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Mathematical modeling of ethanol production by *Saccharomyces cerevisiae* in batch culture with non-structured model

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In this study, batch fermentation of glucose to ethanol by Saccharomyces cerevisiae (ATCC 7754) was carried out using 2.5 dm^3 BioFlo®115 bioreactor. The main objective of this study was to investigate the kinetics of ethanol fermentation by means of the non-structured model. The fermentation process was carried out for 72 h. Samples were collected every 4 h and then yeast growth concentration of ethanol and glucose were measured. The mathematical model was composed of three equations, which represented the changes of biomass, substrate and ethanol concentrations. The mathematical model of bioprocess was solved by means of Matlab/Simulink™ environment. The obtained results from the proposed model showed good agreement with the experimental data, thus it was concluded that this model can be used for the mathematical modeling of ethanol production.

Keywords: mathematical modeling, bioreactor, bioprocess engineering, ethanol production

1. INTRODUCTION

Ethanol (bioethanol) is one of the most promising substitutes of fossil fuels for the economic and environmental reasons (Lee et al., 2017; Phisalaphong et al., 2006). The application of ethanol as a fuel can reduce CO_2 accumulation and NO_x emission in the atmospheric system (Srimachai et al., 2015). The economic reason is that ethanol production can be relatively cheap, when it uses agriculture wastes or lignocellulosic materials (Bai et al., 2008; Srimachai et al., 2015). Ethanol can be produced from any material which contains sugar (Dodić et al., 2012; Nikolić et al., 2017). In the case when sugar comprises polysaccharides, it is previously broken to monosaccharides and then fermented (Cardona and Sanchez, 2007; Dodić et al., 2012).

The ethanol production processes can be realized by different techniques and regimes (batch or continuous), from various materials and in different scale (Muruaga et al., 2016).

The kinetic characteristic of biomass growth and ethanol production are required for effective and efficient performance of the fermentation (Phisalaphong et al., 2006), which is important because of increasing interest in the industrial application of the ethanol fermentation (Bai et al., 2008; Imamoglu and Sukan, 2013). Kinetic models can be used to control the process, reduce it costs and increase product quality (generally to optimize process) (Al-Qodah and Lafi, 2001; de Andreas-Toro et al., 1998; Imamoglu and

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Sukan, 2013). Such models provide also better understanding, designing and controlling of fermentation process (de Andreas-Toro et al., 1998; Imamoglu and Sukan, 2013).

The biological processes are complex, therefore can be affected by many factors (Imamoglu and Sukan, 2013; Rakoczy et al., 2016). Table 1 shows a short review of the selected papers concerning fermentation processes, which take into account different substrates, microorganisms, types of bioreactor and regime.

Table 1. Comparison of selected models from the literature

Ref.	Substrate	Microorganism	Type of bioreactorand process regime
(Phisalaphong et al., 2006)	sugar solution from cane molasses	*Saccharomyces cerevisiae*	batch fermentation in shake flasks
(Germec et al., 2015)	carob pod extract	*Saccharomyces cerevisiae*	repeated-batch ethanol fermentations performed in a 5 L stirred tank biofilm reactor
(Dodić et al., 2012)	sugar beet raw juice	*Saccharomyces cerevisiae*	14 L batch vessel with mixing using using two parallel Rushton turbines (at 150 rpm) and 4 baffles
(Srimachai et al., 2015)	oil palm frond juice	*Saccharomyces cerevisiae*	batch fermentation in 250 ml flasks with shaking at 150 rpm
(Fan et al., 2015)	glucose	*Saccharomyces cerevisiae*	closed-circulating fermentation process with a pervaporation membrane bioreactor
(Imamoglu and Sukan, 2013)	rice hulls (glucose and xylose)	recombinant *E. coli* KO11	100 ml flask shaking at 228 rpm; 2 L, 5 L and 10 L stirred-tank bioreactors at the stirrer rates of 312; 220 rpm

Moreover, the values of the most common parameters in mathematical models describing processes conducted by use of the microorganisms are presented in Table 2.

