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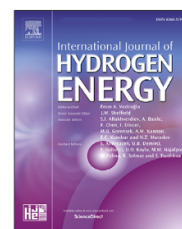
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Development of short chain fatty acid-based artificial neuron network tools applied to biohydrogen production



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HIGHLIGHTS

- Models could predict H₂ production, HPR and H₂ yield with high accuracy ($R^2 > 0.98$).
- Their application in the management of bioH₂ production systems is discussed.
- Mixed fermentations should consider using acetate and butyrate as control parameter.
- Total VFA is recommended to be used in processes using pure cultures.

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ABSTRACT

The biological production of biohydrogen through dark fermentation is a very complex system where the use of an artificial neuron network (ANN) for prediction, controlling and monitoring has a great potential. In this study three ANN models based on volatile fatty acids (VFA) production and speciation were evaluated for their capacity to predict (i) accumulated H₂ production, (ii) hydrogen production rate and (iii) H₂ yield. Lab-scale biohydrogen and VFA production kinetics from a previous study were used for training and validation of the models. The input parameters studied were: time and acetate and butyrate concentrations (model 1), time and lactate, acetate, propionate and butyrate concentrations (model 2), time and the sum of all VFA (model 3) and time and butyrate/acetate (model 4). All models could predict biohydrogen accumulated production, hydrogen production rate and H₂ yield with high accuracy ($R^2 > 0.987$). VFA_T is the input parameter indicated for processes using pure cultures, while for complex/mixed cultures a model based on acetate and butyrate is recommended.

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Introduction

Artificial Neuron Network (ANN) is an artificial intelligence tool supported mainly in algorithmic implementations that uses non-physical systems “computer programs” [1]. The ANN is characterized as a mathematical presentation that mimics the learning process of the human brain using artificial neurons [2], and have been successfully used as a problem resolution algorithm in regression, modeling [3], multivariate data analysis [4], optimization, process control [5], classification, clustering [6] and others.

In recent years, ANN gained importance in bioprocessing for being more reliable than the classical statistical models (such as the response surface methodology) [7,8] and especially due to the non-linearity observed in fermentative and controlling process. In fact, according to Mikulandric [1] artificial intelligence systems are widely accepted as modelling systems that satisfy non-linear processes and are mainly useful in complex systems such as dark fermentation. Because these systems include core functions of different degrees of non-linearity and flexibility in the model, they are also noise-tolerant and even value-constrained because they are derived from advanced statistics [9]. The main advantages of ANN are that it can be approximated to any type of non-linear function and does not require prior specification of the adjustment function, while the designs of response surfaces are limited to square order approximations only [2]. Whiteman & Gueguim [3] showed that ANN more accurately simulates dark fermentation than response surface method. The first showed an imprecision of 15% while the second reached 119%.

The foundations of the neural networks are the artificial neurons or processing elements. The neurons of the first layer have only the function of distributing the input signals for the next layers. After the input layer, the signals flow through the hidden layers (S_i) until the output layer. Considering a neuron “i” of the layer “k”, it receives information from the previous layer, pondered by the weight of the connections. The neuron generates a summation of these pondered inputs and the resulting value is added to an internal activation limit or bias, $W_{i,0}^{(k)}$. The resulting signal of the neuron “i” produces a response $X_i^{(k)}$ in agreement with the activation function or transfer function $f(\bullet)$. Mathematically it can be expressed by equations (1) and (2).

$$S_i^{(k)} = W_{i,0}^{(k)} + \sum_{j=1}^{(n_{k-1})} w_{ij}^{(k)} X_j^{(k-1)} \quad (1)$$

$$X_i^{(k)} = f(S_i^{(k)}) \quad (2)$$

Multilayer Perceptron (MLP) is a neural network structure composed of one input layer, one or more hidden layers of sigmoidal hidden neurons and a layer of linear output neurons. One hidden layer architecture is the most used structure because it possesses nonlinear processing capabilities and universal approximation property [10,11]. Previous research has revealed optimal hidden neurons should be below 20 due to overfitting and loss of performance [12]. Few random input and output values are then used to train the model through a

standard backpropagation learning algorithm [13] based on a gradient descent method. During this “training” process, the correlations between input and output values are defined in form of a mathematical model. ANN can, thus, be defined as mathematical models capable of learning and adapting, recognize, classify and organize data through fast time-variable information processing.

