Effect of Using Paperboard Bacterial Culture on Fermentative Hydrogen Production from Paperboard Mill Wastewater

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ABSTRACT
The effect of paperboard bacterial culture (PBC) supplementation during fermentative hydrogen production under the variation of initial substrate concentration to inoculum (S/X) ratio was studied. The paperboard mill wastewater (PMW) has been used as substrate. The results showed that supplementation of heat-pretreated PBC to PMW progressively improved the hydrogen productivity. Where, the hydrogen yield (HY) increased from 1.72 to 2.63 mmol/$gCOD_{initial}$ by supplementation of PBC to PMW compared to PMW as a sole substrate, with a peak hydrogen production (HP) of 2.4 mmol. Subsequently, different S/X ratios were studied: 0.05, 0.15, 0.3, and 1 gCOD/gVS. In which, HP at S/X ratio of 0.3 gCOD/gVS resulted in the highest value of 3.8 mmol, where lower and higher S/X ratios led to deterioration in HP in the range of (65 to 77%). Meanwhile, the highest % soluble/total carbohydrates and VSS removal of 72.73 and 63.16% % were corresponded to the highest HY (2.1 mmol H_2/gCODinitial) respectively at 0.3 gCOD/gVS. This indicates that most of produced H_2 was generated from the conversion of organic carbon in form of particulates. The main soluble metabolites analysis showed the butyrate fermentation type was occurred with small concentrations of propionate and lactate.

Keywords: Paperboard mill wastewater; Paperboard bacterial culture; Initial substrate concentration to inoculum ratio; Dark fermentation; Hydrogen

1. Introduction

The increased concerns of greenhouse gas emissions and other environmental problems driving research in the area of alternative fuels for sustainable development (Gadhe et al., 2014). Hydrogen (H_2) is emerging as a strong candidate because it has the highest energy content per unit weight (122 kJ/g), and it produces water when combusted (Farghaly and Tawfik, 2015). Moreover, hydrogen appears to be one of the best transportation fuels, the most versatile and efficient fuel (Valdez-Vazquez et al., 2005). Also, H_2 is widely used for the synthesis of ammonia and alcohols, as well as for the hydrogenation of edible oils, petroleum, and shale oil (Zhang et al., 2003).

On the other hand, paperboard industry is one of the most important industries in Egypt. However, the effluents are considered from the major pollutants threats our country given the large wastes volume generation that are certainly harmful to the environment (Farghaly et al., 2015). In particular, the problems associated with paperboard mill wastewater (PMW) are high levels of organic pollutants and suspended solids (Kumar et al., 2014). Fortunately, production of hydrogen by PMW anaerobic processes is accompanied by the breakdown of organic substrates, and appears to be advantageous in converting organic wastes into more valuable energy resources (Chen and Chang, 2001). Therefore, converting low valuable feedstocks such as PMW into hydrogen is undoubtedly a challenging and emerging research area.

The performance of H_2-producing bacteria depends substantially on substrate, inoculum or both. Selection of the ecosystem for efficient hydrogen production is usually started by selecting a particular type of sludge as inoculum followed by its treatment (Mohammadi et al., 2012). In paperboard mills, the sludge produced from clarifiers is typically thickened and dewatered, and then disposed by landfilling or incineration which results in gaseous emissions and water pollution (Bayr et al., 2013). Using these sludge as mixed bacterial culture offers distinct advantages such as increasing H_2 yield and ability to utilize cellulose and hemicellulose present in PMW (Chaganti et al., 2012). As well as, tolerance to indigenous microbes in substrate and capability of producing a wide range of hydrolytic enzymes (Laothanachareon et al., 2014). Nevertheless, a major drawback of using mixed bacterial culture is the rapid consumption of the produced H_2 by methane-producers. Therefore, pretreatment of inoculum used in fermentative H_2 production process permits selective enrichment of specific group of bacteria (Mohan, 2008). According to the literature, cell wall and membrane of the microorganisms are disrupted effectively by heat applied during thermal pretreatment, resulting in a solubilization of the cell components (Assawamongkholsiri et al., 2013). In the same manner, according to (Kan, 2013), the highest H_2 yield was produced by using heat-treated culture followed by heat and acid-treated culture and the heat and base-treated culture.

