Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2011, 23(3) 497–505
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Microbial population dynamics and changes in main nutrients during the acidification process of pig manures

Dongdong Zhang[1], Xufeng Yuan[1], Peng Guo[1], Yali Suo[2], Xiaofen Wang[1], Weidong Wang[1], Zongjun Cui[1,*]

1. College of Agronomy and Biotechnology/Center of Biomass Engineering, China Agricultural University, Beijing 100193, China. E-mail: zhangdonglucky@163.com
2. College of Life Science, Agricultural University of Hebei, Baoding 071001, China

Received 29 March 2010; revised 19 May 2010; accepted 29 June 2010

Abstract

This study evaluated the impact of pig manure acidification on anaerobic treatment and composition of the fecal microbial community. According to the different chemical oxygen demand (COD) in the anaerobic treatment processes, pig manure was diluted 4 times (×4), 16 times (×16), or 64 times (×64) and subjected to acidification. During the acidification process, pH, soluble chemical oxygen demand (SCOD), volatile fatty acids (VFAs), nitrogen (N), phosphorus (P) and potassium (K) were determined along with microbial population dynamics. The pH of the three dilutions first declined, and then slowly increased. The total VFAs of ×4 and ×16 dilutions peaked on day 15 and 20, respectively. The content of acetic acid, propanoic acid, butanoic acid and valeric acid of the × 4 dilution were 23.6, 11.4, 8.8 and 0.6 g/L respectively, and that of the ×16 dilution was 5.6, 2.3, 0.9 and 0.2 g/L respectively. Only acetic acid was detected in the ×64 dilution, and its level peaked on day 10. The results showed that the liquid pig manure was more suitable to enter the anaerobic methanogenic bioreactors after two weeks of acidification. During the acidification process, total P concentration increased during the first ten days, then dropped sharply, and rose again to a relatively high final concentration, while total N concentration rose initially and then declined. Based on the analysis of denaturing gradient gel electrophoresis (DGGE) and 16S rRNA gene clone library, we concluded that the acidification process reduced the number of pathogenic bacteria species in pig manure.

Key words: pig manure; acidification; microbial population dynamics

DOI: 10.1016/S1001-0742(10)60434-2

Citation: Zhang D D, Yuan X F, Guo P, Suo Y L, Wang X F, Wang W D et al., 2011. Microbial population dynamics and changes in main nutrients during the acidification process of pig manures. Journal of Environmental Sciences, 23(3): 497–505.

Introduction

All over the world, swine production has increased more than 3.5-fold during the past 40 years (Song et al., 2010). The increase of swine herds is associated with a large increase of swine manure. Swine waste is a significant source of fecal pollution and can cause serious sanitary problems because of high chemical oxygen demand (COD), high concentrations of suspended solids, nitrogen and phosphorus compounds and *Clostridium perfringens* counts of 40,000 CFU/mL (Mallin et al., 1997). Because swine waste can lead to watershed pollution due to runoff from rainfall or leaching into groundwater systems, it is important to develop an efficient method for storage and disposal of swine waste.

Biogas fermentation technology has been drawing more and more attention due to the removal efficiency of high-strength wastewater. Swine wastewater represents a large unexploited potential for methane production. Due to the production of methane, a readily usable energy source, and relief of burden of environmental pollution, anaerobic digestion has been widely used for treating swine wastewater (Song et al., 2010). However, few studies have tried to evaluate the impact of pig manure acidification on the anaerobic treatment processes. A more systematic analysis of the acidification process of pig manures would greatly aid in the development of a method for the valuable use of pig slurry to produce methane gas.

With regard to microbial population dynamics, and despite the increasing interest now being paid to the understanding of pig slurry microbiology, few studies have tried to evaluate the impact of the acidification process of pig manure on the composition of the fecal microbial community. Previous studies have focused on the fate of pathogenic or fecal indicators by applying cell culture techniques (Munch et al., 1987). A more thorough analysis of microbial population dynamics using molecular methods should further contribute to our understanding of the metabolic transformations that occur during slurry acidification (Peu et al., 2006).

* Corresponding author. E-mail: acuizj@cau.edu.cn

In this study, the impact of the pig manure acidification process on the anaerobic treatment process and the composition of the fecal microbial community were evaluated. Changes in main nutrients that occurred during the acidification process were determined. At the same time, microbial population dynamics were monitored by denaturing gradient gel electrophoresis (DGGE) analysis and construction of a 16S rRNA gene clone library.

