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## RTD/IAD parametric study of liquid and biomass phases in a two-stage bioreactor with cell recycle

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### Résumé

Un bioréacteur bi-étagé avec recyclage cellulaire (BBRC) a été développé afin d'étudier le comportement de *Saccharomyces cerevisiae* en conditions de hautes concentrations cellulaires. La distribution des temps de séjour (DTS) et des âges internes (DAI) des phases gaz, liquide et solide a été décrite par une approche systémique (identification des modèles, étude paramétrique) afin d'étudier le couplage entre le comportement hydrodynamique d'un tel bioréacteur et ses performances biologiques (rendement, productivité). Les différences notables entre les distributions des phases biotique et liquide peuvent s'expliquer par la présence d'une compétition entre la recirculation interne et les taux de dilution quantifiés par un facteur d'excès adimensionnel,  $K$ . Dans cet article, une étude paramétrique est rapportée et discutée. Une hypothèse basée sur ces paramètres adimensionnels et des temps d'adaptation réduits permet de situer les conditions de travail favorables ou non pour le maintien de l'activité biologique du microorganisme (vitesse de croissance, viabilité) et d'identifier les conditions opératoires optimales.

### Abstract

A two-stage continuous bioreactor with cell recycle (TSCB) was developed in order to study the behaviour of *Saccharomyces cerevisiae* in high cell density. Residence time (RTD) and internal age (IAD) distributions of gas, liquid and cell biomass phases have been investigated according to a systemic approach (model identification, parametric study) in order to highlight the complex interactions between hydrodynamics (gas, liquid and solid phases) and biological activity. The noticeable differences between liquid and cell biomass could be explained by the competition between the internal recirculation and dilution rates and described by the dimensionless circulation index,  $K$  [3]. A parametric study is reported and discussed. Assumption based on these dimensionless parameters and reduced adaptation time enable to define acceptable and unacceptable working conditions in order to maintain the biological activity of the microorganism (specific rates and viability) as well as identifying optimal working conditions.

**Mots-clés :** bioréacteur à membrane, DTS, DAI, étude paramétrique, condition opératoire.

**Key-words:** membrane bioreactor, RTD, IAD, parametric study, operating conditions.

## 1. Introduction

The application of three phase reactors, such as membrane bioreactors appeared in the 70th [Smith *et al.*, 1969; Aimar and Daufin, 2004] and was largely used in biotechnology field (pharmacy, food industry) and environment (wastewater treatments). The performance of these bioreactors was particularly investigated in continuous modes processes in order to improve productivity in high cell density

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conditions. Bioreactor configuration is also important to optimize the bioprocess and complex interactions between gas and liquid hydrodynamics as well as the solids (microorganism) activities strongly influenced the performance of bioreactors [Pareek *et al.*, 2001]. In the laboratory, an innovative two-stage bioreactor with cell recycle (TSCB) was previously developed to study the behaviour of *Saccharomyces cerevisiae* under high cell concentration, taking into account the management of growth, ethanol production and cell viability [Aldiguier, 2006; Ben Chaabane *et al.*, 2006]. The first stage was dedicated to cell growth without any oxygen limitation and at a non-inhibitory ethanol concentration. The second stage was dedicated to high ethanol productivity. The high cell density was so obtained by an ultrafiltration module coupled to the second reactor under micro-aeration condition. The innovative part of the bioprocess consists of the use of a recycle loop from the second stage to the first one to manage both cell viability and activity. The TSCB performances have been evaluated through 12 experiments where different parameters (flows, concentrations, pH, temperature, aeration, osmotic pressure, agitation rate...) and physiologic state of microorganisms (viability, cellular activity) were quantified. An intensive ethanol productivity  $41 \text{ kg}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$  was reached by combining: titer, specific rates, growth rate and yield [Aldiguier, 2006; Ben Chaabane, 2006]. However, hydrodynamics of TSCB appeared very complex which induces indeed a distribution of population and requires identification and model of 3 phases flow: gas, liquid and solid (biomass). Only few previous studies led to consider interactions between hydrodynamic interactions of one or two phases systems with biological performances in case of membrane bioreactors used in wastewater treatments and having simpler configuration than TSCB. In preliminary work, an overall hydrodynamic identification (in abiotic conditions) of TSCB was carried out by using residence time (RTD) and internal age (IAD) distributions studies, in order to give new insights in complex hydrodynamic phenomena occurring in the bioreactor, according to a suitable methodology [Ben Gaida *et al.*, 2010]. In the present work, a parametric study is carried out using the proposed model in order to explain how the internal recirculation and dilution rates may interact with biological activity (specific growth rate, viability) considering RTD and IAD within both stages.

