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DARK FERMENTATIVE HYDROGEN PRODUCTION FROM BREWERY EFFLUENT USING THREE PURE ISOLATES

KEYWORDS: Biohydrogen, Brewery effluent, Batch fermentation, Bacillus subtilis, Anaerobic sewage sludge

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ABSTRACT Brewery industries release a large amount of effluent with high COD suitable as a source of substrate for hydrogen production. Organisms isolated from anaerobic sewage sludge, which was found to be a suitable mixed culture for hydrogen production in previous studies, were used in this study. 16S RNA analysis shown that the three organisms isolated were *Bacillus subtilis*, *Enterococcus faecalis* and *Acinetobacter calcoaceticus*. Present studies were conducted in 320 ml batch reactors with a working volume of 250 ml at the pH 6.5. Among the three pure organisms studied, *Bacillus subtilis* has shown a highest H_2 yield of 378 ml/ l effluent after 42 hr at a rate of 9 ml/l/hr. In the volatile fatty acid analysis, it was found that *Bacillus subtilis* and *Enterococcus faecalis* were producing acetic acid majorly, whereas, *Acinetobacter calcoaceticus* produced butyric acid.

INTRODUCTION

There is a dramatic increase in the fuel prices in this century as most of the energy needs are fulfilled by fossil fuels and their continuous depletion. Usage of fossil fuels posing a great damage to environment [1]. Finding the alternative fuels that are environment friendly and can be produced from renewable, largely available, cheap sources is a major objective of many of the scientific projects now. Hydrogen, which is considered as the fuel of the future can be produced from many industrial effluents with biodegradable organic matter. Brewery effluent which is largely available throughout the world with high COD values can be a possible source for hydrogen production in large scale [2]. Hydrogen is being produced mainly from natural gas (40%), crude oil (30%), coal (18%) and water electrolysis (4%) [3]. Biohydrogen is a renewable and sustainable alternative fuel and its low production rate and yield are the areas to be improved by research [4]. Hydrogen production using mixed cultures is advantageous in many respects but the production of same by pure organisms provide an opportunity to understand the metabolic pathways [5].

The current study involved the hydrogen production from brewery effluent using two pure organisms isolated from anaerobic sewage sludge. Batch fermentation process technique is used in small scale to find out various factors affecting hydrogen production.

MATERIALS AND METHODS

Bacterial cultures: *Bacillus subtilis*, *Enterococcus faecalis* and *Acinetobacter calcoaceticus* isolated from the anaerobic sewage sludge collected from anaerobic digester at Hyderabad Metropolitan Water supply and Sewage board (HMWSS), Amberpet, Hyderabad. PCR amplification and 16S RNA gene sequencing were performed to confirm the isolated organisms.

Brewery effluent: Brewery effluent (with 8000 mg/ l COD) used in these studies was collected from beverage industries in and around Sangareddy, Telangana. The effluent was characterized using standard methods [6].

Batch fermentation for Hydrogen production:

Anaerobic sewage sludge was initially pretreated by acid and heat to kill non-spore forming bacteria [7]. Experiments were conducted in 100ml vials with 80ml working volume to find out the optimum pH and temperature for biohydrogen production. Maximum hydrogen production was reported at pH 6.5 and temperature of 55°C.

Further batch experiments with pure isolates were conducted in 320ml vials with 250ml working volume. Each vial containing 245ml of brewery effluent was inoculated with individual culture. Later 5ml of nutrient solution was added to each vial. Final pH in each vial was adjusted 6.5. Reactors were kept at 55°C and agitated at 150rpm.

All the experiments were conducted in batch reactors connected with water displacement systems for quantitative analysis of gases produced. Strict anaerobic conditions were maintained by purging N_2 gas when needed.

Analytical methods:

Characterization of Brewery effluent: Brewery effluent was characterized using standard methods (APHA, AWWA, WEF).

