

**Bio-hydrogen production and optimization of fermentation pH, Initial substrate concentration using Distillery Spent wash as substrate with anaerobic mixed consortium**\*Rajahariharasudhan R<sup>1</sup>, K R Venkatesh<sup>2</sup><sup>1</sup>M.E. Student, Dept. of Civil Engg, Annamalai University, Annamalai Nagar, India- 608002\*<sup>2</sup>Asst. professor, Dept. of Civil Engg, Annamalai University, Annamalai Nagar, India- 608002

**ABSTRACT:-**The various factors have an effect on the Hydrogen production through Dark fermentation process. The interface of various factors and their combined effects must be studied for enhance higher hydrogen yields. Fermentation of organic substrate distillery spent wash as a carbon source on biological Hydrogen production with influence of parameters was studied in batch experiments using heat treated (100°C for 1h) anaerobic sludge used as inoculums. Effective H<sub>2</sub> production maximum at pH 5.5 compared to 6.0 and 5.0. The overall H<sub>2</sub> generation was enhanced by Pre-treatment of anaerobic sludge. Consequently fermentative hydrogen yield were recorded from batch reactors and combined effects of initial pH, initial substrate concentration, and chemical oxygen demand (COD) and HRT. The feed consisting only Distillery Spent Wash as primary substrate to enhance H<sub>2</sub> yield. The work revealed that the Chemical Oxygen Demand (COD) removal efficiency, Initial pH and hydrogen production were determined to be 58%, 5.5 and 128.2 ml. The maximum bio-hydrogen production of 114.8-128.2 ml/l was obtained at initial substrate concentration of 10,000 mg/l with initial pH 5.5 at HRT 8-12hours. As a result the initial pH 5.5 as optimal to enhances the maximum bio-hydrogen production.

**KEYWORDS:** Distillery Spent wash, Dark Fermentation, Batch reactor, Substrate Concentration, Bio-Hydrogen.

**1. INTRODUCTION**

One of the great challenges in the upcoming decade is to get new renewable energy sources, which are eco-friendly and reduce the high dependency on fossil fuels. Fossil fuels (i.e., petroleum, natural gas, and coal), which meet most of the world's energy demand today, are being depleted fast (Jinling Cai et al., 2013). Also, their combustion products are causing the global problems, such as the greenhouse effect, ozone layer depletion, acid rain, and pollution, which are posing great danger to our environment and eventually to all lives in our planet. Most of the scientists think that the solution to these global problems would be to replace the existing fossil fuel system by the hydrogen energy system. Hydrogen is an ideal and clean energy source for the future because of its high conversion, recyclability and non-polluting nature. The hydrogen gas can be generated through thermo chemical, electrochemical or biological processes. Biological hydrogen production processes are environmentally friendly and less energy intensive compared to other chemical processes. Especially wastewater or other biomass could be used as the raw material for substrate. Some biodegradable non-toxic industrial effluents containing carbohydrate rich industrial wastewater such as sugar industry, dairy industry, olive mill, baker's yeast and brewery wastewaters can be used as raw material for bio-hydrogen production (Chiu-YueLin et al., 2012, Periyasamy Sivagurunathan et al., 2015, Jishi Zhang and Lihua Zang 2016) Bio-hydrogen production by dark fermentation of organic wastes can both reduce waste disposal problems and decrease substrate cost. The activities of sugar mills require huge quantum of fresh water for milling processes and subsequently releasing of bulk effluent into the environment and the effluent contains high level of contaminants such as, suspended solids, organic and inorganic matter and chemicals. So their free disposal presents a serious challenge to the natural ecosystem and can cause considerable environmental problems. The main component of sugar industry wastewater is molasses (BingWang et al., 2013) which has a high commercial value due to its use as a carbon source in various fermentations. So the production of hydrogen from sugar industries waste water plays the dual role of waste reduction and energy production (S.G. Won et al., 2013, Gefu Zhu et al., 2013). As compared to the all industrial waste water sugar industry wastewater especially Distillery Spent Wash rich in high carbohydrate content which is more preferable and suitable for fermentative biological hydrogen production hence sugar industry wastewater (molasses) will be adopted for the present study (JinlingCai et al., 2012, Chen-yeon Chu et al., 2015, Gefu zhu et al., 2015). Dark fermentation and Photo-fermentation are two most important types of biological hydrogen production (Antonopoulou G et al., 2010). Continuous dark fermentative hydrogen production can be enhanced by decrease the retention time prior to wash out of hydrogen producing biomass (sivagurunathan p et al., 2016). On the other hand, engineering the wastewater treatment in the path of dark fermentation yields value added by-products with instantaneous waste remediation. Hydrogen (H<sub>2</sub>) production through wastewater treatment the dark fermentation is regarded as a sustainable technology which bring together much interest in recent years (Ren N.Q et al., 2010 Moreno R and Gomex X 2012; Wang A et al., 2008; Venkatamohan S et

