Preprints of the 12th IFAC Symposium on Computer Applications in Biotechnology
The International Federation of Automatic Control
16-18, 2013, December. Mumbai, India

On the Performance of DoDE in a class of *in silico* Fermentation Processes and the Impact of the Input Domain

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Abstract: In a recent publication (Georgakis, 2013), it was shown that a data-driven model obtained through the proposed Design of Dynamic Experiments (DoDE) was able to accurately optimize a penicillin fermentation process without the use a knowledge-driven model. The resulting optimal operation, just after a set of experiments, is almost identical to the one obtained in (Riascos and Pinto, 2004) using the detailed model of the process by B&R (Bajpai and Reuss, 1980). Here we examine *in silico* whether a similar number of DoDE experiments will result in an equally accurate estimation of the optimal process operation of 32 other fermentation processes. This set of fermentation processes is defined by significantly varying the values of 10 key parameters of the initial penicillin model. Only between 3 and 7 of the 32 fermentations require additional experiments to obtain a satisfactory process optimization through a more accurate data-driven model. Furthermore, we examine two different time-evolving domains, A and B, within which the substrate in-flow is varied. One of them, domain A, forces the substrate inflow to be zero at the end of the batch. Domain B removes this constraint but requires more experiments. The obtained optimal operation in domain B is always better than that in domain A; sometimes by as much as 280%. This implies that limiting the number of experiments might also limit optimization gains

Keywords: Pharmaceutical Processes; Optimization; Dynamics and Control, Design Experiment, Batch Fermentation

1. INTRODUCTION

Batch processes are often related to small production rates resulting in processes that are not understood enough to enable the development of an accurate mathematical model describing their inner workings. To accommodate such a lack of detailed understanding we recently introduced a new data-driven approach, the Design of Dynamic Experiments (DoDE) (Georgakis, 2009, Georgakis, 2013), as a generalization of the traditional Design of Experiments (DoE) (Box and Draper, 2007, Montgomery, 2013). The key generalization allows the use of time-varying inputs. The DoDE methodology has been successfully implemented in optimizing some additional batch process (Troup and Georgakis, 2013) (Fiordalis and Georgakis, 2013). It has also been experimentally verified in an industrial hydrogenation reaction (Makrydaki et al., 2010). The DoDE defines a finite number of experiments within an input domain and from the collected data at the end of each batch, develops a data-driven response surface model that enables the optimization of the process. Examining *in silico* the DoDE optimization of the B&R process (Bajpai and Reuss, 1980) through their knowledge-driven model, (Georgakis, 2013) has shown that the data-driven DoDE approach was able obtain an optimal amount of the product that was almost identical to the amount obtained by (Riascos and Pinto, 2004) using a classical model-based optimization approach. We are interested to investigate whether this rather surprising, and very welcomed, accuracy of the DoDE can be reproduced in other fermentation processes, and under what conditions. To this aim, we define *in silico* a set of 32 quite different fermentation processes by systematically varying 10 of the most important parameters of the B&R model over a wide range of values. For each of these processes we design a set of DoDE experiments, estimate an RSM model, calculate the conditions that maximize the amount of product at the end of the batch, and test whether the RSM-predicted optimal run produces *in silico* the expected amount. If the optimal operation produces what is expected, confidence in the accuracy of the DoDE model will be strong and the initial success not accidental.

The selection of the input domain also has a strong effect on the experimental results, the estimated data-driven model and therefore the optimization of the process. The present paper examines *in silico* the impact two choices of input domain, A and B defined below, have on the DoDE optimization of the above set of 32 fermentation processes.

