Control Engineering Perspective Of Fermentation Process From Zymomonas mobilis: Modeling, State Estimation And Control

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Abstract: this work presents a control engineering perspective of ethanol production from biomass. The bacterium Zymomonas mobilis represents an important microorganism used for this purpose but it makes the fermentation process presents high nonlinearities. Last years this continuous fermentation process has been studied and several state estimation and linear and nonlinear control tools have been developed.

Key words: control engineering, bioprocess, modelling, state estimation, control.

1. INTRODUCTION

World energy consumption is increasing due to a burgeoning growth of human population and increase in prosperity, especially in the less developed countries. In recent years, the use of renewable sources as hydropower, geothermal energy, energy from biomass, wind energy (waves energy) and solar energy; for energy production has gained great attention due to limited reserves of traditional non-renewable energy sources, well know as fossil fuels. Growing attention has been devoted to the conversion of biomass into fuel ethanol considered the cleanest liquid fuel alternative to fossil fuels. Significant advances have been made towards the technology of ethanol fermentation. Biomass is seen as an interesting energy source for several reasons. It is mainly because bioenergy could contribute in search of a sustainable living. Resources are often locally available, and the conversion into secondary energy carriers is feasible without high capital investments. One of the most immediate and important applications of biomass energy systems could take place in the fermentation of ethanol from several kinds of substrates. Lignocellulose products are part of the set of the most important renewable energy resources and their consumption have been increasing in recent years because of the development of new technologies designed and implemented for the use of vegetal and agroindustry residues for energy production (ethanol or biodiesel). So far, the main biological processes that could be used for production of liquid energy carriers are fermentations for production of ethanol, and a mixture of acetone, butanol and ethanol (ABE). Moreover, biomass energy can play an important role in reducing greenhouse gas emissions since CO2 that arises from biomass wastes would originally have been absorbed from the air; however, the use of biomass for energy offsets fossil fuel greenhouse gas emissions. Furthermore, since energy plantations (taking into account the fact of preserving the environment at first and keeping the energy source too) may also create new employment opportunities in rural areas, and allowing that it also contributes to the social aspect of sustainability. In addition, application of agro-industrial residues in bioprocesses not only provides alternative substrates but also helps to solve their disposal problem. With the advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology, many new avenues have opened for their utilization. (Karakashev et al, 2007) Nearly all fuel ethanol is produced by fermentation of corn glucose in the US or sucrose in Brazil, but any country with a significant agronomic-based economy can use current technology for fuel ethanol fermentation (Karashev et al, 2007). This is possible because, during the last two decades, technology for ethanol production from nonfood-plant sources has been developed to such an extent that large-scale production will be a reality in the next few years. Therefore, agronomic residues such as corn stover (corn cobs and stalks), sugarcane waste, wheat or rice straw, forestry, and paper mill discards, as well as the paper portion of municipal waste and dedicated energy crops—collectively termed “biomass”—can be converted into fuel ethanol. Many proposals are mooted to generate ethanol from lignocellulosic biomass, but they are not yet at full scale applications.

Respect to microbiology, the desired traits in a microorganism for commercial ethanol production are broad substrate utilization (ability to use both hexoses and pentoses for fermentation), high ethanol yields and productivity, tolerance to inhibitors presented in the hydrolysates and high ethanol tolerance, cellulolytic activity and ability for sugar fermentation preferably at high temperatures (for less contamination and decreased cost for ethanol recovery through distillation). Traditional microorganisms used in fermentation are the Saccharomyces cerevisiae and some bacteria like Thermoanaerobacter, and others genetically modified through recombinant DNA technology. A promising ethanol producer is the bacterium, *Zymomonas mobilis*, which reaches ethanol yields close to the stoichiometrical value of 0.51 g ethanol/g glucose (Lynd et al. 1996). These yields are the highest yields reported in the literature. Furthermore, *Z. mobilis* has a higher optimal temperature than S. cerevisiae which reduces the cost of cooling during fermentation (Claasen et al. 1999). The main disadvantage of the native strains of *S. cerevisiae* and *Z. mobilis* is their inability to utilize pentoses (xylose, arabinose). These microorganisms have the advantage of tolerate the high temperatures for the fermentation. The advantages of ethanol fermentation at elevated temperatures include high productivities and substrate conversions, low risk of contamination, facilitated product recovery, high¬reactor efficiency and utilization of a wide range of substrates.

The paper is organized as follows: Section 2 presents the importance of the ethanol production from fermentation. In Section 3 the fermentation process from Zymomonas mobilis bacteria is presented. Next, in Section 4 a control engineering perspective is developed, whereas Section 5 the conclusions are stated.

