Effect of Mixing Ratios in a Batch Reactor for Biological Hydrogen Production

C. Anantharaj, V. Arutchelvan, N. Ashok Kumar

***Abstract:** This study was to evaluate the cause on the production of bio-hydrogen utilizing Distillery Spent Wash (DSW) and starch mixed wastewater in various mixing ratios under dark fermentation conditions with varying Hydraulic Retention Time in a batch reactor. The pH was kept constantly at 5.5 throughout the study. Thus the hydrogen production investigated in the batch reactor for following Hydraulic Retention Time 24h, 16h, 12h and 8h, the results revealed an extended and steady period of reactor operation with hydrogen percent in the biogas and hinders the methane production. The perfect mixing ratios and Hydraulic Retention Time were seen to be 50:50 (DSW: Starch wastewater) and 16h to 12h HRT with contrasting cumulative hydrogen yields of 3256.5ml and 2674.3ml with specific maximum production of hydrogen rate 468ml/L/d as well as 236.64ml/L/d with the best biomass improvement combination of 4.7g/l and 4.5g/l. The maximum Chemical Oxygen Demand removal rates of 68 and 50 percentage for the optimum HRT of 16h and 12h.*

***Keywords:** Batch reactor; DSW: Starch wastewater; Biomass concentration; COD removal; Hydrogen production.*

I. INTRODUCTION

In current years, non-renewable energy usage has driven specialists to check for optional biofuels. Solid waste could be the obvious choice amongst the most wellsprings of clean biofuel production. Hydrogen could be utilized as an elective biofuel instead of petroleum products, which could be generated using biological hydrogen production methods. Energy content is high in hydrogen; hence it's become energy effective fuel and leaves water as its residue. In room temperature conditions generation of such productive fuel from very low energy contribution would be a huge environmental benefit [1]. So many decades, hydrogen production was achieved from a wide extent of resources, through various generation innovations, where most of it has been generated from non-renewable feedstock, for example, oil, coal, petroleum and gas [2]. Coal gasification and thermolytic burning is most utilized strategy for hydrogen production that contaminates the air due to the by production of ozone depleting substances (GHGs), thus contrarily impacts the supportability of hydrogen as a fuel asset. The finest decision in contrast to these "filthy" production methods is bio hydrogen [3].

Revised Manuscript Received on September 05, 2019.

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A serious concern for most of the nations is the disposal of industrial wastewater in an improper way, where the wastewater is complex in nature produced with the restricted advancements towards evacuate contaminations has further aggravated the problem. Sugar manufacturing industries plays major role in contaminating both the land and water bodies by releasing wastewater with high quantity of solids, COD, BOD_5, Sulfate, Nitrate, Calcium, Chloride and Magnesium[4], [5].

From the sugar manufacturing industries distillery spent wash was found to be the most multifaceted with troublesome wastewater effluent and having strong organic matter with COD ranges from 80000 to 160000mg/l, high bursting debris content, temperature level high, acidity low from pH 3.7 to 4.5 and large amount of in-organic salt in dissolved form [6], [7], [4]. Spent wash generated from sugar industries is the most troublesome surplus wastewater item, which is very much difficult to dispose since the pollutants are environmentally not friendly [9]. These kinds of wastewater pollute the water bodies and hence not suitable for human consumption and amphibian life [5], [10]. In the absence of proper dilution and treatment, sugar industry wastewater when directly used for crop yield water system would fundamentally restrain the seedling development and seed germination [12]. Another case is cassava starch production, where around 5 to 7 L of wastewater generated from 1 kilo of new root [12]. Cassava starch wastewater (CSW) exist as an appropriate substrate in bioreactors for bio-hydrogen production because of its composition of biodegradable starches, nitrogen, magnesium, sodium, phosphorus, calcium, iron, zinc, potassium, nickel and copper [13]. Hydrolysis is a process where the complex typical mixes are changed over (hydrolyze) by fermentative microscopic organisms to basic monomers, for example, unsaturated fats are converted into monosaccharides and amino acids. Next, in acidogenesis process, these monomers included sugars will be converted further to acidic destructive derivation, hydrogen (H2) and carbon dioxide (CO_2) [14]. Bio-hydrogen production utilizing inoculation of mixed anaerobic sludge in the acidogenic reactors comparing with pure cultures. Its having better adjusts to natural stresses which include supplement constraint, pH changes and temperature changes. Anaerobic mixed sludge consists of both hydrogen consuming and hydrogen producing microscopic organisms [15]. For hindering the H_2 consumers during dark fermentation with mixed microbial sludge, the following steps are essential which include homoacetogens, hydrogenotropic methanogens, lactic destructive organisms, sulfate reducers and propionate conveying microorganisms [16]. Hence, pre-treatment is regularly carried out to upgrade the mixed sludge for enhancing the hydrogen producing microscopic organisms in addition to remove the hydrogen utilizing microscopic organisms. Heat pre-treatment of sludge is the well-known method generally utilized [17], [18].

