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Simulated moving bed technology to improve the yield of the biotechnological production of lactobionic acid and sorbitol

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Abstract This work presents an analysis on the suitability of the Simulated Moving Bed (and pseudo-SMB) technology on separating the mixture obtained from the lactose oxidation catalyzed by glucose-fructose oxidoreductase and glucono-α-lactonase enzymes. These enzymes from the *Zymomonas mobilis* bacteria are able to oxidize lactose in presence of fructose to its respective organic acid—lactobionic acid—and sorbitol. Some alternative arrays of chromatographic systems, as fixed bed column, SMB unit, 4 section pseudo-SMB, have been explored to separate the multicomponent mixture, in such way to make possible the product recovery and the recycle of substrates to the enzymatic reactor. This study involved the definition of appropriate operating conditions and the prediction of the performance of the separation units, or arrangement of units, through modeling and simulations tools. To define the proper operating conditions, inequalities from equilibrium theory and the concept of the separation volume analysis have been considered. In this analysis, equilibrium and kinetic parameters for the compounds adsorbing on *DOWEX 50W-X4* resin, in K^+ and Ca^{+2} ion-loadings, have been obtained from chromatographic methods (pulse and adsorption-desorption techniques). The enzymatic kinetic of production of lactobionic acid and sorbitol using permeabilized cells of *Z. mobilis* is shown. The strategy of keeping the highest value of reaction rate by the integration of a chromatographic system proved to be viable when it was found the feasibility to apply the SMB system in cascade.

Keywords Simulated moving bed · Lactobionic acid · GFOR enzyme · Pseudo-SMB configuration

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1 Introduction

The use of the lactose is a challenge for chemical engineers, since such compound accumulates in large amounts in the market (mainly from whey; Marwaha and Kennedy 1988; Coté et al. 2004). Lactose as raw material source for the synthesis of new high-added value products is extremely attractive. For the production of lactobionic acid (*LBA*)—a strong antioxidant with a large commercial interest (Sumimoto and Kamada 1990)—, from lactose oxidation, some different routes have been discussed in literature: electrochemical (Druliolle et al. 1995; Kokoh and Alonso-Vante 2006), catalytic (method used by Solvay Deutschland GmbH), and biological processes (Satory et al. 1997). Regarding biological process, both microbial (Murakami et al. 2002) and enzymatic ways (Baminger et al. 2001; Nordkvist et al. 2007) for oxidizing lactose to lactobionic acid have been studied and, during the last decade, enzymatic methods have received increased attention (Green Chemistry concepts).

One interesting pathway for the *LBA* production was disclosure from lactose and fructose biocatalized with glucose-fructose oxidoreductase (*GFOR*) and glucono-δ-lactonase (*GL*) enzymes (Satory et al. 1997). This route also leads to the formation of sorbitol, which is a non-carcinogenic

substance, widely applied in pharmaceutical and food industries (Bowen 1994). Lactose has a high Michaelis constant (K_m) and, hence, high concentration of substrates is needed to achieve maximum reaction velocity. In this framework, the advantages of simultaneous bio-reaction and separation of products/reactants are well known (reactions occurring at highest substrate concentrations, reduction of inhibitory effects, higher yields, economy in downstream processes, etc.; Cen and Tsao 1993; Ganetsos et al. 1993). There are two main procedures: *in situ* separation of the species and an external recycle between the reactor and the separator. In order to increase the productivity of the mentioned process, the produced quaternary mixture should be separated to allow the recycle of unconverted substrates to the bioreactor and the recovery of lactobionic acid and sorbitol. This could be achieved using the Simulated Moving Bed (SMB) technology.

Multi-component separations have been performed by some alternative SMB configurations, as for example: two (or more) SMB units in cascade (Wankat 2001; Kim and Wankat 2004; Hur and Wankat 2006), SMB with three outlet ports (Navarro et al. 1997; Beste and Arlt 2002; Wang and Ching 2005), eight (and nine) sections SMB (Chiang 1998; Wooley et al. 1998) and pseudo-Simulated Moving Bed (pseudo-SMB) (Ando et al. 1990). Considering the pseudo-SMB, this configuration refers to the JO technology developed by *Japan Organo Co.* (Ando et al. 1990), which has been discussed in some publications: Sayama et al. (1992) have experimentally tested this technology for separation of raffinose from beet molasses, Mata and Rodrigues (2001) have developed a pseudo-SMB model for such process, Kurup et al. (2006) have considered multi-objective optimization of these units, and Borges da Silva and Rodrigues (2006) analyzed JO performances and suggested an extended JO for quaternary separations.

This work is focused on the investigation of suitable separation units linked to the Simulated Moving Bed technology to be used in combination with reactive systems for the production of lactobionic acid and sorbitol. The objective is to promote the simultaneous bioreaction and separation of product in order to make possible the reaction to be conducted at higher concentrations of substrate and to allow improvements at the rate of reaction. Considering the peculiarities of global process, we analyze different configurations to achieve the desired objective: (a) SMB units in cascade (b) batch chromatography in connection to SMB unit, (c) pseudo-SMB unit. This study involves the definition of appropriate operating conditions and the prediction of the performance of the units, or arrangement of units, through modeling and simulations tools.The adsorption parameters for the separation of the mixture of lactose, fructose, lactobionic acid and sorbitol on an ion-exchange resin loaded with calcium and potassium ions are obtained. The bioconversion of the mixture of lactose and fructose to lactobionic acid and sorbitol using permeabilized cells of *Zymomonas mobilis* is carried out to provide information for the evaluation of the global productivity.

2 Description and modeling of the process

The Simulated Moving Bed (SMB) is a separation technology using the principles of continuous counter-current systems, which allows maximizing the mass transfer rates and better utilization of the stationary phase (Ruthven and Ching 1989). Although being one of the most advanced equipment in separation processes, the classic device is limited to two recovery outlets—Extract and Raffinate—allowing only the separation of mixtures into two fractions. Considering the separation of the mixture of lactose (L), fructose (F), lactobionic acid (LBA) and sorbitol (S), one ideal condition would be the collection of the two products in one of the outlets (for further purification) and of the substrates in the another (to be recycled to enzymatic reactor). Our preliminary studies (Pedruzzi et al. 2008) showed that K^+-loaded ion exchange resin is suitable for this quaternary separation with the following affinity: $LBA < L < S < F$ (i.e., product<substrate<product<substrate). The Ca^{2+}-loaded resin also enables this separation, but another affinity sequence occurs: $L < LBA < F < S$ (substrate alternating with product again). This fact will be relevant to define the arrangement of chromatographic systems in cascade, since the choice of stationary phase should take into account the salt of lactobionic acid being produced in the reactor (that depends on the used alkali to maintain the reaction at constant pH). Limitations on the solubility of $Ca(OH)_2$, and also the sugar complexation with calcium ion, make the KOH solution one most appropriate option for the pH control of the reaction, which will produce potassium lactobionate.

Therefore, one alternative considers the operation of two SMB units, in cascade (System 1): the first one works to recover the *LBA* compound and the second one to separate the remaining components. Since the *LBA* will be as potassium lactobionate, columns of the first SMB unit are packed with the ion exchange resin in potassium form (see Fig. 1b). The salt goes to the raffinate port, while other components are collected at the extract port. Afterwards, this mixture of three compounds should be introduced in the second SMB unit (SMB 2 in Fig. 1), which is loaded with resin in calcium form, in order to allow the separation of sorbitol (remaining product) of the other two substrates (lactose and fructose, both compounds are collected at the raffinate). As a particularity, the weak affinity of *LBA* compound to the resin in potassium form, with a separation factor quite large ($\alpha_{L/LBA} \approx 15$; Pedruzzi et al. 2008) that might not justify the use of a SMB unit to perform the separation of this organic acid and the rest of the mixture, lead us to also consider

