H. I Velázquez-Sánchez[1] / G. Lara-Cisneros[2] / R. Femat[3] / R. Aguilar-López[1]

Dynamic Nonlinear Feedback Control Applied to Improve Butanol Production by *Clostridium acetobutylicum*

[1] Departamento de Biotecnología y Bioingeniería. Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional. Av. Instituto Politécnico Nacional No. 2508, Colonia San Pedro Zacatenco, 07360, Ciudad de México, México, E-mail: raguilar@cinvestav.mx
[2] Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí. Av. Dr. Manuel Nava 6, Zona Universitaria, C.P. 78210, San Luis Potosí, S.L.P. México
[3] División de Matemáticas Aplicadas. Instituto Potosino de Investigación Científica y Tecnológica. Camino a la Presa San José 2055. Col. Lomas 4 sección, CP. 78216. San Luis Potosí S.L.P. México

Abstract:
The goal of this work is to present a closed-loop operational strategy in order to improve the butanol production in an anaerobic continuous bioreactor for the called Acetone-Butanol-Ethanol (ABE) process. The proposed control scheme considers a class of feedback signal which includes a nonlinear bounded function of the regulation error. The control scheme is applied to a phenomenological unstructured kinetic model obtained from an experimental and metabolic study of butanol production by *Clostridium acetobutylicum*, which allows the proposed structure to predict several operational conditions from batch and continuous regimes. Numerical experiments using the proposed model considering continuous operation were performed in order to find a feasible operating region for maximum butanol production at open-loop regime. The proposed methodology is applied to regulate the product concentration, manipulating the dilution rate to lead to a higher butanol productivity. The closed-loop behaviour of the bioreactor is analysed, finding that the proposed controller minimizes the response time of the system and allows it to achieve a productivity gain of 55 % over open-loop operation. Further numerical experiments show the satisfactory closed-loop performance of the proposed methodology in comparison with a PI controller.

Keywords: biofuel, butanol, clostridium, process intensification, nonlinear feedback
DOI: 10.1515/ijcre-2017-0034

1 Introduction

Acetone, butanol and ethanol (ABE) fermentation by *Clostridium acetobutylicum* is one of the oldest known industrial fermentations. It was ranked second only to ethanol fermentation by yeast in its scale of production, and is one of the largest biotechnological processes ever known. Butanol is an important industrial solvent and potentially a better fuel extender than ethanol (Wallner, Ickes, and Lawyer 2012). In a typical ABE fermentation using glucose as carbon source, butyric, lactic and acetic acids by *C. acetobutylicum*, in the culture medium the pH drops and undergoes a metabolic shift, and butanol, acetone and ethanol are formed; however the butanol yield from glucose is low, typically around 15 percent w/w (Qureshi and Ezeji 2008). ABE fermentation is severely limited by product inhibition as even butanol concentrations over one percent can significantly impact cell growth and consequently the fermentation process.

Optimizing the ABE fermentation process has long been a goal of the industry. The currently developed pilot processes include cell recycling and cell immobilization reactors in order to increase cell density, productivity, and using *in situ* extractive fermentations to minimize product inhibition. However, despite the above mentioned efforts, the best results ever obtained for ABE fermentations, to date, are still less than two percent in butanol concentration, 4.46 g L^{-1} h^{-1} productivity, and a solvent yield of less than 25 % from glucose, and therefore had proven that they aren't efficient enough in relation to the time and effort required for their implementation (Qureshi, Saha, and Cotta 2007).

An approach to solve the above drawback is to generate novel kinetic models that could allow to design more efficient process schemes. However, as it is well known that biological reactors are typically governed

R. Aguilar-López is the corresponding author.

by highly nonlinear behaviour occurring on both, macroscopic reactor scale and microscopic cellular scale, dynamic simulation is an important and useful tool for model validation, but some drawbacks can be identified; for systems with slow transient behaviour, the dynamic analysis is inefficient and potentially inconclusive. From the above it is important to find new ways to analyse such systems that could provide a more detailed overview of their performance under a wide array of operational conditions.

Bifurcation analysis is a powerful tool for evaluating time depending models applied to continuous bioreactors. The objective of bifurcation theory is to characterize changes in the qualitative dynamic behaviour of a nonlinear system as key parameters are varied, generating a picture of the model behaviour in the form of a bifurcation diagram, which can be used to determine if the model supports the steady-state and dynamic behaviour observed experimentally. When this analysis is applied considering key parameters as the bifurcation ones it's possible to predict the set of operative conditions that could lead the system to a set of high production steady states and discern which one of them is stable or not.

Additionally, a continuous bioreactor control is usually to avoid washout which could cause the reaction to stop so it can maintain a desirable production rate. This may be done by closing one feedback loop and controlling cell mass or substrate concentrations (Zhao and Skogestad 1997).

In this regard, the early successful application control strategy in process control is in the evolution of the PID controller with the traditional Ziegler-Nichols tuning method (Ziegler and Nichols 1942). Till nowadays, a high percent of the controllers implemented in the process industries are PID-type (Nikačević et al. 2012). However since the high-nonlinear behaviour of bioprocess and the adaptive mechanism of the microorganisms, is need to design novel control algorithms for improving the bioreactor yield and ensure a safety operational regime (Spear 2005; Munasinghe and Nakamura 2007; Xu and Yu 2010). Recently, alternative bounded functions have been proposed for estimation and control purposes with satisfactory performance (López-Pérez, Neria-González, and Aguilar-López 2015; Aguilar-López et al. 2016).

Therefore, in this paper a novel nonlinear controller with a class of bounded sigmoid output feedback in order to provide stabilization to a class of continuous bioreactor for butanol production is designed. The controller is aimed to regulate the butanol concentration within the reactor to the corresponding set point and thus lead the butanol productivity to higher values. The results validation was done by comparing the reactor's overall maximum theoretical productivity under open loop, versus the effect of the proposed control law at closed loop operation.

2 Methodology

2.1 Process modelling

Biochemical reactions are generally modelled by the so-called unstructured kinetic models, such structures make use of empirical mathematical structures defined as specific cell growth rates in order to describe cell growth and then, to represent the corresponding substrates consumption and products generation a pseudo-stoichiometric mass balance is used. However, this approach is generally unsuitable to predict accurately the fermentation behaviour in continuous and semi-continuous regimes, as they usually require multiple parametric sets to account for the effect of the switch of operational conditions (Eom et al. 2015; López, Passeggi, and Borzacconi 2015). In order to avoid the above mentioned drawbacks, an alternative modelling approach for biochemical systems, named phenomenological kinetic modelling, which is based on a general analysis of the metabolic pathways to propose particular kinetic models for the selected chemical species is able to predict with most accuracy the dynamic behaviour of batch, continuous and semi-continuous chemical reactors (Velázquez-Sánchez, Montes-Horcasitas, and Aguilar-López 2014). From the above, a novel phenomenological Non-Structured kinetic model was developed to describe the kinetic behaviour of ABE production in batch reactor. The mathematical model is based on classical mass balance approach and it was considered as a benchmark production plant by extending it to simulate continuous operation. The prior assumption is valid as long as the reactor can be operated near perfect mixing conditions, which can be scaled up reliability at least up to pilot level as validated experimentally for similarly modelled systems (Sforza, Enzo, and Bertucco 2014; Ariyajaroenwong et al. 2016):

Biomass reaction rate:

$$\mu_X = \left(\mu_{maxX} * \left(\frac{Sg}{kSg + Sg} \right) * \left(\frac{1}{1 + \left(\frac{But}{kBut} \right)} \right) * \left(1 - \left(\frac{Sg}{ksi} \right) \right) \right) \quad [1]$$

Butanol reaction rate:

$$r_{But} = \left(r_{maxBut} * \left(\frac{Sb}{kSb + Sb}\right)\right) \quad [2]$$

Butyrate reaction rate:

$$r_{Sb} = \left(r_{maxSb} * \left(\frac{Sg}{kSgSb + Sg}\right) * \left(\frac{1}{1 + \left(\frac{But}{kBut}\right)}\right)\right) - \frac{\left(r_{maxBut} * \left(\frac{Sb}{kSb+Sb}\right)\right)}{YButSb} \quad [3]$$

Acetone reaction rate:

$$r_{Ace} = \left(r_{maxAce} * \left(\frac{Act}{kAA + Act}\right)\right) \quad [4]$$

Acetate reaction rate:

$$r_{Act} = \left(r_{maxAct} * \left(\frac{Sg}{kSgAct + Sg}\right) * \left(\frac{1}{1 + \left(\frac{Ace}{kiAce}\right)}\right)\right) - \frac{\left(r_{maxAce} * \left(\frac{Act}{kAA+Act}\right)\right)}{YAceAct} \quad [5]$$

