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Review

Experimental design methods for fermentative hydrogen production: A review

*Jianlong Wang**, *Wei Wan*

Laboratory of Environmental Technology, INET, Tsinghua University, Beijing 100084, PR China

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ABSTRACT

This review summarized the experimental design methods used to investigate the effects of various factors on fermentative hydrogen production processes, including one-factor-at-a-time design, full factorial design, Taguchi design, Plackett–Burman design, central composite design and Box–Behnken design. Each design method was briefly introduced, followed by the introduction of its analysis. In addition, the advantages and disadvantages of each design method were briefly discussed. Moreover, the application of each design method to the study of fermentative hydrogen production was analyzed and discussed. Based on the discussion in this review, an experimental design strategy for optimizing fermentative hydrogen production processes was proposed. In the end, the software packages that can carry out the above mentioned factorial design and analysis were briefly introduced.

1. Introduction

Environmental pollution due to the use of fossil fuels as well as their shortfall makes it necessary to find alternative energy sources that are environmentally friendly and renewable. Hydrogen satisfies the above requirements because it produces only water, when it is combusted as a fuel or converted to electricity. Among various hydrogen production processes, biological method is known to be less energy intensive, for it can be carried out at ambient temperature and pressure. Biological method mainly includes photosynthetic hydrogen production and fermentative hydrogen production. The efficiency of photosynthetic hydrogen production is low and it cannot be operated in the absence of light, while fermentative hydrogen production can produce hydrogen continuously without light using various kinds of substrates such as organic wastes. Moreover, compared with photosynthetic hydrogen production, fermentative hydrogen production has higher hydrogen production efficiency, higher hydrogen production stability, higher feasibility for industrialization, simpler control requirement and lower operating costs. Thus fermentative hydrogen production is more feasible and widely used. In addition, it is of great significance to produce hydrogen from organic wastes by fermentative hydrogen production, because it plays the dual role of waste reduction and energy production. Therefore fermentative hydrogen production has been received increasing attention in recent years [1–5].

A fermentative hydrogen production process can be conducted by using either pure cultures or mixed cultures. However, in a fermentative hydrogen production process using mixed cultures, the hydrogen produced by hydrogen-

* *Corresponding author*. Tel.: +86 10 62784843; fax: +86 10 62771150.
E-mail address: wangjl@tsinghua.edu.cn (J. Wang).

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producing bacteria can be consumed by hydrogen-consuming bacteria. Thus, in order to harness hydrogen from a fermentative hydrogen production process, the seed sludge often needs a pretreatment to suppress as much hydrogen-consuming bacterial activity as possible while still preserving the activity of the hydrogen-producing bacteria [2].

Experimental design can be regarded as a process by which certain factors are selected and deliberately varied in a controlled manner to obtain their effects on a response of interest, often followed by the analysis of the experimental results. According to the number of the factors to be investigated at a time, the experimental design can be classified into two categories: one-factor-at-a-time design (single-factor design) and factorial design (multiple-factor design) [6].

Experimental design is of great importance to a fermentative hydrogen production process, because the process is very complex and influenced by many factors such as hydrogen-producing bacteria, substrates, inorganic nutrients, operational conditions of the bioreactors and so on, thus an appropriate experimental design can be used to study the effects of various factors on the process to make it better understood and even optimized to improve its performance [5].

This review attempts to summarize the experimental design that was 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. Each design was briefly introduced, followed by the introduction of its analysis and application to the study of fermentative hydrogen production.

