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Evaluation of Specific Oxygen Uptake Rates with Reference to Biocatalytic D-Sorbitol Oxidation Systems*

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Summary

Effects of D-sorbitol concentration on the kinetics of D-sorbitol microbial oxidation were studied. The culture of *Gluconobacter suboxydans* S–22 was applied as a biocatalyst. The study was focused on the determination of oxygen uptake rates and the evaluation of factors affecting them. Special emphasis was given to estimations of dissolved oxygen saturation (Monod) constant (K_{sDO}) and critical dissolved oxygen concentration. The estimated values of Monod constant (K_{sDO}) were correlated with different process kinetics parameters and a series of acceptable relationships was established. Excellent correlation coefficients were found for K_{sDO} as a linear function of (*i*) reciprocal viscosity ($R^2 = 0.9848$), (*ii*) relative diffusion coefficient ($R^2 = 0.9983$), (*iii*) cubic root of dissolved D-sorbitol mass concentration ($R^2 = 0.9915$) and (*iv*) oxygen solubility in D-sorbitol solutions of different D-sorbitol concentrations ($R^2 = 0.9967$). The estimated K_{sDO} values were lower than their corresponding critical dissolved oxygen concentrations.

Key words: D-sorbitol microbial oxidation, oxygen uptake, D-sorbitol concentration effects, process relationships

Introduction

Biocatalytic reaction systems of D-sorbitol oxidation showed to be very suitable for a study of mass transfer phenomena, process kinetics and different process relationships (*1–6*). Studies of process kinetics of L-sorbose production oriented on the evaluation of mass transfer parameters (*3,6*) led to the results showing that the process of D-sorbitol biooxidation into L-sorbose could be applied for the estimation of oxygen transfer parameters. It was established that the increase of D-sorbitol concentration caused the linear decrease of oxygen transfer rate volumetric coefficient value (*6*). Studies of other process phenomena (*4–7*) resulted in a series of findings. Thus, it was found that the reductions of oxygen solubility, water activity and diffusion permeability as well as the increase of the viscosity of reaction media appeared due to the increase of D-sorbitol concentration. The expressions

$$\gamma_{DO} / (\mathrm{mg/L}) = 6.865 - 0.01208\, \gamma_S / (\mathrm{g/L}) + 6.954 \cdot 10^{-6}\, (\gamma_S / (\mathrm{g/L}))^2 \qquad /1/$$

and

$$\gamma_{DO} / (\mathrm{mg/L}) = 6.7822 / 1.001867^{\gamma S/(\mathrm{g/L})} \qquad /2/$$

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were found to describe well the oxygen solubility as a function of D-sorbitol concentration in solution (*4*). For a high range of process conditions it was found that the specific rate of L-sorbose formation showed to be proportional to: (*i*) specific oxygen uptake rate; (*ii*) diffusion coefficient; (*iii*) reciprocal viscosity, and (*iv*) difference between genuine and critical water activity (*5*). As reported (*5*), the measuring device composed of the chamber designed as that used by Krieg (*1*), connected with a monitor and a computer, was used to determine specific oxygen uptake rates. Reported values referred to the experimental ranges of constant specific oxygen uptake rates, whereas complete unselected experimental data were collected to be used for a possible more detailed evaluation later (additional information by authors, not published in previous report (*5*)).

The aim of this work was to focus on the study of the effects of dissolved substance concentrations on specific oxygen uptake rates of *G. suboxydans*, taking into account the specific effects on the apparent value of dissolved oxygen saturation (Monod) constant, K_{sDO}. It should be mentioned here that literature data refer more to critical dissolved oxygen concentrations than to Monod constants of dissolved oxygen. One considers that the effect of dissolved oxygen concentration on the specific oxygen uptake rate appears to be of the Michaelis-Menten type (*8,9*). Therefore, one can expect that the relation

$$q_O = q_{Om} \cdot \gamma_{DO} / (K_{sDO} + \gamma_{DO}) \qquad /3/$$

can be applicable for the reaction systems of D-sorbitol oxidation. In the literature (*8,10*) it is clearly demonstrated that the specific oxygen uptake rate increases with the increase in the dissolved oxygen level up to a certain point, referred to as critical dissolved oxygen concentration, above which no further increase in oxygen uptake rate occurs. Some examples of the critical oxygen levels for a series of different microorganisms are reported as well (*8,10*). It is pointed out (*10*) that the critical concentrations commonly appear to be relatively low (of the order of one percent of the saturation value) and that in some microbial cultures with expressed characteristics of a non-homogeneous environment much higher values of critical concentrations, the so-called apparent critical concentrations, can result. Therefore, there is some uncertainty whether the estimation of exact K_{sDO} values will be sufficiently reliable. However, since even the apparent values can be very useful in explaining process events and in developing sophisticated mathematical models of biooxidation processes, an engagement oriented towards the experimental evaluation of apparent K_{sDO} values and the finding of appropriate relationships where K_{sDO} values are taken into account can be recommended.

