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Assessment of Effects of Yeast Extract on Bio-hydrogen Production from Anaerobic Activated Sludge

Liguo Zhang[1*], Qiaoying Ban[1], Jianzheng Li[2] and Yiping Xu[2]
[1]*College of Environment and Resource, Shanxi University, Taiyuan, 030006, P. R. China*
[2]*State Key Laboratory of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, P.R. China*
*For correspondence: zlg_733@163.com; zhangliguo@sxu.edu.cn

Abstract

Biological hydrogen production by fermenting organic wastes is considered as a promising method to obtain sustainable clean energy. However, the yield of hydrogen is still low. To explore effects of yeast extract on biological hydrogen production by fermentation, the efficiency of the bio-hydrogen production under yeast extract of different concentration conditions were investigated by batch cultures in this study. The results explored that H_2 production in all the yeast extract treatments were higher than that of the control (0 $g \cdot L^{-1}$ yeast extract) at initial pH 7.0 and 35ºC. The maximum hydrogen production was 1.45 mol/mol glucose at 0.4 $g \cdot L^{-1}$yeast extract condition, of which major liquid fermentation products were acetate and butyrate. Kinetic analysis showed the maximum hydrogen production rate of 6.49 $mL \cdot h^{-1}$ was obtained by 0.6 $g \cdot L^{-1}$ yeast extract while the maximum accumulative hydrogen production of 46.92 mL was achieved at 0.4 $g \cdot L^{-1}$ yeast extract. It was noteworthy that accumulative H_2 production under high concentration yeast extract conditions (>2.0 $g \cdot L^{-1}$) was decreased at the latter stage of the fermentation. The reduction of hydrogen might be due to the role of hydrogen consumption from homoacetogens in this study.

Keywords: Yeast extract; Bio-hydrogen production; Anaerobic activated sludge; Kinetic analysis; Homoacetogens

Introduction

Bio-hydrogen production from organic wastewater or other wastes has been given considerable attention (Kapdan and Kargi, 2006; Ren *et al.,* 2007; Li *et al.,* 2009; Yang *et al.,* 2010; Zhao *et al.,* 2012). Biological hydrogen production could be divided into at least three processes, including photolysis of water and organics, dark fermentation of organic compounds by algae, PSB (photosynthetic bacteria), fermentation bacteria, respectively (Das and Veziroglu, 2008). In all these processes, the bio-hydrogen production through dark fermentation of organic matter is a paramount promising method because the speed of hydrogen evolution is generally faster than that of photo-hydrogen evolution (Levin *et al.,* 2004). Currently, more interests are concentrated on bio-hydrogen production through fermenting organic waste by anaerobic sludge and some achievements were obtained (Ren *et al.,* 2006; Li and Fang, 2007; Guo *et al.,* 2010a, b). However, the fact that the rate of H_2 production is much lower than theoretical H_2 production rate restricts this technology for practical applications. Further research and development aiming to enhance the rate of bio-hydrogen production and final yield of H_2 is essential (Levin at al., 2004).

The effects of several major factors including inoculum, substrate, reactor configuration, temperature, pH, nitrogen, phosphorus and metal ions, on hydrogen production by fermentation have been studied (Yang and Shen, 2006; Wang and Wan, 2009; Kim *et al.,* 2012). In addition, if some hydrogen-consuming bacteria like methanogens, sulfate reducing bacteria and homoacetogens existed in microflora, the hydrogen yield would be reduced because of their ability of using H_2 to gain energy (Guo *et al.,* 2010a). Therefore, it is vital to enhance the metabolic activity of hydrogen-producing bacteria and meanwhile inhibit the ability of hydrogen-consuming bacteria for improving the efficiency of fermentation hydrogen production.

Yeast extract contains peptide, amino acid, vitamins and trace elements, and these nutrients are important for growth and metabolism of the microorganisms (Bibi *et al.,* 2012). In addition, some vitamins and trace elements are cofactors of certain enzymes in microbial metabolic processes. However, the effects of yeast extract on hydrogen production by fermentation have not been yet studied. The present study was conducted to investigate the effects of yeast extract on the production of hydrogen from the anaerobic sludge by batch cultures. The liquid fermentation products and cumulative hydrogen yields in different yeast extract concentration conditions were compared.

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Materials and Methods

Sludge Sample

The anaerobic sludge from a completely stirred tank reactor (CSTR) was utilized as inoculum. This system was employed in bio-hydrogen production with diluted wastewater from a sugar refinery as substrate. In this study, the anaerobic activated sludge of the 355th day was used as the seed sludge in the batch experiments. The operational conditions were as follows: 35^{o}C, hydraulic retention time of 8 h, influent chemical oxygen demand of 7.0 g·L^{-1}, pH4.5±0.5, the biogas production of 25 L·d^{-1}, hydrogen content of 39%.