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

One-factor-at-a-time design is a traditional design, which investigates one-factor-at-a-time, while keeping the levels of other factors constant. The level of the factor to be investigated is then changed over a desired range to study its effects on a response. After the experimental results are obtained, certain graphs are usually constructed showing how a response is affected by the one factor studied. Since one-factor-at-a-time design is easy to operate and analyze, it has been widely used to study the effects of various factors on fermentative hydrogen production processes. Table 1 summarizes a number of studies using one-factor-at-a-time design to study the effects of various factors on fermentative hydrogen production processes. For example, Kim et al. investigated the effects of sucrose concentration on fermentative hydrogen production using one-factor-at-a-time design, with several graphs being plotted to show the effects of sucrose concentration on hydrogen yield, hydrogen production rate and specific hydrogen production rate, and then concluded that the optimal sucrose concentration for fermentative hydrogen production was 30 g COD/L. Since they investigated only one factor, namely sucrose concentration, at a time in that study, while keeping the levels of other factors constant, it was easy for them to conduct the experimental design and analyze the obtained results [12].

However, one-factor-at-a-time design has two main drawbacks. For one thing, it does not take into consideration the interactions among different factors, which cannot guarantee the optimal conditions identified by it to be optimal, especially when the interactions among different factors are significant. For example, Kim et al. investigated the effects of only one factor, namely sucrose concentration on fermentative hydrogen production using one-factor-at-a-time design, and ignored the interactions between sucrose concentration and other factors such as temperature [12]. For another, it involves a relatively large number of experiments, which makes it laborious and time-consuming to carry out the experiments, especially when the number of factors is large [34]. For example, Chittibabu et al. investigated, respectively, the effects of inoculum size, initial medium pH, initial substrate concentration, temperature and dilution rate on hydrogen productivity using one-factor-at-a-time design, with around 30 runs of experiments being conducted [8].

3. Factorial design

On the contrary, factorial design is able to study the effects of more than one factor at two or more levels. The experimental design generally includes various combinations of different factor levels, which enables it to depict the interactions among different factors and to be more efficient to deal with a large number of factors, compared with one-factor-at-a-time design. Factorial design can be classified into two categories: full factorial design and fractional factorial design [34].

Since coded factor levels provide a uniform framework to investigate the effects of a factor in any experimental context, while the actual factor levels depend on a particular factor to be studied, factorial design is usually given in the form of coded factor levels [35]. One can assign each actual factor level to the corresponding coded factor level of a factorial design when using it. 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 [36]. In this review, the models are expressed based on coded factor levels. Such models can be expressed based on actual factor levels when necessary.

3.1. *Full factorial design*

In a full factorial design, every combination of each factor level is tested. For example, the number of runs for a three-factor full factorial design is $a \times b \times c$, which indicates that, the first factor is tested at a levels, the second factor is tested at b levels, while the third factor is tested at c levels. The number of runs for a full factorial design of n factors, each at a levels is a^n. The most commonly used full factorial design is two-level design, which can be denoted by 2^n when there are n factors [34]. Sometimes, 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.

Table 1 – One-factor-at-a-time design for fermentative hydrogen production processes.

