Hydrodynamic considerations in three-phase internal-loop airlift bioreactors

effect of dual separator and draught tube design

Jaroslav KLEIN^a, Andrej SZÍJJARTO^b, Antonio A. VICENTE^a and José A. TEIXEIRA^a

 ^aCentro de Engenharia Biológica – IBQF, Department of Biological Engineering, University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal
 ^bDept. of Chemical & Biochemical Engineering, Faculty of Chemical Technology Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia e-mail: jarok@deb.uminho.pt

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1. INTRODUCTION

In recent years, there has been a growing interest in bioreactors, which utilise immobilised enzymes and cells in order to improve the bioprocess productivity. In fact, there are many specific advantages of the immobilised systems in comparison with the more conventional systems, in which the bio-agents are suspended freely (cells) or dissolved in a bulk aqueous medium (enzymes):

- high cell densities per unit bioreactor volume, resulting in very high bioconversion rates;
- performance of a bioreactor in the continuous mode of operation; reducing capital costs
- employment of the same biocatalyst (cells) for extended periods of process time;
- easy separation of biocatalyst (cells) from the liquid medium;
- minimised risk of contamination.

The immobilised system usually represents three-phase dispersion, where an intimate contact of gas, liquid and solid phases should be ensured. Three-phase airlift (TPAL) bioreactors provide such suitable environment and together with advantageous combination of controlled mixing and low shear rate, efficient suspension of solids, makes airlift system attractive for bioprocesses, where microorganisms are immobilised at solid carriers (e.g. biofilm particles) or flocculate (e.g. flocculating yeasts S. cerevisiae). In these high-cell density systems, may be solids loading as high as 30-40 % of the total reactor volume, what is necessary for achievement of a high conversion of the continuous bioprocess. This amount of particles can be completely suspended in TPAL with a lower energy requirement comparing to bubble columns [1]. This advantage of ALR results from existence of liquid circulation loop inside the reactor originating from the density difference between the riser and downcomer sections. The liquid circulation velocity is essential parameter in the design of the TPAL reactor because it has crucial effect on various processes – mixing, extent of bubble recirculation, efficiency of solids suspension and distribution of gas and solids holdups. Thus, the knowledge of liquid circulation rate is of particular importance. Together with another hydrodynamic parameters, such as gas and solids holdups, circulation and mixing time, is influenced by air flow rate, solids loading and their properties and reactor design.

Most hydrodynamic studies on bioreactor design investigated the influence of basic reactor dimensions – downcomer to riser cross-sectional area ratio (A_D/A_R) , height of draft tube and column height to diameter ratio (H/D). However, a gas-liquid separator, which may significantly affect the performance of TPAL, is still frequently overlooked. The head zone is usually designed at purpose of a control of the extent of bubble penetration into the downcomer, whereby it determines the magnitude of gas holdup and the driving force for liquid circulation. Consequently, it affects overall hydrodynamic, mass transfer and mixing characteristics of airlift reactors.

The control of intensity of bubble separation is usually achieved by the change of size (i.e.

diameter, height) of the reactor head zone. Most of studies dealt with internal-loop airlift reactor used the reactor configuration without separator, i.e. the column and separator diameters are equals. Significantly fewer papers exist on the impact of the significantly enlarged gas-liquid separator on ALR with two-phase (gas-liquid) system [2-4].

Moreover, the enlarged head zone can act as an efficient sedimentation zone, which can be exploited in the continuous three-phase biosystem, where the efficient separation of the solid particles from the liquid is of particular importance. Despite this fact, only few recent studies covered the investigation of three-phase flow in ALR with such enlarged head zone [5,6].

Despite all mentioned advantages of ALR utilization for three-phase biotechnology processes, its industrial implementation is still very limited. It is mainly due to missing of the reliable detailed description of hydrodynamics, mixing and mass transfer in the ALR with complex gas-liquid solid (G-L-S) system. Recently, the continuous TPAL reactor under name "Circox reactor" was utilised for aerobic purification of wastewater using biofilm particles [7]. Moreover, several successful applications of the continuous TPAL bioreactor at lab scale have been already carried out [8-11], showing future perspectives of that type of bioreactor.