1 Materials and methods

1.1 Pig manure sampling and preparation

Sampling was carried out on an intensive pig-fattening farm located in China Agricultural University. Original pig manure was diluted to 4 times (×4), 16 times (×16), and 64 times (×64) using tap water (Table 1). The diluted pig manure was stored in 15-L plastic buckets at room temperature for acidification. Samples were collected between July 4, 2008 and August 3, 2008. Soluble chemical oxygen demand (SCOD), volatile fatty acids (VFAs), nutrients and microbial communities were analyzed every five days. The pH was measured every day.

Table 1 Proportion of pig manure gradient dilution

	4-times dilution	16-times dilution	64-times dilution
Pig manure (kg)	3	0.75	0.19
Tap water (L)	9	11.25	11.81

1.2 Determination of SCOD and pH of pig manure during the acidification process

Fermented manure (50 mL) was centrifuged at 500 r/min for 1 min, the supernatant was used for determining SCOD using the water quality monitor (LOVIBOND 99731COD, Germany) on day 0, 5, 10, 15, 20, 25, and 30. pH was measured using a HORIBA Compact pH meter (Model B-212, Japan).

1.3 Analysis of the volatile fatty acid content of pig manure

The determination of volatile products was carried out using a GC-MS. On day 0, 5, 10, 15, 20, 25 and 30, samples obtained from the acidizing solution were centrifuged at 8000 r/min for 10 min. The resulting supernatants were filtered through a 0.22 μm pore size filter and analyzed with a GC-MS (model QP-2010, Shimadzu, Japan) on line with a capillary column, CP-Chirasil-Dex CB (25 mm × 0.25 mm). The analytical conditions were as follows: column temperatures, 60°C (for 1 min) → 100°C (for 1 min), 7°C/min → 195°C (for 2 min), 18°C/min; injector temperature, 190°C; ion source temperature, 200°C; carrier gas: He (60 kPa); rate of flow: 34 mL/min; splitter ratio: 1/20; voltage of detector: 0.7 kV; sample volume: 1 μL. The resulting peaks were qualitatively analyzed using the NIST database. Known concentrations of the corresponding compounds were used as standard to confirm the positions of the peaks and for quantitative analysis (Guo et al., 2008).

1.4 Determination of nutrients

Total nitrogen (N) concentration was determined using the Kjeldahl's method. Total phosphorous (P) was extracted by digestion in sulphuric acid and H_2O_2, and its concentration measured by colorimetric analysis (Zeng et al., 2007). Total potassium (K) concentration was analyzed by flame atomic absorption spectrometry (SPECTRAA 55, Varian, USA) (Wei and Jin, 1997).

1.5 Analyses of microbial community using PCR-DGGE

DNA extraction was carried out on day 0, 5, 10, 15, 20, 25 and 30. Fermented manure (20 mL) was centrifuged at 15,000 r/min for 20 min, the supernatant was decanted carefully to obtain the sediment. An extraction buffer was used to preserve the sediment at –20°C. Extraction of DNA was carried out using the benzyl chloride method (Fukumori et al., 1989; Zhu et al., 1993).

16S rDNA PCR amplification was performed using GeneAmp PCR System (Model 9700, Applied Biosystems, USA). The primers used for DGGE were eubacterial universal primers 357F-GC (5′-CCTACGGGAGGCAGCAG-3′) with a GC-clamp (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG-3′), and 517R (5′-ATTACCGCGGCTGCTGG-3′) to amplify the V3 region of 16S rRNA gene (Muyzer et al., 1993). An initial DNA denaturation step was performed at 95°C for 10 min, followed by 30 cycles of denaturation at 93°C for 1 min, annealing at 48°C for 1 min, and elongating at 72°C for 1 min 30 sec, followed by a final elongation step at 72°C for 5 min. The products were examined by electrophoresis on 2% agarose gel (Wang et al., 2006).

Denaturing gradient gel electrophoresis (DGGE) analysis of PCR products was performed with the DCodeTM system (Bio-Rad Laboratories, Hercules, CA, USA) as described by Muyzer et al. (1993) and Haruta et al. (2002). Samples were applied to 1 mm thick, 6%–12% (*W*/*V*) polyacrylamide gradient gels in a 0.5× TAE electrophoresis buffer (20 mmol/L Tris-HCl pH 8.3, 10 mmol/L acetic acid, 0.5 mmol/L EDTA), with a 35%–50% denaturant gradient (where 100% is defined as 7 mol/L urea with 40% formamide). Electrophoresis was performed at a constant voltage of 200 V and a temperature of 61°C for 5 hr. After electrophoresis, the gels were stained with SYBRs Green I (Molecular Probes, Eugene, USA) and photographed, as described previously (Pedro et al., 2001). The bands on DGGE gel were observed under UV 302 nm using the Alpha Imager 2200 Imaging System (Alpha Innotech, USA). The DNA was recovered, and, using the primers 357F (5′-CCTACGGGAGGCAGCAG-3′) and 517R (5′-ATTACCGCGGCTGCTGG-3′) (Haruta et al., 2004), re-amplified using the same program described above. The amplified fragments were purified using the high purity PCR product purification kit (Tiangen Biotech Co., Ltd., China) and sequenced using the ABI 3730XL

DNA Sequencer (Perkin Elmer, USA) at SunBiotech Developing Center. Sequence similarity searches were performed in the GenBank data library using the BLAST Program.

