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Studies on the influence of different feeds for the optimisation of bio hydrogen reactor

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Abstract
The main focus of this experimental investigation is to study the influence of various wastewater as substrates in the production of biohydrogen in suspended growth periodically operated reactors. Two types of chemical wastewater along with designed synthetic feed were used in the process of investigation. The reactor was operated with the optimized conditions established from earlier studies in the BEEC laboratory. The study compares the results obtained in hydrogen production from synthetic and complex feeds undergoing acidogenic reaction in the stirred tank reactor. The objective of the experiment is to optimize the ideal co-substrate on the production of Biohydrogen through Anaerobic Fermentation of Bulk drug effluent.

Keywords: Bio hydrogen reactor, complex feed, industrial effluent, synthetic feed

1. Introduction
Hydrogen is the most plentiful element in the universe, making up about three-fourths of all matter. Atmosphere contains about 0.07% H_2, while the Earth's surface contains about 0.14% H_2. On Earth, hydrogen occurs chiefly in combination with oxygen in water, but it is also present in organic matter such as living plants, petroleum, coal etc. It is present as the free element in atmosphere, but only less than 1 ppm by volume. Hydrogen combines with other elements such as Carbon, Oxygen and Nitrogen to form compounds. Hydrogen is the lightest element with a density of 0.09 g/l while air has a density of about 1.2 g/l. The heating value of Hydrogen is also high, about 3042 cal/m^3. On combustion, Hydrogen yields water vapor as a product. Thus, Hydrogen is regarded as a clean non-polluting fuel [1-5]. Hydrogen, the most abundant element of the universe, is a convincing energy source for clean earth. It constitutes three-quarters of the universal mass comprising about 90% of all the atoms. However, according to the Royal Society of Chemistry, the hydrogen in the atmosphere is extremely limited, and it escapes fast into the earth's gravity. Even though Robert Boyle produced hydrogen gas during his experimentation by irons with acids, it was considered a distinctly recognized element in 1766 only by Henry Cavendish.
Today global energy requirements are mostly dependent on fossil fuels (about 80% of the present world energy demand). This will eventually lead to the foreseeable depletion of limited fossil energy resources. Presently, the utilization of fossil fuels are causing global climate change mainly due to the emission of pollutants like COx, NOx, SOx, CxHx, soot, ash, droplets of tars and other organic compounds, which are released into the atmosphere as a result of their combustion [5-7]. Hydrogen has the highest energy content per unit weight of any known fuel and can be transported for domestic/industrial consumption through conventional means. H2 gas is safer to handle than domestic natural gas. H2 is now universally accepted as an environmentally safe, renewable energy resource and an ideal alternative to fossil fuels that doesn't contribute to the greenhouse effect. The only carbon-free fuel, H2 upon oxidation produces water alone. H2 can be used either as the fuel for direct combustion in an internal combustion engine or as the fuel for a fuel cell. The largest users of H2, however, are the fertilizer and petroleum industries with, respectively, 50% and 37%. Sales of H2 have increased by 6% annually in the last five years, which is closely related to the increased use of H2 in refineries as a result of stricter standards for fuel quality [2]. Processes for biological hydrogen production mostly operate at ambient temperatures and pressures, and are expected to be less energy intensive than thermochemical methods of hydrogen production.

These processes can use a variety of feedstocks as carbon sources. Waste materials can also be used as a carbon source which facilitates waste recycling [8]. However, the rate of H2 production is low and the technology for this process needs further development. Production of clean energy source and utilization of waste materials make biological hydrogen production a novel and promising approach to meet the increasing energy needs as a substitute for fossil fuels [9]. In this study, biological production methods are reviewed. Our economy and lifestyle are heavily dependent on the use of fossil fuels. But over-reliance on fossil fuels has caused serious problems due to their increase in costs, insecurity in their sustainability, as well as their effects on global warming and environmental pollution [10-14] consequently, research into hydrogen production has emerged as a top priority, owing to its zero-carbon emissions and the highest energy-to-mass ratio among all existing fuels. However, the majority of hydrogen production processes currently rely on the use of fossil fuels as raw materials, and there is a need for cost-effective and eco-friendly technologies for producing hydrogen from renewable sources [15]. Biohydrogen production from renewable sources such as wastes, which is considered a green technology, is the most obvious and reliable approach for producing hydrogen compared to the energy-consuming processes, and in recent years has been considered as an approach to promote sustainable development [16-20]. The food and agricultural industries generate a significant amount of waste that is rich in starch, protein, and fat but low in cellulose and semi-cellulose, making them ideal for use as a bioenergy source. Biological methods of hydrogen production, such as dark- and photo fermentation, and hybrid (Dark/photo) fermentation, have shown promise in utilizing organic waste for the production of hydrogen [21-24]. Hybrid fermentation, in particular, has higher hydrogen productivity at a lower fermentation time compared to successive fermentation processes [25].

