Research Article

Biohydrogen Production and Kinetic Modeling Using Sediment Microorganisms of Pichavaram Mangroves, India

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Mangrove sediments host rich assemblages of microorganisms, predominantly mixed bacterial cultures, which can be efficiently used for biohydrogen production through anaerobic dark fermentation. The influence of process parameters such as effect of initial glucose concentration, initial medium pH, and trace metal (Fe2+) concentration was investigated in this study. A maximum hydrogen yield of 2.34, 2.3, and 2.6 mol H2 mol−1 glucose, respectively, was obtained under the following set of optimal conditions: initial substrate concentration—10,000 mg L−1, initial pH—6.0, and ferrous sulphate concentration—100 mg L−1, respectively. The addition of trace metal to the medium (100 mg L−1 FeSO4⋅7H2O) enhanced the biohydrogen yield from 2.3 mol H2 mol−1 glucose to 2.6 mol H2 mol−1 glucose. Furthermore, the experimental data was subjected to kinetic analysis and the kinetic constants were estimated with the help of well-known kinetic models available in the literature, namely, Monod model, logistic model and Luedeking-Piret model. The model fitting was found to be in good agreement with the experimental observations, for all the models, with regression coefficient values > 0.92.

1. Introduction

Fossil Fuels are the primary energy source for the world’s increasing energy consumption. According to a recent survey, total world energy use rises from 524 quadrillion British thermal units (Btu) in 2010 to 630 quadrillion Btu in 2020 and to 820 quadrillion Btu in 2040 [1]. This fossil fuel eventually leads to foreseeable depletion due to limited energy resources; however, in the last few years, research and development activities pertaining to large-scale production of alternate resources of energy such as biodiesel, biohydrogen and bioethanol have risen [2–8]. In the days of fast depleting fossil fuel, biohydrogen has become a promising and viable energy source owing to its inherent advantages: zero-pollution, carbon-free, inexhaustible, recyclable, and highest energy density. However, most of hydrogen is currently produced from non-renewable sources using natural gas (50%), petroleum-derived naphthenes and distillates (30%), coal (18%), and electricity produced from variety of fuels (2%). Since this strategy leads to the depletion of non-renewable energy sources and is considered as a less ecofriendly alternative, it becomes crucial to go in for the production of sustainable energy source.

Biohydrogen production through anaerobic fermentation is a sustainable alternate for the energy crisis and green environment [9–12]. Fermentative hydrogen production processes are technically feasible and economically competitive and have large-scale commercialization possibilities [8, 13–16]. The present work focuses on biohydrogen production by dark fermentative approach using mangrove sediments of Pichavaram (located in Tamil Nadu, India). It is known that no research has been made using the sediments of mangroves, new mixed consortia to produce biohydrogen. Mangrove sediments are inherently rich in organic content [17–19]. The advantages of this sediment can be summarized as follows: flexible substrate utilization and the simplicity
of handling, no major storage problems, no problems with strain degradation, no preculturing required, and sediments are available at low cost.

A kinetic model can adequately describe the relationship among the different state variables and explain the behavior of fermentation quantitatively by providing useful information that can be subsequently used for analysis, design, and operation of any fermentation process [20–22]. The unstructured kinetic models are frequently employed for modeling microbial systems because they are simple, yet can provide useful information about the process [11, 23, 24]. In this study, three unstructured kinetic models, namely, Monod, logistic, and Luedeking-Piret models [25, 26] were used to determine the kinetic parameters.

2. Materials and Methods

2.1. Selective Enrichment on Biohydrogen Producing Mangrove Sediments. The sediments were collected from the mangrove rhizosphere of Pichavaram, Tamil Nadu, India, at a depth of 100 cm, and later stored in sterile polythene bags. Heat shock treatment was done on this sediment sample, by constant heating at 110 °C for 2 h, in order to stimulate spore germination and eliminate all vegetative cells, particularly methanogens. The coarse particles were removed using a stainless steel mesh, while the finer fractions were stored at 4 °C [27].