The biological production of biohydrogen through dark fermentation is a very complex system where the use of an artificial neuron network (ANN) for prediction, controlling and monitoring has a great potential. Fermentative hydrogen processes and technologies are being widely developed in laboratory scale. Scaling up, however, is being a difficult task and requires extensive knowledge at the lab scale level [14]. The technology faces economic viability issues and process modeling is considered one critical requirement for improving our ability to predict the biohydrogen yield [15]. In this context, even little gains may be significant and process optimization/modeling should consider those parameters that cause low effect on the response variable (those generally rejected in traditional statistical optimization tools). To date, the use of ANN in biohydrogen studies mainly focused on the prediction of biohydrogen production based on process conditions such as pH, temperature, substrate concentration, inoculum age [3,7,15–17]. This is different from the Modified Gompertz Model (which uses lag time, H_2 production potential and H_2 production rate to calculate the cumulative hydrogen production and that cannot be used as a predictive model) and especially important because ANN allows to discern synergistic effects of multiple variables ($n > 3$) [18].

Biohydrogen production through dark fermentation occurs concomitantly with the formation of volatile fatty acids (VFA) in the liquid phase, which speciation is closely related to biohydrogen yield. Among the wide range of by-products from diverse anaerobic microbial metabolism, the two pathways producing hydrogen from carbohydrates are associated with acetate and butyrate, resulting in 4 and 2 mols of H_2 per mol of glucose, respectively. The presence of more reduced metabolites (propionic and lactic acids and ethanol, for example) results in lower H_2 production and they are, thus, undesirable. Reduced products can be avoided by controlling process conditions, such as substrate concentration, hydraulic retention time (HRT), H_2 partial pressure, and micronutrients, among others.

One important step in scaling up biohydrogen process is process monitoring and production prediction. Monitoring is generally based on online measurements such as biogas production (gas meter) and offline measurements such as biogas characterization (gas chromatography), substrate consumption (colorimetric methods) and VFAs production (HPLC or gas chromatography) [19]. The prediction of production is generally based on process conditions that should be maintained constant. However, it is not uncommon to have failures in process control (especially in large volumes systems) that causes some kind of turbulence. The time period between the identification of the turbulence and the resolution of the problem can significantly (or definitely) affect the bioprocess. In these cases the prediction of the expected volume of biohydrogen will no longer follow the previously method based on the operating conditions. There must therefore exist a way

of performing this prediction, which will also help in decision-taking on the continuation or interruption of the process. All these become more complex when microbial consortia or mixed cultures are used instead of pure cultures.

The fermentative hydrogen production process has a specific relation between the VFAs and the biohydrogen produced that can be used as a parameter for process monitoring and biohydrogen prediction. In this work we have used process data from our previous work [20] where biohydrogen and VFAs were produced by an identified microbial consortium [21] in a medium composed of sugarcane vinasse to develop an ANN model that correlates biohydrogen production to the profile of VFAs generated with possible application as a prediction tool in process monitoring.

Material and methods

Dark fermentation process

The anaerobic fermentation process for the production of bioH₂ from sugarcane vinasse was described in a previous work [20]. Shortly, pure vinasse was supplemented with sugarcane juice to reach a final concentration of 12 g/L of carbohydrates and the initial pH was 7.0. Dark fermentation was carried in 2L bioreactors with working volume of 1.5L in batch mode at 37 °C for 5 days. The procedure for promoting an anaerobic culture was based on the Balch technique [22]. The removal of oxygen was achieved by boiling the medium under an anoxic ambient (CO₂ atmosphere). Bicarbonate was added at the temperature of 85 °C and cysteine–HCl at 65 °C as reducing agents to lower the redox potential of medium. The inoculum was a microbial consortium originated from a sample of fruit bat feces.

