Flocculation bioreactors – engineering based problems

António A. Vicente - José A. Teixeira

Centro de Engenharia Biológica – IBQF, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal, phone: +351.253604400; fax: +351.253678986; e-mail: jateixeira@deb.uminho.pt

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Abstract

The use of flocculation bioreactors is one of the most interesting ways to provide an increase of the productivity of biotechnological processes.

As other high cell density systems, flocculation bioreactors allow the achievement of high biomass concentrations in each moment inside the bioreactor as well as an improvement of downstream processes.

Besides these advantages, shared by all high cell density systems, flocculation bioreactors are clearly more interesting due to the absence of a support since it is well known that price is a limiting factor for the implementation of those systems.

This being so, there are engineering problems that need to be addressed. In first place, the hydrodynamics of three-phase airlift bioreactors are considered, followed by the evaluation of the mass transfer properties both from gas to liquid and from liquid to solid phases.

Finally, applications of flocculation bioreactors are discussed and examples are presented.

Introduction

The bioreactor is a vessel that should provide the nutritional and physiological environment required for microbial growth: it is the core of a bioprocess.

Immobilised catalysts (cells, enzymes, protoplasts, organelles) can be employed in various types of reactor, depending on the immobilisation technique, as well as on the type of the process. The aim of such systems is to increase catalyst concentration while keeping it inside the bioreactor, in order to increase process productivity. An example of such high cell density systems is given by the production of ethanol using alginate-immobilised yeast (Gough and McHale 1998, Joekes et al. 1998, Nguyen and Shieh 1992, Tyagi et al. 1992) or flocculating yeast (Abate et al. 1996, Sousa et al. 1994a, Jianfeng et al. 1998).

Basic bioreactor configurations are usually considered, namely the stirred tank, the packed bed, the fluidised bed, the bubble column and the airlift. To choose the best reactor type to such high cell density systems studies on mixing, heat and mass transfer between the different phases, as well as an evaluation of operational and maintenance costs have to be made.

With flocculating organisms, suitable hydrodynamic conditions must be provided (especially low shear stress) in order to maintain the adequate floc size, shape and density characteristics necessary to retain biomass inside the bioreactor as much as possible, although keeping the maximum possible activity. For these reasons, stirred vessels and packed beds are not recommended as flocculation bioreactors, although they are suitable for other immobilised cell systems which are mechanically more resistant. The fluidised bed is not very adequate for flocculating cultures, either, as the density difference between flocs and medium is rather small and fluidisation would be achieved at very low air/liquid flow rates. Bubble columns and especially airlift reactors are quite appealing to be used with three-phase systems in processes involving flocculating organisms (Ganzeveld et al. 1995, Kennard and Janekeh 1991, Merchuk et al. 1994, Onken and Weiland 1983, Siegel and Robinson 1992).

The evaluation of the performance of flocculating cultures in airlift reactors depends on the
understanding of the following parameters (Michalski 1992): gas hold-up, gas-liquid interfacial area, volumetric phase distribution, liquid mixing time, liquid circulation velocity, liquid and gas phase axial dispersion coefficients, fluid-wall heat transfer coefficients and cell retention capacity. Especially for this last parameter the flocculation ability of the microorganism is a major help as it contributes significantly to the cell retention capacity of the bioreactor (Sousa et al. 1994a). The overall volumetric mass transfer coefficient as well as liquid-solid and intra-particle mass transfer are phenomena of capital importance, particularly in a flocculation bioreactor, that will be dealt with later on in a separate section. The relations between the variables in terms of their influence in bioreactor hydrodynamic behaviour have been summarised in Figure 1.

![Figure 1: Relations between variables in airlift reactors (adapted from Merchuk et al. 1996).](image)

Anyway, independently of their oxygen transfer capability (which may or may not be needed, depending on the process), mixing (of both liquid and gas) must be adequate in order to minimise mass transfer resistance and to provide the desired homogeneity inside the bioreactor. Mixing time is a parameter directly related with mixing and it is useful to evaluate the degree of mixing in the bioreactor; gas flow rate is a good control variable as it directly influences not only liquid circulation velocity but also gas hold-up both in riser and downcomer. Thus, correct regulation of the gas flow rate will be crucial to keep solids circulating as well as to optimise the hydrodynamic conditions inside the reactor. All the above-mentioned parameters are, in turn, influenced by the design of one or several of the parts that compose an airlift.