Materials and Methods

Microorganism

Gluconobacter suboxydans S-22 (Culture Coll. PLIVA, Res. Institute, Zagreb, Croatia). Microorganism suspension of microbial biomass concentration of 2 g/L was applied.

Microbial cell suspension

An aliquot of the microbial culture prepared in the laboratory bioreactor as described previously (*4*) was used to prepare microbial cell suspension. After 17 h of cultivation the culture was close to the maximal viability and the estimated biomass concentration of 1.8 g/L (estimation on the basis of measured absorbance at 660 nm (*6*)). Microbial biomass was separated from the used microbial culture aliquot and then resuspended in 200 g/L D-sorbitol solution by adjusting the microbial cell suspension to the concentration of 2 g/L of microbial cells. The same microbial cell suspension was applied to perform the same series of dissolved oxygen uptake rate measurements.

Media

Sterile water solutions of different D-sorbitol concentrations (100–800 g/L).

Method of oxygen uptake rate determination

The measuring device consisting of the chamber designed as that published by Krieg (*1*), connected to a monitor (Biological oxygen monitor, model 5300, with Clark type polarographic microelectrode, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio, USA) and a computer (Hewlett-Packard France, PC 486, Grenoble, France) was used to determine specific oxygen uptake rates (Fig. 1). After the saturation of 50 mL of given sorbitol solution with air oxygen, 1 mL of microorganism suspension was injected into the chamber and then the change of dissolved oxygen followed. The incubation temperature was 30 °C. Taking into account the oxygen solubility and the rate of relative dissolved oxygen concentration decrease, the specific oxygen uptake rates were calculated. Already published data were those referring to ranges of practically constant dissolved oxygen decrease rates. The completed unselected experimental data were collected to be used in this work. Calculations were performed as follows:

(*i*) Calculation of specific oxygen uptake rates: Numerical derivation of dissolved oxygen mass concentrations (γ_{DO}) with respect to incubation time was performed. Incubation intervals of 5 s with γ_{DO} values

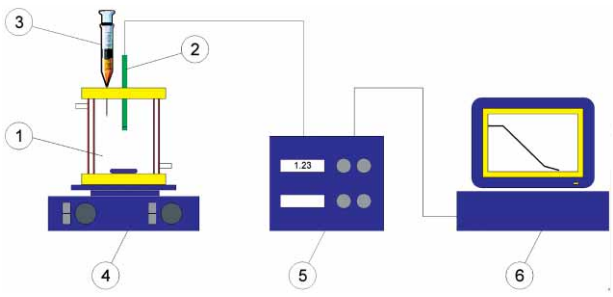

Fig. 1. Scheme of the apparatus for a microbial specific DO uptake rate determination

Legend: 1-biooxidation chamber, 2-polarographic oxygen electrode, 3-injection syringe, 4-magnetic mixer, 5-Amplifier (Oxygen monitor YSI 5300) with analogue signal exit, range 0–1 Volts, 6-PC computer with AD converter, PCL-818H Adventech

monitored every 0.5 s were applied in the calculation of mean dissolved oxygen uptake rates. The second monitored point of the preceding interval was taken to be the starting point for the following interval. The calculated mean values were used to calculate the corresponding specific oxygen uptake rates as well as to evaluate the relationships existing between the mean dissolved oxygen uptake rates and the corresponding mean γ_{DO} values. The values of the starting period of slightly slower changes of dissolved oxygen concentration were neglected in evaluating the mentioned relationships;

(*ii*) Estimation of critical dissolved oxygen concentrations: γ_{DO} values at time points of abrupt change of dissolved oxygen uptake rates were taken as critical dissolved oxygen concentrations.