The present investigation was contemplated to production of bio-hydrogen using mixed Distillery Spent Wash (DSW) and starch wastewater in various mixing ratios under dark fermentation conditions. Diluted DSW and Starch wastewater was utilized as a substrate and inoculums used as pre-treated anaerobic digested sludge. The inoculums to substrate proportion was fixed as 1:5 (Anaerobic sludge: substrate), with HRT maintained from 8h to 24h under mesophilic conditions (35^{o}C). The production of bio hydrogen, COD removal effectiveness along with the optimum HRT was assessed.

II. MATERIALS AND METHODS

A. *Experimental Set-up*

Batch reactor studies were conducted using borosil glass bottles with 1L volume of each bottle. To sustain the reactor in anaerobic conditions, the bottle opening was tightly closed with rubber cork. For biogas collection and sampling, two ports were provided. Water displacement unit was fixed with the reactor for measuring biogas evolved. The reactor was sealed with 100ml of pre-heat treated seed sludge and 600ml of mixed distillery spent wash and Starch Wastewater (different mixing ratios), where the space present above filled sludge and mixed wastewater acts as a gas collection chamber. Batch reactor bottles were purged by Nitrogen to attain anaerobic environment. The experiment studied at constant pH of 5.5 for different HRT and the mixing ratios were optimized at temperature (35±1^{o}C).The parameters pH, COD, Biogas and Biomass concentration were monitored daily.

B. *Seed inoculums*

Mixed anaerobic digested sludge acquired from the anaerobic digester unit treating distillery wastewater was utilized as a seed in the batch reactor. The raw anaerobic digester sludge comprises of vast microflora. The collected anaerobic sludge was exposed to pre-treatment in order to hinder the hydrogen consumer’s activity and also to yield the spore forming anaerobes which are responsible for production of hydrogen. The sludge was heated at (100^{o}C for 2 hours) to harvest sp. Clostridium type bacteria which accountable for the production of hydrogen as well as to suppress methanogens, homoacetogenes, and other unfavorable species of bacteria for hydrogen production [17]. The production of different metabolites associated with the diversity of microorganisms which are existent in the selected microbial consortium once the thermal pre-treatment completed it hinders non spore forming microorganisms. [18], [19]. After the pre-treatment, the seed sludge was inoculated in the reactor using the seed to substrate ratio of 1:5. (The heat shock pre-treatment employed as the main inoculum pre-treatment method when complex wastes used as a substrate [20], [17], [21], [22].

C. *Distillery spent wash and starch wastewater*

The distillery spent wash was collected from sugar industry in Tamil Nadu and to avoid the wastewater from biodegradation due to microbial action; the wastewater was stored at a temperature less than 4^{o}C. The Cassava starch wastewater collected from a cassava flour factory in Tami Nadu.

