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Dark Hydrogen Fermentation From Paper Mill Effluent (PME): The influence of Substrate Concentration and Hydrolysis

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Abstract

Paper mill effluent (PME) was used as an organic feedstock for production of biohydrogen via dark fermentation using heat-shock pretreated anaerobic sludge under mesophilic conditions. The influence of substrate concentration (5, 10 and 15 g-COD/L) and the initial pH (5 and 7) on the efficiency of dark hydrogen fermentation from PME were investigated. The highest hydrogen yield of 55.4 mL/g-COD was obtained at substrate concentration and pH of 5 g-COD/L and 5, respectively. By increasing the concentration of substrate from 5 to 10 and 15 g-COD/L, at fixed initial pH, the hydrogen production efficiency was reduced from 55.4 mL/g-COD to 38.5 and 32.7 mL/g-COD. Furthermore, by increasing pH from 5 to 7, biohydrogen efficiency was reduced up to 40.8%. Different hydrolysis of PME including acidic, acidic-thermal and alkaline hydrolysis prior to fermentation were studied which the alkaline hydrolysis led to the highest hydrogen yield of 62.2 mL/g-COD. Moreover, methane production efficiency of 569 mL/g-COD was obtained at substrate concentration and pH of 5 g-COD/L and 7, respectively.

Keywords: Biohydrogen, dark fermentation, paper mill effluent, hydrolysis.

Introduction

Scientists believe that the dependence of the world's energy production on the fossil fuel sources has caused climate changes, global warming and adverse environmental effects and therefore, numerous studies have been done to find alternative energies which cause no environmental pollutions and can mitigate the global energy demand in a sufficient manner (Cheng et al., 2011; Pawar et al., 2013). Biomass is known as the large and the most sustainable resource of energy which could be sufficiently converted to ethanol, diesel, methane and hydrogen via biological methods under ambient temperature and pressure (Taherdanak et al., 2015). Among these fuels, hydrogen is known as a fully green energy which its combustion only produces steam and energy. Moreover, it has the highest energy content (142 KJ/g) among the known fuels (Penteado et al., 2013; Zilouei & Taherdanak, 2015). The biological hydrogen production methods could be classified in two main categories: light dependent and light independent methods (Bakonyi et al., 2014; L. Wang et al., 2014).

Dark hydrogen fermentation, a light independent method, is known as the most practical method for hydrogen production as it needs no external energy and also has simple operations

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(De Gioannis et al., 2014; Zilouei & Taherdanak, 2015). Moreover, it could deal with a wide variety of organic wastes such as forest residues, agricultural wastes and the organic fraction of municipal and industrial wastes. Since the PME has a high BOD, it could be sufficiently managed and converted to hydrogen via dark fermentation process. Dark fermentation could be performed by using pure or mixed bacterial cultures under anaerobic conditions. Usually it is easier to use mixed microbial culture as inoculum for dark hydrogen fermentation. Since there are hydrogen consumers available in mixed microbial cultures, therefore it could be the bottleneck for their application as inoculum for dark fermentation and makes it necessary to treat such microbial cultures prior to use in the dark fermentation process (Levin & Azbar, 2012b; Zilouei & Taherdanak, 2015).

Commonly dark hydrogen fermentation is considered as the first stage of anaerobic digestion. Anaerobic digestion is mainly composed of four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Taherdanak & Zilouei, 2014). Stage one includes the first three steps and the second stage includes the forth step. During the first step, the substrate is broken into its constituents such as simple sugars. Afterward, the volatile fatty acids and amino acids are produced followed by conversion into acetone, hydrogen and carbon dioxide. In the methanogenesis step, the produced hydrogen and carbon dioxide are consumed by methanogens and finally converted into methane (Levin & Azbar, 2012a; Taherdanak & Zilouei, 2014). Therefore, when the fourth step is eliminated, the main gaseous product of the process would be hydrogen and the process could be considered as dark hydrogen fermentation. Several different methods are proposed to remove the methanogens from the system and to stop the forth step of the anaerobic digestion process (Taherdanak et al., 2016). However, while elimination of the methanogens from the system, some of the hydrolytic bacteria which take part in the hydrolysis step are also eliminated and the hydrolysis step is disturbed too. Therefore, a sufficient hydrolysis of substrate such as enzymatic hydrolysis, acidic hydrolysis and alkaline hydrolysis would be necessary to help the hydrolysis step and to make the substrate ready for dark hydrogen fermentation (Hendriks & Zeeman, 2009; Zhang et al., 2007).

