

## **Evaluation of Kinetic Parameters for Hydrogen Production by Anaerobic Suspended Growth Reactor using Synthetic Feed.**

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### **Abstract**

The objective of the present study is the Evaluation of Kinetic Parameters for hydrogen production by anaerobic suspended growth reactor using synthetic feed. Hydrogen production through anaerobic fermentation of synthetic feed was then studied in an up flow suspended film batch reactor. Synthetic feed consists of specific concentrations of several nutrients required for anaerobic fermentation. The process parameters were set depending on the optimization studies. This process aimed at establishing hydrogen production in a 1 liter suspended reactor. Similar studies were performed in a suspended growth anaerobic system (stirred tank reactor) having an in-built turbine and operated by a magnetic stirrer. The hydrogen production was monitored and sequencing results were used to estimate the kinetic parameters of the reaction. The suspended growth anaerobic system was fed with optimized substrate. The reactor operation was monitored by monitoring the process parameters such as pH, ORP, VFA, etc. The anaerobic stirred tank reactor showed consistency in its results on feeding with the synthetic feed. This set of experiments aims at studying the variation of process parameters on using the optimized co-substrate and nitrogen source as the feed for the reactor.

**Keywords:** Synthetic feed, pH, ORP, Alkalinity ,COD, VFA, HPLC, Bio-hydrogen, Hydraulic retention time (HRT), Kinetic parameters.

### **Introduction**

The energy demand of the world has been continually and increasingly dependent on earth's fossil fuels reserves particularly oil, coal and natural gas. Over 90% of world's

energy demands are made by fossil fuels as they are readily available and convenient to use. However the non-renewability of these resources and their uneven distribution around the globe are the limiting factors. The rate at which we consume these resources will for exceed the rate at which they are being recycled. This will lead to the eventual depletion such as readily accessible reserves, they may well get exhausted by 2030. (Bicelli 1986)

The rising costs of fossil fuels pollution and the finite nature of conventional fuels have forced the world to look for an alternative energy source. The desire for change in the present pattern of energy use stems from the helplessness to curb power consumption and the need for clean energy to eliminate growing environmental threat. The desirable characteristics of such a system are abundant availabilities, low cost, conveniently usable, renewable nature, economically transportable and socially compatible.

The popularity of Hydrogen as a fuel source followed from a crisis that resulted on greater usage of non-renewable fuels. During the Energy crisis of the 1970's, Hydrogen was touted as the "fuel of the future" and a great deal of time and money were put on research on its possible sources and applications. The biological hydrogen production was first seriously considered as a practical capability through a series of sponsored meetings conducted by National Science Foundation, Washington D.C. (Gibbs et. al. 1973)

Studies in laboratory have concentrated on pure substrates including glucose, starch and cellulose, often in batch processes. Volatile Fatty acids have long been recognized as intermediates in anaerobic process and have been proposed as a control parameter. (Ahning et. al., 1997) Major volatile fatty acids detected in the anaerobic process were acetate, propionate and butyrate. Fermentations of hexose to acetate or butyrate produce  $H_2$  and  $CO_2$ . Fermentations to propionate or lactate produces no hydrogen. Reduced fermentation end products such as ethanol and other alcohols contains additional H atoms not present in corresponding acids and therefore alcohol production gives correspondingly low  $H_2$  yields. It is important, therefore, to establish bacterial metabolism resulting in acetate and butyrate as end products. Sustained hydrogen production coupled with bacterial growth was achieved by continuous addition of small amounts of glutamate,  $NH_4^+$  or  $N_2$ . (Fascetti and Todini, 1995) A gradual production of acids depleted the buffering capacity of these substances resulting in a concomitant decline in pH to about 5.5 before hydrogen production began. In stationary growth phase, the acetate production decreased slightly but an increase in butyrate production was noticed. For an optimum pH range of 5.5-5.7, the acetate to butyrate production ratios was found in the range of 3-4 for substrates like sucrose and starch. (Khanal et. al., 2004) Fermentative hydrogen evolution is considered more advantageous than other hydrogen generation processes. It was also found possible to increase the conversion efficiency from 10% to 28% by using proportionate mixtures of various waste materials and effluents from industrial processes, which also facilitated waste recycling. (Kumar et. al., 2001) The efficiency of a hydrogen-producing bioprocess also highly depends upon the optimal control of factors like ratio of substrate concentration to biomass concentration. The ratio significantly influences the metabolic and kinetic characteristics of microorganisms.

(Chudoba et. al., 1992) However, there are still no reports demonstrating the uses of kinetic models and describing and predicting the exact kinetic characteristics of batch anaerobic hydrogen-producing cultures. It is a known fact that normal biological processes are operated at ambient temperatures (30-40<sup>0</sup>C) and normal pressure, which are considered to be less energy intensive. Optimized Hydrogen production from Industrial effluents especially those from the pharmaceutical units through anaerobic technology are considered very important because the real problem in a treatment system is not only the actual content of the wastewater, but also its quantity and quality.

