Optimization of fed-batch fermentation processes with bio-inspired algorithms

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ABSTRACT

The optimization of the feeding trajectories in fed-batch fermentation processes is a complex problem that has gained attention given its significant economical impact. A number of bio-inspired algorithms have approached this task with considerable success, but systematic and statistically significant comparisons of the different alternatives are still lacking. In this paper, the performance of different meta-heuristics, such as Evolutionary Algorithms (EAs), Differential Evolution (DE) and Particle Swarm Optimization (PSO) is compared, resorting to several case studies taken from literature and conducting a thorough statistical validation of the results. DE obtains the best overall performance, showing a consistent ability to find good solutions and presenting a good convergence speed, with the DE/rand variants being the ones with the best performance. A freely available computational application, OptFerm, is described that provides an interface allowing users to apply the proposed methods to their own models and data.

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1. Introduction

In addition to the considerable number of valuable products that have long been produced using fermentation techniques, such as recombinant proteins and antibiotics, Biotechnology has been replacing traditional manufacturing processes in many areas that were until recently dominated by the chemical industry. When compared with conventional production methods, biotechnological processes have relatively low energy requirements and environmental costs, as well as decreased waste generation associated with the possibility of creating biodegradable products and of using renewable raw materials.

Industrial or White Biotechnology can be defined as the use of cells or enzymes for the production of commodity and specialty chemicals. The share of biotechnological processes in the production of various chemical processes is currently raising and this trend is expected to continue. In this new reality, rational fermentation optimization approaches gain a novel fundamental importance since, when compared with pharmaceutical processes, the margins in industrial biotechnological processes are much lower

and competitiveness depends more on product price than on patent protection. In fact, for such processes, an increase in productivity or efficiency can have a direct and strong impact in profitability.

Under this scope, several optimization strategies can be delineated, depending on the defined purposes. The process engineer point of view aims at optimizing the fermentation process by exploiting the maximum capabilities of an already selected microorganism and by manipulating environmental and operational variables, therefore using potentially similar tools, but with a different perspective when compared with optimization strategies that envisage the design of improved strains (Patil, Rocha, Forster, & Nielsen, 2005). The challenge consists in controlling a bioprocess at its optimal state in order to reach its maximum productivity with the lowest possible cost.

In recent years, the efforts devoted to the application of process engineering approaches to optimize biotechnological production processes have focused on the dynamic optimization (open-loop optimal control) of fed-batch bioreactors. This optimization has traditionally been done on the substrate feed rate as key manipulated variable.

For that purpose, the optimization strategies described in the literature include both analytical and numerical methods. It has been shown that for relatively simple bioreactor systems, which are expressed by differential equations models, the optimization problem can be solved analytically by applying the Pontryagin's Minimum Principle (PMP). However, in the majority of the cases

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reported, determination of the optimal feed rate profile is a problem of singular control, because the control variable (feed rate) often appears linearly in the system of differential equations (Shin & Lim, 2006).

Thus, this approach fails to provide a complete solution (Shukla & Pushpavanam, 1998; Tartakovsky, Ulitzur, & Sheintuch, 1995). Nevertheless, in several studies, singular control theory was used to solve the analytical control problem (Lee & Ramirez, 1994; Levisauskas et al., 2003; Modak, Lim, & Tayeb, 1986; Park & Ramirez, 1988), while an unified methodology was proposed by Smets, Claes, November, Bastin, and Impe (2004), the so-called optimal adaptive control strategy, where a theoretically realizable optimum is obtained using the PMP and an adaptive controller is derived based on those results to correct on-line deviations caused by model inaccuracies. However, the analytical approach becomes too complex when the number of state and control variables increases. Moreover, those methods can only be applied to differential equation models and do not satisfy for alternative bioreactor modeling methods such as neural networks or fuzzy models.

Here, numerical methods are required. These can be divided into deterministic (usually local) and stochastic (usually global) methods (Banga, Moles, & Alonso, 2003). Popular techniques that follow the first approach are gradient-based local methods like Sequential Quadratic Programming that are applied to the non-linear programming problem obtained after Control Vector Parameterization (Bapat, Sohoni, Moses, & Wangikar, 2006). The main disadvantages of these methods are the computational cost, as well as premature convergence for local optima, especially if the optimization is started far away from the global solution.

Because of those limitations, some promising approaches regarding methods for bioprocess optimization rely on the use of stochastic metaheuristic algorithms, namely those belonging to the class of Evolutionary Computation (EC). With these methods, although global optimality cannot be guaranteed, good solutions are normally obtained in relatively modest computational times. Popular tools like Genetic and Evolutionary Algorithms (EAs), together with other approaches like Evolution Strategies and Differential Evolution (DE) belong to this class.

