

Original Article

# Examine the Feasibility of Anaerobic Biohydrogen Production Starting from a 25%-75% Mixture of the Complex Feed and the Bulk Drug Wastewater

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**Abstract** - Hydrogen was one of the most promising energy alternatives and was expected to play a significant role in future energy supply because it is clean, CO<sub>2</sub>-neutral, recyclable, and efficient. This study was carried out to investigate the efficiency of Anaerobic Stirred Tank Reactor (ASTR) for Biohydrogen production from Bulk drug wastewater treatment by anaerobic fermentation technology. After inoculating with selectively enriched mixed consortia, the bioreactor was initially operated with a 25%-75% mixture of the complex feed and the bulk drug wastewater as feed at OLR of 5.04 Kg COD/m<sup>3</sup>-day by adjusting the influent pH to 6.0 for 36 days. In this study, the inlet pH (feed) was maintained at six, while the outlet pH did not show any variation in its value of 3.8 to 5.0 throughout the reaction period. The inlet VFA varied within the range of 1500 mg/L to 3500 mg/L, while the outlet VFA values showed similar variations but were higher than the inlet VFA, indicating an acidogenic fermentation process in the system. The alkalinity values decreased gradually, indicating an increased system response to acidogenic fermentation. COD reduction (%) means organic substrate degradation in the culture for hydrogen production. This research article revealed that maximum hydrogen production of 1.42 mmol/hr on the 4th day indicates enhanced system performance. This proves advantageous for understanding the feasibility of the bulk drug wastewater towards hydrogen production.

**Keywords** - Anaerobic Stirred Tank Reactor, Bulk drug wastewater, Complex feed, High power liquid chromatography (HPLC), Hydrogen yield.

## 1. Introduction

Aerobic and Anaerobic systems have been remarkably employed in industrial and municipal wastewater treatment for many years. While most wastewaters have previously been treated in conventional aerobic and anaerobic treatment plants, in recent years, high rate aerobic and anaerobic bioreactors have been increasingly employed for wastewaters with high chemical oxygen demand (COD). Hydrogen can be produced biologically by four different processes: direct and indirect biophotolysis, photo fermentation and dark fermentation. Fermentative hydrogen production provides higher gas production rates than photosynthetic processes, is light-independent and can utilize various carbon sources, including wastewaters[1].The theoretical maximum yield of hydrogen fermentation is reported to be four moles of hydrogen per mole of glucose or eight moles of hydrogen per mole of sucrose if all of the substrate is converted to acetic acid [2]. These values correspond to a theoretical maximum

yield of 0.467 L-H<sub>2</sub>/g-COD. If all the substrate was converted to butyric acid, these values are two and four moles of hydrogen per glucose and sucrose, respectively. In practice, a fraction of the substrate is used for biomass production and other metabolic products are also produced, resulting in a lower hydrogen yield. Hydrogen yields by pure or mixed cultures have been reported to range from 0.37 to 2.0 mole-H<sub>2</sub>/mole-glucose, considering the high theoretical yields; several researchers have begun exploring approaches to increase hydrogen production. Several researchers have used physical methods to increase hydrogen yields by applying a vacuum to the headspace of a bioreactor by sparging the biogas with nitrogen gas [3] by immobilizing cells by vigorous stirring to allow the dissolved hydrogen to escape to the gas phase or by using  $\gamma$ -Alumina, an activated alumina used as desiccant in chemical process industries, to adsorb volatile acids [4].Hydrogen is produced primarily by chemical methods (e.g., steam reforming) in industry. Steam reforming



of natural gas is currently the least expensive method of producing hydrogen, used for about half of the world's hydrogen production. Steam, at a temperature of 700-1,100 °C, is mixed with methane gas in a reactor with a catalyzer at 3-25 bar pressure. Thirty percent more natural gas is required for this process, but new processes are constantly being developed to increase the production rate. Increasing the efficiency to over 85% is possible with an economic profit at higher thermal integration. Many developed countries approved hydrogen as the only known alternative energy for fossil fuel and schemed to improve the hydrogen economic community. However, in most schemes, hydrogen production still depends on the fossil fuel. The increasing cost of fossil fuels and concerns about their impact on our environment have renewed interest in hydrogen as a clean, sustainable alternative. Biohydrogen fermentation is the biotechnology that can transfer the biomass chemical energy into hydrogen energy via biochemical reaction. Many researchers have noticed the promising novel energy technology and have been involved in developing the biohydrogen fermentation technology.

