

Oxygen Solubility and Oxygen Transfer and Uptake Rates as Functions of Water Activity

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Dedicated to the memory of Professor W. D. Deckwer

The first time I met Prof. W.D. Deckwer was long time ago, in Zagreb, during one of the traditional Croatian Meetings of Chemists. Later, I met him several times, in occasions of European Congresses on Biotechnology. I remember him as a communicative friendly person, not speaking too much, but giving the impression that he has an evident sense to recognize that common existing between chemical and biochemical engineering

Marijan Bošnjak

The survival and activities of microorganisms depend on water availability. In this work, relationships expressing the dependence of oxygen solubility and oxygen transfer and uptake rates on water activity are considered. The special emphasis is given to the analysis of the effects of dissolved substance concentrations on solubility of gases. The effects of different dissolved salts, sugars and polyols are mentioned and discussed. More attention is given to experimental data and established relationships referring to the aerobic microbial conversion of D-sorbitol into L-sorbose. The proofs supporting the possibility to define the water activity on the basis of oxygen solubility are given by demonstrating them graphically and by mathematical equations.

Key words:

Oxygen solubility, dissolved substance effects, D-sorbitol concentration, mathematical relationships

Introduction

Historically and actually, in the whole system of Earth, one cannot even imagine the life of any organism without water. Therefore, it is not strangely why one of ancient philosophers, Tales from Milet, considered that everything has originated from water. In the case of microbial population it is known that the water plays the essential role, and that a survival of different microorganisms depends differently on the availability of water. In aerobic microbial processes, in addition to water, the availability of oxygen appears important as well. Quicker et al.¹ pointed out that data on the solubility of oxygen and carbon dioxide are of fundamental meaning in various fields of biosciences. They also mentioned the known findings established by others and showing that in general the gas solubility in fermentation broth depends on the concentration of a variety of dissolved inorganic and

organic substances. Electrolytes, sugars, polyols and a series of compounds with amino and hydroxy groups, are known as substances reducing the solubility of gases. Based on findings and considerations of others^{2–6} and as a continuation of their previous investigations,^{7–9} Quicker et al.¹ studied the effects of dissolved substances on solubility of gases in water reaction systems, in order to improve mathematical models proposed previously and to establish the relationships applicable for a more reliable prediction of oxygen and carbon dioxide solubility. These authors designed the apparatus of properties enabling to determine with high accuracy the gas pressure drop caused by gas dissolving and therefore to estimate exactly the gas solubility. After comparing the experimental data with those theoretical the authors concluded that the mathematical models they developed showed to be acceptable, and that for the gas solubility reduction the logarithmic dependence on dissolved substance concen-

tration can be applied. Since the calculated data for a series of reaction media with different concentrations of various sugars and ions of inorganic salts fitted well to values determined experimentally, one can conclude that the mathematical models developed by these authors¹ can be recommended for the use in further studies of those reaction systems where the water activity plays an important role. In addition, one can consider that the obtained results suggest for gas solubility to be the function of given water activity.

As known and as mentioned by Pirt,¹⁰ the water in living cells has four basic functions: a) as a chemical reactant, b) as a solvent, c) as mechanical agent producing the hydrostatic pressure, and d) as agent having a structural function in hydration of cell components. Pirt¹⁰ pointed out that the activity of water in solution may be expressed in terms of the water vapour pressure in the same way as the activity of a gas. Thus, he proposed to express the water activity of the solution as the ratio of the water pressure of the solution (p_s) to that of pure water (p_w) at the same temperature, i.e. to apply the relation

$$a_w = \frac{p_s}{p_w} \quad (1)$$

By applying appropriate mathematical expressions Pirt¹⁰ explained the dependence of water activity on concentration of solutes. Also, he interpolated relation between water activity and osmotic pressure. However, the expression (1) appeared to be sufficient to support another approach in defining the meaning of water activity, i.e. the approach based on the assumption that oxygen solubility is proportional to free water concentration. When the effects of D-sorbitol concentration on oxygen solubility and microbial oxidation kinetics were studied^{11–13} the expression

$$a_w = \frac{\gamma_w}{\rho_w^*} \quad (2)$$

where, γ_w = free water mass concentration, ρ_w^* = pure water density = free water mass concentration in pure water, was proposed to calculate water activities of solutions.

