Scientific paper

Oxygen Transfer in a Laboratory Stirred Tank Bioreactor During Mammalian Cell Culture Cultivation

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Dedicated to the memory of the late Prof. Dr. Valentin Koloini

Abstract

The influence of power consumption on the volumetric mass transfer coefficient was studied in a 5 liter stirred tank bioreactor during cultivation of a recombinant Chinese Hamster Ovary (CHO) cell line, which requires low aeration and mixing intensity. Under these conditions and at high cell concentrations, oxygen mass transfer coefficient and consequently volumetric oxygen mass transfer rate was not sufficient for the oxygen requirements. This problem was successfully solved by introducing pure oxygen into the bioreactor and the oxygen mass balance was studied. For that purpose, respiration rate as a function of cell concentration, as well as volumetric oxygen mass transfer rate at different operating conditions were measured. Graphical presentation of the results show the process control possibilities and the role of mixing intensity necessary to ensure that there is sufficient oxygen supply for a given cell concentration and respiration rate.

Keywords: Mammalian cell culture, power consumption, oxygen mass transfer

1. Introduction

Several important biopharmaceuticals like interferons, hormones, vaccines and monoclonal antibodies are produced in cell culture systems under aerobic conditions. Due to low solubility in the medium, oxygen supply may become limiting at higher cell concentrations. The gas-liquid oxygen mass transfer depends on liquid physicochemical properties and liquid oxygen concentration. It is also a strong function of energy dissipation, while sensitive animal cells require gentle mixing and aeration. Therefore, bioreactors designed for this purpose differ significantly in reactor geometry and operating conditions compared to those designed for microbial fermentations. In short, low impeller speeds and low aeration rates or consequently low to moderate power inputs are necessary operating conditions. Studies of oxygen transfer under these conditions are much less frequent compared to the extensive number of articles covering microbial cultivations.

Previous extended articles were dedicated to engineering and equipment design principles for large scale cell culture systems.[1–4] In addition, a few papers only focused on oxygen transfer and aeration. For example, agitation, aeration, and perfusion modules were investigated in cell culture bioreactors of various sizes.[5] The influence of power input and aeration on mass transfer has been studied in a laboratory animal cell culture vessel,[6] while a new method for on-line measurement of the volumetric oxygen uptake in animal cell cultures rate was presented.[7] A correlation to predict the liquid-phase oxygen transfer coefficient as a function of Reynolds number has been proposed for animal cell suspension culture.[8] It was shown that the conventional chemical engineering correlations for oxygen transfer correlations are usually out of range and unproven in application for animal cell culture conditions.[9] Oxygen transfer properties of various types of bubbles in aerated stirred tank with animal cell culture media have been studied with three types of spargers at various air flow rates and low power inputs compared to the data about k_La from published correlations. The highest oxygen transfer rate was found for micron-sized bubbles, obtained with a sinter sparger.[10]

Other articles have been dedicated to sparging and agitation-induced injury of cultured animal cells[11] and cell death from bursting bubbles in sparged reactors.[12] Ceramic microsparging system in a pilot scale animal cell culture gave better oxygen transfer rates compared to standard aeration systems.[13]

This study focused on gas-liquid oxygen mass transfer in a laboratory bioreactor during cultivation of mammalian cells at low mixing intensity. As a result, a procedure to control industrial processes according to specific requirements of various cell cultures was obtained.

2. Experimental

2. 1. Bioreactor

Oxygen transfer measurements and cultivation experiments were performed in a 5 liter stirred tank bioreactor system Biostat B (B.Braun Biotech International GmbH, Germany). Inner vessel diameter and height were 160 mm and 345 mm, respectively. Liquid medium was gassed through a ring sparger (diameter 60 mm) with 14 holes (diameter 1mm), placed 20 mm above the bottom. Three two paddle stirrers (pitch 45 °) with a 70 mm diameter and 50 mm height were used for mixing. Distance between stirrers as well as from the bottom was 40 mm. The bioreactor was equipped with a Biostat B Control Unit to control the stirrer speed and with corresponding probes to control, temperature (Pt 100, B.Braun Biotech International GmbH, Germany), pH (405-DPAS-SC-K8S/425, Mettler Toledo GmbH, Germany) and partial oxygen pressure (InPro 6800 Series, Mettler Toledo GmbH, Germany).