Over the past 30 years, the anaerobic wastewater treatment process has become more popular due to its advantages over aerobic processing, including low biomass production, low nutrient requirements, and fuel gas production, such as methane gas and hydrogen. Studies have shown that anaerobic treatment is a stable process under proper operation. However, parameters such as process configuration, temperature, biomass, pH, nutrients, and substrate must be carefully scrutinized to make successful anaerobic treatment. Many process configurations have been investigated. An improvement in the efficiency of anaerobic digestion can be brought about by either digester design modification or advanced operating techniques [5]. Hydrogen gas is mainly produced from fossil fuels, which will soon be depleted. In addition, hydrogen production from fossil fuels normally requires energy-intensive processes such as thermal and chemical processes, which are typically expensive and often cause environmental pollution. Therefore, there is an urgent need to develop more environmentally friendly and cost-effective alternatives to hydrogen production. Concerns about global warming increased the interest in hydrogen as a fuel. Biohydrogen production played an important role in making this interest feasible.

This production process uses bacterial microorganisms that ferment natural wastes and organic compounds into hydrogen and other non-polluting gases [6]. Biological methods for hydrogen production have recently received more attention [7]. Among many types of microorganisms that can be used to produce biological hydrogen, there are two most common ones. The first is anaerobic (i.e., fermentative) bacteria, such as *Clostridium acetobutylicum* [8], *Clostridium butyricum* [9], *Enterobacter aerogenes* [10], *Enterobacter cloacae* [11], etc. In contrast, the second is photosynthetic

bacteria, such as *Rhodobacter sphaeroides* [12], *Rhodobacter capsulatus* [13], etc. The anaerobic bacteria have specific metabolic pathways for the degradation of carbon sources, and the metabolic reactions are usually coupled with the NADH/NAD<sup>+</sup> or Fd/FdH<sub>2</sub> energy-transferring reaction that subsequently drives hydrogen production. They are usually found in the biological sludge commonly used to degrade BOD or COD and simultaneously regenerate hydrogen in wastewater treatment.

Among various hydrogen production processes, biological hydrogen production is less energy-intensive as it can operate under mild conditions. Biological hydrogen production through the dark fermentation process is technically simple, and a high rate of hydrogen evolution has been achieved compared to the photo-fermentation process [14-15]. Anaerobic digestion of wastewater for hydrogen production has been extensively investigated for its low operational cost and effectiveness advantages. Hydrogen production from renewable substrates is rapidly emerging as an alternative to fossil fuels since it has triple the energy yield of hydrocarbon fuels [16] and produces only water with no CO, CO<sub>2</sub>, hydrocarbons, or fine particles when combusted [17]. Hydrogen does not contribute to the greenhouse effect and has a high energy yield of 142 kJ/g, 2.75 times more than other hydrocarbon [18]. Various organic wastes, such as agro-industrial waste containing lignocellulosic materials, could be used as substrates to produce hydrogen through dark fermentation [19].

Nonetheless, the degradation of lignocellulosic biomass is time-consuming since it contains cellulose and hemicelluloses [20]. Hence, enzymatic hydrolysis could convert lignocellulose biomass into monomeric sugars [21]. Today, biological H<sub>2</sub> production processes are becoming important mainly for two reasons: (i) they can utilize renewable energy resources, and (ii) they are usually operated at ambient temperature and atmospheric pressure. However, these processes' reported biohydrogen production rates, stabilities and efficiency are insufficient to make them commercially viable. Biological hydrogen production is a viable alternative to the abovementioned methods for hydrogen gas production.

Under sustainable development and waste minimization issues, bio-hydrogen gas production from renewable sources, also known as "green technology", has received considerable attention in recent years. Production of clean energy sources and utilization of chemical wastewater make biological hydrogen production a novel and promising approach to meet the increasing energy needs as a substitute for fossil fuels. The present study was taken up to evolve a functional, user-friendly, eco-friendly and economical process. This study aimed to examine the feasibility of anaerobic hydrogen production starting from a 25%-75% mixture of the complex feed and the bulk drug wastewater.

## 2. Experimental Methods

### 2.1. Reactor Configuration

The stirred tank reactor, manufactured by Nalgene, consists of a plastic vessel with a curved bottom. The reactor has a magnetic pellet at the centre of a two-axial blade turbine, which rotates about its axis with the help of magnetic force developed by a magnetic stirrer. The reactor has two openings at the top for inlet and outlet purposes. The various design details of the reactor are: Total Capacity: 2.2 litres, Working Capacity: 1 litre, Overall height: 266 mm, Outer Diameter of the reactor: 137 mm shown in Fig 1.



