Grisales Diaz VH, Willis M.

[Kinetic modelling and simulation of batch, continuous and cell-recycling fermentations for acetone-butanol-ethanol production using *Clostridium saccharoperbutylacetonicum* N1-4.](#)

Biochemical Engineering Journal 2018

DOI: <https://doi.org/10.1016/j.bej.2018.05.011>

Copyright:

© 2018. This manuscript version is made available under the [CC-BY-NC-ND 4.0 license](#)

DOI link to article:

<https://doi.org/10.1016/j.bej.2018.05.011>

Date deposited:

16/05/2018

Embargo release date:

15 May 2019

This work is licensed under a

[Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International licence](#)

Accepted Manuscript

Title: Kinetic modelling and simulation of batch, continuous and cell-recycling fermentations for acetone-butan-ol-ethanol production using *Clostridium saccharoperbutylacetonicum* N1-4

Authors: Víctor Hugo Grisales Díaz, Mark J. Willis

PII: S1369-703X(18)30156-6
DOI: <https://doi.org/10.1016/j.bej.2018.05.011>
Reference: BEJ 6948

To appear in: *Biochemical Engineering Journal*

Received date: 13-12-2017
Revised date: 7-4-2018
Accepted date: 13-5-2018

Please cite this article as: Díaz VH, Willis MJ, Kinetic modelling and simulation of batch, continuous and cell-recycling fermentations for acetone-butan-ol-ethanol production using *Clostridium saccharoperbutylacetonicum* N1-4, *Biochemical Engineering Journal* (2018), <https://doi.org/10.1016/j.bej.2018.05.011>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Kinetic modelling and simulation of batch, continuous and cell-recycling fermentations for acetone-butanol-ethanol production using *Clostridium saccharoperbutylacetonicum* N1-4

Víctor Hugo Grisales Díaz* and Mark J Willis

^a School of Engineering, Newcastle University, NE1 7RU, Newcastle upon Tyne, United Kingdom

* victor.grisales-diaz@newcastle.ac.uk; victor.grisales.d@gmail.com

Graphical abstract

Highlights

- A novel kinetic model describing acetone-butanol-ethanol production is developed
- The model accounts for carbon catabolite repression
- The model is validated using both batch and continuous fermentation data
- Butanol inhibited the production of ACoA, acetone, AACoA, pyruvate and biomass
- A productivity up to 16 g l⁻¹ h⁻¹ can be achieved by cell-recycling

Abstract

A kinetic model describing acetone-butanol-ethanol (ABE) production applicable to both single substrate fermentations of glucose and xylose as well as co-fermentation of the substrates has been developed. The model accounts for carbon catabolite repression as well as the inhibition of kinetic rates at high substrate concentrations ($\sim 90 \text{ g l}^{-1}$). Model parameters were obtained through kinetic fitting to previously report experimental data. The model was used to study the design and operation of a continuous ABE fermentation process (with and without recycle of biomass). For continuous operation, it was shown that multiple steady-states exist at low dilution rates. For operation with recycle of biomass, the influence of recycle rate on both biomass concentration and ABE productivity were studied. The results indicate that for a range of recycle and dilution rates, ABE productivity can increase to $16 \text{ g l}^{-1} \text{ h}^{-1}$ (10 times higher than that without biomass recycling), consistent with experimental results.

Keywords: kinetic fitting; co-fermentation of glucose and xylose; carbon catabolite repression; Monte Carlo simulations; model reduction and validation

Nomenclature

CCR, carbon catabolite repression (-)

D, dilution rate (h^{-1})

K_{mj} , kinetic model constant for reaction 'j' (mmol l^{-1})

K_{ma_j} , kinetic model constant for reaction 'j' (mmol l^{-1})

K_{mb_j} , kinetic model constant for reaction 'j' (mmol l^{-1})

I_B , inhibition concentration of butanol (mmol l^{-1})

I_{NH} , inhibition concentration of biomass (mmol l^{-1})

MW_i , molecular weight of species 'i' (g mol^{-1})

n_j , butanol inhibition power term for reaction 'j'

$n_{G/X}$, parameter for carbon catabolite repression

r_j , reaction rate 'j' ($\text{mmol l}^{-1} \text{h}^{-1}$)

R_i , net rate of production / consumption of species 'i' ($\text{mmol l}^{-1} \text{h}^{-1}$)

V, volume of the reactor (l)

$V_{\max_i}$, maximum rate for reaction 'j' (h^{-1})

t, time (h)

[AACoA], concentration of acetoacetyl CoA (mmol l^{-1})

[Acemax], maximum inhibitory concentration of acetone (mmol l^{-1})

[Acetate], concentration of acetate (mmol l^{-1})

[Acetone], concentration of acetone (mmol l^{-1})

[Acmax], maximum inhibitory concentration of acetate (mmol l^{-1})

[ACoA], concentration of acetyl CoA (mmol l^{-1})

[BCoA], concentration of butyryl CoA (mmol l^{-1})

[Bimax], maximum inhibitory concentration of biomass (mmol l^{-1})

[Biomass], concentration of biomass (mmol l^{-1})

[Bmax], maximum inhibitory concentration of butanol (mmol l^{-1})

[Bumax], maximum inhibitory concentration of butyrate (mmol l^{-1})

[Butanol], butanol concentration (mmol l^{-1})

[Butyrate], butyrate concentration (mmol l^{-1})

[C_i], concentration of species 'i' (g l^{-1})

[C_{ik}], concentration of the species 'i' in data set 'k' (mmol l^{-1})

[C_{oi}], inlet concentration of species 'i' (g l^{-1})

[Glucose], glucose concentration (mmol l^{-1})

[Pyruvate], pyruvate concentration (mmol l^{-1})

[Xylose], xylose concentration (mmol l^{-1})

ω , parameter describing the preference of glucose over xylose

σ_i , weighting parameters used in kinetic fitting for species 'i'

1.0 Introduction

Butanol is the primary product of interest in bacterial acetone-butanol-ethanol (ABE) fermentation. It is used in the plastics industry, as a solvent for the manufacture of pharmaceuticals and oils and is considered as a promising biofuel as its energy content and physical properties are closer to gasoline when compared to conventional biofuels (ethanol and biodiesel) allowing use in compression ignition engines [1]. However, biological production of butanol by ABE fermentation is limited by low ABE yield (the theoretical yield of butanol is 41%, 1.25 times lower than that of ethanol) and butanol inhibition at very low concentrations ($<20 \text{ g l}^{-1}$), [2].

