Exploitation of experimental design methods and mathematical modeling for improving fermentative biohydrogen production processes

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Iulian Zoltan Boboescu, Vasile Daniel Gherman, Mariana Ilie, Ion Mirel, Teodor Vintilă, and Gergely Maróti

1 “Politehnica” University of Timisoara, Hydrotechnical Engineering Dept., Timisoara, Romania
e-mail: boboescu.iulian@yahoo.com (IZB), vasile.gherman@hidro.upt.ro (VDG), mariana.ile@mec.upt.ro (MI), ionmirel@bell.net (IM), maroti.gergely@brc.mta.hu (GM)
2 Banat University of Agronomical Sciences and Veterinary Medicine, Timișoara, Romania
e-mail: tvintila@animalsci-tm.ro
3 Hungarian Academy of Sciences, Biological Research Centre, Szeged, Hungary
e-mail: maroti.gergely@brc.mta.hu (GM)
*Corresponding author: phone: 003636308270455, address: Temesvari krt. 62., Szeged, 6726, Hungary

Abstract

Considering the non-renewable nature of today's energy sources, alternative solutions need to be introduced to successfully fulfill the world's energy demands in the future. Biohydrogen production processes coupled to the treatment of different organic wastes might satisfy the requirements of a renewable and environmentally friendly energy carrier. A major drawback of this bioprocess is the low hydrogen production yield, thus, the optimization of the fermentation conditions is imperative for achieving a hydrogen-based economy. The most widely used optimization strategies refer to the design of experimental methods, by which certain factors are selected and deliberately varied in order to obtain the desired effects. In addition, the optimization process can be further improved through mathematical modeling and simulations. Some kinetic models have been proposed to describe the progress of substrate degradation and microbial growth coupled with hydrogen production and some soluble metabolite formation in a batch fermentation-based hydrogen production process. This review attempts to summarize the experimental design methods as well as the kinetic models and simulations that were used to investigate the effects of various factors on fermentative hydrogen production processes and to discuss the advantages and limitations of these optimization approaches.

Keywords: biohydrogen production, dark fermentation, process optimization, design of experiments, mathematical modeling.

1. Introduction

Today's energy demands are permanently growing while the reserves of our primary energy-carriers will be depleted within a few decades, thus, novel and safe energy carriers have to be introduced (D.B. LEVIN & al. [1]). Among the potential new carriers, hydrogen satisfies all the requirements for a clean, alternative fuel (I. MIREL [2]). Among various hydrogen production processes currently used, the biological ways are known to be the least energy intensive. Furthermore, some of these processes, like dark fermentation, can utilize
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various organic wastes as substrate for fermentative hydrogen production. Thus, H₂ production by using wastewater as fermentative substrate along with simultaneous wastewater treatment might be an effective way of tapping clean energy from renewable sources in a sustainable approach. However, none of the described concepts led to a proven and long-term functional, industrial scale bio-hydrogen production system (D. GHOSH & al. [3]).

One of the major impediments of biohydrogen production processes for commercialization is the low hydrogen production yield. Biological production rate of hydrogen and its molar yield - like most other bioprocesses - are dependent on several parameters including the rate of hydrogen-producing and consuming bacteria, substrates, inorganic nutrients, operational conditions of the bioreactors, etc (S. RAY & al. [4]). Thus, the optimization of fermentation conditions, particularly nutritional and environmental parameters are of primary importance for bioprocess development. The most widely used optimization strategy refers to the design of experiment (DOE) method, by which certain factors are selected and deliberately varied in a controlled manner. This approach is often combined with the analysis of the experimental results. Thus, the DOE method is able to predict the analyzed parameters together with the different interactions between them (J. WANG & al. [5]). Thereby, the use of experimental design method for process optimization is of great importance in fermentative hydrogen production processes due to the dynamic and complex nature of these systems. Considering this, appropriate experimental design approaches can be extremely useful to study the effects of various factors on the process to make it better comprehended and even optimized to improve its performance (S.V. MOHAN & al. [6]).