Fig. 1 Anaerobic batch stirred tank reactor

### 2.2. 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 + 20C). The pH was maintained at 6 to ensure the fermentation process did not yield a drastic drop in the Ph value after an 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.

### 2.3. Synthetic Feed Studies (Reactor Operation)

The reactor was started with synthetic feed, which has the composition as shown in Table 1. About 1 litre of artificial feed was taken and fed to the reactor, and the inlet and outlet samples (after an HRT of 24 hours) were collected. They were continuously examined for pH, ORP, VFA, COD and hydrogen gas production. The reactor was analyzed for the various essential 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 models was carried out. The reactor kinetics and substrate conversion efficiency were also calculated using the biomass and substrate concentrations at various time intervals in the sequencing period.

### 2.4. Complex Feed (Reactor Operation)

Complex feed refers to the variable concentrations of nutrients required to enhance the fermentation and hydrogen production process. The complex feed was specified based on optimization studies, as shown in Table 1. The sucrose concentration was calculated to maintain organic loading rates of approximately 5000 mg/l. DAP concentration was based on N: P ratio of 5:1. About 1 litre 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 continuously examined for pH ORP VFA, COD and hydrogen gas production. The reactor was analyzed for the various essential process parameters for the inlet and outlet samples for around three 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 sequencing samples were monitored for pH, VFA, Alkalinity, COD, Sucrose and Hydrogen gas parameters. HPLC for the examples was carried out. The substrate conversion efficiency was also calculated at various time intervals in the sequencing period.

Table 1. Complex feed composition

Nutrients	Composition (g/l)
Di- Ammonium Phosphate	0.5
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
Sucrose (C <sub>11</sub> H <sub>22</sub> O <sub>11</sub> )	3.74

### 2.5. Fermentation of Complex Feed with Industrial Effluent Reactor Operation

The sucrose concentration was calculated to maintain an organic loading rate of approximately 5000 mg/l along with the industrial effluent. About 1 litre of the feed containing 50% complex feed and 50% Industrial effluent was taken and fed to the reactor, and the inlet and outlet samples (after an HRT of 24 hours) were collected. They were continuously examined for pH, ORP, VFA, COD and hydrogen gas production. The reactor was analyzed for the various essential process parameters for the inlet and outlet samples for around three 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 sequencing samples were monitored for pH, VFA, Alkalinity, COD, Sucrose and Hydrogen gas parameters. HPLC for the examples was carried out. The substrate conversion efficiency was also calculated at various time intervals in the sequencing period.

## 2.6. H<sub>2</sub> Producing Mixed Consortia

Anaerobic mixed microflora acquired from an operating laboratory scale-up flow anaerobic sludge blanket (UASB) reactor treating chemical wastewater for the past three years was used as parent inoculum. Before inoculation, dewatered sludge acquired from the UASB reactor was subjected to cyclic pre-treatment sequences (four times) changing between heat-shock (100°C, 2h) and acid { pH 3 adjusted with ortho-phosphoric acid (89%), 24 h} treatment to restrain the growth of methanogenic bacteria, at the same time to enrich the H<sub>2</sub> producing acidogenic bacteria selectively. The resulting improved mixed culture was used as inoculum to start the suspended reactor.

## 2.7. Bulk Drug Waste Water

Bulk drug wastewater pH, 7.4; Color, Yellow; TDS, 16500 mg/L; COD, 6080 mg/L; Sulfides, 25-40 mg/L; BOD, 810 mg/L; Chlorides, 5538 mg/L collected from bulk drug industry (NATCO Pharma Limited, India) was used as substrate. Due to proteins, carbohydrates, and lipids, the wastewater can be considered complex (BOD/COD = 0.49).

After collection, the wastewater was transferred immediately to the laboratory and stored at 4 °C, and the wastewater was not corrected for trace elements deficiency. Wastewater was diluted using tap water to the requisite organic loading rate (OLR) before feeding and pH adjustment.

## 2.8. Reactor Design and Operation

The stirred tank reactor, manufactured by Nalgene, consists of a plastic vessel with a curved bottom. The reactor has a magnetic pellet at the centre of a two-axial blade turbine, which rotates about its axis with the help of magnetic force developed by a magnetic stirrer. The reactor has two openings at the top for inlet and outlet purposes, as shown in Table 2.

