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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Cell growth and product formation kinetics of biohydrogen production using mixed consortia by batch process

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Abstract: The cell growth and product formation kinetics of anaerobic biohydrogen production by mixed anaerobic cultures was investigated using unstructured models in the present study. They described the realtionship between biomass growth and product formation in the hydrogen production process. Experimental results show that the Logistic model and Leudeking- Piret model could be adopted to describe the kinetics of biomass growth and product formation respectively. The kinetic parameters were $K_c = 0.013\ h^{-1}$, simulated $X_{max} = 2.05$ gVSS/L and $Y_{P/X} = 0.793$. The experimental values of kinetic constants were in good agreement with simulated values with $R^2 > 0.90$. Logistic model and Luedeking–Piret model fitted well for the batch system.
Keywords: Biohydrogen- Batch process- Unstructured models- Logistic model-Luedeking–Piret model

Introduction

Energy is crucial for fuelling the growth rate in the near future. The crude oil consumers are United States, with 21%, China with 10.6%, Japan with 5%, and India with 3.9% and Russian Federation with 3.7%[1]. While motivated to bridge our energy deficit, there is need to increase the share of clean, sustainable, new and renewable energy sources. Renewable energy completely replaces fossil fuel that includes wind energy, solar energy, biomass power. Burning the biomass is the easiest, least efficient and oldest method of generating energy. Biohydrogen production is a recent research for alternate for fossil fuels[2-5]. Hydrogen is a high-energy yield which is 2.4, 2.8 and 4 times higher than energy yields of methane, gasoline and coal respectively. A kinetic model is used to illustrate the relationship between the different state variables with useful information[6]. It can be used for analysis, design, and operation of fermentation process[7,8]. Initial COD and biomass concentration determine the metabolic and kinetic characteristics of microorganisms. Several unstructured kinetic models are used for modelling biohydrogen production in a batch system[9-11]. The effect of substrate degradation, substrate inhibition, temperature, biomass concentration and pH were accounted for studying the models. In this study, unstructured kinetic models logistic and Luedeking-Piret models were used to determine the cell growth kinetics and product formation kinetics during the biohydrogen production using anaerobic sludge by batch process.

Materials and Methods

Inoculum

The sludge was collected from Tamilnadu, India and was used as inoculum. One litre of growth medium was prepared using the following composition (g/L). NH_4Cl - 0.5 g; K_2HPO_4 - 0.25 g ; $MgCl_2.6H_2O$ -

0.3 g; $CoCl_2$-0.025 g; $ZnCl_2$-0.0115 g; $CuCl_2$-0.0105 g; $CaCl_2$-0.005 g and $MnCl_2$ - 0.015g; $FeCl_2$ – 0.125[2]. The anaerobic sludge was pre-treated by heat treatment at 110 °C for 1h.

Batch experiment

Batch tests were performed in 1 l Erlenmeyer flasks with a working volume of 700 mL. The initial substrate concentration was varied from 5000 mg COD/L to 40000 mg COD/L. Glucose was used a substrate. The pH was adjusted using 1N HCl or 1N NaOH. The growth medium was inoculated with 500 mg VSS/L of heat pre-treated anaerobic sludge under aseptic conditions, and the flasks were incubated at 35ºC for fermentation.

Analytical methods

Glucose concentrations were estimated by DNS method at a λ_{max} of 550 nm[12]. Biomass concentrations were measured as volatile suspended solids (VSS) and were analyzed according to Standard Methods[13]. The biohydrogen gas was measured using wet gas flowmeter (Toshniwal, India). The gas content was analyzed using a gas chromatograph (Shimadzu, 221-70026-34, Japan) equipped with a thermal conductivity detector (TCD). The column was packed with dual packed column. The hydrogen producing granules were characterized using scanning electron microscope (SEM) (JEOL-JSM, 5300, Japan) at a resolution of 4.5 nm at 15 kVA with a working distance of 8 mm.