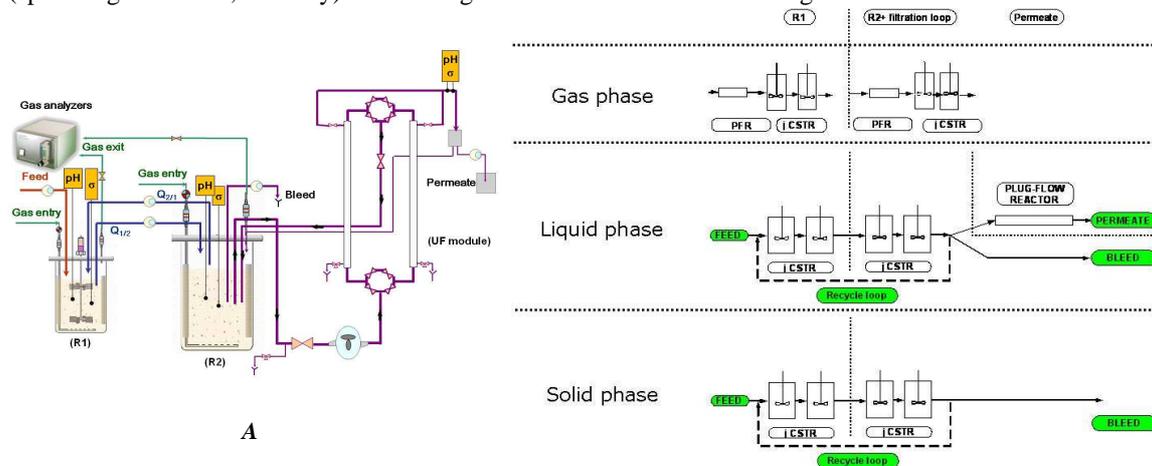


Fig.1. Overview of experimental set-up and specific instrumentation for tracing experiments (A) and of RTD/IAD models for gas, liquid and biomass in TSCB (B)

## 2. Materials and methods

Experiments were carried out using the pilot plant (Fig. 1-A) which was composed of four major parts: the first and second bioreactors (R1 and R2) and the filtration and permeate loops. Description of experimental set-up, as well as tracing experiments (gas, liquid and solid phases), are reported by Ben Gaida *et al.*, 2010. Initial RTD and IAD experiments were conducted in TSCB under abiotic condition. Four operating conditions were selected among 12 experiments established during cell cultures with growth rate and ethanol production [Aldiguier, 2006; Ben Chaabane, 2006]. Three process modes were used: batch, continuous with recycle and without recycle. The TSCB hydrodynamic characteristic parameters were calculated using conventional dimensionless numbers ( $Re$ ,  $Re_{\text{mixing}}$ , recycle rate) and aeration rate VVM. A highly turbulent flow regime was observed inside R1 due to mixing and inside R2

due to the strong flow-rate in the filtration loop. The permeate extraction loop was characterized by a close laminar flow regime. The rate of recirculation between R1 and R2, was almost constant ( $\sim 0.4$ ) for all operating conditions [Ben Gaida *et al.*, 2010].