A Gas Mix Unit for the Biostat B system was used for the preparation of inlet gas mixtures from gas cylinders with pure N_2, O_2, CO_2 and air. Gas was introduced through flowmeters (Q-flow 55, 1.6–16 L/h, Voegtlin Instruments AG, Schweiz, +/– 5% accuracy). MFCS/win supervisory control and data acquisition software package system (B.Braun Biotech International GmbH, Germany) was used for online pO_2, stirrer speed, pH and temperature data logging. This unit is specially tailored to the requirements of fermentation processes and satisfies a broad range of requirements in research, pilot and production area. The configuration can easily be adapted by the user to any change in process requirement.

2. 2. Determination of Power Consumption

A geometrically similar vessel and impellers of the same size were constructed to determine power input in separate experiments on a Benco-ELB system (Bench Scale Equipment Co. USA). Impeller rotational speed was measured using a stroboscope (MA 3905, Iskra, Slovenia, accuracy +/– 1%), while the torque data (accuracy +/– 2%) at various impeller speeds in the range from 60–180 rpm were obtained by measuring the force on the arm attached to the motor housing, which was mounted on top of the low friction ball bearings. Torque measurements in air were subtracted from the measurements in liquid to take into account the friction of ball bearings. All experiments were performed under nonaerated and aerated conditions.

2. 3. Determination of Volumetric Mass Transfer Coefficient

A dynamic gassing method was used to determine the mass transfer coefficient as reported elsewhere.[7,10,14,15] The bioreactor was flushed first with pure nitrogen until the oxygen probe showed zero oxygen partial pressure. Then, gassing of the bioreactor with a constant air flow of 10 L/h started at various impeller speeds ranging from 60–180 rpm. On the basis of the probe response during oxygen saturation the k_La was estimated. The same procedure was repeated at zero impeller speed while varying the air flow from 6–14 L/h. In preliminary experiments it was found that the probe response time does not influence the k_La determination.

2. 4. Determination of Liquid Properties

Medium viscosity at cultivation temperature was determined with a Cannon-Fenske capillary viscometer. Medium density was measured with a DMA 02D (Anton Paar GmbH, Austria) density meter using frequency measurements of a glass tube with a sample in a thermostated air filled glass cylinder.

2. 5. Determination of Oxygen Equilibrium Concentration

The equilibrium oxygen concentration in water for the air-water mixture at cultivation conditions (C^*_{air} = 6,7 mg/L) and in water for the pure oxygen-water mixture (C^*_{O2} = 31,9 mg/L) were calculated using literature data for Henry's coefficient.[7] The equilibrium oxygen concentration in the cultivation medium was calculated by correcting this data with the experimentally determined ratio of the oxygen probe response at saturation conditions in the culture medium and water. This estimation resulted in an oxygen equilibrium concentration in the medium for air of C^*_{air} = 6,5 mg/L and for pure oxygen of C^*_{O2} = 31,0 mg/L.

2. 6. Culture Conditions

Standard cultivation media based on sugars, vitamins, minerals, amino acids, salts and other additives were used. Also a small amount of block copolymer Pluronic F-68 was added to the media to decrease the cell sensitivity to mechanical stress and prevent hydrodynamic da-

mage. Prior to the bioreactor inoculation, the Chinese Hamster Ovary (CHO) cell culture was prepared in a CO_2 incubator at 37 °C and 5% CO_2, using spinner flasks where mixing was achieved with a magnetic stirrer. When the cell concentration reached the value of about 2×10^6 cells/mL, the bioreactor was inoculated with cca. 500 mL of cell culture, aiming at a starting cell concentration of $0{,}2\times10^5$ cells/mL. All the experiments were performed under sterile conditions.

2. 7. Determination of Viable Cell Concentration

Cell concentration and viability were determined by the trypan blue cell exclusion method, using the automated image analysis cell counter Vi-CELL XR (Beckman Coulter, Cell viability Analyzer, USA).

2. 8. Determination of Oxygen Uptake Rate

A dynamic method was used daily for the oxygen uptake rate evaluation at various cell concentrations by following the course of pO_2.[14] The medium was first saturated with oxygen by gassing with air or oxygen enriched gas mixture. Then, the pO_2 value on the control unit was set to 50% of saturation in air to avoid the possible reduction of respiration at low oxygen concentration. The stirrer speed was set to a minimum value of 80 rpm to prevent sedimentation of the cells and gassing was stopped. Putting surface aeration aside and assuming that $k_La \approx 0$ at these conditions, the oxygen uptake rate was evaluated from the slope of the pO_2 curve.