Batches Procedure

Batch experiments were conducted in 150 mL serum bottles. The 10 mL inoculum (1.13 g mixed liquor volatile suspended solids (MLVSS)/L) was inoculated to each bottle. In each experiment, the medium was prepared using glucose of 7.0 g·L^{-1} as the carbon source and anaerobic basic medium. Anaerobic basic medium was prepared according to previously described (Angelidaki and Sanders, 2004). The nutrient solution of 40 mL was added into each bottle. The total liquid volume of each bottle was 50 mL. After adding medium, the yeast extract was added into it. The concentrations of yeast extract (Oxoid, England) were ranged from 0 (control), 0.1, 0.2, 0.4, 0.6 0.8, 1.0, 2.0 and 4.0 g·L^{-1} in the batch experiments. The 4 g·L^{-1} $NaHCO_3$ was added into medium and the initial pH was adjusted to 7.0. The O_2 was eliminated through filling nitrogen to medium. The batch tests were conducted at 35^{o}C and 130 rpm.

Analysis

The H_2 content in biogas was measured with a SP-6800A gas chromatograph (Shandong Lunan Instrument Factory, Zaozhuang, China) (Ban *et al.,* 2013). The volatile fatty acids and ethanol were also analyzed by another gas chromatograph (SP6890, Shandong Lunan Instrument Factory, China) (Ban *et al.,* 2013).

The reducing sugar was measured by the DNS colorimetric method (Chang *et al.,* 2011). The pH and MLVSS were estimated by the standard methods (APHA, 1995). The sludge growth rate (S_r) was evaluated according to formula (1), where M_b (g·L^{-1}) is the MLVSS before batch experiments; M_a(g·L^{-1}) is the MLVSS after batch experiments; R_a (g·L^{-1}) is the reducing sugar before batch experiments; R_a (g·L^{-1}) is the reducing sugar after batch experiments.

$$S_r = \frac{M_a - M_b}{R_b - R_a} \quad (1)$$

Computing Methods

The volume of generated gas was determined by glass syringes, ranged from 10 to 50 mL (Owen *et al.,* 1979). The cumulative H_2 production was calculated as previously described (Yang and Shen, 2006). The cumulative H_2 production profiles were fitted with Gompertz equation for calculating P_{max} (hydrogen production potential), R_{max}, and λ

$$H = P_{max} \times \exp\left\{-\exp\left[\frac{R_{max} \times e}{P_{max}}(\lambda - t) + 1\right]\right\} \quad (2)$$

(lag time) (Lay *et al.,* 1998):

After measuring biogas composition, liquid sample of 3 mL was taken from each batch for determining reducing sugar, VFAs, and alcohols.

Results

Cumulative Hydrogen Production

Batch tests were proceed until the gas production was stopped. During the whole process, there were only H_2 and CO_2 could be detected without any detectable CH_4 and H_2S. Variations of accumulative hydrogen yield with time for different amounts of yeast extract are depicted in Fig. 1. The accumulative hydrogen yield in all the yeast concentration experiments were higher than the control test (0 g·L^{-1} yeast extract) after 48 h fermentation except for 4.0 g·L^{-1} yeast extract condition. Hydrogen production was improved with the yeast extract concentration increased up to 0.4 g·L^{-1}, and the maximum hydrogen yield was 1.45 mol/mol glucose at the 0.4 g·L^{-1} yeast extract condition (Table 1). However, the accumulative hydrogen volume was decreased with the further improvement in the yeast extract content (from 0.6 g·L^{-1} to 1.0 g·L^{-1}). It was also observed that when the yeastextractinthemediumwas2.0 g·L^{-1} or 4.0 g·L^{-1}, the accumulative hydrogen production began to decline after reaching maximum value at 32 h and 16 h, respectively.

Kinetic Analysis

Table 2 shows the kinetic parameters of hydrogen production at different temperature conditions. The detection coefficient (R^2) except for the treatments of 2.0 and 4.0 g·L^{-1} yeast extract, was over 0.99. The longest lag time was 22.6 h with the control (0 g·L^{-1} yeast extract), followedby9.9 h for 0.1, 7.71 h for 0.2, 7.65 h for 0.4, 5.99h for 0.6, 4.34 h for 1.0, 4.04 h for 0.8. The R_{max} of 6.49 mL·h^{-1} was observed for the treatment by 0.6 g·L^{-1} yeast extract. However, the maximum accumulative hydrogen production was 46.92 ml for 0.4 g·L^{-1} yeast extract.