Concerns about climate change and the increase of oil prices have triggered calls to replace fossil fuels with more environmentally sustainable energy sources, such as biofuels [26-27]. In this context, the development and use of new energy sources is already extremely urgent [28]. Among biofuels, biohydrogen is recognized as being one of the most promising because of the highest energy value per unit weight. In addition to its application in fuel cells, biohydrogen can also be used as a component in many fine chemical, petrochemical and food processing industries [28]. Hydrogen production technologies have gained significant attention due to the increased demand for hydrogen as an energy carrier [29]. The only by-product of combustion produced is hydrogen, making it a pollutant-free, alternative form of energy. Hydrolysis of chemical hydrides or metals has recently received attention as an optimal method for the production of hydrogen [30]. However, other chemical hydrogen production methods, such as steam reforming of hydrocarbons and partial oxidation of combustible fossil fuels, are energy intensive and require high temperature, resulting in the release of carbon dioxide and other pollutants as by-products [31]. Under these circumstances, biological hydrogen production from biomass appears to be one of the promising ways to replace traditional methods, as it can produce hydrogen competitively under ambient conditions, without causing pollution problems [32].

Biohydrogen generation from various waste materials is quite promising in renewable energy exploration. Biohydrogen is a cost-effective biofuel that produces both water vapor and energy when burned. However, biohydrogen production is more appreciable in utilizing various waste materials, thereby compromising both socioeconomic and technical strategies of energy exploration. The substrate, inoculum employed and their concentrations, culture kinds, and pretreatment procedure have all been found to be important in biohydrogen production. Physiological variables such as pH, temperature, redox potential, and partial pressure also significantly impact biohydrogen generation. The utilization of several growth factors, mainly the substrate, nitrogen, and phosphorus, also confronts extensive applications during biohydrogen production. This present study explores the influence of different feeds for the optimisation of bio hydrogen reactor.

2. Methodology

2.1 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 [33].

2.2 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.

2.3 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

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 3 min 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).

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

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

2.6 Chemical Oxygen Demand (COD)

The oxygen that is required for oxidation of organic matter in the presence of strong chemical oxidant is called chemical oxygen demand (COD). COD determination has an advantage over BOD determination in that the result can be obtained in about 5 hours as compared to 5 days required for BOD test. The determination was done using COD apparatus employing closed refluxing method as per APHA (2000) method.

The organic matter gets oxidized completely by potassium dichromate ($K_2Cr_2O_7$) in the presence of H_2SO_4 to produce CO_2+H_2O. The excess $K_2Cr_2O_7$ remaining after the reaction is titrated with ferrous ammonium sulfate. The dichromate consumed gives the O2 required for oxidation of the organic matter.

$Cr_2O_7^{2-} + 14\ H^+ + 6e^- \rightarrow 2\ Cr^{3+} + 7H_2O$

When chloride ions concentration is high in water used for COD determination cause serious problem as utilizes the dichromate for its oxidation.

$Cr_2O_7^- + 6Cl^- + 14\ H^+ \rightarrow 3Cl_2 + 3Cr^{3+} + 7H_2O$

To remove chloride ions $HgSO_4$ is added. The Hg^{2+} ion combines with chloride ions to form a poorly ionized $HgCl_2$ complex, which is resistant to oxidation by dichromate. Straight chain aliphatic compounds and certain aromatic compounds are not fully oxidized. That is why, $AgSO_4$ is added as a catalyst. In the present study, closed reflux titrimetric method has been used.

