Increase of Ethanol Productivity in an Airlift Reactor with a Modified Draught Tube

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Static mixers were introduced in the draught tube of a three-phase, concentric tube type airlift bioreactor, resulting in a 30% increase in ethanol productivity during glucose fermentation with a highly flocculent strain of *Saccharomyces cerevisiae*. Increased productivity was obtained as a consequence of the floc size reduction provoked by the new design of the draught tube and of the smaller applied aeration rates, allowing a higher dilution rate to be used. Steady state data at different dilution rates were measured for both systems and the results were compared in terms of specific consumption / production rates and ethanol productivity. An approach based on heterogeneous catalysis principles was used in order to consider the presence of porous yeast flocs. Total glucose conversion was achieved at a dilution rate of 0.34 h^{-1} . The smaller floc size contributed to the higher observed reaction rates by reducing diffusional limitations inside the flocs. The respiratory quotient had a constant value (around 23) at all dilution rates, meaning that the metabolic state of the cells in the flocs remained constant, having a strong fermentative metabolism.

Des mélangeurs statiques ont été introduits dans un tube d'aspiration d'un bioréacteur triphasique à courant ascendant de type tube concentrique, ce qui entraîne une augmentation de 30 % de la productivité de l'éthanol lors de la fermentation du glucose avec une souche hautement floculante de *Saccharomyces cerevisiae*. Une productivité accrue a été obtenue consécutivement à la réduction de la taille des flocs provoquée par la nouvelle conception du tube d'aspiration et les plus petites vitesses d'aération appliquées, ce qui permet d'utiliser une plus grande vitesse de dilution. Les données en régime permanent à différentes vitesses de dilution ont été mesurées pour les deux systèmes et les résultats ont été comparés en termes de vitesses spécifiques de consommation/production et de productivité de l'éthanol. Une approche basée sur les principes de catalyse hétérogène a été utilisée pour tenir compte de la présence de flocs de levure poreux. La conversion totale du glucose a été réalisée à une vitesse de dilution de $0,34 \text{ h}^{-1}$. La taille plus petite des flocs contribue aux plus grandes vitesses de réaction observées en réduisant les limitations diffusielles à l'intérieur des flocs. Le quotient respiratoire a une valeur constante (environ 23) à toutes les vitesses de dilution, ce qui signifie que l'état métabolique des cellules dans les flocs demeure constant, car elles ont un fort métabolisme fermentatif.

Keywords: ethanol productivity, airlift, fermentation, yeast flocs, static mixers.

Higher productivity of fermentative systems with lower maintenance costs may be achieved with continuous processes, when compared to batch or fed-batch systems (Chen et al., 1987; Gyamerah and Glover, 1996; Šmogrovicová et al., 1997; Sousa et al., 1994a). In fact, although it is not the only contributor, fermentation has a large direct effect on overall plant costs (Maiorella et al., 1984).

The application of high cell density systems allows for further improvements on the overall performance of the processes where they are being used (Lima et al., 1992; Nguyen and Shieh, 1992; Roca et al., 1995; Sousa et al., 1994b; Tyagi et al., 1992), especially in the brewing industry (Linko et al., 1997). One of the ways to achieve a high concentration of cells in a bioreactor is to take advantage of the flocculating properties of some microorganisms (Kuriyama et al., 1993; Vicente et al., 1997), which are most effectively grown in airlift bioreactors, as these provide near ideal hydrodynamic conditions for the growth and maintenance of the flocs (Vicente and Teixeira, 1995). Nevertheless, the flocs resulting from the aggregation of microbial cells present some drawbacks when compared with free suspended cells, as diffusional limitations of substrates and products are likely to occur. This problem can be significantly aggravated with an increase of the size of the aggregates (Hamdi, 1995; Pereboom et al., 1990; Sousa and Teixeira, 1991). A considerable impact in the metabolism of the cells forming the aggregate is therefore to be expected, as it will be altered when compared to that of free suspended cells due to the

above mentioned limitations inside of the aggregates (Vicente et al., 1998b).

