

Optimizing in-situ product removal operation in a bioreactor for different production scenarios

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Abstract: Mathematical models and optimization problems can be a valuable tool to optimize the operational design of bioreactors. In the present contribution a bioreactor model, implementing in-situ product removal (ISPR) is presented and used to demonstrate potential real world operational strategies that can be applied to the system. Different optimization objectives are formulated in order to find strategies (i.e. operational designs) that maximize the yield and/or the productivity. The decision variables reflect when feeding pulses should be introduced, how much feeding should be added and when the extraction cycles should take place. The first optimization problem focuses on maximizing the yield of the system by means of a single objective optimization. This solution is the most robust and easiest to implement with no requirements for online measurements or control systems. The optimization problem focusing on maximizing the productivity requires solving a stochastic optimization problem to ensure the robustness of the solution, as trying to maximize the productivity was seen to be very sensitive to model uncertainty. Despite the robustness of the proposed strategy online measurements, monitoring propionate, is advised. Because both yield and productivity are important performance indexes, a multi-objective optimization can be used to consider an acceptable performance of both objectives at the same time. This strategy results in a set of solutions representing the potential compromises among the objectives. However, these solutions certainly require online measurements and control systems to implement them correctly.

Keywords: kinetic modeling, in-situ product removal, biotechnology, optimization problems, operational design

1. INTRODUCTION

Currently, the economic feasibility of many fermentation processes is limited due to low titers in the broth leading to high downstream costs (Woodley et al., 2008). On top of this, many fermentation processes have a limited yield and productivity. A key problem associated with these limitations is the inhibition of the microbial community from either the substrate or product formation (Santos et al., 2021).

To alleviate the problem of product inhibition, in-situ product removal (ISPR) can be applied during the fermentation process. The goal of this technique is to separate the products, during the fermentation, to lower the concentration of inhibiting compounds thereby increasing the potential yield, productivity and titer (López-Garzón and Straathof, 2014). In many cases applying ISPR is not straightforward due to challenges like 1) ensuring long term stability due to longer operational times (compared to batch reactors) resulting in higher risks for contamination, 2) large energy consumption of the extraction procedures and 3) attaining a maximum product recovery as many ISPR techniques do not completely extract the targeted product. As a result of these problems, many ISPR processes do not reach the pilot phase (Woodley et al., 2008; Van Hecke et al., 2014).

Attaining a maximum product recovery, for a batch process applying ISPR discontinuously is not obvious and requires a careful design of the operation. For example, how long should an extraction cycle last, how many extraction cycles should there be, when is the best moment to activate the extraction process to avoid inhibition phenomena, when is the best moment to feed the reactor etc... In other words, the schedule of extraction cycles and external inputs such as spikes, needs to be defined in detail.

To answer these questions a closer look will be taken at a system producing propionate from glucose and employing ISPR discontinuously to enhance the propionate production. The described system can be found in the experimental works of Selder et al. (2020). The aim of this work is to demonstrate how the operational design of a reactor, implementing ISPR can be represented by mathematical programming and solved, depending on the process instrumentation and the user's skills and objectives. Aided by a dynamic model, three optimization techniques will be demonstrated: single objective, stochastic and multi-objective optimization that can lead to the proposal of different operational strategies depending on the user's objectives.

2. MODEL DESCRIPTION

2.1 Experimental system

A mathematical model was made to reproduce the experiments reported by Selder et al. (2020). In this system, the main goal is to produce propionate in a co-culture fermentation while implementing ISPR to reduce potential inhibition caused by organic acids. A stirred-tank bioreactor was fed with a glucose-containing solution with mineral nutrients and yeast extract. The two bacteria used in this co-culture were *Bacillus coagulans*, which converts glucose into lactate (as intermediate) and *Veillonella criceti*, which ferments lactate into propionate and acetate in a 2:1 molar ratio (Fig. 1). Additionally, yeast extract was found to be consumed by both bacteria transforming it into their respective products. The bottleneck of this process lies with *V. criceti* which is inhibited by lactate and propionate (Sabra et al., 2013). It is for this reason that glucose is added in spikes to reduce the accumulation of lactate that could otherwise inhibit the process. Here, ISPR was implemented by means of reverse electro-enhanced dialysis (REED), which selectively removes monovalent organic acids (i.e., lactate, acetate and propionate) through a membrane with an electrical field as driving force. REED was applied discontinuously during the fermentation in cycles of 2 hours because longer extraction cycles could place *V. criceti* in a potentially substrate-deficient state (if the lactate appears in low concentrations in the reactor). As a result, the extraction intervals are set with a fixed duration of 2 hours.