2. ETHANOL FROM FERMENTATION IMPORTANCE

Although procedures for the energy saving will be introduced in the near future; it goes without saying that the global demand for energy is expected to increase. From an environmental point of view, the best solution to meet the increased energy demand is the utilization of renewable sources such as biomass. High oil prices, increasing focus on renewable carbohydrate-based feedstocks for fuels and chemicals, and the recent publication of its genome sequence, have provided continuing stimulus for studies on *Zymomonas mobilis*. However, despite its apparent advantages of higher yields and faster specific rates when compared to yeasts, no commercial scale fermentations currently exist which use *Z. mobilis* for the manufacture of fuel ethanol. This may change with the recent announcement of a Dupont/Broin partnership to develop a process for conversion of lignocellulosic residues, such as corn stover, to fuel ethanol using recombinant strains of *Z. mobilis*. A review of (Rogers et al, 2007) addresses opportunities offered by *Z. mobilis* for higher value products through its metabolic engineering modifications and use of specific high activity enzymes. Previous works (Echeverry et al, 2003, 2004)(Quintero et al, 2004, 2005, 2007, 2008a, 2008b, 2008c, 2008d) about *Z. mobilis* microorganism allow us to take another look at review the dynamic behavior of the bacteria to establish the features to use it as ethanol producer, taking advantage of the natural properties and with the aim to reach the optimum point of productivity.

From estimation and control perspective the knowledge of this microorganism is a challenge, and in future, it will carry us to analyze the possibility to use *Z. mobilis* in medium and large scale fermentations. Many reports of oscillatory behavior in continuous fermentation may be found in the pertinent literature (Chi et al. 1974, Chi e Howell 1976; Borzani 1977; Jöbses et al. 1986, Ghommidh et al. 1989; Daugulis et al. 1997; McLellan et al. 1999; Beuse et al. 1999; Menzel et al., 2000, Pinheiro, 2001; Andersen et al., 2001). Most of the oscillatory phenomenon is associated with *Saccharomyces cerevisiae* and *Zymomonas mobilis*. The oscillation occurrence may favour to the ethanol production rate in creasing or disfavour the possible control strategies with appropiated results to the fermentative processes. In such conditions the microorganism may be under stress and produces a secondary metabolite or inhibit the formation of a primary metabolite. The most developed mathematical model was proposed by Li et al. (1995). The author's studies revealed that the *Zymomonas* growth is influenced by the ethanol concentration rate history instead of cell concentration history. An interesting feature of *Zymomonas mobilis* is that its specific growth rate is negative affected by the ethanol concentration and in at least one of these fermentations, ethanol concentration is higher when a higher glucose concentration feed is used. Ethanol concentration remains constant during the feeding period no matter what Substrate feeding value is when Doubling time (defined as interval after which the inlet flow rate is duplicated in fedbatch fermentations) is 0.5 hours. Respect to the reaction of *Z. mobilis* against substrate, inhibition effect due to the glucose concentration is not as important as that due to the ethanol concentration. As soon as this concentration differs from zero, the specific growth rate reduces and for values close to Ethanol maximum (34.67 g/l) the bacteria grow at very low specific rates. The glucose yield to biomass and to ethanol is negatively affected by glucose accumulation. Thus, the productivity of *Z. mobilis* fed-batch fermentation depends on the feeding strategy. The experiments (Bravo et al, 2000) have also shown that ethanol synthesis is growing rate associated. Because of this a feeding strategy designed to keep the culture growing at constant high rate could improve the ethanol productivity. To design feeding strategies and dynamic actions to improve the *Z. mobilis* performance, some authors have been developed models to explain the dynamic behavior of Z. mobilis (Daugulis et al, 1999) (Bravo et al, 2000) (Echeverry et al, 2004) (Rogers et al, 2007), fixed some model parameters (Raposo et al,

2005) and estimated some biotechnological variables (Quintero et al, 2004, 2005, 2007). It means that *Z. mobilis* has been an important research topic, looking for its possible implementation in large scale ethanol production.