Parametric study aims to investigate how hydrodynamic conditions affect RTD and IAD for liquid and biomass phases under realistic biological constraints. RTD and IAD are considered without biomass growth rate (reaction) in order to analyse the consequence of transport phenomena associated to the 1<sup>st</sup> cell generation. Responses to a pulse lead to the hydrodynamic identification under a transitory regime. Consequently, temporal and spatial distribution in TSCB associated with liquid and biomass phase are identified and may be interpreted in regards with critical time and biological performances.

Several assumptions based on chemical and microbiological engineering criteria are considered:

- Flow regime is permanent and volumes of each reactor are constant ( $V_{R1}=4.5L$  and  $V_{R2}=11.5L$ ),
- Considering the expected high cell densities one liquid (supernatant) and one solid (biomass) phases are assumed [Nishiwaki, 1999; Monbouquette, 1987; Atala *et al.*, 2001],
- Hydrodynamic behaviours are identical for liquid and solid (biomass) phases and described by established models.

- Mass (for solid phase) and volume (for liquid phase) balances are verified in each stage
- Cell growth rate is controlled into R1 ( $\mu_{R1} \geq 0$ , limited to  $\mu_{max}$ ) and is null in R2 ( $\mu_{R2}=0$ ).
- Growth in R1 ( $\mu_{R1}$ ,  $X_{R1}$ ,  $V_{R1}$ ) is simulated by a continuous feed (inlet) in agreement with mass balance:

$$\mu_{R1} \cdot X_{R1} \cdot V_{R1} = Q_{feed} \cdot X_{feed} \cdot$$

- The average growth rate in TSCB is defined by mass balance:  
$$\mu_{TSCB} \cdot (V_{R1} \cdot X_{R1} + V_{R2} \cdot X_{R2}) = \mu_{R1} \cdot V_{R1} \cdot X_{R1} + \mu_{R2} \cdot V_{R2} \cdot X_{R2} \cdot$$
- Biomass volume is equal to considering a cell density constant and equal to 320 g CDW.L<sup>-1</sup> [Jarzesbski *et al.*, 1989].

Our system was defined by 4 degrees of liberty: feed flow rate  $Q_{feed}$  ( $0 \leq Q_{feed} \leq 10 L \cdot h^{-1}$ ), recycle rate  $a$  ( $0 \leq a \leq 1$ ), average growth rate  $\mu_{TSCB}$  ( $\mu_{TSCB}=0.01$  and  $0.05h^{-1}$ ) and biomass concentration  $X_{R2}$  ( $X_{R2}=100g CDW \cdot L^{-1}$ ). Selected parameters were based on real operating conditions. The moment of first order,  $t_s$  and centred moment of second order,  $\sigma^2$  were calculated from RTD and IAD of liquid and biomass phases [Ben Gaida *et al.*, 2010]. The dimensionless circulation index,  $K$ , is defined as the ratio between the holding time and circulation time, and indicates the competition between the internal recirculation and the dilution rates. It also represents the fraction of entity,  $i$ , leaving the bioreactor R2, which is normalized with the total exit of TSCB but partially recycled to R1. The circulation index leads to quantify internal age distribution inside R1. The wort (liquid phase) is composed of supernatant (liquid phase) and catalytic biomass (solid phase). In our conditions we assume that: (i) the wort volume in the entrance and exit of TSCB is conserved, (ii) the supernatant density remains constant and the substrate consumption does not affect the supernatant volume, and (iii) the volume expansions related to biomass growth rates inside R1 and R2 are neglected.

### 3. Results & Discussions

#### 3.1 RTD/IAD model in TSBC

Preliminary tracing experiments were conducted in abiotic conditions using the methodology described by Thereska, 1998. The approach's relevance is based on experimental measurements quality and data interpretation, in relation with physical structure of the reactor and proposition of good and compact model (degree of liberty reduction). Experiments and analytic solutions are followed by a systemic approach using Progepi v.4.2 software [Leclerc *et al.*, 1995]. Our aim is to demonstrate the close agreement between expected RTD model and the physical structure of TSCB coupled with operating conditions. The distribution functions and analytical solutions lead to draw up a first diagnosis of the bioreactor behaviour according to various operating conditions. Each experiment was studied according to process modes (batch, continuous with and without recycle) for each phase (gas and liquid).