Mathematical modeling and simulations have been carried out in attempts to further optimize biohydrogen production. Process models and simulations could be used to determine the optimal values of the important relevant parameters. A variety of models and simulations have been developed that are broadly applicable to a spectrum of diverse fields (J. WANG & al. [7]). Recently, emphasis on using these methods for biohydrogen production process optimization have been made (P.C. HALLENBECK & al. [8]). Some kinetic models have been proposed to describe the progress of substrate degradation, hydrogen producing microbial growth, hydrogen production and some soluble metabolite formation in a batch fermentative hydrogen production process (J. WANG & al. [7]; K. NATH & al. [9]). The insights revealed by these models can provide useful information for the analysis, design and optimization of the fermentative hydrogen production process.

This review summarize the experimental design methods as well as the kinetic models and simulations that were used to investigate the effects of various factors on fermentative hydrogen production processes. The reviewed experimental design included one-factor-at-a-time design, full factorial design, Taguchi design, Plackett–Burman design, central composite design and Box–Behnken design, as well as neural networks and genetic algorithm mathematical representations. The discussed kinetic models describe the general progress of fermentative hydrogen production process, the relationship between microbial growth and substrate degradation rate, as well as different fermentation product formation and process inhibition.

2. Experimental design methods

In order to achieve the required insight and optimization of the fermentative hydrogen production process, a design of experiment (DOE) statistical approach was used in the last years. Through this approach, a certain number of predefined factors are deliberately varied in a controlled manner in order to observe their effects on the output of a process (S.V. MOHAN
An optimization using this approach implies three major steps: i) a screening step, where the important factors that have a significant influence on the output are determined using fractional or full factorial designs, depending on the number of factors to be investigated; ii) an improvement step, where the optimum conditions are searched by repeated change of factor settings using Box or steepest ascent approach; iii) the determination of optimum conditions, where the optimal settings of the factor levels are determined using response surface designs or other approaches.

Principal component analysis and Genetic algorithm based on a neural network model approaches have also been used to some extent in order to optimize the process parameters in fermentative hydrogen production. According to the number of the factors to be investigated at a time, the experimental design can be broadly classified into two categories: one-factor-at-a-time design (single-factor design) and factorial design (multiple-factor design) (J. WANG & al. [5]).

2.1. One-factor-at-a-time design

One-factor-at-a-time design is a straightforward design which investigates just one factor at a time, while keeping the levels of the other factors constant, thus studying all the effects of these factors on a response. Since this method is easy to operate and analyze, it has been widely used to study the effects of various factors on fermentative hydrogen production processes (D. GHOSH & al. [10]; C. HAMILTON & al. [11]). However, one-factor-at-a-time design has the major drawback of ignoring the interactions among different factors. Thus the identified optimal conditions cannot be guaranteed. In addition, this method requires a relatively large number of experiments, which makes it laborious and time-consuming to carry out the experiments when the number of investigated factors is large.

2.2. Factorial design

As opposed to the one-factor-at-a-time design, the factorial design is able to assess the effects of more than one factor at two or more levels, which enables one to depict the interactions among different factors. The analysis and the model-fitting for a factorial design can be performed based on either the coded factor levels or the actual factor levels. However, in almost all situations, the coded factor level analysis is preferable, because in a coded factor level analysis, the model coefficients are dimensionless and thus directly comparable, which make it very effective to determine the relative size of factor effects (D.C. MONTGOMERY [12]). Factorial design can be classified into two categories: full factorial design and fractional factorial design.

2.2.1. Full factorial design

In a full factorial design, every combination of each factor level is tested. An appropriate polynomial model can be used to describe the effects of the factors studied on a response and then optimize the response when necessary (M. KENNEDY & al. [13]). Since all possible combinations of the factor levels can be investigated using a full factorial design, it has been used extensively to study the effects of several factors simultaneously on fermentative hydrogen production processes (Y. KALOGO & al. [14]; F.M. ESPINOZA-ESCALANTE & al. [15]). The main disadvantage of using this design is that the number of runs for a full factorial design increases exponentially with the number of investigated factors. Generally, this results in time-consuming experimental runs which are economically and practically not-feasible.