2.2. Nutrient Medium. The nutrient medium (non-sterilized) used in this study had the following chemical composition (per litre): NH₄Cl—0.5 mg, K₂HPO₄—0.25 mg, MgCl₂·6H₂O—0.3 mg, NiSO₄—0.016 mg, CoCl₂—0.025 mg, ZnCl₂—0.0115 mg, CuCl₂—0.0105 mg, CaCl₂—0.005 mg, and MnCl₂—0.015 mg.

2.3. Batch Experiments. Batch tests were conducted in duplicate, in 1 L Erlenmeyer flasks (working volume: 0.7 L), fitted air-tight with rubber septum, and adequately sealed using commercially available fix gels. The effect of process parameters on biohydrogen yield, namely, the influence of initial substrate concentration (glucose), initial pH, and trace metal, Fe³⁺ concentration, was evaluated by carrying out experiments at different low to high levels of these parameters, and the average values of biohydrogen yield were presented. The pH of the growth medium was adjusted using 1N HCl or 1N NaOH during the start of the experiments. The growth medium was inoculated with 100 g of pretreated sediment under aseptic conditions, and the flasks were incubated at 35 °C for fermentation.

2.4. Analytical Methods. The biohydrogen gas was measured using wet gas flow meter (Toshniwal, India). The gas content was analyzed using a gas chromatograph (Shimadzu, 221-70026-34, Japan) equipped with a thermal conductivity detector (TCD), and the column was packed with dual packed column. The operating temperatures of the column, detector and injector, were 40 °C, 80 °C, and 50 °C, respectively. Biomass concentration was measured as volatile suspended solid (VSS) and analyzed according to Standard Methods [28]. Glucose concentration was measured by DNS method using spectrophotometer (Elico, India) at a λ max of 550 nm [29]. The sludge granules were characterized using scanning electron microscope (SEM) (JEOL-JSM, 5300, Japan) at a resolution of 4.5 nm at 15 kV with a working distance of 8 mm.

3. Results and Discussion

Biohydrogen fermentation reached nearly constant values at the end of 120 h for each batch tests, including their duplicates. Glucose degradation efficiencies, cumulative biohydrogen gas, and hydrogen yields were calculated for each set of experimental condition.

3.1. Effect of Initial Glucose Concentration. For initial glucose concentrations of 4,000, 7,000, 10,000, 13,000, and 16,000 mg L⁻¹, the values of cumulative biohydrogen production and glucose degrading efficiencies were 430, 1190, 2600, 2200, and 2099 mL and 75, 83, 90, 80, and 72%, respectively (Figure 1). The effect of initial glucose concentration was observed when the initial medium pH was kept constant at 6.0 for all the test vials. It was observed that biohydrogen production increased with an increase in glucose concentration from 4,000 to 10,000 mg L⁻¹, and after that the biohydrogen production decreased with further increase in glucose concentration. A maximum biohydrogen yield of 2.34 mol H₂ mol⁻¹ glucose was obtained when initial glucose concentration was 10,000 mg L⁻¹. Furthermore, when initial glucose concentration was increased to 13,000 mg L⁻¹ and 16,000 mg L⁻¹, the hydrogen yield obtained was 2.02 and 1.46 mol H₂ mol⁻¹ glucose, respectively (Figure 1). The decrease in biohydrogen production at higher substrate concentrations might be due to the formation of more volatile fatty acids (data not shown here) which resulted in over-acidification of bacterial cultures, thereby reducing the medium pH, and thus inhibited fermentation. Several reports have shown that although high substrate concentrations showed high biohydrogen production initially, they tend to drop to low levels due to simultaneous acid inhibition, and increased partial pressure of hydrogen in the flask [30, 31]. Maintaining the carbon source levels at an optimum, in bioreactors, is an important parameter during pilot-scale trials and during the continuous production of biohydrogen. Failure to do so could affect the growth rate of the microorganism, its specific substrate utilization rate, enzyme activity, and overall yield of the process itself. Hence, to avoid the formation of volatile fatty acids and the phenomena of substrate inhibitions, the concentration of the substrate (glucose) in the liquid-phase must be maintained at optimal levels.

