Progress of Research on Two-Phase Anaerobic Fermentation of Hydrogen Production and Methanogenesis

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The technology of two-phase anaerobic fermentation of hydrogen production and methanogenesis has attracted wide attention at home and abroad. It is the key of research on two-phase anaerobic fermentation to raise hydrogen productivity of hydrogen fermentor and the conversion rate of biological energy source of two-phase system. This paper summarizes the latest findings in research and application of two-phase anaerobic technology in the latest years, emphatically introduces the use and operation of substrate, superiority of phase separation and superior bacteria between the two phases in two-phase anaerobic fermentation and puts forward suggestions on the prospect of two-phase anaerobic fermentation.

1. Introduction

Environmental pollution and exhaustion of mineral fuel leads people of the whole world to research and explore renewable energy sources and pay close attention to pollution control. The biotransformation of wastes stimulates the generation of renewable biological energy sources in the process of organic matter decrease (Cantrell et al., 2008). It is the typical application of “waste-energy” mode. In this aspect, extensive and intensive research has been done on “green technology” in the past more than 20 years. Two-phase anaerobic fermentation of hydrogen production and methanogenesis is a method that eliminates organic pollutant from wastes/effluent and recycles energy source. It has promising application prospect among several alternative technologies.

2. Introduction of technique

Two-phase anaerobic fermentation process is an anaerobic biological treatment technology developed by Pohland and Ghosh at the beginning of the 1970s (Carotenuto et al., 2016; Calabrò and Panzera, 2017; Pohland et al., 1971). Comparing with single-phase anaerobic technology, two-phase anaerobic technology can get more methane while collecting hydrogen. In addition, degradation rate of waste organic matters is further raised. Therefore, two-phase anaerobic fermentation of hydrogen production and methanogenesis has attracted more and more attention.

Typical two-phase anaerobic fermentation process is comprised of two independent stages, which is show in Figure 1. Through controlling temperature, pH value and hydraulic retention time in the two phases, the growth of acid-producing bacteria and methanogens in their respective optimal proliferation conditions can be adjusted to make tandem two reactors produce acetic acid and methane respectively. In the first stage, hydrolytic bacteria and acid-producing bacteria transform organic polymers, saccharides, protein and lipidsome to be volatile acid, hydrogen, carbon dioxide and other intermediate products in slightly acid environment (pH: 5.0~6.0). Hydraulic retention time is shortened to be 1~3 days. In the second stage, methanogens grow slowly. Long hydraulic retention time (HRT: 10~20 days) is needed. In alkalescent environment (pH: 7.0~8.0), the volatile acid in hydrogen-producing fermentation liquid is transformed to be methane. Typical components of the fermented gas in the first stage and the second stage are 10% H\textsubscript{2}, 30% CO\textsubscript{2} and 60% CH\textsubscript{4} (Cavinato et al., 2011).

Please cite this article as: Bo Wei, Wenhui Liu, 2018, Progress of research on two-phase anaerobic fermentation of hydrogen production and methanogenesis, Chemical Engineering Transactions, 64, 337-342 DOI: 10.3303/CET1864057
**Figure 1: Two-stage fermentation**

### 3. Substrate

The technology of two-phase anaerobic fermentation of hydrogen production and methanogenesis has been successfully applied in fermentation process of many organic wastes, including (1) activated sludge in urban sewage treatment plant, (2) wastes in industrial production such as food garbage and food sewage, (3) urban solid wastes, including household garbage and wastes in kitchen and (4) agricultural wastes and livestock excrement, including maize straw and manure of livestock. Although two-phase anaerobic fermentation is in its developing stage at present, its application in large scale has been reported lately (Borchiellini et al., 2017; Lee and Chung, 2010; Marino et al., 2017).

In some literatures about gas productivity of fermentation system of hydrogen production and methanogenesis and about load of reactor, food wastes, urban solid wastes and high-concentration organic sewage were widely used as the two-phase anaerobic substrate of hydrogen/methane. Besides, the influence of multi-substrate fermentation (Mtz.-Viturtia et al., 1995) and substrate/seed sludge preprocessing (Ghosh et al., 2000) on two-phase anaerobic technology has been widely researched.

It has been approved that increase of substrate’s concentration contributes to enhancing the activity of hydrogen-producing and methane-producing microorganisms in certain degree. The output of hydrogen/methane in the two phases can be increased. However, if the concentration of substrate is high, the output of hydrogen/methane in the two phases will decrease following the increase of the concentration of substrate (Koutrouli et al., 2009; Li et al., 2017; Sakhrieh et al., 2017). Some substances with complex structure are not the ideal substrate of two-phase anaerobic fermentation. Through certain preprocessing, they can be easily used by two-phase anaerobic process. Yangzhiman (2011) used the lipid extractive of microalgae residue as the fermentation substrate of two-phase anaerobic fermentation of hydrogen production and methanogenesis. Through the preprocessing in NaOH solution (8g/L, 100°C) for 8 hours, the output of methane was increased successfully.

