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Effects of Water Activity on Kinetics of Simple and Complex Microbial Bioconversions

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Summary

The effects of water activity on the kinetics of »simple« (biooxidation of D-*sorbitol into* L-*sorbose by means of Gluconobacter suboxydans S-22) and »complex« (biosynthetic conversion of* D-*sorbitol into oxytetracycline by means of Streptomyces rimosus R6) microbial processes were studied. Water activity was defined as a function of oxygen solubility. Experiments with shake flask cultures were used to study the kinetics of both processes. Growth of microorganisms, consumption of* D-*sorbitol and formation of products (*L-*sorbose and oxytetracycline) were monitored. Effects of water activity were more expressed in the process of oxytetracycline biosynthesis than in that of* L-*sorbose formation. Growth kinetics was affected more than product formation kinetics in both processes. The estimated critical values of water activity were 0.35 (G. suboxydans S-22 growth), 0.40 (S. rimosus R6 growth), 0.21 (*L-*sorbose formation) and 0.40 (oxytetracycline biosynthesis) respectively. With decreasing water activity the mass transfer phenomena were observed to become more and more important, especially when considering the liquid-microbial biomass interface as in the case of applying media with* D-*sorbitol concentration above 500 g/L.*

Keywords: water activity, D-sorbitol bioconversion, kinetics

Introduction

There is no doubt that manifestation of life is directly and strongly dependent on water presence in bioreaction systems. It is known *(1)* that water plays a dual role in biocatalytic reaction systems: a) it is required to form and maintain the native, catalytically active conformation of enzyme molecules, and b) most reactions in protein molecules resulting in enzyme inactivation, in particular thermoinactivation, also require water. In the case of enzymatic oxidation of ethanol in the gaseous phase, the alcohol oxidase activity was observed to increase with increasing water activity, while thermostability of the mentioned enzyme was observed to decrease with increasing water activity and *vice versa (1)*.

In microbial processes, the four key factors – microorganism (biocatalyst) concentration, substrate concentration, temperature and mass transfer rate appear most frequently to be the factors of principal importance. However, in the range of very high concentrations of dissolved substances the water concentration, i.e. its activity can become the most important factor (especially in aerobic processes), with an effect dependent on cell properties. In nutrient media, water activity results from water concentration and its dependence on the concentration of dissolved substances, which by their different affinity bind the water, decreasing its availability and causing the vapour pressure of the solution to decrease and the osmotic pressure to increase. This is the basis for water activity definition and determination methods, i.e. as demonstrated by Pirt *(2)*. It is considered that

$$a_w = \frac{p_s}{p_w} \qquad /1/$$

and

$$\Pi = \frac{-RT \ln a_w}{V_w} \qquad /2/$$

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where: a_w = water activity, p_s = solution vapour pressure, p_w = vapour pressure of pure water, Π = osmotic pressure of solution, V_w = molar volume.

Another approach is based on the assumption that oxygen solubility is proportional to free water concentration. The effects of D-sorbitol concentration on oxygen solubility and microbial oxidation kinetics were studied *(3,4)* and the expression

$$a_w = \frac{\gamma_{wo}}{\rho^*_{wo}} \qquad /3/$$

where: γ_w = free water mass concentration, ρ^*_{wo} = pure water density = free water mass concentration in pure water, was proposed to calculate water activities of solutions *(4)*. The basic arguments for the proposed expression resulted from the following findings:

a) Numerical values of diffusion coefficients and reciprocal viscosities were found to be proportional to the difference between genuine and critical water activity when water activity was defined by expression /3/;

b) Both the specific growth rate and specific L-sorbose formation rate were found to be proportional to the difference between genuine and critical water activity, if water activity was defined by expression /3/.

The aim of this work was to compare the effects of water activity on the kinetics of »simple« (biooxidation of D-sorbitol into L-sorbose by means of *Gluconobacter suboxydans* S-22) and »complex« (biosynthetic conversion of D-sorbitol into oxytetracycline by means of *Streptomyces rimosus* R6) microbial processes, when defining the water activity on the basis of oxygen solubility, i.e. when applying the equation /3/. To enable such a comparison, it was necessary to reanalyze known data referring to the influence of water activity on process kinetics of L-sorbose formation and to perform new experiments, mainly concentrating on the process of oxytetracycline biosynthesis. Experiments with shake flask cultures were applied to study kinetics of both processes.

Material and Methods

Microorganisms: *Gluconobacter* (syn. *Acetobacter*) *suboxydans* S-22 (Culture Col. of PLIVA, Research Institute, Zagreb, Croatia) was applied in experiments of D-sorbitol to L-sorbose bioconversion;

Streptomyces rimosus R6 (Culture Col. of PLIVA, Research Institute, Zagreb, Croatia) was applied in experiments of biosynthetic conversion of D-sorbitol into oxytetracycline.

Media:

1) ***L-sorbose production media:***

a) LSM1: sterile media containing: CSL-filtrate 20 g/L, D-sorbitol (0–800 g/L), water – up to 1L;

b) LSM2: sterile water solutions of different D-sorbitol concentrations (100–800 g/L)

2) ***Oxytetracycline biosynthesis media***:

a) OTM1: medium for biomass growth: sterile medium containing, g/L: yeast extract – 8.0, $CaCO_3$ – 5.0, $(NH_4)_2SO_4$ – 4.0, $MgSO_4 \cdot 7H_2O$ – 1.0, $MnSO_4 \cdot H_2O$ – 0.1, $ZnSO_4 \cdot 7H_2O$ – 0.02, $CoCl_2 \cdot 6H_2O$ – 0.006, NaF – 0.0001, unidan (antifoam agent) – 0.2, sorbitol – 0–500;

b) OTM2, medium for oxytetracycline biosynthesis: sterile medium containing, g/L: $CaCO_3$ – 4.0, $(NH_4)_2SO_4$ – 4.0, $MgSO_4 \cdot 7H_2O$ – 1.0, $MnSO_4 \cdot H_2O$ – 0.1, $ZnSO_4 \cdot 7H_2O$ – 0.02, $CoCl_2 \cdot 6H_2O$ – 0.006, NaF – 0.0001, unidan (antifoam agent) – 0.2, sorbitol – 100 – 800;