1.6 Cloning and sequence analysis of 16S rRNA gene in plasmid inserts

The samples taken on day 0 and day 30 (×16) were further analyzed. The total DNA extracted prior to PCR-DGGE analysis was used to amplify the 16S rRNA gene with primer pairs 27F and 907R. The PCR reaction began at 95°C for 10 min, followed by 25 cycles of denaturation at 93°C for 1.5 min, annealiation at 50°C for 1 min, and elongation at 72°C for 1.5 min, followed by a final elongation step at 72°C for 5 min. The PCR products were then ligated into pGEM-T Easy Vector (Promega, USA) according to manufacturer's instruction after purification using the TIANgel Midi Purification Kit (Tiangen, China). A total of 148 white colonies were randomly picked and screened by DGGE profile as described above. Clones that produced a single band with different melting positions were selected for sequence analysis. The insert DNA fragments were sequenced using the primers T7 (5′-AATACGACTCACTATAGGG-3′), 515F (5′-GTGCCAGC(A/C)GCCGCGG-3′) and Sp6 (5′-ATTTAGGTGACACTATAG-3′) (Randazzo et al., 2002).

1.7 Phylogenetic analysis

Sequence similarity searches were performed in the GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) using the blast database. The tree was constructed using CLUSTAL X software program and MEGA 4.0 software, referring neighbor-joining method (Gao et al., 2007).

2 Results and discussion

2.1 Changes in pH during the acidification process of pig manure

Figure 1 shows that the pH of the ×4 manure dilution declined rapidly from an initial value of 6.8 to 5.6 on day 11. The pH increased thereafter, reaching a value of 6.1 on day 21 and remained relatively stable at 5.9 on day 24. The pH of the ×16 manure dilution sharply decreased to 6.3 on day 8, continued to gradually decrease to 6.1 up until day 20 and then rose to 7.0 thereafter. The pH of the ×64 manure dilution slowly declined from 7.1 to 6.8 during the first 4 days, remained relatively stable during the next 10 days, and then began to increase. The variations in pH observed are typical of the acidification period. Numerous VFAs are produced at the beginning of the acidification period, thus causing the pH to decrease, and thereafter, with the consumption of VFAs, the pH begins to increase. Based on the above data, the acidification process of the × 4 manure dilution was the slowest, and it was still incomplete after 30 days. The acidification process of the × 64 manure dilution was the fastest, with its pH beginning to increase on day 15.

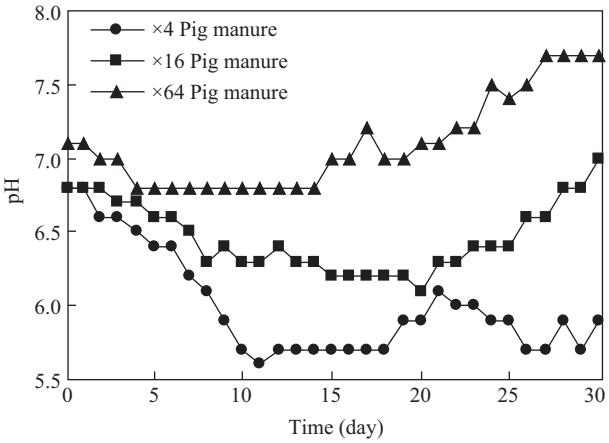

Fig. 1 Changes in pH during the acidification process of pig manures.

2.2 Analyses of changes in volatile products during the acidification process

Results of quantitative analysis of the main volatile products are shown in Fig. 2. Four volatile products were detected in the liquid pig slurry. Among these, acetic acid, propanoic acid, butanoic acid and valeric acid were the more abundant compounds detected. The results of quantitative analysis of the volatile products confirmed that the decrease in pH value of the liquid pig manure was due to the production of VFAs.

The total levels of the volatile products of the ×4 manure dilution increased rapidly, and peaked on day 15. On day 15, the products concentration was as follow: acetic acid, 23.6 g/L; propanoic acid, 11.4 g/L; butanoic acid, 8.8 g/L; valeric acid, 0.6 g/L. The levels of these compounds decreased after day 15.