Table 2. Anaerobic stirred tank reactor operation details [22]

Mode of operation	Sequencing/Periodic discontinuous batch mode
Feed volume	1 litre
Feeding pH (influent)	6
Gas holding capacity	0.28
HRT (Hydraulic retention time)	24 h (single cycle period- Fill-15 min, React-23 h, Settle-30 min, Decant-15 min)
Operating temperature	(29 ± 2°C).
Reactor microenvironment	Anaerobic
Sludge volume	0.28
Total volume	2.2 litres
Organic loading rate(OLR)	5.04 Kg COD/m <sup>3</sup> -day

At the beginning of each cycle, immediately after withdrawal (earlier sequence), a predefined volume (1 litre) was fed to the reactor during the Fill phase. The reactor was kept in suspension during the React phase. The reactor was operated at three OLRs (5.04 Kg COD/m<sup>3</sup>-day). Constant COD removal and H<sub>2</sub> production were considered an indicator for the stabilized performance of the bioreactor; subsequently, the reactor was shifted to a higher OLR. The influent pH was adjusted to 6.0 before feeding using *ortho*-phosphoric acid. The reactor was operated at mesophilic (room) temperature (29±2 °C). Rate of Substrate Degradation (RSD, Kg COD/m<sup>3</sup>-day) was calculated to study the rate and pattern of COD removal during the cycle operation according to Eq. (1), where COD<sub>0</sub> and COD<sub>T</sub> represent COD (mg/l) at '0' and 'T' times, respectively, FR represents feed rate (m<sup>3</sup>/day) and R<sub>v</sub> denotes reactor volume (m<sup>3</sup>).

$$RSD = \frac{[(COD_0 - COD_T) \times FR]}{R_v} \quad (1)$$

## 2.9. Analytical Procedures

The performance of the reactor with complex chemical effluents was assessed by monitoring carbon removal (COD) throughout the reactor operations and the cycle period. In addition, pH, oxidation-reduction potential (ORP), VFA, and Alkalinity were determined during reactor operation to assess the performance of the reactor. The analytical procedures for monitoring the above parameters were adopted from the process outlined in the Standard methods. The method performed to determine physicochemical parameters was adopted from standard American Public Health Association methods. H<sub>2</sub> gas generated in the bioreactor was estimated using a microprocessor-based pre-calibrated H<sub>2</sub> sensor (electrochemical three-electrode H<sub>2</sub> sensor, FMK satellite 4–20mA version, ATMI GmbH Inc., Germany). The output signal displayed the percentage volume of H<sub>2</sub> in the headspace of the bioreactor. The system was calibrated once in two days using a calibration cap provided with the instrument. The sensor measured 0.01–10% H<sub>2</sub> with 5 s response time in a 20–80 °C temperature range. H<sub>2</sub> gas monitoring was conducted under closed conditions to avoid external environmental contamination. Oxidation–reduction potential (ORP) and pH values were determined by a pH meter (Model 20, Denver Instruments Ltd.). Total alkalinities, VSS, VFA, and COD (closed refluxing method) were determined according to standard procedures [23].

## 3. Results and Discussion

### 3.1. Up-scaling study with Anaerobic Stirred Tank Reactor using complex feed and Bulk drug Wastewater

In the first stage of our earlier research, hydrogen production through anaerobic fermentation of synthetic feed was 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-litre suspended reactor. Similar analyses 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 [22,24]. In the second stage, the suspended growth anaerobic system was fed with optimized substrate, co-substrate and nitrogen sources, and several other nutrients called complex feed. This process aims at studying the variations of hydrogen production with nutrient addition. Substrate conversion efficiencies of the difficult feed were analyzed and compared with that of the synthetic feed studied in the previous stages to establish the degree of success of the optimization process [22]. In the third stage, the research has been completed on up-scaling and Sequencing studies with an anaerobic suspended growth reactor for Biohydrogen production using complex feed and Pharmaceutical Effluent (50%-50 %) for up to 33 days [24]. The objective of the present research was further scaling studies with an anaerobic suspended growth reactor for Biohydrogen production using a 25%-75% mixture of the complex feed and the bulk drug wastewater between 34 to 70 days.

