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## Short Communication

Sequential dark and photo fermentation hydrogen production from hydrolyzed corn stover: A pilot test using 11 m<sup>3</sup> reactorQuanguo Zhang<sup>a</sup>, Zhiping Zhang<sup>a</sup>, Yi Wang<sup>a</sup>, Duu-Jong Lee<sup>a,b,c,\*</sup>, Gang Li<sup>a</sup>, Xuehua Zhou<sup>a</sup>, Danping Jiang<sup>a</sup>, Bo Xu<sup>a</sup>, Chaoyang Lu<sup>a</sup>, Yameng Li<sup>a</sup>, Xumeng Ge<sup>a,d</sup><sup>a</sup> Collaborative Innovation Center of Biomass Energy, Henan Agricultural University, Zhengzhou 450002, China<sup>b</sup> Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan<sup>c</sup> Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan<sup>d</sup> Department of Food, Agricultural and Biological Engineering, The Ohio State University/Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691-4096, USA

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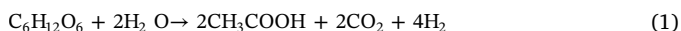
## ABSTRACT

Pilot tests of sequential dark and photo fermentation H<sub>2</sub> production were for the first time conducted in a 11 m<sup>3</sup> reactor (3 m<sup>3</sup> for dark and 8 m<sup>3</sup> for photo compartments). A combined solar and light-emitting diode illumination system and a thermal controlling system was installed and tested. With dark fermentation unit maintained at pH 4.5 and 35 °C and photo fermentation unit at pH 7.0 and 30 °C, the overall biogas production rate using hydrolyzed corn stover as substrate reached 87.8 ± 3.8 m<sup>3</sup>/d with 68% H<sub>2</sub> content, contributed by dark unit at 7.5 m<sup>3</sup>-H<sub>2</sub>/m<sup>3</sup>-d and by photo unit at 4.7 m<sup>3</sup>/m<sup>3</sup>-d. Large variation was noted for H<sub>2</sub> production rate in different compartments of the tested units, revealing the adverse effects of poor mixing, washout, and other inhomogeneity associated with large reactor operations.

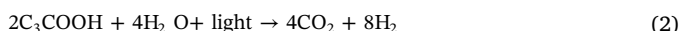
## 1. Introduction

Hydrogen (H<sub>2</sub>) is a clean energy carrier with high energy yield (Nagarajan et al., 2017). Bio-H<sub>2</sub> from lignocellulosic biomass is proposed the promising fuel for future sustainable societies (Cheah et al., 2016). Fermentative production is the most promising pathway to extract H<sub>2</sub> gas from the lignocellulosic biomass (Trchounian et al., 2017). However, up to date the bio-H<sub>2</sub> economy has yet to be realized, partly owing to the fact that the H<sub>2</sub> fuel is still the most expensive amongst all gaseous and liquid competing fuels in the market (Show et al., 2012).

Dark fermentation reaction can be stated as follows (using acetate formation as example):



The photo fermentation to convert acetate to hydrogen can be stated as follows:



The photo fermentation strains can effectively produce hydrogen using the dark fermentation effluent as substrate (Zhang et al., 2017a). For instance, Corona et al. (2017) used a 120 mL bottles with 75 mL liquor (enriched consortium with *Rhodobacter capsulatus*) as reactors at

32 ± 3 °C, pH 6.8 and 3000 lx illumination. The maximum hydrogen production rate was 1340 ± 13 mL/L at light:dark cycles of 30 min:30 min. Ghimire et al. (2016) found that bio-H<sub>2</sub> and poly-beta-hydroxybutyrate (PHB) can be co-produced using photo fermentation with dark fermentation effluent as substrate. If one can have Eqs. (1) and (2) to occur in sequence, the sequential dark and photo fermentation hydrogen production can be stated as follows:



Restated, complete conversion of fed glucose to H<sub>2</sub> can be achieved.