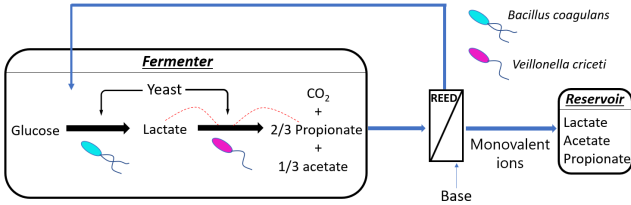


Fig. 1. Schematic representation of the co-culture fermentation in the work of Selder et al. (2020). The red dashes represent the reported inhibition on *V. criceti*.

2.2 Mathematical model

The mathematical model consists of eight ordinary differential equation describing the mass balances of the different components i.e., glucose, yeast, lactate, propionate, acetate, biomass of *V. criceti*, biomass of *B. coagulans* and inert dead biomass in the reactor (Eq.1 -2). Considering the density of the feed and reactor holdup as constant, the flow rate F (gCOD/h)¹ and the mass balances of the different components can be defined by the following equation:

$$\frac{dC(t)}{dt} = D(t)(C^{spike} - C(t)) + R - T \quad (1)$$

Where $C(t)$ is the concentration of the different compounds in the reactor, C^{spike} is the concentration of the spikes in gCOD/L, $R(t)$ is the reaction term in gCOD/L/h and $T(t)$ is the transport flow across the REED extraction membrane in gCOD/L/h. $D(t)$ represents the dilution rate and is zero except when the spike is

¹ The mass of compounds is expressed in terms of the chemical oxygen demand or gCOD to be able to perform accurate electron balances.

added to the fermentation reactor. It is calculated as function of the reactor volume (V) and feed flowrate as:

$$D(t) = \frac{F(t)}{V(t)} \quad \text{with} \quad V(t) = V(0) + \int_0^t F(t') dt' \quad (2)$$

The reaction rate R is calculated by considering the following 5 processes: 1) glucose uptake by *B. coagulans* 2) uptake of yeast extract by *B. coagulans* 3) uptake of yeast extract by *V. criceti* 4) lactate uptake by *V. criceti* and 5) the decay of *V. criceti* (Table 1). These processes were formulated with Monod's kinetics, which are very suitable to describe microbial growth, physiology, and biochemistry of microorganisms (González-Figueroa et al., 2018). The decay of *B. coagulans* was not considered because there was not enough data to accurately determine the process. In Table 1 S_{glu} , S_{lac} , S_{ace} , S_{ye} , X_{ba} and X_{vc} are the concentrations, in gCOD/L, of glucose, lactate, acetate, yeast extract, biomass of *B. coagulans* and biomass of *V. criceti* respectively. The inhibition phenomena experienced by *V. criceti* is expressed by Inh (Eq. 3).

$$Inh = \left(\frac{1}{1 + (S_{lac}/KI_{lac})} \right) \left(\frac{1}{1 + (S_{pro}/KI_{pro})} \right) \quad (3)$$

Where S_{pro} is the concentration of propionate in gCOD/L and KI_{lac}/KI_{pro} are the inhibition constants of lactate and propionate in gCOD/L. Parameters $q1$ and $q2$ are reaction yields equal to 0.78 gCODprop/gCODlac and 0.22 gCODace/gCODlac respectively. By multiplying the kinetic equations, as an array of equations, with the stoichiometric matrix the reaction term R in Eq. 1 can be found for each compound. The values of the parameters in these equations can be found in Table 2 and were either assumed or calibrated from experimental data. Robust calibrated parameters were achieved by embedding the Levenberg-Marquardt and trust-region-reflective methods (using the built-in solver *lsqnonlin* from MATLAB (version r2021a)) in a bootstrap method, as described in the works of Regueira et al. (2021). The mass balances of each component were implemented in MATLAB and solved with built-in numerical solvers (ode45).