Oxygen solubility in reaction systems containing D-sorbitol as the main reactant

Since it was established experimentally for the dependence of oxygen solubility on D-sorbitol concentration¹¹ that the logarithmic relationship

$$\log \gamma_{DO} = 0.831369 - 0.00081 \gamma_S \quad (3)$$

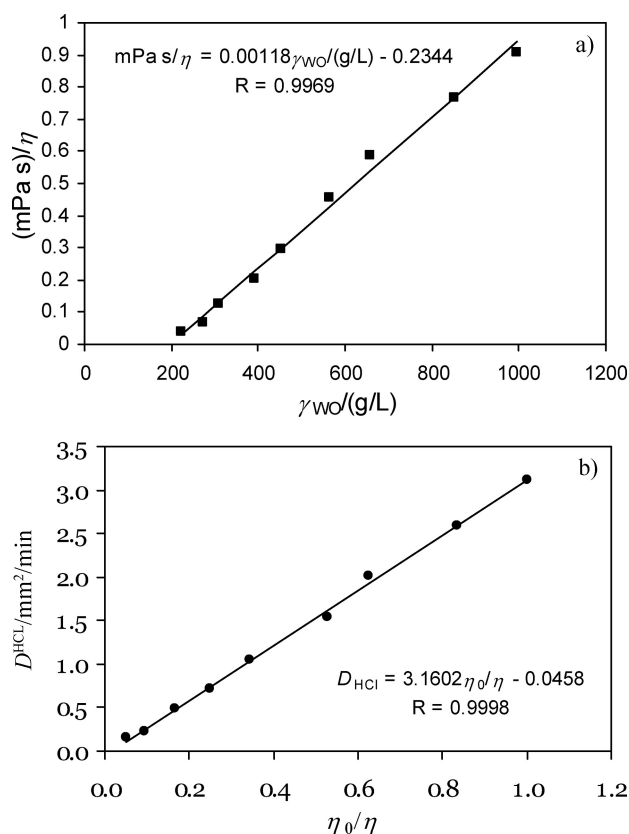


Fig. 1 – a) Effect of free water concentration on solution viscosity, b) Diffusion – viscosity relationship (Data source: Mihaljević et al., 1995)¹²

Table 1 – Specific rate of L-sorbose formation (q_p), as a function of D-sorbitol concentration (γ_S) and medium viscosity (η). Discrepancies between experimental and theoretical data shown in Fig. 4

γ_S /(g/L)	q_p -experiment/h ⁻¹	q_p -theory/h ⁻¹	Standard deviation
0	0.00	0.0000	0
20	15.35	15.2978	0.036911
40	17.17	17.0499	0.084924
70	17.46	17.3146	0.102813
80	17.33	17.1983	0.093126
180	15.10	14.4766	0.440810
190	13.35	14.1410	0.559321
290	11.98	10.7060	0.900854
300	9.03	10.3699	0.950988
390	8.93	7.5405	0.982525
500	4.86	4.7385	0.085913
600	2.32	2.8946	0.406304
700	0.75	1.6543	0.639437
800	0.68	0.8847	0.144745

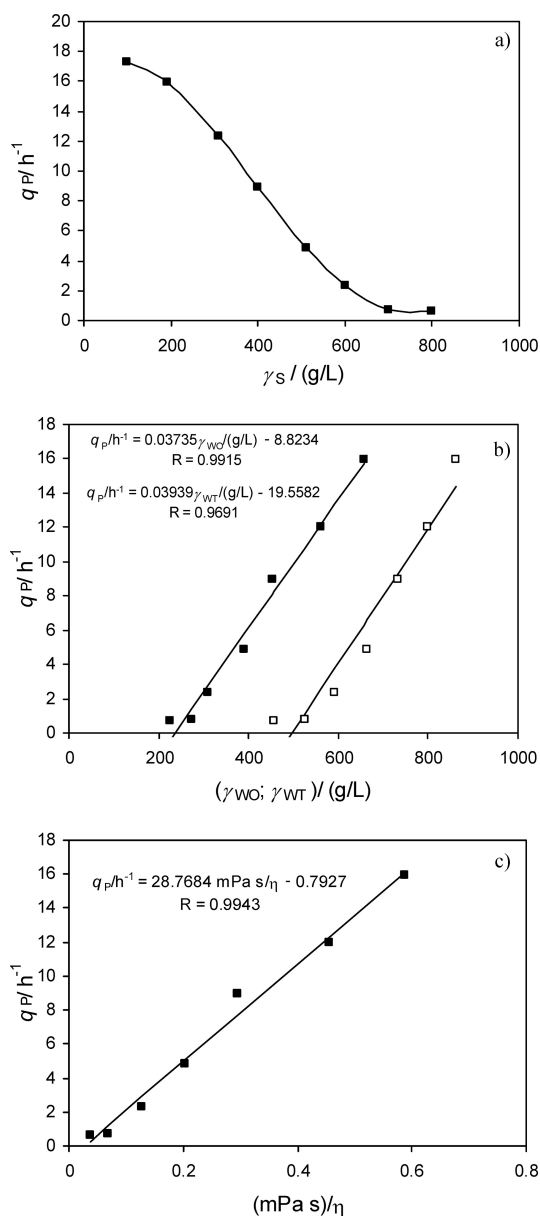


Fig. 2 – The specific L-sorbose production rate (q_p) as a function of a) D-sorbitol concentration (γ_S), b) total and free water concentration (γ_{WT} , γ_{WO}), and c) reciprocal medium viscosity (η_0/η) (Data source: Mihaljević and Bošnjak, 1997)¹³

