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A Review of Enhancement of Biohydrogen Productions by Chemical Addition Using a Supervised Machine Learning Method

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Abstract: In this work, the impact of chemical additions, especially nano-particles (NPs), was quantitatively analyzed using our constructed artificial neural networks (ANNs)-response surface methodology (RSM) algorithm. Fe-based and Ni-based NPs and ions, including Mg2+, Cu2+, Na+, NH4+, and K+, behave differently towards the response of hydrogen yield (HY) and hydrogen evolution rate (HER). Manipulating the size and concentration of NPs was found to be effective in enhancing the HY for Fe-based NPs and ions, but not for Ni-based NPs and ions. An optimal range of particle size (86–120 nm) and Ni-ion/NP concentration (81–120 mg L−1) existed for HER. Meanwhile, the manipulation of the size and concentration of NPs was found to be ineffective for both iron and nickel for the improvement of HER. In fact, the variation in size of NPs for the enhancement of HY and HER demonstrated an appreciable difference. The smaller (less than 42 nm) NPs were found to definitely improve the HY, whereas for the HER, the relatively bigger size of NPs (40–50 nm) seemed to significantly increase the H2 evolution rate. It was also found that the variations in the concentration of the investigated ions only statistically influenced the HER, not the HY. The level of response (the enhanced HER) towards inputs was underpinned and the order of significance towards HER was identified as the following: Na+ > Mg2+ > Cu2+ > NH4+ > K+.

Keywords: biohydrogen (BioH2); nanoparticles; quantitative assessment; artificial neuron networks; process intensifications

1. Introduction

The further rollback of globalization will ultimately reshape the current supply chain block, especially as more and more countries have realized how pivotal it is to have self-sufficient industries to produce strategic products such as medicine, energy, and even toilet paper rolls [1]. Aside from the public health emergency, energy security is another draconian challenge that countries across the world are reluctantly facing, although the price of crude oil did once plunge to USD 25 per barrel (158.98 L) in the middle of 2020 during the COVID-19 pandemic [2]. Whether to take bolder steps in the energy reliance transition from fossil fuel to renewable energy will make a great difference in the world that our children will be able to inherit in the future [3]. Consequently, by 2021, several
developed countries already started to restrict the use of fossil fuels in order to eventually achieve a shift in fuel type [4,5].

Among all sources of energy, hydrogen (H$_2$) is one of the most favorable candidates due to its inherent appealing features: (1) high energy yield (122 kJ kg$^{-1}$), (2) generation of water as a result of combustion, and (3) electricity generation through the fuel cell [6,7]. However, the current predominant H$_2$ generation still comes from fossil-based materials via existing mature industrial chemical processes such as natural gas steam reforming (NGSR), nature gas thermal cracking (NGTC), auto-thermal reforming (ATR), coal gasification, and partial oxidation of heavier-than-naphtha hydrocarbons [8]. Consequently, the paradox of sustainability of H$_2$ utilization and the non-renewability of H$_2$ generation will be encountered, although the development of carbon capture storage and utilization (CCSU) such as via a mature catalytic process like Fischer–Tropsch synthesis might alleviate environmental impacts from H$_2$ generation [9–12].

Apart from the thermal process, the biological hydrogen (BioH$_2$) generation process also plays a supplementary role in H$_2$ generation due to features such as versatile feedstock (lignocellulose, wet kitchen organic waste, and wastewater) and no green-house gas emissions (GHE). Despite the appealing advantages that are mentioned above, BioH$_2$ production is hampered by its relatively lower process performance [13]. To implement BioH$_2$ in different applications either on a decentralized or centralized basis or both, different process intensification approaches have been proposed, such as hydrolysate detoxification, mixed continuous and batch operations, co-fermentation, process optimization, and chemical addition. Among these approaches, chemical addition is considered to be one of the most attractive and practical ones because of its operational simplicity (without any additional modifications) and relatively low energy consumption [14]. However, current reports are limited to focusing on the facilitation of BioH$_2$ production by all types of chemical additives. In contrast, the nanoparticles (NPs) as a potential type of chemical additive still lack research on their addition and the corresponding quantitative relationships, such as hydrogen yield (HY) and hydrogen evolution rate (HER) with detailed incubation conditions, especially the concentration of different metal elements.

In this paper, instead of making a simple BioH$_2$ production enhancement comparison using the addition of NPs across literature reports, the collected data (such as HY, HER, and the substrate concentrations from literature works) were used to construct the data matrix for supervised machine learning algorithm using the developed artificial neural networks (ANNs) coupled with statistical analysis using response surface methodology (RSM) for more insightful and quantitative correlations and analysis. The review of assessing the impact of NPs additions on BioH$_2$ production in form of HY and HER using a developed ANNs-RSM algorithm, to the best of our knowledge, has not been reported before.

