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LIQUID AND GAS RESIDENCE TIME DISTRIBUTION IN A TWO-STAGE BIOREACTOR WITH CELL RECYCLE

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ABSTRACT

Hydrodynamic behaviour of a two-stage bioreactor with cell recycle (TSCB) was investigated. Residence time distribution (RTD) and Internal Age Distribution (IAD) of gas and liquid phases were performed with accurate tracing methods and under specific operating conditions and process modes (batch and continuous modes with and without recirculation between first and second stages). From experiments, RTD and analytical solution were formulated, and systemic analysis carried out. Results demonstrated that the magnitude of mean residence time and variance were strongly different between gas (≈ 1 min) and liquid (≈ 100 min) phases. RTD of gas phase showed that both reactors exhibited independent behaviour, and RTD could be described by two single and simple models (plug flow in series with j CSTR) with constant parameters (α , j) whatever investigated operating conditions (VVM , Re and Re_{mixing}). For liquid phase, experiments will enable to identify mixing time (batch mode) as well as the mean residence time and variance in continuous modes. RTD for permeate and purge flow and IAD in the first and second reactors were obtained and formulated. Under selective assumptions, a systemic analysis of liquid RTD lead to propose a reactor model in close relation with TSCB structure. In a first step, model parameters will be identified under continuous mode without recirculation and then, validated with recycle between two stages.

Keywords: residence time distribution, internal age distribution, tracing methods, liquid, gas, bioreactor, high cell density.

1. INTRODUCTION

The application of three phase reactors (solid, liquid and gas) is well established in a wide variety of chemical processing operations such as, production of fine chemical, wastewater treatment and fermentation processes. For these last cases, increasing cell density in bioreactors is useful to improve the overall productivity of continuous modes processes particularly the bio-ethanol production which has to be competitive according to energetic, economical and environmental criteria. Another determinant factor to optimise the overall performance of biological process is the configuration of the bioreactor. The performance of bioreactors is, however, strongly influenced by the complex interactions between gas and liquid hydrodynamics as well as the solids (microorganism) activities [10].

Theoretical approaches [8, 9] showed that recycle two-stage continuous fermentations with membrane separator would be potentially the most efficient system for improving ethanol productivity in high cell density cultures. An innovative two-stage bioreactor with cell recycle (TSCB) was developed to study the behaviour of *Saccharomyces cerevisiae* under high cell concentration, taking into account the management of growth, ethanol production and cell viability [1, 2]. The first stage of the bioreactor was dedicated to cell growth without oxygen limitation and at non-inhibitory ethanol concentration. The second stage was dedicated to high ethanol productivity. The high cell density was so obtained by an ultra filtration 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 cell viability and activity.

In the present work, a global hydrodynamic study was carried out with the TSCB (Fig. 1). RTD and IAD formulations constitute efficient tools to give new insights in complex hydrodynamic phenomena occurring in the bioreactor. From experiments, RTD and analytical solution were formulated and discussed, and systemic analysis carried out. Reactor models were proposed for gas and liquid phases.

2. DEVELOPMENT

2.1. Theory : RTD and IAD

The residence time distribution (RTD) is a chemical engineering concept introduced by Danckwerts [3]. The concept of RTD can be used to obtain hydrodynamic data and to assess the degree of mixing and flow patterns within any reactors [6]. The models derived from tracer experiments are often limited to simple elementary reactors such as perfect mixing cells in series [7], plug flow with axial dispersion [12], mixing cells in series exchanging with a dead zone [5] or simple compartment models [6].

In our study, analysis of RTD profile can be used to determine the deviations from ideal reactor behaviour and to give indications about the modifications that the system needs to overcome distribution problems.

RTD profiles may be measured by monitoring the evolution of an inter tracer through the bioreactor. The RTD function, $E(t)$, can be evaluated dividing the tracer concentration $c(t)$ in the exit stream of the bioreactor (in our case the exit permeate and bleed) by the total mass of tracer injected in the feed (Eq. 1).