Liquid End Products

The liquid end products are very important for analyzing

Table 1: Kinetic parameters of hydrogen production for different concentration yeast extract treatments

Yeast extract concentration (g/L)	Final pH	H_2 conversion rate (mol H_2/ mol Glucose)	Final MLVSS (g/L)	Sludge growth rate (mg/g Glucose)
Control	5.36[a]	0.51[ab]	4.51[e]	0.61[c]
0.1	5.44[a]	0.79[bcd]	3.60[a]	0.45[a]
0.2	5.37[a]	1.34[g]	3.87[b]	0.50[b]
0.4	5.32[a]	1.45[fg]	4.12[c]	0.54[b]
0.6	5.33[a]	1.21[efg]	4.14[c]	0.55[b]
0.8	5.33[a]	1.07[def]	4.17[c]	0.55[b]
1.0	5.34[a]	1.01[cde]	4.35[d]	0.59[c]
2.0	5.31[a]	0.99[bc]	4.91[f]	0.69[d]
4.0	5.40[b]	0.35[a]	5.52[g]	0.79[e]

The results represent mean values (n=3). There are no significant differences (P>0.05) between samples with the same characters, and significant differences (P<0.05) between samples with different characters.

Table 2: The effect of different yeast extract concentration on pH, H_2 yield and biomass after 48 h cultivation

Yeast extract concentration (g/L)	P_{max}(mL)	R_{max} (mL/h)	λ (h)	R^2
Control	18.28	2.30	22.60	0.9971
0.1	26.64	2.51	9.90	0.9970
0.2	45.79	4.42	7.71	0.9980
0.4	46.92	5.82	7.65	0.9984
0.6	40.50	6.49	5.99	0.9968
0.8	36.80	3.99	4.02	0.9986
1.0	34.51	4.94	4.34	0.9996

hydrogen production characteristics. As shown in Fig.2, there were trace amount of propionate, which was detected in all the batch cultures. For control, concentration of butyric acid, ethanol and acetate were major products (together accounting for 96.2% in total soluble metabolites), indicating mixed-acid type fermentation occurred in this system. Similarly, mixed-acid type fermentation was also formed in the system which was treated by 0.1 $g \cdot L^{-1}$ yeast extract. However, butyric-acid type fermentation was observed in 0.2~4.0 $g \cdot L^{-1}$ of yeast extract treatment experiments, whose major liquid fermentation products were acetate and butyrate (together accounting for 78.7%~87.4% in total soluble metabolites.

pH, Biomass and H_2 yield

Table 2 shows the effect of different yeast extract concentration on pH, H_2 yield and biomass after 48h cultivation. The finial pH was almost same level in the systems and much lower than initial pH because of the production of the VFAs. It can be seen from Table 2 that biomass at the end of experiment was much more than initial inoculum (1.13 g MLVSS/L) in the batches. The highest sludge growth rate of 0.79 $mg \cdot g^{-1}$ glucose was observed for 4.0 $g \cdot L^{-1}$ yeast extract treatment. However, the sludge growth rate was low at 0.4 $g \cdot L^{-1}$ yeast extract condition with the highest hydrogen yield (1.45 mol/mol glucose). This result suggested that the activity of hydrogen