2. DIVERSE *IN SILICO* FERMENTATION PROCESSES

Utilizing the recently introduced Design of Dynamic Experiments (DoDE), (Georgakis, 2013), has shown that a small set of experiments and a related data-driven model was able to accurately optimize a penicillin fermentation process without the use a knowledge-driven model. The resulting optimal is almost identical to the one obtained in (Riascos and Pinto, 2004) using the detailed model of the process by B&R (Bajpai and Reuss, 1980). To examine whether this is also true for other fermentations, we define a set of 32 processes by modifying the initial parameters of the initial model reported by B&R. The diverse parameter set used is given in columns 2-11 of Table 1 below. The last row reports the parameter values for the B&R process, the 33rd process and base case. We do not examine all the possible combinations of the high and low values of the ten parameters varied. This would have resulted in 1024 ($=2^{10}$) cases, a full factorial design. Instead we have designed a resolution IV 2^{10-5} fractional factorial design with 32 cases. The diversity of these fermentations is quite apparent if one looks at processes #13, 20 and 23, for example. Process #13 is characterized by a relative slow growth rate for biomass and product. Contrasting this, process #20 is characterized by fast grow rates for both biomass and product. Process #23 has a slow biomass growth rate and a relative fast one for the product.

3. TWO EXPERIMENTAL DOMAINS

We also study the effect of the DoDE domain by considering two cases A and B. They are depicted in Figure 1. One of the domains (A) assumes that the incoming flow rate of the substrate goes to zero at the end of the fermentation and the second one removes this restriction. Assuming that the substrate incoming flow rate goes to zero at the end of the fermentation introduces an additional constraint and reduces the set of required experiments for estimating a Response Surface Model (RSM). It might be also a reasonable one, as the substrate is a reactant. We are interested to see if it has a negative effect in the optimization of the process. In the second case (B) we remove this constraint and allow the maximum substrate inflow rate to have a finite value. This is, however, smaller than the maximum value allowed at time zero.

We will assume that the initial volume of the bioreactor, V_0, is 7 *lt* and the maximum possible, V_f, is 10 *lt*. We will then need to feed in semi-batch mode ΔV (=3) *lt* of substrate. We first define the reference incoming flow rate, $u_o(t)$, that will serve as the center point of the DoDE design. In the set of DoDE experiments for each of the 32 processes we will vary the initial biomass concentration as well as the batch duration, besides the substrate feeding profile. The batch duration will be varied about a reference batch time, which needs to be appropriate for each process, based on the different set of parameters of said process. This reference batch time is assumed to be depended on the biomass and product growth rates. For each of the 32 processes it is calculated by

$$t_{b,r} = t_{g,r}\frac{\mu_{\max,r}}{\mu_{\max}} + t_{p,r}\frac{\rho_{\max,r}}{\rho_{\max}}, \quad \begin{matrix}\mu_{\max,r}=0.1,\ \rho_{\max,r}=0.0055 \\ t_{g,r}=30hr,\ t_{p,r}=100hr\end{matrix} \qquad (1)$$

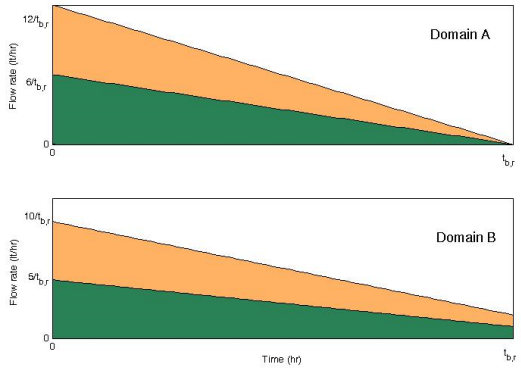

Figure 1: Schematic Representation of the two Domains, A and B, in which the Feeding Profiles of the Substrate Inflow will be Confined

The reference batch time for each process is defined relative to the initial penicillin process of B&R process, where the biomass needed about 30 *hr* to grow and the penicillin growth extended for another 100 hr. Based on the appropriate reference batch time for each process, we define the total volume constraining equation as follows:

$$\int_0^{t_{b,r}} u_0(t)\,dt = V(t_{b,r}) - V(0) = V_f - V_0 \equiv \Delta V. \qquad (2)$$

