SIMULATION AND CONTROL OF ENZYMATIC HYDROLYSIS REACTOR OF SOLUBLE POTATO STARCH BY *ASPERGILLUS NIGER* GLUCOAMYLASE FOR FERMENTATION PROCESS FEED

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ABSTRACT - An existing way to produce glucose uses enzymes to hydrolyzepolysaccharides. *Aspergillusniger* is recognized to produce glucoamylases capable to reduce starch to glucose molecules, used in fermentations processes as substrate. For continuous bioreactors operation mode, the substrate feed is required during bioprocess. Thus, enzymatic reactors able to continuously produce substrate feed are extremely necessary. Although, control strategies are demanded to regulate the variables of enzymatic process for adequate fermentation feed. For this reason, the paper analyses control strategies to regulate glucose concentration in the reactor outlet flow and reaction media volume, manipulating starch and enzymatic inlet solutions flows and reactors outlet flow. Control loop was designed analyzing processes variables responses to disturbances in the manipulated variables. Ratio control was implemented and the results demonstrate its superiority to SISOglucose concentration control.

1. INTRODUCTION

In bioethanol production, substrate preparation is an important part of entire production chainto guarantee product quality and desired process productivity. Process contamination, fermentation inhibition by substrate excess and low bioethanol production in reason of carbohydrates poor broth are some causes of fermentation productivity losses associated to substrate preparation step. In case of sugar-cane as fermentation substrate, is necessary to mill the biomass and extract the broth rich in glucose, which do not requires any chemical post-treatmentfor broth adequationto fermentation process. However, in case of starchy plants as corn, rice and potato,the substrate is founded as starch, a polysaccharide that must be hydrolyzed to glucose that is directly fermentable by yeasts(Nigam & Singh, 1995). Starch hydrolysis is a typical chemical process that can be catalyzed by enzymes as glucoamylases (GA) produced by *Aspergillusniger*, which hydrolyze α-1,4 and β-1,6 glucosidic linkages in saccharides (Polakovic&Bryjak, 2004; Wang *et al.*, 2008; Riaz*et al.*, 2012).

The GAs are of great economic importance, been extensively used in different industry segments as food, beverages and biofuels industry (Boel*et al.*, 1984).As example, for biofuels production the starch hydrolysis is applied to prepare the substrate to fermentation. In case of a CSTR for fermentation, another reactor operating continuously is requiredto starch hydrolysis. Masuda *et al.* (2013) improved the efficiency of continuous starch hydrolysis using a Couette-Taylor flow reactor, but also a CSTR can be used for this process. Thus,

depending on the process operation mode, whether the substrate is totally added in the fermentor load or substrate feeding is continuously, starch hydrolysis control is necessary to do not compromise bioethanol production.

In this work, using CSTR to potato starch hydrolysis by glucoamylases of *Aspergillusniger*, sensibility assay was conducted to identify suitable manipulated and process variables to implement different control strategies to starch hydrolysis process. The control loops were analyzed using performance criterions IAE and ISE, but also control effort of actuators were considered to compare control strategies.

2. MATERIALS AND METHODS

The enzymatic reaction of starch hydrolysis by *Aspergillusniger*glucoamylase was previously studied (Polakovic&Bryjak, 2004) and also simulations were performed to represent this reaction in a CSTR (Ochoa*et al.*, 2010a; Ochoa*et al.*, 2010b). The mass balance for susceptible starch, glucose and enzyme concentrations are represented by Equations 1 to 3 and the enzymatic reaction rateby Equation 4. The process parameters used in simulation are listed in Table 1.

$$\frac{dS_o}{dt} = \frac{F_{s,i}\cdot S_i - F_o\cdot S_o - 1{,}11\cdot K_1\cdot S_o\cdot V}{V} \quad (1)$$

$$\frac{dG_o}{dt} = \frac{1{,}11\cdot K_1\cdot S_o\cdot V - F_o\cdot G_o}{V} \quad (2)$$

$$\frac{dEnz_o}{dt} = \frac{F_{e,i}\cdot Enz_i - F_o\cdot Enz_o}{V} \quad (3)$$

$$K_1 = \frac{K\cdot Enz_o}{K_m\cdot[1+(G_o/K_g)]+S_o\cdot[1+(S_o/K_s)]} \quad (4)$$

In a typical chemical process there are many variables that affect its response with different intensities when manipulated. In the studied process, as manipulated variables the enzymatic and substrate inlet flows, product outlet flow and enzymatic and substrate concentrations in the feed flow were tested. The assays where realized applying steps with same amplitude of steady state value for each variable. For enzymatic solution inlet flow were used steps amplitudes of $\pm 10^{-3} m^3.h^{-1}$; for enzyme concentration amplitudes of $\pm 10^5$ $U.m^{-3}$; for starch concentration $\pm 5 Kg.m^{-3}$ and $\pm 10^{-3} m^3.h^{-1}$for starch feed and output flows.The responses for product, starch and enzyme concentrations and liquid level inferred from the reaction volume were analyzed to identify the variables that more affect these process variables. With this information, it is possible to design efficient control strategy that regulates the process variables with reduced correction actions.