2. Materials and Methods

The literature used in this review was mainly collected from the scientific databases from Web of Science, Google Scholar and Science Direct via keyword search. Various keyword groups were comprised of several words, including “dark fermentation,” “biohydrogen,” and “nanoparticles.” With regard to the possible missing relevant literature, by using the abovementioned searching strategy, an extensive additional search process was conducted with more detailed keywords, including “trace metal,” “transitional metal,” “iron,” “nickel,” “gold,” “copper,” and “metal oxide.” During the additional search, these mentioned keywords were also combined with the keyword “biohydrogen.”

The ANNs (based on Python 2.7 platform) was deployed for data analysis. The detailed schematic diagram of the construction of the ANNs and data collection is shown in Figure S1. In this work, the widely used feed-forward three-layer networks were used. The simplified cross-out method was used for cross-validation during the data training step. The detailed descriptions of the standard procedures for this methodology can be...
found in our previous works [15]. During the data training, the mean square error (MSE) and mean average relative residual (MARR) were computed as follows:

\[
MSE\% = \frac{1}{N_{sam}} \sum_{i=1}^{N_{sam}} \left( \frac{r_{sam}^i - r_{cal}^i}{r_{sam}^i} \right)^2 \times 100\% (1)
\]

\[
MARR\% = \frac{1}{N_{sam}} \sum_{i=1}^{N_{sam}} \left( \frac{|r_{sam}^i - r_{cal}^i|}{r_{sam}^i} \right) \times 100\% (2)
\]

where \(N_{sam}\) is the number of data, and \(r_{sam}^i\) and \(r_{cal}^i\) are actual and calculated values, respectively. The setting for allowable accuracy was 95%. For the ANNs prediction data matrix, the widely used Box–Behnken design (BBD) and the central composite design (CCD) were used to predict the data matrix generation [16]. Once the supervised data learning was complete, the analysis of variation (ANOVA) based on commercial Design Expert® Version 11 software package (Stat-Ease, Inc., Minneapolis, MN, USA) was used for statistical analysis.

3. Literature Survey Comparisons

In this paper, for the convenience of discussion, four different types of NPs (Fe-based, Au-based, Cu-based, and Ni-based) were surveyed across different studies and the results are shown in Figure 1.

Figure 1. Statistics of publications from Scopus and Google Scholar in regard to BioH\(_2\) production by chemical nanoparticle additions.

For each type of NPs, taking Ni-based NPs, for instance, all nickel-related species were included, such as nanoparticles such as zero-valent particles, metal oxide NiO\(_2\), etc. The number of reports on the topic of BioH\(_2\) enhancement by NPs additions has been increasing steadily since 2015. Among different NPs, the number of reports using iron-based NPs has presented a discernible trend in recent years. The impetus underlining this trend is possibly associated with its inherent appealing cost-effective feature compared to other NPs such as gold or nickel. Apart from Fe-based NPs, Ni-based NPs have experienced an appreciable increase in recent years, with an exception in 2015 [17,18]. The research interests that focus
Studies have shown that [FeFe] hydrogenase catalyzes H₂ generation, whereas [NiFe] centers play a pivotal role in the metabolism of proton ion-associated redox reactions. During biological chemical reactions, these enzyme active centers play a pivotal role in the metabolism of proton ion-associated redox reactions. Studies have shown that [FeFe] hydrogenase catalyzes H₂ generation, whereas [NiFe] hydrogenase catalyzes the consumption of H₂. [NiFe] hydrogenase presents a relatively higher tolerance to the existence of oxygen and it widely exists in various types of microbial strains, whereas [FeFe] hydrogenase is relatively strict to the presence of oxygen and only exists in some algae and bacteria [22,23]. Regarding [Fe] hydrogenase, it only strictly exists in some methanogen strains [24–26].

4. Underlying Mechanisms of Metal Ions and Metal-Based Nanoparticles

Many extensively studied metal ions and metal-based nanoparticles are regarded as effective additives in culture medium to facilitate BioH₂ production in the dark fermentation process, including Na⁺, K⁺, NH₄⁺, Mg²⁺, Ca²⁺, Co²⁺, Zn²⁺, Cu²⁺, Fe²⁺/Fe³⁺, and Ni²⁺/Ni³⁺, among others [27–29]. Extensive studies have found that even small changes in the latter may have a significant impact on BioH₂ production; hence, many strategies have been proposed based on them, such as concentration regulation, including concentration manipulation [30], size regulation [17,31], composites fabrication [23,32], and heteroatom doping [33]. In general, the enhancement of NPs addition lies in a few important facts: (i) the controllable release of mental ions that facilitates the passive transport across the membrane [34]; (ii) nanodots that facilitate the electron transport chain during metabolism, such as glycolysis [35]; and (iii) the appropriate level of NPs favorable to the hydrogenase activities (co-enzymes often contain the metal ions in the catalysis center, which ultimately enhances the rate of hydrogen generation [36]. The potential mechanisms of BioH₂ enhancement are summarized in Figure 2. Therefore, in this part, this review will focus on the impact of the latter on BioH₂ production and its mechanisms.