In present study, experiments were performed to determine the liquid circulation velocity, the gas and solids holdups in individual parts of the airlift reactor containing low-density particles. Caalginate beads were used as the model solids system for purpose of to mimic various immobilization carriers as well as flocculating cells with the density close to that of the liquid medium. The techniques for the measurements of all principal hydrodynamic parameters were tested at the TPAL reactor that were suggested especially for application to high-cell biosystem operating in the batch as well as continuous mode. The work was focused on the investigation of an effect of the bioreactor design on hydrodynamics in model three-phase (G-L-S) system, which mimics substantial multiphase fermentation system. Different lengths and diameters of draft tube were tested to show how the dual separator acting as the degassing and sedimentation zone affected hydrodynamic performance of internal-loop airlift reactor. Finally, some conclusions were done to suggest the optimal bioreactor design of three-phase airlift bioreactor with high-cell density system.

2. MATERIALS AND METHODS

The reactor set-up

A 60 L internal-loop airlift reactor with enlarged degassing zone was used for hydrodynamic measurements (see Fig. 1). Three inner tubes of different diameters and lengths were used. The basic dimensions of reactor and variations of configurations are listed in Table 1.

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 top of I	ri <mark>ser tube</mark> , D _S ·	– diameter o	of separato	<i>r zone,</i> H _S ⋅	 height of 	separator	zone.	
Label	D_{c}	H _c	D_R	A_D/A_R	H_{DT}	H_{T}	D_S	H_{S}
	[mm]	[m]	[mm]	[-]	[m]	[m]	[m]	[m]
 1DT	142/150	1.986	92/100	1.2	1.4	0.292	0.442	0.350
2DT	142/150	1.986	92/100	1.20	1.2	0.492	0.442	0.350
3DT	142/150	1.986	62/70	3.97	1.2	0.492	0.442	0.350

Table 1. Basic characteristics of ALR used in experiments.

 D_c – column diameter, H_c – height of column, D_R – riser draft tube diameter, A_R – riser cross-sectional area, A_D – downcomer cross-sectional area, H_{DT} – height of the draft tube, H_T – height of liquid level above top of riser tube, D_c – diameter of separator zone, H_c – height of separator zone

The head section had a shape of reversed cut cone with cylinder overhead. The conical section forms a 51° angle with the main body of the reactor. The reactor was designed especially for a continuous system with solid phase, consequently a construction of drainage tube for liquid overflow and local settler was positioned at the reactor wall at volume level of 50 litres. Thus, the working volume was kept at that constant volume level.

In all experiments water and air were used as the liquid and gas phases, respectively. The experiments were carried out at the average temperature of 19 ° C and atmospheric pressure. The air injection was made 0.061 m below the bottom of the draft tube by means of a perforated plate with a diameter of 0.03 m, with 30 holes of 1 mm each one. The air flow rate was controlled by means of rotameters and ranged from 2 up to 70 Ln/min (1 atm, 20°C) covering most flow rates applying in fermentation processes. In the results the air flow is given as the characteristic superficial velocity referred to the column diameter D_c, U_{GC}. This parameter was calculated according to the air flow rate for the conditions in the geometric centre of the column.



Fig. 1. Scheme of airlift reactor. Belts 1 and 2 indicate a position of measuring coils for the velocity measurement by magnetic tracer method. A. ALR with dual separator - 2DT and 3DT reactor set, B. ALR with separator – 1DT reactor set.

Solid phase

A.

