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ENHANCEMENT OF pH AND SUBSTRATES FOR EFFICIENT PRODUCTION OF BIO-HYDROGEN AND KINETIC STUDIES

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Abstract – The experiment's aim was to optimize substrate concentration (4, 8, 16, 24, 32 and 40 g / L) and pH effect on bioconversion of distillery spent wash into hydrogen by anaerobic mixed sludge. The batch reactor used for this test was about 1 liter filled with 500 mL of diluted Distillery Spent Wash, 100 mL of preheated anaerobic sludge, and the reactor top serves as a biogas collector. The peak rate of hydrogen production was (1720.5 mL/L.d) at 24 g/L and the specific maximum bacterial growth rate of (0.6615 g VSS/ d) was achieved at 16 g / L with pH of 5.5 and 35 ^{o}C temperature. The experimental results have been found to be better suited in Haldane's equation with the correlation coefficient of (R^2=0.92). The experimental results showed that the batch reactor had higher microbial activity and operational stability, suggesting a high substrate utilization rate of 80 percent at 24g / L with initial pH 5.5 and attained a maximum specific growth rate at 16g / L at pH 5. The results showed that the substrate concentration optimized for the development of biological hydrogen and the Maximum specific growth rate of bacteria found between 16-24g / L and pH 5.0-6.0. For the microbial biomass culture, the Anaerobic Sludge Granules SEM analysis was examined in the batch reactor, showing spherical shape may be the presence of *Clostridium* Sp and biogas derived from sludge vents.

INTRODUCTION

Greater part of the world's wealth comes from unsustainable sources of energy, like coal and petroleum (Zhou *et al*., 2016). Nonetheless, such reductions in resources are never again reasonable and present enormous ecological concerns. The consumption of petroleum derivatives has expended the environmental dimensions of carbon dioxide and is the fundamental driver of the outflow of ozone depleting substances (Achinas *et al*., 2017). Barometric concentrations of CO_2 are expected to attain 560 ppm in 2035 through temperature increase of more than 5 ^{o}C (Perera, 2018). To mitigate the above-mentioned problems, Renewable and sustainable energy technologies need to be developed. Hydrogen is considered to be among the most rational energy reserves. Because of its, non-contaminating properties and having high energy yield (122 kJ g-1) which is 2.5 times superior to non-renewable energy sources, it can be developed very well using various techniques including modest procedures and being employed in various modern applications (Sekoai and Daramola, 2015; Sekoai and Yoro, 2016).The production of hydrogen through organic courses is inexhaustible and eco-friendly in nature. Various biomass types, such as crop residues, industrial sewage and urban solid waste, can be used for the production of hydrogen. The different techniques for the conversion of biomass into hydrogen are dark fermentation, light fermentation, Direct photolysis, indirect photolysis, and Microbial electro hydrogen cells (MECs) (Subudhi, 2018). Anaerobic method for digestion of natural substance is Dark fermentation. It takes place in the absence of light and occurs at normal temperatures. Dark fermentation is the most effective and less complex method of bio-hydrogen production. The gain and the rate of hydrogen production are higher if there is a need for dark fermentation as contrasted and other strategies for organic hydrogen supply, i.e. photosynthetic techniques (Das and Veziroglu, 2001). Dark fermentation is an earth-inviting

