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ORIGINAL PAPER

Influence of bioreactor hydraulic characteristics on a *Saccharomyces cerevisiae* fed-batch culture: hydrodynamic modelling and scale-down investigations

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Abstract Yeast is a widely used microorganism at the industrial level because of its biomass and metabolite production capabilities. However, due to its sensitivity to the glucose effect, problems occur during scale-up to the industrial scale. Hydrodynamic conditions are not ideal in large-scale bioreactors, and glucose concentration gradients can arise when these bioreactors are operating in fed-batch mode. We have studied the effects of such gradients in a scale-down reactor, which consists of a mixed part linked to a non-mixed part by a recirculation pump, in order to mimic the hydrodynamic conditions encountered at the large scale. During the fermentation tests in the scale-down reactor, there was a drop in both biomass yield (ratio between the biomass produced and the glucose added) and trehalose production and an increase in both fermentation time (time between inoculation and beginning of stationary phase) and ethanol production. We have developed a stochastic model which explains these effects as the result of an induction process determined mainly by the hydrodynamic conditions. The concentration profiles experienced by the microorganisms during the scale-down tests were expressed and linked to the biomass yields of the scale-down tests.

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Introduction

The production of microorganisms is a difficult task to realize as both a living material and its interaction with the environmental conditions have to be taken into account. Mixing operations are necessary in this production process because agitation enables optimal growth of the microorganism by optimizing such physical phenomena as heat transfer, gas circulation, homogenization, among others [1]. The large-scale production is an essential stage of the development of a bioprocess, but as the volume increases, various limiting phenomena become of importance. One of the consequences of volume increase is the appearance of heterogeneous zones in the reactor, which are mainly concentration gradients in pH, substrate or oxygen. The presence of these gradients in the reactor forces the microorganism to adapt and change its metabolic pathways in accordance with fluctuations in the concentrations of substances in the extracellular environment [2, 3]. These perturbations cause different effects, called the "scale-up" effect, and include a drop in biomass yield (ratio between the biomass produced and the glucose added), loss of viability and an increase in fermentation time (time between inoculation and beginning of stationary phase) and ethanol production [4]. In order to better understand these phenomena, researchers often turn to the "scale-down" principle. Scale-down reactors (SDR) have been described in the literature as a tool allowing the study of the consequences of poor mixing conditions on the growth of microorganisms. Such reactors reproduce the hydrodynamic conditions of industrial reactors [5], so that the

effects of increasing volume can be seen at a laboratory scale [6–8]. They generally consist of a mixed part linked to a non-mixed part by a recirculation pump [9, 10], with the geometry of the non-mixed part depending on the limiting phenomena to be studied [11]. These reactors have already been tested with different microorganisms and found to be suitable for the study of a wide range of effects related to a poor mixing efficiency. They have been used to simulate the effects of oxygen and dextrose concentration gradients [11–17] on the culture of different microorganisms. Various configurations of SDR have been used to determine the effect of dextrose concentration gradients [18, 19] and dissolved oxygen gradient [20–22], or both [23]. The geometry of the plug-flow part of the scale-down reactor has been found to be relevant to stress induction [24]. The scale-down principle can also be used in parallel with numerical analysis to enhance the characterization of the hydrodynamics.

A structured hydrodynamic model is able to simulate the mixing phenomena in a bioprocess with a good resolution [25–27]. In such a model, the reactor is divided into a set of interconnected compartments, considered to be perfectly mixed and linked by flow (mass, heat, among others). The simplest model that has been described comprises one compartment at the agitation stage [28, 29]. The network-of-zones analysis is a more complex model in which a large number of compartments are taken into account [30, 31]. The original version of this model has been extended to simulations of bubble size distributions, gas–liquid transfers, kinetics of bioreactors and multi-agitated systems [26]. The structured model can be developed according to two approaches: deterministic or stochastic. The deterministic method is based on the consideration of differential equations that define the flows between the compartments, while the stochastic approach allows the inclusion of the probabilistic nature of the mixing process and the circulation of particles in the reactor to be traced [32]. The advantage of such a model is that both representation of the circulation of the microorganisms and fluid mixing can be performed using the same structure [33].

The focus of the study reported here was the production of *Saccharomyces cerevisiae* (*boulardii*), which is used on a wide scale at the industrial level because of its capability to produce biomass and metabolites [34]. In this article, we first describe the geometries of the three SDR that we used in our study to reproduce gradient stress experiments at the laboratory scale. We observed different parameters during the culture of *S. cerevisiae* in these SDR (biomass, ethanol or trehalose concentrations) in order to estimate the impact of this stress and then characterized the hydrodynamic conditions of these reactors. We also developed a stochastic model of these conditions.

Materials and methods

Culture of *S. cerevisiae* in scale-down reactors (SDR)

Saccharomyces cerevisiae (MUCL 43340), stored at −80°C before use, was inoculated into a 500-ml preculture medium [dextrose (20 g/l), peptone casein (10 g/l), yeast extract (10 g/l)] and incubated at 30°C for 12 h under orbital agitation. The culture medium was composed of dextrose (5 g/l), peptone casein (10 g/l) and yeast extract (10 g/l). The SDR consisted of a 20-l stirred bioreactor (Biolafitte, France) with two turbines TD4-TD4. The regulation of temperature (30°C), pH (5.5) and dissolved oxygen was ensured by using a direct control system (ABB). The dissolved oxygen level was maintained above 30% saturation by the stirrer speed. Aeration was fixed at 1 vvm. The stirred reactor was connected by a peristaltic pump (Watson-Marlow 325D; Watson-Marlow Bredel, Falmouth, UK) to the appropriate non-mixed part. The three types of SDR are as follows (Fig. 1):

- SDR type A: glass bulb with a diameter of 85 mm, a length of 0.25 m and a capacity of 1 l;
- SDR type B: pipe with an internal diameter of 8 mm, an external diameter of 12 mm, a length of 7.5 m and a capacity of 0.377 l;
- SDR type C: pipe with an internal diameter of 15 mm, an external diameter of 21 mm, a length of 5 m and a capacity of 0.884 l.