As one of the main features of high cell density systems is the high hold-up of the solid phase (that can go up to 50 - 60% v/v of the total bioreactor volume, with the corresponding reduction in liquid-phase volume) a significant research effort has been devoted to optimise the design of several parts of three-phase airlift reactors, namely in those aspects related to their use as high cell density systems. As previously stated the use of flocculent microorganisms requires a surplus of attention when designing a reactor in order to keep flocs with the suitable characteristics for the process (shape, density and size, mainly).

**Hydrodynamics of three-phase internal- and external-loop airlift bioreactors**

The effect of the distributing plate orifice diameter, airflow rate, solids loading and solids density on the hydrodynamic characteristics – gas holdup, circulation time and liquid velocity – of a three-phase external-loop airlift reactor was characterized.

Experiments were performed in a glass wall external-loop airlift reactor with a working volume of 60 l (Figure 2a).
Air was used as the gas-phase and injected through perforated plates with a constant free plate area ratio \( \phi = 0.2\% \) and hole diameters of 0.5 mm, 1.0 mm and 1.6 mm. The airflow rate was varied between 2100 l/h and 11800 l/h corresponding to riser superficial gas velocities, based on the riser cross-section area, between 0.03 m/s and 0.17 m/s, respectively.

Water was used as liquid-phase and Ca-alginate beads, with two different densities, were used as solid-phase simulating yeast cell flocs. The mean diameter and density of the “low density solids” (LD) were \((2.131 \pm 0.102)\) mm and \((1023 \pm 1)\) kg/m\(^3\), respectively, and the values for the “high density solids” (HD) were \((2.151 \pm 0.125)\) mm and \((1048 \pm 1)\) kg/m\(^3\). For each type of solids, solids loading applied was 0%, 5%, 10%, 15%, 20% and 30% (v/v).

It was found that the distributing plate orifices diameter does not have a significant influence on the parameters studied, being only observed a slight changes of riser gas holdup and circulation time, for low airflow rates and low solids loading, and on liquid velocity, for 10% of solids. This reduced effect of the gas distributor in airlift reactors, when compared with bubble columns, can be ascribed to the influence of superimposed liquid flow on the flow pattern and bubble rise velocity in the riser, enhanced due to the presence of the solid phase. On the contrary, airflow rate and solids loading have a great effect on riser gas holdup, circulation time and downcomer liquid velocity. The increase of airflow rate leads to an increase of riser gas holdup and liquid velocity while the circulation time, consequently, decreases. In opposition, the increasing introduction of solids produces a decrease of the gas holdup and liquid velocity and an increase of circulation time. There is not a well defined trend of the effect of solids density, in the range of values studied, on the studied hydrodynamic characteristics of the three-phase external-loop airlift reactor.

In order to evaluate and compare the performance of three-phase internal- and external-loop airlift reactors with the same working volume, the experimental results of riser gas holdup, liquid velocity, circulation and mixing time obtained for the two reactors (working under the same conditions) were compared. The internal-loop airlift reactor used is of the concentric-tube type with an enlarged degassing zone (Figure 2b).

Both reactors showed similar response to the change of the operation parameters. The comparison of the two reactors showed that, in all cases, the external-loop airlift reactor presented higher values of riser gas holdup. However, internal-loop airlift reactor exhibited better circulation and mixing performances.

**Development of an hydrodynamic model**

A mathematical model predicting the hydrodynamic behaviour of three-phase airlift reactors, working with low-density solids and with high solids loading, was developed. The model allows for the prediction of local gas holdup and liquid velocity in airlift bioreactors and was validated for the external- and the internal-loop airlift reactor used in the experimental work.
The riser gas holdup is given by:

\[
\varepsilon_{gr} = \frac{U_{gr}}{C \left( U_{gr} + U_{bt} \right)^{1+\frac{\varepsilon_{gr}}{1-\varepsilon_{gr}}} - \varepsilon_{a} U_{a} + U_{bt}}
\]  

(1)

The riser superficial liquid velocity is obtained by the following equations:

**INTERNAL-LOOP AIRLIFT REACTOR:**

\[
2gH \left( \varepsilon_{gr} - \varepsilon_{sl} \right) = \left( \frac{A_{r}}{A_{d}} \right)^{2} \frac{k_{fb}}{(1-\varepsilon_{gd} - \varepsilon_{sl})} U_{bt}^{2} + \\
\quad + \left[ \frac{D_{r}^{-1.25}}{(1-\varepsilon_{gr} - \varepsilon_{sl})^{75}} \left( \frac{A_{r}}{A_{d}} \right)^{1.75} \frac{D_{r}^{-1.25}}{(1-\varepsilon_{gd} - \varepsilon_{sl})^{75}} \right] H \beta U_{bt}^{1.75}
\]  