technique for hydrogen production and a useful alternative compared to petroleum products frameworks. Dark fermentation requires two groups of micro-organisms that are either optional or obligatory anaerobes. In view of a small amount of oxygen, optional anaerobes can produce hydrogen under anaerobic environment. Facultative anaerobes cannot become oxygen, which even in the following stage, is unnecessarily lethal to the development of facultative anaerobes. Metabolic process for mixed acid exists in dark fermentation, resulting in diverse kinds of volatile unsaturated fats along with alcohols being produced for acetic acid derivation, propionate, butyrate, lactate, acetone, ethanol and butanol. However the organisms is unlikely to follow the acetic acid derivation process, at that point absolute 4 mol hydrogen exist from 1 mol of hexose as well as 2 mol hydrogen will be given if butyrate occurs. No hydrogen will be generated at that point on the unlikely occurrence of ethanol or lactate pathway (Guo *et al*., 2001). Microscopic fermentation species are suitable for fermenting complex substrates contained organic waste converted to H_2 in addition CO_2. Photo fermentation and direct/indirect water photolysis have problems in major with light dependency and lower hydrogen production yields. The most effective technique among each of the methods is dark fermentation by higher-rate actual temperature and output yield of hydrogen. It also has simple and medium processes (Singh and Das, 2019). There is enormous alcohol production worldwide, moreover generate a lot of processing waste; India produces distillery effluent of 40 billion liters per year, for example (Chowdary *et al*., 2018). Distillery and alcoholic wastewaters contain high BODs like organic acids, sugars, dextrin, resins and hemicelluloses. 8-15 L of wastewater generated from Molasses-dependent distilleries for each liter ethanol production. It has around 80-160 g / L of high COD (Nataraj *et al*., 2006). This wastewater can also intend for production of biogas. The further innovation in energy is the distillery spillage burning. Since the effluent contains many nutrients, it has the ability to continue microorganism growth. Using distillery effluent for hydrogen production helps generate fuel and treat waste water. The study's objective is to examine the effects of substrate concentration and pH from Sugarcane Distillery Spent Wash on Sustainable Bio-hydrogen Production. The pH ranges from 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 with the concentration of the substrate from 4, 8, 16, 24, 32 and 40g/L at constant 35 ^{o}C.

MATERIALS AND METHODS

Substrate and Seed sludge

The Anaerobic sludge procured from the molasses treating anaerobic digester unit in sugar industry, Cuddalore. Before loading into the reactor, granular sludge was pre heated at 90-100 ^{o}C for 15-45 minutes (Lay *et al*., 2010; Gadhe *et al*., 2014) and to inhibit the (Seamsirimongkol *et al*., 2011) methanogenic microorganisms. The volatile suspended solids were estimated about 7711mg/L (APHA, 2005). Distillery Spent Wash was obtained in Cuddalore, Tamil Nadu, India from the sugar cane industry and was mixed with tap water. The COD value found to be varying between 4000 mg/L to 40000 mg/L. The dosage of high glucose concentration could inhibit the fermentation process (Singhania *et al*., 2009) and thus, a dosage of 10 g/L practiced as a co-substrate with the distillery spent wash based on co-optimization.

Experimental Set-up

Batch studies carried out in saline bottles of 1L capacity and anaerobic condition was maintained. The bottle necks were sealed air tightly with rubber cork provided with sampling slot for both liquid and gas. The diluted Distillery Spent Wash was loaded into the batch reactor in a ratio of 1:5 (anaerobic pre-treated sludge: Distillery Spent Wash). The batch reactor was one liter the capacity loaded with 100 mL of seed sludge with 500 mL of diluted distilled Distillery Spent Wash and the top space was a gas holder.

Analytical Methods

The studies were conducted by varying the pH and substrate concentration. The pH value of the treated effluent, Chemical oxygen Demand (COD), and Volatile Suspended Solids (VSS) were monitored on regular basis. The hydrogen gas generated during biodegradation was measured by water displacement method and the same was estimated using Gas Chromatography and Auto Gas measuring Sensor kit.

Kinetic Study

Bio kinetics for batch studies conducted with pre-heated anaerobic sludge with initial pH 5.5 and different substrate concentrations.