For domain A, we impose $u_{0A}(t_{b,r}) = 0$ and assume a linear dependence on t, which implies that

$$u_{0A}(t) = 2\Delta V\left(1 - t/t_{b,r}\right)/t_{b,r}\ . \qquad (3)$$

We then let the substrate flow profile to be in the domain $u_{0A}(t) \pm \Delta u_{0A}(t)$, with $\Delta u_{0A}(t) = u_{0A}(t)$. This allows $u(t)$ to vary between zero and $2u_{0A}(t)$. It is depicted in the upper part of Figure 1. For case B we set $u_{0B}(t_{b,r}) = u_{0B}(0)/5$ and domain B, in Figure 1, is defined by

$$u_{0B}(t) \pm \Delta u_{0B}(t);\ u_{0B}(t) = \Delta u_{0B}(t) = \Delta V\left(5 - 4t/t_{b,r}\right)/3t_{b,r} \qquad (4)$$

Again here, this allows $u(t)$ to vary between zero and $2u_{0B}(t)$.

4. DESIGN OF DYNAMIC EXPERIMENTS

We define two traditional factors in the Design of Dynamic Experiments (DoDE). Firstly, the batch time is defined with respect to the reference batch time of each process

$$t_b = t_{b,r} + \Delta t_{b,r} w_1 \text{ with } -1 \le w_1 \le +1 \quad (5)$$

For the original process $\Delta t_{b,r}$ was taken equal to 30 *hr*. The value that we will use in each of the 32 other processes will be $\Delta t_{b,r} = 30 t_{b,r} / 130$. Secondly, the initial biomass concentration varies from 1 to 2 *gr/lt*.

$$x(0) = 1.5 + 0.5 \cdot w_2 \text{ with } -1 \le w_2 \le +1 \quad (6)$$

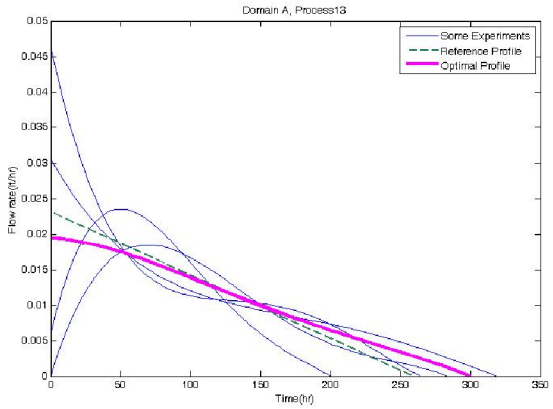

Figure 2: Some of the Feeding Profiles in Domain A for Process #13, along with the Center Point Reference Profile and the Calculated Optimal one

In each DoDE design, we now define the coded time-varying factor, $z(\tau)$, related to the substrate inflow

$$u(\tau) = u_0(\tau) + \Delta u_0(\tau) \cdot z(\tau) \text{ with } -1 \le z(\tau) \le +1 \quad (7)$$

The time dependency within each run is defined in terms of the dimensionless time, $\tau = t/t_b$, for each separate run. Now let $z(\tau)$ be expanded in a series of shifted Legendre polynomials. For each of the 32 processes defined in Table 1, and for the two domains A and B we will now define a set of DoDE experiments. The procedure used is described in (Georgakis, 2009, Georgakis, 2013). We limit the series expansion of $z(\tau)$ to only the first 3 polynomials so that the number of experiments is a reasonable one.

$$z(\tau) = x_1 P_0 + x_2 P_1 + x_3 P_2$$
$$P_0(\tau) = 1, \; P_1(\tau) = -1 + 2\tau, \; P_2(\tau) = 1 - 6\tau + 6\tau^2 \quad (8)$$

Substituting $u(\tau)$ into the total volume constraint in eq. (2) we have:

$$t_b \int_0^1 \left[u_0(\tau) + \Delta u(\tau) z(\tau) \right] d\tau = V(t_b) - V(0) = \Delta V \quad (9)$$