In aerobic fermentations, nutrients are supposed to reach the cells in the centre of the floc by diffusion and a serious depletion of oxygen (Webster, 1981) and other nutrients (Lima et al., 1992) may occur. This will affect the outcome of the whole fermentation process in terms of, e.g., biomass and ethanol productivity (Vicente et al., 1998a).

Controlling the floc size enables a shift of the process towards a higher efficiency, as smaller flocs will reduce diffusional problems (Ananta et al., 1995). In other words, for the same amount of biomass, a reduction of floc size is expected to lead to a higher amount of cells easily reached by nutrients from the medium, increasing substrate conversion. However, the floc size reduction should not alter the cells' retention capacity of the system. The bioreactor should be designed in such a way that disintegrated flocs should be allowed to regenerate in a quiescent zone in order to remain inside the bioreactor.

In the present work the design of the draught tube of an internal loop airlift bioreactor has been altered in order to achieve a size reduction of the flocs during a fermentation of glucose using a highly flocculent strain of *Saccharomyces cerevisiae*. The final aim of such change has been to improve the ethanol productivity of the system by reducing the limitations brought up by the presence of big porous flocs.

Ethanol production was used as the model process, but the results and conclusions obtained here may be applied to other systems and provide valuable insight in the processes making use of immobilized cells, in general, and of cell flocs, in particular. In fact, it shows clearly the advantages

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Figure 1. — Scheme of the static mixers distribution in the altered internal draught tube. The dimensions are in mm and the mixers are equally spaced along the tube.

of using smaller particles (i.e., smaller flocs or smaller immobilization matrices), thus improving the outcome of the processes where they are being used.

Materials and methods

YEAST STRAIN AND CONDITIONS OF CULTIVATION

The microorganism used is a highly flocculent strain of *Saccharomyces cerevisiae* (NRRL Y265), grown continuously in an internal loop airlift bioreactor (working volume: 6.0 dm³) at 30°C and pH 4. The composition of the medium was as follows (kg·m⁻³): KH₂PO₄, 5.0; (NH₄)₂SO₄, 2.0; MgSO₄·7H₂O, 0.4; yeast extract, 1.0; glucose, 130.0. Further details on medium preparation and conditions of operation have been described elsewhere (Sousa et al., 1994b; Vicente and Teixeira, 1995; Vicente et al., 1996).

The airlift reactor is of the concentric draught tube type, made of Plexiglas, with a total height of 90 cm, a down comer with 60 cm of height and an inside diameter of 7 cm. The draught tube's height and diameter are, respectively, 48 cm and 3.2 cm. The thickness of the draught tube is 4 mm and its bottom edge is 3 cm above the bottom of the reactor. The air injection is made immediately below the annulus of the riser by means of a nozzle injector with a diameter of 1 mm. The top section is of the cylindrical conical type to facilitate the degassing and the solids' deceleration. The angle of the conical sector with the main body of the reactor is of 51° and the height and diameter of the cylindrical part are, respectively, 14.5 cm and 19.2 cm (Vicente and Teixeira, 1995).

The reactor was operated at various dilution and aeration rates (see Table 1). For the original system, the dilution rates ranged from 0.03 to 0.25 h⁻¹, with the corresponding aeration rates ranging from 0.2 to 1.0 v.v.m

ALTERATION TO THE DRAUGHT TUBE DESIGN

The new draught tube has the same diameter (3.2 cm) and height (48 cm) as the original one (Vicente and Teixeira, 1995), but six internal static mixers are regularly distributed along its axis. They have been positioned in a way that minimizes the possibility for formation of preferential channels for the flow of gas and liquid (Figure 1). The function of these mixers is to increase shear stresses in the riser in order to decrease average floc size and improve mixing characteristics and mass transfer, without the need of increasing air-flow rate.

STEADY STATE CONDITIONS

Steady state conditions were attained after a period corresponding to 5 residence times. The attainment of steady state was confirmed by a constant value of dissolved oxygen in the medium together with a constant residual value of

glucose concentration in the effluent (Sousa et al., 1994b), lower than 3 kg·m⁻³ (with an approximate density - ρ_l - of 1000 kg·m⁻³). This value corresponds to a glucose conversion of not less than 98%. Once steady state was achieved, samples were withdrawn from the bioreactor and analyzed for glucose, ethanol, biomass and cell viability. Composition of gas outlet stream was also analyzed.

