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Modeling of a simultaneous saccharification and fermentation process for ethanol production from lignocellulosic wastes by *kluyveromyces marxianus*

Modelado de un proceso de sacarificación y fermentación simultanea para la producción de etanol a partir de residuos lignocelulósico utilizando *kluyveromyces marxianus*

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Received: February 14th, 2013. Received in revised form: February 20th, 2014. Accepted: May 19th, 2014

Abstract
This paper presents the modeling of the main dynamics of a Simultaneous Saccharification and Fermentation (SSF) process using lignocellulosic wastes as substrate. SSF experiments were carried out using the yeast *Kluyveromyces marxianus* as the inoculum and oil palm wastes as the substrate, in order to obtain glucose and ethanol concentration data. The experimental data were used for the parameter identification and model validation. The resulting model predictsthe dynamic behavior of glucose and ethanol concentrations very closely. Performing a sensitivity analysis, parameters which have a higher effect in the modelpredictions are recognized, so the model can be re-optimized in particular cases with low computational requirements. The re-optimization strategy improves the model capacity to predict the dynamics of the SSF process.

Keywords: Bio-ethanol; Simultaneous Saccharification and Fermentation; modeling; *kluyveromyces marxianus*; sensitivity analysis.

Resumen
En este trabajo se presenta el modelado de las principales dinámicas de un proceso de Sacarificación y Fermentación Simultaneas (SFS) utilizando residuos lignocelulósicos como sustrato. Experimentos de SSF llevados a cabo con la levadura *Kluyveromyces marxianus* como inóculo y desechos de palma de aceite como sustrato se realizaron para obtener datos de concentración de glucosa y etanol que permitieran identificar parámetros y validar el modelo. El modelo resultante predice el comportamiento general de las concentraciones de glucosa y etanol. Gracias a un análisis de sensibilidad, se definen los parámetros que más afectan el modelo, con el fin de flexibilizar el modelo para que pueda ser optimizado en casos particulares con pocos requerimientos computacionales. Esta estrategia de reoptimización muestra mejorar de manera importante la capacidad del modelo para predecir las dinámicas del proceso SSF.

Palabras clave: Bioetanol; Sacarificación y fermentación simultánea; modelado; *kluyveromyces marxianus*; Análisis de sensibilidad.

1. Introduction

The growing concern generated by the imminent depletion of fossil fuels has led to the search for alternative energy sources to achieve a sustainable society. Ethanol has emerged as one of the first sources that can help significantly to reduce the consumption of fossil fuels and also the emission of gases that promote global warming. Currently, the use of corn and sugar cane for ethanol production creates a major ethical concern in global food security and the rise of food prices[1,2]. That is why in recent years, research towards using lignocellulosic wastes for ethanol production has increased, in a way that is both technically and economically viable. Among the different technologies that can be used for that purpose, the Simultaneous Saccharification and Fermentation (SSF) production process has gained especial attention.

It is known that the success of the introduction of biofuels in each country depends largely on the raw materials used for its production. Colombia is one of the largest global producers of palm oil [3].This industry generates a very large amount of palm residues in the extraction process. Those residues have a very high potential for being use as a substrate in an SSF process for bio-ethanol production as a second generation biofuel [3].

The regulation for the use of ethanol as a fuel in

Colombia started in 2002, with a primary goal to achieve a production capacity of 2.5 million liters per day, in order to add 10% ethanol to the gasoline used for transportation. However, the main five ethanol plants operating in the country, produce only 1.05 million liters per day and the contribution of some small plants does not significantly increase this amount, which is only enough to supply the major cities near the Valle del Cauca, and the capital Bogotá. Therefore, it is necessary to evaluate future technically and economically feasible strategies that allow the ethanol volume of production in the country to be increased and stimulates the development of tools suitable for scaling up the processes for ethanol production from lignocellulosic wastes. Those strategies must be carried out specifically using the kind of residues widely available in Colombia. There have been few of this kind of studies and they have shown that there is a gap in technology and knowledge to overcome the challenge when scaling-up. Therefore, a deep understanding of the phenomenon taking place in the process is still required. For that, the use of modeling tools is a promising approach for gaining that understanding.

In recent years, the development of models for predicting the dynamic behavior of the most important variables in the ethanol production process has been intensified, including the SSF processes [4–7]. However, studies in this field are still scarce and its application in scaling up is restricted. Besides, the models reported so far, have not been developed for alternative processes that uses microorganisms different from *Saccharomyces cerevisiae* and/or processes involving lignocellulosic residues of regional interest. Therefore, it is still necessary to develop a phenomenological-based model to properly predict the dynamic behavior of the different variables involved in the SSF process. In this work, an unstructured mathematical model was developed. The parameter identification and model validation were also carried out, using the experimental data for different SSF processes conducted with oil palm waste as the substrate and *Kluyveromyces marxianus* as the fermentative microorganism. Finally, a sensitivity analysis is proposed to be used in order to improve the parameter identification procedure.

