



**Substrate utilization kinetics of biohydrogen production using Michaelis–Menten model**

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**Abstract**

Kinetics of substrate utilization using unstructured model, Michaelis–Menten model. The kinetics  $v_m$  and  $k_m$  were estimated as 0.106 g COD h<sup>-1</sup> and 32.78 g COD h<sup>-1</sup>, respectively. The regression line had a correlation coefficient of 0.974, suggesting the applicability of the model for study of biohydrogen production.

**Keywords:** Michaelis–Menten model, COD, correlation coefficient.

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**Introduction**

Biohydrogen production by anaerobic digestion of organic rich industrial waste is attracting and effective way of tapping energy (Venkatamohan et al., 2008; Khalid et al., 2011). It results in a dual action of environmental cleanup and energy recovery. Anaerobic digestion has many advantages compared with the aerobic digestion processes (Sridevi et al., 2014). Mathematical models can provide information to analyze and predict the performance of biological hydrogen production by fermentation. Kinetic equations describe the activity of a microorganism on a particular substrate in biochemical processes. For scale-up of the bioreactor, appropriate kinetic model of the process is required. H<sub>2</sub>-producing bioreactors require the mathematical description of several distinct processes such as kinetics of microbial growth (biomass formation) and products (hydrogen, VFA, solvents, etc.) formation in the bioreactor.

**Substrate**

**Distillery wastewater**

Distillery wastewater was collected from Distillery unit, Tamilnadu, India. The wastewater was collected in 20 l plastic cans and stored in a deep freezer (Blue star, India) at – 4 °C. It was used as substrate for biohydrogen production and biodegradation. The characteristics of distillery wastewater were studied.

**Glucose**

Glucose, the simplest monosaccharide, was used as the sole carbon source along with raw wastewater at different concentrations for batch and continuous studies. Glucose was the simplest monosachharide and easily uptaken by the microorganisms for their growth (Lee et al., 2008).

## Anaerobic sludge

The anaerobic sludge collected from anaerobic digester of Distillery unit, Tamilnadu, India. The stones and dust particles in anaerobic sludge were removed and stored in plastic cans. The plastic cans were kept in deep freezer (Blue star, India) for further use.

## Pretreatment of anaerobic sludge

The anaerobic sludge was heat pretreated at 102°C for 1 h to inhibit the methanogens (Mullai et al., 2013a; Mullai et al., 2013b) and also to speed up the rate limiting step, hydrolysis in anaerobic digestion. The heat pretreated sludge was used for biohydrogen production and biodegradation using distillery wastewater.

## Nutrients

Distillery wastewater is one of the agro-industrial wastewater. Due to its high organic content, they lack nutrients. Hence some micronutrients and trace metals are added in low concentrations for the growth of microorganisms for granulation.

## Nutrient medium

All the chemicals used in this study were of analytical grade. The nutrient medium for biomass growth contained the following composition (g/L). NH<sub>4</sub>Cl - 0.5, K<sub>2</sub>HPO<sub>4</sub> - 0.25, MgCl<sub>2</sub>·6H<sub>2</sub>O - 0.3, NiSO<sub>4</sub> - 0.016, CoCl<sub>2</sub> - 0.025, ZnCl<sub>2</sub> - 0.0115, CuCl<sub>2</sub> - 0.0105, CaCl<sub>2</sub> - 0.005, MnCl<sub>2</sub> - 0.015 and FeCl<sub>3</sub> - 0.005 (Mullai et al., 2013a).

## Effect of initial substrate concentration

The effect of initial substrate concentration was studied at different initial substrate concentration such as 5, 000, 10,000, 20,000, 30,000 and 40, 000 mg COD/L at constant ph 6.5 and constant biomass concentration of 1,000 mg VSS/L.

## Substrate utilization kinetics

### Michaelis–Menten equation

It was used to model the substrate degradation as follows where  $v$  (g COD day<sup>-1</sup>) is the substrate degradation rate;  $v_m$  (g COD day<sup>-1</sup>) is the maximum specific substrate degradation rate;  $k_m$  (g COD l<sup>-1</sup>) is

the dissociation constant; and  $S$  (g COD l<sup>-1</sup>) is the substrate concentration.

$$v = \frac{v_m S}{k_m + S}$$

$$\frac{1}{v} = \frac{k_m}{v_m} \frac{1}{S} + \frac{1}{v_m}$$

Linearization of Eq. (1) gives: Plotting 1/v against 1/S, a straight line was obtained with an intercept of 1/v<sub>m</sub> and a slope of k<sub>m</sub>/v<sub>m</sub>

## Substrate Utilization kinetics

The Michaelis–Menten equation was used to model the substrate degradation Plotting 1/v against 1/S, a straight line was obtained with an intercept of 1/v<sub>m</sub> and a slope of k<sub>m</sub> /v<sub>m</sub> from which v<sub>m</sub> and k<sub>m</sub> were estimated as 0.106 g COD h<sup>-1</sup> and 32.78 g COD h<sup>-1</sup>, respectively (Fig.1). The regression line had a correlation coefficient of 0.974, suggesting the applicability( Fig.2). The k<sub>m</sub> value represents the substrate concentration required to achieve 50% of the maximum specific substrate degradation rate. The lower value of v<sub>m</sub> indicates the recalcitrant nature of distillery wastewater (Gadhe et al., 2014) and also indicates the robustness of hydrogen producing microbes under the complex nature of the distillery wastewater. The higher value of Km than the initial substrate concentration indicates that the initial substrate concentration was sufficient and not limiting the specific substrate degradation.

