Tolerance of Anaerobic Granular Sludge to Oxygen

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Abstract - **To study the tolerant capacity of anaerobic granular sludge (AGS) to oxygen using semi-dynamic batch experiment, the aerating time, pH of the basal media, reductive inorganic materials, microorganism, and microorganism metabolite were investigated. When the aerating time was higher or lower than 0.5 h, the producing gas activity of sludge was lower than that of the control. The oxygen tolerance of the experimental sludge was the highest at the initial pH 7.2. The producing gas activity of sludge I was higher than that of sludge II. And storage at 4°C can low the lose of the oxygen tolerance capacity of granular sludge. The producing gas activity of sludge was the highest when KI was added. The growth of aerobic microorganisms and some metabolite could increase the producing gas activity of granular sludge.**

Key words : anaerobic granular sludge, oxygen, tolerance

INTRODUCTION

Although anaerobic granular sludge (AGS) is composed of anaerobic microorganisms, facultative microorganisms and some aerobic microorganisms as well as some other organisms, the anaerobic microorganisms are the crucial organisms (Kato *et al.* 1993). Generally, oxygen is considered as a potential toxic compound during anaerobic treatment, especially for the end-of-food-chain, microorganism, the acetogens and principally the methanogens, which are usually regarded as strict anaerobes (Kato *et al.* 1991). However, some studies have demonstrated that anaerobic microorganisms differ in their ability to withstand exposure to oxygen (Rolfe *et al.* 1978). In fact, the performance of anaerobic reactors seldom is upset when natural mixed cultures of AGS were exposed to air for short periods of time (Shen *et al.* 1996; Wu *et al.* 1989). This may attribute to the rapid utilization of dissolved oxygen by facultative and aerobic microorganisms. At the same time, most of anaerobe located deeper in the granular might be protected in this way from contacting with dissolved oxygen (Wu *et al.* 1989; Kato *et al.* 1991; Kato *et al.* 1993). However, few studies have been conducted to access the dissolved oxygen tolerance of AGS and the effects of some factors.

The purpose of this study was to investigate the tolerant capacity of anaerobic granular sludge (AGS) to oxygen and the effects of some factors by semi-dynamic batch experiment.

MATERIALS AND METHODS

1. Seed sludge

The seed sludge, was taken from a 13.6 L laboratory Up-flow Anaerobic Sludge Bed reactor with a working

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volume of 12 L, operated at 37°C and treating synthetical wastewater with hydraulic retention time (HRT) of 5 h.

2. Basal media

The basal media used in the experiments contained (g L^{-1}): yeast extract, 4; industrial glucose, 4; $CH_3COONa \cdot 3H_2O$, 14. The composition of basal minerals and trace elements for the basal media was followed (mg L^{-1}): NH_4Cl, 110; KH_2PO_4, 150; $MgCl_2 \cdot 6H_2O$, 400; $CaCl_2 \cdot 2H_2O$, 150; KCl, 300; Na_2SO_4, 120; $FeSO_4 \cdot 7H_2O$,7; $CoCl_2c6H_2O$,0.17; $ZnSO_4 \cdot 7H_2O$,0.15; H_3BO_3,0.06; $MnCl_2 \cdot 4H_2O$,0.42; $NiCl_2 \cdot 6H_2O$,0.04; $CuCl_2 \cdot 2H_2O$, 0.027; $Na_2MoO_4 \cdot 2H_2O$,0.025; $NaHCO_3$,1000. The initial pH of basal media was adjusted by 10 M NaOH or 10 M HCl and the value was decided by the experiment. The basal media reservoir was conserved in a constant temperature chamber at 4°C.

3. Experiment setup

The construction of experimental equipment was showed in Fig. 1.

4. Experiment methods

50 mL AGS (treated or not) and 100 mL basal media and the additional were put into reactors. After evenly mixing, the reactors were flaked on an oscillator (170 rpm and 35°C) to aerating. The aerating time was decided by the results of experiments. The gas phase was flushed with nitrogen and the liquid phase was bubbled also with nitrogen to provide an anaerobic environment. Then, the experimental equipments were connected according to Fig. 1. The bath temperature was fixed at 35±1°C. The reactors were shaken every 12 hours to mix the contained of the reactors and enhance the escape of biogas. The reactor was connected to a biogas collection cylinder placed in an acidic saturated salt solution of NaCl with 2% of sulfuric acid.