Besides, the efficiency of hydrogen production is directly related to the initial substrate to inoculum ratio (S/X). Where, the initial substrate concentration represents the carbon and energy source for biosynthesis requirements and other energy purposes (Farghaly and Tawfik, 2015). As reported earlier (Chen et al., 2006), the hydrogen production potential and rate increased with increasing of S/X ratio. However, increased S/X would result in lower H_2 yields that likely have been attributed to the inhibitory effect of higher H_2 partial pressures in the growth medium (Hafez et al., 2010). On the other hand, other researchers reported that lower S/X would inhibit the hydrogen production due to the inadequate food for the higher concentrations of inoculum (Sun et al., 2011). Besides, at lower S/X, the reactions were operated under substrate limiting conditions resulted in lower H_2 productivity (Elsamadony et al., 2015).

Therefore, the objectives of this study were to define the suitability of paperboard bacterial culture as a consortium for hydrogen production from paperboard mill wastewater. In addition, the influence of the initial substrate concentration to inoculum (S/X) ratio effect on the bacteria performance for efficient H_2 productivity, organic pollutants conversion, and the soluble metabolites production were also investigated.

2. Material and Methods

2.1. Mill description and inoculum

The studied mill has a capacity of 60 ton/day of paperboard production where the printing paper wastes are the raw material. The mill generates about 700 m^3/d of PMW that disposed to the sewage networks without any treatment. The end of pipe effluent was transported to the environmental lab for further experiments. The characteristics of PMW are presented in Table 1.

The paperboard bacterial culture (PBC) was collected from the thickener of the same mill. The PBC was further concentrated by settling for 24 h. where the supernatant has been withdrawn. Volatile suspended solids, total suspended solids contents, and sludge volume index (SVI) were 34.5±2.9, 16.2±1.3 g/l, and 28.9±2.6 ml/g-TS, respectively. The sludge was pre-treated at 90 °C for 30 min to harvest spore-forming bacteria and stored for 2 weeks under anaerobic conditions before being used as inoculum.

Table 1. Characteristics of PMW and seed sludge

Parameter	Value	Unit
Total COD	1870	Mg/l
Soluble COD	923	Mg/l
Particulate COD	947	Mg/l
BOD	1120	Mg/l
TSS	1370	Mg/l
VSS	790	Mg/l
pH	7.2	--
Carbohydrates	51	Mg/l
Total VFAs	187	Mg/l
HAc	86	Mg/l
HBu	52	Mg/l
HPr	20	Mg/l
HLa	19	Mg/l
NH4-N	3.2	Mg/l
TKj-N	52.1	Mg/l
C/N	35.9	--

2.2. Experimental set-up

Batch dark fermentation experiments were conducted in a series of 500/400 ml (total/working volume) serum bottles. Prior starting the experiments, pure N_2 gas was flushed into the bottles for 5 min. to eliminate the oxygen from the culture medium and headspace. The experiments were designed to study the effect of PBC supplementation and initial S/X ratio on H_2 production from PMW. Regarding to the 1st experiment, three duplicate batches were performed to study the effect of PBC supplementation to PMW namely: (1) PMW, (2) PBC, and (3) PMW+PBC (inoculums to substrate ratio of 0.32 gCOD/gVS (Yilmaz et al., 2008)). The experiments were conducted for 130 h. While at the 2nd experiment, different S/X ratios of 0.05, 0.15, 0.3, and 1 gCOD/gVS were applied as depicted in Table 2. This was achieved by keeping the inoculum concentration of PBC constant at 16.2±1.3 gVS/l with varying the substrate volume. Distilled water was added to make the working volume of each bottle to be 400 ml. The experiments were conducted at 37 ºC and pH of 7. The bottles were capped tightly with rubber stoppers and aluminum crimp seals. The produced biogas volume has been measured using the water displacement method and corrected to the standard conditions (25 ºC and 1 atm.) as described earlier (Lee et al., 2008).

