A Kinetic Model for Gluconic Acid Production by *Aspergillus niger**

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Fermentation kinetics of gluconic acid production by *Aspergillus niger* was studied in a batch system. The dependence of the specific growth rate on carbon and oxygen substrates was assumed to follow either the Monod or Contois kinetic model. The simulation results revealed that for predicting the fermentation process of gluconic acid production by *A. niger*, the Contois type model was more suitable than that based on the Monod equation. The Luedeking—Piret equation was proposed for gluconic acid production and an equation considering the cell maintenance for glucose and dissolved oxygen consumption. Estimated parameters of this model indicate that the production of gluconic acid was mostly growth-associated.

Gluconic acid and its salts are important materials widely used in pharmaceutical, food, feed, detergent, textile, leather, photographic, and concrete industries [1—3]. There are different approaches available for the production of gluconic acid: chemical, electrochemical, biochemical, and bio-electrochemical processes [4—6]. Fermentation is one of the dominant routes for manufacturing gluconic acid at present [7]. Gluconic acid fermentation by *A. niger* belongs to aerobic fermentations with high oxygen demand. The biotransformation of glucose to gluconic acid represents a simple dehydrogenation reaction without involvement of complex metabolic cell pathways, which is realized with a high selectivity, high rate, and high yield of conversion.

The development of kinetic models consists of comparing assumed models with experimental data in order to obtain more relevant equations. Kinetic models enable the bioengineer to design and control microbial processes [8]. In predicting the behaviour of these processes, mathematical models, together with carefully designed experiments, make it possible to evaluate the behaviour of systems more rapidly than with solely laboratory experiments. Mathematical models for a microbial process can be developed using two different mechanisms. Thus structured and unstructured models can be obtained [9]. Structured models take into account some basic aspects of cell structure, function, and composition. In unstructured models, however, only cell mass is employed to describe the biological system. In this study, an unstructured model for cell growth, product formation, glucose consumption, and dissolved oxygen was found to be convenient to characterize the fermentation process.

In modelling fermentation systems, it is relatively easy to set up a system of differential equations to represent the bioprocess dynamics. The most difficult part in modelling the fermentation process is obtaining the form of the specific growth rate expression μ that best represents the observed kinetic behaviour.

Many investigations have been carried out on the modelling of gluconic acid by *A. niger*. *Takamatsu et al.* [10] reported the structured model to describe gluconic acid fermentation by *A. niger*. *Liu et al.* [11] proposed a simple unstructured model using the logistic equation for growth. *Reuss et al.* [12] developed a comprehensive model, they found that due to the excess of glucose and oxygen during the active growth period, these two components need not be considered in the μ. However, none of them considered the effect of the two important substrates (glucose and dissolved oxygen) in the μ used.

In this study, suitable kinetic models for the production of gluconic acid by *A. niger* in a fermentation process carried out in mechanically stirred bioreactors with the volumes of 2 dm^3 and 5 dm^3 have been suggested taking into account the glucose and dissolved oxygen in the μ.

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THEORETICAL

Assuming constant temperature and a constant gas flow rate, the material balances of the biomass, product, and substrates (glucose and dissolved oxygen) can be written in the following form

$$\frac{\mathrm{d}X}{\mathrm{d}t} = r_{\mathrm{X}} \tag{1}$$

$$\frac{\mathrm{d}P}{\mathrm{d}t} = r_{\mathrm{P}} \tag{2}$$

$$\frac{\mathrm{d}S}{\mathrm{d}t} = r_{\mathrm{S}} \tag{3}$$

$$\frac{\mathrm{d}C_{\mathrm{l}}}{\mathrm{d}t} = k_{\mathrm{l}}a(C_{\mathrm{l}}^{*} - C_{\mathrm{l}}) - r_{\mathrm{O}} \tag{4}$$

with initial conditions at $t = 0$,

$$X = X_0 \quad P = P_0 \quad S = S_0 \quad C_{\mathrm{l}} = C_{\mathrm{l,o}} \tag{5}$$

where rates of formation $r_{\mathrm{X}}, r_{\mathrm{P}}, r_{\mathrm{S}}$, and r_{O} should be expressed by an appropriate kinetic model.