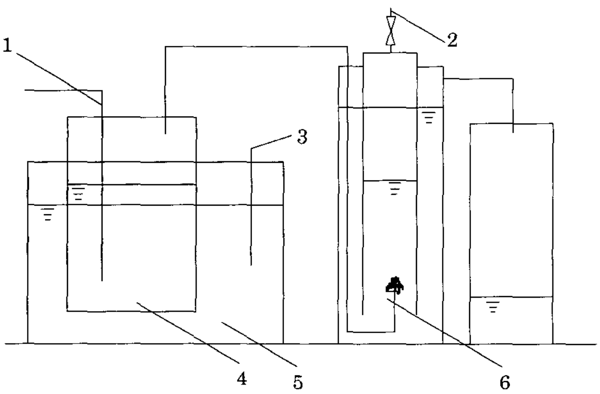

Fig. 1. Schematic diagram of experimental setup.
1. Liquid sampling port; 2. Gas sampling port; 3. temperature control; 4. Reactor; 5. Bath; 6. Gas collection system

5. Analytical methods

The COD, pH and alkalinity of basal media were measured at the start and the end of experiment. The COD, pH and alkalinity tests were conducted in accordance with standard methods (Apha 1992). The gas composition was analyzed by a gas chromatograph (HP 5890A-GC) with a 2-m long and 3-mm inside diameter packed column (Haye-Sep Q, 80/100 mesh) and a thermal conductivity detector (200°C). Helium was used as the carrier gas in the gas chromatography operation with a flow rate of 30 ml/min (Yan and Tay 1997).

RESULTS AND DISCUSSION

1. Tolerant capacity of anaerobic granular sludge to oxygen

In order to study the effects of aerating time on AGS, 0.25, 0.5, 1.0, 2.0, and 3.5 h were investigated. The re-

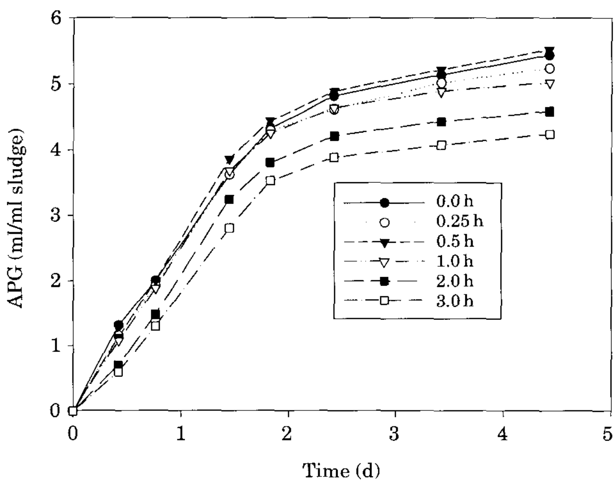

Fig. 2. Determination of aerating time on the APG.

sults are shown in Fig. 2. When the aerating time was 0.5 h, there were few effects on the producing gas activity of granular sludge. The curve of accumulative producing gas (APG) was almost same as that of the control (0 h), and sometime even higher. When the time was higher or lower than 0.5 h, the producing gas activity of sludge was lower than that of the control. The results showed 0.5 h was the optimal aerating time on the oxygen tolerance of the sludge. So, in the later, the aerating time fixed at 0.5 h.

2. Initial pH of basal media

The oxygen tolerance of AGS would be affected by the initial pH of basal media. Therefore, initial pH of basal media were investigated and the results are shown in Fig. 3. From Fig. 3, it was clear that the producing gas activity of the control was the highest, next was pH 8, 6 and 10 in turn. In other words, the oxygen tolerance of the experimental sludge was the highest at the initial pH 7.2. The results also showed the activity of the sludge at pH 8 could recover after about 24 h, while at pH 6 about 48 h. In the later experiments, the initial pH of basal media employs that of the control (7.2).

3. Effects of storages on oxygen tolerance of anaerobic granular sludge

Anaerobic granular sludge was divided into two parts, and one part was stored at 4°C (sludge I), while another was stored at 20°C (sludge II). The storage time was 50 days for each part. Then the stored sludge was used to experiment and the results are shown in Fig. 4. At the first stages (0 ~ 3d), the reactors, which contained sludge I, produced gas was faster than another reactor. The results indicated the producing gas activity of sludge I was higher than that of sludge II. And storage at 4°C can low the lose of the oxygen tolerance capacity of granular sludge. Because of lacking nutrition, the microorganisms of the granular sludge were in the state of dormancy. It is well known that the low temperature makes an important role in the state of dormancy. So the damages of sludge I were lowered than those of sludge II. The results also showed the producing gas activity of granular sludge could keep a long time.

