Brewery Effluent as the Promising Substrate Source for Hydrogen Production Using Various Mixed Microbial Consortia

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Abstract: ***Brewery effluent with COD of 3600 mg/l was used as the substrate for hydrogen production in the present study. Inhibition of methanogenic activity by three pretreatment processes was studied and effective pretreatment, heat and acid treatment, was adopted for all the studies. Hydrogen production efficiencies of three different mixed microbial communities from sewage sludge, mangroves and hot springs are compared. In the pH optimization studies, pH in the range of 4.5 to 7.0 with 0.5 intervals were studied and 6.5 was found to be optimum for sewage mixed consortia (SMC), 6.0 for both mangrove (MAN) and hot spring (HS) mixed consortia. SMC has shown its optimum production at 55°C, MAN at 50°C and using HS, hydrogen yield was increased up to 60°C.Maximum hydrogen production of 293 ml / liter of brewery effluent was obtained with SMC as the inoculum at 55°C and pH 6.5.***

Keywords: Biohydrogen; Brewery effluent; Mixed consortia; Sewage mixed consortia; Mangrove; Hot spring; Batch reactor.

1. Introduction

Present energy needs of the world are depending on fossil fuels causing their depletion. Biodiesel, methane, ethanol and gasoline can be considered as the alternative sources of energy. The usage of these alternative fuels has limitations like the availability, which depends on the location and each fuel require a specific engine technology [1]. Hydrogen can be produced from all these alternative fuels, it has high energy content (143 GJ ton^{-1}) per unit volume and it is the only fuel with zero carbon emissions[2]. Hence hydrogen is the fuel of the future.

To be considered as a cleaner technology, hydrogen has to be produced from non-polluting and largely available sources. In this regard, production of hydrogen from renewable organic wastes like biomass or industrial wastes can be the solution for all the above issues. Brewery industries are available in most of the regions in the world and major effluents from these industries have high COD. As per one estimation for the production of 1 L of beer, 3–10 L of waste effluent is generated depending on the production and specific water usage [3]. These industrial wastes can be the possible sources for hydrogen generation. Mixed cultures do not have sterilization requirements. They are capable of adapting many conditions compared with pure cultures and continuous operation of the fermentation process is possible [4]. Taking all the above facts in to account hydrogen production in dark fermentation from industrial effluents using mixed cultures can be the best way to reach energy requirements.

2. Experiment

2.1 Brewery Effluent

Brewery effluent used in these studies was collected from Brewery industry located in Sangareddy near Hyderabad, Andhra Pradesh, India. The effluent was characterized using standard methods [5] and given in the table 1.

Table 1: Characteristics of the brewery effluent

S.No	*Parameter*	*Value (mg/l)*
1	pH	5.5
2	COD	3600
3	BOD	2420
4	Alkalinity	500
5	TS	2114
6	TDS	1684
7	TSS	430
8	VS	1510
9	VDS	1060
10	VSS	450
11	VFA	650
12	Nitrates	7.613
13	Sulphates	19.91
14	Phosphates	0.829
15	Fluorides	1.602
16	Total hardness	2800

2.2 Inocula

Mixed cultures used in these studies were obtained from three different sources. Sewage mixed consortia (SMC) and hotspring mixed consortia (HS) were collected from anaerobic digester at HMWSS (Hyderabad Metropolitan Water supply and Sewage board), Amberpet, Hyderabad and Taptapani, Orissa respectively. Mangrove mixed culture was brought from Bittarkanica, Orissa.

Effects of acid and heat pretreatments on biogas production were studied on the above three inocula. For the effect of heat treatment study, inocula were kept at 100^0C for 45 min and in acid treatment studies, inocula were incubated at pH 3 for 18hr. Experiments on the effect of the combination of both the pretreatments were conducted by heat treatment followed by acid treatment.