The reactions Eqs. (1) and (2) can be realized in a single reactor, called combined dark and photo fermentation (Zhang et al., 2017c), or in two separate reactors, called sequential dark and photo fermentation (Rai and Singh, 2016; Hitit et al., 2017). The former need extensive operational care since the growth niches for dark-fermentative and photo-fermentative strains are different (Bundhoo, 2017; Zagrodnik and Laniecki, 2017). For the latter, separated dark fermentation with strains (*Clostridium*, *Enterobacter*, *Escherichia coli*, or *anaerobic consortium*) producing hydrogen and volatile fatty acids (VFAs) at around 37 °C in dark, and separate photo fermentative strains (*Rhodospirillum rubrum*, *Rhodobacter*, or *Rhodobium*) producing H<sub>2</sub> in photo-reactor by consuming VFAs at around 30 °C is performed (Supplementary

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## Materials).

Bench-scale tests are relatively easy to perform. However, the adverse impacts of poor mixing, washout, local inhomogeneity in concentration and fluid flow field with industrial applications can only be revealed by pilot tests or above. On dark fermentation, the pilot tests with meter-scale fermenters confirmed the process feasibility of bio-H<sub>2</sub> production from waste (Ren et al., 2006; Lin et al., 2011). Owing to the needs for installation of illumination systems over large reactor volume, pilot-scale tests on photo fermentation H<sub>2</sub> production are rare. Vatsala et al. (2008) presented a pilot-scale, combined dark and photo fermentation H<sub>2</sub> production test in a 100 m<sup>3</sup> reactor with a mix of *Citrobacter freundii*, *Enterobacter aerogenes* and *Rhodospseudomonas palustris*. Zhang et al. (2017b) conducted the first pilot-scale photo fermentation bio-H<sub>2</sub> production test in a 4 m<sup>3</sup> reactor, and noted a contradictory H<sub>2</sub> yield versus hydraulic retention time relationship to the model results assuming a complete-mixed reactor. Up to now, to the authors best knowledge, there is no pilot-scale test on the use of sequential dark and photo fermentation to produce H<sub>2</sub> from crop residues.

As Supplementary Materials lists, the tested reactors in sequential dark and photo fermentation are of size 20 mL–5.55 L. This study aims at developing a pilot-scale sequential dark and photo fermentation system with working volume of 11 m<sup>3</sup> (= 3 m<sup>3</sup> + 8 m<sup>3</sup>) and conducting bio-H<sub>2</sub> production tests in it using pre-hydrolyzed corn stover as the substrate. The facility assembly to this pilot system was fabricated and tested.

## 2. Materials and methods

### 2.1. Substrate preparation

The corn stover was obtained from local farm (Xu Chang, Henan Province, China) and was air-dried and ground in a ball mill (Tai Chi Ring Nano Products Co., Ltd., Qinhuangdao, China) to through a 40-mesh screen. The ground corn stover was stored in sealed plastic bags at room temperature before enzymatic hydrolysis. The characteristics of corn stover were 38.5% cellulose, 22.8% hemicellulose, 10.7% lignin. Enzymatic hydrolysis was conducted in reactor into which 1% w/w ground corn stover, 1% w/w cellulase and citric acid buffer (0.05 M, citric acid/sodium citrate, pH 4.8) were added. The cellulase was purchased from Baolai Corp. (Beijing, China) with an activity of 30 units/mg. The hydrolysis reaction was performed at 50 °C with 150 rpm shaking for 48 h.

### 2.2. Experimental setup

#### 2.2.1. Dark fermentation unit

**2.2.1.1. The reactor.** The dark fermentation unit (3250 mm × 1450 mm × 1500 mm) consisted of a baffle reactor with three series-connected compartments and two post-mix chambers (Fig. 1a). The feed was fed into the reactor via port 1 in Fig. 1a. Dark fermentation occurred in the three baffled reactors with a total working volume of 3 m<sup>3</sup>. The effluent was drawn from port 7 in Fig. 1a. The two post-mix chambers were used for adjusting suspension pH, C/N ratio and removing ammonia nitrogen for subsequent photo fermentation.

The reactor wall is double-layer cuboid with a thermal-protective coating filled in the interlayer as thermal insulators. A cyclical heating system with hot water maintained the reactor temperature.