Samples were withdrawn regularly (10 samples/day), centrifuged (10⁴ xg), filtered (Milipore 0.2 μm) and analyzed for VFAs. Shimadzu Liquid Chromatograph was equipped with an Aminex® HPX-87H 300 × 7,8 mm (Bio-Rad) column and a refractive index detector (RID-10A). The column was kept at 60 °C and a 5 mM H₂SO₄ at 0.6 ml/min was used as the mobile phase. Gas was measured by the water displacement technique. Gas analysis was carried in a Thermo Gas Chromatographer equipped with Petrocol DH150 (50 m × 0.25 mm), DC 200 (1.8 m) and Porapak-N (2.0 m × 1/8"), which were placed in bypass series flow path of gas chromatograph system. The columns were connected to a TCD detector (block temperature: 120 °C, transducer temperature: 120 °C, filament temperature: 190 °C). This system allowed the measurement of oxygen (O₂), nitrogen (N₂), carbon dioxide (CO₂) and methane (CH₄). Hydrogen (H₂) content was then considered as the amount to reach 100%.

ANN model development

In this study, multi-layer perceptron (MLP) architecture of ANNs with one hidden layer of neurons was used with back-propagation (BP) training algorithm implemented in FORTRAN. Models were developed by varying the amount of neurons in the intermediate layer (3–5) and by using 70% of the available data for model training and the remaining 30%

for validation. The number of neurons in the intermediary layer was determined considering the model accuracy.

All models used fermentation time (days) as input parameter. Models differed from the input VFA data. ANN model 1 used acetate and butyrate production (g/L); ANN model 2, the concentration of all the VFA produced fermentation (lactate, acetate, propionate and butyrate); ANN model 3, the sum of VFAs (VFA_T); and ANN model 4, the Butyrate/Acetate ratio. The output parameters evaluated were (i) accumulated biohydrogen production (L_{H_2}/L_{medium}), (ii) Hydrogen production rate (HPR) ($L_{H_2}/L_{medium}/h$) and (iii) H₂ yield. For each model different architectures were tested and the number of intermediary neuron in the best found architecture is presented in Fig. 1.

The range of acetate, butyrate, lactate and propionate used were 0.000–1.331, 0.000 to 4.616, –0.268 to 1.945 and –0.960 to 0.184 g/L, respectively (negative values means that the VFA was consumed during the fermentation, in comparison to the non-fermented medium).

Results and discussion

Very good agreement in the trends between forecasted and validation data was observed for models 1, 2 and 3, where the determination coefficient (R²) was always higher than 0.987 (Fig. 2). This means that biohydrogen accumulated production, HPR and H₂ yield could be mathematically predicted from the produced acetate and butyrate (model 1), VFA_T (model 3) and from the concentration of each VFA produced (model 2). The weights between the input layer and the hidden layer and the hidden layer and the output layer presented in Table 1 represent the strength of the connection between units.

The correlation coefficient (R²) observed in this study is higher than most of the published studies that used ANN as a prediction tool for biohydrogen processes (Table 2). This reinforces the utility of such tool in controlling and monitoring systems. The accuracy of the ANN models proposed can be explained by process uniformity and the close stoichiometric relation between VFAs and biohydrogen production and yield. Although the existence of these correlations is known from a theoretic perspective, the practical mathematical correlation between them had not been explored until then. In a lab - or industrial - scale process where operational parameters are controlled, process turbulences can cause metabolic shift, alteration in the microbial population or process contamination in a way that prediction systems based on operational conditions will lose their consistency. This is especially important in mixed cultures or consortium-based processes where a stable anaerobic microbial community is critical for hydrogen bioproduction [23].

Considering the different parameters used as input values for models 1, 2, 3 and 4, VFA_T (model 2) is the most simple, fast and cost-effective strategy in daily routine analysis. However, the choice of the input parameters in developing ANN models for biohydrogen prediction is a specific case and should be chosen carefully. VFA_T is widely quantified by titration [28] in biogas plants, while VFA speciation can only be identified and quantified through chromatographic methods (Gas or high-pressure liquid chromatography). The titration method is

