# Defining of the Kinetics of Microbial Oxidation Process Events with Reference to L-Sorbose Formation in a Large Range of Culture Conditions

**K. Mihaljević[a,*] and M. Bošnjak[b]**

[a] PLIVA Croatia Ltd., Prilaz baruna Filipovića 25, 10000 Zagreb, Croatia

[b] Croatian Academy of Engineering, Kačićeva 28, 10000 Zagreb, Croatia

Original scientific paper
Received: March 3, 2008
Accepted: October 1, 2008

This work refers to the study of the batch and fed batch cultures of an industrially applicable microbial strain termed *Gluconobacter suboxydans* S-22, capable of converting D-sorbitol into L-sorbose and D-glucose into D-gluconic acid at extremely high substrate mass concentrations. Media based on CSL-filtrate and containing different D-sorbitol concentrations were applied to perform experiments. Studied were the effects of D-sorbitol and L-sorbose concentrations on the kinetics of microbial growth and D-sorbitol conversion into L-sorbose, and of microbial cell metabolism on the oxygen solubility and water activity, on the oxygen transfer and uptake rates and on the bioprocess final L-sorbose yield. In order to define the process relationships and describe the kinetics of all relevant process events in a large range of reaction conditions, a mathematical model based on corresponding differential equations was developed. The applicability of the mathematical model was tested by computer simulation. Data of computer simulations fitted well to experimental data. Changes of biomass, D-sorbitol, L-sorbose and dissolved oxygen concentrations during the batch and fed batch cultures can be well explained by applied mathematical model regardless of whether they refer to the reaction system of commonly dissolved substance mass concentration (close to 200 g $L^{-1}$) or to the reaction system of extremely highly dissolved and total substance concentrations (above 700 g $L^{-1}$). Excellent correlation coefficients (0.99693 – 0.99995) expressing the agreement of the theoretical with experimental data were found for compared biomass, D-sorbitol, L-sorbose and dissolved oxygen concentrations. Results confirmed the hypothesis that the water activity can be expressed as a function of oxygen solubility in the investigated reaction system.

*Key words:*

D-sorbitol oxidation, process kinetics, mathematical modeling, water activity effect, L-sorbose yield

## Introduction

Although a pronounced interest for studying the process of microbial conversion of D-sorbitol into L-sorbose by means of appropriate acetic acid bacteria appeared a long time ago, parallel with the beginning of industrial L-ascorbic acid (vitamin C) synthesis, and although the process in developing methods of industrial L-sorbose production was accompanied with the appearance of a series of corresponding scientific research explaining the metabolic essentials and kinetics of the mentioned process, there is still sufficient interest for further study of the same process. Disregarding the fact that new methods of vitamin C production are already applied industrially, one can consider that advances in the Reichstein-Gruessner method of L-ascorbic acid synthesis could conserve this method as the main method of industrial vitamin C production. Another reason for further study of the process of microbial conversion of D-sorbitol into L-sorbose appears to be the fact that this process can be considered an excellent model of biocatalytic reaction systems applicable for some scientific studies of general importance. The convenience of this reaction system for its application as a model system for a study of process relationships referring to biocatalytic reaction systems can be summarized as follows:

a) The starting nutrient medium as reaction system can be defined so well that it can be considered quite reliable for studying the relationship between growth, substrate uptake and product formation kinetics in the large range of cultivation process conditions;

b) One can apply the cultures of biocatalytically very efficient microbial strains of reliable and sufficiently stable specific properties;

*Corresponding author: E-mail: Kreso.Mihaljevic@pliva.hr; Phone: +385 1 3722 049; Fax: +385 1 3722 802

c) D-sorbitol as a main substrate is extremely well soluble in water and therefore a large range of media with different D-sorbitol concentrations can be prepared. Its high affinity for water molecules causes the decrease of water activity and thus of oxygen solubility depending on the D-sorbitol concentration in the prepared reaction media;

d) L-sorbose as the main biocatalytic process product appears as a result of biochemical reaction of D-sorbitol with dissolved oxygen, catalyzed with specific polyoldehydrogenase of applied microbial culture cells. It also has high affinity for water molecules causing the effects similar to those of D-sorbitol;

e) Since due to extremely low oxygen solubility in water solutions the dissolved oxygen as one of the main reactants can commonly appear as a process kinetics limiting factor, the study of oxygen transfer and uptake rates during the process of D-sorbitol conversion into L-sorbose can lead to the discovery of mass transfer and other process relationships relevant for a series of other microbial processes, especially of those of analogous properties.

Such reaction system properties reflected on the history of advances in process technology. The first method of L-sorbose production was based on the surface culture and the use of media with very low D-sorbitol concentration (2 – 6 %). Fulmer *et al.*[1] advanced this method by increasing D-sorbitol conversion rate and the final L-sorbose yield. The increase resulted due to the increase of medium surface to volume ratio. The more relevant process advancement was achieved by changing the method of surface culture with the method of submerse culture. Wells *et al.*[2,3] applied the rotating bioreactor and air overpressure to convert D-sorbitol into L-sorbose in the media containing 20 – 30 % of D-sorbitol and 0.5 % of yeast extract (primarily) or 0.3 % of CSL (later). It was established that the quality of inoculums and the intensity of aeration were important factors for the bioconversion rate. Further process advancements resulted after the application of conventional bioreactors with stirrers, since such bioreactors enable enhancement of the process, especially with the application of an air stream enriched with pure oxygen.[4] Another process improvement was by applying methods of continuous cultures. Elsworth *et al.*[5] studied the conversion efficiency during the single stage continuous culture of *Acetobacter* (*Gluconobacter*) sp. in aerated and stirred bioreactors. They found that the continuous process was of higher productivity but with outlining the lower conversion yields. To improve the conversion yield the authors proposed addition of a second bioreactor to the system. Bošnjak *et al.*[6] investigated semicontinuous L-sorbose production in pilot plant scale by applying the three interconnected aerated columns as a bioreactor system. It was established that a semicontinuous process can be performed for a long time (for more than 350 hours) with the same yield as in the batch process and with higher productivity than with application of the corresponding batch process. Krieg[7] studied the process of D-sorbitol oxidation with reference to the formation of different metabolites. Special emphasis was given to the process improvements by adding D-sorbitol into the batch culture and by applying the three-stage continuous culture system. He demonstrated experimentally and explained scientifically the advantages of D-sorbitol addition to the batch culture and of the application of continuous culture. Bull and Young[8] advanced the method of continuous cultivation. They investigated the efficiency of microbial biomass recycle. They established that the maximal volumetric rate of L-sorbose production of 111 g $L^{-1}$ $h^{-1}$ could be obtained when applying the biomass recycle of $R$ = 0.49 and the culture dilution rate of $D$ = 0.45 $h^{-1}$ with the medium of D-sorbitol mass concentration of 300 g $L^{-1}$. Application of fed batch culture resulted in L-sorbose production process improvements with respect to the final L-sorbose concentration in the cultivation broth and therefore with respect to the reduction of the cost of crystalline L-sorbose recovery. Mori *et al.*[9] were especially successful. They applied a fed batch culture controlled with dissolved oxygen and obtained L-sorbose concentration of 628 g $L^{-1}$ after 14 hours of cultivation. The repeated fed batch culture could also be applied instead of batch culture method,[10] whereas the gradient fed batch culture appeared to be a convenient method for obtaining high oxidation product yield.[11] However, it was surprising that due to the precipitation of dissolved into crystalline L-sorbose during the process of prolonged fed batch culture,[12] the mass concentration above 770 g $L^{-1}$ (total L-sorbose) and above 200 g $L^{-1}$ of crystalline L-sorbose could be obtained. Obtained results suggested the application of two-compartment fed batch cultivation to advance the process of crystalline L-sorbose production.[13,14] The idea to apply the process of microbial biocatalytic oxidation of D-sorbitol into L-sorbose as the model of biocatalytic oxidations appeared already during the 1970's's, as a consequence of analogous previous proposals referring to the process of biocatalytic oxidation of D-glucose into D-gluconic acid. It was confirmed experimentally[15,16] that the reaction system of microbial oxidation of D-sorbitol to L-sorbose could be efficiently applied in defining oxygen transfer relationships in aerobic microbial cultures.

