

# Mathematical model for *Trametes versicolor* growth in submerged cultivation

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**Abstract** *Trametes versicolor* is a white-rot fungus known as a producer of extracellular enzymes such as laccase, manganese-peroxidase, and lignin-peroxidase. The production of these enzymes requires detailed knowledge of the growth characteristics and physiology of the fungus. Submerged cultivations of *T. versicolor* on glucose, fructose, and sucrose as sole carbon sources were performed in shake flasks. Sucrose hydrolysis catalyzed by the whole cells of *T. versicolor* was considered as one-step enzymatic reaction described with Michaelis–Menten kinetics. Kinetic parameters of invertase-catalyzed sucrose hydrolysis were estimated ( $K_m = 7.99 \text{ g dm}^{-3}$  and  $V_m = 0.304 \text{ h}^{-1}$ ). Monod model was used for description of kinetics of *T. versicolor* growth on glucose and fructose as sole carbon sources. Growth associated model parameters were estimated from the experimental results obtained by independent experiments ( $\mu_{\max}^G = 0.14 \text{ h}^{-1}$ ,  $K_S^G = 8.06 \text{ g dm}^{-3}$ ,  $\mu_{\max}^F = 0.37 \text{ h}^{-1}$  and  $K_S^F = 54.8 \text{ g dm}^{-3}$ ). Developed mathematical model is in good agreement with the experimental results.

**Keywords** *Trametes versicolor* · Invertase kinetics · Biomass growth · Mathematical model

## List of symbols

$D_s$	Diffusion coefficient ( $\text{cm}^2 \text{ s}^{-1}$ )
$K_m$	Michaelis–Menten constant ( $\text{g dm}^{-3}$ )
$K_S$	Monod constant ( $\text{g dm}^{-3}$ )
$r$	Radius (mm)
$r$	Rate of enzymatic hydrolysis ( $\text{g dm}^{-3} \text{ h}^{-1}$ )
$T$	Temperature ( $^{\circ}\text{C}$ )
$t$	Time (h)
$V_m$	Maximal reaction rate ( $\text{h}^{-1}$ )
$Y$	Yield coefficient biomass–substrate ( $\text{g g}^{-1}$ )
$\gamma$	Concentration ( $\text{g dm}^{-3}$ )
$\mu$	Biomass growth rate ( $\text{h}^{-1}$ )
$\mu_{\max}$	Maximal biomass growth rate ( $\text{h}^{-1}$ )
$\Phi$	Thiele module (–)
0	Initial conditions
F	Fructose
G	Glucose
S	Sucrose
X	Biomass

## Introduction

White-rot fungi are basidiomycetes commonly found in the nature. They attack wood and cause its degradation because they possess an extracellular enzymatic system mainly composed from laccase, lignin-peroxidase, and manganese-peroxidase, which function together with  $\text{H}_2\text{O}_2$ -producing oxidases and secondary metabolites [1]. The main characteristic that differentiates white-rot fungi from most other microorganisms is their ability to mineralize all components of lignin to carbon dioxide and water. Possible biotechnological application of white-rot fungi varied from

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degradation of industrial pollutants and other hazardous materials [2] till production of value-added and efficient ligninolytic enzymes [3].

In order to produce ligninolytic enzymes, white-rot fungi can be cultivated either in solid culture (solid-state fermentation), which is the natural way of fungus growth, or in submerged fermentation. Easier and cheaper methods of enzymes isolation and purification can be reached when submerge fermentation is taking place [4]. If cultivated in submerged fermentation, fungi can form either dispersed mycelial filaments or interwoven mycelial masses called pellets, depending of genetic material of the fungal species, the nature of the inoculum, and chemical and physical cultivation conditions. Obtaining uniforming pellets of the same size and shape is not easy and many factors influence on it [5–8].

The enzyme production by white-rot fungi using different methods of submerged and solid-state cultivation has been intensively studied [3, 9–16]. Although there is an increasing interest in using these fungal cultures, there is little published data of the growth of white-rot fungi [17, 18], mostly due to the difficulty of measuring the biomass concentration [19]. Kinetics of white-rot fungi growth was studied using glucose [17–20] and glycerol [21] as carbon sources only.

Microbial growth kinetics is an indispensable tool in physiology, genetics, ecology, and biotechnology [22]. Modeling of biotransformation processes have a large potential in searching for optimal process conditions, development and process design, control, scale-up, and identifying of the process cost structure leading to significant material and cost savings [23, 24]. For that purpose, black box un-structured cell models are commonly used, while they offer significant simplicity providing general understanding of the process at the same time. Estimation of the kinetic parameters in unstructured model is the most sensitive part of the model development, and it is carried out from the batch, fed batch, and continuous experimental results [25]. However, there are two major disadvantages when unstructured models are used. First of all, hard work is needed to identify large amounts of model parameters, although the physiological significance of these parameters is not completely clear. Second, un-structured models cannot really embrace the dynamic changes due to changes in environmental factors, working conditions and batch fermentation runs, therefore, the general prediction and control performance based on the model is limited [26].

In this study, *T. versicolor* growth on glucose, fructose, and sucrose in shake flasks was studied. Mathematical model of the process composed of sucrose hydrolysis catalyzed by intracellular enzyme (probably invertase) and microbial growth on glucose and fructose was developed. Kinetic parameters for invertase-catalyzed reaction and for

biomass growth on glucose and fructose were estimated using experimental results obtained from single substrate experiments. Mathematical model was verified and sensitivity analysis of estimated parameters was performed. To the best of our knowledge, this is the first attempt to be modeled white-rot fungi growth on fructose and sucrose as substrates.