2.2.2. Fractional factorial design

Fractional factorial design provides an alternative when the number of runs for a full factorial design is too large to be applied. This can be achieved because the desired information can often be obtained by performing only a fraction of the full factorial design (J.T. LUFTIG & al. [16]). With a fractional factorial design, the effects of certain factors on a response as well as the interactions among those factors can be studied under economic and
practical conditions. However, the identified solutions may not be guaranteed to be optimal always due to the poor modeling ability of the second-order polynomial model. Plackett–Burman design, Taguchi design, central composite design and Box–Behnken design, coupled with response surface methodology, are the most common used fractional factorial design approaches for fermentative hydrogen production processes.

2.2.2.1. Plackett–Burman design
The first step towards optimizing the hydrogen production process is the identification of factors with significant effects on the process. Plackett–Burman design, which is a two-level fractional factorial design developed by Plackett and Burman, can be successfully used to screen the most significant factors influencing a process, prior to the actual optimization step (M. KENNEDY & al. [13]). Since the main effects have a complicated confounding relationship with two-factor interactions, these designs are suited to study the main effects of influencing factors when it can be assumed that the two-way interactions are negligible. A first-order polynomial model (Eq. (1)) is usually used to describe the effects of various factors on it based on the experimental results from a Plackett–Burman design.

\[ y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i \]  

where \( y \) is the response, \( \beta_0 \) is the constant and \( \beta_i \) is the linear coefficient, and \( X_i \) is the coded factor levels. Based on the analysis of variance (ANOVA) of the estimated model, the significant factors can be identified (P. BAKONYI & al. [17]).

2.2.2.2. Taguchi design
Taguchi design allows the effects of many factors with two or more levels to be studied on a given response, in a relatively small number of runs. This type of fractional factorial design method may provide a powerful and efficient tool to find an optimal combination of factor levels that may achieve a desired optimum (J. ANTONY [18]). Usually, with the aid of statistical tools, the key factors that have significant effects on a response can be identified and the best factor levels for a given process can be determined from the predetermined factor levels. However, the method is not always accurate, as the true optimal factor levels may be different from the pre-determined factor levels (R.S. PRAKASHAM & al. [19]).

2.2.2.3. Method of steepest ascent
The initial estimate of the optimal conditions for a bioprocess is often far from the actual optimum, therefore there is a second step for optimization, after establishing the key factors involved in the process, is to locate the optimal conditions for these key factors. The method of steepest ascent is among the most efficient procedure developed for this purpose. In order to obtain the path of steepest ascent for various factors, a first-order polynomial model (Eq. (1)) is usually used to fit the experimental data obtained from a factorial design such as a Plackett–Burman design or Taguchi design. The path of steepest ascent starts from the design center of the factorial design building the first-order polynomial model and ends until no further improvement can be achieved in the response, thus indicating the region of optimal response (C. LONG & al. [20]).

2.2.2.4. Central composite design and Box–Behnken design
After identifying the region of optimal response for a given process, further characterization of the response in that region is needed. Central composite design and Box–Behnken design can estimate a second-order polynomial approximation to a response in that region through response surface methodology. Central composite design is a five-level fractional factorial design developed by Box and Wilson. The design usually consists of a 2n full factorial design, 2n axial designs and m central designs (G.E.P. BOX & al. [21]). This approach has a relatively high number of factor levels and can contain extreme high or
2.2.2.5. Response surface methodology

For response surface methodology, a second-order polynomial model (Eq. (2)) is usually proposed to describe the effects of various factors on a response based on experimental results from a central composite design or Box–Behnken design.

\[
y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i<j} \beta_{ij} X_i X_j
\]

where \(y\) is the response, \(\beta_0\) is the constant and \(\beta_i\) is the linear coefficient, \(\beta_{ii}\) is quadratic coefficient, \(\beta_{ij}\) is the interactive coefficient and \(X_i\) is the coded factor level.

The estimated second-order polynomial model can be displayed as a surface plot and a contour plot, by varying only two factor levels, while keeping other factor levels constant. The plots will indicate the significance of the studied factors on the investigated response, as well as the interactions between these factors. Using the analysis of variance (ANOVA) on the estimated model, factors and interactions which have significant effects on the response can be determined. In addition, by using the regression model, the optimal response of the key factors together with their interactions, can be estimated (K. NATH & al. [9]; P. MULLAI & al. [23]; Y. XIAO & al. [24]).