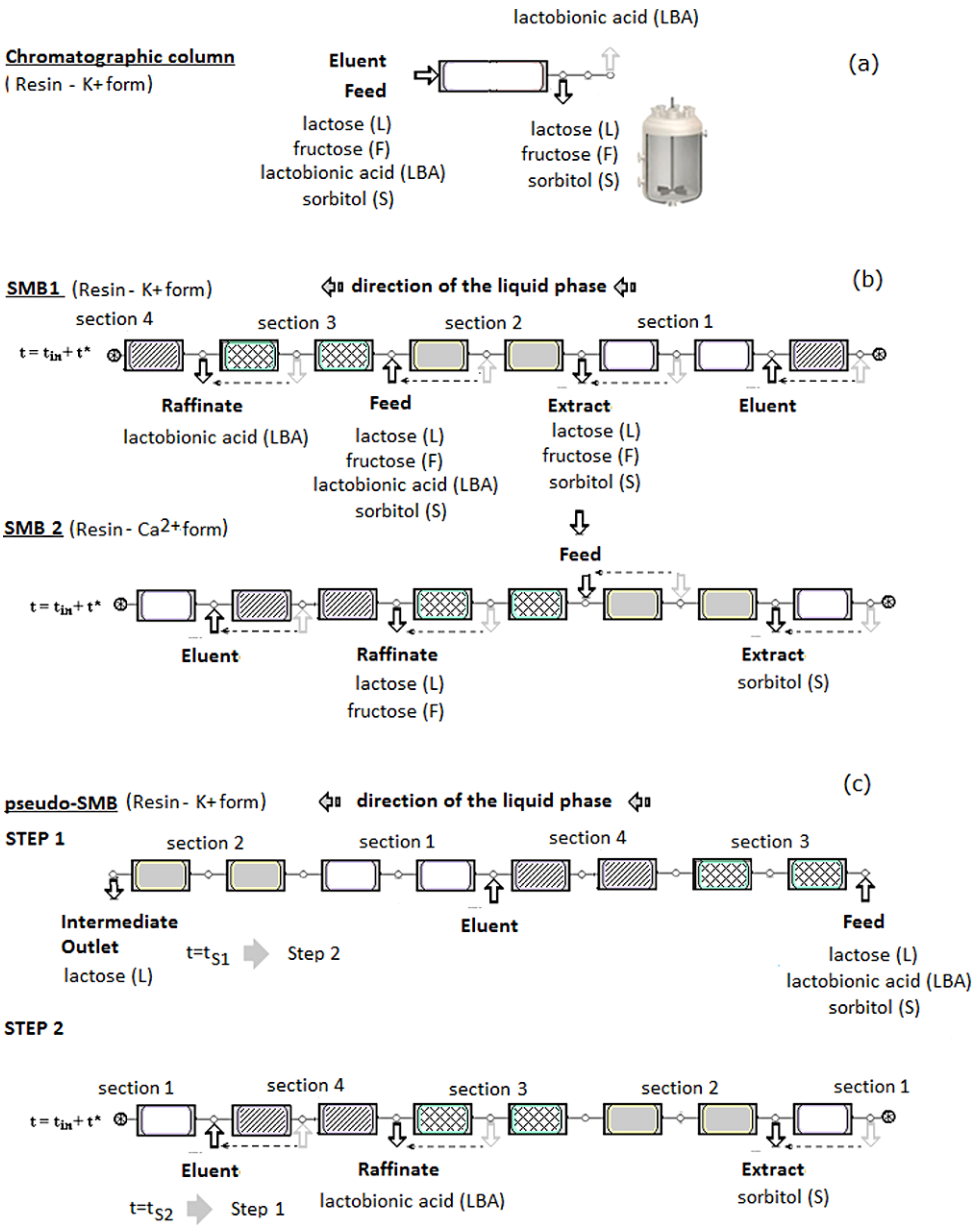

Fig. 1 Strategies of arrangement of chromatographic systems for the separation of mixtures from the enzymatic oxidation of lactose by using *Z. mobilis* cells: (**a**) batch column or (**b**) SMB1 unit (to recover the *LBA*) connected to the SMB2 (to recover the sorbitol and to allow the recycle of substrates); (**c**) 4-section pseudo-SMB unit (to recover the products and to allow the recycle of lactose)

the batch chromatography for the *LBA* recovery from the quaternary mixture. After this discontinuous operation, the ternary mixture (sorbitol, lactose and fructose) accumulated in a storage tank would feed one SMB unit (Ca^{2+} loaded resin as stationary phase), for example, SMB 2 shown in Fig. 1. Regarding the potential of the technology of pseudo-SMB, we can also suggest one 4-section pseudo-SMB unit, consisting of packed columns with the resin in potassium form, to be applied to the current process (Fig. 1c). Its application is justified by the results coming from the reaction data and it will be discussed forward. As expected, the performance of these alternatives should be checked according to productivity, recovery, consumption of eluent and final purity of the collected compounds.

The analysis on the configuration of these systems is accomplished by the mathematical modeling and the dynamic simulation of the process. Of course, adsorption parameters of the compounds on both stationary phases have to be found. The model equations for the different chromatographic systems are described in Table 1, which can be obtained from conservation balances of chemical species in fluid and solid phases. In Table 1, subscript i (= L, F, *LBA* and S) refers to the compounds and k refers to the columns in different sections of the unit (except for the case of the discontinuous process). The concentration in liquid and in adsorbent phase is C and $\bar{q}$ (average concentration over the particle), respectively. The velocity of the liquid and solid

Table 1 Model equations for the different chromatographic systems: batch chromatographic, SMB and pseudo SMB unit

Governing conservation equations

Mass balance in a element volume of the column k (liquid phase):

$$\frac{\partial C_{ik}}{\partial t} + \frac{(1-\varepsilon)}{\varepsilon}\left[\frac{\partial \bar{q}_{ik}}{\partial t} - u_S \frac{\partial \bar{q}_{ik}}{\partial z}\right] + v_k \frac{\partial C_{ik}}{\partial z} - D_k \frac{\partial^2 C_{ik}}{\partial z^2} = 0 \quad (i = L, F, LBA \text{ and } S)$$

Mass balance in the solid phase (LDF model):

$$\frac{\partial \bar{q}_{ik}}{\partial t} = u_S \frac{\partial \bar{q}_{ik}}{\partial z} + k_{mi}\left(f_i^*(C_i) - \bar{q}_{ik}\right) \quad (i = L, F, LBA \text{ and } S)$$

$$u_S = 0 \begin{cases} \text{for pseudo SMB—Step 1} \\ \text{for SMB} \\ \text{for chromatographic column (in batch operation)} \end{cases}$$

(TMB model approach for the step 2 of the pseudo SMB)

Initial conditions:

Batch chromatography and SMB unit:

$t = 0, C_{ik} = \bar{q}_{ik} = 0$

STEP 1—pseudo-SMB:

Cycle 1: $t = 0, C_{ik} = \bar{q}_{ik} = 0$

Cycle θ: $t = (\theta - 1)(t_{S1} + t_{S2})$

$$\begin{cases} C_{ik} = C_{ik}(\text{cycle } \theta - 1, \text{ end of step 2}) \\ \bar{q}_{ik} = \bar{q}_{ik}(\text{cycle } \theta - 1, \text{ end of step 2}) \end{cases} (\theta = 2, 3, 4, \ldots)$$

STEP 2—pseudo-SMB:

$$\text{Cycle 1: } t = t_{S1} \begin{cases} C_{ik} = C_{ik}(\text{cycle 1, end of step 1}) \\ \bar{q}_{ik} = \bar{q}_{ik}(\text{cycle 1, end of step 1}) \end{cases}$$

$$\text{Cycle } \theta\text{: } t = \theta t_{S1} \begin{cases} C_{ik} = C_{ik}(\text{cycle } \theta, \text{ end of step 1}) \\ \bar{q}_{ik} = \bar{q}_{ik}(\text{cycle } \theta, \text{ end of step 1}) \end{cases} (\theta = 2, 3, 4, \ldots)$$

Boundary conditions*:

Liquid phase: $z = 0$ $(t > 0)$: $v_k C_{ik,o} = v_k C_{ik}|_{z=0^+} - D_k \frac{\partial C_{ik}}{\partial z}|_{z=0^+}$

$z = L_C$ $(t > 0)$ $\frac{\partial C_{ik}}{\partial z}|_{z=L_C} = 0$

Solid phase: $z = 0$ $(t > 0)$: $\frac{\partial \bar{q}_{ik}}{\partial z}|_{z=0} = 0$

(only for the step 2 of the pseudo SMB)

Mass Balances at the nodes of the SMB and pseudo-SMB units:

– for the columns within the sections: $C_{ik+1,0} = C_{ik}$ (to the SMB and pseudo-SMB—STEP 1 and 2):

– for the extract and raffinate nodes: $C_{ik+1,0} = C_{ik}$ (to the SMB and pseudo-SMB—STEP 2):

– for the eluent node: $C_{ik+1,0} = (v_4/v_1)C_{ik}$ (to the SMB and pseudo-SMB—STEP 1 and 2):

– for the feed node: $C_{ik+1,0} = (v_{\mathrm{Fe}}/v_3)C_{i\mathrm{Fe}} + (v_2/v_3)C_{ik}$ (to the SMB)

$C_{ik,o} = C_{i\mathrm{Fe}}$ (to the pseudo-SMB unit—STEP 1)

*For the chromatographic column in discontinuous process, subscript k can be omitted from the equations and the inlet concentration of the compound i ($C_{i,o}$) is taken as feed concentration of the compound ($C_{i,\mathrm{Fe}}$)

phase is v (interstitial) and u_s, respectively; D is the axial dispersion and ε is the porosity. The term f_i^* (C_i) represents the equilibrium adsorption isotherm of the compound i and k_m is the mass transfer coefficient. For the step 2 of the pseudo-SMB operation, the TMB approach that treats SMB processes as equivalent true moving systems is employed. Once there is a large number of columns in the unit, this approach can be assumed (Pais et al. 1998).

The steady state of the SMB and pseudo-SMB units is a cyclic steady-state since there is a time dependency of the boundary conditions for each column in the system. The boundary conditions depend on the section of the system and, therefore, it is necessary to include the mass balances at each node of the units. The variables C_{Fe} and v_{Fe} are the concentration of the compounds and the velocity of the feed stream.

The performance of the separation units for the current process is analyzed by purity (Pu) and recovery (Re) of the compounds in their enriched streams, productivity (Pr) and eluent consumption (EC), as defined in Table 2.