Ethanol reaction rate:

$$r_{Et} = \left(r_{maxEt} * \left(\frac{Sg}{kSgEt + Sg}\right)\right) \quad [6]$$

Glucose mass balance:

$$\frac{dSg}{dt} = D\left(Sg_{in} - Sg\right) - \left(\frac{\mu_X \cdot X}{Y_{\frac{X}{Sg}}}\right) \quad [7]$$

Biomass mass balance:

$$\frac{dX}{dt} = -DX + \left(\mu_X - k_{Spo}\right) * X \quad [8]$$

Butanol mass balance:

$$\frac{dBut}{dt} = -D\left(But\right) + r_{But} * X \quad [9]$$

Butyrate mass balance:

$$\frac{dSb}{dt} = -D\left(Sb\right) + r_{Sb} * X \quad [10]$$

Acetate mass balance:

$$\frac{dAct}{dt} = -D\left(Act\right) + r_{Act} * X \quad [11]$$

Acetone mass balance:

$$\frac{dAce}{dt} = -D\left(Ace\right) + r_{Ace} * X \quad [12]$$

Ethanol mass balance:

$$\frac{dEt}{dt} = -D\left(Et\right) + r_{Et} * X \quad [13]$$

The kinetic model is composed of 6 kinetic rates (1–6) and a set of seven differential eqs (7–13) describing the corresponding mass balances for Glucose (*Sg*), Biomass (*X*), Butanol (*But*), Butyrate (*Sb*), Acetate (*Act*), Acetone (*Ace*) and Ethanol (*Et*) respectively. The dilution rate is defined as $D = F/V$, where F stands for the volumetric flow of the feeding solution and V stands for the volume of culture medium within the reactor; and Sg_{in} is the glucose concentration within the feeding solution.

It should be noted that even if the carbon source feeding the bioreactor is obtained from agroindustrial wastes there is always the need to perform saccharification processes in order to obtain assimilable carbohydrates for bacterial growth, such as xylose, fructose, glucose, etc., where the former has been chosen for this

analysis due the wider knowledge about its catabolic pathway within the *Clostridium* cells. Therefore, the analysis of the simplified metabolic pathway of *Clostridium acetobutylicum* reported by Haus et al. (2011) which is shown in Figure 1, was used to propose the mathematical structures representing the reaction rates within the system. It describes that the metabolism of glucose oxidation is carried out in two sequential phases, one phase called acidogenesis, carried out by vegetative cells, which includes from the glycolysis pathway up to the formation of Acetyl CoA and its subsequent oxidation to organic acids such as butyric, lactic and acetic; and another metabolic pathway called solventogenesis, performed in conjunction with the metabolism that triggers the process of sporulation, which starts from the reincorporation of the organic acids mentioned above into the cell and culminates with its transformation into acetone and butanol. In the case of ethanol, it is reported in the literature that its production is constitutive, regardless of the metabolic state of the crop (Jones and Wood 1986). It is also important to note that the strain *Clostridium acetobutylicum* ATCC 824 has not been genetically modified to inhibit its sporulation process, so this effect was also considered within the equation construction.

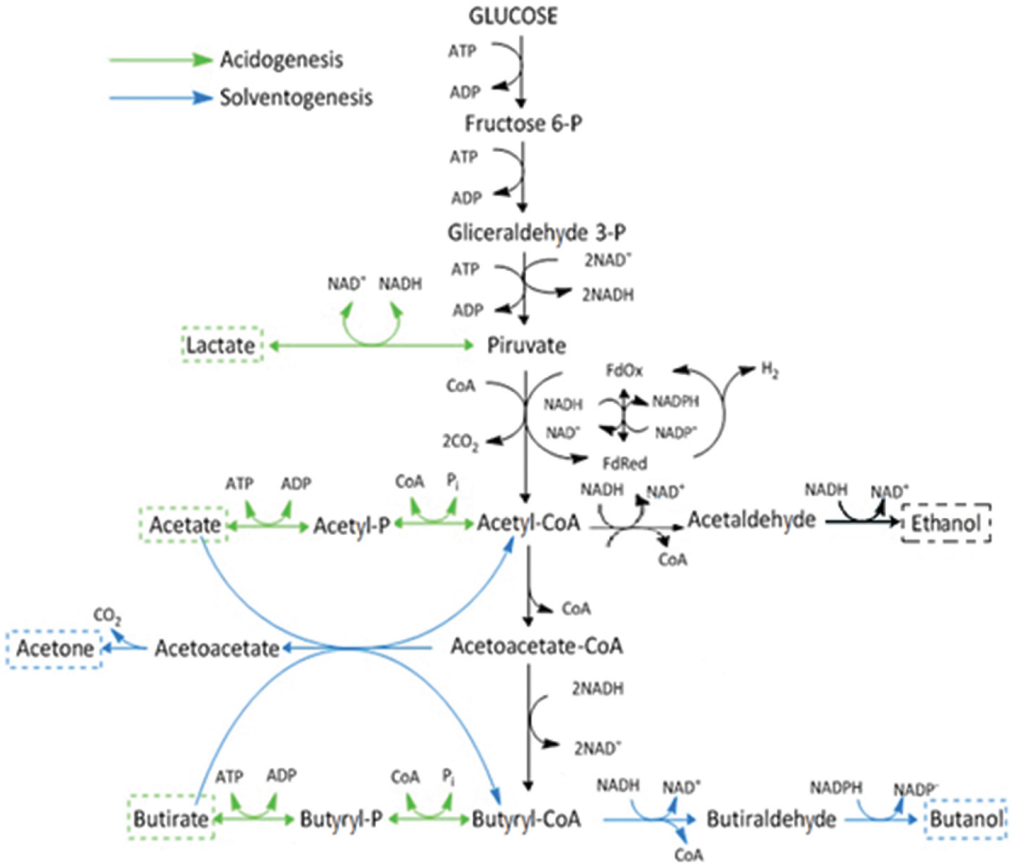

Figure 1: Diagram of the ABE metabolic pathway reported by Haus et al. (2011).

The parametric identification of the proposed model was made via the Marquardt algorithm into the software ModelMaker® 3.0.3. Experimental data was obtained from a fermentation system reported by Yen and Li (2011), which consisted of a stirred tank reactor of 1 L nominal volume working with 600 mL of P2 medium with glucose as the main carbon source and inoculated with cells of *Clostridium acetobutylicum* ATCC 824, operated under two different operating regimes: batch and continuous respectively.

2.2 Design of the proposed controller

Let us to write the system (8)–(13) in the following form:

$$\dot{z} = f(z) + \ell(z)\, U \qquad [14]$$

Here $z \in \mathbb{R}^n_+$ is the corresponding state vector. The system (14) satisfies the Bounded Input Bounded State (BIBS) property (Basting and Dochain 1990), therefore the corresponding trajectories remain bounded. We will assume that the vector field $f(z) : \mathbb{R}^n \to \mathbb{R}^n$ is continuously differentiable in some positive invariant domain $\Omega \subset \mathbb{R}^7$ with; $f(0) = 0$; and $\sup f(z) = L$; $\forall z$, *with* $L < \infty$ and $\sup \ell(z) = G < \infty$.

Where:

$$z = \left[S_g, X, But, S_b, Act, Ace, Et\right] \in \Omega$$

$$f(z) = \begin{bmatrix} -\left(\frac{\mu_X \cdot X}{Y_{\frac{X}{Sg}}}\right) \\ \left(\mu_X - k_{Spo}\right) * X \\ (r_{But} X) \\ r_{Sb} X \\ (r_{Act} X) \\ (r_{Ace} X) \\ (r_{Et} X) \\ \\ \end{bmatrix}$$

$$\ell(z) = \begin{bmatrix} (Sg_{in} - Sg) \\ -X \\ -But \\ (Sb_{in} - Sb) \\ -Act \\ -Ace \\ -Et \end{bmatrix}$$

$$U = D$$

Defining z_{sp} as the required trajectory, where *sp* means set point. Defining the control error as$\varepsilon = z - z_{sp}$, from the above, the expression for the error dynamics is the following, considering a regulation control case, i. e. z_{sp} = constant.

$$\dot{\varepsilon} = f(z) + \ell(z)\, U \qquad [15]$$

Proposition 1. The following control input stabilize the system (14) around a desired set point within the domain physically realizable:

$$\begin{aligned} U &= k_0 g \\ \dot{g} &= -\tfrac{1}{2}\left(\tanh\left(\tfrac{\varepsilon - a_1}{w_1}\right) - \tanh\left(\tfrac{\varepsilon - a_2}{w_2}\right)\right) \end{aligned} \qquad [16]$$

with k_0, w_1, w_2 R.

