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Modeling fixed bed bioreactors for isopropanol and butanol production using *Clostridium beijerinckii* DSM 6423 immobilized on polyurethane foams

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ABSTRACT

Isopropanol-butanol fermentation carried out by *C. beijerinckii* can be improved by immobilizing cells in a fixed bed bioreactor. In this study, a fixed bed bioreactor was developed using polyurethane foams as a solid support for biofilm growth. The dilution rate was increased over time to follow biofilm development while avoiding solvent accumulation within the bioreactor. A phenomenological model was designed and successfully described batch and continuous fixed bed bioreactor dynamics. The parameters regarding biofilm detachment and attachment were estimated using a bioreactor design allowing biofilm sampling during a continuous fermentation. This last experiment was operated during 550 h and reached a maximum productivity of 1.5 $g.L^{-1}.h^{-1}$. The model prediction capacity was tested by describing a continuous fermentation in a fixed bed bioreactor column over 800 h. This latter bioreactor enabled a maximum productivity of 2.5 $g.L^{-1}$ h^{-1} in 650 h of continuous fermentation. A high solvent concentration (13.5 $g.L^{-1}$) with a butanol concentration close to the toxicity threshold (7.5 $g.L^{-1}$) was obtained during the fermentation process. The process enhanced the isopropanol butanol fermentation productivity. The model properly described the batch fermentation and the immobilized cells fermentation. However, a 20% difference between experimental and modeled data was observed. Therefore, the model should be improved to better described such fermentation systems.

1. Introduction

The acetone butanol ethanol (ABE) fermentation, mostly carried out by the strain *Clostridium acetobutylicum*, is a well-known bioprocess that has been used on an industrial scale to produce chemicals from renewable feedstock. However, because it could not compete with the petrochemicals process, this technology was abandoned in most countries after 1960 [1]. One of the major drawbacks of this technology is the production of acetone, which is less valuable than butanol for fuel applications because of its corrosiveness towards rubber in engines [2,3]. To solve this issue, other solventogenic strains, such as *Clostridium beijerinckii*, can be employed. This strain contains a NADPH-dependent secondary alcohol hydrogenase (s-ADH-2) which catalyzes the reaction from acetone to isopropanol [4].

Another major drawback is the inhibition by the butanol. Due to its accumulation during batch processes, bacterial growth is rapidly inhibited and both final solvent concentrations and batch volumetric productivity are low as shown in Table 1. To avoid this inhibition, continuous fermentation can be carried out to remove solvents from the fermentation broth. Nevertheless, the strain has a low growth rate when it produces solvents [7], leading to cell washout at high dilution rates. As shown in the Table 1, continuous fermentation with immobilized cells can be advantageously used to decorrelate immobilized cells residence time from hydraulic residence time. Those systems can therefore be operated at dilution rate higher than the maximal growth rate of the strain and reach higher productivities than classic continuous isopropanol-butanol fermentation.

Bacteria which are adhered to the support can form a structure called a biofilm that is more resistant to environmental conditions than suspended cells [8]. Bacterial adsorption increases the active cell concentration in the reactor and a higher volumetric productivity of up to 5 $g.L^{-1}.h^{-1}$ could be obtained [9]. Specific biofilm bioreactors designs such as fluidized bed bioreactors [10] or fixed bed bioreactors [11,12] have been employed in the literature for acetone and butanol production. A fixed bed bioreactor was selected for this study because it enables a high volume of solid support to be employed in the bioreactor [13] and the best-known productivities for ABE fermentation to be obtained (15.8 $g.L^{-1}.h^{-1}$) [12].

Solid supports must be carefully selected when developing a fixed

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Nomenclature

X	biomass concentration ($g.L^{-1}$)
Xs	suspended biomass concentration ($g.L^{-1}$)
X_b	immobilized biomass concentration ($g.L^{-1}$)
S	residual glucose concentration ($g.L^{-1}$)
S_f	glucose concentration in feed bottle ($g.L^{-1}$)
B	butanol concentration ($g.L^{-1}$)
I	isopropanol concentration ($g.L^{-1}$)
AA	acetate concentration ($g.L^{-1}$)
AA_f	acetate concentration in feed bottle ($g.L^{-1}$)
AB	butyrate concentration ($g.L^{-1}$)
B_{max}	threshold for butanol toxicity ($g.L^{-1}$)
μ_{max}	maximum growth rate (h^{-1})
k_s	saturation constant for glucose ($g.L^{-1}$)
α	growth associated production of acetate (h^{-1})
β	growth associated production of butyrate (h^{-1})
kAA	saturation constant for acetate ($g.L^{-1}$)
kAB	saturation constant for butyrate ($g.L^{-1}$)
Y_{xs}	biomass to substrate yield ($g.g^{-1}$)
Y_{but_s}	butanol to substrate yield ($g.g^{-1}$)
Y_{i_s}	isopropanol to substrate yield ($g.g^{-1}$)
$Y_{I/AA}$	acetate to isopropanol yield ($g.g^{-1}$)
$Y_{B/AB}$	butyrate to butanol yield ($g.g^{-1}$)
k_d	biofilm detachment constant (h^{-1})
k_a	attachment constant (h^{-1})
D	dilution rate (h^{-1})
t	time (h)
PB	batch productivity ($g.L^{-1}.h^{-1}$)
Pc	continuous process productivity ($g.L^{-1}.h^{-1}$)

Table 1
Yield, maximum solvent concentration, and maximal productivity obtained during isopropanol butanol fermentation with C. beijerinckii DSM6423 under different configuration using glucose as carbon source.

	Yields (total solvents/ Glucose)	Maximum solvent concentration ($g.L^{-1}$)	Maximal productivity ($g.L^{-1}.h^{-1}$)	Advantages	Disadvantages	Sources
Batch fermentation	0.34	7.0	0.15	Traceability	Low productivity	[6]
Continuous fermentation	0.29	5.2 (D =0.09 h^{-1})	0.47	Lower expenditure cost	Low total solvent concentration	[5]
Continuous fixed bed bioreactor	0.29	10.9 (D = 0.4 h^{-1})	5.5	High productivity	Blockage risks	[5]

bed bioreactor. The physico-chemical properties of the solid support such as roughness and surface charge play indeed an important role in initial biomass adsorption to the solid support, and can influence the biofilm formation kinetics [14]. To promote the adhesion of *Clostridium acetobutylicum* on linen, Zhuang et al. showed that high hydrophobicity and the increase of positive surface charge of linen improved batch productivity [14]. Moreover, the selected supports should favor bacterial adsorption, have a large specific surface area, be resistant to the process condition, be reusable and affordable [15].

Fixed bed bioreactor processes have been carried out on a laboratory scale to produce solvents by fermentation using a wide variety of supports such as natural wood pulp [16], charcoal [17] or brick [12]. Nonetheless, the solid support colonization rate by cells may vary depending on the chemical characteristics of the solid and the experimental conditions [18]. Polyurethane foams have wide surface area available for biofilm development, open porosity, are not toxic for bacteria and have already been used in fixed bed biofilm bioreactor [19]. In this study, this last material is therefore used as an immobilization support for fixed bed isopropanol butanol fermentation. Because the biofilm growth rate is low in fixed bed bioreactors, start-ups can be time consuming and difficult to operate. Quick biofilm formation is crucial to the industrial scale development of fixed bed biofilm bioreactors because it enables maximum system productivity to be obtained in a limited amount of time [21].

To better operate fixed bed bioreactors and understand biofilm growth, kinetic model could be employed to simulate the process. Those types of models are already used to describe ABE fermentation. Unstructured models are preferred for process design [22–24], while structured models are used to better understand metabolism networks in *Clostridium acetobutylicum* [25,26]. Although extensive work has been carried out on those models, only a few describe isopropanol-butanol fermentations [27–29]. Moreover, kinetic models for continuous biofilm fermentation are often based on steady state experiments and do not take biomass accumulation and biofilm maturity into account [29,30]. Practically, in continuous immobilized fermentation, a steady state is difficult to achieve. Even though biofilm growth is low, the immobilized biomass concentration increases over time and a steady state assumption may not be always correct for fixed bed bioreactor cells processes.

