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Comparative Studies on the Effect of pH And VFA Formation on Bio Hydrogen Production by Anaerobic Suspended Growth Reactor using Synthetic Feed and Complex Feed during Sequencing period

R.Hema krishna[1], S.Venkata Mohan [1], A.V.V.S.Swamy [2],
[1.]Indian institute of chemical technology (IICT), Bio Engineering and Environmental Centre, Hyderabad-500007, Andhra pradesh,India.
[2.]Department of Environmental Sciences, Acharya Nagarjuna Uuniversity,Nagarjuna Nager-522510, Guntur(Dist.) Andhra Pradesh, India.

Abstract
A series of batch tests were conducted to investigate the effects of pH and VFA on biological hydrogen production. The tests were run in The stirred tank reactor, manufactured by Nalgene, and determine the optimal operating conditions to maximize hydrogen production using glucose and sucrose as organic substrates. Apart from hydrogen production , variations in pH, volatile fatty acid concentrations were also monitored. A mixed microbial culture, synthetic feed and complex feed were involved in the fermentation process. The inlet pH was maintained at 6.0 throughout the study while the outlet pH remained almost constant (4.1) The decrease in pH gives a favorable acid formation but it was studied that the production of hydrogen was affected and terminated by low pH. The optimum pH range for hydrogen production being in the range of 5-6. But in this study, though the hydrogen production was found to have a maximum value at the pH of 4.3. This can be attributed to the presence of a reducing atmosphere and a healthy acid formation in the reactor. The initial VFA of the feed varied from 1929 mg/L to 2113 mg/L. However, the VFA conversion showed constant values after a HRT of 24 hours. This consistency in VFA formation provides a perfect atmosphere for the acidogenic activity of the microbial consortia and a greater hydrogen production by the feed. 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 hydrogen gas estimated showed higher variations throughout the experiment. The hydrogen production in the first two days was greater than 3.45 mmol/day but the value decreased on the 3rd day to 1.18 mmol/day indicating a possible system failure. However, the hydrogen values estimated from the anaerobic fermentation of the complex feed was greater than that estimated with the synthetic feed indicating the success of the optimization studies. The hydrogen production rate showed maximum values for fermentation of synthetic feed at the 8th and the 10th hour (0.098 mmol/hr) and complex feed at 10th hour (0.29 mmol/hr). Though, both the feeds showed a decrease in hydrogen values at the end of 24 hours, the fermentation of complex feed showed greater hydrogen production rate at same reactor conditions.

Keywords: *pH, VFA, HPLC, Bio-hydrogen, Synthetic feed, Complex feed* .

INTRODUCTION
The energy sources such as solar, wind, tidal, wave, ocean, thermal, geothermal etc. are available in plenty and environmental compatible, there are still in the experimental stages and are having one (or) the other drawbacks such as productive cost and unfeasible technological Know-How. By contrast Hydrogen is consider as the only conceivable energy source by many researches as an ideal fuel of the future because of its non polluting (The end product of combustion being water) and renewable nature (Auliffe 1973, Gregory 1973).

Increase in industrial processes has been resulting in enormous amount of effluents generation for which selection of appropriate treatment methodology is extremely challenging. At present, hydrogen is mainly produced from fossil fuels, biomass and water. Among these methods, steam-reforming process alone produces about 90% of hydrogen. *(Das and Veziroglu, 2001).*A hydrogen production from biomass resources is important in the environmental and development of energy source research fields. *(Barbosa et. al., 2001; Garcia et. al., 2000; Helena et. al. 2001; Minowa and Inoue, 1999; Wang, 1998)* Some renewable biomass resources are starch, cellulose, sucrose, lactose, and so on. Cellulose is the most abundant biopolymer on the earth and is the

chief component of plant biomass. Plant cell walls contain lignocellulose, which is composed of three major polymers: cellulose, hemicellulose, and lignin. *(Weimer and Zeikus, 1977; Haug, 1993)* These polysaccharides are hydrolyzed to form monosaccharides such as glucose, which can be converted to hydrogen through a new enzymatic pathway. However, in many ecosystems where lignocellulose compounds are degraded, interspecies hydrogen transfer occurs and the hydrogen produced by cellulolytic bacteria is used immediately by methanogens, sulfate reducers and acetogens. *(Wolin and Miller, 1988; Morvan et. al., 1996)* If the activity of hydrogentrophic bacteria contained in anaerobic digested sludge were inhibited, the sludge would posses significant capacity to transform the cellulose to hydrogen. *(Jiunn-Jyi Lay, 2000)* Enrichment procedures, heat-shock, inhibit or kill nonspore-forming bacteria (hydrogen consuming methanogens) and enrich spore-forming bacteria (hydrogen producing acidogens). In the past, much research concerning hydrogen gas production produced a small net amount of hydrogen gas since the methanogens quickly utilized the hydrogen to produce methane. (Lay et. al., 1999) Inhibiting methanogens will enable the hydrogen gas to be recovered. Furthermore, in an anaerobic process producing hydrogen gas from waste, such as a wastewater treatment reactor, the bacterial culture would have to be mixed culture since the wastewater itself contains a mixed culture. Research in anaerobic method has used many mixed cultures sewage, anaerobic digestion sludge, landfill sediments, hydrogen-explosion soyabean silos, and sludge compost. *(Lay et. al., 1999; Roychowdury et. al., 1988; Ueno et. al., 1995; Sparling et. al., 1997)* Biohydrogen generation from industrial effluents is not studied extensively compared to biomass. Unlike other hydrogen forming species such as green algae, production of hydrogen with anaerobic processes is accompanied by breakdown of organic substrates and appears to be advantageous in converting organic wastes in the environment into more valuable energy resources. Fermentative hydrogen production can be maximized through the effective coupling of the factors such as rich source of electron and biochemical electron pump along with an active hydrogenase. *(Classen et. al., 1999)* The carbon source present in industrial and domestic effluents will be converted to hydrogen in the hydrolysis step of anaerobic fermentation.