This paper is concerned to develop multi-variable control strategies applied to the studied process. Thus, the most affecting variables were selected to be manipulated, and the less affecting were selected to be disturbance entries in order to simulate a real situation in an enzymatic reactor system.

Table 1 - Process parameters

Variable	*Description*	*Units*
F_o	Outlet flow	$m^3.h^{-1}$
$F_{e,i}$	Enzymatic solution inlet flow	$m^3.h^{-1}$
$F_{s,i}$	Substrate solution inlet flow	$m^3.h^{-1}$
S_o	Substrate concentration	$Kg.m^{-3}$
S_i	Substrate feed concentration	$Kg.m^{-3}$
Enz_o	Enzyme concentration	$U.m^{-3}$
Enz_i	Enzyme feed concentration	$U.m^{-3}$
G_o	Glucose concentration	$Kg.m^{-3}$
V	Volume	m^3
K	Constant rate	$Kg.U^{-1}.h^{-1}$
K_m	Michaelis constant	$Kg.m^{-3}$
K_g	Product inhibition constant	$Kg.m^{-3}$
K_s	Substrate inhibition constant	$Kg.m^{-3}$
K_1	Enzymatic hydrolysis rate	h^{-1}

As actuators for selected manipulated variables, were considered pumps and valves with linear flow regulation, also lower and upper limits of operation were set. For initial condition, the reactor was in steady state with all process variables in respectiveset-point values, with actuators operating with a 50% value of the range. All initial conditions, parameters limitsand set-points considered in simulation are presented in Table 2.

Considering perfect homogeneity in the CSTR, a cylindrical geometry was proposed with diameter and weigh of 0,66 m and 1,5 m, respectively, totalizing a 2 m³ of volume.

Table2–Parameters limits, initial values and set-points

Parameter	*Lower Limit*	*Initial Condition*	*Upper Limit*	*Set-point*
S_i	50 $kg.m^{-3}$	81,7 $kg.m^{-3}$	100 $kg.m^{-3}$	-
S_o	-	12,1 $kg.m^{-3}$	-	-
Enz_i	4.10^5 $U.m^{-3}$	9.10^5 $U.m^{-3}$	4.10^6 $U.m^{-3}$	-
Enz_o	-	$1,06.10^5 U.m^{-3}$	-	-
G_o	-	60 $kg.m^{-3}$	-	60 $kg.m^{-3}$
$F_{s,i}$	0,015 $m^3.h^{-1}$	0,045 $m^3.h^{-1}$	0,075 $m^3.h^{-1}$	-
$F_{e,i}$	0,002 $m^3.h^{-1}$	0,006 $m^3.h^{-1}$	0,010 $m^3.h^{-1}$	-
F_o	0,017 $m^3.h^{-1}$	0,051 $m^3.h^{-1}$	0,085 $m^3.h^{-1}$	0,051 $m^3.h^{-1}$
V	0 m^3	1 m^3	2 m^3	1 m^3

The PID controllers tuning were performed using a Simulink® functional block named Signal Constraint. The tuning parameters were calculated attempting a response time less than 50 h^{-1} for a ± 2% liquid level and ±1% product concentration errors. This functional block

does an optimization of controller parameters thresholds to obtain the process variable error within the determined constraints.

The time simulation was set to 500 h with steps in disturbance variables each 100 h. To compare the different strategies, classical performance criterions as Integral of Absolute Error (IAE) and Integral of Squared Error (ISE) were calculated to assist analysis.

3. RESULTS AND DISCUSSION

The sensibility assay realized indicates that outlet flow, starch inlet flow and enzymatic solution inlet flow have strong influence in product concentration result, been the most representative interaction attributed to enzymatic flow, as shown in Figure 1. The non-stable result for disturbances in these variables is justified by reactors flooding and drying-out that contributes to product dilution and concentration, respectively.Analog analysis can be done to starch concentration, once it is related to product formation and the same variables disturbances will influence its results.