If the change of oxygen concentration in the gaseous phase flowing through the reactor is negligible, the material balance of oxygen in the gaseous phase can be neglected. This assumption was confirmed by experiments [13].

The model employs rate equations for biomass (X), gluconic acid (P), glucose (S), and dissolved oxygen (C_{l}) to describe the fermentation process.

Experimental findings suggest a high degree of dependence of biomass growth on both the carbon source (glucose) and oxygen as substrates. The dependence of specific growth rate on carbon and oxygen substrates was assumed to follow the Monod kinetic model which considers substrate limitation at low concentration, and the Contois model which assumes biomass inhibition. The biomass growth can be described as

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \mu X \tag{6}$$

where the specific growth rate μ is given by the Monod type model as

$$\mu = \mu_{\mathrm{m}} \frac{S}{K_{\mathrm{S}} + S} \frac{C_{\mathrm{l}}}{K_{\mathrm{O}} + C_{\mathrm{l}}} \tag{7}$$

and by the Contois type model as

$$\mu = \mu_{\mathrm{m}} \frac{S}{K'_{\mathrm{S}} X + S} \frac{C_{\mathrm{l}}}{K'_{\mathrm{O}} X + C_{\mathrm{l}}} \tag{8}$$

The kinetics of gluconic acid formation was based on the Luedeking—Piret equation originally developed for the fermentation of lactic acid [14]. It is an unstructured model, which combines growth- and nongrowth-associated contributions towards product formation. Thus, product formation depends upon both the growth $\mathrm{d}X/\mathrm{d}t$ and instantaneous biomass concentration X in a linear way

$$\frac{\mathrm{d}P}{\mathrm{d}t} = r_{\mathrm{P}} = \alpha \frac{\mathrm{d}X}{\mathrm{d}t} + \beta X \tag{9}$$

where α and β are the Luedeking—Piret equation parameters for growth- and nongrowth-associated product formation, respectively.

A carbon substrate such as glucose is used to form cell material and metabolic products as well as for the maintenance of cells. Therefore, substrate consumption can be described by the following equation

$$\frac{\mathrm{d}S}{\mathrm{d}t} = r_{\mathrm{S}} = -\frac{1}{Y_{\mathrm{XS}}} \frac{\mathrm{d}X}{\mathrm{d}t} - \frac{1}{Y_{\mathrm{PS}}} \frac{\mathrm{d}P}{\mathrm{d}t} - m_{\mathrm{S}} X \tag{10}$$

where Y_{XS} and Y_{PS} are the yield coefficients for biomass and product, respectively, and m_{S} is the specific maintenance coefficient.

Substituting eqn (*9*) into eqn (*10*) yields

$$\frac{\mathrm{d}S}{\mathrm{d}t} = r_{\mathrm{S}} = -\gamma \frac{\mathrm{d}X}{\mathrm{d}t} - \lambda X \tag{11}$$

where γ and λ are the parameters for growth- and nongrowth-associated substrate consumption, respectively, defined as

$$\gamma = \left(\frac{1}{Y_{\mathrm{XS}}} + \frac{\alpha}{Y_{\mathrm{PS}}} \right) \tag{12}$$

$$\lambda = \left(\frac{\beta}{Y_{\mathrm{PS}}} + m_{\mathrm{S}} \right) \tag{13}$$

The oxygen uptake rate r_{O} can be described by the sum of oxygen uptake for cell growth, product formation, and cell maintenance applying, *e.g.* equation considering the cell maintenance

$$r_{\mathrm{O}} = \frac{1}{Y_{\mathrm{XO}}} \frac{\mathrm{d}X}{\mathrm{d}t} + \frac{1}{Y_{\mathrm{PO}}} \frac{\mathrm{d}P}{\mathrm{d}t} + m_{\mathrm{O}} X \tag{14}$$

