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Enhancement of Xylitol Production by Fed-Batch Policy Designed Through Stochastic Search

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In the design of a process to produce xylitol in a stirred tank bioreactor, this work addresses the problem of determining the better feeding policy of sugars by following a stochastic search. Xylitol is a high-value sweetener that can be produced by both chemical and biochemical ways; in the last decades, the second one has been gaining more interest because of the eco-friendly and likely economical advantages of green processes. On the basis of a model that describes a fermentation process of xylose to xylitol by Candida *mogii*, an optimization problem is stated aiming to obtain the highest amount of xylitol with the minimum remaining xylose. The process considers the addition of glucose to drive more amount of xylose to the metabolic pathway xylose-to-xylitol instead of the xylose-to-cell growth one; then the problem implies the determination of two feed flows and the initial load of cells, and sugars. A Genetic Algorithm was implemented, firstly considering the most likely practical case of constant feed flows, and later partitioning the process time in several intervals, along which the feed flows are allowed to change. In both cases, the xylitol concentration obtained is even higher than the one reported in previous works.

1. Introduction

Xylitol is a sweetener that has gained attention because is a sugar substitute for persons with a special diet and its anticariogenic effect, so the commercial market involves a considerable increasing financial rate (Zacharis et al., 2012).

Xylitol is obtained by either a chemical route (Delgado-Arcaño et al., 2020) or a biochemical one (Dasgupta et al., 2017); this latter consists of the fermentation of xylose by a yeast (e.g., the genus *Candida*) in a stirred tank bioreactor at middle temperature and atmospheric pressure. The process conditions are easier to achieve, less dangerous, and cheaper than the ones implied in a chemical way; however, as any bioprocess, the product is obtained in a very diluted broth, therefore the first problem to be addressed is to obtain the highest possible product concentration.

The approaches to achieve a high concentration of xylitol or high conversion of xylose to xylitol considers the selection of microorganisms and their genetic modification (Dasgupta et al., 2017), and the form in which the bioprocess is carried out, even in a model based framework as in the works of Tochampa et al. (2005, 2015). On the use of a strain of *Candida mogii*, in a first work they proposed the addition of glucose to enhance the conversion of xylose to xylitol because the glucose likely takes the place of xylose in the metabolic pathway of sugars to yeast growing; later, they proposed to perform the bioprocess in a fed-batch reactor, achieving a higher concentration of xylitol. In this basis, Koop et al (2017) improved the policy of feeding in a simulated reactor, designing the trajectory of feeding by applying the Genetic Algorithm as optimization approach.

Although the xylitol concentration was significantly increased, a considerable amount of xylose remains. Therefore, in the forward way of designing a feasible xylitol process, this work addresses the optimization problem with the particularities of obtaining the highest xylitol concentration and the lowest remaining xylose concentration, and the raw material feeding policies that can be implemented in practice. As a basis, the process of Tochampa et al. (2015) is considered because the corresponding model has been pretty validated; the application of a stochastic search technique is followed for the sake of mathematical complexities.

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2. The Process of Xylitol Production and its Optimization Problem

The fermentation of xylose in a stirred tank bioreactor at constant temperature is considered, and it is assumed that can be fed with a xylose solution at a certain rate (Figure 1).

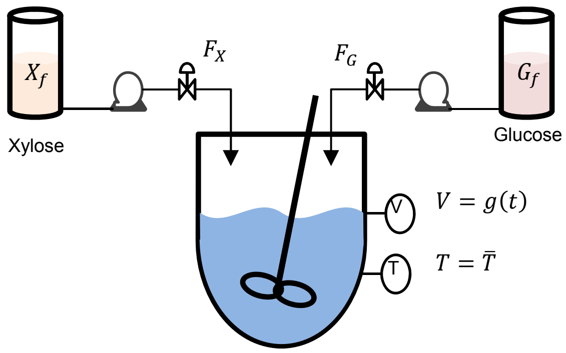

Figure 1: System of bioreaction to produce a maximum xylitol concentration

The particular bioprocess is recalled from Tochampa et al. (2015), which uses *Candida mogii* and proposes the addition of glucose, which is assumed to contribute in the metabolic pathway of glucose to microorganism growth, making to reduce the amount of xylose in its pathway to microorganism growth, so it is driven more amount of xylose in the metabolic pathway of xylose to xylitol. The mathematical model is of the following form:

$$\dot{C} = \mu(\bullet)\cdot C - \frac{F_X + F_V}{V}C, \qquad C(0) = C_0 \tag{1a}$$

$$\dot{X} = -q_X(\bullet)\cdot C + \frac{F_X}{V}X - \frac{F_X + F_G}{V}X, \qquad X(0) = X_0 \tag{1b}$$

$$\dot{G} = -q_G(\bullet)\cdot C + \frac{F_G}{V}G_f - \frac{F_X + F_G}{V}G, \qquad G(0) = G_0 \tag{1c}$$

$$\dot{P} = r_T(\bullet)\cdot C - \frac{F_G + F_X}{V}P, \qquad P(0) = P_0 \tag{1d}$$

$$\dot{R} = (r_F(\bullet) - r_U(\bullet) - r_T(\bullet))\cdot \rho - \mu(\bullet)\cdot R, \qquad R(0) = R_0 \tag{1e}$$

$$\dot{V} = F_X + F_G, \qquad V(0) = V_0 \tag{1f}$$

where *C*, *X*, *G*, and *P* are the concentrations in the fermentation broth of cells, xylose, glucose, and xylitol, respectively, and *R* refers to an intracellular concentration of xylitol; *V* is the volume of the fermentation broth. F_X and F_G are the volumetric flows of streams feeding xylose and glucose, respectively, and X_f and G_f are the concentration of xylose and glucose, respectively, in corresponding feed streams. $\mu(\cdot)$, and $q_X(\cdot)$ and $q_G(\cdot)$ are the functionalities describing the specific growth rate of cells, and the specific uptake rates of xylose and glucose, respectively; besides, $r_F(\cdot)$ describes the mass flux of xylitol per unit of dry cell mass, $r_F(\cdot)$ corresponds to the specific production of xylitol, and $r_U(\cdot)$, its intracellular consumption rate. These functionalities and corresponding parameters, as the density of cells (ρ), are given in detail in Tochampa et al. (2015).

In compact notation, the model is written as,

$$\dot{x} = f(x,u,d), \qquad x(0) = x_0 \quad \text{where } x = [C, X, G, P, R, V]', \quad u = [F_X, F_G], \quad d = [X_f, G_f] \tag{2}$$

The model describes the process in either batch ($F_X = F_G = 0$) or fed-batch (F_X, $F_G > 0$) operation. In either case, the process exhibits a consume of xylose with the simultaneous production of xylitol up to a certain instant (t_{top}) in which the xylitol concentration reaches a maximum (P_{top}); after this point, xylitol is also consumed together with the remaining xylose for the biomass growing. Therefore the process must be opportunely stopped; e.g. in a first instance at t_{top}.

The goal in this process is to obtain a xylitol concentration as high as possible, which in turn implies the design of a policy load of the reducing sugars. In a first instance, the problem means to maximize P_{top}, but at t_{top} the amount of xylose is still considerable; therefore this work also is aimed to search a point t_{stop} in which the xylose be minimum and the xylitol concentration be still high (a little lower then P_{top}). In addition, considering the movements that must be done in practice, the policy load includes the determination of the initial load of cells and reducing sugars.

3. Optimization Approach trough Stochastic Search

The goal of the process, stated above, is mathematically translated to the following optimization statement:

$$\max_{\{x_0, u(t), t_{stop}\}} J = P(t_{stop}) \tag{3a}$$

s.t. $$\dot{x} = f(x,u,d), \qquad x(0) = x_0 \tag{3b}$$

$$X_0 V_0 + \int_0^{t_{stop}} X_f F_X(t)\, dt \le w_X \tag{3c}$$

$$G_0 V_0 + \int_0^{t_{stop}} G_f F_G(t)\, dt \le w_G \tag{3d}$$

$$C_{low} \le C_0 \le C_{up}, \quad 0 \le X_0 \le X_{up}, \quad 0 \le G_0 \le f X_0, \tag{3e}$$

