OPTIMIZATION OF BIOTECHNOLOGICAL PROCESSES. THE ACETIC ACID FERMENTATION. PART III: DYNAMIC OPTIMIZATION

Jorge E. Jiménez-Hornero,[(a)*] Inés M. Santos-Dueñas,[(b)] Isidoro García-García[(b)]

[(a)] Computing and Numerical Analysis Department, University of Cordoba, Spain

[(b)] Chemical Engineering Department, University of Cordoba, Spain

Abstract

Wine vinegar is obtained in a biotechnological process one of the crucial steps in which is the biological oxidation of the starting wine. Such a step is usually performed in a semi-continuous operation mode where a preset fraction of the culture medium is unloaded from the fermenter as product and the remainder left in it as inoculum to facilitate expeditious fermentation of the wine subsequently added to replenish the amount withdrawn. The overall performance of the fermenter can vary markedly depending on the particular operating conditions, and so can the quality of the product and the economy of the process as a result. Identifying the most suitable operating conditions therefore poses a typical optimization problem named as dynamic optimization or open-loop optimal control, which is solved by determining the time profiles for the control variables of the system in order to optimize a given cost function. Such a function represents the goal to be achieved as regards the specific needs of the problem. In part III of this series the previously proposed model in parts I and II has been used for addressing the dynamic optimization of the acetic fermentation process in terms of various objective functions, with special emphasis on productivity.

*Corresponding Author. Tel: +34 957212079; fax: +34 957218630
Address: Edif. Leonardo Da Vinci.
Campus Universitario de Rabanales.
Ctra. Madrid (a) Km. 396.
Postal Code: 14071 – Cordoba (Spain).
Email: jjimenez@uco.es

Keywords: Bioreactor systems; Kinetics; Modelling; Optimization; Acetification; Vinegar.

1. Introduction

Mathematically, dynamic optimization problems are generally solved by maximizing or minimizing the following cost function [1–4]:

$$J(x,u) = \phi\left[x\left(t_f\right)\right] + \int_{t_0}^{t_f} L\left[x(t),u(t),t\right]dt \tag{1}$$

where $x(t)$ is the state vector, $u(t)$ denotes control variables, ϕ is a penalty function for the final state [$x\left(t_f\right)$] and L a function intended to weight the state path travelled and control sequence used.

The previous objective function is essentially subject to the following equality constraint:

$$\varphi\left[x(t),u(t),t\right] = 0 \tag{2}$$

which constitutes the system of differential–algebraic equations (DAEs) of the model representing the dynamics of the system to be optimized. The differential equations result from the mass and energy balances governing the behaviour of the process; by contrast, the algebraic equations can vary in nature (*e.g.*, kinetic equations describing cell growth and death in some bioprocesses). Obviously, one must also know the initial conditions for the state variables, $x\left(t_0\right)$.

Moreover, the solution to the problem may be subject to additional restrictions of widely variable nature. For example, it may be necessary not to exceed specific temperature or pressure limits so as to avoid specific hazards or the end-product may

need to meet certain specifications. All this requires including a variable number of additional equality and inequality constraints on some variables affecting development of the process. Such restrictions can be of the (*a*) point, (*b*) path or (*c*) terminal time types, which are to be met at specific points in time, throughout the process or at its end, respectively, and can be formulated via the following equations and inequations:

$$h\left[x(t),u(t)\right]=0 \tag{3}$$

$$g\left[x(t),u(t)\right]\leq 0 \tag{4}$$

Finally, constraints may include operational bounds or validity ranges for both control and state variables:

$$x_I \leq x(t) \leq x_S \tag{5}$$

$$u_I \leq u(t) \leq u_S \tag{6}$$

A number of methods have been developed over the past few decades for the numerical resolution of open-loop optimal control problems. Despite their apparent variety, such methods can be classified into three broad categories, namely: dynamic programming, indirect methods and direct methods.

Dynamic programming methods rely on Bellman's optimality conditions [5]; these were originally established to solve steady-state problems and later adapted to dynamic problems via the Hamilton–Jacobi–Bellman equation —which usually has no analytical solution. The greatest shortcoming of this procedure is that it involves rather long computational times in practice [6]; in the beginning, however, it gained widespread popularity by virtue of its ability to address this type of problem. Later on, De Tremblay *et al.* [7] and Luus [8] developed Iterative Dynamic Programming (IDP), which, as it implied by its name, applies Bellman's principle in an iterative manner. In fact, IDP performs a backward search (from end to beginning) in order to find the optimum

control profile for the process. Once the search is finished, the procedure is repeated a preset number of times. This methodology has been successfully used with some problems [9–12]. However, its high computational cost [13] for systems involving a large number of DAEs has restricted its use to problems on a smaller scale. In addition, IDP requires fitting some search parameters in order to ensure appropriate convergence. In order to reduce the exponential increase in computational cost with increasing number of decision variables, Valencia *et al.* [14] used dynamic programming in combination with neural networks to obtain slightly better computational efficiency. A comprehensive review of the development and uses of various dynamic programming approaches can be found elsewhere [15,16].

With indirect methods, dynamic optimization problems are solved by using Pontryagin's maximum principle [17,18], which is based on Euler's calculus of variations. The procedure involves constructing the Hamiltonian of the system, which will depend on the particular objective function and on the restrictions associated to it via so-called adjoint variables, and imposing the optimality constraints to be met. This converts the original problem into one governed by the initial conditions for state variables and the final conditions for adjoint variables (*i.e.*, into a Two-Point Boundary Value Problem, TPBVP, which rarely has an analytical solution and requires using a numerical alternative such as the Shooting Method [19,20]). The difficulty involved in numerically solving a TPBVP has led some authors [13,21,22] to devise various transformations. Interested readers can find a description of the use of indirect methods on various chemical and biochemical processes (particularly in reactors operating in a semi-continuous mode) elsewhere [23–25]. Also, Skolpap *et al.* [26], and Roubos *et al.* [27], compared a method of Rocha and Ferreira [28] and several others with Bellman's dynamic programming. The problem was always extremely difficult to solve, especially

in the presence of point or path constraints on state variables. Therefore, indirect methods are extremely complicated to apply in practice.