As can be seen from Tables 1 and 2 there are many studies concerning fermentation processes using various components and process conditions. Models in the literature investigated the influence of temperature (de Andreas-Toro et al., 1998; Phisalaphong et al., 2006), substrate composition (Germec et al., 2015; Imamoglu and Sukan, 2013; Srimachai et al., 2015) and its concentration (Dodić et al., 2012; Srimachai et al., 2015) scale of vessels (de Andreas-Toro et al., 1998; Imamoglu and Sukan, 2013) and agitation parameters (Germec et al., 2015; Imamoglu and Sukan, 2013). Parameters presented in Table 2 are used in many various models e.g.: Monod, Gompertz and Luedeking–Piret models. The values of parameters shown in Table 2 depend on different factors: type of microorganisms, temperature, kind and concentration of the substrate.

In the literature there are many different approaches of the biological process modeling with models describing only one component (biomass or product growth), two components (Fan et al., 2015; Srimachai et al., 2015) or three and more components (in case of several substrates or products) (Imamoglu and Sukan, 2013). Realization of a complex model with many equations interrelated is complicated but necessary in order to understand and predict biological process. However, models which separately describe changes of every component are often used. The most common is mixed approach – some of equations are interrelated some of them are not (Dodić et al., 2012; Staniszewski et al., 2007).

Table 2. Comparison of the parameters in selected models from the literature

Ref.	Model equations	Parameter values and remarks
(Phisalaphong et al., 2006)	comprehensive kinetic model modified from the Monod kinetics model; equations describing changes in the product and substrate concentrations	μ_m = 0.1 – 0.78 1/h $Y_{P/S}$ = 0.011 – 0.059 or 0.22 – 0.51* g/g investigation of temperature effects; * depending on temperature
(Germec et al., 2015)	no model presented but values of the parameters were obtained from the experiment	X_m = 8.68 – 9.98 g/L P_m = 19.24 – 24.51 g/L $Y_{P/S}$ = 0.44 – 0.46 g/g range for different plastic composite supports
(Dodić et al., 2012)	the logistic equation used to model yeast cell growth; modified Gompertz equation employed for modeling the bioethanol	μ_m = 0.194; 0.213 1/h X_0 = 2.576; 2.602 g/L X_m = 8.371; 9.473 g/L P_m = 73.31; 69.85 g/L t_L = 1.04; 2.21 h r_{pm} = 4.39; 4.54 g/(L·h) raw juice/thin juice
(Srimachai et al., 2015)	product described by the modified Gompertz model; the linear form of Monod model used for cell growth descripion; additionally: sugar utilization, ethanol yield, ethanol productivity and fermentation efficiency were calculated	μ_m = 0.11 – 0.32 1/h P_m = 2.34 – 11.50 g/L t_L = 0.12 – 1.04 h r_{pm} = 0.05 – 4.39 g/(L·h) $Y_{P/S}$ = 0.39 – 0.48 g/g range for different media
(Fan et al., 2015)	product concentration described using the modified Gompertz model; equation used for cell growth was fitted for ethanol fermentation in a continuous and closed-circulating fermentation	μ_m = 0.025; 0.031 1/h P_m = 625.2; 763.5 g/L t_L = 1.13; 1.02 h r_{pm} = 3.25; 4.21 g/(L·h) values for two identical processes
(Imamoglu and Sukan, 2013)	the modification of the Monod model to describe cell growth; the formation rate of ethanol and the consumption rate of the substrate were described using the Luedeking–Piret model and modified Luedeking–Piret model	μ_m = 0.57 – 3.65 g/L $Y_{P/S}$ = 0.51 – 0.98 g/g $Y_{X/S}$ = 0.05 – 0.21 g/g scale-up modeling; range for different vessels

The main objective of this study was to investigate the growth kinetic of batch ethanol fermentation by means of the complex, non-structured model. Process was carried out in a commercial bioreactor (BioFlo® 115) using the strain of Saccharomyces cerevisiae (ATCC 7754). The proposed model takes into account changes in the biomass, product and substrate concentrations during the fermentation process. It is based

on the relationship which combines changes in the substrate concentration with the value of the biomass concentration during ethanol production. Additionally, a formula describing product concentration was considered. The model is worked out in the Matlab/Simulink^{TM} environment. The results of simulation are validated with the experimental data and compared to the model available in the relevant literature (Staniszewski et al., 2007).