Table 2 Definition of performance parameters for the chromatographic systems ($i = L, F, LBA$ and S)

Performance Parameter	Batch chromatography	SMB (X = *Ra*, *Ex*)	Pseudo-SMB (X = *Ra*, *Int*, *Ex*)
Average conc., $\bar{C}_{i,X}$ ($g\,L^{-1}$)	$\frac{1}{(t_{fn}-t_{in})}\int_{tin}^{tfn} C_i\,dt$	$\frac{1}{t^*}\int_0^{t^*} C_{i,X}\,dt$	$\frac{1}{t_{S1}}\int_0^{ts1} C_{i,X}\,dt$; $\frac{1}{t_{S2}-t_{S1}}\int_{ts1}^{ts2} C_{i,X}\,dt$
Purity (%)	$\frac{\bar{C}_{i,X}}{\sum_{j=comp.}\bar{C}_{j,X}}100$	$\frac{\bar{C}_{i,X}}{\sum_{j=comp.}\bar{C}_{j,X}}100$	$\frac{\bar{C}_{i,X}}{\sum_{j=comp.}\bar{C}_{j,X}}100$
Recovery (%)	$\frac{Q_{El}(t_{fn}-t_{in})\bar{C}_i}{V_{inj}C_{i,Fe}}100$	$\frac{Q_X\bar{C}_{i,X}}{Q_{Fe}C_{i,Fe}}100$	$\frac{Q_X\bar{C}_{i,X}}{Q_{Fe}C_{i,Fe}}\frac{t_{Sn\ (n=1\text{ or }2)}}{t_{S1}}100$
Productivity ($g\,L^{-1}\,h^{-1}$)	$\frac{Q_{El}\bar{C}_i}{(1-\varepsilon)V_C}\frac{(t_{fn}-t_{in})}{t_{Cycle}}$	$\frac{Q_X\bar{C}_{i,X}}{N_C^{SMB}(1-\varepsilon)V_C}$	$\frac{Q_X\bar{C}_{i,X}}{N_C^{p-SMB}(1-\varepsilon)V_C}\frac{t_{Sn\ (n=1\text{ or }2)}}{t_{S1}+t_{S2}}$
El. Consumption ($L\,g^{-1}$)	$\frac{t_{Cycle}}{(t_{fn}-t_{in})\bar{C}_i}$	$\frac{(Q_{Fe}+Q_{El})}{Q_X\bar{C}_{i,X}}$	$\frac{Q_{El1}t_{S1}+Q_{El2}t_{S2}}{Q_X\bar{C}_{i,X}t_{Sn\ (n=1\text{ or }2)}}$

3 Experimental procedures

3.1 Determination of reaction kinetics

The bioconversion assays were performed in a glass reactor (150 mL) containing 100 mL of the mixture of lactose and fructose and the free permeabilized cells (final cell concentration of 7.19 $g_{cell\ dry}\,L^{-1}$). A magnetic stirrer with temperature control (Stuart model SD 162, UK) was used to maintain the reaction mixture at a constant agitation speed (300–400 rpm) and a controlled temperature (39 °C). The value of pH was maintained at 6.4 by automatic addition of 7.0 $mol\,L^{-1}$ KOH solution expending from solenoid valve (Bio-Chem Valve INC, UK), which was controlled from a pH controller. Samples were withdrawn from the stirred reactor at given time intervals to be analyzed in HPLC.

Strain *Zymomonas mobilis* ATCC 29191 was obtained from Professor M. Silveira, Biotechnology Institute (University of Caxias do Sul), Rio Grande do Sul, Brazil. Inoculums to be added into the bioreactor have been prepared as described elsewhere (Malvessi et al. 2006). Fermentation runs were carried out in a SGI/SET2 bioreactor (SGI, France) with 2 L capacity. The conditions were: pH 5.5 (controlled with 4 M KOH solution), impeller speed around 500 rpm and 30 °C (production media: 150 $g\,L^{-1}$ glucose solution with salts, vitamins, and yeast extract). *Z. mobilis* cells were harvested in the late exponential phase. The medium was centrifuged at 3000 rpm/7 min (Digtor 20, Spain) and each harvested cell was washed twice with deionized water. Afterwards, the permeabilization procedure was carried out and, then, cells were re-suspended around 50 $g\,L^{-1}$ with deionized water to be used as stock solution (kept at 4 °C). For the permeabilization of the cells, a hexadecyltrimethylammonium bromide (CTAB) solution was used. A treatment with glutaraldehyde was carried out in order to prevent enzyme loss through the permeabilized cells.

3.2 Determination of adsorption data

The adsorption equilibrium isotherms and breakthrough curves for the compounds were obtained using a jacketed *Superformance* column 150-26 (Gotec, Germany) packed with the resin. For the stationary phase of *DOWEX 50W-X4* resin (4% DVB; 55 μm average particle size) in potassium form, adsorption-desorption method and frontal analysis were applied to the column of 98.5 mm length and 26 mm ID. The dynamic measurements of breakthrough curves were obtained by collecting samples from the column outlet after a step change from pure eluent to a feed solution of known concentration pumped into the column. The flow rate during these measurements was 6 $mL\,min^{-1}$ and feed concentration up to 130 $g\,L^{-1}$. The parameters of equilibrium adsorption of compounds fructose, lactose and sorbitol adsorbing on *DOWEX 50W-X4* in calcium form were determined using the method of pulses in the *Superformance* column (84.0 mm length ×26 mm ID). Pulses of 500 mL of each compound, at concentrations of 65 $g\,L^{-1}$ and 130 $g\,L^{-1}$, were injected into the column at different eluent flow rate (range of 4 to 13 $mL\,min^{-1}$). The detection of the compounds at the column outlet was accomplished by refraction index measurements. The linear relation obtained between the first moment of the pulse and the ratio of bed volume to the eluent flow rate can provide the Henry constant of the compound. All experimental runs were carried out at 293 K and deionized water was used as eluent. The bed void fraction (ε) was obtained by measuring the retention time of 5 $g\,L^{-1}$ blue-dextran solution pulses through the fixed beds. The axial dispersion was estimated using a fitting procedure from experimental breakthrough curves and calculated using the variance of the experimental curves obtained from the tracer pulse experiments.

3.3 Analytical

The above mentioned compounds in both reaction and adsorption samples were quantified by HPLC using the *Transgenomic ICESep ICE-ION300* (7.8 × 300 mm) ion exchange column connected to the refraction index detector (model 131, Gilson, France). The analyses were performed under the following chromatographic conditions (Pedruzzi

et al. 2007): (i) eluent: 0.450 mM H_2SO_4 solution (pH 3.1); (ii) flow rate: 0.5 mL min^{-1}; (iii) at 75 °C. The conversion of substrates, and formation of products, could be confirmed by the amount of alkali solution (KOH) added to the reactor in order to control the value of pH during the reaction. The cell concentration was determined indirectly by measuring the absorbance of cell suspensions to 560 nm and directly by gravimetric method. All reagents were of analytical grade.

4 Results and discussion

4.1 Enzymatic reaction

Figure 2 shows the kinetics of conversion of lactose and fructose to *LBA* and sorbitol using permeabilized and reticulated cells of *Z. mobilis*, which contain the *GFOR* and *GL* enzymes. In these experimental runs, high concentrations of the substrate are used, since it consists of a more favorable condition to the action of the *GFOR* enzyme (Silveira et al. 1999; Erzinger et al. 2003). One can verify kinetic curves for the initial concentration of around 0.7 mol L^{-1} lactose and initial concentrations of around 0.35 mol L^{-1} and 0.7 mol L^{-1} fructose. It is worth mentioning the value of the initial disaccharide concentration is close to its solubility limit (~30 g/100 g solution; Fox and McSweeney 1998) at 39 °C (the reaction temperature). These results point out there is no considerable effect of the fructose concentration on the initial rate of the enzymatic reaction. Of course, the reaction rate depends on the biocatalyst concentration that, in this study, was 7.2 g of dried cells per liter of solution. The value of the reaction rate can be larger at higher concentrations of the biocatalyst (for example, Malvessi et al. 2002 have used 25 to 30 g L^{-1}). For the case shown in Fig. 2b, with initial condition of 0.7 mol L^{-1} lactose and 0.35 mol L^{-1} fructose, an equimolar mixture of products (*LBA* and sorbitol) and lactose, in excess initially, is accomplished at the end of the bioconversion. The total consumption of fructose could benefit the downstream separation processes, since it reduces the complexity of the formed mixture. In this particular situation, the observed productivity for *LBA* and sorbitol would be 5.614 g L^{-1} h and 2.855 g L^{-1} h, respectively (reaction conditions: 7.2 g L^{-1} cell *Z. mobilis* (in dry basis); 39 °C, pH 6.4, 350 rpm agitation speed).