On the other hand, direct methods use a discretization procedure to convert the original problem, of infinite dimension, into a non-linear optimization problem of finite dimension subject to the constraints imposed by the dynamics of the particular system and several other restrictions which can be solved with a standard optimization algorithm. Some studies [29] have shown that direct methods to be more efficient for solving dynamic optimization problems than are indirect methods and dynamic programming owing to the high practical complexity and computational cost of the latter two. In addition, these procedures find it especially difficult to manage constraints —particularly on state variables. Direct methods afford two different types of parameterization, namely:

(a) Complete Parameterization (CP), also known as the "simultaneous approximation strategy", which discretizes both state and control variables.

(b) Control Vector Parameterization (CVP), also referred to as the "sequential approximation strategy", which discretizes control variables alone.

In its earliest incarnation, the CP method discretized control variables and approximated the differential equations of the model via finite differences. Subsequently, it parameterized the time paths for control and state variables, thereby leading to an Non-linear Programming (NLP) problem under algebraic constraints where decision variables constituted the parameters for each path. The first useful, efficient results provided by this procedure [30,31] were subsequently improved by using various alternative approaches where differential equations in the model where discretized with procedures other than those based on finite differences. Worth special note among such procedures are those of Biegler [32], who uses Lagrange polynomials to define the time

path —which ensured continuity at discretization points—; Renfro *et al.* [33], who employs orthogonal collocation; and Cuthrell and Biegler [34], who uses a finite element approach that improved on previous results. The ensuing NLP problem is frequently solved with the Sequential Quadratic Programming (SQP) algorithm or some variant thereof [32,35]. Additionally, parameterizing state paths substantially increases the dimension of the optimization problem relative to the control parameterization method; the stiffer the system is, the greater is the increase by effect of the growing number of points required to accurately describe all paths involved. This additional requirement further increases computational costs. Interested readers can find applications of this optimization strategy to various processes (particularly of chemical engineering interest) elsewhere [33,35–40]. Also, an excellent review of the state of the art in complete parameterization methods has been published [41].

The foundation of control parameterization methods is also described in detail elsewhere [42–44]. These procedures only discretize and parameterize control variables, which produces an NLP problem that entails solving the system model in each iteration in order to assess the objective function. Therefore, this strategy leads to an external NLP problem combined with an internal Initial Value Problem (IVP) [45–47]. Control paths are parameterized with polynomials —usually Lagrange polynomials— of variable order. However, the problem can usually be solved more efficiently by using a low-order approximation, namely: 0 for constant segments in each discretization interval or 1 for linearly variable segments in the intervals. In addition, to reduce the number of decision variables, these approximations allow one to easily confirm the bounds for the control variables. However, the quality of the resulting solution is extremely dependent on that of the parameterization of the control profile [48]. This strategy has been used in the dynamic optimization of a number of processes. Worth

special note in the chemical engineering domain is the work in this respect of Bojkov *et al.* [49], Vassiliadis *et al.* [46,47], Banga *et al.* [4,50,51], Pushpavanam *et al.* [52], Sorensen *et al.* [53], Ishikawa *et al.* [54] and Mujtaba and Macchieto [55], among many others.

Control Vector Parameterization is the easiest to implement and apply among all direct methods; also, its efficiency and robustness increase with increasing dimension of the target problem. However, if the numerical integrator used to solve the internal IVP is inadequately precise, convergence on the solution for the optimizer employed to solve the outermost problem can be troublesome. At present, this problem is avoided by using numerical integrators based on BDF algorithms such as DASSL [56].

On the other hand, complete parameterization methods have their greatest advantage in the way they handle path constraints on state variables; in fact their paths can be directly subjected to the constraints as the parameters used to describe them constitute decision variables in the optimization problem. However, some authors have developed effective strategies for applying these constraints in CVP methods [57,58]; also, currently available modelling and simulation environments for dynamic systems, which afford handling of such constraints, dispense with the use of CP methods under these conditions. Moreover, the dimension of the NLP problem is greater in a CP method than in a CVP method and so is the computational cost of using the former; this justifies the more frequent use of CVP methods in process engineering problems.

In any case, the NLP problem is subject to the following shortcomings in any type of direct method:

(a) The objective function is usually non-convex or multi-modal (*i.e.*, it exhibits a number of local minima) by effect of its non-linear nature and the potential presence of discontinuities.

(b) Many optimization algorithms require that the objective function and constraints meet specific conditions fulfilment of which may be impossible or simple unverifiable.

Based on the foregoing, efficiently solving a dynamic optimization problem with a direct method entails using a global procedure rather than a classical local optimization algorithm. A description of the application of a deterministic global model (*viz.*, αBB) to this type of problem can be found elsewhere [59,60]. However, stochastic global algorithms are the only ones that afford dealing with an NLP problem as if it were a black-box problem —unlike most deterministic global strategies, it requires no special condition to be met [61]. Banga *et al.* [4] used a hybrid method involving a stochastic global procedure followed by a deterministic local procedure —thereby combining the greatest advantages of both— in order to facilitate convergence on the global solution.

So, the direct control parameterization method CVP constitutes one of the most effective choices for solving dynamic optimization problems and was thus adopted for subsequent work here.

In summary, the main objective of this part is to identify the most suitable operating conditions in order to optimize a given cost function as could be either the productivity or then mean acetification rate. For this purpose, the specific mathematical model used and the experimental data employed in its development, are described in parts I and II of this paper series.

2. Results and discussion

Based on the industrial interest of the target process (acetic acid fermentation) and on our aim to focus on its economic side, the primary goal of our dynamic optimization procedure was to maximize two cost-related functions by using the raw material feed

rate as control variable. Such functions represented production (*viz.*, the mass of acetic acid present in the unloaded volume that was formed per unit time) and the mean fermentation rate (*viz.*, the mass of acetic acid formed per unit volume per unit time), namely:

$$P = \frac{HAc_{cycle} \cdot V_{unloaded}}{t_{cycle}} \tag{7}$$

$$r_{ave} = \frac{P}{V_{mean}} \tag{8}$$

where HAc_{cycle} is the acetic acid concentration at the end of the fermentation cycle (g·L^{-1}), $V_{unloaded}$ the unloaded fermenter volume (L), t_{cycle} the total cycle duration (h), P production (g acetic acid·h^{-1}), r_{ave} the average fermentation rate (g acetic acid·L^{-1} medium·h^{-1}) and V_{mean} the mean volume during the cycle (L).