2.3 Batch fermentation for Hydrogen production

Batch experiments were conducted in 100ml vials with 80ml working volume for pH and temperature optimization. Each vial containing 70 ml of brewery effluent was inoculated with 5 ml of a specific inoculum. Later 5 ml of nutrient solution was added to each vial. Final pH in each vial was adjusted to 4.5, 5, 5.5, 6.0, 6.5, 7.0 and 7.5. All the vials were kept at room temperature for initial pH optimization studies. Temperature optimization studies were conducted at optimized pH in the range of 30 to 60°C with 5°C intervals.

Optimum conditions from these experiments were used for further studies to understand the reactor contents, scale-up possibilities and change in hydrogen production with respect to time. These studies were carried in 5 l batch reactor. Individual efficiencies of SMC, MAN and HS were studied at the optimum pH and temperature. 15% of the working volume of inoculum was inoculated into the batch reactor and was kept on magnetic stirrer.

In all the experiments with different working volumes anaerobic conditions were created by purging with N_2 gas and cultures were agitated at 150 rpm. Experiments were conducted in triplicates and mean of the three results are considered.

2.4 Analytical Methods

Characteristics of the brewery effluent and reactor contents were determined using standard methods.

Biogas was collected using gas-tight syringe (VICI) and analyzedin gas chromatograph (Nucon 5890) equipped with Molecular sieve 5A column and Thermal conductivity Detector (TCD) whereas nitrogen was used as the carrier gas at 30 ml/min flow rate. Temperatures in the injector, oven and the detector were set to 100°C, 80°C and 100°C.For the compositional analysis of volatile fatty acids, the samples were extracted using organic solvent.Composition and quantitative analysis of fatty acid was done by the same GC with FID as detector and Carbowax column by setting the temperatures of the injector, oven and detector to 200°C, 120°C and 200°C.

3. Results and Discussion

3.1 Effect of pretreatment

In the anaerobic digestion process using mixed microbial consortia, hydrogen generated is consumed by the hydrogen utilizing methanogens[6]. The objective of these experiments is to find the effective pretreatment process to kill methanogens. Pretreatment methods viz., heat treatment (H), acid treatment (A) and heat treatment followed by acid treatment (HA) were studied. From the figure 1, it is discernible that the pretreatment of mixed microbial consortia has a momentous effect on the hydrogen production. The combination of heat and acid treatments was found to be effective in killing methanogens and producing more hydrogen (61% with anaerobic mixed consortia, 56% with mangrove mixed consortia and 59% with hot spring mixed consortia) compared to individual effects of acid and heat. No methane production was observed after treating with acid and heat.

3.2 Effect of pH on Hydrogen production

The initial pH of the batch reactor was found to have significant effect on hydrogen yields from three microbial consortia. All the microbial consortia have shown low yield at lower pH conditions. It is evident from these experiments that pH in the range of 6.0 to 6.5 is effectual in hydrogen generation. Increase in pH in 0.5 units has significant effect on hydrogen production. Figure 2 shows the hydrogen yields of various consortia using brewery effluent with variation in the pH. Using anaerobic sewage sludge, H_2 production was increased up to 6.5 after which production decreased showing highest yield at pH 6.5. Mangrove mixed consortia and hot spring mixed consortia have shown maximum hydrogen yields at the pH 6.0.Figure 3 shows the changes in total volatile fatty acid contents with the variation in the pH.