3. FERMENTATION PROCESS FROM *Z. mobilis*

As outlined in the earlier reviews, wild-type strains of *Z. mobilis* (and their mutants) can convert simple sugars to ethanol at faster rates and higher yields compared to yeasts. However, the ethanol industry has traditionally used yeasts, and in spite of the apparent advantages of Z. mobilis, there appears to be little incentive for change with sugar and starch-based raw materials. Some of the reasons lie in the concerns that *Z. mobilis* may be less robust than yeast and more susceptible to contamination in large-scale processes, as well as the lack of ethanol industry experience with large-scale bacterial fermentations. In addition, an established feed market exists for the high protein yeast by-product (as dried distiller's grains) and any new market for a high protein by product from a *Zymomonas* process would need to be established. The key issues and alternative capabilities are presented as follows. Experience with large-scale recombinant bacterial fermentation could provide a future platform well for an increased range of higher value products generated via the metabolic engineering of micro-organisms such as *Z. mobilis* which are capable of both rapid and highly efficient sugar metabolism. Some important features of the bacteria are the following:

- Considerably faster specific rates of sugar uptake and ethanol production (specific rates 2–3 times faster than yeasts).
- Higher ethanol and lower biomass yields compared to yeasts due to different carbohydrate metabolism (Entner–Doudoroff vs. glycolytic pathway).
- Higher reported productivities (120–200 gL–1h–1) in continuous processes with cell recycle (maximum reported values for yeasts are 30–40 gL–1 h–1).
- Simpler growth conditions. Z. mobilis grows anaerobically (not strict anaerobe) and does not require the controlled addition of oxygen to maintain cell viability at high ethanol concentrations.
- Ethanol tolerance comparable is not better than yeasts.
- Ethanol concentrations of 85 gL–1(11% v/v) reported for continuous culture and up to 127 g L–1 (16% v/v) in batch culture.
- Laboratory scale studies with strains of Z. mobilis over many years in controlled fermentations (pH = 5.0, T = 30 ◦C) have not revealed any significant contamination or bacteriophage infection problems.

The wide range of techniques developed for the genetic manipulation of bacteria (such as Escherichia coli) can be applied to developing recombinant strains of *Z. mobilis* and/or their metabolic engineering. Integrant recombinant strains of *Z. mobilis* available for efficient ethanol production from glucose, xylose and arabinose. Ethanol concentrations above 60 g L–1 in 48 h reported for medium containing 65 gL–1 glucose, 65 gL–1 xylose. Sequencing of ZM4 genome now provides information for its metabolic engineering for additional higher value products (e.g., succinic acid). Potential for use of its enzymes for fine chemical biotransformations. The greatest difficulty for the commercial production of such enzymes is the low cell yield of *Z. mobilis* which is typically 0.02–0.03 g g–1 substrate sugar, compared to cell yields close to 0.5 g g–1 for many aerobically grown microorganisms.

4. CONTROL ENGINEERING PERSPECTIVE

4.1 Process:

Fermentation process can be carried in batch, fed batch and continuous process (see Fig. 1). The purpose of fed batch cultures is control the nutrient concentration and to extend the productive phase of the batch process. Fed batch production of the desired metabolite is generally characterized by the relationship between cell growth and nutrient consumption, the dependence of the desired metabolite synthesis dynamics on the feeding nutrient concentration, and the increase in the culture volume. For the production of growth associated products, the synthesis rate is a function of the specific growth rate. In this case the interest is to feed the fermentor in such a way that the specific growth rate remains constant. An example of this is the production of hepatitis-B surface antigen by *Saccharomyces cerevisiae*. For example, in the case of *Z. mobilis* CP4 fed batch fermentations carried out using flow rates higher than 0.11 l/h. In fed batch fermentations substrate concentration will increase if its addition rate is higher than its uptake rate (Bravo et al, 2000).

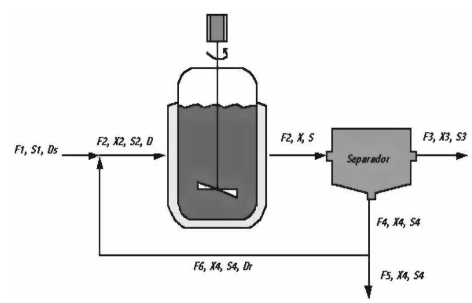


Figure. 1 Continuous fermentation process scheme

The main disadvantage of the native strains of *S. cerevisiae* and *Z. mobilis* is their inability to utilize pentoses (xylose, arabinose). One of the possible disadvantages of *Z. mobilis* is that it has a limited carbon substrate range, as it can only use the simple C6 sugars glucose, fructose and sucrose. As a result early studies on its genetic manipulation focused on extending its substrate range for ethanol production.

