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Studies on the effects of pretreatment on production hydrogen from municipal sludge anaerobic fermentation

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ABSTRACT

Municipal sludge was rich in organic matter, period of natural degradation was long and low efficiency, leachate would pollute underground water. In this paper, a comparative study of the ways of pretreatment with acid alkali treatment, heat digestion and ultrasonic treatment were done. The results showed that the dehydrogenase activity was increased, the SCOD (soluable chemical oxygen demand, SCOD) increased more than 2.47~2.83, 1.70~1.76, 2.6~2.77 times respectively. The hydrogen yield increased more than 11.5~12.2, 24.1~24.7, 34.2~34.9 mL.g^{-1} (VS) respectively. The period of pro-hydrogen was shorten to 7.5, 8.0, 6.5 d respectively. The degradation rate was up to 72.04%, 81.4%, 80% respectively, the methane concentration in the gas was close to "zero" and ultrasonic treatment was better than others. Gompertz model curve fitting on hydrogen production was carried out. All the values of correlation factor R2 were more than 0.97.

Keywords: Municipal Sludge; Pretreatment; Anaerobic Digestion; Biological Pro-Hydrogen

1. INTRODUCTION

The wastesolid treatment mainly includes filleading, compost and incineration. Filleading and heap in brief results in resource waste and pollute the body of water, even endanger the human health. Which don't attach to coincidence method of "circulation economy". The wastesolid anaerobic fermentation producted hydrogen develope a new road of wastesolid resource. And many research workers are interested in it [1,2,3,4,5,6,7]. Gomez [8] has study on the two stages of bio-hydrogen production, hydrogen production and methanogenic, using organic solid waste and slaughterhouse waste as substrate, high temperature activated sludge as inoculum. Levin's study [9] showed the wooden fiber of delignification is a good hydrogen production substrate. Liliana [6] use anaerobic sludge to degraded organic solid waste and synthetic wastewater in UASB whose capacity is 3.85 L, and produce hydrogen successfully. The volume content and hydrogen production rate of H_2 is 47%, 99 N mL.g^{-1}(VS); 51%, 127 N mL.g^{-1}(VS) respectively. Zuo Yi [10] used river sediments as seed sludge, at the optimal condition of the pH of 5.0-5.2, temperature of 35℃, and HRT of 6-8 h, a steady anaerobic bio-hydrogen production was obtained in a lab scale reactor successfully with gluose as substrate. The highest hydrogen production was 6.7 L.d^{-1}. Tai Mulin [11] showed that the optimal initial pH for bio-hydrogen production from sewage sludge was around 11.0, Under this optimal condition, the bio-hydrogen yield of raw sludge was 8.1 mL.g^{-1}, and it would reach to 16.9 mL.g^{-1} when the sludge was pretreated by alkali. Steven W. Van Ginkel [12] used food wastewater as substrate indicated Biogas produced from all four food processing wastewaters consistently contained 60% hydrogen, with the balance as carbon dioxide. Heguang Zhua [13] showed enhanced hydrogen production potential as compared with All combinations of the feedstocks (FW+PS, FW+WAS and FW+PS+WAS). A mixing ratio of 1:1 was found to be the best among the ratios tested and hydrogen yield of 112 mL.g^{-1} volatile solid (VS). M. Krupp and R. Widmann [14] studied Biohydrogen production by dark fermentation, the result showed The gas amount varied with the different OLRs, but could be stabilised on a high level as well as the hydrogen concentration in the gas with 44~52%. Ela Eroglua [15] introduced Biological hydrogen production from olive millwastewater with two-stage processes. In some cases of dark-fermentation, activated sludge was initially acclimatized to the OMW to provide the adaptation of microorganisms to the extreme conditions of OMW. The highest hydrogen production potential obtained was 29 L H_2/LOMW. Dongmin Li [16] used corn straw as substrate, Hydrogen was produced by simultaneous saccharification and fermentation from steam-exploded corn straw (SECS) using *Clostridium butyricum* AS1.209.

Maximum specific hydrogen production rate and maximal hydrogen yield were 126 $mL.g^{-1}$ (VSS) d and 68 $mL.g^{-1}$ SECS, respectively. The yield of soluble metabolites was 197.7 $mg.g^{-1}$ SECS. Acetic acid accounted for 46% of the total was the most abundant product and this shows that hydrogen production from SECS was essentially acetate-type fermentation.

