

## Continuous flocculation airlift bioreactor with high cell loading – hydrodynamic and rheological aspects

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### INTRODUCTION

Cheese whey, a by-product of dairy industry, represents a well-known significant environmental problem due to its very high BOD and COD values. Modern dairy industry, recently more concentrated into less but larger production plants, does not look at whey as a disposal problem any more; cheese whey is seen as a potential source of additional profit. Hence, bioutilization of the cheese whey has become a rapidly growing branch of dairy industry and science. One of the attractive possibilities for whey disposal is a coupled system consisting of ultrafiltration for protein recovery followed by the alcoholic fermentation of lactose from whey permeate. At later step of the process, a continuous high cell density system with flocculating yeast can be efficiently used. A recombinant flocculating strain of *Saccharomyces cerevisiae* containing genes (lactose permease and b-galactosidase) for lactose transport and hydrolysis has been constructed for this purpose (Domingues et al. 1999).

In practice, such a system usually represents a three-phase (gas-liquid-solid) dispersion operating in a continuous mode. For this system, an airlift bioreactor (ALR) with an enlarged head zone seems to be a very attractive option due to a high retention of the solid phase and the advantageous combination of sufficient mixing and low shear stress. Due to the very high solids loading (up to 50-60 % vol.) the maintenance of the solid particles in suspension and circulating is of a particular importance requiring a suitable bioreactor design and a correct regulation of the sole energy input – gas flow rate.

Three basic flow regime in three-phase airlift reactor were defined (Fan et al. 1984): the packed bed regime without suspension of particles and no liquid circulation, the fluidised bed regime with particle suspension exclusively in the riser zone and nonzero liquid circulation and the circulated bed regime with solids distribution throughout the reactor. For high cell density system, the circulated flow regime is highly desirable having the best even solid distribution throughout the ALR. In airlift bioreactors with a well-defined liquid circulation loop, the liquid velocity is the major hydrodynamic parameter, which considerably affects all physical phenomena (Chisti 1989). Despite an extensive number of velocity measuring methods applied to ALRs (Boyer et al. 2002), most of these techniques are not suitable for use in fermentation processes for several reasons (tagging of liquid elements with chemicals due to their interference with the strictly controlled composition of the fermentation medium and sterility problems, visual techniques as Laser Doppler Anemometry (LDA) or Particle Image Velocimetry (PIV) due to the opaqueness of the broth). The use of small flowfollowing particles with non-invasive detection of their movement can be efficiently used in such biosystems. Detection technique using inductive coils and magnetic particle is one of the cheapest and the simplest methods. The technique was already successfully tested to

measure the particle and liquid velocities in an internal-loop airlift bioreactor with two- and three-phase model system (alginate beads) (Klein et al. 2003a) as well as during gluconic acid production (Dolgoš et al. 2001).

Knowing a magnitude of circulation velocity during bioprocess can give us a prompt and useful information on the partial processes occurred in bioreactor – circulation regime of three-phase flow (Klein et al. 2003b), RTD of bioparticles and mixing intensity, mass transfer, shear stress on bioparticles affecting their physical and colloidal properties (e.g. size, shape, viscosity of suspensions) in individual parts of ALR, etc. Moreover, keeping the solid phase in a circulating state is a crucial factor for successful operating high cell density airlift bioreactor. A collapse of net circulation (bioreactor stalling) would have very negative effect on overall functionality and productivity of fermentation. Thus, avoiding the bioreactor stalling applying an optimal bioreactor design and suitable operating conditions (circulation velocity, gas flow rate, biomass concentration) is one of major tasks of biochemical engineer when operating an airlift bioreactor with high cell loading.

The main goal of this work was to study the hydrodynamics of a continuous airlift bioreactor during ethanolic fermentation of lactose using highly flocculating yeast. The magnetic particle-tracer method was used for the assessment of the effect of operating conditions (air flow rate, biomass concentration) on hydrodynamics of the airlift bioreactor as a whole and in its individual sections. Analysis of rheological properties of floc suspension at different concentrations was carried out to give explanation for variations in circulation velocity during the fermentation process.