In the light of the above Comparative studies of pH, VFA formation and hydrogen production by Anaerobic suspended growth reactor using synthetic feed and complex feed during sequencing period were investigated.

MATERIALS AND METHODS

Analytical Procedures

The performance of reactor with complex chemical effluents 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).

Reactor Startup : The reactor was inoculated with biomass acquired from an operating laboratory scale Upflow Anaerobic Sludge Blanket Reactor (UASBR) unit, which has been in operation continuously for 3 years for the treatment of complex chemical effluents. About 300 ml of the anaerobic sludge, Volatile suspended solids (VSS): 3.5 g/liters) from the anaerobic reactor was acquired and fed to the suspended reactor. It was subjected to acid treatment at pH 3.0 adjusted with ortho-phosphoric acid and left undisturbed for 48 hours. Further treatment with 0.2 g/l of 2-bromethansulfonic acid sodium salt ($C_2H_4BeNaO_3S$) for 24 hours was performed to inhibit the methanogenic bacteria present in sludge under aseptic anaerobic conditions.

Reactor Operation : The reactor has a total working volume of 1.3-liter capacity. The hydrogen fermentation was conducted at mesophilic temperature (29 ± 2^0C). 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, decided based on the optimization studies. The suspended reactor was started with synthetic feed, which has the composition as shown in Table 1. About 1 liter 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, VFA, and hydrogen gas production. The suspension was maintained by recirculating the feed through a tube aided by a peristaltic pump operating at 100 rpm. Initial 5 days of operation in upflow feed recirculation mode produced negative results due to absence of suspension. The next 10 days operation was performed by upflow of sludge through the recirculation tube to keep the reactor in suspension. Then, sequencing was done 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, glucose, protein and hydrogen gas parameters. High Power Liquid Chromatography (HPLC) for the samples was carried out.

Table 1. Composition of the Synthetic feed used in the experiments of biohydrogen production

Nutrients	***Composition (g/l)***
NH_4Cl	0.5
KH_2PO_4	0.25
K_2HPO_4	0.25
$MgCl_2.6H_2O$	0.3
$FeCl_3$	0.025
$NiSO_4$	0.016
$CoCl_2$	0.025
$ZnCl_2$	0.0115
$CuCl_2$	0.0105
$CaCl_2$	0.005
$MnCl_2$	0.015
Glucose ($C_6H_{12}O_6$)	3

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 Configuration : The stirred tank reactor, manufactured by Nalgene, consists of a plasticvessel with a curved bottom. The reactor has a magnetic pellet at the center of a 2 axial blade turbine,which rotates about its axis with the help of magnetic force developed by a magnetic stirrer. The reactorhas two openings at the top for inlet and outlet purposes. The various design details of the reactor are:Total Capacity: 2.2 liters; Working Capacity: 1 liter, `Overall height: 266 mm and Outer Diameter of the reactor: 137 mm.

Reactor setup and inlet conditions : The reactor has a total working volume of 1.25-litres. The hydrogen fermentation was conducted at mesophilic temperature (29 $\pm$ 2^0C). 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, VFA, 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, Glucose, and Hydrogen gas parameters. HPLC for the samples was carried out.

Complex Feed (Reactor operation): Complex feed refers to the variable concentrations of nutrients required to enhance fermentation and hydrogen production process. Based on optimization

studies, the complex feed was specified as shown in the Table 2. The sucrose concentration was calculated to maintain an organic loading rate of approximately 5000 mg/l. DAP concentration was based on N: P ratio of 5:1. About 1 liter of the 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, VFA, and hydrogen gas production. The reactor was analyzed for the various important process parameters for the inlet and outlet samples for around 3 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 was maintained. The sequencing samples were monitored for pH, VFA, Alkalinity Sucrose and Hydrogen gas parameters. The analysis of samples were carried out with the help of HPLC. The substrate conversion efficiency was also calculated at various time intervals in sequencing period.