Because the bioreactor was operated in the semi-continuous mode and no effluent was released until the end of the cycle, maximizing the previous two functions need not lead to the same results as the dynamic optimization procedure. In fact, the mean volume of medium present in the reactor would vary with the way the fermenter was loaded (*i.e.*, on the resulting control profile) and also with the unloaded volume; therefore, the peak production level need not coincide with the peak fermentation rate. In any case, the two objective functions were examined in isolation and with provision for no other considerations. On an industrial scale, additional factors must be taken into account depending on the particular needs, goals and policy of each producer. This led us to optimize the fermentation process in accordance with criteria typically shared by vinegar procedures as the core of optimization work on a larger scale.

As noted earlier, the Control Vector Parameterization (CVP) method was chosen to solve the dynamic optimization problem and addressed the ensuing external NLP

problem with a global optimization algorithm (specifically, the Augmented Lagrangian Genetic Algorithm, ALGA, [62,63], which was subsequently combined with a sequential quadratic programming, SQP, algorithm). Therefore, a hybrid strategy which facilitated approximation to the global optimum was used.

The results initially obtained by applying CVP methodology with discretization of the time axis into a preset number of intervals with zero-order polynomials (steps) revealed that all fermenter loading modes could be reasonably accurately approximated with a strategy involving two steps. In the first step, the fermenter was loaded at a constant flow-rate and the volume of medium increased linearly as a result; the model parameters for this step were the flow-rate and duration of the loading process. In the second step, the volume varied in a roughly exponential manner and the sole model parameter involved was the exponent of the function. The choice of an exponential variation for the second step was largely dictated by the discussion and conclusions in a previous paper [64] which emphasized the simplicity of exponential functions for this purpose — a number of other choices, however, could have been made. As shown later on, using this particular strategy reduced the number of decision variables in the target problem.

The following specific mathematical expressions for the feed rate and volume of medium were used:

- First step (continuous loading):

$$F_i = q_1 \tag{9}$$

$$V = q_1 \cdot t \tag{10}$$

- Second step (exponential loading):

$$F_i = \left(V_0 + q_1 \cdot t_1\right) \cdot b \cdot e^{b(t-t_1)} \tag{11}$$

$$V = \left(V_0 + q_1 \cdot t_1\right) \cdot e^{b(t-t_1)} \tag{12}$$

where t is the time elapsed from the beginning of the cycle (h); q_1 ($L \cdot h^{-1}$) and t_1 (h) are the flow-rate and duration of the continuous loading step, respectively; V_0 is the cycle initial volume (L); and b is the exponent reflecting the increase in volume during the exponential loading step. Therefore, the volume at the end of the continuous loading step was ($V_0 + q_1 \cdot t_1$) and the starting flow-rate to be used in the exponential loading step [64] was $(V_0 + q_1 \cdot t_1) \cdot b$.

Parameterizing the loading profile provided q_1, t_1 and b as decision variables for the optimization algorithm. Such variables were expanded with V_0 and —initially— E_0 (ethanol concentration at unload ($g \cdot L^{-1}$)) since, as shown later on, they strongly affect the behaviour of the process and its outcome. The duration of the exponential loading step, t_2, was dictated by the difference between the time needed to reach the working volume —which was dependent on exponent b— and also by t_1. Therefore, the initial number of decision variables was five. Such a small number was a result of using the chosen strategy, and allowed the computational time and identifiability problems with the parameters of the input time profile to be substantially reduced.

The total duration of each cycle was unconstrained and the sole constraint imposed was that the maximum working volume should be about 8 L.

2.1. Optimization of the acetic acid production

Once the loading profile was parameterized, maximization of the acetic acid production [eq. (7)] was addressed. The maximum and minimum values adopted for the respective decision variables were as follows:

$$
\begin{aligned}
q_1 &\in [0.5, 4] \ \left(L \cdot h^{-1}\right) \\
t_1 &\in [0, 14] \ (h) \\
b &\in [0, 0.1] \\
V_0 &\in [1, 7] \ (L) \\
E_0 &\in [0, 93] \ \left(g \cdot L^{-1}\right)
\end{aligned}
\tag{13}
$$

The operational bounds for q_1 afforded flow-rates within the range typically spanned by industrial production processes (note that referred range is for a 8 L fermenter). Based on such bounds, t_1 should vary from 0 in the absence of continuous loading to 14 — which would allow a volume of 7 L to be loaded at the minimum set flow-rate (*i.e.*, the maximum volume to be fed at the lower bound for V_0). Tests involving feeding the fermenter at a controlled ethanol concentration in the medium during the loading step showed that the maximum possible value for *b* was that in eq. (13). Based on the type of reactor usually employed in this process, V_0 cannot fell below 10–15% the total working volume. This led us to adopt 1 L as its minimum value. Also, based on the semi-continuous operational mode used, at least 10–15% of the fermenter volume at the end of each cycle was chosen to be unloaded. Finally, E_0 could obviously never exceed the concentration in the raw material [*viz.*, 93 g·L^{-1}, equivalent to 12 % (v/v)].

Table 1 shows the most salient results obtained after 200 algorithm iterations, in increasing sequence of production. It should be noted that these data were all obtained with a pilot fermenter having a working volume of 8 L.

Case	t_1 **(h)**	q_1 **(L·h^{-1})**	t_2 **(h)**	***b***	V_0 **(L)**	E_0 **(g·L^{-1})**	***P*** **(g acetic acid·h^{-1})**
1	2.0	3.50	0.0	0	1.0	15.4	12.9
2	2.0	2.94	0.2	0.0622	2.0	21.6	14.6
3	2.0	2.20	0.0	0	3.6	23.6	15.9
4	1.6	2.47	0.9	0.0117	4.0	23.6	16.2
5	1.1	0.93	3.0	0.1	5.0	25.0	16.5
6	1.0	2.04	2.4	0.0099	5.8	25.2	16.9
7	1.5	1.32	0.0	0	6.0	26.1	17.0

8	1.5	1.00	0.0	0.0963	6.5	29.6	17.2
9	1.4	0.95	0.0	0	6.7	29.7	17.3
10	1.0	0.98	0.0	0	7.0	29.4	17.5
11	0.2	3.09	0.5	0.0622	7.0	29.2	17.5
12	0.3	3.60	0.2	0.0896	7.0	29.3	17.5
13	0.3	3.71	0.0	0	7.0	29.8	17.5

Table 1: Most salient results obtained in optimizing production

The ethanol concentration for each case listed in Table 1 is shown graphically in Figure 1. As can clearly be seen, the first few cases exhibited a temporal variation of the ethanol concentration differing substantially from one another —as can be seen from Table 1, the presence of differences between ethanol profiles coincided with that between production figures. Also, however, production increased as the ethanol concentration converged on a specific variation pattern. From Table 1 it follows that convergence coincided with the initial increase in volume of each cycle —so much so that the volume reached in cases 10–13 equalled the upper bound for this decision variable— and with an ethanol concentration at unload close to 29.5 $g \cdot L^{-1}$.