Cultivation Methods:

Experiments with shake flask cultures were used to study kinetics of both processes

1) Process of L-sorbose production:

a) Growth of microorganism: conical flasks of 500 mL, each containing 50 mL of sterile medium, were inoculated with the 1 mL of microbial culture in exponential growth phase, and then subjected to the cultivation process on rotary shaker (220 rpm, 30 °C, starting microbial biomass concentration in each flask – 0.119 g/L). Upon 5 hours of cultivation the biomass concentrations in samples were assayed and the mean specific growth rates calculated.

b) L-Sorbose formation: sterile sorbitol solutions of 50 mL in conical flasks of 500 mL were inoculated with corresponding suspension of microbial cells. Experiments with low (0.1–0.2 g/L) and high (1.0–2.0 g/L) biomass concentrations at biooxidation start were performed. To prepare a microbial suspension for inoculation the microbial culture aliquot taken from corresponding laboratory bioreactor was subjected to centrifugation (6000 rpm, 10 min). The separated wet biomass was then suspended in the physiological solution. Diluted suspension (10×) was prepared by diluting that with physiological solution. Upon inoculation the flasks were incubated on rotary shaker of 220 rpm, at 30 °C, for 18 (low biomass concentration) or 9 hours (high biomass concentration). After incubation was finished, L-sorbose concentrations in culture samples were determined and the specific sorbose formation rates calculated.

2) Process of oxytetracycline biosynthesis:

a) Microbial growth kinetics: 500 mL conical flasks, each with 50 mL of medium OTM1, were inoculated with 1 mL of *S. rimosus* R6 inoculum and then subjected to the cultivation process applying a rotary shaking with 220 rpm and a temperature of 30 °C, the starting biomass concentration in each flask being 0.2 g/L. Biomass concentrations in culture samples after 18, 42 and 68 hours of cultivation were determined and the appropriate mean specific growth rates calculated.

b) Kinetics of oxytetracycline biosynthesis: First testing: Two runs were performed. Volumes of 100 mL (1st run) and 20 mL (2nd run), respectively of *S. rimosus* R6 inoculum of supposedly slightly different physiological properties with respect to specific biosynthetic activity *(5)* (1st run: γ_P = 3.48 g/L, $f_\phi = \frac{\gamma_P}{\gamma_X}$ = 0.257; 2nd run: γ_P = 3.47 g/L, f_ϕ = 0.161) were subjected to centrifugation at 5000 min^{-1} for 10 min. Upon eliminating supernatants the biomass sediments were resuspended in sterile sorbitol solutions (solution volumes of 50 mL with different D-sorbitol concentrations). After inoculation, conical flasks of 500 mL, each with 45 mL of reaction mixture, were in-

cubated on rotary shaker of 220 rpm, at 30 °C, for 118 (1st run) or 90 (2nd run) hours. Applied starting biomass concentrations in reaction mixtures were 13.6 (1st run) and 4.32 g/L (2nd run), respectively. Upon determination of oxytetracycline concentrations in corresponding culture samples the mean specific oxytetracycline biosynthesis rates were calculated based on the assumption that biomass concentration was approximately constant during incubation periods.

Second testing: To avoid any effect of differences in biomass physiological properties the same *S. rimosus* R6 cultivation broth ($\gamma_P = 3.52$ g/L, $f_\phi = \frac{\gamma_P}{\gamma_X} = 0.218$) was applied.

Three runs were performed, applying an analogous procedure as in the case of the first testing and incubation time being 90 hours. Applied starting biomass concentrations in reaction mixtures were 16.12 (1st run), 4.84 (2nd run) and 1.61 g/L (3rd run) respectively. The same procedure as in the 1st testing was applied to calculate the mean specific oxytetracycline biosynthesis rates.

Analytical Methods:

1) *G. suboxydans* S-22 biomass determination: Culture samples were diluted with water and the absorbance at 660 nm measured. Experimentally verified mathematical formula

$$\gamma_X = f_D\,(0.323\,A + 0.129\,A^2 - 0.157\,A^3 + 0.121\,A^4) \qquad /4/$$

where f_D = conversion factor, A = absorbance difference (A (sample) – A (sample filtrate)) was used to calculate microbial dry biomass concentration.

2) L-Sorbose determination: Autoanalyser »TRAACS 800« (Bran und Luebbe GmbH, Norderstedt, Germany) and adapted specific analytical methods were applied to determine L-sorbose concentration in samples (S. Gamulin, PLIVA, Res. Inst. internal procedures, adapt, *3,4)*

3) *S. rimosus* R6 biomass determination: Culture samples of 5 mL were acidified with HCl to reach pH of 1.5–2.0. Upon vacuum filtration through weighted filter paper the biomass cake was washed with water, then dried in microwave oven for 20 min and weighted to estimate dry biomass concentration.

Oxytetracycline determination: Culture samples were acidified with HCl to reach pH of 1.8 and after 10 min filtered. Oxytetracycline concentration in filtrate diluted with 0.01 M HCl was assayed applying spectrophotometry, based on absorbance at 354 nm *(6)*. Method was adapted for automatic assays (S. Gamulin, procedure of PLIVA Res. Inst.) and an application of the autoanalyser »TRAACS 800« (Bran und Luebbe GmbH, Norderstedt, Germany).

D-Sorbitol determination: Autoanalyser »TRAACS 800« and adapted specific analytical method were applied *(3)*.

Calculation of Parameters:

a) Mean specific growth rates ($\bar{\mu}$) were calculated applying the formula

$$\bar{\mu} = \frac{1}{t - t_0} \ln\frac{\gamma_X}{\gamma_{X_0}} \qquad /5/$$

b) Mean specific product formation rates ($\bar{q}_P$) were calculated applying formula

$$\bar{q}_P = \frac{1}{t - t_0}\,\frac{\gamma_P - \gamma_{P_0}}{\gamma_{X_0}} \qquad /6/$$

since supposedly the biomass concentration during specific experiments was not changed markedly. In expressions /5/ and /6/ $\bar{\mu}$ = mean specific growth rate, $\bar{q}_P$ = specific product formation rate, γ_X = biomass concentration and γ_P = product concentration in sample after cultivation time t, whereas γ_{X_0} and γ_{P_0} represent values at experiment start, i.e. when $t = t_0$.

