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Mesophilic and thermophilic biohydrogen production using cassava starch wastewater in a CSTR

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ABSTRACT

The objective of this work is to compare and evaluate the production of biohydrogen (bio-H_2) from cassava starch wastewater at mesophilic and thermophilic conditions and to elucidate the metabolic routes involved. This system consisted of a continuous stirred-tank reactor for hydrogen production under the influent organic loading rate (OLRs) of 2.08, 5.05, 12.04, 23.34, and 30.87 kg COD/(m^3·d) for mesophilic temperature and 2.29, 4.47, 12.52, 22.09 and 28.86 kg COD/(m^3·d) for thermophilic temperature with Hydraulic Retention Time of 24, 16, 12 and 8 hours and pH maintained 5 to 6. The steady state of hydrogen production was 3131 ml/L·d obtained at the OLR of 23.34 kg COD/(m^3·d) with the COD removal of 85%, and the hydrogen production of 2007 ml/L.d with a COD removal of % was obtained at the OLR of 28.86 kg COD/(m^3·d). The maximum of 85% of the cassava starch wastewater was converted to hydrogen at the OLR of 23.34 kg COD/(m^3·d) at mesophilic conditions with HRT of 8 h and hence the mesophilic condition favoured the hydrogen production using cassava starch wastewater.

Key words : *CSTR, Cassava starch wastewater, Anaerobic mixed sludge, H_2 production, COD removal.*

Introduction

Dependence on fossil fuels and their shortage have led to complementary, sustainable and low-cost sources of energy being researched. In recent years, commercial electricity generation and transport of renewable energy sources fuel have increased in developing countries producing considerable social, environmental and financial gains (Guo *et al.*, 2015). For this kind of energy transformation, hydrogen is considered to be a promising vector, as it is a clean fuel which releases no carbon dioxide during combustion and it can be used in fuel cells for generating electricity. In addition, in comparison with hydrocarbon fuels, hydrogen has the highest energy value (122 kJ g^{-1}). However, this fuel is created at high temperatures during energy intensive processes such as non-catalytic fossil fuels partly oxidizing methane and hydrocarbon renovation (Kapdan and Kargi 2006). Thus, in recent years, significant attention has been paid to biological hydrogen production, photo and dark fermentation, direct and indirect Photolysis. Bio-H_2 development through Dark Fermentation has become a promising technology. In this regard the use of feed stocks made higher rates of hydrogen production (100-400 ml-$H_2L^{-1}h^{-1}$) than other biological technologies and also the simplicity of the reactor technology is comparatively similar to mature anaerobic digestion technology (Lin *et al.*, 2018). Chemical waste has a high potential for improving the production of biohydrogen by the dark fermentation process. One of the key reasons for effective biohydrogen process is the good use of raw material, cheaper and widely available (Arimi *et al.*, 2015). Cassava starch waste water is carbohydrate-rich and it is a possible substratum for the fermenta-

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tion of hydrogen and maintaining method sustainability. The benefit of this application is that heavily polluted waste water is converted to a renewable energy supply (Cappelletti *et al.,* 2011). The waste water from the processing of starch includes inter alia, carbohydrates, nitrogen and phosphorus (Lucas *et al.*, 2015). According to Urbaniec and Bakker, (2015), at this stage of the advancement of hydrogen fermentation technologies, the discovery of substrates for potential economic applications in industrial scale had become a priority mission. Mixed- culture systems are an enticing alternative to pure/co-cultural systems for food waste and hydrogen production (Kleerebezem and Van Loosdrecht 2007, and Laxman pachapur *et al.*, 2016). In order to inoculate acidogenic reactors for hydrogen processing instead of pure cultures, anaerobic sludge (mixed cultures) was commonly used in order to suit more for environmental stresses, including restriction of Nutrients, pH and temperature increases. The microflora in anaerobic sludge consists typically of both H_2-consuming and H_2-producing bacteria (Shizas and Bagley, 2005). Pre-treatment is widely used to enrich the sludge with bacteria that produce hydrogen and to kill micro-organisms that consume hydrogen. The heat pretreatment of sludge is the most commonly used method (Temudo *et al.*, 2008; Shizas and Bagley, 2005; and Wang and Wan, 2005). The pH of the next stage of batch reactor for base treatment was approximately 5.5, which reduced methanogens activity and was optimally suitable for production of hydrogen (Zhu and Beland, 2006). 5–6 acidic pH is optimal for production of hydrogen and the optimum pH is reduced and increased, resulting in a metabolic change with volatiles fatty acids (VFA) (Laxman Pachapur *et al.*, 2015 and Laxman pachapur *et al.*, 2015).