The selected models were validated on experiments conducted in continuous mode with recirculation between two stages. Once biomass concentrations is defined in the reactors then RTD and IAD functions of gas, liquid (supernatant) and solid (catalytic biomass) phases could be calculated and discussed. The

validation study was realized for experiments where recycle rate is different of zero ( $a \neq 0$ ). Comparison between experimental and simulated data showed good agreements [Ben Gaida *et al.*, 2010].

Both reactors R1 and R2 exhibited independent behaviour and could be simulated by a plug flow reactor in series with  $j$  CSTR with constant parameters ( $\alpha, j$ ) whatever investigated operating conditions range (VVM,  $Re$  and  $Re_{mixing}$ ) for gas phase. For liquid phase, RTD for permeate and purge flow and IAD in the first and second reactors have been obtained and formulated. Under assumptions, the proposed reactor model (Figure 1-B) was in close relation with TSCB physical structure whose parameter values are reported in Ben Gaida *et al.*, 2010. Identified parameters are close to expected values for liquid phase according to physical structure and operating conditions. RTD and IAD catalytic biomass are directly deduced from selected model taking into account the recirculation index,  $K$  and assuming 2 assumptions: (i) the wort flow patterns are common to supernatant and catalytic biomass, and (ii) the retention rate of membranes is of 100% for catalytic biomass.

### 3.2 Parametric study

#### 3.2.1. Overview RTD/IAD studies

The effect of feed flow and recirculation rate on internal age and residence time distributions, reduced variance and associated skewness and spreading in addition to recirculation index could be discussed and quantified in TSCB. The cumulative distributions curves of residence time,  $F(t)$  and internal age,  $J(t)$  for gas, liquid and solid phases in the TSCB are depicted in Fig. 2. The curves time-distribution exhibit significantly different shapes and orders of magnitudes of mean residence time (and dispersion) for gas ( $\approx 1$  min), liquid ( $\approx 100$  min) and solid ( $\approx 1000$  min) phases. This means that in spite of a hydrodynamic behaviour controlled by common parameters; a large uncoupling is observed between gas, liquid and solid behaviours. However, coupling and uncoupling are related to operating conditions and the impacts of feed flow rate and recycle rates were also studied. For biomass, Fig. 3 shows the influence of these parameters on cumulative distribution of internal age in R1. Simulation and interpretation IAD of biomass in R1 appears as a major lock and request the use of dimensionless criteria. Hydraulic and biological performances will result from a complex balance between the dilution and the recirculation rates associated with operating conditions.

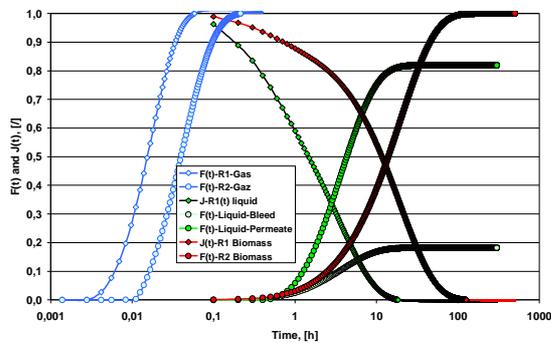


Fig. 2. Cumulative distribution of RTD and IAD for gas, liquid and solid phases ( $Q_{feed}=3L.h^{-1}$ ,  $a=0.6$ ,  $\mu_{TSCB}=0.05h^{-1}$  and  $X_{R2}=100g CDWL^{-1}$ ).