The proposal is to enhance the dilution rate stepwise in order to develop a new start-up strategy which follows the development of biofilm on those solids. An unstructured model is developed to describe the fermentation kinetics during batch processing of isopropanol butanol fermentation and to identify the kinetic parameters of the strain. Two additional parameters related to biomass attachment and detachment from the solid support are added to describe the continuous immobilized fermentation. This model is then used to predict the performance of a packed bed column bioreactor operated with a more complex dilution rate management.

2. Materials and methods

2.1. Microorganism and inoculum preparation

Spores of the natural isopropanol producer *Clostridium beijerinckii* DSM6423 were stored in cryotubes in 20% v/v of glycerol solution at − 80 °C. The spores were heat shocked in boiling water for one minute and cooled over ice for three minutes to destroy vegetative cells and activate the spores. A volume of 250 μL of spore suspension was transferred into 10 mL of CALAM media (250 $g.L^{-1}$ boiled and milled potatoes, 2 $g.L^{-1}$ $CaCO_3$, 2 $g.L^{-1}$ NH_4SO_4, 10 $g.L^{-1}$ glucose; [31]) previously sterilized at 121 °C for 20 min. The CALAM medium containing the spores was incubated at 34 °C in an anaerobic chamber (Bactron, Cornelius, OR, USA) for 24 h. After incubation, the CALAM medium was transferred into 150 mL flasks filled with 90 mL of sterile and anoxic Gapes medium (1.0 $g.L^{-1}$ $MgSO_4.7\ H_20$, 1.0 $g.L^{-1}$ KH_2PO_4, 0.6 $g.L^{-1}$ K_2HPO_4, 2.9 $g.L^{-1}$ CH_3COONH_4, 0.0066 $g.L^{-1}$ $FeSO_4$, 0.1 $g.L^{-1}$ p-aminobenzoic acid, 2.5 $g.L^{-1}$ yeast extract, 60 $g.L^{-1}$ glucose; [32]). The suspension was incubated at 34 °C for 20 h in an anaerobic chamber. The final inoculation volume was 10% v/v of the final bioreactor volume.

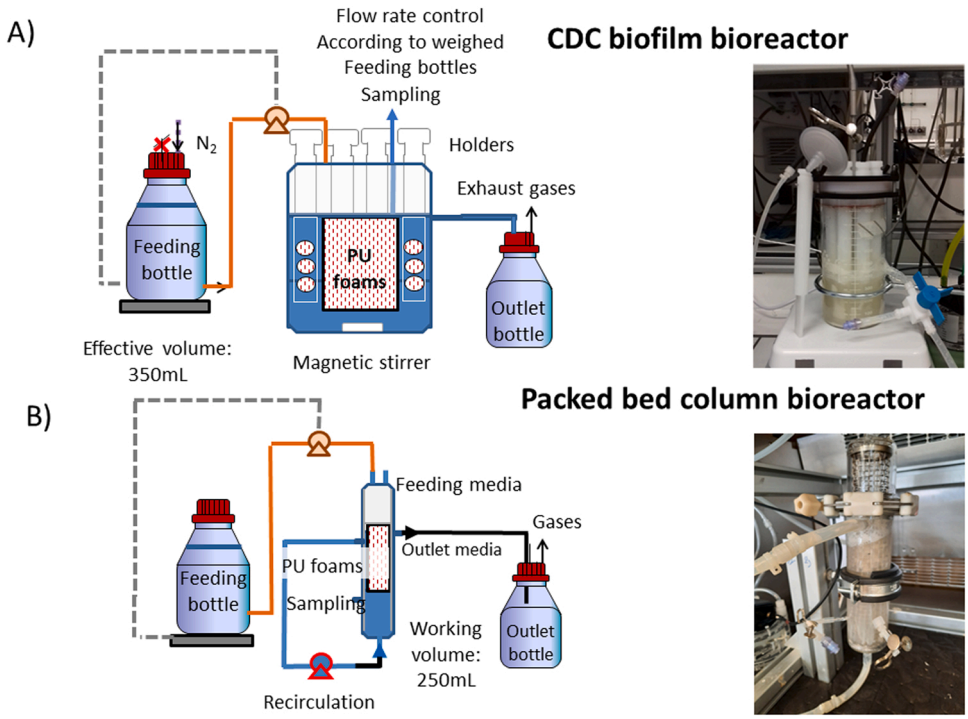

Fig. 1. Schemes and pictures of A) CDC biofilm bioreactor used for identification of attachment and detachment parameters B) packed bed column bioreactor with recycling flow used for continuous biofilm fermentation.

2.2. Batch culture

A Biostat Q bioreactor (Sartorius, Aubagne, France) is a stirred tank bioreactor with a 500 mL total volume and was used for the batch fermentations. The empty bioreactor was sterilized at 121 °C for 20 min. A volume of 405 mL of Gapes medium was sterilized by filtration on a Nalgene membrane filter (ThermoScientific, Walthamn, MS, USA) and transferred into the bioreactor. The temperature control was set to 34 °C using a water-jacket controlled by a thermostat. The agitation was set to 60 rpm and the medium was sparged overnight with oxygen-free nitrogen through a 0.2 μm diameter pore filter at 0.5 vvm before inoculation, to ensure anoxic conditions. Finally, a volume of 45 mL of preculture was transferred into the bioreactor (10% v/v).

2.3. Continuous fermentation

- Solid support
  ISP4452 polyurethane foams (Industrie Schaum Produkte, Limbourg, Germany) with 40 ppi (pores per inch), a density of 40 kg.m^{-3} and dimensions of 20 mm × 20 mm x 7 mm were used as the immobilization support. The effective mass of solid support per volume of bioreactor was the same for all the fermentation systems developed in the present study (7 g.L^{-1}). In the developed fermentation systems, biomass initial adhesion to the polyurethane foams was ensured by adsorption.
- Stirred tank biofilm bioreactor
  A CDC biofilm reactor CBR 90–3 (Biosurface Technologies, Bozeman, MT, USA) is a continuously stirred tank bioreactor with 350 mL of effective volume. The bioreactor has a headplate equipped with eight sampling rods called "holders" which can each contain three coupons of solid support. Those holders can be removed along experiment time to analyze biofilm grown on the coupons. Coupon analysis was carried out by removing the inlet and outlet tubing under sterile conditions and removing the holders within the anaerobic chamber. The CDC biofilm bioreactor headplate is equipped with inlet tubes for feed alimentation and liquid sampling as well as a gas exhaust. A schematic presentation of the bioreactor is presented in Fig. 1-A.
- Packed bed column with recycling flow
  A packed bed bioreactor is a column with an internal diameter of 4.3 cm and height of 20 cm with a working volume of 200 mL and a total volume of 315 mL. A metallic grid is used to keep all the polyurethane foams within the fermentation medium. The column head is designed with a gas exhaust equipped with a sterile 0.2 μm pore diameter filter. A schematic presentation of the packed bed column bioreactor is presented in Fig. 1-B.
- Bioreactor start-up

The polyurethane foam concentration regarding the effective volume of the bioreactors was 7 g.L^{-1} for both of the described systems. Solid supports were placed in the bioreactors, and the empty systems were sterilized at 121 °C for 20 min. The liquid GAPES medium was sterilized by filtration and was transferred into the bioreactors before being reduced. The glucose concentration of the GAPES medium in the feed bottle is presented in the Table 1. The bioreactors were then placed in Multitron cell® incubators (Infors HT, Bottmingen, Switzerland) at 34 °C. Inoculation was carried out from fresh preculture, and was 10% v/v of the final bioreactor volume. Agitation or recirculation was started and a batch incubation of 8 h was carried out before the continuous flow rate was started up. Continuous flow rate enhancement was ensured using the software for the Scilog Chemtec peristaltic pump (Parker Hannifin, Cleveland, OH, USA). The outlet flow rates of both systems were controlled by overflow.