The fed-batch mode was used in all fermentation tests. The dextrose solution was introduced into the non-mixed part. The feeding strategy adopted during each fermentation test first 5 h of culture in batch mode, followed by 12 h of exponential fed-batch mode, with the addition of dextrose controlled by this equation:

$$F = F_0 * e^{(\mu_{\max} * t)} \quad (1)$$

where F is the feed flow rate (ml/min), F_0 is the initial feed flow rate (ml/min), $\mu_{\max}$ is the maximum specific growth rate of the microorganism (1/min) and t is the culture time (min).

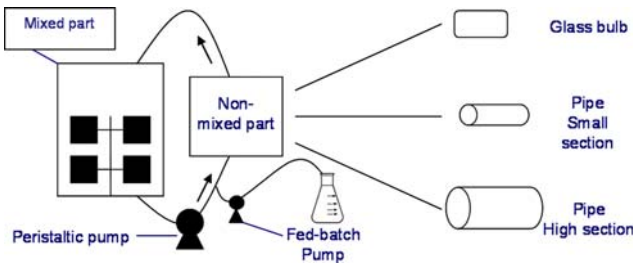

Fig. 1 Description of the three scale-down reactors tested

The parameters μ_{max} (0.005 1/min) and F_0 (0.0058 ml/min) were previously calculated from growth data of *S. cerevisiae* in batch reactor (data not shown). After the exponential fed-batch mode, the feeding was fixed at $Q_{cst} = 3.5$ ml/min until the end of the fermentation, i.e. when the dissolved oxygen goes back to >30%.

Fermentation follow-up

During the fermentation tests, samples were withdrawn at the level of the mixed part of the reactor, and a number of measurements were carried.

The microbial growth of *S. cerevisiae* (*boulardii*) was evaluated by optical density measurements at a wavelength (λ) of 540 nm on a GENESYS 2 spectrometer (Thermo Scientific, Waltham, MA).

Biomass concentration was determined by the correlation between optical density and dry matter.

The dextrose concentration was determined by an enzymatic method (model 2700 Select; YSI, Yellow Springs, OH).

The concentration of ethanol was determined using an enzymatic kit (Enzymatic BioAnalysis; Boehringer Mannheim, Germany).

The intracellular trehalose concentration was determined by extracting trehalose from the cells after washing the samples with water three times. The pellet was then resuspended in 5 ml of distilled water and left for 5 min in a boiling water bath. After centrifugation, the supernatant was recovered and analysed by high-performance anion exchange chromatography coupled with pulse amperometric detection) on a Dionex DX500 chromatographic system operating at 1 ml/min. The volume of injected sample was 25 µl. The stationary phase consisted of a CarboPac PA 100 column (250 × 4 mm) with a pre-column PA 100 (50 × 4 mm) (Dionex, Sunnyvale, CA). The mobile phase consisted of sodium hydroxide (160 mM). The samples were eluted in isocratic mode, followed by a linear gradient with a solution containing both sodium hydroxide (160 mM) and sodium acetate (500 mM). The gradient was completed by washing with 500 mM sodium hydroxide [35, 36].

Mixing time measurement

The mixing time was estimated using a conductometric method. A saline solution (NaCl saturated) was added at the top of the non-mixed part. The conductivity probe was positioned in the mixed part. Relative conductivity (%) was calculated from the conductivity curve. The mixing time was defined as the length of time between the injection of the saline solution and the moment when the relative conductivity reached a value of 85% [37].

Hydrodynamic modelling

In order to understand better the phenomena which take place in the reactor during fermentation, we developed a structured model. The reactor volume was divided into compartments, called "states", which were considered to be perfectly mixed. A stochastic approach was chosen that describes the mixing phenomenon in term of probabilities. The mixed part of the scale-down reactor was divided into 64 states (8 plans, divided into 8 states). Each state was linked to their neighbors by probabilities. The number of states in the non-mixed part depends on the type of scale-down device used (see Results and discussion).

As presented in Fig. 2, three types of probabilities governed mixing in the mixed part: (1) the probability of staying in the present state (P_{stay}), (2) the probability of entering into the circulation flow ($P_{circulate}$) and (3) the probability of diverging from this flow and shifting into another plan (P_{shift}). In the non-mixed part, only the probabilities of staying in the present state or leaving this state and passing into the next state were considered. The passage between the mixed and the non-mixed part is defined by P_{enter}.

The aim of this model was to simulate the dextrose concentration encountered by the microorganisms in the reactor during a fermentation test. As these data are difficult to obtain, two sub-models were considered.

Sub-model 1: fluid mixing

The dextrose concentration gradient that appears in the reactor during the fermentation can be modelled by a Markov chain [24], which is defined as the multiplication of a transition matrix (T) and a state vector (S). The transition matrix is formed by the probabilities presented in Fig. 2 and are proportional to the different flows present in

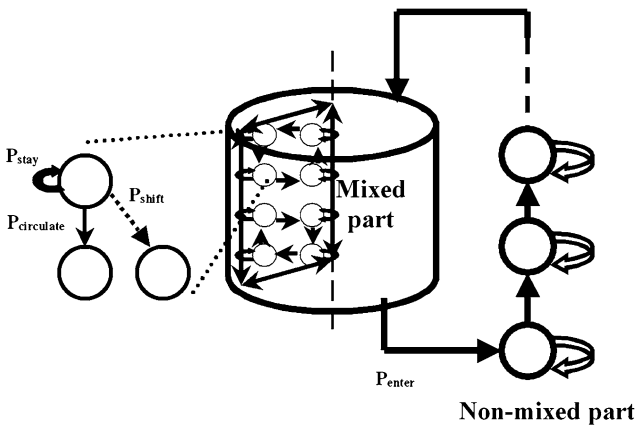

Fig. 2 Structure of the compartment model used to run stochastic simulations. In the case of scale-down reaction (SDR) type A, the non-mixed part comprises three compartments

the reactor. The probability of staying in the circulation flow of the mixed part ($P_{\text{circulate}}$) can be determined from the calculation of the circulation flow rate:

$$Q_c = N_{qc} * N * d^3 \quad (2)$$

$$P_{circulate} = \frac{Q_c}{V_{comp}} * \Delta t \quad (3)$$

where Q_c is the circulation flow (m^3/s), N_{qc} is the pumping number, N is the impeller speed (1/s), d is the impeller diameter (m), V_{comp} is the compartment volume (m^3) and Δt is the time step chosen to run the simulation (in our case, $\Delta t = 1$ s).