Butanol inhibition may be reduced using *in situ* recovery techniques such as e.g. gas stripping, pervaporation, membrane extraction etc. [3–5]. Whilst it is known that these *in situ* recovery techniques increase butanol productivity their design must be performed carefully because of the high cost of the recovery units, i.e. the total cost of the process with *in situ* recovery may not be lower [6]. In order to determine whether *in situ* recovery processes are more economical than conventional reactors and to optimise overall process operation, a reliable kinetic model is required.

One of the main advantages of the microorganisms used to produce butanol (e.g., *Clostridium acetobutylicum*) are their ability to consume several substrates, e.g., hexoses and pentoses [7]. Hence, lignocellulose materials and other residual substrates of low cost can be used in ABE fermentation. Of the alternative microorganisms used to produce butanol, *Clostridium saccharoperbutylacetonicum* N1-4 offers a promising option as it can

consume several sugars with an ABE productivity of up to $0.5 \text{ g}^{-1} \text{ l}^{-1} \text{ h}^{-1}$ [7]. However, given a mixture of substrates this microorganism prefers glucose over other fermentable sugars which inhibits their consumption. This phenomenon is known as catabolite carbon repression (CCR) [8].

Several kinetic models have been proposed in the literature for fermentations [9–11] some of which have considered a mixture of substrates [12,13]. However, to the best of our knowledge, a kinetic model describing ABE fermentation that accounts for CCR has not been proposed. The primary aim of this work is to develop a kinetic model applicable to both single substrate fermentations of glucose and xylose as well as co-fermentation of the substrates.

As a basis for the work, we use the kinetic model for bacterial ABE production using *Clostridium saccharoperbutylacetonicum* N1-4 proposed by Grisales and Tost [6]. This model was shown to predict overall butanol yield with high accuracy (errors lower than 5%), however, it assumed the use of one substrate (glucose or xylose) and the predictive capability of the model given a mixture of substrates was not evaluated. In this paper, the kinetic model is modified to account for CCR. The model is validated using previously reported experimental data of batch and continuous fermentations (with and without biomass recycle), including fermentations with co-substrate and high substrate concentrations (89 g l^{-1}) [8,14–16]. In total ten batch fermentations are used to find the parameters of the model and thirteen continuous fermentations (with and without biomass recycling) are used to validate the model. The kinetic model is then used to study the design and operation of a continuous ABE fermentation process (with and without recycle of biomass).

2.0 Materials and methods

As the experimental data available within the literature has been obtained from both batch, as well as continuous fermentations with and without recycle, the species balance expression (Eq. (1)) is used as the basis for kinetic model development. This equation represents the rate of change of concentration of each one of the biochemical species, shown in Fig. 1 (b), where the metabolic route is that which was proposed in our previous work [6]. Fig. 1 (a) depicts a general representation of the reactor encompassing batch and continuous operation with and without biomass recycle and bleeding. It is assumed that a membrane is used to recycle the biomass, i.e. biomass does not pass through the membrane and is therefore recycled. If the operating mode of the reactor is batch, then the dilution rate (D) in Eq. (1) is zero. In addition, biomass recycle is zero when the dilution rate of permeate (D_p) or the ratio of biomass recirculation (b_r) is zero. The parameter (a_i) is zero if the species does not pass through the membrane (i.e. biomass) and equal to 1 if it does. Finally, the dilution rate of bleeding (D_b) plus dilution rate of permeate is assumed to be equal to the total dilution rate (D), Eq. (3).

$$\frac{d[C_i]}{dt} = R_i \cdot MW_i + D \cdot [C_{o_i}] - (D_p \cdot a_i + D_b) \cdot [C_i] \quad (1)$$

$$b_r = \frac{D_p}{D} \quad (2)$$

$$D = D_p + D_b \quad (3)$$

The molecular weight (MW) of biomass was taken to be 172 g mol^{-1} and assumed to be constant [14]. Using Fig. 1 (b), the net rate of production / consumption of each of the species (R_i) are given by,

$$R_{Pyruvate} = -r_2 + r_1 \cdot \omega + r_{15} \cdot (1 - \omega) \quad (4)$$

$$R_{AcoA} = r_2 + r_3 + r_4 - r_5 - r_6 - r_7 \quad (5)$$

$$R_{AACoA} = r_6 - r_{10} - r_4 - r_{11} \quad (6)$$

$$R_{Acetate} = r_5 - r_4 - r_3 \quad (7)$$

$$R_{Ethanol} = r_7 \quad (8)$$

$$R_{Acetone} = r_4 + r_{11} \quad (9)$$

$$R_{Butyrate} = r_{13} - r_{12} - r_{11} \quad (10)$$

$$R_{BCoA} = r_{10} + r_{11} + r_{12} - r_{13} - r_{14} - r_{16} \cdot (1 - \omega) - r_8 \cdot \omega \quad (11)$$

$$R_{Butanol} = r_{14} \quad (12)$$

The death rate of biomass is considered to be zero for continuous operation. However, for the alternative modes of operation, the death rate of biomass and the net rate of production of biomass, xylose and glucose are given by Grisales and Tost [6],

$$R_{Biomass} = r_{16} \cdot (1 - \omega) + r_8 \cdot \omega - r_{17} \cdot (1 - \omega) - r_9 \cdot \omega \quad (13)$$

$$R_{Biomass\ death} = r_{17} \cdot (1 - \omega) + r_9 \cdot \omega \quad (14)$$

$$R_{Glucose} = r_1 \cdot \omega \quad (15)$$

$$R_{Xylose} = r_{15} \cdot (1 - \omega) \quad (16)$$

In these equations the parameter, ω , is used to model the microorganism's preference for glucose over xylose (which is defined in the next section of the paper).