![Figure 2. Potential mechanism of BioH₂ enhancement by NPs addition.](image-url)
4.1. Fe-Based Ions and Nanoparticles

Iron is an important trace element in the formation of hydrogenases and other enzymes. The pre-addition of Fe in the culture medium is a widely used strategy to enhance BioH\textsubscript{2} production in dark fermentation [37]. As illustrated in Figure 2, first, Fe is the essential element to form the metal content at the active sites of hydrogenase ([FeFe], [FeNi], and [Fe]), thus catalyzing the reduction reaction of H\textsuperscript{+} to H\textsubscript{2} [38]. Second, the presence of Fe-based NPs improves the activity of ferredoxin oxidoreductase by reducing the dissolved oxygen (DO) level and enhancing electron transfer due to the surface and quantum size effects [39,40]. In addition, Fe-based components could participate in enriching the microbial community and enhancing the growth of H\textsubscript{2}-producing bacteria [41]. The oxidative stress increases when there is a higher Fe concentration, which results in the formation of abundant oxidative radicals, thus leading to the deactivation or decomposition of enzymes [17,30].

4.2. Ni-Based Ions and Nanoparticles

Similarly, nickel ions or Ni-based nanoparticles are another widely studied substance that can significantly enhance BioH\textsubscript{2} production in dark fermentation. The mechanisms between Ni-ion/Ni-based nanoparticles and Fe-ion/Fe-based nanoparticles are largely identical but with minor differences. The key mechanisms for Ni include (a) facilitating the synthesis of [FeNi] hydrogenase [42], (b) improving the activity of ferredoxin oxidoreductase [43], and (c) Ni NPs controlling the concentration of Ni\textsuperscript{2+} at the optimum level. In addition, it is worth noting that [NiFe] hydrogenase exists in more bacteria than [FeFe] hydrogenase. Therefore, Ni can promote H\textsubscript{2}-producing bacteria in the dark fermentation process to a certain extent [44].

5. Results

5.1. Impact of Fe-Based Ions and NP Addition

To quantitatively unveil the impact of the concentration of Fe-ion/Fe NPs and size effects upon the HY and HER in BioH\textsubscript{2} generation, the collected values from the literature (Table 1) were statistically analyzed through our previously established ANN-RSM method and the results are shown in Figure 3.

Table 1. Comparison of BioH\textsubscript{2} production with the addition of Fe-based nanoparticles.

| NPs         | Opt/mg L\textsuperscript{-1} | Substrate | SC/g L\textsuperscript{-1} | Size/nm | HY/mmol g \textsuperscript{-1} | HER/mmol L \textsuperscript{-1} h \textsuperscript{-1} | Reference |
|-------------|-------------------------------|-----------|---------------------------|---------|-------------------------------|-------------------------------------------------|-----------|
| Fe (NPs)    | 400                           | Grass     | 10.7                      | 50      | 2.9                           | 5.4                                             | [45]      |
| Fe (NPs)    | 25                            | Starch    | 5                         | 35      | 3                            | -                                               | [18]      |
| Fe (NPs)    | 300                           | Malate    | 3                         | 16      | 20                           | 0.4                                             | [46]      |
| Fe (NPs)    | 50                            | Xylose    | 30                        | 75      | 13.3                         | 2                                               | [47]      |
| Fe (NPs)    | 200                           | MSJ       | 10                        | 50      | 0.9                          | 2.4                                             | [48]      |
| Fe (NPs)    | 200                           | Sucrose   | 7.5                       | 50      | 15.9                         | 10.1                                            | [27]      |
| Fe (NPs)    | 175                           | Glucose   | 7.5                       | 59      | 12.9                         | 5.69                                            | [28]      |
| Fe (NPs)    | 50                            | Starch    | 6                         | 35      | 5                            | -                                               | [43]      |
| Fe (NPs)    | 250                           | Malate    | 4                         | 12      | 24.2                         | 0.8                                             | [44]      |
| Fe\textsuperscript{2}O\textsubscript{3} (NPs) | 50                            | Glucose   | 5                         | 50      | 1.92                         | 2.5                                             | [49]      |
| Fe\textsuperscript{2}O\textsubscript{3} (NPs) | 50                            | CDW       | 15.3                      | 33      | 16.75                        | 102.5                                           | [17]      |
| Fe\textsuperscript{2}O\textsubscript{3} (NPs) | 200                           | DW        | 56                        | 23      | 7.85                         | 62.4                                            | [30]      |
| Fe\textsuperscript{2}O\textsubscript{3} (NPs) | 50                            | Wastewater| 110                       | 6.5     | 1.9                          | 49.4                                            | [50]      |
| Fe\textsuperscript{2}O\textsubscript{3} (NPs) | 200                           | MEG       | 4                         | 100     | 8.4                          | 0.6                                             | [51]      |
| Fe\textsuperscript{3}O\textsubscript{4} (NPs) | 300                           | CAS       | 10                        | 20      | 3.875                        | 1.92                                            | [52]      |
| Fe\textsuperscript{3}O\textsubscript{4} (NPs) | 200                           | Glucose   | 10                        | 20      | 9.2                          | 3.1                                             | [52]      |
| Fe\textsuperscript{3}O\textsubscript{4} (NPs) | 60                            | Glucose   | 6                         | 60      | 1.92                         | 2.5                                             | [49]      |
| Fe\textsuperscript{3}O\textsubscript{4} (NPs) | 10                            | Glucose   | 2.5                       | 100     | 10.1                         | 0.23                                            | [53]      |
| Fe\textsuperscript{3}O\textsubscript{4}(A-C-NPs) | 250                           | Glucose   | 5                         | 30      | 11.658                       | 3.2                                             | [38]      |
| Fe\textsuperscript{3}O\textsubscript{4}(A-C-NPs) | 1000                          | CO        | 1.008                     | 70      | 1.58                         | 0.0662                                          | [54]      |
| Magnetite (NPs) | 200                          | SJ        | 3                         | 50      | 6.7                          | 0.23                                            | [55]      |
| Hematite (NPs) | 200                          | Sucrose   | 12.5                      | 55      | 10.4                         | 6                                               | [56]      |