Several applications of EC algorithms to bioprocesses can be found in the literature. For example, Roubos, van Straten, and van Boxtel (1999) compared the performance of a class of EAs with first order gradient algorithms and with dynamic programming. The results show a good and often superior performance of EAs in comparison with other methods. These approaches were applied to hybridoma cells and to recombinant protein production with *E. coli*. A very similar work (for hybridoma cells) can be found in Nguang, Chen, and Chen (2001).

Integrated methods based on EAs for the maximization of cell mass production in recombinant *S. cerevisiae* and *Aureobasidium pullulans* fed-batch cultures are described in Na, Chang, Chung, and Lim (2002) and Ronen, Shabtai, and Guterman (2002), respectively. In Sarkar and Modak (2003) and Sarkar and Modak (2004), the authors use EAs for the determination of the correct switching structure, as well as the feed rate in the singular intervals obtained by applying the optimal control theory in several case-studies.

Artificial Neural Networks (ANNs), as an alternative modeling scheme, have been used by Zuo and Wu (2000) and Chen, Nguang, Chen, and Li (2004) to represent bioprocesses that are optimized with EAs. A different approach was used by Franco-Lara and Weuster-Botz (2005), in which the feeding profile is represented by means of ANNs that are optimized by EAs. Following a similar approach a hybrid combination of ANNs and DE was presented in Dragoi, Curteanu, Galaction, and Cascaval (2013) with the aim of optimizing the oxygen transfer coefficient in aerobic fermentation processes.

Other stochastic nature-inspired methods have also been applied in the optimization of fed-batch fermentations. In Kookos (2004), the authors used Simulated Annealing to optimize the feeding profile in ethanol and penicillin production, while Jayaraman, Kulkarni, Gupta, Rajesh, and Kusumaker (2001) used the Ant Algorithm for the optimization of the first case study and of protein production in *E. coli*.

Some authors claim that, when compared with EAs, DE is a more appropriate solution for those applications (Banga et al., 2003). In Ros et al. (2013), the authors have compared several stochastic optimization methods in the estimation of the kinetic parameters of an alcoholic fermentation model, and concluded that DE had the best performance, even suggesting their integration in hybrid search procedures.

Among the few works reported, DE has been applied to the optimization of the fed-batch fermentation of *Zymomous mobilis* (Chiou & Wang, 1999), bacteriocin production with lactic acid bacteria (Moonchai, Madlhoo, Jariyachavalit, & Shimizu, 2005), ethanol production in *Saccharomyces cerevisiae* (Wang & Cheng, 1999) and multiple bioprocesses (Kapadi & Gudi, 2004). However, the performance of the different EC and other nature-inspired algorithms has scarcely been compared in an effective way. Moreover, most of the studies described in the literature are based on only one run of the algorithms which, for stochastic methods, is not appropriate.

Therefore, in this work, a more thorough comparison is proposed, that involves several bio-inspired algorithms (from the classes of EAs, DE and Particle Swarm Optimization – PSO) and that goes through an appropriate process of statistical validation of the results. Four case studies were used to compare the performance of the different algorithms, two of them encompassing two distinct feed variables. Each algorithm was allowed to run for a given number of function evaluations and the comparison among the methods was based on their final result and also on the convergence speed of the algorithms, i.e. on the computational time required to obtain a high-quality solution. For every algorithm, 30 runs were conducted for each case study to achieve statistical significance and appropriate statistical tests were performed. The results obtained are clearly favorable to the DE/rand variants, that outperform both EAs and PSO.

As a complement to this work, a computational application with an user friendly interface was developed and is made available for the community. This allows users to validate the proposed algorithms and apply them to their own models. The framework is also fully modular allowing interested researchers to develop their own algorithms or to improve the application with other functionalities.

2. Case studies: fed-batch fermentation processes

In fed-batch fermentations there is an addition of certain nutrients along the process, allowing the achievement of higher product concentrations. During this process the system's states change considerably, from initially low to high biomass and product concentrations. This dynamic behavior motivates the development of optimization methods to find the optimal input feeding trajectories in order to improve the process performance. For the proper simulation of the process, a white box mathematical model is typically developed, based on differential equations that represent the mass balances of the relevant state variables.

The selected four case studies cover a wide range of industrial applications and organisms. These are detailed in the following sections.