2.1 Reaction rate expressions

The rate expressions provided in Table 1 are the structural form of those developed in Grisales and Tost [6] using experimental data reported by Shinto et al. [14,15]. In this model the structure of the reaction rate terms for the consumption of butyrate (r_2), acetate (r_3), ACoA (r_6 , r_7 , r_5), AACoA (r_{10}) and BCoA (r_{13}) are based on Michaelis-Menten kinetics. The

reaction rate terms used to describe glucose and xylose consumption (r_1 and r_{15}) include substrate inhibition terms [14,15], while, the butanol inhibition term (I_B) is of a standard structure. Although, some biochemical reaction network models only relate biomass growth to substrate concentration [17] or acetyl CoA concentration [14], the biomass growth model (reactions r_8 and r_{16} (Table 1)) is assumed to depend on consumed substrate which is activated by the concentration of butyryl-CoA as proposed by Grisales and Tost [6]. The rate of death of biomass (r_9 and r_{17}) in a batch fermentation was assumed proportional to the biomass concentration [14] while the consumption of butyrate was activated by butyrate and butanol [6]. The rate of death of biomass was assumed constant with respect to time. However, it was assumed to depend on type of substrate in the fermentation (similar to growth rate as the growth rate depends on the type of substrate). The reaction rate terms used for acetone production (r_4 and r_{11}) are those proposed by Shinto et al. [14] and the rate term for butanol production (r_{14}) includes activation by butyrate and inhibition by butanol, as was proposed by Shinto et al. [14]. The inhibition of biomass growth (I_{NH}) is modelled using standard inhibition terms which includes inhibition by butanol, acetate, acetone, total biomass, butyrate, glucose and xylose. Note also, that a number of terms are a function of the parameter, F . As described by Shinto et al. [14,15] when the concentration of fermentable sugars is lower than 1 mmol l^{-1} F is zero, otherwise it is one.

In this paper, three modifications to this previous kinetic model are proposed a) the inclusion of a mathematical term to describe CCR, b) a description of butanol inhibition through a modification of the reaction rate terms for acetate, butyrate, ACoA, xylose, glucose and biomass production and c) a reduction in the number of model parameters (and terms) through the use of a model parameter confidence interval analysis.

The first modification is made in order to improve the prediction of the species concentrations given CCR. In our previous work, whilst a weighting factor, ω , was used to represent substrate competition, as proposed by Krishnan *et al.* 1999 [18] this was calculated as the fraction of glucose in relation to the total concentration of substrate. Given that CCR implies a reduction of the rate of xylose consumption, in this work, it is assumed that the weighting parameter, ω , depends on glucose and xylose concentration as follows,

$$\omega = \left(\frac{[Glucose]}{[Glucose] + [Xylose]} \right)^{n_{g/x}} \quad (17)$$

In Eq. (17) the power term, $(n_{g/x})$, is used to model (and predict) CCR. The value of $n_{g/x}$ may be estimated along with the other unknown model parameters via kinetic fitting using several ratios of the substrates as well as a number of individual fermentations. If CCR is not observed, $n_{g/x}$ will tend to 1, while, if a strong CCR is observed, $n_{g/x}$ must tend to 0 indicating the preference of glucose over other fermentable sugars.

The butanol inhibition term (I_B) used is of a standard structure (see table 1, Eq. (18-K)). However, in a second modification, power terms are included as adjustable kinetic parameters, i.e. in the reaction rates terms for production of ACoA (n_3, n_4), acetone (n_{11}), AACoA (n_6), pyruvate (n_{15}, n_1) and biomass (n_{19}) are included. The terms n_1, n_6 and n_{15} are equal to 1 as lineal inhibition was assumed. This is proposed in order to model the strong butanol inhibition at high substrate concentrations (around 90 g l⁻¹). To justify the inclusion of these inhibition terms, two variants of the kinetic model are considered in the results section of the paper. Kinetic model 'B' which includes these terms as kinetic model parameters and kinetic model 'A' which does not.

Given that the proposed kinetic model contains a large number of parameters that have to be estimated (~60 in total) parameter confidence intervals are determined as part of the kinetic fitting procedure. As described in section 3.2 of this paper, this allows a reduction of the number of model parameters through analysis of the calculated confidence bounds.

2.2 Kinetic fitting

For kinetic model A, the model parameters reported by Grisales and Tost [6] were used. However, the additional parameter $n_{g/x}$ was estimated in order to improve model prediction accuracy using the experimental data reported by Noguchi et al. [8]. For kinetic model B, fermentation data provided by Shinto et al. [14,15] and additional laboratory data available in the literature [8,16] for co-substrate fermentation and high substrate concentrations (89 g l⁻¹) were used to determine all the model parameters (apart from the maximum inhibitory concentration of biomass [Bimax] which was fixed at 100 g l⁻¹ as this has been found to be the experimental limit [19]).

In order to perform kinetic fitting and validate the model, the ordinary differential equations (ODEs) (representing the reactor dynamics) are numerically integrated using the initial conditions provided in the original experiments and a given set of estimated model parameters, using the function ode15s in MATLAB 2016a®.

There are two categories of optimisation algorithms that may be used for kinetic fitting, so-called global and local optimisation techniques [20]. Local methods require initial estimates of model parameters, while global methods do not. The use of a combination of both techniques has been suggested as a method to find the global optimum solution [20]. Therefore, a hybrid kinetic fitting method is used in this work which uses several built in MATLAB® functions within the optimisation toolbox (see Fig. 2). In steps 1 to 3 of the

algorithm, model parameters that minimise the cost function (J) are determined using the functions 'ga' (global) and 'fminsearch' (local); where the method that gives the minimum value of J (in steps 1 to 4) will depend on the initial parameter values used by the function 'fminsearch'. The best set of parameters (obtained in step 4) are then used in steps 5 to 8, in an attempt to ensure that a global optimal solution is obtained. In step 5, the parameters obtained using 'nlinfit' (based on Levenberg-Marquardt algorithm) are used as initial parameter estimates in 'fminsearch' (derivative-free method) and if the difference in the minimum value of the objective functions achieved by the two methods is below a threshold (< 0.1 was used in this work), the resulting parameters are assumed to be the global optimum. If the difference is not below the threshold then step 5 is repeated using the previously found best set of parameter values.

The objective function that was minimised was,

$$J = \sum_{k=1} \sum_{i=1} \sigma_i \cdot ([c_{ik}] - [\hat{c}_{ik}])^2 \quad (18)$$

where $[c_{ik}]$ (mmol l⁻¹) is the measured experimental data and $[\hat{c}_{ik}]$ the model estimate (the subscript denotes the concentration of the i^{th} species given the k^{th} set of experimental data). The weighting parameters (σ_i) were fixed to one for all species, except for acetone and acetate which were set as three and two, respectively. The weightings were used in order to reduce the prediction error for these species.

To obtain model parameter confidence bounds the kinetic fitting procedure (Fig. 2) was repeated one hundred times using randomly generated initial estimates of the model parameters (varied by +/- 30% from the best parameter values initially obtained). In addition, to account for experimental errors in the data sets the experimental data was also

varied by the addition of random perturbations of $\pm 10\%$). This allows an upper and lower bound on the parameter estimates to be obtained as well as the mean (taken to be the nominal model parameter values) and the standard deviation of their variation (estimated using the function 'normfit' in MATLAB).