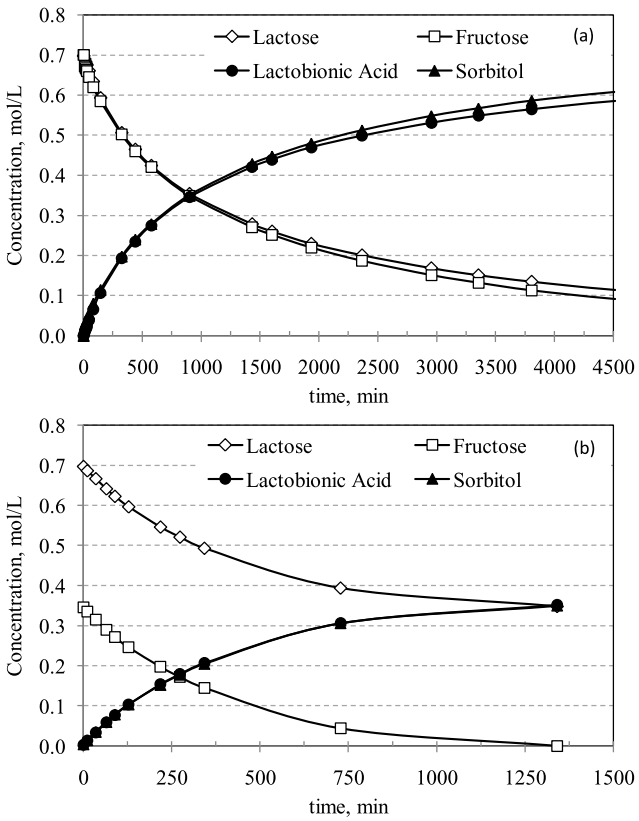

Fig. 2 Production of the lactobionic acid (●) and sorbitol (▲) using permeabilized cells of *Z. mobilis* ATCC 29 191. Initial substrate solution: (**a**) 0.7 mol L^{-1} lactose (◇) and 0.7 mol L^{-1} fructose (□); (**b**) 0.7 mol L^{-1} lactose (◇) and 0.35 mol L^{-1} fructose (□). Conditions: 39°C; pH 6.4, 350 rpm, $C_{cell} = 7.19$ g L^{-1} (cell dry weight)

4.2 Adsorption data

The equilibrium data for the four compounds adsorbing on two ionic forms of the *DOWEX 50W-X4* resin, one loaded with potassium ion and another with calcium ion, are shown in Table 3. The adsorption isotherms are linear in the studied concentration range (up to 130 g L^{-1}), except the lactobionic acid. An unfavorable behavior is verified for the adsorption of *LBA* on K^+ loaded resin, which equilibrium is described by an anti-Langmuir model. Because lactobionic acid was in potassium salt form, the resin has undergone some shrink during the saturation of the column with *LBA* solutions. The shrinking of the resin was only noticeable for the largest salt concentration. Bed porosity increased when shrinking was larger (that means at higher salt concentration). The bed porosity change was considered only to determine adsorption parameters (Pedruzzi et al. 2008). It is known that variations of the porosity during operations may affect the performance of the SMB unit (Mihlbachler et al. 2001; Bae et al. 2006). In the following, to predict the performance of the chromatographic systems, the models employed one fixed bed porosity due to negligible error linked to low values of feed concentration for the *LBA*. In the case of Ca^{2+} loaded resin, the adsorption of *LBA* compound is not considered, since the application of this stationary phase is only subsequent to the recovery of this compound.

Figure 3a shows experimental and simulated breakthrough curves for all compounds adsorbing on ion exchange resin in K^+ form at 20 °C. One can verify the fructose, lactose and sorbitol breakthrough at around 130 g L^{-1}

Table 3 Adsorption parameters for the compounds adsorbing on *DOWEX 50W-X4* resin, in potassium and in calcium form, at 20 °C

Stationary phase	Compound	ε_b	Eq. Isotherm	k_m (s^{-1})[e]
DOWEX 50W-X4	*LBA*	0.32[a]		
K^+ form		0.36[b]	$\frac{0.00446 \cdot 13.7 \cdot C_{LBA}}{1-0.00446 \cdot C_{LBA}}$	[d]
		0.39 [c]		
	Lactose	0.34	$0.48 \cdot C_L$	0.292
	Sorbitol		$0.58 \cdot C_S$	0.405
	Fructose		$0.67 \cdot C_F$	0.403
DOWEX 50W-X4	Lactose	0.40	$0.34 \cdot C_L$	0.407
Ca^{2+} form	Sorbitol		$1.85 \cdot C_S$	0.127
	Fructose		$0.79 \cdot C_F$	0.340

[a] 19.6 g L^{-1} (no shrinking)

[b] 69.1 g L^{-1} (bed length decreased around 2%)

[c] 126.6 g L^{-1} (bed length decreased around 5%)

[d] equilibrium condition (without mass transfer resistance)

[e] Resin in K^+ form: estimation from the best fit procedure of breakthrough data, in which the sum of square residual between the experimental and calculated is minimized; Resin in Ca^{2+} form: estimation from equation of the mass transfer coefficient considering the model of homogeneous particle ($k_{mi} = \frac{15}{r_p^2} \frac{\varepsilon_p D_{mi}}{\tau K_i}$)

feed solution and dimensionless profiles of the *LBA* at three feed concentrations (19.6, 69.1 and 126.6 g L^{-1}). Figure 3b shows the relationship between the stoichiometric time of the component as a function of the bed volume and eluent flow rate ratio, when pulses of the compounds are injected into the column packed with ion exchange resin in Ca^{2+} form. The experimental adsorption kinetic has been simulated using a dispersive plug flow model and the linear driving force approach for the intraparticular mass transfer using a mass transfer coefficient (k_m). This small and homogeneous particle resin exhibits a high kinetic performance, i.e., diffusion rate is quite fast. Concerning the *LBA* breakthrough behavior, one can verify that its diffusional time constant is quite low, i.e., there is no concentration gradient within the particle. In this case, local equilibrium model including the axial dispersion is used to predict breakthrough curves of *LBA* adsorbing on *DOWEX 50W-X4*. The dimensionless breakthrough curves for the uptake of lactobionic acid on Dowex resin are showing a more dispersive profile as feed concentration is larger.

Through the obtained experimental data and solving the set of mathematical models described in Table 1, separation performances of some different configurations, including SMB in cascade, batch chromatography plus SMB or 4-section pseudo-SMB units, applied on the produced mixture are evaluated. The equations of mathematical models describing the operation on mentioned systems were approximated to algebraic equations using the Method of Finite Volumes (Maliska 1995), with the application of the interpolation functions *WUDS* ('Weight Upwind Difference Scheme'). The discretized equations were implemented in computational program (*FORTRAN* language), where the algebraic equations systems were solved for the *TDMA* (Tri-Diagonal Matrix Algorithm) method.

4.3 Performance of the chromatographic systems

In order to make pure compounds from continuous countercurrent chromatographic systems (such as SMB or pseudo-SMB), each compound must move with or the liquid or the solid phase, depending on the considered compound and the unit section, to its respective enrichment outlet. For those systems involving linear adsorption isotherms, the application of the Equilibrium Theory leads to a set of inequalities relating the liquid-phase flow rate of each one of the four SMB sections (Q_j), the switching time t^* (or solid flow rate Q_S) and the linear adsorption constants (Storti et al. 1993). By these inequalities, a separation region or volume can be built. The region of the complete separation in the operating parameter plane m_2 and $m_3 - m_j = (\frac{v_j t^*}{L_C} - 1)\frac{\varepsilon}{(1-\varepsilon)}$, with v_j the liquid-phase velocity in section j—determines one triangular area where the separation of the compounds would be possible (without considering the mass transfer resistance or axial dispersion). In developing the concept of separation volume m_1 versus m_2 versus m_3 established in the work of Azevedo (2001), the mass transfer resistance are taken into account to define a 3D-space for which one can find operating conditions that allow high purity products (Azevedo and Rodrigues 1999).

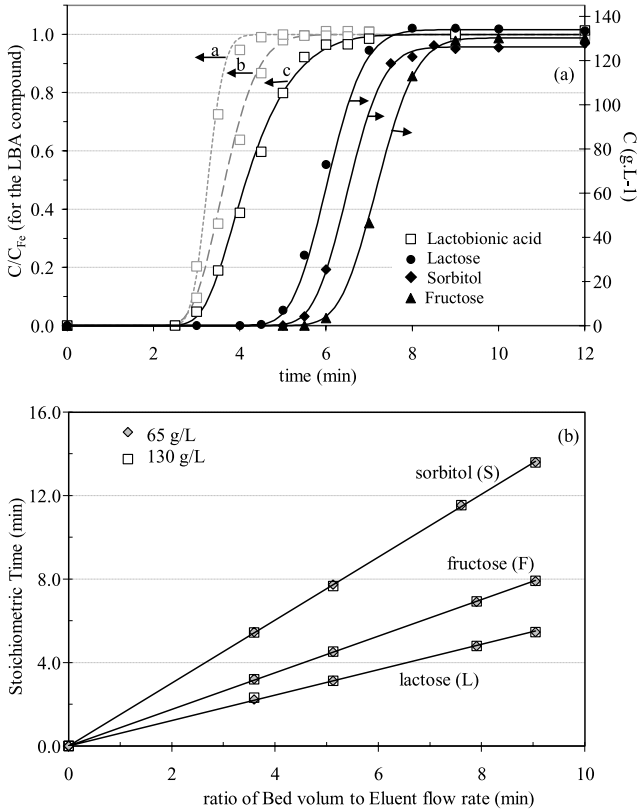

Fig. 3 (**a**) Breakthrough curves for all compounds on *DOWEX 50W-X4* resin in K^+ form at 20 °C. Column: 98.5 mm length and 26 mm I.D.; (6.0 mL min^{-1} flow rate). The *curves a, b and c* correspond to the *LBA* feed solutions of 19.6, 69.1, 126.6 g L^{-1}, respectively (Pedruzzi et al. 2008). (**b**) Stoichiometric time of each component as a function of the bed volume to eluent flow rate ratio. Concentration pulses of the compounds: 130 g L^{-1} and 65 g L^{-1}. Adsorbent: resin *DOWEX 50W-X4* in Ca^{+2} form; eluent: deionized water; at 20°C

Considering the two SMB in cascade for the given multicomponent mixture, the extract stream of the first SMB unit is connected to the feed stream of the second unit ($Q_{Ex}^{\mathrm{SMB1}} = Q_{\mathrm{Fe}}^{\mathrm{SMB2}}$), so there is no buildup in the process. In determining the operating conditions of the units, the geometric parameters of the systems are selected based on characteristics of the columns used in the experimental adsorption assays (SMB1: $L_C = 0.15$ m, $D_C = 0.026$ m; SMB2: $L_C = 0.30$ m, $D_C = 0.031$ m). The maximum possible pressure drop ΔP_{max} in a system is taken as 55 bar. The *Karman-Kozeny* equation can be used to calculate the highest flow rates applied to the system (Sect. 1 of the units). Thus, after defining the flow rate in Sect. 1, the volume of separation $m_1 \times m_2 \times m_3$ may be built for selected value of switching time and a value of m_4 parameter that guarantees the recycle of clean eluent.

