Biohydrogen production using native carbon monoxide converting anaerobic microbial consortium predominantly Petrobacter sp.

Kannan Pakshirajan*, Joyabrata Mal

Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India

Abstract

In this study, anaerobic mixed microbial consortium isolated from a local sewage treatment plant in Guwahati, India, was used to convert carbon monoxide (CO) to hydrogen. The consortium was initially grown in acetate containing medium and later acclimatized to utilize CO as the sole carbon source for hydrogen production. By 16S rDNA analysis, the consortium was identified to be predominantly Petrobacter sp. Statistically designed experiments were then applied to optimize the CO conversion and hydrogen production by the anaerobic mixed consortium. To evaluate the significant factors that influenced the biohydrogen production, Plackett–Burman screening design of experiments was applied, which revealed that temperature and Fe²⁺ influenced the most on hydrogen production with P values less than 0.05 each. The effect due to pH and Ni²⁺ was less with P values 0.120 and 0.132, respectively. Concentration of Fe²⁺ and Ni²⁺ in the medium was then subsequently optimized by using Central Composite Design (CCD) of experiments followed by response surface methodology (RSM) which yielded the optimum value of 213 mg/L for Fe²⁺ and 2.2 mg/L for Ni²⁺. At these optimum conditions, 60.8 mol hydrogen production was achieved which was 8% higher than that observed from the screening experiment.

1. Introduction

Hydrogen (H₂) is one of the most promising sources of clean energy with a very good potential to replace fossil fuels mainly owing to its several technical, socio-economic and environmental advantages. Among the various hydrogen production methods, biological hydrogen production is environmentally favorable and consumes less energy than chemical or electrochemical methods [1]. Further, the discovery of anaerobic bacteria capable of performing the CO conversion to H₂ has opened up a new and an interesting alternative for the production of hydrogen [2]. Anaerobic bioconversion of CO is particularly interesting for CO-rich gases like synthesis gas (a mixture of CO, H₂ and CO₂) that can be obtained by steam reforming of natural gas or thermal gasification of a wide range of types of organic matter, including fossil as well as from biomass [3] or industrial and municipal solid (organic) waste [4]. However, the major limitation of synthesis gas utilization is the presence of CO, which can range from 5% to over 50% [44] and is toxic to most of these anaerobic microorganisms.

CO converting hydrogenogenic microorganisms are capable of utilizing CO as a sole source of carbon and energy and can produce equimolar amounts of CO₂ and H₂ via the water–gas-shift reaction (Eq. (1)) [5].

* Corresponding author. Tel.: +91 361 2582210; fax: +91 361 2690762.
E-mail address: pashki@iitg.ernet.in (K. Pakshirajan).
0360-3199/$ – see front matter Copyright © 2013, Hydrogen Energy Publications, LLC. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijhydene.2013.09.129

Please cite this article in press as: Pakshirajan K, Mal J, Biohydrogen production using native carbon monoxide converting anaerobic microbial consortium predominantly Petrobacter sp., International Journal of Hydrogen Energy (2013), http://dx.doi.org/10.1016/j.ijhydene.2013.09.129
CO + H₂O → CO₂ + H₂,  ∆G° = −20.0 kJ/mol  (1)

The reaction is also thermodynamically favorable to CO oxidation and H₂ synthesis since the equilibrium is strongly to the right of this reaction. Moreover, it is mostly operated at ambient temperature and pressure, thus making the process less energy intensive.

The number of species known to use CO for anaerobic respiration is, however, still limited. Rhodopseudomonas gelatinosa [6,7], later reclassified as Rhodococcus gelatinosa and more recently as Rubrivivax gelatinus was the first reported hydrogenogenic bacteria. Obligate anaerobic bacteria like Thermosinus carboxydivorans [8] and Carboxythermus hydrogenoformans [9] and facultative anaerobic bacteria like Rhodospirillum rubrum [10] and Citrobacter sp. Y19 S [11], are few among them. Thus, there is still a need to explore more potent microorganisms with CO converting capabilities allowing maximum hydrogen production and the optimization of this process. Screening of CO conversion in different anaerobic sludges from wastewater treatment reactors showed that CO conversion capability is ubiquitous in anaerobic bioreactor sludges both at ambient and elevated temperatures [5]. This work was, therefore, aimed at isolation of CO converting native hydrogenogenic microorganisms from a local wastewater treatment plant, its identification and optimization of parameters affecting the biohydrogen production process.