The probability of leaving this loop, diagonally or from one plan to another, is:

$$P_{shift} = 1 - P_{circulate} \quad (4)$$

The probability of remaining within the present stage is calculated using the same formula.

The state vector includes the concentration of tracer in the different states considered by the model. The result of this multiplication is a state vector describing the concentrations in each state at the next time interval:

$$S_I = T * S_{I-1} \text{ or } S_i = T^I * S_0 \quad (5)$$

where S is the state vector at the considered time and T is the transition matrix.

Successive multiplications lead to an understanding of the evolution of the dextrose concentration gradient in the reactor.

In order to calculate accurately the dextrose concentration gradient, the intermittent behaviour of the pump must be considered [33]. Therefore, a pulse matrix was used to represent the discontinuous behaviour of the pump, in which the number of columns corresponded to the number of transitions, and the number of rows to the number of states. The pulse was carried out at the level of the first state of the model, i.e. at the level of the non-mixed part, where the dextrose solution is added during the fermentation tests.

The Markov chain was modified to introduce the S_{pulse} matrix:

$$S_I = T * S_{I-1} + S_{pulse} \quad (6)$$

The concentrations obtained after the application of this model were normalized i.e. brought back to between 0 and 1, which permitted to compare the different profiles obtained.

Sub-model 2: circulation of particles

A stochastic model of "random number" type was used. The displacement of a particle was calculated by comparing a number to the value of the transition probability in order to determine if the cell stays in the present state or switches to the next state.

The structure of the transition matrix was the same as that presented for the fluid mixing sub-model and was not developed during our study.

Superimposition of the two sub-models

The two sub-models were superimposed in order to obtain the dextrose concentration profile encountered by the microorganism during a fermentation test. The profiles form frequency distributions, which allowed the history of the gradient encountered by the microorganisms to be determined. Each bar of the histogram represents the number of microorganisms that were, on average, subjected to the range of concentrations corresponding to the class.

Results and discussion

Fermentation tests in scale-down reactor

The aim of the fermentation test was to demonstrate the impact of the use of a SDR to produce *S. cerevisiae*. The parameters studied were biomass and ethanol and trehalose concentration. The biomass yield was calculated for each test [ratio between the amount of biomass obtained at the end of the fermentation and the amount of substrate (dextrose) involved in the same fermentation].

Tests carried out in the different scale-down configurations were compared on the basis of a reference reactor, i.e. a well-mixed bioreactor ($t_m = 5$ s), without a recirculation part. The dextrose solution was added at the top of the stirred vessel. The biomass yield obtained in these conditions reached 47.9%; in comparison, the ideal yield for a yeast fermentation under fully aerobic conditions is 50% [6, 38]. During the culture, the production of ethanol was detected. It should be noted that the small-scale reactor used as a reference can not be considered to be perfectly mixed, but only as nearly perfectly mixed or well mixed. Tracer experiments were carried out, and a mixing time of about 5 s was determined, which is very low compared with mixing time values found for SDR.

The final biomass concentration in all of the scale-down tests was lower than that of the reference reactor (Fig. 3). The greatest reduction was obtained with SDR type B, with a recirculation flow rate (Q_R) of 39 l/h. The difference between the reference reactor and the scale-down tests appeared after approximately 20 h of culture, when the dextrose solution was added at a constant rate. At this time, the dextrose content in the reactor was close to 0 g/l (data

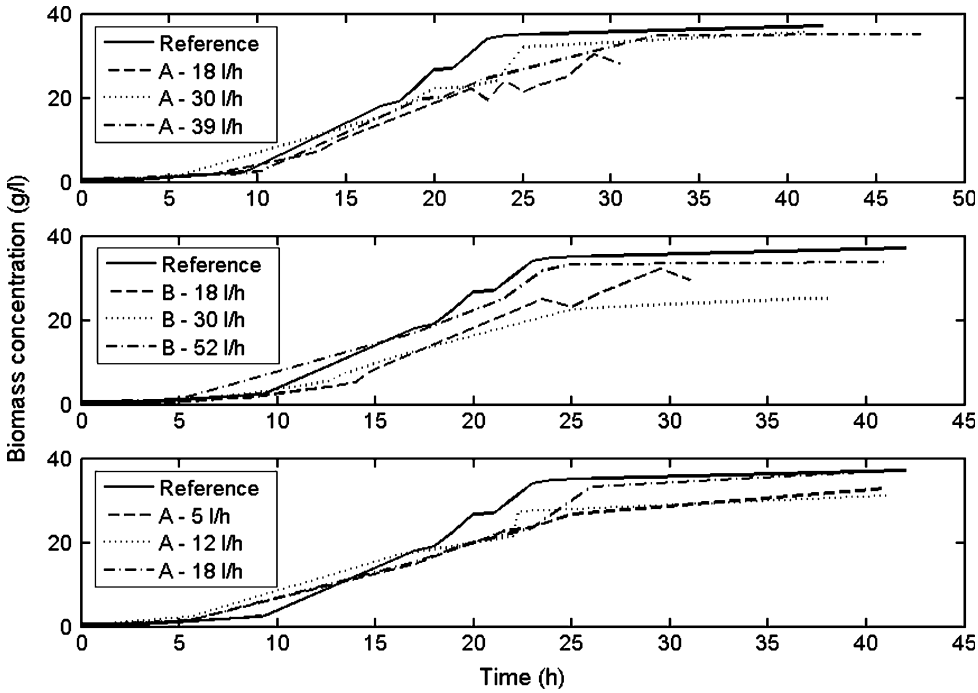

Fig. 3 Evolution of biomass concentration for the three types of SDR. The reference reactor is represented by the *solid line*. **a** SDR type A with $Q_R = 18$, 30 and 39 l/h, **b** SDR type B with $Q_R = 18$, 39 and 52 l/h, **c** SDR type C with $Q_R = 5$, 12 and 18 l/h