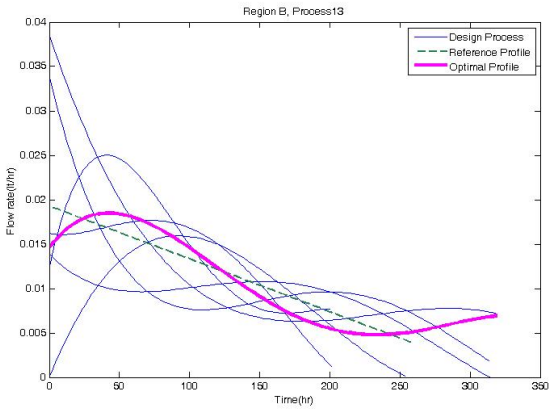

Figure 3: Some of the Feeding Profiles in Domain B for Process #13, along with the Center Point Reference Profile and the Calculated Optimal one

Solving eq. (9), we get w_1 expressed in term of x_1 and x_2

$$w_1 = -4.33 \frac{(3\eta_1 x_1 + \eta_2 x_2)}{9 + (3\eta_1 x_1 + \eta_2 x_2)} \quad (10)$$

where $\eta_1 = 3, \eta_2 = -3$ for domain A and $\eta_1 = 3, \eta_2 = -2$ for domain B. The above relationship reduces the degrees of freedom by one in both A and B cases. For domain A we have an additional constraint at the end of the batch, namely that $\Delta u(\tau = 1) = \Delta u(t = t_b) = 0$. This allows us to set $z(1) = 0$, implying that $x_3 = -(x_1 + x_2)$.

The degrees of freedom in domain A are now further reduced to three. In domain A the DoDE set of runs is obtained by a constrained D-Optimal design with 16 runs. These runs aim in estimating a quadratic Response Surface Model (RSM). Ten of the runs are for estimating the same number of parameters; three additional runs for estimating the Lack-of-Fit (LoF) statistic and three replicate runs at the center point to estimate the normal variability of the process. In domain B the corresponding D-Optimal DoDE, again for a quadratic RSM, has a total of 21 experiments; fifteen experiments for the model parameters, three for the LoF statistic and three replicates at the center point for the estimation of the normal variability of the process. The constraining relationships that both domains need to satisfy are the inequalities of eq. (5) and eq. (6) and the following set of inequalities, which ensure that the constraints in eq. (7) are satisfied.

$$-1 \le x_1 \pm x_2 \pm x_3 \le 1 \quad (11)$$

Table 1. Thirty Two Diverse Sets of Model Parameters Representing a Multitude of Possible Fermentation Processes

