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Hydraulic model of a gas-lift bioreactor with flocculating yeast

E. Roca, C. Ghommidh, J. M. Navarro, J. M. Lema

Abstract The hydraulic model of a gas lift bioreactor, during a continuous alcoholic fermentation by using a strongly flocculating yeast, is analysed. Sucrose at two different concentrations (50 and 100 g/l) was used as substrate and the dilution rate for all the experiments was 1 h^{-1}. The biomass concentrations were between 85 and 110 g dry weight/l. A stimulus response technique was used to obtain the Residence Time Distribution curves, a pulse of a lactose solution being used as the tracer. Mixing time was determined by means of the response to a pulse of an acid tracer. These experiments were carried out by using an on-line data-acquisition system. The bioreactor behaviour is completely homogeneous, except for high substrate and biomass concentrations. A two parameters combined model is necessary, in this case, to fit the experimental data. Mixing times are very low, in the order of 10 seconds.

List of symbols

C_{T1}	Tracer concentration of the tank 1 (g/l)
C_{T10}	Reference tracer concentration (g/l)
C_θ	Normalized tracer concentration (dimensionless)
Q_0	Feed flowrate (l/h)
Q_1	Flow exchanged between tank 1 and 2 (l/h)
$[S]$	Substrate concentration (g/l)
t	Time (s)
t_{mix}	Mixing time (s)
t_c	Circulation time (s)
V	Reactor total volume (l)
X	Biomass concentration expressed as dry weigh (g d.w./l)
α	Fraction of the total volume occupied by the highly agitated region
β	Fraction of the total flow which is exchanged between reactor 1 and 2
τ	Mean residence time (s), $\tau = V/Q_0$
θ	Dimensionless time, $\theta = t/\tau$

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1 Introduction

Increasing cell density in bioreactors is useful to improve the overall productivity of continuous fermentation processes. This can be achieved by immobilization, as well as utilizing the natural ability of several microorganisms for aggregation and flocculation. Nevertheless, flocculation presents many advantages: treating of large substrate volumes, minimizes the contamination risks (because of the non-flocculating contaminants are continuously removed); allows the biomass concentration to be increased (up to 60–120 g d.w./l) without physically confining the microorganisms into a support [1, 2].

Flocculent yeasts have been applied to fermentation processes, mainly in alcohol production [3, 4], although new processes employing flocculent bacteria have also been proposed [5]. Since the first continuous fermentors using flocculent yeasts were developed in 1960, bioreactors have been modified until the current configurations: Columns with external and integrated settler, gaslift bioreactors with integrated settler, etc. Overall alcohol productivities up to 68 g/l h have been obtained, although the results are usually in a range of 10–25 g/l h [6]. The productivity achieved in gas-lift bioreactors depends upon several factors, agitation being an outstanding one. Agitation is usually assured by recycling the CO_2 produced during the fermentation. Low agitation induces the formation of large flocs, creating biomass concentration gradients and also increasing mass transfer resistances.

In this work, the hydraulic model of a gas-lift fermentor was investigated, determining: a) Substrate and product profiles; b) the hydrodynamic behaviour (by RTD analysis); and c) the mixing and circulation time in the bioreactor.

2 Materials and methods

2.1 Microorganism

A strongly flocculating strain of *Saccharomyces cerevisiae* (38A from the IPV collection INRA, Montpellier) was used.

2.2
Fermentation Media

Batch cultures The inoculum (a 20% of bioreactor volume) was cultivated in 5 l Erlenmeyer flasks for 48 h (filled 1/10 of volume) and shaking at 155 oscilations per minute (amplitude 11 cm) in an orbital shaker, at a temperature of 27 °C. Medium was sterilized in autoclave at 121 °C during 15 min. Its composition was: Glucose 140 g/l, YE 10.0 g/l, $(NH_4)_2SO_4$ 5.0 g/l, $MgSO_4$ 2.5 g/l, KH_2PO_4 2.0 g/l.

Continuous cultures Sucrose with a concentration of 50 and 100 g/l was used as a carbon source in the medium fed to the bioreactor (media S50 and S100), except for the bioreactor startup, in which a 10 g/l of glucose concentration was used. Medium composition was as it follows: YE 3.0 g/l, $(NH_4)_2SO_4$ 5.0 g/l, $MgSO_4$ 2.5 g/l and KH_2PO_4 2.0 g/l. Salts solution and YE were sterilized in autoclave during 15 min at 121 °C. Carbon source was prepared concentrated five times and fed to the bioreactor at an adequated flowrate in the feed stream. Tap water was fed to dilute and obtain the desired final concentration.