**2.2.1.2. Inoculum and cultivation medium.** The inoculum used in the dark fermentation was cultivated from the returned sewage sludge obtained from a local sewage treatment plant (Zhenzhou, Henan Province, China). The grits and sands were removed before cultivation. The sewage sludge was then incubated at 40 °C with cow dung and nutrition (NaCl 4.0 g/L, K<sub>2</sub>HPO<sub>4</sub> 1.5 g/L, MgCl<sub>2</sub> 0.1 g/L, CH<sub>3</sub>COONa 2.0 g/L, tryptone 4.0 g/L, and yeast extract 1.0 g/L) for one week, and then was acclimated further for two months with weekly

replenishment of fresh medium. The so-cultivated inoculum was kept at 100 °C for 15 min. The inoculum had the following characteristics: 38.5% total solids, 89.3% volatile solids, 22.8 g/L COD, 10.7% mixed liquor suspended solids.

#### 2.2.2. Photo fermentation unit

**2.2.2.1. The reactors.** The photo fermentation unit with eight compartments, each of 1 m<sup>3</sup> working volume, was installed. The eight compartments were put in two rows, four each as row #1 and row #2, with Fig. 1b showing the schematic of one row of four compartments. The dimensions of photo-fermentation unit (two rows total) are 3680 mm × 2900 mm × 1500 mm. Each compartment has a lift chamber and a fall chamber. The lift chamber is the “photo-reaction area” used for lighting fibers. The length of the lift chamber is 4-fold to that of the fall chamber to provide sufficient mixing at the bottom of the baffle plates. In each compartment, 12 illumination tubes were placed evenly from its top plate into the suspension to provide the light needed (Fig. 2a).

**2.2.2.2. Inoculum cultivation reactor.** The inoculum needed for photo fermentation tests was cultivated in a reactor with three transparent coaxial cylinders connected in a series, light sources and a hot water recirculation system. The combined LEDs light and solar light illumination was utilized (discussed in Section 2.2.2.3), with the lighting pipes inserted into the liquid medium from the top of the columns. The hot water flowed through interlayer to maintain the temperature at 30 °C. In each cylinder there were seven lighting pipes to provide illumination for cell growth. The schematic of the reactor is shown in Fig. 1c. The total working volume for the cultivation reactor is 347 L.

**2.2.2.3. Illumination system.** The solar light was collected by a concentrator, an automatic tracking device and optical fibers. The light concentrator was built by combining matrix lens array with extended reflector array. The illumination system has 24 concentrators (each concentrator has 16 lens). The total area of the illumination system is 2.4 m<sup>2</sup> (the illumination area of each concentrator is 0.1 m<sup>2</sup>), with 384 fibers. The automatic tracking device was three-dimension directional tracking in order to meet the optimal light receiving of the light concentrator (Supplementary Materials). The solar transmission unit was equipped with optical fibers to enable multipoint light distribution in the hydrogen production reactor according to different light transmission paths (Supplementary Materials). An independent photo-voltaic panel was used to generate electricity to be stored in a battery. In cloudy day or at night the battery lights LED bundles set inside photo-compartments as light sources. The internal light intensity was kept at 3000 lx during the biohydrogen production. When the internal light intensity was less than 3000 lx, the LED bundles were turned on to meet the demand of the photo-fermentative bioreactor.

**2.2.2.4. Inoculum and mineral medium.** The mixed photosynthetic bacterial culture used in photo-fermentation is the consortia HAU-M1 (Zhang et al., 2017c). The cultivation medium contained 1 g/L NH<sub>4</sub>Cl, 2 g/L NaHCO<sub>3</sub>, 1 g/L yeast extract, 0.2 g/L KH<sub>2</sub>PO<sub>4</sub>, 4 g/L CH<sub>3</sub>COONa, 0.2 g/L Mg SO<sub>4</sub>·7H<sub>2</sub>O, and 2 g/L NaCl.

### 2.3. Hydrogen production test

The test was composed of the adaption stage (60 d) and production stage (30 d).

At the beginning of the adaption stage, 300 kg of activated sludge was pumped into the dark-fermentation unit (Fig. 1a) with addition of 2 m<sup>3</sup> cultivation medium to mix for 48 h. Then the hydrolysate from hydrolyzed corn stover (Hu et al., 2016), with substrate concentration 10 g COD/L, was fed at a hydraulic retention time (HRT) of 16 h

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