Since the oxygen solubility in culture media depends on the concentration of other dissolved substances present in the culture media, the study focused on the determination of oxygen solubility as a function of concentration of different substances.[17] Based on the obtained results, the idea to define the water activity of different media as a function of oxygen solubility appeared. The oxygen solubility in D-sorbitol water solutions was investigated in order to define the free water concentrations proportional to the dissolved oxygen concentration.[12,18] Based on the obtained results, the applicability of the use of water activity concept defined as the ratio between oxygen solubility in water solution and that in the free water was considered and evaluated.[18,19] Oxygen solubility in D-sorbitol water solutions and oxygen uptake rates of *Gluconobacter suboxydans* S-22 at different D-sorbitol concentrations served as a basis to define relationships between dissolved oxygen saturation constants and other parameters of reaction systems.[20] Finally, experimentally confirmed advances in process technology and established process kinetics relationships suggested a development of corresponding mathematical model applicable for describing adequately the kinetics of the mentioned bioprocess events in a large range of bioprocess conditions. Since one can consider that mathematical models applied previously in describing process kinetics of microbial D-sorbitol oxidation are not quite adequate for such a purpose, the need to develop a more adequate mathematical model sought immediate engagement.

## Mathematical model

In a series of approaches to the mathematical modeling of microbial processes, the deterministic approach is applied frequently. Experience in industrial L-sorbose production, experimental data obtained in laboratory scale, and relevant data already published by the author, suggested that a deterministic approach can be applied to prepare this work as well. Prior to postulating the mathematical model, it appeared necessary to select and group the process events in accordance to their assumed relevance for process kinetics during the range of process conditions planned to be considered in this work. Thus, it was assumed that the following process events should be considered: microbial biomass growth, D-sorbitol uptake for cell constituents and products of energy metabolism, D-sorbitol conversion into L-sorbose, L-sorbose formation, oxygen transfer phenomena, dissolved oxygen uptake, change of media physical properties due to changes in concentration of media constituents, microbial biomass death and autolysis, presence and uptake of other substrates (*e.g.* CSL, yeast extract, mineral salts, etc). The following mathematical model was assumed applicable:

### Microbial growth kinetics

*Batch process:* As known, growth kinetics in the batch process can generally be approximated by the equation

$$\mathrm{d}\gamma_X/\mathrm{d}t = \mu\ \gamma_X \tag{1}$$

The media applied to perform experiments of microbial oxidation contained CSL filtrate as a complex source of growth factors. Therefore, it was supposed that the logistic Verhulst-Pearl equation could be the best starting basis to develop mathematical model expressing microbial growth kinetics. Because *G. suboxydans* S-22 appears to be an obligatory aerobe capable of using D-sorbitol as carbon source, and because the growth and bio-oxidation processes happen in reaction systems where availability of the water plays an important role, the following mathematical equations seem to be suitable for defining the specific growth rate as a function of various parameters

$$\mu = \mu_{max}\ (1 - \gamma_X/\gamma_{Xm})\ (\gamma_S/(K_s + \gamma_S))\ (1 - \gamma_{Xd}/\gamma_X) \cdot \cdot\ (\gamma_{DO}/(K_{sDO} + \gamma_{DO}))\ (\gamma_{WO} - \gamma_{WOcx})/\gamma_{WO} \tag{2}$$

or

$$\mu = \mu_{max}\ (1 - \gamma_X/\gamma_{Xm})\ (\gamma_S/(K_s + \gamma_S))\ (1 - \gamma_{Xd}/\gamma_X) \cdot \cdot\ (\gamma_{DO}/(K_{sDO} + \gamma_{DO}))\ \ln(\gamma_{WO}/\gamma_{WOcx}) \tag{3}$$

For a studied range of bioprocess conditions, the change in concentration of nonviable microbial biomass $\gamma_{xd}$ in eqs. (2) and (3) is neglected.

*Fed batch process:* To describe the growth kinetics, the equation

$$\mathrm{d}\gamma_X/\mathrm{d}t = \mu\gamma_X - q\gamma_X/(V_0 + qt) \tag{4}$$

where, $q$ represents the volumetric medium flow rate, $V_0$ the starting culture volume, $t$ the process time, and where the specific growth rate is expressed by eq. (2) or eq. (3), can be considered adequate.

### Substrate uptake kinetics

*Batch process:* Change in D-sorbitol concentration during the process is generally defined by equation

$$\mathrm{d}\gamma_S/\mathrm{d}t = -q_S\ \gamma_X \tag{5}$$

Then, the specific D-sorbitol consumption rate, $q_S$ can be expressed as follows

$$q_S = (q_{SPm} + q_{SXm}) \cdot (\gamma_S/(K_s + \gamma_S)) \cdot \cdot (\gamma_{DO}/(K_{sDO} + \gamma_{DO})) \cdot (\gamma_{WO} - \gamma_{WOcP})/\gamma_{WO} \quad (6)$$

if one considers that $q_{SPm}$ is the maximal specific rate of D-sorbitol conversion into L-sorbose (product) and that $q_{SXm}$ is the maximal specific D-sorbitol uptake with reference to its use for microbial biomass growth and maintenance.

*Fed batch process:* The equation

$$d\gamma_S/dt = q\,(\gamma_{S0} - \gamma_S)/(V_0 + q\ t) - q_S\,\gamma_X \quad (7)$$

in which the specific D-sorbitol consumption rate is defined by eq. (6) and where $\gamma_{s0}$ represents D-sorbitol mass concentration in the nutrient medium flowing into the culture, should be applied.

## Kinetics of oxygen transfer and uptake

*Batch process:* For changes in dissolved oxygen (DO) concentration the following equation

$$d\gamma_{DO}/dt = k_L a\ (\gamma^*_{DO} - \gamma_{DO}) - q_{ox}\gamma_X \quad (8)$$

is expected to be applicable. In it one considers that the specific oxygen uptake rate is proportional to the specific rate of product (L-sorbose) formation, *i.e.* that the expression

$$q_{ox} = k_{ox}\ q_P \quad (9)$$

can be applied if in the proportionality factor, $k_{ox}$ value, the oxygen consumption for biomass growth and maintenance is included as well.

*Fed batch process:* The equation

$$d\gamma_{DO}/dt = k_L a\ (\gamma^{*'}_{DO} - \gamma_{DO}) - q_{ox}\,\gamma_X + + q\ (\gamma^{*'}_{DO} - \gamma_{DO})/(V_0 + q\ t) \quad (10)$$

where for $q_{ox}$ the expression (9) is applicable and where $\gamma^{*'}_{DO}$ can vary with changes of D-sorbitol and L-sorbose mass concentrations, *i.e.* with changes of $\gamma_{(S+P)}$, is expected to be suitable.

## Product formation kinetics

*Batch process:* L-sorbose formation rate is expected to change as defined by equation

$$d\gamma_P/dt = q_P\,\gamma_X \quad (11)$$

if for the specific L-sorbose formation rate, $q_P$, the expression

$$q_P = q_{Pm} \cdot (\gamma_S/(K_s + \gamma_S)) \cdot (\gamma_{DO}/(K_{sDO} + \gamma_{DO})) \cdot \cdot (\gamma_{WO} - \gamma_{WOcP})/\gamma_{WO} \quad (12)$$

is applied.