Gas analysis: Gas-tight syringe (VICI) was used to collect the biogas generated which was analyzed in gas chromatograph (Agilent 4890). GC-GasPro column was used with nitrogen as the carrier gas at 20ml/min flow rate. Temperatures in the injector, oven and the detector were set to 100°C, 80°C and 100°C.

VFA analysis: The samples were extracted using organic solvent. Quantitative and qualitative analysis of fatty acid was performed in the same GC with FID detector and Carbowax column by setting the temperatures of the injector, oven and detector to 200°C, 120°C and 200°C.

RESULTS AND DISCUSSION

Bacillus subtilis isolated from Anaerobic mixed consortium. In the studies conducted on hydrogen production using anaerobic sewage sludge from brewery effluent [8], maximum hydrogen yield was reported at pH 6.5 and temperature of 55°C. The similar conditions were used in the present studies for *Bacillus subtilis*. The experiments

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were conducted in triplicate for increased accuracy. A maximum of 61% H_2 yield obtained after 42hr and decreased afterwards when no additional substrate was added i.e. in batch mode (figure 1). Hydrogen yield reached 378.2ml/l after 42hr and 58.8% COD degraded, the remaining of which utilized in H_2 production (figure 1). As shown in the figure 2, Hydrogen production rate (HPR) and yield were calculated at various time intervals and a maximum of 3.6mol/kg COD obtained after 42hr of contact time.

Total volatile fatty acids (TVFA) produced were also analyzed and it can be concluded that the organism followed acetic acid pathway majorly to generate hydrogen[9]. The results of TVFA and their composition are shown in figure 3.

In the similar studies performed on *Enterococcus faecalis* and *Acinetobacter calcoaceticus*, maximum hydrogen yields of 2.3mol/kg COD (figure 5) and 2.0mol/kg COD (figure 8) were obtained respectively. The optimum contact time using *Enterococcus faecalis* was 42hr, whereas, it was 36hr for *Acinetobacter calcoaceticus*.

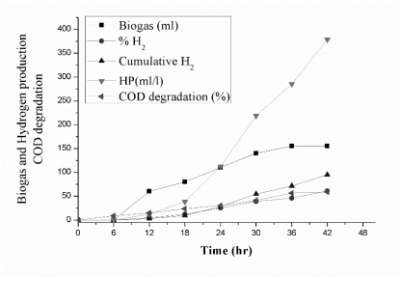

Figure 1: Biogas, Hydrogen production, COD degradation with time *(Bacillus subtilis)*

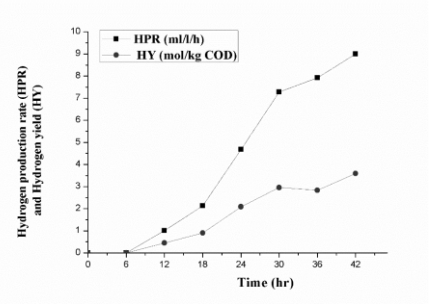

Figure 2: Hydrogen yield and production rate with time *(Bacillus subtilis)*

In the studies with *Enterococcus faecalis,* 2800ml/l TVFAs were obtained (figure 6) in which, acetic acid is a major constituent and with *Acinetobacter calcoaceticus* 3300ml/l TVFAs were obtained (figure 9) in which, butyric acid was the major constituent. These results support hydrogen producing ability of *Enterococcus* sps. shown in some recent studies [10].

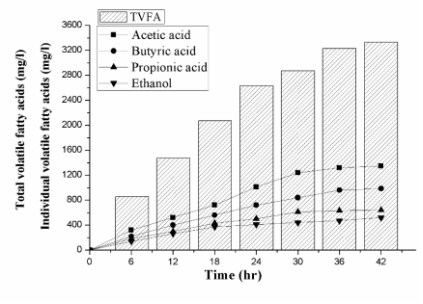

Figure 3: Changes VFAs with time *(Bacillus subtilis)*

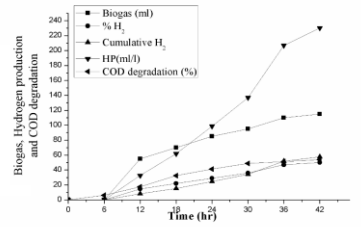

Figure 4: Biogas, Hydrogen production, COD degradation with time *(Enterococcus faecalis)*