Additional data: Some of recently published own data *(3,4,7)* were used to document and complete this work.

Results

Results are presented in Figs. 1 to 11 and in Table 1. Fig. 1 refers to the effects of D-sorbitol and free water concentrations on mean specific growth rates of *G. suboxydans* S-22. As shown for the studied range of D-sorbitol concentrations, the effects could be well described by linear mathematical equations, i.e. the mean specific growth rate decreased linearly with increasing D-sorbitol concentration, above 200 g/L or, it was increasing in proportion of free water concentration increase above the value which could be considered as critical one. In Fig. 2 the effects of D-sorbitol concentration, total and free water concentrations and medium viscosity on mean specific rate of L-sorbose formation are demonstrated. It is evident that relationships between parameters could be well defined by corresponding linear mathematical equations even when considering a relatively large range of parameter values, although a more detailed insight into experimental data suggests S shaped curves would fit better the experimental data than straight lines, if larger parameter range would be considered. Data shown in Fig. 3 support those in Fig. 2 since evidently the reciprocal value of medium viscosity appears to be a linear function of free water concentration or water activity. Here, it seems useful to mention that reciprocal proportionality was established when diffusion coefficient values were related to corresponding media viscosities *(4)*. Therefore, these findings suggest that mathematical models describing parameter relationships more appropriately should be tested and their applicability evaluated. Since the applicability of Monod mathematical model has already been tested and found to be satisfactory for lower sorbitol concentrations *(8)*, a speculation on relationships demonstrated in Fig. 2 and Fig. 3 led to a conclusion that the expression

$$q_P = q_{P_m} \frac{\gamma_S}{K_S + \gamma_S}\,\frac{\eta_0}{\eta} \qquad /7/$$

could be appropriate one. Because the empirical equation

$$\frac{\eta_0}{\eta} = \exp\,(c_0 - c_1\gamma_S - c_2\gamma_S^2) \qquad /8/$$

was found to be adequately describing viscosities of applied D-sorbitol solutions at 30 °C (our reports at 14th Meeting of Croatian Chemists, Zagreb, Feb. 1995, and 7th Eur. Congr. Biotehnol., Nice, Feb. 17–24, 1995) the convenience of mathematical equation /7/ in explaining experimental values was investigated. Experimental data (Fig. 2) suggested that the value of maximal specific sorbose formation rate q_{Pm} could be 24.0 h^{-1} while saturation constant K_S value 7.0 was chosen based on rough experimental estimation and on assumption that it should be close to already reported values *(8)*. Fitting of theoretical to experimental data appeared to be satisfactory enough (Fig. 4).

Concerning the L-sorbose formation, it was interesting to compare specific product formation rates when applying different biomass concentrations. Experimental data presented in Figs. 2 to 4 refer to low biomass concentration. Since it was already established that specific sorbose formation rate q_P is proportional to the specific oxygen uptake rate q_O *(4)* while, as known *(9)*, oxygen transfer rate during the process depends on both oxygen absorption and oxygen uptake rates, i.e. it can be defined by the well known equation

$$\frac{d\gamma_{O_2}}{dt} = K_L a\,(\gamma^*_{DO} - \gamma_{DO}) - q_O\gamma_X \qquad /9/$$

one could expect that the specific sorbose formation rate could become dependent on both oxygen absorption rate

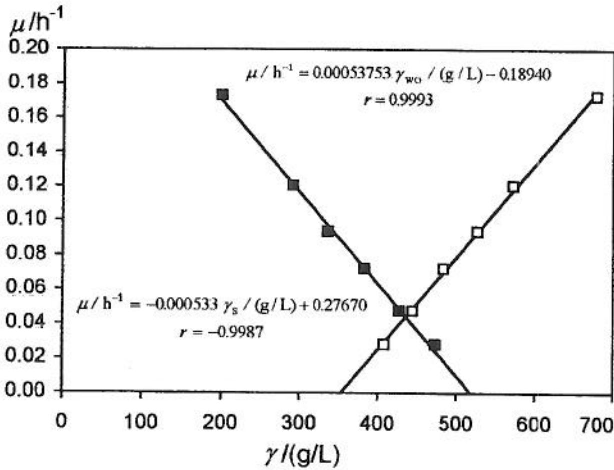


Fig. 1. Effect of D-sorbitol concentration (■) and free water concentration (□) on specific growth rate (μ) of *G. suboxydans* S-22