The masses and types of the polyurethane foam, the volume of liquid media in each bioreactor and the method for reducing the media in both systems are presented in Table 1. This table provides information about the type of agitation as well as dilution rate management for both immobilized cells experiments.

2.4. Bacterial dry mass

To measure the dry mass of the biofilm, prior to fermentation, each

polyurethane foam disk, was placed in individual aluminum plates in an oven at 105 °C overnight and weighed after cooling. After biofilm sampling, the polyurethane foam disks, colonized with biofilm, were placed in the same pre-weighed aluminum plates and dried at 105 °C overnight. The biofilm was weighed after cooling. The dry mass of the immobilized cells was calculated by subtracting the weight of the polyurethane foam before fermentation from the weight of the colonized polyurethane foam after fermentation. The overall biofilm concentration was measured by multiplying the mass per gram of foam by the total foam mass immobilized in the bioreactor. The dry mass was the mean of at least three replicates.

To measure the suspended bacteria dry mass, a volume of 5 mL of suspended cells was filtered on the pre-weighed filters and washed with saline solution (NaCl 9 $g.L^{-1}$).The suspended cellular biomass was obtained by following the same drying protocol as for the biofilm mass. The biomass concentration was the cell mass weighed on the filter divided by the volume of the filtered cell suspension ($g.L^{-1}$).

2.5. Optical density and pH measurements

After sampling, the optical density at 600 nm was measured using a mini 1240 UV spectrophotometer (Shimadzu, Kyoto, Japan). The optical density measured at 600 nm was calibrated with a dry mass of suspended cells taken at five different times during the batch fermentation. One unit of optical density corresponds to 0.52 $g_{cells}.L^{-1}$. The pH was measured with a Mettler Toledo pH-meter.

2.6. Glucose and metabolites concentration

Before analysis, the samples were centrifugated at 5000 rpm for ten minutes. The supernatant was filtered on a 0.2 μm pore diameter membrane and diluted 20-fold in an aqueous solution containing 0.5 $g.L^{-1}$ of 1-propanol which was used as an internal standard.

Residual glucose was analyzed by high performance liquid chromatography (HPLC) on an Ultimate 3000 Series (Thermo Fischer Scientific, Waltham, MS, USA). A volume of 20 μL of the sample was injected into a MetaCarb 87 P column (Agilent, Santa Clara, CA, USA) heated to 80 °C. Water was used as the mobile phase, at a flow rate of 0.4 $mL.min^{-1}$. The analytes were detected using a Varian 350 RI refractive index analyzer. A Micro-Guard De-Ashing Refill Cartridges pre-column (Bio-Rad, Hercules, CA, USA) was added to the chromatographic system for desalting of the samples.

Acetic and butyric acids were analyzed using a CC20A HPLC (Shimadzu, Kyoto, Japan). A volume of 10 μL of the sample was injected into a HPX-87 H column (Bio-Rad, Hercules, CA, USA) heated to 60 °C and eluted with a 0.01 N sulfuric acid solution at a flow rate of 0.6 $mL.min^{-1}$. The HPLC was equipped with a Waters 2487 dual λ UV detector set at 220 nm.

Isopropanol, butanol, acetone and ethanol were analyzed using gas chromatography (Porabond-Q column from Agilent Technologies, 25 m length, 0.32 internal diameter, 5 μm film thickness coupled to a flame ionization detector). Helium was used as a carrier gas at a flow rate of 1.6 $mL.min^{-1}$. The column was gradually heated from 50 °C to 250 °C during a 30 min run.

3. Numerical simulation parameter identification

The computational work was carried out using a computer with an Intel ® Core™ i5–6300 u processor at 25 GHz with 8.0 Gb of RAM. The ordinary differential equations (ODE) were solved using Python software with the Spyder[5] distribution. A Levenberg-Marquard algorithm was computed to find the best set of parameters to minimize the square of the residuals using the optimized package available in Python [33, 34].

The code developed to solve the equations and to find the optimal parameters is available in Supplementary Appendix 1. Goodness of fit was measured using a reduced χ^2 (shown in Eq. (1)), an indicator which describes the sum of the differences between observed and simulated data divided by the variance of the analysis at each point.

$$\chi^2 = \sum_{i=1}^{N} \frac{(y_i - \widehat{y}(x_i, \theta))^2}{\sigma_i^2} \tag{1}$$

If the computed indicator is close to one, the model describes the experimental data well, while if the value of $\chi^2 >> 1$ or $\chi^2 << 1$, the model does not describe the experimental data correctly.

The confidence interval for the estimated parameters was calculated by computing the jacobian matrix with the identified set of p, parameters for n experimental observations, as shown in Eq. (2). The variance-covariance matrix was then computed, as shown in Eqs. (3) and (4).

$$J = \begin{pmatrix} \frac{\partial f(x,\theta)_{t1}}{\partial \theta_1} & \cdots & \frac{\partial f(x,\theta)_{t1}}{\partial \theta_p} \\ \vdots & \ddots & \vdots \\ \frac{\partial f(x,\theta)_{tn}}{\partial \theta_1} & \cdots & \frac{\partial f(x,\theta)_{tn}}{\partial \theta_p} \end{pmatrix} \tag{2}$$

$$\widehat{\sigma}^2 = \frac{\sum_0^n (f(x,\theta) - f(x,\widehat{\theta}))^2}{n-p} \tag{3}$$

$$(J^T.J)^{-1}.\widehat{\sigma}^2 = \begin{bmatrix} Var(\theta_1) & \cdots & Cov(\theta_1,\theta_p) \\ \vdots & \ddots & \vdots \\ Cov(\theta_p,\theta_1) & \cdots & Var(\theta_p) \end{bmatrix} \tag{4}$$

Finally, the confidence interval for each parameter was computed by multiplying the value for the estimated parameter by the Student quantile with n-p degrees of freedom, as shown in Eq. (5). This latter value was multiplied by the square root of the variance parameter to obtain the confidence interval for the identified parameter.

$$IC_{95\%} = \theta_i \pm t_{0.975,\ (n-p)\ \ dof} \cdot \sqrt{Var(\theta_i)} \tag{5}$$

4. Model development

4.1. Model objectives and assumptions

The model developed for the fixed bed bioreactor has several objectives. Firstly, it is intended to be used as a tool to understand which parameters influence fixed bed bioreactor start-up for isopropanol butanol fermentation. Secondly, the model's goal is to describe the process performances and total solvent concentrations during fermentation. Finally, the model can be used for fixed bed bioreactor design and operation.

A kinetic model of isopropanol-butanol batch fermentation using glucose as the carbon source and based on previous existing models has been developed. The equation describing biomass growth rate on glucose was established from a modified Monod law [35]. The effect of butanol toxicity was modeled according to Ghose & Thyagi's equation [36]. The kinetic rates for butanol and isopropanol production are based on Luedeking and Piret's equations [37]. The main assumptions for the batch and fixed bed bioreactor models are as follows:

1) The bioreactors are perfectly mixed.
2) Butanol is the main product inhibitor produced by the studied strain.
3) Glucose is the only limiting carbon source used in the experiments.
4) Biofilm cells have the same molecular composition as suspended cells.
5) The fixed bed bioreactor system does not reach a steady state due to continuous biofilm growth.

Diffusion phenomena may occur when the biofilm develops within the polyurethane foams used in this study. However, the biofilm

thickness may vary in the polyurethane foams as the biofilm grows during the continuous fermentation. The model's goal is to estimate solvent production, glucose consumption and biomass growth during continuous fixed bed biofilm bioreactor. Therefore, a simplifying assumption to describe the system using a kinetic model is to represent the biomass as a homogeneous compartment trapped on a solid support without taking diffusion into account.

Kinetic parameters regarding biomass growth, metabolite uptakes and productions were initially identified during batch fermentations. Kinetic rates for cell attachment and detachment were identified using the fixed bed bioreactor process experimental data. Finally, all the identified parameters were used to simulate the packed bed column bioreactor with recycling flow process.