3.2. Effect of Medium pH. The profile of cumulative biohydrogen gas production at various initial medium pH conditions is shown in Figure 2. The optimum initial glucose concentration of 10,000 mg L⁻¹ was constantly maintained...
Figure 1: (a) Profile of cumulative biohydrogen production at various initial glucose concentrations. (b) Dynamic profile of glucose degradation, biomass concentration, and cumulative biohydrogen production. (c) Biohydrogen yield and glucose degradation efficiency for various initial glucose concentrations.

for these experiments. The substrate degradation efficiencies obtained were 83, 75, 80, 90, and 83%, respectively, at initial pH values of 4.5, 5.0, 5.5, 6.0, and 6.5. The final pH of these test vials at the end of the test period ranged from 1.9 to 3.4. The medium pH is an important operational parameter for hydrogen production, since it affects anaerobic pathways and the activities of hydrogenase enzymes [32]. When the initial medium pH was varied by keeping initial substrate concentration constant at 10,000 mg L$^{-1}$, the maximum hydrogen yield of 2.3 mol H$_2$ mol$^{-1}$ glucose was obtained at an initial pH of 6.0 (Figure 2). Initially, when the medium pH was at 4.5, the lowest hydrogen yield of 0.9 mol H$_2$ mol$^{-1}$ glucose obtained indicated that the higher acidic condition inhibited the fermentation. The hydrogen yield substantially increased to 2.3 mol H$_2$ mol$^{-1}$ glucose at the pH of 6.0. The hydrogen yield decreased to 2.0 mol H$_2$ mol$^{-1}$ glucose at a higher pH value (6.5). It was found that, under near neutral pH condition, a significant amount of substrates was consumed by bacterial growth other than hydrogen production which was verified by the higher biomass concentration at higher pH. Thus, it could be stated that the favourable pH for this mixed bacterial culture was 6.0. Similar results of maximum hydrogen production at the pH of 6.0 were reported [33].

3.3. Effect of Fe$^{2+}$ Concentration. Figure 3 illustrates the effect of fermentation time on the cumulative hydrogen production in batch tests under different Fe$^{2+}$ concentrations. The values of cumulative biohydrogen production for five different Fe$^{2+}$ concentrations: 100, 200, 300, 400, and 500 mg L$^{-1}$ were 3040, 2800, 2610, 2300, and 1180 mL, respectively, and the corresponding substrate degradation efficiencies were 94, 92, 91, 90, and 80%. Hydrogen yields of 2.6, 2.3, 2.1, 1.8, and 0.9 mol
H₂ mol⁻¹ glucose were obtained for various concentrations of iron as illustrated in Figure 3. At 100 mg L⁻¹ of Fe²⁺ concentration, the biohydrogen production was at its maximum (2.6 mol H₂ mol⁻¹ glucose), and it was found to decrease when the Fe²⁺ concentration was increased (Figure 3). Similar trend was obtained by previous researchers [34–36]. The addition/presence of Fe²⁺ concentration in the fermentation medium could influence the fermentative hydrogen production by influencing the activity of hydrogenase enzyme. The literature reports have shown that metal ions affect the microorganisms involved in hydrogen fermentation, beyond a threshold concentration range, and these effects include the
Table 1: Comparison of kinetic parameters for Monod model.