*Fed batch process:* The equation

$$d\gamma_P/dt = q_P\,\gamma_X - q\ \gamma_P/(V_0 + q\ t) \quad (13)$$

should be applicable under the condition that expression (12) is applied to define the specific L-sorbose formation rate.

## Relation between parameters and their changes

In the batch process, no significant change of oxygen solubility during the process is supposed. In the fed batch processes, different situations can appear mainly due to different regimes of feeding the microbial culture with the substrate. In the simplest case, when one feeds the process culture with a medium of constant composition and of the same D-sorbitol mass concentration as in the medium used for the batch process, the eqs. (4), (7), (10) and (13) can be applied without additional prerequisites. Then, in the eq. (10), one can consider the values of $\gamma^*_{DO}$ and $\gamma_{S0DO}$ to be identical (equal). A more complex situation appears in the fed batch processes when feeding the process culture with concentrated D-sorbitol solution. Then, changes in dissolved substance concentration should be taken into account, especially with respect to their effects on oxygen solubility, water activity and mass transfer rate. Fortunately, already established relationships[12,18,19,20] seem to be appropriate in facilitating the finding of an acceptable solution for completing the mathematical modeling. The mentioned established relationships are listed in Table 1. Some of them were selected to form relationships applicable as model supplement:

a) Oxygen solubility as a function of the sum of D-sorbitol and L-sorbose mass concentration

$$\gamma^*_{DO} = 6.7822/1.001867^{\gamma(S+P)} \quad (14)$$

b) Free water concentration as a function of the sum of D-sorbitol and L-sorbose mass concentration

$$\gamma_{WO} = 984.7/1.001867^{\gamma(S+P)} \quad (15)$$

c) Dissolved oxygen saturation constant (Michaelis-Menten-Monod constant) as a function of the sum of D-sorbitol and L-sorbose mass concentration

$$K_{sDO} = 0.000145\gamma_{(S+P)}^{1/3} + 0.076 \quad (16)$$

d) Volumetric oxygen transfer rate coefficient as a function of the sum of D-sorbitol and L-sorbose mass concentration

$$Nk_La_{\gamma}(S+P) = -0.00119\gamma_{(S+P)} + 1.1174 \quad (17)$$

$$k_La_{\gamma(S+P)} = k_La_{100}\ Nk_La_{\gamma(S+P)} \quad (18)$$

where $k_La_{100}$ represents $k_La$ value for the sum of D-sorbitol and L-sorbose mass concentration $\gamma_{(S+P)}$ = 100 g $L^{-1}$

Table 1 – *Relationships established by authors in their previous reports*

| Relationships | References |
|---|---|
| $D_{HCl} = -0.3999\ ln\ \gamma_S + 2.7246$ $D_{NaOH} = -0.2578\ ln\ \gamma_S + 1.7431$<br>$D_{HCl} = 0.0042\ \gamma_{WO} - 0.8657$ $D_{NaOH} = 0.0021\ \gamma_{WO} - 0.4665$ | Mihaljević *et al.* 1995[18] |
| $D_{HCl} = 3.0568\ \eta_0/\eta - 0.0303$ $D_{NaOH} = 1.5424\ \eta_0/\eta - 0.0087$ | Mihaljević *et al.* 1995;[18] Mihaljević and Bošnjak 1997[19] |
| $K_{sDO} = 0.0145\ \gamma_S^{1/3} + 0.076$ $K_{sDO} = -0.0155\ \gamma^*_{DO}{}^{1/3} + 0.2325$<br>$K_{sDO} = -D_{NaOH} + 0.2087$ $K_{sDO} = -0.07688\ \eta_0/\eta + 0.2096$ | Mihaljević & Bošnjak 2003[20] |
| $Nk_La = -0.00119\ \gamma_S + 1.1174$ $Nk_La = 0.76669(\eta_{100}/\eta)^{0.67} + 0.23496$ | Mihaljević and Bošnjak 1997[19] |
| $q_P = 28.7684\ \eta_0/\eta - 0.7927$ $q_P = 0.03735\ \gamma_{WO} - 8.8234$<br>$q_P = q_{Pm}\gamma_S\eta_0/((K_s + \gamma_S)\eta)$ $q_P = 0.03939\ \gamma_{WT} - 19.5582$ | Mihaljević *et al.* 1995;[18] Mihaljević and Bošnjak 1997[19] |
| $\gamma^*_{DO} = 6.865 - 0.01208\ \gamma_S + 6.954 \cdot 10^{-6}\ \gamma_S^2$<br>$\gamma^*_{DO} = 6.7822/1.001867\ \gamma_S$ | Bošnjak and Mihaljević 1993;[12] Mihaljević and Bošnjak 2003[20] |
| $\gamma^*_{DO} = 6.7821\ \exp(-0.0019\ \gamma_S)$<br>$\gamma^*_{DO} = -0.938\gamma_S^{1/3} + 10.104$; valid for $\gamma_S \geq 100$ g L$^{-1}$ | Mihaljević & Bošnjak 2003[20] |
| $\gamma^*_{DP} = 4.6049\ \theta + 468.74\ \eta_0/\eta = \exp(c_0 - c_1\gamma_S - c_2\gamma_S^2)$ | Bošnjak and Mihaljević 1998[13] |
| $\eta_{(30°C)} = \exp(0.08416 + 1.418\ E{-}3\ \gamma_S + 3.25\ E{-}6\ \gamma_S^2)$<br>$\eta_{(37°C)} = \exp(0.1409 + 5.494\ E{-}4\ \gamma_S + 3.77E{-}6\ \gamma_S^2)$ | Bošnjak and Mihaljević 1998[13] |
| $(\eta_0/\eta)_{30\ °C} = 0.001328\ \gamma_{WO} - 0.2728$<br>$(\eta_0/\eta)_{37\ °C} = 0.001285\ \gamma_{WO} - 0.2398$ | Mihaljević *et al.* 1995;[18] Mihaljević and Bošnjak 1997[19] |
| $\mu = -5.333\ E{-}4\ \gamma_S + 0.2767$ $\mu = 5.375\ E{-}4\ \gamma_{WO} + 0.1894$ | Mihaljević *et al.* 1995;[18] Mihaljević and Bošnjak 1997[19] |
| $\mu = \mu_{max}[\gamma_S(\gamma_{WO} - \gamma_{WOcX})/((K_s + \gamma_S)\ \gamma_{WO})]$<br>$\mu = \mu_{max}[\gamma_S/(K_s + \gamma_S)]\ ln(\gamma_{WO}/\gamma_{WOcX})$ | Mihaljević 2005[14] |
| $^{**}k_La = 1.29\ E{-}6\ n^{2.89}$ | Bošnjak *et al.* 1988[11] |

Legend $D_{HCl}$ = diffusion coefficient with reference to HCl, *i.e.* $H_3O^+$ ion; $D_{NaOH}$ = diffusion coefficient with reference to NaOH, *i.e.* $H_3O_2^-$ ion; $K_{sDO}$ = dissolved oxygen saturation constant; $Nk_La$ = normalized volumetric oxygen transfer rate coefficient with reference to oxygen transfer rate coefficient of the medium with D-sorbitol mass concentration of 100 g L$^{-1}$; $q_P$ = specific rate of product (L-sorbose) formation; $q_{Pm}$ = maximal specific rate of product formation; $K_s$ = substrate (D-sorbitol) saturation constant; $\gamma$ indicates mass concentration and refers to: $DO$ = dissolved oxygen, $P$ = product, $S$ = substrate, $WO$ = free water, $WT$ = total water, $X$ = biomass, $^*$ = solubility; $\eta$ = medium viscosity; $\eta_0$ = pure water viscosity; $\mu$ = specific growth rate; $\theta$ = temperature; $^{**}$equation applicability confirmed by De Wulf *et al.*[21]

e) Possible mathematical model simplification can be based on the hypothesis that the effects of changes in $\gamma^*_{DO}$ and $Nk_La$ values can be lumped into effects on changes in $k_La_{\gamma(S+P)}$ values. Also, one can expect that in eq. (10), the first and second term should be of much higher influence on changes in $\gamma_{DO}$ than that of the third term. Relationships established previously[16] can be useful in estimating $k_La$ value as a function of aeration and agitation conditions.