Table 2 . Complex feed composition

Nutrients	***Composition (g/l)***
Di-Ammonium Phosphate	0.5
$MgCl_2.6H_2O$	0.3
$FeCl_3$	0.025
$NiSO_4$	0.016
$CoCl_2$	0.025
$ZnCl_2$	0.0115
$CuCl_2$	0.0105
$CaCl_2$	0.005
$MnCl_2$	0.015
Sucrose ($C_{11}H_{22}O_{11}$)	3.74

pH

The pH of an aqueous solution is defined as negative logarithm of hydrogen ion concentration. pH values from 0 to 7 denote diminishing acidity while 7 to 14 denote increasing alkalinity and 7 is neutral. Increase or decrease in pH by one unit is equivalent to ten-fold decrease or increase in hydrogen ion concentration.

The pH was determined by measurement the emf of a cell comprising an indicator electrode (an electrode responsive to hydrogen ions such as glass electrode) immersed in the test solution and the reference electrode (Calomel electrode). The EMF of this cell was measured with the pH meter. The electrode system was calibrated against standard buffer solutions of known pH. Since buffer solutions might deteriorate as a result of mould growth of contamination, fresh buffer was prepared. pH 4 buffer is best for the single glass electrode. Saturated KCl is required for a calomel and Ag/AgCl reference electrode. Before use, the electrode was removed from storage solutions, rinsed, dried with a soft tissue paper and placed in pH 4 buffer solution and the is potential point was set. Second buffer of 7 or 9 pH was selected and calibrated. Temperature of measurement was recorded and adjusted by setting temperature dial on meter so that the meter indicates pH value of buffer at test temperature. Electrodes were removed from buffer, rinsed thoroughly with distilled water and the electrodes were dried as described above. The purpose of standardization was to adjust response of the glass electrode to the instrument. After standardization the electrodes were immersed in the test sample and the pH was read.

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

Alkalinity of the water is its capacity to neutralize a strong acid and is characterized by the presence of all hydroxyl ions capable of combining with the hydrogen ion. The number of milli-equivalents (meq) of acid used in the titration to combine all the hydroxyl ions is called as total alkalinity. Alkalinity in wastewater results from the presence of hydroxides, carbonates, and bicarbonates of the elements such as Ca, Mg, Na, K and NH_3. The alkalinity in wastewater helps to resist changes in pH caused by acid

addition. Wastewater is normally alkaline from water supply, ground water and materials added during domestic use.

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_2. The sample was cooled immediately for 2min and the sample was titrated with 0.1N NaOH to pH =6.5 (B ml).

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

Alkalinity (mg/l) = (A- B)* dilution factor * 60.

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 4^0C

Procedure

A volume of 0.1 and 0.2ml of sample should be collected into an clean test tube. The volume was made upto 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 upto 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.

Protein Estimation

Protein concentration in the extra-cellular extract was determined using Lowry's method, and for this bovine serum albumin as taken as standard.

Reagents

Reagent A: 2% Na_2CO_3 in 0.1N Sodium hydroxide.

Reagent B: 0.5% CuSO4, 5 H_2O in 1% sodium potassium tartarate.

Alkaline copper solution (reagent C): Mix 50ml reagent A and 1ml of reagent B prior to use

Folins reagent.

Procedure

Pipette out 0.1 and 0.2 ml of sample into two clean test tubes. Make up the volume to 1ml in both the test tubes. A tube with 1ml of water serves as blank. Add 5ml of reagent C to each tube and allow it to stand for 10 minutes. Then add 0.5ml of folins reagent and mix well. Incubate the tubes at room temperature in the dark for 30 minutes. Blue colour will get developed. Take the readings at 660nm. A standard graph has been drawn and the amount of protein was calculated.

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. This is converted to mmol using the calculations as explained below.

The following assumptions are made:

1. Hydrogen gas is considered to be ideal in nature.
2. The air space in the reactor consists only of air and hydrogen. CO_2 composition is considered to be negligible enough to affect the calculations.
3. The temperature and pressure in the reactor is 27^0C and 1 atm respectively.

By Ideal Gas Law,

P * V = N * R * T

Where, P = pressure of hydrogen in the reactor = 1 atm

V = volume of hydrogen in the reactor (ml)

N = number of moles of hydrogen (mol)

T = temperature = 27^0C= 300 K

R = Ideal Gas Constant = 0.0821 L x atm/mol x K

➔ 1 x V = N x 0.0821 x 300

➔ V= N x 24.63 L = Volume of hydrogen

Now, %vol = (volume of hydrogen/volume of air) x 100 = XC (assumption)

Where XC is asuuming percentage of hydrogen per volume

➔ Volume of hydrogen = 0.01 x XC x Volume of air

➔ N * 24.63 = 0.01 x XC x Volume of air

➔ **N= number of moles of hydrogen = 4.06 x 10^{-4} x Volume of air x XC**

For the upflow suspended reactor,

Volume of air = 300 ml = 0.3 L.

