Biological Hydrogen production from Distillery Spent Wash using mixed anaerobic micro flora sludge

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ABSTRACT

The fermentation process was evaluated for wastewater treatment and bio-hydrogen production from acidic Distillery Spent Wash wastewater in a continuous stirred tank reactor (CSTR) with an effective volume of 16.34 L by anaerobic mixed cultures. After inoculating with an aerobically digested sludge, the reactor initially run with Organic Loading Rate of 4.115, 3.72, and 4.21 kg COD/m3.d with an HRT of 24h, 16h, 12h, and 8h. The temperature varied from 35°C, 55°C, and 70°C with 60 rpm, the CSTR reactor achieve stable fermentation after 22 days of operation. When OLR was further increased to maximum 45.92, 41.41, and 43.17 kg COD/m3.d on the 60th day, fermentation dominant micro flora was enhanced. The results revealed that for temperature 35°C, 55°C, and 70°C, the maximum hydrogen production, Effluent pH, VFA/Alkalinity ratio, COD removal percentage, and VSS are (2472, 2054, and 1838 ml/d), (6.0, 6.3, and 5.9), (0.47, 0.45, and 0.46), (68%, 62%, and 62%) and (8.96, 8.76, and 5.62 g/l) were achieved at HRT of 12h in all temperature cases. The SEM analysis of the Anaerobic Sludge granules was examined for Microbial Biomass population in the CSTR. The experimental results illustrated to facilitate the CSTR reactor had better microbial activity and operation stability, which show the way to high substrate conversion rate and hydrogen production capability.

KEY WORDS: Distillery Spent Wash, continuous stirred tank reactor (CSTR), Fermentative hydrogen production, SEM analysis.

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INTRODUCTION

Fossil fuels (i.e., coal, petroleum and natural gas), which convened the majority of the world’s energy scarcity today, are being diminished rapidly\(^1\).\(^2\). Moreover, ignition of fossil fuel products are starting place for the global problems, such as pollution, acid rain, ozone layer depletion and greenhouse effect which are reason for great danger of our environment and ultimately to all lives in our world\(^3\).\(^4\). The scientists thinking is elucidation towards these global problems would be to alternate the existing fossil fuel by the hydrogen energy\(^5\).\(^6\). A broad range of applications with a high-value industrial commodity called as hydrogen. It is a clean and extremely an efficient fuel. From the ignition of hydrogen, it could not produce pollution, greenhouses, acid rain ingredients gases and ozone layer depletion. It can be directly consumed in internal combustion engines or converted into electricity via fuel cells\(^7\). It can also be exercised for the synthesis of alcohols, aldehydes, and ammonia in addition to the hydrogenation of coal, petroleum, shale oil and edible oil.