ANALYTICAL METHODS

Glucose and ethanol concentrations were simultaneously determined by HPLC equipped with a 830-RI (Jasco, Japan) refraction index detector and a 880-PU (Jasco, Japan) pump after sample centrifugation. Commercial standards were used for the calibration of the column (Polyspher® CH CA, Merck, Germany) at 85°C with ultra pure water as eluent (0.5 mL·min⁻¹).

Biomass concentration was determined by measuring optical density at 620 nm of a suspension previously deflocculated by the addition of a solution of 15 kg·m⁻³ of NaCl at pH 4 and correlated to dry weight (Sousa et al., 1994b). Cell viability was determined as the percentage of the count of living cells over total amount of cells after staining with methylene blue.

The analysis of the outlet gas was performed on-line by gas spectrometry (Spectra Mass, Bioquad, U.K.), allowing the determination of CO₂ production and O₂ consumption. Density (ρ_f) of flocs was determined by pycnometry (Vicente et al., 1996) and a value of 1033 kg·m⁻³ was obtained.

STEADY STATE ANALYSIS

An approach based on heterogeneous catalysis principles was used to accurately determine the reaction rates. The option for this approach was taken as this system, containing yeast flocs suspended in a liquid medium, is very similar to a heterogeneous reactor with catalyst particles. In order to calculate the rates of consumption of substrate (q_s) and oxygen (q_O), as well as the rates of production of ethanol (q_E) and carbon dioxide (q_C), some assumptions had to be made:

- the measurements took place in steady state conditions so that the accumulation terms in mass balance equations are equal to zero and, therefore, are not presented;
- glucose can reach all the cells inside the flocs;
- the feed is free from ethanol and biomass ($E_{in} = X_{in} = 0$);
- limitations to mass transfer of gases between the gas and liquid phases are negligible;

$$\dot{Q}_{in} = \dot{Q}_{out} = \dot{Q};$$

- dilution rate is defined as:

$$\dot{D} = \dot{Q}/V_R \dots \dots \dots (1)$$

$$\epsilon_g + \epsilon_l + \epsilon_s = 1 \dots \dots \dots (2)$$

- no reaction occurs in floc pores where only the liquid phase is present;
- reactions take place only in the following volume (V_r) corresponding to the cell volume in the flocs:

$$V_r = (1 - \varepsilon_p) \varepsilon_s V_R \dots\dots\dots (3),$$

where floc porosity (ε_p) was considered to be 0.5 (Teixeira and Mota, 1990) and the volumetric fraction of flocs in the bioreactor (ε_s) is expressed as (see Appendix for derivation):

$$\varepsilon_s = \frac{fX}{(\rho_f - \varepsilon_p \rho_l)} \dots\dots\dots (4)$$

- the reaction rate for the component i is:

$$r_i = q_i X_v \dots\dots\dots (5),$$

Considering all the above, it is possible to establish mass balances for the several components in question. Being so, the mass balance for substrate (glucose) can be written as:

$$\dot{Q}_{in} S_{in} + (-r_s) V_r = \dot{Q}_{out} S_{out} \dots\dots\dots (6)$$

where $(-r_s)$ includes oxidative and fermentative consumption of substrate.

Rearranging Equation (6) with Equations (4) and (5) gives:

$$q_s = \frac{1}{(1 - \varepsilon_p)} \frac{(\rho_f - \varepsilon_p \rho_l)}{fX} \frac{\dot{D}}{X_v} (S_{in} - S_{out}) \dots\dots\dots (7)$$

The mass balance for product (ethanol) is:

$$\dot{Q}_{in} E_{in} + r_E V_r = \dot{Q}_{out} E_{out} \dots\dots\dots (8)$$

where r_E includes ethanol production by fermentation and oxidative ethanol consumption.

Rearranging Equation (8) with Equations (4) and (5) gives:

$$q_E = \frac{1}{(1 - \varepsilon_p)} \frac{(\rho_f - \varepsilon_p \rho_l)}{fX} \frac{\dot{D}}{X_v} E_{out} \dots\dots\dots (9)$$

The mass balance for oxygen can be written as:

$$\dot{G}_{in} O_{in} + (-r_o) V_r = \dot{G}_{out} O_{out} \dots\dots\dots (10)$$

where $(-r_o)$ includes oxygen consumption in all the oxidative processes that take place in the system.