The efficiency of hydrogen production is greatly influenced by particularly nutritional and environmental parameters, such as temperature, pH, nutrient addition, metal ion and substrate concentration [12–16]. Conventional screening and optimization techniques are laborious and time-consuming which as well neither depict the interactive effects among the variables nor guarantee the determination of optimal conditions. On the contrary, statistically designed experiments are more effective and powerful in screening key factors from a multivariable system and optimizing fermentation conditions [17,18]. This method also saves time and yields meaningful results on the parameters effects on a given response of interest [19]. Recently, certain statistical optimization design techniques have been applied to study the effect of different factors on H₂ production and optimization of the process (Table 1).

| Table 1 – Literature reported hydrogen production values obtained using different biomass types and substrates. |
|-----------------|-----------------|-----------------|-----------------|
| Biomass         | Substrate       | H₂              | Reference       |
| Anaerobic sludge| Sucrose         | 17.572 g H₂/L   | [35]            |
| Clostridium sp. Fanp2 | Glucose        | 0.2 g H₂/L     | [36]            |
| Anaerobic sludge| Glucose         | 14.28 g H₂/L   | [37]            |
| Anaerobic sludge| Wheat powder    | 20.13 g H₂/L   | [38]            |
| Anaerobic sludge| Palm oil mill   | 0.02 g H₂/L    | [39]            |
| Anaerobic mixed cultures | Sweet sorghum effluent | 0.33 g H₂/L | [40]            |
| Enterobacter sp. CN1 | Xylose         | 21.4 g H₂/L   | [41]            |
| Digested dairy manure | Liquid swine | 7.8 g H₂/L    | [42]            |
| Klebsiella pneumonia ECU-15 | Glucose      | 0.256 g H₂/L | [16]            |
| Enterobacter sp. (MTCC 7104) | Xylose | 20.75 g H₂/L | [41]            |
| Anaerobic microbial consortium predominantly P. succinatimandens | CO | 64.4 g H₂/L | This study |

2. Materials and methods

2.1. Biomass source

Anaerobic microbial consortium capable of CO conversion in this study was enriched from sludge collected from a local sewage treatment plant at IIT Guwahati in Guwahati, India. The collected sludge was kept at 4 °C in an air-tight jar under anaerobic condition and later used as the seed sludge for CO conversion to hydrogen without any pretreatment.

2.2. Culture medium and enrichment of the anaerobic microbial consortium for CO conversion to H₂

The basal medium used for anaerobic culture of the sludge microorganisms was based on Stams et al. [45] with light modification [20]. The medium composition was (g/L): NaCl (0.3), NH₄Cl (0.2), CaCl₂·2H₂O (0.11), MgCl₂·6H₂O (0.1), KH₂PO₄ (0.1), FeCl₂ (0.945), CuCl₂ (0.013), ZnCl₂ (0.07), CoCl₂ (0.065), Na₂MoO₄ (0.021), MnCl₂ (0.63), NiCl₂ (0.13). The medium was supplemented with 0.5 g/L yeast extract as the nitrogen source and 2 g/L acetic acid as the carbon source.

The medium was buffered using 19.8 mM K₂HPO₄ and the pH was set initially to 7.0 by adding NaOH. For enrichment of the culture as well as for performing batch anaerobic experiments, serum bottles of 100 ml each (Sigma Aldrich, India) were used. Prior to inoculation, the media was purged with nitrogen gas to maintain oxygen free environment. The bottles were then incubated in an orbital incubator shaker set at 35 °C and 120 rpm. At the initial stage of acclimatization, acetic acid was used as the sole carbon source and later, during the acclimatization period and at the end of every batch incubation, it was replaced with CO in a stepwise manner by a simultaneous decrease in the initial acetic acid concentration and increase in the initial CO. This procedure was continued until the sludge microorganisms were capable of utilizing CO as the sole source of carbon and energy. Suitable controls were taken by incubating bottles containing only basal medium with acetate as the sole carbon source and the anaerobic mixed consortium as the inoculum.