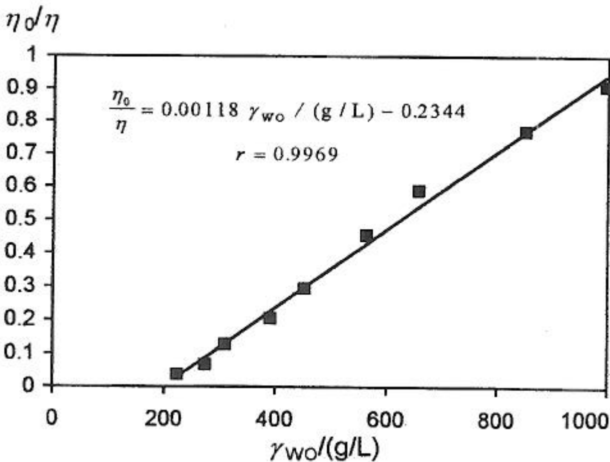
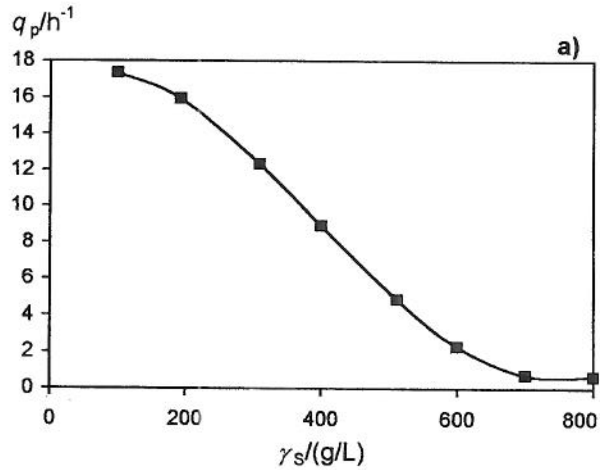
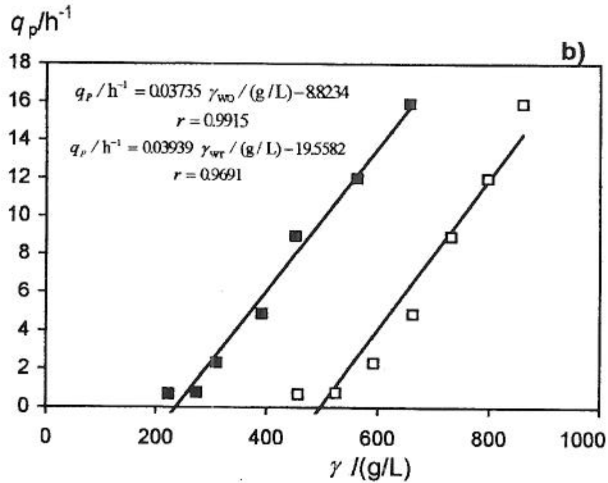


Fig. 3. Effect of free water concentration on solution viscosity





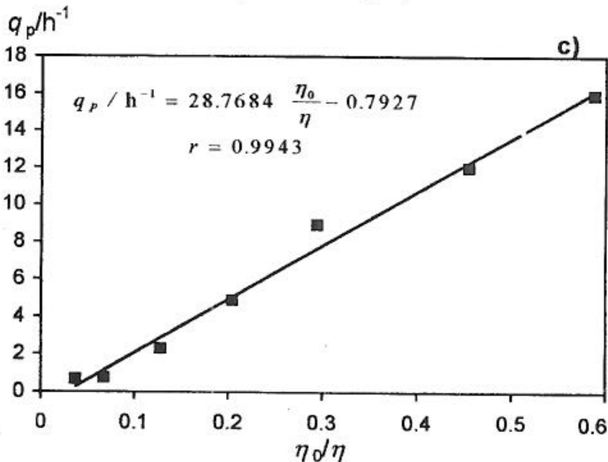


Fig. 2. The specific L-sorbose production rate (q_P) as a function of a) D-sorbitol concentration (γ_S), b) total and free water concentration (□, ■), and c) reciprocal medium viscosity (η_0/η)

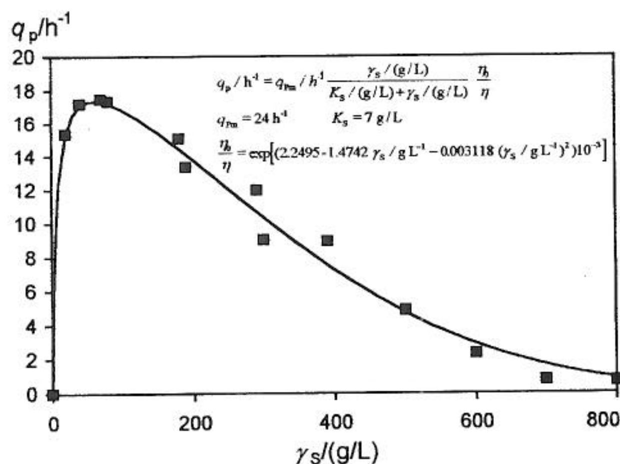


Fig. 4. Specific rate of L-sorbose formation (q_P), as a function of D-sorbitol concentration (γ_S) and medium viscosity (η). Applicability of the mathematical model.

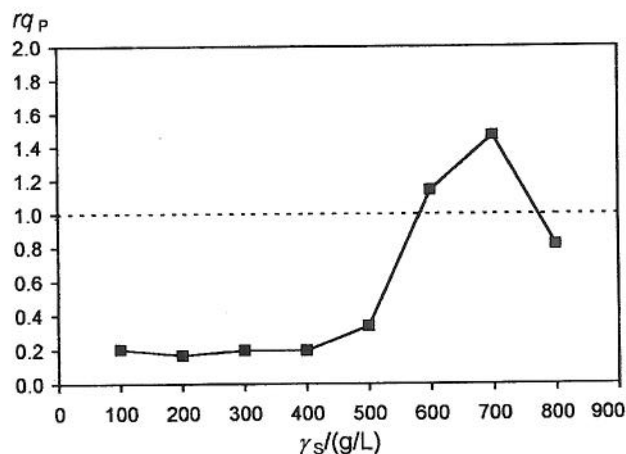


Fig. 5. The relation beetween specific L-sorbose formation rates at high (γ_X) and low (γ_X/10) biomass concentration as a function of D-sorbitol concentration (average values of two runs) $rq_P = q_{PH}/q_{PL}$

and biomass concentration. Therefore, it was reasonable to suppose that the dissolved oxygen concentration was close to the zero level when applying high biomass concentration. Then the relation

$$K_L a \gamma^*_{DO} \approx q_O \gamma_X \quad /10/$$

could be expected to be valid. Otherwise, the relation

$$K_L a\, (\gamma^*_{DO} - \gamma_{DO}) = q_O \gamma_X \quad /11/$$

appears to be the appropriate one. It follows, the value of the ratio rq_P

$$\frac{q_{PH}}{q_{PL}} = rq_P \quad /12/$$

could be useful in explaining some process phenomena because one can consider that

$$rq_P = \frac{\gamma_{XL}}{\gamma_{XH}} \frac{\gamma^*_{DO}}{\gamma^*_{DO} - (\gamma_{DO})_{LB}} \quad /13/$$

In equations /9/ to /13/ $K_L a$ = volumetric coefficient of oxygen transfer rate, γ_X = biomass concentration, γ_{DO} = dissolved oxygen concentration, γ^*_{DO} = oxygen solubility, index H refers to high biomass concentration while index L refers to low biomass concentration.