In addition to these experiments, respiration was also determined with a modified Fenn-Winterstein respirometer to determine the equilibrium oxygen concentration in the cultivation medium.[16] In these experiments, two 500 mL glass flasks with stoppers, connected with pipes on the top and equipped with valves and a 'U' manometer and pipette to measure gas volume were used. Liquid in both flasks was mixed with a magnetic stirrer. To start the experiment, the first flask was filled with purified water (PW) and the second with the cell culture (cell concentration was determined in separate experiment). At known time increments, the volume of added air to equalize the gas pressure was measured. Assuming that this volume equals the volume of the consumed oxygen, the oxygen uptake rate in mL O_2 per minute was evaluated by dividing the volume with the time increment.

3. Results and Discussion

The effect of the mixing intensity on power consumption and consequently on volumetric mass transfer coefficient was investigated in a series of experiments in water and cultivation media without mammalian cells. In Figure 1 the effect of stirrer speed on the volumetric power input during turbulent mixing ($4900 < Re < 14700$) is shown. At these conditions the power input should be proportional to the cube of the stirrer speed.[4] For our experimental conditions, the polynomial extrapolation gave the exponent 2,89, which is in very good agreement with the literature data. It is also shown that there is practically no difference between the results in water and cultivation medium, since the viscosity of the cultivation media (2,7 mPas) vas very similar to water and therefore also turbulent mixing conditions were achieved. Additional experiments performed at a low air flow rate (10 L/h; 0,033 vvm) or equivalent superficial gas velocity ($3{,}5 \times 10^{-5}$ m/s), which was later used during cultivation conditions (results not shown), showed practically no effect on power consumption. Therefore the results from Figure 1 were used in further calculations.

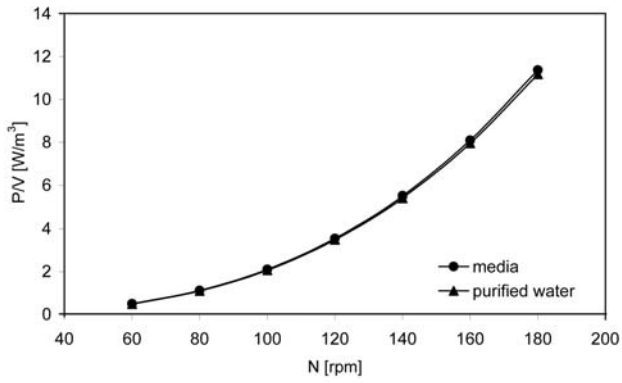

Figure 1. The effect of stirrer speed on power input.

The investigation continued with the dynamic method at various impeller speeds and an aeration rate of 10 L/h to determine the volumetric oxygen mass transfer coefficient as a function of power consumption. A low aeration rate was applied due to possible sparging injury of the mammalian cells.[11,12] Experimental data, which are shown in Figure 2 are correlated with the exponential correlation[4,17] where the exponent over P_g/V is 0,17 (r^2 = 0,98) for purified water and 0,30 (r^2 = 0,99) for cultivation medium. These values are lower than the sparse data from literature describing gentle mixing conditions in small laboratory systems. Let us point out that the power inputs in these systems usually do not exceed 100 W/m^3 to avoid the cell damage. Therefore the well known correlation by Van't Riet,[17] which was obtained for power inputs $P_g/V >$ 400 W/m^3 is out of this range.[4,9,17] For example, Moreira et al[6] found that the exponent over P_g/V is 0,46, while Puthli et al.[14] report that this value is 0,61. The results from the available literature obtained in laboratory and pilot plant reactors during cultivation of mammalian cells are presented in Table 1. It is seen that the experimental

conditions, like reactor configuration as well as impeller speed and aeration rate, are not always in the same range thereby affecting the results. Taking the above into account, our results fit reasonably well with the other data from Table 1.

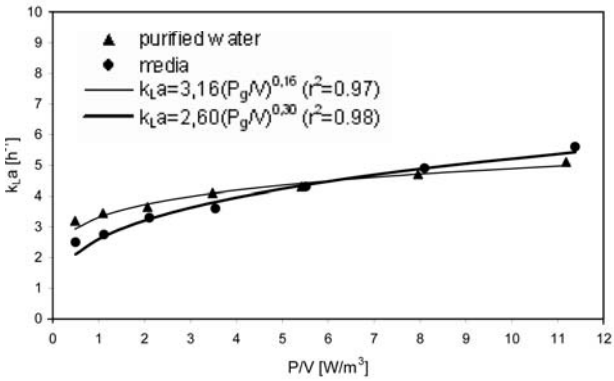

Figure 2. The effect of power input on volumetric mass transfer coefficient.