The present investigation shows the anaerobic stirred tank reactor showed consistency in its results on feeding with the synthetic feed. This set of experiments aims at studying the variation of process parameters on using the optimized co-substrate and nitrogen source as the feed for the reactor.

## **Methodology**

### **Analytical Procedures**

The performance of reactor with synthetic feed was assessed by monitoring carbon removal (COD) throughout the reactor operations and during the cycle period. In addition, pH, oxidation-reduction potential (ORP), VFA, Alkalinity and suspended solids (SS) were determined during reactor operation to assess the performance of the reactor. The analytical procedures for monitoring the above parameters were adopted from the procedure outline in the Standard methods. The method performed for determination of physicochemical parameters was adopted from standard methods of American public health association (APHA, 2000).

### **Glucose Estimation**

Glucose concentration in the culture medium was determined spectrophotometrically by DNS (Dinitro salicylic acid) method basically according to Miller.

**DNS Reagent:** Dissolve by stirring 1g of DNS, 200mg of crystalline phenol and 50mg sodium sulfite in 100ml 1% NaOH. Store at 4<sup>0</sup>C

**Procedure:** A volume of 0.1 and 0.2ml of sample should be collected into a clean test tube. The volume was made up to 1ml with distilled water. 2ml of DNS solution was added to each tube and kept in boiling water bath for 5 minutes. The resulting mix was made up to 10ml with distilled water. The absorbance at 540 was recorded against the blank without glucose. A graph has been plotted against Optical Density Vs concentration.

### **Oxidation-reduction potential (ORP)**

A substance losing electrons is oxidized and the substance that gains e- is reduced. In any system undergoing oxidation or reduction, there is a continual change in the ratio between the materials in the reduced form and those in the oxidized form. When a platinum electrode is immersed in such a system, a potential is developed on it

depending upon the ratio of oxidized and reduced states, is called oxidation-reduction potential (ORP). It is a vital parameter in controlling the biological treatment of wastes. Anaerobic treatment requires a low ORP. It also depends upon pH of the solution; a decrease by 1 unit of pH will be accompanied by a decrease of 0.058 volts. The ORP values are determined using the same pH meter used to measure pH of the samples.

### **Volatile Fatty Acids (VFA)**

Monocarboxylic acids like acetic acid, Propionic acid, butyric acid, etc; and polycarboxylic acids like lactic acid, succinic acid, etc are known as volatile fatty acids (VFA). These acids under anaerobic conditions decompose to give carbon dioxide and methane. If methanogenic bacteria are inhibited and the process of decomposition is controlled at Acidogenesis hydrogen gas is produced.

### **Alkalinity**

**Procedure:** The sample was centrifuged for 5min at a speed of 3000rpm and filtered. 100ml of the centrifuged or filtered sample or a suitably diluted sample containing less than 3 meq/L VFA were taken. The sample was titrated with 0.1N HCl to pH=3 (A ml) using a pH meter. The sample was boiled for 3min in the 250ml flask to remove the CO<sub>2</sub>. The sample was cooled immediately for 2min and the sample was titrated with 0.1N NaOH to pH =6.5 (B ml).

$$\text{VFA (mg/l)} = \frac{(B * 100) - (A + 100)}{99.23} * \text{dilution factor} * 60$$

$$\text{Alkalinity (mg/l)} = (A - B) * \text{dilution factor} * 60.$$

### **Chemical Oxygen Demand (COD)**

#### **Reagents**

1. Standard potassium dichromate (0.25 N): 12.259 g of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was dried at 103°C for 24 hours in distilled water and diluted to 1000 ml.
2. Sulphuric acid reagent: 10 g of AgSO<sub>4</sub> was dissolved in 1000 ml concentrated sulphuric acid, and kept over night for dissolution.
3. Standard ferrous ammonium sulfate- 0.1N: 39.0 g of Fe (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 6H<sub>2</sub>O was dissolved in about 400 ml distilled water. 20ml of concentrated conc. H<sub>2</sub>SO<sub>4</sub> was added. Cooled and made unto 1 L and standardized with K<sub>2</sub> Cr<sub>2</sub>O<sub>7</sub> (0.25N)
4. Ferroin indicator: 1.485 g of 1,10 phenanthrolin monohydrate and 0.695 g FeSO<sub>4</sub> 7H<sub>2</sub>O was dissolved in distilled water and diluted to 100 ml with distilled water.
5. Mercuric sulfate: HgSO<sub>4</sub> crystals (analytical grade) were used.