| Process            | Type of culture                      | Substrate              | $\mu_{\text{max}}$   | $K_s$        | $R^2$       | Author  |
|--------------------|--------------------------------------|------------------------|----------------------|--------------|-------------|---------|
| Batch              | Mixed anaerobic culture              | Sucrose                | 0.078 h$^{-1}$       | —            | —           | [26]    |
|                    | *Clostridium pasteurianum* CH4      |                        | 0.31 h$^{-1}$        | 4.39 g COD L$^{-1}$ | 0.935       | [37]    |
| Batch              | Mixed sludge                        | Glucose                | 0.03 g biomass/g biomass/day | —            | —           | [38]    |
| Sequential batch   | Activated sludge                    | Glucose                | 0.125 h$^{-1}$       | —            | —           | [40]    |
| Batch              | Acidogenic mixed culture             | Glucose                | 0.163 h$^{-1}$       | —            | —           | [41]    |
| Batch              | Acidogenic mixed culture             | Fructose               | 0.108 h$^{-1}$       | —            | —           | [41]    |
| Batch              | Anaerobic acclimatized banana stem sludge | Banana stem waste | 0.111 h$^{-1}$ | 0.330 g/L | 0.902       | [42]    |
| Batch              | Sediments of Pichavaram mangroves    | Glucose                | 0.166 h$^{-1}$       | 0.112 g/L    | 0.971       | Present study |

Table 2: Comparison of kinetic parameters of logistic model.

| Process            | Type of culture                      | Substrate          | $k$ (h$^{-1}$) | $R^2$       | Author  |
|--------------------|--------------------------------------|--------------------|----------------|-------------|---------|
| Batch              | *Rhodobacter sphaeroides*           | Malic acid        | 0.098          | 0.98        | [25]    |
| Batch              | Sludge                              | Glucose           | —              | 0.99        | [26]    |
| Batch              | Sediments of Pichavaram mangroves   | Glucose           | 0.034          | 0.943       | Present study |

following: decreased hydrogen production rate, an increase in lag-phase time, and formation of soluble microbial products [34].

3.4. Kinetics of Biohydrogen Production in Batch Culture

3.4.1. Cell Growth Kinetics as a Function of Substrate. Monod kinetics was applied to study the cell growth kinetics during biohydrogen production. Monod kinetics is given by the following equation:

$$\mu = \frac{1}{\mu_{\text{max}}} \frac{d\mu}{dt} = \frac{\mu_{\text{max}}}{K_s + S}$$

(1)

where $\mu$ is the specific growth rate (h$^{-1}$), $\mu_{\text{max}}$ is the maximum specific growth rate (h$^{-1}$), $x$ is the cell concentration (gL$^{-1}$), and $K_s$ is the substrate consumption rate constant (gL$^{-1}$).

Equation (1) may be linearized, as shown in (2) to estimate the kinetic parameters, and regression analysis is used to find the best fit for a straight line on a plot of $1/\mu$ versus $1/S$ to determine the values of $\mu_{\text{max}}$ and $K_s$ (Figure 4):

$$1 = \frac{K_s}{\mu_{\text{max}}} \cdot \frac{1}{S} + \frac{1}{\mu_{\text{max}}}.$$  

(2)

Table 1 shows the different values of kinetic parameters obtained from Monod model, while Figure 4 shows the correlation between the model fitted and experimental values. The $\mu_{\text{max}}$ and $K_s$ were calculated as 0.166 h$^{-1}$ and 0.112 gL$^{-1}$ respectively.

3.4.2. Cell Growth Rate as a Function of Cell Concentration.
The specific growth rate for the logistic curve relates the change of specific growth rate with respect to change in cell concentration ($x$). The Riccati equation is given by the following equation:

$$\frac{dx}{dt} = kx (1 - \beta x),$$  

(3)

where $\beta = 1/\mu_{\text{max}}$.

On integrating and applying the limits,

$$\int_{x_0}^{x} \frac{dx}{x(1 - \beta x)} = k \int_{0}^{t} dt,$$

(4)

$$e^{-k t} = \frac{x (1 - \beta x_0)}{x_0 (1 - \beta x)}.$$  

Rearranging the above equation, cell concentration $x$ is given by

$$x = \frac{x_0 e^{k t}}{1 - \beta x_0 (1 - e^{k t})},$$  

(5)

$x_{\text{max}}$ and $k$ kinetic parameters are calculated using logistic curve.