Consequently, fermentative bio-hydrogen production technique is at the stage of laboratory research, many hydrogen production bottlenecks binding factors are urgently needed to be solved. This study focused on the factors of fermentative bio-hydrogen production of municipal sludge. In this paper, a comparative study on the effect of pretreatment-acid alkali treatment, heat digestion and ultrasonic treatment on hydrogen production were done, and the optimum pretreatment approach was ascertained, which break new a way for sludge treatment.

2. MATERIALS AND METHODS

2.1. Source and Characteristic of Sludge

Concentrated sludge came from a sewage treatment plant in Guangzhou, China. **Table 1** showed The characteristics of the municipal sludge. In the experiment, the proper complement of N, P and inorganic micronutrients should be added in the sludge. The nutrient solution contained: NH_4HCO_3 2.0 $g·L^{-1}$, $MgSO_4·7H_2O$ 50 $mg·L^{-1}$, NaCl 10 $mg·L^{-1}$, $Na_2MoO_4·2H_2O$ 10 $mg·L^{-1}$, $CaCl_2·2H_2O$ 10 $mg·L^{-1}$, $MnSO_4·7H_2O$ 15$mg·L^{-1}$, $FeCl_2$ 70 $mg·L^{-1}$, KH_2PO_4 10 $mg·L^{-1}$.

2.2. Experimental Equipment

Cylindrical anaerobic reactor (patent number: ZL 20052 0053384.X) with the dimensions: $ø_{diameter}$ = 22 cm, $ø_{external\ diameter}$ = 24 cm, h = 30 cm, effective volume = 11 liters; JY99-IID ultrasonic cell disruptor (Ningbo Xinzhi); XLJ-IIB low-speed tabletop centrifuge (Shanghai an ting Scientific Instruments and Apparatus Co.); SC-15 thermostatic water-circulator bath box (Ningbo Xinzhi); JJ-4 digital display motor stirrer (Jintan City Zhengji Instruments Co. Ltd); BSD0.5 wet-gas flow meter (Shanghai Blue Jewelry); GC-7900 gas chromatograph, thermal conductivity detector, and FID detector (Shanghai Tianmei); ZXZ-1 sliding vane rotary vacuum pump (Zhejiang Huangyanqiujing, modified as shown in **Fig. 1**).

2.3. Experimental Methods

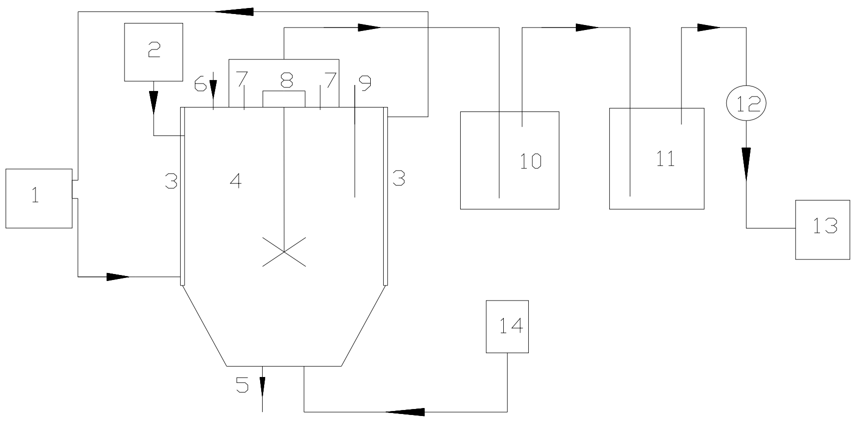
1kg dried sludge was dissolved in 10 L water，stirred uniformly, divided into A, B, C group. This sample would carry out acid alkali treatment, heat digestion and ultrasonic treatment, using 2#, 3#, 4# to mark the sample performed cid alkali treatment, 5#, 6#, 7# to mark the sample performed heat digestion and 8#, 9#, 10# to mark the sample performed ultrasonic treatment. Put 200 mL the liquor in a cone type bottle as reference object which was marked 1#. They were respectively performed anaerobic digestion in shaking table whose rate was 1,050 rpm at 36℃.