#### **Procedure**

1 ml of the sample was taken in 10-ml COD vials and to this 1 ml distilled water, 2ml

$K_2Cr_2O_7$  solution and 4ml  $H_2SO_4$  reagents were added. These vials were kept in COD block digester and refluxed for a period of 2 hours at  $150^\circ C$ . After refluxing was completed the samples were cooled and were transferred into 100 ml beaker. Then 2 to 3 drops of ferroin indicator was added to sample solution and titrated against 0.1 N FAS, until brick red color appeared. COD was calculated by using the following formula. Color, turbidity and silica in the concentration of 500 ppm interfere in this estimation. Filtration can be done to remove color and turbidity.

$$\text{COD (mg/l)} = \frac{(\text{B-S}) * \text{N} * 8000}{\text{ml of sample taken}}$$

Where, B ml is amount of FAS consumed for blank, S ml is FAS consumed for sample, N is normality of FAS( Ferrous ammonium sulphate).

### **Hydrogen production in a stirred tank reactor maintained under suspension**

The inoculum from the suspended reactor was directly transferred to a stirred tank reactor fitted with a 2-blade axial turbine consisting of a magnetic pellet that can be operated with the help of a magnetic stirrer. This reactor maintained a suspension by the movement of the turbine blades, which stirred the microbial culture to move in the working volume in an irregular manner.

### **Reactor start up**

This reactor did not have a startup procedure because the inoculum was taken directly from the suspended reactor, which was recently treated to inhibit methanogenesis.

### **Reactor setup and inlet conditions**

The reactor has a total working volume of 1.25-liter capacity. The hydrogen fermentation was conducted at mesophilic temperature ( $29 \pm 2^\circ C$ ). The pH was maintained at 6 to ensure that the fermentation process does not yield a drastic drop in the pH value after a HRT of 24 hours. This decision was based on the optimization studies. The suspension was maintained by the movement of turbine blades powered by a magnetic stirrer operating at 100 rpm.

### **Synthetic feed studies-Reactor operation**

The reactor was started with synthetic feed, which has the composition as shown in Table 1. About 1 liter of synthetic feed was taken and fed to the reactor and the inlet and outlet samples (after a HRT of 24 hours) were collected and was continuously examined for pH, ORP, VFA, COD and hydrogen gas production. The reactor was analyzed for the various important process parameters for the inlet and outlet samples for around 13 days. After a steady state was attained, the sequencing at the HRT intervals of 1, 2, 4, 6, 8, 10, 12, 24 and 48 Hours of incubation. The samples were regularly monitored for pH, VFA, Alkalinity, COD, Glucose, VSS and Hydrogen gas parameters. HPLC for the samples was carried out. The reactor kinetics and substrate

conversion efficiency was also calculated using the biomass and substrate concentrations at various time intervals in sequencing period.

**Table 1:** Synthetic feed composition.

Nutrients	Composition (g/l)
NH <sub>4</sub> Cl	0.5
KH <sub>2</sub> PO <sub>4</sub>	0.25
K <sub>2</sub> HPO <sub>4</sub>	0.25
MgCl <sub>2</sub> .6H <sub>2</sub> O	0.3
FeCl <sub>3</sub>	0.025
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
<b>Glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>)</b>	<b>3</b>

### Hydrogen Gas Estimation

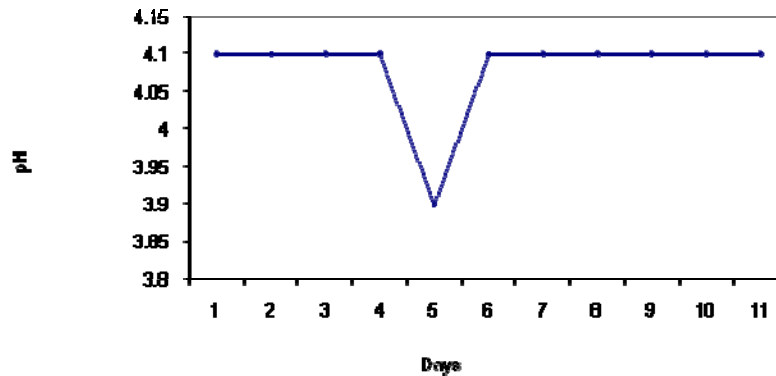
Hydrogen gas produced in the reactor is estimated using a gas sensor, FMK satellite 4-20 mA version (ATMI GmbH Inc.). This equipment is a generic gas-monitoring instrument with microprocessor based electronics interfacing with std. 4 to 20 mA alarm/control systems. Target gas and measuring range depend on type of sensor chosen.

The electrochemical sensors designed for use with the FMK satellite feature an integrated data memory. When a new sensor is fitted, the instrument's electronics will load operating parameters of the sensor into microprocessor's memory. The current flowing through the sensor is amplified electronically, digitized and temperature compensated and resulting concentration value is given as an analog 4 to 20 mA output signal. This output signal usually displays the %volume of hydrogen in the reactor air space.