(2)

**EXTERNAL-LOOP AIRLIFT REACTOR:**

\[
2gH \varepsilon_{gr} = \left[ \frac{(H_{r} + H_{d}) D_{r}^{-1.25}}{(1-\varepsilon_{gr} - \varepsilon_{sl})^{75}} \left( \frac{A_{r}}{A_{d}} \right)^{1.75} \frac{(H_{d} + H_{b}) D_{d}^{-1.25}}{(1-\varepsilon_{sl})^{75}} \right] \beta U_{bt}^{1.75} + k_{pa} U_{bt}^{2} \\
\quad + \left( \frac{A_{r}}{A_{d}} \right)^{2} \left( k_{fd} + k_{fsc} + k_{pa} + k_{p,b} \right) \frac{U_{bt}^{2}}{(1-\varepsilon_{sl})}
\]  

(3)

The optimisation of the parameters \( C, U_{bt}, a, b \) and \( \beta \), allowing for the prediction of values of riser gas holdup and the riser superficial liquid velocity, was done using a developed computer software.

The results obtained show that the model predicts the experimental values of riser gas holdup and downcomer linear liquid velocity obtained, for all the experimental cases studied, with high accuracy (with an error of \( \pm 10\% \) and \( \pm 15\% \), respectively) (Figure 3 a, b).

![Figure 3. Predicted values vs. experimental values of riser gas holdup (a) and downcomer linear liquid velocity (b), for all the experimental conditions (1.6 mm: ○ - LD, ● - HD; 1.0 mm: □ - LD, ■ - HD; 0.5 mm: ◊ - LD, ♦ - HD): LD – “low density solids”; HD – “high density solids”](image)

For both reactors, for the estimated parameters, a similar effect of the solids loading on hydrodynamics was found. The distribution parameter \( C \) presents some oscillations showing that, depending on the amount of solids, the solid-phase affects the flux in different ways. The terminal velocity of a single bubble \( (U_{bt}) \) increases with the increase of solids loading, as a consequence of the increase of coalescence deriving from the increase of the interaction between the bubbles. The parameter \( \beta \) exhibits different trends for the two reactors, resulting from their distinct geometries.
The effect of the presence of ethanol in system hydrodynamics is particularly relevant, since most of the applications of flocculation bioreactors deal with ethanol fermentation. When water is replaced by an ethanol solution (10 kg·m⁻³), a reduction in the surface tension occurs and changes significantly the response of the reactor in terms of the gas and solids hold-up in both riser and downcomer, the circulation and mixing times and the riser and downcomer interstitial velocity (Freitas and Teixeira 1998). In fact, an increase of the riser and downcomer gas hold-up is registered, together with the consequent decrease of solids hold-up in those sections and the resulting decrease of riser and downcomer interstitial velocity. However, the difference between gas and solids hold-up in the riser and in the downcomer remains practically constant when ethanol is added; the driving force for the circulation is thus maintained and, so, the presence of ethanol while increasing mixing time, has no measurable effect on circulation time.

Oxygen mass transfer in a high solids loading three-phase internal-loop airlift reactor

For the previously described internal-loop airlift reactor with an enlarged degassing zone, the volumetric mass transfer coefficient was determined. Airflow rate, solids loading, solids density and liquid-phase were the parameters manipulated.

It was shown that the volumetric mass transfer coefficient diminishes with the increase of solids loading, especially for high airflow rates, due to an increase in bubble coalescence. Reductions between 40% and 70% were obtained with the introduction of 20% and 30% of solids. Solids density also affects $k_L a$. A significant decrease on $k_L a$ resulting from a small increase of solids density (from 1023 to 1048 kg/m³) was observed, as a consequence of the effect of solids density on solids distribution in the reactor. A progressive increase on the concentration of solids in the lower sections of the reactor, as the riser and the downcomer, with the increase of solids density is responsible for the enhancement of coalescence and the consequent decrease on the specific interfacial area.

Airflow rate and the presence of ethanol were shown to enhance the volumetric mass transfer coefficient - airflow rate by primarily increasing gas holdup and ethanol by inhibiting coalescence. The increase on $k_L a$ by the presence of ethanol depends on the amount and type of solids and on the airflow rate. For riser superficial gas velocities up to 0.075 m/s, the augment is very small, while for higher airflow rates, values of $k_L a$ for aqueous ethanol solution are about 1.5 to 2 times the values obtained for water.