# Process	μ_{max} × 10^2	ρ_{max} × 10^3	K_X × 10^3	K_P × 10^5	K_{in} × 10^2	K_m × 10^5	K_d × 10^3	m_s × 10^3	$Y_{X/S}$ × 10^2	$Y_{P/S}$ × 10^2	Domain A				Domain B				% Diff btw Two Domains
											P_pred	PI Width	P_sim	% Diff	P_pred	PI Width	P_sim	% Diff	
Units	h^{-1}	$\frac{g_P}{g_X \cdot h}^{1}$	$\frac{g_P}{g_X}$	$\frac{g_S}{lt}$	$\frac{g_S}{lt}$	$\frac{g_S}{lt}$	h^{-1}	$\frac{g_S}{g_X \cdot h}$	$\frac{g_X}{g_S}$	$\frac{g_P}{g_S}$	g_P	g_P	g_P		g_P	g_P	g_P	-	-
1	5	3	3	5	3	32	32	46	59	151	10.5	1.1	10.7	-1.3	20.4	1.8	20.2	1.0	89.3
2	20	3	3	5	3	3	3	18	37	151	71.1	2.9	66.3	7.2	83.0	7.2	81.2	2.2	22.4
3	5	11	3	5	3	3	3	18	59	95	142.4	11.4	141.8	-0.3	**267.4**	**17.4**	**190.8**	**40.1**	34.6
4	20	11	3	5	3	32	32	46	37	95	62.6	3.6	58.6	6.9	76.4	5.4	75.1	1.8	28.1
5	5	3	12	5	3	3	3	46	37	95	32.6	3.3	30.2	7.7	40.5	4.5	40.3	0.6	33.2
6	20	3	12	5	3	32	32	18	59	95	20.2	1.6	19.7	2.5	**32.3**	**3.7**	**28.2**	**14.2**	43.3
7	5	11	12	5	3	32	32	18	37	151	**76.6**	**10.9**	**61.2**	**25.1**	101.1	14.9	95.4	6.0	55.9
8	20	11	12	5	3	3	3	46	59	151	111.0	6.3	112.0	-0.9	151.8	19.1	154.2	-1.6	37.7
9	5	3	3	20	3	32	32	18	37	95	4.4	0.2	4.4	-1.1	**15.1**	**1.2**	**13.4**	**13.1**	201.9
10	20	3	3	20	3	3	3	46	59	95	**51.4**	**3.7**	**46.1**	**11.6**	**55.2**	**2.8**	**49.3**	**11.9**	7.1
11	5	11	3	20	3	3	3	46	37	151	109.3	14.8	111.1	-1.6	**147.3**	**13.3**	**122.3**	**20.4**	10.1
12	20	11	3	20	3	32	32	18	59	151	70.1	5.1	66.1	5.9	112.6	9.7	111.3	1.2	68.3
13	5	3	12	20	3	3	3	18	59	151	82.5	6.6	76.3	8.1	92.0	12.0	94.5	-2.6	23.8
14	20	3	12	20	3	32	32	46	37	151	2.7	0.1	2.7	0.1	7.1	0.5	6.5	9.5	139.0
15	5	11	12	20	3	32	32	46	59	95	10.1	1.5	10.8	-6.2	38.5	3.1	41.1	-6.3	280.8
16	20	11	12	20	3	3	3	18	37	95	143.1	10.9	137.5	4.0	158.1	9.6	162.3	-2.6	18.0
17	5	3	3	5	32	3	3	18	37	95	93.5	6.3	88.2	6.0	107.0	6.1	102.8	4.1	16.5
18	20	3	3	5	32	32	32	46	59	95	4.8	0.2	5.1	-5.4	10.8	0.4	10.3	4.7	101.0
19	5	11	3	5	32	32	32	46	37	151	33.2	4.4	29.9	11.3	**79.2**	**4.6**	**61.2**	**29.5**	104.9
20	20	11	3	5	32	3	3	18	59	151	258.6	15.9	263.9	-2.0	298.7	27.0	285.0	4.8	8.0
21	5	3	12	5	32	32	32	18	59	151	8.6	0.5	8.3	3.3	19.9	1.5	20.3	-2.3	144.5
22	20	3	12	5	32	3	3	46	37	151	62.5	4.7	60.3	3.7	66.3	5.7	66.7	-0.6	10.7
23	5	11	12	5	32	3	3	46	59	95	169.2	15.9	156.4	8.2	183.0	15.6	186.3	-1.8	19.1
24	20	11	12	5	32	32	32	18	37	95	67.5	6.1	67.0	0.8	97.5	6.3	92.3	5.7	37.8
25	5	3	3	20	32	3	3	46	59	151	**35.6**	**3.3**	**29.6**	**20.3**	41.0	3.9	37.3	9.9	26.3
26	20	3	3	20	32	32	32	18	37	151	12.8	0.7	12.8	-0.2	22.4	0.9	21.9	2.0	71.8
27	5	11	3	20	32	32	32	18	59	95	65.5	8.2	59.8	9.6	117.4	16.0	109.0	7.7	82.3
28	20	11	3	20	32	3	3	46	37	95	108.1	8.1	104.6	3.4	**149.5**	**11.2**	**127.1**	**17.6**	21.5
29	5	3	12	20	32	32	32	46	37	95	5.4	0.3	5.3	0.7	10.6	0.8	10.6	0.4	99.2
30	20	3	12	20	32	3	3	18	59	95	70.7	5.3	65.1	8.6	88.9	9.0	87.6	1.5	34.5
31	5	11	12	20	32	3	3	18	37	151	127.4	15.6	133.5	-4.5	205.8	40.1	196.2	4.9	47.0
32	20	11	12	20	32	32	32	46	59	151	68.5	7.1	67.2	2.0	90.2	5.9	87.6	2.9	30.4
33	10	5.5	6	10	10	10	10	29	47	120	68.9	1.4	65.2	5.7	85.6	8.4	85.3	0.4	30.8