Table 2. The experimental set-up of the initial S/X ratio effect on H_2 production

S/X (gCOD/gVS)	Bioreactor contents (ml)		
	PMW	PBC	Dist. H_2O
0.05	150	250	0
0.15	175	150	75
0.3	250	100	50
1	300	50	50

2.3. Data analysis

Cumulative H_2 production data that generated over the time course was analyzed using the modified Gompertz model (Eq. 1) (Farghaly et al., 2015). Where: HP is the hydrogen production (mmol). H is the cumulative hydrogen production at time (t), P is the H_2 potential (mmol), R_m is the maximum hydrogen production rate (mmol/h), λ is the lag phase required to commence H_2 evolution, and e is the Euler's number. Initial S/X ratio was calculated according to Eq. 2 as described by earlier (González-Fernández and García-Encina, 2009).

$$[1]\ H(t) = P.\exp\left[-\exp\left(\frac{R_m}{P}(\lambda-1)+1\right]\right.$$

$$[2]\ S/X\ (gCOD/gVS) = \frac{Volume_{PMW}\ x\ COD_{PMW}}{Volume_{PBC}\ x\ VS_{PBC}}$$

2.4. Analytical methods

The biogas composition was analyzed using a gas chromatograph (GC-2014, Shimadzu, Japan). It is equipped with a thermal conductivity detector (TCD) and a 0.2 m, 3 mm diameter stainless column packed and Shin carbon (50/80 mesh). The operational temperatures of the injection port, the column oven, and the detector were 100, 120, and 150℃, respectively. Helium was used as the carrier gas at a flow rate of 25 ml/min. Volatile fatty acids (VFAs) concentrations in terms of acetate (HAc), butyrate (HBu), propionate (HPr) and lactate (HLa) were analyzed by high performance liquid chromatography (HPLC) (LC-10AD, Shimadzu, Japan) with ultraviolet detector using a shim-pack HPLC column (4.6x250 mm, VP-ODS, Vertical). The temperature of column oven was 40 °C. 4 mM H_2SO_4 was used as a mobile phase at a flow rate of 0.5 ml/min for 22 min followed by 0.4 ml/min for 8 min. Total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total Kjeldahl nitrogen (TKN), and ammonium nitrogen NH_4-N were determined according to APHA (2005). Soluble COD was determined using filter paper (0.45 μm-Whatman, 7141-104, Japan). The carbohydrate was measured according to the phenol-sulfuric acid method, using glucose as the standard.

3. Results and discussion

3.1. Effect of mixed culture bacteria

The results obtained from this study indicate that it is feasible to enhance the H_2 productivity from paperboard mill wastewater using paperboard bacterial culture as consortium. Fig. 1 shows the cumulative H_2 production from PMW, PBC, and PMW supplemented with PBC (0.32 gCOD/gVS). It was found that, when only PMW or PBC studied as a sole substrate, the hydrogen production (HP) was relatively low (1.6 and 2.0 mmol, respectively). However, the cumulative HP from PMW+PBC peaked at 2.4 mmol, i.e. 1.5 and 1.2 times than those obtained from PMW and PBC, respectively. This coincided to HP rate of 0.2 mmol/h for PMW+PBC. In addition, the produced H_2 content increased from 33.7% (PMW) to 54.3% by inoculating PBC to PMW. Meanwhile, the methanogens present in the PBC could be completely inhibited as a result of thermal pretreatment. Therefore, the PBC supplementation not only improved the HP but also accelerated the HP rate. This may be attributed to that the plentiful cellulosic substrate contained in PBC could be feasibly utilized for H_2 production by anaerobic digestion process (Chairattanamanokorn et al., 2012).

The results revealed that, the hydrogen yield (HY) increased to 2.63 mmol/$gCOD_{initial}$ by the PBC addition. Where, the HY was found lower using PMW (1.73 mmol/$gCOD_{initial}$) as sole substrate. Furthermore, the conversion efficiency would be observed to be enhanced using PBC supplementation. In specific, the COD R% amounted to 59.9% at the end of fermentation process, while 26.89% was registered to PMW. Accordingly, the PBC supplementation to PMW increased the microbial growth and substrate utilization, thereby enhancing the H_2 productivity.

