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Modelling and prediction of bioethanol production from intermediates and byproduct of sugar beet processing using neural networks

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ABSTRACT

The aim of this work was to model and predict the process of bioethanol production from intermediates and byproduct of sugar beet processing by applying artificial neural networks. Prediction of one substrate fermentation by neural networks had the same input variables (fermentation time and starting sugar content) and one output value (ethanol content, yeast cell number or sugar content). Results showed that a good prediction model could be obtained by networks with single hidden layer. The neural network configuration that gave the best prediction for raw or thin juice fermentation was one with 8 neurons in hidden layer for all observed outputs. On the other side, the optimal number of neurons in hidden layer was found to be 9 and 10 for thick juice and molasses, respectively. Further, all substrates data were merged, which led to introducing an additional input (substrate type) and defining all outputs optimal network architecture to 3-12-1. From the results the conclusion was that artificial neural networks are a good prediction tool for the selected network outputs. Also, these predictive capabilities allowed the application of the Garson's equation for estimating the contribution of selected process parameters on the defined outputs with satisfactory accuracy.

1. Introduction

Interest in upgrading bioethanol production has increased in the past decades both for ecological and economic reasons, primarily for its use as an alternative to fossil fuels [1]. Implementing the concept of coproducing ethanol represents an attractive option for sugar production facilities, as it provides flexibility in terms of varying produced quantities of sugar and ethanol, depending on the conditions prevailing on the market. Sugar factories are operating at full capacity only during the campaign of sugar beet. By introducing the concept of simultaneous sugar and ethanol production one plant could work at full capacity throughout the year, leading to better utilization of equipment and manpower. Furthermore, these industrial plants would have an advantage in terms of transport due to the fact that the feedstock (raw, thin and thick juice as intermediates and molasses as byproduct of sugar refining technologies) would be available on site [2]. The decision about which substrate to use for ethanol production would depend on the time of the year, i.e. whether there is an on-going annual campaign of sugar beet processing, when all fresh intermediate and byproducts are available, or there are only stored raw materials [3].

The economic analysis of producing ethanol in such a way showed that the process needs optimization, since it could take away its environmental benefits and could make this biofuel too expensive to produce and distribute on a large scale. The first step toward an optimal production would be modelling the process which is still a challenging problem. Fundamental approach, largely considered as the most rigorous, cannot be applied in fermentation kinetic modelling due to inherent non-linearity, lack of information, experimental inaccuracy, deviations from ideal conditions the fundamental approach, [4]. There is a wade range methods for modelling non-linear systems, such as multiple regression, artificial neural network (ANN), genetic algorithm, fuzzy logic and ant algorithm among others [5]. Because of their simplicity, ANN's are suitable for modelling the main variables (biomass, substrate and product concentrations) in alcohol fermentation processes as a function of fermentation time and initial substrate concentration.

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In many previous studies ANN's have shown to be a better predictive tool compared to traditionally applied statistical methods. However, they are considered as "black box" models because they provide little explanatory insight into relative influence of independent variables in the prediction process. For research purposes this lack of explanatory power is a major drawback, since it is necessary to understand the relationships between dynamic biochemical phenomena. For this reason, numerous methods (e.g. connection weights, Garson's algorithm, sensitivity analysis, etc.) have been used for interpreting the connection and contribution of variables in neural networks [6].

The aim of this study was to investigate the possibility of predicting the ethanol content, yeast cells number and residual sugar content by applying the artificial neural networks on data collected during batch fermentation process by free *Saccharomyces cerevisiae* cells on intermediates and by-products of sugar beet processing industry. Also, the Garson equation has been applied in order to understand and interpret the influence of variables on the fermentation process.

2. Materials and methods

2.1. Microorganisms and substrates

Commercial baker's yeast *S. cerevisiae* (Alltech, Senta, Serbia) has been chosen as the producing microorganism. The reason for this was its well predictable industrial performances such as superior ethanol tolerance and high ethanol yield [7]. The yeast was suspended in a small quantity of culture medium (1/10 of the total working volume), so as to give an initial cells number of 10^8 cells mL^{-1}, which is approximately 10 g fresh baker's yeast (30% dry matter) per 1000 mL of medium. This suspension was directly used for inoculation.

