

# Producing Sustained Renewable Energy and Removing Organic Pollutants from Distillery Wastewater using Consortium of Sludge Microbes

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**Abstract**—Distillery wastewater in the form of spent wash is a complex and strong industrial effluent, with high load of organic pollutants that may deplete dissolved oxygen on being discharged into aquatic systems and contaminate groundwater by leaching of pollutants, while untreated spent wash disposed on land acidifies the soil. Stringent legislative measures have therefore been framed in different countries for discharge standards of distillery effluent. Utilising the organic pollutants present in various types of wastes as food by mixed microbial populations is emerging as an eco-friendly approach in the recent years, in which complex organic matter is converted into simpler forms, and simultaneously useful gases are produced as renewable and clean energy sources. In the present study, wastewater from a rice bran based distillery has been used as the substrate in a dark fermenter, and native microbial consortium from the digester sludge has been used as the inoculum to treat the wastewater and produce hydrogen. After optimising the operational conditions in batch reactors, sequential batch mode and continuous flow stirred tank reactors were used to study the best operational conditions for enhanced and sustained hydrogen production and removal of pollutants. Since the rate of hydrogen production by the microbial consortium during dark fermentation is influenced by concentration of organic matter, pH and temperature, these operational conditions were optimised in batch mode studies. Maximum hydrogen production rate (347.87ml/L/d) was attained in 32h dark fermentation while a good proportion of COD also got removed from the wastewater. Slightly acidic initial pH seemed to favor biohydrogen production. In continuous stirred tank reactor, high H<sub>2</sub> production from distillery wastewater was obtained from a relatively shorter substrate retention time (SRT) of 48h and a moderate organic loading rate (OLR) of 172 g/l/d COD.

**Keywords**—Distillery wastewater, hydrogen, microbial consortium, organic pollution, sludge.

## I. INTRODUCTION

**D**ISTILLERIES are one of the major polluting industries as the distillery spent wash has a very high organic load and dark colour, which have to be treated properly before being discharged in order to meet the stringent legislative regulations of its disposal. It has therefore become absolutely essential to look for technologies to process this effluent efficiently and economically. With increasing emphasis on waste minimization, resource recovery and energy generation,

efforts are underway to utilize various types of wastes as a resource and produce energy. Integrated biohydrogen production coupled wastewater treatment has been reported earlier using cyanobacteria for textile waters [1]. Since distillery spent wash is rich in organic matter, it is important to reduce its organic matter and also explore its potential for production of some renewable energy. From pollution control and resource recovery point of view, it would be ideal if we could convert the organic pollutants in distillery wastewater into hydrogen. Distillery wastewater in the form of spent wash is a complex industrial effluent, which possesses high concentration of biodegradable organic material. Spent wash is toxic to aquatic organisms and when disposed into water bodies, it may result in the complete depletion of dissolved oxygen. If disposed untreated on land, it reduces alkalinity of the soil, and crops may be affected. Ground water contamination problem may also be caused by leaching down of the pollutants.

The present study aims at assessing the potential of a rice bran based distillery spent wash for utilization as a substrate by using microbial consortium present in the ETP sludge and examining the degradation of organic pollutants along with hydrogen production to derive dual benefits of energy generation as well as wastewater treatment. In order to have sustained and enhanced energy production, it is important to optimise various operational parameters influencing substrate degradation and hydrogen formation. Concentration of organic matter in the distillery spent wash would be an important parameter affecting the hydrogen yield in dark fermentation since low substrate concentrations cause rate limitations, while high concentrations may result in substrate and product inhibitions [2]. Hydrogen production would also depend on factors such as inoculum type, pH, temperature, and type of reactor used. In anaerobic digestion process, regulation of pH is very important as it influences hydrogenase, the activities of hydrogen producing bacteria and the metabolic pathway for fermentative hydrogen production [3]. Observations of some earlier studies indicate that pH optimum for fermentative hydrogen production is dependent upon type of substrate, type of seed sludge and operating conditions adopted [4]. Besides the other factors, temperature is also considered as an important parameter influencing biohydrogen production from wastewater, and a general acceptability is for operating the reactor in mesophilic temperature range as that would be cost effective and easy to control at industrial scale. Inoculum dose is important to ensure that the organic substrate is involved

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more in hydrogen production than in microbial biomass formation.

