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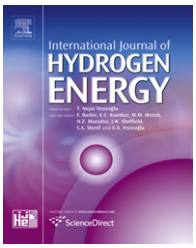

Estimation of hydrogen production in genetically modified *E. coli* fermentations using an artificial neural network

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ABSTRACT

Biological hydrogen production is an active research area due to the importance of this gas as an energy carrier and the advantages of using biological systems to produce it. A cheap and practical on-line hydrogen determination is desired in those processes. In this study, an artificial neural network (ANN) was developed to estimate the hydrogen production in fermentative processes. A back propagation neural network (BPNN) of one hidden layer with 12 nodes was selected. The BPNN training was done using the conjugated gradient algorithm and on-line measurements of dissolved CO_2, pH and oxidation-reduction potential during the fermentations of cheese whey by *Escherichia coli* Δ*hycA* Δ*lacI* (WDHL) strain with or without pH control. The correlation coefficient between the hydrogen production determined by gas chromatography and the hydrogen production estimated by the BPNN was 0.955. Results showed that the BPNN successfully estimated the hydrogen production using only on-line parameters in genetically modified *E. coli* fermentations either with or without pH control. This approach could be used for other hydrogen production systems.

1. Introduction

Hydrogen is considered as a good choice as future energy carrier since it has the highest energy content per weight unit and its use either via combustion or fuel cells results in pure water [1]. Among the hydrogen production processes, the biological production is an attractive method because it is carried out at ambient pressure and temperature, therefore consumes less energy than chemical or electrochemical processes [2]. The fermentative hydrogen production is a promising method since it has the higher production rate; it does not need light and utilizes a wide range of carbon sources [2–5]. In the dark fermentation, several microorganisms can use carbohydrate rich substrates. From the enterobacteria, *Escherichia coli* is the main microorganism used for studies of hydrogen production, since its genetic and metabolism are well documented [6–12]. Under anaerobic conditions and in absence of external electron acceptors *E. coli* converts sugars to pyruvate that may be converted to lactate or broken into formate and acetyl-coenzyme A (acetyl-CoA), which is converted to acetate or ethanol, whereas formate is metabolized to hydrogen and CO_2 (Fig. 1).

The on-line hydrogen determination is strongly desired to establish feedback or feed forward control algorithms.

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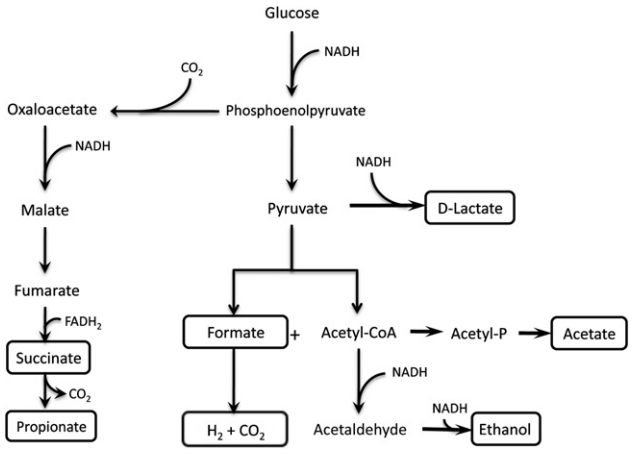

Fig. 1 – Schematic representation of the fermentative pathways in *Escherichia coli*. Final products are framed.

However, the most common method to determine hydrogen is by gas chromatography (GC) off-line [13–19]. This method is very useful, accurate and sensitive to determine hydrogen, but requires equipment and specific installations. Another method used is the gas displacement using a solution of NaOH, however the solution could be saturated and confirmation by GC is still needed [20–24]. Massanet-Nicolau *et al.* [25] measured the composition of the gas produced by the fermentation of sewage biosolids with hydrogen, CO_2 and CH_4 sensors. Ferchichi *et al.* [26] used a solution of 30% of KOH to remove CO_2, and the residual gas was channeled into a bubble counter for the measurement of hydrogen and it was confirmed by a specific hydrogen sensor. The counter was linked to a computer and the on-line hydrogen production was recorded.