2.3 Model simulation studies

The model simulation studies reported in this section are performed for two purposes. First, given that the continuous fermentation experiments reported by Tashiro et al. [19] were at the same pH, temperature and media as the experimental batch fermentation data, the data allows the kinetic models to be further validated. Therefore, simulations of the steady-state conditions of the continuous fermentation model (with and without recycling) were made (using the reported [18] inlet concentrations of glucose and acetate of 50 g l^{-1} and 3 g l^{-1} , respectively). The stable steady-state concentrations, i.e., when all the eigenvalues of the Jacobian of the solutions of the steady-state model of Eq. (1) are negative, were found using the function 'fsolve' in MATLAB 2016a[®] by setting the derivatives of the concentrations of the chemical species in Eq. (1) equal to zero. Using the standard deviation of each model parameter, one hundred Monte Carlo simulations are performed (one hundred randomly generated sets of parameter values within the upper and lower bounds of each model parameter) in order to determine the effect of these model parameter variations on the calculated steady-state of the continuous fermentation. The expectation being that the model will display the same qualitative behaviour as that observed experimentally.

The second purpose of the simulation studies is to study the design characteristics with biomass recycling. The effects of bleeding in the reactor with biomass recirculation was studied because a high value of biomass recirculation can be used to give high productivity.

3.0 Results and discussion

3.1 kinetic fitting to the batch fermentation data

The estimated parameters of the two kinetic models are presented in Table 2. For kinetic model A, the optimal value of $n_{g/x}$ was found to be 0.17, confirming the strong CCR in the ABE fermentation by *Clostridium saccharoperbutylacetonicum* N1-4. When compared to the assumption that $n_{g/x}$ is equal to one [6], the coefficients of determination (R-squared values) between the estimated and measured glucose, xylose and butanol concentrations in a fermentation of mixed sugars was improved from 0.938, 0.78, and 0.95 to 0.997, 0.973 and 0.986, respectively. Although strong CCR was predicted using only one additional parameter (Fig. 3 (a)), it may be observed that kinetic model A still had a low prediction accuracy for glucose consumption and acetone production at high concentrations of substrate (89 g l⁻¹, Fig. 3 (b)). This indicates that butanol production and glucose consumption had a stronger inhibition than that reported previously.

When compared to kinetic model A, the optimised values of the parameters of kinetic model B provided improved coefficients of determination between measured and estimated butanol and acetone concentrations (initial glucose 89 g l⁻¹), with values increasing from 0.967 and 0.934 to 0.991 and 0.997, respectively. Fig. 3 (d, e, f) show the measured species profiles and model estimates using kinetic model B for a fermentation using a mixture of glucose and xylose and high concentrations of glucose, respectively.

In comparison to kinetic model A, the parameter determining the maximum inhibition of glucose growth ([G_{max}]) was ~2-fold lower than that of kinetic model B. This reduction was caused by the use of the high substrate concentration data (89 g l⁻¹) and indicates that the adverse effects of substrate concentration on growth will be underestimated if glucose

concentrations of less than 60 g l^{-1} are used for the kinetic fitting. Furthermore, higher butanol inhibition was observed in the acetate consumption for acetone production ($n_4=2.8$) and in the biomass growth ($n_{19}=1.7$). A similar effect was also observed for acetone production via butyrate consumption, however, only when the butanol concentration was greater than 11.6 g l^{-1} . Interestingly, the power term associated with the expression for butanol inhibition on xylose consumption was found to be lower than 1 (0.55). Hence, this and the strong CCR would appear to indicate that the lower ABE productivity of xylose in relation to glucose was mainly caused by the fact that xylose is a poor substrate for biomass growth. A summary of all the calculated R-squared values (using kinetic model B) is provided in Table 3. The concentration profiles of butanol, glucose, xylose and acetone at concentrations of substrate lower than 60 g l^{-1} are shown in Fig. 4. Whilst, the concentrations profile of acetate, biomass and butyrate are shown in Fig. 5. It may be noted that biomass, acetate, and butyrate were the species with the lowest average R-Squared values of 0.867, 0.907 and 0.856, respectively. These results are consistent with the performance of previous kinetic models reported in the literature [6,14,15].

3.2 Model reduction

The model parameter confidence intervals obtained for kinetic model B are reported in Table 4. Using these bounds allows the modification of the kinetic model. First, observe that the upper and lower bounds for the xylose and glucose inhibition terms tend to plus and minus infinity, i.e. these parameters have a high degree of uncertainty. Therefore the xylose and glucose inhibition terms (K_{ma1} and K_{ma12}) obviously do not affect model prediction accuracy and may therefore be removed from kinetic model B. Secondly, it may be observed that the terms for acetate production (reaction 5) also tend to infinity. In other

words, it may be concluded that given the currently available experimental data it is not possible to accurately model acetate production and these parameters may also be removed from the model.

In addition, the parameter describing the inhibition of acetone also has high uncertainty (the bounds of $[Ac_{max}]$ tend to plus and minus infinity). This is possibly because acetone does not inhibit biomass growth given the concentrations of the experimental data used as it is known that acetone totally inhibits ABE fermentations at high concentrations (around 60 g l⁻¹ [23]). Whilst it is not possible to reach these concentrations in a conventional reactor, in alternative fermentation configurations, acetone concentration may be high, e.g. in a fermentation with *in situ* recovery by liquid-liquid extraction and high substrate concentrations [4].

Finally and interestingly, although butanol inhibited the production of ACoA, acetone, AACoA, pyruvate and biomass, butanol did not inhibit its own production rate (the bounds of $[K_{m_{b14}}]$ tend to plus and minus infinity). These results are at variance with those reported by Shinto et al. [14] (i.e., a term for butanol inhibition is necessary to increase the prediction of butanol production).

3.3 Model simulation studies - continuous fermentation

The results shown in the Fig. 6 show the steady-state outlet concentration of biomass as well as the ABE productivity (calculated as the product of ABE concentration (acetone plus butanol and ethanol) in the reactor at the stable steady-state and the total dilution rate) and ABE yield (calculated as the ratio of ABE concentration (g l⁻¹) and the substrate feed (glucose plus acetate) to the reactor (53 g l⁻¹)) as a result of the Monte-Carlo simulations. The blue 'dots' are the values of biomass, ABE productivity and yield obtained for a

particular dilution rate as a result of model parameter variations and the black 'dots' are the values obtained using the nominal model, i.e. when there is no variation in the model parameters. It may be observed that the majority of experimental data (biomass and ABE concentration) is in the range of the simulation results obtained as a result of the model parameter perturbations.