Results and Discussion

Physico- chemical parameters of anaerobic sludge

The anaerobic sludge contained high organic content and high microbial biomass. The characteristics of the sludge were observed as follows: colour – dark brown; pH- 4.3; COD (mg/L) - 6838 mg/l; total solids (TS) - 1, 00,000 mg/L, VSS - 46,879 mg/L. SEM image of anaerobic sludge is shown in Fig.1.

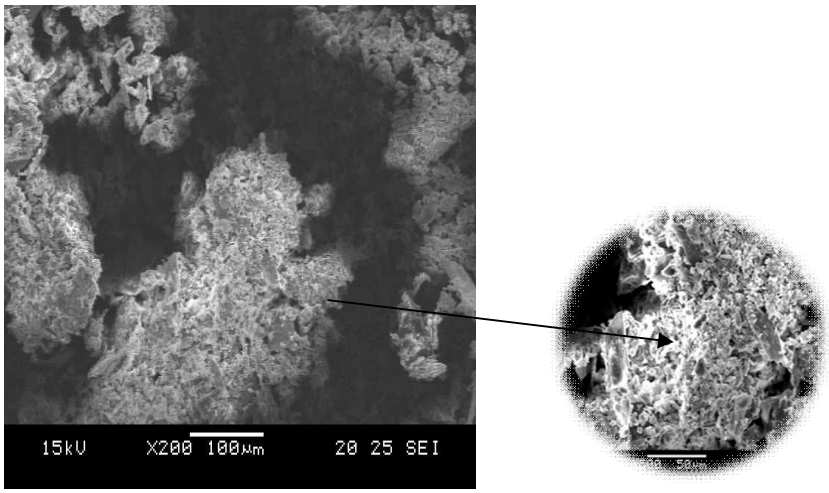

Fig. 1 SEM image of anaerobic sludge

Biohydrogen production

The biohydrogen production started after 24 h. Fig. 1 showed the profile of cumulative biohydrogen production at constant pH 5.5. At different initial substrate concentrations, 5000, 10000, 20000, 30000 and 40000 mg L^{-1}, the corresponding cumulative biohydrogen production were 900, 2850, 2750, 2600 and 2500 mL, respectively. The VSS concentrations at corresponding initial substrate concentrations were 600, 1500, 8200, 24200 and 17600 mg/L (Fig.2). Thus maximum cumulative biohydrogen production of 2850 mL was obtained when the initial substrate concentration was 10000 mg/L. The initial substrate concentrations, VSS concentrations and cumulative biohydrogen production were used to evaluate the kinetic parameters in cell growth and product formation kinetics.

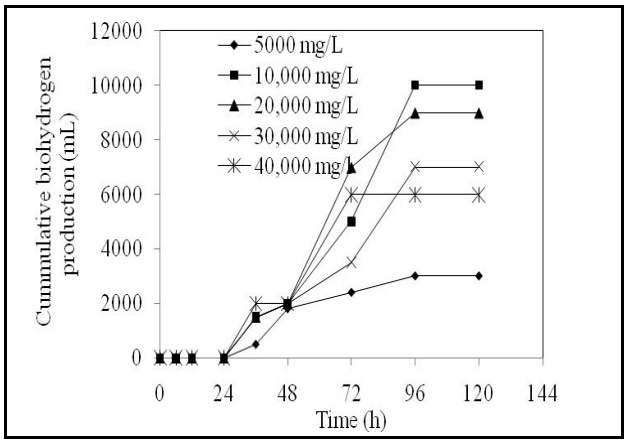

Fig. 2 Biohydrogen production at various initial substrate concentrations

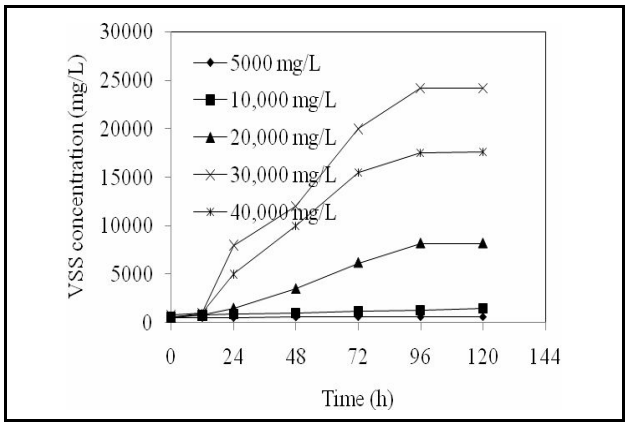

Fig. 3 VSS concentration at various initial substrate concentrations

Cell growth kinetics

The specific cell growth rate was studied using logistic model and is given by (Eq. 1)