Table 1 Characteristics of mixed distillery spent wash and starch wastewater

Para meters	10;90	20;80	30;70	40;60	50;50	60;40	70;30	80;20	90;10
pH	5.20	4.86	4.85	4.83	4.72	4.65	4.62	4.53	4.50
COD	16800	16500	16700	16800	19200	18200	18600	17600	17200
TS	11300	11500	11600	11700	18200	17600	13800	12600	12300
TSS	4900	4800	4600	4800	10900	9200	8200	7200	5600
TDS	6400	6700	7000	6900	7300	8400	5600	5400	6700
MLSS	6800	6200	6500	6300	8600	7800	6300	6200	6800
MLVSS	5200	4700	4200	4600	6800	5800	4200	4700	4200

All Parameters are in mg/L except pH

D. *Analytical procedure*

The biogas production rate and pH was regularly monitored, the samples from influent and effluent were collected and examined for pH, total solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), COD as per the standard procedure [24]. Stable state condition was achieved for each HRT and it was vindicated with measured biogas and COD effluent. To estimate the process performance of the Batch reactor systems, the experimental data arrived from the steady state was considered. Water displacement method was employed to measure the total biogas evolved with biological hydrogen production percentage estimated by GAS chromatography.

III. RESULTS AND DISCUSSION

A. *Hydrogen production vs COD removal in different mixing ratios*

During the first series of experiment, the effects of mixing ratios with respect to HRT were investigated. The Batch acidogenic reactor was operated to optimize the mixing ratios with respect to HRT. The reactor was initially started with 24h HRT and further the HRT was gradually reduced to 16h, 12h, and finally 8h HRT ensuring that in each HRT the steady state was reached. It is also noted that during the operation of the reactor no methane was detected as confirmed by GAS measuring sensor unit which consists of H_2, CO_2 and CH_4 measuring sensor.

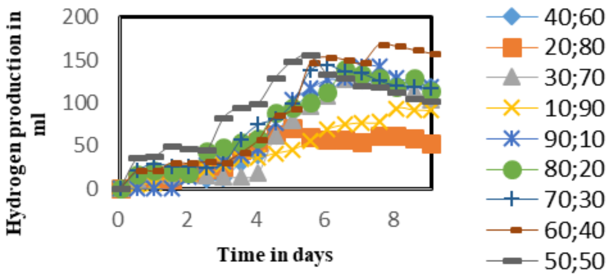

Fig.1 Hydrogen production in different mixing ratio at 24h HRT

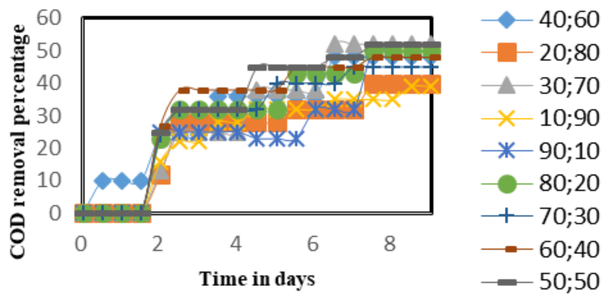

Fig.2 COD removal percentage in different mixing ratio at 24h HRT

In figure 1 and 2 the hydrogen production rate with respect to COD removal percentage at 24h HRT with different mixing proportion were presented. The hydrogen production rates increase when the HRT decreases. At an HRT of 24h, the cumulative hydrogen production of 1754.63ml and maximum rate of specific hydrogen production was about 156.6ml/L/d were achieved with 52% COD removal which gradually increases while the HRT decreased. Thus, the highest production of hydrogen and COD removal percentage were observed at HRT 16h with cumulative H2 production of 3256.4ml and maximum rate of specific hydrogen production 468ml/L/d on 7^{th} day of batch reactor operation. This is maximum when with the previous study of [25], where about 429.72ml/L/d rate of specific hydrogen production achieved. The results from the batch reactor runs with HRT16h exist to be high when compared with the reactor runs with HRT 24h, 12h and 8h as mentioned in the figure 3 and 4.

