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Evaluation of artificial neural networks for modelling and optimization of medium composition with a genetic algorithm

Ezequiel Franco-Lara *, Hannes Link, Dirk Weuster-Botz

Lehrstuhl für Bioverfahrenstechnik, Technische Universität München, Boltzmannstr. 15, 85748 Garching, Germany

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Abstract

A new concept was evaluated for experimental multi-objective medium optimization using a genetic algorithm which is supported by an artificial neural network (ANN). The ANN is used to model objective functions with the medium components as variables each time a new data set has been produced. An appropriate topology of the ANN was first identified with simulation studies using a multi-dimensional test function (De Jong's function). The performance of this ANN model was validated from generation to generation with the data of an experimental optimization of a medium with 13 medium components for *Synechococcus* PCC 7942. Objective functions were the simultaneous maximization of biomass concentration and conversion of pentafluoroacetophenon (PFAP) for asymmetric synthesis of (*S*)-(−)1-(pentafluorophenyl)-ethanol. The mean absolute error of the ANN simulation was within the experimental estimation error after six from eight generations for one of the two objective functions (PFAP conversion). This artificial neural network supported genetic algorithm (ANNSGA) can thus be implemented at the end of a stochastic optimization procedure to reduce the experimental effort.

Keywords: Bioprocess design; Stochastic search; Biotransformation; Cyanobacteria; Neural networks; Media formulation

1. Introduction

The reduction of the invested time and costs for development and improvement of a bioprocess is a fundamental demand in today's biotechnology. In the specific application of fermentation media formulation, the aim of the optimization is to determine the most suitable reaction conditions (pH, temperature, etc.) and specially medium component concentrations which maximize or minimize economically or technologically important process variables (product concentration, yield, selectivity, raw materials costs, etc.) [1]. However, due to the metabolic complexity of microorganisms and the usually large number of variables involved, the development of rigorous models for a given biological reaction system on physical and chemical basis is still a critical challenge. This is mainly due to the non-linear nature of the biochemical network interactions and, in some cases, the incomplete knowledge about the kinetics involved in such systems.

Some recent innovations are notable as they incorporate the use of advanced mathematical tools in a data-driven form to model bioprocesses. As stated by Kim and Lewis [2] an important goal is to formalize humanlike decision-making, behaviour and performance into a rigorous system theory. According to this concept, artificial neural network (ANNs) can be considered under such techniques. ANNs have been utilized with high success for system design, modelling, optimization and control due mainly to their capacity to learn, filter noisy signals and generalize information through a training procedure [2–6]. ANNs are used commonly as ''black box'' models of key variables whose relationship to other process entities are neither formally described nor mathematically established, but are assumed to occur. Training is referred as the minimization of an error norm, which is usually the least squared criterion with respect to the output of the network and the desired output. Through this training procedure the parallel processing units of the ANN (parameters) are adapted iteratively enabling thus function approximation. Validation is done while presenting the network a dataset not used for training and evaluating the system performance under this situation.

Concerning the experimental optimization of media formulation, the use of stochastic search procedures based

* Corresponding author. Present address: Institut für Bioverfahrenstechnik, Technische Universität Braunschweig, Gaußstr. 17, 38106 Braunschweig, Germany. Tel.: +49 531 3917656; fax: +49 531 3917652.

E-mail address: e.franco-lara@tu-bs.de (E. Franco-Lara).

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on genetic algorithms (GAs) has been lately applied in an efficacious manner compared to other methods, like statistical design of experiments [1,7–11]. The success of this approach is specially associated with the recent advances in the application of miniaturisation and parallelization techniques to bioreactors, allowing the implementation of a large number of simple batch experiments which can be carried out simultaneously. GAs are capable to explore large variable spaces with the additional advantage of an evolutionary adaptation through selection, information exchange and mutation. The strategy ''survival of the fittest'' is applied according to the optimization objectives.

Some attempt have been made to use ANNs for optimising recipes formulations, but their application has been restricted to the use of 2–3 components [4,12–16], with an exceptional maximum of six components reported by Ref. [17]. In their work, the authors describe the incorporation of an ANN model into a GA for the optimization of the xylitol production. However, the authors do not give any clues or particulars belonging to the ANN topology, activation functions or validation procedure with independent experiments, which complicates the conception of an adequate ANN prototype or its extension to new processes.

Moreover, the effect of experimental errors or noisy measurements is either not taken into account or simply ignored by many reports. As stated by Zuzek et al. [10], robust handling of experimental error is a most critical issue for optimization success and a major challenge in media formulation no matter which design method is applied.