➔ **N = 0.122 x (%vol) mmol**

For anaerobic contact stirred reactor,

Volume of air = 850 ml = 0.85 L.

➔ **N = 0.3451 x (%vol) mmol**

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 5th 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.

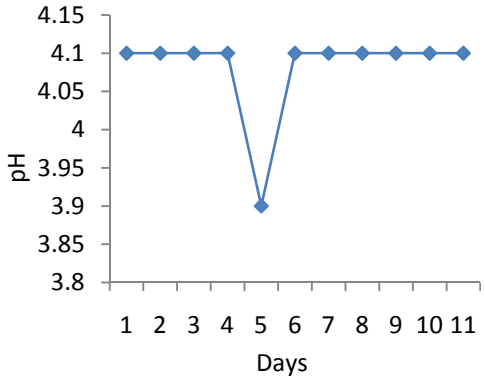


Fig.1. Study state condition of pH in a suspended growth reactor

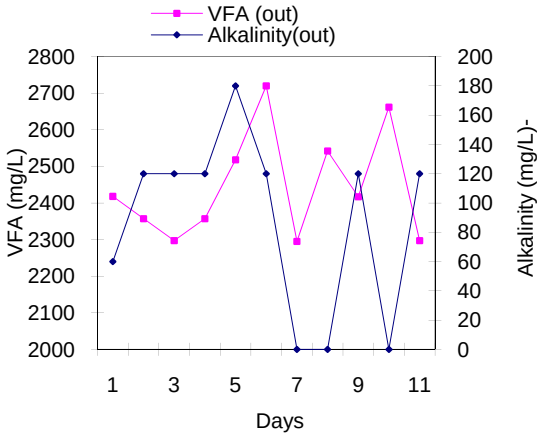


Fig.2 Variation of VFA-concentration and alkalinity of inlet and outlet of Synthetic feed at different HRTs.

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 7th day and a maximum of 2720 mg/L on the 6th day. VFA showed an increase of 300-400 mg/l after a HRT (Hydrogen 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 hydrogen production rate is calculated is shown in figure 3. 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

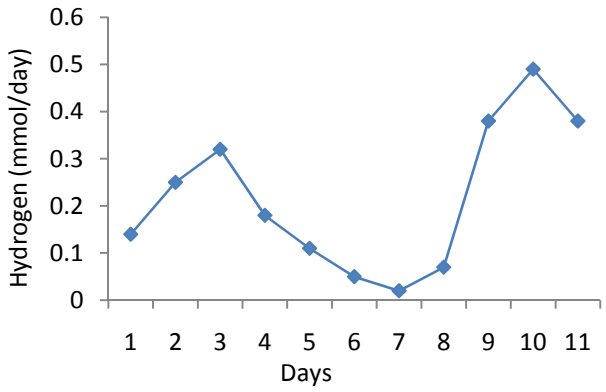


Fig.3 Variation of hydrogen production rate at different HRTs.

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. The variation of pH as depicted in figure .4 shows a drop from 5.7 at the 0th hour to 4 at the end of 8th hour followed by a small increase to 4.1 at the end of 10th 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.

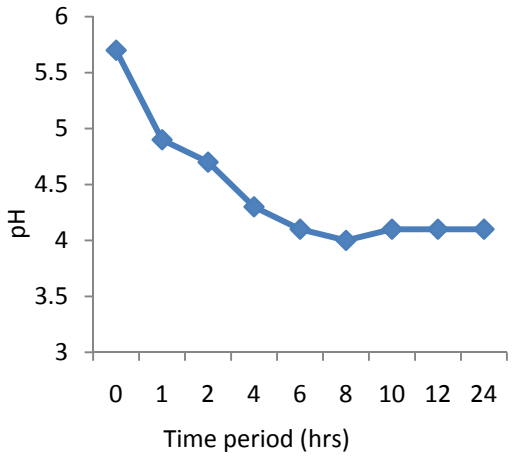


Fig. 4 Variation of pH of synthetic feed at different HRTs

The variation in VFA as shown in figure .5 showed a constant increase from 2052 mg/L at the 0th hour to around 2661 mg/L at the end of 12th hour followed by a decrease in the VFA to 2417 mg/L at the end of 24th hour. The VFA of the synthetic feed showed a maximum value at the end of 12th hour (2661 mg/L). The VFA variation indicates a stable performance of the system till the 12th 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 0th hour to 0 mg/L at the end of 2nd hour after which stability remained indicating consistent VFA

formation until the 12th hour. Then the alkalinity values also started increasing finally showing 60 mg/L at the end of the reaction period.