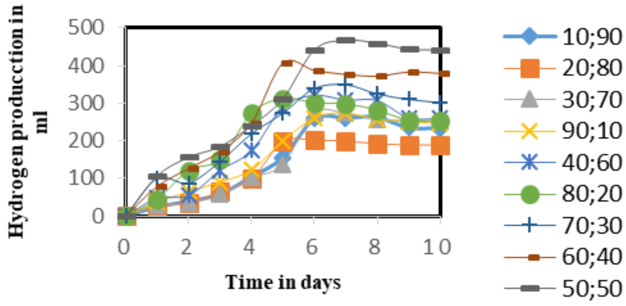

Fig.3 Hydrogen production in different mixing ratio at 16h HRT

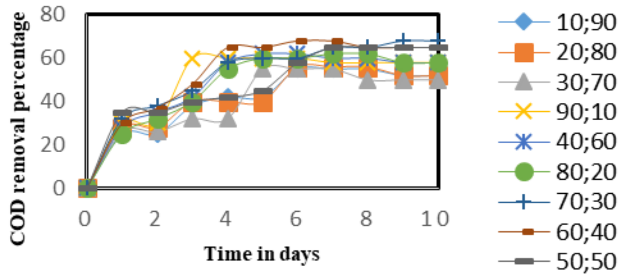

Fig.4 COD removal percentage in different mixing ratio at 16h HRT

Batch reactor runs with the nine mixing ratios of 10;90, 20;80, 30;70, 40;60, 50;50, 60;40, 70;30, 80;20, 90;10 were carried out, out of which the maximum cumulative production of hydrogen was achieved at the mixing proportion 50:50 (DSW: Starch wastewater) followed by 60:40, 70:30. This pattern of hydrogen production from the mixing ratios was similar in all cases of the HRT throughout the experimental study. The greatest hydrogen production was achieved while the reactor runs with 16h HRT with mixing ratio 50:50 (DSW: Starch wastewater). The cumulative production of hydrogen was about 3256ml and the maximum specific hydrogen rate of 468ml/L/d with COD removal of 65%. During the mixing ratio of 60:40, the cumulative H2 production was 2929.8ml and specific H2 rate was 406.9ml/L/d with 68% of COD removal and for 70:30, the cumulative H2 production was2450.6ml and specific H2 rate was349.2ml/L/d and 68% removal rate of COD as shown in the figure 3 and 4.

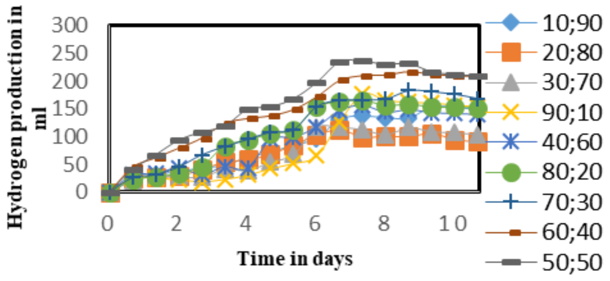

Fig.5 Hydrogen production in different mixing ratio at 12h HRT

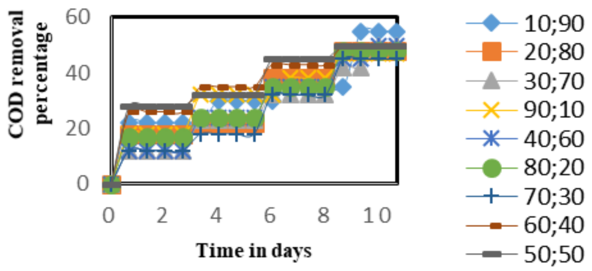

Fig.6 COD removal percentage in different mixing ratio at 12h HRT

After the reactor runs with the HRT of 24h and 16h, the reactor starts with the reducing HRT to 12h and 8h. The hydrogen production maximum with specific maximum hydrogen production rate of 2674.3ml and 236.64ml/L/d at HRT of 12h under the mixing ratio of 50:50 where COD removal rate was of 50% as shown in figure 5and 6. This reveals the fact that hydrogen production rate increases, although the COD removal efficiency decreases at low HRTs. Finally the reactor runs with the 8 h HRT and the results obtained was 1691.95ml of cumulative hydrogen production and 132.75ml/L/d of specific maximum hydrogen production with COD removal of 43%, which is less as compared with the other HRTs which implies that hydrolysis occurs in acidogenic reactor at low HRT.