Rearranging Equation (10) with Equations (4) and (5) gives:

$$q_O = \frac{1}{(1 - \varepsilon_p)} \frac{(\rho_f - \varepsilon_p \rho_l)}{fX} \frac{1}{X_v V_R} (\dot{G}_{in} O_{in} - \dot{G}_{out} O_{out}) \dots\dots\dots (11)$$

The mass balance for carbon dioxide is:

$$\dot{G}_{in} C_{in} + r_C V_r = \dot{G}_{out} C_{out} \dots\dots\dots (12)$$

where r_C refers to the overall production of carbon dioxide.

Figure 2 — Glucose (▲), ethanol (●) and viable biomass (■) bulk concentrations vs. dilution rate at pseudo-steady-state.

Rearranging Equation (12) with Equations (4) and (5) gives:

$$q_C = \frac{1}{(1 - \varepsilon_p)} \frac{(\rho_f - \varepsilon_p \rho_l)}{fX} \frac{1}{X_v V_R} (\dot{G}_{out} C_{out} - \dot{G}_{in} C_{in}) \dots\dots\dots (13)$$

In conclusion, the specific rates of substrate consumption/production for the present system are given by Equations (7), (9), (11) and (13), and can be calculated using experimentally determined parameters.

Results and discussion

An alteration of the draught tube design was made in order to investigate the consequences of the changes in the flow characteristics and shear stresses caused by the introduction of static mixers. The other reactor design characteristics and operational conditions were maintained as in Sousa et al. (1994b).

Measurements of the ethanol and glucose concentrations in the bulk and of the carbon dioxide and oxygen concentrations in the outlet gas were performed after the attainment of pseudo-steady-state conditions (i.e., when the concentrations of the mentioned components reached constant values during one day, but biomass concentration in the bulk did not).

Figure 2 shows the evolution of glucose, ethanol and viable biomass bulk concentrations with the dilution rate at pseudo-steady-state. Glucose bulk concentration was, in all cases, kept below 2.5 kg·m⁻³, corresponding to quite high conversion values (> 98%), except for the highest dilution rate (0.40 h⁻¹). At this value, although no bioreactor washout occurred, glucose conversion started to decrease. These results compare very well with the work of Sousa et al. (1994b) where residual concentrations were kept below 3 kg·m⁻³. However, the maximum dilution rate at which the glucose outlet concentration was below that value was, in the present work, of 0.34 h⁻¹, whereas in the work previously presented by Sousa et al. (1994b), it was of 0.25 h⁻¹. Above that value, glucose conversion started to decrease as the glucose consumption rate of the system was already at its maximum.

Under identical dilution and aeration rates, the alteration introduced in the draught tube induced a two-fold decrease of the floc size (from 1.75 ± 0.45 mm to 0.98 ± 0.35 mm).

Figure 3 — Specific rates of oxygen consumption (x) and carbon dioxide (♦) production vs. dilution rate at pseudo-steady-state.

In smaller flocs the diffusional limitations are not so severe as in flocs with bigger size (Fontana et al., 1992). This is especially true in when oxygen (often limiting), glucose and ethanol (feedback inhibitor) concentrations are concerned (e.g. high ethanol concentrations inside the flocs due to poor mass transfer will inhibit further ethanol production by the cells). This will influence the overall behaviour of the system, as will be shown by the data presented below.

Viable biomass bulk concentration increased linearly with the dilution rate ($\bar{D}$) until the value of $\bar{D} = 0.40 \text{ h}^{-1}$; higher values caused reactor washout and also did not comply with the condition of having conversion values $> 98\%$. Total biomass bulk concentration reached the maximum value of around $70 \text{ kg} \cdot \text{m}^{-3}$, whilst viability varied from 52% to 92%. In the original (unmodified) system, the maximum total biomass bulk concentration obtained was around $78 \text{ kg} \cdot \text{m}^{-3}$ and the corresponding viability was of the same order of magnitude (around 62%).