The average residence time was given by the first moment of the RTD function $E(t)$, I^1 also called mean residence time, t_s . This parameter may be calculated by determining the area under the curve of a plot of $t E(t)$ as a function of time (Eq. 2). The theoretical mean holding time, τ was calculated as the ratio of the reactor volume, V to the volumetric flow rate, Q (Eq. 3)

$$E(t) = \frac{c(t)}{\int_0^{\infty} c(t) \cdot dt} \quad \text{Eq. 1, } I^1 = t_s = \int_0^{\infty} t \cdot E(t) \cdot dt \quad \text{Eq. 2 and } \tau = \frac{V}{Q} \quad \text{Eq. 3}$$

In this study, the results where the values of τ differed significantly from the mean residence time, t_s (above 10%) were rejected.

The general equation (Eq. 4) defines the central moments of order i . The higher order centred moments, I^i , can provide significant information about the behaviour of the function $E(t)$. For example, the second central moment, I^2 is referred to as the variance, σ^2 which describes the spread of the RTD curve. The centred moments of third order, I^3 , represent deviation from a symmetrical distribution (skewness) and of fourth order, I^4 , spreading of the RTD. However, the quality of signals led to non significant or reliable results for centred moments of third and fourth order.

Internal age distribution, $I(t)$ was also defined to describe the reactor contents. This function has a similar definition as $E(t)$: the fraction of fluid within the reactor with an age of t is $I(t) dt$. The relation between $E(t)$ and $I(t)$, and between $F(t)$ and $J(t)$ can be determined from the mass balance (Eq. 6 and 7):

$$\Gamma^j = \int_0^{\infty} (t - t_s)^j \cdot E(t) \cdot dt \quad \text{Eq. 4 and } I(t) = \frac{1}{\tau} \left(1 - \int_0^t E(t) \cdot dt \right) \quad \text{Eq. 5}$$

$$\text{With } E(t) = -\tau \frac{dI(t)}{dt} \quad \text{Eq. 6 and } J(t) = \tau \cdot I(t) = 1 - F(t) \quad \text{Eq. 7}$$

2.2. Simulation of complex compartment models

A software package (DTS Progepi v4.2 software) has been developed to simulate the response to an input of any complex network of elementary reactors properly interconnected [5]. Processes with multiple inlets or outlets can be modelled by convolution and optimization procedures. The software may also be used to determine the parameters of the different models giving the same response, and the subsequent test of the physical soundness of these parameters leads to the choice of a realistic model. In

addition, automatic generation of RTD needs both analysis of the RTD curves (tail, number of peaks, etc.) and physical information about the process itself (presence of agitator, dead volume, etc.). A very good knowledge of the geometry of the reactor is thus necessary in order to establish a model as faithful as possible to the real flow, and permitting to draw some useful information for future experiments.

3. MATERIALS AND METHODS

3.1. Experimental set-up

Experiments were carried out using the pilot plant (Fig. 1) which was composed of four major parts: the first and second bioreactors, the filtration and permeate loops. The TSCB included a first, R1 (Chemap CMF100, 5 l) and a second bioreactor, R2 (SGI, 8L) controlled by a CB5 control unit (B. Braun Biotech International, pH, dissolved oxygen, mixing, temperature). The second reactor (R2) was connected to a filtration loop (6.5L, $\varnothing=25.4\text{mm}$) composed of two parallel modules (Rhodia - Orelis company - Miribel, France, Kerasep-K01-B-X2, $\text{Al}_2\text{O}_3\text{-TiO}_2$ ceramic membrane, \varnothing_{int} 6mm, 7 channels, \varnothing_{ext} 25mm, 1178mm length, UF cut-off: 150kDa, unit surface: 0.155m^2) and a tubular heat exchanger including temperature regulation. Transmembrane pressure was adjusted to get an accurate permeate flow-rate by a counter pressure valve. Permeate was extracted and partially recycled in R2 reactor.