not shown), so these differences cannot be explained by a difference in a dextrose consumption rate.

Saccharomyces cerevisiae is a Crabtree-positive microorganism. As such, when the cells are exposed to high concentrations of dextrose, they produce ethanol [39]. This production can cause the biomass yield to fall to 39% [6], whereas the ideal yield is 50%. In the SDR, the ethanol concentration reached during the test was greater than that reached in the reference reactor (Fig. 4). This increase ethanol production is due to the fact that cells were exposed to high dextrose concentrations in the non-mixed part and subsequently switched their metabolism to the fermentation process, producing ethanol. Scale-down reactors types B and C were shown to be systems in which cells produce more ethanol than SDR type A, with type C producing the highest concentration, with $Q_R = 18$ l/h.

Trehalose is a reserve carbohydrate used by yeast cells to survive during nutrient limitation or starvation, but it is also a cryoprotectant that provides protection to the plasma membrane during, for example, freeze-drying. A high cellular content of trehalose is linked to a high capacity to resist conservation treatments. The results presented here show that the final trehalose content was influenced by the use of the SDR (Fig. 5). It was higher for the reference reactor than for SDR type C. The recirculation flow also had an impact on Trehalose content in that as the recirculation flow increased, the trehalose content increased, approaching the content of the reference reactor. Trehalose is synthesized by cells when nutrient conditions are favourable, and it can be reassimilated under conditions of nutrient starvation. In our SDR, glucose was added at the level of the nonmixed part; therefore, starvation tended to occur at the level of the mixed part. This last part represents about 90% of the reacting volume, and the starvation effect is thus very important. Trehalose reassimilation is therefore expected to occur when the residence time at the level of the nonmixed part is higher.

The biomass yield was calculated for each SDR and determined as a function of the recirculation flow rate (Fig. 6). In Fig. 6, the values of the reference reactor are indicated by the broken line in order to provide a basis of comparison with the results of the scale-down tests. The biomass yield of the reference was found to be superior to that of the SDR, with the greatest difference being observed for the SDR type B with $Q_R = 39$ l/h. Biomass yield depends on the recirculation flow rate, as have almost all of the parameters discussed so far, with the yields increasing with the recirculation flow rates. Three tests showed a yield close to that of the reference reactor (SDR type A with $Q_R = 30$ and 39 l/h and SDR type C with $Q_R = 18$ l/h).

An assessment of the different parameters reveals that a SDR mimics the scale-up effects observed in a large-scale bioreactor in that they induce an increase of by-product (ethanol) excretion and a reduction in biomass concentration, biomass yield and final trehalose concentration. Similar observations have been reported for a fed-batch culture of *E. coli* in a large-scale bioreactor (20 m^3) and different SDR [2, 23]. Various hypotheses can be formulated to gain an understanding of these effects. The first hypothesis is based on the appearance of fluctuations in temperature, pH or dissolved oxygen in the non-mixed

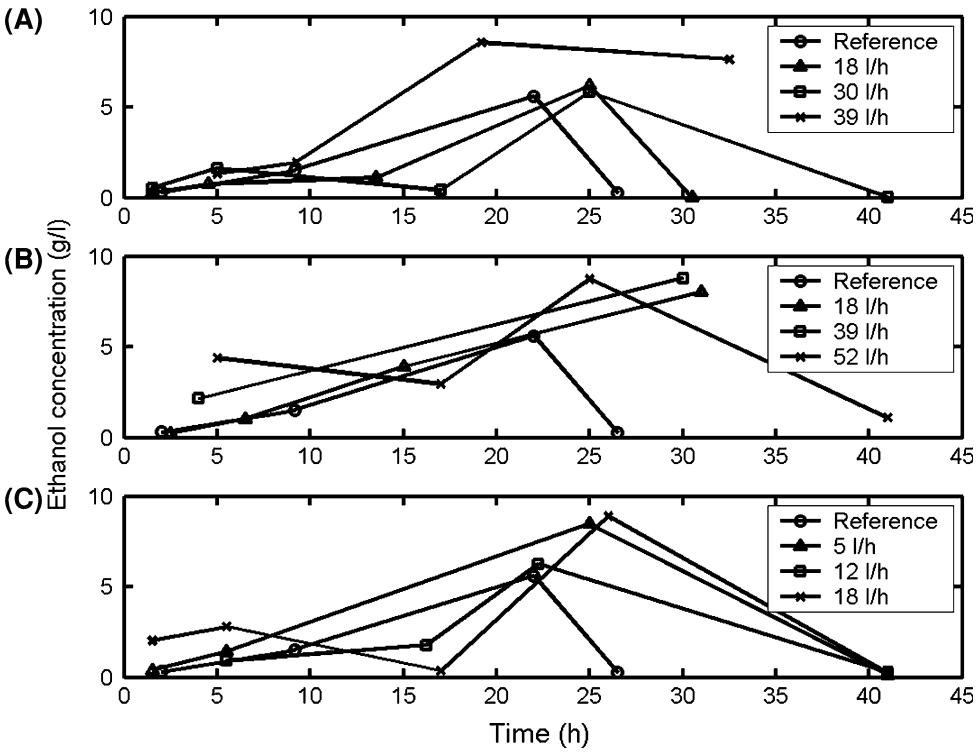

Fig. 4 Evolution of ethanol concentration for the different fermentation tests realized. **a** SDR type A with $Q_R = 18$, 30 and 39 l/h, **b** SDR type B with $Q_R = 18$, 39 and 52 l/h, **c** SDR type C with $Q_R = 5$, 12 and 18 l/h