< Figure 1 >

The variation pattern for the ethanol concentration in cases 8–13 was very similar, even though the loading process was conducted somewhat differently; thus, cases 11 and 12 included an exponential loading step (notice that t_2 in Table 1 is different to zero), whereas case 13 did not. This suggests that the influence of the loading mode on the evolution of the system weakens as the volume of medium unloaded between cycles is reduced. This effect can be explained as follows: if little medium is unloaded, then the residual mass will contain a substantial concentration of highly active biomass; also, whatever the loading procedure, the maximum possible ethanol concentration and acidity of the medium can cause no substantial cell growth inhibition or death during the cycle under these conditions. Therefore, whatever the loading mode, the system will evolve roughly identically. Worth additional note is the fact that the ethanol

concentration at unload was virtually the same (*ca.* 29–30 $g \cdot L^{-1}$) in cases 8-13 and that the initial volume in each cycle was 7 L (*i.e.*, close to the upper bound set for this decision variable).

The previous results suggest that production increases as changes in the conditions of the culture medium become slighter; this situation mimics steady-state operating conditions (*i.e.*, the typical conditions under continuous operation). This led us to examine the behaviour of the system in the continuous operation mode by converting the problem into one of static optimization —the constraints were now the algebraic equations of the model for steady-state conditions— where the sole decision variable was the raw material feed rate ($(F_i)_{steady}$). The new objective function to be maximized was

$$(P)_{steady} = (HAc_o)_{steady} \cdot (F_o)_{steady} \tag{14}$$

where $(P)_{steady}$ denotes production (g acetic $acid \cdot h^{-1}$); $(HAc_o)_{steady}$ the acetic acid concentration in the effluent ($g \cdot L^{-1}$); and $(F_o)_{steady}$ the effluent flow-rate ($L \cdot h^{-1}$), which coincided with $(F_i)_{steady}$ in practice. The results obtained for these parameters are: $(P)_{steady}$ = 17.9 g acetic $acid \cdot h^{-1}$, $(E_0)_{steady}$ = 35.2 $g \cdot L^{-1}$, $(HAc_o)_{steady}$ = 74.7 $g \cdot L^{-1}$ and $(F_i)_{steady}$ = 0.24 $L \cdot h^{-1}$. As can be seen, production under these conditions was better than in any of the cases shown in Table 1, at the expense of a low acidity and a relatively high concentration of ethanol in the effluent. Therefore, aiming at a high production leaves a high proportion of fed ethanol unreacted in the fermenter, which is uneconomical as regards substrate use. In addition, the industrial process must meet some legal requirements concerning the maximum ethanol content allowed in the product. Consequently, the use of a single fermenter to obtain high acetic acid

concentrations while maximizing ethanol use is incompatible with the obtainment of high production figures. Meeting both requirements at once therefore entails using some alternative such as introducing a second fermenter in order to deplete unreacted substrate from the first.

2.2. Optimization of the fermentation rate

On the other side, the maximization of the mean fermentation rate [eq. (8)] was addressed. To this end, the same loading profile parameterization and optimization algorithm as in maximizing production as well as the same lower and upper bounds for the decision variables were used.

Table 2 shows the most salient results obtained after 200 algorithm iterations, in increasing sequence of mean fermentation rate.

Case	t_1 (h)	q_1 ($L \cdot h^{-1}$)	t_2 (h)	b	V_0 (L)	E_0 ($g \cdot L^{-1}$)	r_{ave} (g acetic acid $\cdot L^{-1} \cdot h^{-1}$)
1	2.7	2.16	0.0	0	2.2	24.6	1.90
2	1.2	1.87	9.1	0.0108	5.1	34.2	2.10
3	1.5	1.68	18.4	0.0154	3.5	25.6	2.16
4	0.1	0.36	15.0	0.0912	2.0	34.2	2.25
5	0.4	0.32	15.3	0.062	3.0	36.3	2.36
6	0.1	0.65	20.2	0.068	2.0	36.3	2.40
7	0.1	0.78	20.1	0.0547	2.6	37.8	2.45
8	0.4	0.24	20.2	0.0547	2.6	36.6	2.46
9	1.8	0.41	23.2	0.0358	2.7	34.4	2.46
10	0.1	0.48	20.4	0.0409	3.4	38.7	2.47
11	0.2	0.3	22.1	0.0469	2.8	37.4	2.51
12	0.2	0.3	23.9	0.0459	2.6	37.7	2.54

Table 2: Most salient results obtained in optimizing the fermentation rate

Figures 2 and 3 show the variation of the ethanol concentration in cases 1–6 and 7–12, respectively. As can be seen from Figure 2 and, especially, Figure 3, and also from Table 2, the highest fermentation rates (cases 10–12) were obtained when the ethanol concentration varied within a relatively narrow range (see scale on Figure 3). This may be why the ethanol concentration is kept constant during the industrial loading step

based on the results of much trial-and-error testing. Also, the minimal differences in fermentation rate between cases 7 to 12 were a result of the small differences in their operating conditions (*viz.*, mean volume of medium present in the fermenter – see table 3 –, ethanol concentration at unload, unloaded volume and loading profile – see table 2 –).

< Figure 2 >

< Figure 3 >

Table 3 shows the mean fermentation rates and volumes obtained in each case. As can be seen, the fermentation rate increases with decrease in the mean volume.