Sketch of Proof. In order to prove the closed-loop system's stability, let us to consider the dynamic equation of the control error ε, as follows:

$$\begin{aligned} \dot{\varepsilon} &= f(z) + \ell(z)\, k_0 g \\ \dot{g} &= -\tfrac{1}{2}\left(\tanh\left(\tfrac{\varepsilon - a_1}{w_1}\right) - \tanh\left(\tfrac{\varepsilon - a_2}{w_2}\right)\right) \end{aligned} \qquad [17]$$

Now a Lyapunov based formulation for the stability analysis of the dynamics of system (15). The concept of stability in the sense of Lyapunov is closely related to that of continuity of solutions of dynamical systems. An equilibrium is stable if all solutions starting at nearby points stay nearby; otherwise, it is unstable. It is asymptotically stable if all solutions starting at nearby points not only stay nearby, but also tend to the equilibrium point as time approaches infinity.

For several classes of dynamical systems described by of ordinary differential equations, the existence of the named Lyapunov functions is a necessary and sufficient condition for stability. Whereas there is no general techniques for constructing Lyapunov functions for ordinary differential equations, in several specific cases, the construction of Lyapunov functions can be known. For instance, quadratic-type functions suffice for dynamic systems related with conservation laws can often be used to construct Lyapunov functions for physical systems (Khalil 1996).

The following Lyapunov candidate function:

$$L = \varepsilon^T J \varepsilon = \varepsilon_J^2, J = J^T >; 0 \qquad [18]$$

Note that the proposed Lyapunov function is a standard quadratic form of the control error, where J is a positive definite symmetric matrix; therefore $L(0) = 0$ and $L(\varepsilon) >; 0$ for $\varepsilon \neq 0$ as required (Bacciotti and Rosier 2005).

In general, the time derivative along the trajectories of (15) is calculated as:

$$\dot{L} = \dot{\varepsilon}^T J\varepsilon + \varepsilon^T J\dot{\varepsilon} \quad [19]$$

In order to assure stability to the system (15), must be proved that eq. (19) is negative semi-definite in accordance with the Lyapunov criteria (Haddad and Chellaboina 2008):

Now, replacing eq. (15) in eq. (19):

$$\dot{L} = (f(z) + \ell(z)u)^T J\varepsilon + \varepsilon^T J(f(z) + \ell(z)u) \quad [20]$$

From the symmetric characteristics of the algebraic terms of eq. (20), the following can be obtained:

$$\dot{L} = 2J\varepsilon f(z) + 2\varepsilon^T J\ell(z)u \quad [21]$$

$$\dot{L} = 2[f(z) + \ell(z)u]J\varepsilon \quad [22]$$

Now replacing the proposed control law (16), and considering the corresponding integral form for the term g, in eq. (22):

$$\dot{L} = 2\left[f(z) - \ell(z)\left(k_0\int_0^t\left(\frac{1}{2}\left[tanh\left(\frac{\varepsilon - a_1}{w_1}\right) - tanh\left(\frac{\varepsilon - a_2}{w_2}\right)\right]\right)d\sigma\right)\right]J\varepsilon \quad [23]$$

Now, let us to consider the following auxiliary differential equation:

$$\dot{L}_m = 2[L - Gk_0]\varepsilon_J \quad [24]$$

With L and G are quadratic matrix, can be observed that eq. (24) take in account the upper bounds of eq. (23). Here, considering that $sup\int_0^t\left(\frac{1}{2}\left[tanh\frac{\varepsilon-a_1}{w_1} - tanh\frac{\varepsilon-a_2}{w_2}\right]d\sigma\right) = 1$, this is a consequence of the above integral term is a class of sigmoid function and $\|^\circ\|$ is defined as the standard Euclidian norm.

Note that k_0 is the control´s gain, if it is chosen as:

$$Gk_0 > L \quad [25]$$

or:

$$k_0 > (G)^{-1}L \quad [26]$$

Then:

$$\dot{L}_m = 2[L - \mathrm{k_0G}]\varepsilon_J \leq 0 \quad [27]$$

From the comparison theorem of differential equations (Budincevic 2010; Reid 1969; Kirkiliones and Walcher 2004; McNabb 1986), it assert particular properties of solutions of a differential equation (or of a system) provided that an auxiliary equation/inequality (or a system) possesses a certain property, as stability in this case, therefore the following holds:

$$2\left[f(z) - \ell(z)\left(k_0\int_0^t\left(\frac{1}{2}\left[tanh\frac{\varepsilon - a_1}{w_1} - tanh\frac{\varepsilon - a_2}{w_2}\right]\right)d\sigma\right)\right]J\varepsilon < 2[L - Gk_0]\varepsilon_J \quad [28]$$

Therefore is concluded that:

$$\dot{L} < \dot{L}_m \quad [29]$$

Finally, from eq. (27):

$$\dot{L} \leq 0 \quad [30]$$

The above, when $\dot{L}_m$ considers the gains selection given by eq. (26). Note that this results show that the closed-loop behaviour of the system (14) under the controller (16) presents stable behaviour for the system trajectories who lies in the conic sector provided by eq. (18), in accordance with the Lyapunov second method criteria (Bacciotti and Rosier 2005). Therefore can be concluded that the proposed controller provide semi-global closed-loop stability.

2.3 Numerical simulations

All the numerical simulations were made into a PC equipped with an Intel® Xeon© E5450 processor and 8 GB of RAM into the 64-bits version of the MATLAB® 2016a software, first using the ODE Solver library's command ode15s to solve the system's set of differential equations at an undefined time-span assuming continuous mode operation until steady state was observed, then for bifurcation analysis the system was solved employing the Matcont 5p0 toolbox for the generation of the phase diagrams considering the reactor's dilution rate (D) and glucose feeding concentration (Sg_{in}) as bifurcation parameters.

3 Results and discussion

3.1 Model validation

Table 1 summarizes the values obtained by the results of the parametric identification. The validation of the kinetic model was made by numerical simulation considering the following initial conditions: $X_0 = 0.2$ Kg m^{-3}, $Sg_0 = 60$ Kg m^{-3}, $But_0 = 0.01$ Kg m^{-3}, $Sb_0 = 0.01$ Kg m^{-3}, $Ace_0 = 0.01$ Kg m^{-3}, $Act_0 = 0.01$ Kg m^{-3} and $Et_0 = 0.01$ Kg m^{-3}, and operational conditions for $D = 0.054$ h^{-1} and $Sg_{in} = 60$ Kg m^{-3} (Yen and Li 2011), where the mathematical model represents the dynamic behaviour of the analysed variables with a linear correlation index $r^2 = 0.9952$ and $r^2 = 0.9710$ over experimental data for batch and continuous culture respectively, and a p-value < 0.001 which ensures there is not a significant difference between the predicted behaviour described by the proposed model and the experimental data (Sellke, Bayarri and Berger, 2011). The results are showcased in Figure 2.

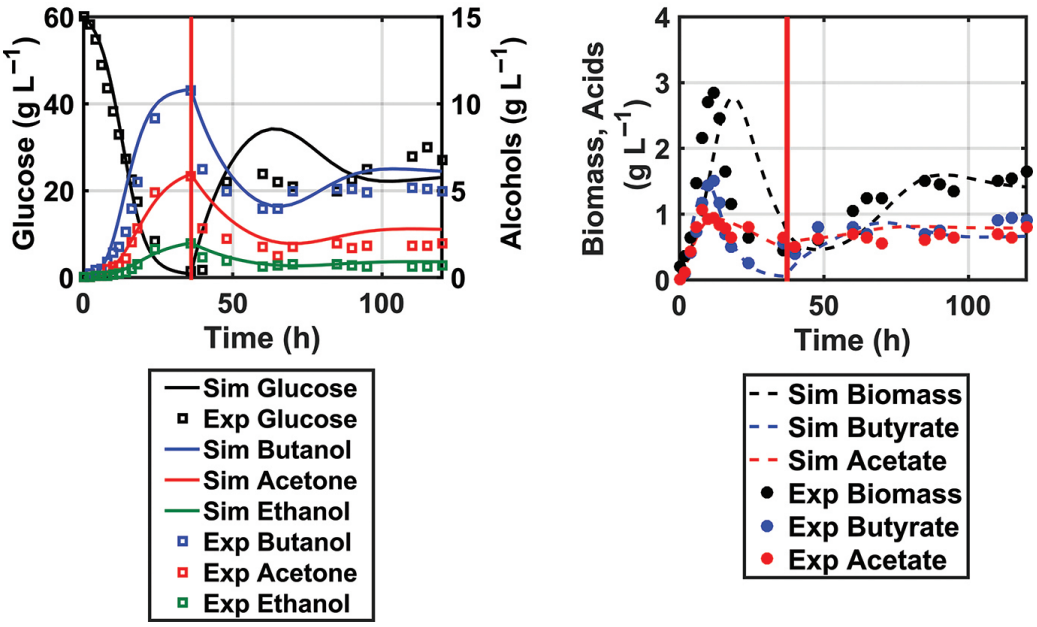

Figure 2: Validation of the proposed kinetic model versus *Clostridium acetobutylicum* ATCC 824 experimental data reported by Yen and Li (2011): Batch fermentation, $r^2 = 0.9995$ and continuous fermentation, $r^2 = 0.9710$. Horizontal line indicates the switch between batch and continuous regime (36 h).