In this table, MEG refers to mono ethylene glycol, SC refers to substrate concentration, MSJ denotes Macroalgea Saccharina Japonica, NMBL refers to R. sphaeroides NMBL-02 and E. coli NMBL-04, MC refers to mixed consortia, BA refers to Bacillus anthracis PUNAJAN 1, CP refers to C. pasteurianum, EA refers to E. aerogenes ATCC13408, EC refers to E. cloacae, CI refers to Clostridium, Ca refers to C. acetobutylicum NCIM 2337, SJ refers to sugarcane juice, CAS refers to cassava starch.
4.1. Fe-Based Ions and Nanoparticles
Iron is an important trace element in the formation of hydrogenases and other enzymes. The pre-addition of Fe in the culture medium is a widely used strategy to enhance BioH2 production in dark fermentation [37]. As illustrated in Figure 2, first, Fe is the essential element to form the metal content at the active sites of hydrogenase ([FeFe], [FeNi], and [Fe]), thus catalyzing the reduction reaction of H+ to H2 [38]. Second, the presence of Fe-based NPs improves the activity of ferredoxin oxidoreductase by reducing the dissolved oxygen (DO) level and enhancing electron transfer due to the surface and quantum size effects [39,40]. In addition, Fe-based components could participate in enriching the microbial community and enhancing the growth of H2-producing bacteria [41]. The oxidative stress increases when there is a higher Fe concentration, which results in the formation of abundant oxidative radicals, thus leading to the deactivation or decomposition of enzymes [17,30].

4.2. Ni-Based Ions and Nanoparticles
Similarly, nickel ions or Ni-based nanoparticles are another widely studied substance that can significantly enhance BioH2 production in dark fermentation. The mechanisms between Ni-ion/Ni-based nanoparticles and Fe-ion/Fe-based nanoparticles are largely identical but with minor differences. The key mechanisms for Ni include (a) facilitating the synthesis of [FeNi] hydrogenase [42], (b) improving the activity of ferredoxin oxidoreductase [43], and (c) Ni NPs controlling the concentration of Ni2+ at the optimum level. In addition, it is worth noting that [NiFe] hydrogenase exists in more bacteria than [FeFe] hydrogenase. Therefore, Ni can promote H2-producing bacteria in the dark fermentation process to a certain extent [44].

5. Results
5.1. Impact of Fe-Based Ions and NP Addition
To quantitatively unveil the impact of the concentration of Fe-ion/Fe NPs and size effects upon the HY and HER in BioH2 generation, the collected values from the literature (Table 1) were statistically analyzed through our previously established ANN-RSM method and the results are shown in Figure 3.