In section 2, a description of the methodology for the SSF experiments and the development of the mathematical model is presented. In section 3 the results of the model optimization and sensitivity analysis are shown, and the role of the different parameters is discussed. Also, the results of re-identification for the sensitive parameters, and its implication in the model performance are presented. Finally in the section 4, some conclusions are summarized.

2. Methodology

2.1. Pretreatment of the lignocellulosic waste

The oil palm wastes were donated by the CENIPALMA investigation center, obtained in an oil extraction factory located in Santander, Colombia. The dry wastes were milled in the Industrial Biotechnology Laboratory of the Universidad Nacional de Colombia, to obtain particles with a diameter of 1.5mm or less, and then a pretreatment with sulfuric acid was carried out (2%V/V, 20% W/V of solid load and 121°C during 80 minutes). The material was then dried for 12 hours in an oven at 50°C in the Biotechnology Laboratory of the Universidad de Antioquia. After that, an alkali pretreatment was performed (121°C, in a solution of NaOH 1%V/V, 10% W/V of solid load during 30 minutes). Finally the material was washed with distilled water several times, dried in an oven at 50°C for 12 hours and stored in a fresh place.

2.2. Yeast strain

The yeast Kluyveromyces marxianus ATCC 36907, a thermotolerant yeast, was used in this work. The strain was kept at 4°C, in a solid medium containing Glucose 20g/L, Peptone 5g/L, yeast extract 3g/L, malt extract 3g/L and Agar 20g/L. The pH of the solid medium was adjusted to 5.0. Every three months a new culture was made. Before using the microorganism in the SSF process, and in order to reactivate it, a colony was taken from the culture in the solid medium and inoculated in a 250ml flask containing 100 ml of MGYP growth medium (20g/L glucose, 5g/L peptone, 3 g/L yeast extract and 3 g/L malt extract) with an initial pH of 4.8±0.05. The flask was kept in a shaker at 38°C and 150 rpm overnight. Finally, a new culture in solid medium was made in a Petri dish, and it was incubated for 48h at 38°C.

2.3. SSF inoculum preparation

A 1L flask, containing 460ml of MGYP growth medium (pH of 4.8±0.05) enriched with ammonium sulfate 3g/L, magnesium sulfate 1g/L and monobasic potassium phosphate 2g/L. It was autoclaved at 121°C, 15 Psi for 20 min. Then, a loop of the reactivated yeast in the solid medium was added in sterile conditions. The flask was incubated in a rotatory shaker at 38°C and 150 rpm overnight. When the concentration of the yeast was close to 1g/L, achieved after 10-12 hours of incubation, at the end of the exponential phase, the inoculum was added to the SSF reactor.

2.4. Saccharification Enzyme

In the SSF process, the enzymatic complex Acellerase 1500®, purchased from Genencor®, was used. The measured activity of this enzyme was 80 FPU/mL following a modified procedure of the protocol reported by Adney and Baker[8]. This activity was stable for more than 8 month while keeping the enzyme at 4°C.

2.5. SSF experiments

A description of the experiments to obtain the data for identifying the parameters and validating the model is shown in Table 1. Experiments were carried out in a 7 liter

Table 1.
Experimental Design for the Simultaneous Saccharification and Fermentation experiments, using oil-palm wastes as the substrate.

Experiment	Conditions		Data usedfor:	
	Agitation (rpm)	Solid load (%w/v)	Identification	Validation
SSFa	300	6		X
SSFb	150	8	X	
SSFc1	300	8	X	
SSFc2	300	8	X	
SSFc3	300	8		X
SSFd1	500	8	X	
SSFd2	500	8	X	
SSFe	300	10		X

Newbrunswick Bioflo 110 bioreactor with 5L of working volume. The saccharification enzyme, and 500 ml of the inoculums were added to the reactor containing 4.5 L of citrate buffer 0.5M, pH 4.8 (previously autoclaved at 121°C, 15 psi, 20 min), in order to achieve a final concentration of 15 FPU/(g of substrate) and 0.1g/L respectively. The medium also contained peptone 5g/L, yeast extract 3g/L, malt extract 3g/L, ammonium sulfate 3g/L, magnesium sulfate 2g/L and monobasic potassium phosphate 1g/L. the substrate (pretreated oil palm waste) was added at different solid loads (see Table 1). All the steps above were carried out in sterile conditions. The temperature of the process was controlled at 38°C. The pH and dissolved Oxygen concentration (DO) in the reactor were monitored. Different values of the agitation velocity were used (150, 300 or 500 rpm) in order to evaluate whether it has an important effect in the SSF process, and for it to be described in the mathematical model. Table 1 shows the experimental arrangements with their role (data used for parameter identification vs. used for model validation). In order to take into account experimental errors, a triplicate for one of the SSF experiments (randomly selected) was carried out. The standard deviation in this experiment was considered the same as the others. The SSF process was monitored for 72 h, taking samples periodically and keeping them in a freezer at -20°C for less than a week, until they were analyzed.