The investigation continued under the cultivation conditions during cell growth. A dynamic method was used to determine the oxygen respiration rate at different cultivation times and consequently, different cell concentrations. Results are shown in Figure 4. According to the increasing

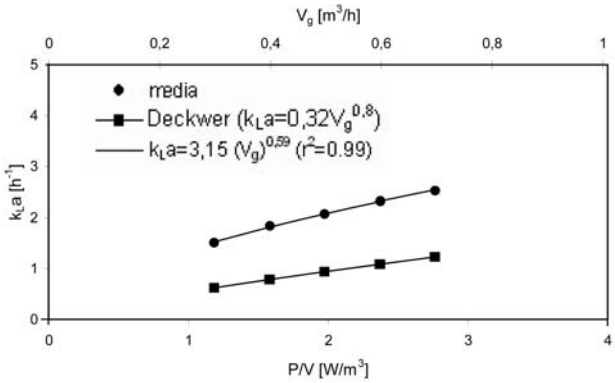

Figure 3. The effect of aeration linear velocity on volumetric mass transfer coefficient.

Since the impeller power input was low, some experiments to determine the effect of only aeration on k_La were performed. These results are presented in Figure 3. Here, the calculation of power input by aeration was done considering only the isothermal expansion of the gas.[18] While comparing the results with literature data, it should be mentioned that the correlation by Deckwer was obtained for bubble columns where usually H/T > 5. Here H/T = 2,2 and surface aeration probably has a pronounced effect and, as a consequence, k_La values are higher. Aeration had a strong effect on k_La, which is in accordance with the literature.[9,18] Comparison of the k_La values at an aeration of 10 L/h without mixing (k_La = 2,0 h^{-1}) and N = 100 rpm (k_La = 3,4 h^{-1}) from Figures 2 and 3 gives an interesting result: the contribution of only aeration to the oxygen mass transfer is more than 50%.

cell concentration also the respiration rate increases and reaches its maximal value of 50 mg/lh at a cell concentration of $2,5 \times 10^7$ cells/ml. Values are consistent with the literature data. For example 6,4 mg/lh at a viable cell concentration of 5×10^5 cells/ml and 11 mg/lh at a viable cell concentration around 10^7 cells/ml are reported.[7,5]

The data about oxygen consumption due to respiration are needed to evaluate the necessary oxygen supply or volumetric oxygen mass transfer rate with aeration and mixing. In Figure 5, the oxygen supply k_La (C* – C) is presented on the left Y axis, while the oxygen uptake rate is given on the right Y axis and the values on the horizontal lines are compared. It is clearly seen that gassing with air is sufficient only at the initial stage of cultivation. The maximal volumetric oxygen mass transfer rate obtained under the highest mixing intensity 180 rpm and gassing

Table 1. The effect of mixing intensity on volumetric mass transfer coefficient for cell culture bioreactors.

V L	Configuration	N min^{-1}	P_g/V W/m^3	Aeration,vvm min^{-1}	k_La h^{-1}	Reference
2	AB; 1S,	60			1,6	Fenge[5]
10	AB, PS;1S					Fenge[5]
35	AB, PS,RS;1S					Fenge[5]
1,5	HS; 2S	100–350			1,0–3,1	Lavery[19]
1,8	PS; 1–3S; H/T=1,7	300–600		0,3–1,0	10–60	Puthli[15]
2	RS,PS;H/T=1,8;1S	40–120		0,05–0,3	10–100	Zhang[10]
2	SA, 1S	50–250		0,23	0,6–1,6	Ducommun[7]
2	RS, SA, 2S	50–350	1,5–250	0,01–0,04	1–10	Moreira[6]
5	PS,1S,H/T=1,5	50–100		0,05–0,1	9,4–10,4	Nehring[13]
100	PIS;H/T=1–1,3;1S		2–10		1–6	Langheinrich[8]
5	RS, 3S, H/T=2,2	60–180	0,5–11,4	0,03	2,5–5,6	this work

Legend to Configuration: RS – ring sparger, PS-porous sparger, PIS-pipe sparger, AB – aeration basket, HS – hollow shaft, SA – surface aeration, nS – number of stirrers, H/T – vessel height-diameter ratio.