In contrast to pure culture, the mixed cultures contain various types of bacteria, which may have synergistic, antagonistic or non-interactive nature, such as hydrogen-producing bacteria encapsulated with spores, methane-producing bacteria and acid-producing bacteria. In a mixed culture system under anaerobic condition, the hydrogen produced by bacteria, such as *Clostridium* or *Enterobacter*, is often readily consumed by other hydrogen-consuming bacteria. The elimination of hydrogen-consuming bacteria would, therefore, be important in biohydrogen production to favor the growth of hydrogen producing bacteria, which is generally achieved by some pre-treatment of the seed sludge [5]. The results of pretreatment vary with sludge type.

In the present study, therefore, hydrogen production potential of the sludge based microbial consortium using distillery wastewater was examined under optimised conditions of various operating parameters in batch studies followed by continuous mode operation of the bioreactor exploiting the dark fermentation metabolic pathway of the consortium.

## II. MATERIALS AND METHODS

### A. Feedstock and Inoculum

Wastewater was obtained from a local distillery, located in Haryana, India that produces alcohol using rice bran as raw material. Characteristics of the distillery wastewater used for the fermentative hydrogen production in the present study showed pH 3.3, electrical conductivity (EC) 3.4 dS/m, COD 230 g/L, carbohydrates 75g/L, proteins 31g/L, and volatile fatty acids 385 mg/L. The anaerobic sludge was obtained from wastewater treatment plant of the distillery unit and the native microbial consortium was used as inoculum for the study. The seed sludge was stored at 4 °C till its use. The pH of the seed sludge was 7.4, EC was 9.8 dS/m, and dry weight was 60 g/L. The sludge seed was used directly as inoculum that had the native microbial consortium. Our earlier detailed experiments on pre-treatment of the sludge suggested that we could use it effectively even without any treatment.

### B. Batch Experiments

Batch experiments were conducted in serum bottles (110 ml) placed in an incubator shaker at 37.5 °C at 110 rpm, and anaerobic environment was created by purging argon in the head space of the bottles. Using wastewater (COD 180 g/L) and substrate: inoculum ratio 9:1, a broad range of pH varying from 5.5 to 9.0 was tested. The wastewater was diluted to varying strengths (COD 46 to 230 g/L), and hydrogen production capacity was evaluated. Biohydrogen production was studied both in mesophilic (35, 37.5 °C) and thermophilic range (40 °C). COD was estimated by refluxometric titration.

### C. Bioreactor Studies

A continuous stirred-tank reactor (CSTR) made of thick glass (4L volume) covered with dark paper was used with continuous stirring at 250 rpm, 37.5 °C, controlled pH and

placed in a dark room batch studies were carried out in the bioreactor at optimised conditions to evaluate the potential of hydrogen production by the microbial consortium from the spent wash used as a substrate. Sequential batch studies conducted at three substrate retention times (SRTs) of 48h, 72h, and 96h were also carried out in the bioreactor using 50 percent replenishment of substrate after each cycle. Continuous mode studies were carried out at three organic loading rates (OLRs) of 115, 172, and 230 g COD/l/d to examine continuous hydrogen production in the bioreactor.

### D. Hydrogen Estimation

The hydrogen gas evolved on fermentation of the substrate was analyzed using gas chromatograph (GC Agilent 6890N) fitted with thermal conductivity detector (TCD) and mole sieve column. Nitrogen was used as a carrier gas at a flow rate of 24.8 ml/min. Temperature of the injector, oven, and detector were maintained at 100, 50, and 200 °C, respectively.

## III. RESULTS AND DISCUSSION

Production of renewable energy in the form of hydrogen from distillery wastewater was optimized by varying parameter conditions.

### A. Effect of Initial pH

Microbial consortium is known to be influenced by the initial pH of the substrate that it has to degrade and this in turn influences generation of renewable energy. It is therefore important to determine the optimum conditions of pH along with the other operational factors while using a new substrate for hydrogen production. Also, when single species is used, the pH optima are generally known, but when mixed species are used, it becomes more important to know at which pH the microbial community would give maximum H<sub>2</sub> production. In the present study, hydrogen production by the microbial consortium was studied under a wide range of pH (5.0-9.0) using distillery wastewater as substrate and wide variations in hydrogen production at different pH was observed (Table I). The results showed that pH in slightly acidic to neutral range (pH 6.5-7.0) favoured more hydrogen production, while it tended to decline at alkaline pH, and very acidic pH completely inhibited hydrogen formation.