The volumetric mass transfer coefficient was correlated to the riser superficial gas velocity ($u_{gr}$) and the solids loading ($\varepsilon_s$), for the two types of solids and for the two liquids, water and ethanol solution. Agreements between the calculated values and the experimental data of ±10%, for water, and of ±20%, for aqueous ethanol, were obtained (Figure 4 a,b,c and d). The obtained correlations were:

Water/"Low density solids":

$$k_L a = -0.93 u_{gr}^2 + 1.33 u_{gr} - 0.012 \times (-0.0000016 \varepsilon_s^2 - 0.00099 \varepsilon_s + 0.054)$$  (4)

Water/"High density solids":

$$k_L a = -0.33 u_{gr}^2 + 0.43 u_{gr} - 0.0064 \times (0.000080 \varepsilon_s^2 - 0.0056 \varepsilon_s + 0.17)$$  (5)

Ethanol/"Low density solids":

$$k_L a = -0.95 u_{gr}^2 + 1.34 u_{gr} - 0.021 \times (-0.000072 \varepsilon_s^2 + 0.00079 \varepsilon_s + 0.075)$$  (6)

Ethanol/"High density solids":

$$k_L a = -0.78 u_{gr}^2 + 1.20 u_{gr} - 0.021 \times (-0.000074 \varepsilon_s^2 - 0.0035 \varepsilon_s + 0.081)$$  (7)

where

$$0 < u_{gr} (m/s) < 0.5$$
$$0 < \varepsilon_s (% v/v) < 30$$
Mass Transfer of Solute in the Flocs

Vicente et al. (1998a) studied mass transfer characteristics (effective diffusivity, $D_e$, and external mass transfer coefficient, $K_e$) of glucose and oxygen in yeast flocs (0.90 mm to 2.42 mm in diameter) of $S.\, cerevisiae$ using inactivated cells in a modified diffusion cell (Vicente et al. 1997) developed in order to avoid floc destruction. $D_e$ and $K_e$ were calculated using two methods: a classical one, based on analytical solutions of Fick’s law of diffusion and a numerical one, based on general mass balances of a component in flocs and bulk phase. Diffusion coefficients were found to be, for glucose, 17% of the diffusivity in water and, for oxygen, between 0.2% to 1% of the diffusivity in water, which is in agreement with the data from Ananta et al. (1995) if the size is considered. $K_e$ values increased with the agitation rate, as expected, and have values which range from $7.5\times10^{-9}\, \text{m}\cdot\text{s}^{-1}$ to $15\times10^{-9}\, \text{m}\cdot\text{s}^{-1}$. These values indicate that not only the mass transfer inside flocs, but also the one outside them, may be a limiting step in this process.

The values obtained were then used to estimate the penetration depth of the solutes in flocs by modelling diffusion-reaction phenomena. Concentration profiles of glucose inside aggregates of $S.\, cerevisiae$ were simulated and calculations were made for different possible sizes of the yeast flocs, considering also the presence or the absence of a polymeric additive (Vicente et al. 1998b) (Figure 5).

The presence of a polymeric additive that enlarges the space between cells inside flocs proved to be effective in reducing mass transfer limitations.
Figure 5. Comparison of dimensionless glucose concentration profiles for flocs grown with (dot-dashed lines) and without (solid lines) a flocculation additive, with a radius of 0.8 mm (the parameters near the lines identify the values of the bulk concentration to which each line corresponds).

Applications of flocculation bioreactors

Besides all the other factors pointed out through the preceding pages, the operational conditions under which a given bioreactor can be operated are also largely dependent on the flocculation ability of the microbial strain involved in the fermentation. If a highly flocculent strain is used, its continuance inside the bioreactor poses no problem even if “tough” operational conditions are used, such as high aeration and dilution rates (Vicente et al. 1999). However, when a given strain has weak flocculation abilities, the reactor design plays a fundamental role, being an airlift one of the most appropriate, especially if equipped with a sedimentation zone (either at the top of the reactor, for an internal loop airlift, or at the top of the downcomer, for an external loop airlift). In Figure 6 it is possible to follow the operation of an external loop airlift with a sedimentation zone, a volume of 1.2 L and fed at different dilution rates, as shown (Teixeira and Mota 1992).

Figure 6. Flocculation bioreactor response to increasing dilution rate (D) with a feed lactose concentration of 57.2 g L⁻¹; ⬤ - biomass concentration in the bioreactor (X); ○ - biomass concentration in the effluent (X); ■ - lactose concentration (S); ▲ - ethanol concentration (P).

