Biohydrogen Production from Co-Digestion of High Carbohydrate Containing Food Waste and Combined Primary and Secondary Sewage Sludge

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ABSTRACT

In this paper, FW (Food Waste) and SS (Sewage Sludge) were co-digested for biohydrogen production. After characterization both FW and SS were found as better option for biohydrogen production. FW was rich in carbohydrate containing specially rice, which was added as more than 50% and easily hydrolyzable waste. FW is considered as an auxiliary substrate for biohydrogen production and high availability of carbohydrate in FW makes it an important substrate for the production of biohydrogen. On the contrary, SS was rich in protein and has a high pH buffering capacity, which makes it appropriate for co-digestion. Adequate supplementation of inorganic salts, the addition of hydrogen producing inoculums, protein enrichment and pH buffering capacity of SS and carbohydrate content in FW increases the hydrogen production potential. Various experiments were performed by considering different mixing ratios like 90:10, 80:20, 70:30, 60:40 and 50:50 of FW and SS. The 50:50 and 90:10 mixing ratio of FW and SS were found as best among all other co-digested ratios. The maximum specific hydrogen yield 106.7 mL/g VS added was obtained at a waste composition of 50:50 followed by 92.35 mL/g VS added from 90:10 of FW to SS. The optimum pH and temperature for operating this process were in the range of 5.5-6.5 and 35°C. The production of clean energy and waste utilization in anaerobic co-digestion process makes biohydrogen generation a promising and novel approach to fulfilling the increasing energy needs as a substitute for fossil fuels.

Key Words: Biohydrogen, Food Waste, Sewage Sludge, Volatile Solids.

1. INTRODUCTION

Fossil fuels are nonrenewable sources of energy and cause pollution. The quantity of fossil fuels is limited, thus they should be substituted with renewable energy sources which are nonpolluting sources of energy [1]. Hydrogen is used as an alternative to fossil fuels that can be used in place of various kinds of fossil fuels [2]. Environmental impacts and cost of fossil fuels in recent years have developed an interest in sustainable, renewable and clean energy alternatives [3]. Hydrogen is a sustainable and clean fuel and has a very high energy yield of 122 kJ/g. Such a high yield makes it energy carrier of the future, and also viable alternative to...
fossil fuel. Hydrogen is not only utilized as a source of energy but also used as a feedstock in the production of chemicals, steel processing, hydrogenation of oil and fats, reformulation and desulfurization of gasoline in refineries and for the production of electronic devices. In recent years significant attention has been gained by efficient and cost-effective hydrogen production technologies as a need for hydrogen energy is increasing [4]. Conventional hydrogen production methods are not cost-effective because they require high energy like steam reforming of hydrocarbons, electrolysis of water and non-catalytic partial oxidation of fossil fuels [5]. Due to the current oil crisis and pollution causing effects of nonrenewable energy sources advancement in renewable and eco-friendly energy technologies have been increased [6].

Among all renewable and inexhaustible technologies anaerobic co-digestion of renewable fuel sources like waste plays major role in the production of clean energy and also in the treatment of waste that on the other way would be contributing to global environmental problems [7-9]. Biohydrogen production by using waste as raw material has been considering attention as a sustainable and eco-friendly process that does not require fossil fuel [10]. Productions of hydrogen through biological processes have more advantages than chemical methods [9]. Biohydrogen production has been considered as clean, renewable and environmentally friendly technique. Biohydrogen is produced biologically by bacteria and algae [11-13]. Biohydrogen can also be produced through biomass with either butyrate or acetate or both [14].