4.2. Batch fermentation

Mass balance analysis was carried out to model the fermentation process. Biomass growth can be described by Eq. (6) below:

$$\mu = \mu_{max}\left(\frac{S}{k_s + S}\right).\left(1 - \left(\frac{B}{B_{max}}\right)\right) \tag{6}$$

$$\frac{dX}{dt} = \mu.X \tag{7}$$

Glucose is the limiting carbon source of the medium and its uptake is correlated to bacterial growth, as shown in Eq. (8) below.

$$\frac{dS}{dt} = \frac{-\mu.X}{Y_{xs}} \tag{8}$$

Butanol and isopropanol are produced during bacterial growth and are produced with the kinetic rates described in Eqs. (9) and (10).

$$\frac{dB}{dt} = \mu.X.\left(\frac{Y_{but_s}}{Y_{x_s}}\right) \tag{9}$$

$$\frac{dI}{dt} = \mu.X.\left(\frac{Y_{i_s}}{Y_{x_s}}\right) \tag{10}$$

Acid production and consumption were modeled using equations developed by Luedeking and Piret [37]. Production is associated with biomass growth. Uptake rates were represented as being dependent on the concentration of acetate and butyrate available in the medium and butanol and isopropanol production rates, as previously described in the model developed in reference [28]. The parameters $Y_{I/AA}$ and $Y_{B/AB}$ represent the stoichiometric conversion of acetate into isopropanol and butyrate into butanol, respectively. Acetate and butyrate mass balances are presented in the Eqs. (11) and (12). The first term of the Eqs. (11) and (12) represent the carboxylic acid production associated with the growth phase of the bacteria during batch fermentation. The second term of the equation describes the carboxylic acid consumption to produce solvent during the solventogenic production phase. In the presented equations, the acid consumption rate is linked to the solvent production rate as both acetate and butyrate are consumed by the bacteria to produce isopropanol and butanol respectively.

$$\frac{dAA}{dt} = \alpha.\mu.X - \frac{dI}{dt}.\frac{\left(\frac{AA}{AA+k_{AA}}\right)}{Y_{I/AA}} \tag{11}$$

$$\frac{dAB}{dt} = \beta.\mu.X - \frac{dB}{dt}.\frac{\left(\frac{AB}{AB+k_{AB}}\right)}{Y_{B/AB}} \tag{12}$$

Batch process productivity (P_b), shown in Eq. (13), was calculated by dividing the final total solvent concentration by the total fermentation period (t_f).

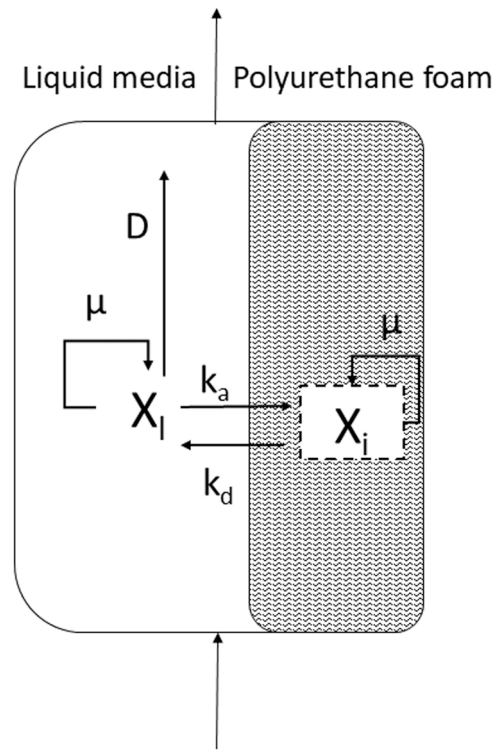

Fig. 2. Scheme for biomass mass balance for fixed bed bioreactor.

$$P_b = \frac{(B+I)}{t_f} \tag{13}$$

4.3. Continuous biofilm fermentation

The dilution rate (D, expressed in h^{-1}) was calculated by dividing the volumetric rate (F expressed in $L.h^{-1}$) by the effective volume of the bioreactor (V in L) used for the fermentation experiments.

$$D = \frac{F}{V} \tag{14}$$

For the experiment carried out with the CDC biofilm bioreactor, the dilution rate at all times was simulated by computing a linear regression with the data obtained in order to find the best fit for the parameters in accordance with Eq. (15) below. The regression coefficient found was 0.99 and the parameters are presented in Table 1.

$$D(t) = a.t + b. \tag{15}$$

For the packed bed column bioreactor, the dilution rate was increased continuously during the experiment according to the measured total solvent concentration at the bioreactor outlet. This strategy is detailed in Table 1. The dilution rate operation was thus more complex to predict and was estimated at all times with the following Eq. (16). The determination coefficient for the regression was 0.97 and the values for the parameters are presented in Table 1.

$$D(t) = c.t + d.t^2 + e.t^3 + f.t^4 \tag{16}$$

The total solvent productivity in continuous fermentation systems (P_c) was calculated at all times by multiplying the total solvent concentration by the dilution rate, as shown in Eq. (17).

$$P_c = D.(B+I) \tag{17}$$

Biomass attachment and release phenomena were considered when drawing up the mass balance, based on previously developed model structures for fixed bed biofilm bioreactors [38]. On the one hand, the

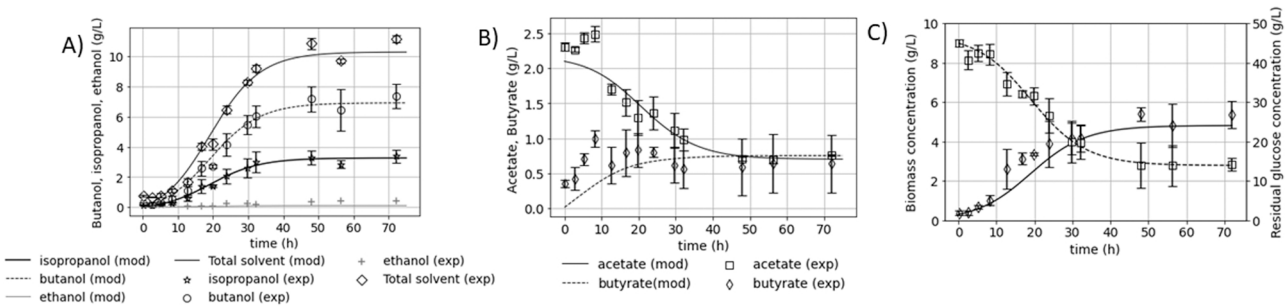

Fig. 3. Parameter estimation set for IBE batch process against time with 45 g.L^{-1} initial glucose concentration of A) experimental and simulated isopropanol (open stars and solid line), butanol (open circles and dashed line) and total solvent (open diamonds and solid line) concentration; B) experimental and simulated acetate (open squares and solid line) and butyrate (open diamonds and dashed line) concentration; and C) biomass (open squares and solid line) and residual glucose (open diamonds and dashed line) concentrations (g.L^{-1}) modeled data (mod), experimental data(exp).