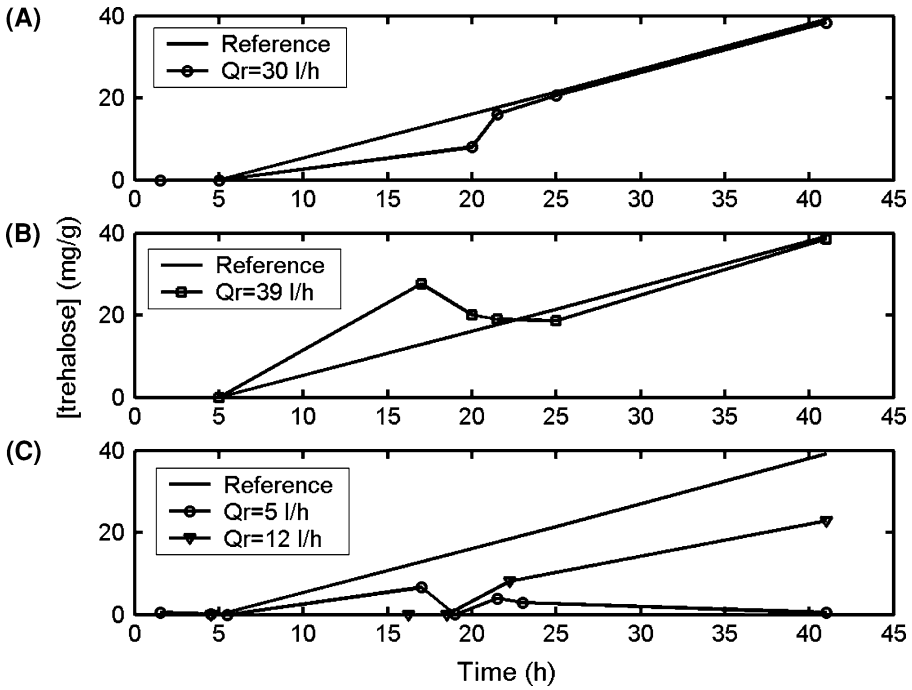

Fig. 5 Evolution of trehalose concentration for the different fermentation tests realized. **a** SDR type A with $Q_R = 30$ l/h, **b** SDR type B with $Q_R = 52$ l/h, **c** SDR type C with $Q_R = 5$ l/h, **d** SDR type C with $Q_R = 12$ l/h

part, although some measurements showed that it was not the main explanation [40]. Our working hypothesis is therefore: the yield drop is due to the passage of microorganisms into the non-mixed part, in which the dextrose concentration is submitted to high fluctuations. Thus, microorganisms have to adapt their metabolism and their enzymatic systems to these continually changing conditions [41]. Even if the key stages of the metabolism can respond to fluctuations in substrate within <1 min [9], these continuous changes slow down the normal growth of yeast. These adaptation phenomena cause a lack of efficiency in biomass production and thus a reduction in yield.

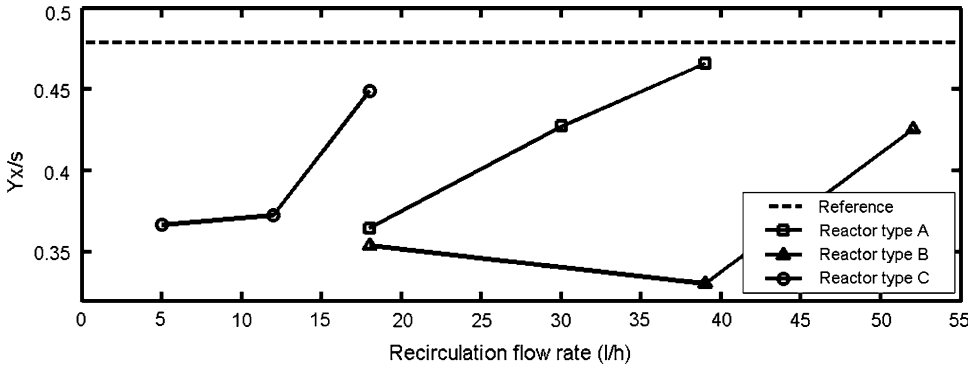

Fig. 6 Comparison of the biomass yield between the scale-down reactors tested

Mixing efficiency of scale-down reactors

All of the tests realized in a SDR showed a reduction in biomass yield, but the same reduction was not found in all test. A hydrodynamic characterization is essential to explain the differences observed. A mixing time calculation can provide information on the mixing efficiency in a reactor. This parameter was measured for each fermentation test realized in the SDR.

The evolution of the relative conductivity in the mixed part was different for each type of SDR (Fig. 7). In the case of types A and C, the mixing phenomenon is in two steps in terms of homogenization: the saline solution is dispersed into the non-mixed part, following which it seeps out gradually into the mixed part. The curve increases slowly as the tracer comes gradually into the mixed part. The curve profiles are completely different for SDR type B, which is governed by a plug-flow section. There is a low fluid dispersion in the non-mixed part, and the perturbation caused by the addition of saline solution is propagated in one pulse into the mixed part. The geometry explains the quasi instantaneous increase in relative conductivity. An increase in the diameter of the non-mixed part was associated with a decrease in plug-flow section effect, which gave way to dispersion in the scale-down reactor of types A and C.