Substituting eqn (*9*) and eqn (*14*) into eqn (*4*) gives

$$\frac{\mathrm{d}C_{\mathrm{l}}}{\mathrm{d}t} = k_{\mathrm{l}}a\,(C_{\mathrm{l}}^{*} - C_{\mathrm{l}}) - \delta \frac{\mathrm{d}X}{\mathrm{d}t} - \phi X \tag{15}$$

where δ and ϕ are the parameters for growth- and nongrowth-associated oxygen uptake, respectively, defined by

$$\delta = \left(\frac{1}{Y_{\mathrm{XO}}} + \frac{\alpha}{Y_{\mathrm{PO}}} \right) \tag{16}$$

$$\phi = \left(\frac{\beta}{Y_{\mathrm{PO}}} + m_{\mathrm{O}} \right) \tag{17}$$

EXPERIMENTAL

All experiments were carried out in mechanically agitated bioreactors with a volume of 2 dm^3 and 5 dm^3 (Biostat, B. Braun, Germany). The bioreactor was equipped with a pH electrode, pH titrator, O_2 electrode, an outlet gas analyzer (Tandem Dual gas sensor by Adaptive Biosystems), and a thermostat.

During the fermentation process the following parameters were measured and recorded on-line on a PC: concentration of dissolved oxygen in the medium, temperature and pressure of inlet and outlet gas streams and their composition (O_2 and CO_2 content). Moreover, the temperature of the medium inside the reactor, the air flow rate, and pH were measured and controlled on-line. Agitation was set at 300 rpm and was kept constant for all experiments.

The microorganism *Aspergillus niger* CCM 8004 with a high activity of glucose oxidase and catalase [15] was used in this study. The mycelium grew in a pellet form. The inoculum was prepared in shaked flasks for 24 h. The bioreactor with a volume of 2 dm^3 or 5 dm^3 was inoculated with 2 vol. %. The reactor temperature was kept at 30 °C. Air flow rate of 8 dm^3 min^{-1} (at 298 K and atmospheric pressure) was chosen as the gas phase for all experiments.

The fermentation medium contained 150 g dm^{-3} glucose, 0.59 g dm^{-3} $(NH_4)_2SO_4$, 0.25 g dm^{-3} KCl, 0.25 g dm^{-3} KH_2PO_4, 0.25 g dm^{-3} $MgSO_4 \cdot 7H_2O$, 1.0 g dm^{-3} $Ca(NO_3)_2 \cdot 4H_2O$, and 1.5 cm^3 dm^{-3} of a 50 % corn-steep liquor.

The pH of the culture was maintained at the value of 6.5 during the growth phase and at 5.5 (maximum glucose oxidase activity) during the production phase by the addition of 11.7 M-sodium hydroxide. The parameters of the PID-controller of pH were adjusted to take into account the volume capacity of the reactor in order to avoid an undesirable increase of pH above 7.

During experiments under nongrowth conditions solution containing 150 g dm^{-3} of glucose with pH of 5.5 and an appropriate amount of washed biomass was employed. Different biomass concentrations and air flow rates have been applied to investigate their effect on the production rate of gluconic acid.

The concentration of biomass was calculated from the mycelial dry weight, which was determined by a gravimetric method. The mycelial suspension was filtered, several times washed with distilled water and dried to a constant weight at 90 °C. The filtrate was used for HPLC analysis.

Gluconic acid concentration was determined by HPLC analysis (Knauer, Germany, chromatographic column polyester OA Hy, 300 mm × 6.5 mm^2 Id-E. Merck, Germany) with UV detector.

The concentration of glucose was determined using HPLC technique (Knauer, Germany, chromatographic column Eurokat Pb, 300 mm × 8 mm^2) with a differential refractometer detector.

To determine the kinetic parameters, the values of k_la should be known from independent experiments. *Klein et al.* [13] described the method of k_la determination from the experiments of gluconic acid production at nongrowth conditions, under which the dissolved oxygen was consumed merely by the bioreaction of glucose to gluconic acid

$$C_6H_{12}O_6 + \frac{1}{2}O_2 \rightarrow C_6H_{12}O_7 \qquad (A)$$

Considering that the dynamics of fermentation process is much slower than that of oxygen transport through the liquid film, a steady value of concentration of dissolved oxygen in the liquid can be achieved during the experiment after a reasonable time. It means that the accumulation term on the left-hand side of eqn (*4*) can be neglected and the equation rewritten as follows