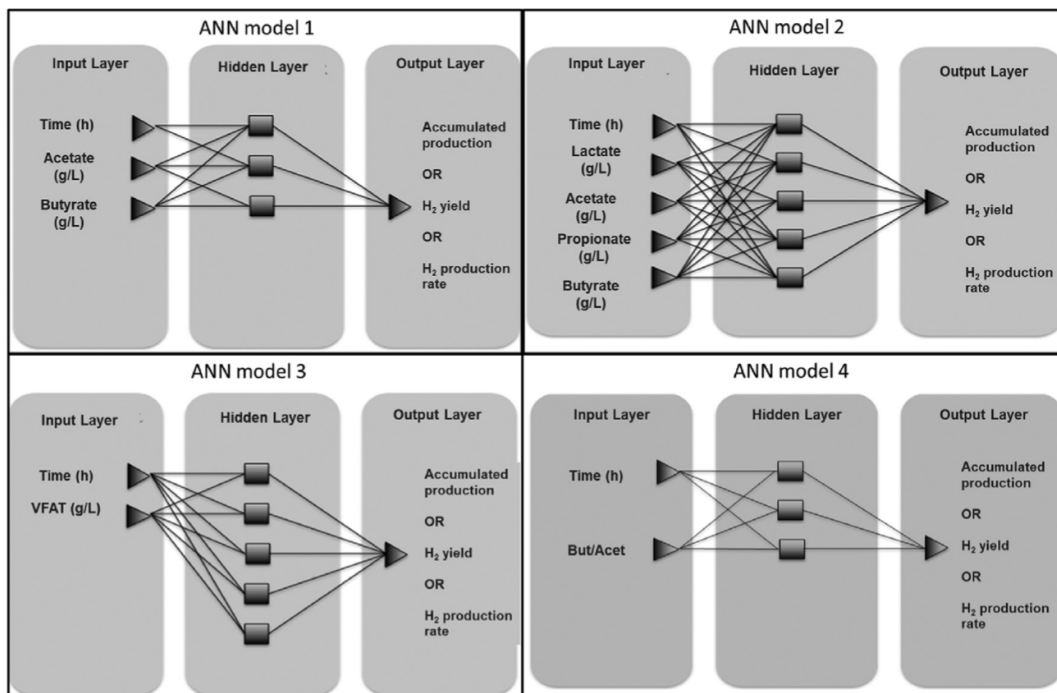


Fig. 1 – Architecture of each ANN model proposed for the prediction of accumulated biohydrogen production. The input and output parameters and the number of neurons in the hidden layer are presented.