The flow rate in the Sect. 1 of the first SMB is calculated to be 95.81 mL min^{-1} ($Q_{1,\mathrm{SMB1}}$). In order to construct the separation volume of this unit, the switching time is estimated based on the minimum limit given by the retention time of the more retained compound on the adsorbent phase under the highest flow rate in unit (Sect. 1). In this operation, the switching time is chosen as equal to 1.0 min (retention time of fructose at $Q_{1,\mathrm{SMB1}}$ is 0.65 min). The composition of the quaternary mixture to feed the SMB unit is established from the results of enzymatic kinetics. If the enzymatic reaction is interrupted after six hours (Fig. 2), when the decrease of the reaction rate becomes more pronounced, about 0.2 mol L^{-1} of both substrates is consumed. Consequently, one mixture containing approximately 0.5 mol L^{-1} lactose (171.2 g L^{-1}), 0.15 mol L^{-1} fructose (27 g L^{-1}) and 0.2 mol L^{-1} for each product (i.e., 71 g L^{-1} *LBA* and 36.4 g L^{-1} sorbitol) can be taken. To avoid the data extrapolation on the adsorption isotherms (as in the case of lactose), we have decided to limit the total concentration of compounds in the mixture to about 180 g L^{-1}. Therefore, keeping the substrate to product ratio linked to the maximum total concentration, feed composition is 100.7 g L^{-1} lactose, 42 g L^{-1} *LBA*, 21.4 g L^{-1} sorbitol and 16 g L^{-1} fructose.

Depending on the objective function that one requires to maximize (as productivity) or to minimize (as eluent consumption or degree of dilution of the compounds on enriched outlets), distinct operating conditions should be selected. Figure 4a shows the volume of separation $m_{1\mathrm{SMB1}} \times m_{2\mathrm{SMB1}} \times m_{3\mathrm{SMB1}}$ for the first SMB unit (purity above 99.9% in both extract and raffinate)—with $m_{4\mathrm{SMB1}} = 0.058$ ($t^* = 1.0$ min)—to separate the fraction of sorbitol, fructose and lactose, which is collected at the extract, of *LBA* (product recovered at raffinate). The lower values of m_1 parameter, the smaller triangular region for the total separation (only under lower feed throughput for the unit, adsorbent at Sect. 1 could be regenerated). As known, the value of the parameter m_4 affects the recycle flow rate of the unit. A higher fraction of eluent can be recycled if higher possible value of m_4 is selected (taking into account the maximum value to ensure the recycling of the clean eluent). The set of parameters m providing the best performance in terms of productivity in the extract stream (to reduce spending on downstream processes such as evaporation or membrane) is ($m_{1\mathrm{SMB1}} = 0.8$; $m_{2\mathrm{SMB1}} = 0.07$; $m_{3\mathrm{SMB1}} = 0.37$; $m_{4\mathrm{SMB1}} = 0.058$) with $t^* = 1.0$ min. The performance parameters are shown in Table 4 (SMB1/Op.C.1). One could also observe in Fig. 4b the profiles of average concentration of compounds in sections of the SMB unit under the operating conditions given by referred set of m parameters ($Q_{El\mathrm{SMB1}} = 38.77$ mL min^{-1}; $Q_{\mathrm{FeSMB1}} = 15.67$ mL min^{-1}; $Q_{Ra\mathrm{SMB1}} = 16.30$ mL min^{-1}; $Q_{Ex\mathrm{SMB1}} = 38.14$ mL min^{-1}; $Q_{4\mathrm{SMB1}} = 30.43$ mL min^{-1}).

4.3.1 Discontinuous process for the recovery of LBA

Now, let us consider the discontinuous process for the separation of *LBA* of other components (L + F + S). Some studies on the comparison between batch and SMB systems

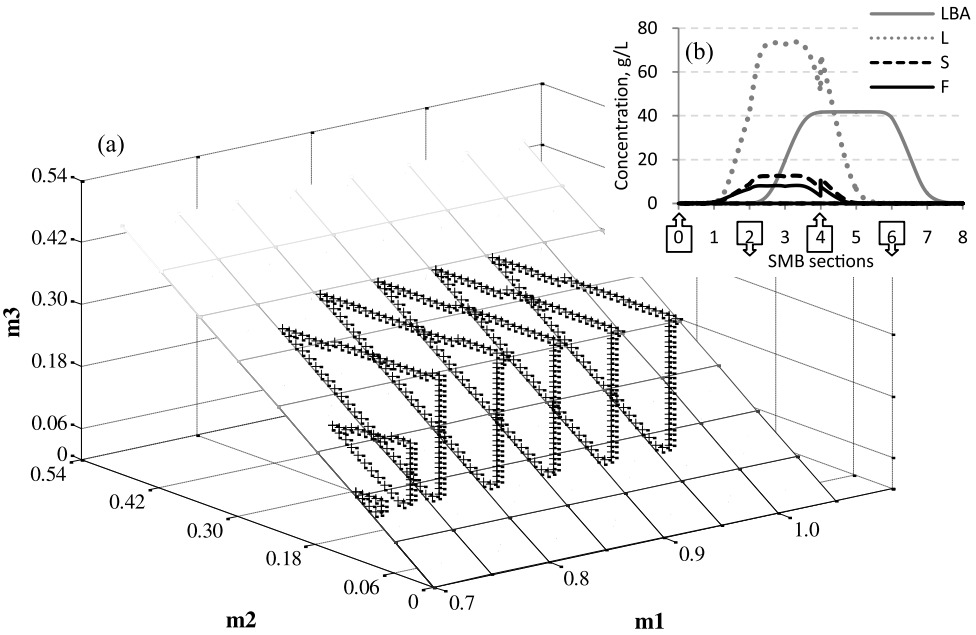

Fig. 4 (**a**) Separation volume $m_1 \times m_2 \times m_3$ (purity above 99.9%), for values of $t^* = 1.0$ min and $m_4 = 0.058$, of the SMB1 unit for the separation of *LBA* (at raffinate) of the fraction containing sorbitol, fructose and lactose (collected at the extract). (**b**) Profile of average concentration of the compounds in SMB, at cyclic steady state, under the conditions: $(m_1; m_2; m_3; m_4) = (0.8; 0.07; 0.37; 0.058)$ with $t^* = 1$ min (*LBA* recovered at raffinate port)

Table 4 Operating and performance parameters for the separation of *LBA* of the fraction containing lactose, fructose and sorbitol in two chromatographic systems: SMB1 unit and batch column

Characteristics		Batch Column	SMB1 unit	
			Op.C. 1	Op.C. 2
L_C (m) $\times D_C$ (m)		1.2 × 0.026	0.15 × 0.026	0.15 × 0.026
N. Columns/Configuration		1 (–)	8 (2/2/2/2)	8 (2/2/2/2)
t_{inj} (min)		2.27	*	*
t_{cycle} (min)		27.23	*	*
Q_{Fe} (mL min^{-1})		*	15.67	2.08
Q_{El} (mL min^{-1})		25.0	38.77	4.56
Q_{Ex} (mL min^{-1})		*	38.14	4.24
Q_{Ra} (mL min^{-1})		*	16.30	2.40
t^* (min)		*	1.0	7.5
Performance variables				
Purity (%)	*LBA*	99.9	99.9	99.9
	L+S+F	99.9	99.9	99.9
Recovery (%)	*LBA*	99.5	97.6	99.9
	L+S+F	99.5	99.9	99.8
Productiv. (g L^{-1} h^{-1})	*LBA*	17.89	92.22	12.58
	L+S+F	58.76	309.96	41.35
El. Consumption (L g^{-1})	*LBA*	0.286	0.085	0.076
	L+S+F	0.061	0.025	0.023
Product Dilution	*LBA*	2.57	1.07	1.15
	L+S+F	5.79	2.44	2.04

have been published (Heuer et al. 1997; Strube et al. 1998; Jupke et al. 2002). It is widely known that the productivity from SMB processes is larger than that achieved by elution chromatography, and, moreover, compounds are recovered with lower degree of dilution. However, many issues related to the decision to operate one or another mode still emerge when one reflect on other aspects linked to the overall separation process (system flexibility, initial investment, labor costs, adsorbent and eluent costs, etc.). The definition of the more advantageous process will depend on very thorough studies of several variables that might be relevant in a particular process, using tools as numerical simulation and op-