Zymomonas mobilis that reaches ethanol yields close to the stoichiometrical value of 0.51 g ethanol/g glucose (Lynd et al. 1996). These yields are the highest yields reported in the literature. Furthermore, *Z. mobilis* has a higher optimal temperature than S. cerevisiae which reduces the cost of cooling during fermentation (Claasen et al. 1999). On the other hand, the continuous alcoholic fermentation process of *Z. m*obilis can provide high ethanol performance, but it has an oscillatory behavior on the state variables of the process. From the control perspective, it represents a challenge due to the difficulties to measure some of these states with the aim to be used as feedback signals. A model of the process (Oliveira 2005; Daugulis et al. 1997; Tano et al. 2000) is represented by the following differential and algebraic equations: the change in biomass concentration can be obtained from (Daugulis et al. 1997), by a set of algebraic considerations and working with the defined flows in (Echeverry et al. 2003), the biomass term will be expressed as function of *D,* which is the total dilution rate. The total dilution rate is $D_s + D_r$, where D_r is the dilution rate associated to biomass recycle *R* and substrate dilution rate D_s.

$$\frac{dX}{dt} = \mu X + \left[RD/4 + D_s\right](R-1)X \qquad (1)$$

where μ is the specific speed growth. The change in substrate concentration is given by,

$$\frac{dS}{dt} = \left(-\frac{1}{Y_{p/s}}\right)\left(Q_p X\right) + DS_{in} - DS \qquad (2)$$

where $Y_{p/s}$ is the substract/product performance coefficient, Q_p is the specific ethanol production rate, *D* is the total dilution rate, and S_{in} is the substrate concentration on the input flow. The change in product concentration is given by

$$\frac{dP}{dt} = Q_p X - DP \qquad (3)$$

The weighted average of the ethanol concentration rate is,

$$\frac{dZ}{dt} = \beta(I - Z) \qquad (4)$$

where β is a weighted historic parameter for the ethanol concentration rate and *I* is an intermediate variable auxiliary for the inhibition effect determination:

$$\frac{dI}{dt} = \beta\left(Q_p X - DP - I\right) \qquad (5)$$

For further information about the physical meaning of inhibition variables see (Daugulis et al. 1997) (Echeverry et al, 2003). The dynamic effect of the ethanol concentration rate on the biomass growth is given by,

$$f\mu = \frac{1}{2}\left(1 - \frac{e^{\lambda Z-\delta} - e^{-\lambda Z-\delta}}{e^{\lambda Z-\delta} + e^{-\lambda Z-\delta}}\right) \qquad (6)$$

where δ and λ are the parameters associated with the inhibition factor of the ethanol concentration rate.

The biomass growth rate is given by,

$$\mu e = \frac{\mu_{\max} S\left(1 - \left(\frac{P}{P_{ma}}\right)^a\right)\left(1 - \left(\frac{P - P_{ob}}{P_{mb} - P_{ob}}\right)^b\right)}{K_s + S + \frac{S(S - S_i)}{K_i - S_i}} \qquad (7)$$

where $\mu_{\max}$ is the maximum value of the specific growth speed, P_{ma} and P_{ob} are factors of the ethanol inhibition for the specific growth rate expressed in (g/L), P_{mb} is the factor related to the maximum ethanol inhibition for the cells growth expressed in (g/L), *a* and *b* are inhibition exponents for the ethanol production rate, K_s is the substrate saturation coefficient, and K_i is a substrate inhibition. The following conditions are considered:

$$\begin{aligned} &\left(\frac{P - P_{ob}}{P_{mb} - P_{ob}}\right) = 0, P \le P_{ob}, S - S_i = 0, S \le S_i \\ &\left(\frac{P - P_{ob}}{P_{mb} - P_{ob}}\right) = 1, P > P_{mb} \end{aligned} \qquad (8)$$

The dynamic growth speed is defined by:

$$\mu = f\mu * \mu e \qquad (9)$$

And finally, the specific rate to the ethanol production is given by:

$$Qp = Qp_{\max}\left(\frac{S}{K_{mp} + S}\right)\left(1 - \left(\frac{P}{P_{me}}\right)^{\alpha}\right) \qquad (10)$$

For this process, it is very important to reach an accurate estimation of the non-measurable system states with the purpose of using them for control. Differences between reported and estimated parameter values in the mathematical model would be due to the different *Z. mobilis* strain and he culture conditions (pH and temperature) used in fermentation runs. Reported parameters set are in the work of (Daugulies et al, 1997)(McLellan et al, 1999)(Bravo et al, 2000)(Echeverry et al, 2003)(Raposso et al, 2005). Model can be used to define those feeding strategies that improve the system productivity. From the kinetics proposed, in which the specific growth rate is negatively affected by the ethanol concentration that increases during the process, a constant glucose concentration in the culture would not result in a constant specific growth rate.