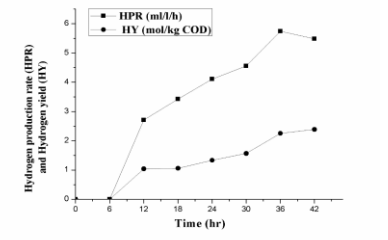

Figure 5: Hydrogen yield and production rate with time *(Enterococcus faecalis)*

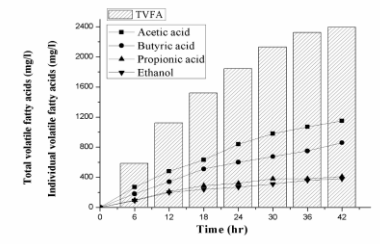

Figure 6: Changes VFAs with time *(Enterococcus faecalis)*

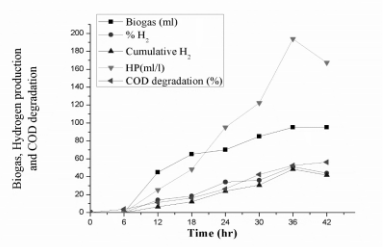

Figure 7: Biogas, Hydrogen production, COD degradation with time *(Acinetobacter calcoaceticus)*

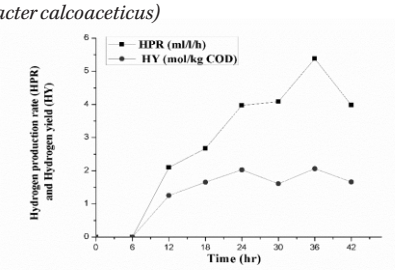

Figure 8: Hydrogen yield and production rate with time *(Acinetobacter calcoaceticus)*

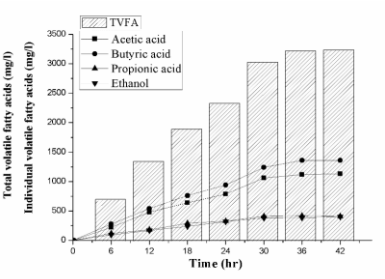

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Figure 9: Changes VFAs with time (*Acinetobacter calcoaceticus*)

Total volatile fatty acids were increased gradually in the reactors and therefore reducing the pH in the vials. COD degradation was observed in the three sets of experiments (Figures 1, 4 &7) and the part degraded COD was utilized in hydrogen production and VFA generation. COD degradation is above 50% by using all the three organisms and maximum COD degradation was obtained with *Bacillus subtilis* that correlated with highest hydrogen and VFA productions. Volume of biogas and cumulative hydrogen produced were also highest with *Bacillus subtilis.*

CONCLUSIONS

As concluded in the previous studies, this study also suggests that brewery effluent can be a probable source for hydrogen production. The organisms obtained from the anaerobic sewage sludge, which found to be a suitable mixed inoculum, have shown a considerable hydrogen yields. *Bacillus subtilis* was found to be the most appropriate organism for hydrogen production among the three compared. It produced a maximum of 3.6mol H_2/kg COD at 55^{0}C, pH 6.5, 150rpm when brewery effluent with 8000 mg/l COD used as a substrate. *Bacillus subtilis* can also increase starch degradation by producing amylase enzyme [9]. The present study confirm the hydrogen producing capability of *Bacillus* sps. which was reported in various earlier studies [10,11,12]. Usage of mixed cultures or mixed culture combinations in hydrogen production is more economical and does not involve the laborious isolation and sterilization techniques. But, hydrogen production studies by pure organisms provide the opportunity in understanding metabolic pathways involved in hydrogen generation and can be used in metabolic engineering for better yields.

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