Samples were taken at regular intervals during this fifty-day acclimatization period and analyzed for chemical oxygen demand (COD). The gas produced in the headspace was sampled using air-tight glass syringe (Hamilton 1000, Sigma Aldrich, India) and analyzed by gas chromatography (GC, Varian 450, Netherlands) to monitor the conversion of CO and the composition of the produced gas.

All chemicals used were of analytical grade and purchased from either Merck (India) or SRL (India). The CO (purity 99.9%) and N₂ (purity 99.9%) gases used were obtained from Vadilal...
nucleotides by online BLAST tool (http://www.ncbi.nlm.nih.gov). These sequences were further aligned with the closest matches found in the GenBank Database with the CLUSTALW function of MEGA version 5.2 [21]. The neighbor-joining phylogenetic tree was constructed to represent the relationship between the hydrogen producing strain PJ1 and related genera. A bootstrap analysis with 500 iterations was carried out to check the robustness of the tree.

The morphology of the hydrogen producing microbial community was analyzed using a scanning electron microscope (Zeiss Sigma, USA).

2.4. Screening and optimization of parameters for maximum hydrogen production by the CO converting anaerobic microbial consortium

2.4.1. Screening of parameters using Plackett–Burman design

Plackett–Burman experimental design consisting of a set of 13 experiments was used to determine the relative significance of six factors that influenced the hydrogen production by the CO converting anaerobic microbial consortium. The factors considered for this study included three physico-chemical variables (temperature, pH and inoculum volume), and three nutritional variables (initial concentration of Fe^{2+}, Ni^{2+}, and nitrogen to phosphate ratio (N/P)). Each factor level was coded as: −1 for low level and +1 for high level. According to this design, all the experimental runs were performed in duplicate. Table 2 presents the combination of each factor and their levels along with the responses (A and B) obtained in each of these experimental runs.

All the experiments were performed under batch conditions using serum bottles as mentioned before. For maintaining anaerobic condition, the bottles were purged with nitrogen gas following inoculation and incubated at different temperatures (as per the experiment design shown in Table 2) and under agitated condition (120 rpm).
2.4.2. Optimization of Fe\(^{2+}\) and Ni\(^{2+}\) employing Central Composite Design (CCD) followed by response surface methodology (RSM)

For achieving a maximum \(\text{H}_2\) production from CO, Central Composite Design (CCD) was employed by choosing initial Fe\(^{2+}\) and Ni\(^{2+}\) concentration as the significant variables in this study.

Response surface methodology (RSM) was then used to determine the optimum levels of these two variables (Fe\(^{2+}\) and Ni\(^{2+}\)) and their mutual interaction on hydrogen production by the anaerobic mixed consortium. All experiments in this optimization study were conducted as described earlier and by varying the initial levels of Fe\(^{2+}\) and Ni\(^{2+}\) in each experimental run as per the design (Table 3). The other variables were fixed at 20 °C temperature, pH 5, 5% v/v inoculum volume and N/P ratio of 5.

For statistical analysis purpose, the levels of the two variables were coded according to Eq. (2)

\[
X_i = \frac{x_i - x_i^c}{\Delta x_i}
\]

where, \(X_i\), \(x_i\), and \(x_i^c\) are the coded, uncoded and center point value of the \(i\)th test variable, respectively, and \(\Delta x_i\) is the step change. Thus, the experimental design consisted of a total of 11 experiments with three replicates at the center point level of the variables (Table 3).

The statistical software package Minitab version 16 (Minitab Inc., Pennsylvania, USA) was used for the experimental design and analysis of the results obtained.