For liquid level, only variables related to inlet and outlet flows would influence the reaction volume resultant inside the reactor, once mass balance has to be respect. In fact, the results shown in Table 3 demonstrate it.In the case of enzyme concentration, it was also expected to be more affected by both enzymatic solution concentration and inlet flow, whatisconfirmed with results in Table 3.Thus, the product concentration and reaction volume were selected as process variables. It is necessary that glucose concentration in outlet flow attempts continuous fermentation requirements, also the liquid level in the reactor has to be controlled to avoid reactors dry-out and flood. Instrumented with liquid level sensor to determine the reaction volume, its set-point was set to half of total volume and product concentration set-point to 60 $kg.m^{-3}$.

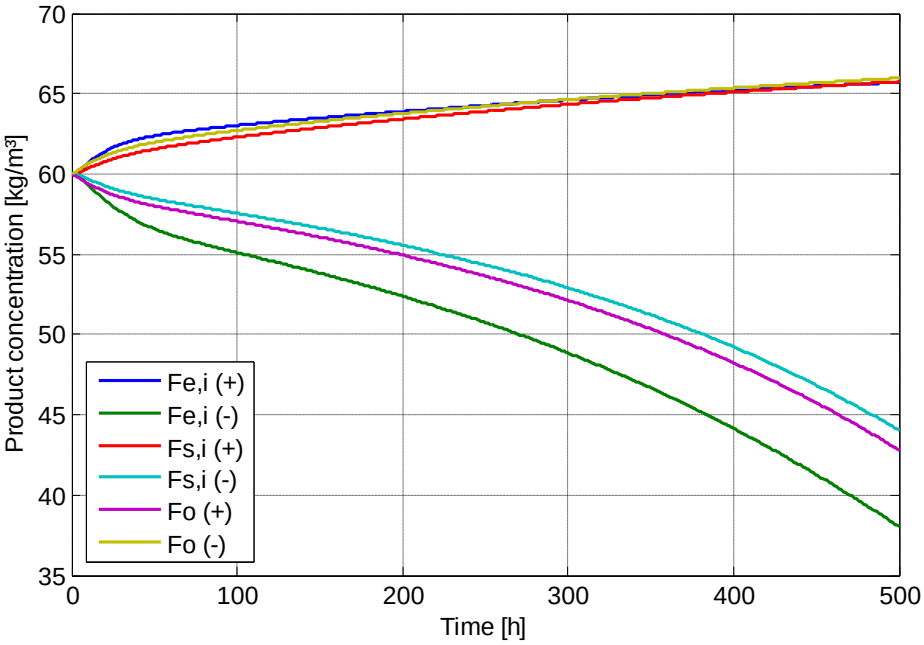

Figure 1 - Product concentration results for sensibility assays

For liquid level, a single-input-single-output (SISO) control loop was implemented in reason that it is a simple and reliable strategy for level control and was also successfully applied by Ochoa *et al.* (2010a).

Table 3 - Process variables values at time 500 h for sensibility assays

Variable	*Step*	G_i[kg.m^{-3}]	S_i[kg.m^{-3}]	V[m³]	Enz_i[U.m^{-3}]
$F_{e,i}$	+	65,7	6,38	1,5	1,235.10^5
	-	38,04	34,04	0,5	8,824.10^4
Enz_o	+	61,43	10,66	1	1,176.10^5
	-	58,16	13,92	1	9,412.10^4
$F_{s,i}$	+	65,75	7,94	1,5	1,059.10^5
	-	44,01	26,47	0,5	1,059.10^5
S_o	+	62,67	13,83	1	1,059.10^5
	-	57,19	10,48	1	1,059.10^5
F_o	+	42,81	27,89	1,5	1,038.10^5
	-	65,97	7,56	0,5	1,080.10^5

Two different strategies were tested for product concentration, the first based on a SISO loop, comparing the process variable with set-point and actuating in enzymatic solution inlet flow because it is the most affecting variable to glucose concentration. The second strategy suggested is a ratio control. To control the same process variable, starch and enzymatic inlet flow were manipulated to maintain proportionality between both. The second control loop is demonstrated in Figure 2. In steady state for initial conditions, when glucose concentration is in set-point value as mentioned before, the ratio starch:enzyme flow is 7.5:1, therefore this value was used as set-point to enzymatic solution flow.