Experiments differing 10 times in biomass concentration were performed and rq_P values are presented in Fig. 5. It is obvious that rq_P values differed depending on D-sorbitol concentration. Low values and their slow increase appear to be characteristic for media with D-sorbitol concentrations lower than 500 g/L. Above this concentration values increased abruptly and could be explained by supposing $(\gamma_{DO})_{LB}$ values close to that of γ^*_{DO} and/or $(\gamma_{DO})_{HB}$ markedly higher than zero. This may imply that microbial cell structure and physiology and mass transfer rate at cell – liquid interface appear to be factors of dominant influence on biooxidation rate, when media with higher D-sorbitol concentrations are applied. If one calculates relative oxygen transfer coefficient values $NK_L a$ (with respect to oxygen transfer coefficient when γ_S = 100 g/L is applied, based on L-sorbose formation rates when applying higher biomass concentration) the relationship as shown in Fig. 6 can be obtained. Evidently, by increasing sorbitol concentration the values of $NK_L a$ decreased, the effects being clear in concentration range below 500 g/L. Above the mentioned concentration range the $NK_L a$ values deviate from expected trend and, presently, a reliable explanation of deviations cannot be suggested before the corresponding new experiments giving the information on oxygen concentration would be performed. Possibly, another oxygen transfer mechanism (e.g. more direct oxygen transfer via some kind of »gas-microbial cell (biocatalyst) clustering«) becomes more relevant in concentration range above 500 g/L. It is known that viscosity affects oxygen transfer rate and in the case of the reaction system of D-sorbitol microbial oxidation it was found for $K_L a$ to be roughly proportional to the 2/3 power of reciprocal viscosity *(9)*. For a dissolved substance concentration range below 500 g/L (Fig. 6) data clearly confirm the previous observation *(9)* and suggest that more direct oxygen transfer mechanisms markedly participate in biooxidation process kinetics when the resistance to oxygen absorption becomes critical. Therefore, it is important to point out that analysis of various factors affecting D-sorbitol process kinetics led to the conclusion that relationships applicable to cultivation conditions where microorganism is capable of growing (substrate concentration range below 500 g/L) strongly differ from those relationships which could be applied to the process conditions where a microorganism cannot express its growth capability (substrate concentration range above 500 g/L) although retaining its main biocatalytic activity with respect to substrate oxidation.

Oxytetracycline biosynthesis is a more complex process than that of L-sorbose formation and similarities between the two processes with respect to process kinetics relationships cannot be expected in every case. However, one can suggest similar experiments. Then some of the possible similarities can be observed, as can be dem-

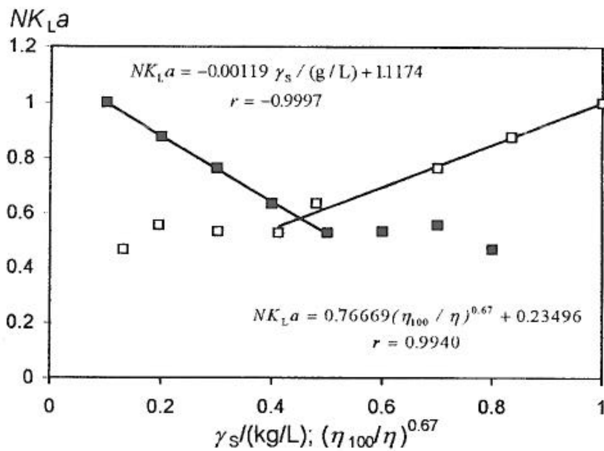


Fig. 6. Effect of solution viscosity (η) and D-sorbitol concentration (γ_S) on relative oxygen transfer coefficient values (NK_La)

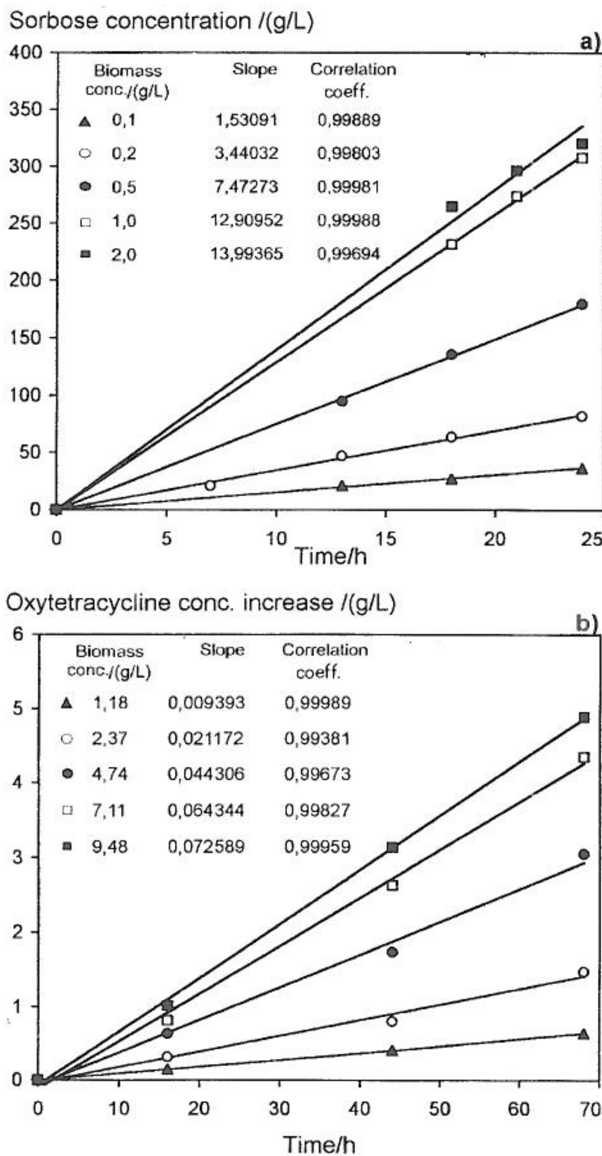


Fig. 7. Effect of biomass concentration on kinetics of product formation a) L-sorbose formation (laboratory bioreactors), b) oxytetracycline formation (shake flasks, dextrin used as a carbon source)

onstrated by the following example. A previous study of the effects of biomass concentration on L-sorbose and oxytetracycline formation rates led to similar findings *(7)*. Some typical data presented in Fig. 7 undoubtedly show that in relatively high range of biomass concentration both L-sorbose and oxytetracycline formation rates were proportional to biomass concentration, whereas decreased biomass activities were observed at too high biomass concentrations. Therefore, some other similarities between corresponding relationships of two processes could be expected as well.