Peristaltic pumps (Masterflex model, Q ranging from 0 to 10 l/h) were used to enable the circulation of the liquid phase as feed Q_w , circulation Q_{12} and Q_{21} , second stage bleed Q_{pg} and permeate Q_p . Flow rate in filtration loop was achieved with a volumetric pump (PCM pump, Moineau M6000C4, $Q_{\text{max}}=5\text{m}^3/\text{h}$). Two mass flow meters (Micromotion serie R, Coriolis effect) measured the total flow in the filtration module and the permeate flow. Each reactor was equipped with an inlet air flow meter (Bronkhorst high-tech, Instrutec type F, $Q_{\text{max-R1}}=5\text{NI}/\text{min}$ and $Q_{\text{max-R2}}=2.5\text{NI}/\text{min}$). The experimental set-up was also connected to a computer with homemade software performing the on-line acquisition of the controlled parameters (stirring rate, pH, temperature, inlet air flow, dissolved oxygen, liquid level regulation), and monitored the peristaltic pumps. The control of feed flow, Q_{Feed} , circulation flow from R1 to R2, Q_{12} and outlet flow, Q_{Bleed} and Q_{Permeate} , allowed us to keep the working volume constant.

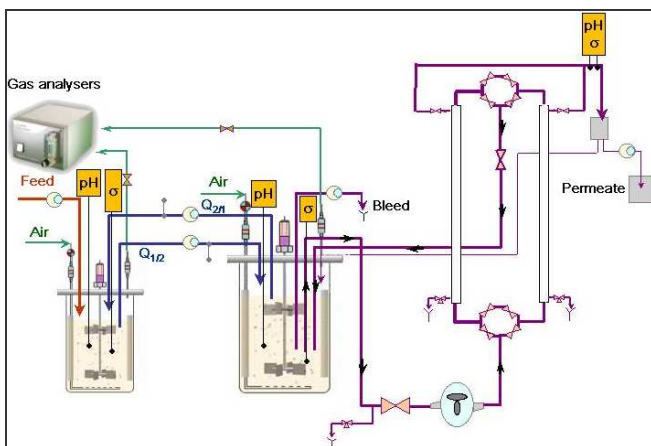


Fig. 1 : Overview of experimental TSCB set-up and specific instrumentation for RTD investigation

Parameters	Units	Exp(a)	Exp(b)	Exp(c)	Exp(d)
Q_{21}	[l/h]	1	4.1	1	4
Q_{12}	[l/h]	2.56	9.11	2.56	9.79
Q_{Loop}	[l/h]	2500	2600	1100	2600
Q_{Permeate}	[l/h]	1.38	4.95	1.38	5.7
Q_{Bleed}	[l/h]	0.18	0.06	0.18	0.09
Q_{Feed}	[l/h]	1.56	5.01	1.56	5.79
Rpm_{R1}	[tr/min]	700	900	850	900
Q_{Air_R1}	[l/min]	1.5	1.7	0.15	2.6
Q_{Air_R2}	[l/min]	2	3.8	0.6	0.8
V_{R1}	[L]	4.5	4.5	4.5	4.5
$V_{(R2+Loop)}$	[L]	5+6.5	5+6.5	5+6.5	5+6.5
T	[°C]	30	30	30	30
a	[/]	0.39	0.45	0.39	0.44
$Re_{\text{mixing-R1}}$	[/]	36400	46800	44200	46800
$Re_{\text{loop-R2}}$	[/]	46600	48700	20700	48000
Re_{perm}	[/]	1800	1900	1400	2300
$VVM-R1$	[1/min]	0.33	0.38	0.03	0.58
$VVM-R2$	[1/min]	0.17	0.33	0.05	0.07

Table 1: Average operating conditions in experiments a, b, c and d.

3.2. Operating conditions for tracing experiments

RTD and IAD experiments were carried out under 4 operating conditions (a, b, c and d, Table 1), identified and selected among 8 experiments established during microorganism culture and ethanol production [1, 2], and 3 process modes: batch, continuous with recycle and without recycle ($Q_{21}=0$). Experimental RTD and IAD were conducted with TSCB without biological activity and following the methodology described by Thereska [11].