Lower aeration rates will certainly lower production costs and improve system stability. In fact, high aeration rates, together with high dilution rates, may provoke washout in three-phase airlift bioreactors (Sousa et al., 1994b). Due to the smaller size of the flocs in the modified system, the aeration rates needed to maintain liquid circulation were lower than those needed in the original system, and this is the reason why it was possible to achieve higher dilution rates in the present work without the occurrence of reactor washout.

Ethanol bulk concentration diminished slightly with the dilution rate, oscillating around $50 \text{ kg} \cdot \text{m}^{-3}$, with a maximum of $55 \text{ kg} \cdot \text{m}^{-3}$. Outlet ethanol concentrations around $40 \text{ kg} \cdot \text{m}^{-3}$ have been reported (see Fontana et al., 1992) for a high cell density bioreactor with a highly flocculent strain of *Saccharomyces cerevisiae*.

The evolution of the specific consumption rate of oxygen and the specific production rate of biomass and carbon dioxide is shown in Figure 3. The significant difference between q_C and q_O indicates the occurrence of mainly fermentative metabolism in the bioreactor, while the slight decrease in the values corresponding to the highest dilution rate points to the proximity of reactor washout. As can be clearly seen, both q_C and q_O are well adjusted by linear regression. This means that the ratio between the slopes of the fitted lines is a constant. As that ratio is proportional to the respiratory quotient this value is also a constant (around 23), which means

Figure 4 — Comparison between specific rates of glucose consumption (▲, Δ) and ethanol production (●, ○) obtained in the present work (solid symbols) with those obtained with the original system (open symbols) vs. dilution rate.

Figure 5 — Comparison between ethanol productivity (●, ○) obtained in the present work (solid symbols) with that obtained with the original system (open symbols) vs. dilution rate.

that the metabolic state of the cells in the flocs remained constant at all dilution rates and in these operational conditions.

As it is clear from Equation (5) cell viability was included in the calculations of specific consumption/production rates (Equations 7, 9, 11 and 13). Usually, viability is assumed to be 100%, but that was not certainly the case in the present work, with values varying from 52% to 92%. This could introduce a serious discrepancy between the calculated and the real reaction rates and was, therefore, considered in the calculations.

In Figure 4, specific rates of glucose consumption and ethanol production obtained in this work (solid symbols) are compared with those obtained in the original system by Sousa et al. (1994b) (open symbols). In fact, values for q_S are two-fold higher when compared with q_S' and values for q_E are more than 1.5 times higher than q_E' . Even if the total biomass concentration (instead of the viable biomass concentration) was used for the calculation of specific rates, the increases would still be at least of 50 to 60%. However, the main difference is observed when comparing the maximum dilution rates achieved in each work: 0.25 h^{-1} in the previous work (Sousa et al., 1994b) (vertical dashed line) and 0.40 h^{-1} in the present work (vertical solid line) before reactor washout. This allowed achieving even higher specific consumption and production rates with the modified system.

TABLE 1
Dilution Rates and the Respective Aeration Rates for the Present System

Dilution rate (h ⁻¹)	Aeration rate (v.v.m.)
0.033	0.15
0.069	0.15
0.144	0.15
0.206	0.40
0.253	0.60
0.284	0.87
0.334	1.17
0.398	1.32

As can be seen in Figure 5, ethanol productivity values before (open symbols) and after (solid symbols) the alterations were similar until the point at which the reactor washout in the original system occurred. From then on, ethanol productivity was increased due to the possibility of working at a higher dilution. Further, as defined here, the dilution rate ($\bar{D}$) was calculated per working volume of reactor. Nevertheless, because of the presence of a high amount of solid phase (flocs), the real liquid volume was significantly lower than the reactor working volume. Being so, the effective dilution rates, defined per liquid volume, would be even higher than those reported here.

Conclusions

In conclusion, the alteration to the draught tube design permitted the reactor's operation at higher dilution rates, as well as provided a smaller mean floc size. The higher dilution rates allowed the achievement of higher productivity values (an increase of around 30%) and, furthermore, made the system less prone to contamination by other microorganisms, as they would easily be dragged out of the bioreactor. The floc size reduction, in turn, also contributed to higher reaction rates (substrate consumption and product formation), mainly because of the reduced diffusional limitations (Hamdi, 1995; Vicente et al., 1998a,b), and also due to a decrease of the air flow rate needed to keep the circulation inside the bioreactor; as the flocs were smaller they were less prone to cause reactor stalling. All these are reasons to expect an improved performance of the modified system when compared to the original one.