**Reactor start-up:** After inoculating with selectively enriched mixed consortia, the bioreactor was initially operated with a 25%-75% mixture of the complex feed and the bulk drug wastewater as feed at OLR of 5.04 Kg COD/m<sup>3</sup>-day by adjusting the influent pH to 6.0 for 36 days shown in Table 3.

### 3.2. P<sup>H</sup> Studies in Reactor Operation

The pH values remained very low at the end of the experimental period, indicating a decrease in the system's performance in the acidogenic fermentation process. The reduction in pH gives a favourable acid formation, but it was studied that hydrogen production was affected and terminated by low P<sup>H</sup>. The optimum pH range for hydrogen production is in the field of 5-6. In this study, the inlet pH (feed) was maintained at six while the outlet pH did not show any variation in its value of 3.8 to 5.0 throughout the reaction

period (Fig 2.). The primary aim of maintaining a reactor-operating environment at pH 6.0 was to facilitate the inhibition of Methanogenic bacteria while creating a conducive environment for the effective functioning of Acidogenic bacteria. The most effective way to enhance H<sub>2</sub> production from the anaerobic culture is to restrict or terminate the methanogenesis process by allowing H<sub>2</sub> to become an end product in the metabolic flow. In this study, it was observed that adoption of low operating pH inhibited Methanogenic bacteria. This facilitated the generation of H<sub>2</sub> as a terminal product of anaerobic fermentation due to the suppression of the formation of CH<sub>4</sub>. The adopted HRT of 24 h further helped to control the methanogenic reaction. The sequencing batch operation mode of the reactor used might also have influenced the H<sub>2</sub> evolution. The sequencing/periodic discontinuous batch mode operation facilitates controlled unsteady-state conditions, and exposure time, frequency of exposure and substrate concentration can be set independent of inflow conditions. This enabled the microorganisms to be exposed to defined process conditions periodically and helped select generally more robust organisms that could withstand shock loads [26].

### 3.3 Studies of VFA and Alkalinity:

VFA production has always been associated with converting organic fractions to acid intermediates in the anaerobic microenvironment with the help of a specific group of bacteria. It represents the total of all acids generated during the acidogenic fermentation step. VFA production showed a consistent variation with the function of operating pH. Maximum VFA production (3490 mg/l) was observed at operating pH 6.0. The high VFA yield observed at operating pH 6.0 might be attributed to the prevailing acidophilic conditions inhibiting the methanogenic activity required for VFA breakdown. The inlet VFA varied from 1500 mg/L to 3500 mg/L. Still, the variation was found to show an alternate decrease and increase in the VFA values, indicating a sort of Instability present initially in the system. While the outlet VFA values showed similar variations but were higher than the inlet VFA, displaying the acidogenic fermentation process in the system is shown in (Fig. 3).

**Table 3. Details of hydrogen production from the experimental variations studied**

S.No	Industrial Wastewater	Organic-loading-rate (OLR) (Kg COD/cum-day)	Hydrogen Production (mmol/day)	References
1	Designed synthetic wastewater	3.80	0.486	[24]
2	Complex chemical wastewater	4.67	3.45	[22]
3	Complex chemical wastewater + pharmaceutical wastewater(1:1 )	5.90	2.95	[25]
4	Complex chemical wastewater + Bulk drug wastewater (1:3).	5.04	3.49	(Present study)