2. MATERIALS AND METHODS

2.1. Experimental apparatus

The fermentation process was carried out in BioFlo®115 bioreactor, which was treated as a fully baffled stirred vessel with two six-blade Rushton turbines. Figure 1 presents the sketch of BioFlo®115 bioreactor.

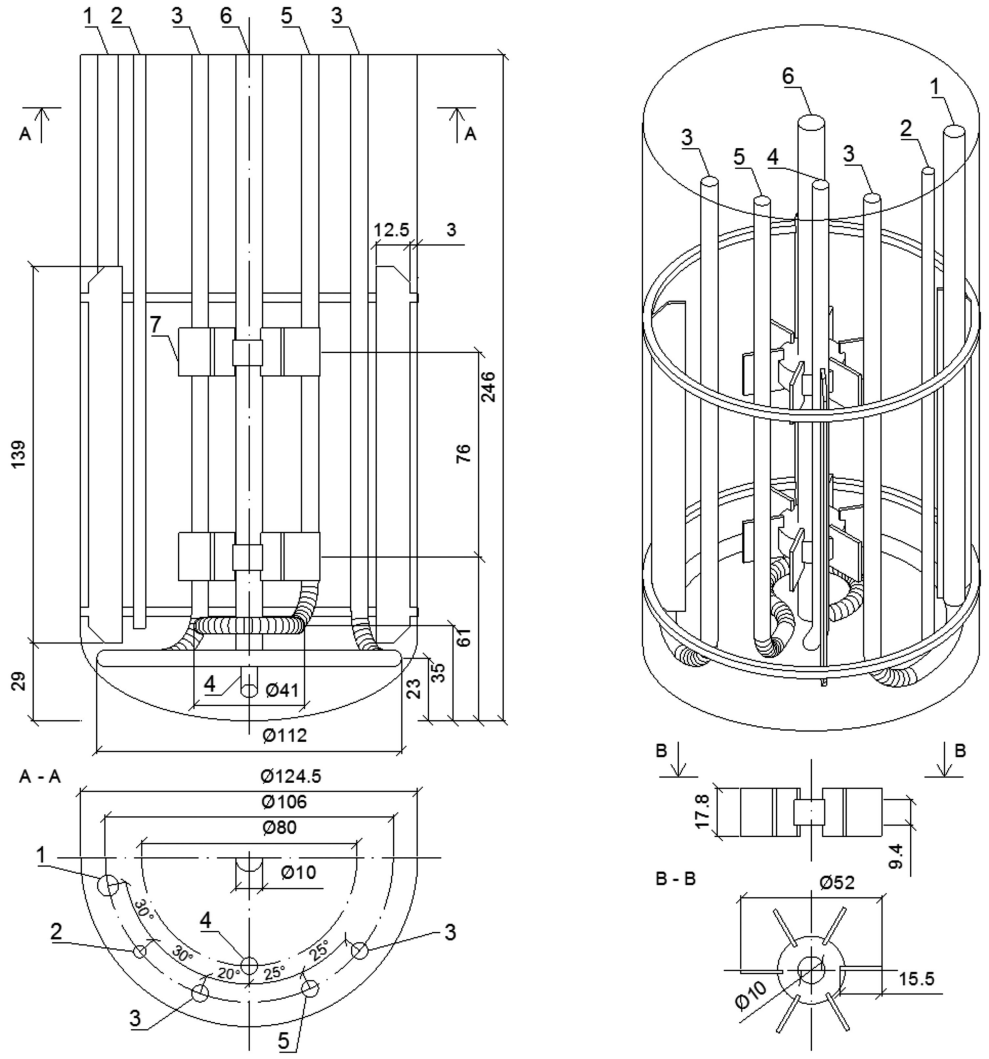

Fig. 1. Sketch of BioFlo®115 bioreactor: 1 – tube for temperature sensor; 2 – sampling tube; 3 – thermostatic circuit; 4 – blowdown connection; 5 – sparger; 6 – shaft; 7 – impeller