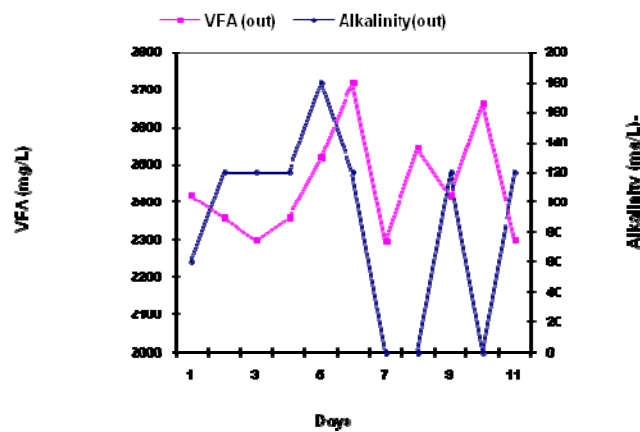
## Results and discussion

### Anaerobic suspended growth reactor using synthetic feed

The reactor operation was monitored by monitoring the process parameters such as pH, ORP, VFA, etc. The inlet pH was maintained at 6.0 throughout the study while the outlet pH remained almost constant (4.1) with only variation of 3.9 at the 5<sup>th</sup> day as shown in figure 1. Little variation was found in pH representing a steady state condition achieved by the reactor resulting in better performance in comparison with the up flow reactor studies.



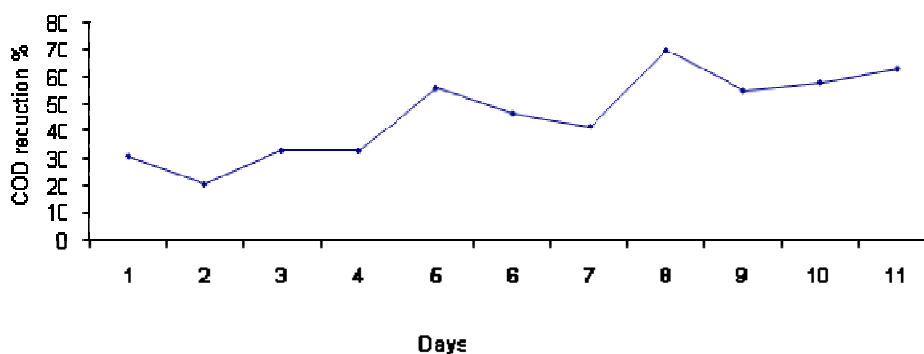
**Figure 1:** Study state condition of pH in a suspended growth reactor.



**Figure 2:** Variation of VFA-concentration and alkalinity of inlet and outlet of synthetic feed at different HRTs.

The inlet VFA concentration as shown in figure. 2, lies within the range of (1987 to 2416 mg/L) while outlet recorded a minimum of 2295 mg/L on the 7<sup>th</sup> day and a maximum of 2720 mg/L on the 6<sup>th</sup> day. VFA showed an increase of 300-400 mg/l after a HRT (Hydraulic Retention Time intervals) of 24 hours. There was a regular increase in the outlet VFA value representing the VFA accumulation within the system, which is responsible for the relative decrease in the pH within the system. The variation in VFA explicitly proves the consistent performance of the system further aided by the study on the hydrogen production rate. In this system, the alkalinity values are well within the range of 0 to 840 mg/L with a number of readings constant at 120 mg/L. The zero alkalinity indicates the increase in buffering capacity of the system to counteract VFA formation and maintain equilibrium. The zero alkalinity was found only on days 7, 8 and 10 where the VFA conversion was greater. The studies indicate a highly varying COD reduction %. The COD reduction % values varied between a range of 21% to a high value of 70% indicating greater performance by the system. The COD reduction % values shown in figure 3, however, showed an

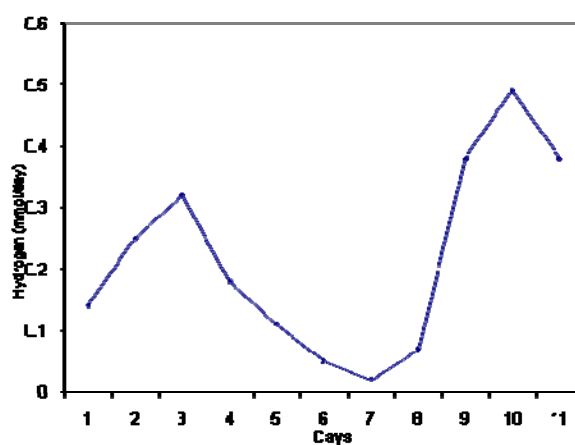
average of around 30% throughout the experimental period. The inlet COD was maintained at an average of around 5000 mg/L.