The mixing time was calculated for each test (Table 1). These were different for each type of SDR, type B giving the shortest mixing times. The system that gave the worst mixing efficiency, at the particular recirculation flow rate tested, was SDR type C, partially due to the increase in volume of the non-mixed part and to the increase in the number of possible ways the tracer can reach the detector. Moreover, SDR type C had very low recirculation flow rates compared to the other two SDR types. Scale-down reactor type A, the system in which the dispersion phenomenon was the strongest, gave longer mixing times than SDR type B.

The yield in biomass was found to depend on mixing time (Fig. 8), with the yield decreasing with increasing mixing time. The highest yield drop appeared for SDR type B, which is surprising because it leads to the shortest mixing time and so to the best mixing efficiency. One possible explanation is that in this type of reactor, in which the non-mixed part consists of a pipe with a small section, the arrival of the cell in the pipe at the same time as a dextrose pulse would cause the cell to be exposed to high dextrose concentrations as long as it is in the pipe. Therefore, the cell has to change its metabolic pathways and adapt itself to the new conditions. The "Hydrodynamic modelling" section will provide other explanations.

Hydrodynamic modelling of scale-down reactors

The differences observed between the three types of SDR appeared after 15 h of culture, i.e. when the feed solution was added at a constant flow. We therefore carried out the simulation when the pump adds dextrose into the systems at 5-s intervals. The exposure time to the pulse was 1000 s, and the number of cells was 3500. It is clear that these values are far from reality, but they allow us to develop a good idea of the hydrodynamic conditions in the reactors. These considerations have been discussed by Delvigne et al. [42].

The number of states considered in the model was different for each non-mixed part to allow a clear differentiation between the different hydrodynamic conditions observed with the conductometric tests. The probability of passing in the non-mixed part from one state to another was defined as:

$$P_{plug} = \frac{Q_R}{V_{comp}} \tag{7}$$

where P_{plug} is the probability of passing from state I to state $I + 1$, Q_{R} is the recirculation flow rate (l/h) and V_{comp} is the state volume (l).

As plug flow was predominant for SDR types B and C, we modelled the non-mixed part using a high number of compartments placed in series in order to mimic the progression of a tracer in the pipe, as observed in tracer tests.

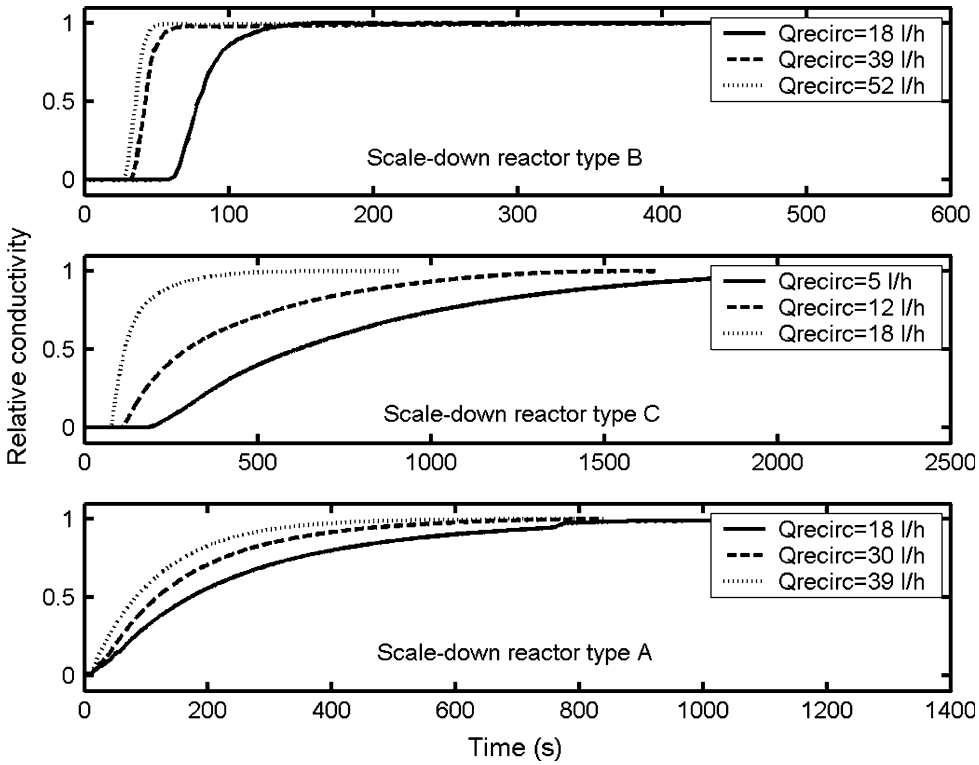

Fig. 7 Variation in the relative conductivity when a pulse of saline solution is injected into the non-mixed part of the reactor. **a** SDR type A with $Q_R = 18$ (*solid line*), 30 (*dashed line*) and 39 l/h (*dotted line*), **b** SDR type B with $Q_R = 18$ (*solid line*), 39 (*dashed line*) and 52 l/h (*dotted line*), **c** SDR type C, with $Q_R = 5$ l/h (*solid line*), 12h (*dashed line*) and 18 l/h (*dotted line*)

Table 1 Synthesis of the different parameters characterizing the hydrodynamic conditions of scale-down reactors

Reactor type	Recirculation flow rate (l/h)	Yx/s	Mixing time (s)	Relative number of stressed cells (%)	Fluctuation magnitudes in dextrose concentration
A	18	0.352	487	2.09	0.06
	30	0.451	306	0.54	0.1
	39	0.457	213	0.14	0.13
B	30	0.354	101	0	0.7
	39	0.331	50	0	0.95
	52	0.425	40	0	1
C	5	0.418	1305	9.29	0.007
	12	0.396	748	0.94	0.16
	18	0.481	214	0.17	0.25

The plug flow effect decreased from SDR type B to C, with SDR type A governed by dispersion. Consequently, the number of cells in SDR type B was 15, in type C, ten, and in type A, three.