2.3
Bioreactor

A gas lift bioreactor with internal cell recycle was used (see Fig. 1). The overall reactor volume was 2 l (Riser and Downcomer diameters are 6.5 and 4 cm respectively and the bioreactor height is 48 cm, the settler is 23 cm high and 16 cm as diameter). Fresh medium was fed to the bioreactor by means of three membrane dosifier pumps. The effluent overflowed through a tube disposed in the settler zone, around the gas collecting chamber, at the top of the bioreactor. Mixing was obtained by partial recycling of fermentation gases (2 VVM) allowing the fluidization of the bed.

The regulation systems allow: Temperature control at 28–30 °C; foam level control by addition of antifoam (Rhodorsil); and pH control by automatic addition of a 2N NaOH solution, the setpoint being fixed at pH 5. The pH was initially adjusted by addition of H_3PO_4.

Feed flowrate was kept constant at 2 l/h for all the experiments (dilution rate of 1 h^{-1}). The high flowrate and the relative selectivity of the recycling system was sufficient to avoid contamination problems.

2.4
Analysis

Biomass Biomass concentration was determined, as dry weight, by filtering on 0.45 μm membranes. The weight difference was measured after drying for 24 h in an oven at 104 °C.

Sugars Sucrose, glucose and fructose were determined by HPLC (pump Shimadzu LC6A, differential refractometer Knauer, column Amino Spheri 5, 4.6 mm · 20 mm).

Ethanol Ethanol was measured by gas Chromatography using an Intersmat IGC 121 DFL apparatus equipped with a FID. The temperatures in the column (Porapak Q, 80–100 mesh, 75 cm), injector and detector were 150, 160 and 180 °C respectively.

Lactose Lactose concentration was determined using Boehringer enzymatic kits (Boehringer Mannheim N°176303), measuring absorbances in a Spectrophotometer UV Bausch & Lomb Spectronic 21, at a wave length of 340 nm.

Gas hold-up The overall gas hold-up of the bioreactor was determined manometrically, as described by Chisti, 1989 [7].

2.5
RTD curves

RTD experiments are useful to determine the deviations from a behaviour and to give indications about the modifications that the system needs to overcome distribution problems.

RTD curves were obtained using a stimulus-response technique. Lactose (94–186.5 g/l) was chosen as the tracer for several reasons: a) It has very similar density and viscosity to the medium, which implies no mixing problems; b) Its molecular size and shape are quite similar to sucrose, therefore the diffusional mass transfer resistances of sucrose and lactose in the flocs are of the same order of magnitude; c) Lactose did not react with the medium and it is not metabolized by *S. cerevisiae*.

In each experiment, a small volume of the tracer (2 ml) was injected at the inlet of the bioreactor. The determination of tracer concentration in the exit stream of the bioreactor allows the C curve to be obtained, and from that the residence time distribution [8]. This curve was obtained by using a mixing-cups procedure.

2.6
Mixing time

To determine the mixing time, a tracer is injected into a significative section of the bioreactor and the evolution of its concentration allows the mixing and turnover time to be determined. Short time scale models are used for this purpose [8].

Mixing times of the bioreactor were determined by injecting an acid tracer (1 ml of concentrated H_2SO_4), and observing the pH evolution. To do this a pH electrode was placed at the top of the reactor loop and connected to a S.G.I. meter which was connected to a microcomputer APPLE II GS, through a 12 bits A/D converter board.

3
Results

3.1
Concentration profiles in the bioreactor

Three continuous fermentation experiments were performed with the media S50 and S100, results are showed in Table 1. The bioreactor performance was kept stable throughout 70 days. To keep the biomass concentration constant, it was necessary to periodically purge the biomass. Productivities up to 40 g/l h were reached during the fermentation process, using a sucrose concentration of 100 g/l.

In order to analyse possible concentration gradients, ethanol and sugars (fructose, glucose and sucrose) were determined at several points along the bioreactor. The sample points (Fig. 1) were: A and B, placed at the bottom of the bioreactor (at the final part of the biomass recycle loop); C, on the other side, all of them at 1.5 cm from the feed entrance. The sample points D, E and F were placed at distances of 8, 20 and 34 cm from the bottom, respectively, and S was the sample point corresponding to the exit stream.