The tracing methods (Fig. 1) were respectively a step of oxygen or nitrogen concentration as gas tracer, injected at the inlet of the reactor R1 or R2, and a pulse (Dirac) of NaCl/NaOH concentration as liquid tracer, injected rapidly with a syringe at R1 inlet. In each experiment, the quantity of tracer injected was controlled. For gas phase, oxygen/nitrogen concentration at R1 and R2 outlets were simultaneously monitored with two gas analyzers (Innova Air Tech Instruments, Type 1313). Percentage of dissolved di-oxygen inside R1 and R2 reactors and permeate loop were measured using 3 dissolved oxygen electrodes (Hamilton type Oxyferm FDA120, OxyProbe Serie Dissolved Oxygen Sensor and Ingold n°322 756800/83264). For liquid phase, the tracer concentration was measured simultaneously by conductivity (Kemotron type 9147, Conducell 4USF-PG215 and PG325) and pH (Hamilton type Easyferm plus VP/120, pH FermProbe F-615-B130-DH and F-635-B325-DH) electrodes located on different zones of the bioreactor (R1, R2 and permeate extraction). All sensor signals were electrically conditioned and collected using an on-line data-acquisition system (Agilent technologies, Loveland, Colorado, USA, type 34970A with specific cards 34901A and 34907A). A PC (Dell, Intel(R) Core(TM)2 CPU, T56001@ 1.83GHz) equipped with a software driver (Benlink Data Logger, version 3.00.02) were used for the configuration and the acquisition of data.

Hydrodynamic conditions in TSCB were firstly characterized by conventional dimensionless numbers (Re , Re_{mixing} , $recirculation\ rate$) and VVM . These parameters were related to operating conditions (i.e. mixing rate, gas flowrate, liquid flowrate, recirculation).

$$Recirculation\ rate: \quad a = \frac{Q_{21}}{Q_{12}} \quad Eq. 8 \quad \text{and} \quad \text{Aeration rate: } VVM = \frac{Q_{air}}{V_{liq}} \quad Eq. 9$$

$$Reynolds\ numbers: \quad Re_{mixing} = \frac{\rho \cdot N \cdot d^2}{\mu} \quad Eq. 10 \quad \text{and} \quad Re = \frac{\rho \cdot U \cdot d_h}{\mu} \quad Eq. 11$$

Two flow regimes were observed, (i) a highly turbulent flow regime inside R1 due to mixing ($36000 < Re_{Mixing-R1} < 47000$) and inside R2 due to flow-rate in the filtration loop ($20000 < Re_{Loop-R2} < 50000$) and (ii) a close laminar flow regime inside the permeate extraction loop ($1400 < Re_{Permeate} < 2400$). It was also deduced that the rate of recirculation between R1 and R2, was almost constant for all operating conditions whereas VVM exhibits a wide range of value in both reactors.

4. RESULTS AND DISCUSSIONS

4.1. Analytical solution: RTD and IAD formulation

In a first step, data analysis and RTD formulation were realized, inlet and outlet RTD signals, $x(t)$ and $y(t)$ were formulated. Measured parameters (electrical conductivity, gas percentage) were converted into concentration values. Concentration profiles, $c(t)$ were obtained as a function of time and reduced and normalized signals established, $E(t)$ and $E(\theta)$. Then, experimental residence time distributions, $E(t)$ or cumulative, $F(t)$ and the internal age distribution, $I(t)$, under different operating conditions and process modes could be compared and characterized by a set of moments (mean residence time) and centred moments of order i .

4.1.1. RTD of gas phase

Experiments demonstrated that R1 and R2 exhibit independent behaviour. None gas passage between R1 and R2 was observed as shown in Figure 2A. For an infinite time (300-400s), $F_{R1}(t)$ tends to unit value whereas $F_{R2}(t)$ remains null after a nitrogen step in R1. Holding time, τ , mean residence time, t_s and reduced variance, β_{exp}^2 were determined (Table 2), and demonstrated different hydrodynamic behaviour between R1 and R2, under investigated conditions. A larger spread of RTD signal was noticeable in R2 which seems correlated to physical structure of filtration loop associated with R2. A gas retention inducing liquid hold-up was corroborated by holding time and mean residence time ratio of 1.5 in R1 and 1.8 in R2. Under highly turbulent flow or mixing, these ratios stand at constant values whatever VVM .