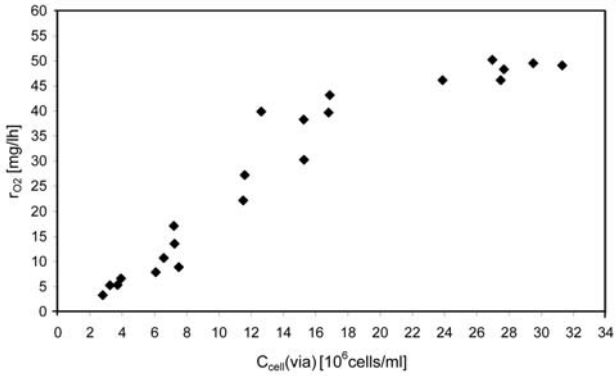

Figure 4. Oxygen respiration rate as a function of cell concentration.

with air in Figure 5 is only 20 mg/lh. The insufficient oxygen supply at higher cell concentrations is mainly due to the necessary gentle mixing and aeration and consequently low mass transfer coefficients. This problem can be solved by adding pure oxygen to the inlet gas stream,[5,7] when a few fold increase in oxygen supply can be achieved due to the substantially higher oxygen concentration difference (C* – C) at the same gentle mixing conditions. The estimated results for gassing with pure oxygen, also shown in Figure 5, record a sufficient oxygen supply of 50 mg/lh can be achieved already at 60 rpm and at a set concentration of 50% air oxygen saturation.

The main result of this investigation is shown in Figure 6, which allows the estimation of operating parameters such as impeller speed and set oxygen concentration to satisfy the oxygen needs of the culture. For a given value of the respiration rate and oxygen transfer rate on the vertical line between the left and right Y axis and the selected set media oxygen concentration on the X axis, the impeller rotational speed can be selected at the cross section of the lines. It is clearly seen that at the same mixing intensity, an increased magnitude of oxygen supply is achieved by gassing with pure oxygen, compared to gassing

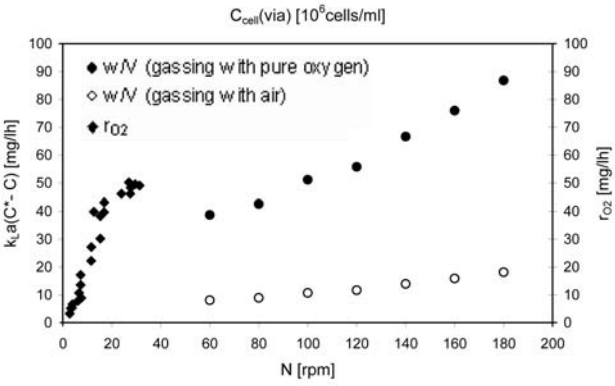

Figure 5. Volumetric oxygen mass transfer rate as a function of impeller speed during gassing with air and pure oxygen and cell respiration rate as a function of cell concentration.

with air only. For example, an oxygen supply of around 65 mg/lh of oxygen can be achieved at the lowest mixing intensity used (60 rpm) and 80% air oxygen saturation set point, which is higher than the oxygen consumption 50 mg/lh at the highest cell concentration.

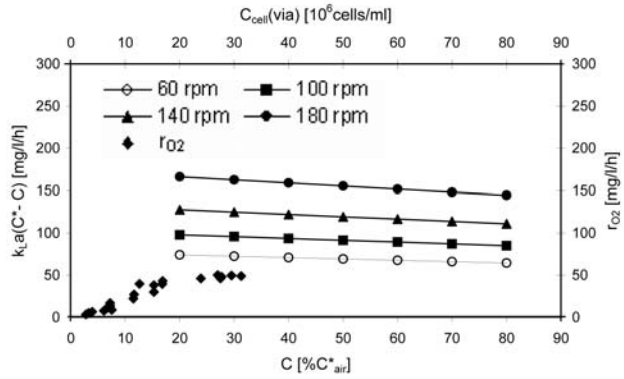

Figure 6. Volumetric oxygen mass transfer rate as a function of various set media oxygen concentration C and cell respiration rate as a function of cell concentration.