The feedstock (intermediates and by-products of sugar beet processing) was obtained from local sugar factory, located in Senta, Serbia, and stored at −18 °C until use. Since raw and thin juice have an initial sugar concentration of ≈130 g kg^{-1}, this concentration together with two other concentrations (50 and 100 g kg^{-1}), obtained by diluting them with distilled water, were used in the experiments. On the other hand, thick juice and molasses, which have a higher starting sugar concentration, were diluted to give 50, 100, 150, 200 and 250 g kg^{-1} of sugars. The upper limit of the initial sugar concentration was 250 g kg^{-1} because of the osmotic stress of the producing strain. Thus prepared substrates were adjusted to pH 5.0 with 10% sulphuric acid.

2.2. Fermentation

Fermentations were carried out in 2-L bench-scale bioreactors (BIOSTAT Aplus, Sartorius AG, Germany) with a working volume of 1.5 L. The bioreactors filled with the aforementioned substrates were sterilized by autoclaving them at 121 °C and overpressure of 1.2 bar during 30 min. Immediately after inoculation gaseous N_2 was purged into the bioreactors through the aeration system in order to reach anaerobic conditions, which were confirmed by pO_2 electrode. After attaining anaerobic conditions, the introduction of N_2 into the bioreactor was ceased. The fermentation was carried out in batch mode for 48 h at 30 °C and mixing speed of 200 rpm.

2.3. Analytical methods

Sampling of culture medium in order to perform its analysis was performed aseptically at the beginning of the process (immediately after inoculation) and at the predetermined time intervals: 4, 8, 12, 24, 30, 36 and 48 h from the moment of inoculation.

Biomass analysis was performed by counting the present yeast cells in a determined volume of fermentation medium using the Neubauer Haemocytometer under an optical microscope at × 400 magnifications (Wild M20, Heerbrugg, Gais, Switzerland).

The pH value was measured on-line with the pH electrode rinsed into the fermentation medium.

Taken samples of the substrates and cultivation media were centrifuged at 4000 rpm for 15 min. Sugar content, in term of the sum of glucose, fructose and sucrose, in the supernatant was determined by high pressure liquid chromatography (Jasco, Inc, Easton, MD, USA, pump PU-980, detector RI-930, sampler AS-950, 20 μL injection loop, column sugar KS-801, eluent: water at flow rate of 0.6 mL/min and elution time 30 min). The standards of glucose, fructose and sucrose were purchased from Supelco (Bellefonte, PA, USA). All of the chemicals used were of reagent grade or better.

Ethanol content in the distillates of fermented media was determined by gas chromatography (GC), using a HP 5890 Series II GC (Agilent Technologies Inc, Santa Clara, CA, USA) equipped with a flame ionization detector, a Carbowax 20 M column at 85 °C and helium as the carrier gas. Injector temperature was maintained at 250 °C, while the detector temperature was set to 300 °C. For constructing the ethanol calibration curve standard aqueous solutions of ethanol (1%, 3%, 5%, 8%, 10%, 15% and 20% (v/v)) in distilled water were made, and analysed by the GC.

2.4. Calculations

Data analysis, after cultivation, was performed using the software Matlab neuralnetwork toolbox, The Math Works Inc., Natick, MA. The ANN implemented in this study consisted of an input layer, one hidden layer with varied node number (1–12) and an output layer with one node. Standard training procedure was applied for network training: feedforward of the input training pattern, calculation and back propagation of the associated error, and the adjustment of the weights. The ANN output corresponding to the input values were then compared with the target values and the weights were adjusted to reduce the sum of squares of errors. The Levenberg–Marquardt algorithm was employed for training with an intermediate learning rate of 0.05.

3. Results and discussion

3.1. Data generation

The process of ethanol production by fermentation is favourable if it can produce high concentrations rapidly, while maintaining high yields. These conditions are necessary to minimize capital costs and energy required for distillation [3]. Another important factor in fermentation processes is substrate concentration. High substrate concentration inhibits cell growth and product formation and may distort the metabolism of the producing strain. When it comes to ethanol fermentation, previous studies have shown that substrate inhibition becomes significant somewhere in the range of 5–25% of sugar with complete inhibition of growth at 40% of glucose [8,9].

In accordance with the above mentioned data, fermentation time and substrate concentration were defined as input variables for the set of experiments conducted with each substrate separately. After the analysis, the obtained data for all substrates were merged and substrate type was defined as additional input. Since the complexity of the model depends on the number of applied process variables, it is important to define whether it is possible to use one artificial neural network for predicting the ethanol content, yeast cells number or residual sugar content during fermentation