2.3. Neural network and genetic algorithm

Recently, the mathematical representation of the neurological function of the brain was developed. This model is able to describe the interactive effects of various factors of a multivariate non-linear process. It has been suggested that for the modeling of complex non-linear systems like the biohydrogen fermentation process, a neural network model is more accurate than a second-order polynomial model (J. REN & al. [25]). However, using this model, different optimization techniques are required, as well as a slightly larger number of experimental runs. Genetic algorithm, which is based on the principles of evolution through natural selection, has established itself as a powerful search and optimization technique to solve problems characteristic to nonlinear systems characteristic to complex bioprocesses.

2.4. Multiple-response optimization

When two or more different responses are to be optimized, the optimization of one response often interferes with the optimization of the other responses. Because of this, a simultaneous optimization of multiple responses is required. This step first involves building an appropriate model for each response and then trying to keep them both in the desired ranges. The simultaneous optimization of several responses can be achieved by determining the maximum of the overall desirability, thus the simultaneous optimization of several responses can be reduced to maximizing a single response (S. PINZI & al. [26]).

3. Mathematical models and simulations

Mathematical models and simulations of different biological, physical and chemical aspects of biological hydrogen production systems based on dark fermentation can provide a valuable insight upon analyzing and predicting the performance of these processes. In order to achieve accurate system predictions, appropriate kinetic models describing different stages of the process are required (Fig. 1). Modeling such a complex system requires the mathematical
description of a number of distinct stages and factors which influence the biohydrogen production process, such as kinetics of microbial growth (biomass formation), initial microbial composition and substrate degradation as well as metabolic product (hydrogen, VFA, solvents, etc.) formation and their inhibitor actions on the overall system. Additionally, a few models are developed to describe the effects of several physicochemical parameters on biohydrogen production, like temperature, pH, dilution rate etc., together with the interactions among them (K. NATH & al. [9]; C. NEAGU & al. [27]).

3.1. Progress of $H_2$ production process

Hydrogen producing fermentation under dark conditions is a dynamic and continuous changing process. Although such systems are very complex in nature, some kinetic models have been developed and adapted in order to describe these changes. One of the earliest models used is the modified Gompertz model (Eq. (3)). Developed by M.H. ZWIETERING & al. [29], the model was widely used to describe the progress of substrate degradation, bacteria growth, hydrogen production and certain metabolic products generation in batch fermentative hydrogen production processes (J. CAI & al. [30]; X. LIU & al. [31]).

\[
X = \frac{X_0\exp(k_c t)}{1-(X_0/X_{\text{max}})(1-\exp(k_c t))}
\]

(3)

\[
H = H_{\text{max}}\exp\left\{-\exp\left[\frac{R_{\text{max}}q}{H_{\text{max}}}(y - t) + 1\right]\right\}
\]

(4)

where $H$ and $H_{\text{max}}$ denote the cumulative degraded substrate value and the maximum degraded substrate value, respectively (when used to describe the progress of substrate degradation), or the cumulative bacterial growth value and the maximum bacteria growth value, respectively (when used to describe the progress of bacterial growth).

Based on the Logistic model (Eq. (4)), used mostly to describe the progress of bacteria growth in batch fermentation studies, the modified Logistic model (Eq. (5)) was developed and used in the last years to describe the progress of hydrogen production using model systems (Y. MU & al. [32]). Compared with the Logistic model, the modified Logistic model can obtain the lag time of bacteria growth directly by fitting the experimental data. In addition, the modified Logistic model was also used to describe the progress of bacteria growth in batch fermentation conditions (J.L. WANG & al. [33]; Y. MU & al. [34]). Furthermore, some studies compared the ability of the modified Gompertz, modified Logistic
and modified Richards models (Eq. (6)) to describe the progress of bacteria growth in batch tests, concluding that the modified Gompertz model was the most suitable to predict the process development (Y. MU & al. [34]).

\[
H = \frac{H_{\text{max}}}{1 + \exp\left[4R_{\text{max}}(y-t)/H_{\text{max}}+2\right]}
\] (5)

\[
S = S_0 \left\{ 1 - \left\{ 1 + (m - 1)e^{m \times \exp\left[ \frac{m}{S_0} \left( \frac{m}{m-1}(y - t) \right) \right]} \right\}^{1/(1-m)} \right\}
\] (6)

The anaerobic digestion model No. 1 (ADM1) developed by the International Water Association (IWA) task group was modified and used to describe the progress of glucose degradation, bacterial growth, and the productions of hydrogen and some metabolites in batch tests (V. GADHAMSHETTY & al. [35]; I. NTAIKOU & al. [36]). One of the major drawbacks is the complexity of this model, which can be a limiting factor for its applications.