The effects of NPs size and NPs concentration together with the binary combined impact upon the HY and HER were extensively explored. Regarding HY, it was found that the size of the NPs together with the concentration of NPs were both statistically significant to the H2 yield amongst the surveyed literature’s reports of experimental conditions. From Figure 3A, it is indicated that the HY tended to approach the highest value in the range of NP size (81–100 nm) and NP concentration (406–604 mg L\(^{-1}\)). For HER, it was found that the size of NPs, the concentration of NPs, and Fe\(^{2+}\)/Fe\(^{3+}\) were all significant to HER. For the combined effects (NP size and concentration), on the other hand, these effects were found to be statistically insignificant to HER. The 3D plot of HER versus NPs size and NPs concentration (Figure 3B) also tended to show the highest region of HER located at the size range of 81–100 nm. Among the collected literature reports, the HER seemed to be more appreciably and directly related to the relatively larger size of the particle, which might be quite contradictory to some findings. This indicates that the manipulation of NPs ideally in size range of 81–100 nm is favorable for both high HY and HER. Reducing the size of NPs could improve the quantum dot effect, thus improving the electron transport. In contrast, the electron transport phenomena in extracellular media during cultivation is quite complicated and some factors such as osmosis condition and the activity of the fermentation broth might be counter-effective to the nanoparticle size effect for enhancing BioH2 generation. Currently, very few works have been done to elucidate the mechanisms of this size impact upon selective enhancement of HY and HER. From our statistical analysis, a reasonable explanation for the ideal size effect is that the nanoparticle size of 81–100 nm is more thermodynamically stable than NPs with a smaller size during fermentation, since Fe-based NPs with smaller size are easier to agglomerate and form large Fe-based particles and deteriorate the electron transport performance in extracellular conditions. The fabrication of composites (e.g., Fe@graphene) is a promising strategy to enable the stable existence of small-sized nanoparticles; however, it has not been widely investigated.
5.2. Impact of Ni-Based Ions and NP Addition

The impact of Ni-based ions and NPs upon HY and HER is summarized in Table 2 and the statistical analysis results are shown in Figure 4.

Table 2. Comparison of BioH\textsubscript{2} production with the addition of Ni-based nanoparticles.

| Nanoparticles | Opt/mg L\textsuperscript{-1} | Substrate  | SC/g L\textsuperscript{-1} | Size/nm | HY/mmol g\textsuperscript{-1} | HER/mmol L\textsuperscript{-1}h\textsuperscript{-1} | Reference |
|---------------|-----------------|------------|-----------------|--------|-----------------|-------------------|----------|
| Ni (NPs)      | 5.7             | Glucose    | 14.01           | 13.6   | 14.1            | 11.5              | [57]     |
| Ni (NPs)      | 32              | Starch     | 8               | 80     | 2.4             | 10.3              | [18]     |
| Ni (NPs)      | 60              | MEG        | 4.7             | 60     | 1.11            | 1.5               | [23]     |
| Ni (NPs)      | 10              | Glucose    | 1               | 25     | 9.5             | 30                | [32]     |
| Ni (NPs)      | 1               | Glucose    | 2.5             | 100    | 11.7            | 0.28              | [53]     |
| Ni (NPs)      | 4.3             | Glucose    | 13.92           | 28     | 12.7            | 10.4              | [57]     |
| Ni (NPs)      | 2.5             | Glucose    | 5               | 42.5   | 10.8            | 1.3               | [58]     |
| Ni (NPs)      | 25              | Starch     | 10              | 40     | 2.7             | 11.5              | [18]     |
| Ni (NPs)      | 11              | Glucose    | 2.7             | 120    | 1.21            | 0.22              | [59]     |
| NiO (NPs)     | 20              | MEG        | 4               | 100    | 7.25            | 0.5               | [51]     |
| NiO (NPs)     | 10              | CDW        | 15.3            | 23     | 15.7            | 44.9              | [17]     |
| NiO (NPs)     | 1.5             | Wastewater | 9.6             | 23.6   | 0.5             | 12                | [31]     |
| Ni (NPs)      | 100             | CS         | 20              | 50     | 20              | 0.27              | [60]     |

In this table, MEG refers to mono ethylene glycol, CS: cornstalk.

Figure 4. Statistical analysis of HY and HER. (A) Particle size and nanoparticle concentration versus HY, (B) particle size and nanoparticles concentration versus HER.

Among the collected literature reports, the size and concentration of NPs together with their combined effect were not statistically significant to either HY or HER according to the calculated \textit{p}-value. Regarding HY (Figure 4A), it was found that both too low and too high levels of NPs size and concentration were not favorable. Indeed, an optimal range existed if the NP size and concentration were manipulated within 86–120 nm and (81–120 mg L\textsuperscript{-1}, respectively. Similarly, the HER also presented the same variation patterns as those of HY. An optimal range of particle size (86–120 nm) and Ni-ion/NPs concentration (81–120 mg L\textsuperscript{-1}) existed for HER. Unlike Fe, Ni presented more consistent responding patterns between HY and HER in regards to the variation in the size and concentration of NPs. In addition, studies have indicated that Ni-based ions and NPs tend to selectively enhance some BioH\textsubscript{2} generation pathways, such as enhancing the acetate pathway while suppressing or inhibiting butyrate and propionate pathways. However, discrepancies still exist due to different strains of microbes inoculated, cultivation medium, experimental
uncertainties, etc. Although the size of NPs was significant to the HER, the combined effects (NP size and concentration) were found to be insignificant. Among the collected literature reports, the HER seemed to be directly related to the relatively larger size of the particles. This indicates that the manipulation of NPs ideally in size range of 81–100 nm is favorable for both HY and HER. This might contradict the first impression that the reduction of NPs size significantly enhances the quantum dot effect that subsequently boosts electron transport. However, the preparation and large-scale deployment of small-sized NPs that can stably exist in the cultivation medium has always been a substantial challenge, which will inevitably increase fixing and operating costs. Fortunately, the enhancement of BioH$_2$ generation seems to be linked to an ideal range of NPs at the size of 81–100 nm; therefore, blindly pursuing small nanoparticles may be meaningless.