In general, there are four accessible basic processes for the hydrogen production from non-fossil primary energy sources: 1) biologic process; 2) radiolysis process; 3) thermo chemical process, and 4) water electrolysis. Among these various processes of hydrogen production, the process of biologic hydrogen production is sustainable, cost-effective, and environmentally friendly\(^8\).\(^9\). The process which is used for year’s waste treatment and energy production known as anaerobic digestion process\(^10\).\(^11\). Volatile Fatty Acids, Carbon dioxide, hydrogen, and infrequently alcohols are produced in the acid genesis of organic wastes. At various laboratories by using acid genesis process the organic wastes convert into hydrogen has been widely revealed. Biohydrogen production from biomass during fermentation is considered as a most promising route for renewable and sustainable hydrogen production\(^12\). Potentially the attractive and broadly used renewable resource for biofuel production recognized as Biomass. The capability to convert any biomass into hydrogen with maximum efficiency is one of the biggest confront in bio-hydrogen production\(^13\). In recent years it has been raising thought as the anaerobic fermentative hydrogen production process plays the dual role of energy production and waste reduction\(^14\).\(^15\). Further, the sugar factories produce huge volumes of high strength wastewater that is of severe environmental concern. Distillery Spent Wash is the main component of sugar factory wastewater which is used as a carbon source in various fermentations and has a high commercial value due to its utilization. The wastewater is exemplified by low pH, dark brown color and strong odor apart from this it's having extremely high (COD) Chemical Oxygen Demand and biochemical oxygen demand (BOD) (5000–10000 mg·L\(^{-1}\)) and (BOD\(_5\)) (4000–7000 mg L\(^{-1}\)). They can cause significant environmental problems; hence their free disposal presents a stern test to the natural ecosystem\(^16\). Anaerobic treatment technology application
has been found to be highly efficient due to the high BOD5 of the wastewater \(^{17,18}\). Conversely, the strongly acidic wastewater will reduce the methanogenic activities due to their high pH sensitiveness. Therefore it is very hard to attain satisfying treatment efficiency\(^{19}\). One of the major successful ways of producing hydrogen gas from sugars at low pH is an ethanol-type dark-fermentation process\(^{20}\). Continuous dark fermentative hydrogen production can be enhanced by shortening the retention time prior to washing out of hydrogen producing biomass\(^{21}\). Availability of large quantity of wastewater and high organic load may be considered as a potential resource for the production of bio-hydrogen by anaerobic fermentation. Hence anaerobic fermentative hydrogen production will be carried out by using acidic sugar factory wastewater. However, so far little information is available concerning simultaneous bio-hydrogen production and wastewater treatment using sugar wastewater in the literature. Thus the purpose of this study is to investigate the characteristics of simultaneous H2 production and wastewater treatment utilizing Distillery Spent Wash by continuous experiments using mixed culture. The Continuous Stirred Tank reactor performance was utilized and hydrogen production from Distillery Spent Wash wastewater with the results of operating parameters was collectively assessed.

**EXPERIMENTAL METHODS**

### 2.1 seed sludge

The reactor was inoculated with sludge taken from an Anaerobic Digester treatment Unit situated in Sugar Industry wastewater treatment plant, Tamilnadu, India. The sludge concentration of the CSTR system after inoculation was 6.92g/l, 6.95g/l and 4.61g/l for 35°C, 55°C, and 70°C. The sludge was preheated at 100°C for 1 hr to enhance the hydrogen producing microorganisms and to inhibit the methanogenic microorganisms.

### 2.2 Experimental Set Up

![Schematic diagram of Continuous Flow Stirred Tank Reactor](image)

*Fig. 1 Schematic diagram of Continuous Flow Stirred Tank Reactor*
The continuous fermentative bio-hydrogen producing reactor used in this study is a continuous flow stirred-tank reactor as shown in figure 1 and 2.

Fig. 2 Real Set up of Continuous Flow Stirred Tank Reactor

The schematic representation and overview of the Continuous Stirred Tank Reactor have been shown in figure 1 and 2 respectively. The system includes four automated units, feeding tank, main body of reactor, gas measuring sensor and automated temperature control system. The temperature was automatically maintained at 35°C, 55°C and 70°C. The influent flow rate was controlled by a feed pump to regulate the HRT and organic loading rate (OLR) in the reactor. It was constructed from stainless steel and the feed tank has the feed supply volume of 10 L and the total volume of the reactor was 21.783 L out of which 5L volume meant for gas collection chamber located at the top of the reactor and 16.34 L as working volume to perform the bioconversion. The bioreactor dimensions were measured as the diameter of 0.215 m and height of 0.6 m. The entire bioconversion mechanism took place in 4 different segments of the bioreactor namely, seed sludge introduction area at the bottom, substrate configuration part at the middle, bio-film placed in between the substrate and the gas collection chamber at the top of the reactor.