2.6. Analytical techniques

Samples of 5ml were taken periodically during the 72h of the SSF experiments. After centrifugation (6000rpm, 10 min, 4°C) and filtering the supernatant with a cellulose filter of 0.2 µm, the sample was analyzed by duplicate in an HPLC. The analysis for glucose and ethanol were carried out in a Supelcol-gel® Column at flux conditions of 1.2 ml/min and 80°C, with sulfuric acid 5mM as the mobile phase. The yeast concentration was not measured.

2.7. Mathematical model

Mass balances were performed for the SSF system, applying principles of conservation, considering the desired model resolution for making the adequate assumptions in order to describe the main process dynamics. The dynamic equations that provide valuable information are chosen and combine with the constitutive equations that complement the first principles model. Fig. 1 shows the proposed mechanism of ethanol production from lignocellulosic wastes in the SSF process.

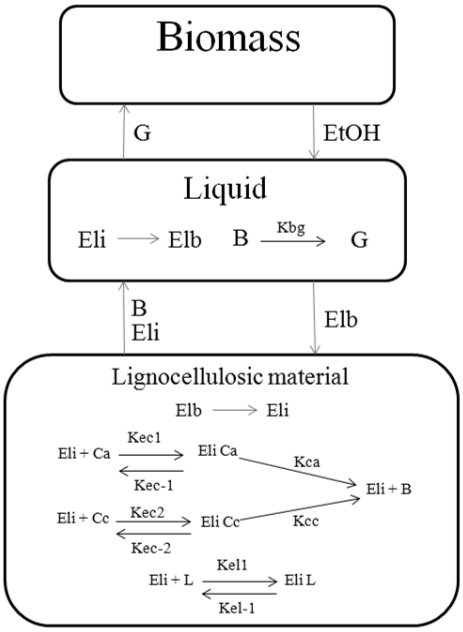

Figure 1.Proposed mechanism of ethanol production from lignocellulosic wastes in the SSF process.

The equations for the proposed model in this work and the respective assumptions are presented. During the SSF process it is necessary for the enzyme to diffuse into the solid phase to react with the substrate, hence a distinction can be made between 2 types of enzymes. The first is the free enzyme in the bulk of the liquid (Elb). The ability of this enzyme to react changes for two reasons, because its diffusion to the solid phase and because its inactivation due to unknown phenomena. Eq.(1) describes this dynamic behavior.

The second is the enzyme that has accessed the vicinity of the solid particles (*Eli*) whose concentration depends on the mass transfer of the enzyme from the bulk liquid and the formation of complexes with the fractions of the lignocellulosic material. This is expressed by Eq.(2)

$$\frac{dEl_b}{dt} = -Ka_p(El_b - El_i) - K_{ed}El_b \qquad (1)$$

$$\frac{dEl_i}{dt} = Ka_p(El_b - El_i) - \left(\frac{dEl_iCa}{dt} + \frac{dEl_iCc}{dt} + \frac{dEl_iL}{dt}\right) \qquad (2)$$

Cellulose is considered to be composed of two fractions, one easily-hydrolysable amorphous cellulose and the other, a fraction of crystalline cellulose that is highly organized and whose hydrolysis takes place more slowly. The change in the concentration of these fractions over time, and of the complexes that they form with the enzymes is presented in Eqs.(3)-(6). It is considered that there is a decrease of

amorphous or crystalline cellulose (equations 3 and 4 respectively) when the enzyme diffused to the solid phase is adsorbed on a part of the cellulose fraction of the material. This fraction is represented by α for the amorphous cellulose (*Ca*) and β for crystalline cellulose (*Cc*). It is also assumed that these fractions are kept at the same proportion throughout the process. Furthermore, the cellulose for each fraction, will reappear again when the respective enzyme-cellulose complex is dissociated.

$$\frac{dCa}{dt} = -a_p \alpha K_{ec1}[El_i][Ca] + K_{ec-1}[El_i Ca] \quad (3)$$

$$\frac{dCc}{dt} = -a_p \beta K_{ec2}[El_i][Cc] + K_{ec-2}[El_i Cc] \quad (4)$$

The complexes between cellulose fractions and the enzyme that has accessed the substrate are formed and dissociate as explained in the preceding paragraph, but these complexes also disappear when the saccharification reaction occurs. This reaction is inhibited by the presence of cellobiose and ethanol[9]. Accordingly, expressions for the change over time of the amorphous cellulose enzyme complex (EliCa) and crystalline cellulose enzyme complex (EliCc) are given in Eq.(5) and Eq.(6) respectively.