$$k_la = \frac{r_O}{C_l^* - C_l} \qquad (18)$$

The dissolved oxygen is depleted only by the biotransformation of glucose to gluconic acid. Thus, the oxygen consumption rate r_O is proportional to the amount of NaOH necessary for neutralization of gluconic acid. From stoichiometry of the bioreaction (*A*) and the neutralization reaction, the resultant equation can be derived for calculation of the oxygen mass transfer coefficient k_la as follows

$$k_la = \frac{r_P \dfrac{M_O}{M_{GA}}}{C_l^* - C_l} \qquad (19)$$

RESULTS AND DISCUSSION

For the determination of the values of k_la, the experiments under nongrowth conditions were performed in a 2 dm^3 fermentor, using different concentrations of biomass and rates of aeration. From these data, value of k_la was evaluated according to the procedure described in [13] using eqn (*19*). The dependence of the k_la values on the applied air flow rate at different biomass concentrations during nongrowth conditions is shown in Fig. 1.

It should be noted that the overall oxygen mass transfer coefficient k_la varies with both the air flow rate and the biomass concentration. During the growth fermentation experiments the speed of agitation and the air flow rate were constant (300 rpm and 8 dm^3 min^{-1}, respectively). As shown in Fig. 1, at 8 dm^3 min^{-1} the biomass concentrations have a significant effect on the values of k_la. In case where the k_la varied within the range of 45—80 h^{-1}, the influence of the k_la values on the estimated kinetic parameters

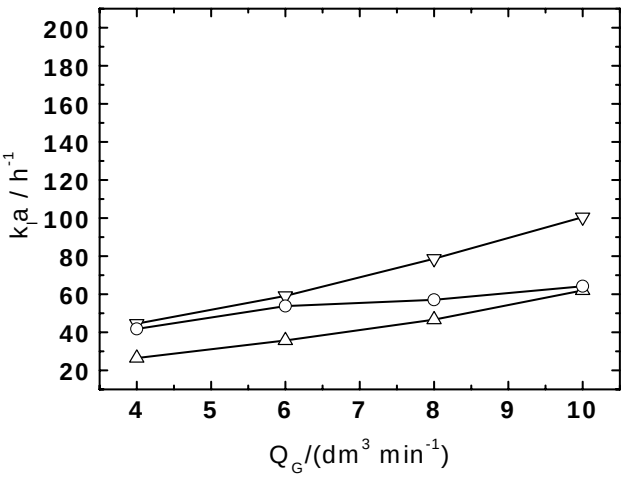

Fig. 1. The effect of the gas flow rate on the volumetric oxygen transfer coefficient at different biomass concentrations (○ 0.720 g dm^{-3}, ▽ 1.94 g dm^{-3}, △ 5.90 g dm^{-3}) under nongrowth conditions.

was negligible within that range. Therefore, an average value of 63 h^{-1} was assumed.

The model contains a total of nine kinetic parameters that must be determined in order to simulate the process, these values were evaluated by fitting the mathematical models (eqns (*6*), (*9*), (*11*), and (*15*)) with Monod and Contois type models (eqn (*7*) and eqn (*8*), respectively), to the experimental data (carried out in the 2 dm^3 CSTR), using Athena software package (nonlinear optimization) [16], in order to minimize the sum of squares of the deviations between the experimental measurements and the model predictions. Table 1 presents the values of the nine parameters obtained for modelling this fermentation process.

Fig. 2 clearly shows that the Contois type model allows to fit very well the behaviour of this fermentation process. The sum of squares of residuals (SSR) was better with Contois type model, being lower than half the value obtained with Monod type model.

Although the Monod type model can represent the product and substrate profiles, it failed to represent the biomass and dissolved oxygen profile. As shown in Figs. 2*B* and 2*D* the prediction is very good at the beginning of fermentation (during the first 10 h) where the biomass concentration is low. After 10 h of fermentation, however, the model failed to represent the dissolved oxygen concentration and biomass concentrations. This may be attributed to the fact that Monod equation did not consider the biomass inhibition. Therefore, the Monod equation is generally more suitable for describing substrate limited growth with low cell populations.