3. OPTIMIZATION OBJECTIVES

Depending on the objective of the operator, a certain performance index of a bioreactor (such as final titer) can be prioritized over others. Two typically important indexes are the product yield and productivity. The yield is an especially important metric when the substrate needs to be utilized in the most efficient way possible because either the obtained product or the used substrate is a very valuable resource. On the other hand, productivity is favored in cases where the obtained product is sold cheaply and large bulks of product needs to be sold to ensure a healthy profit margin with respect to the capital investments. In reactions with product inhibition, high product yield and productivity are often conflicting objectives. This is because a high yield, in these cases, require long reaction (or residence) times whereas quick reactor turnover (i.e., a high productivity) is associated with incomplete substrate consumption. These two objectives are defined as functions of the decision variables as $f_{yield}(z)$ and $f_{prod}(z)$, expressed in gCOD_{prop}/gCOD_{glu} and gCOD_{prop}/L/h respectively and are calculated by Eq. 4-5.

$$f_{yield}(z) = \frac{C_{pro}^{res} \cdot V_{res}}{C_{glu,0} \cdot V_{reac} + V_{spikes} \cdot C_{glu}^{spike}} \quad (4)$$

Table 1. Stoichiometric matrix and the vector of kinetic expressions. 1) Glucose uptake 2) Yeast extract consumption *B. coagulans* 3) Yeast extract consumption *V. criceti* 4) Lactate uptake 5) Decay of *V. criceti*

Process	Compounds						Biomass B.	Biomass V.	Inert mass	Kinetic equations
	Glucose	Lactate	Yeast	Propionate	Acetate					
1)	-1	(1-Y _{glu})	0	0	0	Y _{glu}	0	0	$\mu_{glu} = km_{ba} \left(\frac{S_{glu}}{KS_{glu} + S_{glu}} \right) X_{ba}$	
2)	0	(1-Y _{yeb})	-1	0	0	Y _{yeb}	0	0	$\mu_{yeb} = km_{ba} \left(\frac{S_{yeb}}{KS_{yeb} + S_{yeb}} \right) X_{ba}$	
3)	0	0	-1	(1-Y _{yev})	0	0	Y _{yev}	0	$\mu_{yev} = km_{lac} \left(\frac{S_{yev}}{KS_{yev} + S_{yev}} \right) Inh \cdot X_{ve}$	
4)	0	-1	0	(1-Y _{lac})*q1	(1-Y _{lac})*q2	0	Y _{lac}	0	$\mu_{lac} = km_{lac} \left(\frac{S_{lac}}{KS_{lac} + S_{lac}} \right) \cdot Inh \cdot X_{ve}$	
5)	0	0	0	0	0	0	-1	1	$\mu_{dec} = kdec_{X_{lac}} * X_{ve}$	

Table 2. Parameter abbreviations, values, confidence intervals, units and source of the parameters.

Abbreviation	Value	CI	Units	Source
km _{ba}	3.41	[3.22, 3.84]	h ⁻¹	Calibrated
km _{lac}	10.3	[9.11, 11.73]	h ⁻¹	Calibrated
KS _{glu}	0.10	/	gCOD L ⁻¹	Assumed
KS _{lac}	0.27	/	gCOD L ⁻¹	Assumed
KS _{yeb}	0.10	/	gCOD L ⁻¹	Assumed
KS _{yev}	0.50	/	gCOD L ⁻¹	Assumed
kdec _{X_{lac}}	0.04	[0.04, 0.09]	h ⁻¹	Calibrated
Y _{glu}	0.07	[0.07, 0.08]	gCOD _X gCOD ⁻¹	Calibrated
Y _{lac}	0.03	[0.02, 0.04]	gCOD _X gCOD ⁻¹	Calibrated
Y _{yeb}	0.07	/	gCOD _X gCOD ⁻¹	Assumed
Y _{yev}	0.02	/	gCOD _X gCOD ⁻¹	Assumed
KI _{lac}	10.0	[8.42, 12.01]	gCOD L ⁻¹	Calibrated
KI _{pro}	7.34	[5.33, 10.67]	gCOD L ⁻¹	Calibrated
q1	0.78	/	gCOD _{pro} gCOD _{lac} ⁻¹	Calculated
q2	0.22	/	gCOD _{ace} gCOD _{lac} ⁻¹	Calculated

$$f_{prod}(z) = \frac{C_{pro}^{res} \cdot V_{res}}{t_{end}} \quad (5)$$

In these objective functions C_{pro}^{res} is the concentration of propionate in the reservoir in gCOD/L, V_{res} is the volume of the reservoir in L, V_{reac} the volume of the reactor in L, V_{spike} is the total added volume of the spikes in L, C_{glu}^{spike} the concentration of the glucose spikes in gCOD/L, $C_{glu,0}$ the initial glucose concentration in gCOD/L and t_{end} the operation time in h. Both objective functions are dependent on the decision variables z as further elaborated in the following section.