The aim of this study was to produce hydrogen and methane from PME via dark fermentation and anaerobic digestion, respectively. The impact of operational conditions including substrate concentration and the initial pH of the system on the efficiency of dark hydrogen fermentation was investigated. Moreover, the impact of substrate hydrolysis on the efficiency of dark hydrogen fermentation was investigated.

Material and Methods

Substrate and inoculum preparation

The PME was obtained from the Sepehr Paper Industry, Yazd, Iran, and its characteristics were analyzed and are presented in Table 1.

The anaerobic sludge was obtained from a wastewater treatment plant which was located at Isfahan. It was filtered through a 1 mm screen to remove its sands. Then the filtrate was collected and used as inoculum for methane production via anaerobic digestion. In order to prepare the required inoculum of the dark hydrogen fermentation, the filtered mixed culture was heat-shock pre-treated at 95 °C for 45 min. Afterward it was immersed in cold water to decrease its temperature to 35 °C. The pre-treated inoculum was placed in incubator overnight. After the pre-treatment, the totals solids (TS) and the volatile solids (VS) of sludge were changed from 54 g/L and 28 g/L to 38 g/L and 24 g/L, respectively.

Table 1. The characteristics of the PME

Parameter	Value	Unit
pH	7	-
COD	30,000	mg/L
BOD	20,500	mg/L
TDS	15,808	mg/L
TSS	4,540	mg/L
Total nitrogen	1,032	mg/L

Substrate hydrolysis

Sulfuric acid (1 N) and sodium hydroxide (1 N) were used to adjust the required pH of substrate to the specified amounts. Through acidic hydrolysis, the pH of the PME was adjusted to 3 and the mixture was kept at 4 °C overnight, followed by neutralization. The alkaline hydrolysis was done by increasing the pH of PME up to 12, and the mixture was kept at 4 °C overnight followed by neutralization. In order to perform the acidic-thermal hydrolysis, the pH of the PME was adjusted to 3, and the mixture was boiled at 120 °C for 60 min. Then, the mixture was neutralized and kept at 4 °C overnight.

Dark fermentation and anaerobic digestion procedure

The gas production processes were performed in 118 mL glass bottles with the working volume of 80 mL. The bottles were charged by the certain concentrations of PME (5, 10 and 15 g-COD/L), 20 mL inoculum, 40 mL nutrient solution and a certain amount of distilled water. The pH of each bottle was adjusted to its specified amount (5 or 7) using sulfuric acid/sodium hydroxide solutions (1 N). Blank samples containing only 20 mL of inoculum, 40 mL of nutrient solution and 20 mL of distilled water were prepared to define the bio-methane/bio-hydrogen potential of inoculum alone. In order to prepare the required anaerobic conditions, each bottle was capped tightly and its head space was purged using nitrogen gas for 3min. The bottles were prepared in triplicates and were placed in incubator at 37 °C without shaking.

The used nutrient solution had the following composition: $CuSO_4.5H_2O$, 10 mg/L; $NaHCO_3$, 4000 mg/L; $MnSO_4.4H_2O$, 30 mg/L; $MgCl_2.6H_2O$, 200 mg/L;$CoCl_2.6H_2O$, 0.25 mg/L; $MnSO_4.4H_2O$, 30 mg/L; $FeSO_4$, 10 mg/L and $NiSO_4$, 10 mg/L (Zhao et al., 2013).