On the other hand, the Contois type model fitted the fermentation process very well. This model is an extension of the Monod equation considering an inhibitory effect of cells concentration on growth and it is usually better for high cell density cultures. Numer-

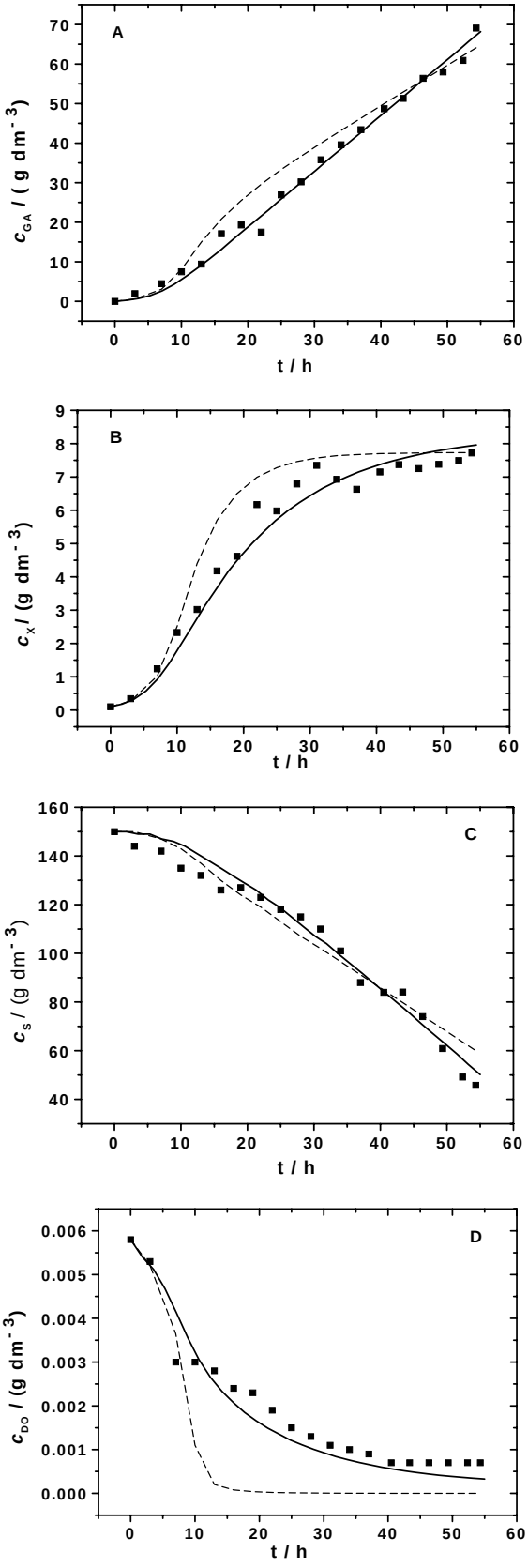

Fig. 2. Comparison of the calculated values and experimental data (in a 2 dm^3 batch reactor). *A* – gluconic acid, *B* – biomass, *C* – substrate, *D* – dissolved oxygen. Monod type model (dashed line), Contois type model (solid line).

Table 1. Parameter Evaluation Using Batch Reactor Data

Parameter	Monod type model	Contois type model
μ_m/h^{-1}	0.668	0.361
$K_S/(g\ dm^{-3})$	130.902	–
K'_O	–	0.001061
K'_S	–	21.447
$K_O/(g\ dm^{-3})$	0.000363	–
α	2.9220	2.5800
β/h^{-1}	0.1314	0.1704
γ	2.1241	2.17680
λ/h^{-1}	0.2320	0.29370
δ	0.27824	0.2724
ϕ/h^{-1}	0.00487	0.0425
SSR	**1262**	**590**

ous authors have studied gluconic acid production by *A. niger*, none of them considering the effect of two substrates (glucose and oxygen) on the specific growth rate μ. *Liu et al.* [11] using a logistic model for simulation of their own data and those of *Takamatsu et al.* [10] found that μ_m was 0.22 h^{-1} and 0.33 h^{-1}, respectively. *Reuss et al.* [12] used the following empirical specific growth rate equation without considering the effect of glucose and DO concentration

$$\mu = \mu_m \left(1 - \frac{P}{P_m}\right) \quad \text{for} \quad P \leq P_m \qquad (20)$$

They found from their results that μ_m at pH = 4.5 was equal to 0.21 h^{-1}.