TABLE I  
MAXIMUM SPECIFIC HYDROGEN PRODUCTION RATE (SHPR) FROM  
DISTILLERY WASTEWATER AT VARYING pH

pH	Maximum SHPR (ml/g/d)
5.0	ND
5.5	ND
6.0	0.82±0.08
6.5	1.82±0.07
7.0	1.73±0.07
7.5	1.23±0.06
8.0	0.92±0.02
8.5	0.85±0.07
9.0	0.54±0.04

ND = Not detectable

Hydrogen production by microbes has been studied under a wide range of initial pH, varying from pH 4 to 9 by different researchers [6]-[8]. Using different substrates, different pH optima were observed in different cases. The optimal pH range for bio-hydrogen production from spent wash found in this study coincides with the pH optima of hydrogenase enzyme.

#### B. Effect of Organic Load in the Wastewater

Concentration of organic load (COD) in the wastewater is likely to influence hydrogen production as the organic matter gets fermented under dark anaerobic conditions. Between COD concentration of 46 g/L to 92 g/L, the rate of hydrogen production increased with rising concentration, but COD higher than this reduced the production rates. Table II shows the variations in specific hydrogen production rate (ml/g/d) while using various concentrations of distillery wastewater at pH 6.5.

TABLE II  
MAXIMUM SPECIFIC HYDROGEN PRODUCTION RATE (SHPR) AT VARYING STRENGTH OF DISTILLERY WASTEWATER

Wastewater Concentration	Maximum SHPR (ml/g/d)
20%	7.96 ± 0.69
40%	4.40 ± 0.25
60%	2.83 ± 0.29
80%	1.65 ± 0.30
100%	1.09 ± 0.15

At low concentrations substrate and nutrient limitation seem to have a limiting effect on the microbial metabolism and degradation. Higher wastewater concentration, however, resulted in less cumulative hydrogen formation due to substrate and product (VFA) induced inhibition. Cumulative hydrogen, however, decreased significantly, above 136 g/L (60% concentration) of COD caused apparently by substrate inhibition, yielding very little hydrogen. The reason seems to be that higher substrate concentration enhances the production of VFA's, which acts as substrate for CH<sub>4</sub> formation [9], [10]. Thus, COD concentration of 46-136 g/L corresponding to 20 to 60% of distillery wastewater concentration was found to be more suitable for efficient hydrogen production.

#### C. Effect of Temperature

Temperature influences microbial growth, metabolism and enzyme activities. Hence, it is likely to influence hydrogen generation from the system. As may be seen in Table III, hydrogen production enhances as mesophilic to thermophilic conditions are created. Working at higher temperatures results in better utilization of the substrate producing more hydrogen, but it involves energy input, and therefore, it also increases the operation cost.

The hydrogen production of the present system was appreciably higher than that (71 ml/l/h) reported by [11] who used synthetic wastewater as substrate. When we compare the hydrogen production at 37.5 °C and 40 °C, we find that H<sub>2</sub> production rates are quite good even at 37.5 °C and almost approach the rate of production obtained at 40 °C. This

provides a definite advantage, because the ambient temperature in the tropical climate is around this range, and thus, it would not require additional energy inputs.

TABLE III  
MAXIMUM SPECIFIC HYDROGEN PRODUCTION RATE (SHPR) FROM DISTILLERY WASTEWATER AT DIFFERENT TEMPERATURES

Temperature (°C)	Maximum SHPR (ml/g/d)
35 °C	3.03±0.01
37.5 °C	3.92±0.08
40 °C	4.11±0.06

The mixed microbial consortium (5% concentration) is found to be very efficient in degrading the wastewater having COD in the range of 92 to 136 g/L, by managing the initial pH in slightly acidic to neutral range (pH 6.5-7.0). Removal of COD from the wastewater corresponds well with the H<sub>2</sub> production. Thus, it is possible to produce biological hydrogen from distillery wastewater, and simultaneously, to remediate the wastewater by reducing its organic load.