## Mathematical model

*Trametes versicolor* growth (X) was studied in shake flasks during its submerged cultivation on glucose (G), fructose (F), and sucrose (S) as sole carbon sources. When sucrose is used as carbon source, reaction scheme given by Eq. 1–3 was used for model development. First, sucrose is enzymatically hydrolyzed to glucose and fructose followed by biomass growth simultaneously using glucose and fructose.



For simplification, invertase-catalyzed sucrose degradation is considered as one-step enzymatic reaction. Viscosity of reaction media remains constant during cultivation. Taken these basic assumptions into accounts, mathematical model composed of *T. versicolor* growth on glucose and fructose and enzymatic hydrolysis of sucrose by intracellular invertase was developed. The Monod type model was used for the description of biomass growth on glucose (Eq. 4) and fructose (Eq. 5), while Michaelis–Menten type kinetics was applied for the description of invertase-catalyzed sucrose degradation (Eq. 6)

$$\mu_G = \mu_{\max}^G \cdot \frac{\gamma_G}{K_S^G + \gamma_G} \quad (4)$$

$$\mu_F = \mu_{\max}^F \cdot \frac{\gamma_F}{K_S^F + \gamma_F} \quad (5)$$

$$r = V_m \cdot \frac{\gamma_S \cdot \gamma_X}{K_m + \gamma_S} \quad (6)$$

where  $\mu$  is biomass growth rate,  $\mu_{\max}$  is maximal biomass growth rate,  $\gamma$  is concentration,  $K_S$  is Monod constant,  $r$  is rate of enzymatic hydrolysis,  $V_m$  is maximal reaction rate, and  $K_m$  is Michaelis–Menten constant. Overall biomass growth rate is given as sum of biomass growth rate on glucose and on fructose (Eq. 7). Glucose and fructose are the only limiting substrates. All other nutrients, such as nitrogen, phosphate, and other growth factors are not limiting.

$$\mu = \mu_G + \mu_F \quad (7)$$

The mass balances of biomass (Eq. 8), glucose (Eq. 9), fructose (Eq. 10), and sucrose (Eq. 11) in batch reactor are represented by the following differential equations:

$$\frac{d\gamma_X}{dt} = \mu \cdot \gamma_X \quad (8)$$

$$\frac{d\gamma_G}{dt} = r - \frac{1}{Y_{X/G}} \cdot \mu_G \cdot \gamma_X \quad (9)$$

$$\frac{d\gamma_F}{dt} = r - \frac{1}{Y_{X/F}} \cdot \mu_F \cdot \gamma_X \quad (10)$$

$$\frac{d\gamma_S}{dt} = -r, \quad (11)$$

where  $Y$  is yield biomass–substrate and  $t$  is reaction time.

## Materials and methods

### Materials

#### Microorganism

*Trametes versicolor* MZKI G-99 was kindly donated by the Microbial culture collection of the National Institute of Chemistry, Ljubljana, Slovenia. The culture was cultivated at 30 °C, stored at 4 °C on medium containing 2% malt-extract agar and 2% agar–agar, and transferred at one-month intervals.

#### Chemicals

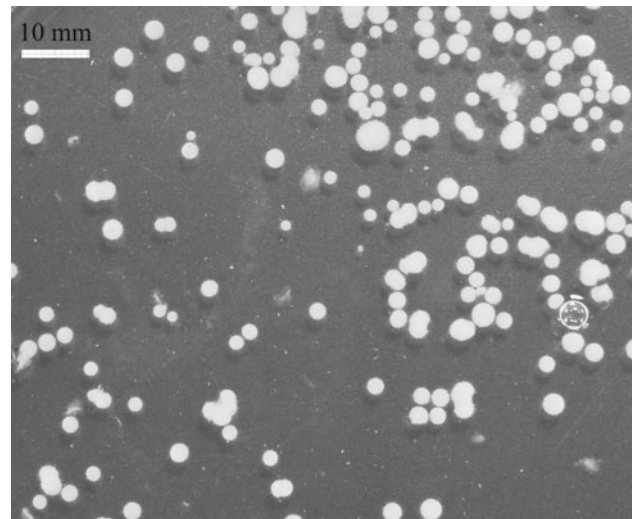
Fructose was purchased from Sigma-Aldrich (Steinheim, Germany); sucrose was purchased from Kandit (Osijek, Croatia); glucose, Na<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>HPO<sub>4</sub>, agar–agar, and citric acid were purchased from Kemika (Zagreb, Croatia); MgSO<sub>4</sub>·7H<sub>2</sub>O and CaCl<sub>2</sub>·2H<sub>2</sub>O were purchased from Merck (Darmstadt, Germany); peptone, malt-extract agar, and yeast extract were purchased from Difco–Becton, Dickinson and Co (New Jersey, USA).

### Methods

#### Analytcs

The biomass concentration was determined by dry weight method. The culture medium was filtered through 0.45 μm filter paper (5 H/N, Munktell & Filtrak GmbH, Barenstein, Germany). Retained biomass was washed with distilled water and dried at a temperature of 80 °C for 24 h.

The concentration of glucose, fructose, and sucrose were determined by high-pressure liquid chromatography (Shimadzu, Kyoto, Japan). The HPLC was equipped with a C<sub>18</sub> column (Carbohydrate Ca<sup>2+</sup>, 300 × 6.5 mm,



**Fig. 1** Mycelial pellets of *T. versicolor* used as inoculum in kinetic measurements and batch experiments

CS-Chromatographie service GmbH, Germany) and an index refraction detector. The analysis was performed at 80 °C with the flow of mobile phase (redistilled water) of 0.5 cm<sup>3</sup> min<sup>-1</sup>. Retention times for sucrose, glucose, and fructose were 7.6, 9.1, and 10.6 min, respectively.