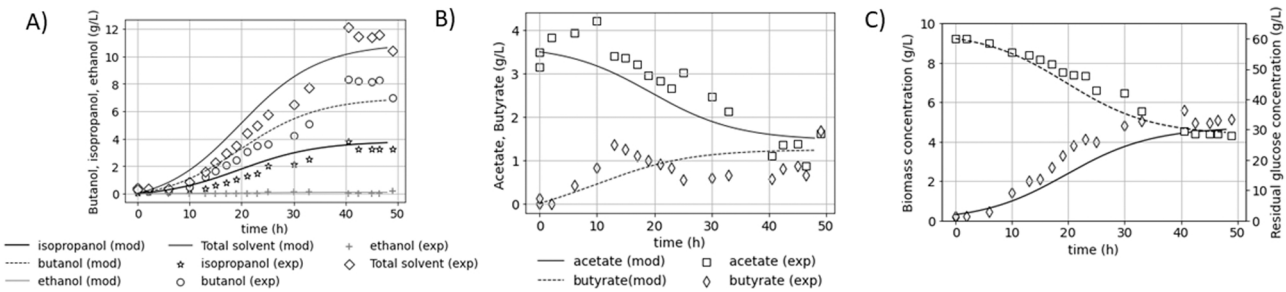

Fig. 4. Validation of parameter set for IBE batch process against time with 60 g.L^{-1} initial glucose concentration of A) experimental and simulated isopropanol (open stars and solid line), butanol (open circles and dashed line) and total solvent (open diamonds and solid line) concentration; B) experimental and simulated acetate (open squares and solid line) and butyrate (open diamonds and dashed line) concentration; and C) biomass (open squares and solid line) and residual glucose (open diamonds and dashed line) concentrations (g.L^{-1}) modeled data (mod), experimental data(exp).

initial immobilized biomass was set to zero and cells adsorb onto the porous support at a rate, k_a, assumed constant, which is proportional to the suspended biomass concentration.

$$\frac{dX_b}{dt} = \underbrace{\mu \cdot X_b}_{bio\ film\ growth} - \underbrace{k_d \cdot X_b}_{bio\ film\ detachment} + \underbrace{k_a \cdot X_s}_{suspended\ cells\ attachment} \tag{18}$$

On the other hand, biomass detachment occurs at the biofilm surface and in the present system was modeled by a first order kinetic law proportional to the immobilized biomass concentration. Only suspended biomass is washed out of the bioreactor during the entire process. A scheme for the biomass fluxes is presented in Fig. 2.

$$\frac{dX_s}{dt} = \mu.X_s + k_d.X_b - k_a.X_s - D.X_s \tag{19}$$

The specific glucose uptake rate follows the same kinetics for both immobilized and suspended biomass. Butanol, isopropanol, acetate, and butyrate specific production rates are considered to be the same for immobilized and suspended biomass. The mass balance equations for those compounds are presented in Eqs. (20)–(24).

$$\frac{dS}{dt} = \frac{-\mu.(X_b + X_s)}{Y_{xs}} + D.(S_f - S) \tag{20}$$

$$\frac{dB}{dt} = \mu.(X_b + X_s).\left(\frac{Y_{but_s}}{Y_{x_s}}\right) - D.B \tag{21}$$

$$\frac{dI}{dt} = \mu.(X_b + X_s).\left(\frac{Y_{i_s}}{Y_{x_s}}\right) - D.I \tag{22}$$

$$\frac{dAA}{dt} = \alpha.\mu.(X_b + X_s). - \frac{dI}{dt}.(X_b + X_s).\frac{\left(\frac{AA}{AA+k_{AA}}\right)}{Y_{I/AA}} + D.(AA_f - AA) \tag{23}$$

$$\frac{dAB}{dt} = \beta.\mu.(X_b + X_s) - \frac{dB}{dt}.(X_b + X_s).\frac{\left(\frac{AB}{AB+k_{AB}}\right)}{Y_{B/AB}} - D.(AB) \tag{24}$$

5. Results

Experimental results obtained from batch cultures presented in Fig. 3 were first used to determine kinetic parameters of the model. The identified parameters were then validated on another batch dataset, presented on the Fig. 4, to verify the model accuracy. Two parameters were then added to the biomass mass balance to describe dynamic cell attachment to the support and biofilm detachment from the support. Those latter parameters were identified by means of experiments carried out in the continuous biofilm bioreactor. The latter model was finally used to predict the kinetics of a continuous immobilized cell culture in a packed bed column with flow recirculation.

5.1. Batch process

The final isopropanol and butanol concentrations, shown on Fig. 3-A, were 3.4 ± 0.4 g.L^{-1} and 7.4 ± 0.8 g.L^{-1} respectively after 72 h of fermentation when the initial glucose concentration was 45 g.L^{-1}. The total solvent yield obtained with glucose as carbon source during the batch fermentation was 0.35 ± 0.04 g.g^{-1}. The data in Fig. 3-B show that fermentation begins with butyrate production. The concentration of this latter compound was increased from 0.35 ± 0.06 g.L^{-1} to 1.0 ± 0.24 g.L^{-1} during the first ten hours of the batch process. Both acetate and butyrate were then consumed from glucose to produce butanol and isopropanol. The total consumption of acetate and butyrate was 1.55 ± 0.45 g.L^{-1} and 0.36 ± 0.1 g.L^{-1} respectively for the total experimental time. The total glucose consumption is shown in Fig. 3-C and reached 31.4 ± 2.1 g.L^{-1}. Finally, bacterial growth stopped after

Table 2
Summary of start-up conditions for both continuously stirred tank biofilm reactor and packed bed column with recirculation.

System parameters	Continuously stirred tank biofilm reactor	Packed bed column
Polyurethane foam	24 disk coupons (12.7 mm × 3.8 mm) disposed in holders 2.5 g of polyurethane foams placed in liquid media (20 mm x 20 mm x 7)	1.5 g of polyurethane foams cut in four equal pieces placed in metallic grid within column
Volume of medium transferred into bioreactor after sterilization	315 mL	180 mL
Media reduction	Anaerobic chamber overnight	Nitrogen flow (0.5 vvm) passing through 0.2 µm pore diameter membrane within column
Agitation	Magnetic stirring at 200 rpm	Recirculation with liquid flow of 1000 mL.h^{-1}
Dilution rate management	Set at 0.03 h^{-1} then continuously enhanced at 2.0.10^{-4} h^{-2}	Set at 0.03 h^{-1} then continuously enhanced at 3.0.10^{-4} h^{-2} Dilution is enhanced by 20% if total solvent concentration is above 12.5 g.L^{-1} It is decreased by 20% if total solvent concentration is below 10 g.L^{-1}
Glucose concentration in the feed bottle	45 g.L^{-1}	60 g.L^{-1} between 0 h and 300 h 45 g.L^{-1} after 300 h

Table 3
Values for parameters used for dilution rate prediction during continuous fermentation carried out in a fixed bed.

Parameters	Value
a	2.0.10^{-4}
b	0.025
c	-8.10^{-10}
d	1.0.10^{-6}
e	-1.1.10^{-4}
f	0.05

40 h of fermentation due to butanol inhibition and reached a maximum concentration of 5.4 ± 0.2 g.L^{-1} (Fig. 3-C). The maximum productivity was 0.15 g.L^{-1}.h^{-1} under the conditions of the study.

The fermentation data presented in Fig. 3 were used to identify parameters regarding the initial conditions of: X_0: 0.2 g.L^{-1},S_0: 45 g.L^{-1}, AA_0: 2.3 g.L^{-1} AB_0: 0.1 g.L^{-1} B_0:0.2 g.L^{-1} and I_0: 0.1 g.L^{-1}. The identified parameters are shown in Table 2. The confidence intervals vary between 2.5% and 57% of the identified parameter value. Those results are indicative of a good identification quality. The kinetic parameters regarding acetate and butyrate production rates were taken from the model developed by Velázquez-Sánchez et al. [28] since the model of the presented study followed a similar mathematical structure.

As shown in Fig. 3, the model used in this study (Eq. (6) to Eq. (12)) with the identified parameters successfully describes the batch fermentation kinetics. The reduced χ^2 was 0.47 and indicates that the model predictions are close to the experimental data. To estimate the goodness of the predictions for each state variable, determination coefficients of the linear regression between the experimental and simulated data were computed and are shown in Table 3. The model shows a good agreement for butanol and isopropanol concentrations, biomass growth and substrate consumption ($R^2 > 0.95$), whereas the butyrate concentration was not well predicted in the first dataset ($R^2 = 0.67$).