## **MATERIALS AND METHODS**

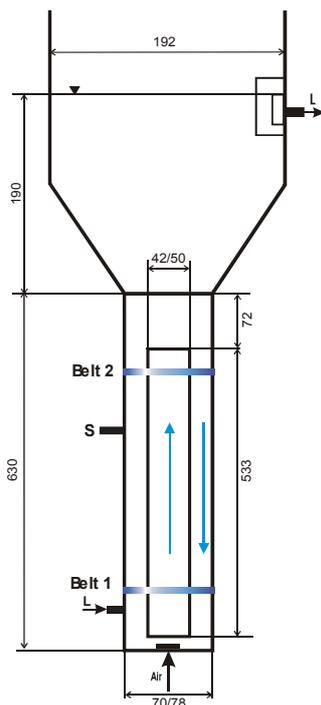
### **YEAST STRAIN AND CONDITION OF CULTIVATION**

A recombinant *Saccharomyces cerevisiae* NCYC869-A3/T1 flocculent strain expressing the LAC4 (coding for  $\beta$ -galactosidase) and LAC12 (coding for lactose permease) of *Kluyveromyces fragilis* was used. The construction of the recombinant strain was described by Domingues et al. (1999).

The composition of the semi synthetic medium used for cultivation and fermentation was as follows (in g.dm<sup>-3</sup>): Lactose, 50; KH<sub>2</sub>PO<sub>4</sub>, 5; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.4 and yeast extract, 1. Foam level was controlled by adding Antifoam 204 (SIGMA, Germany). The inoculum was cultivated the first in 100 ml of the media in a 250 ml Erlenmeyer flask for 24 hours and then in a 1 litre of the media in a 2 l Erlenmeyer flask for another 24 hours at a rotary shaker at 150 rpm and 30 °C. A 1 litre of media was finally passed aseptically into a 6-litre airlift reactor where it was kept in the batch mode under aeration (0.1 vvm) until 90% of lactose was consumed. The temperature was maintained at 30 °C, the pH automatic adjusted at 4.0 by automatic addition of ammonia solution.

### **BIOREACTOR AND OPERATIONAL CONDITIONS**

A 6 L internal loop airlift reactor with a dual enlarged degassing zone made of Plexiglas was used. The head zone had the shape of a reversed cut cone with a cylindrical overhead. The conical section formed a 50° angle with the main body of the reactor. The dimensions of the reactor are given in Fig. 4. The diameter of the draft tube was 42/50 mm, and the A<sub>D</sub>/A<sub>R</sub> ratio was 1.36. The air injection was made via perforated plate with the diameter of 16 mm, with 10 holes each of 0.5 mm in diameter and 25 mm below the bottom of inner draft tube. The wash-out of biomass was minimised by means of an efficient sedimentation barrier around a drainage tube for liquid outlet.



**Figure 1.** Scheme of a 6 dm<sup>3</sup> continuous airlift bioreactor. Symbols: S - sample port, L feed and outlet. Belts 1 and 2 indicate a position of measuring coils for the liquid velocity measurement by magnetic tracer method.

The temperature inside the reactor during continuous operation was maintained at  $30 \pm 0.5$  °C by means of a heating coil connected to a thermostat. The pH control using a pH electrode (METTLER TOLEDO, Switzerland) and pH controller (HANNA, Portugal) was realised by automatic addition of ammonia solution, being the set point fixed at  $4 \pm 0.1$ . An efficient condenser was located at the top of the bioreactor in the line of outlet gas to ensure entering dry gas into the gas analyser (Tandem, ADAPTIVE BIOSYSTEMS, UK).