However, for the purposes of batch hydrogen production experiments, where the initial substrate concentrations and the inoculation volume are kept constant, the logistic model is only a fair approximation of the growth curve. From Figure 5, kinetic parameters were estimated and their values were as follows: $k = 0.061$ h$^{-1}$; $x_{\text{max}} = 30.74$ gVSS L$^{-1}$. Table 2 shows the comparison of different kinetic parameters for the logistic model. The experimental and model fitted specific growth rates were significant with high regression coefficient values. From Figure 5, it could be inferred that the model performed well during the simulation of batch reactors performance, with respect to the glucose and biomass concentration.
\[ \mu_{\text{max}} = 0.166 \text{ h}^{-1} \]
\[ K_s = 0.112 \text{ g VSS L}^{-1} \]

\[ \frac{1}{\mu} = \frac{1}{S} \]

\[ R^2 = 0.976 \]

**Figure 4**: Monod model for substrate utilization kinetics.

\[ k = 0.034 \text{ h}^{-1} \]

\[ x_{\text{max}} = 30.74 \text{ g VSS/L} \]

\[ R^2 = 0.9431 \]

**Figure 5**: Logistic model for cell growth kinetics.
### Table 3: Comparison of kinetic parameters of Luedeking-Piret model.

| Process       | Type of culture               | Substrate    | $Y_{P/x}$ | $R^2$   | Author      |
|---------------|-------------------------------|--------------|-----------|---------|-------------|
| Batch         | Clostridium butyricum CGS5    | Xylose       | 0.041     | 0.910   | [37]        |
| Batch         | Mixed microflora              | Wheat stalk  | —         | >0.855   | [43]        |
| Batch         | Sediments of Pichavaram mangroves | Glucose     | 11.04     | 0.999   | Present study |

![Graph](image1.png)  

**Figure 6:** Luedeking-Piret model for product formation kinetics.

3.4.3. Cell Growth Rate as a Function of Product Formation.  
The Luedeking-Piret model shown in (6) has been widely used to describe the relationship between hydrogen producing bacterial growth rate and product formation rate:

$$\frac{dp}{dt} = Y_{P/x} \frac{dx}{dt} + \beta x, \quad (6)$$

where $dp/dt$ is the product formation rate (h$^{-1}$), $dx/dt$ is the specific growth rate (h$^{-1}$), $P$ is the product (biohydrogen production), $x$ is the cell concentration (g L$^{-1}$), $Y_{P/x}$ is the growth associated product yield coefficient, and $\beta$ is the non-growth associated product yield coefficient.

Table 3 shows the values of different kinetic parameters estimated for this model. A plot of specific growth rate versus product formation rate, as shown in Figure 6, indicates that hydrogen is purely a growth associated product. The growth associate product yield coefficient ($Y_{P/x}$) was calculated by plotting specific hydrogen production rate versus specific growth rate, and the value was found to be 11.04. From Figure 6, it could be inferred that the model performed well with $R^2$ value of 0.999.

3.4.4. Microscopic Examination of Hydrogen Producing Granule. Scanning electron micrographs showed that the granules had multiple cracks with cavities on the surface (Figure 7). These cavities were likely to facilitate the passage of nutrients and substrate as well as the release of hydrogen. Bacterial cells were distributed all over the granules.

Furthermore, considering the practicality of this research work, microbiological analyses are warranted at this stage to characterize the dominant anaerobic consortium responsible for biohydrogen production. In general, kinetic models are applied in order to study and assess the metabolic features of defined cultures. Further studies in this field should be aimed at the following aspects: optimization studies with different inocula, substrates and process parameters, evaluation of the performance, and economics of a continuous biohydrogen production processes (bioreactors).

4. Conclusions  
The results from batch tests showed that initial substrate (glucose) concentration, medium pH, and Fe$^{2+}$ concentration had influence on the biohydrogen yield. Maximum biohydrogen yields were found to be 2.34, 2.3, and 2.5 mol H$_2$ mol$^{-1}$ glucose at the following conditions: initial substrate concentration—10,000 mg L$^{-1}$, medium pH—6.0, and Fe$^{2+}$ concentration—100 mg L$^{-1}$, respectively. The addition of trace metal to the medium at a concentration of 100 mg L$^{-1}$ was found to enhance biohydrogen production although higher metal ion concentrations reduced biohydrogen production. The kinetics of batch anaerobic hydrogen production was estimated by fitting the experimental data
to the well-known unstructured kinetic models. The Monod model, logistic model, and Luedeking-Piret model were used to describe the kinetics of cell growth rate as a function of substrate, cell concentration, and product formation, respectively, in the hydrogen production process, and the corresponding kinetic constants were estimated. The results showed that high regression co-efficient values ($R^2$) were obtained between the model fitted and the experimental observations for the different models, namely, as 0.976, 0.943, and 0.999, respectively.