Analytical methods

The characteristics of PME including COD, BOD, TDS, TSS as well as those of inoculum (TS and VS) were measured according to APHA standard method (APHA, 1995). The pH measurement was done using a pH meter (Model 526, Germany). The produced bio-methane/bio-hydrogen was analyzed using a gas chromatograph (Sp-3420A, Beijing Beifen Ruili Analytical Instrument CO) which was equipped with a thermal conductivity detector (TCD) and a packed column (Propack Q). Nitrogen was used as the gas carrier with the flow rate of 30 ml/min. The operating temperatures of the column, injection port, and the detector were adjusted to 40, 100 and 150 °C, respectively. A pressure lock syringe was used to inject the certain amount of the produced gas into the gas chromatograph. The volumes of the gasses were calculated based on the ideal gas law.

Results and discussion

The effect of the operational conditions on the batch dark hydrogen fermentation from PME was investigated in batch tests. After the definition of the optimum operational conditions, the PME was hydrolyzed and used as substrate for hydrogen fermentation under the defined optimum conditions.

The effect of the substrate concentration on the efficiency of dark hydrogen fermentation is shown in Fig. 1. It could be seen that almost for all samples no hydrogen is produced after 5 days of experiments. It is observed that the maximum hydrogen yield was obtained for the initial substrate concentration of 5 g COD/L at initial pH of 5, which is equivalent to 55.4 mL/g-COD.

At initial pH of 5, as the concentration of the substrate was increased from 5 to 10 and 15 g-COD/L, the hydrogen yield was reduced from 55.4 mL/g-COD to 38.5 and 32.7 mL/g-COD, respectively, and that might be due to the inhibitory effect of higher concentrations of substrate or other compounds present in the PME on the efficiency of dark hydrogen fermentation. This result was in agreement with other studies (Sivaramakrishna et al., 2014). Moreover, extreme substrate concentration would result in the system acidification and accumulation of VFA which might inhibit the activity of hydrogen-producing bacteria. Furthermore, partial pressure of hydrogen in the bottles was raised with the increasing of substrate concentration (Xing et al., 2010).

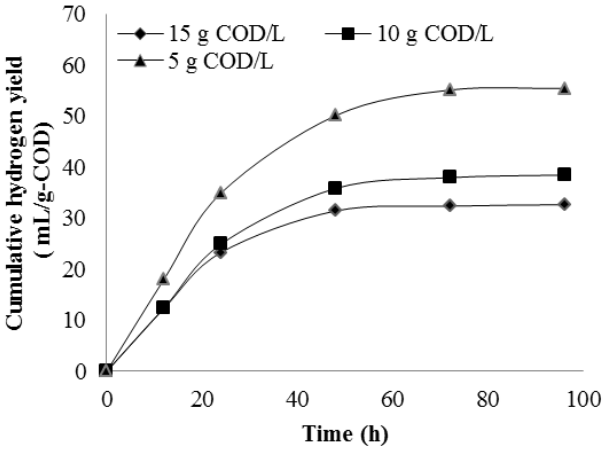

Fig. 1. The effect of substrate concentration on the hydrogen yield at constant initial pH of 5.

By comparison of Fig. 1 and Fig. 2, it is demonstrated that at constant substrate concentrations of 10 and 15 g-COD/L, increasing pH caused reduction in the hydrogen production at all levels of COD concentration. At substrate concentrations of 10 and 15 g-COD/L, increasing pH to 7 caused reduction in the hydrogen fermentation efficiency to 40.06% and 40.81%, respectively. These results are in agreement with Lin et al. and Khanal et al., who demonstrated that increasing of initial pH, caused decrease in hydrogen production yield (Lin et al., 2008; Khanal et al., 2004) . It was occurred due to effect of pH variation on hydrogen-producing microbial and hydrogen fermentation process metabolic, so that ability of these bacteria was reduced by pH rising (Ghimire et al., 2015; J. Wang & Wan, 2009).