The experimental data shows a maximum biomass concentration at dilution rates of around 0.1 and 0.15 h^{-1} and the maximum ABE productivity is achieved at around 0.2 h^{-1} . The simulation (using the nominal model) also indicates a maximum biomass concentration at similar dilution rates, whereas the prediction of the maximum ABE productivity was 1.4 $\text{g}^{-1} \text{h}^{-1}$ at a dilution rate of 0.16 h^{-1} . The variability in the experimental results may be because the actual fermentation time used was lower than that required to achieve a stable state-state condition in the fermenter. Finally, it may also be noted that multiple steady-state conditions were found at dilution rates lower than 0.05 h^{-1} and it is possible that a significant number of stable and unstable steady-state conditions may be found using a more detailed bifurcation analysis.

3.3 Model simulation studies - continuous fermentation with biomass recycling

Continuous production of butanol by fermentation with biomass recycling or biomass immobilization is known to achieve the highest productivities [24,25]. In order to investigate this, Eq. (1) was simulated using kinetic model B for various values of the biomass recirculation parameter (br). The simulation used a fixed dilution rate of 0.85 h^{-1} which corresponds to the experimental data provided by Tashiro et al. [19] who present experimentally determined values of biomass concentration and ABE productivity at three distinct recirculation rates, $br = 0.84, 0.87$ and 0.89 . It may be observed in Fig. 7 that when

biomass recycling was used ABE productivity can increase to $16 \text{ g l}^{-1} \text{ h}^{-1}$ which was around 10 times higher than the maximum productivity achieved without biomass recycling. Given the limited amount of experimental data, the simulation results demonstrated a relatively good predictive performance (Fig. 7), with the majority of the data (biomass and ABE productivity) being in the range of random variations of the model parameters.

Fig. 8 demonstrates the effects of changing the recycle rate on the biomass concentration and ABE productivity for a range of different dilution rates and b_r (for clarity, only the nominal model has been used to generate these results). As may be observed in Fig. 8, the simulation predicts that the same productivity can be achieved using biomass recycling at a dilution rate of 0.85 h^{-1} and b_r of 0.99 (Fig. 7 (b)) or using a dilution rate of 2 h^{-1} and b_r of 0.93 (Fig. 8 (b)). A high b_r increased the ABE productivity and yield because the biomass concentration increased. However, it is known that high biomass concentrations reduces the ability to control the volume of the reactor due to the high viscosity and the presence of heavy bubbling [19]. The maximum biomass concentration achieved experimentally in a continuous fermentation using *Clostridium saccharoperbutylacetonicum* is $\sim 40 \text{ g l}^{-1}$ [26]. Hence, the efficiency and the capacity of volume control with biomass concentrations higher than 40 g l^{-1} still requires be demonstrated experimentally.

Although high productivity is important to reduce the reactor capital costs, the maximum productivities with r_b lower than 0.93 were achieved with an ABE yield lower than 0.2 g/g (Fig. 6 (b)). Given that the ABE yield is the most important factor in an economic evaluation of butanol production [6], determining the maximum productivity is not recommended as a basis to select the operating conditions of the reactor with biomass recycling and an economic optimisation of the reactor is still required. It may also be noted from Fig. 8, that

as with the continuous fermentation without recycle, multiple stable steady-states were found at low dilution rates ($< 0.5 \text{ l}^{-1}$).

4.0 Conclusions

In this paper, the development of a kinetic model describing bacterial ABE production applicable to both single substrate fermentations of glucose and xylose as well as co-fermentation of the substrates has been described. Two variants of the model were developed and it was found that a model incorporating terms for CCR as well as the strong butanol inhibition at high substrate concentrations (around 90 g l^{-1}) to the rates of acetate butyrate, ACoA, xylose and biomass production was the most accurate (kinetic model B). To obtain the parameters of the model we used data available in the literature and it was observed that data sets with initial concentrations of substrate higher than 60 g l^{-1} (e.g. 90 g l^{-1}) are necessary in order to develop a reliable kinetic model. This is because concentrations of substrate lower than 90 g l^{-1} underestimated the butanol and substrate inhibition in the data fitting procedure. As a consequence of the experimental data used, it was found that the kinetic model was not able to predict acetate production as well as acetone inhibition. It was also found that glucose and xylose did not inhibit their respective consumption rates. The developed models were validated and used to consider the design and operation of continuous ABE fermentation and a continuous fermentation system with recycle of biomass. Monte Carlo simulations based upon model parameter confidence bounds gave model predictions that represented the behaviour of the experimental data. The model was therefore used to study the design characteristics of continuous operation with biomass recycling and bleeding. Using the model it was found that when biomass recycling is used, ABE productivity can increase to $16 \text{ g}^{-1} \text{ l}^{-1} \text{ h}^{-1}$ which is around 10 times higher than the

maximum productivity achieved without biomass recycling, however, the ABE yield is low <0.2 g ABE g⁻¹ substrate. Hence, future work should consider an economic optimisation to select the best operational conditions.

For the continuous fermentation system it was shown that multiple steady-states exist at low dilution rates. A more detailed bifurcation analysis may reveal the existence of additional stable and unstable steady state conditions. This is one area we wish to pursue as future work. Finally, it should be noted that whilst the results presented in this paper demonstrate the accuracy of the developed models, they did not include variables such as temperature and pH, hence, their inclusion should also be studied in future work.

References

- [1] R. Mayank, A. Ranjan, V.S. Moholkar, Mathematical models of ABE fermentation: review and analysis, Crit. Rev. Biotechnol. (2012) 1–29. doi:10.3109/07388551.2012.726208.
- [2] V.H. Grisales Diaz, G. Olivar Tost, Energy efficiency of acetone, butanol, and ethanol (ABE) recovery by heat-integrated distillation, Bioprocess Biosyst. Eng. 41 (2018) 395–405. doi:10.1007/s00449-017-1874-z.
- [3] V. Outram, C.-A. Lalander, J.G.M. Lee, E.T. Davis, A.P. Harvey, A comparison of the energy use of in situ product recovery techniques for the acetone butanol ethanol fermentation, Bioresour. Technol. 220 (2016) 590–600. doi:10.1016/j.biortech.2016.09.002.
- [4] V.H. Grisales Díaz, G. Olivar Tost, Techno-economic analysis of extraction-based separation systems for acetone, butanol, and ethanol recovery and purification, Bioresour. Bioprocess. 4 (2017) 12. doi:10.1186/s40643-017-0142-z.
- [5] X. Kong, A. He, J. Zhao, H. Wu, J. Ma, C. Wei, W. Jin, M. Jiang, Efficient acetone-butanol-ethanol (ABE) production by a butanol-tolerant mutant of *Clostridium beijerinckii* in a fermentation-pervaporation coupled process, Biochem. Eng. J. 105 (2016) 90–96. doi:10.1016/j.bej.2015.09.013.
- [6] V.H. Grisales Díaz, G. Olivar Tost, Butanol production from lignocellulose by simultaneous fermentation, saccharification, and pervaporation or vacuum evaporation, Bioresour. Technol. 218 (2016) 174–182. doi:10.1016/j.biortech.2016.06.091.
- [7] D. Yao, S. Dong, P. Wang, T. Chen, J. Wang, Z.B. Yue, Y. Wang, Robustness of *Clostridium*