$$\frac{dEl_i Ca}{dt} = -K_{ec-1}[El_i Ca] + a_p \alpha K_{ec1}[El_i][Ca] - \frac{K_{ca}[El_i Ca]}{1+\frac{B}{K1b}+\frac{EtOH}{Ke}} \quad (5)$$

$$\frac{dEl_i Cc}{dt} = -K_{ec-2}[El_i Cc] + a_p \beta K_{ec2}[El_i][Cc] - \frac{K_{cc}[El_i Cc]}{1+\frac{B}{K1b}+\frac{EtOH}{Ke}} \quad (6)$$

The interaction of the enzyme with lignin is expressed in Eq.(7)and Eq.(8). The formation of the enzyme-Lignin complex (*EliL*) occurs by reversible adsorption of the enzyme on a portion of the lignin fraction (γ) of the material.

$$\frac{dEl_i L}{dt} = a_p \gamma K_{el1}[El_i][L] - K_{el-1}[El_i L] \quad (7)$$

$$\frac{dL}{dt} = -a_p \gamma K_{el1}[El_i][L] + K_{el-1}[El_i L] \quad (8)$$

It is considered that the area of the substrate particles decreases with time due to the hydrolysis of cellulose. Assuming spherical particles of area a_p (Eq. 9) it can express the decrease of the radius of the particles according to Eq.(10), which takes into account the hydrolysis of cellulose, the density of the material of the particles (ρp) and the number of particles in the reactor (*Np*).

$$a_p = N_p(4\pi r_p^2) \quad (9)$$

$$\frac{dr_p}{dt} = -\frac{\left(\frac{K_{cc}[El_i Cc]+K_{ca}[El_i Ca]}{1+\frac{B}{K1b}+\frac{EtOH}{Ke}}\right)V}{N_p \rho_p (4\pi r_p^2)} \quad (10)$$

The saccharification process, specifically the hydrolysis of the fractions of cellulose, leads to the production of cellobiose (B), as expressed by Eq.(11). This equation takes into account the inhibition effects of the hydrolysis of cellulose in the presence of cellobiose and ethanol.

$$\frac{dB}{dt} = \frac{K_{cc}[El_i Cc]+K_{ca}[El_i Ca]}{1+\frac{B}{K1b}+\frac{EtOH}{Ke}} - r_{gp} \quad (11)$$

On the other hand, there is a phenomenon of hydrolysis of cellobiose that leads to glucose production (Eq. 12). This hydrolysis is inhibited by the product, i.e. by the presence of glucose in the medium[6,9]. Glucose is consumed by the yeast for growth and maintenance (Eq. 13). The dynamics of glucose is then given by Equation 14.

$$r_{gp} = \frac{K_{bg} B El_b}{K_{sgp}+[B]+\frac{[G]}{K_{1g}}} \quad (12)$$

$$r_{gc} = \frac{\mu X}{Y_{xs}} + m_s X \quad (13)$$

$$\frac{dG}{dt} = r_{gp} - r_{gc} \quad (14)$$

Finally, the yeast growth and ethanol production are described by Eq.(15) and Eq.(16) respectively, whereas the expressions for the specific growth rate (assumed to be Monod kinetics with a correction for inhibition by ethanol)[4,10]and the specific rate of ethanol production are defined in Eq.(17) and Eq.(18) respectively.

$$\frac{dX}{dt} = \mu X - K_d X \quad (15)$$

$$\frac{dEtOH}{dt} = q_p X \quad (16)$$

$$\mu = \left(\frac{\mu_{\max G}}{K_S+G}\right)\left(1-\frac{EtOH}{K_{iEtOH}}\right) \quad (17)$$

$$q_p = \frac{\mu}{Y_{xp}} \quad (18)$$

The proposed model consists of 13 ordinary differential equations, five algebraic equations and a total of 22 parameters. Finally, the effect of agitation was not included in the model, as the experimental results at different stirring velocities showed no significant difference.

2.8. Parameter identification

Using data from five different experimental setups (Table 1) the parameter identification was performed in the software Matlab, using the MIPT algorithm described by Ochoa et al. [11]. For the identification procedure, an objective function was defined (Eq. 19), consisting of the summation of the absolute average error of the experimental values of ethanol and glucose from the chosen SSF experiments. The calculation of the absolute average error for each set of data was performed according to Eq.(20), where *AAE* is the absolute value of the average error, *n* is the number of experimental data points, *Exp* indicates the experimental value and *Pre* the value predicted by the model. *Expmax* is the maximum value of the experimental data that are being used for the calculation of the *AAE*, and in turn the *Expmin* is the minimum value of the same data. The optimization problem to solve during the parameter identification is given by Eq.(21), where *x* is the vector of parameters to be identified, *lb* (lower bounds) is the vector of minimum acceptable values of the parameters, *ub* (upper bounds) is the vector of maximum acceptable values for the parameters and *fobj* is the objective function to be minimized (Eq. 19).