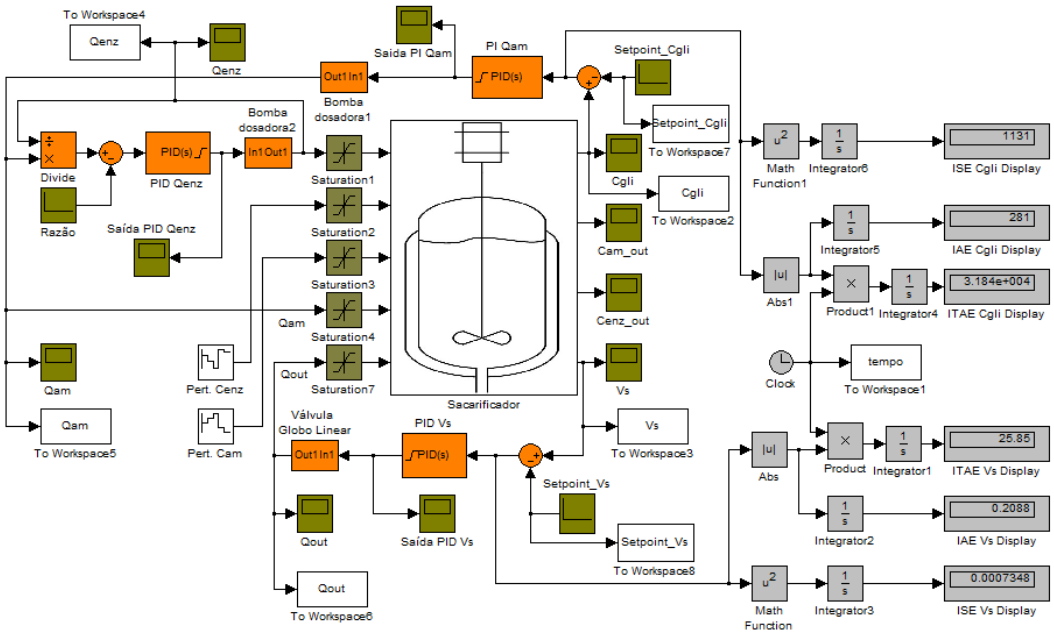

Figure 2 - Enzymatic reactor simulation applying ratio control loop

With tuning procedure, the PID parameters obtained with Signal Constraint block to all controllers implemented in strategies proposedare presented in Table 4. As it is observed, a PI

controller was used to actuate in starch inlet flow instead a PID when applying the ratio control strategy. In this case, the reason is that derivative action was unnecessary to reach a process variable response which satisfies the determined constraints. The steps value on enzyme concentration and starch concentration of inlet flows with respective times are presented in Table 5.

Table4–Controllers parameters for SISO and ratio control strategies

Controller	PID_SISO	PI_Fs,i	PID_Fe,i	PID_V
Parameter P	2,6736	0,0187	1,9043	21,5673
Parameter I	0,1818	3,2377	1,0104	3,7428
Parameter D	0,1849	-	1,8547	0,9248

In the first 100 hours of time simulation, the observed variations in process variables are consequences of controller initialization. Its output starts in lower limit value, been necessary time simulation to process variable reach steady state.

Table5–Time and value of steps on disturbs variables

Time (h)	100	200	300	400
Enz_0 (U/m³)	8.10^5	7.10^5	1.10^6	$9{,}5.10^5$
S_0(Kg/m³)	82,7	83,7	80,7	79,7

For SISO control strategy, the process variables were poorly regulated if compared to the other tested control loop. Although the glucose concentration had a high overshoot in first 100 hours to ratio control shown in Figure 3a, itstabilized around set-point value. Single output strategy was not capable to regulate product concentration in set-point value, been strongly affected by disturbances. Whereas, applying ratio control was possible to regulate this process variable without oscillation. Moreover, also the liquid level control loop had better performance with ratio control for product concentrationthan SISO,as can be seen in Figure 3b for reaction volume. Even with a high ISE for G_o control loop justified by initial overshoot, the less values of performance criterions calculated to ratio control presented in Table 6is a proof that actuating in both reactors feed flows is more effective to glucose concentration and reaction volume control.