Research has focused on the biodegradation of Distillery Spent Wash and its derivatives through microbial cultures. The most important issues addressed in these studies are the analysis of the kinetics of organism growth and degradation. Growth kinetics is important for understanding the micro-organisms ' ability to degrade and operate the treatment units (Beydilli and Pavlostathis, SG 2005; Arutchelvan *et al*., 2006; Bhunia and Ghangrekar, 2008; Sponza and Uluko, 2008). The following equation can be used to Kinetics model of cell growth in a batch reactor:

$$K_dX = \mu_{net}X \quad .. (1)$$

For substrate,

$$\frac{ds}{dt} = -\frac{1}{Y}\left(\frac{dx}{dt}\right) \quad .. (2)$$

Where, S - concentration of substrate mg/L, X – concentration of biomass mg/L, µg – Specific rate of microorganism growth h^{-1}, µnet- Specific rate of microorganism growth h^{-1}, t – time h, Kd – Endogenous decay coefficient, Y – true yield coefficient g/g, μ_g is a S function. There are two perspectives on make use of the formulas related in the direction of the common growth rate µg to concentration of Distillery Spent Wash. One is the Distillery Spent Wash called non-inhibitory for a mixed microbial population and substituted by the non-inhibitory formula of Monod as shown below.

$$\mu_g = \mu_{max}\, S/ (K_s+S) \quad .. (3)$$

The other view is that the Distillery Spent Wash is an inhibitory compound for development. Several kinetic models were fitted to the experimental data to select the best models to describe the growth kinetics of inhibitory compounds. Due to its mathematical simplicity and wide acceptance, Haldane's model was used out of the models to describe the growth kinetics of inhibitory substrates. The inhibitory kinetics formula for growth of the Haldane is as follows:

$$\mu_g = \mu_{max} S / [K_s + S + (S^2/K_i)] \quad .. (4)$$

or

$$1/\mu_g = [1/\mu_{max}] + [S/(K_i\mu_{max})] \quad .. (5)$$

The equation 4 and 5 are linearized Haldane's equation. Where μ_{max} – specific growth rate of biomass Maximum h^{-1}, K_i – substrate inhibition coefficient. From the batch process the bio-mineralization kinetics behavior of anaerobic bacteria consortium at 35 °C was studied attributed to optimum hydrogen production and COD removal efficiencies. Maximum efficiency of the batch process was achieved at pH 5.5. The results of the maximum hydrogen production and COD removal efficiency and pH were used for calculating kinetic constants (refer Fig 38-43) and it has been tabulated as follows:

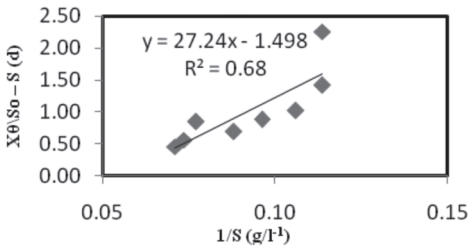

Fig. 1. Evolution of substrate utilization rate maximum k & half velocity constant k_s at pH 5.5 with substrate concentration of 16000 mg/l

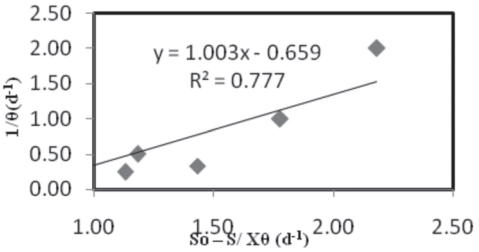

Fig. 2. Evolution of decay coefficient K_d & maximum yield coefficient Y at pH 5.5 with substrate concentration of 16000 mg/L

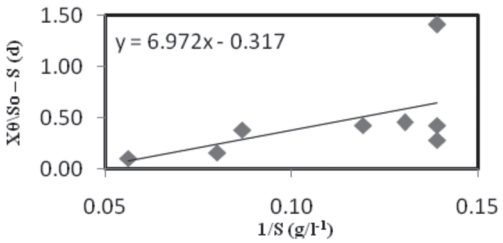

Fig. 3. Evolution of substrate utilization rate maximum k & half velocity constant k_s at pH 5.5 with substrate concentration of 24000 mg/L