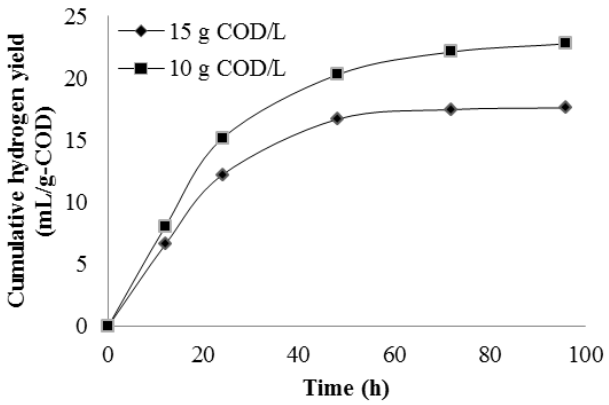

Fig. 2. Cumulative hydrogen yield at initial pH of 7 at two different substrate concentrations.

The impact of the substrate hydrolysis on the efficiency of dark hydrogen fermentation from PME is shown in Fig. 3. Acidic and acidic-thermal hydrolysis had no positive impact on the efficiency of dark fermentation process while the alkaline hydrolysis led to the hydrogen yield of 62.2 mL/g-COD which was nearly 14% higher than that of the raw substrate. These results were in low consistent with other studies. Lakshmidevi et al. (Lakshmidevi & Muthukumar, 2010) demonstrated the enzymatic hydrolysis of PME using *Trichoderma reesei* followed by biohydrogen production using *Enterobacter aerogenes* from the hydrolysate. The highest hydrogen yield of 2.03 mol /mol-sugar was obtained. Ramprakash et al. (Ramprakash & Muthukumar, 2014) investigated acid hydrolysis and enzymatic hydrolysis on the rice mill wastewater to optimize hydrogen production. The hydrogen yields of 1.74 and 1.40 mol /mol-reducing sugar were obtained from the hydrolysate obtained from enzymatic and acid hydrolysis, respectively. So the less favorable impact of hydrolysis method in this study might be due to the inhibitory effect of salt production during pH adjustment. Moreover, as it shown in Table 1, characterization of used PME showed that the major organic content of the substrate was VFA which is not fermented in dark fermentation, so that hydrolysis wasn't an efficiency enhancement method for this wastewater type.

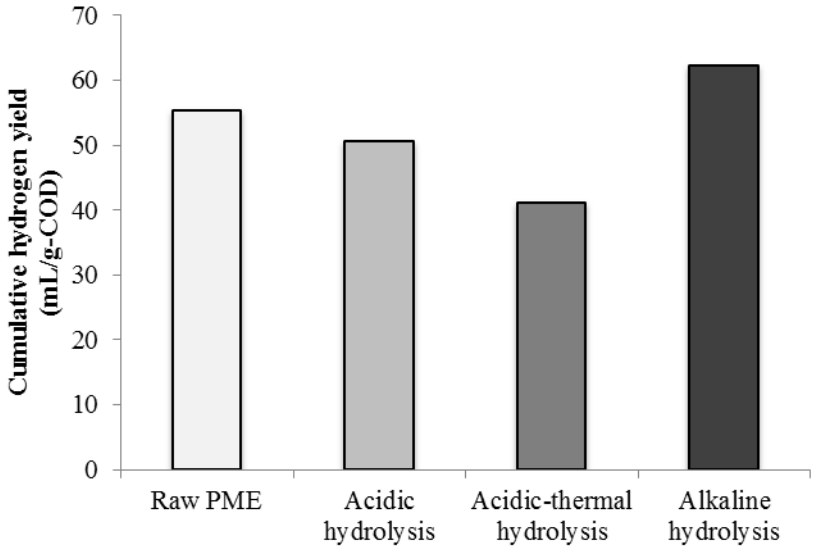

Fig. 3 Hydrogen potential of PME in respect to the type of hydrolysis.