Reagents

- Standard potassium dichromate (0.25 N): 12.259 g of $K_2Cr_2\ O_7$ was dried at 103 °C for 24 hours in distilled water and diluted to 1000 ml.
- **Sulphuric acid reagent:** 10 g of $AgSO_4$ was dissolved in 1000 ml concentrated sulphuric acid, and kept over-night for dissolution.
- **Standard ferrous ammonium sulfate- 0.1N:** 39.0 g of Fe $(NH_4)_2SO_4$ $6H_2O$ was dissolved in about 400 ml distilled water. 20ml of concentrated conc. H_2SO4 was added. Cooled and made unto 1 L and standardized with $K_2\ Cr_2O_7$ (0.25N)
- **Ferroin indicator:** 1.485 g of 1, 10 phenanthrolin monohydrate and 0.695 g $FeSO_4$ $7H_2O$ was dissolved in distilled water and diluted to 100 ml with distilled water.
- **Mercuric sulfate:** $HgSO_4$ 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 ºC. 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.

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

Where, B ml is amount of FAS consumed for blank, S ml is FAS consumed for sample, N is normality of FAS.

Biochemical Oxygen Demand (BOD)

The BOD test is similar to the COD test in that the consumption of the oxidant of organic material is measured. However, in the conventional BOD test the oxidant used is dissolved oxygen and for the red ox reaction with organic material to take place, mediation by bacteria is necessary. If insufficient bacteria are present in the sample, these should be added along with nutrients. The concentration of the nutrients should not constitute a limiting factor for oxygen consumption. While in the BOD test the oxidation of the organic material takes several weeks. As this is unpractical, a standard period of five days has been introduced for the BOD test. To eliminate the influence of temperature on the oxidation rate, a constant value of 20 ^{0}C is maintained. Thus, unless specified differently, the BOD of a wastewater is its biological oxygen consumption over a five- day incubation period at 20 ^{0}C. In the case of wastewaters with a large range

of organic compounds, an extra difficulty in using the BOD, as quantitative parameter is that the rate of oxidation of organic material depends on the nature and size of its molecules. Small molecules are readily available for use by bacteria, but macromolecules and colloidal and suspended matter can only be metabolized after preparatory steps. It is therefore not possible to establish a general relationship between the experimental five- day BOD and the ultimate BOD of a sample, i.e. the oxygen consumption after several weeks. The BOD test is carried out less frequently, mostly to establish an empirical relation between COD and BOD. If the relation between the two is known, the BOD is estimated from the result of the COD test. The COD test offers another advantage over the BOD test. The biodegradability of organic material in the BOD test is assessed for the aerobic environment. The result is not necessarily indicative of the biodegradability in an anaerobic wastewater system; first the maximum amount of organic material that can be removed in an anaerobic environment should be determined. BOD denotes the amount of oxygen required for microorganisms for stabilization of organic matter under aerobic conditions.

$$\text{Organic matter} + O_2 \xrightarrow[\text{5 days at 20ºC temp}]{\text{Microorganisms}} CO2 + H2O + \text{cell mass}$$

Procedure

The samples are diluted with oxygenated water which contains trace amounts of nutrient materials and minerals (N, P, S, Fe, and Ca), but which does not consume oxygen. In all cases a blank is incubated consisting only the dilution water, which has been subjected to the same pretreatment as the samples. Seeding is required. Then for these samples DO is determined initially by using DO probe (YS1 5010, USA) and samples are kept in BOD incubator for 5 days at 20ºC. During the incubation the DO decreases and the final DO_5 was determined. The 5 days BOD at 20 ºC is equal to the difference in the DO values.

$$BOD_5 \text{ mg/L} = \frac{(DO_0S - DO_5S) - (DO_0B - DO_5B)}{\text{Decimal Volumetric fraction of the sample}}$$

Where,
DO_0S = Initial DO of the sample
DO_5S = Final DO of the sample
DO_0B = Initial DO of the blank
DO_5B = Final DO of the blank
Decimal Volumetric fraction of the sample = volume of the sample taken/volume of the bottle

Total solids (TS)

A well-mixed sample is evaporated in a weighed dish and dried to constant weight in an oven at 103 to 105 ^{0}C. The increase in weight over that of the empty dish represents the total solids.