2.4. Analysis Methods

Gas components were detected using a gas chromatograph (model: GC-7,900). A flame ionization detector (FID) and a 2-m stainless steel column packed with 5A

Table 1. Characteristics of condensed sludge from municipal wastewater treatment plan.

pH	SS (g/Kg)	Water content (%)	TN	COD
6.7~7.9	13~27	78.7~90	1750~2000	3900~5000



1. SC-15 water bath; 2. pH adjusting port; 3. organic reactor; 4. agitating blade; 5. reaction substrate outlet; 6. material inlet; 7. vent; 8. digital stirrer; 9. thermometer; 10. CO2 removal; 11. desiccant; 12. wet gas flowmeter; 13. GC-7900 gas chromatography; 14. nitrogen purging port.

Figure 1. Equipment and sequence of steps in the anaerobic digestion of sludge.

Molecular Sieve were used to analyze the methane concentration. The temperatures of the injector, detector, and packed column were, respectively, 150, 180, and 100°C. H_2 was used as the carrier gas at a flow rate of 30 $mL \cdot min^{-1}$. The N_2 flow rate was 30 $mL \cdot min^{-1}$ and the air velocity 260 $mL \cdot min^{-1}$. The hydrogen concentration was analyzed using a thermal conductivity detector (TCD) and a 2-m stainless steel column packed with 5A Molecular Sieve. The temperatures of the injector, detector, and packed column were, respectively, 180, 200, and 100°C. N_2 was used as the carrier gas at a flow rate of 29 $mL \cdot min^{-1}$. The injection volume was 10 μL. Quantitative analysis was carried out using external standards.

Otherwise, The quantity of chemical oxygen demand in sludge supernatant fluid was used to estimate the performance of sludge disintegration. The value of TCOD was equal to that of waste activated sludge supernatant fluid. The value of SCOD was equal to that of COD of sludge supernatant fluid which has been treated by centrifugal separation and filtration [17]. Determination of COD followed the standard methods [18]. The centrifuge worked for 20 min at 1,050 rpm, COD was determined according to International Standard [3]. Dehydrogenase activity of sludge was determined according to the method reported in the literature [19,20].

2.5. Cumulative Hydrogen Yield

Cumulative hydrogen yield was estimated using the following equation [21,22]:

$$V = V_0\gamma_i + \sum V_i\gamma_i \quad (1)$$

where V is the cumulative hydrogen yield (mL), V_0 the volume above the liquid level in the reactor (mL), V_i the volume of gas extracted in phase i (i=1,2,3...) (mL), and γ_i the concentration of hydrogen in the gas extracted in phase i (i = 1, 2, 3...) (i = 1, 2, 3...)(%).

2.6. Kinetic Model of Hydrogen Production

The Gompertz equation was used in the regression analysis of the anaerobic hydrogen production data in order to determine the lag time of hydrogen production, the hydrogen production potential, and the hydrogen production rate [22,23]:

$$H = P_s \exp\left\{-\exp\left[\frac{R_s e}{P_s}(\lambda - t) + 1\right]\right\} \quad (2)$$

where H is the cumulative hydrogen yield (mL), P_s the maximum hydrogen yield (mL), R_s the maximum hydrogen yield rate ($mL.h^{-1}$), and λ the lag time of hydrogen production (h).

3. RESULTS AND DISCUSSIONS

3.1. Effect of Acid and Alkali Treatment on Hydrogen Production

Under normal temperature, the SCOD value of different sludge changed. 2#, 3#, 4# were used to mark the sludge pH = 10, 11, 12, respectively. 1# is control group, it is neutral. **Fig. 2** showed the changes of SCOD value of sludge treated by acid alkali treatment. **Fig. 3** showed the state of sludge anaerobic digestion bio-hydrogen production. **Fig. 2** showed that the SCOD value of 2#, 3#, 4# changed with time in the same regular but in different level: 9,266, 9,477, 10,624.5 $mg.L^{-1}$. Compared with 1#, The SCOD value of 2#, 3#, 4# were separately increased 2.47, 2.53, 2.83 times, The data indicated the dissolution of organic increased because of acid alkali treatment. The value of SCOD reached maximum at 24th hour, beginning to decrease at about 24-28th hour. The degradation rate was up to 72.04% from 60.4%, increased by 12%. The reason was that after the sludge was treated by acid alkali treatment, most of the organic has been dissolved, some of the difficult dissolved or-