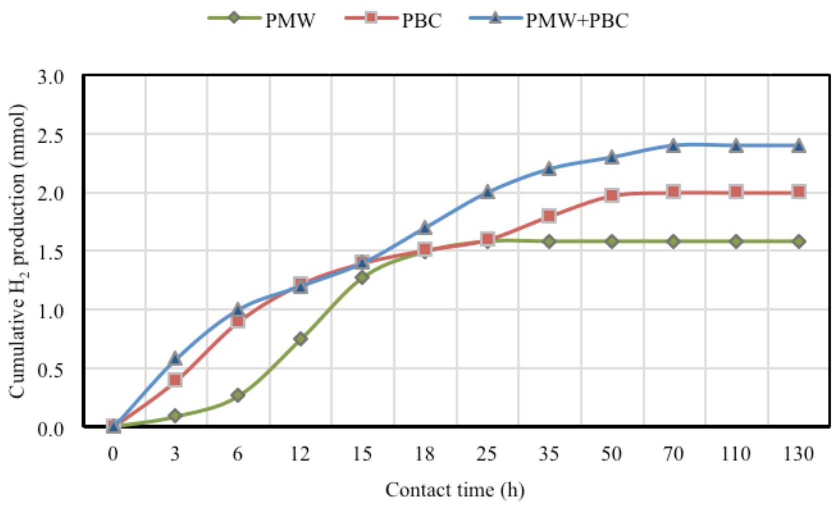

Figure 1. Effect of paperboard bacterial culture supplementation to PMW on cumulative H_2 production

3.2. Effect of initial S/X ratio

Fig. 2 shows the cumulative HP at different initial S/X ratios using PBC as inoculum and PMW as substrate. The results obtained indicated that increasing initial S/X ratio from 0.05 to 0.3 gCOD/gVS enhanced the cumulative HP from 0.9 to 3.8 mmol, respectively. Nevertheless, at a higher S/X ratio of 1 gCOD/gVS, the cumulative HP dropped to 1.3 mmol. In addition, cumulative H_2 production was fitted using Gompertz equation of which kinetics parameters were determined by regression analysis. The correlation coefficient between the experimental and simulated data was relatively high (R^2>0.978) as shown in Table 2. The kinetic results showed that the hydrogen potential (P) and the maximum hydrogen production rate (R_m) were S/X-dependent. In particular, the highest R_m and P were 0.19 mmol/h and 3.8 mmol at initial S/X ratio for 0.3 gCOD/gVS. The lower HP at S/X ratio of 0.05 gCOD/gVS was mainly due to the substrate limiting conditions of batch cultivation given that the most of substrate were consumed for bacterial growth. This behaviour was likely due to the biomass accumulation which further led to nutrients consumption and substrate inhibition (Sun et al., 2011). However, at the higher S/X ratio, a microbial shift occur resulting in increasing the biomass yield that is not related to hydrogen producers (Hafez et al., 2010).