Case	V_{mean} **(L)**	r_{ave} **(g acetic acid·L^{-1}·h^{-1})**
1	7.7	1.90
2	7.5	2.10
3	6.9	2.16
4	6.2	2.25
5	6.1	2.36
6	5.7	2.40
7	5.6	2.45
8	5.6	2.46
9	5.5	2.46
10	5.6	2.47
11	5.4	2.51
12	5.3	2.54

Table 3: Mean volumes and fermentation rates obtained in each selected case

Based on the data of Table 2, the operating modes that maximize the mean fermentation rate also involve leaving unreasonably high residual ethanol concentrations for the substrate to be efficiently used.

Irrespective of the previous results, a comparison of the production figures for cases 7–12 (Table 4) with those obtained in the continuous mode or the best cases shown in Table 1 exposes significant differences. The primary origin of the differences was the mean volume during the fermentation cycle in each case.

Case (Fermentation rate)	P (g acetic acid·L^{-1})
7	14.0
8	14.0
9	14.1
10	14.3
11	13.9
12	13.6

Table 4: Production obtained in cases 7–12 (optimization of the fermentation rate)

2.3. Optimization of acetic acid production with maximal substrate use.

The conclusions drawn as regards the loading modes that maximized both production and the mean fermentation rate raised the question as to the most suitable mode for industrial purposes (i.e., that affording maximal production and substrate use) what would be. In order to answer it, the system was subjected to dynamic optimization by imposing an ethanol concentration at unload, E_0, of 3.9 g·L^{-1} in order to ensure that the substrate concentration in the output effluent would be minimal.

Identical procedures were used to perform 200 iterations of the optimization algorithm. The most salient results are summarized in Table 5, in increasing order of production.

Case	t_1 (h)	q_1 (L·h^{-1})	t_2 (h)	b	V_0 (L)	P (g acetic acid·h^{-1})
1	0.6	2.97	3.1	0.1	4.0	13.6
2	2.9	1.58	0.7	0.1	3.0	13.9
3	2.2	2.27	0.2	0.0844	3.0	13.9
4	2.0	2.36	0.7	0.0525	2.9	13.9
5	1.8	2.80	0.9	0.0195	2.8	13.9
6	0.8	3.71	2.1	0.0975	3.5	13.9
7	2.0	2.54	0.0	0.0509	3.1	14.0

Table 5: Selected results obtained with E_0 = 3.9 g·L^{-1} (optimization of production)

The ethanol concentration for each case is shown in Figure 4.

< Figure 4 >

Based on eq. (7), production under these conditions is exclusively influenced by the ratio of the unloaded volume to the cycle duration; in fact, the acid concentration in the

effluent, as well as the rest of the operating conditions, were quite similar in all cases – see table 5 –, leading to the same optimum (*viz.*, a production of ca. 14 g acetic acid·h^{-1}, which is lower than under continuous operation but uses the substrate much more efficiently).

The need to unload the fermenter to such a low ethanol concentration resulted in high unloaded volumes relative to those of Table 1. Otherwise, the process would always operate under conditions of ethanol deficiency and excessive acidity, which would severely restrict microbial growth.

So, depending on the goal, the initial volume used in each cycle as well as the way the reactor is loaded must be different because the resulting ethanol concentration and acidity may exert a strong negative influence on the bacterial activity.

4. Conclusions

In part III of this series, dynamic optimization strategies to the acetic acid fermentation process were applied and the ensuing results were analysed. Thus, the optimum input profile (specifically, the raw material feed rate) was used to maximize two objective functions of interest as regards process economy, namely: acetic acid production and fermentation rate. For this purpose, because of its ease of implementation and compatibility with standard optimization algorithms, the direct method known as Control Vector Parameterization (CVP) was used. In fact, the procedure followed was a combination of a global evolutionary strategy and a local deterministic algorithm (SQP).

Production was maximal with continuous or steady-state operation. In fact, this result could be expected after the conclusions obtained working on a semi-continuous operation mode: an increasing production with decreasing volume of unloaded medium

in each cycle; this ensured retention of a substantial volume of highly active biomass between cycles and lead to convergence at a specific ethanol concentration. Also, the loading profile had virtually no influence on the performance on the process by virtue of the large residual volume remaining after unloading. The optimum production value obtained in the continuous operation mode was about 18 g acetic acid·h^{-1}, all with a constant ethanol and acetic acid concentration of *ca.* 35 g·L^{-1} and 75 g·L^{-1}, respectively. Despite the good production obtained, the excessive ethanol concentration present in the product makes the process industrially unproductive. Therefore, the continuous operation mode appears to be unsuitable for industrial purposes except in isolated, specific cases.

In the absence of other restrictions, the maximum fermentation rate was found to be obtained by unloading the fermenter to an ethanol concentration in the region of 35 g·L^{-1}; this entailed unloading ca. 60% of the total volume and re-loading it in such a way as to keep the ethanol concentration within the approximate range 38–46 g·L^{-1}. For the above-stated reasons, however, this ethanol concentration level at unload is too high by industrial standards. Therefore, the optimum operating mode adopted in terms of fermentation rate is industrially impractical in theory if the substrate has to be efficiently used.

Based on the foregoing, the operating conditions that would maximize production at a given substrate uptake efficiency (*viz.*, by unloading the fermenter to a concentration of 3.9 g ethanol·L^{-1} each time) were sought. The study conducted to this end revealed that it is necessary unloading around ca. 60% of total volume, being recommended a quick loading step. The highest production level achieved in this way was in the region of 14 g acetic acid·h^{-1}, which is less than the typical values for continuous operation.

5. References

[1] E.B. Lee, L. Markus, Foundations of optimal control theory, John Wiley (1967).

[2] D.E. Kirk, Optimal control theory: an introduction, Prentice–Hall (1970).

[3] B. Srinivasan, S. Palanki, D. Bonvin, Dynamic optimization of batch processes: I. Characterization of the nominal solution, Comp. Chem. Eng. 27 (2003) 1–26.

[4] J.R. Banga, E. Balsa, C.G. Moles, A. Alonso, Dynamic optimization of bioprocesses: efficient and robust numerical strategies, J. Biotechnol. 117 (2005) 407–419.

[5] R. Bellman, Dynamic programming, Princeton University Press (1959).

[6] G.M. Siouris, An engineering approach to optimal control and estimation theory, John Wiley & Sons (1996).

[7] M. De Tremblay, M. Perrier, C. Chavarie, J. Archambault, Optimization of fed batch culture of hybridoma cells using dynamic programming: single and multi feed cases, Bioprocess Eng. 7 (1992) 229–234.