Considering the need to develop new and simpler fermentation systems and the suitability of the airlift bioreactor for cultures using flocculating microorganisms, a 5.4 L internal loop airlift bioreactor was tested and compared with the previous system (Sousa et al. 1994a) using a highly flocculating strain of *S. cerevisiae* growing on glucose. A comparison was made in terms of start-
up evolution, overall performance and power costs. The best ethanol productivity was obtained for
the concentric tube airlift reactor (12.9 g·L\(^{-1}\)·h\(^{-1}\)), but both systems behaved in a similar way and the
productivity values were about seven times higher than in commercial systems. There was also a
clear indication of a higher cell activity in the concentric tube airlift bioreactor when compared to
the external loop airlift, thus compensating for the lower cell retention capacity of the former. The
work proceeded, then, with the concentric tube airlift (Sousa et al. 1994b), by studying the evolution
of fermentation parameters of the same flocculent strain of \textit{S. cerevisiae} during the start-up of a
continuous fermentation. A strong influence of the dilution and aeration rates was found on both
biomass and ethanol concentrations and kinetic parameters. The operating parameters, in turn, do
not seem to affect glucose consumption rates but affect, instead, the stoichiometry of its conversion
to either biomass or ethanol, suggesting a shift in the metabolic mechanisms as biomass builds up.

One of the shortcomings of flocculation bioreactors, already mentioned, is the presence of
mass transfer limitations inside the flocs and it has been pointed out in the previous section that a
reduction in floc size could be expected to bring a reduction in mass transfer limitations, leading to
an increase of productivity. Vicente et al. (1999) introduced static mixers in the draught tube of the
internal loop airlift bioreactor used by Sousa et al. (1994a,b), achieving an effective reduction of the
floc size (3 mm to 1 mm in diameter). Steady state data at different dilution rates were measured for
both systems (the original and the modified bioreactor) and the results were compared in terms of
specific consumption/production rates and ethanol productivity. A 40% increase was obtained in the
maximum dilution rate at which a glucose conversion higher than 98% could be achieved. The
respiratory quotient had a constant value (around 23) at all dilution rates, meaning that the
metabolic state of the cells in flocs remained constant, having a strong fermentative metabolism.

The floc size reduction contributed to the higher observed reaction rates, not only by means
of an increased dilution rate, but also because of reduced diffusional limitations, leading to a 30%
increase of ethanol productivity when compared with the original system.

One of the first commercial applications of the sedimentation characteristics of flocculating
microorganisms was made by the brewing industry, back in 1971, in order to facilitate the
separation of the yeast cells from beer at the end of the process (Greenshields and Smith 1971).
Still, it is mostly in the brewing industry that flocculation bioreactors are widely used. However, in
this case, flocculation is essentially a separation technique and not a way to immobilise cells in
continuous high cell density systems. Most brewing companies and brewing research groups have
several research works running using high cell density bioreactors, mostly with airlift configuration,
in order to investigate their potential use in continuous beer fermentation, with the advantages
pointed out throughout the preceding text (Dillenhöffer and Röhn 1996, Dömeny et al. 1998, Linko
Nevertheless, none of these works actually deals with flocculating cultures, though some mention
them as a possible alternative to the existing processes, in particular for beer maturation (Linko
et al. 1997).