The waste activated sludge containing abundant organic matters in sewage treatment plant is the potential substrate of the two-phase anaerobic process. Through acidification, sterilization, freezing/unfreezing and sonic wave treatment, some preprocessing methods (freezing/unfreezing) can increase output of hydrogen; some other preprocessing methods (sonic wave) can increase output of methane (Hafez et al., 2010). Different substrate preprocessing method has different features. Researchers have reported several methods of preprocessing substrate in two-phase anaerobic process (Yang et al., 2011).
4. Superiority of Phase Separation

4.1 Characteristics of phase separation

In the first stage, hydrolysis and acid-producing bacteria transform the organic matters in waste water to be volatile fatty acids and other intermediate products. In the second stage, methanogenesis bacteria transform preceding volatile fatty acids to be biological energy source (methane) or biological chemicals (such as biological fertilizer, biological coagulant etc.). The strategy of phase separation not only guarantees more organics can be degraded but also proves the high productivity and high purity of target product.

Firstly, through mixed cultivation in the first stage, complex wastes can be degraded to be targeted intermediate products which can be easily utilized. The two-phase system becomes stable and many available substances can be got. The influence on the compounds (ammonia, long-chain fatty acid, sulfide etc.) that restrain functional microbes is relieved. In addition, the intermediate products can be further biologically transformed in the next stage. In research report, methanogens in anaerobic system are more sensitive to change of environment than other anaerobes (Lier et al., 1993). Generally speaking, in use of competitive substrate, it is secondary to other anaerobes represented by sulfate (Pender et al., 2004). Therefore, in acid production phase, acidification process of wastes can be controlled through adjusting hydraulic retention time. Overload of methanogenesis phase can be effectively prevented. In addition, the process preliminarily degrades compounds with rejection capability and enhances stability of the whole system (Ghosh et al., 2000).

Secondly, through proper process control and specific microorganisms utilizing volatile acid, the recycling of biological energy source can be maximized. The optimization of temperature, pH value and hydraulic retention time in process control can especially improve the fermentation performance of high-concentration organics (Kyazze et al., 2007). A prominent character of two-phase anaerobic fermentation is to recycle energy from waste organics. The optimization control of process is to recycle more energy and eliminate more organics (Demirer and Chen, 2005).

Finally, phase separation is widely applicable. Most organics in waste water can degrade to be volatile acid with micro-molecules. They further transform to be valuable terminal products in respective phase.

4.2 Comparison between two-phase operation and single-phase operation

In single-phase anaerobic fermentation process, the H2 produced previously can be transformed to be CH4 and CO2 by methanogens rapidly. Therefore, the proportion of H2 is low in biogas. The available bioenergy is methane. In two-phase anaerobic fermentation process, H2 and CH4 can be collected in respective reactor. Therefore, two-phase anaerobic fermentation is more attractive in the aspect of energy recycling.

1. Output of CH4. Pakarinen (2011) operated batch test. Hydrochloric acid and water are used to preprocess corn substrate and explore the output of gas in single-phase (CH4) and two-phase (H2+CH4) anaerobic fermentation in different substrate preprocessing. In the research, the output of methane after hydrochloric acid preprocessing is 312ml/g VS in single-phase methanogenesis in the conditions of 35°C and pH=7; in the same environmental conditions in two-phase (H2+CH4) anaerobic fermentation process, the output of H2 and CH4 is 20.5 and 368ml/g VS, respectively. The result indicates that phase of hydrogen production has the function of increasing the output of methane in subsequent methanogenesis phase comparing with single-phase anaerobic fermentation. In addition, comparing with single-phase anaerobic fermentation, the initial productivity of methane rises. Above conclusion is also shown in the studies of Yang (2011).

2. Removal rate of organics. Viñas (1993) used the waste water (pH value=4.7, VFA 1400 mg/L and COD 8600 mg/L) in paper pulp processing as substrate. The effect of single-phase anaerobic fermentation and two-phase anaerobic fermentation is compared. Load rate of single-phase anaerobic fermentation is 7000 mg COD/(m3·d); productivity of CH4 is 0.3m3/(kg·COD); removal rate of COD is 75%. Load rate of two-phase anaerobic fermentation is 11000 mg COD/(m3·d); productivity of CH4 is 0.34m3/(kg·COD); total removal rate of COD reaches 90%. Therefore, two-phase anaerobic fermentation has higher volumetric loading rate, productivity of CH4 and removal rate of organics that is higher for 17%.