This work also presents an approach based on mass balances for heterogeneous reaction systems, considering that reactions take place only in the volume occupied by the cells in the flocs. This is not a common practice, but it is our opinion that in high cell density systems such an approach should be used instead of the usual treatment based on homogeneous reaction systems, as it represents the real system more closely.

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Appendix: Derivation of Equation (4)

In Equation (3), only ε_s cannot be directly determined. Therefore, there is the need to define it as a function of experimentally determined parameters.

For each floc, the following mass balance holds:

$$m_f = m_b + m_l \dots \dots \dots (A-1),$$

where m_f is the total mass of the floc, m_b is the mass of cells in the floc and m_l is the mass of liquid medium in pores. Rearranging Equation (A-1) and multiplying by the total number of flocs present in the bioreactor (n), gives,

$$n V_f \rho_f = n m_b + n \varepsilon_p V_f \rho_l \dots \dots \dots (A-2),$$

where V_f is the volume of one floc, ρ_f is the floc density and ρ_l is the liquid medium density.

The total volume of flocs in the bioreactor ($n \cdot V_f$) can be expressed in terms of the product of floc hold-up (ε_s) and the working volume of the bioreactor (V_R):

$$n V_f = \varepsilon_s V_R \dots \dots \dots (A-3)$$

The total concentration of biomass in the bioreactor ($n \cdot m_b / V_R$) can be expressed as the product of the dry biomass concentration (X), a measured variable, and a factor (f) which is the ratio between the total mass of a cell and its dry mass, here considered to be approximately 10/3, corresponding to about 70% of water in the cell (Alberts et al., 1994):

$$\frac{n m_b}{V_R} = f X \dots \dots \dots (A-4)$$

From Equations (A-3), (A-4) and (A-2), one can obtain:

$$\varepsilon_s = \frac{f X}{\rho_f - \varepsilon_p \rho_l} \dots \dots \dots (A-5).$$

Nomenclature

C	= carbon dioxide, carbon dioxide concentration, (kg·m ⁻³)
$\bar{D}$	= dilution rate, (h ⁻¹)
E	= ethanol, ethanol concentration, (kg·m ⁻³)
f	= ratio between the total mass of a cell and its dry mass, (—)
G	= volumetric gas flow rate, (m ³ ·h ⁻¹)
n	= number of flocs present in the bioreactor, (—)
O	= oxygen, oxygen concentration, (kg·m ⁻³)
q_C	= specific carbon dioxide production rate, (kg·(kg _{biomass}) ⁻¹ ·h ⁻¹)
q_E	= specific ethanol production rate, (kg·(kg _{biomass}) ⁻¹ ·h ⁻¹)
q_O	= specific oxygen consumption rate, (kg·(kg _{biomass}) ⁻¹ ·h ⁻¹)
q_S	= specific substrate consumption rate, (kg·(kg _{biomass}) ⁻¹ ·h ⁻¹)
$\bar{Q}$	= volumetric liquid flow rate, (m ³ ·h ⁻¹)
r	= overall reaction rate, kg·(m ³ ·h ⁻¹)
S	= glucose, glucose concentration, (kg·m ⁻³)
V_f	= volume of one floc, (m ³)
V_f'	= volume available for reaction, (m ³)
V_R	= working volume of the bioreactor, (m ³)
X	= biomass, total biomass concentration, (kg·m ⁻³)
X_v	= viable biomass concentration, (kg·m ⁻³)

Greek letters

ε_g	= gas hold-up, (—)
ε_l	= volumetric fraction of liquid, (—)
ε_s	= volumetric fraction of flocs, (—)
ε_p	= floc porosity, (—)
ρ_f	= floc density, (kg·m ⁻³)
ρ_l	= liquid medium density, (kg·m ⁻³)

Subscripts

i = component, (C, E, O, S)
in = inlet
out = outlet

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