The reactor was operated at various dilution rates from 0.08 to  $0.4 \text{ h}^{-1}$  and aerations from 0.63 to  $2.76 \text{ l} \cdot \text{min}^{-1}$  (referred to 20 °C and 1 atm) or from 0.1 to  $0.45 \text{ vvm}$  (normal volume of air per volume of reactor per minute) The air flow rates were controlled by means of a rotameter and filtered by a microbiological filter (ZANDER, Germany). The  $\text{pO}_2$  was measured by a  $\text{pO}_2$  electrode (METTLER TOLEDO, Switzerland) connected to oxymeter (BROADLEY TECHNOLOGIES, USA).

Data acquisition of all process parameters (composition of outlet gas- $\text{CO}_2$ ,  $\text{O}_2$ , pH,  $\text{pO}_2$ , inlet pressure and temperature) was done by an A/D converter *API III* connected to PC software (SAMPLE, Slovakia).

## ANALYSIS METHODS

### *Biomass concentration*

Gravimetric method was used to monitor the dry weight cell concentration. A sample of approximately 15 ml of biomass suspension was taken through the sample port in the centre point in the downcomer and then filtrated by  $0.45 \mu\text{m}$  filter paper (PALL CORPORATION, USA). The biomass filtrate cake was dried at 105 °C until constant weight. It was shown in the previous work (Klein et al. 2003a) that it is reasonable to assume for low-density particles (e.g. alginate beads, yeast flocs) that the even distribution in both main ALR sections (riser and downcomer). Thus, biomass concentration measured in the downcomer was used in hydrodynamic analysis of ALR as a representative value of biomass quantity. Since the net circulation flow takes place only in the main

vertical reactor sections, a lower biomass concentration in head enlarged zone was not taken into account.

#### *Density of biomass suspension and liquid phase*

Density of biomass suspension and liquid phase was determined by pycnometry using a 100 ml pycnometer. Well-mixed suspension was transferred into the pycnometer and was thermostated to reach a constant temperature equal to that in bioreactor (30 °C). After determining the density of biomass suspension, the flocs were removed and the density of liquid medium was measured by the same procedure. It was found a linear dependence between the yeast suspension and dry biomass concentration:  $\rho_{FL} (kg.m^{-3}) = (1000.43 \pm 0.98) + (0.246 \pm 0.018) c_x$ . The suspension density was then used for a calculation of effective settling velocity of tagged particle necessary for determination of liquid velocity.

#### *Floc density*

Floc density was determined measuring the weight and volume of flocs. Approximately 1 l of biomass suspension was taken out of the bioreactor. The biomass was several times washed with distilled water then the flocs were inserted into the  $500 \pm 0.24$  ml and  $1000 \pm 0.4$  ml volumetric flasks. The flasks were filled with the known volume of distilled water. From the volume and weight of distilled water the corresponding volume and weight of flocs were calculated to obtain floc density. The floc density ( $\rho_S$ ) appeared constant during the whole fermentation with average value of  $1066.37 \pm 2.18$  kg.m<sup>-3</sup>.

#### *Total solid hold-up*

From known values of floc density, liquid-phase density and dry biomass concentration it is possible to calculate volumetric fraction of flocs,  $\varepsilon_S$ , in the CALR as follows (Vicente et al. 1999):

$$\varepsilon_S = \frac{f c_x}{\rho_S - \varepsilon_p \rho_L} \quad (1)$$

Here the following symbol represent:  $f$  ratio between the total weight of a cell and its dry weight,  $c_x$  dry biomass concentration,  $\varepsilon_p$  floc porosity and  $\rho_S$  and  $\rho_L$  densities of flocs and liquid phase, respectively.

The factor  $f$  was considered to be approximately 10/3, corresponding to about 70% of water in the cells (Vicente et al. 1999) and  $\varepsilon_p$  was considered to be 0.50 (Teixeira and Mota 1990).

### **HYDRODYNAMIC MEASUREMENT**

A magnetic tracer method (Klein et al. 2000) was used to determine liquid velocity in the airlift reactor. The method makes use of the principle of a magnetic metal locator and flowfollowing technique. Two inductive coils were fixed around the column in a mutual vertical distance of 441 mm to detect the transition of the magnetic tracer particle (see Fig. 1). The measuring technique allow to determine liquid velocities, circulation velocity and residence times of tagging particle in individual sections of the airlift reactor. The overall circulation velocity ( $V_{LC}$ ) was determined as average velocity in two main reactor sections (riser and downcomer) assuming that tagged particle does not reside in the head separator zone.