RTD curves of gas phase showed that there is not any significant difference between RTD curves, $E(t)$ versus process mode (batch, continuous with and without recycle) and between normalized RTD curves, $E(\theta)$ whatever operating conditions (Figure 2B). From these results, R1 and R2 under investigated conditions demonstrated that both reactors exhibit a single hydrodynamic behaviour. However, dissolved oxygen sensors located in R1 and R2 indicated that a short delay exists between inlet and outlet in both reactors.

In conclusion, both reactors behaviour could be assumed as a plug flow reactor associated with j CSTR whose model parameters should remain constant whatever the investigated operating conditions. In addition, gas volume and retention could be estimated through model parameters.

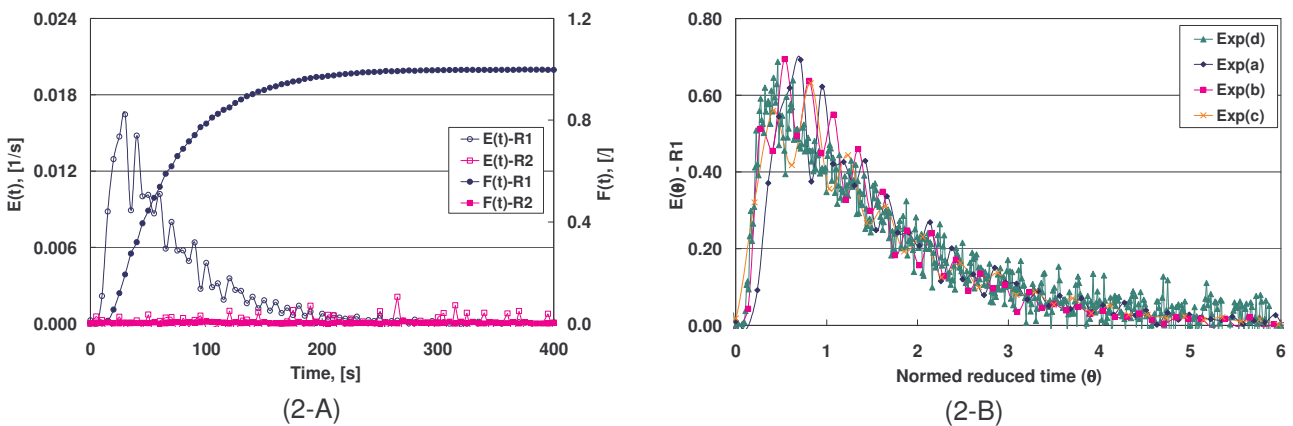


Fig. 2 : Residence time distribution, $E(t)$ and cumulative, $F(t)$ of gas phase in R1 and R2 (Fig. 2-A), (experiment a) and reduced RTD, $E(\theta)$ in TSCB (experiments a, b, c and d) in continuous mode with recycle.

4.1.2. RTD and IAD of liquid phase

Hydrodynamics of liquid phase appeared complex in TSCB and required an appropriate methodology to identify several parameters (mixing time, mean residence time and variance, impact of recycle). In the future, experiments will lead to (i) determine the mixing time in batch mode, (ii) identify model parameters in continuous mode without recycle, (iii) validate models in continuous mode with recycle and (iv) investigate different operating conditions.

Both reactors (R1 and R2) and outlets (bleed and permeate) were considered. In figure 3-A, the experimental residence time distribution, $E(t)$ or cumulative, $F(t)$ and the internal age distribution cumulative, $J(t)$ were reported for experiment (a) in continuous mode without recycle. Mass balance between internal age distribution and cumulative residence time distribution was verified. RTD and IAD curves exhibited high spread value, which should be carefully considered during bioreaction. In a first step, only R1 reactor was considered. The evolution of normalized and reduced liquid RTD at the outlet of R1 had a single behaviour whatever various operating conditions (Figure 3-B). R1 behaviour was close to a CSTR with a reduced variance equal to 0.97 ± 0.16 .