The cumulative methane production from PME is shown in Fig. 4. The initial pH and the substrate concentrations were 7 and 5 g-COD/L, which led to the methane yield of 569 mL/g-

COD. Zwain et al. (Zwain et al., 2013) proved that modified anaerobic baffled reactor (MABR) in treating recycled PME at batch phases could indicate methane production efficiency of 27 mL/g-COD. Moreover, Lin et al. (Lin et al., 2011) demonstrated methane yield in similar batch system of 200 mL/g-VS). Furthermore, Jantsch et al. (Jantsch et al., 2002) showed that maximum methane yield of 130 mL/g-COD was obtained from spent sulphite liquor (SSL), which is a type of chemical pulping effluent. In the another study, Saha et al. (Saha et al., 2011) investigated different pretreatment methods to enhance methane potential of pulp mill wastewater treatment sludge and showed the highest methane production efficiency of 290 mL/g-VS_{added}. These results were lower than methane production efficiency in this batch study (569 mL/g-COD). The high methane potential of the PME in this study, indicates to the availability of the biodegradable organics in it, so that the major organic content of the substrate was VFA which could be sufficiently converted into methane via methanogenesis stage at anaerobic digestion process.

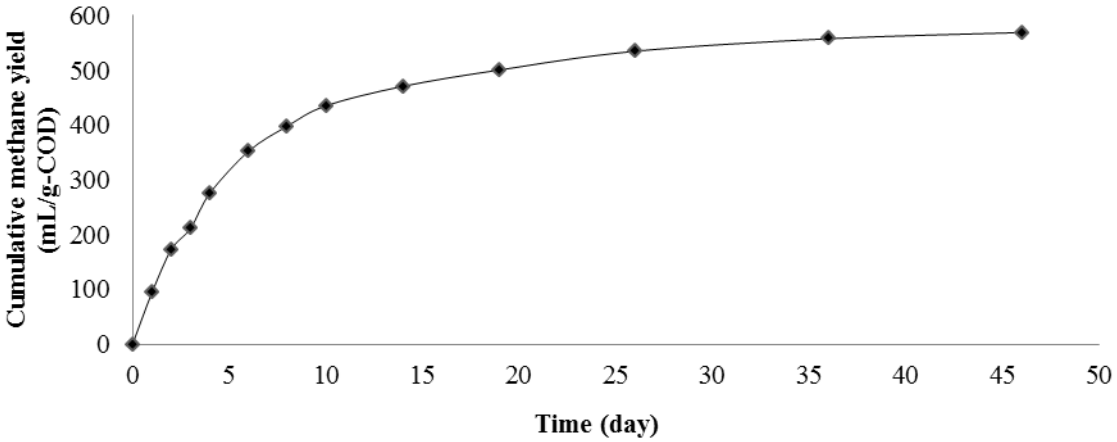

Figure 4. Cumulative methane yield of PME at substrate concentration of 5 g-COD/L and pH=7.

Conclusion

The high BOD content of the PME indicated to its high potential for energy production via biological methods. The substrate concentration and the pH of 5 g-COD/L and 5 led to the highest hydrogen yield (55.4 mL/g-COD). At fixed initial pH, as the concentration of the substrate was increased from 5 to 10 and 15 g-COD/L, the hydrogen yield was decreased from 55.4 mL/g-COD to 38.5 and 32.7 mL/g-COD. Furthermore, at constant substrate concentrations, increasing pH from 5 to 7 caused reduction in the hydrogen fermentation efficiency up to 40.81%. In addition, three substrate hydrolysis including acidic, acidic-thermal and alkaline hydrolysis were investigated which the alkaline hydrolysis of PME improved the hydrogen yield by 14%. The high methane potential of PME (569 mL/g-COD) indicated to the high potential of PME for bio-energy production too.

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