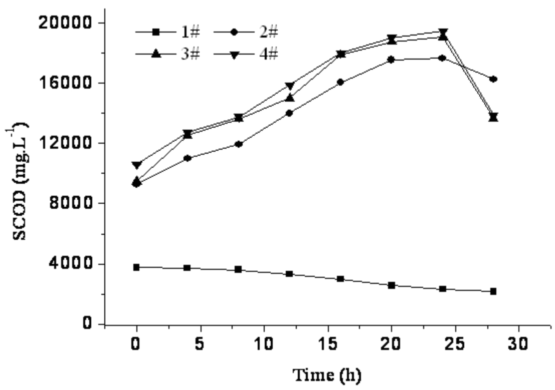


Figure 2. Change of SCOD about Sludge for anaerobic digestion.

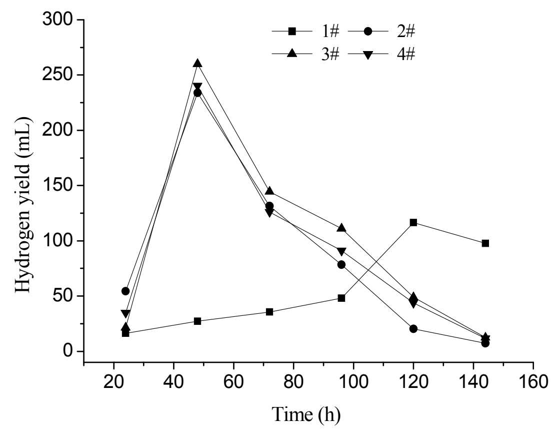


Figure 3. Change of hydrogen production about Sludge for anaerobic digestion ed and modified , and then solidified.

ganic lignose, cellulose and hemicellulose etc had structural changes, hydrolyzed by cellulase, ligninase, etc. 24h after start-up, almost all the organic absorbed by sludge has dissolved. The rate of organic dissolution was faster than that of bio-degradation result in the accumulation of organic in liquor, therefore, SCOD value rose obviously. 28~32 h after start-up, the number of biomass and live bacteria is max in the system. Plenty of carbon source was needed to maintain biological metabolism, so the anaerobic digestion speeded up, the organic was consumed by anaerobic bacteria as nutrient, and the COD started to decline. At the moment a large number of small bubbles attached to the conical flask because of biohydrogen bacterium starting to produce enormous hydrogen. In order to facilitate gas emissions, turn down the rate of shaking table to 1,050 rpm to reduce gas-liquid interface pressure. The peak hydrogen productionin was observed at 32−48th hour, then the sludge hydrogen production ability weakened gradually, and the hydrogen content began to decline. 7.5 d after start-up, litter hydrogen was produced. The hydrogen production yield of 2#, 3#, 4# were 11.5, 12.2, 11.7 $mL.g^{-1}$ (VS) respectively. A little gas was produced at the first day in 1#, and the hydrogen content was low, Less than 30%. The amount of hydrogen started to increase linearly in the second day and reach its peak at the sixth day, but the maximum rate of hydrogen production lasted no more than 2 h. Until the 13th day the gas was too little to collected, therefore the hydrogen production period is 13d. hydrogen production yield is 9.2 $mL.g^{-1}$ (VS). Consequently, after the sludge was treated by acid alkali treatment, the period of hydrogen production was shortened obviously, acid alkali treatment is an effective solution to the problem that the hydrogen production period is too long in sludge anaerobic digestion system. After acid pretreatment, the amount of dissolved organic in sludge increased, which is the same as the effect of alkali pretreatment. The difference between acid pretreatment and alkali pretreatment was that acid pretreatment provided methanogen a good growth condition because of the acidification of substrate and the formation of menthanogenic phase, in order to maintain the systems ability of hydrogen production, it is needed to control the pH value or to use methanogen inhibitor such as acetylene, BES to make the system pro-hydrogen instead of methane.