### **RHEOLOGICAL MEASUREMENT**

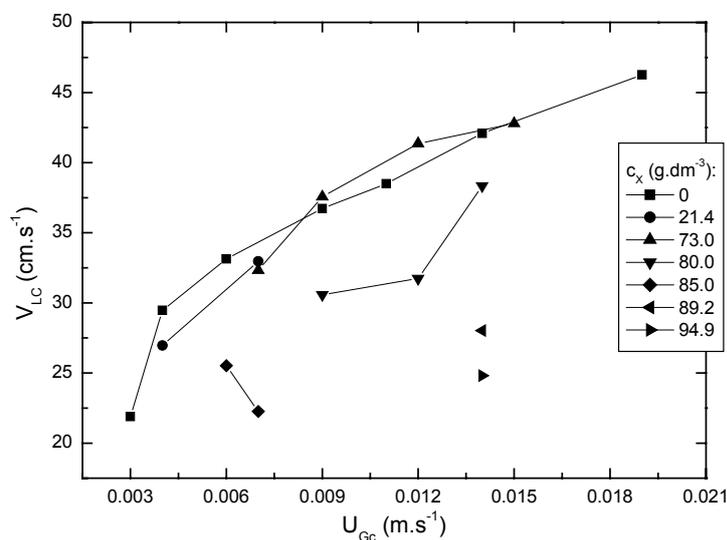
The viscosity of the floc suspension at different biomass concentration was measured by Modular Compact Rheometer Physica MCR 300 (Paar-Physica). Controlled shear-stress measurements were done using plate-plate system at constant temperature of 30 °C. The plate used had 2.5 cm in diameter. According to preliminary tests with various values of gap between plates, gap of 2 mm was chosen for all measurements. The shear stress in the interval of 0.1-200 Pa in logarithmic scale was applied. The sample with desirable concentration was prepared and homogenized a priori measurements, then appropriate volume was placed on base plate and was left

for few minutes to thermostat on constant temperature. Then, rheological measurement was done three times for one value of biomass concentration using always fresh sample.