[8] R. Luus, On the application of iterative dynamic programming to singular optimal control problems, IEEE Trans. Autom. Control 37 (1992) 1802–1806.

[9] B. Bojkov, R. Luus, Application of iterative dynamic programming to time optimal control, Chem. Eng. Res. Des. 72 (1994) 72–80.

[10] S.A. Dadebo, K.B. McAuley, Dynamic optimization of constrained chemical engineering problems using dynamic programming, Comp. Chem. Eng. 19 (1995) 513–525.

[11] A. Tholudur, W.F. Ramírez, Obtaining smoother singular arc policies using a modified iterative dynamic programming algorithm, Int. J. Control 68 (1997) 1115–1128.

[12] C. Sayer, G. Arzamendi, J.M. Asua, E.L. Lima, J.C. Pinto, Dynamic optimization of semicontinuous emulsion copolymerization reactions: composition and molecular weight distribution". In S. Pierucci, European Symposium on Computer Aided Process Engineering-10, Elsevier (2000).

[13] J. Lee, H. Lim, Y. Yoo, Y. Park, Optimization of feed rate profile for the monoclonal antibody production, Bioprocess Eng. 20 (1999) 137–146.

[14] C. Valencia, N.S. Kaisare, J.H. Lee, Optimal control of a fed-batch bioreactor using simulation-based approximate dynamic programming, IEEE Trans. Contr. Systems Technol. 13 (2005) 786–790.

[15] D.P. Bertsekas, Dynamic programming and optimal control, Vol I. Athena Scientific, Belmont (1995).

[16] D.P. Bertsekas, Dynamic programming and optimal control, Vol II. Athena Scientific. Belmont (1995).

[17] L.S. Pontryagin, V.G. Boltyanskil, R.V. Gamkrelidge, E.F. Mishchenko, The mathematical theory of optimal processes, Interscience (1962).

[18] A.E. Bryson, Y.C. Ho, Applied optimal control, Hemisphere Pub. Corp. (1975).

[19] W.H. Ray, J. Szekely, Process optimization, John Wiley (1973).

[20] A.E. Bryson, Dynamic optimization, Addison–Wesley (1999).

[21] A. Jayant, S. Pushpavanam, Optimization of a biochemical fed-batch reactor – transition from a non-singular to a singular problem, Ind. Eng. Chem. Res. 37 (1998) 4314–4321.

[22] H.J. Oberle, B. Sothmann, Numerical computation of optimal feed rates for a fed-batch fermentation model, JOTA 100 (1999) 1–3.

[23] J. Modak, H. Lim, Y. Tayeb, General characteristics of optimal feed rate profiles for various fed-batch fermentation processes, Biotechnol. Bioeng. XXVIII (1986) 1396–1407.

[24] S. Park, W.F. Ramírez, Optimal production of secreted protein in fed-batch reactors, AIChE J. 34 (1988) 1550–1558.

[25] J.F. Van Impe, B.M. Nicolai, P.A. Vanrolleghem, J. Spriet, B. Moor, J. Vandewalle, Optimal control of the penicillin fed-batch fermentation: an analysis of a modified unstructured model, Chem. Eng. Comm. 117 (1992) 337–353.

[26] W. Skolpap, J.M. Scharer, P.L. Douglas, M. Moo-Young, Optimal feed rate profiles for fed-batch culture in penicillin production, Songklanakarin J. Sci. Technol. 27 (2005) 1057–1064.

[27] J. Roubos, C. de Gooijer, G. van Straten, A.V. Boxtel, Comparison of optimization methods for fed-batch cultures of hybridoma cells, Bioprocess Eng. 17 (1997) 99–102.

[28] I. Rocha, E.C. Ferreira, Optimisation methods for improving fed-batch cultivation of E. coli producing recombinant proteins. In Proceedings of the 10th Mediterranean Conference on Control and Automation (2002).

[29] E. Balsa, J.R. Banga, A. Alonso, V.S. Vassiliadis, Efficient optimal control of bioprocesses using second order information, Ind. Eng. Chem. Res. 39 (2000) 4287–4295.

[30] C.P. Neuman, A. Sen, A suboptimal control algorithm for constrained problems using cubic splines, Automatica 9 (1973) 601–613.

[31] T.H. Tsang, D.M. Himmelblau, T.F. Edgar, Optimal control via collocation and nonlinear programming, Int. J. Control 21 (1975) 763–768.

[32] L.T. Biegler, Solution of dynamic optimization problems by successive quadratic programming and orthogonal collocation, Comp. Chem. Eng. 8 (1984) 243–248.

[33] J.G. Renfro, A.M. Morshedi, O.A. Asbjornsen, Simultaneous optimization and solution of systems described by differential/algebraic equations, Comp. Chem. Eng. 11 (1987) 503–517.

[34] J.E. Cuthrell, L.T. Biegler, On the optimization of differential–algebraic process systems, AIChE J. 33 (1987) 1257–1270.

[35] A. Cervantes, L.T. Biegler, Large-scale DAE optimization using a simultaneous NLP formulation, AIChE J. 44 (1998) 1038–1050.

[36] J. Villadsen, M.L. Michelsen, Solution of differential equation models by polynomial approximation, Prentice–Hall (1978).

[37] P. Terwiesch, M. Agarwal, D.W.T. Rippin, Batch unit optimization with imperfect modelling –A survey, J. Process Contr. 4 (1994) 238–258.

[38] J.W. Eaton, J.B. Rawlings, Feedback control of nonlinear processes using on-line optimization techniques, Comp. Chem. Eng. 14 (1990) 469–479.

[39] J.E. Cuthrell, L.T. Biegler, Simultaneous optimization and solution methods for batch reactor control profiles, Comp. Chem. Eng. 13 (1989) 49–62.

[40] A. Cervantes, S. Tonelli, A. Brandolin, A. Bandoni, L.T. Biegler, Large-scale dynamic optimization for grade transitions in a low density polyethylene plant, Comp. Chem. Eng. 24 (2000) S983–S990.

[41] L.T. Biegler, A. Cervantes, A. Wätcher, Advances in simultaneous strategies for dynamic process optimization, Chem. Eng. Sci. 57 (2002) 575–593.

[42] C.J. Goh, K.L. Teo, Control parametrization: a unified approach to optimal control problems with general constraints, Automatica 24 (1988) 3–18.