Biological methods of hydrogen production are divided into two categories. The first is by anaerobic bacteria and other is by photosynthetic bacteria [10]. The types of bacteria involved in biohydrogen production are Escheria coli, Enterobacter and Clostridium [15-16]. These hydrogen producing bacteria widely found in wastewater sludge, soil, sediments manure and compost [17]. In photosynthetic process, algae utilize water and carbon dioxide in the production of hydrogen and in fermentation process anaerobic bacteria consume organic acids like acetic, butyric and lactic acid for the production of hydrogen [9]. In the fermentative process of biohydrogen production, organic wastes may become less expensive and abundant source; in which stabilization and reduction of organic waste take place [8]. Co-digestion of FW with sewage sludge produces biohydrogen because FW and SS were known as necessary and auxiliary substrate for hydrogen production. Some experimental results were reported using food waste [13,18-20], waste activated sludge [21-22], municipal solid waste [18,23-25]. FW is suitable for the production of hydrogen as it is rich in carbohydrate and hydrolyzable waste [26].

An important part is that it is an easily degradable waste with high potential of biogas production [27]. Although this waste is also a major source of vermin attraction, ground water contamination, gaseous emissions and odor emanation due to high organic content. FW is carbohydrate rich waste and SS is rich in protein [28]. Protein enrichment by mixing SS enhances the potential of hydrogen production. For effective hydrogen production appropriate amount of minerals and pH buffer solution are used to maintain the nutrient balance and required pH [29]. A previous study indicates that addition of SS with food provides a balanced carbohydrate/protein ratio [30]. Co-digestion of carbohydrate rich waste with SS is not only suitable for the treatment of waste but also energy intensive. This concept of anaerobic co-digestion of SS and FW for biohydrogen production is provided to be significant, as it also contribute to the management of solid waste [28].

From the literature it was conceived that the previous studies work was carried on the substrate having low carbohydrate whereas present study was aimed to produce hydrogen by anaerobic co-digestion of high
carbohydrate containing FW and SS. In this study, the substrate was characterized and co-digested in the batch type cultivation system under various mixing ratios to produce hydrogen and find out the best mixing ratio for co-digestion of high carbohydrate containing FW and combined primary and secondary sludge on the basis of VS (Volatile Solids) added into the reactors.

2. METHODOLOGY

2.1 Materials

Materials used for biohydrogen production are FW, primary sludge and secondary sludge (waste activated sludge) [29]. These wastes are complimentary for biological hydrogen production. FW collected from dining hall contains a variety of beans and vegetables like rice, wheat bran, potatoes, pulse, cabbage, beans and peas [18-19,23,29]. The maximum content of carbohydrates makes FW an auxiliary feedstock for the production of biohydrogen. Biohydrogen was produced by Lay et. al. [25] from the OFMSW (Organic Fraction of Municipal Solid Waste). Kim et. al. [28] produced hydrogen by using FW as the main substrate. Shin [31] produced hydrogen from FW under thermophilic conditions, Ginkel et. al. [32] produced biohydrogen from food processing industrial wastewater effluent, and Tanisho et. al. [33] produced hydrogen from molasses.

Primary sludge was collected from the sewer main hole. SS is also rich in protein and polysaccharides and hydrogen yield were reported by Wang et. al. [21-22, 34]. The microorganisms responsible for fermentative reaction include the members of Clostridium, many of them are found in SS [35]. Additionally, effluent from the hydrogen reactor can be used as a feedstock for photo fermentative biohydrogen production from SS [36]. Waste activated sludge was taken from anaerobic digester. The Volatile suspended solids, alkalinity and pH of activated sludge were 6.5 g/L, 3.23 g CaCO₃/L and 7.2, respectively. This sludge was digested and enriched with bacterial growth. The study is limited to the FW having high carbohydrate containing specially rice, which was added as more than 50% and was acidic in nature with the cumulative moisture content of 80-84%. The SS used in the present study having only 3% of TS (Total Solids) and was neutral in the nature. Moreover, the co-digestion was carried out at mesophilic temperature range of 35°C.