## Materials and methods

### Microorganism

*Gluconobacter* (*syn. Acetobacter*) *suboxydans* S-22, the strain name used in: "PLIVA Croatia – Research Institute microorganism collection" (The name applied when the experiments were performed).

### Cultivation media

*Medium for inoculums preparation (MIP)*

D-sorbitol (PLIVA, Zagreb, HR) 200 g L$^{-1}$

CSL (50 %) (Roquette Freres, Lestrem, F), filtrate 20 g L$^{-1}$

pH after sterilization: 4.0 – 4.2

*Medium for the batch culture (MBC)*

D-sorbitol 220 g L$^{-1}$

CSL (50 %), filtrate 20 g L$^{-1}$

pH after sterilization: 4.5 – 4.6

*Initial medium for fed batch culture (IMFBC)*

D-sorbitol 220 g L$^{-1}$

CSL (50 %), filtrate 40 g L$^{-1}$

pH after sterilization: 3.8 – 4.0

*D-sorbitol solution for culture feeding*

D-sorbitol mass concentration 1000 g $L^{-1}$

Note: All media were sterilized 40 min at 110 °C

## Bioreactors

a) Rotary shaker ("Mlinostroj", Domžale, SLO) with rotation speed of $n = 220$ $min^{-1}$ was used for *G. suboxydans* S-22 propagation in order to prepare inoculums for seeding the reaction media in laboratory bioreactors;

b) Laboratory glass bioreactors "Microferm MF-214" (New Brunswick Scientific Co. Inc., USA), cylindrical stirred glass vessels of 14 L total volume, supplied with dissolved oxygen analyzers and applied in previous works,[14,16] were used for inoculum preparation as well as for processes of D-sorbitol conversion into L-sorbose by applying batch and fed batch cultures.

## Cultivation methods

### *Seed culture preparation*

To prepare seed cultures of *G. suboxydans* S-22, sterile Erlenmeyer flasks of $V = 500$ mL, each containing 50 mL of sterile medium MIP were used. After medium inoculation with 5 mL of *G. suboxydans* S-22 culture, the flasks were subjected to 20 hours incubation at 30 °C on the rotary shaker with rotation speed of $n = 220$ $min^{-1}$. The seed culture prepared on such a way was used ($\varphi = 3$ %) for the inoculation of 6 L of the sterile medium MIP in the laboratory bioreactor where cultivation proceeded at 32 °C applying the aeration intensity of $q = 15$ L $min^{-1}$ and an agitation defined by stirrer rotation speed of $n = 1200$ $min^{-1}$. After 16−17 hours of cultivation, the microbial biomass concentration of 1.6 to 1.9 commonly was attained. Such a seed culture was stored in a deep freezer at −20 °C in order to be used as inoculum for seed culture preparation, biomass production and/or D-sorbitol to L-sorbose oxidation (conversion).

### *Processes of D-sorbitol to L-sorbose biooxidation*

Experiments of D-sorbitol to L-sorbose biooxidation were performed applying shaken flasks cultures, and cultures in aerated and agitated laboratory bioreactors. The batch, fed batch and prolonged fed batch cultures were applied. The effects of pH, temperature, concentrations of biomass, D-sorbitol and growth factors on process kinetics were studied. The series of obtained results has already been published (see Table 1), whereas the results of this work mainly refer to the effects of D-sorbitol and total substance concentration on process kinetics.

### *Determination of the effect of D-sorbitol mass concentration on the growth kinetics*

Sterile nutrient media of different D-sorbitol concentrations were seeded with the microbial biomass taken from the batch culture exponential phase. At the moment when seeding started, the biomass concentration was found to be of $\gamma_X = 0.119$ g $L^{-1}$. Cultivation process was performed at $\theta = 30$ °C, in conical flasks of 500 mL containing 55 mL of the culture, on a rotary shaker rotating with $n = 220$ $min^{-1}$. After 5 hours of cultivation, the biomass concentrations were determined and the mean specific growth rates calculated.

### *Determination of the effects of D-sorbitol concentration on the specific L-sorbose formation rate*

Sterile D-sorbitol solutions of $\gamma_S = 100$, 200, 300, 400, 500, 600, 700 and 800 g $L^{-1}$ were seeded with small amounts (the final biomass concentration in D-sorbitol solutions was $\gamma_X = 0.1$ g $L^{-1}$) of microbial biomass prepared in the following manner: The biomass was separated from the batch culture by centrifugation, washed with a physiological water solution, and then suspended in the same. The seeded D-sorbitol solutions were incubated at $\theta = 30$ °C for 20 h, using Erlenmeyer flasks of $V = 500$ mL and rotary shaker with a rotation speed of $n = 220$ $min^{-1}$. After incubation, the L-sorbose concentrations in samples were determined and the specific L-sorbose formation rates estimated.

## Analytical methods

### *Determination of biomass concentration during D-sorbitol into L-sorbose conversion*

Biomass concentration was assayed by spectrophotometry at $\lambda = 660$ nm. Previously,[14] the relationship between biomass concentration and corresponding absorbance of given sample was established. It was found[14] that the following empirical expression can be applied:

$$\gamma_X = (f_D\ (0.121\ A^4 - 0.157\ A^3 + \\ + 0{,}129\ A^2 + 0.323\ A)) \qquad (19)$$

In the expression (19), $A$ is the difference between the absorbance of the sample ($A_{spl}$) and the absorbance of the sample filtrate ($A_{splf}$), whereas $f_D$ refers to the applied sample dilution.

### *Determination of L-sorbose concentration*

The method based on the reduction of $Cu^{2+}$ ion into $Cu^{+}$ ion followed by iodometric titration with sodium tiosulphate was applied. The expression

$$\gamma_P = (4.22147\ V + 0.0109684\ V^2) \qquad (20)$$

was applied to estimate L-sorbose mass concentration in the sample, taking into account the eventual sample dilution. In the expression (20) $V$ refers to the difference between volumes of sodium tiosulphate solution consumed for the titration of treated and untreated sample.

*Determination of total dissolved substance concentration (the sum of D-sorbitol and L-sorbose mass concentrations)*

Total dissolved substances were estimated on the basis of the refraction index of investigated solution. Therefore, it was found[14] that the following mathematical expression can be used

$$\gamma_{(S+P)} = (9.57881\ n + 0.05594\ n^2) \qquad (21)$$

If $n$ refers to the measured value of the refraction index ("Refractometer RF3", Zrak, Sarajevo, BH). When the mass concentration of L-sorbose is known, the mass concentration of D-sorbitol can be estimated on the basis of the difference between the total dissolved substance mass concentration and L-sorbose concentration.

*Determination of the oxygen solubility and the specific oxygen uptake rate*

The methods described in the previous papers[14,20] were applied.