The continuous experiments were started up by inoculating continuous stirring tank bioreactors (CSTR) with 4 L sludge. The inoculum was obtained from other lab-scale CSTR operated for biohydrogen production from cassava wastewater. The experiment was conducted at various concentrations, HRTs of 24, 16, 12 and 8 h, mesophilic (35 °C) and thermophilic (55 °C) temperature and pH 5 to 6.

Materials and Methods

Reactor

The schematic representation and overview of the Continuous Stirred Tank Reactor have been shown in the Figure 1. The system includes four automated units, feeding tank, main body of the reactor, gas measuring sensor unit and automated temperature control system. The temperature was automatically maintained at 35 °C and 55 °C with agitation speed of 120 rpm. 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. It has the feed supply volume of 10 L and the total volume of the reactor was 21.78 L. Out of which 5 L 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 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.

Substrate and inoculums

The Cassava starch wastewater collected from a cassava flour factory in Tamil Nadu, to avoid the wastewater from biodegradation due to microbial action; the wastewater was stored at a temperature less than 4 °C. The sludge used as inoculum was collected from a pilot scale anaerobic reactor, treating effluent from starch production. The sludge was pre-treated by boiling at 95 °C for 15 minute (Sreethawong *et al.*, 2010) to suppress the activities of hydrogen-consuming bacteria. The reactors were inoculated with 4L of anaerobic sludge with Volatile Suspended Solids (VSS) of 4.5 g/l and the working volume was completed with cassava starch wastewater.

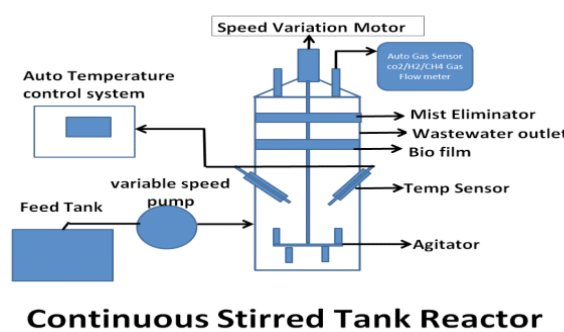

Fig. 1. Schematic diagram of Continuous Flow Stirred Tank Reactor

Results and Discussion

Mesophilic and thermophilic bio H_2 production comparison

Initial usage of OLR 2.08 and 2.29 kg COD / m^3d, bacteria should use the carbon source primarily for the growth of biomass and not for the production of hydrogen, and thus the production of minimum hydrogen Zhang *et al*., 2007a. The steady state production rates for hydrogen were 3039, 3102, 3131, 3201 and 2991 ml/d on 49, 50, 51, 52 and 53rd day for 35°C, 1939 ml/d on 50th day, 1988 ml/d on 53rd day, 1990 ml/d on 53rd day, 1963 ml/d on 54^{tht} and 1971 ml/d on 55th day ml/d obtained for 55 °C respectively as shown in Figure 2. Hydrogen was derived from anaerobic degradation of organic matter, so the production rate of hydrogen was in tandem with OLR. Therefore, the increase in OLR from 2.08 and 2.29 Kg COD/m^3d to 30.87 and 28.86 Kg COD / m^3d also increased the production of hydrogen. The maximum hydrogen production at 35 °C was 3131 ml/d; it is comparatively higher than 2007 ml/d obtained at 55 °C. The variation in the hydrogen production rate can be attributed to variation in the microbial population and OLR (Hussy *et al.*, 2005).

During dark fermentation, the medium endure pH changes that can significantly affect bio-H_2 production. Thus, in continuous process with pH control a distinction between initial and operational pH must be done. The final pH reached during this study was 5 to 6 for both temperature regimes. This value was in agreement with the optimal ones reported for other industrial wastewaters such as rice winery wastewater (Yu *et al.*, 2002), food industry (Chu *et al.*, 2013), and dairy wastewater (Gadhe *et al.*, 2013). (Table.1). During the bio-H_2 production from cheese whey wastewater at 36 °C and 55 °C. They observed a higher bio-H_2 yield at 36 °C (206 ml-H_2 $gCOD^{-1}$) than 55 °C (178 ml-H2 $gCOD^{-1}$). (Lee *et al.*, 2008) and (Borges *et al.*, 2018) also reported that the temperature of 37 °C is preferable compared to 55 °C for the bio-H_2 production from cassava starch. Finally, it was reported that the mesophilic (35 °C) H_2 production from starch wastewater was more efficient than thermophilic (55 °C) in anaerobic Continuous Stirred Tank Reactor.