Similarly, kinetic constants v<sub>m</sub> and k<sub>m</sub> as 0.40 g COD g VSS<sup>-1</sup> h<sup>-1</sup> and 15.77 g COD L<sup>-1</sup> respectively with a R<sup>2</sup> value of 0.975 was obtained by Gadhe et . (2014) using dairy wastewater as substrate and anaerobic seed sludge as inoculums. Mu et. (2006) also used Michalis-Menten equation to study the kinetics of substrate utilization. The constants v<sub>m</sub> and k<sub>m</sub> were found to be 0.28 g g VSS<sup>-1</sup> h<sup>-1</sup> and 13.5 g/L with a regression co-efficient of 0.963. Lo et al., 2008 studied the kinetics of hydrogen production by *C. butyricum* strain CGS5 using xylose. Constants v<sub>m</sub> and k<sub>m</sub> were found to be 0.15 L/h/g VSS and 0.5 g COD/L respectively. Similarly for sucrose, the constants v<sub>m</sub> and k<sub>m</sub> were 0.29 L/h/ g VSS and 3.7 g COD/L.

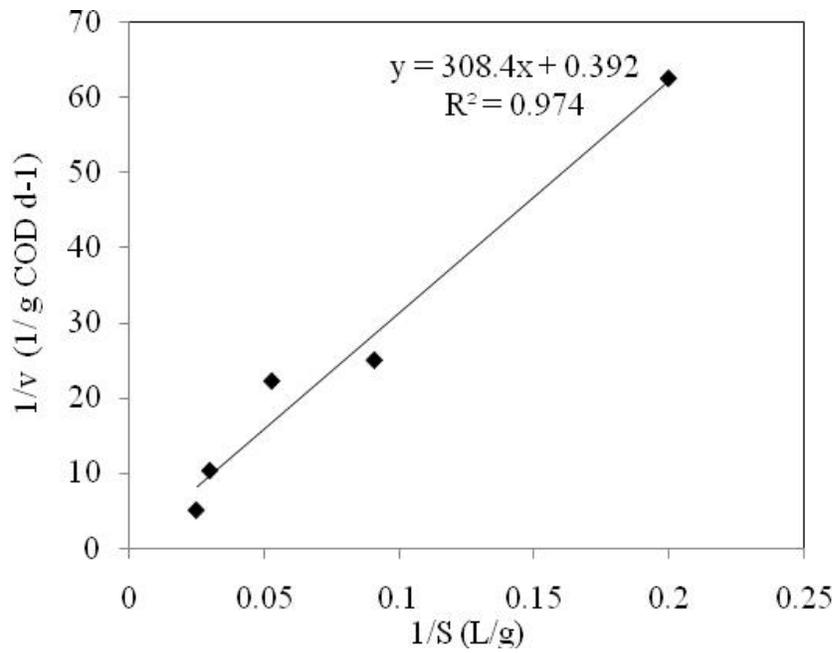


Fig.1 Michaelis–Menten model for substrate utilization kinetics

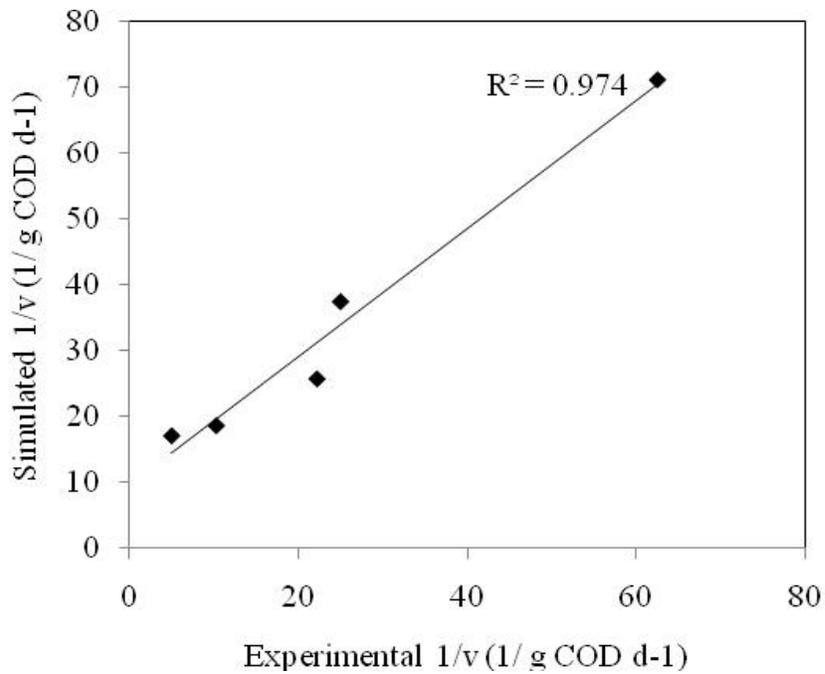


Fig.2 Correlation chart between experimental and simulated specific substrate degradation rate using Michaelis-Menton model

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