

Effect of sulphate on hydrogen fermentation process by using distillery wastewater

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Abstract

The effect of sulphate on hydrogen production by fermentation of distillery wastewater was studied in small scale batch experiments at 37.5 °C with an initial pH of 6.5 and adding sulphate varying in concentrations from 0-3000 mg/L. Hydrogen yield and substrate degradation efficiency was estimated at various concentrations of sulphate. The results showed that the hydrogen production and substrate degradation efficiency decreased with increase in sulphate concentrations from 0 to 3000 mg/L. Maximum hydrogen yield of 3.8 ml/g was found when distillery wastewater (having 1892 mg/L sulphate) was used without any further addition of sulphate. Since in the presence of high sulphate additions of 1000- 3000mg/L, substrate degradation and hydrogen production sharply declined, the fermentative hydrogen production technology using distillery effluents, which have high sulphate concentrations may not be practicable and wastewaters with low sulphates should be utilized for fermentative hydrogen production.

Keywords: Hydrogen production, substrate degradation, fermentation, wastewater, COD

1. Introduction

In view of environmental pollution problems caused due to burning of fossil fuels, it has become necessary to find alternative energy sources that are renewable and environment friendly (Kapdan, Kargi, 2006; Winter, 2005) ^[5, 12]. Hydrogen is expected as a major energy carrier in the future, essentially due to its non-polluting nature, cost effective production and versatility of application as fuel. Among various hydrogen production processes, biological method is known to be less energy intensive, for it can be carried out at ambient temperature and pressure. Biological method mainly includes photosynthetic hydrogen production and fermentative hydrogen production. Photosynthetic hydrogen production cannot be operated without light, while fermentative hydrogen production can produce hydrogen continuously without light. It is of great significance to produce hydrogen from organic substrates by fermentative hydrogen production, because it plays a dual role of waste reduction and energy production (Wang, Wan, 2008; Li and Fang, 2007) ^[11, 6]. Thus fermentative hydrogen production is more feasible and widely used. Fermentative hydrogen production using mixed culture of microbes more favourable as compared to pure ones. Organic wastewater and wastes that contain a variety of components require various microorganisms to perform the degradation process. Hydrogen production from organics has been studied using anaerobic hydrogen-producing sewage and compost sludge as the microbial sources (Badiei *et al.*, 2011; Tawfik, EL-Qelish, 2012) ^[2, 10]. Microbial hydrogen production from waste using fermentative bacteria is more favourable compared to conventional thermo-chemical and electro-chemical processes, because it does not require additional energy input and can simultaneously achieve energy recovery and environmental cleanup (Li, 1999) ^[7].

Sulphate is a common constituent of many industrial wastewaters. Sulphate is found in some wastes produced from pulp/paper, sea-food processing and the alcohol fermentation

industry. However, high sulphate content has been reported to adversely affect anaerobic digestion (Lin and Chen, 2006) ^[8]. Since fermentative hydrogen production involves biochemical anaerobic digestion, the hydrogenic microbes might be readily affected by the presence of sulphate, decreasing hydrogen production efficiency (Lin and Chen, 2006) ^[8]. High sulphate may lead to the production of H₂S by sulphate reducing bacteria that inhibit activity of hydrogen producing microorganisms (Chen *et al.*, 2008) ^[3]. The production of hydrogen sulphide gas in the biogas becomes a concern when bio hydrogen is used for fuel cells because this gas is toxic to certain fuel cell materials. Under anaerobic environments, sulphate is reduced to sulphite and then to sulphide, depending upon pH.

Distillery wastewater is rich in organic matter and it also contains a high amount of sulphate, which shows wide variations and may be as high as 9,000 mg/l (Mohana *et al.*, 2009) ^[9]. The sulphate content of distillery wastewater is dependent on the type of raw material used in the process. In the present study, distillery wastewater was used as a substrate for hydrogen production and effect of sulphate on fermentative hydrogen production was studied by addition of sulphate in different quantities, along with studies on changes in pH, EC, volatile fatty acid production and substrate degradation.

2. Materials and methods

2.1 Feedstock and Inoculum

Wastewater was collected from a distillery, located in Haryana, which produces alcohol using rice bran as a raw material. The chemical oxygen demand of distillery wastewater used as feedstock for the fermentative hydrogen production in the present study was 120g/l, while sulphate concentration was 1892 mg/l.

Anaerobic sludge that was used as inoculum was collected from wastewater treatment plant of the distillery unit that had

near neutral pH (7.4), high electrical conductivity (9.8 dS/m), total dissolved solids (TDS) 4.9 g/L and dry weight of the sludge was 60 g/L. The collected sludge was stored in refrigerator till use.