For the sensitivity analysis, the approach of sensitivity index described by Ochoa et al. [12] was followed in order to evaluate how the model results are affected with the variation of each parameter. The procedure of parameter identification and sensitivity analysis is presented in Fig. 2.

$$\boldsymbol{F_{obj} = AAE_{Glucosa} + AAE_{Etanol}} \qquad (19)$$

$$\boldsymbol{AAE = \frac{\sum_{i=1}^{n}\left|\frac{Exp_i - Pre_i}{Exp_{max} - Exp_{\min}}\right|}{n}} \qquad (20)$$

$$\min_{\mathbf{x}} Fobj \quad s.to.\ \mathbf{lb} < \mathbf{x} < \mathbf{ub} \qquad (21)$$

The initial values for the set of parameters were taken from values reported in the literature by several authors (see Table 2). The identification of Parameters for the proposed model (Eqs. 1-18) was made by solving the optimization problem proposed in Eq.(21) and the experimental data as shown in Table 1. The sensitivity index with respect to the identified set of parameters was calculated as described in Eq.(22). Where Si^k is the sensitivity index for the kth parameter and Po^k is the optimized value of the Kth Parameter. Sensitive parameters were defined as those whose sensitivity index was higher than an established tolerance. This tolerance was chosen in a way that it would be at least one order of magnitude of difference between the sensitivity index of the parameters considered sensitive and those considered non-sensitive. When first principles based models are developed for describing the dynamic behavior of complex processes (like the case study addressed in this paper), usually the number of parameters is high and there are not enough experimental data available for reliable parameter identification. Usually, the number of experimental runs is limited to a couple of experiments, where different experimental conditions are analyzed (according to the design of experiments carried out). However, not all the possible conditions can be tested due to economic concerns. On the other hand, it is important to notice that if, the developed model is a first principles based model, and not an empirical one, the model uses some constitutive equations which have empirical bases. That is precisely why some parameters of the model must be re-identified when the model is tested using new experimental conditions. However, not all the parameters must be re-identified, and that is why the main objective of this paper is to propose a methodology for finding the best set of parameters under different experimental conditions, using lower computational time (which means, reducing the number of parameters that must be re-identified). Specifically, in this work the use of a re-optimization routine separately for each dataset is presented and analyzed, recalculating only the parameters classified as sensitive and keeping constant the set of non-sensitive parameters.

$$\boldsymbol{S_i^K = \int_{P_o^K - 0.1P_o^K}^{P_o^K + 0.1P_p^K} \left| F_{obj}(P^K) - F_{obj}(P_o^K) \right| dP} \qquad (22)$$

2.9. Model validation

The validation of the model was performed by comparing the dynamic behavior of the main variables predicted by the model against experimental data obtained for these variables. Also we calculated the objective function (measurement of the error) to check the model performance. Table 1 shows the experimental set-ups used for validation.

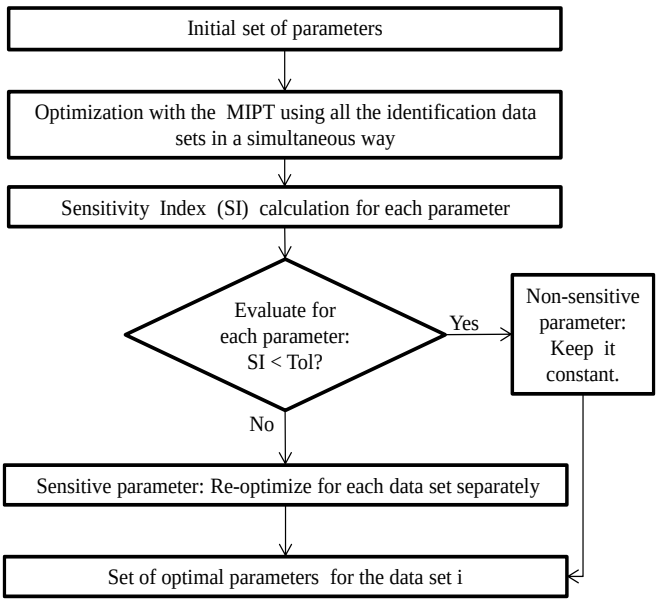

Figure 2.Parameter identification procedure.

3. Results and discussion

In Fig. 3 the dynamic behavior of glucose and ethanol can be observed. Experimentally, at the beginning of the process (the first 5 hours) a very fast increase of the glucose concentration takes place due to the high hydrolysis rate. However, after glucose starts to be available, a high glucose consumption rate is reached. This effect causes a decrease in the total glucose concentration. Such a decrease is motivated by the cellular growth. Although the glucose concentration goes to low values rapidly, a continuous production of ethanol is observed until reaching 6g/L approximately. This evidences the fact that the hydrolysis reaction occurs during the whole process and not just at the beginning.