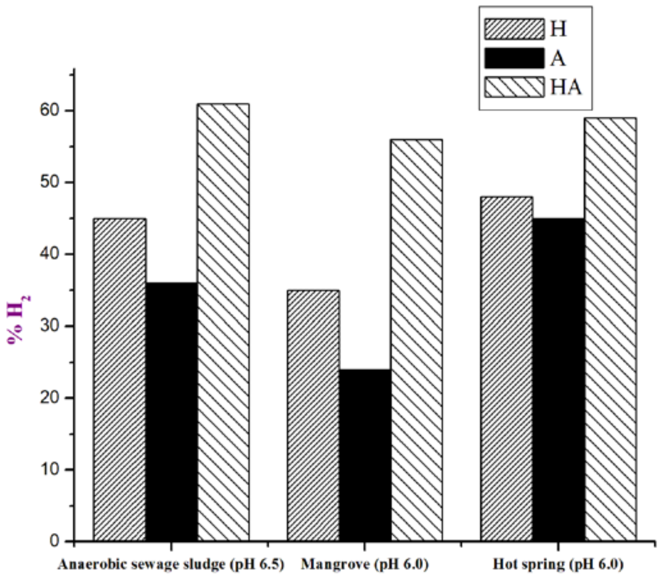

Figure 1: Effect of pretreatment on hydrogen production (Substrate: Brewery effluent, pH: 6.5 for ASS, 6.0 for Man, 6.0 for HS; Temp: 28±2)

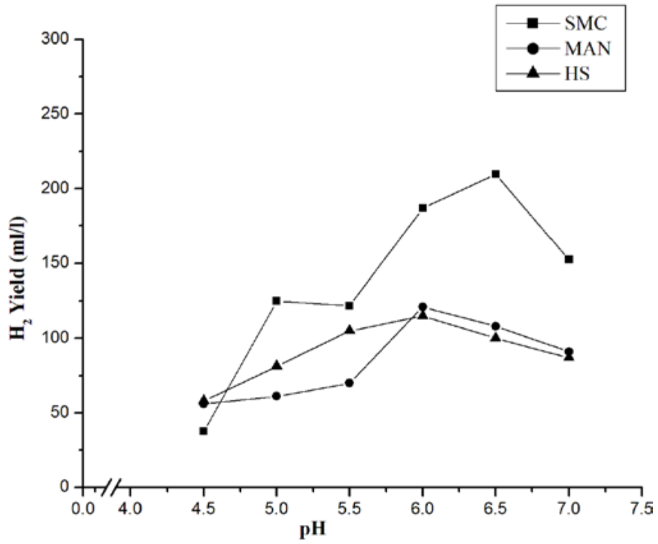

Figure 2: Effect of pH on Hydrogen production (Substrate: Brewery effluent, Temp: 28±2°C, Anae: Anaerobic mixed consortia, Man: Mangrove mixed consortia, HS: Hot spring mixed consortia)

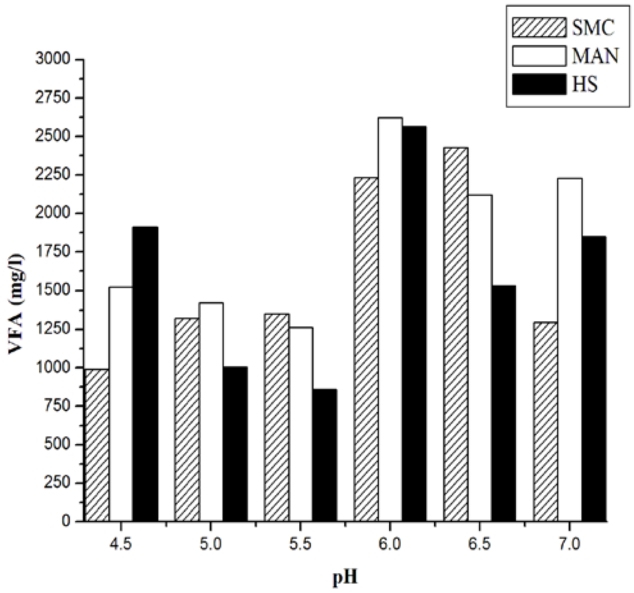

Figure 3: VFAs formed in pH optimization studies (Substrate: Brewery effluent, Temp: 28±2°C, Anae: Anaerobic mixed consortia, Man: Mangrove mixed consortia, HS: Hot spring mixed consortia)

3.3 Effect of temperature on hydrogen production

It is evident from the figure 4 & 5 that the hydrogen production is sensitive to the operating temperature. In this study, it was observed that hydrogen production was increased with temperature. Significant hydrogen production was observed at room temperature, which can be considered economically feasible process. The hydrogen production with HS was increased up to 60°C. The optimum temperature for MAN was found to be 50°C and 55°C for SMC.