#### Inoculum preparation

Stock culture was inoculated into 500 cm<sup>3</sup> shake-flask containing 50 cm<sup>3</sup> medium (pH 6.13) with 10 g dm<sup>-3</sup> glucose, 0.2 g dm<sup>-3</sup> peptone, 0.3 g dm<sup>-3</sup> yeast extract, 0.8 g dm<sup>-3</sup> KH<sub>2</sub>HPO<sub>4</sub>, 0.2 g dm<sup>-3</sup> Na<sub>2</sub>HPO<sub>4</sub>, and 0.5 g dm<sup>-3</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O. The cotton-stopped flasks were incubated in an orbital shaker (Innova 4330, New Brunswick Scientific, Edison, NJ, USA) at 140 rpm and 27 °C. After 72 h of cultivation, cultures were harvested by filtration (5 H/N, Munktell & Filtrak GmbH, Barenstein, Germany). Image analysis technique (Motic Images Plus 2.0 ML, Motic China Group Co. Ltd., 1999–2004) was used for determination of size and shape of produced pellets. Only pellets of the same size (diameter of 3 ± 0.5 mm) and shape (spherical) were used for all further experiments (Fig. 1).

#### Kinetic measurements

Kinetic measurements were carried out in 250 cm<sup>3</sup> glass bioreactor containing 50 cm<sup>3</sup> of reaction media. Reaction media was composed of 10 g dm<sup>-3</sup> or 20 g dm<sup>-3</sup> of sucrose, 0.8 g dm<sup>-3</sup> of peptone, 2 g dm<sup>-3</sup> of yeast extract, 0.8 g dm<sup>-3</sup> of KH<sub>2</sub>HPO<sub>4</sub>, 0.2 g dm<sup>-3</sup> of Na<sub>2</sub>HPO<sub>4</sub>, 0.5 g dm<sup>-3</sup> of MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.05 g dm<sup>-3</sup> CaCl<sub>2</sub>·2H<sub>2</sub>O, and 0.15 g dm<sup>-3</sup> citric acid. Reaction was started with addition of 4.3 g dm<sup>-3</sup> of mycelial pellets (dry weight).

**Table 1** Initial conditions for batch experiments

Experiment	Sugar	$\gamma_{\text{sugar}, 0}$ (g dm <sup>-3</sup> )	$\gamma_{X, 0}$ (g dm <sup>-3</sup> )
1	Glucose	11.0	0.083
2		24.0	0.078
3		5.7	0.113
4		12.1	0.667
5		36.0	0.074
6	Fructose	10.5	0.408
7		10.5	0.075
8	Sucrose	21.1	0.076

Reaction mixture was stirred on magnetic stirrer at 200 rpm and thermostated at 27 °C. Samples were taken every 15 min for 2 h, centrifuged at 9,000 rpm for 10 min (Centric 150, Tehtnica, Železniki, Slovenia), filtered (0.25  $\mu\text{m}$ , Macherey–Nagel GmbH & Co. KG, Düren, Germany) and analyzed on HPLC for determination of glucose, fructose, and sucrose concentrations.

#### Batch cultivation

Experiments were carried out in 500 cm<sup>3</sup> Erlenmayer flasks on orbital shaker (Innova 4330, New Brunswick Scientific, Edison, NJ, USA) at  $T = 27$  °C and 140 rpm. Reaction mixture contained sole carbon source (glucose or fructose or sucrose), 0.8 g dm<sup>-3</sup> of peptone, 2 g dm<sup>-3</sup> of yeast extract, 0.8 g dm<sup>-3</sup> of KH<sub>2</sub>PO<sub>4</sub>, 0.2 g dm<sup>-3</sup> of Na<sub>2</sub>HPO<sub>4</sub>, 0.5 g dm<sup>-3</sup> of MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.05 g dm<sup>-3</sup> CaCl<sub>2</sub>·2H<sub>2</sub>O, and 0.15 g dm<sup>-3</sup> of citric acid. The initial concentration of glucose, fructose, sucrose, and biomass (dry weight) for different batch experiments are given in Table 1. Reaction was started by addition of mycelial pellets.

Each experiment was conducted simultaneously in 14 flasks. At irregular intervals, a sample from each flask was taken, filtered, and analyzed on HPLC for the concentration of sugar. When significant change of sugar concentration was experimentally observed, content of two flasks was

quantitatively transferred on filter paper, and biomass was quantified as described above. The seven cycles for determination of biomass concentration were carried out during one experiment.

#### Data handling

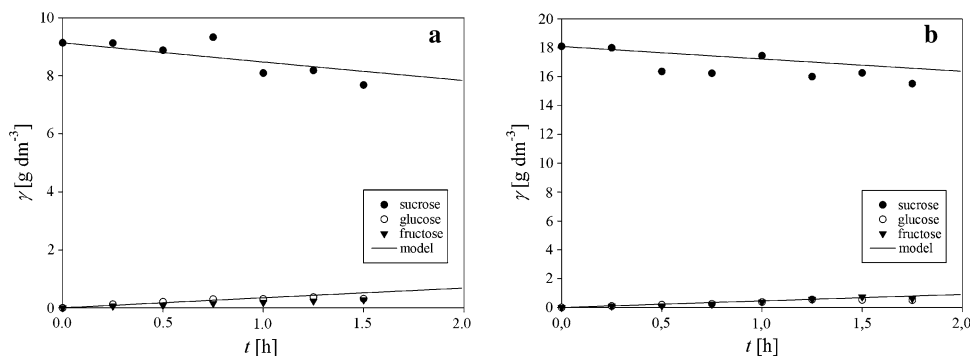
Model parameters were estimated by nonlinear regression analysis using Simplex and Least Squares method implemented in the SCIENTIST software (MicroMath<sup>®</sup>, Salt Lake City, USA). They were evaluated by fitting the model to the experimental data for sucrose hydrolysis or from batch cultivation experiments for biomass growth. The calculated data were compared with the experimental data, recalculated in the optimization routine, and fed again to the integration step until a minimal error between experimental and integrated values was achieved. The residual was defined as the sum of the squares of the differences between the experimental and the calculated data. “Episode” algorithm for the stiff system of differential equations, implemented in the SCIENTIST software was used for simulations of batch reactor mathematical models.