To validate the identified parameters, another data set was used, with a change of the initial acetate and glucose concentrations only (AA_0: 3.5 g.L^{-1}, S_0: 60 g.L^{-1}). The results are presented in Fig. 4 and show that the model could better predict the consumption and production of butyrate for the second set of experimental data used for model validation ($R^2 = 0.82$). To model those variables, the kinetic equations and parameters were taken from Velazquez et al. study [28]. The parameters values identified by these authors might be more suitable for butyrate kinetic prediction under the studied conditions of the second data set, where both initial glucose and acetate concentration are higher than in the first data set. All the other variables were predicted well by the model (R^2>0.95).

5.2. Continuous stirred biofilm bioreactor

Another model structure (shown in Eqs. (18)–(24)) was used to describe the fixed bed bioreactor process. Two parameters were proposed to describe the suspended biomass attachment rate to the support: k_a, and the biomass detachment from the biofilm: k_d. These latter parameters were identified using the CDC biofilm bioreactor experiment operated in continuous mode for 550 h. Values and confidence intervals for the two parameters are described in Table 1.

After inoculation, batch fermentation was initially carried out for 8 h to ensure mixing and bacterial adhesion to polyurethane foams. The dilution rate was then set to 0.03 h^{-1} and was continuously increased over the fermentation period at a linear rate, as described in Fig. 5-A, to reach 0.13 h^{-1} at 550 h of fermentation. The butanol concentration, shown in Fig. 5-A, remained stable at 5.5 g.L^{-1} between 72 h and 350 h of fermentation. This concentration increased to 7.2 g.L^{-1} after 350 h of fermentation. The isopropanol concentration also increased from 4 g.L^{-1} after 300 h of fermentation to 6 g.L^{-1} at 480 h. Consequently, the total solvent concentration remained stable for the first 300 h of the test between 9.5 g.L^{-1} and 10 g.L^{-1} and increased continuously between 300 h and 450 h. The total solvent yield over glucose remained constant at 0.3 $g_{solvent}.g_{glucose}^{-1}$ over the experimental period, despite continuous increase of the dilution rate.

The acetate concentration was between 1.0 g.L^{-1} and 1.5 g.L^{-1} during the process period, whereas the butyrate concentration increased slightly from 0.5 g.L^{-1} to 0.7 g.L^{-1} (Fig. 5-B). Due to the continuous increase of the dilution rate, the overall process productivity, shown on Fig. 5-C, was continuously enhanced and reached a maximum of 1.7 g.L^{-1}h^{-1} after 480 h of fermentation. This continuous increase of productivity can be caused by the higher residence time of immobilized productive cells while suspended cells are gradually washed out. The suspended biomass (Fig. 5-D) grew until it reached a concentration of 4 g.L^{-1} after 72 h of fermentation. The suspended biomass concentration then decreased due to the constant dilution rate enhancement and reached 2 g.L^{-1} 550 h after inoculation. The immobilized biomass was weighed at 48 h and at 550 h to check biofilm development during the studied fermentation period. The initial immobilized biomass concentration was 1.9 ± 0.4 $g.L_{bioreactor}^{-1}$ at 47 h of fermentation and reached 35.3 ± 0.8 $g.L_{bioreactor}^{-1}$ after 480 h of continuous fermentation. Residual glucose concentration, shown in Fig. 5-D, stabilized after 72 h of continuous fermentation to reach 17 g.L^{-1}. The observed residual glucose concentration decreased to 5 g.L^{-1} after 400 h of continuous fermentation.

The model structure developed here can describe the solvent and process productivity dynamics during a fixed bed bioreactor process using *C. beijerinckii* DSM6423. Biofilm growth dynamics were estimated, and the model successfully predicted the total amount of immobilized biomass trapped within the polyurethane foams (47 $g.L_{bioreactor}^{-1}$ simulated, compared to 35.3 ± 0.8 $g.L_{bioreactor}^{-1}$ measured experimentally). However, the suspended biomass concentration was underestimated at the beginning of the experiment under the studied conditions. The model presented here also describes the dynamics of glucose consumption well, but struggles to predict the increase of

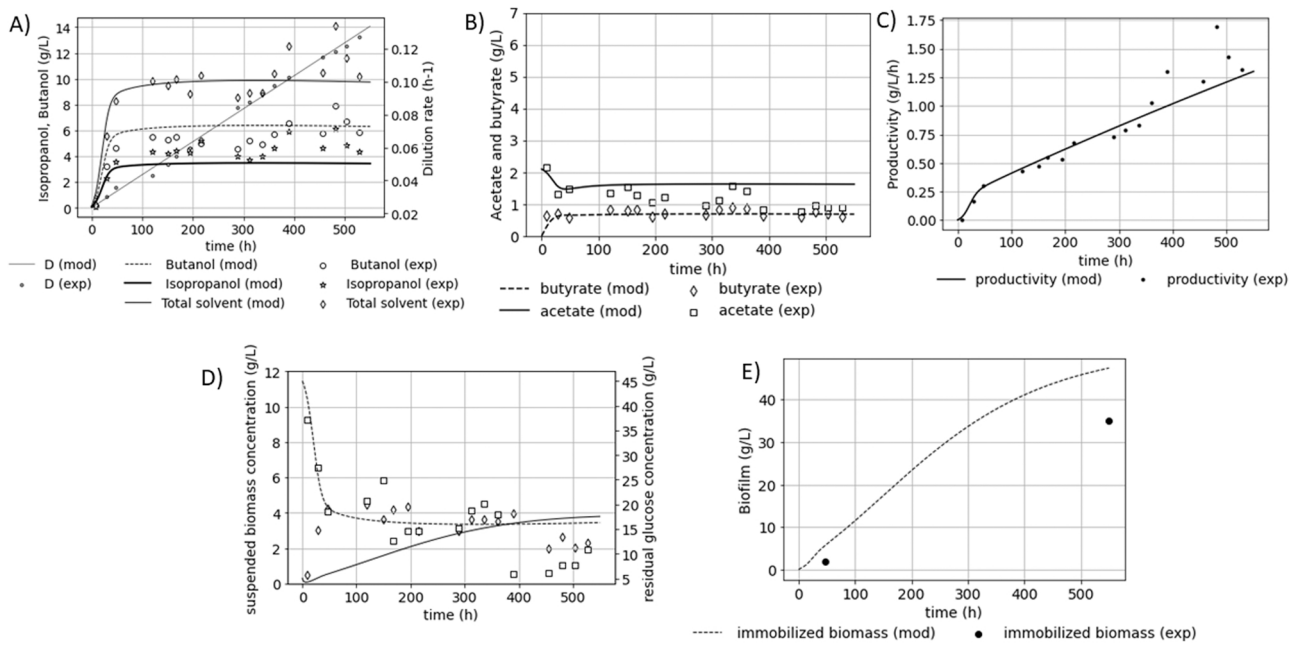

Fig. 5. Fermentation kinetics for experimental (exp) and modeled (mod) data during a CDC biofilm bioreactor process time: A) dilution rate (circle and dashed line), butanol (open circles and solid line), isopropanol (open stars and solid line) and total solvent (open diamonds and solid line) concentration; B) Simulated and experimental concentration of acetate (open squares and line) and butyrate (open diamonds and solid line); C) Volumetric productivity (solid points and line); D) residual glucose concentration (open squares and solid line) and suspended cell concentration (open diamonds and lines); E) immobilized cells (dashed line) concentration.