3.4 Batch reactor analysis studies at optimum conditions

5 l reactor was operated in batch mode to study the biohydrogen production efficiency from brewery effluent with all the three mixed consortia. All the three different mixed consortia emulate the same pattern in hydrogen and VFA production, pH and alkalinity change and also the COD degradation. Batch reactor was studied up to 36 hr time where a drop in the H_2 yield was observed.

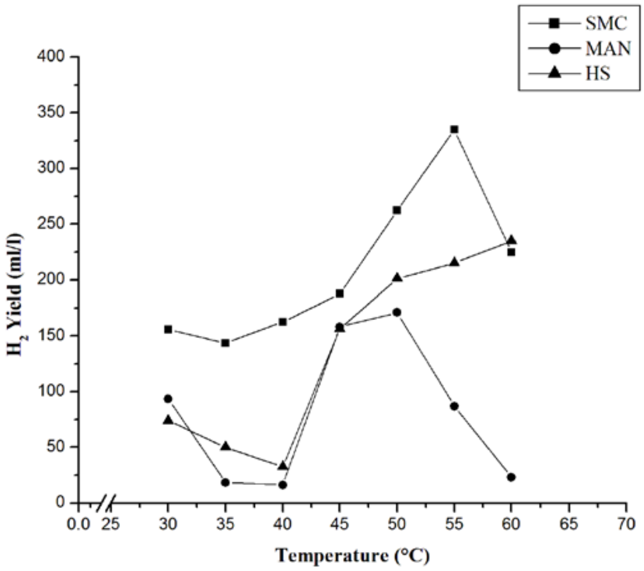

Figure 4: Effect of temperature on Hydrogen production (Substrate: Brewery effluent, Anae: Anaerobic mixed consortia, Man: Mangrove mixed consortia, HS: Hot spring mixed consortia)

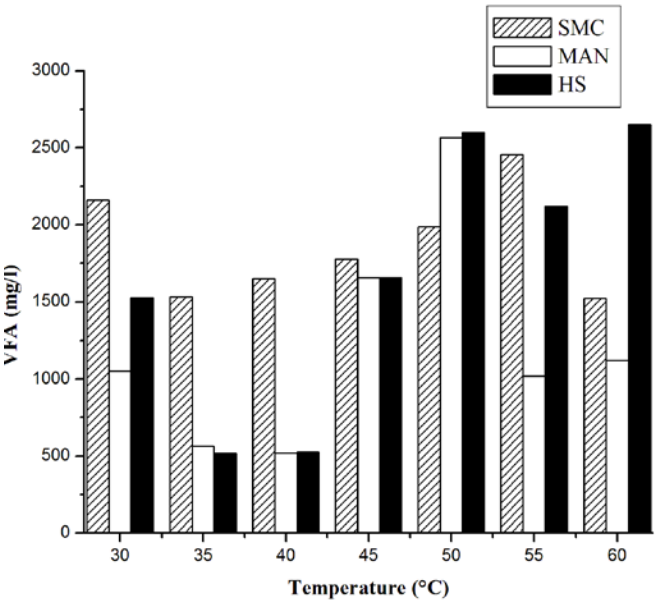

Figure 5: VFAs formed in temperature optimization studies (Substrate: Brewery effluent, Anae: Anaerobic mixed consortia, Man: Mangrove mixed consortia, HS: Hot spring mixed consortia)

All the three mixed consortia have started production within 6 hr of time. Although there is a steady increase in the biogas production with time, more amount of CO_2 started accumulating than hydrogen. Using SMC (figure 6), H_2 content in biogas increased from 56% at 6hr to 68% at 18 hr. The maximum H_2 content of 68% in the biogas was observed at 18 hr, where CO_2 content was around 32%. From 18 hr to 36 hr the CO_2 content was increased to 32% to 44% and the H_2 content was decreased from 68% to 56%.