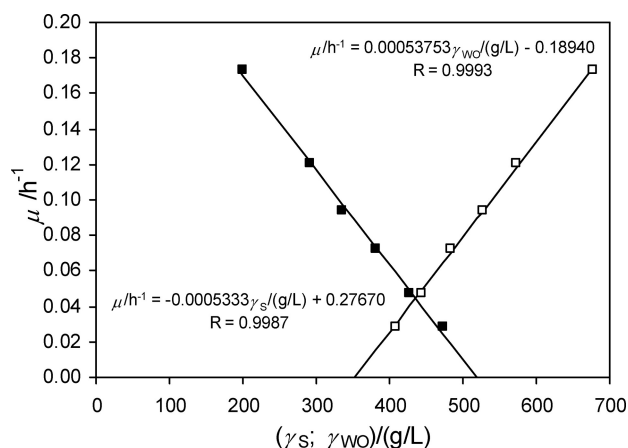


Fig. 3 – Effect of D-sorbitol concentration (γ_S) and free water concentration (γ_{WO}) on specific growth rate (μ) of *G. suboxydans* S-22 (Data source: Mihaljević and Bošnjak, 1997)¹³

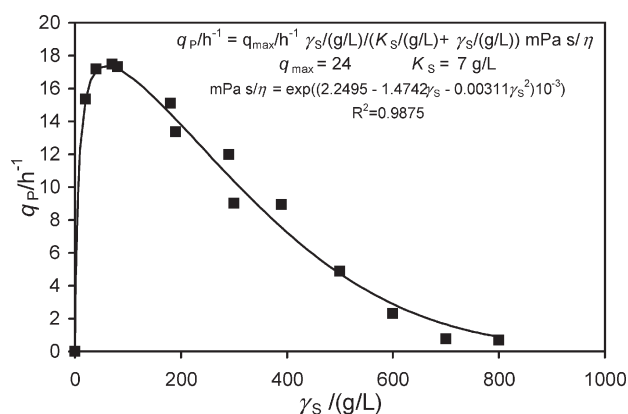


Fig. 4 – Specific rate of L-sorbose formation (q_p), as a function of D-sorbitol concentration (γ_S) and medium viscosity (η). Applicability of the mathematical model (Data source: Mihaljević and Bošnjak, 1997)¹³

Remark: Experimental data resulted from two series of experiments. Series of experiments slightly differed one to other by slight differences in physiological age of applied biomass samples.

Table 2 – Effects of D-sorbitol mass concentration (γ_S) on the maximal oxygen uptake rate (q_{Om}), critical dissolved oxygen concentration (γ_{crDO}) and dissolved oxygen saturation constant (K_{sDO}). Estimated values based on experiments performed with microbial suspension in D-sorbitol solutions of different D-sorbitol mass concentrations (Data source: Mihaljević and Bošnjak, 2003)¹⁵

$\gamma_S/(g/L)$	q_{Om}/h^{-1}	$\gamma_{crDO}/(mg/L)$	$K_{sDO}/(mg/L)$	$q_{Om}/h^{-1}/K_{sDO}/(mg/L)$	$\gamma_{crDO}/(mg/L)/K_{sDO}/(mg/L)$
100	1.8555	0.66	0.1407	13.1874	4.6907
200	1.6626	0.57	0.1635	10.1710	3.4870
300	1.2642	0.52	0.1744	7.2486	2.9815
400	0.9363	0.48	0.1833	5.1074	2.6183
500	0.5472	0.45	0.1922	2.8476	2.3418
600	0.2805	0.43	0.1993	1.4072	2.1572
700	0.1107	0.41	0.2022	0.5476	2.0280

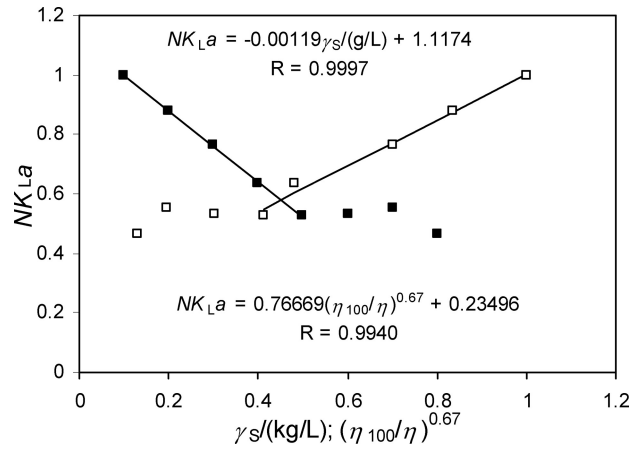


Fig. 5 – Effect of solution viscosity (η) and D-sorbitol concentration (γ_s) on relative oxygen transfer coefficient values (NK_{La}) (Data source: Mihaljević and Bošnjak, 1997)¹³