Nomenclature

- $\mu$: Specific growth rate (h$^{-1}$)
- $\mu_{\text{max}}$: Maximum specific growth rate (h$^{-1}$)
- $x$: Microbial concentration (g VSS L$^{-1}$)
- $x_0$: Initial microbial concentrations (g VSS L$^{-1}$)
- $K_s$: Substrate consumption rate (g L$^{-1}$)
- $k$: Apparent specific growth rate (h$^{-1}$)
- $x_{\text{max}}$: Maximum microbial concentration (g VSS L$^{-1}$)
- $P$: Cumulative biohydrogen production (mL)
- $Y_{\text{p/x}}$: Growth associate product yield coefficient
- $\beta$: Non-growth associated product yield coefficient.

Conflict of Interests

The authors declare that there is no conflict of interests.

Authors’ Contribution

The authors of this research article contributed to a similar extent overall and agreed to submit the paper.

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References

[1] International energy outlook, “U.S. Energy Information Administration,” pp. 300, 2013.
[2] D. B. Levin, L. Pitt, and M. Love, “Biohydrogen production: prospects and limitations to practical application,” International Journal of Hydrogen Energy, vol. 29, no. 2, pp. 173–185, 2004.
[3] S. Venkata Mohan, Y. Vijaya Bhaskar, and P. N. Sarma, “Biohydrogen production from chemical wastewater treatment in biofilm configured reactor operated in periodic discontinuous batch mode by selectively enriched anaerobic mixed consortia,” Water Research, vol. 41, no. 12, pp. 2652–2664, 2007.
[4] M. T. Skonieczny, Biological hydrogen production from industrial wastewater with Clostridium beijerinckii [M.S. dissertation], McGill University, 2008.
[5] G. E. Diwani, N. K. Attia, and S. I. Hawash, “Development and evaluation of biodiesel fuel and by-products from jatropha oil,” International Journal of Environmental Science and Technology, vol. 6, no. 2, pp. 219–224, 2009.
[6] H. Le Man, S. K. Behera, and H. S. Park, “Optimization of operational parameters for ethanol production from korean food waste leachate,” International Journal of Environmental Science and Technology, vol. 7, no. 1, pp. 157–164, 2010.
[7] H. N. Abubackar, M. C. Veiga, and C. Kennes, “Biological conversion of carbon monoxide: rich syngas or waste gases to bioethanol,” Biofuels, Bioproducts and Biorefining, vol. 5, no. 1, pp. 93–114, 2011.
[8] P. Mullai, M. K. Yogeswari, and K. Sridevi, “Optimisation and enhancement of biohydrogen production using nickel nanoparticles—a novel approach,” Bioresource Technology, vol. 141, pp. 212–219, 2013.
[9] P. Mullai, K. Sampath, and P. L. Sabarathinam, “Kinetic models anaerobic digestion of penicillin-G wastewater,” Chemical Engineering World, vol. 38, no. 12, pp. 161–164, 2003.
[10] P. Mullai, S. Arulselvi, H.-H. Ngo, and P. L. Sabarathinam, “Experiments and ANFIS modelling for the biodegradation of penicillin-G wastewater,” Bioresource Technology, vol. 100, pp. 4085–4091, 2009.
of penicillin-G wastewater using anaerobic hybrid reactor,” *Bioresource Technology*, vol. 102, no. 9, pp. 5492–5497, 2011.