## Results and discussion

### Kinetic of invertase-catalyzed sucrose degradation

Invertase-catalyzed sucrose degradation was studied in batch reactor. It is considered as one-step enzymatic reaction with glucose and fructose as products (Eq. 1). Experiments were carried out at high initial concentration of mycelial pellets (4.3 g dm<sup>-3</sup>) at two different initial concentration of sucrose (10 g dm<sup>-3</sup>—Fig. 2a, and 20 g dm<sup>-3</sup>—Fig. 2b). The high initial concentration of mycelial pellets was used to decrease the influence of the biomass growth on produced glucose and fructose on total biomass concentration. During the reaction time of 2 h, the consumption of glucose and fructose are not supposed to be seen.

**Fig. 2** Intracellular invertase-catalyzed sucrose consumption for different initial sucrose concentration of (a)  $\gamma_{S,0} = 10$  g dm<sup>-3</sup> and (b)  $\gamma_{S,0} = 20$  g dm<sup>-3</sup> at 27 °C



**Table 2** Estimated kinetic parameters for invertase-catalyzed sucrose degradation and for biomass growth on glucose and fructose

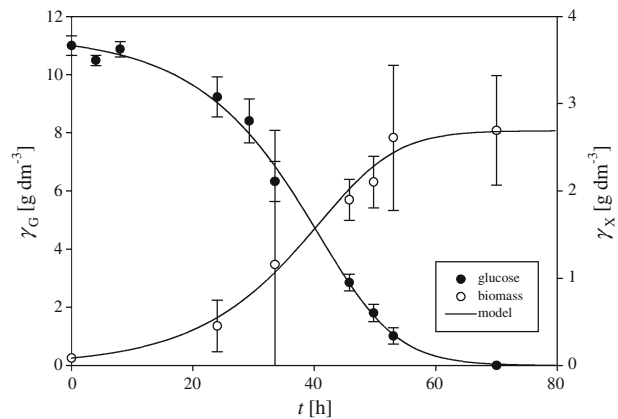
Parameters	Unit	Value
$K_m$	$\text{g dm}^{-3}$	7.99
$V_m$	$\text{h}^{-1}$	0.304
$\mu_{\max}^G$	$\text{h}^{-1}$	0.14
$K_S^G$	$\text{g dm}^{-3}$	8.06
$\mu_{\max}^F$	$\text{h}^{-1}$	0.37
$K_S^F$	$\text{g dm}^{-3}$	54.8

Obviously, the fungus *T. versicolor* has intracellular invertase as its constituent, while sucrose biotransformation to glucose and fructose was experimentally proved. The proposed kinetic model for the intracellular invertase-catalyzed sucrose degradation (Eq. 6) was fitted to experimental data obtained at lower initial sucrose concentration  $\gamma_{S,0} = 10 \text{ g dm}^{-3}$  (Fig. 2a). Kinetic parameters  $K_m = 7.99 \text{ g dm}^{-3}$  and  $V_m = 0.304 \text{ h}^{-1}$  were estimated (Table 2). This was first attempt to estimate  $K_m$  and  $V_m$  for sucrose degradation catalyzed by whole cells of *T. versicolor* as a source of intracellular invertase. Estimated  $K_m$  for intracellular invertase is in accordance with reported values for free invertase from baker's yeast ( $K_m = 8.21 \text{ g dm}^{-3}$ ; [27]) and significantly lower in comparison with immobilized invertase from *Cladosporium cladosporioides* ( $K_m = 153 \text{ g dm}^{-3}$ ; [28]). Estimated parameters were validated for the higher initial sucrose concentration  $\gamma_{S,0} = 20 \text{ g dm}^{-3}$  at same process conditions (Fig. 2b). Developed kinetic model describes experimental data quite well even for higher initial sucrose concentration. Glucose and fructose consumption was experimentally not observed at reaction period of 2 h.

#### Biomass growth on glucose

Kinetics of biomass growth on glucose as a sole carbon source was investigated in batch experiments. Experiments were performed at different initial concentrations of glucose and different initial concentrations of mycelial pellets (Table 1). Kinetic parameters of the Monod model (Eq. 4) were estimated from the experiment performed at  $\gamma_{G,0} = 11.0 \text{ g dm}^{-3}$  and  $\gamma_{X,0} = 0.083 \text{ g dm}^{-3}$ . Model simulation results and experimental data are shown in Fig. 3, while estimated model parameters are given in Table 2. Yield coefficient biomass–glucose was calculated from experimental results for each initial concentration of glucose and biomass.