Inoculum	Substrate	Factor studied	Reference
Digested sludge	Glucose	Fe^{2+} concentration	[1]
Digested sludge	Glucose	Inoculum pretreatment method	[2]
Digested sludge	Glucose	Ni^{2+} concentration	[3]
Digested sludge	Glucose	Substrate concentration	[4]
Pure cultures	Glucose	Inoculum type	[7]
Recombinant *Escherichia coli* BL-21	Glucose	Inoculum size	[8]
Escherichia coli MC13-4	Glucose	Cell density	[9]
Anaerobic sludge	Glucose	Temperature	[10]
Thermoanaerobacterium thermosaccharolyticum PSU-2	Carbohydrate	Substrate type	[11]
Anaerobic digester sludge	Sucrose	Substrate concentration	[12]
Cracked cereals	Citric acid wastewater	Organic loading rate	[13]
Clostridium butyricum CGS5	Carbohydrate	Medium composition	[14]
Anaerobic digester sludge	Cheese whey permeate powder	Food to microorganism ratio	[15]
Cracked cereals	Starch	Nitrogen concentration	[16]
Cracked cereals	Starch	Iron concentration	[16]
Cracked cereals	Starch	Initial pH	[16]
Cracked cereals	Starch	Substrate concentration	[16]
Sewage digester sludge	Glucose	Nitrate concentration	[17]
Wasted activated sludge	Sucrose	C/N ratio	[18]
Citrobacter sp. Y19	Glucose	Phosphate concentration	[19]
Digester sludge	Sucrose	Iron concentration	[20]
Fermentative bacteria B49	Glucose	Magnesium concentration	[21]
Fermentative bacteria B49	Glucose	Iron concentration	[21]
Fermentative bacteria B49	Glucose	Sparging gas type	[21]
Pseudomonas sp. GZ1	Wastewater sludge	Substrate pretreatment method	[22]
Escherichia coli MC13-4	Glucose	Immobilized gel bead size	[23]
Ruminococcus albus	Glucose	Formate concentration	[24]
Anaerobic sludge	Glucose	Butyrate concentration	[25]
Compost	Sucrose	Initial pH	[26]
Anaerobic sludge	Glucose	pH	[27]
Municipal sewage sludge	Xylose	Temperature	[28]
Clostridium butyricum CGS2	Hydrolyzed starch	Hydraulic retention time	[29]
Clostridium paraputrificum M-21	N-acetyl-D-glucosamine	Agitation speed	[30]
Clostridium thermolacticum	Lactose	Dilution rate	[31]
Wastewater sludge	Sucrose	Liquid reflux	[32]
Wastewater sludge	Sucrose	Gas reflux	[32]
Enterobacter cloacae IIT-BT 08	Glucose	Recycle ratio	[33]

Since with a full factorial design, all possible combinations of the factor levels can be investigated, it has been used a lot to study the effects of several factors simultaneously on fermentative hydrogen production processes. Table 2 summarizes a lot of studies using full factorial design to study the effects of various factors on fermentative hydrogen production processes. For example, Chou et al. investigated the effects of pH (at 4 levels) and stirring speed (at 6 levels) on fermentative hydrogen production using full factorial design with 24 runs of experiment, with two second-order polynomial models being constructed to describe the effects of the two factors on hydrogen yield and specific hydrogen production rate, and then concluded that the optimal pH and stirring speed for fermentative hydrogen production were 6.0 and 120 rpm, respectively. Since they examined every combination of each pH and stirring speed level, the interactions between the two factors were depicted [54].

The number of runs for a full factorial design increases geometrically as the number of factors increases. For example, Espinoza-Escalante et al. investigated the effects of alkalinization, thermical treatment and sonication (each at 2 levels) on fermentative hydrogen production using full factorial design. If they examined the effects of only two factors on fermentative hydrogen production using full factorial design, 2^2 runs of experiment were required, and if they examined the effects of the three factors on fermentative hydrogen production using full factorial design, 2^3 runs of experiment were required, that is when a factor with 2 levels was added to the full factorial design, the runs of experiment doubled [53]. In many instances, when the effects of a large number of factors are to be studied simultaneously, a great many runs of experiment are required. Generally, this will constitute a larger experiment that is not economically and practically feasible [6].

3.2. *Fractional factorial design*

It turns out, however, that when the number of runs for a full factorial design is relatively large, the desired information can often be obtained by performing only a fraction of the full factorial design, which is often referred to as fractional factorial design to distinguish it from the full factorial design. In other words, fractional factorial design provides an alternative when the number of runs for a full factorial design is too large to be practicable. With a fractional factorial design,

Table 2 – Full factorial design for fermentative hydrogen production processes.