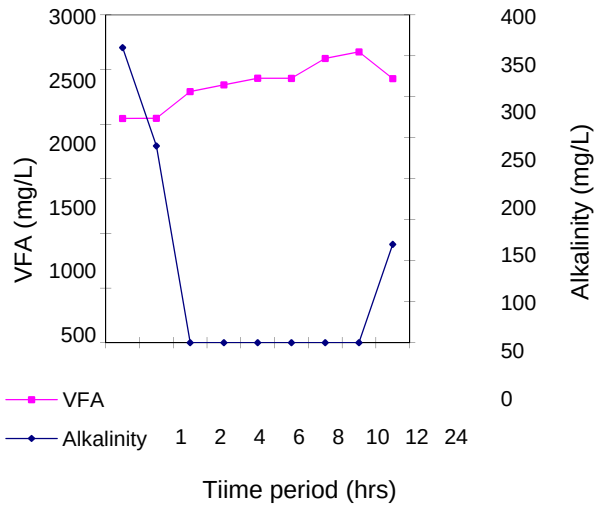


Fig.5. Variation of VFA-concentration and alkalinity of inlet and outlet of synthetic eed at different HRTs.

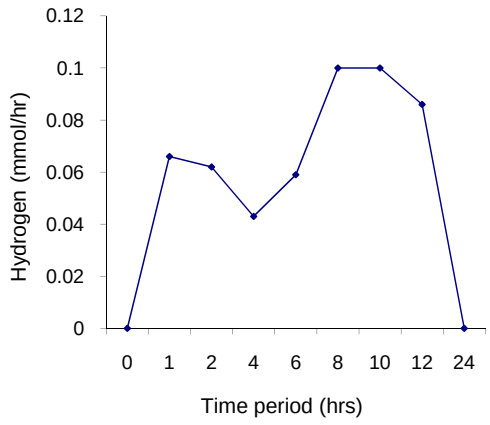


Fig.6. Variation of hydrogen production rate at different HRTs

The variation in hydrogen production complies with that discussed under the variation of VFA and alkalinity. The hydrogen production as shown in figure.6 rate decreased to 0 mmol/hr at the end of 24 hours from a value of 0.086 mmol/hr measured in the 12th hour. This indicates a decrease in system's performance. However, the hydrogen production rate increased from 0 mmol/hr in the 0th hour to 0.1 mmol/hr at the 8th and 10th hours before starting to decrease to lower values. The system's performance required kinetic study to establish conclusions.

Upscaling Anaerobic suspended growth reactor using complex feed

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. The complex feed containing sucrose and DAP was calculated to maintain a COD value of 5000 mg/L.

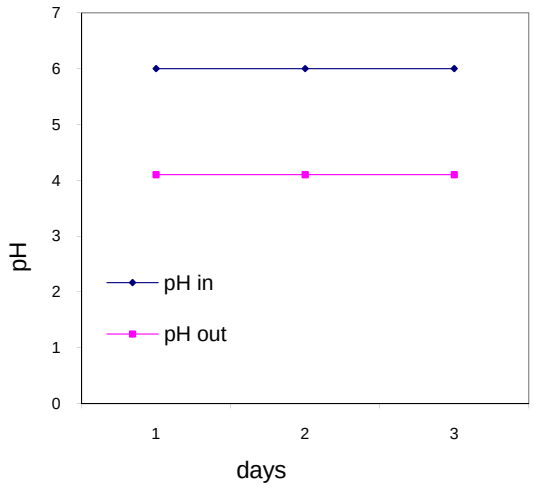
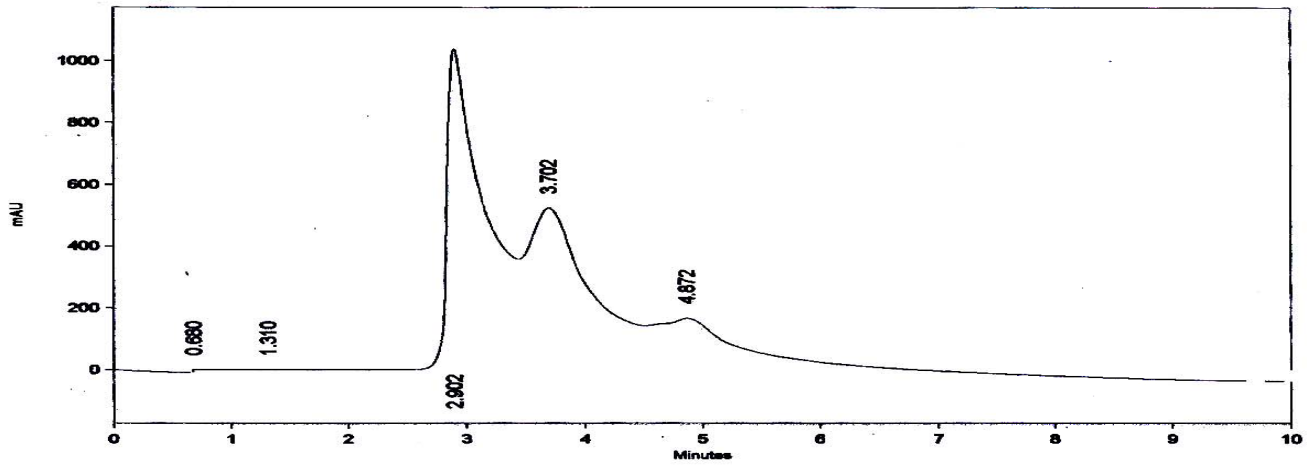