As it can be noticed, the operation conditions are bounded through a maximum total amount of xylose (w_X) and glucose (w_G) to be charged (3c, 3d). Considering the starting up of the fermentation process, the initial loads of cells, xylose and glucose are also bounded (3e); C_{low} considers that always must exist a certain number of cells, and C_{sup}, that the process can be inhibited by a great number of cells; X_{up} regards inhibition by an excess of sugar, but also can be related to the practical aspect of xylose solubility. Glucose is loaded in an amount much smaller than xylose, and it is practical to consider G_0 as a fraction f of X_0.
The maximum xylitol concentration to obtain, $P(t_{stop})$, is related to P_{top} through the following relationship:

$$P(t_{stop}) = P_{top} - penalty, \quad penalty = 10\sum_{j=1}^{3} \beta_j p_j \tag{4a}$$

where $$p_1 = \bar{z}_p - z_P, \qquad p_2 = V(t_{stop}) - V_R, \qquad p_3 = P_{min} - P_{top}, \qquad z_P = \frac{P}{X+G+P}, \tag{4b}$$

and $\beta_j = 0$, if $p_j < 0$, $\beta_j = 1$, if $p_j > 0$ (4c)

In this way, through p_1 with the mass fraction of xylitol free of cells (z_p), the high conversion of xylose and glucose is driven; through p_2, it is guaranteed that the broth volume will be lower than the reactor volume (V_R), and that there will be at least a P_{min} of xylitol concentration, through p_3.
The optimization problem was addressed by the application of the Genetic Algorithm in Matlab®. Since it is a search technique, the determination of a continuous trajectory of the feed flows is pretty unfeasible; rather the values of the feed flows in intervals resulted from the partition of the process time are searched. Therefore, the constraints (4b) and (4c) are time discretized,

$$X_0 V_0 + \sum_{i=1}^{N} X_f F_{X,i} \Delta t \le w_X, \qquad G_0 V_0 + \sum_{i=1}^{N} G_f F_{G,i} \Delta t \le w_G \tag{5}$$

where N is the number of intervals in which is partitioned the process time, Δt is the interval size, and i corresponds to the interval number. In this sense, $F_{X,i}$ and $F_{G,i}$ are constant flows along the interval i. N is a freedom degree; e.g. if N = 0, the problem to solve corresponds to the determination of the load for a batch process; if N = 1, the problem corresponds to the determination of an initial load together with a constant feed flow.
An operative aspect in the solution process worthy to remark is that in every scenario (x_0, $\{F_{X,i}, F_{G,i}\}$, i = 1, …,N) set by the Genetic Algorithm, the evaluation of objective function J (3), implies the following steps:
1. Solution of the process model (1) up to a final time t_f. This time is another freedom degree together with N.

2. The determination of the P_{top} and t_{top} along the P trajectory.
3. The calculus of z_P (4b) along the trajectories of X, G and P; next, the determination of the time-point in which z_P is maximum (z_{Pstop}). This time-point is t_{stop} for the particular scenario.

4. Results

On the test of the optimization approach, as mentioned above, the system of Tochampa et al. (2015) was recalled, and the following features for the optimization statement (3, 4) were considered:

$$C_{min} = 0.1 \text{ g/L}, \quad C_{up} = 6 \text{ g/L}; \quad X_{up} = 70 \text{ g/L}; \quad f = 0.1; \quad P_{min} = 10 \text{ g/L}; \quad \overline{z}_P = 0.99, \quad V_R = 1.6 \text{ L} \tag{6}$$

4.1 Previous work result

For comparison purposes, the results obtained by Tochampa et al. (2015) in the design of their fed-batch process are given in Table 1; it can be noticed that the remaining xylose is of the same magnitude as xylitol.

Table 1. Maximum xylitol concentration obtained in Tochampa et al. (2015)

Time (h)	Final Concentration (g/L)				Spent substrate per litre of fermentation broth (g/L)		Volumetric productivity (g/L/h)	Xylitol mass fraction (biomass free)
	Biomass	Xylose	Glucose	Xylitol	Xylose	Glucose		
40	18.20	25.30	0.08	25.59	75	7.5	0.640	0.502
Initial Conditions: (C_0, X_0, G_0, P_0, R_0, V_0) = (6 g/L, 0, 0, 0, 0, 2.5 L)								

4.2 Fed-Batch Process at Constant Feed Flows

The first task consisted of the design of the fed-batch process with constant feeding flows. This considers a practical framework in which the automation platform is limited; besides, this operation case is the easiest to implement. Then, it was set N = 1. In addition, this task was useful to set in a first instance the process time t_f. The scenarios tested were generated according to the process characteristics given in Table 2, with a V_0 = 1 L. This initial volume value was considered for further reference.

Table 2. Parameters tested in the process design at constant feed flow.