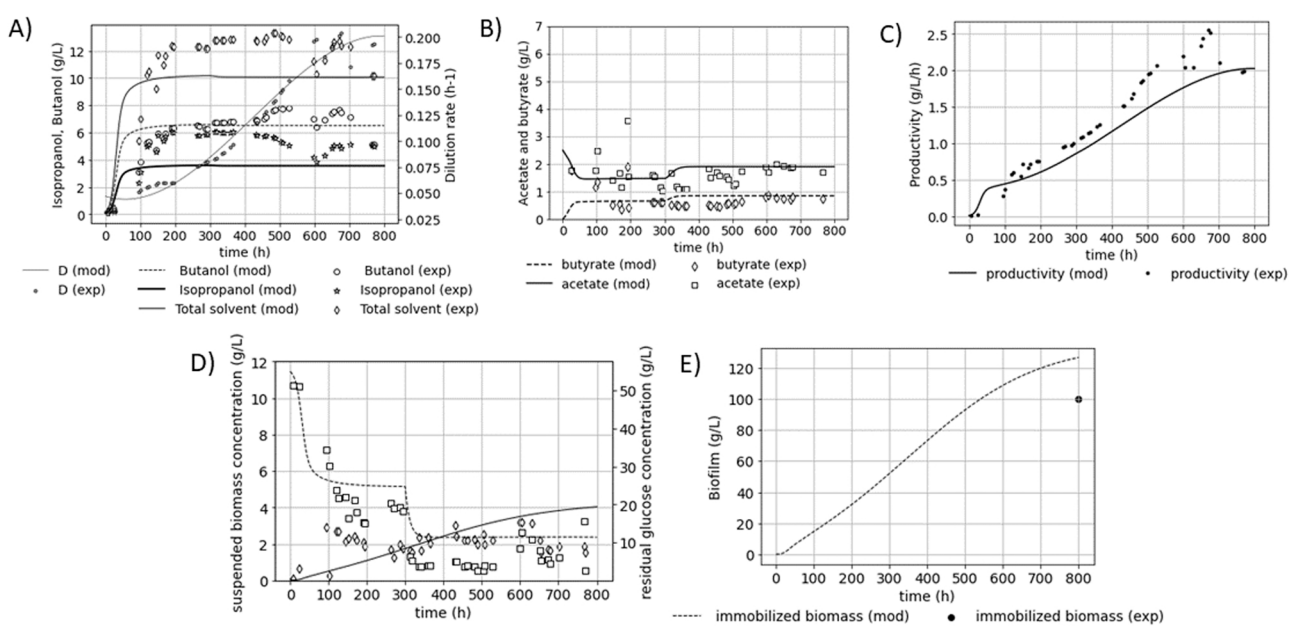

Fig. 6. Fermentation kinetics for experimental (exp) and modeled (mod) data during packed bed column bioreactor process period: A) dilution rate (circle and dashed line), butanol (open circles and solid line), isopropanol (open stars and solid line) and total solvent (open diamonds and solid line) concentration; B) Simulated and experimental concentration of acetate (open squares and line) and butyrate (open diamonds and solid line); C) Volumetric productivity (solid points and line) D); residual glucose concentration (open squares and solid line) and suspended cells concentration (open diamonds and lines); E) immobilized cells (dashed line) concentration.

glucose consumption after 400 h of continuous fermentation. The simulated butanol and isopropanol dynamics were close to those observed experimentally. Butyrate production and uptake are well described, whereas acetate uptake was underestimated during the first 300 h of the process. The computed reduced χ^2 was 6.5, which is acceptable for such a dynamic process.

5.3. Model prediction capacity

Kinetic parameters regarding the attachment and detachment were identified using the CDC biofilm bioreactor. However, in order to study the prediction capacity of the model developed here, the identified parameters were used to describe a more complex dilution rate management of an experiment carried out with a packed bed column bioreactor.

As shown in Fig. 6-A, the dilution rate was increased stepwise during the experiment but did not follow a linear curve, as was previously described with the CDC biofilm bioreactor. The glucose concentration in the feed bottle also was changed from 55 $g.L^{-1}$ to 44 $g.L^{-1}$, after 300 h of continuous fermentation.

In this bioreactor configuration, the total solvent concentration quickly increased to 13 $g.L^{-1}$ and remained stable for the studied fermentation period (Fig. 6-A). The butanol yield over glucose increased (from 0.16 $g.g^{-1}$ to 0.22 $g.g^{-1}$), while the isopropanol one decreased (from 0.16 $g.g^{-1}$ to 0.13 $g.g^{-1}$) from 450 h of fermentation. The total solvent yield on glucose increased slightly from 0.32 $g.g^{-1}$ to 0.35 $g.g^{-1}$. Fig. 6-B shows that the acetate concentration was decreased from 2 $g.L^{-1}$ to 1 $g.L^{-1}$ between 24 h and 350 h of continuous fermentation. The concentration was then increased back to 2 $g.L^{-1}$ between 350 and 800 h of the process. The butyrate concentration was increased continuously from 0.5 $g.L^{-1}$ to 0.85 $g.L^{-1}$ between 150 h and 800 h of continuous fermentation. The maximum productivity of 2.5 $g.L^{-1}.h^{-1}$ was obtained after 650 h (as shown in Fig. 6-C) at a dilution rate of 0.2 h^{-1}. The glucose consumption, shown in Fig. 6-D, was stable over the fermentation period (about 35–40 $g.L^{-1}$). While the dilution rate was increased continuously, the suspended biomass concentration (Fig. 6-D) was stable during the experiment and reached 2 $g.L^{-1}$. The final measured immobilized biomass concentration is shown in Fig. 6-E and was 100 $g.L_{bioreactor}^{-1}$ after 800 h of continuous fermentation.

The developed model used with previously identified parameters with both the batch fermentation and the CDC biofilm bioreactor was also used to simulate a packed bed column bioreactor with recycling flow. Fig. 6-A shows that both the butanol and isopropanol concentrations were underestimated by the model. In addition, the solvent selectivity during continuous fermentation was not described well due to the fixed stoichiometric parameters used for solvent production. However, the solvent production dynamics were well described, and the biofilm biomass at the end of fermentation was 20% higher than the experimental measurements (120 $g.L_{bioreactor}^{-1}$ simulated and 100 $g.L_{bioreactor}^{-1}$ weighed experimentally). The concentrations of acetate and butyrate were estimated well by the model, as along with the suspended biomass concentration during the process period. The overall process productivity over time was underestimated, whereas its dynamics were predicted well. The computed reduced χ^2 was 12.5, which indicates that the results of the simulation were poorly correlated with the experimental data.

6. Discussion

6.1. Start-up strategies and process performance

In the present study, two strategies were tested for dilution rate increase over time. The main objective of those strategies was to allow both biofilm growth and solvent production at the same time during the process start-up. High solvent concentration during the whole production process could indeed ease the downstream process design. Therefore, the start-up strategy consisted in setting the dilution rate low enough without reaching butanol inhibition to keep sufficient solventogenic biomass within the bioreactor. In this context, the regulation of pH during startup phase was not necessary because no excess of acids was produced.

Firstly, constant dilution rate enhancement was tested at a rate of $2.0.10^{-4}$ h^{-2} in a CDC biofilm bioreactor setup. Surprisingly, the solvent concentration was drastically enhanced after 350 h of fermentation. This result suggests that the biofilm growth rate is not linear in the developed fermentation system. This phenomenon has been observed by Meraz et al. [39] which described growth kinetic of anaerobic biofilm formed on polyethylene supports during continuous fermentation. Exponential biofilm growth was observed only after 360 h of fermentation in their study.

Therefore, another start-up strategy was tested in the present study using a packed bed column bioreactor. The dilution rate was enhanced according to the butanol concentration measured in the bioreactor. This latter operational mode allowed a constant total solvent concentration within the bioreactor, whereas the dilution rate enhancement was not fixed. The dilution rate increase was higher after 450 h of fermentation, suggesting that the biofilm growth rate was increased after 400 h of continuous fermentation.

Napoli et al. developed a start-up strategy based on a fixed pH (5) and high dilution rate (0.4 h^{-1} followed by 0.8 h^{-1} and finally 2.4 h^{-1} after 140 h of fermentation). With this operation, the suspended cells were washed out and biofilm could grow on a solid support without suffering from butanol inhibition. In this configuration, the biofilm formed by *C. acetobutylicum* on Tygon™ rings took 9 days to grow [11]. During this phase, the lactose consumption (between 5 and 10 $g.L^{-1}$) and solvent concentration (near 0 $g.L^{-1}$) were low compared to what was obtained in our system.