The Luedeking–Piret model (Eq. (7)) and its modified form Eq. (8)) are widely used to describe the relationship between the growth rate of hydrogen producing bacteria and product formation rate (K. NATH & al. [9]). In addition, a number of authors used Eq. (9) and (10) to describe the relationship between the substrate degradation rate and hydrogen, acetate and butyrate production rates, as well as bacterial growth rates (Y. MU & al. [34]).

\[
\frac{dP}{dt} = \frac{Y_{P/X}}{X} \frac{dx}{dt} + \beta X
\] (7)

\[
\frac{dP}{dt} = \frac{Y_{P/X}}{X} \frac{dx}{dt}
\] (8)

\[
\frac{dP}{dt} = \frac{Y_{P/S}}{S} \frac{ds}{dt}
\] (9)

\[
\frac{dx}{dt} = -\frac{Y_{X/S}}{S} \frac{ds}{dt}
\] (10)

As a conclusion, the modified Gompertz model has the ability to describe a large range of factors influencing the batch fermentative biohydrogen production process. In addition, using the modified Logistic model, similar predictions can be achieved as the modified Gompertz model. The development and the application of the modified ADM1 model are very complex, which may limit its applications. More studies are recommended using the Luedeking–Piret model and its modified form in continuous fermentation systems. Using Eq. (9) to describe the relationship between the rate of substrate degradation and the production rates of other metabolites, as well as using Eq. (9) and (10) to describe the relationship between substrate degradation rate and some product formation rates by pure and mixed cultures is also recommended. Studies on the comparison of the ability of different models to describe the progress of a continuous fermentative hydrogen production process are limited, thus more efforts are recommended to compare them.

### 3.2. Microbial growth and substrate degradation

The biohydrogen fermentation substrates contain carbohydrates that can provide carbon and energy sources for hydrogen producing bacteria, thus directly influencing the growth of biohydrogen production rate of these organisms. Some kinetic models have been successfully used to describe the mechanisms of substrate degradation, biohydrogen producing microbial growth and hydrogen production (K. NATH & al. [9]).

Until recently, classical Monod model (Eq. (11)) was the most commonly applied model used to describe microbial growth in relation with growth-linked substrate utilization (N. KHANNA & al. [37]). Additional models were developed in the last years in order to
describe more accurately these phenomena. A number of studies compared the classical Monod model with the Andrew model (Eq. (12)) and the modified Andrew model (Eq. (13)). These describe the progress of glucose degradation in relation with microbial growth in batch tests, concluding that the Andrew and the modified Andrew models are more suitable than the classical Monod model (K. NATH & al. [38]). These models took into consideration the effects of substrate inhibition as well, bringing the model system closer to the real systems.

\[
\frac{dS}{dt} = \frac{-1}{Y_{X/S} K_S + S} R_{\text{max}} S \frac{X}{S(S_0 - S)}
\]

where \( X = X_0 + Y_{XS}(S_0 - S) \) and \( R_{\text{max}} \) is the specific microbial growth rate.

When a substrate inhibits the microbial growth and thus the fermentative hydrogen production process at high concentrations, the modified Monod model (Eq. (14)) can be used to describe the effects of substrate concentrations on the hydrogen production rate and specific microbial growth rate. In addition, two models developed from a modified Monod model (Eqs. (15) and (16)) incorporating low pH inhibition and the biomass decay, were used to describe the progress of glucose degradation rate and microbial growth in batch tests (I. NTAIKOU & al. [39]; P.Y. LIN & al. [40]). These models could also be used to describe the progress of hydrogen production together with some soluble metabolite production.

\[
I_{pH} = \exp \left[ -3 \left( \frac{pH - pH_{UL}}{\Delta pH_{UL} - pH_{LL}} \right)^2 \right]
\]

\[
\frac{dS}{dt} = \frac{-1}{Y_{X/S} K_S + S} R_{\text{max}} S X I_{pH}
\]

\[
\frac{dX}{dt} = \frac{R_{\text{max}} S}{K_S + S} X I_{pH} - k_d X
\]

where \( R_{\text{max}} \) is the specific HPB growth rate.