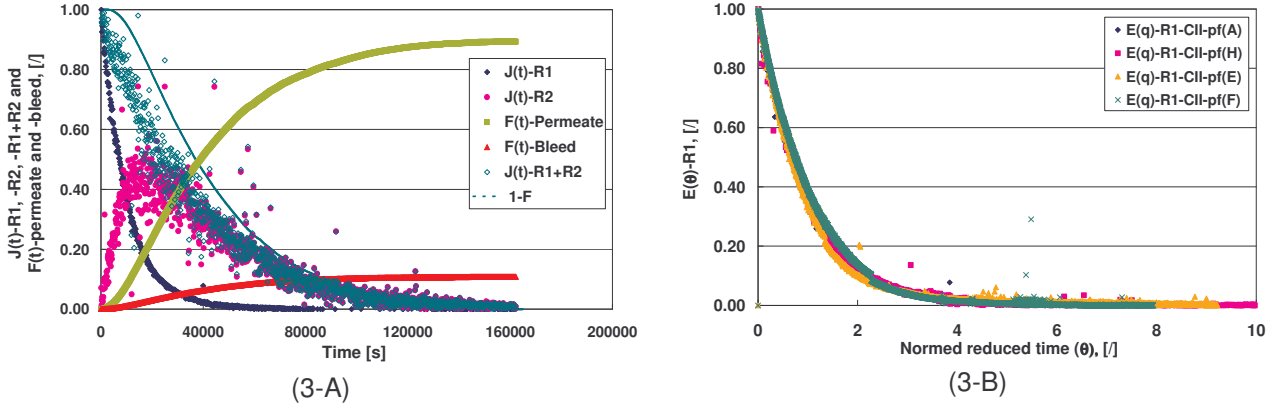


Fig. 3: IAD, $J(t)$ and RTD, $F(t)$ of liquid phase in TSCB (Exp. A-Fig. 3-A) and liquid normalized and reduced RTD, $E(\theta)$ in R1 (Exp. A, b, c and d, fig.3-B) under continuous mode without recycle.

4.2. Systemic analysis of RTD

In a second step, the reactor behaviour was described with a RTD analysis based on DTS Progepi v4.2 software [4]. RTD formulation stands as an efficient tool to give new insights in hydrodynamic phenomena occurring in TSCB. In order to quantify the degree of mixing and to investigate the hydrodynamic behaviour of the reactor for gas and liquid phases, a systemic analysis was performed. Two different models for gas and liquid phases were assumed based on (i) physical structure of the CSTR, (ii) experimental RTD and IAD results and (iii) assumptions about hydrodynamic behaviour. For each experiment, curves fitting of both models and parameters identification were realized by minimizing the sum of square residual (SSR). Model parameters were discussed versus operating conditions and process modes.

4.2.1. Gas phase

For gas phase, a single model was assumed for R1 and R2 (Figure 4) and structured as a cascade of PF reactor (τ_{PFR}) in series with j CSTR (τ_{CSTR} , j). This model was chosen because it is simple and closely correlated to the physical structure of the process and perfectly fit experimental data (Figure 5).