The advantage of biomass retention in bioreactors makes the use of these systems
particularly attractive in continuous fermentations. This is the case of flocculation bioreactors. In
Table 1, a summary of works with flocculation bioreactors is presented, proving an emerging
interest for this type of systems. As can be seen, the majority of the work presented deals with
flocculating microorganisms for continuous ethanol production, which is not surprising since, for
the moment, continuous high cell density systems are adequate for high volume low added value
products. As also indicated, most of the studies on flocculation bioreactors have been done in
bench-scale apparatuses due to costs and complexity involved in larger scale research. Being so,
further information on hydrodynamics and mass transfer needed for reactor scale-up is still missing
and it is not surprising that, so far, industry still hesitates to select a flocculation-based process for
commercial purpose, in spite of the operational advantages of these systems.
Table 1. Works with flocculation bioreactors.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Reactor type</th>
<th>Volume [L]</th>
<th>Main substrate</th>
<th>Main product</th>
<th>Productivity [g.L^{-1}.h^{-1}]</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae</td>
<td>bubble column</td>
<td>1-0.5</td>
<td>wort</td>
<td>beer</td>
<td>-</td>
<td>Smith and Greenshields 1974</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>bubble column</td>
<td>-</td>
<td>molasses</td>
<td>ethanol</td>
<td>25-30</td>
<td>Kida et al. 1989</td>
</tr>
<tr>
<td>K. marxianus</td>
<td>external airlift loop</td>
<td>1.2</td>
<td>lactose</td>
<td>ethanol</td>
<td>24.4</td>
<td>Teixeira et al. 1990</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>external airlift loop</td>
<td>2</td>
<td>glucose/sucrose</td>
<td>ethanol</td>
<td>68</td>
<td>Fontana et al. 1992</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>CSTR</td>
<td>3</td>
<td>molasses</td>
<td>ethanol</td>
<td>5</td>
<td>Kida et al. 1992</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>series of 2 CSTR</td>
<td>0.5 +</td>
<td>glucose</td>
<td>ethanol</td>
<td>9.3</td>
<td>Kuriyama et al. 1993</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>internal airlift loop</td>
<td>5.4</td>
<td>glucose</td>
<td>ethanol</td>
<td>-</td>
<td>Sousa et al. 1994b</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>internal + external airlift loop</td>
<td>1.2 + 5.4</td>
<td>glucose</td>
<td>ethanol</td>
<td>12.9</td>
<td>Sousa et al. 1994a</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>fluidised bed</td>
<td>10</td>
<td>molasses</td>
<td>ethanol</td>
<td>15 - 20</td>
<td>Wieczorek and Michalski 1994</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>external airlift loop</td>
<td>2</td>
<td>sucrose</td>
<td>ethanol</td>
<td>-</td>
<td>Roca et al. 1995</td>
</tr>
<tr>
<td>S. diastaticus</td>
<td>external airlift loop</td>
<td>2</td>
<td>Jerusalem artichoke extract</td>
<td>ethanol + inulin</td>
<td>-</td>
<td>Schorr-Galindo et al. 1995</td>
</tr>
<tr>
<td>Z. mobilis + Saccharomyces sp.</td>
<td>agitated conical flasks</td>
<td>1</td>
<td>-</td>
<td>ethanol</td>
<td>1.5</td>
<td>Abate et al. 1996</td>
</tr>
<tr>
<td>Rhodiola sachalinensis</td>
<td>internal airlift loop</td>
<td>10 - 100</td>
<td>-</td>
<td>salidroside</td>
<td>-</td>
<td>Jianfeng et al. 1998</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>internal airlift loop</td>
<td>6</td>
<td>green beer</td>
<td>maturated beer</td>
<td>-</td>
<td>Mafra et al. 1997</td>
</tr>
<tr>
<td>Schizosaccharomyces pombe</td>
<td>external airlift loop</td>
<td>1.2</td>
<td>grape musts</td>
<td>deacidified grape musts</td>
<td>-</td>
<td>Sousa et al. 1993</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>internal airlift loop</td>
<td>1000</td>
<td>deproteinised cheese whey</td>
<td>ethanol</td>
<td>-</td>
<td>Unpublished data</td>
</tr>
</tbody>
</table>

A particular reference must be made to the work of Domingues et al. (1999), who describe the use of a recombinant flocculating strain of *S. cerevisiae* expressing the *LAC4* (coding for β-galactosidase) and *LAC12* (coding for lactose permease) genes of *K. marxianus* to perform alcoholic fermentation of lactose with the previously described airlift bioreactor. In continuous operation, an ethanol productivity of 11 g.L^{-1}.h^{-1} was obtained (with a feed lactose concentration of 50 g.L^{-1} and a dilution rate of 0.55 h^{-1}), being seven-fold larger than the one in conventional continuous systems. Despite of the flocculence instability of the recombinant strain, a high biomass concentration was achieved inside the bioreactor as its design allowed for a selection of the most flocculating cells from a mixed culture, contributing thus with a selective pressure for the maintenance of the flocculating cells inside the bioreactor. The most direct application of this work is the high-productivity fermentation of the lactose present in cheese whey to produce ethanol, not only contributing for the bioremediation of this by-product of the dairy industry produced in large amounts, but also allowing for the production of a useful fuel.

There is the need for more research on high cell density systems, in general, and on flocculation bioreactors, in particular, in order to gather the necessary information to make them an interesting alternative to the processes used nowadays, which are in most cases very well established.
and studied. The potential surely exists in this new technology, but it has to be demonstrated before the industry risks investing largely in it.

References