Table 1: Parametric identification of the proposed model considering experimental data reported by Yen and Li (2011).

Parameter	Value	Units	Parameter	Value	Units
k_{AA}	4.3115 ± 13.446	Kg m^{-3}	v_{maxAce}	0.6687 ± 0.1764	h^{-1}
k_{But}	8.5 ± 2.7447	Kg m^{-3}	v_{maxAct}	0.4716 ± 0.1078	h^{-1}
k_{Spo}	0.099 ± 0.0615	h^{-1}	v_{maxBut}	0.9949 ± 0.3656	h^{-1}
k_{iAce}	25 ± 2.9482	h^{-1}	v_{maxEt}	0.0340 ± 0.0025	h^{-1}
k_{Sb}	2.25 ± 1.2741	Kg m^{-3}	v_{maxSb}	0.9949 ± 0.1162	h^{-1}
k_{Sg}	24.9999 ± 2.9178	Kg m^{-3}	μ_{max}	0.6 ± 0.1211	h^{-1}

k_{SgAct}	0.5942 ± 0.0542	Kg m^{-3}	Y_{AceAct}	0.2681 ± 0.0775	Kg Kg^{-1}
K_{SgEt}	0.0535 ± 0.0045	Kg m^{-3}	Y_{ButSb}	0.6 ± 0.0941	Kg Kg^{-1}
k_{SgSb}	9.9962 ± 3.2172	Kg m^{-3}	Y_{XSg}	0.1107 ± 0.0417	Kg Kg^{-1}
k_{si}	250.9999 ± 17.7785	Kg m^{-3}			

The high parametric uncertainty observed in some estimated values can be attributed to the fact that experimental data is measured with finite accuracy and only a subset of the state variables is accessible experimentally in an *in-line* manner, as samples must be obtained from the culture and processed *off-line*, this coupled with the inherent non-linearity nature of biological systems can be the main causes for such results (Vanlier et al. 2013); however key parameters like the maximum specific growth rate (μ_{max}) are indeed within values reported into literature for *Clostridium* bacteria growing using glucose as main carbon source (Procentese et al. 2014; Raganati et al. 2015).

To further reinforce the validation of the proposed kinetic model for its use as an adequate basis for the analysis of ABE fermentation additional numerical simulations were made to compare its performance against experimental values reported by Yen and Li (2011) considering a bioreactor operating in continuous culture under different feeding glucose concentrations (Table 2) and also varying the reactor's dilution rate (Table 3), where it should be noted that the model is able to reproduce the phenomenological behaviour of the culture for butanol production, total acid production and total solvent production.

Table 2: Performance comparison of the proposed kinetic model against experimental data reported by Yen and Li (2011) for butanol, total acids and total solvent production under continuous regime at different feeding glucose concentrations at D = 0.027 h^{-1}.

	Feeding glucose concentration			
	60 Kg m^{-3}		**80 Kg m^{-3}**	
	Yen and Li (2011)	This work	Yen and Li (2011)	This work
Total ABEs	17.25	13.54	17.44	18.58
Butanol	8.94	7.68	9.94	10.63
Total Acids	1.07	2.50	1.63	2.46

Table 3: Performance comparison of the proposed kinetic model against experimental data reported by Yen and Li (2011) for butanol, total acids and total solvent production under continuous regime at different dilution rates at Sg_a = 60 Kg m^{-3}.

	Dilution rate			
	D = 0.054 h^{-1}		**D = 0.107 h^{-1}**	
	Yen and Li (2011)	This work	Yen and Li (2011)	This work
Total ABEs	12.9	12.79	11.28	11.34
Butanol	8.07	7.33	6.82	6.62
Total Acids	1.35	2.57	1.72	2.71

It is noteworthy that the proposed kinetic model attained an acceptable reproduction performance of the fermentation system using only the parameter set reported at the beginning of this section, which showcases that the mathematical representation of the metabolic pathway is more than adequate to cope with changes in the operational conditions of the bioreactor, however as industrial processes are moving towards the use of agroindustrial waste as raw material for ABE fermentation systems there is also the need to ensure that the structure could remain as a viable choice under such scenarios. To assess such issue Jiang et al. (2014) and Raganati et al. (2015) reported that *Clostridium* cultures are indeed affected by changes in the utilised carbon source, but the main effect was over butanol yield and fermentation time caused by a different affinity for every substrate different to glucose which mostly impacted just into its observed μ_{max}, which suggests that the proposed structure can be adapted to function considering xylose, sucrose or sugarcane juice as substrates by just the re-identification of the kinetic parameters.

3.2 Bifurcation analysis

As a first step in the construction of the aforementioned bifurcation diagrams, numerical simulations were performed to find steady state points on which the continuation algorithms of the MATCONT software could perform the calculation of the equilibrium points reached by the fermentation system under different operational conditions. The final concentrations of the modelled variables by extending the simulation time under continuous regime until steady state was reached, considering Yen and Li's (2011) reported operational conditions and experimental data. The results for said simulation were 23.4361, 1.4188, 6.0305, 0.6742, 2.7217 and 0.8916 Kg m^{-3} for residual sugar, biomass, butanol, acetate, acetone and ethanol respectively after 200 h.

With the results obtained with the prior simulations there was applied a bifurcation analysis over the dilution rate to identify operational range values for the controller to avoid washout into the reactor and also to find the theoretically maximum glucose feeding concentration (Sg_{in}) that the biological system could handle without compromising its performance due product accumulation or low substrate uptake efficiency. In this experiment the aim was to determine the final concentration of the desired metabolites at steady state and to identify the reactor's washout rate (D_W), the maximum butanol concentration and maximum butanol productivity of the fermentation system (Figure 3). From the results the identified D_W was equal to 0.2251 h^{-1}, where it is noteworthy that under the experimental conditions reported by Yen and Li (2011) the maximum dilution rate evaluated was equal to 0.107 h^{-1} and under said scenario the products concentration at steady state compared with the ones obtained at 0.054 h^{-1} were 16 % lower on average, indicating that the result obtained by simulation for the determined D_W can be considered as accurate. Additionally it must be noted that even if the correlation between Sg_{in} and residual glucose within the reactor is always directly proportional, the behaviour of the butanol titer against the dilution rate is not. There is a region which can be identified in the diagram showcased in Figure 3 that suggest there is a local maximum for butanol productivity approximately equal to 0.5 Kg m^{-3} h^{-1}, with a final butanol concentration of 7.15 Kg m^{-3}, at a D = 0.075 h^{-1} and Sg_{in} values between 110 and 120 Kg m^{-3} but not higher. Said behaviour is consistent with the fact that *Clostridium* growth rate is not only inhibited by the accumulation of the produced organic solvents, but also by substrate feeding concentrations over 150 Kg m^{-3} (Lee et al. 2008).

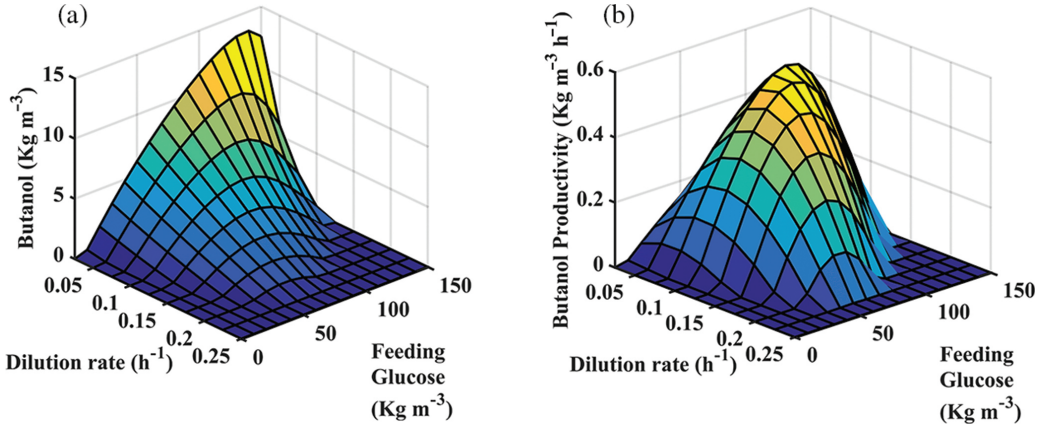

Figure 3: Dynamics of the ABE fermentation system of *Clostridium acetobutylicum* ATCC 824 operating as CSTR with respect to changes in dilution rate (D) and glucose feed concentration (Sg_{in}): (a) Butanol concentration and (b) Butanol productivity.