Process characteristic	Tested Values
Simulation time	20 h, 30 h, 40 h
Concentration of sugars in feeding tanks ($X_f = G_f$)	220 g/L, 330 g/L
Ration between initial glucose and initial xylose (f)	14%, 30%
N (for varying feed flows)	10, 20, 40

For each scenario generated from Table 2, 10 runs were executed through the Genetic Algorithm, each one starting with a different initial guess set of solution. The better outcomes are given in Table 3.

Table 3. Better scenarios for the fed-batch process with a constant feed flow of sugars.

t_f (h)	Dispenser concentration (g/L)		f	P_{stop} (g/L)	z_P	Volumetric productivity (g/L/h)	Added substrates (g)		Initial conditions (g/L)		
	X_f	G_f					Xylose	Glucose	C_0	X_0	G_0
40	220	220	0.14	28.29	0.663	0.707	75.00	9.89	3.060	69.972	6.1E-03
40	220	220	0.30	28.30	0.679	0.707	75.00	13.40	2.703	69.900	2.7E-03
30	220	220	0.14	28.45	0.661	0.948	75.00	10.00	6.000	69.275	1.6E-03
30	220	220	0.30	28.60	0.680	0.953	75.00	13.80	6.000	69.980	6.1E-03
40	330	330	0.14	28.89	0.666	0.722	75.00	10.00	3.032	69.963	4.4E-03
40	330	330	0.30	29.06	0.685	0.726	75.00	14.78	2.425	69.952	1.0E-03
30	330	330	0.14	29.10	0.668	0.970	75.00	10.00	5.999	69.511	2.7E-08
30	330	330	0.30	29.30	0.691	0.977	75.00	15.44	5.914	69.899	1.3E-03

It is worthy to recall that the approach, besides the search of feeding flows, it searches for the initial load of cells and sugars. In any scenario of Table 3, the xylitol concentration obtained is greater than the one of Tochampa et al. (2015) (P_f = 25.59 g/L). The greatest xylitol concentration (P_{stop} = 29.30 g/L) corresponds to the scenario in which the both sugar dispensers have the greatest concentration of sugar, consequently the input streams as well, and the final time is the lowest; however, it implies the greatest amount of glucose added. In other hand, a scenario with the lowest concentration of sugars in the input streams yields a little lower xylitol concentration (P_{stop} = 28.29 g/L), but requires the lowest glucose added, and the lowest initial cells; then, although economic aspects are beyond this work, considering the glucose cost, this scenario could be preferred in practice. The evolution of this latter scenario is illustrated in Figure 2; it can be noticed in the xylitol trajectory the point in which P reaches its maximum value, and the point where the process is stopped because z_P reaches its maximum value. This t_{stop} coincides with t_f, meaning that the optimization approach seeks for the best trajectory that can be extended along the complete process time t_f.

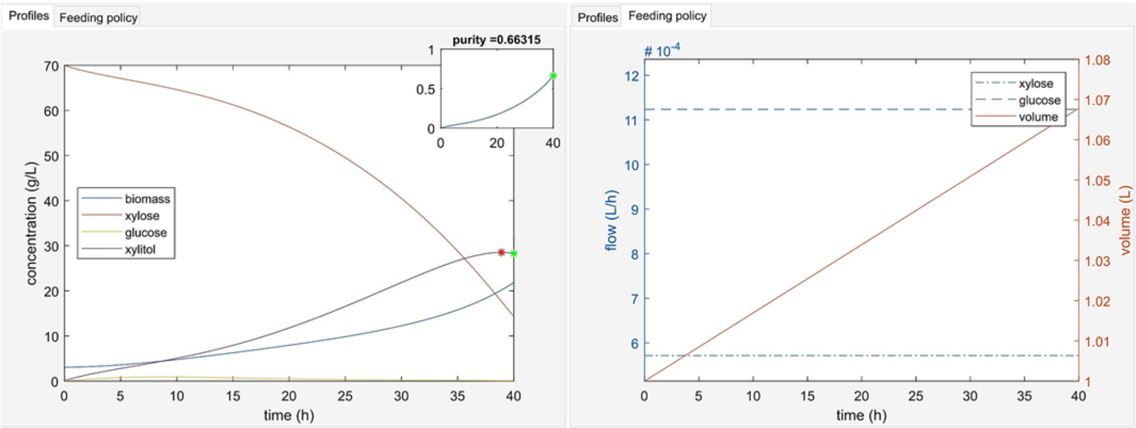

Figure 2. Process evolution with a constant feed flow of sugars for the likely most economic process (t_f = 40 h, f = 0.14, total glucose added = 9.89 g).