Fig.7. Inlet and out let pH values of complex feed composition

The initial pH was maintained at 6. The final pH showed a constant value (4.1) indicating consistency in anaerobic fermentation of the feed. Figure .7 shows that even though the final pH was low, it did not prove inhibitory for hydrogen production and VFA formation due to greater air space and lower partial pressure of hydrogen.



Detector A (210nm) Retention Time	Area	Area Percent	Height
0.680	167959	0.28	8204
1.310	289689	0.48	10050
2.902	24887926	41.26	1054261
3.702	21063138	34.92	540721
4.872	13912499	23.06	187583
Totals	60321211	100.00	1800819

Fig 9.VFA evaluation through high power liquid chromatography- spectrum

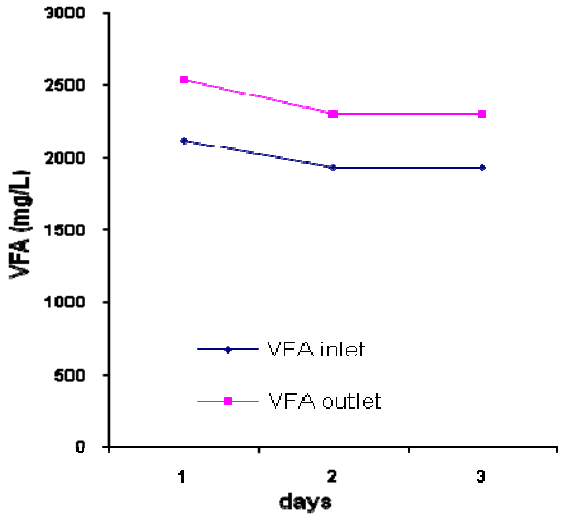


Fig.8. Variation of VFA-concentration of inlet and outlet of complex feed at different HRTs.

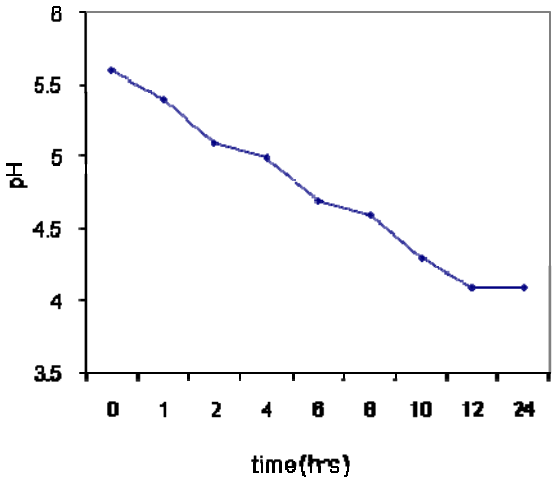


Fig. 10 Variation of pH of complex feed at different HRTs

The initial VFA of the feed varied from 1929 mg/L to 2113 mg/L as shown in figure 8. However, the VFA conversion showed constant values after a HRT of 24 hours. This consistency in VFA formation provides a perfect atmosphere for the acidogenic activity of the microbial consortia and a greater hydrogen production by the feed.

VFA evaluation through HPLC indicated presence of acetic acid within the system which could be the possible substrate for hydrogen production is shown in figure 9

The sequencing procedure also showed a number of variations in the process parameters especially the hydrogen production rate. The pH values varied from 5.6 on the 0th hour to 4.1 at the 24th hour. The variation in the pH was a constant decrease till the 8th hour (4.1) and further onwards, a constant pH value was witnessed till the end of experiment.

The decrease in pH gives a favorable acid formation but it was studied that the production of hydrogen was affected and terminated by low pH. The optimum pH range for hydrogen production being in the range of 5-6 (Ueno et. al., 1995; Venkata Mohan et al., 2005). But in this study, though the hydrogen production was found to have a maximum value at the pH of 4.3 as shown in figure.10. This can be attributed to the presence of a reducing atmosphere and a healthy acid formation in the reactor. The VFA values increased throughout the sequencing period till 12 hours indicating a consistent anaerobic fermentation of the feed by the microbial consortia. The VFA value at the 0th hour (2173 mg/L) is more than in the 1st hour (2115 mg/L) due to presence of some amount of acids in the reactor, which adds on during sampling. However, the VFA value dipped from 3334 mg/L at the 12th hour to 2539 mg/L at the end of 24th hour as shown in figure .11 indicating the fermentative inhibition of the pH discussed above.