Data shown in Figs. 8 to 11 and in Table 1 refer to the process of oxytetracycline biosynthesis. The effects of D-sorbitol and free water concentrations on *S. rimosus* R6

Table 1. D-sorbitol consumption during 72 hours of oxytetracycline biosynthesis. *S. rimosus* R6 biomass concentration, γ_X= 13.6 g/L

Applied D-sorbitol conc./(g/L)	Consumed D-sorbitol/ (g/L)	Oxytetracycline increase/ (g/L)	Ratio: Consumed D-sorbitol/Oxytetracycline increase
100	27.1	2.41	11.24
200	7.4	0.70	10.57

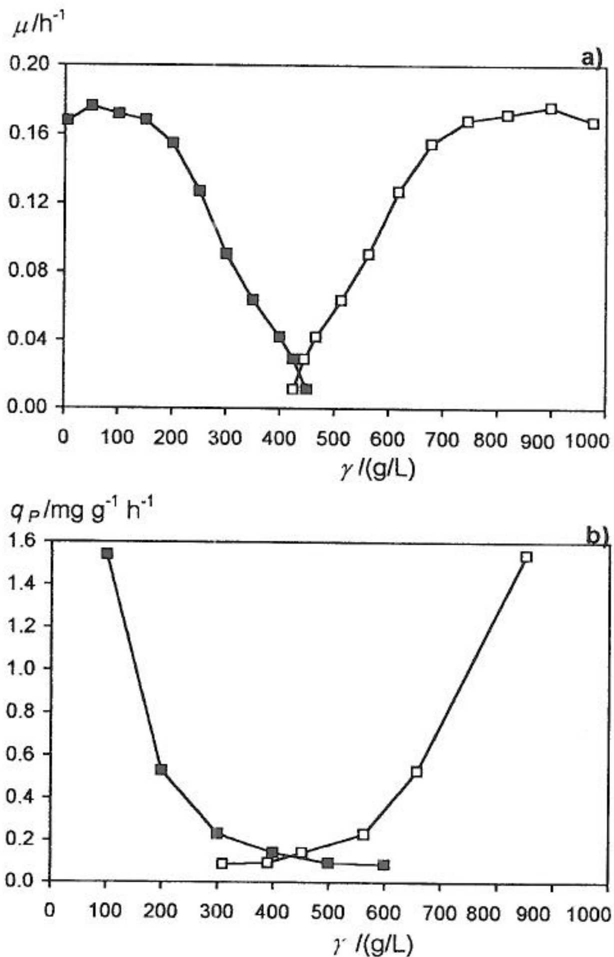


Fig. 8. Effects of D-sorbitol (γ_S) and free water (γ_{WO}) concentration on a) specific growth rate (μ) of *S. rimosus* R6 and b) specific oxytetracycline biosynthesis rate (q_P)

growth and oxytetracycline biosynthesis are demonstrated in Fig. 8. As can be observed, to some extent the effects were similar to those found in the process of L-sorbose production, but the process of oxytetracycline biosynthesis seems to be more sensitive to D-sorbitol concentration increase and water concentration decrease. However, to have a better insight into process relationships the selected data from Fig. 8 were subjected to mathematical analysis which led to the results presented in Fig. 9. Evidently the effects on specific growth rate can be described by linear equations with coefficient values very close to those referring to *G. suboxydans* S-22 (Fig. 1). Here, one should mention that in addition to D-sorbitol the media for *S. rimosus* R6 growth and oxytetracycline biosynthesis contain mineral salts which by their effects decrease water activity as well. For the given media one could suppose the net effect to be equivalent to D-sorbitol concentration of roughly 10 g/L (estimation based on oxygen solubility in D-sorbitol *(3,4)* and mineral salts water solutions *(10)*). Therefore, values of critical water activities for growth of *G. suboxydans* S-22 and *S. rimosus* R6 seem to be even closer than it is shown by estimated values 0.35 and 0.40, respectively. In contrast to growth kinetics data, the selected data on specific rates of oxytetracycline biosynthesis can be described by exponential rather than linear function. Nevertheless,

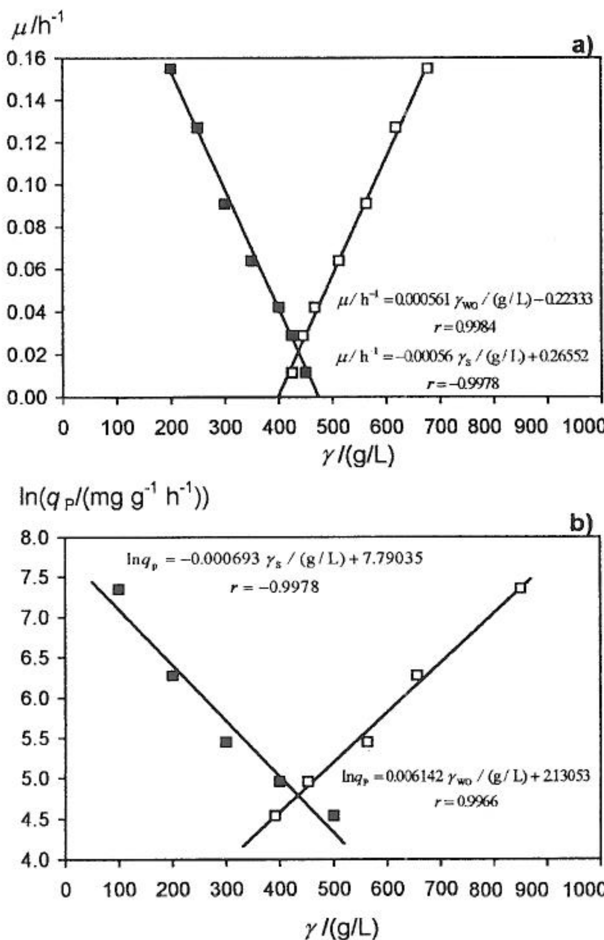


Fig. 9. Mathematical evaluation of parameter relationships (selected data from Fig. 7) a) growth kinetics, b) oxytetracycline biosynthesis kinetics; γ_S ■, γ_{WO} □

critical water activity for oxytetracycline biosynthesis could be estimated to be close to 0.40, the similar value as for growth.