Equipment

Computer: Compaq, Type Deskpro EN, Serial No. 8122FHGZ0D2F

OS: Microsoft Windows 2000 (5.00.2195)

Software: Microsoft Excel 2000 (9.0.4402 SR-1); Microsoft Word 2000 (9.0.4402 SR-1)

Source of other data: Selected data from our previous publications (*4–6*) were used to enable an adequate interpretation of the results treated in this work.

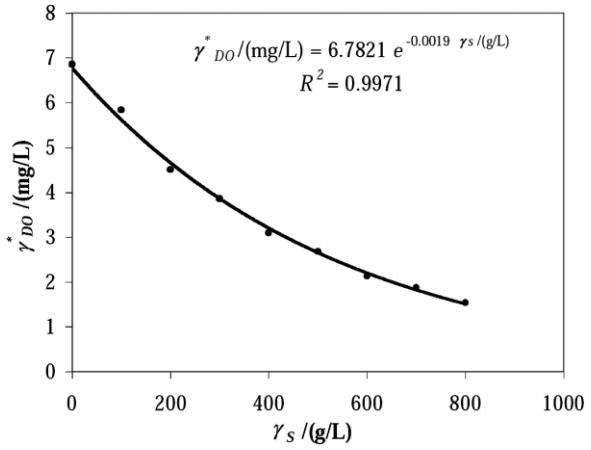

Fig. 2. Effect of D-sorbitol mass concentration (γ_s) on oxygen solubility (γ^*_{DO})

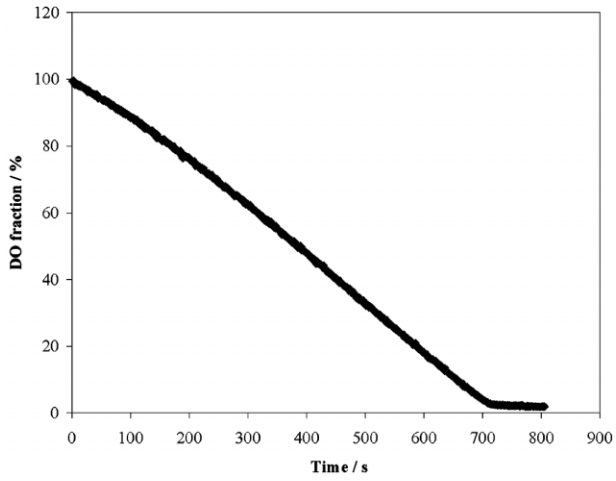

Fig. 3. Kinetics of oxygen uptake in the microbial suspension of *G. suboxydans* S-22 in 100 g/L D-sorbitol solution. (Typical example of the process performed in the measuring chamber)

Results

To improve the insight into the obtained results and to facilitate the understanding of particular relationships, the already published data referring to the effect of D-sorbitol concentration on oxygen solubility (*4*) are reinterpreted. Fig. 2 demonstrates how experimental data fit to those theoretical if the relationship /2/ is applied. The agreement of experimental with theoretical data is evidently excellent. Since it is known that the relationship /1/ can also be applied (*4*), one can consider the presented data to be reliable enough for further use in interpreting process phenomena. A typical example of the changes of dissolved oxygen concentration in cell suspension of *Gluconobacter suboxydans* S–22 present in the measuring chamber during the experiment of specific oxygen uptake rate determination is shown in Fig. 3. The curve enables to distinguish quite easily the two characteristic ranges. The range where oxygen uptake rate reduced continuously and depended on dissolved oxygen concentration is obviously characterised by a very low dissolved oxygen concentration, and one can estimate the critical dissolved oxygen concentration to be of the order of several percent of the saturation level. Table 1 contains the

Table 1. 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 (Monod) constant (K_{sDO}). Estimated values based on experiments performed with microbial suspensions in D-sorbitol solutions of different D-sorbitol mass concentrations