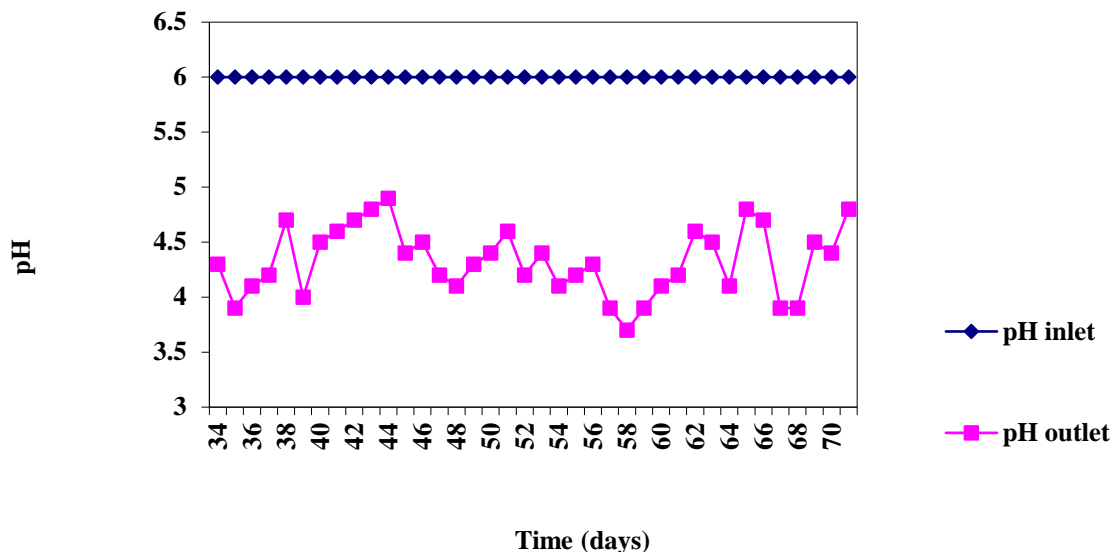


Fig. 2 Study state condition of pH inlet and outlet in a suspended growth reactor

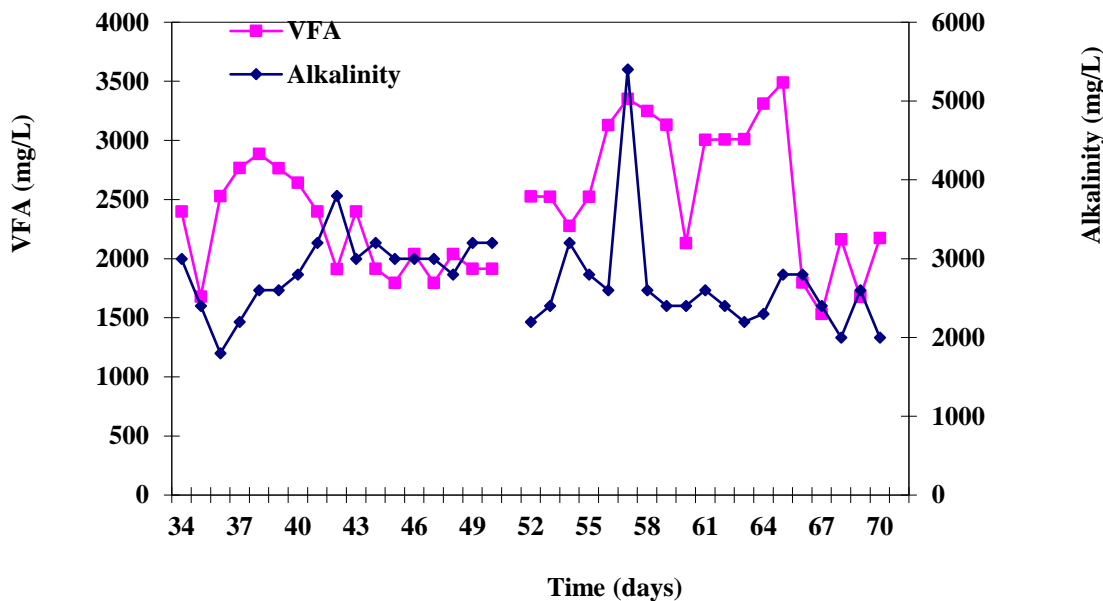


Fig. 3 Variation of VFA-concentration and Alkalinity of inlet and outlet of industrial effluent feed at different HRTs

Alkalinity in an anaerobic microenvironment was considered as an index of volatile acid generation in alliance with the existing buffering capacity (Alkalinity) of the system. Alkalinity plays a vital role in restricting the organic acid accumulation, leading to a balanced pH level within the reactor to enable hydrogen production and substrate removal during the reaction. Alkalinity during the bioreactor operation was monitored to understand the buffering activity of the reactor system [27-28]. The variation of Alkalinity lies within

the range of 1800 mg/L to 5400 mg/L, which is considered to be on the higher side, indicating the presence of greater buffering capacity in the system. The Alkalinity of the system recorded indicates healthy VFA accumulation and a relatively good resistance offered by the system against imbalance. As shown in (Fig. 2), the alkalinity values decreased gradually, indicating an increased system response to the acidogenic fermentation process.