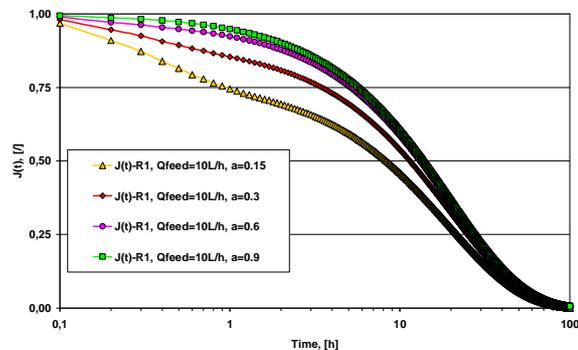


Fig. 3. Cumulative distribution of IAD of biomass ( $Q_{feed}=10 L.h^{-1}$ ,  $a=0.15$  to  $0.9$ ,  $\mu_{TSCB}=0.05h^{-1}$  and  $X_{R2}=100g CDWL^{-1}$ ).

#### 3.2.2. Simulation

The hydrodynamic behaviour of the system is discussed through the evolution of reduced residence times (ration of mean residence time / holding time),  $t_{S-R1}/\tau_{TSCB}$  and  $t_{S-R2}/\tau_{TSCB}$ , respectively for R1 and R2 and reduced variances,  $\sigma_{R1}^2/t_{S-R1}^2$  and  $\sigma_{TSCB}^2/t_{S-TSCB}^2$  respectively for R1 and TSCB. Fig. 4 and 5 represent the evolution of these parameters and dimensionless circulation index,  $K$  as a function of dilution and recycling rates respectively for the liquid and biomass phases. Theoretical analyse of TSCB leads to establish an expression of reduced variance as a function of stage behaviour (CSTR) and volume ratio of each phase:

For liquid and biomass phases in R1, we observed that if  $Q_{feed}$  increases,  $t_S/\tau$  increases whereas  $\beta^2$  decreases. For biomass phase, this trend is strongly observed between  $0.1 < a < 0.7$ . A critical feed flow rate

(around  $7L \cdot h^{-1}$ ) is noticeable. Above its value, no significant impact of this parameter is observed and for  $a > 0.3$ ,  $t_s/\tau$  and  $\beta^2$  tends to 1. This observation is strengthened with a reducing specific growth rate  $\mu_{TSCB}$ . For  $a=0$  (two stage bioreactor in series without recycle),  $\beta^2$  is equal to 1 in R1 and R2 in agreement with a CSTR model and to 0.56 in TSCB in agreement with Eq.1 for liquid phase. For biomass phase, R1 concentration is a function of  $Q_{feed}$  and  $\mu_{TSCB}$  (mass balance). However  $V_{X-R1}$  is negligible compared to  $V_{X-R2}$  and induces  $\beta_{TSCB}^2 \approx \beta_{R2}^2 \approx 1$ . For  $a > 0$ ,  $t_s/\tau$  provides an information on IAD and induces  $\beta_{P1}^2$  and  $\beta_{P2}^2$  superior to 1. An extremum ( $\beta_{P1}^2$  equal to 1.6 and 1.8 for  $a=0.3$  with liquid phase and to 2.0 and 2.5 for  $a=0.15$  with biomass phase respectively with  $\mu_{TSCB} = 0.01$  and  $0.05h^{-1}$ ) is observed for  $a$  ranging between 0.2 and 0.5 for liquid phase, and between 0.1 and 0.3 for biomass phase. If  $Q_{feed}$  increases,  $t_s$ .  $t_{TSCB}/\tau_{TSCB}$  remains equal to 1 (whatever  $a$ ) and  $\beta_{TSCB}^2$  almost constant showing a perfect macro-mixing in agreement with identified models and calculated mixing times in both reactor. If  $a$  increases,  $\beta^2$  increases from 0.5 up to 1 respectively for  $a$  equal to 0 and 1, corresponding with 2 limit conditions: 2 CSTR in series and 1 perfect CSTR ( $Q_{12} \approx Q_{21} \rightarrow +\infty$  and  $Q_{12} \gg Q_{feed}$ ). These results could be checked by calculating  $\beta^2$  knowing volume and behaviour of each reactor. Attention should be paid to simulated conditions corresponding to theoretical cases inducing unrealistic specific growth rate in R1 with  $\mu_{R1} > \mu_{max} = 0.24h^{-1}$ .