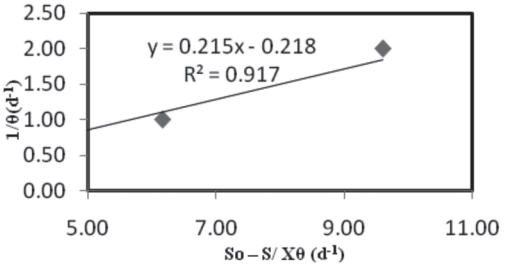

Fig. 4. Evolution of decay coefficient K_d & maximum yield coefficient Y at pH 5.5 with substrate concentration of 24000 mg/L

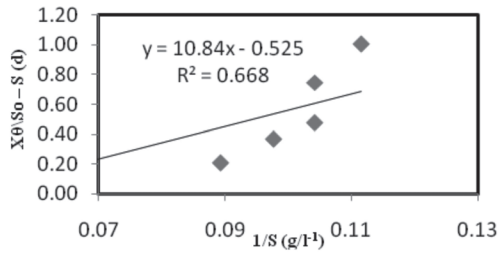

Fig. 5. Evolution of substrate utilization rate maximum k & half velocity constant k_s at pH 5.5 with substrate concentration of 32000 mg/L

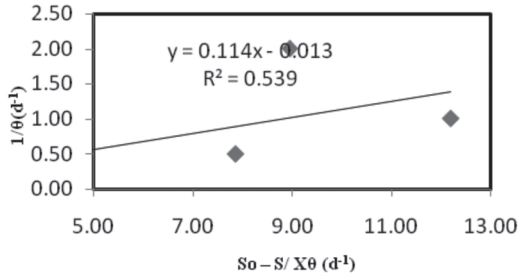

Fig. 6. Evolution of decay coefficient K_d & maximum yield coefficient Y at pH 5.5 with substrate concentration of 32000 mg/L

RESULTS AND DISCUSSION

Effects of Substrate Concentration on Bio hydrogen Production

Distillery Spent Wash of various substrate was tested at an initial pH of 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 at 35 °C. Hydrogen production improved from 4g/L to 40g/L and limited to a higher concentration of substrate 40 g/L. Degrading efficiencies are impaired through a strong efficiency of removal (80 per cent) at 24 g/L, Whereas the substrate concentration increased further, the substrate degradation efficiency decreased significantly and 75% of substrate degradation achieved by increasing substrate concentration of 40 g/L. The peak HPR was reached at 24 g / L at the initial pH of 5.5 as 1720.5mL/L-d. This can be understood because the enhanced metabolic flux with higher substrates keeps changing the rate of hydrogen production process (Argun and Das, 2017; Ghimire *et al.*, 2015). The optimal concentration of substrates for achieving maximum output of hydrogen from substrates differed from one another. For example, (Shi *et al.*, 2010), Noted that 6.05 g COD/L appropriate for brewery wastewater hydrogen production, however (Wu and Lin, 2004) stated soluble condensed molasses of 40g COD/L. Furthermore, 2.93 g COD/L of substrate contributed by Hay plus pulp and paper mill effluent led to the largest production of bio-hydrogen. These results showed that mixed culture favored lower concentrations of substrates (24 g / L) and further increased concentrations of substrates influenced hydrogen development by diverting the metabolic flux to solventogenesis rather than hydrogen synthesis (Lee *et al.*, 2008; Chu *et al.*, 2013). In Fig.7, the hydrogen production rate of different concentrations of Distillery Spent Wash was demonstrated. The expected Ks value is 21,963 mg / L and the R^2=0.9175 regression coefficient. The value of Ks obtained from Distillery Spent Wash (21,963 mg / L) was quite lower than the value reported by (Prakash *et al.*, 2014) but the value obtained was higher than the value reported by (Metcalf and Eddy 2003).