4. Conclusions

Volumetric oxygen transfer rates or oxygen supply during cultivation of mammalian cells must reach up to 50 mg/lh. This is not easy to attain with air since at the necessary gentle mixing and low aeration conditions, only low mass transfer coefficients are obtained. For example, the k_La value is roughly 5 h^{-1} at Pg/V = 10 W/m^3 and the corresponding oxygen supply with air can reach only 20 mg/lh. Higher values can be ensured by introducing pure oxygen to the inlet gas stream sparged into the medium. A graphical presentation of the oxygen consumption and supply gives an overview of the process control possibilities and provides an easy method to find the necessary mixing conditions to ensure sufficient oxygen supply for a given cell concentration and respiration rate. The results of this investigation and the presented control strategy are successfully applied during cultivation process on industrial scale.

5. References

1. Kelley B. D., T. W. Chiou, M. Rosenberg, D. I. C. Wang, Industrial Animal Cell Culture, In Biotechnology, Volume 3, (Eds: Rehm, H. J., Reed, G., Puhler, A., and Stadler, P.), VCF, Weinheim, **1993**, pp23–38
2. Ozturk S.S., Cytotechnology, **1996**, *22*, 3–16
3. Marks D.M., Cytotechnology, **2003**, *42*, 21–33
4. Nienow A.W., Cytotechnology, **2006**, *50*, 9–33
5. Fenge C., C. Klein, C. Heuer, U. Siegl, E. Fraune, Cytotechnology, **1993**, *11*, 233–244

6. Moreira J. L., P. E. Cruz, P. C. Santana, A. S. Feliciano, J. Chem. Tech. Biotechnol, **1995**, *62*, 118–131
7. Ducommun P., P. A. Ruffieux, M. P. Furter, I. Marison, U. Von Stockar, Journal of Biotechnology, **2000**, *87*, 139–147
8. Yu P., T. S.Lee, Y. Zeng, H. T. Low, Computer Methods and Programs in Biomedicine, **2007**, *85*, 59–68
9. Langheinrich C., A. W. Nienow, T. Eddleston, N. C. Stevenson, A. N. Emery, T. M. Clayton, N. K. H. Slater, Trans IchemE, **2002**, *80*, 39–44
10. Zhang,S, A. Handa-Corrigan and R. E. Spier, Biotechnology and Bioengineering, **1992**, *40*, 252–259
11. Michaels J. D., A. K. Mallik, E. T. Papoutsakis, Biotechnology and Bioengineering, **1996**, *51*, 399–409
12. Meier S. J., T. A.Hatton, D. I. C.Wang, Biotechnology and Bioengineering, 1999, 62, 368–478
13. Nehring D., P. Czermak, J. Vorlop, H. Lubben, Biotechnol. Prog., **2004**, *20(6),* 1710–1717
14. Taguchi H., A. E. Humphrey, J. Ferment. Technol., **1966**, *44*, 881–889.
15. Puthli M. S., V .K.Rathod, A. B.Pandit, Biochemical Engineering Journal, **2005**, *23*, 25–30
16. Ghiese, A.C., Cell Physiology, 4th Ed., Department of Biological Sciences, Stanford University, W.B.Saunders Company, California, **1973**, p 437–463.
17. Van't Riet K., Ind. Eng. Process. Des. Dev., **1979**, *18*, 357–364.
18. Deckwer W. D., Bubble Column Bioreactors, Biotechnology, Vol 2., Fundamentals of Biochemical Engineering, Verlag Chemie, Weinheim, **1983**, pp445–455.
19. Lavery M, A. W. Nienow, Biotechnology and Bioengineering, **1986**, *30*, 368–373.

Povzetek

V 5-litrskem mešalnem bioreaktorju smo proučevali vpliv vnosa moči z mešali na volumenski koeficient prenosa kisika k_La med gojenjem rekombinantnih sesalskih celic, ki zahtevajo neintenzivno mešanje in prezračevanje. Ugotovili smo, da pri teh obratovalnih pogojih in visoki koncentraciji celic dobava kisika z zrakom ne zadošča celični porabi kisika. Ta problem smo uspešno rešili z uvajanjem čistega kisika v bioreaktor. Pri raziskavah smo proučevali snovno bilanco kisika tako, da smo izmerili hitrost respiracije v odvisnosti od koncentracije celic in snovni prenos kisika v brozgo pri različnih vrtljajih mešala. Z grafično predstavitvijo rezultatov smo prikazali možnost kontrole in vodenja procesa ter intenzivnost mešanja, ki zagotavlja zadostno dobavo kisika pri podani koncentraciji celic in njihovi porabi kisika.