Inoculum	Substrate	Factors studied	Reference
Municipal sewage sludge	Glucose	Cultivation pH and enrichment pH	[37]
Anaerobic sludge	Sucrose	Reactor condition and pH	[38]
Municipal sewage sludge	Sucrose	Temperature and initial pH	[39]
Anaerobic digester sludge	Cellulose	Cellulose concentration and sludge density	[40]
Sewage digester sludge	Sucrose	Hydraulic retention time and calcium concentration	[41]
Sludge compost	Garbage slurry	Hydraulic retention time and pH	[42]
Thermotoga elfii	Glucose	Glucose, yeast extract and tryptone concentrations	[43]
Anaerobic sludge	Starch	Iron concentration and initial pH	[44]
Mixed cultures	Organic solid waste	Inoculum type, inoculum pretreatment method and cultivation temperature	[45]
Cracked cereals	Sucrose	Temperature and iron concentration	[46]
Mixed cultures	Carbohydrate	Substrate type and inoculum type	[47]
Clostridium thermocellum 27405	Cellulosic biomass	Substrate type and concentration	[48]
Sewage digester sludge	Glucose	Solid retention time and pH	[49]
Dewatered and thickened sludge	Glucose	Ammonia concentration and pH	[50]
Mixed and pure cultures	Starch residue	Substrate concentration and inoculum type	[51]
Municipal sewage sludge	Sucrose	Substrate concentration and cell immobilization method	[52]
Digested sludge	Tequila's stillages	Alkalinization, thermical treatment and sonication	[53]
Compost	Spent grains	pH and stirring speed	[54]
Mixed cultures	Glucose	Inoculum type and heat-shock time	[55]
Municipal sewage sludge	Carbohydrate	Hydraulic retention time and substrate type	[56]
Municipal sewage sludge	Carbohydrate	Upflow velocity and substrate type	[57]

the effects of certain factors on a response can be studied under an economical and practical condition [6].

Taguchi design, Plackett–Burman design, central composite design and Box–Behnken design are fractional factorial designs that were used a lot for fermentative hydrogen production processes [58–78]. Table 3 summarizes some studies using fractional factorial design to study the effects of various factors on fermentative hydrogen production processes.

3.2.1. *Taguchi design*

Taguchi design, which is a fractional factorial design using orthogonal array, allows the effects of many factors with two or more levels on a response, to be studied in a relatively small number of runs. In addition, the orthogonal array facilitates the analysis of the design. When used properly, Taguchi design may provide a powerful and efficient method to find an optimal combination of factor levels that may achieve optimum. Usually, with the aid of range analysis, analysis of

Table 3 – Fractional factorial design for fermentative hydrogen production process.

Inoculum	Substrate	Design	Factors studied	Reference
Wasted activated sludge	Sucrose	Taguchi	A nutrient formulation, 3 carbonate sources, and 3 phosphate sources	[58]
Wasted activated sludge	Sucrose	Taguchi	Concentrations of 13 nutrients	[59]
Clostridium sp. Fanp2	Glucose	Plackett–Burman	Concentrations of 7 nutrients and initial pH	[60]
Mixed cultures	Sucrose	Central composite	Initial pH and substrate concentration	[61]
Anaerobic sludge	Wheat powder	Central composite	C/N and C/P ratio	[62]
Mixed cultures	Food residues and manure	Central composite	Hydraulic retention time, temperature and N_2-flow rate	[63]
Anaerobic digester sludge	Food waste with residual blood	Central composite	Solid content in the feed, proportion of residues and hydraulic retention time	[64]
Compost	Food wastes	Central composite	PO3-4, Fe^{2+} and NH^{4+} concentrations	[65]
Mixed cultures	Organic municipal solid waste	Central composite	Amounts of hydrogen-producing bacteria, pretreated anaerobic digestion sludge and organic municipal solid waste	[66]
Anaerobic digested sludge	Starch	Central composite	Hydraulic retention time and pH	[67]
Anaerobic sludge	Sucrose	Central composite	Substrate concentration and hydraulic retention time	[68]
Cow dung compost	Sucrose	Central composite	Substrate concentration and initial pH	[69]
Anaerobic sludge	Palm oil mill effluent	Central composite	Fe^{2+} concentration, C/N ratio and C/P ratio	[70]
Anaerobic sludge	Sucrose	Central composite	pH, temperature and substrate concentration	[71]
Anaerobic sludge	Sucrose	Central composite	pH, temperature and substrate concentration	[72]
Clostridium sp. Fanp2	Glucose	Box–Behnken	Glucose, phosphate buffer and vitamin concentrations	[60]
Enterobacter aerogenes	Glucose	Box–Behnken	pH, temperature and substrate concentration	[73]

variance or analysis of signal-to-noise ratio, 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 pre-determined factor levels [76].