The alkalinity values also decreased throughout the experiment and showed an increase at the 24th hour indicating an increase in buffering capacity of the system. This aided in decrease of acid formation and hydrogen production even though the pH value lied in an acidic range.

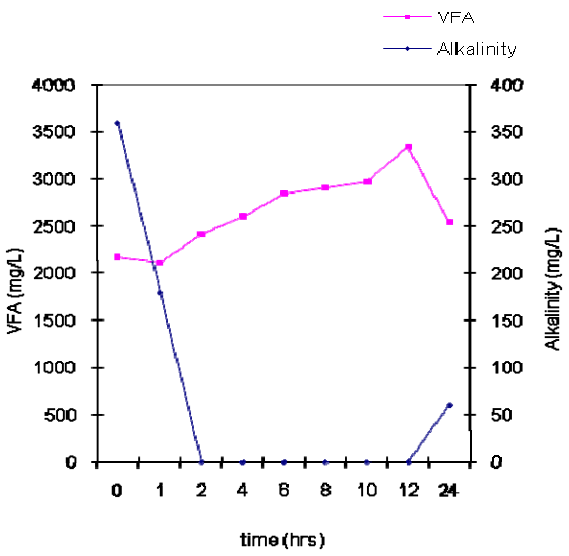


Fig.11 Variation of VFA-concentration and alkalinity of inlet and outlet of complex Feed at different HRTs.

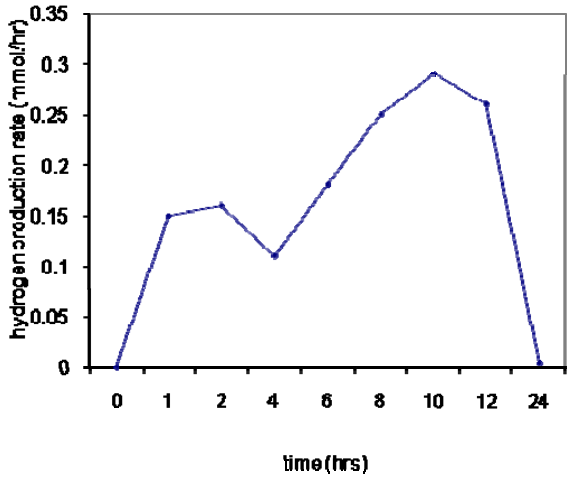


Fig.12. Variation of hydrogen production rate at different HRTs.

The hydrogen production rate values increased till the 2nd hour and showed a small decrease at the end of 4th hour followed by an increase till the 10th hour where it recorded the maximum value of 0.29 mmol/hr is shown in figure.12 Then the hydrogen values dropped indicating an inhibitory process due to drop in pH and other factors between the 12th and 24th hours.

Comparative studies of pH, VFA formation and hydrogen production by synthetic feed and complex feed during sequencing period

Comparison of the important process parameters obtained during experiments in the

anaerobic stirred tank reactor with synthetic and the complex feed aids in proposing the degree of success of the up scaling studies using optimized parameters.

The pH values during sequencing of synthetic feed in a stirred tank suspension reactor showed a drop in values from 5.7 at the 0th hour to 3.9 at the end of 24 hours. However, it was studied that the hydrogen production is inhibited at lower pH of 4 $\pm$ 0.1 (Ueno et al., 2001) which can be seen from the graph. However, the pH variation of complex feed, although being similar in profile, was a little higher (pH range of 5.6 to 4.1 after 24 hours HRT) than that obtained through synthetic feed fermentation as in figure 13.

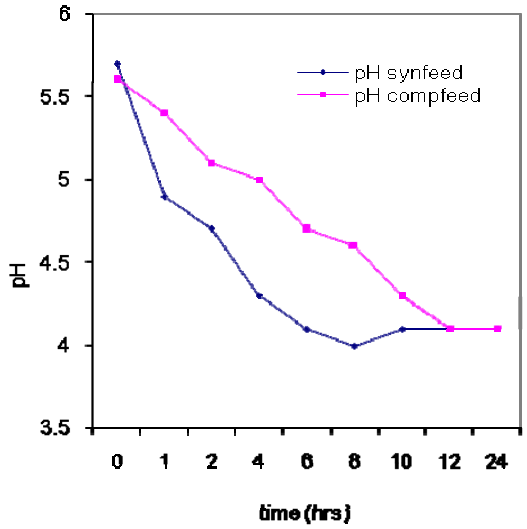


Fig. 13. pH variation in synthetic feed and complex feed at different HRTs

This comparative study on hydrogen production rate with time for fermentation of synthetic and complex feeds is done to indicate the best substrate for acidogenic fermentation process in a 2.1 liters stirred tank suspension reactor as shown in figure 14 .The hydrogen production rate showed maximum values for fermentation of synthetic feed at the 8th and the 10th hour (0.098 mmol/hr) and complex feed at 10th hour (0.29 mmol/hr). Though, both the feeds showed a decrease in hydrogen values at the end of 24 hours, the fermentation of complex feed showed greater hydrogen production rate at same reactor conditions. Thus, the VFA and hydrogen production rate comparisons for both the feeds indicates a better prospective for sucrose as a better carbon source and nitrogen source for hydrogen production from anaerobic bacterial consortia through inhibition of methanogenic organisms.