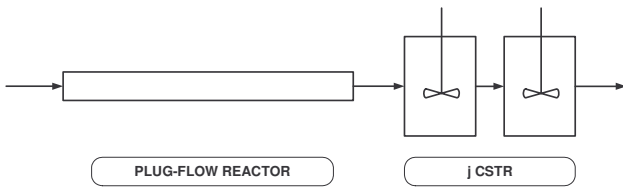


Fig. 4: Reactor model for RTD of gas phase in R1 and R2

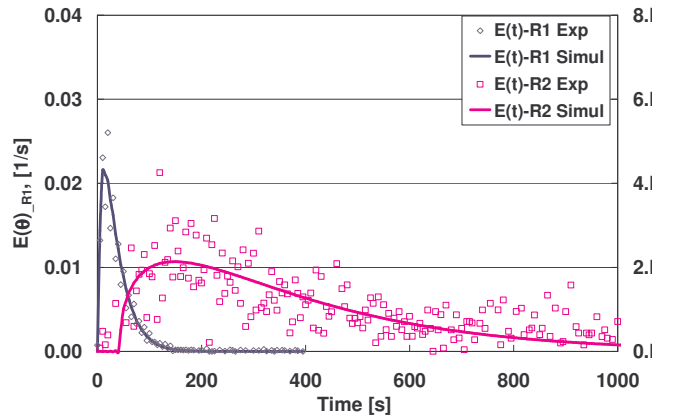


Fig. 5 : Experimental and simulated $E(t)$ in R1 and R2 for gas phase, continuous mode with recycle (exp.a)

The corresponding expression of the transfer function $G(s)$ and $E(t)$ were formulated by Eq.12 and Eq.13.

$$G(s) = \frac{\exp(-s \cdot \tau_{PFR})}{\left(1 + \left(\frac{s \cdot \tau_{CSTR}}{j}\right)\right)^j} \quad \text{Eq. 12}, \quad E(t) = H(t - \tau_{PFR}) \cdot \left(\frac{j}{\tau_{CSTR}}\right)^j \cdot \frac{(t - \tau_{PFR})^{j-1} \cdot \exp\left(\frac{-j \cdot (t - \tau_{PFR})}{\tau_{CSTR}}\right)}{(j-1)!} \quad \text{Eq. 13}$$

With Van der Laan's relations (Eq. 14), a simple relation between α and β^2 was established. The most advantage of this simple model is that only two parameters j and α are used, if the knowledge of mean residence time is assumed to be equal to holding time, τ . It is the plug reactor contribution, in term of residence time (Eq. 15) from which reduced variance may be deduced (Eq. 16).

$$\Gamma^1 = -G'(0) = (\tau_{PFR} + \tau_{CSTR}) = \tau$$

$$\Gamma^{2,1} = G''(0) = \tau^2 + \frac{\tau_{CSTR}^2}{j}$$

Eq. 14, $\alpha = \frac{\tau_{PFR}}{\tau_{PFR} + \tau_{CSTR}}$ Eq. 15 and $\beta^2 = \frac{(1-\alpha)^2}{j}$ Eq. 16

In table 2, the holding time, τ , experimental data, t_s , β_{exp}^2 and model parameters, τ_{PFR} , τ_{CSTR} and j were reported and differences between experiments and models were calculated. Experimental and model mean residence times exhibited close values for R1 and R2 (difference < 5% except for the experiment (d) in R2). In the same way, calculated reduced variances showed a good agreement with experimental data for R1 (average error < 10%) which was not the case for R2 in spite of a good fitting with experimental data.

Experiments	Model										Deviations [%]	
	Exp	τ [s]	t_s [s]	β_{exp}^2 [l]	τ_{PFR} [s]	τ_{CSTR} [s]	j [l]	τ_{Tot} [s]	α [l]	β^2 [l]	$\frac{t_s - \tau_{Tot}}{t_s}$	$\frac{\beta_{exp}^2 - \beta_{calc}^2}{\beta_{exp}^2}$
R1	(a)	42.1	67.7	0.54	6.9	55.7	1.66	62.6	0.11	0.48	7.4	12.6
	(b)	37.1	52.6	0.58	5.1	45.6	1.47	50.8	0.10	0.55	3.6	5.0
	(c)	420.8	646.3	0.61	52.4	571.6	1.46	624.0	0.08	0.57	3.4	5.4
	(d)	24.3	37.8	0.72	2.4	34.0	1.45	36.3	0.07	0.60	4.0	15.8
R2	(a)	90	148.0	1.53	36.2	141.9	1.19	178.1	0.20	0.53	3.2	65.3
	(b)	47.4	98.1	1.73	14.6	78.8	1.41	93.4	0.16	0.51	4.8	70.8
	(c)	300	505.7	1.61	64.2	412.0	1.75	476.2	0.13	0.43	5.8	73.4
	(d)	225	437.9	1.61	42.8	341.7	1.45	384.5	0.11	0.54	12.2	66.3

Table 2: Experimental and identified model parameters for gas RTD in R1 and R2.