2.2 Batch Experiments

Batch tests using the feedstock for hydrogen fermentation were conducted in 110 ml serum bottles. The feed stock was spiked with different concentrations of sulphate (0 to 3000 mg/L). Final volume of culture tubes was maintained at 50 ml with distillery wastewater containing 5% of the seed sludge as inoculum. The anaerobic environment was established by purging argon gas in head space of the tubes. Then the serum tubes were placed under controlled temperature of 37.5° C in incubator shaker at 110 rpm. Initial pH of this system was adjusted to 6.5. Each batch test was performed in triplicate. Data shown are the average values of results from independent tests.

2.3 Analytical methods

2.3.1 Determination of hydrogen

Hydrogen production was analyzed quantitatively 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.

Estimation of various parameters of the substrate was done following standard methods (APHA, 1995). COD estimation was done using open reflux method, and VFA's were estimated using distillation method. Sulphate was estimated using spectrophotometric methods.

3. Results and discussion

3.1 Effect of sulphate on fermentative hydrogen formation

Hydrogen production from each experimental vial was estimated after 24h. Fig. 1 shows the influence of added sulphate on hydrogen production by fermentation of distillery wastewater. Production was found to decrease with the addition of increasing concentration of sulphate into the feedstock, and a sharp decrease occurred when sulphate concentration added was 1500 mg/L. This demonstrates that increase in sulphate concentration leads to reduction in hydrogen production. In an anaerobic environment, sulphate is converted into hydrogen sulphide by sulphate reducing bacteria, and production of sulphide in a reactor results in toxicity to hydrogen producing microbes, thus resulting in less hydrogen production.

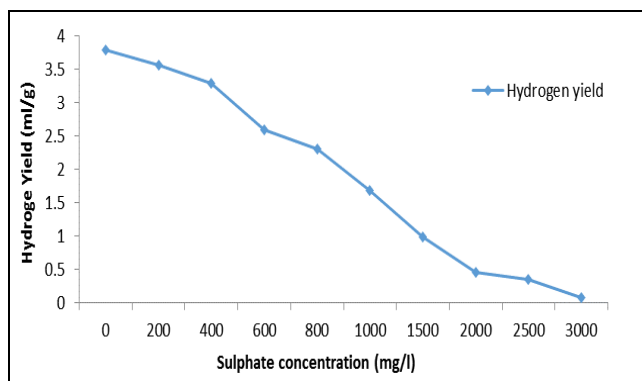


Fig 1: Hydrogen yield at various concentrations of sulphate

Maximum hydrogen yield of 3.8 ml/g was found using distillery wastewater without addition of sulphate. Hydrogen yield was minimum (0.08 ml/g) when there was addition of 3000 mg/L of sulphate to the distillery effluent. The results demonstrate that sulphate addition into distillery wastewater leads to decrease in the hydrogen production, which is attributed to an inhibitory effect on hydrogen producing bacteria. Zuhair *et al* (2008) [13] showed that sulphate has an inhibitory effect on bacterial growth. Lin and Cheng (2006) [8] found that during dark fermentation using enriched mixed micro flora, sulphate inhibited hydrogen production by changing the metabolic pathway from a butyrate to ethanol fermentation. This inhibition could however, be overcome by lowering the operating pH to a level that is unfavourable for the growth of sulphate-reducing bacteria but, favours active hydrogen producing bacteria. Hwang *et al* (2009) [4] reported that in an acidogenic hydrogen production reactor, sulphate concentration and pH variation had significant influence on hydrogen production. They reported decreased hydrogen production at pH 5.8 and 6.2 as compared with that at pH 5.5, which was presumably due to increase of sulphate reducing bacterial activity leading to reduced activity of hydrogen producing bacteria.

3.2 Effect of sulphate on pH, EC, VFAs and substrate degradation

Table 1 shows variations in pH, EC, VFAs and substrate degradation efficiency as determined after 24 h of the batch experiments in the presence of different concentrations of added sulphate. Though pH declined on addition of 200 mg/l of sulphate into the feedstock, but difference in pH was not significant with further addition of sulphate.

Table 1: Effect of Sulphate concentration on pH, EC, VFAs and substrate degradation efficiency

Concentration of added sulphate mg/l)	pH	EC	VFAs	Substrate degradation efficiency %
0	5.12	9.5	1200	40
200	4.24	9.5	1045	28
400	4.16	9.6	428.57	25
600	4.15	9.9	1011.43	23
800	4.14	9.9	754.28	23
1000	4.14	9.9	857.14	18
1500	4.14	10.4	1028.57	18
2000	4.11	10.7	1060	17
2500	4.09	11.2	1011.43	13
3000	4.06	14.3	1024.56	13

*Sulphate concentration of distillery wastewater (feedstock) = 1892 mg/l

The pH varied from 4.24 to 4.06 in the presence of 200 to 3000 mg/L sulphate (Table 1). The results show that with increase in the concentration of sulphate, pH change results in more acidic environment. Extremely low pH has negative effect on the growth of hydrogen producing microbes and the hydrogen production tends to stop. Hwang *et al* (2009) [4] stated that for maximum hydrogen production, it is important to keep the operational pH at 5.5 or to maintain low sulphate concentration at neutral pH ranges.