Table 1. Concentration profiles along the gas-lift bioreactor

S50 Medium (X=100 g d.w./l)				
	D	E	F	S
Ethanol (g/l)	23.5	24.0	23.0	22.4
S100 Medium (X=85–90 g d.w./l)				
	A	B	C	S
Fructose (g/l)	7.0	7.1	6.7	6.5
Glucose (g/l)	3.7	3.7	3.5	2.9
Sucrose (g/l)	5.0	5.0	5.6	2.4
Ethanol (g/l)				35.0

S100 Medium (X=100–110 g d.w./l)							
	A	B	C	D	E	F	S
Fructose (g/l)	7.2	5.2	7.6	4.2	4.2	4.1	4.5
Glucose (g/l)	5.2	3.1	5.8	2.1	2.0	1.9	2.2
Sucrose (g/l)	0.4	0.4	0.4	0	0	0	0
Ethanol (g/l)							40.0

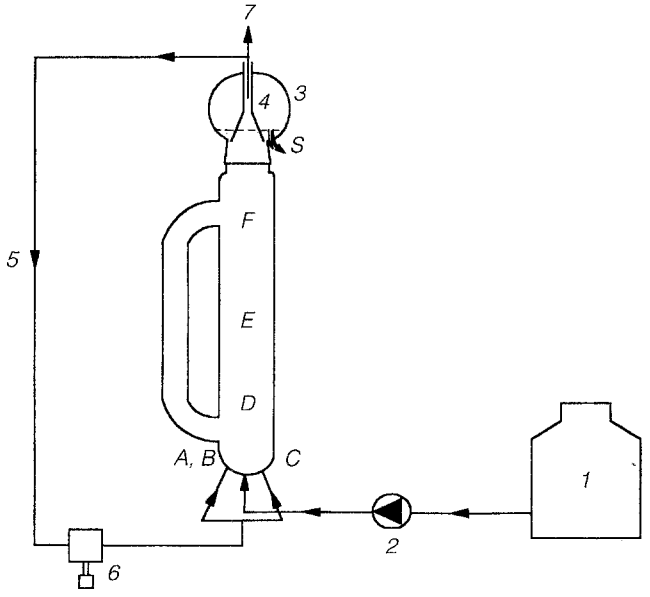

Fig. 1. Scheme of gas lift bioreactor: 1 Feeding tank; 2 Pump; 3 Settler; 4 Gas collecting chamber; 5 Gas recycle; 6 Compressor; 7 Gas purge. A, B, C, D, F Sample points. S Exit

As it can be seen in Table 1, ethanol concentration was almost constant for all the operating conditions. Nevertheless, using the medium S100 and biomass concentrations of 90 and 103 g d.w./l, a zone placed in the bottom of the bioreactor can be detected (at a distance of 1.5 cm from the feed stream) where the sucrose concentration was twice as high as in the effluent, being 5 g/l in the final experiment. The overall sugar concentration for experiments with 90 g d.w./l of biomass and the S100 medium was 15.8 g/l at the bottom of the bioreactor, 75% of this value being at the exit. Using the S100 medium and 103 g d.w./l of biomass, the overall sugar concentration of the bioreactor was one half of that obtained at the bottom.

3.2
Curves of residence time distribution

In order to study the macroscopic behaviour of the bioreactor and the influence of biomass and substrate concentrations, several RTD experiments were performed.

Two RTD's were carried out using the medium S50 and employing biomass concentrations of 85 and 108 g d.w./l; the mean size of the flocs were 1 mm and 2 mm respectively. Experimental data were fitted to the CSTR model. From these experiments the void fraction determined were 75 and 62% of the overall volume corresponding to a mean residence time of 44.6 and 35.3 min respectively. The overall gas hold-up was 5% of the total volume.

However, the hydraulic model of the fermentation with the S100 medium corresponds to the combined model of the two CSTR's with a flow exchange. The model equation is as it follows:

$$\frac{C_{T1}}{C_{T10}} = \frac{(\alpha \cdot m_1 + \beta + 1) \cdot e^{m_2 \cdot \theta} - (\alpha \cdot m_2 + \beta + 1) \cdot e^{m_1 \cdot \theta}}{\alpha \cdot (m_1 - m_2)} \cdot$$

$$|m_1| < |m_2| \qquad (1)$$

Where: α is the rate between the volume of the highly agitated region and the overall volume ($\alpha = V_1/V$); β is the fraction of the total flow exchanged between the two reactors of the model ($\beta = Q_1/Q_0$); and θ the dimensionless time ($\theta = t \cdot Q_0/V$).

α and β were determined by using the method described by Fogler, 1992 [9]. In Fig. 2, the good fitting of the experimental data to the model can be observed. The value of α is 0.95, which indicates a region with a low exchange rate with the rest of the bioreactor (5% of the overall one). β has a value of 0.014, the exchanged flow being between the two regions of the model $28.5 \cdot 10^{-3}$ (l/h).

From these results and from those obtained before for concentration profiles, it can be deduced that a region exists, probably placed between the feeding point and the biomass recycle loop, in which mixing is less efficient. Nevertheless, this region implies a small reaction volume (100 ml) as it was stated above.