The experimental measurements of the fermentation process were realized using a glass cylindrical vessel with a liquid height to vessel diameter ratio equals to 1.45 (H_L = 180 mm; D = 124.5 mm; working volume V = 2 dm^3). Single impeller speed rotation, 150 rpm, was used in this study.

2.2. Batch fermentation

Batch fermentation of glucose to ethanol by *S. cerevisiae* (ATCC 7754) was carried out using 2.5 dm^3 of BioFlo®115 bioreactor for 72h. One colony forming unit (CFU) of the yeast *S. cerevisiae* was transferred into Yeast Peptone Dextrose (YPD) broth and incubated for 24 h at 28 °C with shaking (200 rpm). In the next step, cultures were diluted in YPD broth to obtain the same optical density (OD) equal to 0.5 at 540 nm for yeast inoculum. Obtained microorganism suspension was mixed using a vortex mixer, dispensed into working volume of the bioreactor and cultivated under the experimental conditions described above. The fermentation process was carried out for 72 h. Samples were collected every 4 h and yeast growth, concentration of ethanol and glucose were measured.

2.3. Analytical methods

The OD of yeast culture, which indirectly reflects cellular growth and number of microorganisms was measured at the wavelength of 600 nm in 96 well plates. The OD values were converted to biomass concentration. To convert optical density to dry cellular weight conversion factor from the literature (Liu et al, 2018) was used. It should be noticed that 1 unit of OD is 0.241 g/L of dry cellular weight. The measurements of glucose were realized by means of the enzymatic method. Concentration of remaining glucose was determined by colorimetric method using spectrophotometer at the wavelength of 510 nm (D-Glucose Assay Kit, GOD Method)). Ethanol concentration was determined by a gas chromatography system (Shimadzu Model GC-2014). All parameters were measured three times in each time step.

2.4. Mathematical modeling

The mathematical model of ethanol production was developed with the following main assumptions (Dodić et al., 2012): (1) the bioreactor was well mixed, the conditions inside the bioreactor were uniform in each point; (2) the yeast cells were viable or dead; (3) the agitation speed was enough to provide a uniform substrate availability. The mathematical model was composed of three equations, which represented the changes of biomass, substrate and ethanol concentrations. The formulas used in this model with short descriptions are presented below. Usually the Monod kinetic model or logistic function is used to describe the microbial growths of many different systems (Dodić et al., 2012). The logistic equation was fitted to experimental data of the biomass growth. This equation describes the microorganism growth (X) as a function of the initial biomass concentration (X_0), time of growth (t), the specific growth rate (μ_m) and the final (maximum) biomass concentration (X_m). During the ethanol production the yeast viability is decreased by the product, when the product concentration (ethanol) is higher than 15 w/w% (Dodić et al., 2012). In this study the concentration of ethanol was lower than 15 w/w%. For this reason, the term describing inhibition was not considered. In the current study the following logistic function was used to represent the biomass cell growth during the batch fermentation process (Dodić et al., 2012; Wachenheim et al., 2005):

$$X = \frac{X_0 \cdot \exp(\mu_m \cdot t)}{1 - \left(\frac{X_0}{X_m}\right) \cdot (1 - \exp(\mu_m \cdot t))} \tag{1}$$