As shown in Table 3, among the reviewed studies, two studies used Taguchi design. For example, Lin and Lay studied the effects of 13 nutrient concentrations on fermentative hydrogen production using a Taguchi design. Based on the analysis of the experimental results, they determined that magnesium, sodium, zinc and iron were important trace metals affecting hydrogen production and identified the best nutrient levels for the fermentative hydrogen production process from the pre-determined factor levels [59]. However, the true optimal factor levels may not be guaranteed using Taguchi design, because the true optimal factor levels may be different from the corresponding pre-determined factor levels [76].

3.2.2. *Plackett–Burman design*

In reality, there may be a great number of factors influencing a process, but it does not mean that all the factors have significant effects on it. More often than not, the factors that influence the process greatly may be paid greater attention than those that influence it slightly, because the former are essential to the successful operation of the process. Thus, the first step to optimize a process is to identify which factors have significant effects on the process.

Plackett–Burman design, which is a two-level fractional factorial design developed by Plackett and Burman, has been extensively used to screen important factors for further investigation [34]. In addition, the number of runs for a Plackett–Burman design is equal to a multiple of 4. Plackett–Burman design can examine up to $n = N - 1$ factors in an experiments with N runs and it works for all such N up to 100, except for 92 [35]. If the number of factors to be examined is less than $n = N - 1$, a subset of Plackett–Burman design for N runs can be used. Sometimes, some replications are performed to estimate the experimental errors.

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.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i \qquad (1)$$

where y is the response, β_0 is the constant and β_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 [74,75].

As shown in Table 3, among the reviewed studies, only the study by Pan et al. used Plackett–Burman design to study the effects of 8 factors on fermentative hydrogen production and then screened 3 factors (glucose, phosphate buffer and vitamin solution) that had significant effects on the specific hydrogen production potential for further study based on analysis of the experimental results [60].

3.2.3. *Method of steepest ascent*

Frequently, the initial estimate of the optimal conditions for a bioprocess is far from the actual optimum. Thus, the second step for optimization is to locate the region of factor levels that produce optimal conditions. The method of steepest ascent is a simple and economically efficient procedure developed to move the experimental region of a response in the direction of the maximum change toward the optimum. Of course, if minimization of a response is desired, then this method is referred to as the method of steepest descent. The factors screened by the Plackett–Burman design can be further investigated using this method.

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. The path of steepest ascent is perpendicular to the contour plots of the response based on the estimated first-order polynomial model, and moves β_i units in the x_i direction for every β_j units in the x_j direction. Equivalently, the path has a movement of β_j/β_i units in x_j for every 1 unit movement in x_i. Fig. 1 shows the contour plot of a response with varying only two factor levels, while keeping other factor levels constant, and the corresponding path of steepest ascent [35].

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, which indicates that the region of optimal response is in the neighborhood of that condition [35].

Among the reviewed studies, only the studies by Pan et al. and Lay used the method of steepest ascent to search the region of factor levels that produce optimal conditions for further optimization of fermentative hydrogen production processes [60,67]. For example, Pan et al. used the method of steepest ascent to find the design centers of glucose, phosphate buffer and vitamin solution for further optimization [60].

3.2.4. *Central composite design and Box–Behnken design*

Once the region of optimal response is identified by the method of steepest ascent, it is often necessary to characterize the response in that region. Central composite design