Therefore, as the main objective of this paper is to improve the system's butanol productivity but taking into account that the majority of its process costs are due the recovery stage, it's crucial for the implementation of closed-loop production schemes outside laboratory scale that butanol concentration could lie between 8 and 19 Kg m^{-3}, as Khöler et al. (2015) report that purification costs by distillation are inversely proportional of each other within such region.

3.3 Proposed controller performance

Table 4 indicates the obtained values for the proposed controller tuning, using the considerations mentioned into (14) and (26). For determining the controller's set-point, which corresponds to the butanol concentration into the reactor, it was resorted to using parallel simulations at different proposed values within the range between 9 Kg m^{-3} to 10 Kg m^{-3}, considering that the bifurcation analysis showed that within such product titters the productivity of the system would maintain at least 85 % of the maximum productivity reported in

Section 3.2 while keeping the butanol concentration in the outlet as high as possible within the operational region of the reactor. The selection criterion was based on the characterization of the control effort, determined as the fluctuation in the control input (D) with respect to process time and the steady state concentration of the products of interest. The value established based on the analysis described above corresponded to a value of 9.5 Kg m^{-3}, as it generates the greatest product concentration while resorting to the least control effort.

Table 4: Proposed controller tuning values obtained by the theoretical considerations made into (14) and (26).

Parameter	Value	Units
k_0	0.00275	—
a_1	0	Kg m^{-3}
a_2	0	Kg m^{-3}
w_1	1	m^3 Kg^{-1} h^{-1}
w_2	2	m^3 Kg^{-1} h^{-1}

For the performance analysis versus traditional control structures, a kinetic disturbance in the open-loop system via a 10 % increment to the dilution rate was made in order to tune the parameters of a PI controller, which would be used as a standard reference for comparison. The tuning parameters were obtained by the Internal Model Control (IMC) criteria reported by (Rivera, Morari, and Skogestad 1986) and are shown in Table 5.

Table 5: PID controller tuning values obtained by the empirical method described by Rivera, Morari, and Skogestad (1986).

Parameter	Value	Units
ΔU	0.054	h^{-1}
Kp	0.0712	—
t_i	280.5	h

Figure 4 shows the proposed controller stabilises the butanol concentration into the proposed set-point in approximately 400 h, versus the PI controller that requires more than 1000 h to attain such condition, as even if the proposed structure causes a higher overshoot of the controlled variable it also does converge faster. Furthermore an integral time absolute error (ITAE) analysis (Smith and Corripio 1985) was performed to compare the proposed structure behaviour with the application of the PI structure and tracking of the ITAE versus time is shown in Figure 5. Once again, it's seen that the proposed structure attains lower ITAE values than the PI one by more than an order of magnitude and such behaviour is maintained throughout the temporal spectrum evaluated in this essay (1000 h).

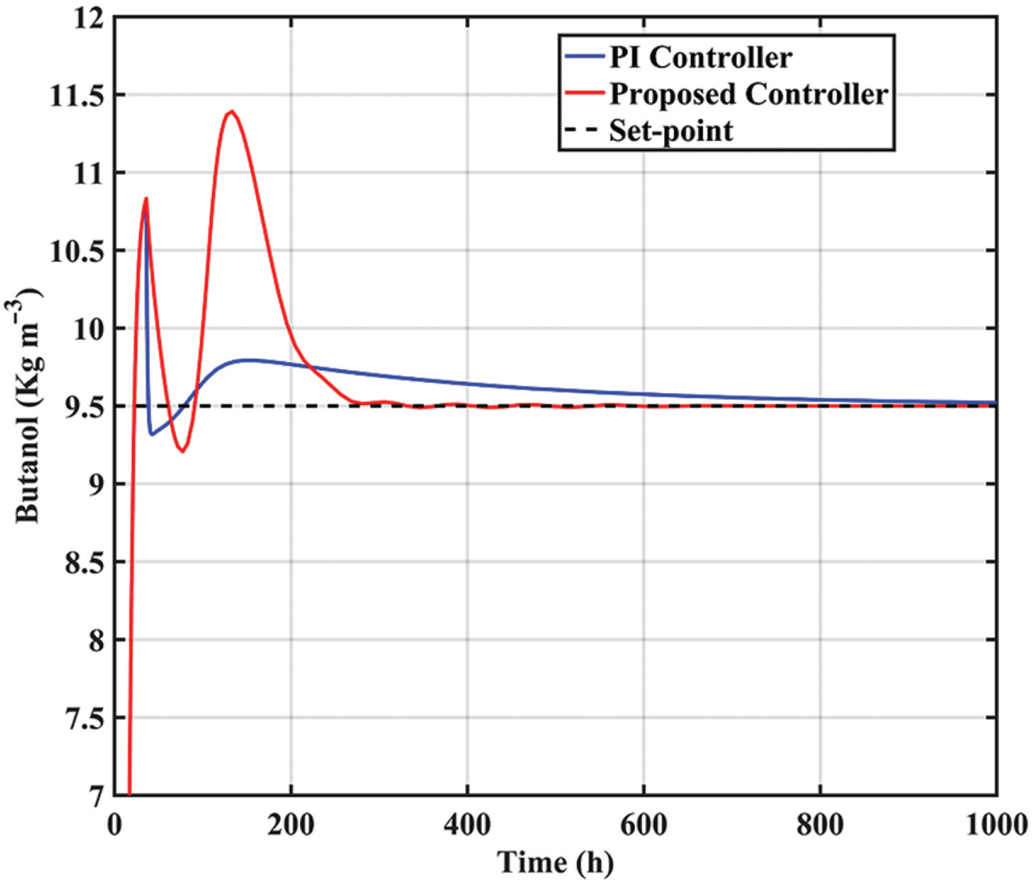

Figure 4: Comparison of the butanol concentration (controlled variable) dynamic under closed-loop operation between the proposed controller and a PI one.