**Figure 3:** The % of COD reduction values in the experimental period at different HRTs.

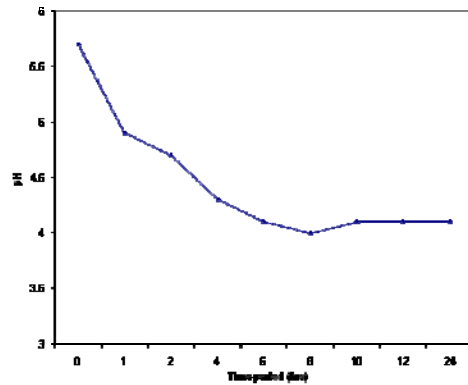
The hydrogen production rate is calculated is shown in figure 4.4. The hydrogen production rate increased in the first 3 days to around 0.32 mmol/day that is considered very good taking into account that only 3 days were given for the acclimation of the culture to the system. Then, the hydrogen rates slowly decreased to 0.02 mmol/day at the end of 7 days showing depletion in the performance of the system. Further, the culture acclimatized to the system increasing hydrogen production rates to 0.49 mmol/day with consistency being maintained in the other process parameters.

The sequencing experiment aimed at deciding the success ratio of the experiment with an anaerobic stirred tank reactor. The parameters analyzed during the sequencing procedure were used to calculate the kinetic model parameters for hydrogen production through the anaerobic fermentation of glucose.



**Figure 4:** Variation of hydrogen production rate at different HRTs.

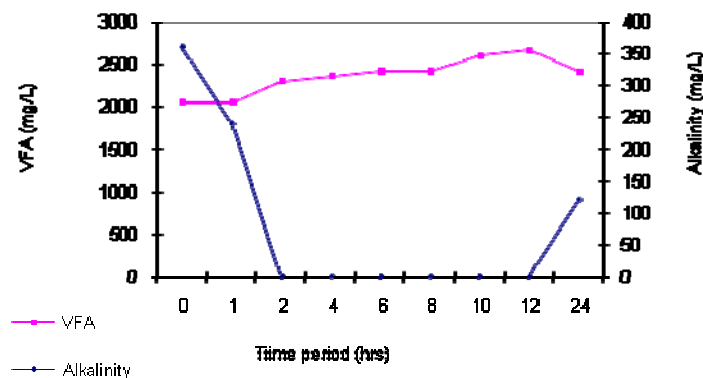




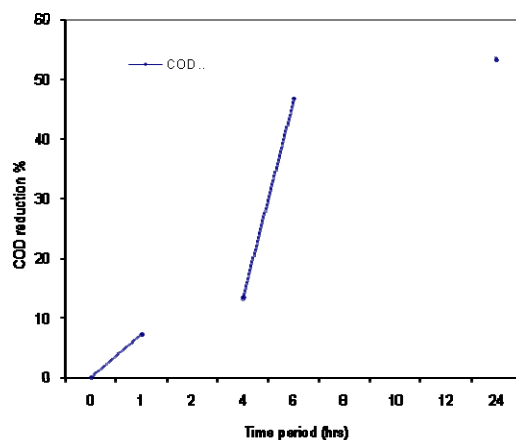
**Figure 5:** variation of pH of synthetic feed at different HRTs.

The variation of pH as depicted in figure .5 shows a drop from 5.7 at the 0<sup>th</sup> hour to 4 at the end of 8<sup>th</sup> hour followed by a small increase to 4.1 at the end of 10<sup>th</sup> hour and remained constant until the end of sequencing period. The pH values remained very low at the end of the sequencing period indicating the decrease in system's performance in acidogenic fermentation process.

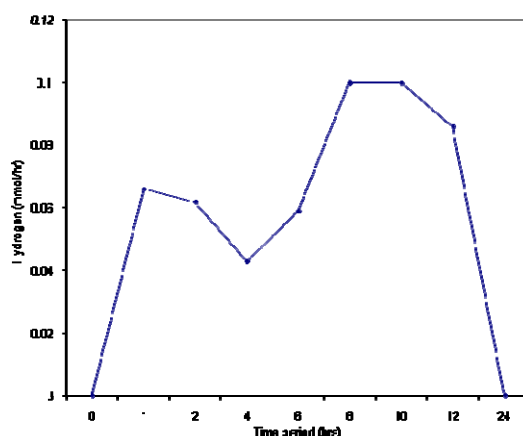
The variation in VFA as shown in figure .6 showed a constant increase from 2052 mg/L at the 0<sup>th</sup> hour to around 2661 mg/L at the end of 12<sup>th</sup> hour followed by a decrease in the VFA to 2417 mg/L at the end of 24<sup>th</sup> hour. The VFA of the synthetic feed showed a maximum value at the end of 12<sup>th</sup> hour (2661 mg/L). The VFA variation indicates a stable performance of the system till the 12<sup>th</sup> hour where the process encounters inhibitory action finally ending up with a comparatively reduced performance. The alkalinity values also decreased from 360 mg/L at the 0<sup>th</sup> hour to 0 mg/L at the end of 2<sup>nd</sup> hour after which stability remained indicating consistent VFA formation until the 12<sup>th</sup> hour. Then the alkalinity values also started increasing finally showing 60 mg/L at the end of the reaction period. The COD reduction % values indicated multiple variations in its values as shown in figure 7. This caused difficulties in discussing the variation of the COD reduction % values. But it can be stated that an increase in COD reduction % was witnessed at the end of the reaction period.