### Calculation of experimental mean specific rates

*Mean specific growth rates*

a) Batch culture:

$$\overline{\mu} = 2\ (\gamma_{Xt} - \gamma_{X0})/((t - t_0)(\gamma_{Xt} + \gamma_{X0})) \qquad (22)$$

b) Fed batch culture:

$$\overline{\mu} = (1/(t - t_0))\ \ln\ (\gamma_{Xt}\ V_h/\gamma_{X0}\ V_l) \qquad (23)$$

*Mean specific rates of L-sorbose formation*

a) Batch culture:

$$\overline{q}_P = 2\ (\gamma_{Pt} - \gamma_{P0})/((t - t_0)(\gamma_{Xt} + \gamma_{X0})) \qquad (24)$$

b) Fed batch culture:

$$\overline{q}_P = 2(\gamma_{Pt}\ V_h - \gamma_{P0}\ V_l)/((t - t_0)(\gamma_{Xt}\ V_h + \gamma_{X0}\ V_l)) \qquad (25)$$

### Computer simulation

Based on the developed mathematical model and initial parameter values estimated on the basis of our own published experimental data (see Table 1), simulation program was developed in *MicroMath Scientist Ver. 2.0* software package and a series of simulations were performed on PC.

## Results

Results are presented in Fig. 1 to Fig. 8 and in Table 2. Since two ways of expressing the effects of free water concentration on the specific growth rate were recommended in the proposed mathematical models, it appeared useful to demonstrate graphically the differences between the recommended two ways. Thus, Fig. 1 refers to the demonstration of the mentioned differences. Evidently, both ways proved acceptable, and therefore either can be used for calculations.

The main goal of this work was to see whether the proposed mathematical model could explain the experimental data with reference to all batch, fed batch and prolonged fed batch cultures. A series of experiments was performed and the most convenient examples (those having enough experimental data with reference to all considered process parameters) were selected for inspection by computer simulation. The example referring to the batch culture was considered the first and its process course is presented in Fig. 2. Evidently, for applied computer simulation parameters, the agreement of computer simulation with experimental data appeared excellent. Therefore, the applicability of the proposed mathematical model for batch culture was confirmed, and based on the findings one can calculate the values of specific rates of particular process events during the whole process. Indeed, calculations led to the results demonstrated in Fig. 3. The process course demonstrated in Fig. 4 refers to the fed batch culture. Again, the applicability of the mathematical model was confirmed with undoubtedly excellent agreement of computer simulation with experimental data. Here, one should point out that data refer to larger concentration range of dissolved substances in the reaction system. Similar to Fig. 3, Fig. 5 refers to the calculated values of specific rates of particular process events. However, although it was expected, special emphasis should be given to the data demonstrated in Fig. 6, since they refer to the prolonged fed batch culture and therefore to the extremely large concentration range of dissolved substances. The simulation curves with no evident differences in the quality of fitting simulated to experimental data were obtained, when different values of maximal specific growth rate and different expressions describing the effects of free water concentration on the growth rate (Fig. 1) were applied. Extremely low differences become evident if one compares values of correlation coefficients (Table 2), but evidently, such differences can be neglected. As in the case of batch and fed batch cultures, the specific rates of particular events were also calculated for prolonged fed batch culture. The course of changes of mentioned specific rates is presented in Fig. 7.

Table 2 – *Agreement of mathematical model and experimental data for all presented examples (Equation of linear regression: $x_{calc} = a\ x + b$)*

| Cultivation method | Quantities | Simulation concentration range $\gamma$/g L$^{-1}$ | Slope $a$ | Intercept $b$ | Correlation coefficient $R$ | Determination coefficient $R^2$ |
|---|---|---|---|---|---|---|
| Batch Culture (BC) | $\gamma_X$ | 0.132 – 1.917 | 0.9937 | 0.0055 | 0.99975 | 0.99950 |
| | $\gamma_S$ | 0.001 – 195.0 | 0.9979 | −0.2409 | 0.99993 | 0.99986 |
| | $\gamma_P$ | 27.0 – 218.2 | 0.9875 | 0.9510 | 0.99992 | 0.99984 |
| | $\gamma_X$, $\gamma_S$, $\gamma_P$ | 0.001 – 218.2 | 0.9943 | 0.1477 | 0.99995 | 0.99990 |
| Fed Batch Culture (FBC) | $\gamma_X$ | 0.419 – 1.886 | 0.9873 | 0.0314 | 0.99773 | 0.99546 |
| | $\gamma_S$ | 0.001 – 157.0 | 0.9913 | −1.0363 | 0.99895 | 0.99790 |
| | $\gamma_P$ | 55.0 – 460.5 | 0.9880 | 3.3474 | 0.99970 | 0.99940 |
| | $\gamma_X$, $\gamma_S$, $\gamma_P$ | 0.001 – 460.5 | 0.9973 | −0.2284 | 0.99985 | 0.99970 |
| *Prolonged Fed Batch Culture (PFBC) | $\gamma_X$ | 0.606 – 1.683 | 0.9762 | 0.0345 | 0.99693 | 0.99387 |
| | $\gamma_S$ | 60.5 – 225.0 | 0.9880 | 3.7219 | 0.99698 | 0.99397 |
| | $\gamma_P$ | 84.0 – 713.9 | 1.0164 | −6.8989 | 0.99978 | 0.99956 |
| | $\gamma_X$, $\gamma_S$, $\gamma_P$ | 0.606 – 713.9 | 1.0037 | 0.6635 | 0.99992 | 0.99984 |
| **Prolonged Fed Batch Culture (PFBC) | $\gamma_X$ | 0.606 – 1.671 | 0.9976 | −0.0107 | 0.99802 | 0.99604 |
| | $\gamma_S$ | 59.6 – 225.0 | 0.9918 | 4.2254 | 0.99751 | 0.99503 |
| | $\gamma_P$ | 84.0 – 702.3 | 1.0020 | −3.6250 | 0.99981 | 0.99962 |
| | $\gamma_X$, $\gamma_S$, $\gamma_P$ | 0.606 – 702.3 | 0.9937 | 1.5445 | 0.99993 | 0.99986 |

*Eq. 2 was applied to define values of specific growth rate.
**Eq. 3 was applied to define values of specific growth rate.

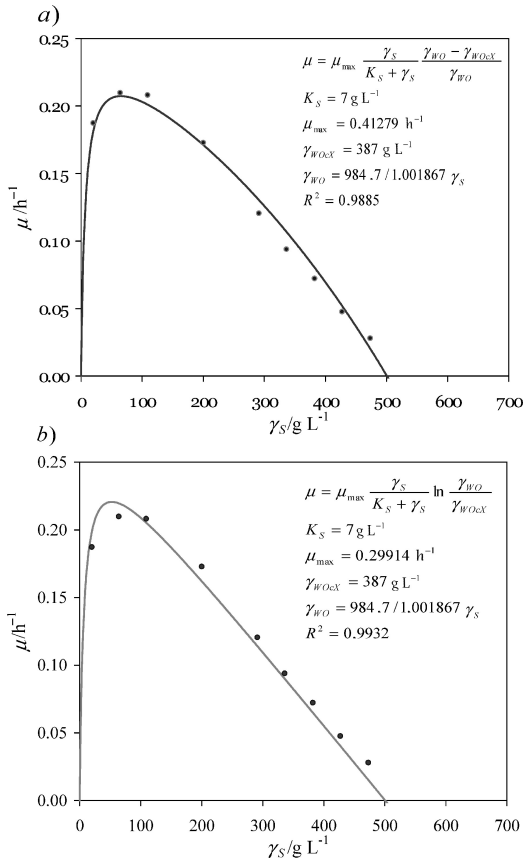



Fig. 1 – *Specific growth rate ($\mu$) of G. suboxydans S-22 as the function of D-sorbitol ($\gamma_S$) and the free water ($\gamma_{WO}$) concentrations. Applicability of mathematical models: a) $\mu = \mu_{max}[\gamma_S(\gamma_{WO} - \gamma_{WOcX})/(K_s + \gamma_S)\gamma_{WO}]$; b) $\mu = \mu_{max}[\gamma_S/(K_s + \gamma_S)\ln(\gamma_{WO}/\gamma_{WOcX})]$.*

