Two-stage anaerobic process treating high concentration methanol wastewater and the acid resistance of methanogenic bacteria

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Abstract
In our study, two-stage anaerobic system was employed to treat high concentration methanol wastewater. The results showed the COD removal efficiency was over 90% at the volume loading of 26.8kgCOD/(m$^3$.d) and two-stage anaerobic digestion of methanol wastewater was more efficient and stabler than one stage. The pH of the two UASBs was always lower than 6.0, which wasn’t the suitable condition that common methanogenic bacteria could exert its degradability. Obviously, the methanogenic bacteria of the system had some acid resistance. Static experiments were conducted to investigate it. It was found that methanogenic bacteria, viz. Methanosarcina barkeri, could still live and degraded organic matter at pH5.0. If trained further, their acid resistance would be improved somewhat.

Keywords
high concentration methanol wastewater; two-stage UASB process; methanogenic bacteria; acid resistance

Introduction
As an important material of chemical plants, high concentration methanol always occurred in some chemical wastewater. It is well known that methanol has two fermentation patterns, one is that it can be converted directly by methanogenic bacteria and the other is that it can be translated into acetate firstly and then into methanogenic. Generally, the former is the important reaction and acetate-producing relies on the existence of CO$_2$ or HCO$_3^-$ and trace element(Gonzalez-Gil et al.,1999; Weijma et al.,2001; Liu and Suflita,1994). Because CO$_2$-producing is unavoidable and trace element is important equally for methanogenic-producing, the anaerobic reactor could supply the conditions for acetate-producing from methanol(Lettinga et al.,1981). So some researchers suggested to treat high concentration methanol wastewater by two-phase anaerobic biological system(Lettinga et al.,1979; Zhao Hongbo,1989). Until now, methanol wastewater are treated by aerobic treatment process, anaerobic treatment process, chemical process etc..

In this study, two-stage anaerobic process was adopted to treat high concentration methanol wastewater in order to reduce acidification risk. Through the long term running of the process, we analyze the system’s treating efficiency, stability and acidification conditions. In view of the results obtained from bench-scale experiment, there was a need for a further investigation into the acid resistance of methanogenic bacteria.
Methods

Two laboratory-scale upflow anaerobic sludge blanket (UASB) reactors were used to treat methanol wastewater and their flow schematic was given in Figure 1. The first UASB reactor named U1 was 10 l, 4.1 l in total volume and net volume respectively and the second one named U2 was 18.4 l, 12.5 l respectively. The two reactors were operated at a constant temperature of $35 \pm 1^\circ C$. The UASB reactors were fed with a synthetic methanol wastewater, diluted by pure methanol used in chemical industries. The volume loading of the anaerobic system was changed by the influent COD levels and the hydraulic loading.

When the UASB reactors were steady at $25 \text{kgCOD/m}^3\cdot \text{d}$ for twenty days, granule sludge samples were taken from the second reactor for methanogenic bacteria acid resistance tests. As illustrated in Figure 2, the static experiments were conducted in glass vials with 150ml working volume. Ten vials used for each test were divided evenly into two groups named A and B. Sludge of about 3.7g taken from the second UASB reactor was added directly to the vials along with 75ml feed solution. The feed solution was prepared by pure methanol used in chemical industry and its COD was kept at 6000 mg/l. In the vials of B group, there were several beadings. After finishing the former step, the vials of B group were placed in a shaking bed at a rotate speed of 300rpm. The vials of B group were shaken for 15 minutes in order to break the granule sludge into dispersive bacterium. The pH of the ten vials was set at 4.0, 4.5, 5.0, 5.5, 6.0, respectively. After all of this, the vials were immediately flushed with nitrogen and then sealed with a rubber plug. The vials were placed in a water bath with temperature controlled at $35^\circ C$. The volume of biogas production was measured by aerometer at regular intervals for 8 hours.