5.3. Impact of Other Metal and Non-Metal Nanoparticle Addition

The impact of other metal and non-metal NPs addition upon BioH$_2$ generation is summarized in Table 3.

| NPs       | Opt/mg L$^{-1}$ | Substrate | SC/g L$^{-1}$ | Size/nm | HY/mmol g$^{-1}$ | HER/mmol L$^{-1}$ h$^{-1}$ | Reference |
|-----------|-----------------|-----------|---------------|---------|------------------|----------------------------|-----------|
| Ag        | 0.002           | Glucose   | 12.5          | 15      | 13.8             | 10.5                       | [61]      |
| Cu        | 2.5             | Glucose   | 2.5           | 97      | 2.8              | 5.4                        | [62]      |
| Pd        | 5               | Glucose   | 10            | 100     | 8.1              | 6.7                        | [63]      |
| Au        | 0.002           | Sucrose   | 15            | 5       | 7.5              | 7.3                        | [64]      |
| Co        | 1               | Glucose   | 2.5           | 100     | 4.85             | 0.16                       | [53]      |
| CoO       | 1               | POME      | 76.5          | 17      | 22.5             | 0.7                        | [31]      |
| TiO$_2$   | 100             | Xylose    | 30            | 30      | 12               | 1.8                        | [47]      |
| ZnO       | 10              | MEG       | 4             | 100     | 7.3              | 0.58                       | [51]      |
| MgO       | 1               | Glucose   | 100           | 100     | 4.3              | 0.1                        | [53]      |
| Cu/SiO$_2$| 0.064           | Glucose   | 5             | 2.5     | 5.8              | 0.54                       | [65]      |
| Ag/SiO$_2$| 0.107           | Glucose   | 5             | 2.5     | 5.4              | 0.5                        | [65]      |
| Pd/SiO$_2$| 0.207           | Glucose   | 5             | 2.5     | 5.4              | 0.52                       | [65]      |

The addition of NPs was found to be effective at improving BioH$_2$ generation due to the fact that NPs can facilitate electron transport in extracellular cultivation medium during fermentation [66,67]. With regard to the HY and HER, it was quite hard to find one individual NPs that positively enhanced both HY and HER simultaneously. This reflects the complex features of the BioH$_2$ generation process, which generally involves many different steps of sub-metabolic pathways [43,68]. Among the investigated collected literature, CoO-NPs addition was among the most appreciable enhancement for HY and Ag-NPs addition was the most influential factor for HER enhancement. In addition, the impact of adding NPs prepared from hybrid approaches such as combining two different kinds of NPs, i.e., Cu and SiO$_2$, was marginal. The correlation between BioH$_2$ generation values (HER and HY) and the corresponding size of the NPs added to the fermentation broth was constructed and is plotted in Figure 5. The corresponding HY and HER varied from 0–30 (mmol g$^{-1}$) and 0–80 (mmol L$^{-1}$ h$^{-1}$), respectively. Regarding to the enhancement of HY, some reported that smaller size (less than 42 nm) surely increased HY from 10 to 20–25 mmol g$^{-1}$. On the other hand, for the enhancement of HER, some reported that a relatively bigger size of 40–50 nm seemed to significantly increase the H$_2$ evolution rate. However, by considering the numbers of reports, the majority of works showed (i) the size of NPs seems to be more effective in enhancing HY than HER, and (ii) the rate of H$_2$ evolution seems to be less responsive to the size of NPs, though some literature reported exceptionally higher values of HER after NPs (40–50 nm) addition.
The addition of NPs was found to be effective at improving BioH₂ generation due to different kinds of NPs, i.e., Cu and SiO₂, was marginal. The correlation between BioH₂ generation and HER BioH₂ generation, ions including Mg²⁺, Cu²⁺, Na⁺, NH₄⁺, and K⁺ were selected and all data are summarized in Table S1.