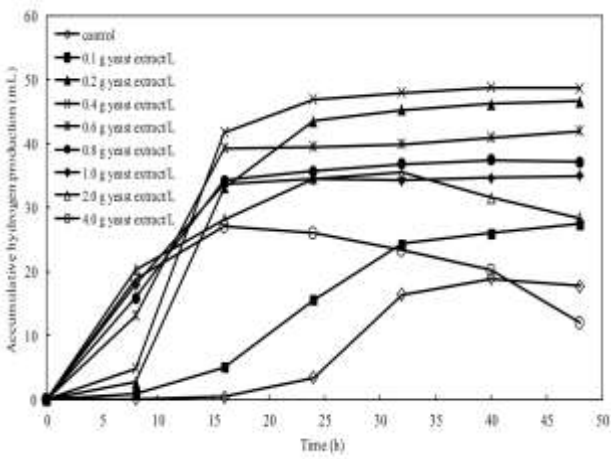

Fig. 1: Cumulative hydrogen volume versus corresponding fermentation time

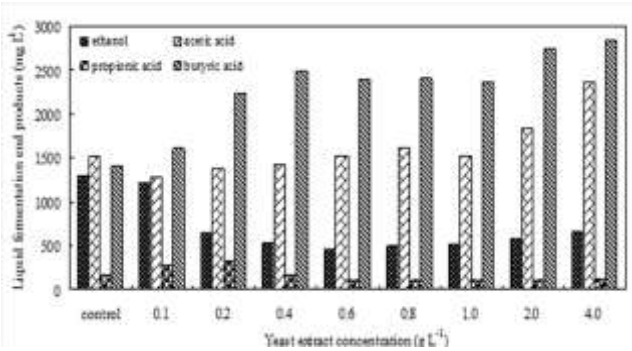

Fig. 2: Effect of different concentration yeast extract on the liquid fermentation end product

production was high at 0.4 $g \cdot L^{-1}$ yeast extract condition. The hydrogen yields of all the tests were lowered than the theoretical maximum yield of 4 mol H_2/mol glucose, but the biomasses were increased significantly, indicating that most of the released electron was used to cell synthesis.

Discussion

It is vital to enhance the activity of hydrogen-production bacteria and meanwhile inhibit the activity of hydrogen-consumption bacteria for further improving the efficiency of hydrogen production by fermentation. The results indicated that the yeast extract of low concentration (less than 1 $g \cdot L^{-1}$) inhibited the activity of hydrogen-consuming bacteria while enhanced the activity of hydrogen-producing bacteria. But the yeast extract of high concentration (more than 2.0 $g \cdot L^{-1}$) might promote the role of hydrogen consumption at the latter part of the fermentation. These hydrogen-consuming bacteria might be homoacetogens as result of no methane and H_2S were detected during the fermentation process. Indeed, the previous study found that the homoacetogens may be existed

in the inoculated sludge (Li *et al.*, 2011). The similarity of 16S rRNA gene sequences is 99% and 94%, respectively between this bacterium and *Eubacterium limosum*, *Butyribacterium methylotrophicum*, which can convert H_2/CO_2 into acetate through Acetyl-CoA pathway (Li *et al.*, 2011; Drake *et al.*, 2008; Diekert and Wohlfarth, 1994). Parameswaran *et al.* (2011) also found there are homoacetogen in the bio-hydrogen fermentation system. Liu and Suflita (1995) reported that H_2 depletion and growth was delayed for *E. limosum* at yeast extract of 0.2~1.0 $g \cdot L^{-1}$, but H_2 consumption and growth was accelerated at more than 1.0 $g.L^{-1}$ (Liu and Suflita, 1995). Homoacetogens as heterotrophic bacteria firstly utilized glucose when the medium contained glucose. They could also utilize H_2/CO_2 to produce acetate in the glucose-free medium, especially when the hydrogen partial pressure was high in the fermentation system (Loubiere *et al.*, 1992; Zavarzin *et al.*, 1994).

Furthermore, the H_2 production ended earlier (<24 h) than that of control at the 0.2~4.0 $g \cdot L^{-1}$ of yeast extract conditions. These results seemed to indicate that the addition of yeast extract into the medium cause H_2 evolution to rapidly happen and end. Yang and Shen (2006) also found this phenomenon in a research about improving the performance of bio-hydrogen production, while other studies showed the H_2 production ended simultaneously (Chang *et al.*, 2011; Argun *et al.*, 2009; Lee and Hwang; 2009).

The composition of liquid end products showed that the fermentation type was changed when the yeast extract was added into the medium. From Fig.1 and Fig.2, it was known that the butyrate content was consistent with accumulative hydrogen production except for 2.0 and 4.0 $g \cdot L^{-1}$ yeast extract treatments, indicating butyric acid pathway more favored efficient H_2 production. Yang and Shen (2006) also found the high butyrate proportion corresponded high hydrogen production. It is noteworthy that acetate content was increased, but hydrogen was decreased in later fermentation at 2.0 $g \cdot L^{-1}$ and 4.0 $g \cdot L^{-1}$ yeast extract (Fig.1 and 2), indicating hydrogen consumption could be caused by homoacetogenesis.

It can be concluded from the above results that: (1) Yeast extract could improve the yield of the fermentation hydrogen production with the highest hydrogen yield of 1.45 mol/molglucose was achieved at the 0.4 $g \cdot L^{-1}$ yeast extract condition, (2) Hydrogen-consuming function occurred in the latter of the fermentation when the yeast extract concentration was greater than 2.0 $g \cdot L^{-1}$, (3) the yeast extract might cause H_2 evolution rapidly happen and end, and (4) Butyric acid fermentation was more favorable for bio-hydrogen production.

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