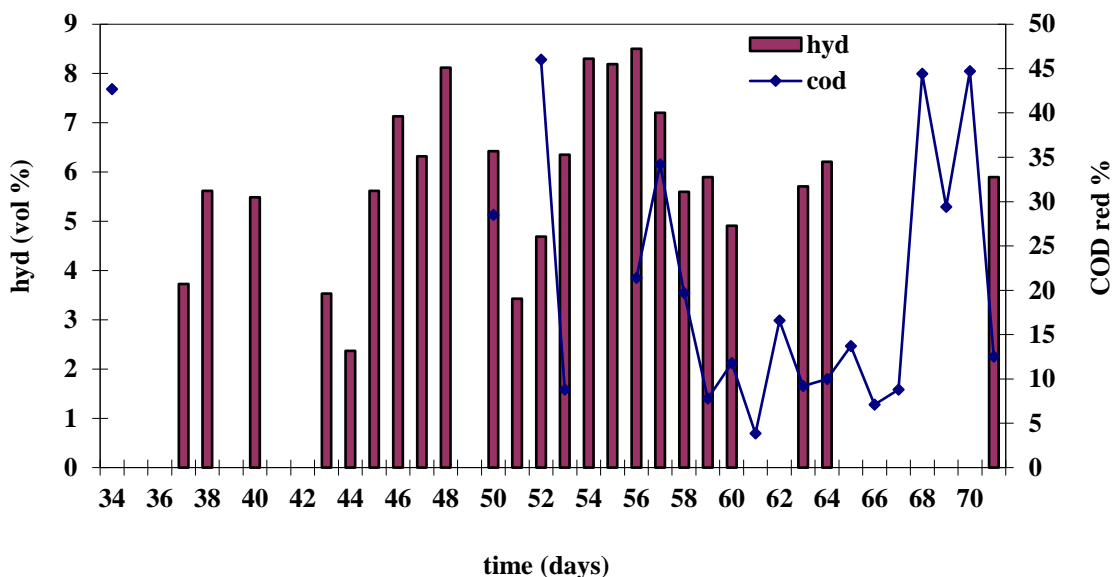


Fig. 4 Variation of COD reduction % and hydrogen production with different HRTs

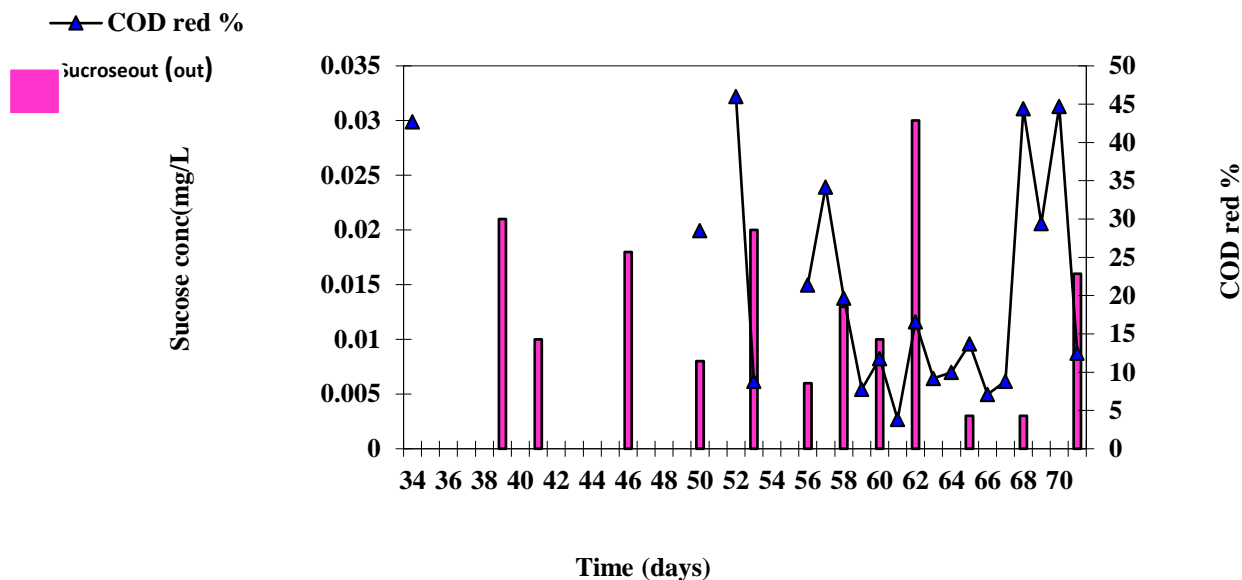


Fig. 5 Variation of sucrose concentration at different HRTs with %COD

### 3.4. Studies on COD and H<sub>2</sub> Production

The variation of COD reduction (%) indicates a multitude of variations as the experiment proceeds, indicating perfect degradation of the organic substrate present in the culture aimed towards hydrogen production(Fig .3). This set of experimentation was continued till the system attained maximum hydrogen production on a steady scale and further was stepped up by increasing the Bulk drug wastewater composition in the feed. The hydrogen production rate