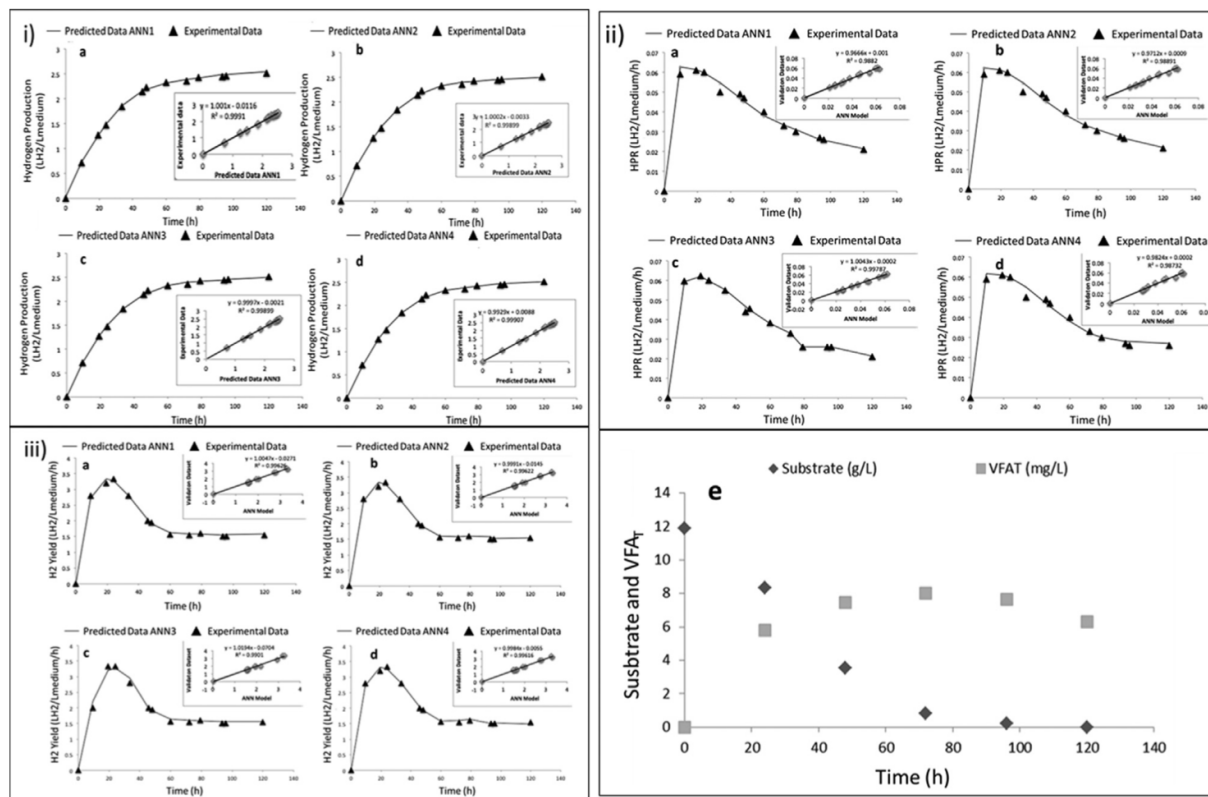


Fig. 2 – ANN models and validation dataset for (i) biohydrogen production, (ii) hydrogen production rate and (iii) H₂ yield from (a) acetate and butyrate concentrations (model 1), (b) every individual VFAT produced (model 2), (c) VFAT_T (model 3) and acetate/butyrate (model 4). The kinetics of substrate consumption and VFAT_T production is also presented (e).

Table 1 – Connections' weights between neurons (j) of the ANN models according to equation 1.

Accumulated Hydrogen Production				Hydrogen Production Rate				H ₂ Yield			
Model 1											
W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k
j = 1	0.44	1.43	3.87	0.43	1.65	0.96	0.39	0.16	5.77	6.21	0.61
j = 2	1.97	*	-0.35	0.31	-1.14	-0.36	0.91	-0.1	1.65	-0.34	0.35
j = 3	-0.06	*	1.34	3.17	-0.63	1.18	-0.34	0.35	2.32	-2.54	1.35
j = 4	-0.24	*	-0.8	-0.57	0.75	-2.54	1.35	3.2	2.58	-2.54	1.35
j = 5	-0.82	*	-1.59	1.85	3.94	1.35	-0.76	-6.5	1.66	3.94	1.35
Model 2											
W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k
j = 1	0.44	1.43	3.87	0.43	1.65	0.96	0.39	0.16	5.77	6.21	0.61
j = 2	1.97	*	-0.35	0.31	-1.14	-0.36	0.91	-0.1	1.65	-0.34	0.35
j = 3	-0.06	*	1.34	3.17	-0.63	1.18	-0.34	0.35	2.32	-2.54	1.35
j = 4	-0.24	*	-0.8	-0.57	0.75	-2.54	1.35	3.2	2.58	-2.54	1.35
j = 5	-0.82	*	-1.59	1.85	3.94	1.35	-0.76	-6.5	1.66	3.94	1.35
Model 3											
W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k
j = 1	0.3667	42.073	-0.0765	0.3534	18.369	j = 2	1.62	*	0.77	5.24	21.217
j = 2	40.693	*	80.580	0.2383	24.208	j = 3	15.72	*	0.18	-8.94	37.603
j = 3	-19.908	*	0.5142	68.471	19.906	j = 2	1.81	*	2.38	0.81	19.906
j = 4	-0.0025	*	-10.947	0.6912	2.23	j = 3	15.73	*	2.23	-7.21	4.86
j = 5	66.270	*	102.366	-99.139	2.63						3.94
Model 4											
W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k	W_{0j}^{k+1}	W_{0j}^k
j = 1	-0.47	0.009	9.36	4.69	4.86	j = 1	-1.86	6.87	-1.35	-9.93	37.603
j = 2	11.04	*	1.11	0.74	3.94	j = 2	1.81	*	2.38	0.81	19.906
j = 3	2.19	*	-1.87	-9.10	2.63	j = 3	15.73	*	2.23	-7.21	4.86

* There is no associated value.