## RESULTS AND DISCUSSION

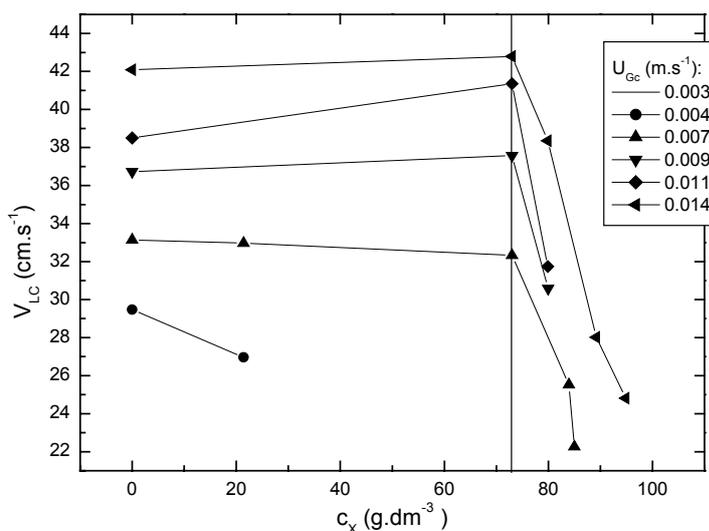
### HYDRODYNAMICS IN AIRLIFT BIOREACTOR

The particle with high relative magnetic permeability was aseptically inserted into the bioreactor at beginning of fermentation; thus available for velocity measurements at any process time. As the biomass was being accumulated during the whole fermentation, hydrodynamic measurements have been carried out to obtain information how circulation velocity varied with biomass concentration. Moreover, short-term changes of air flow rate were applied to determine its effect on bioreactor hydrodynamics. The effect of superficial gas velocity,  $U_{Gc}$ , on overall circulation velocity,  $V_{LC}$ , at different biomass concentrations is shown in Fig. 2. As expected, the circulation velocity increased with  $U_{Gc}$  following a common logarithmic curve. It is evident from the graph that the increase of gas flow rate improves the net circulation flow up to cca. 80 g.dm<sup>-3</sup> of biomass concentration. Unfortunately, few experimental data were available at higher  $c_X$  concentrations; thus, any further conclusions on effect of  $U_{Gc}$  at the highest  $c_X$  would be only speculative.



**Figure 2.** Effect of superficial gas velocity,  $U_{Gc}$ , on overall circulation velocity,  $V_{LC}$ , at different biomass concentrations in the bioreactor,  $c_X$ . A tap water was used for measurements at zero  $c_X$ . A range of  $U_{Gc}$  applied corresponds to the range of air flow rates  $Q_G$  from 0.1 to 0.45 vvm

The effect of biomass concentration on hydrodynamics during the fermentation process is shown in Fig. 3. The results revealed that the dependence of the overall circulation velocity on biomass concentration does not present a monotonous behaviour for the tested concentration values as was expected. Surprisingly, as the biomass accumulates the velocity  $V_{LC}$  was kept at approximately constant value for any value of air flow rate. This initial plateau on the velocity-biomass concentration curve at lower  $c_X$  concentrations was followed by a sudden steep decline indicating a strong degradation of net circulation flow. A dramatic decrease of liquid velocity beyond the critical biomass concentration  $c_{Xcrit}$  equal to 73 g.dm<sup>-3</sup> corresponding to 42.8 % vol. of solids fraction (see solid vertical line in Fig. 3) was found for all air flow rates applied, in an average by 20 % from 73 to 80 g.dm<sup>-3</sup>.



**Figure 3.** Effect of biomass concentration,  $c_x$ , on overall circulation velocity,  $V_{LC}$ , at different superficial gas velocity,  $U_{Gc}$ . A solid line marks a critical value of biomass concentration,  $c_{xcrit}$ . A range of  $U_{Gc}$  applied corresponds to the range of air flow rates  $Q_G$  from 0.1 to 0.45 vvm.

This breakpoint was found to be a crucial parameter for the operation of a high cell density airlift bioreactor, representing the onset of potential appearance of bioreactor stalling (stopping the net circulation). There was also an attempt to experimentally determine the maximal biomass concentration allowing operating the airlift bioreactor in a circulated three-phase flow regime. During the whole fermentation no natural regulation of biomass concentration inside the bioreactor was observed. The system continuously accumulated biomass up to a point when the reactor stalling occurred. The biomass started to accumulate at the bottom part of downcomer resulting in the break up of the net circulation. The maximal available concentration of biomass ( $c_{xmax}$ ) was found to be higher than the critical value and linearly dependent on air flow rate (e.g. for highest air flow rate of 0.45 vvm  $c_{xmax}$  was higher than the critical value by 40 %).

It is worth to notice another important fact implying from the velocity curves in Fig 3. The critical value of biomass concentration at the breakpoint was found to be independent of the gas flow rate. It means that on one hand an increase of air flow rate will increase circulation flow rate but on the other hand it will not help ever to increase the critical limit for biomass concentration to avoid the strong decrease of the circulation velocity.