[11] P. Mullai, H. H. Ngo, and P. L. Sabarathinam, “Substrate removal kinetics of an anaerobic hybrid reactor treating pharmaceutical wastewater,” *Journal of Water Sustainability*, vol. 1, no. 3, pp. 301–312, 2011.

[12] R. Kothari, D. P. Singh, V. V. Tyagi, and S. K. Tyagi, “Fermentative hydrogen production—an alternative clean energy source,” *Renewable and Sustainable Energy Reviews*, vol. 16, no. 4, pp. 2337–2346, 2012.

[13] F. R. Hawkes, R. Dinsdale, D. L. Hawkes, and I. Hussy, “Sustainable fermentative hydrogen production: challenges for process optimisation,” *International Journal of Hydrogen Energy*, vol. 27, no. 11-12, pp. 1339–1347, 2002.

[14] N. Ren, J. Li, B. Li, Y. Wang, and S. Liu, “Biogas hydrogen production from molasses by anaerobic fermentation with a pilot-scale bioreactor system,” *International Journal of Hydrogen Energy*, vol. 31, no. 15, pp. 2147–2157, 2006.

[15] S. Robaire, *Biological hydrogen production using Citrobacter amalonaticus Y19 to catalyze the water gas shift reaction* [M.S. dissertation], McGill University, 2006.

[16] K. Vijayaraghavan and M. A. M. Soom, “Trends in biohydrogen generation—a review,” *Environmental Sciences*, vol. 3, pp. 255–271, 2006.

[17] K. Kathiresan, “A review of studies on Pichavaram mangrove, Southeast India,” *Hydrobiologia*, vol. 430, no. 1–3, pp. 185–205, 2000.

[18] K. Kathiresan, “Why are mangroves degrading?” *Current Science*, vol. 83, no. 10, pp. 10–25, 2002.

[19] D. Zhu, G. Wang, H. Qiao, and J. Cai, “Fermentative hydrogen production by the new marine Pantoea agglomerans isolated from the mangrove sludge,” *International Journal of Hydrogen Energy*, vol. 33, no. 21, pp. 6161–6123, 2008.

[20] J. Wang and W. Wan, “Kinetic models for fermentative hydrogen production: a review,” *International Journal of Hydrogen Energy*, vol. 34, no. 9, pp. 3313–3323, 2009.

[21] R. Zhao, H. Liu, H. Hu et al., “A fundamental research on combustion chemical kinetic model’s precision property,” *Science China Technological Sciences*, vol. 53, no. 8, pp. 2222–2227, 2010.

[22] P. Mullai, E. R. Rene, H. S. Park, and P. L. Sabarathinam, “Adaptive network based fuzzy interference system (ANFIS) modeling of an anaerobic wastewater treatment process,” in *Handbook of Research on Industrial Informatics and Manufacturing Intelligence: Innovations and Solutions*, M. A. Khan and A. Q. Ansari, Eds., pp. 252–270, IGI, New York, NY, USA, 2012.

[23] J. E. Bailey and D. F. Ollis, *Biochemical Engineering Fundamentals*, Tata McGraw-Hill, New Delhi, India, 1986.

[24] K. Nath and D. Das, “Modeling and optimization of fermentative hydrogen production,” *Bioresource Technology*, vol. 102, no. 18, pp. 8569–8581, 2011.

[25] H. Koku, I. Eroğlu, U. Gündüz, M. Yücel, and L. Türker, “Kinetics of biological hydrogen production by the photosynthetic bacterium Rhodobacter sphaeroides O.U. 001,” *International Journal of Hydrogen Energy*, vol. 28, no. 4, pp. 381–388, 2003.

[26] Y. Mu, G. Wang, and H.-Q. Yu, “Kinetic modeling of batch hydrogen production process by mixed anaerobic cultures,” *Bioresource Technology*, vol. 97, no. 11, pp. 1302–1307, 2006.

[27] J. Niessen, F. Harnisch, M. Rosenbaum, U. Schröder, and F. Scholz, “Heat treated soil as convenient and versatile source of bacterial communities for microbial electricity generation,” *Electrochemistry Communications*, vol. 8, no. 5, pp. 869–873, 2006.