4.3 Fed-Batch Process at Varying Feed Flows

In the design of a fed-batch with varying feed flows, the parameters given in Table 2 to set testing scenarios were used as well. For this task, taking in account the results of the constant feed flows case, the initial guesses of the feed flows were set constant flows, and the sugar concentration in the feeding tanks were set the lowest values.

The better outcomes are given in Table 4. As can be seen, the xylitol concentration obtained is a little lower than in the case of constant feed flow (Table 3); however, the time is quite lower (t_{stop} = 20 h), and the amount of glucose added is lower in the most of scenarios. In other hand, with a lower N, it is obtained a better process performance, which goes in hand with the result that with N = 1 (i.e., at constant feed flow) the best performance is obtained. In this way, it seems that the only advantage of varying feed flows, with respect to constant feed flows, lies on that the process time is pretty lower.

Table 4. Better scenarios for the fed-batch process with varying feed flow of sugars.

N	P_{min} (g/L)	P_{stop} (g/L)	z_P	Volumetric productivity (g/L/h)	Added substrates (g)		Initial conditions (g/L)		
					Xylose	Glucose	C_0	X_0	G_0
10	26	27.07	0.559	1.354	75.00	10.00	5.9976	69.9956	6.6505
20	27	25.30	0.510	1.265	75.00	10.00	5.9990	63.5133	2.3366
40	26	26.65	0.546	1.332	75.00	10.00	5.9957	69.9987	6.2861

The process evolution with varying feed flows is illustrated in Figure 3, where in addition it can be seen that the variation of feed flows along the process are considerable, implying a likely additional disadvantage with respect to the constant feed flows. In other hand, in the xylitol trajectory, it can be observed that the time points of P_{top} and P_{stop} coincide. Finally, the purity of the product is pretty lower (Figure 2).

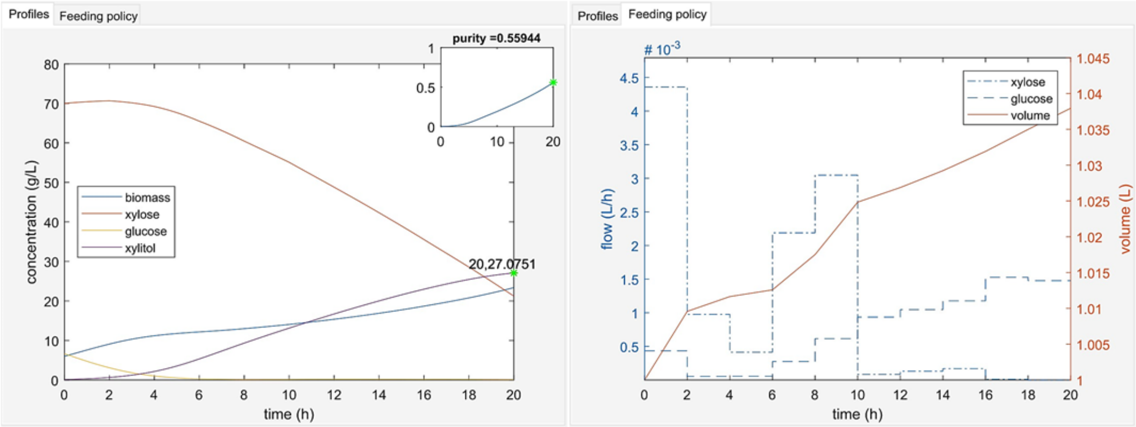

Figure 3. Process evolution with varying feed flows (N = 10).

5. Conclusions

In this work, a fed-batch process of xylose fermentation to produce xylitol was enhanced in the sense that not only a high concentration of xylitol was obtained but a reduced remaining xylose; this was achieved based on an optimization problem that encloses a penalty term that considers the fraction of xylitol with respect to remaining sugars. The solution of the problem through Genetic Algorithm resulted that constant feed flows of the sugars yield the highest concentration of xylitol, but varying feed flows provide faster fermentation, up to twice, with the advantage that the yielded xylitol concentration just is a little lower than the one obtained in constant feed flows. Addressing the optimization problem through Genetic Algorithm was of direct technique application.

Acknowledgments

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