2.5. Analytical methods

For COD analysis, a combination of reactor digestion method and colorimetric method was used which is equivalent to the Standard Method 5220D: Closed Reflux, Colorimetric Method [22]. In this method, samples were digested at 150 °C for 2 h using a digital reactor block (DRB 200, HACH, USA) with potassium dichromate (Cr\(^{7+}\)) to form green colored chromic ion (Cr\(^{3+}\)). After allowing the resultant mixture to cool down to room temperature, the amount of green colored Cr\(^{3+}\) complex formed was measured using a UV–Visible spectrometer (Cary 100, Varian, Australia) set at 600 nm wavelength.

The \(\text{H}_2\) gas produced in the headspace of the serum bottles used in the experiments was measured using a gas chromatograph (GC, Varian 450, Netherlands) equipped with a thermal conductivity detector (TCD) and a molecular sieve column (Mole sieve 5A, mesh 80/100, 6 ft × 1/8 in). Pure nitrogen gas was used as the carrier gas at a constant flow rate of 30 ml/min and the temperature of the injector, column and detector was 50 °C, 90 °C and 105 °C, respectively [23].

2.6. SEM analysis

For SEM analysis of the CO acclimatized anaerobic microbial consortium, a 50 µl sample of the biomass was taken and diluted to 50 ml with a 0.1 M phosphate buffer solution (pH 7.2). The diluted sample was filtered through 0.2 µm membrane filter, and the filtered biomass was fixed by treating with phosphate buffer solution containing 2.5% glutaraldehyde for 2 h. The fixed sample was then dehydrated in a stepwise manner using a graded series of water/ethanol solution and later critical point dried with carbon dioxide. Finally, the dried sample was sputter coated with gold prior to SEM analysis.

3. Results

3.1. Acclimatization of the anaerobic microbial consortium for CO utilization

Fig. 1 shows the time course of COD concentration in the media during the acclimation period, both in presence and in absence of CO. During the initial period of acclimatization and upon complete depletion of the initial COD in the media, acetic acid was added to check the efficiency of the consortium to further remove the added COD in the medium. The difference in COD removal efficiency of the culture in presence and in absence of CO is apparent from Fig. 1, which

![Fig. 1 – COD concentration and COD removal efficiency profiles during the acclimatization period. Symbols used: (●) COD concentration in presence of CO, (--) COD concentration in absence of CO and (--) COD removal efficiency.](image-url)
reveals that the COD removal from the media without CO is higher and reached a maximum value of 95.11%. Maximum COD removal efficiency of the culture in presence of CO was slightly less at 83.97% at the end of the 50-day acclimatization period. These results also suggest that in presence of CO the culture required more time to remove the initial COD in the media than that required in the absence of CO, which, however, needs to be ascertained by further experiments.

3.2. Isolation and identification of the predominant strain

The predominant hydrogenogenic microorganism in the CO acclimatized anaerobic microbial consortium yielded two distinct colonies on nutrient agar plate and these colonies were designated as strain PJ1 and PJ2. However, partial 16S rDNA sequencing of these two colonies yielded similar results with 1405 base pairs (bp) each indicating that these colonies could be from the same strain with a capability toward CO conversion to H₂. The sequence has been submitted to the Gene Bank public database with the accession number KF000349.1. Gene analysis by online BLAST tool indicated that the bacterial isolate contains sequence that is specific to the members of the β subdivision of the family Proteobacteria, more specifically the strain belongs to the member of the genus Petrobacter sp. and has closest relation (99%) to Petrobacter succinatimandens strain 4BON. The phylogenetic tree shown in Fig. 2 was prepared using neighbor-joining method based on near-full-length 16S rDNA gene sequences recovered from the isolated strain and other sequences obtained from the GenBank database.

SEM image of the acclimatized microbial consortium at different magnifications (Fig. 3a and b) revealed the presence of filamentous, long-rod shaped microorganisms confirming that the morphology of these microorganisms is due to P. succinatimandens [24].