The modified Gompertz model is widely used to model product formation. This equation takes into account: the lag time (t_L), the maximum production rate (r_{pm}) and the maximum product concentration (P_m) (Dodić et al., 2012; Fan et al., 2015). The modified Gompertz equation, which was used, is presented as follows (Dodić et al., 2012; Fan et al., 2015):

$$P = P_m \cdot \exp\left(-\exp\left(\left(\frac{r_{pm} \cdot \exp(1)}{P_m}\right) \cdot (t_L - t) + 1\right)\right) \tag{2}$$

The substrate was consumed to form biomass, products, and was also used for the maintenance. (Imamoglu and Sukan, 2013; Krzystek, 2010; Panda, 2011). The changes of glucose concentration (S) were calculated using yield factor ($Y_{X/S}$), which correlates the mass of cells formed from the mass of substrate consumed and the coefficient describing the mass of glucose consumed by the yeast cells (m) (Panda, 2011):

$$-\frac{dS}{dt} = \frac{1}{Y_{XS}} \cdot \frac{dX}{dt} + m \cdot X \tag{3}$$

The mathematical model of bioprocess, consisting of Equations (1), (2) and (3) was solved in Matlab/Simulink^{TM} environment. Figure 2 shows an overview of the block model built in the Simulink based on the equations described above. Connections between the equations are realized as combinations of proper blocks.