Apparatus

1. Evaporating dishes of 100ml capacity made of porcelain.
2. Drying oven for operation at 103 to 105 ^{0}C.
3. Desiccator.
4. Analytical balance, capable of weighing to 0.1 mg.

Procedure

A measured volume of well-mixed sample was pipetted to a pre-weighed dish. The sample was evaporated to dryness on a steam bath or in a drying oven. The sample was stirred with a magnetic stirrer during transfer. The evaporated sample was dried for at least 1h in an oven at 103 to 105 ^{0}C. The dish was cooled in dessicator to balance temperature, and weighed. The cycle of cooling, desiccating and weighing was repeated until a constant weight was obtained.

$$\text{Total solids in mg/l} = \frac{(W2 - W_1) * 1000}{\text{Volume of sample in ml}}$$

Where:
W_1 = Weight of empty dish in mg
W_2 =Weight of dish + Weight of dried residue in mg

Total suspended solids (TSS)

A well-mixed sample is filtered through a weighed filter paper (Already dried previously at 103 to 105 ^{0}C for 1h) and the residue retained on the filter paper is dried to a constant weight at 103 to 105 ^{0}C. The increase in weight of the filter paper represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, it may be necessary to increase the diameter of the filter or decrease the sample volume.

Apparatus

1. Filter paper of appropriate diameter.
2. Filtering apparatus
3. Distilled water
4. Aluminum weighing dishes

Procedure

Filtering apparatus and filter paper were assembled and suction was started. The filter paper was wet with a small volume of reagent-grade water to seat it. The sample was stirred with a magnetic stirrer at a speed to shear larger particles. Filter was washed with three successive 10 ml volumes of reagent-grade water, allowing complete drainage between washings, and suction was continued for about 3 min after filtration completed. Samples with high dissolved solids might require additional washings. The filter paper was carefully removed from the filtration apparatus and transferred to an aluminum-weighing dish as a support. The sample was dried for at least 1h at 103 to105 ^{0}C in an oven, cooled in a dessicator to balance temperature, and weighed. The cycle of drying, cooling, desiccating and weighing was repeated until a constant weight was obtained.

$$\text{Total suspended solids in mg/l} = \frac{(W2 - W1) * 1000}{\text{Volume of sample in ml}}$$

Where, W_1=Weight of filter paper in mg just before using it for filtering sample; and
W_2 = Weight of filter paper + dried residue in mg.

Volatile Suspended Solids (VSS)

The residue obtained above (TSS) could be used for volatile suspended solids. The residue obtained after oven drying was ignited in muffle furnace at 550± 50 ºC for 20 minutes. Silica crucible containing residue was taken out from the furnace. The crucible was cooled in a dessicator and weighed to a constant weight (W_4).

$$\text{Volatile suspended Solids (VSS) (mg/l)} = \frac{(W4 - W_3) * 1000}{\text{Volume of sample in ml}}$$

Where, W_3 = Initial weight of crucible in mg; and W_4 =Weight of crucible + residue after ignition in mg.

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 ^{0}C

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 $_2$ O 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.

Results and Discussion

Innoculum development for hydrogen production

Properly pretreated mixed anaerobic sludge for process startup was procured from a lab scale UASB reactor used in treating chemical wastewater for almost 3 years. The innoculum was subjected to heat treatment at 80^{o}C for 24 hours followed by acid treatment at pH 3 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 [34]. A 20ml of anaerobic Innoculum was added to the anaerobic reactor in aseptic anaerobic conditions to a mixture of 33ml effluent and 22ml of sewage. The characteristics of the anaerobic innoculum was as follows

a) Suspended Solids - 13,500 mg/l
b) Volatile Suspended Solids - 7600 mg/l
c) pH (1:1 dilutions)- 6.85

Table 1: Characteristics of wastewater

Parameter	Concentration
pH	7.4
Color	Yellow
TDS	16,500 mg/l
COD	6080 mg/l
Sulfides	25-40 mg/l
BOD	810 mg/l
Chlorides	5538 mg/l

3.1 Anaerobic Sludge Characteristics

The sludge used in the experiment was characterized for the following parameters:

1) pH and ORP: At 1:10 dilutions, pH: 7.7 and ORP: -62.1mV
2) VFA: At 20 dilutions VFA was found to be 21,199mg/ml
3) COD: At 100 dilutions, COD was found to be 4000mg/ml
4) Total solids: 74.25 mg / ml sludge
5) Total Organic Carbon = 22.92
6) Organic Matter Estimation (% carbon- 13.48% organic matter: 23.48)

Reactor start up

The reactor was innoculated with biomass acquired from an operating laboratory scale upflow anaerobic sludge blanket reactor (UASB) unit, which has been in operation continuously for 3 years for the treatment of complex chemical effluents. About 300ml of the anaerobic sludge (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 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$\pm$2 ^{0}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 suspended reactor was started with synthetic feed, which has the composition as shown in Table 2. 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, ORP, VFA, COD 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 up flow feed recirculation mode produced negative results due to absence of suspension. The next 10 days operation was performed by up flow 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, COD, Glucose, Protein and Hydrogen gas parameters. HPLC for the samples was carried out.

Table 2: Synthetic feed composition

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

3.2 Hydrogen production in a stirred tank reactor maintained under suspension

The innoculum 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 plastic vessel 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 reactor has 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
- **Outer Diameter of the reactor:** 137 mm

Fig 1: Anaerobic batch stirred tank Reactor

Reactor start up

This reactor did not have a startup procedure because the innoculum 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 ^{0}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. (Figure 1)

Synthetic feed studies

Reactor operation

The reactor was started with synthetic feed, which has the composition as shown in Table 2. 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 [35]. 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.

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 3. 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, ORP, VFA, COD and hydrogen gas production [36]. 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. The sequencing samples were monitored for pH, VFA, Alkalinity, COD, Sucrose and Hydrogen gas parameters. HPLC for the samples was carried out. The substrate conversion efficiency was also calculated at various time intervals in sequencing period.

Table 3: 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

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 liter 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 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 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. The sequencing samples were monitored for pH, VFA, Alkalinity, COD, Sucrose and Hydrogen gas parameters. HPLC for the samples was carried out. The substrate conversion efficiency was also calculated at various time intervals in sequencing period.

4. Conclusion

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, co-substrate and nitrogen sources along with several other nutrients, which is referred to as complex feed. This process aims at studying the variations of hydrogen production with nutrient addition. Substrate conversion efficiencies of the complex feed was studied and compared with that of the synthetic feed studied in the previous stages, to establish the degree of success of the optimization process.

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6. References

1. Fermentation from food waste by tagged 16S rRNA gene pyrosequencing. Journal of Environmental Management. 2014;144:143-51. DOI: 10.1016/j.jenvman.2014.05.019.
2. Laxman V, *et al*. Biohydrogen production by co-fermentation of crude glycerol and apple pomace hydrolysate using co-culture of *Enterobacter aerogenes* and *Clostridium butyricum*. Bioresource Technology. 2015;193:297-306. DOI: 10.1016/j.biortech.2015.06.095.
3. Lee DJ, Kuan-yeow S, Ay S. Dark fermentation on biohydrogen production: Pure culture. Bioresource Technology. 2011;102(18):8393-402. DOI: 10.1016/j.biortech.2011.03.041.
4. Liu BF, Ya-ruo J, Qing-fen C, Guo-jun X. Photo-fermentation hydrogen production by *Rhodopseudomonas* sp. nov. strain A7 isolated from the sludge in a bioreactor. International Journal of Hydrogen Energy. 2015;40(28):8661-8. DOI: 10.1016/j.ijhydene.2015.05.001.
5. Marone A, Izzo G, Mentuccia L, *et al*. Vegetable waste as substrate and source of suitable microflora for bio-hydrogen production. Renewable Energy. 2014;68:6-13. DOI: 10.1016/j.renene.2014.01.013.
6. Mohanakrishna G, Venkata SG, Venkata MS. Adaptation of biohydrogen-producing reactor to higher substrate load: Redox-controlled process integration strategy to overcome limitations. International Journal of Hydrogen Energy. 2011;36(15):8943-52. DOI: 10.1016/j.ijhydene.2011.04.138.
7. Moreno CEL, Cano QDY, Cortés MEA. Generation of biohydrogen by anaerobic fermentation of organic wastes in Colombia. In: Zhen F, editor. Liquid, gaseous and solid biofuels conversion techniques. Rijeka: Intech; c2013. p. 377-400.
8. Mthethwa NP, Nasr M, Kiambi SL, Bux F, Kumari S. Biohydrogen fermentation from *Pistia stratiotes* (Aquatic weed) using mixed and pure bacterial cultures. International Journal of Hydrogen Energy. 2019;44(33):17720-31. DOI: 10.1016/j.ijhydene.2019.05.152.
9. Sarangi PK, Nanda S. Biohydrogen production through dark fermentation. Chem Eng Technol. 2020;43:601-12.
10. Nath K, Das D. Modeling and optimization of fermentative hydrogen production. Bioresource Technology. 2011;102(18):8569-81. DOI: 10.1016/j.biortech.2011.03.108.
11. Ortigueira J, Pinto T, Gouveia L, Moura P. Production and storage of biohydrogen during sequential batch fermentation of *Spirogyra* hydrolyzate by *Clostridium butyricum*. Energy. 2015;88:1-9. DOI: 10.1016/j.energy.2015.05.070.
12. Prabakar D, Manimudi VT, Suvetha SK, *et al*. Advanced biohydrogen production using pretreated industrial waste: Outlook and prospects. Renewable and Sustainable Energy Reviews. 2019;96:306-24. DOI: 10.1016/j.rser.2018.08.006.
13. Dong L, Zhenhong Y, Yongming S, Longlong M. Evaluation of pretreatment methods on harvesting hydrogen producing seeds from anaerobic digested organic fraction of municipal solid waste (OFMSW).