4.2 Information availability

Into the bio process field, there is a lack of real information of chemical and biological variables such as: biomass concentration, specific bacterial activity, intermediate products concentration among others. Frequently, these variables constitute the states of the bio process and they are very important for its monitoring and control. The observer or a state estimator choice depends inherently on the particular problem specifications. In practice, this choice is mainly influenced by the availability of a sufficiently representative model of the process, and the reliability of experimental data. When an adequate model is available, an Extended Kalman Filter (EFK), High Gain observers or several estimators that use the model of the process (generally based on first principles) to do the estimation of variables can be used. On the contrary, if a model is not representative enough, asymptotic observers can be developed, whose dependency of the model is not too strict, but their convergence depend on the operation conditions. Besides, observers based on artificial intelligence, for example neural networks and fuzzy logic can be implemented. These observers are built as black box models. In general, when the priori knowledge about the plant or the model is incomplete, different techniques of approximation may be used, looking for the state estimation from the data information input/output. In the literature, several proposes for the state estimation on bio process can be founded, the most representative ones are: the work of (Dochain, 2002), (Dochain, 2003), (Boillereaux and Flaus, 2000), (Leal, 2001), (Adilson and Rubens, 2002) and finally ref (Rallo et al, 2002).

The *Zymomonas mobilis* micro-organisms show a highly non linear and oscillatory kinetic behaviour; besides, some states of the process are difficult or impossible to measure, they are: biomass concentration and intermediate variables that represent the rate of ethanol production and to determine the inhibition effect. In (Quintero et al, 2004) and (Quintero et al, 2005), the possibility of to use the Kalman filter and the extended Kalman filter, to do the estimation of the biomass concentration into this fermentation was explored. As a result, the estimations obtained were not satisfactory due to the strong non linearity present in all the process states. In general, the optimum filtering techniques are used to do the estimation of the states of a dynamic system whose inputs and outputs are observed by measurements disturbed by noise. "System states" is defined as the minimum requirements of information in time that in conjunction to the inputs value defined in all time from $t \geq t_0$; allow determining the behavior of the system to any time $t \geq t_0$. The measurements are in general uncertain, because of that, they are called "measurements noise" and, even if the real states of the system are known, the measurements are not a deterministic function of the states mentioned, and they have a random component. In this context, the time evolution of the states is modeled through a dynamic system perturbed by a stochastic process (state noise), by using a stochastic differential equation. The noise or states disturbance is incorporated into the model to represent the uncertainties of the dynamic system, and it can be not only from random nature, but also signals or dynamics not considered in the model. In accordance with the Bayesian paradigm, the solution of the optimal filtering problem in time consists in to obtain the conditional probability distribution of the states, respect to the observations available information until time. Specifically in this work, variations of a bayesian recursive filter SIR (Sampling Importance Resampling) are developed, and different resampling schemes were applied to reduce the effect of the "sampling Impoverishment" 15, (Doucet et al, 2001), (Doucet et al, 2006), (de Freitas, 2001) .

The application of this technique over the bio process is justifiable due to the high non linear features of the fermentation, its non Gaussian statistical features and as previously mentioned, other estimation techniques applied did not give us satisfactory results. In addition, the random nature of biochemical reaction at the molecular scale has been mentioned and studied by many authors, see (Gillespie, 2000). At a macroscopic scale, (Kutz, 1987) modeled the overall effect of these individual reactions on the global concentrations, by an additive noise term of variance proportional to the reaction kinetics. In this context, the state (Biomass, Substrate, Product and also the inhibition variables) is then a Markov process satisfying the Langevin chemical equation (Johannides et al, 2005). All the previously mentioned features make the bio process an attractive application to the use of non linear filtering tools for the state observer design.

In previous works, some techniques for state estimation in *Z.m* have been explored. The work of Quintero et al. (2004) presented a control scheme in closed loop with a virtual sensor based on a fuzzy model. The estimator performs well in simulation, in spite of that, it is not reliable, and its performance depends on the data used for training. After that, Quintero et al. (2005) used the Kalman Filter and Extended Kalman Filter for the same purpose. These results are less representative than those presented in Quintero et al. (2007), Quintero et al. (2008a), Quintero et al. (2008d); even if they are obtained in simulation, by the use of models developed and validated with real data by (Raposso et al. 2005). Those works present a state estimator for a continuous bioprocess. To this aim, the Non Linear Filtering theory based on the recursive application of Bayes rule and Monte Carlo techniques was used. Recursive Bayesian Filters of Sampling Importance Resampling (SIR) had been employed, including different kinds of resampling. An important remark is that the filter follows the model properly, and as approach to the real problem, the performance to online implementation was tested. The modeled dynamics were according to the real behavior and the robustness against disturbances of modeling and uncertainties was shown in simulation. It was necessary to apply the SMC Particle Filtering methodology to the assumption of a sampled data model for the SDE´s; this way, the set of equations are posed as a new and improved model that includes uncertainties and disturbances. SIR Filters are satisfactory, but even if this is a novel application of the SMC, it may require a more advanced SMC method to the real data problem solution (Briers et al. 2004, 2005; Briers 2006). Figures 2 and 3 show the particle filter performance obtained with real sampled data, complete results to be published.

