Bioresource Technology 217 (2016) 44–49

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Biogas production and microbial community shift through neutral pH control during the anaerobic digestion of pig manure

Jun Zhou [a,1], Rui Zhang [a,1], Fenwu Liu [b], Xiaoyu Yong [a], Xiayuan Wu [a], Tao Zheng [a], Min Jiang [a], Honghua Jia [a,*]

[a] College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Nanjing 211816, China
[b] Environmental Engineering Laboratory, College of Resource and Environment, Shanxi Agricultural University, Taigu 030801, China

HIGHLIGHTS

- Biogas production and methane content in pH 7.0 system were higher than others.
- Organic matter degradation rate was highest in pH 7.0 system.
- The major genus at pH 7.0 was *Methanocorpusculum*.
- The major genus at pH 6.0 and 8.0 was *Methanosarcina*.

ARTICLE INFO

Article history:
Received 30 December 2015
Received in revised form 17 February 2016
Accepted 19 February 2016
Available online 26 February 2016

Keywords:
Anaerobic digestion
Biogas
pH
DGGE
Microbial community

ABSTRACT

Laboratory-scale reactors, in which the pH could be auto-adjusted, were employed to investigate the mesophilic methane fermentation with pig manure (7.8% total solids) at pH 6.0, 7.0, and 8.0. Results showed that the performance of anaerobic digestion was strongly dependent on pH value. Biogas production and methane content at neutral pH 7.0 were significantly higher (16,607 mL, 51.81%) than those at pH 6.0 (6916 mL, 42.9%) and 8.0 (9739 mL, 35.6%). Denaturing gradient gel electrophoresis fingerprinting and Shannon's index indicated that the samples contained highly diverse microbial communities. The major genus at pH 7.0 was *Methanocorpusculum*, compared with that was *Methanosarcina* at both pH 6.0 and 8.0. Our research revealed that cultures maintained at pH 7.0 could support increased biogas production, which has significant implications for the scale-up biogas engineering.

1. Introduction

Energy crisis and environmental pollution are among the most pressing global issues. Traditional energy structures worldwide continue to rely mostly on oil, coal, and natural gas; as a consequence, these structures have caused severe pollution (Manyi-Loh et al., 2013). In China, the dependency on fossil fuels should be reduced by creating sustainable energy essential for sustainable economic development. This objective can be achieved by increasing the use of biogas. With the rapid development of intensive pig farming in China in the last two decades, about 209.3 million tons of pig manure is produced annually, and more than 50% of this amount is directly discharged into the environment (Zhang et al., 2014). Pig manure waste causes serious environmental problems because of its high contents of chemical oxygen, suspended solids, nitrogen, and phosphorus compounds. Therefore, effective and economical methods should be developed to treat massive amounts of pig manure to minimize waste and to recover bioenergy.

Anaerobic digestion is widely considered as an optimum approach to treat organic wastes because this process produces sustainable energy; in addition, the slurry and residues produced through anaerobic digestion can be used as agricultural fertilizers (Jiang et al., 2011; Poschl et al., 2010). The produced biogas is commonly used as a source to generate heat and power or as vehicle fuel after upgrading (Petersen et al., 2007). Thus, the utilization of pig manure in the production of clean and renewable energy significantly contributes to environmental protection and energy demand reduction.

Anaerobic digestion involves hydrolysis, acidogenesis, acetogenesis, and methanogenesis; various microorganisms also

* Corresponding author at: College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University, Box 77, No. 30 South Puzhu Road, Nanjing 211816, Jiangsu Province, China. Tel./fax: +86 25 58139929.
E-mail address: hhjia@njtech.edu.cn (H. Jia).
[1] These authors contributed equally to the article.

http://dx.doi.org/10.1016/j.biortech.2016.02.077
0960-8524/

participate in these processes. These microorganisms can be divided into three functional groups: hydrolysing and fermenting bacteria, obligate hydrogen-producing acetogenic bacteria, and methanogenic archaea (Ahring, 2003). The performance of anaerobic digestion systems can be influenced by substrate characteristics (Chandra et al., 2012), incorporated microbial communities (Zhang et al., 2012), and operational conditions, such as pH and temperature (Wang et al., 2012). The pH level suitable for methanogenesis ranges from 6.8 to 7.2 (Ward et al., 2008); the pH levels appropriate for hydrolysis and acidogenesis are 5.5 and 6.5, respectively (Khalid et al., 2011). Furthermore, the effects of pH should be elucidated to increase the biogas production of livestock waste during anaerobic digestion. However, studies about the effects of pH on anaerobic digestion have focused on the pretreatment of pig manure. Lin et al. (2013) investigated the effects of initial pH adjustment (pH 3–12) on the mesophilic hydrolysis and acidification of pig manure, and they found that SCOD and VFAS concentrations in suspensions are enhanced in an alkaline environment. Zhai et al. (2015) investigated the effects of different initial pH on the lab-scale anaerobic co-digestion of kitchen waste with cow manure, and results found that initial pH 7.5 was the best in the co-digestion. The optimal initial pH was 6.81 in the anaerobic co-digestion of swine manure and maize stalk (Zhang et al., 2015) system. However, the microbial activity in complex systems usually alters the pH levels during anaerobic digestion, and the initial pH adjustment may be insufficient to examine the relationship between biogas production effects and microbial communities.