The maximum H_2 content of 63% was observed at 12hr with MAN culture and 63% of maximum hydrogen content was observed at 24 hr with HS culture.

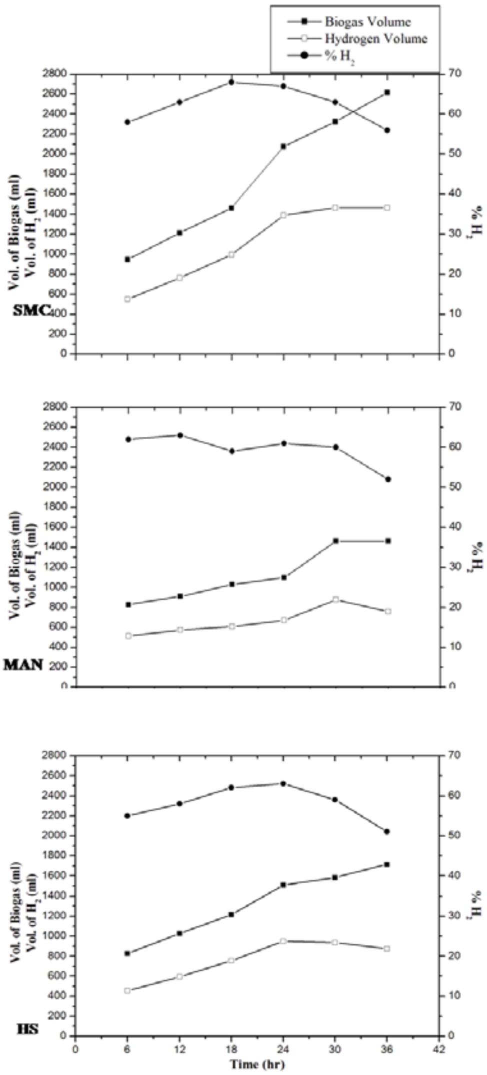

Figure 6: Change in H_2 content with time (Substrate: Brewery effluent, SMC: Sewage mixed consortia, Man: Mangrove mixed consortia, HS: Hot spring mixed consortia)

As depicted in figure 7, at 6 hr the hydrogen yield was 110 ml/l with SMC, 102 ml/l with MAN and 91 ml/l with HS. After 12 hr the cumulative hydrogen yield was 153 ml/l with SMC, 114 ml/l with MAN and 119 ml/l with HS. At these two stages, there is little or no change in the pH in all the three reactors. For every 6 hr the yields were calculated, reactor contents were analyzed and it was found that maximum yields were obtained after 24 hr time. SMC and MAN have shown maximum yields of 293 ml/l and 175 ml/l respectively at 30 hr. HS has shown maximum yield of 190 ml/l, after 24 hr. COD degradation was in between 21-71% with SMC, 16-61% with MAN and 12 to 67% with HS (figure 7). 60 to 70% of reduction in the COD (reflecting the available substrate) was observed at the end of 36 hr with all the consortia. There is an increase in the H_2 yield when COD degradation was in between 58% and 68%. This suggests that COD, which reflects available substrate in the reactor, is one limiting factor for H_2 production. Increasing the COD by adding substrate is one way of increasing the H2 production after this stage.