Until now, there are few parameters for on-line monitoring in bioreactors, the most frequents are temperature, pH, oxidation-reduction potential, dissolved oxygen and dissolved CO_2. Therefore, a useful approach is the use of mathematical models with these on-line determinations for the estimation of the fermentative products. For this purpose, the artificial neural networks (ANNs) have been successfully used, since they are based on the connectivity of biological neurons that have an incredible capability for emulation, analysis, prediction, association and adaptation [6,27]. For instance, Poirazi *et al.* [28] used pH, temperature and NaCl concentration to predict the maximum specific growth rate and bacteriocin production using feed forward ANNs in *Streptococcus macedonicus* ACA-DC 198 cultures. Chen *et al.* [27] used the dissolved oxygen, feed rate and liquid volume to determine the biomass concentration in *Saccharomyces cerevisiae* cultures using a recurrent neural network. Escalante-Minakata *et al.* [29] used the oxidation-reduction potential and a back propagation neural network to estimate the ethanol and biomass production in non-axenic cultures.

The aim of this work is to develop an ANN to estimate the hydrogen production in genetically modified *E. coli* fermentations based on the on-line measurements of the oxidation–reduction potential, pH, and dissolved CO_2.

2. Materials and methods

2.1. Strain and culture media

E. coli Δ*hycA* Δ*lacI* (WDHL) a hydrogen overproducing strain was used in this study. A complete description of this strain can be found elsewhere [14]. For hydrogen production, inocula were grown overnight in Luria Bertani (LB) medium at 37 °C and shaken at 200 rpm, afterwards added to fresh LB medium and cultured in closed twist cover bottles at 37 °C for 48 h. Fermentations were done in hydrogen production (HP) medium described elsewhere [14]. HP medium was pasteurized at 65 °C during 25 min and chilled 20 min on ice. Cheese whey powder (Land O'Lakes, Arden Hills, Minnesota) at 20 g L^{-1} was used as carbon source.

2.2. Batch cultures in bioreactor

Pre-inocula was harvested, washed once and inoculated into 1 L bioreactor (Applikon, Foster City, CA) equipped with two six-blade Rushton turbines. Oxidation-reduction potential (ORP), pH and dissolved CO_2 (DCO_2) were monitored using autoclavable electrodes (Applikon) connected to Bioconsole ADI 1035/Biocontroller ADI 1030 (Applikon). The ORP and DCO_2 electrodes were calibrated according to the manufacturers at 215 mV using the reference solution HI7020 (Hanna Instruments, Armazem, Portugal) and using 100% of CO_2 gas saturation at atmospheric pressure, respectively. BioXpert 1.3 software (Applikon) for data acquisition was used. The cultures were performed at 37 °C and stirred at 175 rpm. Culture samples were periodically taken from the bioreactor, and centrifuged at 11,500*g* for 5 min. The supernatants were filtered through a 0.22 μm filter (Millipore) before the analysis of fermentation products.

2.3. Analytical methods

The gas was measured by water displacement in an inverted burette connected to the bioreactor with rubber tubing and a needle. The hydrogen content in the gas phase, was determined in a Gas Chromatograph 6890 N (Agilent Technologies, Wilmington, DE) as described elsewhere [30]. Ethanol was measured by GC as described by De Leon-Rodriguez *et al* [31]. Organic acids and carbohydrates were analyzed by isocratic liquid chromatography using a Waters 600 HPLC system and UV-Vis 2487 detector (Waters) at wavelenght-190 nm. Samples of 20 μL were separated on a Rezex ROA H^+ column (300 mm × 7.8 mm, 8 μm) from Phenomenex (Torrance, CA) at 60 °C and using 0.005N H_2S0_4 at 0.6 mL/min as mobile phase.

2.4. Structure of ANN

To predict the hydrogen production through the on-line measurements of pH, dissolved CO_2 and ORP, a back propagation neural network (BPNN) was chosen. The model was structured as follows:

$$H_2 = F(pH,\ DCO_2,\ ORP;\ W)$$

where ORP is the oxidation-reduction potential in mV, DCO_2 is the % of dissolved CO_2, pH is the H^+ potential and W is the vector of adjustable parameters of the network or weight. The