4. OPTIMIZATION METHODS

4.1 Decision variables

To find optimal operational strategies regarding the scheduling of the extraction cycles and spikes that maximize the objective function, the following 9 decision variables were considered: the concentration of the glucose spikes (z_1), the time at which *V. criceti* is inoculated (z_2), the time at which the first extraction cycle is activated (z_3) and the 6 time intervals between the spikes and the start of an extraction cycle (z_4 to z_9). For each spike the same concentration (as determined by z_1) and volume is introduced to the system (24 ml). Fig. 2 illustrates the decision variables in relation to a typical operation of the system. In this system, the spikes are always introduced between extraction cycles.

4.2 Single objective optimization

In the early stages of development, where detailed cost assessment is often not available, single objective optimizations often

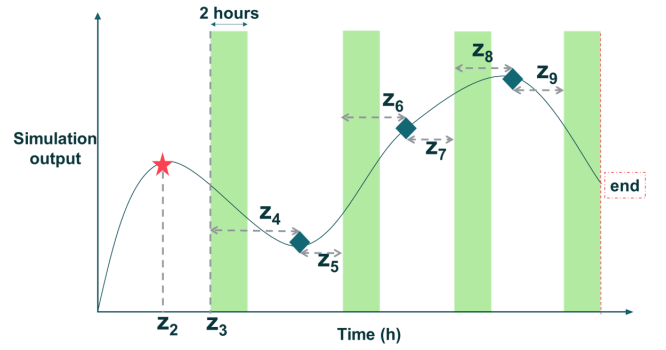


Fig. 2. Depiction of decision variables (z_i) in a typical operation: inoculation of *V. criceti* (★), extraction cycles (■) and glucose spikes (◆).

refer to maximizing the yield or productivity. The problem is formulated as seen in Eq. 6-7:

$$\max_{z \in \Omega} f(z) \quad (6)$$

$$\Omega = \{z : g(z) \leq 0, a \leq z \leq b\} \quad (7)$$

Where $f(z)$ is the objective function and Ω represents the feasible space of the decision variables z . In this case Ω is defined by the (non-)linear inequality constraints $g(z)$ and the lower and upper variables bounds a and b , respectively. The inequality constraints are put in place to ensure a minimum yield or productivity is achieved. The bounds of the decision variables (a and b) make sure that the spikes and extraction cycles always follow each other up sequentially, that spikes are not added during the extraction cycles and that the extraction cycles of last for 2 hours. To solve these single objective problems the built-in function of MATLAB *patternsearch* was used which uses derivative-free methods, called generalized pattern search (Conn et al., 2009). This algorithm was chosen due to its efficiency at finding global optima where many local minima exist and its relatively quick computational speed compared to other derivative-free methods.

4.3 Stochastic optimization

The operational strategy attained by single objective optimization might, in some cases, be very sensitive to uncertainty in the model parameters which could cause issues to correctly implement the solution. To design a process which is more robust against uncertainty, a stochastic optimization problem can be solved. In this case the *Here and now* algorithm from Diwekar and Rubin (1991) was used to solve the optimization problem.

In this algorithm, the objective function and constraints are expressed in terms of a probability distribution, which was obtained by Monte Carlo simulations. During these simulations

the parameters of the model were varied according to their uncertainty (parameter uncertainty was estimated during model calibration from experimental data, not shown here). Different percentiles or moments of the distribution can be taken as the objective to maximize. Conservative solutions would try to maximize low percentiles (“maximize worst cases”) or minimize distribution variance (“minimize variability”). In this work, the lower 10th percentile was taken as the objective to maximize the productivity ensuring a more conservative and robust strategy. The optimization problem is defined as followed:

$$\max_{z \in \Omega} F(z, u) = P_1(f(z)) \quad (8)$$

$$\Omega = \{z : P_2(g(z), u) \geq 0, a \leq z \leq b\} \quad (9)$$

Where u is the vector of uncertain parameters which were characterized during the model calibration, while P_1 and P_2 represent the probabilistic representation of the objective and constraint function. I.e., the tenth percentile from the distribution of Monte Carlo simulations.