$$\mu = \frac{dx}{dt} = K_c \left(1 - \frac{X}{X_{\max}}\right) \qquad (1)$$

Where, K_c is the apparent specific growth rate (h^{-1}), and X_{max} is the maximum cell dry weight concentration (g L^{-1}). By integrating Eq. (1), the following equation for cell concentration is obtained (Eq. 2):

$$X = \frac{X_0 \exp\left(K_c\, t\right)}{1 - \left(X_0 / X_{\max}\right)\left(1 - \exp\left(K_c t\right)\right)} \qquad (2)$$

Where, X_0 (g L^{-1}) is the initial microbial concentration (g VSS L^{-1}), and X_{max} (g L^{-1}) is the maximum microbial concentration (g VSS L^{-1}). From Fig. 3, Kinetic parameters were estimated as follows: K_c = 0.013 h^{-1}, simulated X_{max} = 2.05 gVSS L^{-1}. The experimental X_{max} was found to be 1.5 gVSS L^{-1}. The experimental and simulated specific growth rates were significant which is evident with high regression coefficient values, R^2 = 0.947 (Fig.4). Similarly, Mullai et al.(2013b) reported higher simulated X_{max} than experimental X_{max} in biohydrogen production using mangrove sediments by batch process[3].

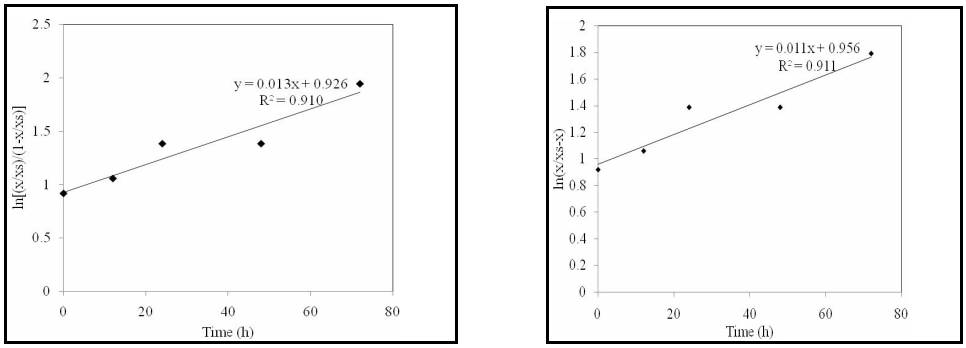

Fig. 4 Logistic model for growth of hydrogen producing anaerobes

Product formation kinetics

The Luedeking-Piret model shown in Eq. (3) has been used to describe the relationship between hydrogen producing bacterial growth rate and product formation rate.

$$\frac{dP}{dt} = Y_{P/X}\frac{dX}{dt} + \beta X \qquad (3)$$

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 VSS L^{-1}), $Y_{P/X}$ is the growth associate product yield coefficient, and β is the non-growth associated product yield co-efficient. A graph between specific growth rate vs product formation rate was plotted as shown in Fig. 5. The yield coefficient ($Y_{P/X}$) was 0.79, which indicates that hydrogen is a growth associated product. The experimental and model fitted specific hydrogen production rates were significant with high regression coefficient values, R^2 = 0.997 (Fig. 6). Similar R^2 were also reported in batch kinetics of biohydrogen production using mixed cultures and pure cultures [3,7,8,10].