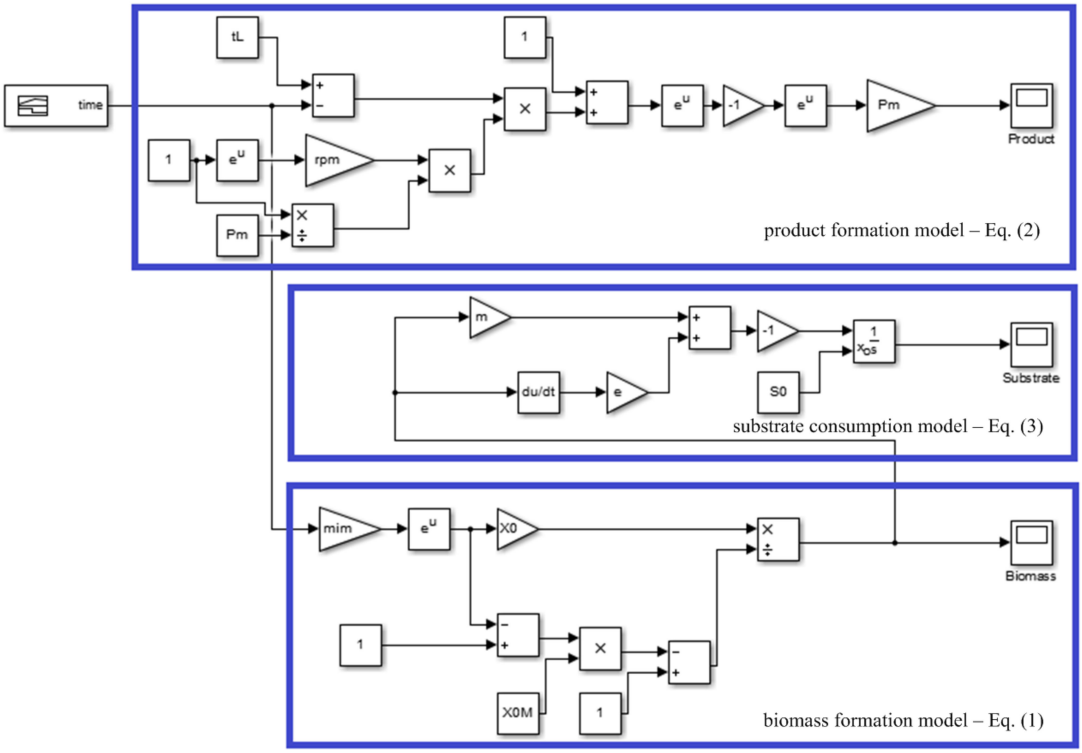

Fig. 2. Mathematical model in Matlab/Simulink^{TM} environment

3. RESULTS AND DISCUSSION

The production of ethanol, carried out in BioFlo® 115 by the strain of *S. cerevisiae* (ATCC 7754), was described by means of the mathematical model (system of Eqs. (1)–(3)). The coefficients, occurring in these equations were determined on the basis of the experimental data (initial biomass concentration (X_0) and maximum biomass concentration (X_m)) or were taken from the literature (maintenance coefficient (m) (Krzystek, 2010) and optical density conversion to dry cellular weight factor (Liu et al, 2018). Moreover, some of them (maximum specific growth rate (μ_m), potential maximum product concentration (P_m) and maximum production rate (r_{pm})) were estimated using the least squares method.

The value of yield coefficient of biomass (see Eq. (3)) from substrate was calculated as a ratio between biomass increment (ΔX) and substrate consumption (ΔS) as shown by following equation (Krzystek, 2010):

$$Y_{XS} = \frac{\Delta X}{\Delta S} \tag{4}$$

Based on the experimental data, we can obtain the averaged value of this coefficient. In the case of this work the yield coefficient of biomass was 0.03 g_X/g_S.

The Table 3 presents the established parameters from the relevant literature and obtained values of parameters used to solve the system of Eqs. (1)–(3).

Table 3. Model parameters for the ethanol production

Parameter	Symbol	Value	Remarks and references
biomass – Eq. (1)			
Initial biomass concentration	X_0	0.015 [g/L]	experimental results
Maximum biomass concentration	X_m	0.3378 [g/L]	
Maximum specific growth rate	μ_m	0.4 [1/h]	estimated by using the least squares method
product – Eq. (2)			
Potential maximum product concentration	P_m	5.0542 [g/L]	estimated by using the least squares method
Maximum production rate	r_{pm}	0.1086 [g/(L·h)]	
Lag phase	t_L	1.05 [h]	
substrate – Eq. (3)			
Yield of biomass from substrate	$Y_{X/S}$	0.03 [g_X/g_S]	calculated according to eq. (4)
Maintenance coefficient	m	0.015 [g/(g·h)]	(Krzystek, 2010)

The obtained results were compared to data from the literature (Staniszewski et al., 2007). In this paper the derived model concerns data for yeast cells growing on glucose and lactose. This model takes into account biomass cell growth, ethanol production and substrate consumptions. The system of the equations used in this paper is presented below.

The cell growth rate was described with the extended Monod Eq. (5):

$$\mu = \mu_m \frac{S}{K_S + S} \frac{K'_s}{K'_S + S} \left(\frac{P}{P'}\right)^a \tag{5}$$

Rates of substrate consumption and product formation were calculated according to the Eqs. (6) and (7), respectively:

$$q_S = m_S + \frac{\mu}{Y_{XS}} \tag{6}$$

$$q_P = \mu \frac{Y_{PS}}{Y_{XS}} \tag{7}$$

The model has the form of three ordinary differential equations describing changes of biomass, product and substrate concentrations with time. These formulas are presented below as Eqs. (8), (9) and (10):

$$\frac{dX}{dt} = \mu X \tag{8}$$

$$\frac{dP}{dt} = q_P X \tag{9}$$

$$\frac{dS}{dt} = -q_S X \tag{10}$$

In Table 4 a comparison of the original parameter values from the literature (Staniszewski et al., 2007) and adapted values used in the present work are presented.

Table 4. Comparison of the original parameters from the literature with the parameters applied in the present study

Parameter	Symbol	Value in (Staniszewski et al., 2007)	Value in proposed model	Remarks and references
Maximum specific growth rate	μ_m	0.115 [1/h]	0.4 [1/h]	estimated using the least squares method
Michaelis–Menten constant for biomass	K_s	1.7 [kg/m^3]	5 [g/L]	
Substrate inhibition constant for cell growth	K'_s	112 [kg/m^3]	35 [g/L]	
Ethanol concentration at which cell growth was inhibited	P'	–	5.0542 [g/L]	
Exponent in term for inhibition of cells by ethanol	a	–	0.22 [–]	(Imamoglu and Sukan, 2013)
Substrate utilization rate directed towards the maintenance of the vital processes of cells	m_S	–	0.015 [g/(g·h)]	(Krzystek, 2010)
Yield of biomass from substrate	$Y_{X/S}$	0.07 [kg/kg]	0.03 [g_X/g_S]	experimental results
Yield of product from substrate	$Y_{P/S}$	0.39 [kg/kg]	0.4 [g_X/g_S]	