Variations in EC were not significant (9.9-9.7 units) upto 1000 mg/l concentrations of sulphate. However, increase in EC (10.4 to 14.3 dS/m) was found, as the sulphate concentration increased from 1500 to 3000 mg/l. Volatile fatty acids were produced by the degradation of substrates by anaerobic bacteria during the fermentation process. A significant trend was not found between concentrations of sulphate and VFA's produced during the fermentation. Maximum VFA concentration (1200 mg/L) was observed when distillery wastewater was used without sulphate. Addition of sulphate into the feedstock reduces degradation of substrate, due to which concentration of VFAs was also found low. Phowan and Danvirutai (2014) clearly evidenced that the high hydrogen productivity occurs concurrently with the formation of VFAs and alcohols.

The substrate degradation efficiency in batch tests decreased consistently with increase in added sulphate concentration (200 to 3000 mg/L). Maximum substrate degradation efficiency of 40% was obtained using distillery wastewater without the addition of sulphate. Addition of sulphate (200 mg/l) led to reduced substrate degradation efficiency (28%). A very sharp fall in substrate degradation efficiency was seen when sulphate concentration reached 1000 mg/L. Only 13% reduction in substrate degradation efficiency was found using sulphate in the concentration of 3000 mg/L. The distillery wastewater used in the present study had a low sulphate concentration of 1892 mg/L, while many of the reports indicate higher sulphate concentration in other distillery wastewater samples. Hydrogen production from such wastewaters with 5000 to 9000 mg/l of sulphates may not be suitable for high hydrogen production.

4. Conclusion

High sulphate concentrations in the distillery wastewater used as feedstock reduce substrate degradation and H₂ production. A decline in pH also results when sulphate concentration is high. Fermentative hydrogen production from distillery wastewater would be favoured only when its sulphate concentration is low as in the present study.

5. References

1. APHA, Standard methods for the examination of water and wastewater. 19th Ed. New York, USA, 1995.
2. Badieli M, Jahim JM, Anuar N, Abdullah SRS. Effect of hydraulic retention time on biohydrogen production from palm oil mill effluent in anaerobic sequencing batch reactor. *Int. J Hydrogen Energy*, 2011; 36:5912-5919.
3. Chen CC, Chen HP, Wu JH, Lin CY. Fermentative hydrogen production at high sulphate concentration. *Int J Hydrogen Energy*, 2008; 33:1573-8.
4. Hwang JH, Choi JA, Shanab RAlA, Bhatnagar A, Min B, Song H, Kumar E, Choi J, Lee ES, Kim YJ, Um S, Lee DS, Jeon BH. Effect of pH and Sulphate concentration on

hydrogen production using anaerobic mixed microflora. *Int J Hydrogen Energy*, 2009; 34:9702-9710.

5. Kapdan IK, Kargi F. Bio-hydrogen production from waste materials. *Enzyme Microbial Technol*, 2006; 38(5):569-82.
6. Li CL, Fang HHP. Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. *Crit Rev Env Sci Technol*, 2007; 37:1-39.
7. Li JZ. The bio-producing hydrogen technology of organic wastewater fermentation (in Chinese with English abstract). Ph.D. Thesis. Harbin University of Architecture and Engineering. Harbin, China 1999.
8. Lin CY, Chen HP. Sulfate effect on fermentative hydrogen production using anaerobic mixed microflora. *Int J Hydrogen Energy*, 2006; 31:953-960.
9. Mohana S, Acharya BK, Madamwar D. Distillery spent wash: Treatment technologies and potential applications. *Journal of Hazardous Materials*, 2009; 163:12-25.
10. Tawfik A, El-Qelish M. Continuous hydrogen production from co-digestion of municipal food waste and kitchen wastewater in mesophilic anaerobic baffled reactor. *Bioresource Technology*, 2012; 114:270-274.
11. Wang JL, wan W. Comparison of different pretreatment methods for enriching hydrogen producing cultures from digested sludge. *Int. J Hydrogen Energy*, 2008; 33:2934-41.
12. Winter CJ. Into the hydrogen energy economy-milestones. *Int J Hydrogen Energy*, 2005; 30:681-85.
13. Zuhair SA, Naas MHE, Hassani HA. Sulphate inhibition effect on sulphate reducing bacteria. *Journal of Biochem Tech*. 2008; 1(2):39-44.