In Figures 2 and 3 some of the feeding profiles of the substrate are given for domains A and B, respectively and for Process #13. All the feeding profiles in Figure 2 are ending with a zero value at the end of the batch, while this is not the case with the feeding profiles in Figure 3. We see that the batch time and the incoming flow profiles vary considerably between runs in each of the DoDE sets.

4. RESULTS

For each of the 32 sets of fermentation processes, defined by the 32 different of parameter values, we implement *in silico* the two sets of DoDE experimental runs designed, for domains A and B. To the simulated amount of total grams of product produced at the end of the fermentation we add an experimental error of 4% to represent the normal variability of the process. We then calculate, by linear regression, the quadratic Response Surface Models (RSMs) for each domain. Through the data-driven RSM models obtained, we calculate the optimum initial biomass concentration, the optimum batch time and the optimum feeding profile of the substrate that will produce the maximum amount of product at the end of the batch. The predicted maximum amount of product (P_pred) is reported in column 12 for domain A and in column 16 for domain B. We also report the width of the prediction interval (PI Width) in column 13 for domain A and in column 17 for domain B. For example for process #1 it is predicted that the optimum amount of product to be produced is 10.5 ± 1.1 *gr* for domain A and 20.4 ± 1.8 *gr* for domain B. The calculated optimal experiment is then verified by a follow-up *in silico* fermentation. The resulting amount of product (P_sim) is reported in columns 14 and 18 for domains A and B, respectively. The percent difference between the predicted (P_pred) and simulated values (P_sim) are given in columns in columns 15 and 19 for domains A and B, respectively. In most processes the grams of product produced is less than those predicted, which is expected due to the approximate character of the DoDE approach. The opposite happens in ten processes in domain A and seven in domain B. We note with bold face if this difference is more than 10% and, at the same time the amount produced is outside the prediction interval. This happens three times in domain A (#7, #10, and #25) and for seven processes in domain B (#3, #6, #9, #10, #11, #19, and #28). For these cases we conclude that the quadratic RSM in not sufficient to represent the process dependence on the varied factors accurately. In response to this observation we append the initial DoDE design with additional experiments so that a cubic RSM is estimated. The process optimization is repeated, the predicted optimum is lower than with the quadratic RSM. The simulated optimum, though, is not that different from the one reported in Table 1 and within the prediction interval of the cubic RSM.

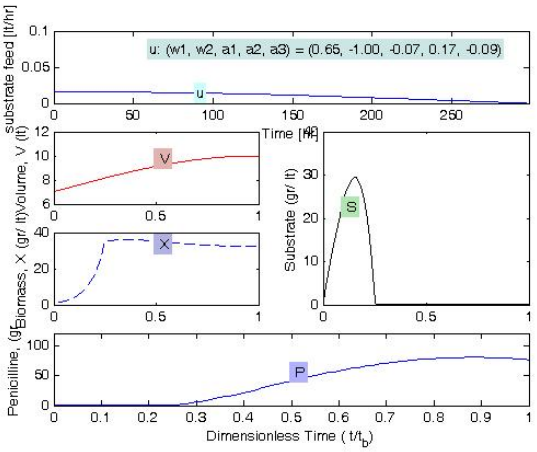

Figure 4: The Time Evolution of the State and Input Variables of the Optimum Operation for Process 13 in Domain A