Identifying a first set of parameters using simultaneously all the data sets (ssfb, ssfc1, ssfc2, ssfd1 and ssfd2 in Table1), the objective function value decreased from 10.72 to 2.23, which indicates an improvement in the model performance due to the optimization process. A sensitivity analysis was performed to analyze which parameters mostly affected the model results, when their values vary. Table 2 shows the sensitivity index of each parameter calculated as explained in the methodology section. It was observed that 11 of the 22 parameters affect significantly the model results. Firstly it is important to realize that the parameters related to the metabolic capabilities of the yeast, specifically μmax, Y_{XS}, Y_{XP} and *Ks* are the parameters to which the model is most sensitive.

This result indicates that the use of a different microorganism in the SSF process can strongly affect the results, and in turn justifies the current interest of many researchers for testing various microorganisms with different capabilities to get better results in the SSF processes [13,14]. Something similar may be said about the parameter *'Ms'*, which indicates the glucose consumption for maintenance, which may vary among different microorganisms and conditions. In contrast we found that the parameter *Kd* of cell death, does not significantly affect the model results. Furthermore, the optimized value found for this parameter is very low(close to zero), which might suggest that the effect of cell death proposed in the model could be neglected, at least for a time up to 72 hours of cultivation.

However, it is possible for *Kd* to become an important parameter in processes that take a longer time to be completed. Furthermore, it is observed that the parameters, *Kcc* and *Kca*, which are related to the hydrolysis of cellulose fractions for producing cellobiose, significantly affect the model, as the parameter *Ke*, which is related to the inhibition of cellobiose production due to presence of the ethanol. According to this result, the hydrolysis of cellulose and the consequent production of cellobiose have a significant influence on the results of the SSF processes performed with lignocellulosic materials. This suggest the importance of using cellulases, which are able to maintain a good catalytic activity and at the same time are less sensitive to inhibition, when aiming to optimize the results of an SSF process.

Table 2.
Results of the first optimization and the sensitivity analysis

Parameter	Initialvalue*	Identifiedvalue	Sensitivityindex	Sensitive	Non-sensitive
Kd(h^{-1})	0.0020	0.0006	0.00007		X
KietOH(g/l)	50.000[4]	12.7500	0.00769	X	
K1g (g/l)	3.1500[14]	0.9630	0.00009		X
K($m^{-2}h^{-1}$)	0.0050	0.0055	0.00560	X	
Kel1(m^{2}*l/fpu*h)	0.0092[4]	0.0069	0.00006		X
Kel_1(h^{-1})	7.2000[5]	6.8818	0.00006		X
Yxp(g/g)	0.2500[15]	0.2913	0.16672	X	
umax(h^{-1})	0.4010[5]	0.2807	0.06998	X	
Yxs(g/g)	0.4850[5]	0.1750	0.11411	X	
Ks(g/l)	2.1840[5]	1.1842	0.02912	X	
Kec1(m^{2}*l/fpu*h)	0.0368[5]	0.0275	0.00422	X	
Kec_1(h^{-1})	0.0092[14]	0.0016	0.00014		X
Kec2(m^{2}*l/fpu*h)	0.0106[5]	0.0056	0.00010		X
Kec_2(h^{-1})	0.0027[14]	0.0015	0.00001		X
Ke(g/l)	50.3500[4]	60.2035	0.00276	X	
Ked(h^{-1})	0.0020	0.0013	0.00003		X
Kca(h^{-1})	0.0057[14]	0.0029	0.05061	X	
Kcc(h^{-1})	0.0017[14]	0.0001	0.00255	X	
Ms(h^{-1})	0.0064	0.0072	0.00682	X	
Kbg(h^{-1})	0.2000	0.1390	0.00012		X
K1b(g/l)	0.0860	0.0973	0.00005		X
Ksgp(g/l)	0.1229	0.1255	0.00003		X
Fobj**	10.72	2.2263	-	-	-

*Initial value of the parameters. Those referenced were taken from the literature. The others were based on previous knowledge.

In general it was found that the parameters related to the formation of the cellulose-enzyme complex, do not strongly affect the model. The only one of these parameters that affects the model results was *Kec1*. This indicates that in the saccharification of lignocellulosic materials, the hydrolytic capacity of the cellulases can be more important than its ability to bind themselves to the substrate; however, no information was found in the literature to support this fact.

On the other hand, the *KietOH* parameter significantly affects model results. This confirms what was stated before concerning the importance of the microorganism's capabilities, specifically in this case, the ability to resist high ethanol concentrations.

Finally, it was found that the mass transfer coefficient (*K*) significantly affects the model, indicating that when carrying out an SSF process, the access of the enzymes to the lignocellulosic material is an important fact that must be taken into account.

Since the use of different stirring velocities does not significantly affect the SSF process, such accessibility must be improved by other methods such as decreasing the size of the substrate particles or changing the properties of the medium, by for example, adding surfactants to the bioreactor. Some studies have already shown that by doing so, it is possible to improve the results of the SSF process[17,18].