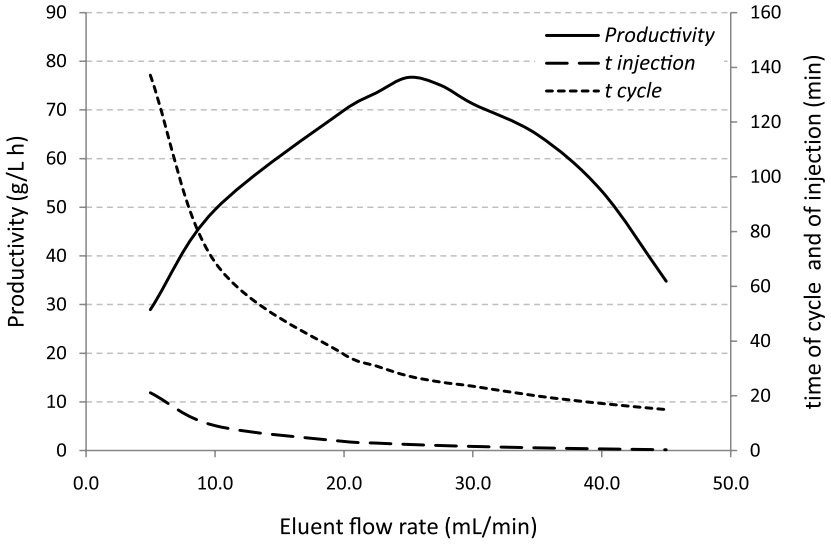

Fig. 5 Productivity for optimal values of t_{cycle} and t_{inj} as function of the eluent flow in batch process for the separation of lactobionic acid and the fraction containing lactose, sorbitol and fructose

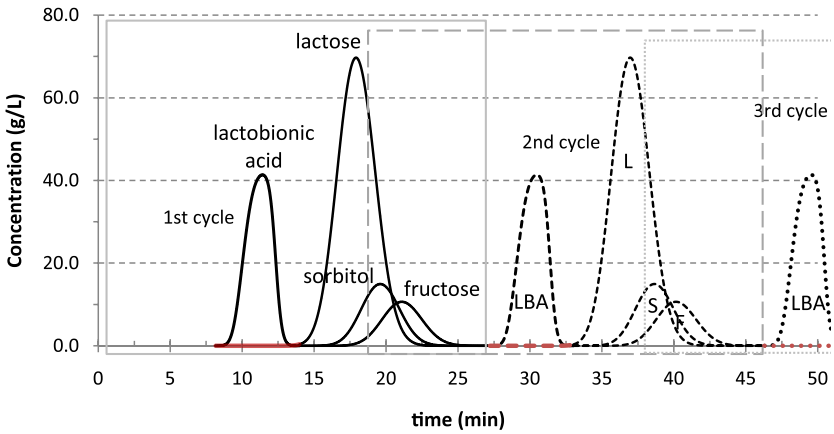

Fig. 6 Concentration profiles of the compounds of the quaternary mixture during the cycles of batch operation. Operating conditions: $t_{\text{cycle}} = 27.23$ min; $t_{inj} = 2.27$ and $Q_{El} = 25.0\ \text{mL min}^{-1}$ (Feed condition: $100.7\ \text{g L}^{-1}$ lactose, $42\ \text{g L}^{-1}$ *LBA*, $21.4\ \text{g L}^{-1}$ sorbitol and $16\ \text{g L}^{-1}$ fructose)

timization. Here, an optimization procedure on the operation of a chromatographic column for the given quaternary separation is performed. The optimal conditions can be used to compare the separation performance of using these two technologies: batch and SMB.

The parameters to be optimized in a process performed in chromatographic column are geometric parameters (length, L_C and diameter, D_C), time of injection (t_{inj}), period of time to collect the fractions and elution flow rate. In general, in the optimization of batch processes, a compromise among the maximum yield, high recovery and high purity of the compounds is the major challenge, since the maximum values of each one of these performance variables are achieved at the expense of others. The chromatographic column evaluated in this system is defined as being the SMB unit in open looping. Thus, the volume of adsorbent and the columns remain the same in both operations. The feed concentration of each compound is that defined previously.

The performance parameters for the separation of component i through the batch process are described in Table 2. The objective function is the sum of productivity of the two collected fractions (fraction 1: *LBA*; fraction 2: L + S + F). The two fractions are subject to the following restriction: $Pu_l - Pu_l^{required} \geq 0$, where the variable $Pu_l^{required}$ refers to the purity required in the recovery of the fraction l (defined as 99.9% for both fractions). The recovery is also constrained by defining concentration limits at the beginning and at the end of the time to collect each product fraction. This separation process in the batch column is optimized in respect to the variables time of cycle (t_{cycle}) and time of injection (t_{inj}). The numerical problem is solved with the aid of the optimization subroutine IPOPT 3.5.4 (Kawajiri and Biegler 2006).

Figure 5 shows the optimized values of t_{cycle} and t_{inj} as function of the elution flow rate applied to the chromatographic column. One can verify there is an elution flow rate where the sum of productivities reaches a maximum value. In spite of the ratio Q_{El}/t_{cycle} is monotonically increasing, the amount injected into the column (and recovered at the end of the cycle) becomes much smaller at high flow rates, causing a significant decrease on the productivity. Under lower elution flow rates, the feed load on the injection time can be larger, but the time of cycle becomes quite high resulting in reduced productivity. Fig-

ure 6 presents the concentration profile of the compounds of the quaternary mixture during the process under the following conditions: $t_{\text{cycle}} = 27.23$ min, $t_{inj} = 2.27$ min and $Q_{El} = 25.0\,\text{mL min}^{-1}$. In this scheme, while the withdrawal of the second fraction is still occurring, the column is being already loaded with a new injection of the feed solution. This strategy is used to maximize productivity by reducing cycle time of a batch process (touching band assumption).

Table 4 presents the operating parameters and performance achieved by the first SMB and the chromatographic column for the separation of *LBA* and the fraction containing the lactose, fructose and sorbitol. It should be considered the relationship between the feed load admitted, continuously, in the SMB and the one introduced discontinuously, in a cycle of the batch process, such that $Q_{\text{Fe}}^{\text{SMB1}} C_{\text{Fe}} = Q_{\text{Fe}}^{Batch} (t_{inj}/t_{\text{cycle}}) C_{\text{Fe}}$. When comparing the performance achieved by chromatography column, under optimized conditions, with the performance of the SMB operating under the best operating conditions acquired previously (conditions listed as Op.C.1), one can notice the superiority of SMB technology: (i) its productivity is five-fold that the one of the batch process; (ii) compounds from the mixture are recovered 2.4-fold more concentrated; (iii) its eluent consumption is approximately three times less to recover the *LBA* and twice to recovery the rest of the other compounds.

On the other hand, when the SMB unit operates with equal feed throughput of the chromatographic column, the best operating conditions (corresponds to the condition Op.C 2 in Table 4) lead to lower productivity than the batch system, about 1.4 times lower. This fact has also been verified by Strube et al. (1998) in assessing the separation of glucose and fructose. The numerical results showed if batch process does not operate on the principle of touching band assumption, this difference does not occur and productivities from both systems are almost the same. Despite this noticeable difference on the productivity, SMB performance with regard to the eluent consumption and degree of dilution is still higher under the operating conditions obtained with the conditions Op.C 2.

4.3.2 *Second SMB in the separation of substrate and sorbitol*

The second SMB unit should be considered (feed condition—41.3 g L^{-1} lactose, 8.8 g L^{-1} sorbitol and 6.6 g L^{-1} fructose—from the extract outlet of the SMB 1/Op.C 1). Following the previously procedure for the first SMB, when its operating conditions were established from the maximum possible pressure drop in such system, one can define the value for the switching time of 2.72 min (time retention of sorbitol—now, the more retained compound—, under the maximum possible flow rate in Sect. 1, is 2.12 min).

Figure 7a corresponds to the separation region m_2 versus m_3 (purity above 99.9% in both raffinate and extract)

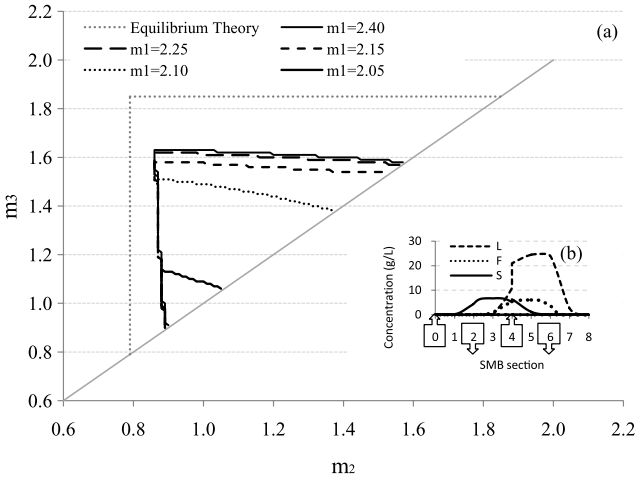

Fig. 7 Separation region m_2 versus m_3 (purity above 99.9% in both raffinate and extract), with different values of m_1 and fixed values of m_4 (= 0.31) and t^*, for the second SMB to recover the sorbitol (extract) and the fraction of fructose and lactose (raffinate). (**b**) Profile of average concentration of the compounds in SMB, at cyclic steady state, under the conditions: $(m_1; m_2; m_3; m_4) = (2.4; 0.86; 1.63; 0.31)$ with $t^* = 2.72$ min (sorbitol recovered at extract port) (Feed condition: 41.3 g L^{-1} lactose, 8.8 g L^{-1} sorbitol and 6.6 g L^{-1} fructose)