#### RHEOLOGY OF FLOC SUSPENSION

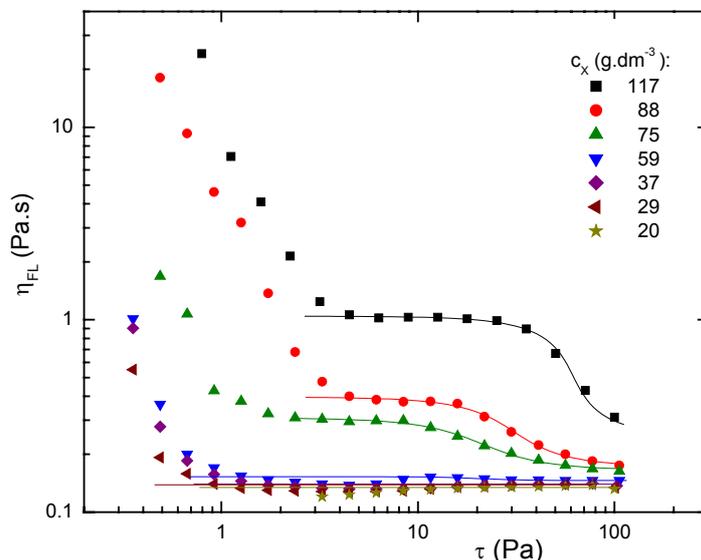
One of possible reasons of such sudden decrease in circulation velocity with biomass concentration could be a change of viscosity of yeast floc suspension. Hence, the rheological analysis of floc suspension in the whole range of biomass concentrations was carried out. Then, appropriate rheological model was suggested to describe viscosity curves. The Cross viscosity model was found to fit best the experimental data, which has a capability of handling Newtonian regions of shear-thinning fluids at low and high shear rates:

$$\eta_{FL} = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{(1 + m \dot{\gamma}^n)} \quad (2)$$

Here  $\eta_{FL}$  is apparent viscosity of floc suspension,  $\eta_0$  and  $\eta_{\infty}$  are viscosities of Newtonian regions for low and high shear rates respectively,  $\dot{\gamma}$  is shear rate,  $m$  and  $n$  are correlation coefficients.

Experimental viscosity curves with fitting lines are depicted in Fig. 4. The first points at low shear stresses were not considered in the regression analysis due to not fully developed flow. One

can see that the rheological behaviour of suspension of yeast flocs significantly changes with its concentration. At the highest  $c_X$  values, the suspension possesses the pseudoplastic behaviour with two Newtonian regions. However, at biomass concentration lower than  $59 \text{ g.dm}^{-3}$  constant viscosity was observed indicating only the Newtonian behaviour of floc suspension.



**Figure 4.** Viscosity curves as a function of shear stress  $\tau$  for different biomass concentrations  $c_X$ . The lines represent the fittings using the Cross model. All measurements were done at  $30^\circ\text{C}$ .

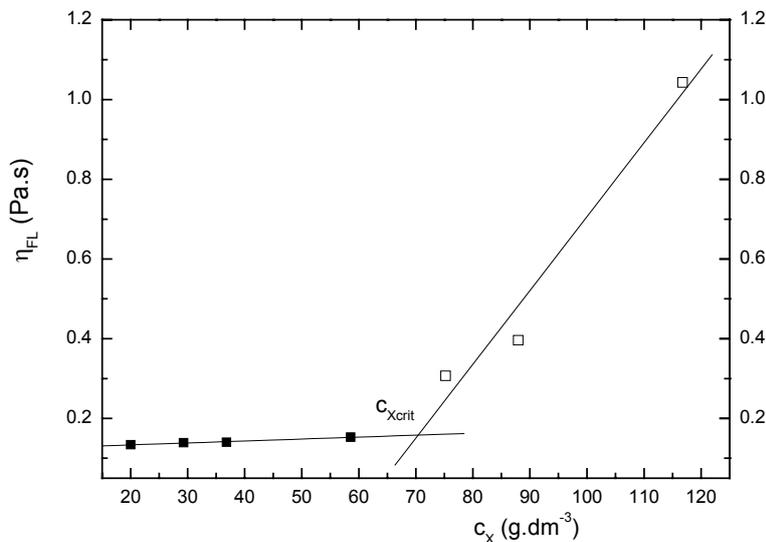
Several authors reported viscoelastic behaviour of yeast suspensions. Labuza et al. (1970) reported shear-thinning behaviour of baker's yeast (*S. cerevisiae*) in the range of  $1$  to  $100 \text{ s}^{-1}$  at yeast concentrations above  $10.5\%$  (w/w). The power-law model was successfully applied. More recently, Mancini and Moresi (2000) measured rheology of baker's yeast too using different rheometers in the concentration range of  $25$  to  $200 \text{ g.dm}^{-3}$ . While Haake rotational viscometer confirmed Labuza's results on the pseudo-plastic character of yeast suspension, dynamic stress rheometer revealed definitive Newtonian behaviour. This discrepancy was attributed to the lower sensibility of Haake viscometer in the range of viscosity tested ( $1.5$ - $12 \text{ mPa.s}$ ). Speers et al. (1993) used controlled shear-rate rheometer with plate-cone system to measure viscosity of suspensions of flocculating and nonflocculating strains of *S. cerevisiae* and *S. uvarum*. They derived "cell floc" model, which used the Bingham model for description of viscoplastic flow behaviour of cell suspension:

$$\eta = \eta_\infty + \frac{\tau_0}{\dot{\gamma}} \quad (3)$$

Here  $\tau_0$  is yield stress.

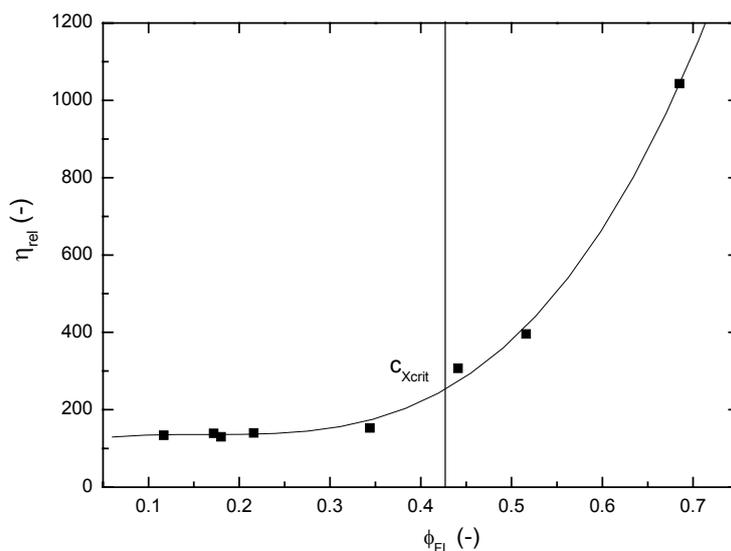
The viscosities observed were in range of  $4.6$  to  $26.9 \text{ mPa.s}$  for shear rates up to  $1000 \text{ s}^{-1}$  and concentration of  $2.5 \times 10^9 \text{ cells/ml}$  measured at  $15^\circ\text{C}$ . They also discovered important fact: the flocculating strains had higher both apparent viscosity and yield stress than nonflocculating ones. It indicates that the morphology of yeast suspension have a significant impact on its flow behaviour. It is hardly possible to compare quantitatively the viscosities of yeast suspension due to strong relation of the viscosity of yeast suspension to flocculating properties of yeast strain and other factors reported e.g. in work of Speers et al. (1992). However, it can be concluded from available literature that yeast suspension below certain concentration displays the Newtonian flow behaviour, whereas above this limit the flow behaviour changes to either shear-thinning or viscoplastic ones.

**RELATION OF HYDRODYNAMICS TO RHEOLOGY**



**Figure 5.** Dependence of viscosity of floc suspension on biomass concentration.  $c_{Xcrit}$  is critical value of biomass concentration determined from an intersection of two regression lines.