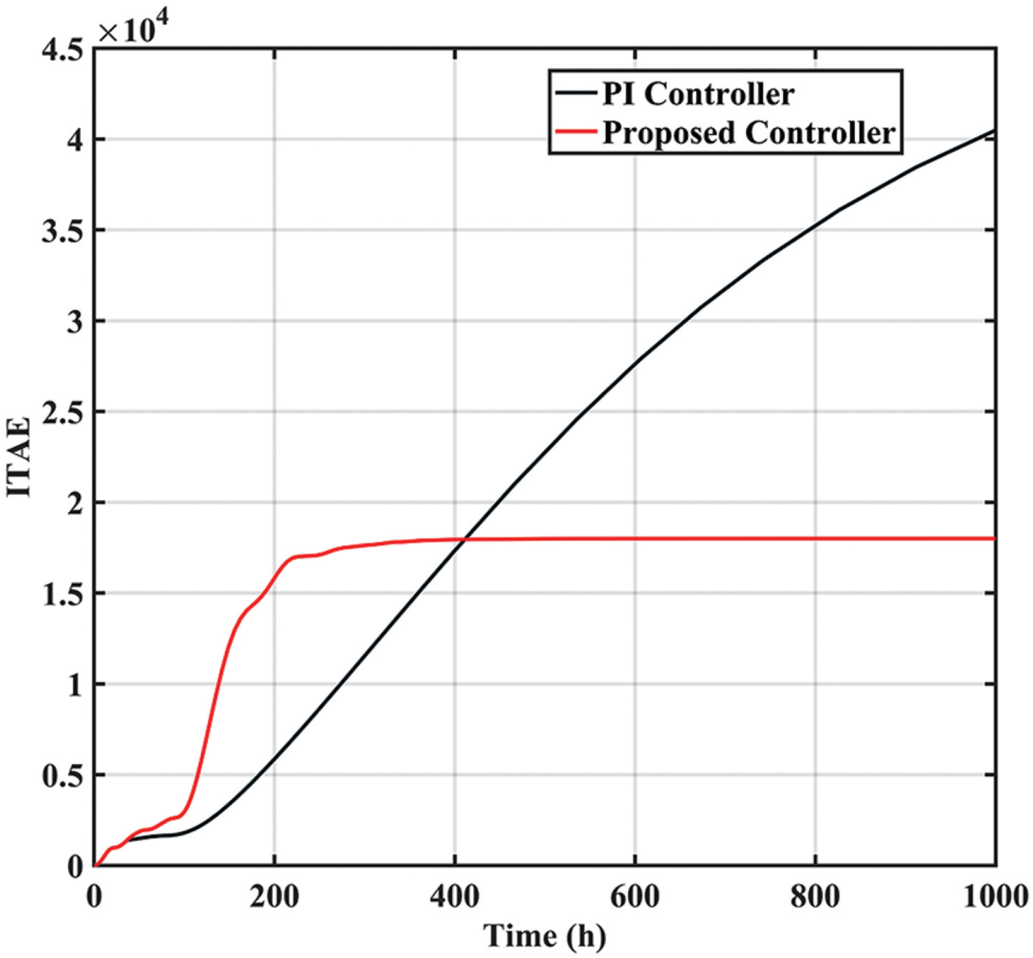

Figure 5: Evolution of the Integral Time Absolute Error performance index over time of both the proposed feedback structure and a PI controller.

In order to evaluate the system's performance, monitoring of the control effort was made, which consisted in the tracking of the dynamics of both the non-controlled variables (Figure 6) and of the dilution rate over time (Figure 7). In both cases it's shown that the control effort does indeed stabilize without visible delay after the set-point was reached and that the remaining variables do exhibit a stable behaviour, which could be inferred due the absence of critical points predicted by the model into the bifurcation analysis stage.

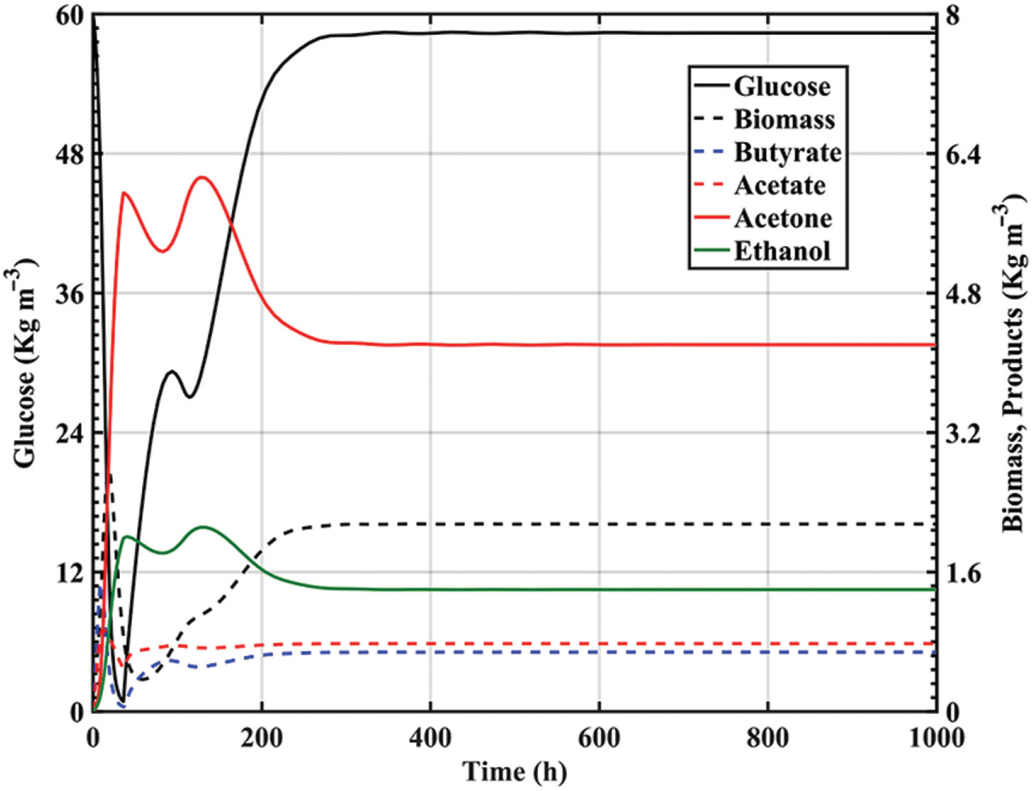

Figure 6: Dynamic of the non-controlled variables of the system under closed-loop operational regime using the proposed controller.

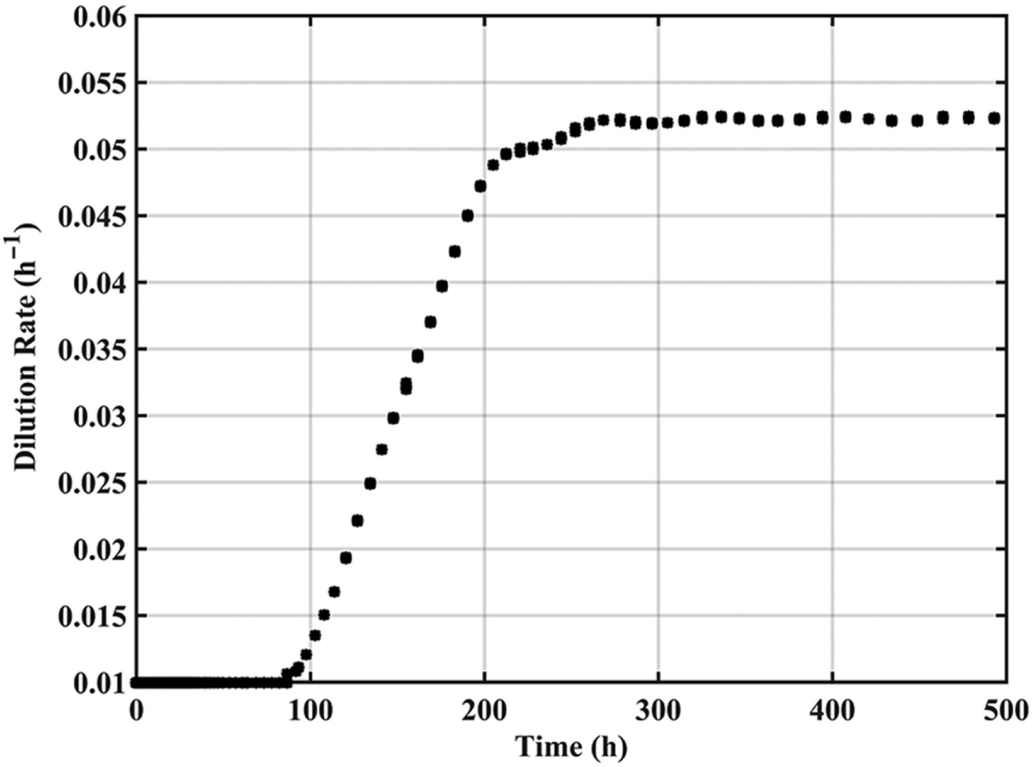

Figure 7: Dynamic of the control effort (D) of the butanol production system under closed-loop operational regime using the proposed controller.

Finally, further simulations made considering the closed-loop system demonstrated this operational regime provided both the best butanol concentration (Figure 8) and productivity results compared with the ones obtained by the open-loop simulation (Figure 9).