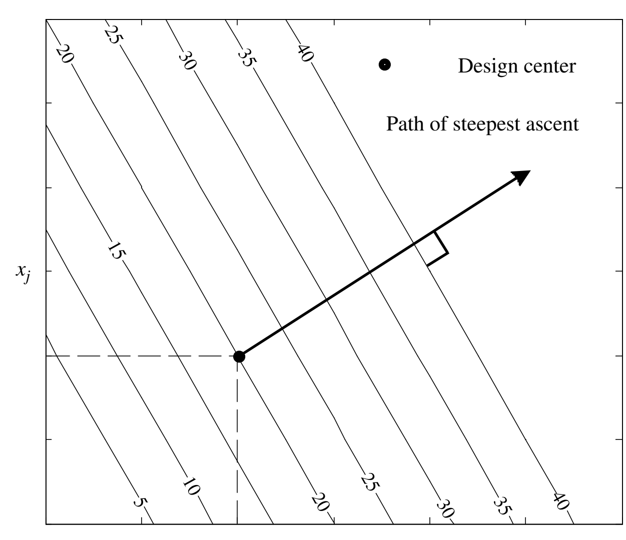

Fig. 1 – Contour plot of a response and the path of steepest ascent.

and Box–Behnken design are widely used experimental designs for response surface methodology to estimate a second-order polynomial approximation to a response in that region.

Central composite design is a five-level fractional factorial design developed by Box and Wilson [77]. The design usually consists of a 2^n full factorial design, $2 \times n$ axial designs and m central designs. The axial design is identical to the central design except for one factor, which will take on levels either above the high level or below the low levels of the 2^n full factorial design [35]. For example, Thong et al. studied the effects of Fe^{2+} concentration, C/N ratio and C/P ratio on fermentative hydrogen production using a central composite design. They concluded that the presence of 257 mg Fe^{2+}/L, C/N ratio of 74 and C/P ratio of 559 were optimal for simultaneous hydrogen production and COD (chemical oxygen demand) removal, and Fe^{2+} concentration and C/N ratio had the greatest interactive effect on hydrogen production, while C/N and C/P ratio gave more profound interactive effect on COD removal [70].

Box–Behnken design is a three-level fractional factorial design developed by Box and Behnken [78]. The design can be thought of as a combination of a two-level factorial design with an incomplete block design. In each block, a certain number of factors are put through all combinations for the factorial design, while other factors are kept at the central levels. It usually includes some central designs. For example, Pan et al. studied the effects of glucose, phosphate buffer and vitamin solution on fermentative hydrogen production using a Box–Behnken design. They concluded that glucose and vitamin solution, and glucose and phosphate buffer had interactive effects on hydrogen production and the optimal conditions were glucose 23.75 g/L, phosphate buffer 0.159 mol/L and vitamin solution 13.3 mL/L [60]. Box–Behnken design provides an economical alternative to the central composite design, because it has less factor levels than the central composite design and does not contain extreme high or extreme low levels. For example, Pan et al. studied the effects of 3 factors, namely glucose, phosphate buffer and vitamin solution (each at 3 levels), on fermentative hydrogen production using a Box–Behnken design in 15 runs of experiment [60], while Thong et al. studied the effects of 3 factors, namely Fe^{2+} concentration, C/N ratio and C/P ratio (each at 5 levels), on fermentative hydrogen production using a central composite design in 20 runs of experiment [70].

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.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j} \beta_{ij} x_i x_j \tag{2}$$

where y is the response, β_0 is the constant and β_i is the linear coefficient, β_{ii} is quadratic coefficient, β_{ij} is the interactive coefficient and x_i is the coded factor level.

As shown in Fig. 2, 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 surface plot and contour plot will visually show the response over a region of interesting factor levels. In addition, they will indicate how sensitive the response is to the change of each factor levels and to what degree the factors interplay as they affect the response.

Based on the analysis of variance (ANOVA) of the estimated model, terms which have significant effects on the response can be determined. In addition, with the aid of the regression model, the optimal response can be estimated by calculating the derivatives of the model.

For example, Jo et al. investigated the effects of glucose concentration, temperature and pH on the hydrogen production using a Box–Behnken design for response surface methodology. A second-order polynomial model was used to describe the effects of the three factors on the hydrogen production rate. Several surface plots and contour plots were plotted to visually show the effects of the three factors on the hydrogen production rate. Based on the analysis of variance of the estimated model, they concluded that glucose concentration, temperature and pH all had interactive effects on the hydrogen production rate. In addition, with the aid of the regression model, the optimum conditions obtained by them were glucose concentration 118.06 mmol/L, temperature 38 °C and pH 6.13 [73].