Effects of pH on bio hydrogen production

pH is a basic control parameter for the production of bio-hydrogen. The low pH inhibits the growth of the bacteria and causes changes in the production of metabolic products. The optimum range of pH for hydrogen production utilizing Waste Water ranged from 4.5 to 9.0 (Lin *et al.*, 2012, Stavropoulos *et al.*, 2016). The medium pH changes influenced the CHP and HY. The hydrogen production rate not exaggerated at pH 5.5 to 6.5 and gets deficiency at the pH of 5.0 and 7.0. The results indicate that

Table 1. Kinetics Constant for Different Concentrations of Substrate with pH 5.5

Description	Constants	Different Substrate Concentration with initial pH 5.5		
		16000 mg/L	24000 mg/L	32000 mg/L
Substrate utilization rate maximum (COD/mg VSS.d)	K	0.6673	3.15	1.904
Half velocity constant (mg COD/L)	K_s	18.1785	21.963	20.6488
Maximum Yield Coefficient	Y	1.0037	0.2151	0.1146
Endogenous Decay Coefficient (g VSS/g VSS.d)	K_d	0.6591	0.2183	0.0138
specific growth rate maximum (g VSS/g VSS.d)	μ_m	0.6615	0.04695	0.00158

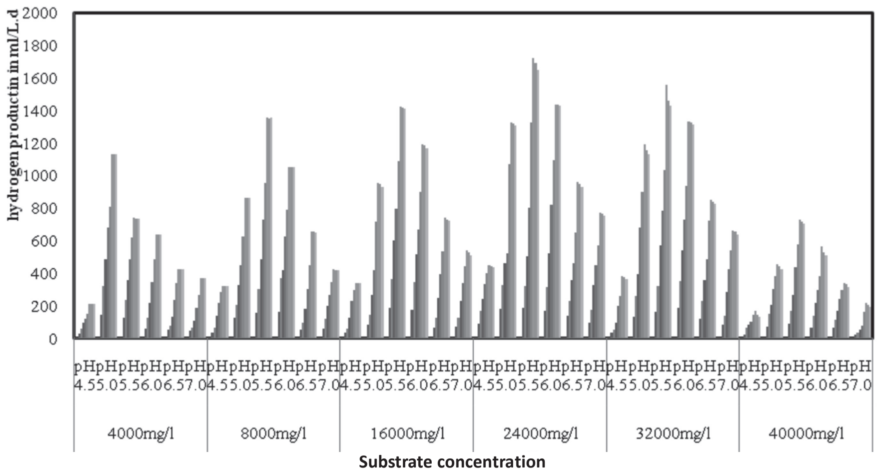

Fig. 7. Hydrogen production vs. different substrate concentrations

hydrogen production differs with changes in the initial pH. It was prominent to facilitate the enhancement of hydrogen is pH- dependent; the initial pH of 5.0 decreases the production of hydrogen owing to lower the biomass growth and a decline in the final pH 3.6 where accumulation of organic acids limits the substrate use and could serve as a barrier in mixed. The pH higher than 5.5 demonstrates improved substrate utilization efficiency as well as higher biomass production. The mixed anaerobic seed inoculums showed adaptation to growing conditions above pH 5.5, at which the initial pH value lasted longer to reach a final pH below 4.5, leading to increased biomass growth and hydrogen efficiency. The average production of hydrogen was lower than the peak production of HY and CHP 1.30 mol / mol hexose, 2983 mL/L respectively at pH 5.5 and pH 6.5, but the optimum pH was 5.0-6.0 respectively. This observation resembled a previous report (Sen and Suttar, 2012). The peak hydrogen production capacity of 1720.5 mL/L. d attained at pH 5.5 as shown in Fig.7, and the maximum normal growth rate of bacteria was obtained at pH 5.5, respectively. It was observed that the lag-phase period ranged from 8 to 10 at pH 5.5 to 7.0, while 4.6 was observed at pH 5.0. A similar report (Kumar and Lin, 2013) indicated that the lag phase duration was not directly associated with hydrogen production process. The long lag-phase from pH 5.5 to 7.0 could be attributed during acidic environments through the adaptation of enriched hydrogen producers.