3.3. Plackett–Burman screening of parameters affecting H₂ production

Table 2 clearly indicates that H₂ production by the anaerobic microbial consortium varied depending upon the combination level of the six factors. However, no clear understanding of the role played by these factors on the biohydrogen produced could be ascertained from this result. Therefore, statistical analysis of the results in the form of Analysis of Variance (ANOVA) and Student t-test was performed. ANOVA of the H₂ production presented in Table 4 indicates that the...
main effects of the factors in the regression model used to describe the system were highly significant (P < 0.01). This table also shows a term for error, the MS value of which indicates that the amount of variation in the response data that is left unexplained by the model is less confirming that the regression model used to explain the experimental data set is valid for further analysis of the results obtained. The t-value shown in Table 5 indicates a negative effect for all the five factors (temperature, inoculum volume, Fe\textsuperscript{2+}, Ni\textsuperscript{2+} and N/P) on hydrogen production, but only for pH it was positive. Further, the effect of temperature was the most significant (P < 0.01) followed by Fe\textsuperscript{2+} (P < 0.01), pH (P = 0.12) and Ni\textsuperscript{2+} (P = 0.132). Inoculum volume and N/P ratio did not show a significant effect on the biohydrogen produced as revealed by a very high P value (0.577 and 0.908, respectively). Pareto chart better illustrates these effects of the six different variables on H\textsubscript{2} production by the anaerobic microbial consortium in the study (Fig. 4). Although temperature showed the maximum effect on H\textsubscript{2} production, it was negatively significant with a maximum H\textsubscript{2} production value obtained at a low temperature of 20 °C. At a higher temperature, the production value decreased (Table 2). Fig. 4 as well reveals that the effect due to pH or nickel was almost similar, but the pH effect was positive compared to the negative effect shown by nickel (Table 5).

Based on these results, both Fe\textsuperscript{2+} and Ni\textsuperscript{2+} were chosen further for maximizing the H\textsubscript{2} production from CO using the anaerobic microbial consortium.

### 3.4. Optimization of Fe\textsuperscript{2+} and Ni\textsuperscript{2+} employing RSM

The results of H\textsubscript{2} production obtained by simultaneously varying the levels of Fe\textsuperscript{2+} and Ni\textsuperscript{2+} in this optimization study are shown in Table 3, which reveals optimum hydrogen production value at the center point value of these two variables. These results were subjected to ANOVA for a better understanding of the role played by these two factors on the biohydrogen production.

The ANOVA of the quadratic regression model used to describe the biohydrogen production indicated that the model was highly significant with an F value of 23.07 (Table 6). The determination coefficient (R\textsuperscript{2}) value of 0.958 due to the model also indicates that the quadratic regression model could explain up to 95.84% of the variability in the response. It also indicates the good agreement between the experimental and predicted values of the response. Further, the coefficients of linear and square terms for Fe\textsuperscript{2+} in the model are found to be significant on the hydrogen production with P value < 0.01. Coefficient of the squared effect due to Ni\textsuperscript{2+} also showed significant effect on H\textsubscript{2} production (P = 0.026). On the other hand, coefficient of linear effect due to Ni\textsuperscript{2+} and interaction effect between Fe\textsuperscript{2+} and Ni\textsuperscript{2+} did not show significant effect on the production of biohydrogen from CO (Table 6).

In order to further investigate the mutual interaction between Fe\textsuperscript{2+} and Ni\textsuperscript{2+} on hydrogen production, three-dimensional response surface and two-dimensional contour plots were obtained and are illustrated in Fig. 5. In general, the response surface plot (Fig. 5a) represents the effect of the two independent variables (Fe\textsuperscript{2+} and Ni\textsuperscript{2+}) and the shape of the corresponding contour plot (Fig. 5b) indicates whether the mutual interaction between these two variables is significant or not. As shown in Fig. 5a the response surface plot of hydrogen production indicates an optimum hydrogen production value over 60 mol. It also suggests that a significant increase in hydrogen production is achieved with an increase in concentration of Fe\textsuperscript{2+} from 50 mg/L to 188 mg/L. Above this value, the production slightly decreases suggesting some inhibitory effect due to Fe\textsuperscript{2+}. Ni\textsuperscript{2+} also showed inhibitory effect beyond its concentration at 2.89 mg/L. From the nature of the contours, whether elliptical, circular or saddle point, interaction between the variables may be predicted [25]. Thus, slightly elliptical nature of the response surface contours (Fig. 5b) indicates some significant interaction between the variables Fe\textsuperscript{2+} and Ni\textsuperscript{2+} which is consistent with the Student t-test of regression coefficients for H\textsubscript{2} production (Table 6).