Table 2 – Comparative modeling studies using ANN for biohydrogen prediction with focus on the correlation coefficient (R^2).

Input parameters	Output parameter(s)	Topology	R^2	Reference
Recycle ratio, HRT, sucrose, biomass, pH, alkalinity, ORP, ethanol, acetate, butyrate, propionate	Hydrogen production rate	12-20-1	0.906	[24]
Time, COD, pH, VFA_T	Hydrogen production rate	4-20-1	0.870	[25]
Inoculum type, temperature, pH, substrate types, substrate concentration	Hydrogen production rate	5-7-7-1	0.900	[26]
ORP, pH, dissolved CO_2	Hydrogen production rate	–	0.96	[27]
pH, substrate concentration, inoculum, temperature, time	Hydrogen production rate	5-6-4-1	0.98	[15]
HRT, immobilized cell volume, temperature	Biohydrogen yield	3-4-4-2	0.99	[8]
Time, iron, vitamin, molybdenum, light intensity, pH	Biohydrogen production rate	6-9-1	0.939	[12]
Time, acetate, butyrate	Biohydrogen production rate	3-3-1	0.999	This study
Time, acetate, butyrate, propionate, lactate	Biohydrogen production rate	5-5-1	0.999	This study
Time, VFA_T	Biohydrogen production rate	2-5-1	0.998	This study

less precise than chromatographic ones because interference can be caused by some possible components of the medium (ammonia, sulfide, phosphate, bicarbonate) [29].

The use of VFA_T as input parameter in ANN models for biohydrogen prediction is indicated to pure culture where the metabolic pathway involved is well determined and there is little VFA profile variation (process turbulence may not affect definitely VFA speciation). When this is not the case, Butyrate/Acetate (B/A) ratio, VFA speciation and production should be considered. Among the VFAs produced during fermentation, acetate and butyrate pathway results in the production of H_2 , while propionate and lactate do not. For this reason, the similarity between model 2 and 3 were expected. A prediction/controlling/monitoring model based on acetate and butyrate concentrations in the fermentation liquid phase is then more suitable due to its lower complexity and cost of analysis. The B/A ratio is another parameter often used as indicator of biohydrogen production but controversial results are described in the literature [31]; it must be considered that some homoacetogens can produce acetate from H_2 and CO_2 , lowering H_2 yield. Moreover, B/A ratio should not be considered by itself because in the case of a proportional reduction in acetate and butyrate and increase of more reduced VFAs, B/A is maintained constant but H_2 release is significantly decreased.

In a hypothetical scenario where a temperature increase/pH drop/redox imbalance, variation in the feed rate (in the case of continuous fermentation of complex substrate, hydraulic retention time adjustments) occurs and is controlled, a definitive metabolic shift (for example due to changes in the microbial community) will affect VFA production and speciation and alter H_2 production. At this moment, maintaining operational process in the original conditions does not guarantee process correction. If the correlation between VFAs and biohydrogen production is known, one can predict how the turbulence will modify the expected production and decide whether it is worth keeping it running (or if it is worth trying to recover it). Having an adaptive and non-linear control system is, thus, an interesting option for biological process control and can be used as a predictive controller and monitoring parameter for management and operation of biohydrogen production systems.

The perception of the need to valorize the VFAs and the development of technologies for their application in

integrated industrial systems will likely make VFA analyzes routine and the application of VFA-based ANN models for process control and monitoring will be strategic.

Conclusion

The proposed ANN models based on VFA speciation and quantity could predict with high accuracy the accumulated biohydrogen production, HPR and H_2 yield. Although the data presented in this work indicate that it is possible to develop an ANN biohydrogen prediction tool based on VFA production and profile, especially applied after the occurrence of turbulences to the bioprocess, future studies evaluating the adaptability of ANNs to process turbulences are needed to pave the way for its use in realistic scale. The choice of input parameters is crucial for the construction of the model and must consider the metabolic pathways followed by the microorganism(s) and VFAs speciation. VFA_T is the input parameter indicated for processes using pure cultures, while for complex/mixed cultures a model based on acetate and butyrate is recommended.

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