This paper addresses the evaluation of ANN supported GA applied to a multi-objective optimization problem of media design. The simultaneous maximization of the biomass concentration and conversion of pentafluoroacetophenon (PFAP) with *Synechococcus* PCC 7942 is discussed. For both variables a functional relation is supposed to exist between the 13 media components, which is represented by two ANNs. To establish an appropriate topology for the ANN architecture, i.e. the number of nodes in the input and hidden layers and to select a proper training procedure, the De Jong test function was examined. The function presents multi-dimensional symmetry for the design variables, which resemble a pure quadratic statistical design. The original De Jong test function was modified adding a noisy signal to simulate error measurement.

2. Materials and methods

2.1. Experimental set-up

The strain *Synechococcus* PCC 7942 was provided by the Pasteur Culture Collection of Cyanobacteria, Paris. Innoculum preparation, media set-up (Table 1) and cultivation conditions have been published elsewhere [18]. All optimization experiments were performed under constant temperature of 20 °C and constant incident light of 26 μEinstein m^{-2} s^{-1} at the surface of parallel operated 1 ml glass reactors (32 mm × 11 mm, VWR, Germany) with a sterile 0.2 μm PTFE filter cap on the top. They were charged with 1 ml of each medium to be tested. A maximum set-up of 80 reactors was incubated on a rotary shaker with an eccentricity of 3 mm at 600 rpm. The initial cell concentration was adjusted to 0.85 g_{dcw}/L.

After 72 h cultivation the optical density was measured and evaporation was determined gravimetrically for each micro reactor. Subsequently the evaporated volume and sample volume for optical density measurement were compensated with the respective original medium. After gas tight sealing of the reactor, 1.4 mM 2′-3′-4′-5′-6′-Pentafluoroacetophenon (Interchim, France) was added trough a septum using a syringe. The reaction mixtures were incubated at 20 °C and 600 rpm on a rotary shaker with an eccentricity of 3 mm for 24 h in the dark and product formation was analyzed by chiral gas chromatography according to [19].

The dry cell weight (dcw) was estimated photometrically at 730 nm (Genesis 20, Thermo Spectronic) and calculated using the correlation, $dcw = 0.2699 \times OD_{730}$.

2.2. Modelling media formulation with artificial neural networks

Two artificial neural networks (ANN) were used as models associating the concentrations of the various medium components to the biomass concentration after 72 h and to the conversion of PFAP after 24 h, respectively.

The election of a suitable architecture and the activation functions was based on explorative experiences carried out to assess the performance of the ANN to describe the test function. The architecture of both ANNs was fixed to three layers having 13 inputs. The number of nodes was varied initially from 1 to 15 for the first and hidden layers and afterwards fixed to 4 and 15, respectively (see Section 3.1). Each input component was normalized in such a way, that its corresponding concentration value entering the ANN lay between 0 and 1. Accordingly, the output signal was de-normalized to the real value range of

Table 1
Composition of modified BG11 medium and maximum variation of each component in media recipes

Numbers	Media component	Reference concentration (g/L)	Range of variation in media recipes (g/L)
1	$NaNO_3$	1.5	1.5×10^{-1} to 1.5×10^{2}
2	K_2HPO_4	3.1×10^{-2}	3.1×10^{-3} to 3.1×10^{-1}
3	$MgSO_4 \cdot 7H_2O$	7.5×10^{-2}	7.5×10^{-3} to 7.5×10^{-1}
4	$CaCl_2 \cdot 2H_2O$	3.7×10^{-2}	3.7×10^{-3} to 3.7×10^{-1}
5	Citric acid	6×10^{-3}	6×10^{-4} to 6×10^{-2}
6	Ferric ammonium citrate (18% Fe)	6×10^{-3}	6×10^{-4} to 6×10^{-2}
7	Na_2CO_3	4×10^{-2}	4×10^{-3} to 4×10^{-1}
Trace metals solution			
8	H_3BO_3	2.86×10^{-3}	2.86×10^{-4} to 2.86×10^{-2}
9	$MnCl_2 \cdot 4H_2O$	1.81×10^{-3}	1.81×10^{-4} to 1.81×10^{-2}
10	$ZnSO_4 \cdot 7H_2O$	2.22×10^{-4}	2.22×10^{-5} to 2.22×10^{-3}
11	$Na_2MoO_4 \cdot 2H_2O$	3.9×10^{-4}	3.9×10^{-5} to 3.9×10^{-3}
12	$CuSO_4 \cdot 5H_2O$	7.9×10^{-5}	7.9×10^{-6} to 7.9×10^{-4}
13	$Co(NO_3)_2 \cdot 6H_2O$	4.94×10^{-5}	4.94×10^{-6} to 4.94×10^{-4}