γ_S/(g/L)	q_{Om} /h^{-1}	γ_{crDO}/(mg/L)	K_{sDO}/(mg/L)	q_{Om} /h^{-1} / K_{sDO}/(mg/L)	γ_{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

data showing how the estimated critical dissolved oxygen concentrations depended on the concentration of D-sorbitol in the tested reaction media and varied from 11.7 % (at D-sorbitol concentration of 100 g/L) to 22.3 % (at D-sorbitol concentration of 700 g/L) of the saturation levels. The estimated values are not quite in accordance with the expectations, if literature data (*8,10*) are taken as a basis. One can consider that K_{sDO} values should be markedly below the estimated dissolved oxygen critical values. In addition, Table 1 demonstrates the relationships between maximal specific DO uptake rates and corresponding critical DO concentrations and K_{sDO} values. D-sorbitol concentration affected the values of all parameters shown in Table 1. Since it is known (*9*) that in the range above critical DO concentration and strongly above K_{sDO} values the specific rates of DO uptake are practically constant, whereas in ranges where DO concentrations are markedly below K_{sDO} values the specific rates of DO uptake are proportional to the actual DO concentrations and q_{oxm}/K_{sDO} ratios, it can be concluded that the effects of the increase of D-sorbitol concentration were more pronounced in the ranges below DO critical concentrations than in those above them. Supposing the relation /3/ to be quite applicable for the mentioned range of low dissolved oxygen concentrations, the Lineweaver-Burk form of relation /3/ was applied for the evaluation of relations between the estimated values of

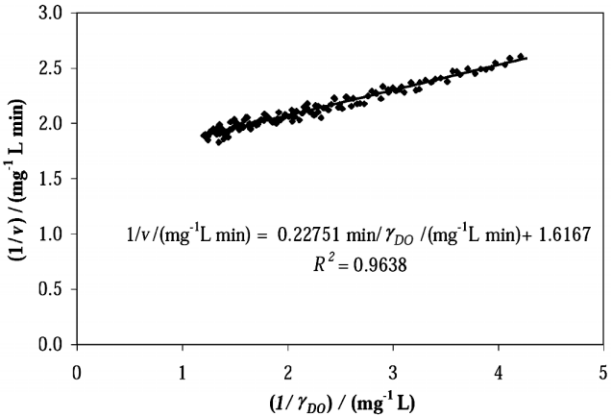

Fig. 4. Typical Lineweaver-Burk plot referring to the data of the experiment demonstrated in Fig. 3. Statistical evaluation of K_{sDO} value

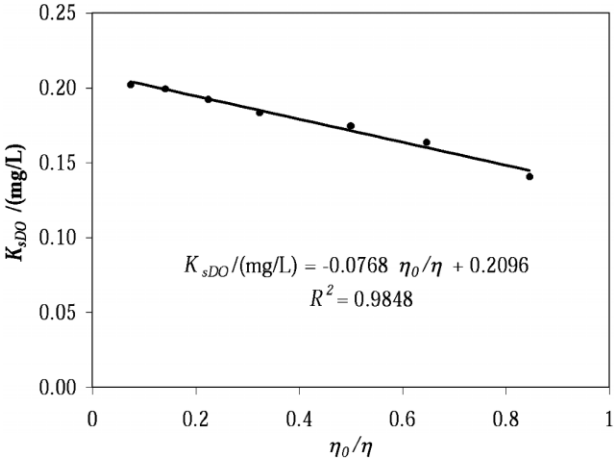

Fig. 5. Effect of D-sorbitol solution reciprocal viscosity on K_{sDO} values

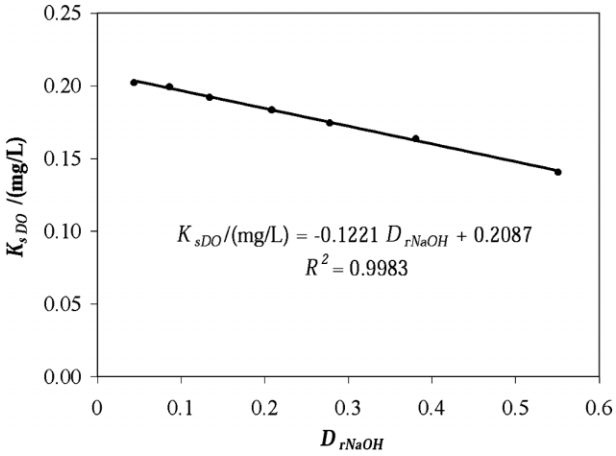

Fig. 6. K_{sDO} value as a function of diffusion coefficient values (D_{rNaOH}) determined applying agar layer plates with holes containing NaOH solution (*5*)