International Journal of Hydrogen Energy. 2010;35(15):8234-40. DOI: 10.1016/j.ijhydene.2009.12.058
14. Elsamadony M, Tawfik A. Dry anaerobic co-digestion of organic fraction of municipal waste with paperboard mill sludge and gelatin solid waste for enhancement of hydrogen production. Bioresource Technology. 2015;191:157-65. DOI: 10.1016/j.biortech.2015.05.017
15. Elsharnouby O, Hisham H, George N, Hesham MEN. A critical literature review on biohydrogen production by pure cultures. International Journal of Hydrogen Energy. 2013;38(12):4945-66. DOI: 10.1016/j.ijhydene.2013.02.032
16. Escamilla-Alvarado C, Ponce-Noyola T, Ríos-Leal E, Poggi-Varaldo HM. A multivariable evaluation of biohydrogen production by solid substrate fermentation of organic municipal wastes in semi-continuous and batch operation. International Journal of Hydrogen Energy. 2013;38(28):12527-38. DOI: 10.1016/j.ijhydene.2013.02.124
17. Ghimire A, Frunzo L, Pontoni L, d'Antonio G, Lens PNL, Esposito G, *et al*. Dark fermentation of complex waste biomass for biohydrogen production by pretreated thermophilic anaerobic digestate. Journal of Environmental Management. 2015;152:43-48. DOI: 10.1016/j.jenvman.2014.12.049
18. Gomez-Romero J, Gonzalez-Garcia A, Chairez I, Torres L, García-Peña EI. Selective adaptation of an anaerobic microbial community: Biohydrogen production by co-digestion of cheese whey and vegetables fruit waste. International Journal of Hydrogen Energy. 2014;39(24):12541-50. DOI: 10.1016/j.ijhydene.2014.06.050
19. Hernández MA, Rodríguez SM, Yves A. Use of coffee mucilage as a new substrate for hydrogen production in anaerobic co-digestion with swine manure. Bioresource Technology. 2014;168:112-18. DOI: 10.1016/j.biortech.2014.02.101
20. Hidaka T, Wang F, Tsumori J. Comparative evaluation of anaerobic digestion for sewage sludge and various organic wastes with simple modeling. Waste Management. 2015;43:144-51. DOI: 10.1016/j.wasman.2015.04.026
21. Jiang J, Zhang Y, Li K, Wang Q, Gong C, Li M. Volatile fatty acids production from food waste: effects of pH, temperature, and organic loading rate. Bioresource Technology. 2013;143:525-30. DOI: 10.1016/j.biortech.2013.06.025
22. Laothanachareon T, Kanchanasuta S, Mhuanthong W, Phalakornkule C, Pisutpaisal N, Champreda V. Analysis of microbial community adaptation in mesophilic hydrogen production. International Journal of Hydrogen Energy. 2014;39(26):14232-43. DOI: 10.1016/j.ijhydene.2014.07.051
23. Reungsang A, Sreela-or C, Plangklang P. Non-sterile bio-hydrogen fermentation from food waste in a continuous stirred tank reactor (CSTR): Performance and population analysis. International Journal of Hydrogen Energy. 2013;38(35):15630-37. DOI: 10.1016/j.ijhydene.2013.03.138
24. Robledo-Narváez PN, Muñoz-Páez KM, Poggi-Varaldo HM, Ríos-Leal E, Calva-Calva G, Ortega-Clemente LA, *et al*. The influence of total solids content and initial pH on batch biohydrogen production by solid substrate fermentation of agro-industrial wastes. Journal of Environmental Management. 2013;128:126-37. DOI: 10.1016/j.jenvman.2013.04.042