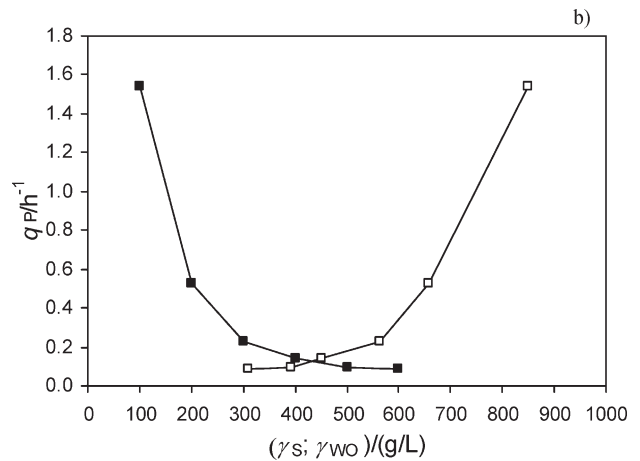
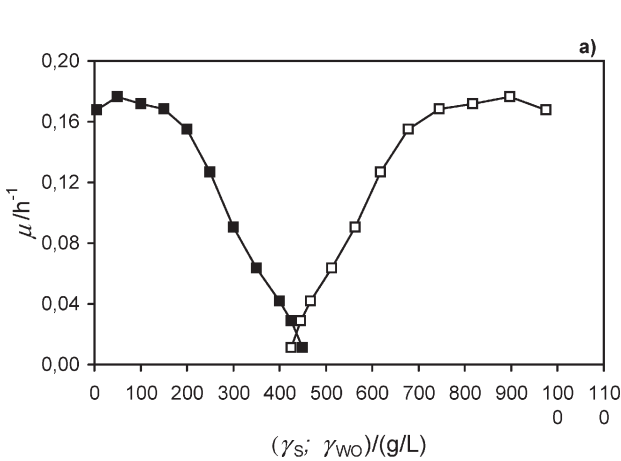


Fig. 6 – Effects of D-sorbitol (γ_s) and free water (γ_{w0}) concentration on a) specific growth rate (μ) of *S. rimosus* R6 and b) specific oxytetracycline biosynthesis rate (q_p) (Data source: Mihaljević and Bošnjak, 1997)¹³

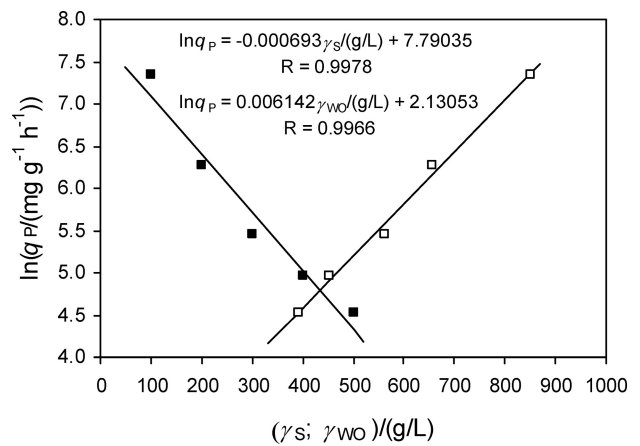
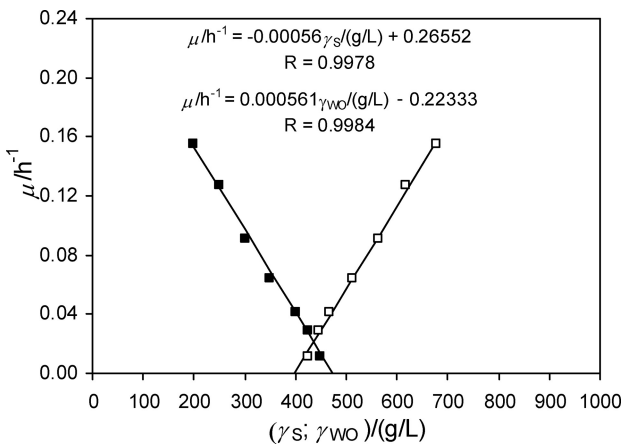


Fig. 7 – Mathematical evaluation of parameter relationships (selected data from Fig. 6.) a) growth kinetics, b) oxytetracycline biosynthesis kinetics (Data source: Mihaljević and Bošnjak, 1997)¹³