In the present study, the value of maximum specific growth rate of 0.361 h^{-1} was calculated. This discrepancy may be caused by different properties of the strain used and the process conditions like agitation and aeration. In addition, *Liu et al.* [11] did not consider the effect of the dissolved oxygen in their model. Since the magnitude of the nongrowth-associated parameter ($\beta = 0.1704$) is lower than that of the growth-associated parameter ($\alpha = 2.58$), this fermentation appears to be mostly growth-associated.

The biomass growth, product formation, substrate uptake, and dissolved oxygen models were tested using the parameters evaluated from the Contois type model (Table 1). A comparison of the calculated values of biomass, substrate, product, and dissolved oxygen with experimental data obtained with a 5 dm^3 batch reactor with the same *A. niger* strain, is given in Fig. 3. This figure reveals that the model can describe the gluconic acid fermentation process very well.

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SYMBOLS

c	concentration	g dm^{-3}

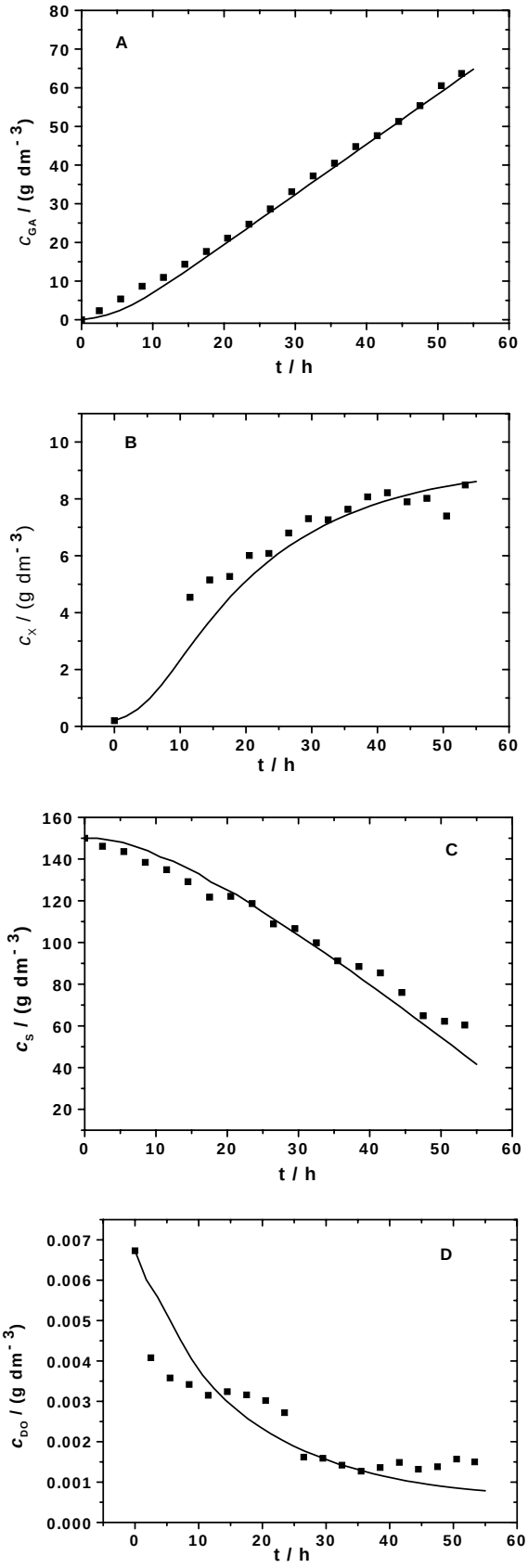

Fig. 3. Comparison of the values calculated by the Contois type model and experimental data (in a 5 dm^3 batch reactor). *A* – gluconic acid, *B* – biomass, *C* – substrate, *D* – dissolved oxygen.