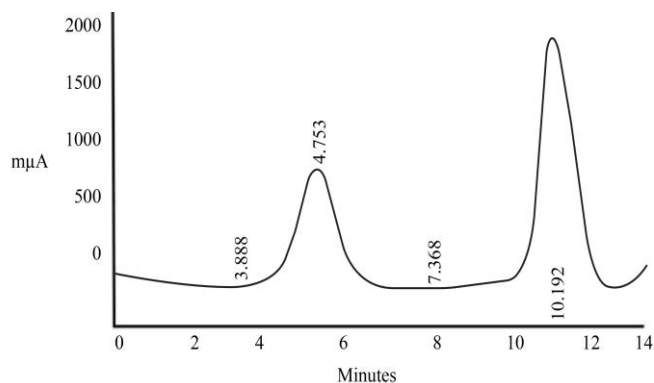
decreased drastically on the 44th day of the experiment (Fig .4). The values then soared to maximum hydrogen production of 1.42 mmol/hr on the 4th day, indicating an enhanced system performance. This proves advantageous for understanding the feasibility of bulk drug wastewater towards hydrogen production.

A parallel study of the residual sucrose concentration indicated the lowest concentrations of the compound at the

end, indicating good hydrogen production shown in (Fig. 5). However, there was an increase in the residual sucrose concentration, indicating the requirement of further time for the completion utilization of feed and attainment of highest hydrogen production.

### 3.5. Volatile fatty acids (VFA) Evaluation by HPLC Analysis

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). Under anaerobic conditions, these acids decompose to give carbon dioxide and methane. If methanogenic bacteria are inhibited and the decomposition process is controlled at acidogenesis, hydrogen gas is produced [29, 30]. Reactor samples during experiments were collected and analyzed for the composition of VFA. Chromatographic analysis of samples showed a higher acetic acid concentration and a relatively lower butyric acid concentration. This suggests that the acid-forming pathway dominated the metabolic flow during H<sub>2</sub> generation with the occurrence of acidogenesis instead of solventogenesis. High specific H<sub>2</sub> production was reported to be associated with a mixture of acetate and butyrate fermentation products. Low specific H<sub>2</sub> production was observed with propionate and reduced end products (alcohols, lactic acid) [31, 32]. This phenomenon observed in the reactor under acidophilic conditions could be considered the optimum environment for effective H<sub>2</sub> generation. In this study, VFA evaluation through HPLC indicated the presence of acetic acid within the system, which could be the possible substrate for hydrogen production (Fig 6).



Detector A(210 nm) Retention time	Area	Area percent	Height
3.888	34423	0.03	2109
4.753	33808090	27.41	684539
7.368	751335	0.61	15563
10.192	88734957	71.95	1673822
Total	123328825	100.00	2376033

Fig. 6 VFA- Evaluation through high-power liquid chromatography

## 4. Conclusion

- Biological H<sub>2</sub> production is the most challenging area of bioengineering and biotechnology to environmental problems. A challenging problem in establishing biohydrogen as an energy source is the renewable and environmentally friendly generation of large quantities of H<sub>2</sub> gas. However, two significant aspects need indispensable optimization: a suitable renewable biomass/wastewater and ideal microbial consortia that can convert this biomass efficiently to H<sub>2</sub>.
- The study demonstrated the feasibility of H<sub>2</sub> generation from Bulk drug wastewater treatment by anaerobic fermentation in a suspended growth bioreactor using anaerobic mixed inoculum. However, the process of H<sub>2</sub> generation was found to be dependent on the organic loading- rate applied. Integration of suspended configuration with sequencing/ periodic discontinuous batch operation was highly flexible and could influence the microbial system by selectively enriching the specific group of microflora. Extensive research was in progress 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 Anaerobic Technology.
- Comparative studies on the available processes indicate that biohydrogen production requires more significant improvement, mainly to H<sub>2</sub> yield from the cheaper raw materials. The future of biological H<sub>2</sub> production depends not only on research advances, i.e., the improvement in efficiency through genetically engineered microorganisms and the development of bioreactors but also on economic considerations (the cost of fossil fuels), social espousal and the development of H<sub>2</sub> energy systems.

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