- saccharoperbutylacetonicum* for acetone-butanol-ethanol production: Effects of lignocellulosic sugars and inhibitors, *Fuel*. 208 (2017) 549–557. doi:10.1016/j.fuel.2017.07.004.
- [8] T. Noguchi, Y. Tashiro, T. Yoshida, J. Zheng, K. Sakai, K. Sonomoto, Efficient butanol production without carbon catabolite repression from mixed sugars with *Clostridium saccharoperbutylacetonicum* N1-4, *J. Biosci. Bioeng.* 116 (2013) 716–721. doi:10.1016/j.jbiosc.2013.05.030.
- [9] R.-D. Li, Y.-Y. Li, L.-Y. Lu, C. Ren, Y.-X. Li, L. Liu, An improved kinetic model for the acetone-butanol-ethanol pathway of *Clostridium acetobutylicum* and model-based perturbation analysis, *BMC Syst. Biol.* 5 (2011) S12. doi:10.1186/1752-0509-5-S1-S12.
- [10] E.A. Buehler, A. Mesbah, Kinetic study of acetone-butanol-ethanol fermentation in continuous culture, *PLoS One*. 11 (2016) 1–21. doi:10.1371/journal.pone.0158243.
- [11] F. Raganati, A. Procentese, G. Olivieri, P. Götz, P. Salatino, A. Marzocchella, Kinetic study of butanol production from various sugars by *Clostridium acetobutylicum* using a dynamic model, *Biochem. Eng. J.* 99 (2015) 156–166. doi:10.1016/j.bej.2015.03.001.
- [12] J. Varner, D. Ramkrishna, Application of cybernetic models to metabolic engineering: Investigation of storage pathways, *Biotechnol. Bioeng.* 58 (1998) 282–291. doi:10.1002/(SICI)1097-0290(19980420)58:2/3<282::AID-BIT24>3.0.CO;2-D.
- [13] D.S. Kompala, D. Ramkrishna, N.B. Jansen, G.T. Tsao, Investigation of bacterial growth on mixed substrates: Experimental evaluation of cybernetic models, *Biotechnol. Bioeng.* 28 (1986) 1044–1055. doi:10.1002/bit.260280715.
- [14] H. Shinto, Y. Tashiro, M. Yamashita, G. Kobayashi, T. Sekiguchi, T. Hanai, Y. Kuriya, M. Okamoto, K. Sonomoto, Kinetic modeling and sensitivity analysis of acetone-butanol-ethanol production., *J. Biotechnol.* 131 (2007) 45–56.
- [15] H. Shinto, Y. Tashiro, G. Kobayashi, T. Sekiguchi, T. Hanai, Y. Kuriya, M. Okamoto, K. Sonomoto, Kinetic study of substrate dependency for higher butanol production in acetone–butanol–ethanol fermentation, *Process Biochem.* 43 (2008) 1452–1461. doi:10.1016/j.procbio.2008.06.003.
- [16] S. Tanaka, Y. Tashiro, G. Kobayashi, T. Ikegami, H. Negishi, K. Sakaki, Membrane-assisted extractive butanol fermentation by *Clostridium saccharoperbutylacetonicum* N1-4 with 1-dodecanol as the extractant., *Bioresour. Technol.* 116 (2012) 448–52. doi:10.1016/j.biortech.2012.03.096.
- [17] N. Leksawasdi, E.L. Joachimsthal, P.L. Rogers, Mathematical modelling of ethanol production from glucose / xylose mixtures by recombinant *Zymomonas mobilis*, *Biotechnol. Lett.* 23 (2001) 1087–1093. doi:10.1023/A:1010599530577.
- [18] M.S. Krishnan, N.W. Ho, G.T. Tsao, Fermentation kinetics of ethanol production from glucose and xylose by recombinant *Saccharomyces* 1400(pLNH33)., *Appl. Biochem. Biotechnol.* 77–79 (1999) 373–388. doi:10.1385/ABAB:78:1-3:373.
- [19] Y. Tashiro, K. Takeda, G. Kobayashi, K. Sonomoto, High production of acetone-butanol-ethanol with high cell density culture by cell-recycling and bleeding, *J. Biotechnol.* 120 (2005) 197–206.

- [20] J. Almquist, M. Cvijovic, V. Hatzimanikatis, J. Nielsen, M. Jirstrand, Kinetic models in industrial biotechnology – Improving cell factory performance, *Metab. Eng.* 24 (2014) 38–60. doi:10.1016/j.ymben.2014.03.007.
- [21] R. Flowers-Cano, R. Ortiz-Gómez, J. León-Jiménez, R. López Rivera, L. Perera Cruz, Comparison of Bootstrap Confidence Intervals Using Monte Carlo Simulations, *Water.* 10 (2018) 166. doi:10.3390/w10020166.
- [22] E. Koehler, E. Brown, S.J.-P.A. Haneuse, On the Assessment of Monte Carlo Error in Simulation-Based Statistical Analyses, *Am. Stat.* 63 (2009) 155–162. doi:10.1198/tast.2009.0030.
- [23] D.T. Jones, D.R. Woods, Acetone-butanol fermentation revisited., *Microbiol. Rev.* 50 (1986) 484–524. <http://www.ncbi.nlm.nih.gov/pubmed/3540574>.
- [24] N. Qureshi, J. Schripsema, J. Lienhardt, H.P. Blaschek, Continuous solvent production by *Clostridium beijerinckii* BA101 immobilized by adsorption onto brick, *World J. Microbiol. Biotechnol.* 16 (2000) 337–382.
- [25] H.G. Moon, Y.S. Jang, C. Cho, J. Lee, R. Binkley, S.Y. Lee, One hundred years of clostridial butanol fermentation, *FEMS Microbiol. Lett.* 363 (2016). doi:10.1093/femsle/fnw001.
- [26] S.-I. Baba, Y. Tashiro, H. Shinto, K. Sonomoto, Development of high-speed and highly efficient butanol production systems from butyric acid with high density of living cells of *Clostridium saccharoperbutylacetonicum*., *J. Biotechnol.* (2011) 4–11.
- [27] J. Zheng, Y. Tashiro, T. Yoshida, M. Gao, Q. Wang, K. Sonomoto, Continuous butanol fermentation from xylose with high cell density by cell recycling system, *Bioresour. Technol.* 129 (2013) 360–365. doi:10.1016/j.biortech.2012.11.066.