The validation of both models was performed by means of coefficient of determination (R^2). This parameter shows information about the goodness of fit of the models. A value of R^2 equaling 1 indicates perfect match and R^2 of 0 means that there is no fit. The comparison of the coefficient of determination for the proposed model and the description of the process production form the relevant literature (Staniszewski et al., 2007) is given in Table 5. This comparison was presented separately for the biomass, product and substrate concentrations.

Table 5. Comparison of the coefficient of determination for the model tested in this work and the mathematical description according to the literature

Model	Coefficient of determination (R^2)		
	Biomass	Product	Substrate
(Staniszewski et al., 2007)	0.7740	0.9711	0.5818
presented model	0.9938	0.9498	0.7662

Figure 3 shows a fit of proposed model (solid line) and model from the literature (dashed line) (Staniszewski et al., 2007) with experimental data (points).

In this figure, the experimental data and prediction of the both models are presented. Models describing changes in the biomass concentration are shown in Fig. 3a. The proposed model better represents prediction

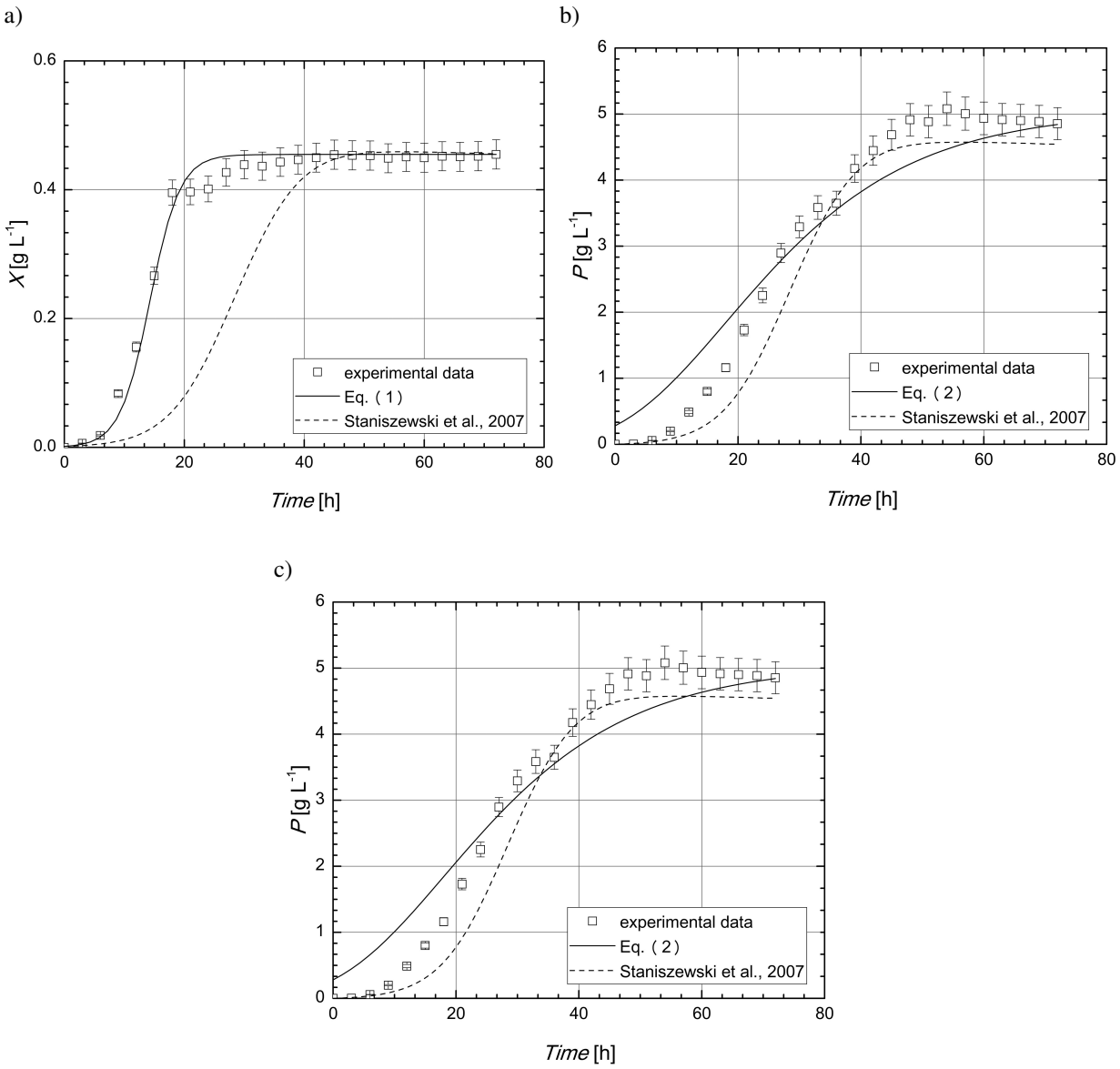

Fig. 3. Fit of the proposed model and model from the literature with experimental data

of biomass concentration than model from the literature (Staniszewski et al., 2007), which was confirmed by the values presented in Table 5. Figure 3b presents changes in the product concentration (ethanol concentration) with time. As can be seen in Fig. 3b and Table 5 both models show good agreement. Figure 3c presents experimentally determined changes of substrate during the process and predicted values from both models. According to this figure and the values in Table 5 better prediction was achieved in the proposed model than in the model from the literature (Staniszewski et al., 2007).

4. CONCLUSIONS