#### D. Bioreactor Based Hydrogen Production from Distillery Wastewater

**Batch studies:** After the batch studies conducted in small serum bottles, up-scaling was done for batch mode hydrogen production using a continuously stirred tank reactor (CSTR). For this, 2 L of feed stock spiked with 5% inoculum of anaerobic microbial consortium from the sludge was introduced into the reactor from top. Distillery wastewater having 120 g/L COD was used as substrate. After inoculation, the bioreactor was operated for 90h with continuous stirring at 250 rpm. The results suggested that fermentation of distillery wastewater for 40h to 50h is suitable for maximum hydrogen production and degradation of substrate using distillery wastewater.

##### 1. Sequential Batch Studies

A laboratory scale anaerobic sequencing batch reactor with a total volume of 4 L and working volume of 2 L was used in the sequential batch study using different SRTs. The reactor was operated for a total period of 31 days. In the first cycle, the reactor was operated at 48 h SRT for a period of 10 days until the hydrogen production stabilized. At the end of 10<sup>th</sup> day, the SRT in the reactor was increased to 72h for the second cycle for a period of nine days. After 19 days of operation, in the third sequential cycle, the SRT in the reactor was further increased to 96 h and the third cycle continued for 12 days. At the beginning of the 2<sup>nd</sup> and 3<sup>rd</sup> cycle, half of the spent substrate was replaced by fresh substrate.

Specific hydrogen production rate from the wastewater in the three sequential batch cycles is depicted in Fig. 1. At low SRT of 48h, the rate of hydrogen production was higher as compared to that at higher SRTs of 72h and 96h, which suggests that long retention of substrates can cause accumulation of liquid products like volatile fatty acids (VFAs) that are likely to cause over-acidification of bacterial cultures leading to inhibition of hydrogen formation. Thus, specific hydrogen production was found to decrease with the

increase in SRT as substrate retention time in a reactor is in inverse relation to food-to-microbe ratio [12], which implies that more and more substrate keeps on degrading at longer SRT and thus availability for microbial degradation and subsequent hydrogen production declines. Thus, we find a decline in specific hydrogen production from 2 ml/g COD/d in 1<sup>st</sup> cycle at 48h SRT to a maximum of 1ml/g COD/d in 2<sup>nd</sup> cycle at 72h SRT, which further declines to half, i.e. just 0.5 ml/g COD/d in the 3<sup>rd</sup> cycle at 96h SRT.

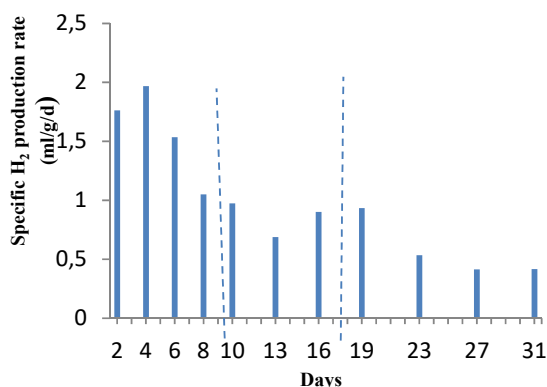


Fig. 1 Specific hydrogen production rates obtained from dark fermentation of distillery wastewater in three sequential cycles of 48, 72, and 96h SRTs

## 2. Continuous Mode Studies

In order to examine the possibility of producing hydrogen from the wastewater in a sustained manner, continuous mode studies were conducted using different OLRs of 115, 172, and 230g/l/d in the bioreactor. At operating OLR of 172 mg/l d, the reactor showed a maximum cumulative hydrogen production of 21.43 L by the end of 66 hours of operation. At an OLR of 172g/L, hydrogen production was the highest, while that at 115 and 230 g/L/d OLRs were almost comparable as may be seen in Table IV.

An OLR of 172 g/l/d was found to be most suitable for generating renewable H<sub>2</sub> energy and for simultaneous removal of COD from distillery wastewater using the sludge microbial consortium at ambient 37.5 °C temperature.

TABLE IV  
CUMULATIVE HYDROGEN PRODUCTION AND COD REMOVAL DURING 66H DARK FERMENTATION AT DIFFERENT OLRs

OLR (g/L/d)	Cumulative H <sub>2</sub> Production (ml)	COD removal (%)
115	14.2±0.08	45±3.2
172	21.4±0.03	48±4.5
230	13.2±1.0	35±3.1

In continuous mode, low OLR in the CSTR leads to reduction in hydrogen formation due to limitation of substrate, while high OLR also inhibits hydrogen formation due to toxicity caused by excessive production of VFAs during the fermentation process.