2.3 Feed and Medium Composition

The Distillery Spent Wash was collected from the sugar industry at Cuddalore, Tamilnadu, India and it was diluted with tap water. The COD range from 4000 mg/l to 40000 mg/l. High glucose concentration could inhibit the fermentation process\(^22\). Hence glucose10 g/l was added as a co-substrate with the distillery spent wash.
Table 1 “physico chemical characteristics of Distillery Spent Wash wastewater”

<table>
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<th>Parameters</th>
<th>Value</th>
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</thead>
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</tr>
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<td>COD</td>
<td>43146</td>
</tr>
<tr>
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<td>BOD</td>
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<td>7</td>
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<tr>
<td>8</td>
<td>MLSS</td>
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<tr>
<td>9</td>
<td>MLVSS</td>
<td>7711</td>
</tr>
<tr>
<td>10</td>
<td>TOC</td>
<td>2261 (ppm)</td>
</tr>
</tbody>
</table>

All values are in mg/l except pH and TOC

2.4 Analytical Methods

Chemical analysis was performed for both influent as well as effluent. The parameters like Total Solids (TS), Volatile Solids (VS), Suspended Solids (TSS), COD and BOD5 were determined based on the standard methods\(^23\). The total volume of biogas evolved was measured by using Gas Measuring Sensor Unit which is comprised of three Sensors (H2, CO2, and CH4) it helps to sense and measures the amount of biogas generated from the reactor and reading will show in the LED display in ppm. After the biogas passed through the sensor unit, it reaches the water displacement unit. For confirmation of the biogas, it was injected into the GC using the syringe and biogas composition was analyzed by Gas Chromatography (GC 7410) equipped with a Thermal Conductivity Detector (TCD) and Stainless steel column packed with nitrogen gas was used as a carrier gas for biogas analysis. The temperature of the injector and column was maintained at 80\(^\circ\)C.

3. 0 RESULTS AND DISCUSSION

3.1 Acidogenic Fermentation

After inoculation of the enriched mixed consortia the CSTR was operated with Distillery Spent Wash at OLR of 4.115, 3.72, and 4.21 kg COD m\(^3\).d with various temperatures of 35\(^\circ\)C, 55 \(^\circ\)C, and 70 \(^\circ\)C with 24 hour HRT by adjusting the influent pH value to 5.5 to 6.0. 22 days later CSTR has reached the stabilized state then the reactor was operated at higher organic loading rate for 35\(^\circ\)C (6.106, 8.45, 20.15, and 41.4 Kg COD m\(^3\).d), 55 \(^\circ\)C (8.45, 20.15, 37.14, and 41.41 Kg COD m\(^3\).d), and for 70 \(^\circ\)C (10.1, 24.06, 43.17 Kg COD m\(^3\).d) with HRT of 16h, 12h, and 8h for 44 days. The CSTR presents satisfactory process efficiency on COD removal rate as represented in figure 3, 4, and 5.
In the initial days of the startup of the CSTR period, the COD removal efficiency was lower due to the adaptation of the microorganisms to the Distillery Spent Wash wastewater. The average COD removal efficiency was 13%, 23% and 14% in the first 5 days and then gradually increased to 68%, 62% and 62% in 43, 44 and 40th day of operation under stable conditions after 30 -40 days. At higher OLR (41.4, 41.41 and 43.17) the system documented a maximum COD removal efficiency of (68%, 62%, and 62%) in the CSTR system during this phase of stable operation. Sugar industry
wastewater consists of Distillery Spent Wash mainly sucrose which can be converted to methane in a
traditional anaerobic wastewater treatment process by a sequence of four steps: hydrolysis, acid
 genesis, acetogenesis, and methanogenesis. After the hydrolysis of complex sugars converted into
simple sugars and further degradation is identified as to continue through simultaneous steps by
rapidly growing and pH-insensitive acidogenesis bacteria to organic acids (butyric, propionic and
acetic acids), CO2 and H2. In the next step gradually growing and pH-sensitive bacteria further
oxidize the higher acids to acetic acid, carbon dioxide, and hydrogen. Methanogenesis involved the
reduction of carbon dioxide to methane using hydrogen by relatively rapid growing pH sensitive
autotrophic bacteria. Furthermore, Methanogens also catalyze the reduction of acetic acid to
methane. Conversely in the CSTR system where acidogenic bacteria were dominant COD was
removed during the cytogenesis and gas releases (mainly CO2 and H2), whereas a considerable
amount of COD was converted to liquid intermediate products (e.g., ethanol, acetate, butyrate, and
propionate) and remained in the system24. Hence COD removal efficiency in this system was lower
than traditional anaerobic process.