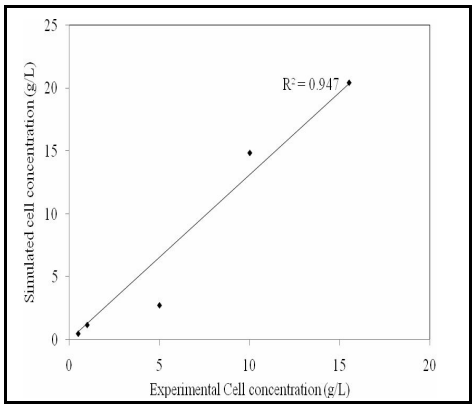

Fig. 5 Correlation chart between experimental and simulated cell concentration

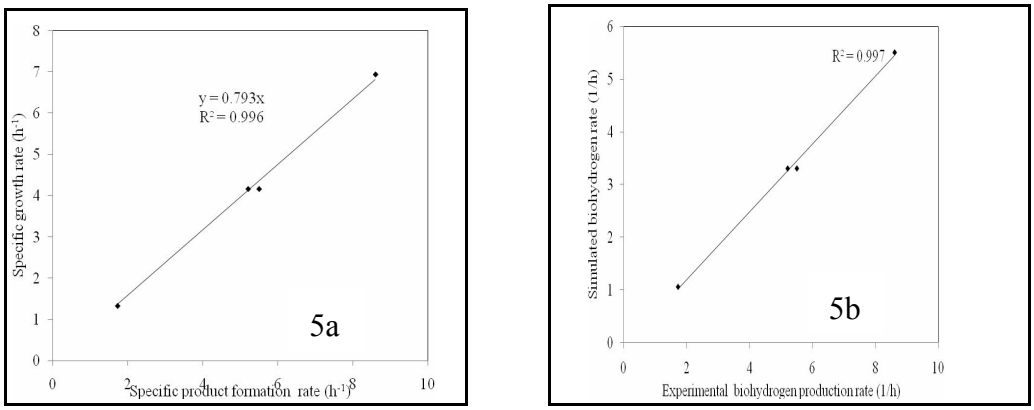

Fig. 6 (a) Luedeking-Piret model (b) Correlation chart between experimental cumulative biohydrogen production rate and simulated biohydrogen production rate

Microscopic analysis of hydrogen producing granule

Figure. 7 shows SEM image of hydrogen producing granule. Bacterial cells were distributed on the surface of granules. The granules had several cracks on its surface which indicated the channelling of nutrients and release of hydrogen.

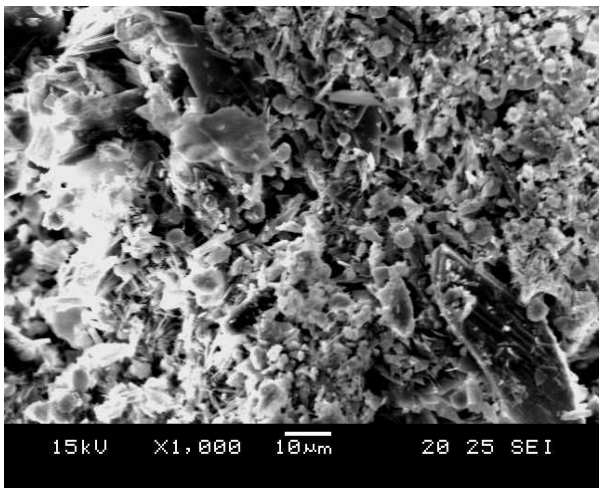

Fig. 7 SEM image of hydrogen producing granule

Conclusions

A kinetic model of batch process describes the production of fermentation products, the growth of biomass and degradation of substrates. Product and substrate profiles were calculated to study the bio hydrogen production process and its kinetics constants. The kinetics of batch anaerobic hydrogen production was studied with experimental data using unstructured kinetic models, logistic model and Luedeking–Piret model. They described the kinetics of cell growth rate as a function of cell concentration and product formation respectively, in the hydrogen production process. The regression co-efficient values (R^2) obtained between the experimental and simulated observations for the models, logistic model and Luedeking–Piret model were 0.947 and 0.997 respectively. Numerical results showed good agreement between experimental values and simulated values.

Acknowledgments

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