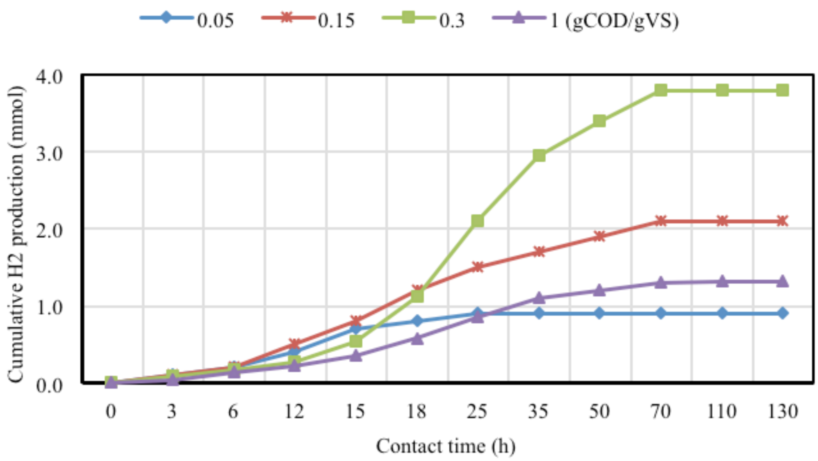

Figure 2. Effect of initial S/X ratio on cumulative H_2 production

The conversion efficiency of organic pollutants in PMW was highly affected by initial S/X ratio as shown in Table 3. Given that, it was observed that COD removal efficiency (% R) and HY increased from 43.63 to 51.66% and 0.5 to 2.1 mmol/gCOD$_{initial}$ with increasing S/X ratio from 0.05 to 0.3 gCOD/gVS, respectively. Furthermore, the solubility of organic matter at the end of fermentation was found S/X ratio-dependent, where the peak COD solubility of 22.94% was at S/X ratio of 0.3 gCOD/gVS, which was accorded to the highest HP. This ratio decreased at lower and higher S/X ratio of 7.45 and 14.18% at 0.05 and 1 gCOD/gVS, respectively. The increasing solubility at the end of fermentation indicated that a portion of organic matter was acidified and dissolved during the reaction due to the volatile fatty acids generation which is accompanied with H_2 production (Zhou et al., 2013).

Table 3. Performance of initial S/X ratio of fermentative H_2 production and conversion efficiency

S/X (gCOD/gVS)	Model simulation				HY	COD R%	Solubility %
	P (mmol)	R_m (mmol/h)	λ (h)	R^2	(mmol/gCOD$_{initial}$)		
0.05	0.9	0.10	8	0.98	0.50	43.63	7.45
0.15	2.1	0.13	9	0.98	1.16	45.78	20
0.3	3.8	0.19	9	0.96	2.10	51.66	22.94
1	1.3	0.07	10	0.99	0.73	31.37	14.18

On the other hand, the carbohydrates conversion was also studied given the substrate affinity of the enriched hydrogen producing bacterial culture depends on carbohydrate content (Chen et al., 2006). In particular, it was observed that the peak carbohydrates removal efficiency was 48.25% at S/X ratio of 0.3 gCOD/gVS. Where, lower and higher S/X significantly decreased the removal efficiency to 32.05 and 15.5% at S/X of 0.05 and 1 gCOD/gVS, respectively. Besides, it was found that the highest HY (2.1 mmol H_2/gCOD$_{initial}$) was accorded to the highest % soluble/total carbohydrates removal (72.73%) as shown in Fig. 3. Moreover, this is coincided to the highest % removal of volatile suspended solids (63.16%). This indicates that most of produced H_2 was generated from the conversion of organic particulates in PMW. From the aforementioned calculations, it is concluded that the ability of PBC to convert COD into hydrogen is greatly influenced by the initial S/X ratio.

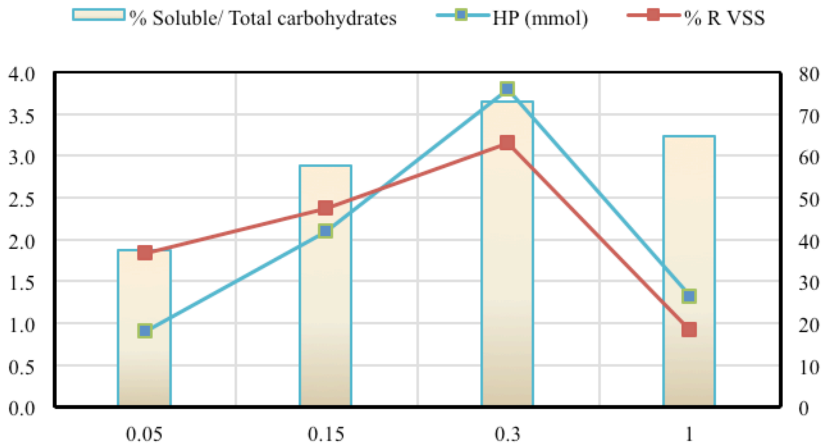

Figure 3. The relationship between HP and the percent of total suspended solids and carbohydrates removal