Estimated maximal specific growth rate on glucose,  $\mu_{\max}^G = 0.14 \text{ h}^{-1}$  is significantly higher than values reported for *T. versicolor* growth on glucose as a sole carbon source ( $0.034\text{--}0.036 \text{ h}^{-1}$  for growth in shake flasks and


**Fig. 3** Model simulation and experimental results for biomass growth on glucose at  $\gamma_{G,0} = 11.0 \text{ g dm}^{-3}$ ,  $\gamma_{X,0} = 0.083 \text{ g dm}^{-3}$ , and  $Y_{X/G} = 0.24 \text{ g g}^{-1}$  (experimental data shown as average value of two data sets with confidence interval calculated using significance level of 0.05)

$0.032\text{--}0.052 \text{ h}^{-1}$  for growth in stirred tank reactor). On the other hand, estimated Monod constant  $K_S^G = 8.06 \text{ g dm}^{-3}$ , and calculated yield coefficient  $Y_{X/G} = 0.24 \text{ g g}^{-1}$  are similar to the literature data [17, 19]. Assumed kinetic model (Eq. 4) for *T. versicolor* growth on glucose as a sole carbon substrate was validated using experimental results obtained in shake flasks for different initial glucose concentrations and initial biomass concentrations. Experimental data and model simulation results for specified initial process conditions (Table 1, Experiments 2, 3 and 4) are presented in Fig. 4.

Proposed mathematical model is able to describe process behavior for a wide range of different initial concentration of glucose ( $5.7\text{--}24 \text{ g dm}^{-3}$ ) and initial concentrations of biomass ( $0.078\text{--}0.667 \text{ g dm}^{-3}$ ; Fig. 4a–c). For higher initial concentration of glucose ( $36 \text{ g dm}^{-3}$ ), model cannot quantitatively describe dynamic of glucose consumption (Fig. 4d). This is probably due to inhibition by glucose or unknown product. On the other hand, biomass concentration change obtained experimentally was in good correlation with model prediction results. Experimentally obtained yield coefficients biomass–glucose did not change significantly for different initial process conditions concerning glucose and biomass ( $Y_{X/G} = 0.17\text{--}0.27 \text{ g g}^{-1}$ ).

#### Biomass growth on fructose

In order to evaluate kinetic parameters for biomass growth on fructose as a sole carbon source, batch experiments in shake flasks were performed. Experiments were carried out for two different initial concentrations of mycelial pellets (Table 1, Experiment 6 and 7). The kinetic model (Eq. 5) was fitted to experimental data obtained from the experiment performed at  $\gamma_{F,0} = 10.5 \text{ g dm}^{-3}$  and  $\gamma_{X,0} = 0.408 \text{ g dm}^{-3}$ .

**Fig. 4** Model simulation and experimental results for biomass growth on glucose in (a)

Experiment 2

( $\gamma_{G,0} = 24.0 \text{ g dm}^{-3}$ ,  
 $\gamma_{X,0} = 0.078 \text{ g dm}^{-3}$ , and  
 $Y_{X/G} = 0.18 \text{ g g}^{-1}$ ), (b)

Experiment 3

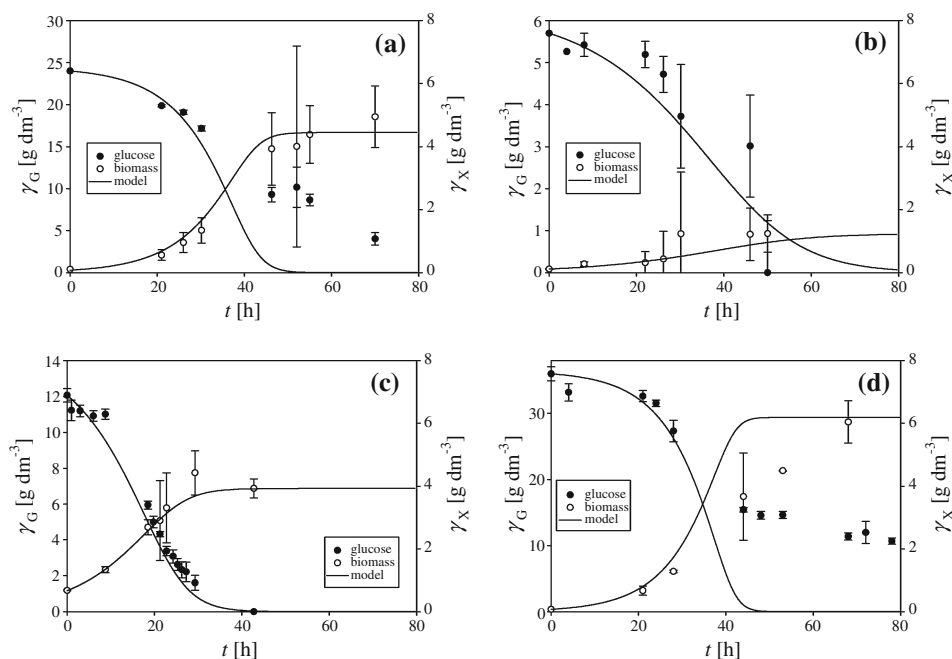
( $\gamma_{G,0} = 5.7 \text{ g dm}^{-3}$ ,  
 $\gamma_{X,0} = 0.113 \text{ g dm}^{-3}$ , and  
 $Y_{X/G} = 0.20 \text{ g g}^{-1}$ ), (c)

Experiment 4

( $\gamma_{G,0} = 12.1 \text{ g dm}^{-3}$ ,  
 $\gamma_{X,0} = 0.667 \text{ g dm}^{-3}$ , and  
 $Y_{X/G} = 0.27 \text{ g g}^{-1}$ ), and (d)