2.1.1 Preparation of Substrate

The substrate was prepared by using FW, primary Sludge and waste activated sludge. FW an aerobically crushed by blender and the slurry was prepared by mixing the blended FW with distilled water. For the preparation of slurry 10 ml distilled water was added in one gram of FW sample [29]. The digested sludge taken from anaerobic digester was heated for 15 minutes at 90°C for harvesting hydrogen producing bacteria [14]. Then all substrate including waste activated sludge, primary sludge and FW were filtered through stainless steel sieve of Mesh No.8 with sieve opening size of 2.68 mm.

2.1.2 Preparation of Hydrogen Producing Inoculum

The inoculum was prepared using a sucrose medium as substrate and digested sludge as a source of organisms [17]. The medium contains inorganic salts, phosphate buffer and 10 g/L sucrose. Cellulose powder can also be used as substrate for hydrogen production [10], the maximum hydrogen yield obtained was 102 mL/g cellulose [37] and 2.18 mol/g cellulose [38]. Hydrogen production from sucrose has been widely used due to high potential of hydrogen production like 4.52 mol of Hydrogen/mol sucrose [39], 3.47 mol of Hydrogen/mol sucrose [40] and 1.5 mol of Hydrogen/mol sucrose [41], and the yield from glucose was only 0.91 mol/mol glucose [32]. Inorganic
salts in medium are 3.2 g/L Na₂HPO₄·7H₂O, 1.5 g/L KH₂PO₄, 4.2 g/L NH₄Cl, 0.18 g/L MgCl₂·6H₂O [10]. The 20 mL digested sludge and 80 mL sucrose medium were added to the reactor. Before the bottle was capped the head space of the reactor bottle was flushed with nitrogen gas for one minute.

2.1.3 Preparation of Gas Measuring Jars

The reactors are connected to gas measuring jar which were filled with the 3M NaOH solution and thymolphthalein indicator. The 3M NaOH solution was prepared by dissolving 120 g of NaOH in one liter of distilled water. The thymolphthalein indicator was prepared by adding 20 mg of thymolphthalein indicator powder in 45 ml ethanol.

2.1.4 Operating Procedure for Batch Reactors

The tests were conducted using 500 mL glass reactor bottles. Total five bottles with different volumetric ratios of FW and SS were added according to achieve a final volume of 200 mL in each reactor as shown in Fig. 1. Five ratios were made and the mixing ratio of FW and SS in each reactor was designed accordingly as given in Table 1.

All reactor bottles were inoculated with hydrogen producing inoculum and are supplemented with chemicals [25]. The 20 ml hydrogen producing inoculum, appropriate amount of substrate which were designed accordingly and varying amount of phosphate buffer solution was added to each reactor bottle. The buffer solution was prepared by using 1 liter distilled water 0.2 g KCl, 8 g NaCl, 4.4 g Na₂HPO₄·0.24 g KH₂PO₄. The inorganic salts along with their quantity are given in Table 2. Then all reactors were purged with N₂ for one minute and capped tightly. The Gas produced anaerobically in all reactor bottles and was collected in the gas measuring acrylic glass jar. The produced gas was a mixture of CO₂ and H₂ and for the separation of hydrogen from the mixture of gases 3M NaOH solution was used in the gas measuring jar.

2.1.5 Gas Sample Analysis

For measuring the quantity of gas produced, the water displacement technique was used. The gas was transferred with the help of pipe to the gas collection jar containing NaOH solution in it, the quantity of NaOH solution was displaced by the produced gas. Hence, the quantity of NaOH solution in the inner tube is equal to the quantity of biohydrogen produced. Volume of gas produced in mL was calculated by using Equation (1).

\[ V = \frac{\pi}{4} d^2 (h + H) \]  

Where, V is the volume of hydrogen in ml, d is the diameter of the inner tube of the gas measuring jar in cm, h is the height of the NaOH solution raised in the inner tube and the H is the height of the NaOH solution raised in the outer tube of the gas jar. The measured volume of the hydrogen gas was converted into the standard pressure and temperature by using Equation (2).

\[ V_{STP} = V \times \frac{P}{101.325} \times \frac{273}{273 + T} \]  

Where \( V_{STP} \) is the volume of hydrogen in mL at standard temperature and pressure, V is the volume of hydrogen in mL, P is the atmospheric pressure and T is the temperature of the gas measuring jar.