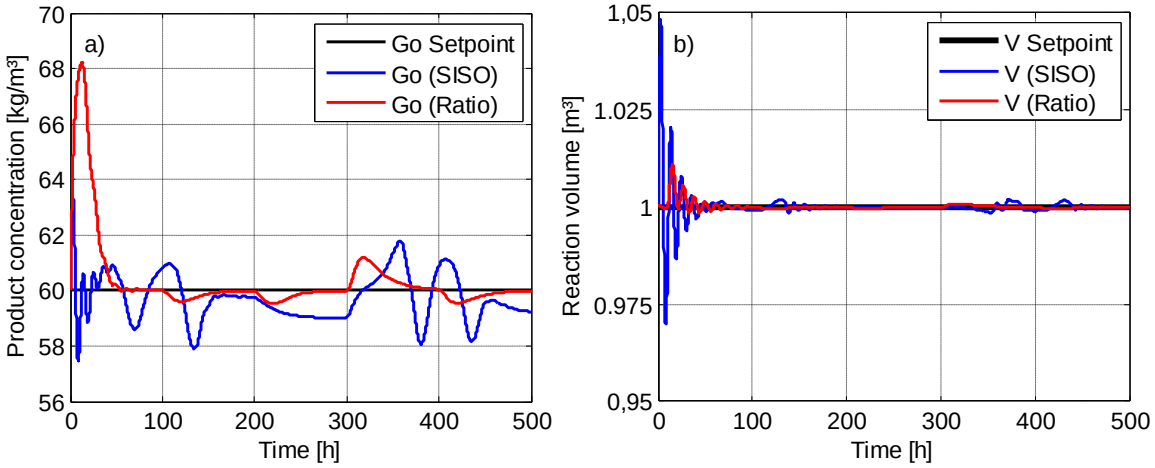

Figure3 -Process variables response in regulative control of a) product concentration and b) reaction volume

In manipulated variables actuation point of view, the ratio control was again better performed in comparison to SISO. Keeping starch feed flow constant, the others manipulated variables were extremely penalized. The Figure 4a shows that,as opposite toratiocontrol performance, starch feed flow was intensively modified during SISO control and the same analysis can be done to output flow as presented in Figure 4c.

Table 6 - Calculated performance criterions IAE and ISE

Process Variable	Strategy	IAE	ISE
G_o	SISO	384,1 $kg.s.m^{-3}$	426,1 $kg^2.s.m^{-6}$
	Ratio	281 $kg.s.m^{-3}$	1131 $kg^2.s.m^{-6}$
V	SISO	0,6761 $m^3.s$	0,01121 $m^6.s$
	Ratio	0,2088 $m^3.s$	$7,35.10^{-4}$ $m^6.s$

Ratio control strategy resulted in not only more effective process variables control, but in less manipulation of substrate and product flow too.

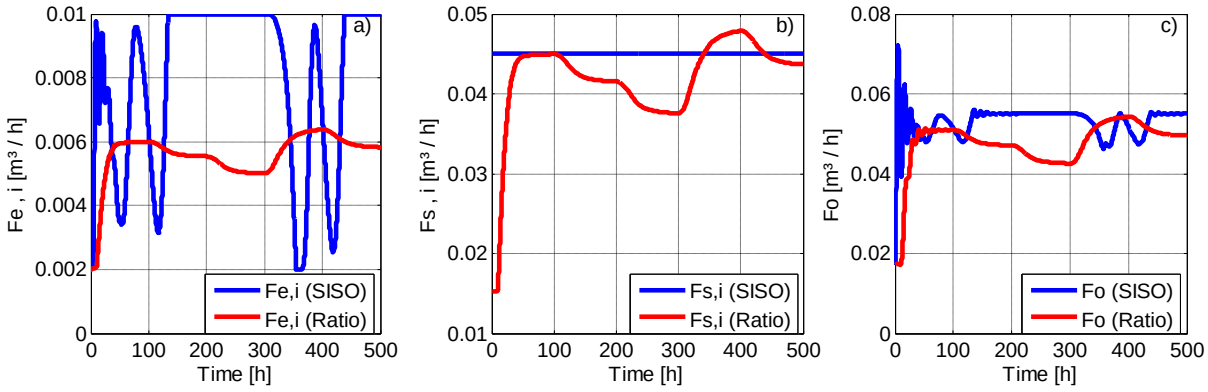

Figure 4 - Control variation of a) enzymatic solution inlet flow, b) starch inlet flow and c) output flow.

4. CONCLUSION

Process sensibility assay identified that reactor inlet and outlet flows affect with more intensity the glucose concentration and reactor liquid level, therefore the inlet and outlet flows were selected to be manipulated variables for product concentration and liquid level control loops. And theenzymatic hydrolysis reactor simulation demonstrated that a ratio control is more efficient in product concentration control. Comparing ratio control loop to SISO, the IAE criterion reduction of 26,84% for glucose concentration and 69,12% reduction for reaction volume proves that actuating in starch feed flow and enzymatic solution feed flow with a ratio strategy control results in better process regulation and also less actuation effort manipulating feed and output flows.

5. REFERENCES

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