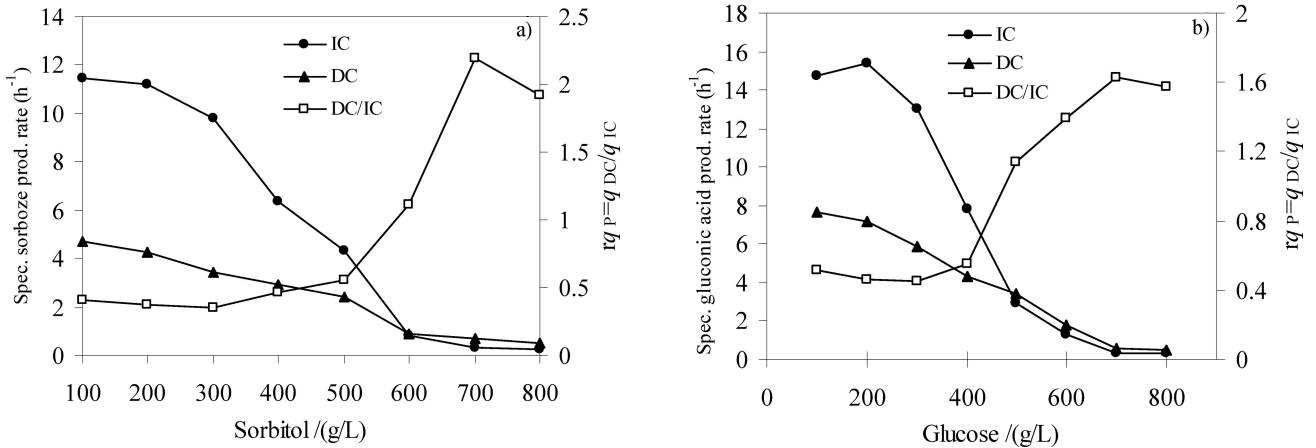


Fig. 8 – Similarity between concentration effects of different substrates on kinetics of corresponding product formation a) D-sorbitol conversion catalysed with biocatalysts of different specific activity, b) D-glucose conversion catalysed with biocatalysts of different specific activity (IC refers to *G. suboxydans* S-22 whole cell biomass; DC refers to *G. suboxydans* S-22 disintegrated cell biomass material, i.e. to the biocatalyst resulting after microbial cell biomass ultrasonic treatment; r_{qP} refers to the ratio of corresponding specific product formation rates), (Data source: Zrinka Pervan, Diplomski rad, Faculty of Food Technology and Biotechnology, Zagreb 1997)¹⁶, (also: Bošnjak and Mihaljević 1998)¹⁴

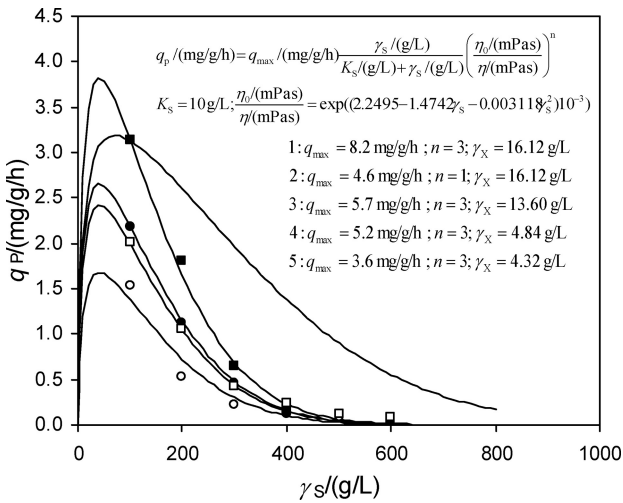


Fig. 9 – Specific rate of oxytetracycline biosynthesis as a function of D-sorbitol concentration. Mathematical model application (Data source: Mihaljević and Bošnjak, 1997)¹³

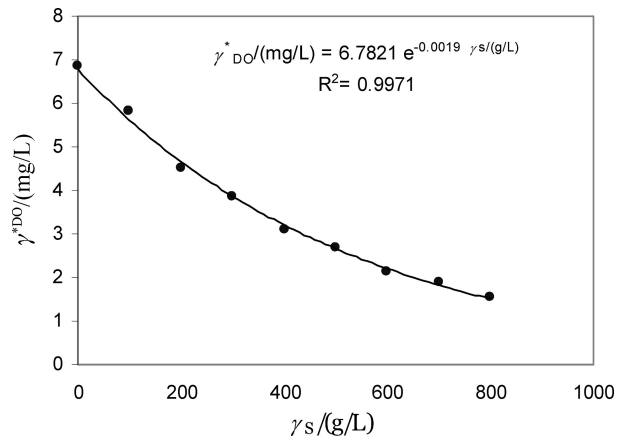


Fig. 10 – Effect of D-sorbitol mass concentration (γ_S) on oxygen solubility (γ_{DO}^*) (Data source: Mihaljević and Bošnjak, 2003)¹⁵