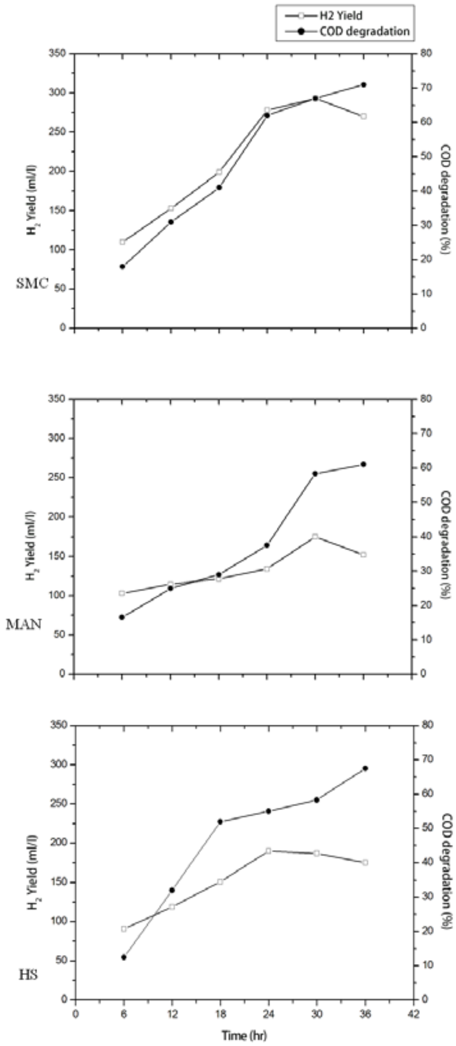

Figure 7: Changes in H_2 Yield and COD degradation with time (Substrate: Brewery effluent, SMC: Sewage mixed consortia, Man: Mangrove mixed consortia, HS: Hot spring mixed consortia)

Initial pH of the SMC reactor was set to 6.5, in which a drop in the pH up to 5.4 was observed at the end of 36 hr (figure 8). pH was also dropped to 5.13 in case of MAN and HS reactors with initial pH of 6.0 after 36 hr of operation. In SMC batch reactor, H_2 production was observed till the pH reaching 5.7 (at 30 hr) after which there is a drop whereas, with MAN and HS it is 5.2 (30 hr) and 5.78 (24 hr) respectively. Alkalinity was decreased in all the three reactors with three different consortia. Among the three consortia studied, MAN has shown more alkalinity (200 mg/l) at the end of 36 hr where a lesser drop in the pH (5.2 to 5.13) was observed. As the pH and alkalinity are important factors in H_2 production, controlling these can be one solution for increasing the yield.

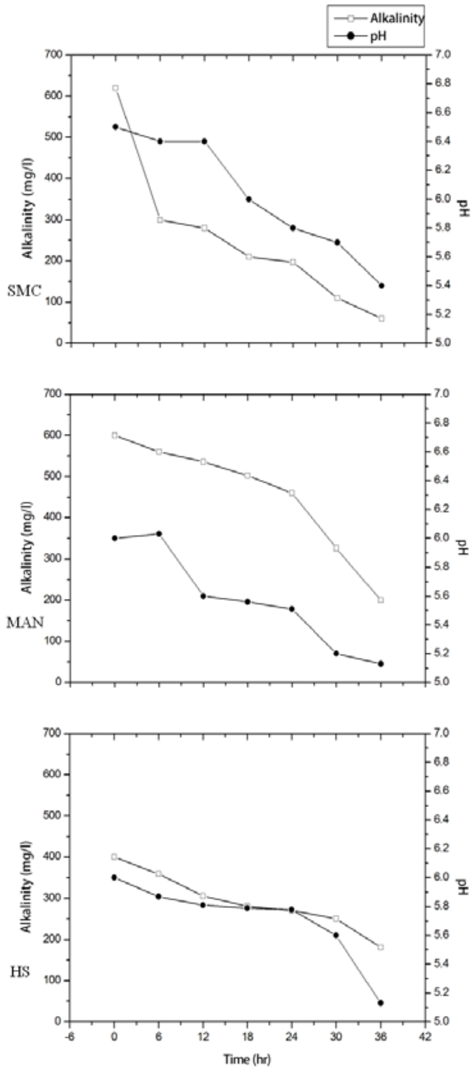

Figure 8: Changes in pH and Alkalinity with time

In addition to hydrogen and CO_2, other soluble metabolic products were observed viz. acetic acid, butyric acid, propionic acid and ethanol. VFAs contributed for most of the soluble metabolic products followed by ethanol. Volatile fatty acids were accumulated altering the operating pH of the reactor towards more acidic. Figure 9 shows an increase in the total volatile fatty acids with time. Maximum hydrogen yields can be expected with acetic acid pathway, but a mixture of volatile fatty acids is produced indicating other pathways. Acetic acid and butyric acid were predominant among the VFA produced in all the three cultures as they have contributed up to 85 % of the total soluble metabolic products. VFA accumulation is another factor, which is limiting the H_2 production.