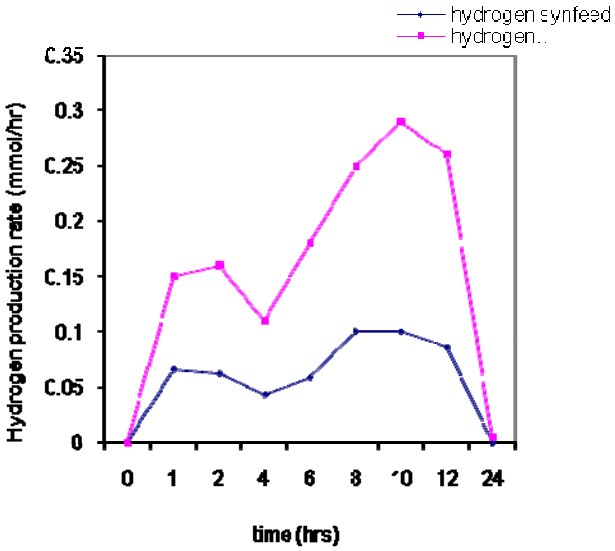


Fig. 14. Variation of Hydrogen production rate by synthetic feed and complex feed at different HRTs

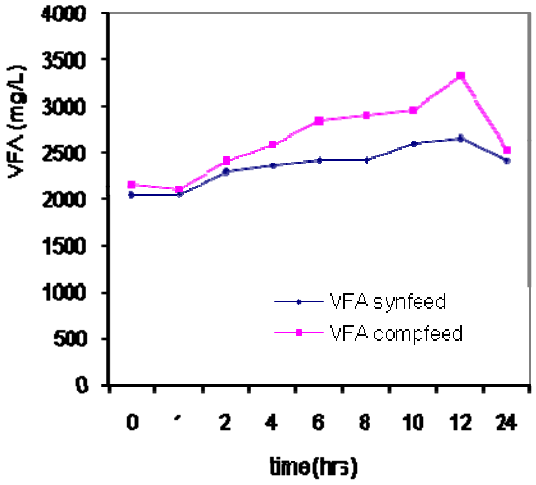


Fig. 15. VFA variation in synthetic feed and complex feed at different HRTs

The VFA values for synthetic feed showed a constant increase till the 12th hour and then a decrease at the 24th hour as in case of complex feed. The VFA value reached its maximum at 12th hour (2661 mg/L), which is low compared to that produced by the complex feed (3334 mg/L) at the same time with the same reactor conditions is shown in figure 15.

SUMMARY AND CONCLUSION

The consistency in VFA formation provides a perfect atmosphere for the acidogenic activity of the microbial consortia and a greater hydrogen production by the feed.

The decrease in pH gives a favorable acid formation but it was studied that the production of hydrogen was affected and terminated by low pH. The optimum pH range for hydrogen production being in the range of 5-6. But in this study, though the hydrogen production was found to have a maximum value at the pH of 4. 3 .This can be attributed to the presence of a reducing atmosphere and a healthy acid formation in the reactor.

The hydrogen gas estimated showed higher variations throughout the experiment. The hydrogen production in the first two days was greater than 3.45 mmol/day but the value decreased on the 3rd day to 1.18 mmol/day indicating a possible system failure. However, the hydrogen values estimated from the anaerobic fermentation of the complex feed was greater than that estimated with the synthetic feed indicating the success of the optimization studies.

This comparative study on hydrogen production rate with time for fermentation of synthetic and complex feeds is done to indicate The hydrogen production rate showed maximum values for fermentation of synthetic feed at the 8th and the 10th hour (0.098 mmol/hr) and complex feed at 10th hour (0.29 mmol/hr). Though, both the feeds showed a decrease in hydrogen values at the end of 24 hours, the fermentation of complex feed showed greater hydrogen production rate at same reactor conditions. Thus, the VFA and hydrogen production rate comparisons for both the feeds indicates a better prospective for sucrose as a better carbon source and nitrogen source for hydrogen production from anaerobic bacterial consortia through inhibition of methanogenic organisms.

Extensive research was in progress in Bioengineering and Environmental Centre (BEEC) laboratory of Indian Institute of Chemical Technology (IICT), Hyderabad regarding bioprocess monitoring during hydrogen production, reactor configuration optimization, characterization of hydrogen producing bacteria, bioaugmentation strategy, etc. in the direction of biohydrogen production from industrial wastewater using periodic discontinuous process operation..

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