![Fig.1. schematic diagram of two-stage UASB process](image1)

![Fig.2. Set-up of static experiment](image2)

Results and discussion

Results of the two-stage anaerobic process

As illustrated in Figure 3, the system of two stages ran normally and its COD removal efficiency was higher than 90%, when the system volume loading was 26.8 kgCOD/(m$^3$.d). If the volume loading exceeded 26.8 kgCOD/(m$^3$.d) for a period of time, the COD removal efficiency of the system would fall gradually and the formation of acetate would be maintained at relatively high level.

The pH of the UASB reactors was always below 6.0, which seemed unfitted for methanogenic bacterium to live and exert them. As seen in Figure 4, the pH of the first UASB reactor changed from 4.9 to 5.8 and the second’s from 5.5 to 6.2. Results showed that the bacterium of the system could perform well at these pH levels.

There were two species of bacteria named acidogenic bacteria, methanogenic bacteria respectively in the two
UASB reactors. Methanogenic bacteria were more important than the other, which consisted mainly of Methanosarcina barkeri and mainly existed in interior space.

Acid resistance of granule sludge and methanogenic bacteria
Effect of pH on the granule sludge and methanogenic bacteria could be described by the gas-producing rate and COD removal efficiency within a period of time. Figure 5 presented the results of static experiments by vials.

Fig. 3. The performance of two-stage anaerobic system
Fig. 4. System’s pH at various phase

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(c) pH 4.0
(d) Effect of pH on COD removal
(in the plots: A: granule sludge, B: methanogenic bacteria. SS=49.22g/L, VSS/SS=0.59)

Fig. 5. Effect of pH on methanogenic bacteria
As illustrated in Figure 5a, there was a difference of acid resistance between granule and methanogenic bacteria. When the pH was between 5.5 and 6.0, the gas-producing rate of granule sludge varied a little and the COD removal efficiency within 8h was almost the same, while the gas-producing rate of methanogenic bacteria at pH6.0 was obviously higher than that at pH5.5. Though the difference of methanogenic bacteria at pH 5.5 and 6.0 wasn’t so distinct, methanogenic bacteria was still affected a little by pH. Along with the pH decreasing from 5.5, the gas-producing rates of granule sludge and methanogenic bacteria were all reducing and the later was quicker than the former, which were presented in Figure 5b. The methanogenic bacteria stopped producing gas at pH4.5 three hours later, while granule sludge could still keep gas-producing rate to a certain extent. At pH4.0, the gas production of all the vials was severely inhibited.

It was evident from Figure 5 that granule sludge could protect the methanogenic bacterium within its body against the impact of acid environment and make them degrade organic matter at pH4.5. The acid resistance of granule sludge was more relevant to the structure of itself. Moreover, the methanogenic bacteria itself had good performance of resisting to acid environment. They degraded organic matter at a high speed when the pH was between 5.5 and 6.0. At pH5.0 they could maintain 30% gas-producing rate of that at pH6.0. If cultivated in acid environment, the methanogenic bacteria would adapt to lower pH.

**Conclusion**

1. The maximum volume loading of two-stage anaerobic process was 26.8kgCOD/(m$^3$.d), moreover, which was higher than one-stage process. Moreover its operating stability and ability of resisting to impulsive loading were apparently better than one stage.
2. In the well digesting two-stage system, the pH was always lower than 6.0 because of methanol fermentation. The pH of the first UASB changed from 4.9 to 5.8 and 5.5 to 6.2 for the second reactor. Apparently, these weren’t the advisable pH levels that common methanogenic bacteria could accept.
3. At pH 4.5, the anaerobic granule sludge could still degrade organic matter with a certain extent, which was attributed to its structure, bacteria species the distributing of bacterium inside the granule.
4. The methanogenic bacteria, especially Methanosarcina barkeri, could adapt to a lower pH of 5.0. If acclimatized further, its acid resistance would be improved apparently.

**References**