## IV. CONCLUSION

The present study shows that the wastewater of distilleries, which are considered to be highly polluting can be successfully used for producing the clean and renewable hydrogen energy. Under optimal pH of slightly acidic to neutral range, the native microbial consortium of the sludge gives almost two folds higher H<sub>2</sub> production rates at short SRT of 48h. When loaded with medium strength wastewater, the microbes produced about 1.5 times more cumulative hydrogen in continuous mode in bioreactors. The high ambient temperature of tropics (35-38 °C) are found favorable for the microbial consortium of sludge, which ferment the organic pollutants present in the distillery wastewater, reduce the COD and produce clean renewable hydrogen. There are small to medium distillery units in most developing countries, which produce large quantities of wastewater to be treated. The hydrogen energy produced from the wastewater by dark fermentation can be used appropriately, such as to feed fuel cells and be used in the industrial complex itself to supplement energy requirements in an economical and environmentally sound manner.

## REFERENCES

- [1] Kaushik, A. Mona, S., Kaushik, C. P., " Integrating photobiological hydrogen production with dye-metal bioremoval from simulated textile wastewater" *Bioresource Technology* vol. 102 no. 21, pp 9957-9964, Aug. 2011.
- [2] Fan, K. S., Chen, Y. Y., "H<sub>2</sub> production through anaerobic mixed culture: effect of batch S<sub>0</sub>/X<sub>0</sub> and shock loading in CSTR" *Chemosphere* vol. 57, pp 1059-68, Jan. 2004.
- [3] Chu, C. F., Li, Y. Y., Xu, K. Q., Ebie, Y., Inamori, Y., Kong, H. N., "A pH-and temperature-phased two stage process for hydrogen and methane production from food waste. *Int J Hydrogen Energy*," vol. 33, pp. 4739-46, July, 2008.
- [4] Luo, G., Xie, L., Zou, Z., Zhou, Q., Wang, J. Y., "Fermentative hydrogen production from cassava tillage by mixed anaerobic microflora: effects of temperature and pH", *Appl. Energy*, vol. 87, no. 12, pp. 3710-17, Sept. 2010.
- [5] Valdez-Vazquez, I., Poggi-Varaldo, H. M., "Hydrogen production by fermentative consortia", *Renew. Sust Energy Review*. Vol. 13, no. 5, pp. 1000-1013, June, 2009.
- [6] Noike, T., "Biological hydrogen production of organic wastes—Development of the two-phase hydrogen production process, *Proceedings International Symposium on Hydrogen and Methane*", *Fermentation of Organic Waste*, Tokyo, March, 2002, pp. 31–39.
- [7] Li, D., Chen, H., "Biological hydrogen production from steam-exploded straw by simultaneous saccharification and fermentation", *Int J Hydrogen Energy*, vol. 32, no. 12, pp. 1742-48, Aug., 2007.
- [8] Doi, T., Matsumoto, H., Abe, J., Morita, S., "Feasibility study on the application of rhizosphere microflora of rice for the biohydrogen production from wasted bread", *Int J Hydrogen Energy*, vol. 34, no.4, pp. 1735-43, May, 2009.
- [9] Argun, H., Kargi, F., Kapdan, I. K., Oztekin, R., "Batch dark fermentation of powdered wheat starch in to hydrogen gas: effects of initial substrate and biomass concentration", *Int J Hydrogen Energy*, vol. 33, pp. 6109-15, Nov., 2008.
- [10] Kumar, G., Lin, C. Y., "Bioconversion of de-oiled Jatropha waste (DJW) to hydrogen and methane gas by anaerobic fermentation: influence of substrate concentration, temperature and pH", *Int J Hydrogen Energy* vol. 38, pp. 63-72, Jan., 2013.
- [11] Skonieczny, M. T., Yargeau, V., "Biohydrogen production from wastewater by *Clostridium beijerinckii*: effect of pH and substrate concentration", *Int J Hydrogen Energy*, vol. 34, no. 8, 3288-94, May, 2009.
- [12] Mohammadi, P., Ibrahim, S., Annur, M. S. M., Ghafari, S., Vikineswary, S., Zinatizadeh, A. A. "Influence of environmental and operational factors on dark fermentative hydrogen production: A

Review”, *Clean- Soil, Air, Water*, vol. 40 no. 11, pp. 1297-1305, Nov., 2012.