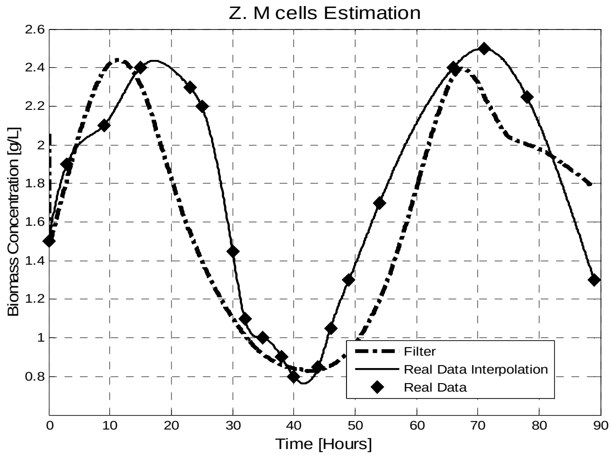


Figure 2. Estimator Performance: The dotted line describes the estimated biomass concentration [g/L] by the SIR filter and the solid line describes the value from real data [g/L] interpolation. The second test was performed with deviation of the mean in diffusions terms.

4.3 Control Strategy

Biochemical engineering is concerned with the industrial production of biologically based products such as foods and beverages, pharmaceuticals, commodity, and especially agricultural chemicals. The biochemical manufacturing industry is rapidly growing due to dramatic advancements in biotechnology and the high value of biochemical products such as pharmaceuticals (Lee, 1992). Process control has played a rather limited role in the biochemical industry as the economic incentive for improved process operation is often dwarfed by costs associated with research and development. This situation is likely to change with the expiration of key patents and the continuing development of global competition. Another obstruction to process control has been the lack of on-line sensors for critical process variables. While this will remain an important issue for the foreseeable future, recent advancements in biochemical measurement technology make the development of advanced process control systems a realistic goal. These trends suggest that biochemical processes will emerge as an important application area for control engineers (Daoutidis, and Henson., 2002).

In the literature, the use of benchmark of a continuous fermentation process by the use of models with 2 state variables has been registered. The state variables like Biomass and Substrate, in conjugation with growth rates equations that couple theses variables, make the models mentioned presents high non linearities.

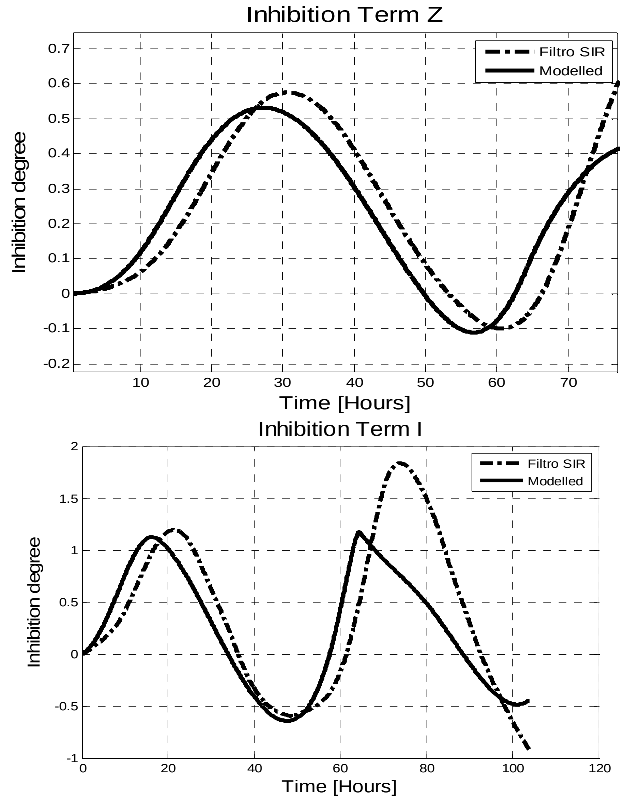
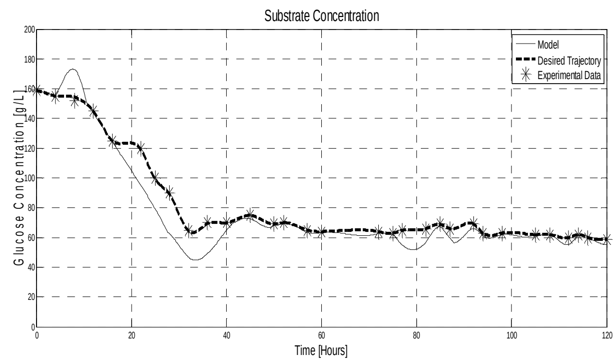