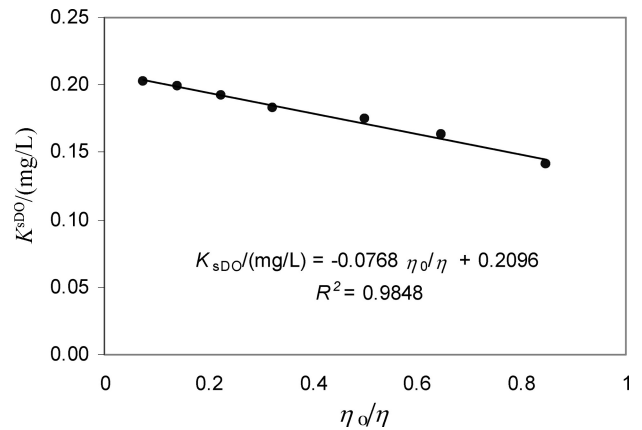


Fig. 11 – Effect of D-sorbitol solution reciprocal viscosity on K_{sDO} values (Data source: Mihaljević and Bošnjak, 2003)¹⁵

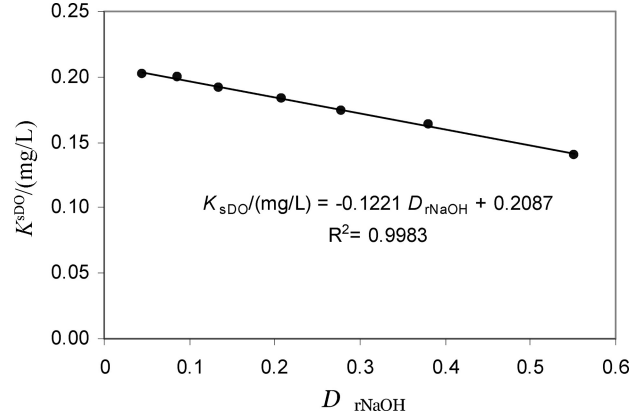


Fig. 12 – K_{sDO} values as a function of diffusion coefficient values (D_{rNaOH}) determined applying agar layer plates with holes containing NaOH solution (Mihaljević et al. 1995). (Data source: Mihaljević and Bošnjak, 2003)¹⁵

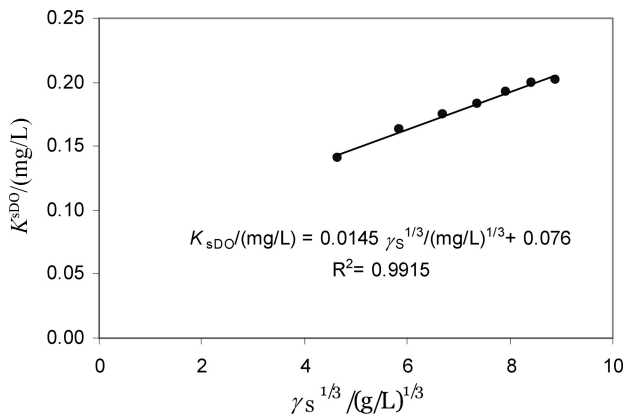


Fig. 13 – Relationship between cubic root of D-sorbitol mass concentration ($\gamma_S^{1/3}$) and dissolved oxygen saturation constant (K_{sDO}) (Data source: Mihaljević and Bošnjak, 2003)¹⁵

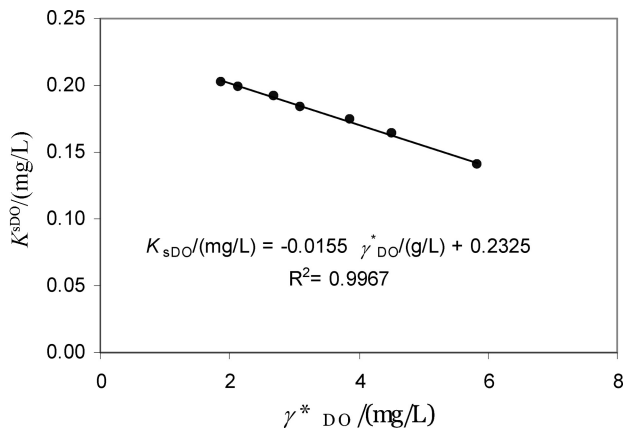


Fig. 14 – Effect of oxygen solubility (γ_{DO}^*) on dissolved oxygen saturation constant (K_{sDO}) (Data source: Mihaljević and Bošnjak, 2003)¹⁵

could be applicable (correlation coefficient $R = 0.99856$), the corresponding exponential relationship

$$\gamma_{DO}^* = \frac{6.782}{1001867\gamma_S} \quad (4)$$

could also be applicable.