25. Sekoai PT, Gueguim Kana EB. A two-stage modelling and optimization of biohydrogen production from a mixture of agro-municipal waste. International Journal of Hydrogen Energy. 2013;38(21):8657-63. DOI: 10.1016/j.ijhydene.2013.04.130
26. Yang G, Wang J. Biohydrogen production from waste activated sludge pretreated by combining sodium citrate with ultrasonic: Energy conversion and microbial community. Energy Conversion and Management. 2020;225:113436. DOI: 10.1016/j.enconman.2020.113436
27. Singh L, Wahid ZA. Methods for enhancing bio-hydrogen production from biological process: A review. Journal of Industrial and Engineering Chemistry. 2015;21:70-80. DOI: 10.1016/j.jiec.2014.05.035
28. Sreela-or C, Imai T, Plangklang P and Reungsang A. Optimization of key factors affecting hydrogen production from food waste by anaerobic mixed cultures. International Journal of Hydrogen Energy. 2011;36(21):14120-14133. DOI: 10.1016/j.ijhydene.2011.04.136
29. Srikanth S, Venkata Mohan S. Regulating feedback inhibition caused by the accumulated acid intermediates during acidogenic hydrogen production through feed replacement. International Journal of Hydrogen Energy. 2014;39(19):10028-10040. DOI: 10.1016/j.ijhydene.2014.04.152
30. Tawfik A, El-Qelish M. Key factors affecting on bio-hydrogen production from co-digestion of organic fraction of municipal solid waste and kitchen wastewater. Bioresource Technology. 2014;168:106-111. DOI: 10.1016/j.biortech.2014.02.127
31. Tyagi VK, Angériz Campoy R, Álvarez-Gallego CJ, Romero García LI. Enhancement in hydrogen production by thermophilic anaerobic co-digestion of organic fraction of municipal solid waste and sewage sludge--optimization of treatment conditions. Bioresource Technology. 2014;164:408-415. DOI: 10.1016/j.biortech.2014.05.013
32. Yang G, Wang J, Shen Y. Antibiotic fermentation residue for biohydrogen production using different pretreated cultures: Performance evaluation and microbial community analysis. Bioresource Technology. 2019;292:122012. DOI: 10.1016/j.biortech.2019.122012
33. APHA-AWA-WPCF. 2000. Standard methods for the examination of water and wastewater, Washington, DC, American Public Health Association.
34. Hema Krishna R, Venkata Mohan S, Swamy AVVS. Bio Hydrogen Production from PharmaceuticalWaste Water Treatment by a Suspended Growth ReactorUsing Environmental Anaerobic Technology. Ameri ChemSci J. 2013;3(2):80-97.
35. Yasin NHM, Mumtaz T, Hassan MA, Abd Rahman N. Food waste and food processing waste for biohydrogen production: A review. Journal of Environmental Management. 2013;130:375-385. DOI: 10.1016/j.jenvman.2013.09.009
36. Saratale GD, Saratale RG, Banu JR, Chang JS. Biohydrogen production from renewable biomass resources. In Biohydrogen; Elsevier: Amsterdam, The Netherlands; c2019. p. 247-277.