As shown in Table 3, central composite design has been used more widely for fermentative hydrogen production processes, compared with Box–Behnken design. Since Box–Behnken design provides an economical alternative to the central composite design, using it in the study of fermentative hydrogen production is recommended.

3.2.5. *Neural network and genetic algorithm*

In recent years, as a mathematical representation of the neurological functioning of a brain, neural network, which is

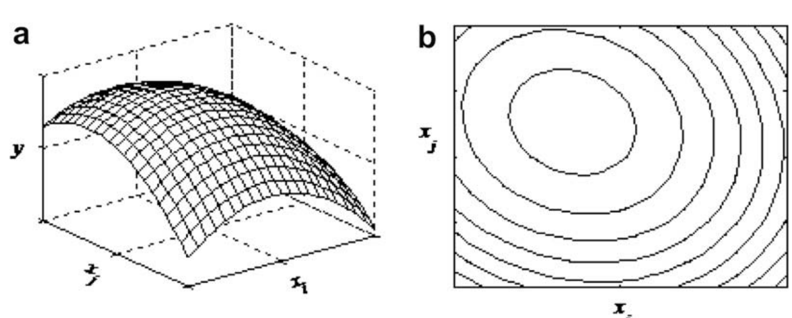

Fig. 2 – Surface plot (a) and contour plot, (b) for a response.

able to describe the interactive effects of various factors on a complicated process, has been applied successfully in a multivariate non-linear process as a useful tool to construct models. It has been shown that a neural network model is more accurate than a second-order polynomial model as it represents the non-linearities in a much better way [79,80].

A neural network model can be considered as the objective function for the purpose of optimization. However, using conventional optimization techniques such as gradient-based methods to optimize a neural network model is not a simple task because it is difficult to calculate the derivatives of the model. Genetic algorithm, which is based on the principles of evolution through natural selection, that is, the survival of the fittest strategy, has established itself as a powerful search and optimization technique to solve problems with objective functions that are not continuous or differentiable. In recent years, genetic algorithm based on a neural network model has been applied successfully to optimize complicated bio-processes [79,80].

In addition, Nagata and Chu showed that the optimal solution identified by response surface methodology was not guaranteed to be optimal due to the poor modeling ability of the second-order polynomial model, while a neural network model had a much higher modeling ability than it, and the optimal solution identified by the genetic algorithm based on a neural network model was much better than that identified by response surface methodology [80].

In a word, the genetic algorithm based on a neural network model is a better optimization method than response surface methodology. To the best of our knowledge, however, the genetic algorithm based on a neural network model has not been used to optimize a fermentative hydrogen production process, thus using it for such purpose is recommended.

3.2.6. *Multiple-response optimization*

Moreover, many experiments involve the optimization of two or more conflicting responses, that is, the optimization of one response usually worsens the optimization of other responses. Simultaneous optimization of multiple responses involves first building an appropriate model for each response and then trying to find a set of operating conditions that in some sense optimizes all responses or at least keeps them in desired ranges.

One useful approach to multiple-response optimization is the method of desirability function [36]. The general approach is to first convert each response y_i into an individual desirability function d_i that ranges from 0 to 1. If the response y_i is at its goal or target, then $d_i = 1$, while if the response is outside an acceptable range, then $d_i = 0$. Then the design factor levels are chosen to maximize the overall desirability D (Eq. (3)), which is the geometric mean of all the individual desirability functions.

$$D = (d_1 \times d_2 \times \cdots \times d_m)^{1/m} \tag{3}$$

In other words, 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: the overall desirability.