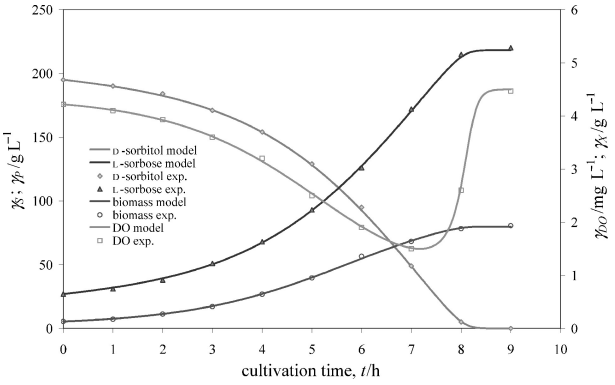



Fig. 2 – *Batch process kinetics of G. suboxydans S-22 culture, and fitting of computer simulation to experimental data. Simulation parameters: maximal specific growth rate, $\mu_{max}$ = 1.33 h$^{-1}$; maximal microbial biomass concentration, $\gamma_{Xm}$ = 2.55 g L$^{-1}$; substrate saturation constant, $K_s$ = 7.0 g L$^{-1}$; starting non viable biomass concentration, $\gamma_{Xd}$ = 0.07 g L$^{-1}$; dissolved oxygen saturation constant, $K_{sDO}$ = 0.000165 g L$^{-1}$; volumetric coefficient of oxygen transfer rate, $k_La$ = 1600 h$^{-1}$; maximal specific substrate uptake rate with reference to product formation, $q_{SPm}$ = 50.25 h$^{-1}$; oxygen uptake coefficient, $k_O$ = 0.1046; specific substrate uptake rate with reference to biomass growth and maintenance, $q_{SXgm}$ = 1.0 h$^{-1}$; critical free water mass concentration with reference to biomass growth, $\gamma_{WOcX}$ = 387 g L$^{-1}$; critical free water mass concentration with reference to product formation, $\gamma_{WOcP}$ = 195.8 g L$^{-1}$; expression for calculation of free water mass concentration: $\gamma_{WO} = 984.7/1.001867^{\gamma(S+P)}$; initial biomass concentration, $\gamma_{X0}$ = 0.123 g L$^{-1}$; initial D-sorbitol concentration, $\gamma_{S0}$ = 195 g L$^{-1}$; initial L-sorbose concentration, $\gamma_{P0}$ = 27 g L$^{-1}$; initial dissolved oxygen mass concentration, $\gamma_{DO0}$ = 0.0043 g L$^{-1}$. Remark: eq. (3) was applied to define values of specific growth rate.*

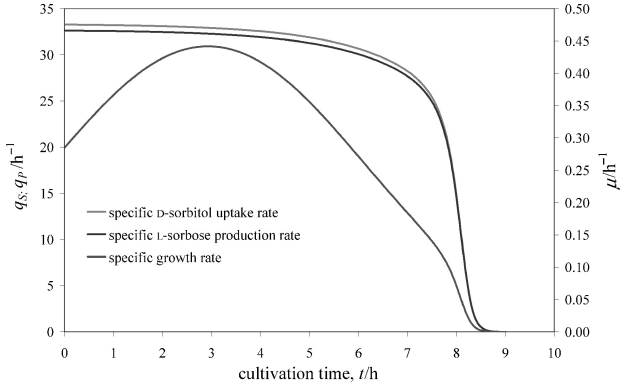



Fig. 3 – *Simulated specific biomass growth, substrate uptake and product formation rates during the batch process shown in Fig. 2*

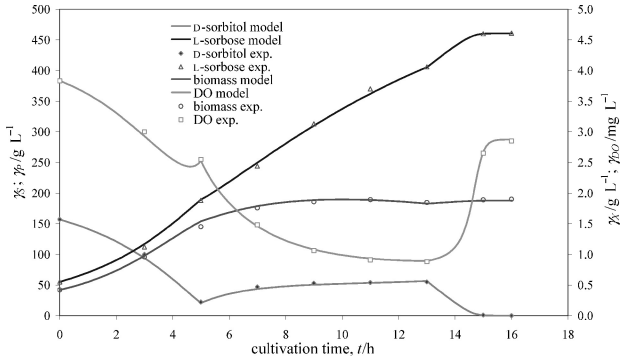



Fig. 4 – *Fed batch process kinetics of G. suboxydans S-22 culture, and fitting of computer simulation to experimental data. Simulation parameters: maximal specific growth rate, $\mu_{max}$ = 1.147 $h^{-1}$; maximal microbial biomass concentration, $\gamma_{Xm}$ = 2.66 g $L^{-1}$; substrate saturation constant, $K_s$ = 7 g $L^{-1}$; non viable microbial biomass concentration, $\gamma_{Xd}$ = 0.20 g $L^{-1}$; equation for calculation of dissolved oxygen saturation constant: $K_{sDO} = 0.0000145\gamma_{(S+P)}^{1/3} + 0.000076$ g $L^{-1}$; expression for volumetric coefficient of oxygen transfer rate calculation: $(k_La)_{\gamma(S+P)} = (k_La)_{100}*(Nk_La)_{\gamma(S+P)}$; applied value of $(k_La)_{100}$ = 2200 $h^{-1}$; maximal specific substrate uptake rate with reference to product formation, $q_{SPm}$ = 49.422 $h^{-1}$; specific substrate uptake rate with reference to biomass growth and maintenance, $q_{SXgm}$ = 1.0 $h^{-1}$; oxygen uptake coefficient, $k_O$ = 0.1046; critical free water mass concentration with respect to biomass growth, $\gamma_{WOcX}$ = 387 g $L^{-1}$; critical free water concentration with reference to product formation, $\gamma_{WOcP}$ = 196 g $L^{-1}$; expression for calculation of free water mass concentration: $\gamma_{WO} = 984.7/1.001867^{\gamma(S+P)}$; volumetric medium flow rate, $q_{(5-13h)}$ = 0.2422 L $h^{-1}$; substrate concentration in the feed medium, $\gamma_{fmS0}$ = 1000 g $L^{-1}$; initial culture volume, $V_0$ = 4.0 L; initial biomass concentration, $\gamma_{X0}$ = 0.419 g $L^{-1}$; initial D-sorbitol concentration, $\gamma_{S0}$ = 157 g $L^{-1}$; initial L-sorbose concentration, $\gamma_{P0}$ = 55 g $L^{-1}$; initial dissolved oxygen concentration, $\gamma_{DO0}$ = 0.00475 g $L^{-1}$. Remark: eq. (3) was applied to define values of specific growth rate.*

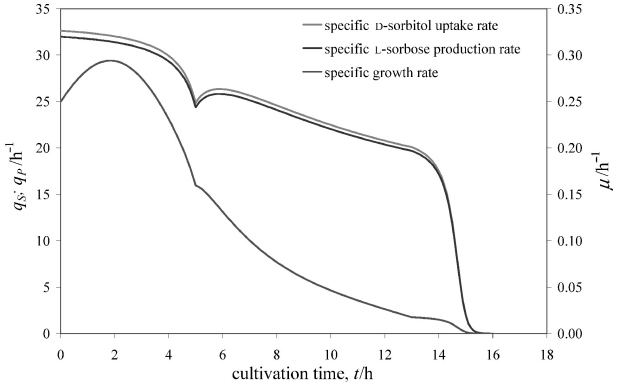



Fig. 5 – *Simulated specific biomass growth, substrate uptake and product formation rates during the fed batch process shown in Fig. 4*