Ca-alginate beads were used as solid phase, which mimic well-immobilised carriers of yeast flocculation particles. They have been prepared according to the procedure described by Vicente et al. [5]. Ca-alginate beads with immobilised cells (killed compressed baker's yeast) had a mean diameter of 2.15 \pm 0.13 mm and density of 1048 \pm 1 kg.m⁻³. Three different solids loadings were applied: 10, 20 and 30 %(v/v) corresponding to the true solids loading of 7.2, 14.2 and 21.3 %(v/v), respectively. However, in the experimental set using the draft tube 3DT (see Table 1), the highest solids loading was only 19.2 %. In all experiments, the labelling of the sets with different solids loading and draft tubes will be used as follows:

Table 2. Labeling of experimental sets									
Draft tube	Loading	Loading	Loading	Loading					
DT	0%	10%	20%	30%					
true volume	0	7.2	14.2	21.3					
1DT	1H50	1T1	1T2	1T3					
2DT	2H50	2T1	2T2	2T3					
3DT	3H50	3T1	3T2	3T3					

Liquid velocity

A magnetic tracer method [12] was used to determine liquid velocity in the internal-loop ALR. The method makes use of the principle of a magnetic metal locator and flowfollowing. A magnetic particle with a high magnetic permeability and diameter of 1.1 cm was used as the flowfollower. The particle density was adjusted very closely to the liquid density, which resulted in very low terminal settling velocity (up to 1 cm/s). It utilises non-invasive technique without need of a direct impact on the fluid inside the reactor, e.g. an injection of tracer of electrolyte. The method was developed for purpose of using in fermentation systems with multiphase dispersion and was already successfully tested in two-phase (air-water) system [12]. Moreover, the measuring technique allow to determine liquid velocities, circulation velocity and residence times of tagging particle in individual sections of the airlift reactor.

Gas and solids holdup

A method for simultaneous measurement of gas and solids holdups in gas-liquid-solid (G-L-S) multiphase contactors suggested by Wenge et al. [13] was used. This method was chosen because of its advantage for use in the three-phase fermentation system, where a direct outside contact with fermentation broth should be avoided as much as possible because of risk of contamination. Sidewall samplers are known of giving erroneous measurements of concentration and particle size distribution apart from very small particles with density very close to that of liquid fluid, such as bacteria or yeast.

The method makes use of measurements of hydrostatic pressures in the three-phase dispersion followed by interruption of gas flow, complete gas disengagement, and a second measurement in the resulting two-phase (solid-liquid) dispersion. This measurement period has to be short enough to avoid significant sedimentation of the solid particles. The quick-response differential pressure transducers (Shaewitz Sensors, USA) were used for manometric measurements of pressure differences between two places in the riser, downcomer and separator of the ALR. Signal from the pressure sensors was sampled every 1 s. The positions of measuring points were properly chosen in order to avoid the effect of a liquid acceleration at the bottom and the top of the draft tube [14]. Two values of pressure differences, expressed by the values of the liquid level difference in the manometer Δh_a and Δh_b , are read from digital pressure readouts for all three parts of the reactor (see example in Fig. 2) Then, a calculation of solids holdup in two-phase L-S slurry ϕ_S , solids (ε_S) and gas (ε_G) holdups in the G-L-S dispersion can be done from balance equations of the hydrostatic pressure between two measuring places.



Figure 2. Example of signal acquired from the measurement of pressure differences in the riser (Δh_R) , downcomer (Δh_D) and separator (Δh_S) sections of the TPAL reactor. A vertical line indicates the air supply interruption at 70 s.

First, the solids holdup ϕ_S in the riser, downcomer and separator sections is calculated using the steady value of Δh_b , which corresponds to the manometric reading during a period of the aeration

interruption:

$$\Phi_s = -\frac{\rho_L}{\rho_s - \rho_L} \frac{\Delta h_b}{\Delta z} \tag{1}$$

Here ρ_L and ρ_S are densities of liquid fluid and solid particles, respectively. Δz is a distance between two measuring places at the reactor.

Then, the gas holdup ε_G is calculated using the steady value of the manometric reading Δh_a during the period of the aeration:

$$\varepsilon_{G} = \frac{(\rho_{S} - \rho_{L})\phi_{S} + (\rho_{L}\Delta h_{a}/\Delta z)}{(\rho_{S} - \rho_{L})\phi_{S} + (\rho_{L} - \rho_{G})}$$
(2)

Finally, the solids holdup in the G-L-S dispersion is calculated:

$$\boldsymbol{\varepsilon}_{S} = \boldsymbol{\phi}_{S} \left(1 - \boldsymbol{\varepsilon}_{G} \right) \tag{3}$$

It was observed during measurements that the curve of pressure difference in the separator zone did not exert any local minimum, which corresponds to the volume fraction of solids in the L-S slurry after aeration interruption (see Fig. 2). This was caused by the fact that as soon as all bubbles escaped the head zone, most of particles were already located on the wall of conical part of separator and afterwards moved down along it into the lower sections of the ALR. Thus, the hydrostatic pressure difference was nil corresponding to the liquid only and the solids holdup in the separation zone ε_{SS} was calculated from known values of solids holdup in the whole reactor and in the riser and downcomer zones.