The degradation of carbohydrate or proteins or lipids may have taken place in the reactor at all HRT values tested. The importance of the pH on hydrogen production has been revealed by many experimental studies, the activities of the various enzymes would be inhibited at low pH especially the hydrogenase enzyme [26], [27].The batch reactor was operated at constant pH 5.5. Particularly in the dark fermentation progression, the optimum pH range was kept as 5.5-6.0 in order to avoid the methanogensis and solventogenesis [28]. The low pH ranges of 4.5-6 leads to high in acetic and butyric acids concentration, the soluble metabolites which there by affects the H2 production pathways [29], [30], [31], [32], [33]. Hence, mesophilic dark fermentation, the optimum initial pH was considered as 5.5-6.0, where several studies have reported the same. The production rate of hydrogen and yield of hydrogen using pre heat treated anaerobic sludge was found to be maximum [34] as compared with pre-treated algal biomass with production rate of hydrogen and hydrogen yield was 62ml/l/d with 9.5ml/g VSS added.

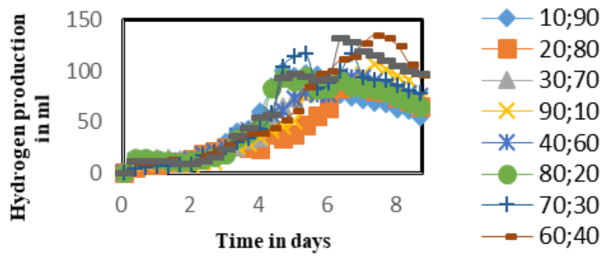

Fig.7 Hydrogen production in different mixing ratio at 8h HRT

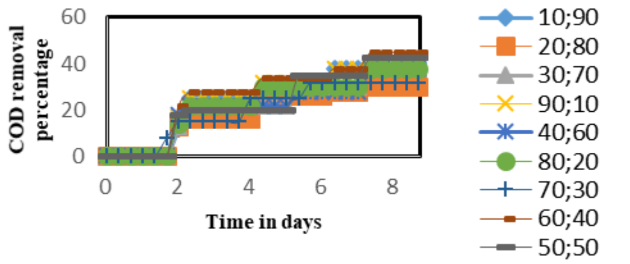

Fig.8 COD removal percentage in different mixing ratio at 8h HRT

B. Biomass concentration in different mixing ratios at different HRT

MLVSS Mixed Liquid Volatile Suspended Solids measured as biomass concentration for various wastewater mixing proportions with different HRT 24h, 16h, 12h and 8h. It was identified that in all the HRT cases, the COD removal was achieved with gradual increase in the biomass growth. The degradation takes place with an initial biomass of 4.3 to 6.3g/l for 24h HRT, 4.2 to 6.8 g/l for 16h HRT, 4.3 to 7.2 g/l for 12h HRT and finally 4.5 to 6.5 for the batch reactor runs with 8h HRT. For the first two to three days, the biomass growth rates were low which may be due to new environmental conditions and new. Later the period of lag phase time, the microorganisms were capable to adapt to the new environmental condition and the growth of biomass was gradually improved and lastly it reaches the stable state conditions as presented in figure 9 to figure 10. The maximum biomass growth was gained at various mixing ratios 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10 for 24h HRT were estimated. The maximum biomass growth was about 5.8g/l, for 16h HRT it was 6g/l, 5.8g/l for 12h HRT and finally 5.2g/l for 8h HRT.