The glass syringe was used for collecting the gas sample [42]. Hydrogen content was determined by gas chromatography (Shimadzu AOC-20i) using a thermal conductivity detector and column Rt-Q-BOND (30 m length, 0.53 mm ID and 20 µm DF) with nitrogen as the carrier gas at a pressure of 30 psi in the column operated with
column temperature of 50°C injector and detector temperature was 80°C, respectively. The Senso direct 150 multi-meter instrument was used to monitor the pH of the sample. TSs, VSs, Alkalinity, COD (Chemical Oxygen Demand) and NH3-N were determined by following APHA standards [43].

3. RESULTS AND DISCUSSION

3.1 Characteristics of Food Waste and Sewage Sludge

The characteristics of substrate are given in Table 3. The FW contains alkalinity as low as 40 mg/L as CaCO3 while alkalinity contains in SS was high 1550 mg/L as CaCO3, which shows that it allowed fermentation to establish pH 5.5-6.0 that is conductive for biohydrogen production [29]. The pH values of SS and FW were 7 and 6.2. All reactor bottles were adjusted to initial pH 7 [29]. As the FW fraction of the substrate increases the decline in pH increased, for that phosphate buffer solution were added to reactor bottles and least decline was observed in reactors where sludge fraction is more in the substrate. The concentration of COD in FW and SS was 10, 90, 10, 810, 2, 20, 80, 20, 610, 4, 30, 70, 30, 410, 6.

![Image of reactor bottles](image-url)

**FIG. 1. REACTOR BOTTLES OF VARIOUS RATIOS OF FOOD WASTE AND SEWAGE SLUDGE ALONG WITH GAS MEASURING JARS**

**TABLE 1. MIXING RATIOS OF FOOD WASTE AND SEWAGE SLUDGE IN TERMS OF PERCENTAGE AND VOLUME**

| Name of Reactor | Food Waste (%) | Sewage Sludge (%) | Food Waste (mL) | Sewage Sludge (mL) |
|-----------------|----------------|-------------------|-----------------|-------------------|
| 1               | 90             | 10                | 180             | 20                |
| 2               | 80             | 20                | 160             | 40                |
| 3               | 70             | 30                | 140             | 60                |
| 4               | 60             | 40                | 120             | 80                |
| 5               | 50             | 50                | 100             | 100               |

**TABLE 2. THE QUANTITY OF CHEMICAL USED AS SUPPLEMENT IN ALL BIOHYDROGEN REACTORS**

| No. | Chemical Formula | Quantity Added (mg) |
|-----|------------------|---------------------|
| 1   | CaCl2·2H2O       | 2                   |
| 2   | Na2MoO4·4H2O     | 2                   |
| 3   | MgCl2·4H2O       | 4                   |
| 4   | K2HPO4           | 4                   |
| 5   | FeCl2·4H2O       | 10                  |
| 6   | K2HPO4           | 200                 |
28000 and 15000 mg/L, respectively and previous study has reported that the majority of the volatile fraction of the SS is proteinaceous materials and proteinaceous COD is more than 50% of the total COD in the SS [28]. The result indicates that FW contains high concentration of carbohydrate, which suggests the availability of FW as suitable and auxiliary substrate for hydrogen production [28,31-33].

Both substrates contain significant concentrations of ammonia nitrogen as 1322 mg/L in SS and 605 mg/L in FW. This high concentration of ammonia nitrogen in SS suggests that SS has a high concentration of protein in it[29], and addition of SS with food provides a balanced carbohydrate/protein ratio [30]. The FW contains TS and VS concentration as 16120 and 15650 mg/L, respectively. Although, TS and VS in SS were 29500 and 11500 mg/L, respectively. Moreover, VS in SS indicate high organic matter as compared to FW.