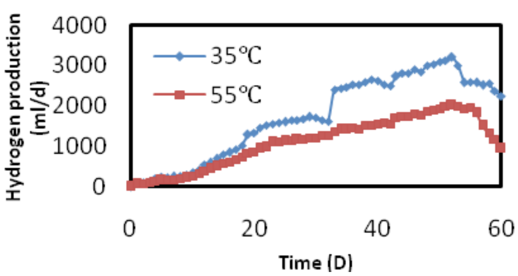

Fig. 2. Biological hydrogen production at 35°C and 55°C

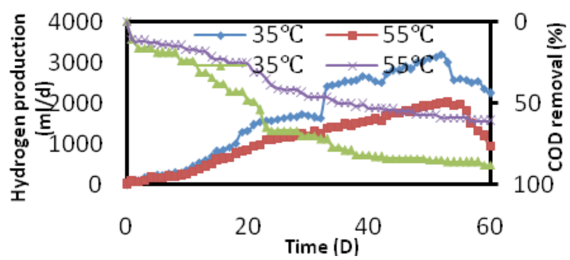

Fig. 4. Hydrogen production vs COD removal in a CSTR runs at 35°C and 55°C

Substrate degradation in mesophilic and thermophilic bio H_2 production

The steady state COD removal efficiencies obtained at 35°C were 85% on 51st to 52nd day, 88% was obtained on 59th to 60th day respectively. Whereas, the

Table 1. Comparison of biohydrogen production from different industrial wastewater

Substrates	COD concentration (g/l)	Temperature (°C)	pH	Hydrogen yield (ml-H_2/g COD)	Reference
Tequila vinasses	27	35/55	5.5	73.4/62.4	Alma Toledo-Cervantes *et al.*, 2020
Rice winery wastewater	34	55	5.5	234	Yu *et al.*, 2002
Rice slurry	5.5	37	4.5	326	Fang *et al.*, 2006
Dairy wastewater	15.3	37	5.5	303	Gadhe *et al.*, 2013
Brewery wastewater	6	35.9	5.95	149.6	Shi *et al.*, 2010
Food industry wastewater	40	35	5.5	165	Chu CY *et al.*, 2013
Cassava starch	24	37	6.0	179	Lee *et al.*, 2008
Cassava starch wastewater	2.08-30.87/2.29-28.86	35/55	5-6	107.3/73.5	This study

maximum COD removal of 59% obtained on 52nd to 56th day and 61% was achieved on 57th to 60th for 55 °C were presented in figure.4. The lowest efficiency in COD removal in both mesophilic and thermophilic temperature studies may be attributed to the minimal acclimatization period available (Mullai *et al,* 2013a). As the concentration of the biomass increased by the use of the substratum with the maximum COD removal of 88% achieved on 60th day for 35 °C.

The COD removal efficiency was found to be higher at 35 °C, as compared to 55 °C. Gradual growth in the OLR created a new environment for micro-organism adaptation, resulting in a fluctuation before achieving stable efficiency in COD removal (Chang and Lin, 2014).

Metabolic pathways involved in bio H_2 production

The VFA concentrations of acidogenic reactor were presented in the Figure 5, acetic and propionic acids make up the bulk of volatile acids accumulated inside the reactor (Show *et al*., 2004). The maximum VFA concentration was found at HRT of 16 hours with the hydrogen production of 1726 ml/d and minimum VFA concentration was found at HRT of 24 hours with hydrogen production of 84 ml/d at 35 °C. However, the VFA production of 1526 to 2805 mg/l with hydrogen production of 1203 ml/d occurs at VFA concentrations of 2805 mg/l at 55 °C. VFA concentration defines the hydrogen production rate with respect to the reactor pH as illustrated in Figure 5. VFA plays a major role in the production of hydrogen and also to maintain the reactor in the acidic condition. Hydrogen production by acidogenic microorganisms is always accompanied by the production of VFA. Therefore, changes in the VFA production reflect changes in the metabolism of the microorganisms.

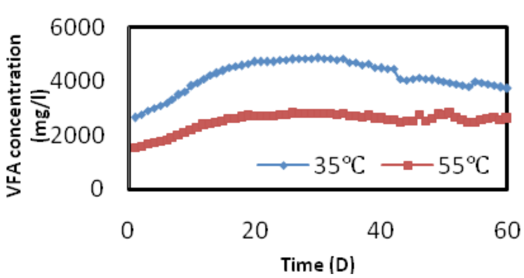

Fig. 5. VFA concentration in a CSTR runs at 35°C and 55°C

Moreover, pH has been confirmed as an important factor influencing the activities of hydrogen producing bacteria because it may affect the hydrogenase activity as well as metabolic pathways (Wang and Wan 2009).