The assumption that oxygen solubility appeared to be proportional to the concentration of free water in the solution led to the conclusion that concentrations of free water could be calculated applying the expression

$$\gamma_{WO} = \frac{\rho_W}{\gamma_{WDO}^*} \gamma_{DO}^* = \frac{9960}{6.86} \gamma_{DO}^* \quad (5)$$

Consequently, concentration of the water engaged in maintaining D-sorbitol dissolved (γ_{WE}) should be calculated as a difference between total water concentration (γ_{WT}) and that equivalent to the saturation dissolved oxygen concentration in

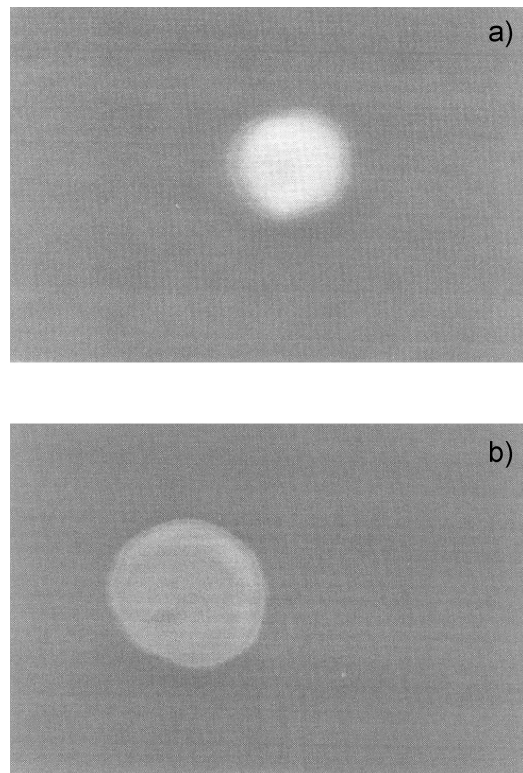


Fig. 15 – Effect of D-sorbitol concentration on size and morphology of *Gluconobacter suboxydans* S-22 cells. Electronic microscope photos of microbial cells. a) cell living in the medium containing the high dissolved substance concentration (700 g/L), b) cell living in the medium of usually applicable dissolved substance concentration of 200 g/L (Data source: Z. Pervan, 1997; K. Mihaljević, PhD Thesis, Faculty of Food Technology and Biotechnology, Zagreb, 2005)^{16,17}

given D-sorbitol solution, i.e. by applying the expression

$$\gamma_{WE} = \gamma_{WT} - \gamma_{WO} \quad (6)$$

In D-sorbitol solutions the homologous and heterologous associations of water and D-sorbitol molecules are possible. Thus, the relation

$$\gamma_{WE} = c_1\gamma_S - c_2\gamma_S^2 \quad (7)$$

was investigated for its applicability, and it was established for parameter values $c_1 = 1.082311$ and $c_2 = 0.0010095$ that experimental data fitted well to those calculated on the basis of relation /7/ and mentioned c_1 and c_2 values¹¹. Therefore, another possibility for expressing the dependence of oxygen solubility on D-sorbitol concentration appeared, i.e. that based on the resulting expression

$$\gamma_{DO}^* = 6.865 - 0.01208\gamma_S + 6.945 \cdot 10^{-6}\gamma_S^2 \quad (8)$$

Excellent correlation coefficients resulted (above 0.998) when theoretical with experimental data were compared, disregarding whether the ex-

pression (3) or the expression (8) were applied¹¹. Such results provoked the further studies and a series of papers containing scientifically interesting data appeared.^{12–15} Effects of water activity on growth and product formation kinetics were investigated in processes of simple and complex microbial bioconversions. Reaction systems with different main substances (D-sorbitol, D-glucose) and different microbial strains (*Gluconobacter suboxydans* S-22, *Streptomyces rimosus* R6) as biocatalysts were applied. Series of experiments were performed and a discovery of different process relationships resulted. Representative part of obtained results is demonstrated in Figs 1–15 and in Table 1. Evidently, one can say for the experimental data that they can be explained well by established mathematical relationships. As shown in Fig. 1, the linear relationships are applicable when relating the medium viscosity with the free water concentration or with corresponding diffusion coefficients. The similar statement can be said concerning the effects of free water and total water concentrations, or of medium viscosity, on specific rate of L-sorbose formation. (Fig. 2). However, one should point out that much better correlation coefficient results with reference to free water concentration than with reference to total water concentration. For a well defined limited range of substrate concentrations the linear relationships can be also applied when expressing the effects of D-sorbitol and free water concentrations on specific growth rate (Fig. 3). More complex relationships should be applied to express combined effects of medium viscosity and substrate concentration on the specific product formation rate (Fig. 4). As shown, the discrepancy between the experimental and theoretical data in such a case appeared to be higher, suggesting the higher degree of uncertainty (Table 1). Since the experimental data resulted from two series of experiments slightly differing in physiological age of applied microbial biomass with reference to particular series, one can suppose that more pronounced discrepancies appeared mainly due to such slight physiological differences of applied biomass samples. Effects of solution viscosity and D-sorbitol concentration on relative oxygen transfer coefficient values are demonstrated in Fig. 5. Two ranges can be distinguished: the range of linear relationship applicability and the range where the effects cannot be explained simply. Data demonstrated in Fig. 6 and Fig. 7 refers to the more complex microbial process, the process of oxytetracycline biosynthesis. Evidently, there is an analogy with the process of D-sorbitol conversion into L-sorbose, if one compares the effects of D-sorbitol and free water concentrations on specific growth rates in these processes, whereas the compared two processes differ markedly concerning the

