic diagram describing integrative SSF model. This integrative SSF modelling approach describing the enzyme kinetics together with the dynamics, time-dependence involved in the cell metabolism is capable of accurately predicting ethanol fermentation profiles by *S. cerevisiae* during SSF process. The model is considered a useful guiding tool for predicting fed-batch SSF process performance under various solid substrate, enzyme and yeast cell feed profiles permitting a systematic optimization of feeding strategies for efficient fed-batch SSF with maximized product yield, titer and productivity (Unrean et al. 2015).

Future prospect

The utilization of lignocellulosic feedstocks as substrate in bio-based processes has increased considerably in recent years for a sustainable development of bio-based economy. Design and optimization of lignocellulosic bioprocesses to improve yield, titer and productivity of desired bioproducts is key to the success of bioprocesses and biorefineries. Model-based bioprocess design and optimization appears as a promising approach that can be used, in combination with genetic engineering and fermentation control, to facilitate the systematic design and optimization efforts aimed at rapidly improving efficiency of lignocellulosic biomass process for the production of value-added products. Integrative cell and fermentation kinetic modelling can assist in designing fermentation strategies or identifying genetic modification candidates for enhanced lignocellulose-based bioprocess efficiency to meet the current technical and economical demand. However, the current models do not include the regulatory and signalling network or stress response mechanisms of the cell when being cultured in lignocellulosic hydrolysate which also play important roles in determining the process efficiency. Inclusion of high-throughput omics data to describe cellular regulation and genome-wide kinetics is a future trend to further improve the accuracy of the integrative modelling framework for lignocellulosic bioprocess design, optimization and scaling up.

Nomenclature

C_{S_i}	concentration of sugar S_i
C_{S_j}	concentration of sugar S_j
$C_{S_i,\text{feed}}$	concentration of sugar S_i in feed media
C_P	concentration of end product P
$C_{P,\text{max}}$	maximum concentration of end product P
C_{m_j}	concentration of intracellular metabolite m_j
C_{mexj}	concentration of extracellular metabolite $m_{ex,j}$
$C_{mexj,\text{feed}}$	concentration of metabolite $m_{ex,j}$ in feed media
I_i	concentration of competitive inhibitor i
I_j	concentration of non-competitive inhibitor j
I_k	concentration of inhibitor k
X	biomass concentration
t	fermentation time
μ_{S_i}	specific cell growth rate
μ_{max,S_i}	maximum specific growth rate on sugar S_i
D	dilution rate of continuous culture mode
q	metabolite flux vector of enzymatic reaction
q_{S_i}	specific uptake rate of sugar S_i
q_{P_i}	specific production rate of product P_i
q_{ATP}	synthesis rate of ATP
V_{max,S_i}	maximum rate of sugar S_i uptake
V_{max,P_i}	maximum specific production rate of product P_i
$v_{\text{T}}^{\text{max}}$	maximum specific transformation rate
K_{m,S_i}	saturation constant of sugar S_i uptake
K_{S_j}	non-competitive inhibition rate constant of sugar S_j on sugar S_i
K_{mu,S_i}	saturation constant for growth on sugar S_i
K_{P_i}	saturation constant of product P_i
K_i	competitive inhibition rate constant of inhibitor i
K_j	non-competitive inhibition rate constant of inhibitor j
K_{trf}	specific transformation rate from type I to type II cells
k_{T}	inhibitor saturation constant of type I–type II cell transformation
n	exponential constant of ethanol inhibition to growth on sugar S_i
m_{S_i}	maintenance coefficient for growth on sugar S_i
Y_{X,S_i}^{max}	maximum cell yield on sugar S_i
Y_{P_i,S_i}	yield of product P_i based on consumed sugar S_i
A	frequency factor for Arrhenius equation
E	activation energy for Arrhenius equation
T	culture temperaturef
a	ethanol death coefficient
b	ethanol death rate constant
H	coefficient for cooperative transformation from type I to type II cells
S	m by n stoichiometric matrix of metabolite m in enzymatic reaction n
J	vector of accumulation and exchange rates
E	set of intracellular metabolites with externally determined exchange flux
N	set of intracellular metabolites with no accumulation
M	set of extracellular metabolites based on

experimental measurement

r_i specific rate of reaction i for synthesis or degradation of metabolite

r_{ek} synthesis rate of enzyme k

k_i rate constant of reaction i

ε_i relative concentration of enzyme catalysing reaction r_i

K_{m_j} saturation constant of metabolite m_j

e_k concentration of enzyme k

β_k first-order degradation constant of enzyme k

v_i cybernetic variable for activity of enzyme i

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