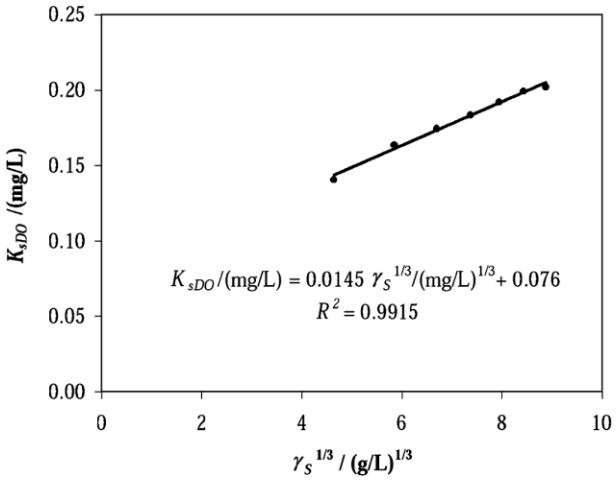

Fig. 7. Relationship between cubic root of D-sorbitol mass concentration ($\gamma_s^{1/3}$) and dissolved oxygen saturation constant (K_{sDO})

dissolved oxygen concentration and their corresponding estimated specific oxygen uptake rates. The data were analysed statistically, as demonstrated by an example represented by Fig. 4 and the obtained set of calculated values was correlated with the corresponding set of dissolved substance concentrations (γ_S), or other reference data, applying different mathematical relations (Figs. 5–9). Good correlations were found for a few of them, *e.g.* for K_{sDO} as a linear function of: (*i*) reciprocal viscosity ($R^2 = 0.9848$, Fig. 5), (*ii*) relative diffusion coefficient ($R^2 = 0.9983$, Fig. 6) and (*iii*) cubic root of dissolved substance concentration ($R^2 = 0.9915$, Fig. 7). Because of the established good correlation coefficients, the following relationships

$$K_{sDO} = -0.0768\ \eta_0/\eta + 0.2096 \qquad /4/$$

$$K_{sDO} = -0.1221\ D_{rNaOH} + 0.2087 \qquad /5/$$

$$K_{sDO} = 0.0145\ \gamma_S^{1/3} + 0.076 \qquad /6/$$

can be accepted. The correlation between K_{sDO} values and D_{rNaOH} values was investigated since it is supposed that the diffusion rate of HO^- ions, *i.e.* $H_3O_2^-$ ions

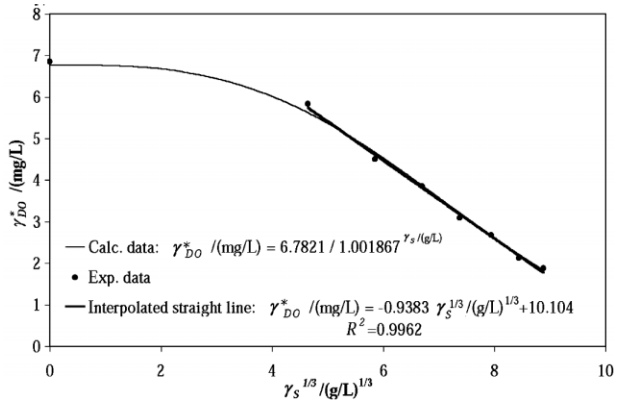

Fig. 8. Oxygen solubility (γ^*_{DO}) as a function of cubic root of D-sorbitol mass concentration ($\gamma_s^{1/3}$); Range of the linear relationship applicability

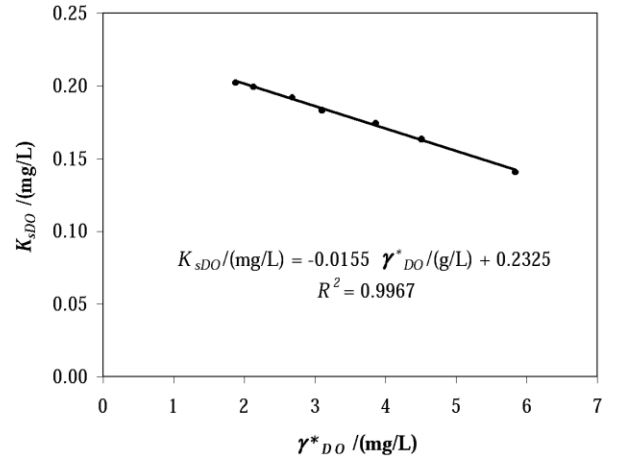

Fig. 9. Effect of oxygen solubility (γ^*_{DO}) on dissolved oxygen saturation constant (K_{sDO})

($M_r(H_3O_2^-) = 35$), could be similar to that of dissolved oxygen molecules ($M_r(O_2) = 32$).