$$\beta_{TSCB}^2 = \beta_{R1}^2 \cdot v^2 + \beta_{R2}^2 \cdot (1-v)^2 = \frac{1}{j_{R1}} \cdot v^2 + \frac{1}{j_{R2}} \cdot (1-v)^2 \text{ with } v = \frac{V_{R1}^X}{V_{TSCB}^X} \text{ or } \frac{V_{R1}^L}{V_{TSCB}^L} \quad (1)$$

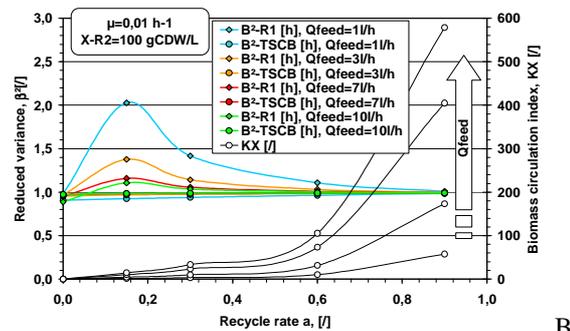
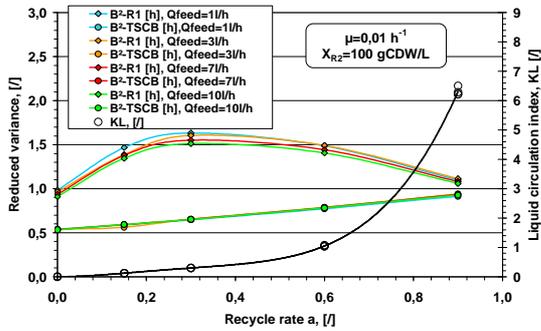
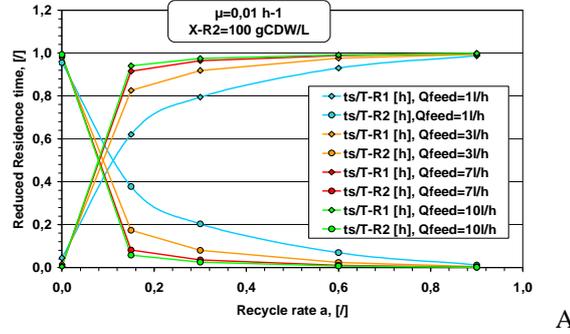
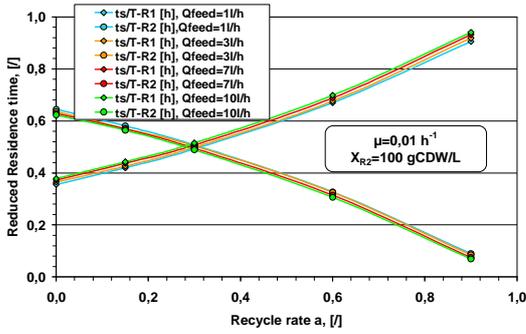


Fig. 4. Evolution of reduced residence time (A) and reduced variance and dimensionless circulation index (B) as a function of dilution and recycling rates for liquid phase ( $\mu_{TSCB}=0.01h^{-1}$ ,  $X_{R2}=100g CDW \cdot L^{-1}$ ).

Fig. 5. Evolution of reduced residence time (A) and reduced variance and dimensionless circulation index (B) as a function of dilution and recycling rates for biomass phase ( $\mu_{TSCB}=0.01h^{-1}$ ,  $X_{R2}=100g CDW \cdot L^{-1}$ ).