It is worth noting that some metal ions inevitably introduced into the culture medium due to the use of NPs addition are not in the scope of discussion. It was quite challenging to find out the detailed concentration ranges in each study due to the factor that many reports did not specify the detailed cultivation steps. Although this could be difficult for estimating the level of those ions during the cultivation, the type of defined and undefined cultivation media used in the studies could be utilized to indirectly estimate the range of those different ions accordingly. The level of different ions upon HY and HER BioH₂ generation are summarized in Tables S2 and 4, respectively, and the collected values from the literature were statistically analyzed through our previously established ANNs-RSM method.

By comparing the p-values, the impact of the variations in ion concentrations upon HY and HER of BioH₂ generation could be identified accordingly [16,69]. It was found that the variations in the investigated ions only statistically influenced HER, but not HY. This suggests pivotal guidance for process intensification for BioH₂ generation. The manipulations of ion concentrations in cultivation media can effectively improve or inhibit the rate but not the potential limit of BioH₂ generation. In other words, the kinetics of BioH₂ generation can be altered by varying some level of ionic concentration. The statistically significant impact of metal ion addition on HER is shown in Figure 6. Among the investigated ions, the single factor included Mg²⁺, Cu²⁺, and Na⁺ (Figure 6A,B) and the combined factor included Mg²⁺/Cu²⁺, Cu²⁺/Na⁺, Na⁺/NH₄⁺, Na⁺/K⁺, and NH₄⁺/K⁺ (Figure 6C–E) as the most influential factors for HER. The responding patterns of HER towards different kinds of ions appeared to be appreciably different. These effects can be broadly classified as counter-effective and synergistic. For instance, for the counter-effective impact, the binary Mg²⁺/Cu²⁺ belongs to this category, as does the binary NH₄⁺/K⁺ (Figure 6A,E). For the synergistic effect, the binary Cu²⁺/Na⁺, Na⁺/NH₄⁺, and Na⁺/K⁺ fall into this category (Figure 6B–D). These different ions will act as essential nutritious elements during metabolism at different stages of the growth of microbes [70–72]. For the growth pattern of microbes, there will normally be lagging, exponential, stationary, and death phases [73–75]. After inoculation, the microbes will experience a lagging phase with different duration [76,77]. The length of the lagging phase depends on many factors, such
as the harshness of cultivation media, which contains lignocellulosic precursors and high levels of salt concentration [78–80].

Table 4. ANOVA analysis for the effect of ion concentration upon HER.

| Source | Sum of Squares | DF | Mean Square | F-Value | p-Value |
|--------|----------------|----|-------------|---------|---------|
| Model  | 38,286.08      | 20 | 1914.30     | 4.16    | 0.0005  |
| A-Mg^{2+} | 2467.73      | 1  | 2467.73     | 5.36    | 0.0291  |
| B-Cu^{2+} | 1729.50      | 1  | 1729.50     | 3.75    | 0.0640  |
| C-Na^{+}  | 7543.84       | 1  | 7543.84     | 16.38   | 0.0004  |
| D-NH^{4+} | 496.57        | 1  | 496.57      | 1.08    | 0.3091  |
| E-K^{+}   | 261.49        | 1  | 261.49      | 0.56    | 0.4582  |
| AB       | 7903.35       | 1  | 7903.35     | 17.16   | 0.0003  |
| AC       | 1957.27       | 1  | 1957.27     | 4.25    | 0.0498  |
| AD       | 513.91        | 1  | 513.91      | 1.12    | 0.3009  |
| AE       | 1109.51       | 1  | 1109.51     | 2.41    | 0.1332  |
| BC       | 41.84         | 1  | 41.84       | 0.09    | 0.7656  |
| BD       | 330.26        | 1  | 330.26      | 0.71    | 0.4052  |
| BE       | 16.50         | 1  | 16.50       | 0.04    | 0.8514  |
| CD       | 4919.66       | 1  | 4919.66     | 10.68   | 0.0031  |
| CE       | 2100.83       | 1  | 2100.83     | 4.56    | 0.0427  |
| DE       | 1719.79       | 1  | 1719.79     | 3.73    | 0.0647  |
| A^2      | 801.66        | 1  | 801.66      | 1.74    | 0.1990  |
| B^2      | 3897.09       | 1  | 3897.09     | 8.46    | 0.0075  |
| C^2      | 2148.80       | 1  | 2148.80     | 4.67    | 0.0406  |
| D^2      | 387.39        | 1  | 387.39      | 0.84    | 0.3679  |
| E^2      | 1539.54       | 1  | 1539.54     | 3.34    | 0.0795  |
| Residue  | 11,515.22     | 25 | 460.61      |         |         |
| Lack of fit | 11,515.22    | 20 | 575.76      |         |         |
| Pure Error | 0.0000       | 5  | 0.0000      |         |         |
| Cor total | 49,801.31     | 45 |             |         |         |

In this table, r^2 = 0.94, adjusted r^2 = 0.93, predicted r^2 = 0.93, and adequate precision (AP) = 15.