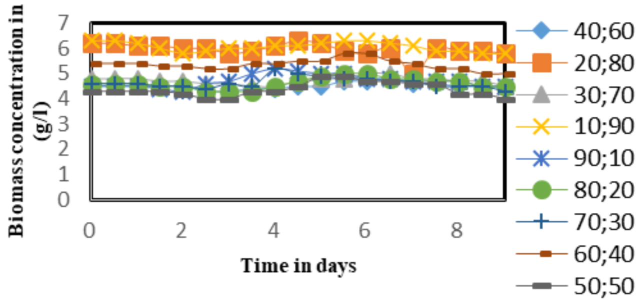

Fig.9 Concentration of biomass in different mixing ratio at 24h HRT

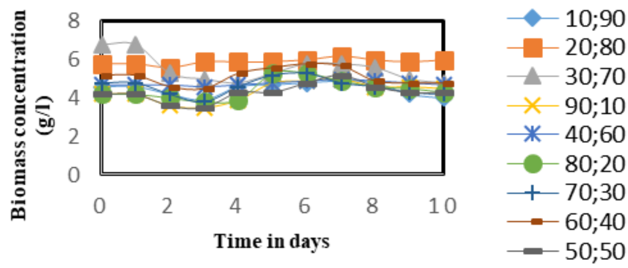

Fig.10 Concentration of biomass in different mixing ratio at 16h HRT

Retrieval Number: K19240981119/2019©BEIESP
DOI: 10.35940/ijitee.K1924.1081219

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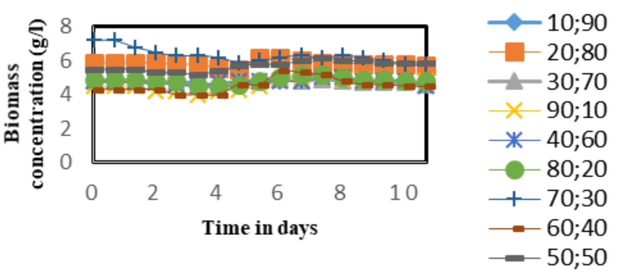

Fig.11 Concentration of biomass in different mixing ratio at 12h HRT

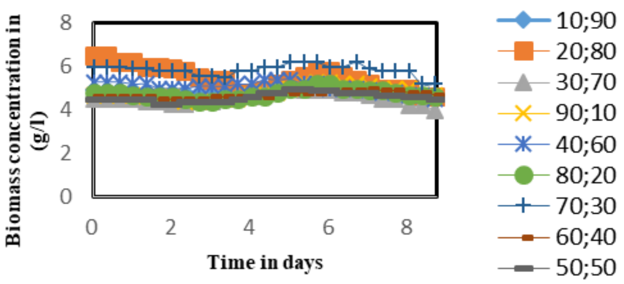

Fig.12 Concentration of biomass in different mixing ratio at 8h HRT

IV. CONCLUSION

Dark fermentation of carbohydrate rich waste are a viable source of hydrogen production and also reducing environmental pollutions Dark fermentative hydrogen production of mixed sugar DSW: starch wastewater was investigated in a batch reactor under various mixing ratios, various HRT and constant pH 5.5. The heat treated anaerobic mixed sludge was practiced as seed inoculum towards enhance the production rate of biological hydrogen. The optimal HRT range was between 16h-12h and the optimum mixing ratio for maximum hydrogen production was 50:50 (DSW: Starch wastewater). The highest hydrogen production was obtained during 16h HRT was 3256.4ml of hydrogen production with 468ml/L/d of maximum specific hydrogen and COD removal rate of 68% and maximum biomass concentration of 4.3g/l. Hence it proves that dark fermentative hydrogen production is an admirable way intended for production of hydrogen as compared to other biological systems. Though by these methods, exclusive bioenergy generation cannot be optimized, the waste discharged to the environment can be decreased.

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Blue Eyes Intelligence Engineering
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