The value of 0.076 mg/L (the intercept on the y-axis, equation /6/) refers to K_{sDO} value in the pure water. Therefore, the increase in the concentration of dissolved substance evidently affects the oxygen transfer and uptake rates by: (*i*) reducing oxygen solubility (*4,5*) and oxygen transfer rate volumetric coefficient (*6*) as well as (*ii*) reducing the maximal oxygen uptake rate and increasing the value of dissolved oxygen saturation constant, K_{sDO}. Relation /6/ seems to be in agreement with the observations of N. Bošnjak *et al.* (*11,12*) who studied the structural origin of gas chromatographic (*GC*) retention data of alkanes and cycloalkanes. They correlated the experimental *GC* retention indices of the mentioned hydrocarbons with the corresponding structural parameters and found that *GC* retention indices correlated well with cubic roots of Wiener numbers of the investigated substances. The increase of D-sorbitol concentration means the increase of the concentration of corresponding volumetric equivalent of D-sorbitol. The increased D-sorbitol concentration is accompanied with reduced free water concentration and therefore with reduced oxygen solubility in the given solution. For experimental data in the D-sorbitol concentration range of 100–700 g/L an excellent correlation was found when relating cubic roots of D-sorbitol concentration against oxygen solubilities in given solutions ($R^2 = 0.9962$, Fig. 8), *i.e.* when applying the relationship

$$\gamma^*_{DO} = -0.9383\ \gamma_S^{1/3} + 10.104 \qquad /7/$$

In equation /7/, the extrapolated too high value for oxygen solubility in the pure water may imply that the effect of D-sorbitol on oxygen solubility in the concentration range 0–100 g/L cannot be explained by cubic root law. If one inspects the equation /1/ then undoubtedly one can conclude that the effect of D-sorbitol on the reduction of water activity appeared to be relatively more pronounced at lower than at higher D-sorbitol concentrations *(4)*. Perhaps a speculation that the range of lower D-sorbitol concentrations is the range where relationships applicable for chaotic systems appear to be appropriate ones can be accepted. Then, the range of higher D-sorbitol concentrations can be considered as a range where relationships valid for order systems (systems with lower degree of freedom) can be applicable. Good correlations of K_{sDO} with oxygen solubility ($R^2 = 0.9967$, Fig. 9), reciprocal viscosity and diffusion coefficient probably mean that dissolved oxygen diffusion rate through the solution and especially through the microbial cell surrounding laminar layer appears to be the factor strongly limiting the biooxidation rate at a very high D-sorbitol concentration. Of course, the reduction of cell biocatalytic activity at extremely high dissolved substance concentrations is also an important factor. Some experimental observations *(7)* support such an explanation. The dependence of K_{sDO} on oxygen solubility can be expressed by equation

$$K_{sDO} = -0.0155\ \gamma^*_{DO} + 0.2325 \qquad /8/$$

The extrapolated maximal K_{sDO} of 0.2325 mg/L refers to the water free medium, *i.e.* to the dry (pure) D-sorbitol, where the oxygen is supposedly insoluble.

Discussion

As mentioned in the Introduction, the aim of this work was to complete the previous information on studies of process phenomena and relationships referring to reaction systems of biocatalytic D-sorbitol oxidation. Thus, this work was focused on the determination of specific oxygen uptake rates with an emphasis on the evaluation of the effects of dissolved substance (D-sorbitol) concentration on values of the apparent dissolved oxygen saturation (Monod) constant, K_{sDO}, and critical dissolved oxygen concentration, γ_{crDO}. As shown in the Results, the efforts were fruitful. Experimentally found values for critical dissolved oxygen concentration and specific dissolved oxygen saturation (Monod) constant seem to be enough reliable and accurate. Estimated K_{sDO} values were lower than the corresponding critical dissolved oxygen concentrations, which appeared to be in accordance with the expectations, if the media with lower D-sorbitol concentrations were considered. However, one should point out that for the media with