After the sensitivity analysis, the re-identification of the sensitive parameters was carried out for each data set

individually (ssfa,ssfc3,ssfe) and the objective function value decreased considerably (see Table 3), which indicates the improvement of the model performance due to the coupling of the sensitivity analysis and the re-optimization process.

Fig. 3 shows the results for the model fit when performing the re-optimization using each experimental set separately, for identifying just the 11 parameters considered as sensitive.

In general an improvement is observed in the fit of the data of glucose and ethanol. This improvement, when comparing the fit of the model before and after optimization, leads to a better prediction of the trends for each case in particular and a reduction in the value of the objective function of 8%, 19% and 10% for the validation data of SSFa, SSFc3 and SSFe respectively (Table 3). Nevertheless, for the data of the SSFe experiment (Fig. 3c), where the prediction of the values and the trends of the variable are still close to the experimental data, the variation in the production of glucose in the first hours of the process is underestimated.

It is important to notice that the change in the value of almost all the parameters is not even of one order of magnitude after re-optimization. Most of the parameters that had the biggest change are kinetic parameters related to the reactions for producing the cellobiose and for the formation of the complexes enzyme-cellullose. This fact shows that those parameters are affected by the initial solid load. According to these results, it might be possible to state that the reaction kinetics in the mentioned reactions are of a superior order, and not of order one as assumed in the development of the model.

Other variables for which experimental data were not taken had a realistic behavior when simulations were performed, giving more confidence in the model performance (data not shown).

Table 3.
Reidentified parameters for each experiment.

Parameter	Value in firstidentification	Valuefor SSFa	ValueforSSFc3	ValueforSSFe
KietOH(g/l)	12.7500	10.0400	3.5850	6.7200
K($m^{-2}h^{-1}$)	0.0055	0.0017	0.0059	0.0053
Yxp(g/g)	0.2913	0.2687	0.2965	0.2867
Umax(h^{-1})	0.2807	0.3439	0.4763	0.2096
Yxs(g/g)	0.1750	0.1826	0.1390	0.2773
Ks(g/l)	1.1842	1.5019	1.7730	1.9186
Kec1(m^2*l/fpu*h)	0.0275	0.0048	0.0258	0.0412
Ke(g/l)	60.2035	13.5039	23.5940	53.2854
Kca(h^{-1})	0.0029	0.0006	0.0039	0.0019
Kcc(h^{-1})	0.0001	0.0016	0.0006	0.0003
Ms(h^{-1})	0.0072	0.0011	0.0004	0.0075
Fobj in optimization	-	0.258	0.473	1.959
Fobj in re-optimization	-	0.237	0.384	1.767

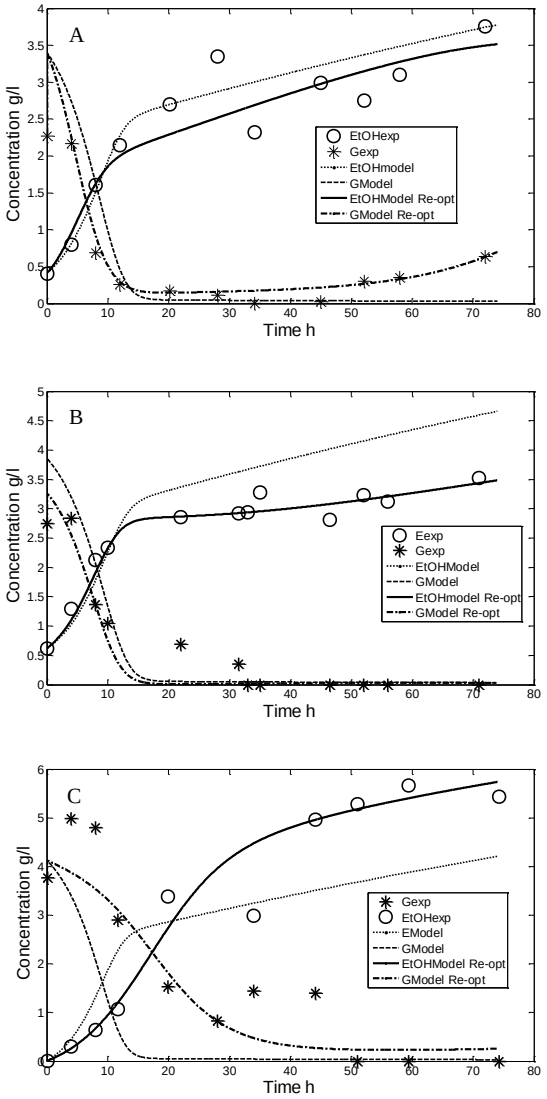

Figure 3.Model fit for the validation data: A) Ethanol and Glucose for the SSFc3 data, B) Ethanol and Glucose for the SSFa data, C) Ethanol and Glucose for the SSFe data. For the three Figures, the nomenclature is: Experimental Glucose (*), Experimental Ethanol (o), Ethanol (---) and Glucose (- - -) predicted by the model with the parameters found using all the identification data simultaneously (see Table 2, column 3) and Ethanol ( ) and Glucose (-.-.-) predicted by the model with the parameters found using re-optimization (see Table 3, columns 3-5)
Data points represent the mean value from at least three separate experiments (the minimum standard deviation for ethanol was between 0.09 and the maximum was 0.99. For glucose the minimum standard deviation was 0.001 and the maximum was 0.8) Error bars are omitted for reasons of clarity