All of the microbial cells were present in the first state of the non-mixed part at the initial time. The frequency distributions obtained from the model enabled us to observed the appearance of the dextrose concentration gradient in the reactor. The different parameters are discussed here to provide a better understanding of the yield drop observed in the scale-down tests.

The profiles of the frequency distributions differed depending on the type of SDR (Figs. 9, 10 and 11). In Fig. 9, the profiles of the frequency distributions of SDR type A decrease regularly. The frequency distributions of SDR type B (Fig. 10) show that the cells in the reactor are particularly exposed to small relative concentrations of dextrose. The frequency distributions of SDR type C (Fig. 11) show that there were a large number of cells that encountered relatively small dextrose concentrations but also cells that were exposed to relatively high concentrations. The recirculation flow rate is an important parameter of the model. The distribution seems to be much more compact for high recirculation flows than for low recirculation flows. Therefore, a higher number of cells would be exposed to high dextrose concentrations if a low recirculation flow were to be used.

The profiles of the distributions of SDR type A and C are similar. This result agrees with the results obtained for mixing time tests, which show that the cause of

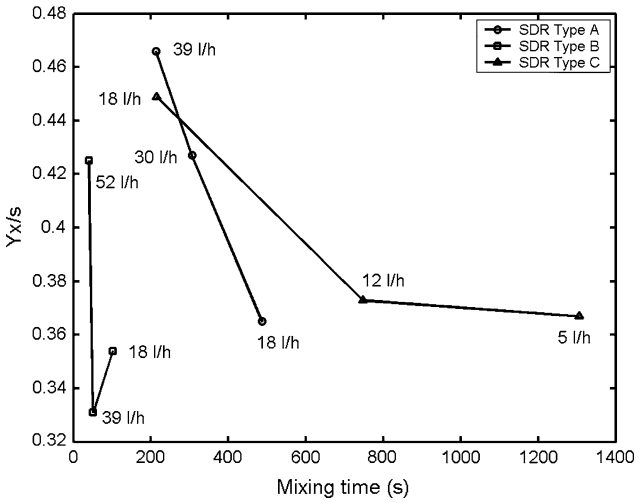

Fig. 8 Relationship between mixing time and biomass yield

homogenization of the tracer in these two types of SDR is dispersion. However, the distributions of SDR type C seem to be divided into two parts: for the relatively small concentrations in dextrose, the profiles resemble those of SDR type B; when the relative concentrations increase, the profiles become closer to those of SDR type A. This result corroborates the results of the mixing time tests.

The SDR of type B show the shortest mixing times. The profiles of the relative concentrations encountered by cells indicate that the majority of cells are exposed to relatively small concentrations. However, this type of SDR shows the highest reduction in biomass yield. To explain this difference in biomass yield, we propose the following parameters to be relevant.

First, it is possible to count the number of cells that have been exposed to relative concentrations higher than a critical relative concentration—in our case, 0.5. The number of cells is expressed relative to the total number of cells in the population (3500) (expressed in percentage) (Table 1). This relative number of cells decreases, whereas $Y_{X/S}$ increases. This means that in the case of SDR with a high yield drop, a higher number of cells was exposed to relatively high dextrose concentrations. Therefore, the relative concentrations encountered by cells have an impact on the yield of the culture. The high dextrose concentration imposes the condition that the cells have to adapt their metabolism to withstand these fluctuations, resulting in a decrease in the biomass yield of the culture. These high concentrations are located in the non-mixed part of the SDR. This reasoning verifies our working hypothesis: the yield drop is due to the passage of the cells through the non-mixed part where they are exposed to high concentrations of dextrose.

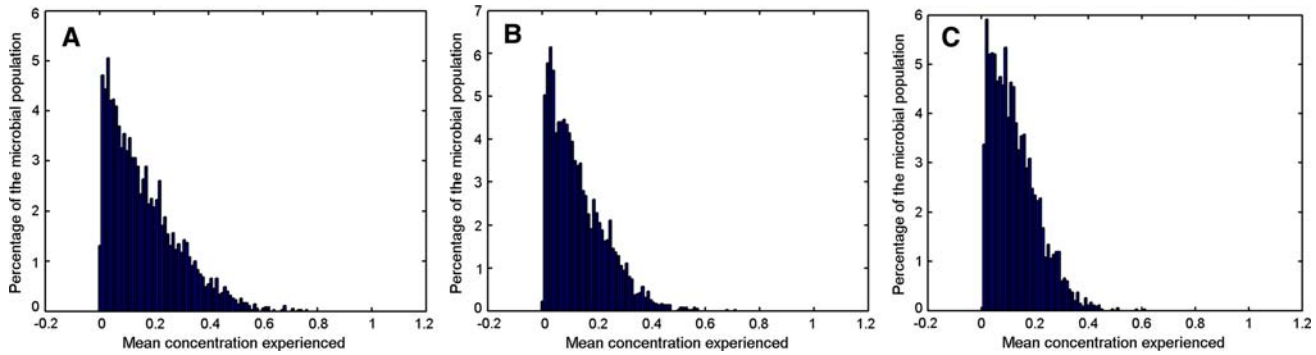

Fig. 9 Frequency distributions for scale-down reactors type A: **a** $Q_R = 18$ l/h, **b** $Q_R = 30$ l/h, **c** $Q_R = 39$ l/h