[43] K.L. Teo, C.J. Goh, C.C. Lim, A computational method for a class of dynamical optimization problems in which the terminal time is conditionally free, IMA J. Math. Contr. Inform. 6 (1989) 81–95.

[44] T.F. Edgar, D.M. Himmelblau, L. Lasdon, Optimization of chemical processes, McGraw–Hill (2001).

[45] V.S. Vassiliadis, Computational solution of dynamic optimization problems with general differential–algebraic constraints, PhD Thesis, Imperial College, University of London (1993).

[46] V.S. Vassiliadis, C.C. Pantelides, R.W.H. Sargent, Solution of a class of multistage dynamic optimization problems. 1. Problems without path constraints, Ind. Eng. Chem. Res. 33 (1994) 2111–2122.

[47] V.S. Vassiliadis, C.C. Pantelides, R.W.H. Sargent, Solution of a class of multistage dynamic optimization problems. 2. Problems with path constraints, Ind. Eng. Chem. Res. 33 (1994) 2123–2133.

[48] J.S. Logsdon, L.T. Biegler, Accurate solution of differential–algebraic optimization problems, Ind. Eng. Chem. Res. 28 (1989) 1628–1639.

[49] B. Bojkov, R. Hansel, R. Luus, Application of direct search optimization to optimal control problems, Hungar. J. Ind. Chem. 21 (1993) 177–185.

[50] J.R. Banga, W.D. Seider, Global optimization of chemical processes using stochastic algorithms. In C.A. Floudas, P.M. Pardalos, (Eds.), State of the art in global optimization, Kluwer Academic Publishers (1996) 563–583.

[51] J.R. Banga, A. Alonso, R.P. Singh, Stochastic dynamic optimization of batch and semicontinuous bioprocesses, Biotechnol. Prog. 13 (1997) 326–335.

[52] S. Pushpavanam, S. Rao, I. Khan, Optimization of a biochemical fed-batch reactor using sequential quadratic programming, Ind. Eng. Chem. Res. 38 (1999) 1998–2004.

[53] E. Sorensen, S. Macchieto, G. Stuart, S. Skogestad, Optimal control and on-line operation of reactive batch distillation, Comp. Chem. Eng. 20 (1996) 1491–1498.

[54] T. Ishikawa, Y. Natori, L. Liberis, C.C. Pantelides, Modelling and optimisation of an industrial batch process for the production of dioctyl phthalate, Comp. Chem. Eng. 21 (1997) S1239–S1244.

[55] I.M. Mujtaba, S. Macchieto, Efficient optimization of batch distillation with chemical reaction using the polynomial curve fitting technique, Ind. Eng. Chem. Res. 36 (1997) 2287–2295.

[56] K.E. Brenan, S.L. Campbell, L.R. Petzold, Numerical solution of initial-value problems in differential–algebraic equations, Elsevier Science Publishers (1989).

[57] W.F. Feehery, Dynamic optimization with path constraints, PhD Thesis, MIT (1998).

[58] W.F. Feehery, P.I. Barton, Dynamic optimization with state variable path constraints, Comp. Chem. Eng. 22 (1998) 1241–1256.

[59] I. Papamichail, C.S. Adjiman, Global optimization of dynamic systems, Comput. Chem. Eng. 28 (2004) 403–415.

[60] W.R. Esposito, C.A. Floudas, Deterministic global optimization in nonlinear optimal control problems, J. Global Opt. 17 (2000) 97–126.

[61] J.R. Banga, E. Balsa, C.G. Moles, A. Alonso, Dynamic optimization of bioreactors: a review, Proc. Indian Natn. Sci. Acad. 69 (2003) 257–265.

[62] A.R. Conn, N.I.M. Gould, P.L. Toint, A globally convergent augmented lagrangian algorithm for optimization with general constraints and simple bounds, SIAM J. Numer. Anal. 28 (1991) 545–572.

[63] A.R. Conn, N.I.M. Gould, P.L. Toint, A globally convergent augmented lagrangian barrier algorithm for optimization with general inequality constraints and simple bounds, Math. Comput. 66 (1997) 261–288.

[64] I. García-García, D. Cantero-Moreno, C. Jiménez-Ot, S. Baena-Ruano, J.E. Jiménez-Hornero, I. Santos-Dueñas, J. Bonilla-Venceslada, F. Barja, Estimating the mean acetification rate via on-line monitored changes in ethanol during a semi-continuous vinegar production cycle, J. Food Eng. 80 (2007) 460–464.

Figure captions

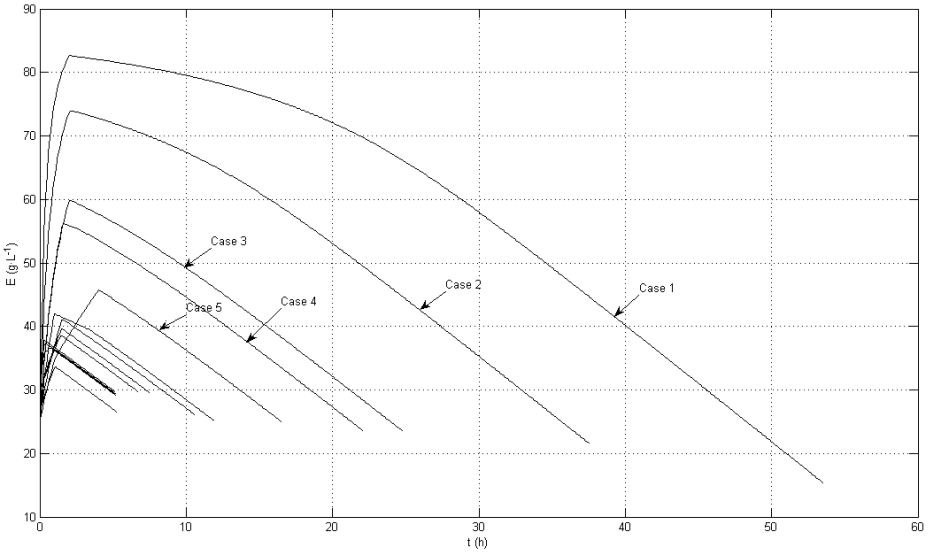

Figure 1: Comparison of ethanol concentrations for the most salient cases (optimization of production).