effects of same parameters on specific product formation rates. How differences in biocatalyst specific activity reflected on the concentration effects of different substrates one can observe in Fig. 8. In the case of oxytetracycline biosynthesis, differences in D-sorbitol concentration reflected on applicable values of mathematical model parameters (Fig. 9). Therefore, there is no doubt that the oxygen solubility as well as the oxygen transfer and uptake rates appear to be the well defined functions of water activity. If one compares these findings with those of Quicker et al.¹ one can conclude that there is an acceptable agreement between them, although they refer to different reaction systems, different methods of experimental data estimation and different mathematical models for calculations of theoretical values. Special emphasis should be given to different methods of oxygen solubility measurements. Quicker et al.,¹ applied the physical method and the apparatus designed by them, whereas in the case of oxygen solubility measurement in D-sorbitol solutions the chemical, long time known Winkler method (1888), was applied. This method was convenient for application to investigated D-sorbitol solutions, but its applicability for a series of other reaction systems can be debatable. Therefore, one can consider the method applied by Quicker et al.¹ could be more convenient for other reaction systems. However, more light concerning oxygen solubility in D-sorbitol solutions certainly could be given after comparative application of both mentioned methods to reaction systems of D-sorbitol solutions. Since, based on the data of Quicker et al.,¹ De Wulf et al.¹⁸ estimated for a 20% D-sorbitol/L-sorbose medium the oxygen solubility to be 5.53 mg/L at 30 °C, one can expect a satisfactory agreement between the results obtained after applying the mentioned two methods. Because L-sorbose is less soluble than D-sorbitol, its effect on the reduction of oxygen solubility supposedly is lower than that of D-sorbitol. Therefore, one can expect results showing the values quite close to those already established for D-sorbitol solutions^{11,12}.

The recent report¹⁵ refers to the study of D-sorbitol concentration effects on parameters of oxygen uptake rate and represents a relevant source of data. Part of them is demonstrated in Figs. 10–14 and Table 2. An insight into demonstrated data suggests the conclusion that the water activity has well defined influence on parameters of oxygen uptake kinetics. With decreasing the water activity, e.g. the estimated critical dissolved oxygen concentrations decrease, whereas the estimated values of dissolved oxygen saturation constants increase (Table 2).

Final discussion and conclusions

If one compares different processes on the basis of demonstrated data then one can conclude that the differences in water activity effects depended on microorganism properties, reaction system similarity and process complexity. However, the effects in every case can be relatively well defined by mathematical models. In addition, data give the evidence (Fig. 15) that the water activity undoubtedly influences microorganism morphology and therefore has an additional effect on process kinetics *via* microorganism morphology changes.

Since D-sorbitol conversions were realized with morphologically very distinguishable microbial species (*Streptomyces rimosus* and *Gluconobacter oxydans*) an extrapolation of some conclusions to processes catalysed by morphologically more different microbial species can be suggested, especially with reference to corresponding already published data. D-glucose can be converted into D-gluconic acid by different microorganisms. It is known that in addition to bacterial *Gluconobacter oxydans* strains, some strains of the fungus *Aspergillus niger* can also be applied as efficient biocatalysts. *A. niger* can convert D-glucose into D-gluconate in fed batch culture even at very low water activity.¹⁹

ACKNOWLEDGMENT

Advices of prof. Ivan Butula are well appreciated.

List of Symbols

- a_w – water activity, dimensionless
 c_1 – proportionality coefficient, dimensionless
 c_2 – proportionality coefficient, L/g
 NK_{La} – relative volumetric oxygen transfer rate coefficient, dimensionless
 P_{sol} – solution vapour pressure, Pa
 P_w – vapour pressure of pure water, Pa
 γ_{DO}^* – oxygen solubility, saturation dissolved oxygen concentration, mg/L
 γ_S – D-sorbitol mass concentration, g/L
 γ_W – free water mass concentration, g/L
 γ_{WDO}^* – concentration of oxygen solubility in pure water, mg/L
 γ_{WE} – water engaged in maintaining D-sorbitol dissolved, g/L
 γ_{WO} – free water mass concentration calculated on the basis of oxygen solubility, g/L
 γ_{WT} – total water concentration, g/L
 ρ_w^* – pure water density, g/L
 – Indexes

- DO – refers to dissolved oxygen
 K_{La} – refers to volumetric oxygen transfer rate coefficient
 S – refers to substrate (D-sorbitol)
 sol – refers to solutions
 W – refers to water
 WDO – refers to dissolved oxygen in pure water
 WE – refers to water engaged in dissolving D-sorbitol
 WT – refers to total water

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