3. RESULTS AND DISCUSSION

In terms of the shape of the top section, the airlift reactor can be classified as follows – airlift reactor without a separator (the separator diameter is equal to the diameter of the outer column) and with a separator (the separator diameter is larger than the diameter of the outer column) [4]. In this work, the airlift reactor with an enlarged head zone was used. However, different separator configurations can be achieved by adjusting the length of the draft tubes. Thus, two basic constructions of the ALR are considered:

- A. The top of the draft tube is located exactly at/above the opening of enlarged zone, the separator consists of only the enlarged section (see Fig. 1B), which acts as bubble separation zone as well as particle settler. Such reactor configuration represents airlift reactor with separator (present configuration 1DT)
- B. The top of the draft tube is located below the opening of enlarged zone inside the downcomer column, the separator consists of two parts (see Fig. 1A) a lower part acting as bubble separation zone with very high mixing intensity and upper enlarged zone acting as the particle settler. This configuration of the head zone was entitled as "dual" separator (present configurations 2DT and 3DT). According to our knowledge, only recently the firsts Vincente et al. [5] and Freitas et al. [15] were used the ALR with this construction of the separator zone for studying the three-phase hydrodynamics.

Effect of solids loading and gas flow rate

The influence of the solids loading and gas flow rate was investigated on all principal operating parameters, which are necessary for the characterisation of the hydrodynamic behaviour of the TPAL – liquid velocity, gas and solids holdup in individual parts of the reactor and total circulation time. Since overwhelming majority of the data hydrodynamics handle with TPAL reactor operating in the batch mode, the first experiments were carried out to test how the continuous ALR differ from the batch one. The measurements were done in the G-L as well as G-L-S system applying the value of the dilution rate of 0.6 h^{-1} . That value represents a conventional upper limit in the high-cell density systems ensuring both an ability of operation and economic feasibility of the bioprocess [16]. The results did not show any relevant differences in the values of all principal hydrodynamic

parameters. For all that, the hydrodynamics of the continuous TPAL reactor can be satisfactorily treated by model equations acquired from the batch ALR systems. All experiments were carried out only in the batch system.

The effect of the solids loading and air flow rate on gas holdups in the riser, downcomer and separator sections is shown in Fig. 3.



Figure 3. The gas holdups in individual sections of the ALR versus air superficial velocity U_{Gc} for different solids loadings of values 0% (xH50), 7.2% (label xT1), 14.2% (label xT2) and 21.3% (label xT3). For explanation of reactor configurations 1DT, 2DT and 3DT see Table 1 and 2.

As it can be seen in Figure 3, all partial gas holdups increased, as expected, in the whole range of air superficial velocity U_{Gc} applied. The gas holdups in the riser ε_{GR} as well as in the separator ε_{GS} grew monotonously with increase of the flow rate immediately from lowest values, however, for high flow rates the growth of the riser holdup slowed down because of high values of liquid velocity and bubble coalescence. The same trend at high U_{Gc} could be seen at the curves of the gas holdup in downcomer ε_{GD} . They display an initial plate due to the fact that higher values of the air flow rate were needed for entrainment of bubbles into downcomer. It can be clearly seen that a significance of this occurrence grew from the configuration 1DT through 2DT to 3DT with dual separator (see Fig. 3A-C).