C_l	dissolved oxygen concentration	g dm^{-3}
C_l^*	equilibrium dissolved oxygen concentration	g dm^{-3}
K'_O	Contois oxygen limitation constant	
K_O	Monod oxygen limitation constant	g dm^{-3}
K'_S	Contois saturation constant	
K_S	Monod saturation constant	g dm^{-3}
$k_l a$	overall oxygen mass transfer coefficient	h^{-1}
M_{GA}	gluconic acid molar mass	g mol^{-1}
M_O	molar mass of oxygen atom	g mol^{-1}
m_O	maintenance coefficient; substrate mass per cell mass per hour	h^{-1}
m_S	maintenance coefficient; substrate mass per cell mass per hour	h^{-1}
P	product concentration	g dm^{-3}
Q_G	gas flow rate	dm^3 min^{-1}
r_O	oxygen uptake rate	g dm^{-3} h^{-1}
r_P	production rate	g dm^{-3} h^{-1}
r_S	substrate uptake rate	g dm^{-3} h^{-1}
r_X	biomass growth rate	g dm^{-3} h^{-1}
S	substrate concentration	g dm^{-3}
SSR	sum of squares of residuals	
t	time	h
X	biomass concentration	g dm^{-3}
Y_{PO}	yield constant; product mass per oxygen mass	
Y_{PS}	yield constant; product mass per glucose mass	
Y_{XO}	yield constant; product mass per oxygen mass	
Y_{XS}	yield constant; product mass per glucose mass	

Greek Letters

α	growth-associated product formation coefficient	
β	nongrowth-associated product formation coefficient	h^{-1}
γ	growth-associated parameter in eqn (*11*) for substrate uptake; substrate mass consumed per biomass mass grown	
δ	parameter in eqn (*15*) for oxygen uptake; oxygen mass consumed per biomass mass grown	
λ	nongrowth-associated parameter in eqn (*11*) for substrate uptake; substrate mass consumed per biomass mass grown per hour	h^{-1}
μ	specific growth rate	h^{-1}
μ_m	maximum specific growth rate	h^{-1}
ϕ	parameter in eqn (*15*) for oxygen uptake; oxygen mass consumed per biomass mass grown per hour	h^{-1}

Subscripts

DO	dissolved oxygen
GA	gluconic acid
m	maximum
S	substrate (glucose)
X	biomass

REFERENCES

1. Das, A. and Kundu, P. N., *J. Sci. Ind. Res. 46,* 307 (1987).
2. Sawyer, D. T., *Chem. Rev. 64*, 633 (1964).
3. Milsom, P. E. and Meers, J. L., in *Comprehensive Biotechnology,* Vol. *3*, pp. 681—700. (Moo-Young, M., Editor.) Pergamon Press, Oxford, 1985.
4. Rao, D. S. and Panda, T., *Bioprocess. Eng. 8*, 203 (1993).
5. Dirkx, J. M. H. and van der Baan, H. S., *J. Catal. 67,* 1 (1981).
6. Laane, C., Pronk, W., Franssen, M., and Veeger, C., *Enzyme Microb. Technol. 6*, 165 (1984).
7. Bao, J., Furumoto, K., Fukunaga, K., and Nakao, K., *Biochem. Eng. J. 8*, 91 (2001).
8. Moser, A. and Steiner, W., *Eur. J. Appl. Microbiol.* 1, 281 (1975).
9. Sinclair, C. G. and Kristiansen, B., *Fermentation Kinetic and Modeling.* Open Univ. Press, Milton Keynes, UK, 1987.
10. Takamatsu, T., Shioya, S., and Furuya, T., *J. Chem. Technol. Biotechnol. 31*, 697 (1981).
11. Liu, J. Z., Weng, L. P., Zhang, Q. L., Xu, H., and Ji, L. N., *Biochem. Eng. J. 14,* 137 (2003).
12. Reuss, M., Frohlich, S., Kramer, B., Messerschmidt, K., and Pommerening, G., *Bioprocess. Eng. 1,* 79 (1986).
13. Klein, J., Rosenberg, M., Markoš, J., Dolgoš, O., Krošlák, M., and Krištofíková, Ľ., *Biochem. Eng. J. 1*, 3568 (2002).
14. Luedeking, R. and Piret, E. L., *J. Biochem. Microbiol. Technol. Eng. 1*, 363 (1959).
15. Rosenberg, M., Šturdík, E., Gomory, J., Stanek, S., Kacina, R., and Škárka, B., *Czechoslov.* 262877 (1988).
16. Athena Visual Workbench, version 8, Stewart and Associates Engineering Software, copyright 1997—2001.