In order to investigated the relationship between the most maximum methane production and the pH values during the anaerobic digestion, this study was performed (1) to examine the effects of different constant pH levels (6.0, 7.0, and 8.0) on biogas production during mesophilic anaerobic digestion with pig manure as the sole substrate and (2) to reveal the microbial community structure at different constant pH during anaerobic digestion through denaturing gradient gel electrophoresis (DGGE).

2. Methods

2.1. Materials

Raw pig manure was used as a substrate in laboratory-scale reactors and was sampled from an ecological farm located in Nanjing, Jiangsu Province, China. Characteristics of the pig manure used in the experiment were as follows: total solids (TS) 25.0 ± 0.2%, volatile solids (VS) 62.6 ± 0.3%, pH value 7.46 ± 0.01, and *C*/*N* ratio 10.4:1. The raw manure was initially stored at 4 °C. Afterward, the raw manure was mixed and diluted with sterilized water to achieve 7.8% total solid (TS) concentration. The diluted manure was then added to the reactors.

2.2. Anaerobic digestion

Batch experiments were conducted in six 1000 mL anaerobic flasks with ground stoppers at a working volume of 750 mL. pH 6.0, 7.0, and 8.0 were obtained by adding 6 mol/L of sodium hydroxide or hydrochloric acid. The pH of the bottles was adjusted simultaneously every day under a nitrogen atmosphere. After the pH was adjusted, each bottle was closed tightly. And then a hole was allotted for biogas collection and sampling. The six bottles were placed in an electro-thermal incubator at 38 ± 1 °C for fermentation, which lasted 20 days. Gas samples were collected using 1 mL injectors daily before the pH was adjusted. Liquid samples were obtained and placed in a 10 mL sterilized volumetric flask every 4 days. The gas samples were immediately analyzed. The liquid samples were stored at −20 °C. All the experiments were conducted in triplicate.

2.3. Chemical analysis of anaerobic digestion

Each collected sample (6 mL) was used to detect TS degradation during anaerobic digestion. Another amount (4 mL) was centrifuged at 10,000 rpm for 15 min, and the supernatant was used to detect total organic carbon (TOC). Sediments were stored at −20 °C for subsequent DNA extraction. The TOC was analyzed using a TOC analyzer (Shimadzu model TOC-5000A). The supernatant was diluted 200 times with deionized water and then auto-sampled in the analyzer. Biogas production and methane content were monitored daily via water displacement method and gas chromatography, respectively. A 500 mL graduated cylinder was used. The gas chromatograph (SP-6800A) was fitted with a HayeSep Q (2 m × ϕ 3 mm) packed column and TCD detector. Experimental data were expressed as the average of triplicate tests.

2.4. Genomic DNA extraction

The 20-day anaerobic digestion samples were collected. Total DNA was extracted from these samples by using an Ultra Clean™ soil DNA isolation kit (Mo BIO Laboratories, Inc., Carlsbad, CA, USA); the extracted DNA was then stored at −20 °C. The procedures were performed in accordance with the manufacturer's instructions. The DNA extracts were electrophoresed in 1% agarose gel and visualized through UV transillumination (Gel Doc 2000, BioRad, Hercules, USA).

2.5. PCR amplification of 16S rRNA gene fragments

The DNA extracts were subjected to PCR in an Eppendorf C1000 Touch thermal cycler (Eppendorf, Hamburg, Germany). The primers are listed in Table 1. The PCR products with approximately 200 bp fragments of 16S rDNA genes were used for DGGE. The PCR mixture consisted of 5 μL of 10× PCR buffer (Mg^{2+} free), 4 μL of 25 mM $MgCl_2$, 4 μL of 2.5 mM dNTP mixture, 0.2 μM of each primer, 1 μL of 10–15 ng DNA template, and 0.5 μL of *Taq* polymerase (Takara, Japan). The solution was filled with sterile water to obtain a final volume of 50 μL. A touchdown PCR was performed to reduce the non-specificity of amplification and to prevent the formation of spurious byproducts. The PCR conditions were as follows: initial temperature of 94 °C for 5 min; 20 cycles of 94 °C for 1 min, annealing at temperatures decreasing from 65 °C to 55 °C at a rate of 0.5 °C per cycle for 1 min, and extension of 72 °C for 1 min; 10 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min; and a final extension step at 72 °C for 10 min. A total of 30 cycles were performed. The PCR products were characterized through electrophoresis on 1.5% (w/v) agarose gel at 120 V, and the DNA was visualized by using BIOimaging Systems (UVP BioImaging Systems, UVP, Inc., Upland, CA, USA).

2.6. DGGE analysis

The PCR products were subjected to DGGE by using CBS Scientific DGGE-2401 System (C.B.S. Scientific Company, Inc., Del Mar, CA, USA). The products were separated on 8% (w/v) polyacrylamide gels by using a denaturing gradient from 30% to 60% (for bacteria and archaea), where 100% denaturant contained 7 mol/L urea and 40% (v/v) formamide. Electrophoresis was run for 12 h at 100 V in 1× TAE (40 mmol/L Tris base, 40 mmol/L glacial acid acetic, and 1 mmol/L EDTA) at a constant temperature of 60 °C. After electrophoresis was conducted, the gels were stained in 150 mL of 1× TAE buffer containing ethidium bromide for 20 min. Visualization and photography were performed as described in the previous