4.4 Multi-objective optimization

Strategies can also be found using multi-objective optimization and is especially interesting to apply if the various objectives are conflicting. The solution to multi-objective optimization is not a single solution but rather a whole set of solutions called the nondominated set (also known as the Pareto set) representing the potential compromise solutions among the objectives (Diwekar, 2020). For this specific case the multi-objective optimization can be considered as a collection of single objective optimizations but where the second objective is an inequality constraint. The advantage of a multi-objective optimization is that a complete and unbiased (as the constraints do not need to be chosen) search of possible solutions is carried out. This optimization problem was solved in MATLAB using the built-in function *paretosearch*. This algorithm uses the aforementioned *generalized pattern search* but instead of updating a single point per iteration, it updates an iterate list of nondominated points (i.e. the points that have the best rank and are closest to the Pareto front) (Custódio et al., 2011). The multi-objective optimization can be defined as followed:

$$\max_{z \in \Omega} F(z) = (f_{yield}(z), f_{prod}(z)) \quad (10)$$

$$\Omega = \{z : a \leq z \leq b\} \quad (11)$$

5. RESULTS

Different optimization problems are used to maximize different performance indexes of the reactor. In the following section why a certain optimization problem was chosen, the results of the optimization and how best to implement them will be further discussed.

5.1 Optimizing the yield

If only the yield is chosen to be optimized then the best optimization problem to formulate would be a single objective optimization. This is because the resulting strategy from this optimization (Fig. 3) can be implemented in a conservative way. The obtained simulation results in a high yield (0.69 gCOD/gCOD) which is close to the theoretical maximum of the system (0.72 gCOD/gCOD). In this optimization a constraint was placed so a

minimum productivity of 0.60 gCOD/L/h is achieved. The strategy relies on the fact that lactate is almost completely depleted during each extraction cycle and converted to propionate. This strategy can thus be made very robust by simply taking more time before activating the extraction cycles to ensure all the lactate is depleted. This strategy is also the easiest to implement as it does not require any sophisticated online monitoring to carry out.

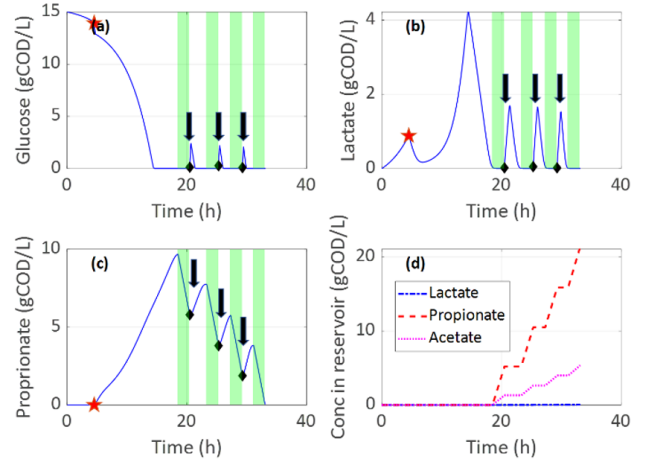


Fig. 3. Simulation of the concentration profiles in the reactor (a-c) and the reservoir (d), from the single objective optimization maximizing the yield. \star is the inoculation of *V. criceti*, \downarrow indicates a spike and \blacksquare indicates an extraction cycle.

5.2 Optimizing the productivity

Excellent yields can be found using the solution found in the previous section but for a relatively cheap commodity chemical like propionate, the productivity is likely to be a more important performance index to consider. A single optimization problem could be used to find a strategy to maximize the productivity, however the robustness of the solution can be put in to question. The simulation resulting from a single objective optimization schedules the extraction cycles and spikes very close to each other and starts the first extraction cycle very early resulting in a very high productivity of 1.13 gCOD/L/h (Fig. 4). In this optimization a constraint was placed so a minimum yield of 0.60 gCOD/gCOD is achieved. Because of the uncertainty in the model parameters and the inherent variability of biological systems (e.g. variations in initial bacterial concentration, slight changes in consumption rates, etc.), propionate might actually not be present in the reactor when the first extraction cycle starts. Only the intermediate (i.e., lactate) would then be extracted in this case, resulting in a significant loss of yield and productivity.