**Figure 6:** Variation of VFA-concentration and alkalinity of inlet and outlet of synthetic feed at different HRTs.



**Figure 7:** The % of COD reduction values in the experimental period at different HRTs.



**Figure 8:** Variation of hydrogen production rate at different HRTs.

The variation in hydrogen production complies with that discussed under the variation of VFA and alkalinity. The hydrogen production as shown in figure.8 rate decreased to 0 mmol/hr at the end of 24 hours from a value of 0.086 mmol/hr measured in the 12<sup>th</sup> hour. This indicates a decrease in system's performance. However, the hydrogen production rate increased from 0 mmol/hr in the 0<sup>th</sup> hour to 0.1 mmol/hr at the 8<sup>th</sup> and 10<sup>th</sup> hours before starting to decrease to lower values. The system's performance required kinetic study to establish conclusions.

### Kinetic Parameters Evaluation

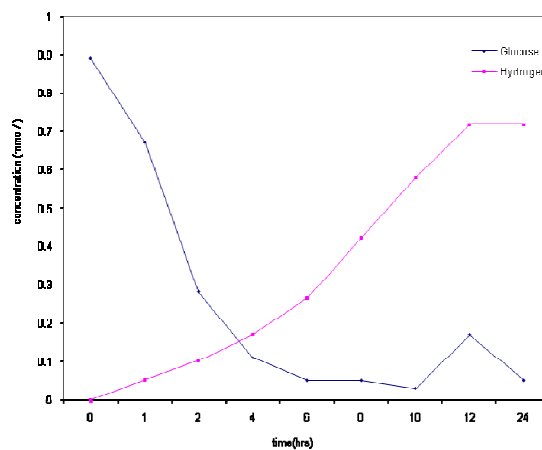
For a batch reactor operated with mixing, the control volume consists of the entire reactor. Components in the reactor are distributed uniformly throughout the reactor so that concentration of any component is the same at any location within the reactor at

any time. We select components as the bacteria (anaerobic mixed culture) and their rate-limiting substrate, which is frequently the electron donor, as our choice here. We assume that all the other bacterial requirements like the electron acceptor and nutrients are sufficiently high in concentration, as they impose no limitations on organism growth rate.

Kinetic model parameters are usually based on the substrate consumption rate and bacterial cell growth rate. Kinetic model parameter for a 24 hr HRT (by sequencing procedure) has not been attempted in the past because of its invariably low results. Theoretically, the cell growth rate is expressed as

$$\rightarrow dX/dt = \mu * X \quad (1)$$

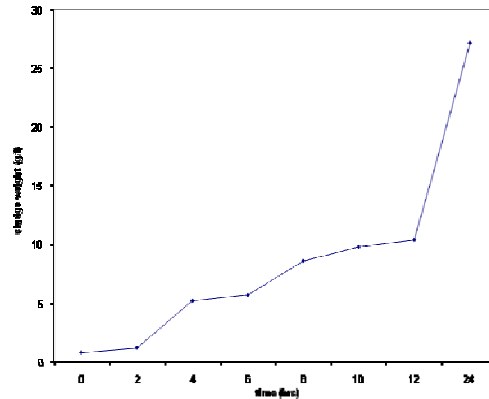
Where, X is the cell dry weight concentration (g/l) and  $\mu$  is the specific growth rate ( $h^{-1}$ ), which might depend on substrate concentration and the other factors. Several models provide an expression for  $\mu$ , the Monod expression being the most common.



**Figure 9:** Hydrogen concentration and glucose utilization with time.

The profile of hydrogen concentration and glucose utilization with time shown in figure.9 indicates that the kinetics of product formation and disappearance of reactants is in acceptance with the stoichiometry of the reaction. The graph of substrate concentration and biomass concentration with respect to time was also plotted to show whether the reaction stoichiometry has been satisfied (Koku et. al., 2003). The  $\mu$  values calculated from procedure are given in the Table 2.

The bacterial culture in the stirred tank suspension reactor is considered to be in the exponential phase through the study of variation of sludge dry weight (VSS) with time studied during sequencing period. The VSS values showed a small increase in the first 2 hours followed by an exponential growth till the 12<sup>th</sup> hour is shown in figure 10. The VSS of the sludge than recorded the maximum value ( $X_{max}$ ) of 27.2 g/L at the end of 24<sup>th</sup> hour.