Figure 3. Filter estimation performance. The dotted lines represent the dynamics of Inhibition variables Z and I estimated by the SIR filter while the solid lines are the modelled dynamics of the system, considered as real for estimation purposes.

Also, the complex dynamics of bio reactors have been researched, for the design of different non linear stabilizant control techniques. These have been applied to relatively simple continuous fermentation process, using to reach this aim, the linear as reference to non linear methods. Bio reactors control has been studied by many researchers, including the schemes based on adaptive control (Aguilar, R. et al, 2001), optimum control and Neural Networks control (Onder et al, 1998). Nevertheless, the investigations in this field remain in a latent stage and it seems that its needed long time to find a control scheme well developed to be implemented into practical bio reactors with high performance. The present work fronts the challenge to control a continuous fermentation process in which a micro organism with high kinetic complexities is used. It makes that, as the modelled system presents highly non linear dynamics, the process become very hard to control. Continuous bio reactors are critical unit operations in a wide variety of biotechnological processes. While they can be viewed as chemical reactors, bio reactors offer unique modelling and control challenges due to the complexity of the underlying biochemical reactions and the distributed properties of the cell population. Most of the chemical processes are non-linear in nature. The dynamic behaviour of continuous bio reactors can be strongly affected by variations between individual cells that are captured only with cell population models (Wang et al, 2005). From control perspective, for effective control and operation of nonlinear process, low dimensional linear models are highly desirable. It is not always possible to represent a non-linear process by a single linear model. Consequently, a multiple model approach has attracted increased attention in recent years applied to a variety of areas (Murray-Smith and Johansen, 1997). In the conventional multiple model approach, a complex, non-linear model is reduced to a set of localized, linear sub-models. The overall model is the weighted combination of the local models (Shorten et al, 1999); these results can be too much conservative (Bartholomaus, 2001). The previously mentioned approximations to the control problem solution are all valid, but we are looking for a more simple, understandable, feasible and easy to generalise solution into bio process field.

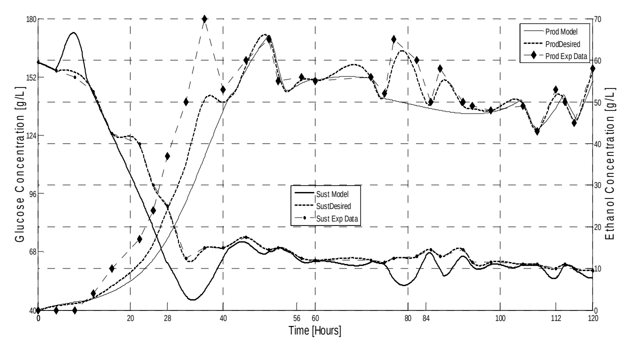
Consequently, we proposed to use numerical methods, not only to simulate the evolution of fermentation process, but also to find the control actions that allow state variables to go from current state to the desired next one. The result is that controllers for Substrate and Product concentration are obtained; later a Biomass controller is developed. In (Quintero et al, 2008b), (Quintero et al, 2008e), two controller based on Numerical Methods were built for their application on a continuous alcoholic fermentation process from *Zymomonas mobilis* bacteria. The control structures can be designed and implemented without great difficulty, because standard algebraic-numerical techniques are used. Simulation results of the developed controller designed for a *Z.m* continuous fermentation have been also addressed. Through the analysis of these experiments, it can be concluded that the trajectory error between the desired and the real trajectory of the fermentation is very small. We conclude that the proposed methodology is quite simple for selecting the parameters of the controller in order to achieve a good performance of the system. This methodology for the controller design can be applied to other types of systems. The required precision of the proposed numerical method for the system approximation is smaller than the one needed to simulate the behavior of the system. Thus, the approach is used to find the best way to go from one state to the next one, according to the availability of the system model. Real data trajectories showed that the controller is feasible and can be easily implemented with control actions bounded to the needed specifications of the real process. The term of recycle (R) added in this controller represents the likelihood to use biomass recycle as a control variable for continuous fermentation, and improves the dynamic behavior. Figures 4, 5 and 6 show the controller behavior.