Among the reviewed studies, Espinoza-Escalante et al. and Cuetos et al. used the method of desirability function to optimize several responses simultaneously for fermentative hydrogen production processes [53,64], while most other studies optimized several responses separately for fermentative hydrogen production processes. For example, Espinoza-Escalante et al. optimized several responses, namely COD increment, total sugar consumption, acetic acid increment rate, propionic acid increment rate, butyric acid increment rate and hydrogen accumulated production simultaneously for a fermentative hydrogen production process using the method of desirability function. Several second-order polynomial models were used to describe the effects of alkalinization, thermical treatment and sonication on the above responses, and then each response was converted into an individual desirability function. Subsequently, the geometric mean of the individual desirability functions was built to form the overall desirability. In the end, it was observed that the higher overall desirability value was achieved when Tequila's stillages were pretreated at the alkalinization of 7, thermical treatment of 150 °C/30 min and sonication of 47 kHz/30 min, which were the global optimal conditions for the above responses obtained by them [53]. Otherwise, without multiple-response optimization, they would have had to optimize the above responses separately.

Thus, when there are many responses to be optimized, using the method of desirability function to optimize several responses simultaneously for fermentative hydrogen production processes is highly recommended.

4. Recommended experimental design strategy

From the above analysis in this review, the following experimental design strategy for optimizing a fermentative hydrogen production process is highly recommended.

First of all, Plackett–Burman design is used to screen the key factors of a fermentative hydrogen production process for further study. And then, the method of steepest ascent is used to approach the vicinity of the optimal conditions. Subsequently, central composite design or Box–Behnken design for response surface methodology can be used to estimate the relationship between a response and these key factors at the vicinity of optimum and then locate the optimal conditions based on a second-order polynomial model [36].

Among the reviewed studies, only the study by Pan et al. first used Plackett–Burman design to study the effects of 8 factors on fermentative hydrogen production and then screened 3 key factors (glucose, phosphate buffer and vitamin solution). And then they used the method of steepest ascent to find the design centers of the three factors for Box–Behnken design. Subsequently, they used Box–Behnken design for response surface methodology to study the effects of the three factors on fermentative hydrogen production and concluded that the optimal conditions for fermentative hydrogen production were glucose 23.75 g/L, phosphate buffer 0.159 mol/L and vitamin solution 13.3 mL/L [60].

Moreover, the genetic algorithm based on a neural network model can be used for optimizing a fermentative hydrogen production process when necessary. In addition, if there are many responses to be optimized for the process, optimizing simultaneously these responses is highly recommended.

5. Software packages for factorial design and analysis

So far, several commercial software packages such as Design-Expert (Stat-Ease, Inc., USA), Minitab (Minitab, Inc., USA) and so on are able to conduct the above mentioned factorial design such as Taguchi design, Plackett–Burman design, central composite design and Box–Behnken design and their analysis.

Take using Minitab for example, as for the Plackett–Burman design, one can first use Minitab to generate a Plackett–Burman design with the corresponding high levels and low levels for each factor. And then one can perform the experiment and collect the response data. After that, one can fit the response data using a first-order polynomial model and then analyze the model to determine which factors have significant effects on the responses for further optimization. As for the Box–Behnken design, one can first use Minitab to generate a Box–Behnken design with the corresponding high levels and low levels for each factor. And then one can perform the experiment and collect the response data. After that, one can fit the response data using a second-order polynomial model and then analyze the model to determine which factors have significant effects on the response. If one tries to optimize one response or multiple responses at the same time, one can first set the goal (such as maximum and minimum) for each response to be optimized and then conduct the optimization.

For example, Pan et al. conducted a Plackett–Burman design and analysis, as well as Box–Behnken design and analysis using Minitab [60]. Each software package has its unique character, thus it is up to the user to decide which one is more suitable.

The training of a neural network and the optimization of a fermentative hydrogen production process by genetic algorithm based on a neural network model can be performed by the software package of Matlab (Mathworks, Inc., USA) using its neural network toolbox and genetic algorithm toolbox, respectively.

In addition, multiple-response optimization for response surface methodology by the method of desirability function can be performed either by the software package of Design-Expert or the software package of Minitab.

Furthermore, multiple-response optimization for several responses based on neural network models can be carried out by genetic algorithm using the software package of Matlab.

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