**Figure 10:** Variation of sludge weight with different HRTs.

The growth rate in the exponential phase is assumed to be constant. Therefore, (1) gives,

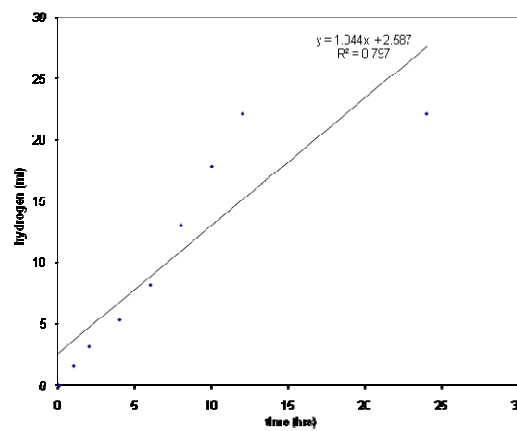
$$\mu_c = \ln (X_2/X_1) / (t_2-t_1) \quad (2)$$

Where,  $\mu_c$  is the specific growth rate in the exponential phase and  $X_1$ ,  $X_2$  are the two distinct cell concentrations in the exponential phase (g/l).

Now, a logistic model is used, which has the additional benefit of representing the entire growth curve, including the lag phase (if present), the exponential growth and the stationary phases. The specific growth rate for the logistic model is

$$\mu = k_c (1 - X/X_{max}) \quad (3)$$

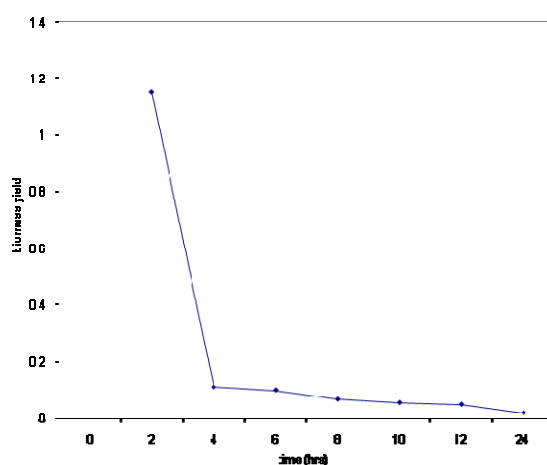
The logistic model parameters ( $k_c$  and  $X_{max}$ ) and  $\mu_c$  values were then recorded in a table. The graph of total hydrogen production versus time was plotted and the  $R^2$  value was used as a measure of goodness of fit is shown in figure 11.



**Figure 11:** variation of hydrogen production with different HRTs.

The profile of hydrogen produced (ml) versus time (hrs) gave a variance curve for which a curve is fitted to measure the  $R^2$  value. The  $R^2$  value, which quantifies the dispersion of distribution from the mean, is used as a measure of goodness of fit. It was found that the  $R^2$  value for the model was 79% indicating a close agreement of the model to experimental data. However, the  $R^2$  values for fermentation of glucose using a pure culture of photosynthetic bacteria was reported above 98% (Koku et. al., 2003)

Two conventions were used for expressing the gas production rate in this study. The first is the average gas production rate per bacterial dry weight ( $r'_g$ ), which is calculated by time averaging of individual rates and has the unit of l/g/h. The individual rates obtained for a certain period were calculated by dividing the volume increment of the gas produced by average cell concentration and by the duration of that period. The second convention is the average gas production rate per culture volume ( $r_g$ ), which is calculated by dividing the total volume of gas produced by the volume of culture and by the duration of gas production, and has the unit l/l/h (Koku et. al., 2003). These two parameters were calculated and recorded in the Table 2.



**Figure 12:** variation of biomass with different HRTs.

Another important variable necessary for kinetic study is the biomass yield as shown in 12. The variation of biomass yield ( $Y$ ) with time showed a drastic decrease at the end of 2<sup>nd</sup> hour followed by a gradual decrease indicating that the biomass used less amount of substrate for its growth when compared with that utilized for hydrogen production. This can be measured by determining the substrate conversion efficiency ( $\eta$ ). A particular useful parameter for characterizing microbial hydrogen production is the substrate conversion efficiency, which is the ratio of the actual amount of hydrogen evolved to the amount expected through stoichiometric conversion of the substrate (Hillmer and Gest, 1977). This is due to the belief that the portion of substrate initially utilized for biosynthesis can eventually end up as a substrate for hydrogen production (Koku et. al., 2003).