The physiochemical stability of the reactor was continuously monitored by measuring alkalinity in the reactor. Figure.6 shows the alkalinity of acidogenic reactor; it was clear that the alkalinity in the acidogenic reactor was between 1322 to 9861 mg/l at 35 °C and 918 to 6848 mg/l at 55 °C was attained. The maximum hydrogen production of 2007 ml/d gained at alkalinity concentration of 6022 mg/l at 35°C with CSTR.

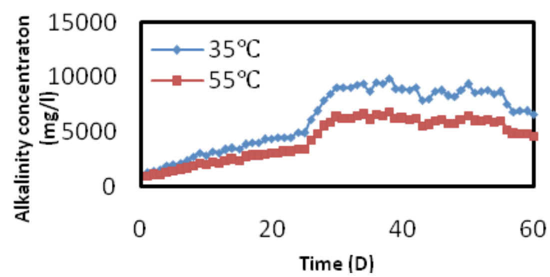

Fig. 6. Alkalinity concentration in a CSTR runs at 35 °C and 55 °C

Figure.7 shows the VFA/Alkalinity ratio for acidogenic reactors run at 35 and 55 °C; it ranges between 2-0.4 and 1.7-0.4. Hence, the effluent VFA/Alkalinity ratio of the acidogenic reactor was more or less equal to 2, which demonstrate the proper functioning of the anaerobic process. Simpson (1960) reported that the Volatile fatty Acid/Alkalinity ratio must be very low in range for stable anaerobic digester. It reveals that throughout the experimental period, volatile fatty acid accumulation was under the control and the reactor was in a stable condition.

In a stable reactor, Volatile Fatty Acid content will be low in proportion to the available alkalinity.

Biomass concentration and microbial identification

The biomass concentration at different substrate

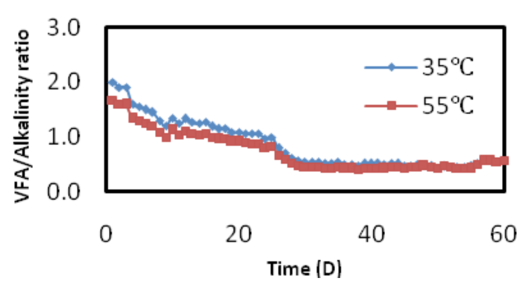

Fig. 7. VFA/Alkalinity ratio in a CSTR runs at 35 °C and 55 °C

concentrations after the cessation of hydrogen production, are presented in Figure.8, It was apparent from the figures that the final biomass level concentration increased with an increasing COD concentration. The initial VSS concentration of 5.45 g/l was continuously increasing and decreasing in the biomass concentration and finally reduced in the VSS concentration. The maximum hydrogen production occurs at the biomass concentration of 5.84 g/l at 35 ºC and 5.24 g/l at 55 ºC with constant pH maintained between 5 and 6 experimented by (Anantharaj *et al*., 2020). However, a lower final pH was observed at a higher COD concentration. It reflects that the removed mixed wastewater was used by hydrogen-producing bacteria for their growth and organic acid production. This finding was in close agreement with that of (Heyndrickx *et al.*, 1987).

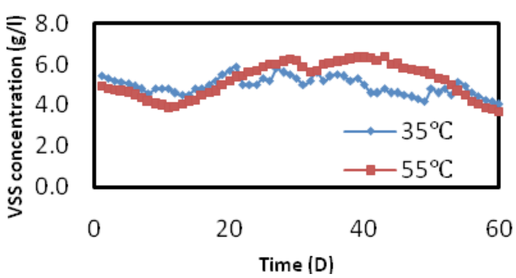

Fig. 8. VSS concentration in a CSTR runs at 35 ºC and 55 ºC

Conclusion

Hydrogen produced from cassava starch wastewater using a CSTR runs at mesophilic and thermophilic temperature with HRT of 24, 16, 12 and 8 h and pH maintained in the range 5 to 6. The steady state hydrogen production of 3201 ml/L·d was obtained at the OLR of 23.34 kg COD/(m^3·d) with a COD removal of 85% at 35 ºC and hydrogen production of 2007 ml/L.d with COD removal of 59% was obtained at the OLR of 28.86 kg COD/(m^3·d) at 55 ºC. Through the continuous hydrogen production, a maximum 61% of the starch wastewater was converted to hydrogen in the mesophilic temperature (35 ºC). This study provides suitable operational conditions for industrial application of the continuous process to maximize energy recovery from cassava starch wastewater under mesophilic condition.

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