al., 2009). Dark fermentation involves mostly development of volatile fatty acids (VFA) during acidogenesis process and secondarily formation of acetate, CO<sub>2</sub> and H<sub>2</sub> by acetogenesis (Ren N.Q et al., 2008; Lay CH et al., 2012; Barreta m et al., 2015). For sustainable fermentative process pre-treatment of anaerobic microbial consortia earlier to using it as inoculums was felt prerequisite for enhanced bio hydrogen production (Ren N.Q et al., 2008). The objectives of the present study to find out optimum pH, initial substrate concentration, HRT, inoculums and enrichment procedure for hydrogen-producing batch reactor.

## 2. Materials and Methods

Seed sludge and Distillery Spent Wash used in all experimental sets was anaerobic digester Sludge, obtained from the anaerobic digesters of the sugar industry wastewater treatment plant Cuddalore, Tamilnadu. It is common practice to apply pretreatment to anaerobic sludge to eliminate methanogens and select acidogenic hydrogen producers Guo XM et al., 2010. Heat treatment has been mentioned to be successful in inactivation of hydrogen consumers and supporting Clostridium-like hydrogen producers Kim SH et al., 2006. Therefore, the seed sludge used in this study was heat-treated at 105 °C for an hour, Distillery Spent Wash was used as substrate for bio-hydrogen production. It is one of the agro-industrial wastewater Ozkan L et al. 2010. The characterization of the sugar wastewater and experimental laboratory scale anaerobic reactor model was conducted using different pollutant parameters namely pH, solids, mixed liquor suspended solids, mixed liquor volatile suspended solids, total organic carbon, biochemical oxygen demand, chemical oxygen demand were measured during the experiment for the purpose of monitoring the treatment process (APHA, 2005).

**Table 1 Physico chemical characteristics of anaerobic sludge**

S.No	Parameters	Values
1	pH	4.9
2	TS	23463.3
3	TSS	12320
4	TDS	11143.3
5	VS	6224.67
6	MLVSS	10306.6
7	MLSS	14133.3

All values are in mg/l except pH

**Table 2 Physico-chemical characterization of Distillery Spent Wash**

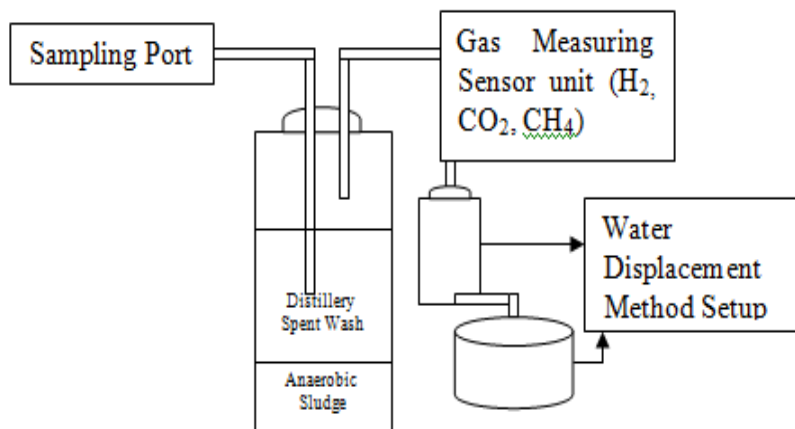
S.No	Parameters	Values
1	pH	4.5
2	COD	47233.3
3	BOD	27533.33
4	TS	10753.3
5	TSS	8456.6
6	TDS	2296.6
7	VS	10573.3
8	MLVSS	5640
9	MLSS	8266.6
10	TOC	2611.6 (ppm)

All values are in mg/l except pH and TOC

### 2.1 Batch reactor

The reactor about 1000 ml capacity with the effective volume of 700ml and top 300 ml space for gas collecting chamber, two consecutive glass rod tube were placed in the reactor one is for sampling port to analyze the parameters pH, COD, Temperature and the another glass rod tube which is connected to the Gas Measuring Sensor Unit. Gas Measuring Sensor Unit which is having three Sensors (H<sub>2</sub>, CO<sub>2</sub>, and CH<sub>4</sub>) which help to sense the biogas from the reactor and the

reading will show in ppm. After the biogas passed through the sensor it reach the water displacement method unit (Vincenzo Piemonte et al., 2014, Nabilah AminahLutpi et al., 2015).