Recently, a number of studies used two extended Monod models, namely the Han–Levenspiel model (Eq. (17)) and a modified Han–Levenspiel model (Eq. (18)), to describe the effects of glucose and sucrose concentrations on hydrogen production rate in batch tests, concluding that these models fit better to describe such processes than the classical Monod model (M.R. BONI & al. [41]).

\[
R = \frac{R_{\text{max}} S (1 - \frac{S}{S_{\text{Crit}}})^m}{S + K_S (1 - \frac{S}{S_{\text{Crit}}})^n}
\]

\[
R = \frac{R_{\text{max}} S (1 - \frac{S}{S_{\text{Crit}}})^m}{S + K_S}
\]

In general, the modified Monod model with some additional terms such as various inhibitions or biomass decay, can be used easily to describe the effects of substrate concentrations on the rates of substrate degradation, bacterial growth and hydrogen production. Furthermore, a comparison of the different modified Monod models is
recommended in order to determine the most suitable model for a specific fermentative hydrogen production process, as well as to describe the effects of substrate concentrations on some soluble metabolite production rate during fermentative hydrogen production.

3.3. Product formation and inhibition

It has been confirmed that some metabolic products and salts, or even hydrogen accumulation in the head space, may interfere with the energy requirement of the hydrogen producing organisms or even inhibit some specific enzymes related to fermentative hydrogen production. Thus, these combined factors can inhibit microbial growth as well as the fermentative hydrogen production.

So far, some kinetic models have been used to describe the inhibitory effects of some salts and hydrogen concentrations on the fermentative hydrogen production process. Among them, the modified Han–Levenspiel model (Eq. (18)) was extensively used. In addition, further models have been proposed (Eq. (19), (20) and (21)) to describe the inhibitory effects of sucrose, sodium acetate and butyrate concentrations on the specific rates of sucrose degradation and hydrogen production, as well as microbial growth rates in batch and fed-batch tests (Y. Wang & al. [42]).

\[ R = \frac{R_{\text{max}}}{1 + (C/K_C)^m} \]

\[ R = \frac{R_{\text{max}}K_C}{K_C + C} \]

\[ R = R_{\text{max}} \frac{S}{K_s + S} \left( \frac{1 - \frac{S}{S_{\text{crit}}}}{1 - \frac{C}{C_{\text{crit}}}} \right)^n \]  

Temperature is considered to be one of the most important factors influencing fermentative hydrogen production because of its direct effect on the activity of hydrogen producing microorganisms by influencing the activity of essential enzymes such as hydrogenases. One of the first models used to describe the effects of temperatures on hydrogen production rate and microbial growth rate is the Arrhenius model (Eq. (22)). In addition, the Arrhenius model can also be used to describe the effects of temperatures on the substrate degradation rate and on the production rate of certain soluble metabolites (M.R. Boni & al. [41]).

\[ R = A e^{\frac{-E_a}{R g T}} \]

One limitation of the Arrhenius model is that it cannot take into account the decrease in the R with increasing temperatures above the optimal temperatures. In an attempt to overcome this problem, the Ratkowsky model (Eq. (23)) was developed. This model can describe the effects of temperature on fermentative hydrogen production throughout the entire temperature range. Thus, the model can describe the temperature curve from Tmin to Tmax, having the optimum range at the Topt (J.L. Wang & al. [43]).