3. Productivity of energy sources. Elbeshbishy (2011) researched two-phase and single-phase anaerobic reactor processing food residues (TSS, VSS and TCOD are 42.5±2.67, 28.9±2.1 and 91.7±4.75 g/L, respectively). In the same conditions, average productivity of methane in two-phase anaerobic fermentation and single-phase anaerobic fermentation is 2.3 and 1.6L/m2·d respectively; removal rate of TCOD is 54% and 43%, respectively. According to hydrogen heat energy 286KJ/mol and methane heat energy 891KJ/mol,
the overall energy productivity of two-phase anaerobic fermentation and single-phase anaerobic fermentation is 462 and 288KJ/d, respectively. Kvesitadze (2012) used municipal solid waste containing 35~37% lignocellulose as the substrate. In his calculation, for 1 ton of municipal solid waste containing 230kg lignocellulose processed in up-flow bioreactor at temperature 55°C in biogas plant, overall energy output of two-phase hydrogen/methane fermentation is heat energy 2652MJ and electric energy 1203MJ. In the same conditions, overall energy output of single-phase methane fermentation is heat energy 2170MJ and electric energy 980MJ. Lee (2010) and Pakarinen (2011) got similar conclusion in research.

5. Microorganisms

The microorganisms that play the key role in the two-phase anaerobic fermentation process of hydrogen production and methanogenesis are hydrolysis and acid-producing bacteria and methanogens. In existing researches, the reports on two-phase anaerobic microorganisms focus on the superior bacteria groups of hydrogen production and methanogenesis. Table 1 summarizes the microbial populations obtained from the analysis of two-phase anaerobic mixed bacterial strains.

Table 1: Microbial populations obtained from the analysis of two-phase anaerobic mixed bacterial strains reported

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reactor type</th>
<th>Temp. (°C)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitchen waste</td>
<td>Acid reactor MR</td>
<td>55°C</td>
<td>Bacillus coagulans</td>
</tr>
<tr>
<td></td>
<td>Methane reactor MR</td>
<td>55°C</td>
<td>Clostridium spp. Lactobacillus spp. Methanoculleus thermophilus Methanosarcina thermophilis</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>CSTR CSTR</td>
<td>37°C</td>
<td>Klebsiella pneumonia Clostridium pasteurianum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37°C</td>
<td>Clostridium acetobutyricum Thermoanaerobacter siegellii</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caloramator fervidus Thermoanaerobacterium sp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Caldanaerobacter subteraneus Thermodesulfivibrio sp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Thermoanaerobacterium- Thermosaccharolyticum Lactobacilluskeferi</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saccharomyces barnetti Saccharomyces cariocanus</td>
</tr>
<tr>
<td>Garbage slurry</td>
<td>CSTR IRPR</td>
<td>60°C</td>
<td>Methanothermobacter thermautrophicus Methanothermobacter defluvi Methanosarcina mazei</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55°C</td>
<td>Parabacteroides distasonis</td>
</tr>
<tr>
<td>Rice straw residues</td>
<td>Hydrogen bioreactor Methane bioreactor</td>
<td>35°C</td>
<td>C. Perfringens C. botulinum</td>
</tr>
</tbody>
</table>

For analyzing superior bacteria groups of hydrogen production and methanogenesis phase, the methods of PCR-DGGE, PCR-gel electrophoresis and Clone libraries are used. Some scholars once reported the superior bacteria groups of hydrogen production and methanogenesis phase. They further explained the growth of superior bacteria groups in their most suitable conditions in the two phases from micro perspective. The microorganisms being analyzed indicated that H2 could be produced in methanogenesis phase. The H2 produced was consumed by methane bacteria (Kongian et al., 2011). The analysis on the sludge in methanogenesis phase indicated that hydrogen-nutrient methane bacteria and acetic acid- nutrient methane bacteria could exist simultaneously (Park et al., 2010).

6. Conclusion

Since two-phase anaerobic fermentation was put forward, it can degrade most organic pollutants in organic waste water and solid wastes and get renewable energy sources-hydrogen and methane. Comparing with
single-phase anaerobic fermentation, two-phase anaerobic fermentation has high organic load rate, high rate of organics removal and corresponding recycling of heat and electric energy, which especially attracts attention of researchers. In the latest research on the technology, when the fermented liquid of methane phase recycles to hydrogen production phase, appropriate pH value can be maintained without extra alkali addition (Cavinato et al., 2011). However, productivity of hydrogen is low in the first stage of two-phase anaerobic fermentation. The corresponding contribution rate of the energy recycled in the form of H2 is still low in the total high removal rate of COD. Besides, some researches show that two-phase anaerobic fermentation is not suitable for some kinds of waste processing. At the same time, technology shall be chosen based on C/N of substrate. At present, two-phase hydrogen/methane fermentation technology is in its testing stage. It has higher energy productivity comparing with single-phase anaerobic fermentation, but its operation cost is higher because of complex technological process. In this case, economic benefit of two-phase anaerobic fermentation is doubted. With the further research on superior microorganisms and increase of economic benefit, two-phase anaerobic fermentation will be applied widely and the transfer from lab to application in large scale will be accelerated.

Acknowledgments

This paper was supported by Doctoral Scientific Research startup fund of Xinjiang University (BS160253)

Reference