higher D-sorbitol concentrations the estimated K_{sDO} and γ_{crDO} values showed to be dependent on D-sorbitol concentration, and good correlations were found for K_{sDO} as a linear function of: (*i*) reciprocal viscosity, (*ii*) relative diffusion coefficient and (*iii*) cubic root of dissolved substance mass concentration. The observation that the relationship expressing K_{sDO} as a linear function of cubic root of dissolved substance mass concentration in microbial culture showed to be analogous to that expressing *GC* retention indices of alkanes as a function of cubic root of their Wiener numbers could be accepted, although the biocatalytic system of microbial D-sorbitol oxidation where oxygen molecules move through the medium and become adhered on biocatalyst (microorganism) surface does not seem to be quite analogous to the system of *GC* where volatile alkanes move through the *GC* column and simultaneously adhere to the surface of column particles. Since in an ideal case of spherical particles the cubic root of particle volume is practically proportional to the value of the ratio between the particle volume and its surface area, which increases with the increase of the particle size, partial analogy between the two mentioned systems can be observed. Wiener number reflects the molecule size and form to high extent, whereas the volume of dissolved D-sorbitol determines also the volume of free water which is decisive for the dissolving of oxygen, its movement through the solution and its adhesion to the biocatalyst surface. Specific affinities between different particles and the obstacles to particle movement play an important role with respect to particle adhesion and release rates in both systems. Therefore, one can consider the mentioned *GC* data (*11,12*) support the validity and applicability of the relationship /6/.

The data of this work can be exploited for different purposes. Together with already discovered relationships and other appropriate data published previously *(3–7)*, the relationships defined in this work can be used for the development of the mathematical model which describes appropriately the process events during D-sorbitol biocatalytic oxidations even when different cultivation methods are applied. Improved process control systems can be developed as well. A special benefit of this work appeared due to the fact that the relationships discovered in this work could facilitate the finding of optimal methods of L-sorbose production. As proven experimentally *(2,4,7,13)*, a fed batch culture and a repeated fed batch culture appeared to be the most convenient methods for L-sorbose production, especially when one tends to produce efficiently the microbial culture broths with extremely high L-sorbose concentrations *(4,7)*. One of the recent publications *(14)* confirmed again the advantages of the fed batch culture for L-sorbose production.

Acknowledgement

The kind advice of professor emeritus Pavao Mildner referring to the improvement of the text is well appreciated.

List of symbols

Symbol Unit Meaning

D_{rNaOH}	relative diffusion coefficient referring to the performed experiment
$f\eta$	relative reciprocal viscosity, ratio between viscosities of water and reaction medium, dimensionless
q_O	specific oxygen uptake rate
q_{Om}	maximal specific oxygen uptake rate
R	correlation coefficient, dimensionless
K_{sDO}	specific dissolved oxygen saturation (Monod) constant, mg/L
γ_{crDO}	critical dissolved oxygen mass concentration, mg/L
γ_{DO}	dissolved oxygen mass concentration, mg/L
γ^*_{DO}	oxygen solubility in D-sorbitol water solution, mg/L
γ_S	D-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
η_0	viscosity of pure water, mPa s

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Procjena specifičnih brzina potrošnje kisika u sustavima biokatalitičke oksidacije D-sorbitola

Sažetak

Proučavan je utjecaj koncentracije D-sorbitola na kinetiku mikrobne oksidacije D-sorbitola. Mikrobna kultura soja *Gluconobacter suboxydans* S-22 primijenjena je kao biokatalizator. Proučavanje je usredotočeno na određivanje brzina potrošnje kisika i vrednovanje faktora koji na njih utječu. Poseban je naglasak bio na procjenama konstante zasićenosti otopljenim kisikom (Monodove konstante, K_{sDO}) i kritične koncentracije otopljenoga kisika. Procijenjene vrijednosti Monodove konstante (K_{sDO}) uspoređene su s različitim parametrima procesne kinetike, te su ustanovljene prihvatljive zakonitosti. Nađeni su izvrsni korelacijski koeficijenti za K_{sDO} kao linearnu funkciju: (*i*) recipročne viskoznosti ($R^2 = 0.9848$), (*ii*) relativnog difuzijskog koeficijenta ($R^2 = 0.9983$), (*iii*) trećeg korijena masene koncentracije otopljenog D-sorbitola ($R^2 = 0.9915$) i (*iv*) topljivosti kisika u otopinama D-sorbitola različitih koncentracija ($R^2 = 0.9967$). Procijenjene vrijednosti K_{sDO} bile su niže od njima pripadajućih kritičnih koncentracija otopljenoga kisika.