The increase of specific growth rate  $\mu_{TSCB}$  slightly affects the liquid phase behaviour but strongly modified the hydrodynamic of biomass (Fig. 6). The differences between liquid and biomass behaviours in regards with  $t_s/\tau$  and  $\beta^2$  could be explained by the competition between the internal recirculation and dilution rates and associated dispersion. The dimensionless circulation index  $K$  defined as the ratio between the holding time and circulation time enables to quantify this competition. The order of magnitude for  $K$  remains below 10 for liquid and may exceed 500 for biomass under investigated working conditions. The absolute value of  $K^X$  in addition to  $t_s/\tau$  and  $\beta^2$  are valuable indicators leading to compare both hydrodynamic and biological performances in TSCB.

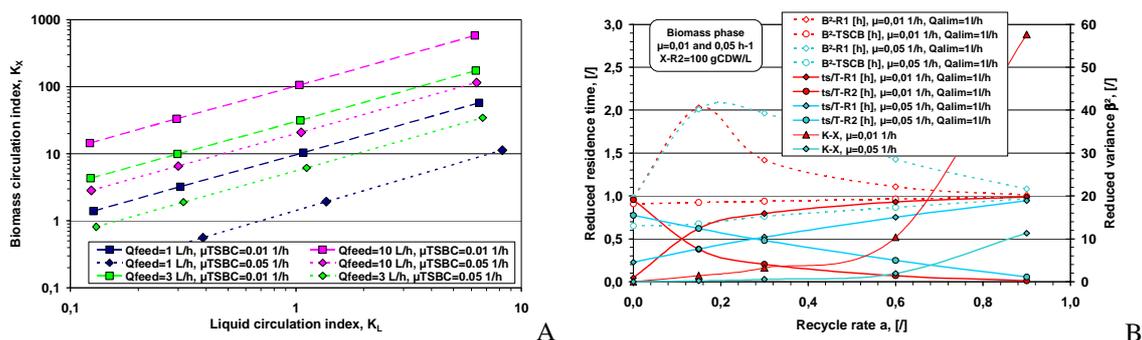


Fig.6. Evolution of biomass and liquid circulation index (A) and reduced residence time and variance for biomass (B) ( $\mu_{TSBC}=0.01$  and  $0.05$   $h^{-1}$ ,  $Q_{feed}=1$   $L.h^{-1}$ ,  $X_{R2}=100$ g/L CDWL).

#### 4. Conclusion

Bioprocesses are considered as an important operation of agro-chains engineering for food and no food transformation industries with specific and optimized biocatalysts application (microorganisms, enzymes). Scientific advances of this study are in keeping with bioprocess performances intensification with a better knowledge of physical and microbiological interactions. A parametric study enabled to investigate the complex hydrodynamic behaviour of the liquid and biomass phases through the evolution of reduced residence times and associated variances. Biotic experiments are characterized by total biomass,  $X_t$ , including viable,  $X_v$ , and inactive biomass ( $X_t - X_v$ ) and biological activity (ethanol production rate, specific growth rate). One assumption is based on an adaptation time (to different environmental conditions) of cells coming from the second into the first stage. This adaptation time could be a complex function of time and environment encountered into the second stage [Sanchez-Gonzales *et al.*, 2009]. In TSCB, the biological performances will be improved by an optimal control of biomass in R1 in regards with an adaptation time. It means that highest  $t_s/\tau$  and lowest  $\beta^2$  stand as experimental aims and imply high values of  $K^x$ . These dimensionless parameters and a reduced adaptation time enable to define acceptable and unacceptable working conditions in order to maintain biological activity (specific growth rate and/or viability) of micro-organism and thus to identify optimal working conditions. In the future, several actions will be conducted or reported on (i) comparison of established models with passive tracer (lactose) under biotic conditions and (ii) simulation of experiments carried out under biotic conditions in order to interpret performances (concentration, viability, ethanol production, growth rate).

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