The strategies for how to improve and shorten the length of the lagging phase will contribute to the improvement of the duration of the lagging phase [81]. For microbes to initiate their metabolism, elements such as Mg^{2+}, Na^{+}, NH_{4}^{+}, and K^{+} are essential [82–84]. These elements usually act as the major components of active centers in many enzymes [85–87]. Ensuring a sufficient amount of these necessary elements will facilitate the smooth and fast transition from the lagging phase to the growth phase [88–90]. It is commonly accepted that BioH_{2} generation will occur mainly in the exponential and stationary phases [91,92]. Clearly, these investigated literature reports provide useful guidance for the levels of these necessary ion elements in the cultivation media. More importantly, through statistical analysis from our developed ANN-RSM algorithm, the level of the response (the enhanced HER) was underpinned. In addition, the order of significance for HER was also identified as the following: Na^{+} > Mg^{2+} > Cu^{2+} > NH_{4}^{+} > K^{+}. From a holistic point of view, all the steps involved in BioH_{2} generation metabolism could be targeted as steps to enhance BioH_{2} generation (HY and HER). Two major metabolic pathways, namely, butyrate and acetate, are mainly associated with the activities of hydrogenase and the generation of H_{2} during dark fermentation [93–95]. From a stoichiometric perspective, the metabolic route towards acetate generates two times that of butyrate pathways [96,97]. From a process intensification point of view, the facilitation of the metabolic pathway towards an acetate pathway is favorable. From our statistical analysis, of all the investigated ions among the literature reports, the yield of BioH_{2} generation for these chemical additions of ions is not significant, suggesting that the enhancement of BioH_{2} generation by simple chemical additions of ions might be ineffective at further improving the ceiling value of BioH_{2} generation yield. For the sake of skewing the delicate balance between butyrate and acetate pathways, the combination of other chemical additions such as acti-
vated carbon, biochars, or porous adsorbents will be more effective in enhancing BioH₂ generation [98–100].

Figure 6. ANNs-RSM analysis of statically significant ion concentrations for HER. (A) Mg²⁺/Cu²⁺ nanoparticle concentration versus HER, (B) Na⁺/Mg²⁺ nanoparticle concentration versus HER, (C) Na⁺/NH₄⁺ nanoparticle concentration versus HER, (D) Na⁺/K⁺ nanoparticle concentration versus HER, (E) NH₄⁺/K⁺ nanoparticle concentration versus HER.

6. Conclusions

The statistical significance of these different NPs and ion additions were rigorously and quantitatively analyzed through a well-developed ANNs-RSM algorithm. As a result, this work provided effective guidance for the size optimization of NP additions and concentration regulation of ion additives in practice. For Fe-based NPs and ions, both the size of NPs and their corresponding concentration are statistically significant to HY. For HER, it was found that the combined effect of NP size and concentration is insignificant to HY. For Ni-based NPs and ions, neither size nor concentration is statistically significant to HY and HER, respectively. The variation in the size of NPs for the enhancement of
HY and HER behaved differently. The smaller (less than 42 nm) were found to definitely improve HY. Simultaneously, for HER, most reported literature indicated that manipulating the size of NPs is ineffective. It was found that variations in the investigated ions only statistically influenced HER, but not HY. This discovery suggests very pivotal guidance for process intensification for BioH₂ generation. Using the constructed algorithm, the level of responses (enhanced HER) towards inputs (other ion additions) was underpinned, and the order of significance towards HER was also identified as the following: Na⁺ > Mg²⁺ > Cu²⁺ > NH₄⁺ > K⁺. However, the number of relevant literature reports is currently limited; with the support of more experimental data, the results predicted by the ANNs-RSM algorithm will be more credible.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/en14185916/s1, Figure S1: Schematic diagram of methodology: (A) The procedures flowchart, (B) ANNs construction: feed forward three layers networks; Table S1: Ion comparison upon BioH₂ generation—refers to all data missing as, for convenience of calculation, the missing value was replaced by the averaged value during the artificial neuron network learning process; Table S2: ANOVA analysis for the effect of ion concentration on HY, where r² = 0.94, adjusted r² = 0.93, predicted r² = 0.93, and adequate precision (AP) = 15.

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Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ANOVA        | Analysis of variation |
| ANNs         | Artificial neural networks |
| ATR          | Auto-thermal reforming |
| BioH₂        | Biological hydrogen |
| BBD          | Box–Behnken design |
| CCSU         | Carbon capture storage and utilization |
| CCD          | Central composite design |
| DO           | Dissolved oxygen |
| GHE          | Greenhouse gas emission |
| H₂           | Hydrogen |
| HER          | Hydrogen evolution rate |
| HY           | Hydrogen yield |
| MSE          | Mean square error |
| NGSR         | Natural gas steam reforming |
| NGTC         | Nature gas thermal cracking |
| NPs          | Nanoparticles |
| RSM          | Response surface methodology |
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