3.2 Bio-hydrogen production

The hydrogen yield and specific hydrogen production rate comprise normally been
considered as the significant indices to assess the bio-hydrogen producing processes25. The result of
hydrogen yields in the process of startup stage and sludge acclimatization. Due to the activated
sludge was in the stage of adjusting and acclimatizing itself to their inner environment of the reactor,
the biogas productivity and hydrogen content were low at the beginning of the startup (the first 10-20
days). The operation time went on the activated sludge acclimatized slowly and the biogas
productivity improved. Once the sludge acclimatization was done by 22nd day and the hydrogen
production with HRT of 24h, 16h, 12h and 8h was 1.92L/d, 2.0L/d, 2.12L/d and 2.47L/d for CSTR
run with 35oC, 1.2L/d, 2.0L/d, 1.93L/d and 0.98L/d for CSTR run with 55°C, 1.24L/d, 1.51 L/d, 1.83
L/d and 1.62 L/d for CSTR run with 70°C. Once the OLR of the system was improved after 23rd
day, the hydrogen productivity increased and reached to the maximum of 2.472L/d, 2.054L/d and
1.838L/d for 35°C, 55°C, and 70°C. The produced biogas consists of hydrogen and carbon dioxide
and free of methane. It is visible from the experimental data that the OLR has revealed considerable
influence on both H2 production and substrate removal. The variation in the hydrogen production
rate can be attributed to variation in the microbial population and OLR26. In the stable operation of
the reactor confirmed stable performance with respect to biogas production and substrate
degradation. This signifies that the Distillery Spent Wash participated as the primary carbon source
in metabolic reactions involving molecular H2 generation in this present study.
3.3 Bio-hydrogen production process evaluation

Several parameters such as HRT, VFAs, pH, Temperature and alkalinity were investigated in this present study for evaluating the performance of this fermentative bio hydrogen production process. Figure 9 – 13 illustrates the variation in effluent VFA and the relationship between pH and liquid end products concentration in the CSTR system during the bioreactor operations. The difference of liquid products recommended that the system had undergone a switch of fermentation.
types. Observable fermentation phenomenon takes place in the CSTR system after the bioreactor startup.

3.4 Bio-hydrogen process evaluation for CSTR runs with varied temperature 35°C, 55°C, and 70°C with 60 rpm

In all temperature ranges the pH 5.0-5.5 was found to be optimum for hydrogen production, thus the results suggested that ethanol type fermentation process is suitable for bio-hydrogen production from sugar industry Distillery Spent Wash wastewater treatment. Figure 9-13 shows that the changes in VFA, pH, Alkalinity and VFA/Alk ratio in the CSTR, the changes of four factors affected not only the anaerobic hydrogen ability but also the microbial community and fermentation types. It was found that the bioreactors underwent significant variation in pH, Alk, VFA/Alk ratio in the first 22 days of operations. After 22 days the pH of the bioreactor stabilized at 5.5-6.0. It was evident that the typical anaerobic mixed cultures could not produce H\textsubscript{2} it was a methanogenesis process by allowing H\textsubscript{2} to become an end product in the metabolic flow. The experimental results illustrated that the biogas was composed of hydrogen and carbon dioxide and free of methane. It can be finalized that the low effluent pH suppressed the methanogenesis activity. Alkalinity is an important parameter in biohydrogen production reactor and it’s a key parameter that influence greatly on the stability and hydrogen yields. Alkalinity and Volatile Fatty Acids (VFA) concentration in an anaerobic system was determined by the mixed liquor pH. Since alkalinity was influenced by the balance between [CO2] and [HCO\textsubscript{3}], and the majority of alkalinity was [HCO\textsubscript{3}] at pH lower than 5, low pH and alkalinity were estimated at high VFA concentration due to the utilization of HCO\textsubscript{3}. After 22 days, both hydrogen production and VFAs increased representing anaerobic bacteria had adapted to the CSTR system. With additional CO\textsubscript{2} being produced, [HCO\textsubscript{3}] became higher and alkalinity increased in the same way. A higher alkalinity improved the system neutralization capability for VFAs and directed to a stable pH value. Thus, pH can be stabilized at 5.5–6.0 however more VFAs were produced after the 22\textsuperscript{nd} day when OLR was increased in the CSTR. In comparison with VFA production from wastewater is generally conducted under acidic condition with optimum pH range from 5.25 to 6.0. Thermophillic temperature 60°C could direct to more rapidly biological acclimatization and further active acidogenesis as evaluate to mesophillic temperature 35°C, thus leading to a higher VFA yield. Temporarily the production of VFA at extreme hyper thermophilic temperatures 70-80°C.