Supposing the equation /7/ could be appropriate also in describing the effects of D-sorbitol concentration on oxytetracycline biosynthesis, calculations were done applying $K_S = 10$ g/L *(11)* and appropriate values of q_{Pm}. Since the effect of D-sorbitol concentration appeared to be more pronounced on oxytetracycline biosynthesis than on L-sorbose formation a more pronounced effect of medium viscosity was expected as well. Based on this assumption better fitting to experimental data was expected when applying a proportionality factor $f_\eta = (\frac{\eta_0}{\eta})^n$ instead of $f_\eta = \frac{\eta_0}{\eta}$. Fig. 10 demonstrates how experimental data fit to those theoretical. No doubt the fitting of theoretical to experimental data becomes satisfactory if cubic instead of linear dependence for f_η is applied.

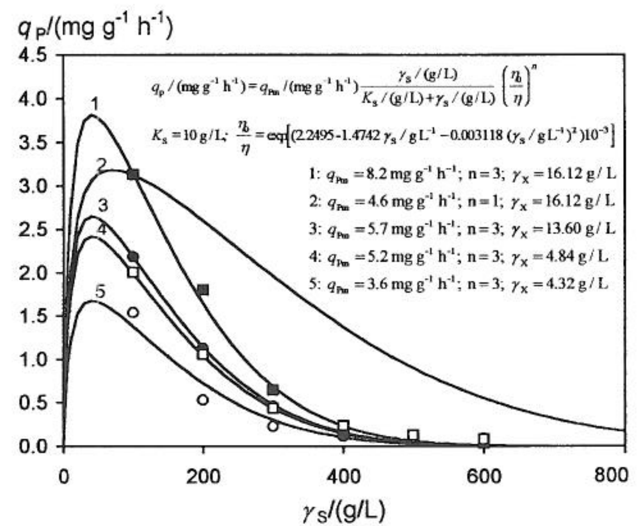


Fig. 10. Specific rate of oxytetracycline biosynthesis as a function of D-sorbitol concentration. Mathematical model application.

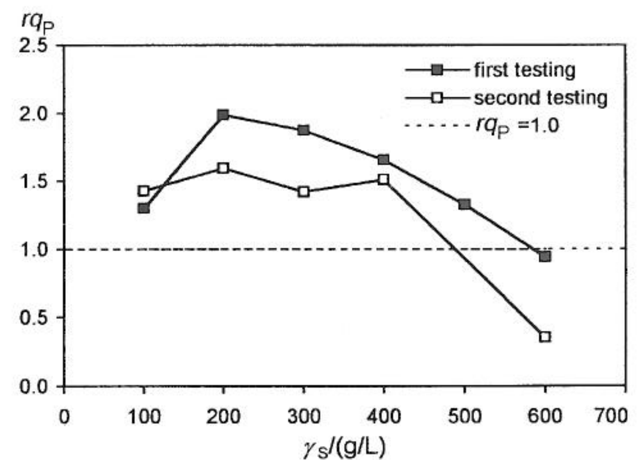


Fig. 11. The relation between specific oxytetracycline biosynthesis rates at high and low biomass concentration as a function of D-sorbitol concentration ($rq_P = q_{PH}/q_{PL}$). At very low biomass concentration (1.61 g/L) oxytetracycline biosynthesis was not observed.

As in the case of specific rates of L-sorbose formation, the effect of biomass concentration on specific rate of oxytetracycline biosynthesis was studied as well, and the resulting rq_P values are presented in Fig. 11. It is clear that the shown relationship markedly differs from that shown in Fig. 5. Since oxytetracycline biosynthesis is much slower than simple conversion of D-sorbitol into L-sorbose, the relation /13/ could not be recommended for application in explaining data presented in Fig. 11. Moreover, because of faster substrate consumption at higher biomass concentration the cultivation conditions could become more convenient for oxytetracycline biosynthesis, at least due to some increase of water activity. Therefore, higher specific product formation rates, i.e. higher rq_P values in the range of lower D-sorbitol concentrations are quite in accordance with the expectation. Data in Table 1 support such an explanation. However, it appears necessary to ask: why q_P values were higher when higher biomass concentrations were applied. The satisfactory answer could be: the separated biomass used as biocatalyst was not treated to eliminate accompanied »impurities« i.e. substances with extracellular biocatalytically relevant factors in proportion to biomass concentration. The net effect of these factors was favourable for bioconversion and therefore higher specific bioconversion rates resulted when higher biomass concentrations were applied. The fact that at very low biomass concentration (1.61 g/L) the biosynthetic effect was not observed at all supports such an explanation. It seems that the influence of slight differences in biomass physiological properties (difference in f_ϕ) could be neglected, since similar relationships resulted (2nd testing) when the inocula of equal physiological properties were applied (Fig. 11, curve 2). Another question is how to explain rq_P values above 1.0. In the case of oxytetracycline biosynthesis these values refer to the concentration range 100–500 g/L (Fig. 11) and could be explained by assuming that the oxygen limitation was not playing a role in this concentration range, whereas in the case of L-sorbose formation rq_P values above 1.0 belong to a higher concentration range (Fig. 5) and probably could be explained supposing: a) an inhibitory effect of some sorbitol impurities which reflected relatively more against lower number of microbial cells and/or b) higher cell autolysis at higher biomass concentration enabling the biocatalyst to become more easily available. However, one should bear in mind that the effect of experimental errors could be relatively higher in the higher substrate concentration range. It seems that the new specific experiments should be performed to verify whether such an explanation could be accepted.