Experiment 5

( $\gamma_{G,0} = 36.0 \text{ g dm}^{-3}$ ,  
 $\gamma_{X,0} = 0.074 \text{ g dm}^{-3}$ , and  
 $Y_{X/G} = 0.17 \text{ g g}^{-1}$ )  
 (experimental data shown as  
 average value of two data sets  
 with confidence interval  
 calculated using significance  
 level of 0.05)



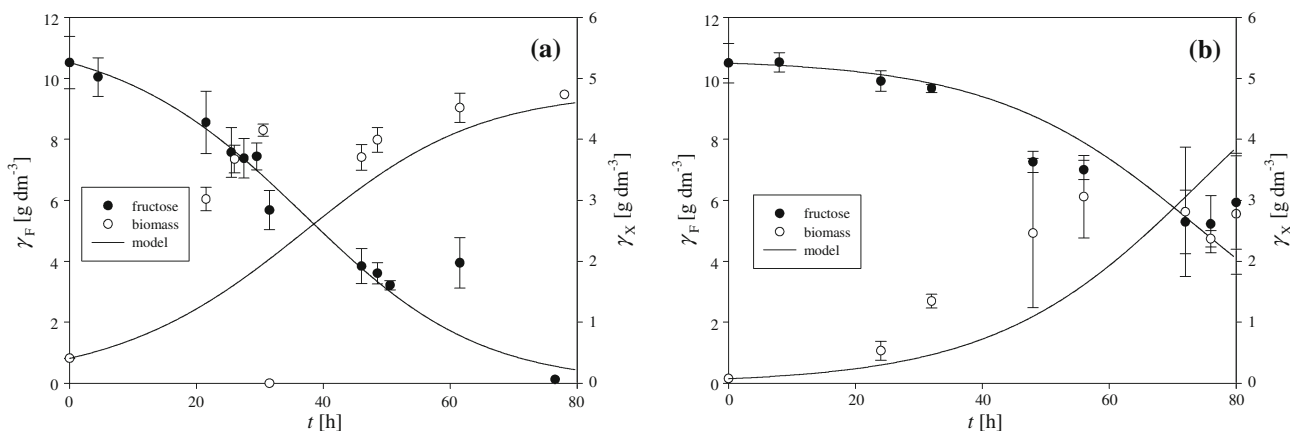
The kinetic parameters for biomass growth on fructose are presented in Table 2, while the comparison of the model with the experimental data for different initial concentration of biomass is presented in Fig. 5. Yield coefficient biomass–fructose was calculated from experimental results for both experiments.

Kinetic parameters  $\mu_{\max}^F = 0.37 \text{ h}^{-1}$  and  $K_S^F = 54.8 \text{ g dm}^{-3}$  for *T. versicolor* growth on fructose as a sole carbon source are estimated for the first time to our best knowledge. Obviously, *T. versicolor* shows higher affinity toward glucose as a growth substrate, which is indicated by lower estimated  $K_S$  value (Table 2). Estimated kinetic parameters were validated using experimental results obtained for lower initial biomass concentration (Fig. 5b).

Assumed Monod model (Eq. 5) describes experimental data quite well. Experimentally obtained yield coefficients of biomass–fructose was significantly influenced by different initial concentration of biomass ( $Y_{X/F} = 0.41 \text{ g g}^{-1}$  in Experiment 6 and  $Y_{X/F} = 0.59 \text{ g g}^{-1}$  in Experiment 7), which was not the case with experiments that were performed with glucose as sole carbon source.

Mass-transfer resistance during pellet's growth

In batch experiments of *T. versicolor* cultivation on glucose and fructose as a sole carbon source, before measuring biomass concentration, pellets were photographed and analyzed using an image analysis technique as described in



**Fig. 5** Model simulation and experimental results for biomass growth on fructose in (a) Experiment 6 ( $\gamma_{F,0} = 10.5 \text{ g dm}^{-3}$ ,  
 $\gamma_{X,0} = 0.408 \text{ g dm}^{-3}$ , and  
 $Y_{X/F} = 0.41 \text{ g g}^{-1}$ ), (b) Experiment 7

( $\gamma_{F,0} = 10.5 \text{ g dm}^{-3}$ ,  $\gamma_{X,0} = 0.113 \text{ g dm}^{-3}$ , and  
 $Y_{X/F} = 0.59 \text{ g g}^{-1}$ )  
 (experimental data shown as average value of two data sets with  
 confidence interval calculated using significance level of 0.05)

“Inoculum preparation”. In order to investigate the influence of mass-transfer resistance during pellet’s growth by calculating Thiele module, two representative experiments with glucose and fructose as sole carbon source were chosen (Experiment 3 and Experiment 7, Table 1). Changes in the average radius of all the pellets and in the radius of the largest pellets during fungi cultivation were calculated according to the results of area and the parameters obtained by an image analysis technique. Thiele module ( $\Phi$ ) was calculated according to the equation  $\Phi = \frac{V_p}{A_p} \cdot \sqrt{\frac{(\mu_{max} \cdot c_s)}{D_s \cdot (Y_{X/G} \cdot K_S)}}$ , for both, average and maximal values of radius using previously estimated kinetic parameters (Table 2) and diffusion coefficient  $D_s = 0.57 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  [29]. The results are presented in Table 3.

In both experiments, when using the average pellet’s radius, mass resistance was not significant, and the calculated Thiele module was below the value of the three. According to the criteria for assessing the magnitude of mass-transfer effects on overall kinetics when Thiele module is higher than three, diffusion is limiting rate process [29]. Only when the maximum radius of pellets was taken into account (Experiment 3 with glucose as the sole carbon source), the maximum value of Thiele module from 3.31 to 3.52 during the exponential phase of growth were calculated, indicating the influence of mass-transfer resistance to the growth of biomass.