[28] APHA, *Standard Methods for the Examination of Water and Wastewater*, American Public Health Associations, New York, NY, USA, 1995.

[29] G. L. Miller, “Use of dinitrosalicylic acid reagent for determination of reducing sugar,” *Analytical Chemistry*, vol. 31, no. 3, pp. 426–428, 1959.

[30] J. Wang and W. Wan, “The effect of substrate concentration on biohydrogen production by using kinetic models,” *Science in China B*, vol. 51, no. 11, pp. 1110–1117, 2008.

[31] W. M. Alalayah, M. S. Kalil, A. A. H. Kadhum, J. M. Jahim, and N. M. Alauji, “Effect of environmental parameters on hydrogen production using Clostridium saccharoperbutylacetonicum Nl-4 (ATCC 13564),” *American Journal of Environmental Sciences*, vol. 5, no. 1, pp. 80–86, 2009.

[32] S. K. Khanal, W.-H. Chen, L. Li, and S. Sung, “Biological hydrogen production: effects of pH and intermediate products,” *International Journal of Hydrogen Energy*, vol. 29, no. 11, pp. 1123–1131, 2004.

[33] M. Ferchichi, E. Crabbe, G.-H. Gil, W. Hintz, and A. Almadidy, “Influence of initial pH on hydrogen production from cheese whey,” *Journal of Biotechnology*, vol. 120, no. 4, pp. 402–409, 2005.

[34] Y. J. L. Young Joon Lee, T. Miyahara, and T. Noike, “Effect of iron concentration on hydrogen fermentation,” *Bioresource Technology*, vol. 80, no. 3, pp. 227–231, 2001.

[35] H. Yang and J. Shen, “Effect of ferrous iron concentration on anaerobic bio-hydrogen production from soluble starch,” *International Journal of Hydrogen Energy*, vol. 31, no. 15, pp. 2137–2146, 2006.

[36] J. Wang and W. Wan, “Effect of Fe2+ concentration on fermentative hydrogen production by mixed cultures,” *International Journal of Hydrogen Energy*, vol. 33, no. 4, pp. 1215–1220, 2008.

[37] Y.-C. Lo, W.-M. Chen, C.-H. Hung, S.-D. Chen, and J.-S. Chang, “Dark H2 fermentation from sucrose and xylose using H2-producing indigenous bacteria: feasibility and kinetic studies,” *Water Research*, vol. 42, no. 4–5, pp. 827–842, 2008.

[38] Y. Sharma and B. Li, “Optimizing hydrogen production from organic wastewater treatment in batch reactors through experimental and kinetic analysis,” *International Journal of Hydrogen Energy*, vol. 34, no. 15, pp. 6171–6180, 2009.

[39] P. Kongian, B. Min, and I. Angelidaki, “Biohydrogen production from xylose at extreme thermophilic temperatures (70°C) by mixed culture fermentation,” *Water Research*, vol. 43, no. 5, pp. 1414–1424, 2009.

[40] F. J. Fernández-Morales, J. Villaseñor, and D. Infantes, “Modeling and monitoring of the acclimatization of conventional activated sludge to a biohydrogen producing culture by biokinetic control,” *International Journal of Hydrogen Energy*, vol. 35, no. 20, pp. 10927–10933, 2010.

[41] F. J. Fernández, J. Villaseñor, and D. Infantes, “Kinetic and stoichiometric modelling of acidogenic fermentation of glucose and fructose,” *Biomass and Bioenergy*, vol. 35, no. 9, pp. 3877–3883, 2011.

[42] N. Zainol, “Kinetics of biogas production from banana stem waste,” in *Biogas*, S. Kumar, Ed., p. 408, InTech, Europe, 2012.

[43] X. Yuan, X. Shi, P. Zhang, Y. Wei, R. Guo, and L. Wang, “Anaerobic biohydrogen production from wheat stalk by mixed microflora: kinetic model and particle size influence,” *Bioresource Technology*, vol. 102, no. 19, pp. 9007–9012, 2011.