To obtain a strategy that anticipates these types of uncertainties a stochastic optimization problem was solved resulting in a simulation with a productivity of 0.97 gCOD/L/h (Fig. 5). In this optimization a constraint was placed so that, from the resulting distribution of yields from the Monte Carlo simulations, a minimum average yield of 0.63 gCOD/gCOD was achieved. Compared to the deterministic optimization (Fig. 4), the stochastic solution takes a bit more time to activate the first extraction cycle and schedules the following cycles slightly more apart, thus giving the reactor more time to form propionate.

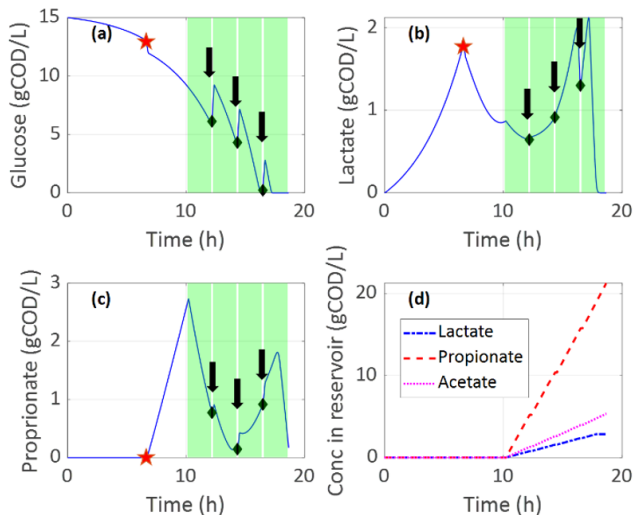


Fig. 4. Simulation of the concentration profiles in the reactor (a-c) and the reservoir (d), from the single objective optimization maximizing the productivity. \star is the inoculation of *V. criceti*, \downarrow indicates a spike and \blacksquare indicates an extraction cycle.

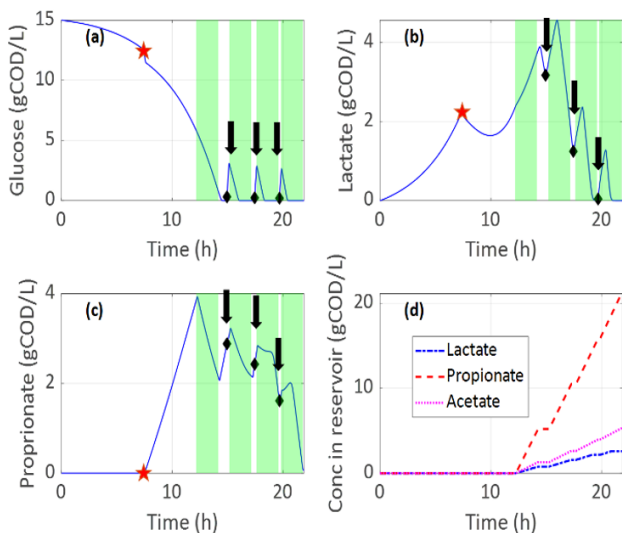


Fig. 5. Simulation of the concentration profiles in the reactor (a-c) and the reservoir (d), from the stochastic optimization maximizing the productivity. \star is the inoculation of *V. criceti*, \downarrow indicates a spike and \blacksquare indicates an extraction cycle.

Although uncertainty is taken into account, online monitoring is still advised so corrective actions can be taken if necessary (e.g. delaying the extraction cycle if no propionate is formed).

5.3 Optimizing the productivity and yield simultaneously

As mentioned earlier the most desired solution is the one that maximizes the productivity and yield simultaneously. However, for most biological reactions maximizing these two performance indexes is conflicting: either the reactor is operated with a quick turnover time to prioritize the productivity, which leaves part of the substrate unconsumed, or the reactor operation time is long to maximize substrate consumption, sacrificing the productivity. In cases like these applying a multi-objective

optimization is useful as it allows us to see the potential compromises among the objectives (i.e., yield and productivity). In Fig 6 these solutions can be seen and an example of a trade-off solution from the Pareto front is shown in Fig. 7 resulting in a yield of 0.67 gCOD/gCOD and a productivity of 0.85 gCOD/h. If a trade-off solution is desired a careful economical evaluation of the reactor should be performed to choose the correct compromise solution.