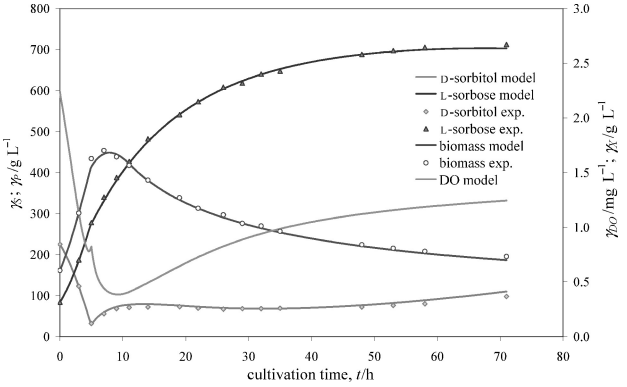
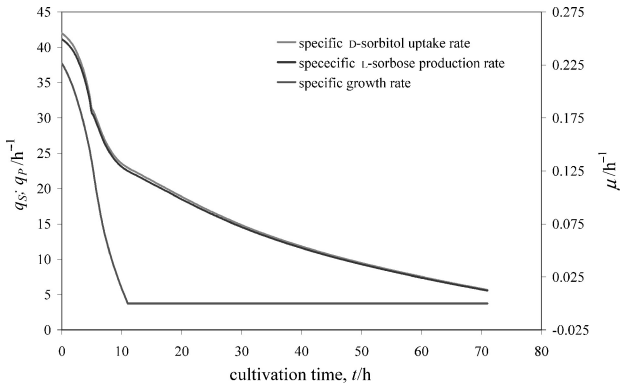
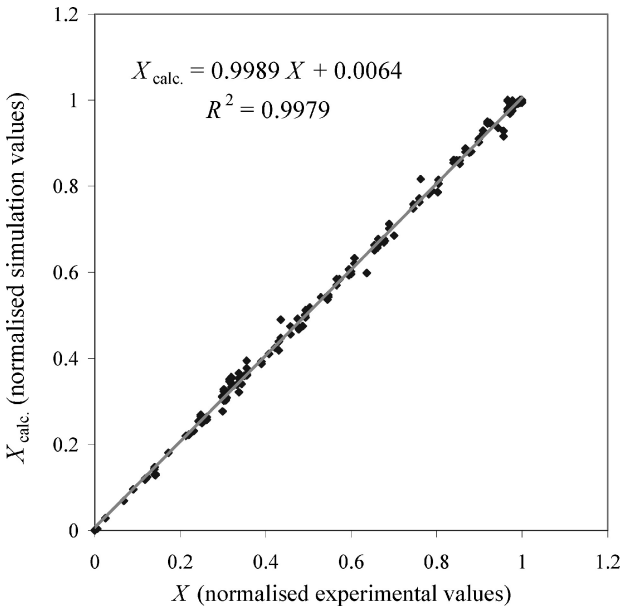



Fig. 6 – *Prolonged fed batch process kinetics of G. suboxydans S-22 culture, and fitting of computer simulation to experimental data. Simulation parameters: maximal specific growth rate, $\mu_{max}$ = 0.819 $h^{-1}$; microbial biomass maximal concentration, $\gamma_{Xm}$ = 4.80 g $L^{-1}$; substrate saturation constant, $K_s$ = 7.0 g $L^{-1}$; non viable biomass concentration, $\gamma_{Xd}$ = 0.01 g $L^{-1}$; equation for dissolved oxygen saturation constant calculation: $K_{sDO} = 0.0000145\gamma_{(S+P)}^{1/3} + 0.000076$ g $L^{-1}$; expression for volumetric coefficient of oxygen transfer rate calculation: $(k_La)_{\gamma(S+P)} = (k_La)_{100}*(Nk_La)_{\gamma(S+P)}$; applied value of $(k_La)_{100}$ = 2200 $h^{-1}$; maximal specific substrate uptake rate with reference to product formation, $q_{SPm}$ = 70.786 $h^{-1}$; specific substrate uptake rate with reference to biomass growth and maintenance, $q_{SXgm}$ = 1.4 $h^{-1}$; oxygen consumption coefficient, $k_O$ = 0.1046; critical free water mass concentration with reference to biomass growth, $\gamma_{WOcX}$ = 387 g $L^{-1}$; critical free water mass concentration with reference to product formation, $\gamma_{WOcP}$ = 195.77 g $L^{-1}$; expression for the calculation of free water mass concentration during the process: $\gamma_{WO} = 984.7/1.001867\ \gamma_{(S+P)}$; nutrient medium volumetric flow rate, $q_{(5-71h)} = -0.141 \ln t + 0.638$ L $h^{-1}$; substrate concentration in the feed medium, $\gamma_{fmS0}$ = 1000 g $L^{-1}$; initial culture volume, $V_0$ = 4 L; initial biomass concentration, $\gamma_{X0}$ = 0.606 g $L^{-1}$; initial D-sorbitol concentration, $\gamma_{S0}$ = 225 g $L^{-1}$; initial L-sorbose concentration, $\gamma_{P0}$ = 84 g $L^{-1}$; initial dissolved oxygen concentration, $\gamma_{DO0}$ = 0.00224 g $L^{-1}$; Remark: eq. (3) was applied to define values of specific growth rate.*




F i g . 7 – *Simulated specific biomass growth, substrate uptake and product formation rates during the prolonged fed batch process shown in Fig. 6*




F i g . 8 – *Correlation between normalized simulation and experimental biomass, substrate and product values with reference to performed BC, FBC and PFBC*

Table 2 and Fig. 8 in general show the excellent agreement of theoretical (simulated) with experimental data for all presented examples. Excellent correlation coefficients ($R^2$ = 0.99693 to 0.99995) and theoretically very acceptable line coefficients (gradient values very close to 1.0, *i.e.* $a_{lr}$ = 0.9762 to 1.0164) and intercept values resulting from linear regression analysis support such an impression.

## Conclusion

Although the processes of biocatalytic conversion of D-sorbitol into L-sorbose by means of aerobic microbial strains belonging to the genera of acetic acid bacteria have long been known, along with continuous advances concerning the process efficiency and the information on process biochemistry as well as the determination of process relationships, not enough attention is given to the effects of water activity with respect to oxygen solubility and oxygen transfer rate, and therefore to the general process relationships valid for much larger range of process conditions of this reaction system. In contrast to previous less complete mathematical models, the mathematical model developed for purposes of this work is markedly advanced, more sophisticated, and with confirmed excellent applicability. The confirmation of mathematical model applicability was performed by computer simulation and refers to the batch, fed batch and prolonged fed batch cultures of microbial strain *Gluconobacter suboxydans* S-22. One can expect that the same or similar mathematical model might be applied for microbial processes of similar characteristics. However, perhaps some questions concerning the excellent agreement of theoretical with experimental data should be asked, such as: Was something overlooked, or were eventual experimental deviations neglected? The answer could certainly be: deviations were not observed, whereas for an overlooking of something one could say: there is no significant probability for overlooking something relevant to the considerations of the present approach to the description of process kinetics of studied reaction system. Experience of authors obtained during their long time engagement in the successful L-sorbose production performed in the laboratory, pilot-plant or plant scale supports such an answer. It is also relevant to point out that the process of microbial D-sorbitol conversion into L-sorbose belongs to those processes that are considered technologically relatively simple, since it can be performed without difficulties with respect to foam formation and changes in fermentation broth rheological properties.