From this study the VFA concentration achieved maximum 5994mg/l at 35°C which is lower than 4470 mg/l and 3390 mg/l reactor run with 55°C and 70°C similar with the acid forming enzymes
activities at thermophilic temperature 55°C were lower than that at mesophilic temperature 37°C. As an outcome the total VFA concentration achieved at 55°C was 40% lower than that at 37°C.33.

Fig. 9 Effluent VFA concentration (mg/l) at 35°C

Fig. 10 Effluent Alkalinity concentration (mg/l) at 55°C

Fig.11 CSTR VFA/Alkalinity ratio for various temperatures
3.5 Microbiology and Biomass Concentration

SEM images figure 14 of the anaerobic mixed culture obtained from experiments envisaged scattered, slightly bent and short chain rods. Images of mixed consortia illustrated the proliferation of morphologically similar groups of bacteria. The discerning enrichment procedure adopt in this study might result in the enrichment of specific group of bacteria capable of producing H2. Figure 15 shows the progression of the sludge concentration in the fermentative bio-hydrogen production reactor. In the start-up period, the sludge concentration increased with time. The biomass at 35°C, 55°C and 70°C reached 7.62, 7.53, and 4.59gVSS/l on the 22nd day in the CSTR system. The increase in sludge concentration was due to the efficient anaerobic operating conditions (pH, temperature and loading rate) for the anaerobic bacteria. When the OLR was further increased after 22nd day, the sludge was washed out because of the hydraulic shock, thus the biomass concentration decreased slightly. After a week, the VSS stabilized 35°C, 55°C and 70°C of 8.0-8.5gVSS/l, 8.2-8.7gVSS/l and 5.5-5.7gVSS/l respectively. The maximum biomass concentration of 8.96gVSS/l for 35°C on 43rd day, 8.76gVSS/l for 55°C and 4.59gVSS/l for 70°C. Based on the results at the stable period, the hydrogen productions were 2472ml/d, 2054ml/d and 1838ml/d. Therefore, the specific hydrogen production was higher in the CSTR in system run with 35°C.
CONCLUSION:

The key objectives of this current study are to study viability of simultaneous wastewater treatment and bio-hydrogen production using sugar industry wastewater with various temperature 35°C, 55°C, and 70°C. The CSTR system attained stable ethanol type fermentation after 22 days of acclimatization in all the temperature cases studied. Particularly CSTR run with 35°C showed the better results with acclimatization OLR 3.72 kg COD/m3.d and HRT of 24h. When the OLR was further increased from 3.72 to 6.10, 8.45, 20.15, and 41.4 with HRT of 16h, 12h, and 8h after 22 days, the reactor showed a maximum COD removal efficiency of 70% and hydrogen production of 2472ml/d with VSS of 8.96g/l in the system during the stable operation of 30 - 40 days). Effluent pH, alkalinity, VFA, and VFA/Alkalinity are 5.9, 12726, 5981 and 0.47 respectively. A low pH, ethanol type fermentation process is an effective dark fermentation method for producing hydrogen from acidic sugar factory wastewater. After this period of operation, the reactor showed a stable COD removal efficiency for 35°C, 55°C, and 70°C are 68%, 62%, and 62% with hydrogen production of 2472ml/d, 2054ml/d and 1838ml/d in the system. Hence for biological hydrogen production and wastewater treatment CSTR run with 35°C with 60 rpm is suitable in future.
REFERENCES