\[ R = [A(T - T_{\text{min}})]^2 \{1 - e^{[B(T - T_{\text{max}})]} \}^2 \]

pH is considered another important factor influencing fermentative hydrogen production, affecting mostly the activity of the hydrogenase enzymes together with the overall microbial growth and development. The Andrew model (Eq. (12 and 24)) was used in a number of research studies to describe the effects of H⁺ concentration on the specific hydrogen production rate. In addition, the Andrew model can be used to describe the effects of H⁺ concentration on other factors like the rates of substrate degradation, microbial growth
and some soluble metabolite production (D. FRASCARI & al. [44]). It is also possible to model the pH as well rather than H⁺ concentration using this model. The Ratkowsky model (Eq. (25)) may also be a good candidate to describe the effects of pH on different process parameters (J. WANG & al. [45]).

\[
R = \frac{R_{\text{max}}[H^+]}{K_a + [H^+] + [H^+]^2/K_b}
\]

\[
R = [A(pH - pH_{\text{min}})]^2(1 - \exp[B(pH - pH_{\text{max}})])^2
\]

Dilution rate can also be an important factor influencing the ability of hydrogen-producers to degrade the substrate, thus influencing the fermentative hydrogen production process. Some studies used the single-substrate models with and without biomass decay, as well as the dual substrate model with biomass decay (based on Eqs. (26-29)) to describe the effects of dilution rates on hydrogen production together with the concentration of glucose, sucrose, peptone, biomass, ammonium nitrogen, formate, acetate, propionate, butyrate and ethanol in a continuous stirred tank reactor for hydrogen production. They concluded that the dual-substrate model with biomass decay was the most suitable one to accurately describe the effects of these factors on biohydrogen yields (J. WANG & al. [45]). Other authors used Eq. (30) to describe the effects of dilution rates on the specific sucrose degradation rate in an up-flow anaerobic sludge blanket reactor for hydrogen production (F.Y. CHANG & al. [46]).

\[
S = \frac{DK_S}{R_{\text{max}} - D}
\]

\[
S = \frac{(D + k_d)K_S}{R_{\text{max}} - D - k_d}
\]

\[
X = Y_{X/S}(S_0 - S)
\]

\[
P = Y_{P/X}X
\]

where \( R_{\text{max}} \) is the specific microbial growth rate.

\[
R = \frac{D + k_d}{Y_{X/S}}
\]

In conclusion, most of the studies concerning the metabolites production together with the inhibitory factors on the dark fermentation biohydrogen production process using these models were mostly performed in batch tests, thus, the description of the inhibitory effects for continuous tests using these models is recommended. Only the modified Han–Levenspiel model was used to describe the inhibitory effects of salt and hydrogen concentrations on hydrogen production rate. In future studies, the model can be used to describe the effect of these factors on substrate degradation, hydrogen producing microbial growth as well as the production rate of different metabolites. In addition, more focus should be directed towards modeling the production of different metabolites as well as their inhibitory effects on the fermentative hydrogen production process in continuous reactors in the future.

4. Conclusions

One-factor-at-a time optimization approaches are economically unfeasible as well as time-consuming. In addition, these methods are insensitive to the interactive effects among the studied variables, thus cannot guarantee the determination of optimal conditions. On the
contrary, the statistically based factorial design approaches are able to analyze a much larger number of influencing factors in a shorter time, thus revealing the influence together with the interactions which may occur among these factors. Therefore, these approaches are cost-effective as well as have the ability to minimize the error in determining the effect of the studied parameters. Most of these statistical methods have been successfully adapted and used for the optimization of different biohydrogen production processes. However, until now, there is a limited amount of research papers dealing with genetic algorithm based on neural network models, together with simultaneously multiple response optimizations, necessary for a system which couples biohydrogen production with biological waste treatments.

Regarding the mathematical modeling and simulation of the fermentative biohydrogen production processes, several directions were discussed, concerning the progress of biohydrogen production, microbial growth and substrate degradation as well as the formation of different metabolic products and their inhibitory effects on the biohydrogen yields. Each of these models have advantages as well as limitations. Thus, it is recommended to focus on the ability of certain models to accurately describe the studied effects on the fermentative hydrogen production. In addition, comparative studies of these models are also required. Most of the modeling studies were performed under batch mode conditions. Different types of batch and continuous hydrogen production systems have different properties and perform under the influence of different factors, thus using these models to investigate the effects of the influencing factors on such different fermentation systems is also recommended.

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