Sensitivity analysis of the process model

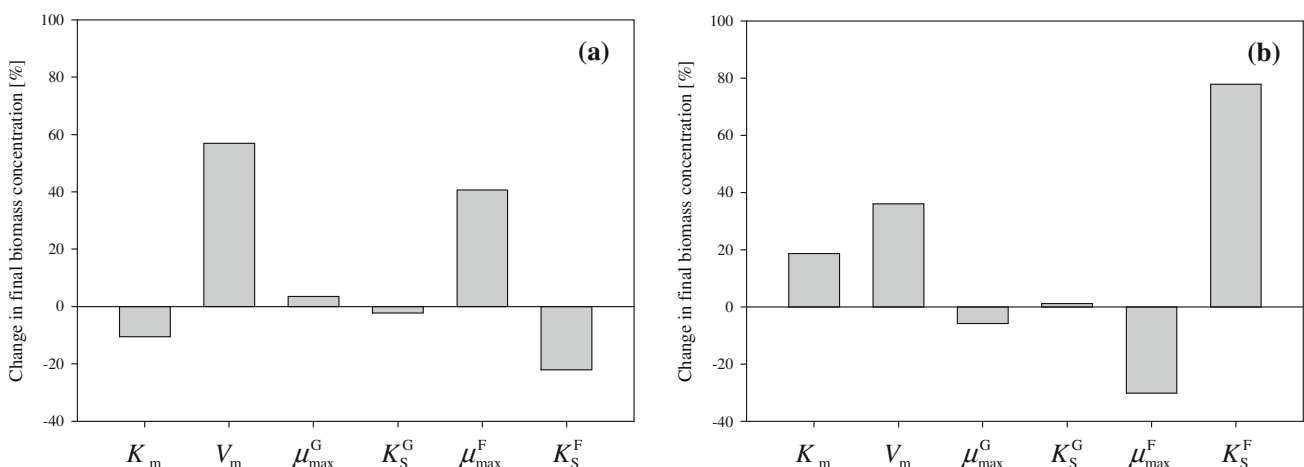
In order to qualify the sensitivity of model predictions with respect to kinetic parameters of *T. versicolor* growth on sucrose as substrate, sensitivity analysis has been performed. For this purpose, the mathematical model parameters ( $K_m, V_m, \mu_{max}^G, K_S^G, \mu_{max}^F, K_S^F$ ) were varied in the range of  $\pm 50\%$ , and their effect on the final biomass

**Table 3** Thiele module calculation for *T. versicolor* growth on glucose and fructose

Experiment	t (h)	r average (mm)	$\Phi_{av.r.}$ (-)	r maximal (mm)	$\Phi_{max.r.}$ (-)
3	0 (inoculum)	1.78	1.16	–	–
	4	2.92	1.91	3.52	2.29
	8	1.03	0.67	3.25	2.12
	22	1.12	0.73	4.41	2.88
	26	1.39	0.91	5.27	3.44
	30	1.00	0.65	4.41	2.88
	46	1.68	1.09	5.08	3.31
7	0 (inoculum)	1.78	0.57	–	–
	24	4.69	1.51	5.60	1.80
	32	4.09	1.32	4.10	1.32
	48	2.09	0.67	6.17	1.99
	56	1.96	0.63	5.71	1.84
	72	1.72	0.55	6.17	1.99
	76	2.09	0.67	5.90	1.90
	80	2.92	0.94	7.40	2.38

concentration was determined (Fig. 6). All simulations were performed for a period of 80 h.

The process is significantly influenced by kinetics of invertase-catalyzed hydrolysis of sucrose, while sucrose degradation participates in the biomass growth. Change the parameters related to the growth of *T. versicolor* on glucose has less impact on the final biomass concentration. On the other hand, kinetic parameters have great influence on the growth of *T. versicolor* on fructose. This is because the yield coefficient biomass-substrate is 2.5 times higher for fructose.



**Fig. 6** Sensitivity analysis of the process model (a) 50% enhancement of the parameters, (b) 50% decrease of the parameters

## Batch experiment with sucrose

Developed mathematical model of the process (Eq. 4–11), which is composed of sucrose hydrolysis catalyzed by the intracellular invertase from *T. versicolor*, and its growth on glucose and fructose has been validated in batch experiment using sucrose as carbon source. Experiments were performed in duplicate with the following initial conditions of sucrose and biomass:  $\gamma_{S,0} = 21.1 \text{ g dm}^{-3}$  and  $\gamma_{X,0} = 0.076 \text{ g dm}^{-3}$ , respectively. Kinetic parameters (Table 2) and yield coefficients, which were estimated in independent experiments with glucose (Experiment 2) and fructose (Experiment 6) as the sole carbon sources were used for model simulations. Experimental results and model simulation results are presented in Fig. 7a.