4. Conclusions

Regarding the results of the fermentation process, it can be concluded that the rapid production of glucose during the first moments of the process, decreased drastically possibly due to the formation of ethanol. Likewise it is noted that even though the hydrolysis could be affected by the presence of ethanol, it is maintained throughout the process time, which is an important result for the development of this type of process.

A new unstructured, first principles based model for predicting the main dynamics in the ethanol production process from lignocellulosic wastes using the Simultaneous Saccharification and Fermentation technology was developed. The proposed model contains some new features such as: a) an approach for describing the enzymatic action on a lignocellulosic substrate, considering it to consist of spherical particles whose radius decreases as the saccharification takes place, b) the formation of different enzyme-substrate complexes, c) Mass transfer issues.

From a sensitivity analysis, it was found that from the 22 parameters present in the model, only 11 parameters appear to have a significant effect on the model behavior, most of them associated with characteristics related to the yeast used, while others were found to be associated with enzyme's properties and the mass transfer in the system. The re-identification of these 11 parameters, allows one to reduce the value of the objective function. This fact suggests that such a procedure for sensitivity analysis can improve the parameter identification process, resulting in a greater flexibility when implementing the model.

Acknowledgements

The authors acknowledge the support given by the Industrial Biotechnology research group at the Universidad Nacional de Colombia Sede Medellín, where the pretreatment of the samples was carried out. Also, the academic and financial support by the Academic Environmental Corporation of the Universidad de Antioquia and the CODI committee at Universidad de Antioquia are gratefully acknowledged.

Notation

Notation	Units	Definition
Ca	g/l	Amorphous cellulose concentration
Eaca	g/l	Complex adsorbed enzyme-Amorphous cellulose concentration
Cc	g/l	Crystalline cellulose concentration
Eacc	g/l	Complex adsorbed enzyme-Crystalline cellulose concentration
L	g/l	Lignin concentration
EaL	g/l	Complex adsorbed enzyme-lignin concentration
El	FPU/l	Free enzyme concentration
Ea	FPU/l	Adsorbed enzyme concentration
B	g/l	Cellobiose concentration
G	g/l	Glucose concentration
X	g/l	Yeast concentration
EtOH	g/l	Ethanol concentration
r_p	m	Particle radius
a_p	m^2	Particle area
Np	-	Number of particles
V	l	Reactor volume
ρ_p	g/m^3	Particle density
Kd	h-1	Cell death constant
KietOH	g/l	Growth Inhibition constant by ethanol
Kel1	m^2l/fpu *h	Rate constant of adsorbed enzyme-lignin complex formation
Kel_1	h^{-1}	Rate constant of adsorbed enzyme-lignin complex separation.
Yxp	g/g	Cell biomass yield by ethanol
μmax	h^{-1}	Maximum Specific rate of cell growth
Yxs	g/g	Cell biomass yield by glucose
Ks	g/l	Saturation constant for growth using glucose as substrate
Kec1	m^2l/fpu *h	Rate constant of adsorbed enzyme-amorphous cellulose complex formation
Kec_1	h^{-1}	Rate constant of adsorbed enzyme- amorphous cellulose complex separation.
Kec2	m^2l/fpu *h	Rate constant of adsorbed enzyme-Crystalline cellulose complex formation
Kec_2	h^{-1}	Rate constant of adsorbed enzyme-Crystalline cellulose complex separation.
Ke	g/l	Inhibition constant of cellobiose production by ethanol
Ked	h^{-1}	Inactivation rate of the free enzyme
Kca	h^{-1}	Reaction rate constant for cellobiose formation using amorphous cellulose
Kcc	h^{-1}	Reaction rate constant for cellobiose formation using crystalline cellulose
Ms	h^{-1}	Glucose consumption for maintenance constant.
Ksgp	g/l	Saturation constant for glucose production using cellobiose as substrate
K1g	g/l	Inhibition constant of the free enzyme by glucose
Kbg	g/fpu* h	Rate constant for glucose production using cellobiose as substrate
K1b	g/l	Inhibition constant of cellobiose production by cellobiose
K	$1/m^2$*h	Mass transfer coeficient

Notationfor figures

EtOHModel	Ethanol predicted y the model
GModel	Glucose predicted by the model
'O'EtOHexp	Experimental ethanol
'*'Gexp	Experimental Glucose

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