The VFA in FW were 2040 mg/L shows that acid genesis phase has started while in SS no VFA were detected. Moisture content in FW and SS indicates that the rapid gas generation will take place due to easy and rapid degradation of co-substrate. The pH buffering capacity and protein enrichment of SS with FW which contains complimentary nutrient composition, suggests the appropriate potential of co-digestion for maximum hydrogen production.

### 3.2 Co-Digestion of Food Waste and Sewage Sludge

FW and SS both are the appropriate sources for the biological production of hydrogen. Availability of carbohydrates in carbon rich FW, Protein enrichment and pH buffering capacity of SS and supplementation of inorganic salts enhance the hydrogen production potential.

In fermentation process pH influence the metabolic pathway for the conversion of carbohydrate and for the production of hydrogen. Although, in each reactor the initial pH was maintained as 7 and final pH varied from 3.8-6.5. The sludge fraction of feedstock has least decline in pH due to pH buffering capacity and a decline in pH increases as the FW fraction of the feedstock increased [29]. This pH lowering of FW also inhibits methanogenic activity. Previous studies reported that the suitable pH for the biological production of hydrogen from anaerobic co-digestion of FW and SS was in the range of 5.5-6.0 [29]. For maintaining the required pH in all reactor bottles adequate amount of pH buffer solution were added. SS has higher alkalinity as compared with FW and therefore has more pH buffering capacity [29,30]. This pH buffering capacity of SS was useful for the co-digestion of waste in mixture.

| Parameter                     | Food Waste | Sewage Sludge |
|-------------------------------|------------|---------------|
| Alkalinity (mg CaCO3/L)       | 40         | 1550          |
| pH                            | 6.2        | 7             |
| COD (mg/L)                    | 28000      | 15000         |
| NH3-N (mg/L)                  | 605        | 1322          |
| Total Solids (mg/L)           | 16120      | 29500         |
| Volatile Solids (mg/L)        | 15650      | 11500         |
| Volatile Fatty Acids (mg/L CH3COOH) | 2040      | 0             |
| Moisture Content (%)          | 83.88      | 97.05         |

| TABLE 3. CHARACTERISTICS OF SUBSTRATES |
In Reactor-1, the 180 mL of FW was mixed with 20 mL of SS. Initial pH was maintained at 7 while the final value varied. Due to the maximum content of carbohydrate in FW the hydrogen yield from this mixture was 92.35 mL/g VS. Based on the availability of carbohydrates from FW in Reactor-1, the volumetric ratio 90:10 produces maximum hydrogen and results of this mixture are in agreement to the Kim et. al. [28].

In the study presented, the maximum specific hydrogen was observed as 106.74 mL/gVS$_{added}$ from the volumetric ratio of 50:50. Initial pH was maintained at 7 and final pH was 6.5 of this 50:50 mixture of FW and SS. This mixing ratio has maximum hydrogen production due to carbohydrate content in FW, protein enrichment and pH buffering capacity of SS which was added here 100 mL. The factor impacting hydrogen production is the availability of carbohydrate, pH and protein. This 50:50 mixing ratio was found as best among all other co-digestion ratio, in which carbon rich FW was added with SS that favored the conditions for maximum hydrogen production by influencing the metabolic conversion of carbohydrate for the formation of biohydrogen. The results of the present study are in comparison to the results of Zhu et al. [29]. According to Zhu et. al. [29] the hydrogen yield of 104 mL/gVS$_{added}$ and 112 mL/gVS$_{added}$ were produced at a volumetric ratio of 3:1 and 1:1 of FW to SS.