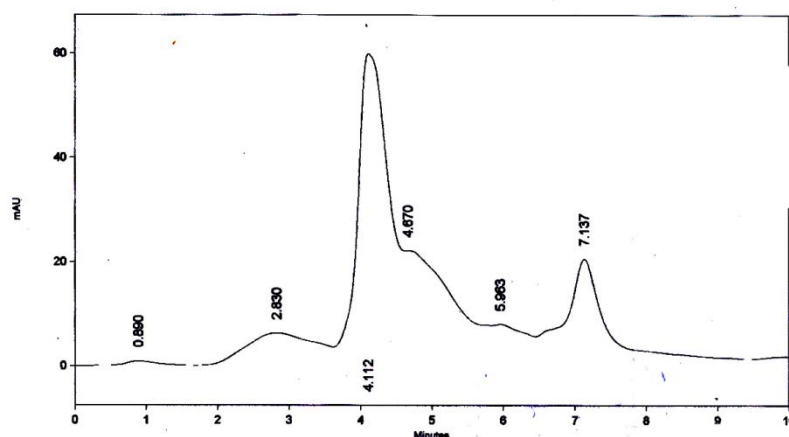
For glucose, which is the primary carbon substrate used in this study, 4 moles of hydrogen are expected to be produced per mole of glucose utilized, so the substrate conversion efficiency ( $\eta$ ) is  $\eta = 100 * P / 4 * V * S_0$

Where, P is the moles of hydrogen produced till that time and V is the culture volume in liters (Koku et al., 2003). The substrate conversion efficiency attained maximum value at the end of 24<sup>th</sup> hour with a value of 6.34%. The substrate utilization rate ( $k_s$ ) is also calculated and listed in the Table 2. The substrate conversion efficiency of glucose assuming 4 mol of hydrogen per mole of glucose consumed was studied to show a maximum value of 23% using pure cultures. However, the experiments were conducted for around 8 to 15 days. No previous experiments have been conducted to find the substrate conversion efficiency during the sequencing period of 24 hours.

**Table 2:** Kinetic Model Parameters.

Time (hrs)	Hydrogen (ml/hr)	VSS (g/L)	$\mu$ ( $\text{hr}^{-1}$ )	$k_c$ ( $\text{hr}^{-1}$ )	$k_s$ ( $\text{hr}^{-1}$ )	Y	$r_g * 10^{-3}$ (l/l/hr)	$r'_g * 10^{-3}$ (l/g/hr)	$\eta$
0	0	0.8	-	-	-	-	-	-	0
2	3.15	1.2	0.2	0.21	1.16	1.15	2.52	7.88	0.91
4	2.64	5.2	0.47	0.58	0.81	0.11	2.11	0.6	1.51
6	4.08	5.7	0.33	0.42	0.66	0.1	3.26	0.83	2.34
8	6.51	8.6	0.3	0.44	0.49	0.068	5.21	0.88	3.73
10	8.93	9.8	0.25	0.39	0.46	0.056	7.14	0.99	5.12
12	11.1	10.4	0.21	0.34	0.24	0.05	8.88	1.16	6.34
24	1.84	27.2	0.15	0	0.16	0.02	1.47	0.07	6.34

VFA evaluation through HPLC indicated presence of acetic acid and propionic acid within the system, which could be the possible substrate for hydrogen production is shown in figure 13



Detector A (210nm) Retention Time	Area	Area Percent	Height
0.890	25465	0.59	878
2.830	402647	9.36	6064
4.112	1876492	43.62	59415
4.670	988863	22.98	21533
7.137	779279	18.11	19586
<b>Totals</b>	<b>4072746</b>	<b>94.66</b>	<b>107476</b>

Figure 13

### Summary and conclusions

1. Kinetic model parameters for hydrogen production from synthetic feed also formed an objective for this study. The anaerobic suspended batch reactor was supplied with the pre treated anaerobic mixed culture and was fed with synthetic feed containing glucose as co-substrate. The anaerobic stirred tank reactor showed consistency in its results on feeding with the synthetic feed. This set of experiments aims at studying the variation of process parameters on using the optimized co-substrate and nitrogen source as the feed for the reactor.

**Table 3:** Details of hydrogen production from the experimental study.

S.No	Industrial Wastewater	Organic loading rate (OLR) (Kg COD/cum-day)	Hydrogen Production (mmol/day)
1	Designed synthetic wastewater	3.80	0.486

2. It is evident from the data that the suspended growth configuration has yielded hydrogen production without process inhibition, however the process of hydrogen production seems to dependent on the type of wastewater and organic loading rate. Though efficient research study has been affected in this field with a high degree of success in achieving the established objectives and wastewater characteristics plays a major role in determining the stability in system performance especially in case of hydrogen production through anaerobic fermentation.

This interest will be motivated by their broad field of application, ranging from their use in wastewater treatment to biological hydrogen production .Implementation of these applications in our daily life will require the collaboration of biologists, chemists and engineers to integrate their knowledge from diverse fields.

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