Discussion

The data here presented clearly show that water activity has a significant effect on the kinetics of microbial bioconversions disregarding the degree of process complexity. However, differences can be observed with respect to consequences of water activity decrease, i. e. with respect to specific water activity effects on the rate of particular events. Experimental data suggest that microbial biomass catalytic activity, morphology, cell size and structure play a significant role. It seems that the choice of both process examples was quite adequate to observe key differences and similarities when comparing »simple« and »complex« processes. No doubt, the analogy is evident when comparing main relationships expressing the effects of D-sorbitol concentration and water activity on growth kinetics of both »simple« and »complex« processes. However, differences in biocatalyst properties could be considered to be a cause of pronounced specific responses of microbial biomass on changes of its environment. In the case of the process of D-sorbitol oxidation into L-sorbose by means of *G. suboxydans* S-22 the biochemical conversion is relatively simple and catalyzed by well separated and suspended individual microbial cells of relatively small size and very high specific biocatalytic activity. In such a reaction system a biomass – liquid interface area is relatively very large and with an interface layer thickness as minimal as possible, i.e. the resistance to water passing towards cells and therefore to the transfer of reaction substances into and out of cells is relatively small for a given medium. Quite contrary, in the process of oxytetracycline biosynthesis where the biocatalyst is a mycelial microorganism *S. rimosus* R6, which grows in the form of filaments and agglomerates (pellets) of different morphology and size, the biomass – liquid interface area is relatively much smaller and probably with larger interface layer thickness. Biochemical conversion is much »complex« at least because of much more reaction steps, and slower for a given substrate i. e. one can consider that the biomass specific catalytic activity is relatively much lower than in the case of *G. suboxydans* S-22 biomass. In such a reaction system one can expect the resistance to water passing into cells and transferring necessary nutrients will be higher than in the case of simpler process of L-sorbose production. Because of slower specific oxygen uptake the oxygen limitation could be expected if applying biomass concentration much higher than that applied in this work. Therefore, differences between the two mentioned processes with respect to rq_P values could be considered to be in accordance with expectations.

It is important to point out that the two processes also differ with respect to critical values of water activity. They were lower for the process of L-sorbose production (0.21 for L-sorbose formation and 0.35 for *G. suboxydans* S-22 growth, respectively) than for that of oxytetracycline biosynthesis where the value of 0.40 seems to be critical for both *S. rimosus* R6 growth and oxytetraxycline biosynthesis by this microorganism. To conclude, water activity defined on the basis of oxygen solubility, i.e. by applying expression /3/ appears to be an appropriate parameter in defining cultivation media properties. Theoretically, one could discuss whether such a definition is correct and whether it could be accepted in general. Practically, problems could appear when estimating oxygen solubility since chemical methods are of limited applicability while physical methods depend on reliability of corresponding measurement devices.

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List of symbols

Symbol Unit Meaning

a_w water activity, dimensionless

c_0, c_1, c_2 polynomial coefficients, dimension reciprocal to term dimension

f_D conversion factor, dependent on reaction mixture dilution, g/L

f_ϕ physiology factor, ratio between product and biomass mass concentrations, dimensionless

f_η relative viscosity, ratio between viscosities, dimensionless

A absorbance difference (A (sample) – A (sample filtrate)), dimensionless

K_La volumetric oxygen transfer rate coefficient, h^{-1}

K_S saturation constant, g/L

NK_La relative volumetric oxygen transfer rate coefficient, dimensionless

p_S solution vapour pressure, Pa

p_w vapour pressure of pure water, Pa

q_P mean specific product formation rate, h^{-1}

q_{Pm} maximal specific product formation rate, h^{-1}

r correlation coefficient, dimensionless

R gas constant, J mol^{-1} K^{-1}

rq_P ratio between specific product formation rates, dimensionless

T absolute temperature, K

t cultivation time, h

V_w molar volume, L mol^{-1}

γ_{DO} dissolved oxygen mass concentration, mg/L

γ_P product mass concentration, g/L

γ_S sorbitol mass concentration, g/L

γ_{WO} free water mass concentration, g/L

γ_X microorganism mass concentration, based on dry biomass, g/L

η viscosity, mPa s

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

Π osmotic pressure of solution, Pa

ρ^*_{WO} pure water density, g/L

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Utjecaj aktivnosti vode na kinetiku jednostavne i kompleksne mikrobne biokonverzije

Sažetak

Proučavan je utjecaj aktivnosti vode na kinetiku »jednostavnog« (biooksidacija D*-sorbitola u* L*-sorbozu s pomoću bakterije Gluconobacter suboxydans S-22) i »složenog« (biosintezna pretvorba* D*-sorbitola u oksitetraciklin s pomoću bakterije Streptomyces rimosus R6) mikrobnog procesa. Aktivnost je vode definirana kao funkcija topljivosti kisika u reakcijskom mediju. Za proučavanje kinetike obaju procesa primijenjeni su pokusi s tresenim kulturama. Tijekom pokusa praćeni su rast mikroorganizama, potrošnja* D*-sorbitola i tvorba produkata (*L*-sorboza i oksitetraciklin). Utjecaj aktivnosti vode jače se očitovao na proces biosinteze oksitetraciklina nego na proces tvorbe* L*-sorboze. U oba procesa utjecaj je bio jači na kinetiku rasta nego na kinetiku tvorbe produkta. Procijenjene kritične vrijednosti aktivnosti vode bile su 0,35 (rast G. suboxydans S-22), 0,40 (rast S. rimosus R6), 0,21 (tvorba* L*-sorboze) i 0,40 (biosinteza oksitetraciklina). Sa smanjenjem aktivnosti vode postaju sve važnije pojave oko prijenosa tvari, poglavito ako se promatra razmeđe biomase s reakcijskom tekućinom, kao npr. u primjeni podloga s koncentracijom sorbitola iznad 500 g/L.*