RTD of gas phase in R1 and R2 could be described by a simple reactor model whose parameters α and j remained almost constant under investigated conditions (Table 3).

	α , [l]	j , [l]	Re , [l]	VVM , [min ⁻¹]
R1	0.09	1.51	36400 < Re_{mixing} < 46800	0.03 < VVM_{R1} < 0.58
R2	0.15	1.45	20700 < Re_{loop} < 48700	0.05 < VVM_{R2} < 0.33

Table 3: Average identified parameters for RTD of gas phase in R1 and R2.

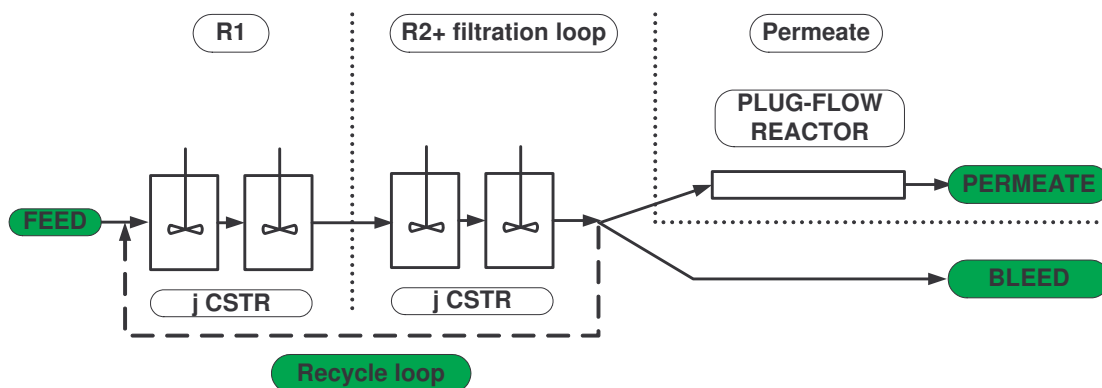


Fig. 6: Considered reactor model for liquid phase in TSCB

4.2.2. Liquid phase: proposal of reactor model

From experiments carried out in continuous mode without recycle and the first RTD and IAD curves, a reactor model could be proposed. Reactor model was based on the physical structure of TSCB and integrated 2 successive j CSTR in series with exchange loop, followed by a plug flow reactor (Fig. 6). This reactor model had been validated for experiment (a) and comparison between experimental and simulated data showed good agreements. However, others operating conditions need to be investigated in the hydrodynamic behaviour understanding in order to get insight of liquid phase in TSCB.

5. CONCLUSION

RTD and IAD formulations for liquid and gas phases stand as an efficient tool to give new insights in hydrodynamic phenomena occurring in TSCB and to investigate interaction between physical and biological phenomena. From experiments, RTD and analytical solution were formulated, and systemic analysis carried out. Results demonstrated that the magnitude of mean residence time and associated variance were strongly different for gas (≈ 1 min) and liquid (≈ 100 min). Reactors model have been proposed for gas and liquid phases. RTD of gas phase showed that both reactors exhibited independent behaviour, and could be simulated by a single model (plug flow in series with j CSTR) with constant parameters (α, j) whatever investigated operating conditions range (VVM, Re and Re_{mixing}). For liquid phase, RTD for permeate and purge flow and IAD in the first and second reactors have been obtained and formulated. Under assumptions, a systemic analysis of liquid IAD and RTD led to propose a reactor model in close relation with TSCB structure. In the future, model parameters will be fully identified under continuous mode without recirculation and so that the proposed model could be applied with recycle between two stages. These parameters will then be confronted to experiments carried out with biological activity (cells concentration and viability, ethanol production rate, specific growth rate).

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