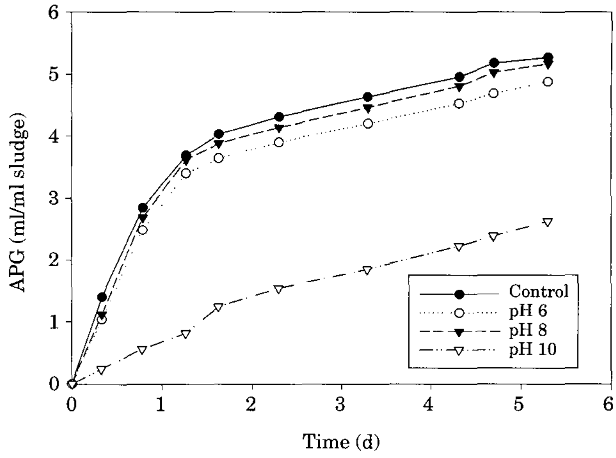

Fig. 3. Effect of pH on the APG.

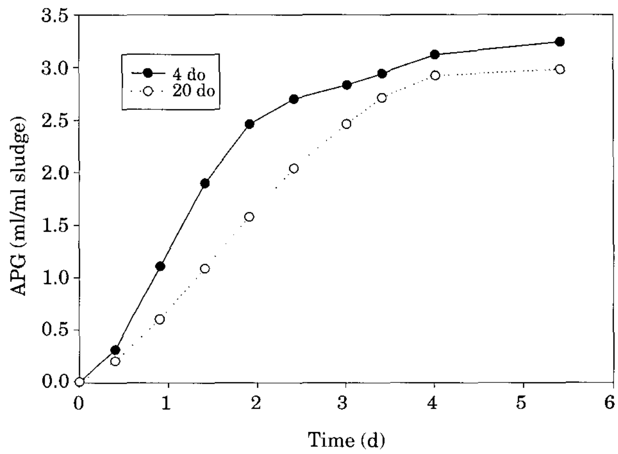

Fig. 4. Effect of storage temperature on the APG.

4. Effects of reductive inorganic material on oxygen tolerance

In order to investigate the effects of these reductive inorganic materials on the oxygen tolerance of AGS, 0.5 g of KI, 0.5 g of $NaHSO_3$ and 0.5 g of Na_2S were added into the reactors respectively. The results are shown in Fig. 5. The producing gas activity of sludge was the highest when KI was added, which suggested the adding of KI could increase the oxygen tolerance of granular sludge. On the other hand, in the case of the use of Na_2S and $NaHSO_3$ both resulted in negative effects. That is, the adding of them would decrease the oxygen tolerance of granular sludge. The results showed some reductive inorganic material could reduce the free oxygen into chemical oxygen and create a relative anaero-

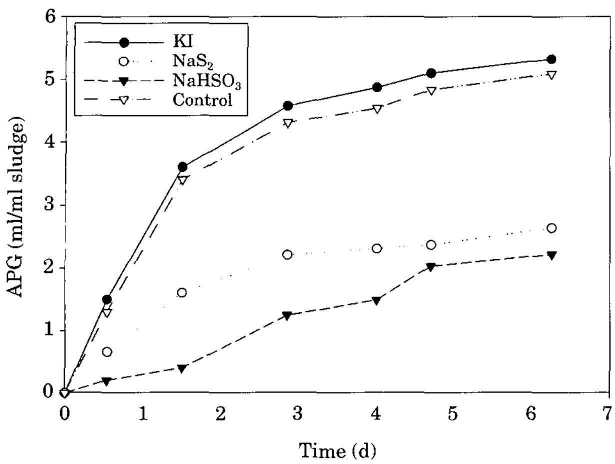

Fig. 5. Effect of reductive inorganic meterial on the APG.

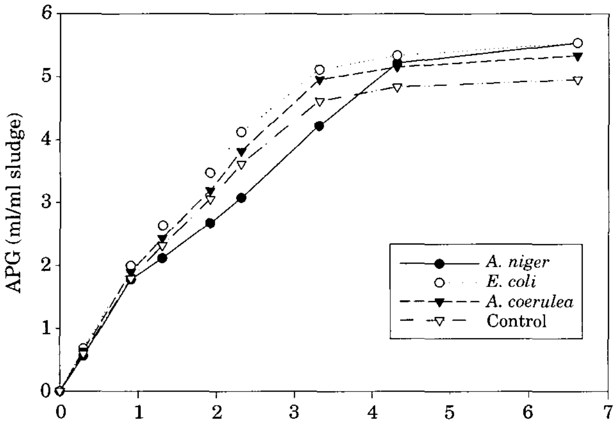

Fig. 6. Effect of various microorganisms on the APG.

bic microenvironment, while some could not. The cause for latter may be those material could not get rid of the dissolved oxygen and maybe would hold up or kill some microorganisms, which are important to remove dissolved oxygen in the liquid phase. At the same time, those materials maybe can change the validity of organic material in the environment.

