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

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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 (~90 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 g l^{-1} h^{-1} (10 times higher than that without biomass recycling), consistent with experimental results.

1. 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 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 g^{-1} l^{-1} 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

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Nomenclature

CCR	carbon catabolite repression (-)
D	dilution rate (h^{-1})
Km_j	kinetic model constant for reaction 'j' ($mmol\,l^{-1}$)
Kma_j	kinetic model constant for reaction 'j' ($mmol\,l^{-1}$)
Kmb_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' ($mmol\,l^{-1}\,h^{-1}$)
R_i	net rate of production/consumption of species 'i' ($mmol\,l^{-1}\,h^{-1}$)
V	volume of the reactor (l)
V_{maxi}	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' ($g\,l^{-}$)
[c_{ik}]	concentration of the species 'i' in data set 'k' ($mmol\,l^{-1}$)
[C_{oi}]	inlet concentration of species 'i' ($g\,l^{-}$)
[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'

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. 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 (Dp) 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 (Db) plus dilution rate of permeate is assumed to be equal to the total dilution rate (D), Eq. (3).

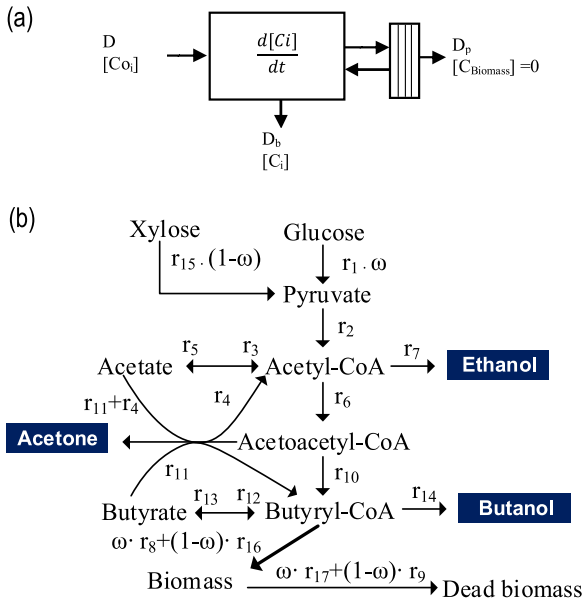

Fig. 1. The reactor and biochemical network. a) A general representation of the reactor for batch and continuous operation with and without biomass recycle and bleeding. b) The proposed biochemical reaction network for simultaneous saccharification and ABE fermentation. In the network there are thirteen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

$$\frac{d[Ci]}{dt} = R_i \cdot MW_i + D \cdot [Co_i] - \left(D_p \cdot a_i + D_b\right) \cdot [Ci] \quad (1)$$

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

$$D = Dp + Db \quad (3)$$

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,

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

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

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

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

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

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

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