From these results, the optimal values of Fe\textsuperscript{2+} and Ni\textsuperscript{2+} are found to be 189 mg/L of Fe\textsuperscript{2+} and 2.90 mg/L of Ni\textsuperscript{2+} and the corresponding maximum predicted value of hydrogen production is 62.96 mol. The predicted maximum was further confirmed by performing triplicate experiments at these optimum settings of Fe\textsuperscript{2+} and Ni\textsuperscript{2+}, which yielded 60.8 mol H\textsubscript{2}.
productions. The good correlation between these two results verifies the model validation and the existence of an optimal point [25].

4. Discussion

During the initial acclimation period, COD removal efficiency in presence of acetic acid in the media was consistently good (Fig. 1) mainly because acetate is a known carbon source that can be well utilized by anaerobic microorganisms [26]. At a later time period, when acetic acid was gradually replaced with CO, the COD removal profile (Fig. 1) showed that the culture required a slightly long time to acclimatize to this change in the substrate. This could be mainly attributed to the fact that CO is toxic to microorganism, particularly at a high concentration, even if it possesses the enzyme CODH required to utilize this substrate for its uptake [27].

The high bootstrap support of the phylogenetic tree shown in Fig. 2 derived from the 16S rDNA analysis demonstrated that strain PJ1 is a member of the β subdivision of the family Proteobacteria. R. gelatinosus was the first hydrogenogenic microorganism belonging to the β subdivision of the family Proteobacteria [7,28]. Although such members of the β subdivision of the family Proteobacteria have been reported to possess hydrogen production capacity from CO, this is the first study which reports Petrobacter sp. for hydrogen production from CO.

From the results of the Plackett–Burman screening study, temperature showed the most significant effect on H2 production from CO by the anaerobic microbial consortium (Fig. 4, Table 5), which is mainly due to the temperature dependent hydrogenase enzyme secreted by such microorganisms. It has been demonstrated that within a suitable range, an increase in the temperature can enhance the ability of this enzyme for the production of hydrogen. However, elevated temperature levels can be detrimental to the enzyme activity resulting in a reduced hydrogen production capability of these microorganisms [29,30]. In this study, lower temperature (20 °C) showed a highly positive effect on hydrogen production by the anaerobic microbial consortium, a feature which can be applied for converting CO to H2 under psychrophilic conditions (≤20 °C) where the main concern lies in avoiding an excess of hydrogen-utilizing methanogens (especially Methanospirillum) [31]. In fact, this is particularly true for this study because no methane was detected in the headspace of the culture bottles incubated at a lower temperature (20 °C) or even at a higher temperature (50 °C) (data not shown). This clearly indicated either the absence of methanogens in the CO converting anaerobic microbial consortium or inhibition of methanogens under these temperature conditions.

Iron and nickel are two well-known metals required for the activation or proper functioning of many enzymes and co-enzymes needed for normal cell growth and maintenance of anaerobic microorganisms [32]. In case of hydrogenogenic CO converting microorganisms, whereas hydrogenase can be classified into mainly two groups, i.e. [NiFe]-hydrogenases and [FeFe]-hydrogenases, CODH responsible for CO conversion is a Ni-containing enzyme. Therefore, these metals constitute the active site of [NiFe]-hydrogenase responsible for H2 production by the anaerobic microbial consortium. An increase in Fe2+ and Ni2+ concentration thus yielded an increase in the hydrogen production in the optimization study (Fig. 5). However, both these metals Fe2+ and Ni2+ at a much higher concentration were found to be detrimental to the H2 production (Fig. 5) probably due to their inhibitory effect on the anaerobic biomass particularly at their high concentrations tested in this study [14,32,33]. Moreover, iron is a fundamental component of ferredoxin which plays an important role in hydrogen production, and only within a suitable concentration range iron can increase H2 production by increasing the activity of hydrogenase. Thus, it is quite likely a much higher or lower concentration of iron does not result in a desired maximum activity of hydrogenase in the organism [13,34].