SEM Assessment

Morphology of bacteria obtained from the reactor in the sludge granules. The sludge samples were first set by soaking 6 percent glutaraldehyde in the same volume for 2 hours. It was centrifuged and washed in a phosphate buffer at least three times after fixation and kept at 4 °C overnight. The samples are sequentially dehydrated using concentration-increasing ethanol solutions: 10%, 15%, 30%, 50%,

Table 2 Comparative study on Kinetics Coefficients

	Substrate utilization rate maximum (COD/mg VSS.dK	Half velocity constant (mg COD/l) Ks	Endogenous Decay Coefficient (g VSS/g VSS.d) Kd	Maximum Yield Coefficient Y	Specific growth rate maximum (g VSS/g VSS.d)μm
Prakash *et al*., 2014	3.125	24.0	0.05	0.5	1.563
Metcalf and Eddy 2003	2-10	10-60	0.06-0.15	0.3-0.6	-
Talaie *et al*., 2010	9.39	169.3	0.107	0.882	-
This study					
16000 mg/L	0.6673	18.1785	0.6591	1.0037	0.6615
24000 mg/L	3.15	21.963	0.2183	0.2151	0.04695
32000 mg/L	1.904	20.6488	0.0138	0.1146	0.00158

75%, and 90%, and subsequently twice exposed to 100% ethanol wash. This specimen has been dried for two days at around 37 °C. The specimens were placed on the SEM sample holder and in a sputter coating unit was coated with gold. These samples were then examined at various magnifications and using Scanning Electron Microscope the related SEM micrographs were taken. Scanning Electron Microscopic (SEM) of batch sludge granules figure 8 showed rough and uneven surface. The granules typically have a complex layered structure. Most granules appeared to be release of gases. *Clostridium* Sp. (densely packed spherical shaped) were found to be the dominant species in the granules.

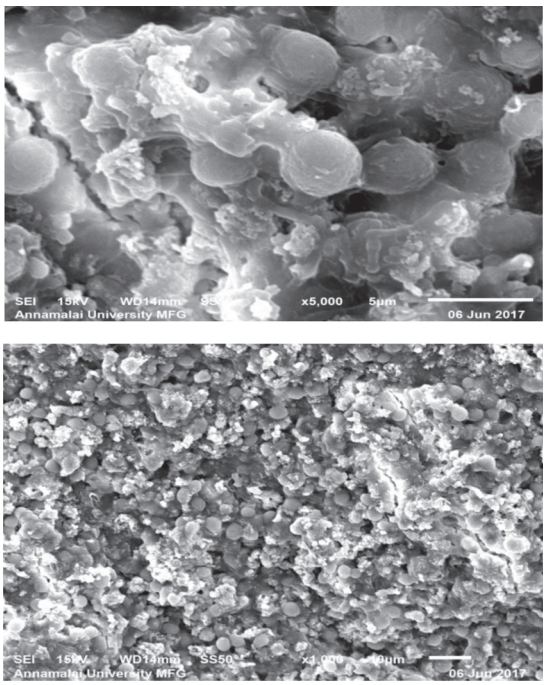

Fig. 8. SEM Image of Typical Sludge Granule

CONCLUSION

The results revealed from the batch study the maximum biological hydrogen production of 1720.5ml/L.d obtained at substrate concentration of 24 g/L with 80% substrate utilization rate at initial pH of 5.5, while maximum specific growth rate occurred at substrate concentration of 16g/L with COD removal of 75%. Hence the pH ranges between 5.0-6.0 suitable for biological hydrogen production and substrate between 16-24g/L suitable for maximum hydrogen production. In future the optimized pH and substrate concentration will be considered to run both the bench and pilot scale studies.

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