In this study a simple non-structured mathematical model was used to describe biomass growth, product formation and substrate consumption. Results of the model simulations showed good agreement with experimental data. The biomass growth was described by the logistic equation, the production of ethanol by modified Gompertz function and substrate consumption was expressed by equation considering biomass formation and microorganism maintenance. The model can explain fermentation kinetics of biomass

growth, ethanol production and substrate consumption. The fitting results of the model showed good agreement with experimental data, thus the model can be used for further development of ethanol production and for prediction of biomass, ethanol and substrate concentrations during the fermentation process realized in BioFlo®115 bioreactor.

SYMBOLS

a exponent in term for inhibition of cells by ethanol,
C concentration (of biomass, product or substrate), $g \cdot L^{-1}$
K_S Michealis–Menten constant for biomass, $g \cdot L^{-1}$
K'_S inhibition constant for cells by substrate, $g \cdot L^{-1}$
m maintenance coefficient, $g_s \cdot (g_x \cdot h)^{-1}$
m_s substrate utilization rate directed towards the maintenance of the vital processes of cells, $kg \cdot (kg \cdot h)^{-1}$
P product concentration, $g \cdot L^{-1}$
P' ethanol concentration at which cells stop growth, $kg \cdot m^{-3}$
q_S specific reaction rate of substrate, h^{-1}
r_{pm} maximum ethanol production rate, $g \cdot (L \cdot h)^{-1}$
S substrate concentration, $g \cdot L^{-1}$
t_L the lag phase or the time from the beginning of fermentation to exponential bioethanol production, h
X biomass concentration, $g \cdot L^{-1}$
Y_{AB} yield of A from B, $g_A \cdot g_B^{-1}$

Greek symbols

μ_m maximum specific growth rate, h^{-1}
ΔS the substrate concentration changes
ΔX the biomass concentration changes

Subscripts

0 initial value
calc calculated
exp experimental
m maximum value
P product
S substrate
X biomass

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