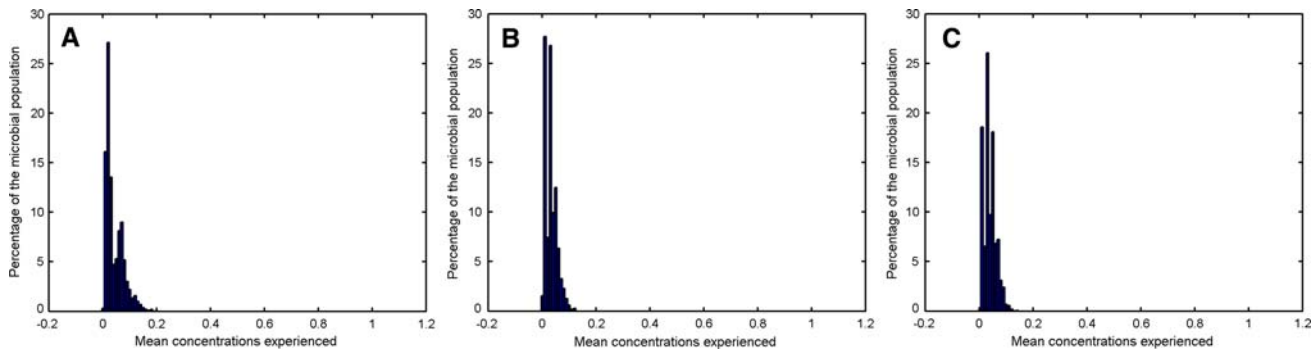

Fig. 10 Frequency distributions for scale-down reactors type B: **a** $Q_R = 18$ l/h, **b** $Q_R = 39$ l/h, **c** $Q_R = 52$ l/h

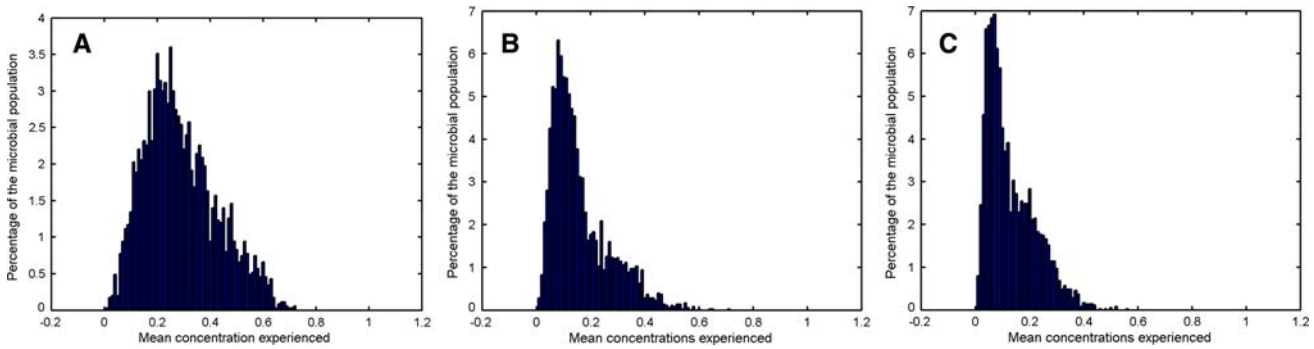

Fig. 11 Frequency distributions for scale-down reactors type C: **a** $Q_R = 5$ l/h, **b** $Q_R = 12$ l/h, **c** $Q_R = 18$ l/h

The number of cells that are exposed to high dextrose concentrations alone cannot explain the anomalies observed for SDR type B. The fluctuation in the magnitude of the dextrose concentrations experienced by the cells when they enter the non-mixed part can also be considered to be an explanation of the anomaly observed for SDR type B (Table 1). This fluctuation magnitude is higher in

SDR type B. In the non-mixed part of SDR type B, the cells experience high fluctuations in dextrose concentration, and they therefore have to alter their metabolic pathways to withstand these changes. These permutations disturb the growth of cells, with the consequence that the quantity of biomass obtained at the end of fermentation is lower in this SDR type than in other types.

The hydrodynamic modelling presented here enables the three scale-down systems used in our fermentation tests to be differentiated. In combination with other parameters, our modelling can explain the scale-down effect.

Conclusion and perspectives

The scale-up of a bioprocess is difficult to realize because limiting phenomena appear in the reactor that induce a drop in yield or the production of undesirable metabolites. We have presented the results of our study on three types of SDR with the aim of representing, at the laboratory scale, the hydrodynamic conditions of the industrial scale. The scale-down effects observed in our study were a decrease in biomass concentration and trehalose production and an increase in the fermentation time and ethanol production during the culture. All of these parameters were influenced by the recirculation flow rate. The increase in ethanol production reveals that the cells were exposed to high concentrations of dextrose when they passed into the non-mixed part, leading to the synthesis of ethanol.

The hydrodynamic conditions that developed in the SDR were characterized by our calculation of mixing time, which revealed that mixing in the three types of SDR was governed by two different phenomena: dispersion in the case of SDR types A and C and plug flow section in the case of SDR type B. A relation between increasing mixing time and decreasing biomass yield was also established.

To provide an explanation of why the yield decreases in the case of scale-down fermentation, we have presented a hydrodynamic modelling procedure. The model is able to describe the concentration profile encountered by the cells during the fermentation due to its division into two parts: the circulation of microorganisms in the reactor and the appearance of dextrose concentration gradients in the system. The model also takes the pulses generated by the discontinuous operating of the pump into consideration. The results of the simulation provide a partial explanation of the yield drop, revealing that this drop is correlated to an increase in the number of cells exposed to high concentrations of dextrose. The drop in yield is thus related to the passage of microorganisms within the zones of high concentrations of dextrose created by the scale-down system. This relationship is very clear for reactor types A and C but not for type B. In this latter case, the yield drop observed can be explained by the fluctuation magnitudes in dextrose concentrations encountered by yeast cells when they enter into the non-mixed part.

This model constitutes a very promising tool for the scale-up of fermentation processes. However, it does not allow us to clarify all of the limiting phenomena. The poor hydrodynamic conditions cannot explain the scale-down effect on its own . It would be interesting to obtain information on the manner in which the microorganisms experience fluctuations in dextrose concentrations. This physiological component is missing in our model. We are currently working on a model of microbial growth which could yield knowledge of the behaviour of a cell as a function of the concentration of dextrose actually experienced by this cell.

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