In most cases, the solids loading had a negative effect on the gas holdup in all reactor sections. And reduced the gas holdup in the riser for all reactor configurations. Similar trends have been achieved by Verlaan et al. [17] and Lu et al. [18] in external-loop and internal-loop ALR, respectively. The solids affect the building-up the gas holdup in the riser and downcomer by two ways: the first, the presence of solids promote bubble coalescence and the second, an addition of solids reduces the flow area of the gas and liquid phases what decrease the gas holdup. The downcomer gas holdup curves in the reactor configurations with the dual separator 2DT and 3DT displayed the same trends with change of the solids loading as well as the air flow rate, except the air-water system in the reactor set 3DT (3H50, see Fig. 3C). Opposite to the 2DT set, pretty high air supply rate ($U_{Gc} = 0.03 \text{ m.s}^{-1}$) was necessary to draw first bubbles into downcomer in the 3DT set. However, the gas holdup grew very fast at higher U_{Gc} values and reached soon much higher values in comparison with all three-phase experiments. This difference originates from distinct crosssectional area ratios A_D/A_R (see Table 1). For that, the liquid velocity in the riser V_{LR} is about four times higher than that in downcomer V_{LD} at the 3DT reactor set. At low air flow rates, the V_{LR} value is already high enough to take out majority of bubbles out lower part of separator coming from the riser. At higher flow rate more than 0.03 m.s⁻¹, an influence of lower separator part is getting dominating over the drifting force of liquid coming from riser section resulting in building-up the gas holdup in the downcomer. In three-phase system, the downcomer gas holdup grows up at much lower air supply rates. It is due to the reduction of possibility of bubbles to escape lower part of separator due to the particle presence. The solids slow down or stop moving up the bubbles and enable them to be entrained into the downcomer.

In the case of the reactor set 1DT, a situation was anomalous. The downcomer gas holdup was much lower (two –three times) than in the case of the reactor with the dual separator (2DT and 3DT set). At first, the ε_{GD} curves rose up with the increase of solids loading, reached maximum at the value of 14.2 % and after the holdup fell down almost at the level of two-phase dispersion. This surprising fact results from a distinct distribution of bubble size in the downcomer for this reactor set (ALR with separator). In comparison with the reactor set with dual separator (either 2DT or



Figure 4. The gas holdups in downcomer versus solids loading $\phi_{s,tot}$ equal to 0% (xH50), 7.2% (label xT1), 14.2% (label xT2) and 21.3% (label xT3). For explanation of reactor configurations 1DT, 2DT and 3DT see Table 1 and 2.

3DT), where a lower narrow part of the separator enables to draw substantial amount of bubbles of different sizes, only fine bubbles (with diameter of about 2-4 mm) are entrained into downcomer section. The magnitude of the liquid circulation velocity is enough to recirculate most of them back to the riser. Thus, a residence time of bubbles is short resulting in low gas holdups (see Fig. 3A and 4). In this situation, a presence of solids supports bubbles coalescence events and helps to keep them inside the downcomer. This mechanism works up to a certain border of the solids loading (14.2%). With high solids concentration, the solids promote large bubbles enabling them to rise up and escape the downcomer.

The gas holdup in the separator ε_{GS} is almost independent from the amount of solids loading. It is probably due to a low concentration of particles in this section, even at highest values of the air flow rates. However, in case of 2DT and 3DT reactor set, the slight reduction of ε_{GS} was observed with the rise of solids loading, which was more pronounced in the 3DT set.

The linear liquid velocities in the riser and downcomer V_{LR} and V_{LD} rose up with the increase of the air flow rates and the velocity curves showed an expected logarithmic shape. At another hand, an independence of linear velocity on the change of solids loading was observed. At first glance, it is a surprising fact that the linear

liquid velocity did not change despite expected increasing bubble coalescence events and friction loss what should result in decrease of velocity. Nevertheless, as solids holdup increases with the increase of the solids loading in main reactor sections, free cross sectional area for liquid flow decreases. The magnitude of resulting true velocity thus will be dependent, which effect will be dominant – either decrease of free area for liquid flux or increase of friction loss and decrease of driving force. In order to find out how the liquid flux varies with solids loading, the superficial liquid velocities in main reactor sections U_{LR} and U_{LD} were calculated.