### List of symbols

$a$ – relative gas-liquid interface surface area, m$^2$ m$^{-3}$

$D$ – dilution (feed) rate, h$^{-1}$

$d\gamma_{DO}/dt$ – rate of change of dissolved oxygen mass concentration, g L$^{-1}$ h$^{-1}$

$d\gamma_P/dt$ – rate of L-sorbose (product) formation; rate of change of product concentration, g L$^{-1}$ h$^{-1}$

$d\gamma_S/dt$ – rate of D-sorbitol (substrate) conversion (uptake); rate of change of substrate concentration, g L$^{-1}$ h$^{-1}$

$d\gamma_X/dt$ – microorganism growth rate; rate of change of microbial biomass concentration, g L$^{-1}$ h$^{-1}$

$K_s$ – saturation constant (Michaelis-Menten-Monod constant) with respect to D-sorbitol concentration, g L$^{-1}$

$K_{sDO}$ – saturation constant (M-M-M constant) with respect to dissolved oxygen concentration, g $L^{-1}$

$k_L$ – linear coefficient of oxygen transfer rate

$k_La$ – volumetric coefficient of oxygen transfer rate, $h^{-1}$

$(k_La)_{100}$ – volumetric coefficient of oxygen transfer rate with reference to D-sorbitol/L-sorbose total mass concentration of 100 g $L^{-1}$, $h^{-1}$

$(k_La)_{\gamma(S+P)}$ – volumetric coefficient of oxygen transfer rate with reference to D-sorbitol/L-sorbose total mass concentration of $\gamma_{(S+P)}$ g $L^{-1}$, $h^{-1}$

$k_{ox}$ – proportionality factor expressing the relation between specific oxygen uptake rate and specific L-sorbose formation rate

$n$ – rotation speed, $min^{-1}$

$(Nk_La)_{\gamma(S+P)}$ – normalized volumetric oxygen transfer rate coefficient; ratio of $(k_La)_{\gamma(S+P)}/(k_La)_{100}$

$q$ – volumetric medium flow rate, L $h^{-1}$

$q_{ox}$ – respiration coefficient; specific oxygen uptake rate, $h^{-1}$

$q_P$ – specific product formation rate; product formation quotient, $h^{-1}$

$\overline{q}_P$ – mean specific product formation rate; product formation quotient, $h^{-1}$

$q_{Pm}$ – maximal specific product (L-sorbose) formation rate, $h^{-1}$

$q_S$ – specific substrate (D-sorbitol) uptake rate; substrate uptake quotient, $h^{-1}$

$q_{SPm}$ – maximal specific substrate (D-sorbitol) into L-sorbose conversion rate, $h^{-1}$

$q_{SXm}$ – maximal specific substrate (D-sorbitol) into biomass conversion rate, $h^{-1}$

$t$ – cultivation time after time $t$, h

$t_0$ – cultivation time at process start, h

$V$ – culture volume, L

$V_0$ – culture starting volume, L

$V_l$ – fed batch culture lower volume, L

$V_h$ – fed batch culture higher volume, L

## Greek letters

$\gamma_{DO}$ – dissolved oxygen mass concentration, g $L^{-1}$

$\gamma^*_{DO}$ – oxygen mass solubility in the reaction medium, g $L^{-1}$

$\gamma_P$ – product (L-sorbose) mass concentration, g $L^{-1}$

$\gamma_S$ – substrate (D-sorbitol) mass concentration, g $L^{-1}$

$\gamma_X$ – microbial biomass concentration, g $L^{-1}$

$\gamma_{X0}$ – microbial biomass concentration at process start, g $L^{-1}$

$\gamma_{Xt}$ – microbial biomass concentration at process time $t$, g $L^{-1}$

$\gamma_{Xd}$ – non-viable microbial biomass concentration, g $L^{-1}$

$\gamma_{Xm}$ – maximal microbial biomass concentration, g $L^{-1}$

$\gamma_{(S+P)}$ – summarized substrate and product mass concentrations, g $L^{-1}$

$\gamma_{WO}$ – free water mass concentration defined by oxygen solubility, g $L^{-1}$

$\gamma_{WOcP}$ – critical free water concentration with reference to product, g $L^{-1}$

$\gamma_{WOcX}$ – critical free water concentration with reference to biomass, g $L^{-1}$

$\mu$ – specific growth rate, $h^{-1}$

$\mu_{max}$ – maximal specific growth rate, $h^{-1}$

$\overline{\mu}$ – mean specific growth rate, $h^{-1}$

## Abbreviations

BC – batch culture

FBC – fed batch culture

IMFBC – initial medium for fed batch culture

MBC – medium for batch culture

MIP – medium for inoculums preparation

PFBC – prolonged fed batch culture

## References


1. *Fulmer, E. I., Dunning, J. W., Guyman, J. F., Underkofler, L. A.*, J. Am. Chem. Soc. **58** (1936) 1012.
2. *Wells, P. A., Stubbs, J. J., Lockwood, L. B., Roe, E. T.*, Ind. Eng. Chem. **29** (1937) 1385.
3. *Wells, P. A., Lockwood, L. B., Stubbs, J. J., Roe, E. T., Porges, N., Gastrock, E. A.*, Ind. Eng. Chem. **31** (1939)1518.
4. *Yamada, S., Wada, M., Chibata, I.*, J Ferment. Technol .**56** (1978) 29.
5. *Elsworth, R., Telling, R. C., East, D. N.*, J. Appl. Bact. **22** (1959) 138.
6. *Bošnjak, M., Kapetanović, E., Veble, D.*, Kem. Ind. **9** (1965) 769.
7. *Krieg, P.*, Doctors Natw ETH Diss Nr 4675, Zuerich: Juris Druck, 1972.
8. *Bull, D. N., Young, M. D.,* Biotechnol. Bioeng. **23** (1981) 373.
9. *Mori, H., Kobayashi, T., Shimizu, S.*, J. Chem. Eng. Jpn. **14** (1981) 65.
10. *Bošnjak, M.,* Repeated fed batch culture and its applications efficacy, in Continuous Culture, FEMS symp. No. 41, *Kyslik, P., Dawes, E. A., Krumphanzl, V., Novak, M.* (Eds.) Academic Press Inc. London, Ltd., (1988), pp. 215–228.
11. *Bošnjak, M., Pintar, P., Gomerčić, K.*, Gradient fed batch culture as efficient microbial oxidation method, in Med. Fac. Landbouww. Rijksuniv. Gent, **56** (4a), Proc. 5th FAB, Gent, (1991), pp. 1723–1730.
12. *Bošnjak, M., Mihaljević, K.,* Efficiency of D-sorbitol to crystalline L-sorbose bioconversion, in Med. Fac. Landbouww. Univ. Gent, 58/4b, Proc. 7th FAB, Gent, (1993), pp. 1939–1948.
13. *Bošnjak, M., Mihaljević, K.,* Prolonged fed batch cultivation, in *Berovič, M.* (Ed.) Bioprocess engineering course, National Institute of Chemistry, Ljubljana, 1998, pp. 344–369.
14. *Mihaljević, K.,* Ph. D. Thesis, Faculty of Food Technology and Biotechnology, Zagreb, 2005.
15. *Bošnjak, M.,* Mikrobiologija (Yu) **8** (1971) 127.

16. *Bošnjak, M., Gomerčić, K., Pintar, P., Mikačić, D., Matošić, S.*, Chem. Biochem. Eng. Q. **2** (1988) 218.
17. *Quicker, G., Schumpe, A., Koenig, B., Deckwer, W. D.*, Biotechnol. Bioeng. **23** (1981) 635.
18. *Mihaljević, K., Gamulin, S., Pavlović, N., Bošnjak, N., Bošnjak, M.*, Prehrambeno-tehnol. Biotehnol. Rev. **33** (1995) 7.
19. *Mihaljević, K., Bošnjak, M.,* Food Technol. Biotechnol. **35** (1997) 89.
20. *Mihaljević, K., Bošnjak, M.,* Food Technol. Biotechnol. **41** (2003) 203.
21. *De Wulf, P., Soetaert, W., Vandamme, E. J.*, Biotechnol. Bioeng. **69** (2000) 339.