3.2. Effect of Heat Digestion Pretreatment on Bio-Hydrogen Production

Fig. 4 showed the amount of SCOD in 5#, 6#, 7# rose linearly with the heat digestion time extended and the temperature rose. When the sludge temperature is 80~100℃, the amount of dissolved organic increased obviously. The dissolution rate reached the peak at 93℃, however the rate decreased when the temperature rose

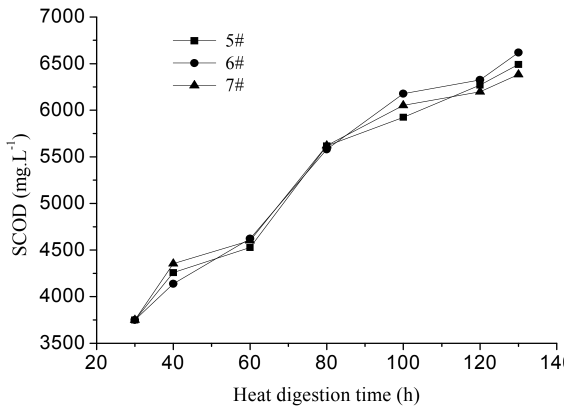


Figure 4. Change of SCOD about Sludge for heat digestion.

to 120℃. There was refractory fiber as well as easily degradable organic in the sludge. **Fig. 5** showed the structure of the fiber. When the temperature was higher than 120℃, the fiber structure was destroyed, decomposing into cellobiose and penta-disaccharide, and then transforming into glucose, degraded by bacteria finally. Therefore, the SCOD value increased slowly. The amount of SCOD in 5#, 6#, 7# were separately increased 1.73, 1.76, 1.70 times. The changes of fermentative bio-hydrogen production were depicted in **Fig. 6** hydrogen production of 5#, 6#, 7# increased considerably 27 h after start-up, and reached the peak in 40~48h, then declined. Little gas could be collected at the 8th day, which was considered as the end of cycle period. The hydrogen production yield of 5#, 6#, 7# is 24.7, 24.1, 24.1 $mL.g^{-1}$ (VS). Heat digestion dissolved the de-lipid of cell, weakened the tolerance ability of cell wall against heat, promoting the hydrolysis of sludge. It was observed that the color of sludge mixed liquor turned into reddish-brown, and the liquor was covered by a layer of film because of the effect of heat. the reason maybe a part of microbiology protein dissolve.

3.3. Effects of Ultrasound Treatment on Anaerobic Sludge Digestion Hydrogen Production

Fig. 7 illustrated changes of anaerobic sludge digestion hydrogen production. On the conditions of P = 1,800 W and f = 35 kHz, the sludge samples 8#, 9#, 10# are treated with ultrasound for 20 min respectively. An increase of dissolved chemical oxygen demand of sludge was observed. The rate of organic matter dissolution can be calculated by Equation (2).

$$DDCOD = \frac{SCOD_t - SCOD_{t0} - SCOD_{pH}}{TCOD - SCOD_0} \times 100\% \qquad (3)$$

In the equation:
DDCOD——rate of organic matter dissolution, %;

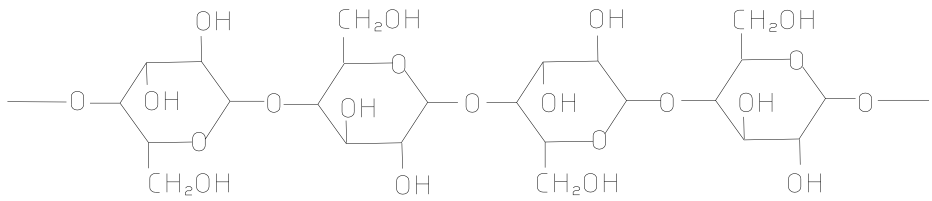


Figure 5. The schematic diagram about the structure of the cellulose.