**Figure 1 Schematic diagram of Batch Reactor**



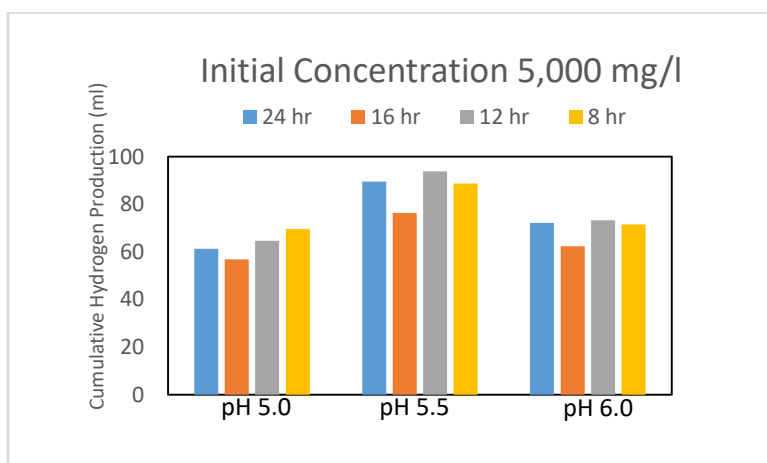
**Figure 2 Experimental set up of Batch Reactor**

The batch reactor is made up of Borosil glass bottle in which the rubber stopper is used to make the reactor as air tight. The silica tube is used for transferring the biogas from reactor to the gas collection chamber. Water collection chamber is placed next to the gas collection chamber in which the water displacement occurs depending upon the amount of biogas collected (Figure 1 and Figure 2). The physical dimension of the batch reactor was assessed in empirical approach for effective volume of the reactor is 0.7 liters. A laboratory scale Batch reactor made from a Borosil Glass bottle of 10cm diameter. The overall height of the reactor is 22.5cm and effective height about 12cm, two opening at the top of the reactor was provided for collection of gas and sampling port.

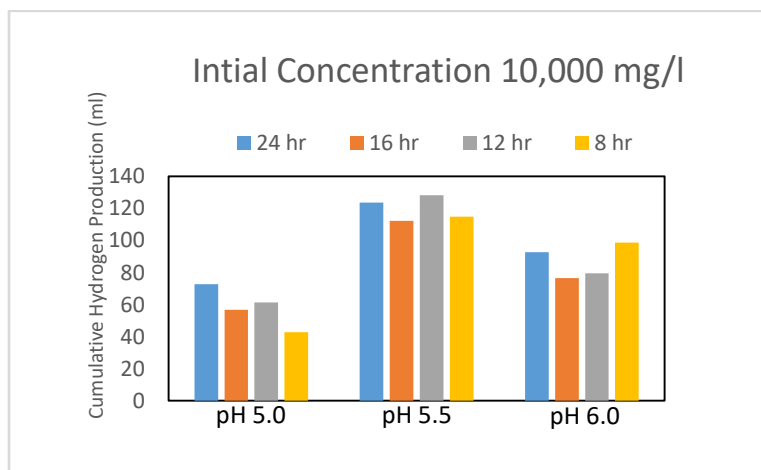
### 3. Results and Discussions

The yield of bio-hydrogen and metabolic pathway gets affected by the important environmental factor pH. In the conventional studies via pure or mixed cultures of bacteria the optimum pH range to attain the maximum hydrogen yield or specific hydrogen production rate was found between 5.2 and 6.0 (Oh et al., 2004a; Zhang et al., 2008a). Hydrogen production from liquid swine manure addition with glucose by mixed cultures in an anaerobic sequencing batch reactor 107.5 mL-H<sub>2</sub>/g COD with average hydrogen content of 41-44% at 35°C. The yield of bio hydrogen and removal efficiency of