As reported in the literature, the initial (S/X) ratio significantly affects the metabolic production through the process (Chen et al., 2006). It was observed that the main VFAs produced were acetate (HAc) and butyrate (HBu) as shown in Fig. 4. Where, the HAc and HBu increased from 112.2 and 54 mg/l to 172.12 and 71.6 mg/l, respectively. Nevertheless, HAc and HBu decreased with increasing S/X ratio (1 gCOD/gVS) to 115.4 and 61.8 mg/l, respectively. As can be seen from Fig. 4, the highest HAc/HBu of 2.4 was coincided to the highest HP and HY. Comparable trends were observed elsewhere (Elsamadony et al., 2015), where HAc and HBu increased from 1.28±0.11 and 2.2±0.27 g/l to 3.82±0.33 and 2.91±0.12 g/l with increasing S/X ratio from 1.7 to 9.2 using organic fraction of municipal solid waste as a substrate. Accordingly, HAc and HBu are the desirable volatile fatty acids as they are indicative of hydrogenogenic metabolic pathway (Reungsang et al., 2013). On the other hand, the production of propionate (HPr) and lactate (HLa) was likely due to that there is such consumption of H_2 that may be caused by the lactic acid bacteria (Eqs. 3 and 4). Besides, the discrepancies in HPr and HLa trends with varying S/X ratios are possibly due to the polysaccharides behaviour in the cellulosic content of the PMW, which affected the VFAs fermentation process (Farghaly et al., 2015). The polysaccharides have resulted from the bleaching chemicals used in the manufacturing process of PMW, such as slimicides that control bacteria and fungi that produce polysaccharides in the finished product (Ozaki et al., 2004).

[3] $\text{Glucose} + 4H_2O \longrightarrow HLa^- + 2HCO_3^- + 4H^+$

[4] $HLa^- + H_2 \longrightarrow 2CH_3CHOHCOOH + H_2O$

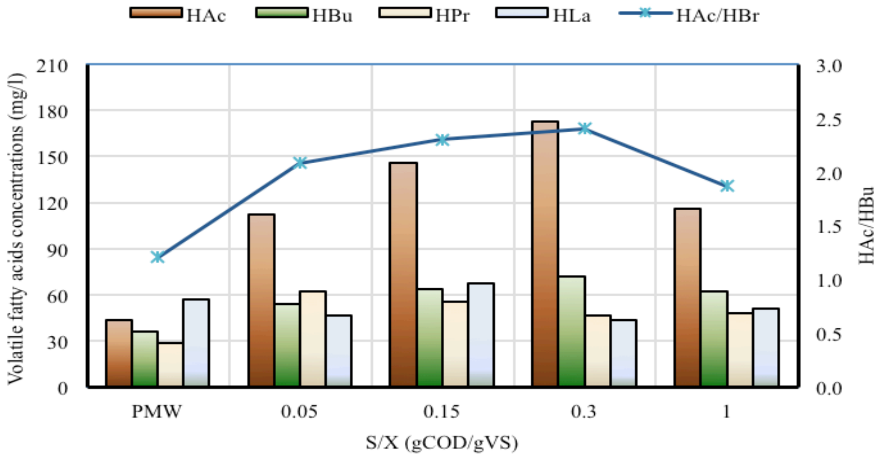

Figure 4. Effect of S/X ratio on the produced volatile fatty acids concentrations

4. Conclusions

The results obtained from this study showed the positive effect of PBC supplementation as consortium for fermentative H_2 production using paperboard mill wastewater. In addition, it was found that the HP and biodegradation process were strongly initial S/X-dependent. In particular, inoculating PBC to PMW resulted in HP and HY of 1.5-fold higher than those produced from PMW as a sole substrate. Moreover, HP and HY at S/X ratio of 0.3 gCOD/gVS resulted in the highest values of 3.8 mmol and 2.1 mmol/gCOD$_{initial}$, respectively. This was coincided to considerable organic pollutants removal in terms of COD, carbohydrates, TSS, and VSS of 51.6, 48.25, 82.1, and 63.16 %, respectively. Meanwhile, the volatile fatty acids analysis indicated that HAc-HBu fermentation pathway has been occurred accompanied with HPr and HLa production.

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