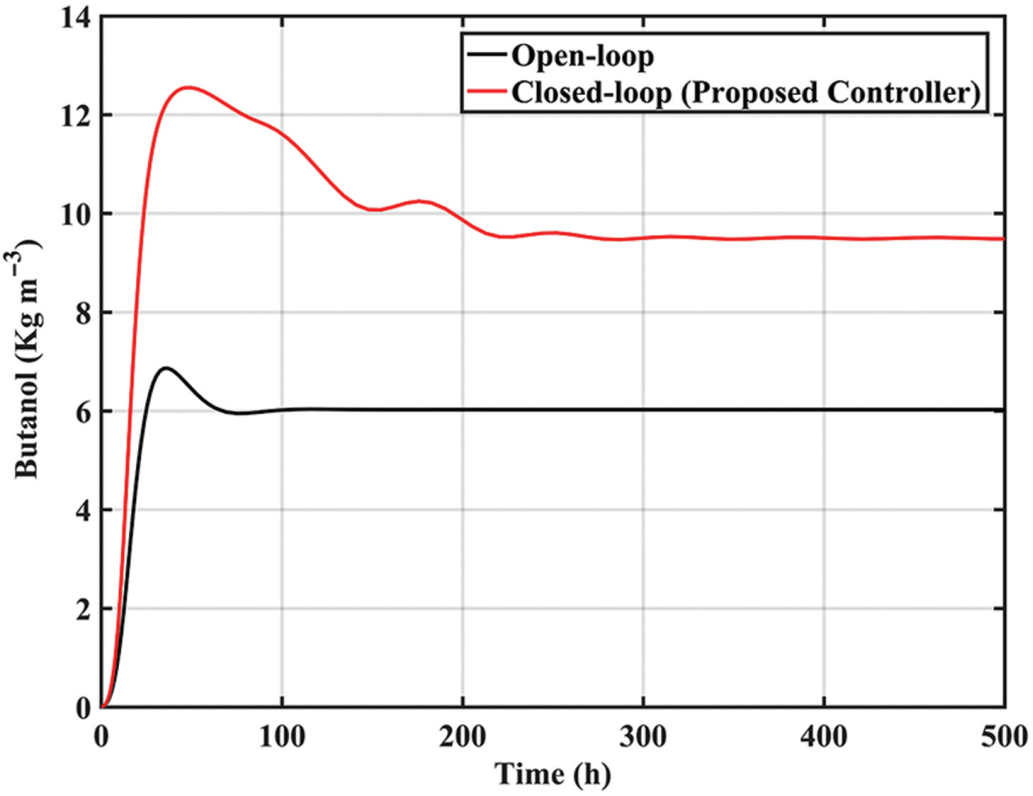

Figure 8: Comparison of the dynamic of butanol concentration between the system under open-loop and closed-loop operation.

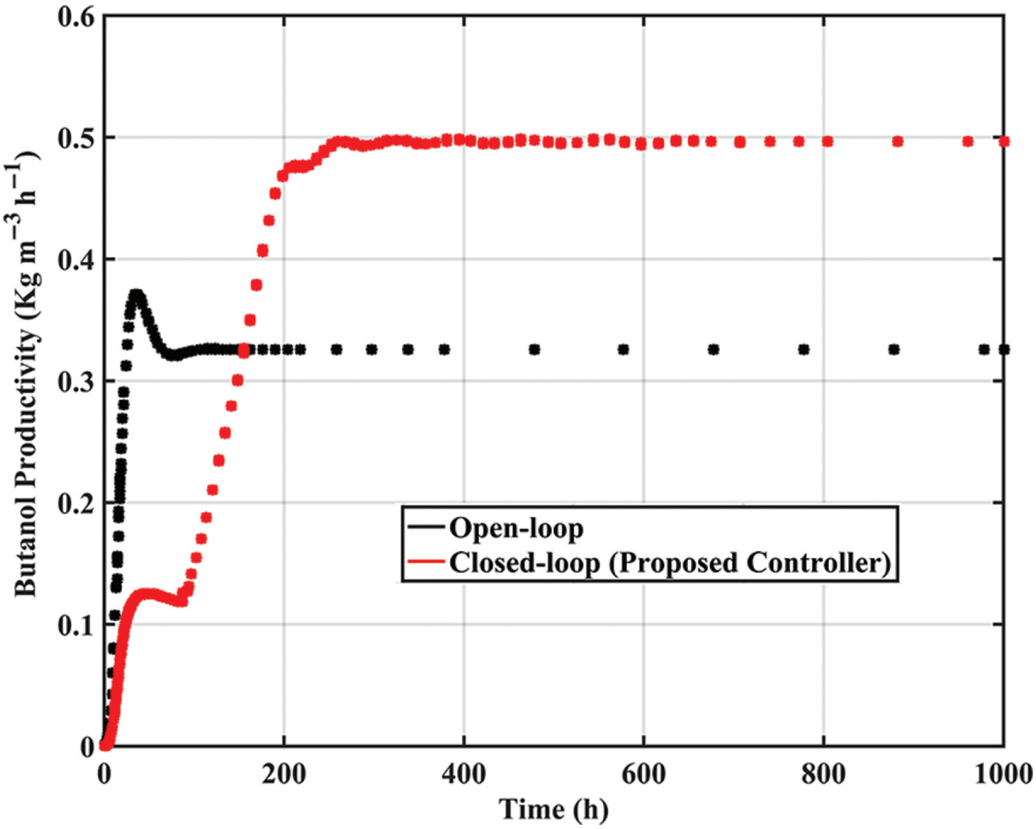

Figure 9: Comparison of the dynamic of butanol productivity between the system under open-loop (continuous line) and closed-loop (discontinuous line) operation.

As a last remark to validate the viability of the obtained results Table 6 compares the performance of the proposed closed-loop system of this work versus experimental and industrial results published into recent literature.

Table 6: Performance comparison considering butanol concentration and productivity between previously reported experimental data and this work.

Reactor configuration	Butanol concentration (Kg m^{-3})	Butanol productivity (Kg m^{-3} h^{-1})	Reference
Open-loop Industrial Fed-Batch Reactor	12.95	0.1799	Jiang et al. (2015)
Open-loop CSTR	6.01	0.3245	Yen and Li (2011)
Closed-loop CSTR	9.50	0.4995	This work

4 Conclusions

The design of a sigmoid controller is proposed to improve the butanol production in a class of anaerobic continuous bioreactor, to boost its productivity up to 56 % at high substrate feeding conditions. The proposed controller provides robustness against external disturbances, and ensure thereby conferring it a stable behaviour for long periods of time. By numerical experiments it shown that the proposed feedback structure provides a better performance compared to classical PI control. Also the controller allows the system to converge at a stable operating point with high productivity in a low settling time.

Nomenclature

k_0 Proposed controller's gain
K_{AA} acetate-acetone affinity constant, Kg m^{-3}
K_{BA} butyrate-acetone affinity constant, Kg m^{-3}
K_{But} butanol growth inhibition constant, Kg m^{-3}
K_d specific cell death rate, h^{-1}
K_{SA} acetate-glucose affinity constant, Kg m^{-3}
K_{Sb} butanol-butyrate affinity constant, Kg m^{-3}
K_{SbSg} butyrate-glucose affinity constant, Kg m^{-3}
K_{SE} ethanol-glucose affinity constant, Kg m^{-3}
K_{Sg} glucose affinity constant, Kg m^{-3}
K_{SgBut} butanol-glucose affinity constant, Kg m^{-3}
r_{maxAce} maximum acetone specific production rate, h^{-1}.
r_{maxAct} maximum acetate specific production rate, h^{-1}.
r_{maxBut} maximum butanol specific production rate, h^{-1}.
r_{maxEt} maximum ethanol specific production rate, h^{-1}.
r_{maxSb} maximum butyrate specific production rate, h^{-1}.
$Y_{Ace/Sg}$ acetone per glucose mass yield, Kg Kg^{-1}
$Y_{But/Sb}$ butanol per butyrate mass yield, Kg Kg^{-1}
$Y_{Et/Sg}$ ethanol per glucose mass yield, Kg Kg^{-1}
$Y_{Ace/X}$ acetone per biomass yield, Kg Kg^{-1}
$Y_{Act/X}$ acetate per biomass yield, Kg Kg^{-1}
$Y_{But/X}$ butanol per biomass yield, Kg Kg^{-1}

$Y_{But/Sg}$ butanol per glucose mass yield, Kg Kg^{-1}

$Y_{Et/X}$ ethanol per biomass yield, Kg Kg^{-1}

$Y_{Sb/X}$ butyrate per biomass yield, Kg Kg^{-1}

$Y_{X/Sg}$ biomass per glucose mass yield, Kg Kg^{-1}

Greek symbols

μ_{maxX} maximum specific cell growth rate h^{-1}

Acknowledgments

H. I. Velázquez-Sánchez is very grateful with the CONACyT for the financial support via a postgraduate scholarship No. 277760 and with CINVESTAV-IPN for supplying the research facilities to develop this work.

References

Aguilar-López, R., R. V. Gómez-Acata, G. Lara-Cisneros, and R Femat. 2016. "Nonlinear and Robust Control Strategy Based on Chemotherapy to Minimize the HIV Concentration in Blood Plasma ." *Journal of Control Science and Engineering* 9: 29–35.

Ariyajaroenwong, P., P. Laopaiboon, A. Salakkam, P. Srinophakun, and L Laopaiboon. 2016. "Kinetic Models for Batch and Continuous Ethanol Fermentation from Sweet Sorghum Juice by Yeast Immobilized on Sweet Sorghum Stalks ." *Journal of the Taiwan Institute of Chemical Engineers* 66: 210–216.