Figure 5 shows the dependence of apparent viscosity of floc suspension on biomass concentration. It clearly demonstrates a dramatic increase of viscosity at  $c_X$  in the interval of 60 to 75  $g.dm^{-3}$ . Determining accurately the point of the strongest increase of viscosity, intersection of two regression lines for low and high ranges of biomass concentration was calculated. The value of 70.4  $g.dm^{-3}$  was found, which is in a very good agreement with the critical biomass concentration determined from the hydrodynamic measurements (3.7 % of experimental error). This finding explains a strong decrease of circulation velocity when the biomass concentration overruns its critical value. If biomass accumulation continues, the net circulation slows down having a significant negative impact on all important phenomena of the bioprocess (mass and heat transfer, mixing, distribution of solid phase, kinetics) and increasing the risk of clogging of bioreactor.



**Figure 6.** Polynomial regression of relative viscosity of floc suspension  $\eta_{rel}$  as a function of floc volumetric

fraction  $\phi_{FL}$ .  $c_{Xcrit}$  is critical value determined from velocity measurements as a point of the biggest decline of  $V_{LC}$ .

The experimental data were used to describe the dependence of yeast suspension viscosity on biomass concentration. The relative viscosity  $\eta_{rel}$  in dependence on the volumetric solid fraction  $\phi_{FL}$  (% vol.) can be satisfactorily correlated by polynomial regression as follows:

$$\eta_{rel} = \frac{\eta_{FL}}{\eta_L} = 109.3 + 500.5 \phi_{FL} - 3096.6 \phi_{FL}^2 + 6353.2 \phi_{FL}^3 \quad (4)$$

The regression curve is shown in Fig. 6, where  $c_{Xcrit}$  is critical value determined from velocity measurements as a point of the biggest decline of  $V_{LC}$ .

## CONCLUSIONS

The hydrodynamic analysis of high cell density airlift bioreactor during the alcoholic fermentation showed dramatic changes in circulation velocity. Gas flow rate and especially biomass concentration were found to mostly affect the bioreactor hydrodynamics. Measurements of liquid circulation velocity showed the existence of a critical value of biomass concentration, at which a dramatic deceleration of net liquid flow appeared with increasing biomass quantity. Rheological analysis revealed a direct dependence of net circulation velocity in airlift bioreactor on apparent viscosity of yeast floc suspensions.

From the practical point of the use of airlift bioreactor for high cell density system, the bioreactor should operate below the critical biomass concentration to ensure a safe operation in desirable circulated bed flow regime. The ALR can be operated with higher solids loading up to maximal solids loading; however, having negative effect on transport phenomena, mixing and solid distribution in the bioreactor. A prompt information on hydrodynamics in the airlift bioreactor can be used a priori or during the bioprocess to optimise operational parameters in order to avoid any occurrence of undesirable bioreactor stalling and to maximize the process productivity.

Further experiments with different reactor configuration and at bigger scale will be performed to verify and generalize these findings in relation of airlift bioreactor hydrodynamics to rheology of floc suspension.

## Acknowledgment

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## NOMENCLATURE

A	cross-sectional area, (m <sup>2</sup> )
c	concentration, (g.dm <sup>-3</sup> )
f	factor corresponding to about 70% of water in the cells
m	regression coefficient
n	regression coefficient
$U_{Gc}$	superficial gas velocity referred to the riser diameter (averaged at geometrical centre of column), (m.s <sup>-1</sup> ),
$V_{LC}$	overall circulation velocity, (m.s <sup>-1</sup> )

### Greek letters

$\varepsilon_p$	floc porosity
$\dot{\gamma}$	shear rate, (s <sup>-1</sup> )
$\eta$	viscosity, (Pa.s)
$\eta_0$	suspension viscosity at low shear rates, (Pa.s)
$\eta_\infty$	suspension viscosity at high shear rates, (Pa.s)
$\Phi$	volumetric solid fraction, (-)
$\rho_s$	density of flocs, (kg.m <sup>-3</sup> )

$\rho_{FL}$	density of flocs suspension, ( $\text{kg}\cdot\text{m}^{-3}$ )
$\tau$	shear stress, (Pa)

#### Subscripts

C	overall circulation
D	downcomer
crit	critical value
G	gas
L	liquid
max	maximal value
R	riser
rel	relative
X	biomass dry weight

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