In the Reactor-2, the volumetric ratio 80:20 was prepared by using 160 mL FW with 40 mL of SS. In this mixture the final pH was 4 and gas production was 81.95 mL/g VS$_{added}$. In the Reactor-3, the 140 mL of FW was mixed with 60 mL of SS and mixing ratio 70:30 was prepared. Initial pH was adjusted to 7 and final pH was 4.2. Due to lower final pH value, the availability of suitable substrate was limited in the reactor. Thus, the mixture generate hydrogen of only 76.80 mL/g VS$_{added}$. The mixing ratio 60:40 which contains 120 mL FW and 80 mL SS was initially adjusted at pH 7 and final pH of this mixture was observed 5.5. Due to the high pH buffering capacity of SS which was added here 80 mL the specific hydrogen yield 82.91 mL was obtained at this mixing ratio.

The results of these mixing ratios and hydrogen yield indicates that gas was produced in each reactor at all mixing ratios, but highest yields from this study 106.74 mL/gVS$_{added}$ and 92.35 mL/gVS$_{added}$ were obtained at mixing ratio 50:50 and 90:10 of FW to SS. The 50:50 mixing ratio has most hydrogen production potential and this was substantially due to the suitable pH value and the presence of carbohydrate from FW. The cumulative hydrogen production obtained from all reactors at different ratios of FW to SS is shown in Fig. 2. The maximum hydrogen production 289.8 mL observed from FW to SS ratio of 50:50, followed by 281.4, 242.9, and 221,232 from the ratios of 90:10, 80:20, 70:30, and 60:40, respectively.

Flow rates of hydrogen from all mixing ratios are given in Fig. 3. Hydrogen production was analyzed on a daily basis for five days. Hydrogen production was started from day one and maximum hydrogen was observed at day one and then it was gradually decreased on subsequent days due to decline in pH and microbial conversion of organic compounds into hydrogen.

| Name of Reactor | VS$_{added}$ (mg VS) | H$_2$ (mL) | Specific H$_2$ (mL/gVS$_{added}$) |
|-----------------|----------------------|-----------|----------------------------------|
| 1               | 304.7                | 281.4     | 92.35                            |
| 2               | 296.4                | 242.9     | 81.95                            |
| 3               | 288.1                | 221       | 76.70                            |
| 4               | 279.8                | 232       | 82.91                            |
| 5               | 271.5                | 289.8     | 106.74                           |

TABLE 4. SPECIFIC HYDROGEN PRODUCTION PER GRAM OF VS$_{added}$
4. CONCLUSION

In this study five batch tests were conducted to evaluate the potential of biohydrogen production at various volumetric ratios of FW and SS by anaerobic co-digestion. The study is limited to the FW having high carbohydrates containing specially rice, which was added as more than 50% and was acidic in nature with the cumulative moisture content of 80-84%. The SS used in the present study having only 3% of TS and was neutral in nature. Moreover, the co-digestion was carried out at mesophilic temperature range of 35°C. The maximum specific hydrogen yield was obtained at a waste composition of 50:50 followed by 92.35 mL/g VS added from 90:10 of FW to SS. The results of this research demonstrate that FW and SS were suitable and useful for hydrogen production. Co-digestion of primary sludge and waste activated sludge with carbohydrate rich waste is an economically feasible approach for biological production of hydrogen on the basis of availability of the cheaper substrate. Biological production of hydrogen is also suitable for the treatment of FW and SS as it considerably decreases the organic matter present in the waste. Carbohydrate availability and pH are the principal factors impacting hydrogen generation. FW shows maximum hydrogen production due to larger availability of carbohydrate in it. On the other hand, the addition of SS with food waste enhance hydrogen production by providing pH buffering capacity to establish pH.
A pilot study may be carried out to check the feasibility of co-digestion of FW having high carbohydrates and SS and to carry out the energy balance of the system. Moreover, the energy used for heating and stirring may be obtained from the renewable sources of energy as solar and wind energies.

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