Tables

Table 1

Reaction rate terms

Rate term	Eq.	Rate term	Eq.
$r_1 = \frac{V_{max1}[Glucose][Biomass]}{Km_1 + [Glucose] + Km_1 \cdot ([Glucose]/Kma_1)^2} \cdot I_B^{n1} \cdot F$	(1-K)	$r_{10} = \frac{V_{max10}[AACoA][Biomass]}{Km_{10} + [AACoA]} \cdot F$	(10-K)
$r_2 = \frac{V_{max2}[Pyruvate][Biomass]}{Km_2 + [Pyruvate]} \cdot F$	(2-K)	$r_{11} = \frac{V_{max11}[AACoA][Butyrate][Biomass]}{([AACoA] + Kmb_{11})([Butyrate] + Kma_{11})} \cdot I_B^{n11}$	(11-K)
$r_3 = \frac{V_{max3}[Acetate][Biomass]}{Km_3 + [Acetate]} \cdot F \cdot I_B^{n3}$	(3-K)	$r_{12} = \frac{V_{max12}[Butyrate][Biomass]}{Km_{12}(1 + Kma_{12}/[Butyrate] + Kmb_{12}/[Butanol]) + [Butyrate]} \cdot F$	(12-K)
$r_4 = \frac{V_{max4}[Acetate][AACoA][Biomass]}{(Kma_4 + [Acetate]) \cdot (Kmb_4 + [AACoA])} \cdot F \cdot I_B^{n4}$	(4-K)	$r_{13} = \frac{V_{max13}[BCoA][Biomass]}{Km_{13} + [BCoA]} \cdot F$	(13-K)
$r_5 = \frac{V_{max5}[ACoA][Biomass]}{Km_5 + [ACoA]} \cdot F$	(5-K)	$r_{14} = \frac{V_{max14}[BCoA][Biomass]}{Km_{14}(1 + Kma_{14}/[Butyrate]) + [BCoA](1 + [Butanol]/Kmb_{14})} \cdot F$	(14-K)
$r_6 = \frac{V_{max6}[ACoA][Biomass]}{Km_6 + [ACoA]} \cdot F \cdot I_B^{n6}$	(6-K)	$r_{15} = \frac{V_{max15}[Xylose][Biomass]}{Km_{15} + [Xylose] + Km_{15} \cdot ([Xylose]/Kma_{15})^2} \cdot F \cdot I_B^{n15}$	(15-K)
$r_7 = \frac{V_{max7}[ACoA][Biomass]}{Km_7 + [ACoA]} \cdot F$	(7-K)	$r_{16} = \frac{V_{max16}[Xylose]}{Km_{16}(1 + Kma_{16}/[BCoA]) + [Xylose]} \cdot F \cdot I_{NH}$	(16-K)
$r_8 = \frac{V_{max8}[Glucose][Biomass]}{Km_8(1 + Kma_8/[BCoA]) + [Glucose]} \cdot I_{NH}$	(8-K)	$r_{17} = V_{max17}[Biomass]$	(17-K)
$r_9 = V_{max9}[Biomass]$	(9-K)	$I_B = \left(1 - \frac{[Butanol]}{[Bumax]}\right)$	(18-K)
$I_{NH} = \left(1 - \frac{[Acetate]}{[Acemax]}\right) \left(1 - \frac{[butyrate]}{[Butmax]}\right) \left(1 - \frac{[Biomass] + [Biomass\ dead]}{[Bimax]}\right) \left(1 - \frac{[Acetone]}{[Acemax]}\right) \left(1 - \frac{[Glucose]}{[Glumax]}\right) \left(1 - \frac{[Xylose]}{[Xymax]}\right) \cdot I_B^{n19}$			(19-K)

*The parameters of the model are defined in the nomenclature

Table 2

Model parameters (kinetic models A and B)

Parameter	Vmax		Km		Kma		Kmb		n	
Reaction	Kinetic		Kinetic		Kinetic		Kinetic		Kinetic	
	A	B	A	B	A	B	A	B	A	B
r ₁	1.2	1.16	0.09	0.050	54.3	4×10^{12}			1	1
r ₂	27	33.1	22	17.5						
r ₃	1.1	0.84	170	113					0	1
r ₄	156	34.1			312	198	17	8.40	0	2.75
r ₅	2.7	6.2×10^8	16.1	2×10^{10}						
r ₆	6.9	2.46	0.004	0.001					0	1
r ₇	144	50.4	1	2.24						
r ₈	0.68	0.92	71.9	83.7	0.0018	0.0013				
r ₉	0.042	0.034								
r ₁₀	79.1	59.8	8	3.6						
r ₁₁	0.19	0.23			0.081	0.062	0.0027	0.002	0	1
r ₁₂	59.3	38.3	1	1.36	290	113	1070	679.3		
r ₁₃	12.7	6.18	2.11	2.06						
r ₁₄	268	146	2.6	3.65	562	520	5.4	1.6×10^9		
r ₁₅	2.4	1.72	0.14	0.248	175.4	1×10^{13}			1	0.52
r ₁₆	0.13	0.14	0.96	1.47	65.1	14.02				
r ₁₇	0.0072	0.0088								
Additional parameters of kinetic model A and B										
Par.	A	B	Par.	A	B	Par.	A	B		
[Bumax]	212.0	210.7	[Acmax]	746.0	142.3	[Butmax]	120.50	87.48		
[Bimax]	581	581	[Glumax]	1829	916	[Acemax]	746.0	1.1×10^9		
[Xymax]	1940	3367	n ₁₉	1.00	1.73	ng/x	0.17	0.096		

^a n₁₁ was equal to 0.068 ± 0.006 if [Butanol] < 11.6 ± 1.1 g/l

Table 3

Coefficients of determination (R-Squared values) between the predicted species concentrations from kinetic model B and the experimental data reported by [7,13–15]