The first contribution of those works is that complex calculations to get the control signal are not necessary; it makes this solution an easily implementable answer to the control challenge (Scaglia, 2006), (Scaglia et al, 2006), (Scaglia et al, 2007). Finally, in Quintero et al, 2008c, a close loop with a recursive Bayesian State Estimator and a controller based on Numerical Methods was built, for a *Zymomonas mobilis* continuous alcoholic fermentation process. The set point was selected according to the real behavior of bacteria and by following the purpose of maximizing its productivity. Another important remark is that the initial conditions used to simulate the *Z.m* oscillatory open loop behavior, correspond to real data. To test the controller performance against disturbances, a scenario composed by a set of extreme additive disturbances in input flows was generated. In the literature it is cited that a frequent source of disturbance in this kind of systems is an augmenting or decreasing of input flow (or in Batch systems, constants parameters with variations decreasing Batch to Batch).

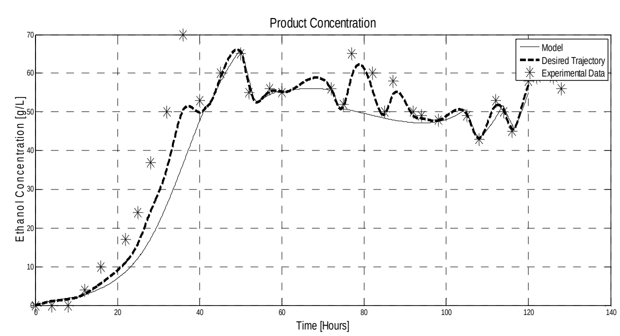


a.

Fig. 4 Simple Controller performance by the use of Real data based trajectory. Against disturbances in times 30 and 90 hours. Recycle fixed at 10%. a. Glucose concentration.



a.



b.

Fig. 5 Simple Controller performance by the use of Real data based trajectory. Against disturbances in times 30 and 90 hours. Recycle fixed at 10%. a. Substrate and Product time evolution. b. Ethanol concentration.

In order to be close to the real behavior of a controller implemented on line, an experimental trajectory was selected (Raposso et al, 2005), and as study case, these experimental data were interpolated to obtain a continuous reference trajectory for Substrate and Product. The controller followed the pre defined trajectory very well, and corrects the inherent oscillations of the real fermentation. It was observed that, the application of the use of particle filtering as Biomass, and Inhibition variables estimator is acceptable, feasible and of viable implementation. The use of the estimation tool allows solving the problem of the lack of on line biomass estimation, and other important variables into a continuous process, due to its reliability and admissible computational cost to the real problem sample times. Its performance was satisfactory into the control loop.

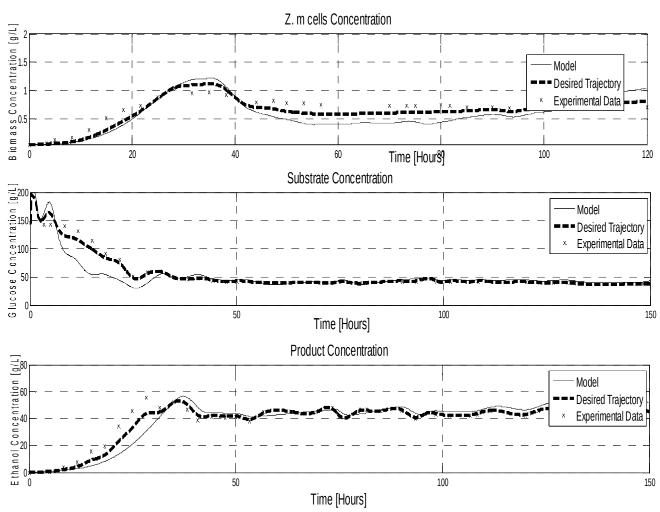


Figure 6 Complex controller performance by the use of Real data trajectory.

5. CONCLUSIONS

Control engineering plays an important role in the biochemical industry. The nonlinearities presented in many biological and biochemical reactions allows to develop and improve several strategies of information availability and control such as state estimators and non linear controls.

Fermentation process for ethanol production involves many of these nonlinearities and represents a current topic of study for many researches that look for the biofuels industry development.

State estimation tools based in nonlinear filtering and nonlinear control based on numerical methods approach are an example of the contributions in this topic.

Consequently, the control engineering approach presented represents an advance for bioprocess and biofuels industry development in global community.

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