Table 6 – ANOVA of hydrogen production in the optimization study.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>5</td>
<td>1627.28</td>
<td>1627.28</td>
<td>325.53</td>
<td>23.07</td>
<td>0.002</td>
</tr>
<tr>
<td>Fe2+</td>
<td>1</td>
<td>245.14</td>
<td>980.04</td>
<td>980.04</td>
<td>69.45</td>
<td>0.000</td>
</tr>
<tr>
<td>Ni2+</td>
<td>1</td>
<td>58.63</td>
<td>45.70</td>
<td>45.70</td>
<td>3.24</td>
<td>0.132</td>
</tr>
<tr>
<td>Fe × Fe</td>
<td>1</td>
<td>1120.75</td>
<td>1290.43</td>
<td>1290.43</td>
<td>91.44</td>
<td>0.000</td>
</tr>
<tr>
<td>Ni × Ni</td>
<td>1</td>
<td>178.15</td>
<td>178.15</td>
<td>178.15</td>
<td>12.62</td>
<td>0.026</td>
</tr>
<tr>
<td>Fe × Ni</td>
<td>1</td>
<td>25.00</td>
<td>25.00</td>
<td>25.00</td>
<td>1.77</td>
<td>0.241</td>
</tr>
<tr>
<td>Residual error</td>
<td>5</td>
<td>70.56</td>
<td>70.56</td>
<td>14.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure error</td>
<td>2</td>
<td>6.33</td>
<td>6.33</td>
<td>3.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-Sq</td>
<td></td>
<td>95.84%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Fig. 5 – Interaction effect plot between Fe2+ and Ni2+ on hydrogen production in the optimization study (a) 3D response surface plot and (b) 2D contour plot.](image-url)
The maximum H₂ obtained at optimum Fe and Ni concentration was almost 8.0% higher than that obtained from the screening experiments, which clearly reveals the effectiveness of such non-conventional, statistically based design techniques in screening and optimization of variables affecting the biohydrogen production by the anaerobic microbial consortium.

Compared to the literature reports on H₂ production using different biomass types and substrates (Table 1), this study has shown much superior results. It has, therefore, revealed a very good potential for application in syngas conversion to H₂ at ambient temperature and without the need for any expensive chemical catalysts. However, more research on the effect of varying CO concentration, fermentative production of volatile fatty acids (VFA) such as acetic acid, butyric acid etc. and their effect on final H₂ yield from CO using this anaerobic microbial consortium still needs to be carried out to further establish its applicability.

5. Conclusions

Anaerobic mixed microbial consortium from a local wastewater treatment plant was able to produce H₂ and no methane from CO. The phylogenetic analysis of the predominant strain in the anaerobic consortium revealed a close relation (99%) with P. succinatimandens strain 4BON which is reported for the first time in microbial CO conversion to H₂. Among the various factors investigated for their effect on H₂ production by the anaerobic microbial consortium, temperature and Fe²⁺ were found to be the most significant. Under optimal concentration of 183 mg/L of Fe²⁺ and 2.67 mg/L of Ni²⁺, maximum hydrogen production obtained was 60.2 mol. From the results obtained in the study, the potential of the anaerobic mixed microbial consortium P. succinatimandens can be implicated further for syngas conversion to H₂.

Acknowledgments

The authors thankfully acknowledge the Department of Biotechnology and Centre for Energy, Indian Institute of Technology (IIT) Guwahati, India, for providing the necessary facility for carrying out this research work. The authors thank Dr. Sasidhar Gummna, Department of Chemical engineering, IIT Guwahati, for providing the CO gas in this study and Mr. Abhijit Sharma Roy, Centre for Environment, IIT Guwahati, for his help in DNA purification.

REFERENCES