Initial xylose (g l ⁻¹)	Initial glucose (g l ⁻¹)	Xylose	Glucose	Butanol	Acetone	Acetate	Biomass	Butyrate	Average
-	53		0.964	0.957	0.959	0.917	0.739	0.838	0.896
-	22		0.995	0.992	0.994	0.957	0.871	0.735	0.924
-	13		0.968	0.974	0.991	0.975	0.880	0.817	0.934
-	7		0.998	0.996	0.994	0.985	0.980	0.846	0.966
-	89		0.995	0.991	0.997	-	0.946	-	0.982
44	-	0.998	0.984	0.998	0.952	0.841	0.676	0.949	0.900
17	-	0.978	-	0.987	0.958	0.674	0.808	0.809	0.847
10	-	0.981	-	0.977	0.956	0.960	0.969	0.953	0.963
6	-	0.956	-	0.997	0.961	0.950	0.953	0.900	0.952
32	31	0.978	0.999	0.990	0.957	-	0.851	-	0.933
Average		0.983	0.984	0.986	0.972	0.907	0.867	0.856	0.930

Table 4

Parameter confidence intervals (kinetic model B)

Parameters	Vmax		Km		Kma		Kmb		n	
Reaction	LB	HB	LB	HB	LB	HB	LB	HB	LB	HB
r1	1.15	1.17	0.049	0.051	-4×10^{11}	$8.E+12$			1 ^a	1 ^a
r2	32.5	33.6	17.4	17.5						
r3	0.82	0.85	112	115					1 ^a	1 ^a
r4	33.3	34.9			192	203	8.22	8.57	2.67	2.84
r5	-6×10^8	2×10^9	-2×10^{10}	5×10^{10}						
r6	2.4	2.5	0.0009	0.001					1 ^a	1 ^a
r7	49.4	51.3	2.20	2.28						
r8	0.91	0.9	82.3	85.1	0.0013	0.0013				
r9	0.033	0.03								
r10	58.5	61	3.52	3.64						
r11	0.23	0.24			0.06	0.063	0.0019	0.002	1 ^a	1 ^a
r12	37.6	39.1	1.32	1.40	112	113	669	690		
r13	6.1	6.3	2.03	2.09						
r14	143.7	148.8	3.59	3.70	510	530	-7×10^8	4×10^9		
r15	1.70	1.74	0.20	0.30	-5×10^{12}	3×10^{13}			0.51	0.53
r16	0.14	0.15	1.20	1.74	11	18.4				
r17	0.006	0.0075								
Additional parameters (Par.) of kinetic model B										
Par.	LB	HB	Par.	LB	HB	Par.	LB	HB		
[Bumax]	210	212	[Acmax]	141	144	[Butmax]	85.5	89.4		
[Bimax]	581 ^a	581 ^a	[Glumax]	905	926	[Acemax]	-1×10^9	3×10^9		
[Xymax]	3087	3647	n ₁₉	1.7	1.8	ng/x	0.09	0.10		

^a Parameters were fixed in kinetic fitting^b LB, lower bound; UB upper bound.

Figures

Fig. 1. The reactor and biochemical network. a) A general representation of the reactor for batch and continuous operation with and without biomass recycle and bleeding. b) The proposed biochemical reaction network for simultaneous saccharification and ABE fermentation. In the network there are thirteen species (Glucose, xylose, acetyl-CoA, butyryl-CoA, Pyruvate, acetoacetyl-CoA, acetate, butyrate, biomass, dead biomass, acetone, butanol and ethanol) and seventeen rate expressions, r_i .

Fig. 2. Algorithm used to try ensuring a minimum global

Fig. 3. Species concentration profiles for fermentations that used mixed substrates and high glucose concentration. The continuous lines show the predictions obtained using kinetic model A (a, b and c) and kinetic model B (d, e, f). Experimental data [8,16]: Circles (butanol (a, b, d and e) and biomass (c, f)), diamond (glucose), triangle (acetone (C and D) and xylose (A and B)). Biomass concentration was calculated in relation to the optical density (OD) reported by Noguchi et al. [8] and Tanaka et al. [16]. OD of 1.0 was equivalent to 0.301 g biomass l⁻¹ [27]

Fig. 4. Concentration profiles for glucose (a, b and c) and xylose (d, e and f) fermentations.

Individual glucose (F1, F2, F3 and F4) and xylose (F5, F6, F7 and F8) fermentations. Initial concentrations of substrate F1 (53.1 g l^{-1}), F2 (22 g l^{-1}), F3 (12.7 g l^{-1}), F4 (6.5 g l^{-1}), F5 (43.8 g l^{-1}), F6 (17.4 g l^{-1}), F7 (9.9 g l^{-1}) and F8 (6.1 g l^{-1}). Experimental data [14,15]: diamond (F1 (60) and F5), triangle (F2 and F6), open circle (F3 and F7), and closed circle (F4 and F8).

Continuous and dashed lines are the predictions obtained using kinetic model B

Fig. 5. Concentration profiles for acetate, butyrate and biomass. Xylose fermentations (a, b and c) and glucose fermentations (d, e and f). Experimental data [14,15]: diamond (F1 and F5), triangle (F2 and F6), open circle (F3 and F7), and closed circle (F4 and F8). Continuous and dashed lines are the predictions obtained using kinetic model B

Fig. 6. Prediction of biomass concentration and ABE productivity using kinetic model B (continuous fermentation) for a range of dilution rates. The substrates were glucose (50 g l^{-1}) and acetate (3 g l^{-1}). Points are simulations and diamonds are the experimental data reported by [19]. Black points are the simulations using the nominal parameter values for kinetic model B. The light blue points are calculated by randomly varying the model parameters between their respective standard deviations. The dark blue line is the prediction interval.

Fig. 7. Prediction of biomass concentration and ABE productivity using kinetic model B (continuous fermentation with recycle) for a range of biomass recirculation rates. The substrates are glucose (50 g l⁻¹) and acetate (3 g l⁻¹). The dilution rate was 0.85 h⁻¹. The parameters of kinetic model B were varied randomly between their respective standard deviations. The dark blue line is the prediction interval. The points are simulations and diamonds are the experimental data reported by [19].

Fig. 8. Model predictions of biomass concentration, ABE productivity and yield using kinetic model B (continuous fermentation with recycle) for a range of biomass recirculation rates and dilution rates. The substrates are glucose (50 g l^{-1}) and acetate (3 g l^{-1}). The nominal model is used to generate these results, i.e. the parameters reported in Table 2 for kinetic model B. The arrows are used to indicate which b_r was used in every simulation and experiment.