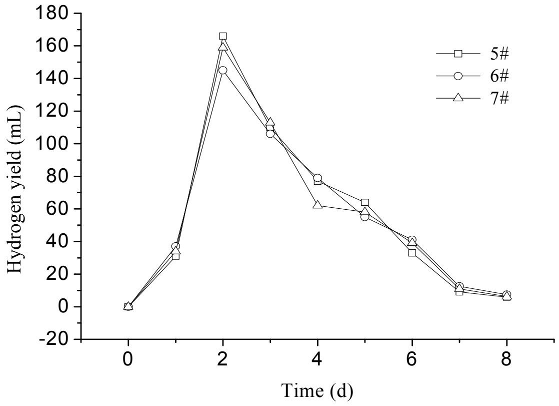


Figure 6. production hydrogen of anaerobic digestion from sludge by heat digestion.

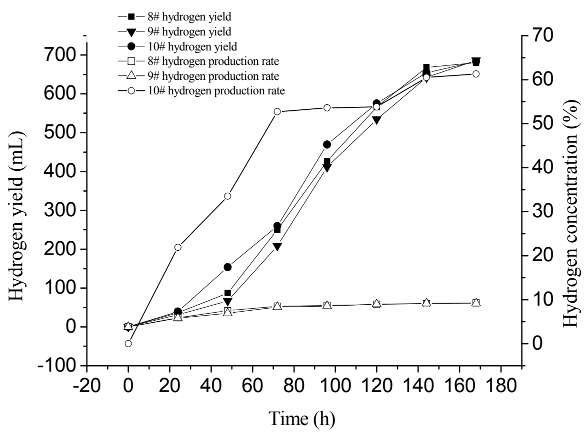


Figure 7. production hydrogen of anaerobic digestion from sludge by ultrasonic wave.

TCOD——the COD of supernatant obtained from the sludge solution, mg/L;

$SCOD_{pH}$——the COD of filtering supernatant of sludge solution under pH, $mg \cdot L^{-1}$;

$SCOD_{t0}$——the COD of supernatant obtained from the sludge solution been centrifuged, $mg \cdot L^{-1}$;

$SCOD_t$——the COD of filtering supernatant of sludge solution under different radiation time, $mg \cdot L^{-1}$.

The SCOD value was 2.70, 2.77, 2.64 times than that of 1# respectively, organic solution rate was 48~65%. Adaptation time of hydrogen-producing bacteria is 17.4 h, and logarithmic phase time is 9 h. The growth and reproduction of microorganisms went into stationary phase after 26 h, which has the largest biomass and the most hydrogen production. Simultaneously, hydrogen production of 8#, 9#, 10# went into the peak phase, and the production of 10# reached 210 $mL.d^{-1}$, then the production began to decline. 7 days after start-up, hydrogen production was less than 10 $mL.d^{-1}$ reaching almost zero at the end of the cycle period. Degradation rate of COD is more than 80%, and the hydrogen production yield is 34.2, 34.9, 34.5 $mL.g^{-1}$ (VS) respectively, and the methane concentration close to "zero". The analysis showed that sludge solution was affected by ultrasonic energy experience dynamic processes of vibration, growth, collapse and closure. At the moment of the collapse of the bubble, high-temperature and high pressure will be created in a very small space around the bubble, which will destruct the floceulent structure of sludge and crush the cell of microorganisms. Intermolecular hydrogen bond of Cellulose which is refractory broke by ultrasonic irradiation, producing organic matter easily biodegradable such as sugar. Consequently the dissolved organic, which provided enough carbon source for the growth and reproduction of anaerobic microbe, multiplied in sludge solution. It was also found that after ultrasonic irradiation, the permeability of cell membrane and cell wall have changed, and the looseness of extracellular polymers increased, which promoted biological mass-transfer and improved the enzyme activity, so the TF (The activity of dehydrogenase was evaluated by the amount of TF which generated by the reaction between per unit mixture liquid sludge and TTC in unit time, the unit is $mg \cdot L^{-1} \cdot h^{-1}$) value rose. **Fig. 7** showed the change trend of dehydrogenase activity. because of the ultrasound pretreatment, the catalysis of dehydrogenase and nitrogenase etc. was improved, as well as the decomposition and absorption ability of anaerobic bacteria and facultative anaerobe [24]. Therefore the degradation of organic speed up. In the stage of peak hydrogen production lots of bio-hydrogen heterotrophic bacteria were observed, such as clostridium, enterobacter, Escherichia coli, Citrobacter, Bacillus, Thiobacillus, etc. by microscopic examination, the most bacteria were enterobacter aerogenes, candida maltose. Synergistic effect between strain is good, which inhibited the accumulation of metabolites and then provided a good environment for hydrogenogens, therefore it was given full play to hydrogenogens, the hydrogen production yield rose.