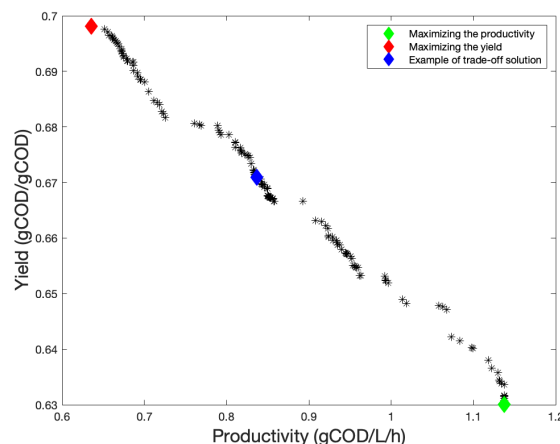


Fig. 6. The estimated nondominated set of solution from the multi-objective optimization represented on the Pareto front

These solutions are not optimized to consider the uncertainty of the model or anticipate experimental variations, which means that there is a possibility that the reactor does not behave as the model solution. To effectively face the mismatch of the model and the inherent variability of co-cultures during the batch operation, online monitoring and control needs to be applied. For example in Fig. 7, for the first extraction cycle, propionate must be present in the reactor while for the following 3 extraction cycles lactate is almost completely depleted. If no monitoring or control mechanisms are put in place it is possible that no extraction of propionate happens for the first extraction cycle or that lactate is extracted in the following extraction cycles leading to a severe loss in productivity and yield.

There are a few online sensors capable of selectively measuring propionate, lactate and acetate. For example, Near-infrared spectroscopy (NIR) has been reported as a suitable method to follow carboxylic acids in aqueous solutions online (Jacobi et al., 2009). Additionally, Cabaneros Lopez et al. (2021) have demonstrated, in a real bench-scale reactor, how NIR probes combined with state estimation (in this case the Extended Kalman Filter) can effectively monitor such bioreactors. Future works in this area should consider online monitoring possibilities to improve the operational strategy.

6. CONCLUSION

In this contribution, three optimization techniques are successfully applied to find 3 different operational strategies to run the described co-culture fermentation implementing ISPR. This contribution demonstrates that the optimization problem needs to be carefully chosen to fit the objective of the user but also that the solution needs to be critically examined according to the uncertainty of the solution and its practical implementation. E.g.

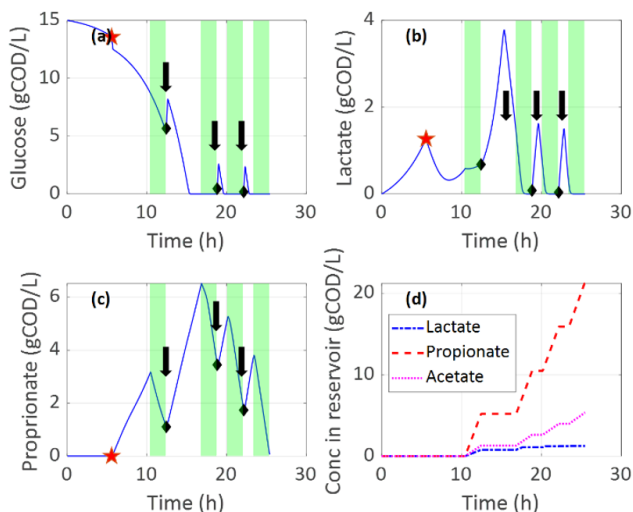


Fig. 7. Simulation of the concentration profiles in the reactor (a-c) and the reservoir (d), from the multi-objective optimization. ★ is the inoculation of *V. criceti*, ↓ indicates a spike and ■ indicates an extraction cycle.

how real time monitoring is to be implemented. Furthermore, future works should focus on developing monitoring strategies to effectively apply control mechanisms in those cases where the optimized solution is uncertain (i.e., for the solutions of the multi-objective optimization).

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