These studies suggest that various factors like available substrate, VFA concentration, pH and alkalinity can be attributed to the change H_2 production with time.

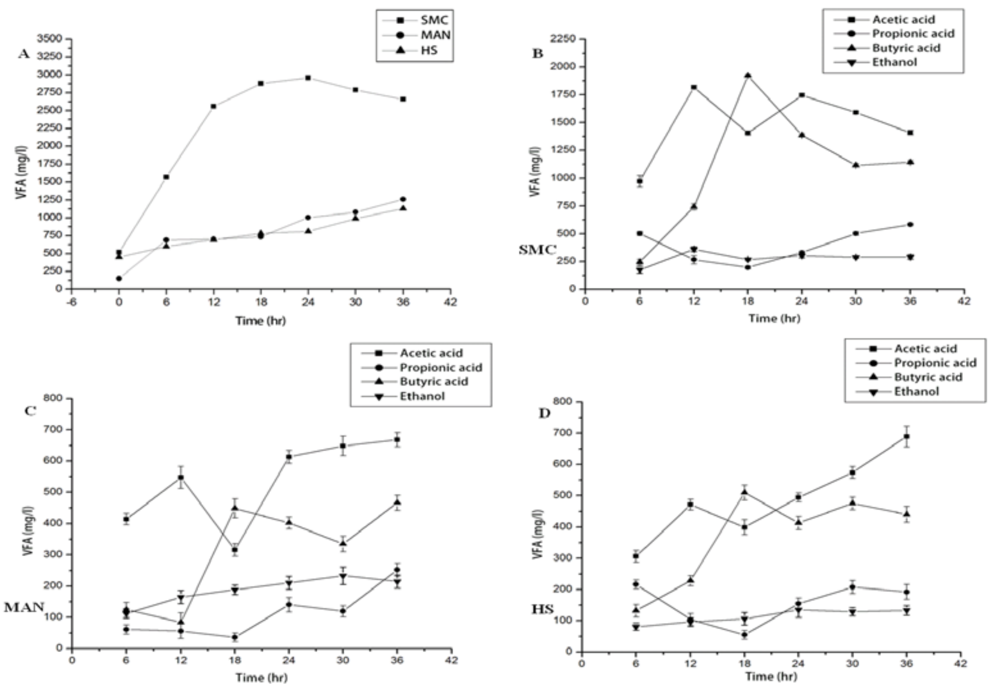

Figure 9: Change in the VFAs with time
(Substrate: Brewery effluent, SMC: Sewage mixed consortia, Man: Mangrove mixed consortia, HS: Hot spring mixed consortia)

4. Conclusion

Brewery effluent is the promising substrate due to many reasons. Firstly, it is largely available in many regions of the world. Secondly, the pH of the effluent (in the range of 5.5 to 6.5) is optimum pH for several hydrogen producing organisms. Availability of substrate with near optimum pH and operation of the reactor at room temperature are economically more feasible. Moreover, brewery effluent using three microbial sources has significant hydrogen production. Therefore, most of the mixed microbial cultures depending on the availability can be used in hydrogen production. No additional carbon source was added and effluent obtained directly was used in these studies. Maximum of 210 ml/l of H_2 was under mesophilic conditions, 293 ml/l of H_2 under thermophilic conditions was obtained repeatedly at pH 6.5 using sewage mixed consortia (SMC), which has large availability in Hyderabad.

5. Acknowledgements

This work was supported by the financial assistance from Ministry of New and Renewable Energy (MNRE), INDIA, under National Mission mode project on “Hydrogen production through Biological routes”.

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