3.4. Effect of Dehydrogenase Activity by Pretreatment Sludge

Dehydrogenase activity is defined as the TF volume per unit time, with the unit mg·L^{-1}·h^{-1} [25]. From **Fig. 8**, the initial TF was 60.6 mg·L^{-1}·h^{-1}, after pretreatment TF were 71.2, 69.9, 78.8 mg·L^{-1}·h^{-1} respectively.12h later, TF declined to 32, 31.2, 27.7 mg·L^{-1}·h^{-1} respectively. The result showed Dehydrogenase activity was increased with pretreatment. the permeability of cell membrane and cell wall changed because of pretreatment, which promoted the mass transfer, the production and activity of cell enzyme, so the metabolism speeded up. Moreover, NAD+ or NADP+ regenerated by cell can absorb and transport substrate or TTC effectively, therefore the amount of TF increased [26].

3.5. Analyzing Kinetic Model of Hydrogen Production Bacteriaons

Fig. 3, **Fig. 6**, and **Fig. 7** showed hydrogen production closely related to the microbial growth regularity. The change of hydrogen yield contain four phases: lag phase, beginning of hydrogen production, continuous hydrogen production and attenuation of hydrogen production. The lag phase was short (0~11 h). There was no hydrogen production until a stable hydrogen production flora formed after acclimation, cultivation and propagation. Subsequently, hydrogen yield increased gradually with the exponential increase of bacteria. Hydrogen content rose when the growth rate of bacteria was maximum. About 27 h later, the organic content of substrate declined because of the rapid bacteria propagation consuming considerable organic material. Meanwhile, the accumulation of metabolites poisoned bacteria, and bacteria death rate rose. When the growth rate balanced the death rate, the amount of bacteria in the system was maximum. After 80h nutrition was exhausted, the bacteria performed endogenous respiration and even formed spore, hydrogen yield declined until the end. The reaction period was about 160 h. No methane was observed during the reaction. Gompertz model curve fitting on hydrogen production was carried out. All the values of correlation factor R2 were more than 0.97. Therefore the fitting effect of Gompertz model on describing the biohydrogen production process was good.

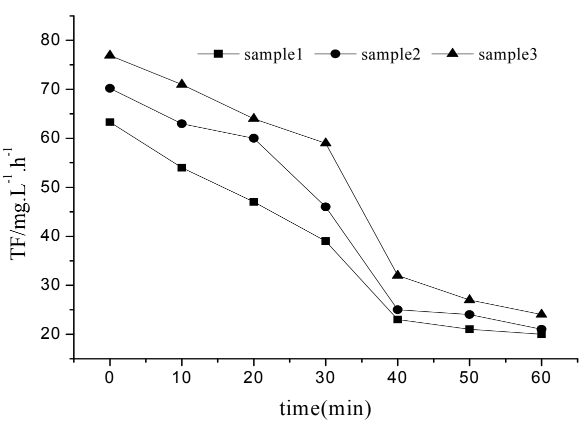


Figure 8. Variation of dehydrogenase activity with pretreatment.

4. CONCLUSIONS

The sludge been treated by acid and alkali, heat digestion, ultrasonic treatment, most of the refractory organic transformed into easily degradable carbohydrate. Compared with control group, the dehydrogenase activity was increased, the SCOD was increased 2.47~2.83, 1.70~1.76, 2.6~2.77 times respectively, the hydrogen production yield were 11.5~12.2, 24.1~24.7, 34.2~34.9 mL.g^{-1}(VS) respectively, the period of hydrogen production was shorten to 7.5, 8.0, 6.5 d respectively. Remove of the COD was up to 72.04%, 81.4%, 80% respectively. the methane concentration in the gas was close to "zero". The hydrogen concentration can reach 99.3% after the bio-gas was purified by $Ca(OH)_2$ saturated solution. Gompertz model curve fitting on hydrogen production was carried out. All the values of correlation factor R2 were more than 0.97.

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