for the second SMB, with different values of m_1 and fixed values of m_4 (= 0.31) and t^*, in order to separate the fraction of fructose and lactose, collected on the raffinate, of sorbitol, which is recovered in the extract. The limitations of mass transfer of compounds between the stationary and liquid phases in this adsorptive process lead to a reduction of the triangular region given by equilibrium theory. For the switching time of 2.72 min, values of $m_{1\text{SMB2}}$ larger than 2.4 would lead to flow rates above the highest allowed given by the maximum pressure drop; while the application of values lower than 2.05 (with increment of 0.05) does not allow the recovery of compounds with purity equal to, or above, 99.9% at both outlet ports. The larger productivity for the product collected at the extract is achieved for the set of parameters ($m_{1,\text{SMB2}} = 2.4$; $m_{2,\text{SMB2}} = 0.86$; $m_{3,\text{SMB2}} = 1.63$; $m_{4,\text{SMB2}} = 0.31$) with $t^* = 2.72$ min – $Pr_{Ex,\text{SMB2}} = 18.47\ \text{g L}^{-1}$ h; $Q_{\text{Fe SMB2}}/Q_{El\ \text{SMB2}} = 0.368$ (considering the investigated range of m_4: [0.344; 0.270]). At this point, compounds in the raffinate, which will be recycled to the enzymatic reactor, are recovered at low degree of dilution (= 1.72). The performance parameters are: (i) Raffinate: $Pr = 101.37\ \text{g L}^{-1}$ h, $EC = 0.078\ \text{L g}^{-1}$, $Re = 99.9\%$, *Product dilution* = 1.72; (ii) Extract: $EC = 0.42\ \text{L g}^{-1}$; $Re = 99.3\%$, *Product dilution* = 2.02. Using the operating conditions defined by the mentioned set, the profile of average concentration of compounds in sections of the SMB, at the cyclic steady state, is shown in Fig. 7b.

Table 5 Operating conditions and performance for the separation of ternary mixture composed of *LBA*, lactose and sorbitol with the pseudo-SMB unit ($L_C = 0.384$ m, $D_C = 0.026$ m; 12 columns)

Step 1	Step 2		
Optimized variables			
t_{S1}	4.68 min	t_{S2}	87.08 min
Inlet operating parameters			
Q_{Fe}	21.37 mL min^{-1}	Q_{Ra}	13.94 mL min^{-1}
Q_{El1}	33.06 mL min^{-1}	Q_{Ex}	3.56 mL min^{-1}
		Q_{El2}	17.49 mL min^{-1}
$C_{\mathrm{Fe},LBA}$, $C_{\mathrm{Fe},L}$, $C_{\mathrm{Fe},S}$	125.4, 119.8, 63.7 g L^{-1}	Q_S	35.46 mL min^{-1}
Performance parameters			
	LBA (*Ra*)	Lactose (*Int*)	Sorbitol (*Ex*)
Purity (%)	99.9	99.0	99.0
Recovery (%)	99.9	99.6	98.2
Productivity (g L^{-1} h^{-1})	5.11	4.86	2.55
El. Consumption (L g^{-1})	0.13	0.14	0.26

4.3.3 *Pseudo-SMB for the ternary mixture from the enzymatic process*

In the case of the substrate fructose is completely consumed in the enzymatic oxidation of lactose, the resulting mixture would be composed of *LBA*, sorbitol and lactose not converted. As the levels of lactose concentration is kept high relative to fructose (leading to the highest enzyme activity), lactose is not completely consumed by the total consumption of fructose. This situation allows exploiting the potential of the pseudo-simulated moving bed technology, when one single separation unit is able to recover the components of the mixture produced in the enzymatic process. The pseudo-SMB permits the separation of three compounds in individual outlet ports, since it includes, besides raffinate and extract, an intermediate port to recover the component of intermediate affinity with the stationary phase (Ando et al. 1990; Borges da Silva and Rodrigues 2006). Hence, the pseudo-SMB unit, containing columns packed with potassium-loaded resin, can be applied to the recovery of *LBA* (in raffinate), lactose (at the intermediate outlet) and sorbitol (in extract). However, it is worth mentioning that this option would have its overall productivity (considering both reaction and separation processes) adversely affected due to the longest time of a reaction cycle linked to the complete consumption of fructose. In this study, the characteristics of the pseudo-SMB unit are: $L_C = 0.384$ m, $D_C = 0.026$ m, 12 columns; configuration: 3/3/3/3 (same packing volume used in the two SMBs in cascade before).

In this procedure, the objective function is given by the sum of productivity at each enrichment outlet, (i.e., $\sum_{i,m,n} Pr_{i,m,n};\ i,m,n = LBA,\ Ra, 2;\ L,\ Int, 1;\ S, Ex, 2$). The restriction is imposed on the values of purity of the compounds at the pseudo-SMB outlets, such that $Pu_{i,m,n} - Pu_{i,m,n}^{required} \geq 0$, where the variable $Pu_{i,m,n}^{required}$ refers to the required purity of component i at its respective enriched stream m in step n (defined as 99% for all compounds). The set of design parameters concerns: system characteristics (column diameter, column length, total number of columns and its configuration, maximum pressure drop), kinetics and equilibrium adsorption parameters, composition of feed mixture and operating conditions. The operating conditions are: (i) for the step 1: t_{S1}, Q_{El1}, Q_{Fe} ($Q_{1/2,S1}$; $Q_{3/4,S1}$); (ii) for the step 2: t_{S2}, t^*, Q_{El2}; Q_{Ex}; Q_{Ra} ($Q_{1,S2}$; $Q_{2/3,S2}$; $Q_{4,S2}$). Table 5 shows the optimized values of t_{S1} and t_{S2} considering the definition of other operating conditions. These operating conditions are determined by the design methodology described in Borges da Silva and Rodrigues (2008). The performance parameters of the 4 section pseudo-SMB to separate the ternary mixture are also listed in Table 5.

In respect to only the separation process, if the productivity of the pseudo-SMB system to separate the two products—*LBA* and sorbitol—are compared with those ones of two SMBs in cascade (already considering the total volume of adsorbent used in the two units), one can verify the latter leads to higher values (as well as other process performance parameters). Although, it should be noted that the feed throughput in SMB system was not determined under the condition of equal time average feed flow rate of the pseudo-SMB (because there is only feed flow rate during the step 1 in pseudo-SMB operation), but it was fixed as function of the ΔP_{max}. Under the application of the maximum possible pressure drop, the best arrangement to be integrated to the reactive process has been the two SMBs in a row. Nevertheless, if the decision of the best system is not based only on the productivity, conclusions could be different. One should be cautious, however, when deciding on the

best configuration for the separation of the mixture coming from the reactive process and the inclusion of a cost function is compulsory. Besides Jupke et al. (2002) has shown that it is not sure that maximizing the productivity leads to an economic process performance.

The strategy of keeping the highest value of reaction rate by the integration of a chromatographic system to allow the fractionation of the produced mixture proved to be viable when it was found stationary phases to promote the adsorptive separation of compounds and the feasibility to apply the SMB system in cascade. The advantages of simultaneous bio-reaction and separation of products/reactants can be well explored.

5 Conclusion

The process of lactose oxidation integrated to separative systems, including the SMB and pseudo-SMB technologies, was analyzed as one alternative to allow the enzymatic reactions occur at highest substrate concentrations and, as result, to improve its productivity for the formation of lactobionic acid and sorbitol. The conversion of lactose and fructose to *LBA* and sorbitol was carried out by using free and permeabilized *Z. mobilis* cells, which contains *GFOR* and *GL* enzymes. The kinetics results have shown the initial fructose concentration has no considerable effect on the initial rate of the enzymatic reaction.

In order to allow the recycle of substrate non-converted and the recovery of pure products, different arrangements of chromatographic units were investigated: batch column connected to SMB unit, SMB in cascade and pseudo-SMB (this last is applied in the case of total consumption of fructose). To integrate the enzymatic reaction to these adsorptive systems in an external looping fashion, it is convenient the first unit packed with resin in the ionic form of potassium to separate the lactobionic acid from the mixture (since pH is controlled by the addition of potassium hydroxide at the reactive process, potassium salt is formed). The adsorption kinetics and equilibrium parameters of the compounds adsorbing on calcium- and potassium-loaded resins were obtained. In general, fast adsorption kinetic and linear equilibrium isotherms (except to the *LBA*) were verified when using the *DOWEX 50W-X4* resin in both ionic forms.

As a result of this work, we have shown that two units in cascade, among the studied arrangements of chromatographic units, are suitable to perform the intended separation and, then, to promote highest values of reaction rate by the integration of this chromatographic system with the reactive system.