Bacciotti, A., L. Rosier 2005. *Lyapunov Functions and Stability in Control Theory. Communications and Control Engineering Series.*, edited by E. D. Sontag, et al.,Lecture Notes in Control and Information Sciences Vol. 267, 2nd ed. London: Springer.

Bastin, G., and D. Dochain. 1990. *On-Line Estimation and Adaptive Control of Bioreactors*. Amsterdam: Elsevier ISBN: 0-444-88430-0.

Budincevic, Mirko 2010. "A Comparison Theorem of Differential Equations ." *Novi SAD Journal of Mathematics* 40 (1): 55–56.

Eom, M. H., B. Kim, H. Jang, S. H. Lee, W. Kim, Y. A. Shin, and J. H. Lee. 2015. "Dynamic Modelling of a Fermentation Process with Ex Situ Butanol Recovery (ESBR) for Continuous Butanol Production ." *Energy & Fuels* 29: 7254–7265. DOI:10.1021/acs.energyfuels.5b01031.

Haddad, W., and V. S. Chellaboina. 2008. "Nonlinear Dynamical Systems and Control: A Lyapunov-Based Approach, 1–948." Princeton: Princeton University Press ISBN: 0691133298.

Haus, S., S. Jabbari, T. Millat, H. Janssen, R. J. Fischer, H. Bahl, J. R. King, and O. Wolkenhauer. 2011. "A Systems Biology Approach to Investigate the Effect of pH-Induced Gene Regulation on Solvent Production by Clostridium acetobutylicum in Continuous Culture ." *BMC Systems Biology* 5: 5–10.

Jiang, M., J.-n. Chen, A.-y. He, H. Wu, X.-p. Kong, J.-l. Liu, C.-y. Yin, W.-f. Chen, and P. Chen. 2014 (8). "Enhanced acetone/butanol/ethanol production by Clostridium beijerinckii IB4 using pH control strategy ." *Process Biochemistry* 49 (8): 1238–1244. DOI:10.1016/j.procbio.2014.04.017.

Jiang, Y., J. Liu, W. Jiang, Y. Yang, and S. Yang. 2015. "Current status and prospects of industrial bio-production of n-butanol in China ." *Biotechnology Advances* 33 (7): 1493–1501. DOI:10.1016/j.biotechadv.2014.10.007.

Jones, D., and D. Wood. 1986. "Acetone-Butanol Fermentation Revisited ." *Microbiological Reviews* 50: 484–524.

Khalil, H. K. 1996. *Nonlinear Systems*. NJ: Prentice Hall Upper Saddle River.

Khöler, K. A. K, J. Rühl, L. M. Blank, and A. Schmid. 2015. "Integration of Biocatalyst and Process Engineering for Sustainable and Efficient n-Butanol Production ." *Engineering in Life Sciences* 15: 4–19.

Kirkiliones, M., and S. Walcher. 2004. "On Comparison Systems for Ordinary Differential Equations ." *Journal of Mathematical Analysis and Applications* 299: 157–173. DOI:10.1016/j.jmaa.2004.06.025.

Lee, S. Y., J. H. Park, S. H. Jang, L. K. Nielsen, J. Kim, and K. S. Jung. 2008. "Fermentative Butanol Production by Clostridia ." *Biotechnology and Bioengineering* 101: 209–228. DOI:10.1002/bit.22003.

López, I., M. Passeggi, and L. Borzacconi. 2015. "Variable Kinetic Approach to Modelling an Industrial Waste Anaerobic Digester ." *Biochemical Engineering Journal* 96: 7–13. DOI:http://dx.doi.org/10.1016/j.bej.2014.12.014.

López-Pérez, P. A., M. I. Neria-González, and R. Aguilar-López. 2015. "Increasing the Biohydrogen Production in a Continuous Bioreactor Via Nonlinear Feedback Controller ." *International Journal of Hydrogen Energy* 40 (48): 17224–17230. DOI:10.1016/j.ijhydene.2015.09.106.

McNabb, A. 1986. "Comparison Theorems for Differential Equations ." *Journal of Mathematical Analysis and Applications* 119: 417–428.

Munasinghe, S. R., and M. Nakamura. (2007).Hyperbolic Tangent Function Based Force-Position Compliant Controller for Robotic Devices . International Conference on Control, Automation and Systems, October 2007, Seoul, South Korea. 2397–2401.

Nikačević, N. M., A. E. M. Huesman, P. M. J. Van Den Hof, and A. I. Stankiewicz. 2012. "Opportunities and Challenges for Process Control in Process Intensification ." *Chemical Engineering and Processing: Process Intensification* 52: 1–15. DOI:10.1016/j.cep.2011.11.006.

Procentese, A., F. Raganati, G. Olivieri, M. E. Russo, P. Salatino, and A. Marzocchella. 2014. "Continuous Xylose Fermentation by Clostridium acetobutylicum – Kinetics and Energetics Issues Under Acidogenesis Conditions ." *Bioresource Technology* 164: 155–161.

Qureshi, N., and T. Ezeji. 2008. "Butanol, a Superior Biofuel Production from Agricultural Residues (renewable biomass): Recent Progress in Technology ." *Biofuels, Bioproducts & Biorefining* 2: 319–330. DOI:10.1002/bbb.85.

Qureshi, N., B. C. Saha, and M. A. Cotta. 2007. "Butanol Production from Wheat Stray Hydrolysate Using Clostridium beijerinckii ." *Bioprocess and Biosystems Engineering* 30: 419–427. DOI:10.1007/s00449-007-0137-9.

Raganati, F., A. Procentese, G. Olivieri, P. Götz, P. Salatino, and A. Marzocchella. 2015. "Kinetic Study of Butanol Production from Various Sugars by Clostridium acetobutylicum Using a Dynamic Model ." *Biochemical Engineering Journal* 99: 156–166.

Reid, W.T. 1969. "Comparison Theorems for Non-Linear Vector Differential Equations ." *Journal of Differential Equations* 5: 324–337.

Rivera, E. D., M. Morari, and S. Skogestad. 1986. "Internal Model Control: PID Controller Design ." *Industrial & Engineering Chemistry Process Design and Development* 25: 252–265. DOI:10.1021/i200032a041.

Sellke, T., M. J. Bayarri, and J. O. Berger. 2001. "Calibration of p Values for Testing Precise Null Hypotheses ." *The American Statistician* 55 (1): 62–71.

Sforza, E., M. Enzo, and A. Bertucco. 2014. "Design of Microalgal Biomass Production in a Continuous Photobioreactor: An Integrated Experimental and Modelling Approach ." *Chemical Engineering Research and Design* 92: 1153–1162.

Smith, C. A., and A. B. Corripo. 1985. *Principles and Practice of Automatic Process Control*., 1st ed. New York: Wiley.

Spear, M. 2005. "Time to Turn Up the Speed? Process Control Developments Continue to Outrun Their Uptake by Industry ." *Chemical Processing* 23: 345–349.

Vanlier, J., C. A. Tiemann, P. A. J. Hilbers, and N. A. W. Van Riel. 2013. "Parameter Uncertainty in Biochemical Models Described by Ordinary Differential Equations ." *Mathematical Biosciences* 246: 305–314.

Velázquez-Sánchez, H. I., M. C. Montes-Horcasitas, and R. Aguilar-López. 2014. "Development of a Phenomenological Kinetic Model for Butanol Production Using Clostridium beijerinckii ." *Revista Mexicana de Ingeniería Química* 13: 103–112.

Wallner, T., A. Ickes, and K. Lawyer. (2012).Analytical Assessment of C2-C8 Alcohols as Spark-Ignition Engine Fuels . Proceedings of the FISITA 2012 World Automotive Congress, 27–30 November 2012, Beijing, China. 15–26.

Xu, F., and P. Yu. 2010. "Chaos Control and Chaos Synchronization for Multi-Scroll Chaotic Attractors Generated Using Hyperbolic Functions ." *Journal of Mathematical Analysis and Applications* 362: 252–274. DOI:10.1016/j.jmaa.2009.03.041.

Jiang, Min 2014.

Yen, H. W., and R. J. Li. 2011. "The effects of dilution rate and glucose concentration on continuous acetone-butanol-ethanol fermentation by Clostridium acetobutylicum immobilized on bricks ." *Journal of Chemical Technology & Biotechnology* 86 (11): 1399–1404. DOI:10.1002/jctb.2640.

Zhao, Y., and S. Skogestad. 1997. "Comparison of Various Control Configurations for Continuous Bioreactors ." *Industrial Engineering Chemical Researcher* 36: 697–705. DOI:10.1021/ie960123l.

Ziegler, J. G., and N. B. Nichols. 1942. "Optimum Settings for Automatic Controllers ." *Transactions ASME* 64: 759–765.