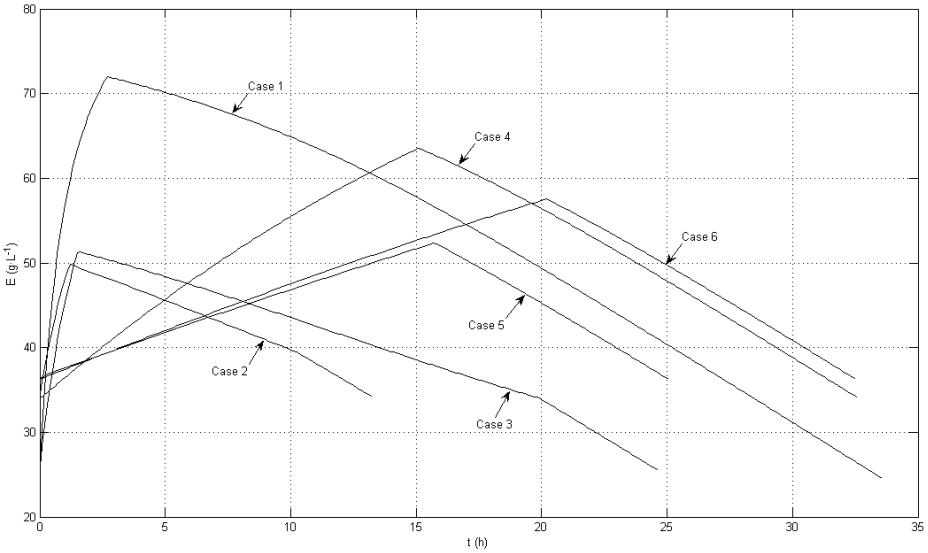

Figure 2: Comparison of ethanol concentrations for cases 1–6 (optimization of the mean fermentation rate).

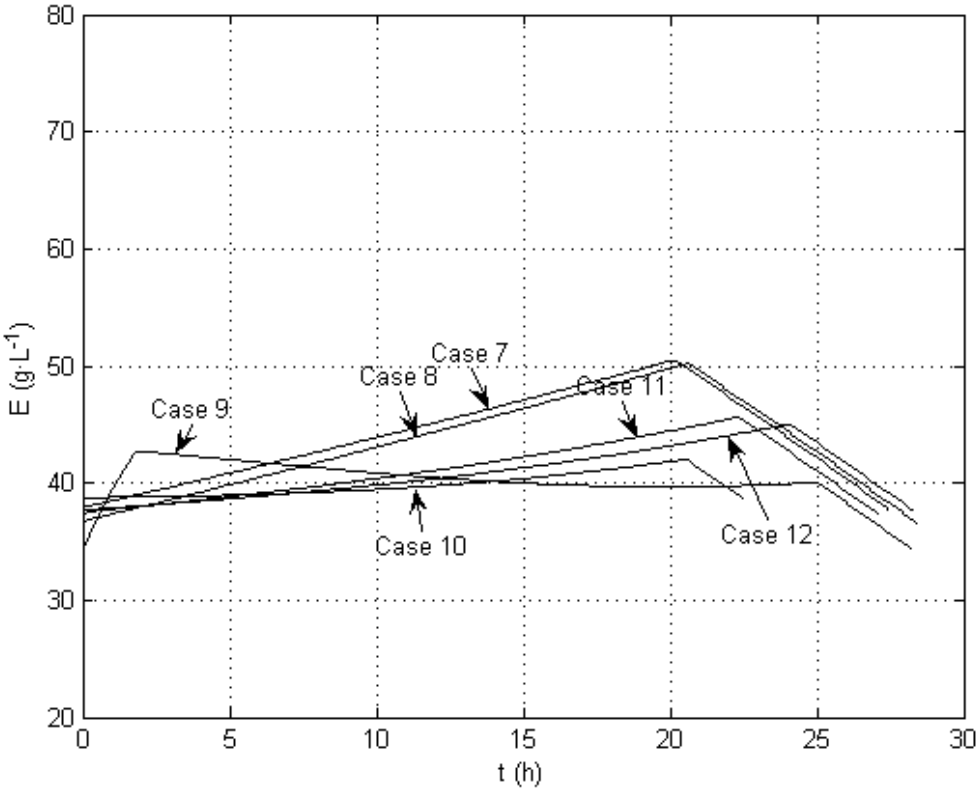

Figure 3: Comparison of ethanol concentrations for cases 7–12 (optimization of the mean fermentation rate).

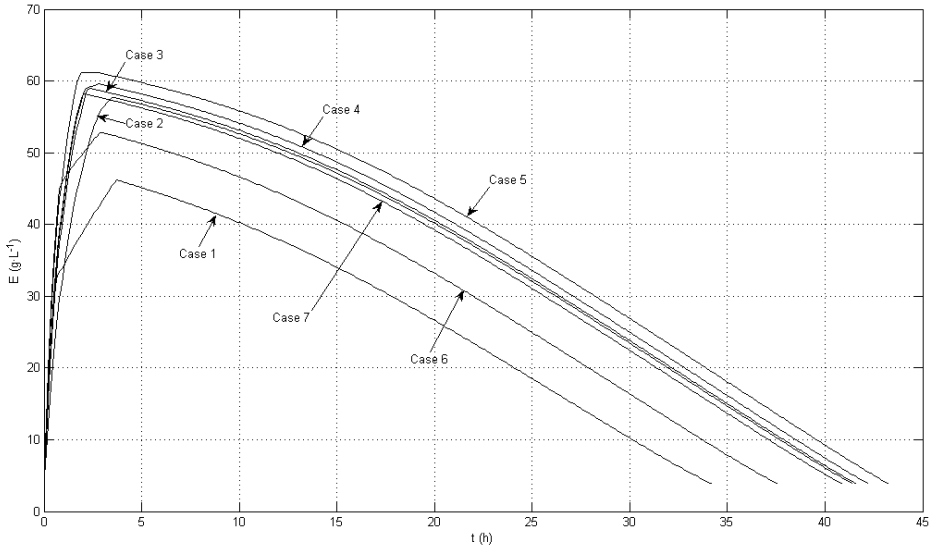

Figure 4: Comparison of ethanol concentrations for the most salient cases (optimization of production when unloading at 3.9 $g \cdot L^{-1}$ ethanol).