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References

Ando, M., Tanimura, M., Tamura, M.: Method of chromatographic separation. U.S. Patent 4,970,002 (1990)

Azevedo, D.C.S.: Separation/reaction in simulated moving bed. Ph.D. thesis, University of Porto, Porto, Portugal (2001)

Azevedo, D.C.S., Rodrigues, A.E.: Design of a simulated moving bed in the presence of mass-transfer resistances. AIChE J. **45**(5), 956–966 (1999)

Bae, Y.S., Moon, J.H., Lee, C.H.: Effects of feed concentration on the startup and performance behaviors of simulated moving bed chromatography. Ind. Eng. Chem. Res. **45**(2), 777–790 (2006)

Baminger, U., Ludwig, R., Galhaup, C., Leitner, C., Kulbe, K., Haltrich, D.: Continuous enzymatic regeneration of redox mediators used in bio-transformation reactions employing flavoproteins. J. Mol. Catal. B **11**, 541–550 (2001)

Beste, Y.A., Arlt, W.: Side-stream simulated moving-bed chromatography for multicomponent separation. Chem. Eng. Technol. **25**(10), 956–962 (2002)

Borges da Silva, E.A., Rodrigues, A.E.: Design of chromatographic multicomponent separation by a pseudo-simulated moving bed. AIChE J. **52**(11), 3794–3812 (2006)

Borges da Silva, E.A., Rodrigues, A.E.: Design methodology and performance analysis of a pseudo-simulated moving bed for ternary separation. Sep. Sci. Technol. **43**(3), 533–566 (2008)

Bowen, W.H.: Food components and caries. Adv. Dent. Res. **8**(2), 215–220 (1994)

Cen, P., Tsao, G.T.: Recent advances in the simultaneous bioreaction and product separation processes. Sep. Purif. Technol. **3**, 58–75 (1993)

Chiang, A.S.T.: Continuous chromatographic process based on SMB technology. AIChE J. **44**(8), 1930–1932 (1998)

Coté, A., Brown, W.A., Cameron, D., van Walsum, P.: Hydrolysis of lactose in whey permeate for subsequent fermentation to ethanol. J. Dairy Sci. **87**, 1608–1620 (2004)

Druliolle, H., Kokoh, K.B., Beden, B.: Selective oxidation of lactose to lactobionic acid on lead-adatoms modified platinum-electrodes in Na_2CO_3+$NaHCO_3$ buffered medium. J. Electroanal. Chem. **385**(1), 77–83 (1995)

Erzinger, G.S., Silveira, M.M., Lopes da Costa, J.P.C., Vitolo, M., Jonas, R.: Activity of glucose-fructose oxidoreductase in fresh and permeabilised cells of *Zymomonas mobilis* grown in different glucose concentrations. Braz. J. Microbiol. **34**(4), 329–333 (2003)

Fox, P.F., McSweeney, P.L.H.: Lactose. In: Fox, P.F., McSweeney, P.L.H. (eds.) Dairy Chemistry & Biochemistry, pp. 21–66. Kluwer Academic/Plenum, New York (1998)

Ganetsos, G., Barker, P.E., Ajongwen, J.N.: Batch and continuous chromatographic systems as combined bioreactors-separators. In: Ganetsos, G., Barker, P.E. (eds.) Preparative and Production Scale Chromatography. Dekker, New York (1993)

Heuer, C., Kniep, H., Falk, T., Seidel-Morgenstern, A.: Comparison of various process engineering concepts of preparative liquid chromatography. Chem. Ing. Tech. **69**(11), 1535 (1997)

Hur, J.S., Wankat, P.C.: Two-zone SMB/chromatography for center-cut separation from ternary mixtures: Linear isotherm systems. Ind. Eng. Chem. Res. **45**(4), 1426–1433 (2006)

Jupke, A., Epping, A., Schmidt-Traub, H.: Optimal design of batch and simulation moving bed chromatographic separation processes. J. Chromatogr. A **944**(1–2), 93–117 (2002)

Kawajiri, Y., Biegler, L.T.: Optimization strategies for simulated moving bed and PowerFeed processes. AIChE J. **52**(4), 1343–1350 (2006)

Kim, J.K., Wankat, P.C.: Designs of simulated-moving-bed cascades for quaternary separations. Ind. Eng. Chem. Res. **43**(4), 1071–1080 (2004)

Kokoh, K.B., Alonso-Vante, N.: Electrocatalytic oxidation of lactose on gold nanoparticle modified carbon in carbonate buffer. J. Appl. Electrochem. **36**(2), 147–151 (2006)

Kurup, A.S., Hidajat, K., Ray, A.K.: Optimal operation of a pseudo-SMB process for ternary separation under non-ideal conditions. Sep. Purif. Technol. **51**(3), 387–403 (2006)

Maliska, C.R.: Transferência de Calor e Mecânica dos Fluidos Computacional. LTC—Livros Técnicos e Científicos S.A., Rio de Janeiro (1995)

Malvessi, E., Concatto, K., Silveira, M.M.: Biotransformação de frutose e lactose em sorbitol e ácido lactobiônico por células de *Zymomonas mobilis*. In: Proceeding of 5° Brazillian seminary of Enzymatic Technology—Enzitec, 2002, Brasília (DF), Brasil (2002)

Malvessi, E., Concatto, K., Carra, S., Silveira, M.M.: Formulation of medium for growth and production of ethanol and intracellular enzymes by *Zymomonas mobilis*. Braz. Arch. Biol. Technol. **49**, 139–144 (2006)

Marwaha, S.S., Kennedy, J.F.: Whey-pollution problem and potential utilization. Int. J. Food Sci. Technol. **23**(4), 323–336 (1988)

Mata, V.G., Rodrigues, A.E.: Separation of ternary mixtures by pseudo-simulated moving bed chromatography. J. Chromatogr. A **939**(1–2), 23–40 (2001)

Mihlbachler, K., Fricke, J., Yun, T., Seidel-Morgenstern, A., Schmidt-Traub, H., Guiochon, G.: Effect of the homogeneity of the column set on the performance of a simulated moving bed unit. I. Theory. J. Chromatogr. A **908**(1–2), 49–70 (2001)

Murakami, H., Kawano, J., Yoshizumi, H., Nakano, H., Kitahata, S.: Screening of lactobionic acid producing microorganisms. J. Appl. Glycosci. **49**, 469–477 (2002)

Navarro, A., Caruel, H., Rigal, L., Phemius, P.: Continuous chromatographic separation process: Simulated moving bed allowing simultaneous withdrawal of three fractions. J. Chromatogr. A **770**(1–2), 39–50 (1997)

Nordkvist, M., Nielsen, P.M., Villadsen, J.: Oxidation of lactose to lactobionic acid by a *Microdochium nivale* carbohydrate oxidase: Kinetics and operational stability. Biotechnol. Bioeng. **97**(4), 694–707 (2007)

Pais, L.S., Loureiro, J.M., Rodrigues, A.E.: Modeling strategies for enantiomers separation by SMB chromatography. AIChE J. **44**(3), 561–569 (1998)

Pedruzzi, I., Malvessi, E., Mata, V.G., Silva, E.A.B., Silveira, M.M., Rodrigues, A.E.: Quantification of lactobionic acid and sorbitol from enzymatic reaction of fructose and lactose by high-performance liquid chromatography. J. Chromatogr. A **1145**(1–2), 128–132 (2007)

Pedruzzi, I., da Silva, E.A.B., Rodrigues, A.E.: Selection of resins equilibrium and sorption kinetics of lactobionic acid, fructose, lactose and sorbitol. Sep. Purif. Technol. **63**(3), 600–611 (2008)

Ruthven, D.M., Ching, C.B.: Counter current and simulated counter-current adsorption separation processes. Chem. Eng. Sci. **44**(5), 1011–1038 (1989)

Satory, M., Furlinger, M., Haltrich, D., Kulbe, K.D., Pittner, F., Nidetzky, B.: Continuous enzymatic production of lactobionic acid using glucose-fructose oxidoreductase in an ultrafiltration membrane reactor. Biotechnol. Lett. **19**(12), 1205–1208 (1997)

Sayama, K., Kamada, T., Oikawa, S., Masuda, T.: Production of raffinose—a new by-product of the beet sugar-industry. Zucker-Ind. **117**(11), 893–898 (1992)

Silveira, M., Wisbeck, E., Lemmel, C., Erzinger, G., Lopes da Costa, J., Bertasso, M., Jonas, R.: Bioconversion of glucose and fructose to sorbitol and gluconic acid by untreated cells of *Zymomonas mobilis*. J. Biotechnol. **75**, 99–103 (1999)

Storti, G., Mazzotti, M., Morbidelli, M., Carra, S.: Robust design of binary counter-current adsorption separation processes. AIChE J. **39**(3), 471–492 (1993)

Strube, J., Haumreisser, S., Schmidt-Traub, H., Schulte, M., Ditz, R.: Comparison of batch elution and continuous simulated moving bed chromatography. Org. Process Res. Dev. **2**(5), 305–319 (1998)

Sumimoto, R., Kamada, N.: Lactobionate as the most important component in UW solution for liver preservation. Transplant. Proc. **22**(5), 2198–2199 (1990)

Wang, X., Ching, C.B.: Chiral separation of beta-blocker drug (nadolol) by five-zone simulated moving bed chromatography. Chem. Eng. Sci. **60**(5), 1337–1347 (2005)

Wankat, P.C.: Simulated moving bed cascades for ternary separations. Ind. Eng. Chem. Res. **40**(26), 6185–6193 (2001)

Wooley, R., Ma, Z., Wang, N.H.L.: A nine-zone simulating moving bed for the recovery of glucose and xylose from biomass hydrolyzate. Ind. Eng. Chem. Res. **37**(9), 3699–3709 (1998)