5. Cooperated efficiencies between microorganisms and anaerobic granular sludge

Since the dissolved oxygen of environment can be removed by chemical means, whether the same results can be obtained by biological means? Three microorganisms (*Esherichia coli* (*E. coli*), Absidia coerulea (*A. coerulea*) *Aspergillus niger* (*A. niger*)) was used in the experiment to studied the possibility of achieving an

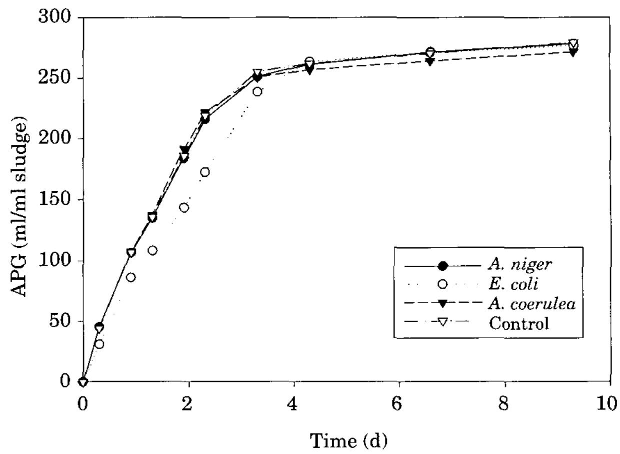

Fig. 7. Effect of various microorgansims metalite on the APG.

anaerobic environment by the metabolism of aerobic microorganisms. The results are shown in Fig. 6. These microorganisms were cultivated under certain condition, under which them to grow well. Then some cultures were mixed with AGS to process the experiment. Though the affecting stages were different, all three microorganisms could accelerate and promote the sludge to produce gas. The affecting stage of *E. coli* was almost the start (1-3d), while A. coerulea the middle (2~4d), A. niger also the middle (3-5d). The results indicated that it is possible to improve the anaerobic activity of sludge by biological means, which can be achieved by the growth of proper microorganism. In addition, there may be exist cooperative effects, which need further study.

6. Effects of microorganism metabolite on oxygen tolerance of anaerobic granular sludge

From the former study, it can be known that the adding of proper aerobic microorganism can improve the oxygen tolerance of sludge. But it could not be decided whether the growth of microorganisms or the metabolite plays the key role. After the microorganisms were autoclaved and mechanically broken, they were mixed with AGS to process the experiment in order to make clear that and the results are shown in the Fig. 7. Comparing Fig. 6 and Fig. 7, it would be found that the difference only lay in the affecting stage, which was from 1st day to 4th day in Fig. 6, while from 3rd day to

6th day in Fig. 7. The results revealed the growth of aerobic microorganism and some metabolite could increase the producing gas activity of granular sludge. However, which metabolite can play a role needs further investigations.

CONCLUSION

The special composition and structure of AGS cause the sludge has certain oxygen tolerance. AGS would tolerance 0.5 h aerates without obvious activity loses. When environment pH was natural (pH 7.2), the oxygen tolerance was the highest. Some reductive materials could improve the oxygen tolerance of the AGS. Storage under 4°C would lower the lost of the oxygen tolerance of anaerobic granular sludge. The oxygen tolerance of AGS could be improved by some live microorganisms and some metabolites.

REFEERENCE

Apha AW. 1992. Standard Methods for the Examination of Wastewater, 18th ed, Washington, D.C., U.S.A.

Kato MT, JA Field and G Lattinga. 1991. Methanogenesis in Granular Sludge Exposed to Oxygen. FEMS Microbiol. Lett. 144:317-324.

Kato MT, JA Field and G Lattinga. 1993. High Tolerance of Methanogens in Granular Sludge to Oxygen. Biotech. Bioeng. 42(11):1360-1366.

Shen CF and SR Guiot. 1996. Long-Term Impact of Dissolved O_2 on the Activity of Anaerobic Granular. Biotech. Bioeng. 49(6):611-620.

Syutsubo K, H Harada and A Ohashi. 1998. Granulation and sludge Retainment during Start-up of a Themophilic UASB Reactor. Wat. Sci. Tech. 38(8-9):349-357.

Rolfe RD and DJ Hentges. 1978. Factors Related to the Oxygen Tolerance of Anaerobic Bacteria. Appl. Environ. Microbiol. 36(2):306-314.

Wu W, X Hu and Y Zhao. 1989. Cultivation of Anaerobic Granular Sludge in UASB Reactors with Aerobic Activated Sludge as Seed. Wat. Res. 21:789-799.

Yan Y and J Tay. 1997. Characterisation of the Granulation Process during UASB Start-up. Wat. Res. 31(7): 1573-1580.

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