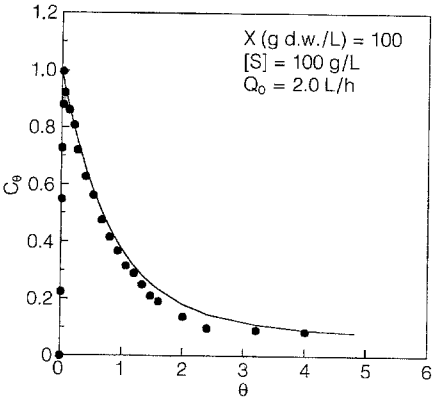

Fig. 2. RTD curve obtained injecting a pulse of a lactose solution (94 g/l), S100 medium and a biomass concentration of 100 g d.w./l and fitted by using a combined model of two CSTR with flow exchange

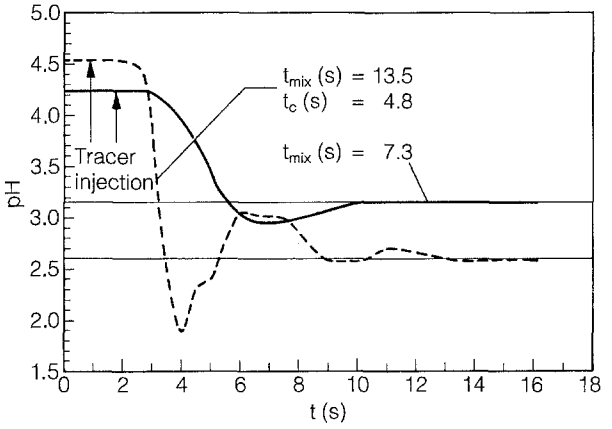

Fig. 3. Response curves to determine the mixing time: (----) Tracer injection in the settler of the bioreactor. (———) Tracer injection in the feed stream of the bioreactor

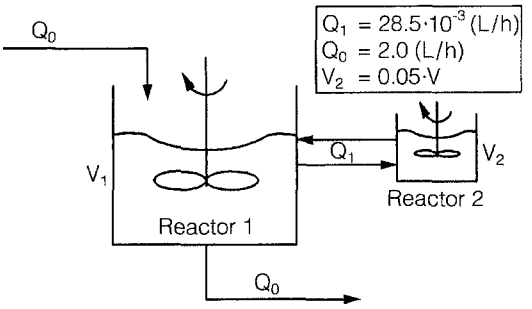

Fig. 4. Scheme of the proposed model to fit the experimental data, showing the parameters

3.3
Determination of mixing time

This experiment was carried out by using the S100 medium and a biomass concentration of 99 g d.w./l, the tracer being concentrated sulfuric acid. The response time of the electrode was determined from a step disturbance in the pH from 6 to 4, obtaining a value of 1.5 s for 95% of the full response.

Figure 3 shows the response curves to the tracer pulse introduced in the settling zone and in the feed stream. As it can be seen, when the tracer was check injected into the feed stream, the mixing is almost instantaneous, only 7.3 s being required for the complete mixing, oscillations in the tracer concentration were not observed. Nevertheless, several circulations of the tracer in the bioreactor appeared when it was check injected into the settler. In this case the mixing time and the circulation time, measured from the response curve, were 13.5 and 4.8 seconds respectively. These values are in agreement with those obtained by using the Bello equation [10] (circulation time 6.2 s). These values suggest a limited mixing zone placed in the biomass settler.

4
Conclusions

The results obtained, using the three different methods, show that the gas-lift bioreactor presents a hydraulic behaviour close to a complete mixing reactor, this fact being more evident from the analysis of RTD's curves. In fermentations with low substrate concentrations (50 g/l) the hydrodynamic behaviour can be predicted by means of a complete stirred tank reactor model, including the operation at high biomass concentrations (108 g d.w./l). However, from the substrate concentrations profiles, a very small region with respect to the overall volume can be detected. This region is placed at the bottom of the bioreactor where the sugar concentration is higher than in the rest of the bioreactor, being in some cases twice as high. Nevertheless, the substrate concentration in this zone is notably lower than in the feed stream.

Increments of the substrate concentration up to 100 g/l has a larger influence on homogenization process than increments of the biomass concentration, being necessary to fit the data to a combined model consisting of two stirred tanks with flow exchange. This special behaviour using a substrate concentration of 100 g/l can be justified by the different viscosity of the medium provoking a defficient mixing in the settler or/and also a limited diffusion to the interior of the flocs. The parameters of the model permit the determination, between the two reactors of the model, of a dead zone, with a volume of 100 ml and a exchange flow of $28.5 \cdot 10^{-3}$ l/h, a scheme of the proposed model is showed in Fig. 4. The dead zone, probably located in the settler, accounts for 5% of the overall volume. This close to complete mixing behaviour is also in agreement with other results that have been found in literature where dead volumes between 4–7% of the reactor volume as a function of the recirculation flow rate [11], but not for other models with higher complexity [1]. This fact is also supported by the mixing time experiments.

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