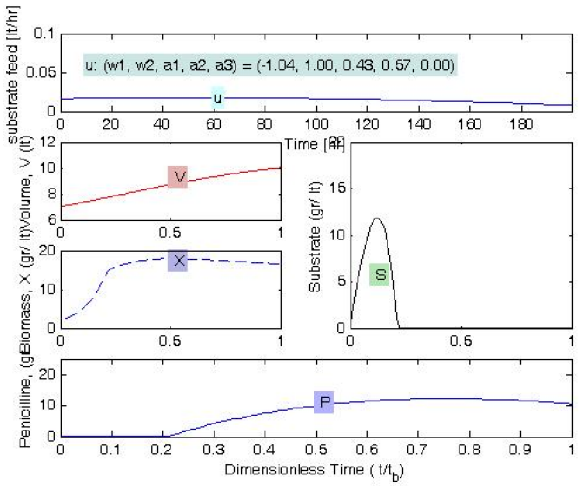

Figure 5: The Time Evolution of the State and Input Variables of the Optimum Operation for Process 13 in Domain B

We also calculate the percent difference in the amount of product produced by the optimal operation in domain B versus the optimal operation in domain A, using for all processes the quadratic RSM. This is reported in the last column. In all 32 processes examined the optimum obtained in domain B was better than that obtained in Domain A. The maximum percent difference was 280.8% for process #15 and the minimum was 8.00% for process #20. The average difference was 60.9% and the median was 36.2%.

The time evolution of the state and input variables of process #13 in domain A is given in Figure 4, while Figure 5

presents the corresponding optimal profiles of process #29 in domain B.

Table 2. Differences between the Predicted and Simulated Optimum grams of Product through Quadratic and Cubic RSMs

		Quadratic RSM		Cubic RSM	
Domain	Process #	P_pred	P_sim	P_pred	P_sim
		g_P	g_P	g_P	g_P
A	7	76.6	61.2	69.4	62.0
A	10	51.4	46.1	51.1	46.9
A	25	35.6	29.6	31.2	28.9
B	3	267.4	190.8	211.7	200.8
B	6	32.3	28.2	29.3	28.2
B	9	15.1	13.4	13.7	13.7
B	10	55.2	49.2	50.6	51.3
B	11	147.3	122.3	138.6	139.6
B	19	79.2	61.2	66.5	61.2
B	28	149.5	127.1	134.9	129.3

One might observe that the product composition reaches a maximum before the end of the fermentation; at 75% of the total batch duration for process #13 in Figure 4 and about the same fraction of the batch duration for process #29 in Figure 5. This is due to the product degradation term in the used model. Because we avail ourselves measurements only at the end of the batch, this is not obvious experimentally. Nevertheless, this is not a better optimum as the total grams of product than can be harvested then is smaller than at the end of the batch since volume of the reacting mixture is less than the maximum volume, reached at the end of the batch. Here we maximize the total grams of product at the end of the batch, independently of how long it might last. Maximizing the process productivity, e.g the ration of product grams divided by the batch duration, is also possible in the DoDE framework. For a simpler example see (Troup and Georgakis, 2013). Comparison with the model-based optimum for all 32 processes is still in progress.

A generalization of DoDE methodology to incorporate online date during the fermentation is under development and will be reported elsewhere.

5. CONCLUSIONS

The success of the recently developed DoDE methodology in correctly estimating the optimal operation of the B&R penicillin fermentation process with just a set of well-designed time-varying experiments has been also demonstrated in 32 other fermentation processes *in silico*. The calculated optimal operation, utilizing a quadratic RSM, was confirmed for almost all processes with an *in silico* operation under the optimal conditions. Among the two domains considered, A and B, within which the inflow of the substrate is constrained, the second one, B, always produces, after the DoDE optimization, more product than domain A. In process #15 this is as much as 280% better. It appears that domain A, which forces the substrate inflow to zero at the end of the fermentation, restricts the maintenance consumption by the product.

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