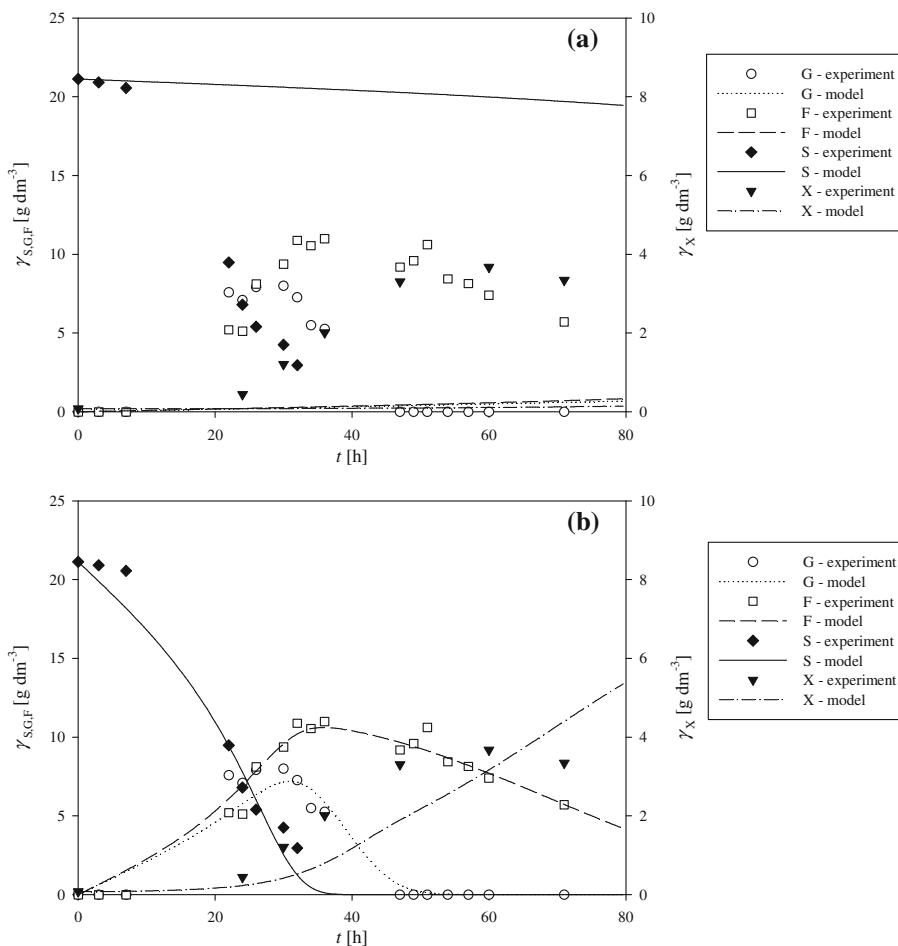
The simulated results, in Fig. 7a, are not in qualitative agreement with the experimental observations. Deviation of the predicted and measured values was probably the results of the use of kinetic parameters estimated from the independently performed experiments with glucose and fructose as sole carbon sources for model simulation. However, developed model seems to have a good predictive ability for

**Table 4** Kinetic parameters estimated from the results of batch experiment performed with sucrose as carbon source

Parameters	Unit	Value
$V_m$	$\text{h}^{-1}$	7.48
$\mu_{\max}^F$	$\text{h}^{-1}$	0.27
$Y_{X/G}$	$\text{g g}^{-1}$	0.09
$Y_{X/F}$	$\text{g g}^{-1}$	0.62

the simulation. Model sensitivity analysis showed that  $V_m$  and  $\mu_{\max}^F$  have a significant influence on the process. Therefore, these parameters were estimated directly from the experimental data of batch experiment performed on sucrose. Additionally, when cultivated on sucrose, biomass growth occurs simultaneously on two substrates, glucose and fructose, respectively. Therefore, yield coefficients  $Y_{X/G}$  and  $Y_{X/F}$  were also estimated from the data of the same batch experiment. Re-estimate model parameters are given in Table 4, while the experimental results and model simulation results, which were calculated using parameters given in Table 3, are shown in Fig. 7b.

**Fig. 7** Model simulation and experimental results in batch experiment with sucrose as carbon source (a) using kinetic parameters from Table 2 and for  $\gamma_{S,0} = 21.1 \text{ g dm}^{-3}$ ,  $\gamma_{X,0} = 0.076 \text{ g dm}^{-3}$ ,  $Y_{X/G} = 0.24 \text{ g g}^{-1}$ , and  $Y_{X/F} = 0.41 \text{ g g}^{-1}$ , (b) using kinetic parameters from Table 3 and for  $\gamma_{S,0} = 21.1 \text{ g dm}^{-3}$ ,  $\gamma_{X,0} = 0.076 \text{ g dm}^{-3}$





## Conclusion

Developed mathematical model accurately correlates sucrose hydrolysis catalyzed by intracellular invertase and microbial growth of *T. versicolor* on glucose and fructose in submerged fermentation performed in shake flasks. Hydrolysis of sucrose into glucose and fructose, which was assumed to exist, was experimentally confirmed and verified with the model. Kinetic parameters  $K_m = 7.99 \text{ g dm}^{-3}$  and  $V_m = 0.304 \text{ h}^{-1}$  for invertase-catalyzed hydrolysis of sucrose were estimated and verified, respectively. The proposed assumption of simultaneous growth of *T. versicolor* on glucose and fructose was also experimentally confirmed. Monod type kinetics of biomass growth on glucose and on fructose was assumed, and kinetic parameters were estimated ( $\mu_{\max}^G = 0.14 \text{ h}^{-1}$ ,  $K_S^G = 8.06 \text{ g dm}^{-3}$ ,  $\mu_{\max}^F = 0.37 \text{ h}^{-1}$  and  $K_S^F = 54.8 \text{ g dm}^{-3}$ ). The highest biomass yield ( $Y_{X/F} = 0.59 \text{ g g}^{-1}$ ) was obtained in experiment performed with fructose as sole carbon source. Additionally, glucose uptake ends before consumption of fructose, which is in accordance with higher affinity of *T. versicolor* to glucose as a growth substrate.

Therefore, based on experiments and the model developed in this study, we could assume that *T. versicolor* has an enzyme for the degradation of sucrose, even before it starts to grow on glucose and fructose.

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