Figure 5. The superficial liquid velocity in main sections of the ALR U_{LR} and U_{LD} and circulation time t_C versus air superficial velocity U_{Gc} for different solids loadings of values 0% (xH50), 7.2% (label xT1), 14.2% (label xT2) and 21.3% (label xT3). For explanation of reactor configurations 1DT, 2DT and 3DT see Table 1 and 2.

Figure 5 shows the change of superficial liquid velocity in main reactor sections U_{LR} and U_{LD} and circulation time t_C with the air flow rate and the solids loading for different ALR configurations. The Figure displays decline of liquid flux with the solid loading in all investigated cases. The curves of liquid velocities U_{LR} and U_{LD} for all reactor configurations exhibit the break point at lower air flow rates, where a slope of the curve suddenly decreases. This point corresponds well to the point of a transition of two-phase circulation regime, i.e. entrainment of bubbles into downcomer appears [19]. This is more distinct in the ALR configuration with dual separator (2DT and 3DT). Moreover, the breakpoint was more pronounced in the system without solids and low solids loading; for higher solids loadings the regime transition was damped and the onset of penetration of bubbles into downcomer was gradual.

The effect of the solids loading on the circulation time was negligible; no evident increase of overall circulation velocity could be indicated.

Solid distribution in the ALR

The change of the solids holdup in all sections of ALR with the solids loading and air flow rate is shown in Figure 6.

For all ALR configurations investigated, the solid holdup in the riser ε_{SR} fell down with increase of the air flow rate. This decline deepened with growth of the solids loading. At highest air flow rates; the steady values of ε_{SR} were attained for solid loading besides the highest one. As was expected, the ε_{SR} grew with the increase of solids loading; however, one exception was observed in the configuration with long draft tube 1DT – the holdups ε_{SR} were very similar for two highest solids loadings (14.2 and 21.3 %). The same was valid for values of solids holdup in the downcomer. It means that this increased amount of solids inside the reactor was distributed solely into separator zone. The solids holdup in the downcomer exhibited the similar dependence on change air flow rate and solids loading and their values have been lower than that in the riser. The significantly lowest values of solids holdup were attained in the enlarged separator zone, where the amount of solids rose up gradually with growth of air flow rate.



Figure 6. The superficial liquid velocity in main sections of the ALR U_{LR} and U_{LD} and circulation time t_C versus air superficial velocity U_{Gc} for different solids loadings of values 0% (xH50), 7.2% (label xT1), 14.2% (label xT2) and 21.3% (label xT3). For explanation of reactor configurations 1DT, 2DT and 3DT see Table 1 and 2.

4. CONCLUSION

The ALR with dual separator can be successfully applied to the batch/continuous three-phase system, where efficient separation of particles from the liquid phase and keeping the bubbles inside the reactor is profitable. Moreover, the lower part of the dual separator acts as efficient mixer, which can significantly help to improve the overall mixing in the ALR.

The results showed that hydrodynamics of the three-phase ALR operating in the continuous mode in the range of the dilution rates used in the fermentation technology can be satisfactorily described with model equations of batch ALR. The measuring techniques suggested for a characterisation of hydrodynamics, allowed us to acquire various important information on the multiphase flow and distribution of gas and solid phases in the ALR. The results showed similar the solids distribution in the riser and downcomer, however, even distribution was achieved only at high flow rates, particularly when the reactor operated with highest solids loading equal to 21.3 % (v/v). Measurements of solids holdup in the enlarged separator zone revealed very low solids concentrations and its low sensitivity to the magnitude air supply rates.

According to the results with different separator and draft tube configurations, hydrodynamic considerations on an optimal bioreactor design have been done. The best solids distribution was achieved in the ALR configuration with dual separator 2DT and cross-sectional area ratio A_D/A_R equal to 1.2, where highest steady values of solids holdup in the separator and equal solids holdup in the riser and downcomer at lower air flow rates were attained. In comparison with the ALR configuration 1DT with longer draft tube and absence of lower separation part, significantly higher gas holdup in the downcomer was observed. Moreover, comparing with the reactor set 3DT with high ratio A_D/A_R equal to 4.0, gentler hydrodynamic environment is provided for application in bioprocesses using particles that are sensitive to shear.

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