However, once the biofilm growth phase had been carried out, their system achieved high productivities (4.4 $g.L^{-1}.h^{-1}$) obtained at dilution rate of 0.97 h^{-1} after 616 h of continuous fermentation. In the present study, high productivity was achieved (2.5 $g.L^{-1}.h^{-1}$) using packed bed bioreactor with recycling flow operated at a dilution rate of 0.2 h^{-1} reached after 650 h of continuous fermentation. However, solvents were continuously produced during the experimental period and no pH regulation was required for biofilm development. The present study did not reach a steady state as biofilm kept on developing in the polyurethane foams used as the solid support.

Yang et al. obtained a maximum productivity of 1.0 $g.L^{-1}.h^{-1}$ with a high solvent concentration (12.1 $g.L^{-1}$) at a dilution rate of 0.085 h^{-1} using another natural isopropanol producer strain: *Clostridium optinoii* [20]. This bacterium was immobilized in a packed bed column bioreactor on ceramic rings. Survase et al. successfully produced a maximum solvent productivity of 5.6 $g.L^{-1}.h^{-1}$ at a dilution rate of 0.6 h^{-1} using the same strain as this study with glucose as the main carbon source and using wood pulp as the immobilization support in a continuously stirred tank bioreactor [5].

Numerous parameters can influence the productivity observed in packed bed bioreactors for A/IBE fermentations. Process performances for A/IBE production mainly rely on strain characteristics and the fermentation medium used. However, in the case of immobilized cells in a packed bed bioreactor, other process characteristics can have an impact on performances. Solid support hydrophobicity as well as specific surface area and pore sizes are indeed known to play a role in biomass adhesion [40]. The more the active biomass is adhered onto solid support, the higher is the productivity that can be achieved. Shear rate influences the cell attachment and detachment kinetics in a packed bed bioreactor [41]. This phenomenon also has an impact on the amount of biomass that can be attached to the solid support for long term operations and may vary as a function of the packed bed bioreactor design and process operation. Those phenomena could explain why such productivity differences are observed between our study and the other developed processes for isopropanol and butanol production in packed bed bioreactors.

In the present study, the use of fixed bed bioreactors improved cells concentration within the developed fermentation system. The productivity was multiplied by 11 compared to batch fermentation. This system could lower the capital expenditure for the process development and improved the competitivity compared to petrochemical based process. However, its operation is still challenging due to continuous biofilm growth which could lead to blockages, and kinetic modeling could help to control such process.

6.2. Kinetic modeling of the packed bed bioreactors start-up

A kinetic model can be used to better understand and optimize the operation of the developed fixed bed bioreactor without testing every process condition experimentally. What are known as unstructured

models are highly suitable for process design purposes because of their simplicity and their capacity to describe biochemical processes with a reduced number of parameters [42].

The model developed in the present study was based on Ghose and Thyagi model to describe biomass growth and Luedeking and Pirret to describe metabolites production [36,37]. Acids consumption were modeled based on kinetics described by Velázquez-Sánchez et al. [28], and therefore kinetic parameters regarding acids production rates from this study were used in our model. The other kinetic parameters related to the studied strain were successfully identified and validated on two series of batch experiments. Both kinetic structure and parameters values were used to model biomass growth and metabolites production in the developed fixed bed bioreactors.

The goal of the model developed in this study is to describe reaction rates and biofilm development as a function of dilution rate and glucose concentration within feeds over time. To our knowledge, such a model has never been proposed for isopropanol-butanol fermentation processes. To model the packed bed bioreactor behavior during the start-up phase, biomass attachment and detachment from solid support should be modeled. Brück et al. [38] developed a kinetic model structure for *Bacillus subtilis* biofilm growth, attachment and detachment kinetics on metal supports. The mass balance structure based on this previous study was therefore used, and both immobilized and suspended cells growth kinetics were described. The model presented here can describe biomass growth within polyurethane foams. However, the total solvent concentration is underestimated by the model by 20% compared with experimental data. Attachment and detachment rates are indeed influenced by numerous parameters, such as shear rates in the bioreactor and those phenomena are not described in the presented model. The attachment rate could be modeled as function of solid support physico-chemicals properties and might change as more biomass accumulate within the solid support. On the other hand, the detachment rate could be modeled as dependent of the biofilm thickness and the shear rate within the bioreactor as described by Stewart et al. [43].

The model developed by Raganati et al. [30] did not describe biofilm growth during packed bed bioreactor startup but rather focuses on biomass composition at observed steady state. The model presented in this study can describe immobilized and suspend cells dynamic during continuous fermentation with fixed bed. The model developed by Raganati et al. could nevertheless be employed to describe biofilm composition in terms of acid- and solvent- producing cells as a function of observed reaction rates in the presented study.

The model presented in our study could be useful to optimize start-up phase of fixed bed biofilm bioreactors applied to isopropanol-butanol fermentation. However, more experimental points would be needed in order to validate the biofilm's growth kinetic. The sampling protocol can be enhanced by sampling more biofilm but requires great attention to avoid oxygen and contaminations. Model could also be used to investigate the effect of other carbon source on the strain's growth and on the biofilm development. This would be relevant to develop a fermentation process with lignocellulosic substrates although the kinetic would need some modifications for this purpose. The model could be improved to account for hydrodynamic impact on biomass detachment and attachment kinetics. The model could also describe mass transfer phenomena such as diffusion in the polyurethane foams used in this study.

7. Conclusion

The employ of the fixed bed bioreactor multiplied by 11 butanol and isopropanol productivity compared to batch fermentation in this study. The polyurethane foams used were suitable for efficient biofilm development in the two systems presented in this study. A new dilution rate management was proposed and allowed stable solvent concentration, despite continuous biofilm growth. To better understand and operate those systems a kinetic model was developed. Kinetic parameters regarding metabolites production and substrate consumption were estimated and successfully validated using batch experiment. Kinetic parameters regarding biomass attachment and detachment from the polyurethane foams were estimated using data obtained during continuous fermentation using CDC biofilm bioreactor. Simulated data were compared to experimental results obtained with fixed bed column bioreactor performed with another dilution rate management. However, important differences between experimental and modeled data were obtained. The developed model should therefore be enriched to better described attachment and detachment phenomena as well as diffusion that could occur within biofilm depth (Tables 4 and 5).

Table 4
Values and 95%confidence intervals for identified parameters of the proposed batch model.

Parameter names	Value	95% confidence interval	Units	Source
μ_{max}	0.14	±0.004	h^{-1}	This study
Y_{xs}	0.14	±0.009	$g.g^{-1}$	This study
Y_{buts}	0.22	±0.01	$g.g^{-1}$	This study
Y_{es}	0.004	±0.006	$g.g^{-1}$	This study
Y_{is}	0.12	±0.01	$g.g^{-1}$	This study
k_s	3	±0.56	$g.L^{-1}$	This study
B_{max}	6.9	±0.31	$g.L^{-1}$	This study
k_{AA}	0.10	±0.002	$g.L^{-1}$	This study
k_{AB}	0.74	±0.32	$g.L^{-1}$	This study
α	1	–	h^{-1}	[28]
β	0.5	–	h^{-1}	[28]
Y_{B_AB}	0.7	–	$g.g^{-1}$	[28]
$YI_{_AA}$	1		$g.g^{-1}$	[28]
k_a	2.01	±0.54	h^{-1}	This study
k_d	0.072	±0.038 h^{-1}	This study	

Table 5
Determination of coefficient of linear regression between simulated and experimental data for batch experiments in respect of two different initial conditions.

Variable	S0 = 45 $g.L^{-1}$	S0 = 60 $g.L^{-1}$
X	0.99	0.97
S	0.99	0.96
I	0.99	0.99
B	0.99	0.99
AA	0.95	0.99
AB	0.67	0.82

Author contributions

M.C and JC.G conceptualized the research work, designed the methodology and performed the experiments. M.C created the kinetic models, the python code used for kinetic parameters identification and performed data analysis. M.C wrote the original draft. JC.G, H.V and F. BC reviewed and edited the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bej.2022.108355.

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