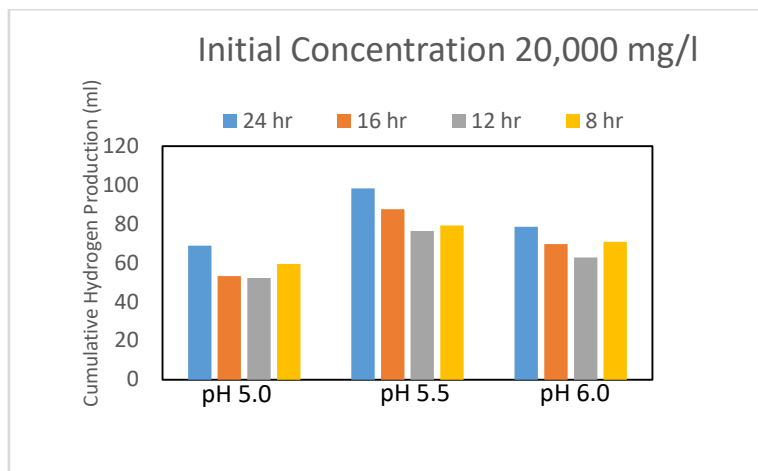
COD from the waste stream depends upon various factors moreover both the phenomenon was maximized in this study by finalizing the most favorable operational conditions based on trial and error. The influence of different parameters and their impact has been signified in this section. pH 5 as optimal at 37°C with a yield of 1.48 L-H<sub>2</sub>/L and the effect of pH values ranging from 4.7 to 5.9 on H<sub>2</sub> production (Lin CY et al., 2012). In mixed culture on sugar and starch substrates the highest specific hydrogen production at pH between 5.5 and 5.7 (Khanal SK et al., 2004). The above results are stated that for hydrogen production in an anaerobic environment, the optimum pH 5-6 found to be favorable for hydrogen production. This study illustrated that the hydrogen production at pH 5.5 with initial concentration of 10,000mg/l found to be 128.2ml at 12 hours HRT of batch reactor operation which is almost similar to (Chyi-How Lay et al., 2013) showed that pH 5.5-6.0 as the optimum for peak H<sub>2</sub> production of 129 ml and hydrogen yield of Previous studies from batch experimentation reported that the optimum initial pH for hydrogen production assorted based on different substrates, e.g., pH from 5.5 to 5.7 for sucrose (Van Ginkel S et al., 2001; Khanal SK et al., 2001; Wang G et al., 2005), pH of 6.5 for xylose (Lin CY and Cheng CH 2006), for starch (Zhang T et al., 2003) and for cheese whey (Ferchichi M et al., 2005) pH of 6.0. In continuous process, in the early investigations stated that a pH of 5.5 for glucose (Fang HHP and Liu H 2002), for starch pH of 5.2 (Lay JJ 2000), and for beer processing wastes pH of 5.8 (Lay JJ et al., 2005) were optimum for hydrogen production. These studies imply that vaguely acidic pH from 5.1 to 5.5 assist to enhance the fermentative hydrogen production. Fermentative hydrogen production was feasible at even lesser controlled pH without decline based on our investigation. (Fang HHP et al., 2006) reported that the initial pH of 4.5 was the optimal condition for hydrogen fermentation from rice slurry. Operating the batch reactor at pH of 5.5 is probably more advantageous in terms of the inhibition of hydrogen consumers.



**Figure 3 Hydrogen productions at various HRT with COD 5000mg/l**



**Figure 4 Hydrogen productions at various HRT with COD 10000mg/l**



**Figure 5 Hydrogen productions at various HRT with 20000mg/l**

### Conclusion:

The effects of initial substrate and initial pH were studied and examined that the effect of initial substrate concentration and initial pH for biological hydrogen production using three sets of batch reactors. Hydrogen production at pH 5.5 found to be 128.2 ml with initial concentration of 10,000mg/l at HRT 8-12 hours, which is higher as compared to 93.8 ml and 76.4 ml with initial substrate concentration of 5,000mg/l and 20,000mg/l. pH 5.5 was found to be optimal for maximum hydrogen production in all batch reactors with HRT of 8-12 hours and 58% of COD removal rate from the overall batch reactor run. The results revealed that the effect of initial pH 5.5 on bio-hydrogen production and maximum COD removal efficiency and maximum cumulative bio-hydrogen production was attained.

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