The total levels of VFAs of the ×16 manure dilution were at the highest on day 20. The concentration were as follow: acetic acid, 5.6 g/L; propanoic acid, 2.3 g/L; butanoic acid, 0.9 g/L and valeric acid, 0.2 g/L.

Only acetic acid was detected in the ×64 manure dilution. Its level peaked on day 10, and the concentration was 1.3 g/L. Both the type and the concentration of VFA decreased in pig manure at ×64 dilution.

The liquid pig manure produced organic compounds of low molecular weight via the acidification process. All these compounds represent good materials for biogas industry. Propanoic acid, butanoic acid and valeric acid can be degraded to acetic acid and hydrogen by hydrogen-producing acetogenic bacteria during the acetic acid production stage of anaerobic treatment processes. Furthermore, methanogens can utilize acetic acid and hydrogen to produce methane (Ren and Wang, 2004). Therefore, in wastewater treatment systems, the more organic acids the liquid pig manure contains, the more methane is produced by methanogens. This indicates that the pig manure dilutions of ×4, ×16 and ×64 should be used as substrates for anaerobic methanogenic bioreactors on day 15, 20 and 10, respectively.

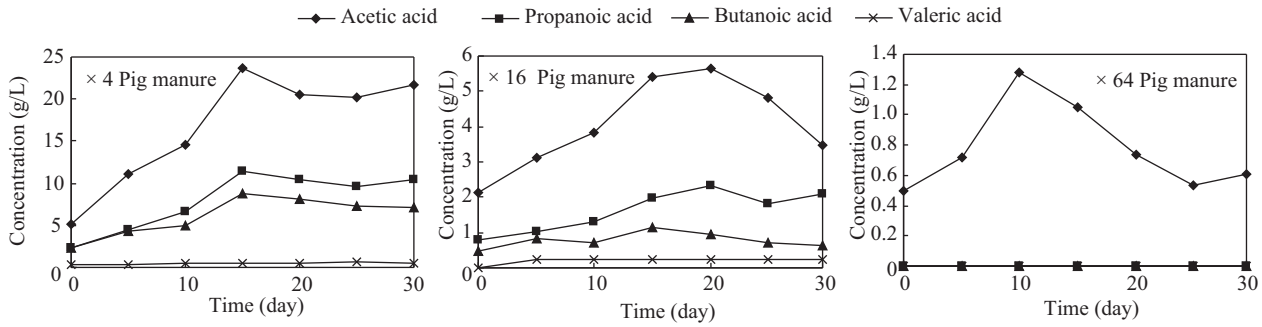

Fig. 2 Quantitative analysis of major volatile fatty acids by GC-MS.

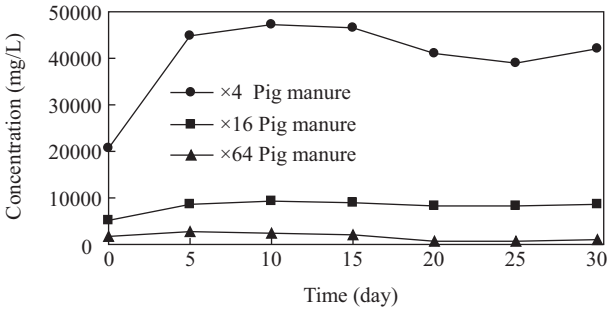

Fig. 3 Concentrations of total SCOD.

2.3 Changes in SCOD during the acidification process of pig manure

Figure 3 shows that SCOD increases substantially at the beginning. On day 10, the SCOD of the ×4 and ×16 manure dilutions peaked at 47,350 and 9395 mg/L, respectively. The SCOD of the ×64 manure dilution peaked at 2777.5 mg/L on day 5.

During the acidification process, the increase of SCOD is due to the accumulation of small molecule organic acids. Generally speaking, SCOD can be expressed by the contents of VFA (Chen et al., 2008). Therefore, swine wastewater is more suitable to enter anaerobic methanogenic bioreactors when the SCOD is at its maximum.

Based on the SCOD data presented above, the liquid pig manure should be treated by anaerobic wastewater treatment system after two weeks of acidification. This correlates with the analytical results abotained for the VFAs.

2.4 Changes of nutrient contents during the acidification process

In order to analyze the dynamic change of nutrient composition in pig manure during the acidification process, the concentrations of N, P and K in swine wastewater were determined.

As Fig. 4a shown, total nitrogen concentrations of the ×16 and ×64 manure dilutions reached the maximum value on day 25 (710 and 180 mg/L respectively), while that of the ×4 manure dilution reached the maximum value on day 20 (3200 mg/L). The dynamic changes of total phosphorus concentrations are shown in Fig. 4b. Total P concentration increased rapidly during the first ten days, then dropped sharply, and rose again to reach a relatively high final concentration. On day 10, total P concentrations

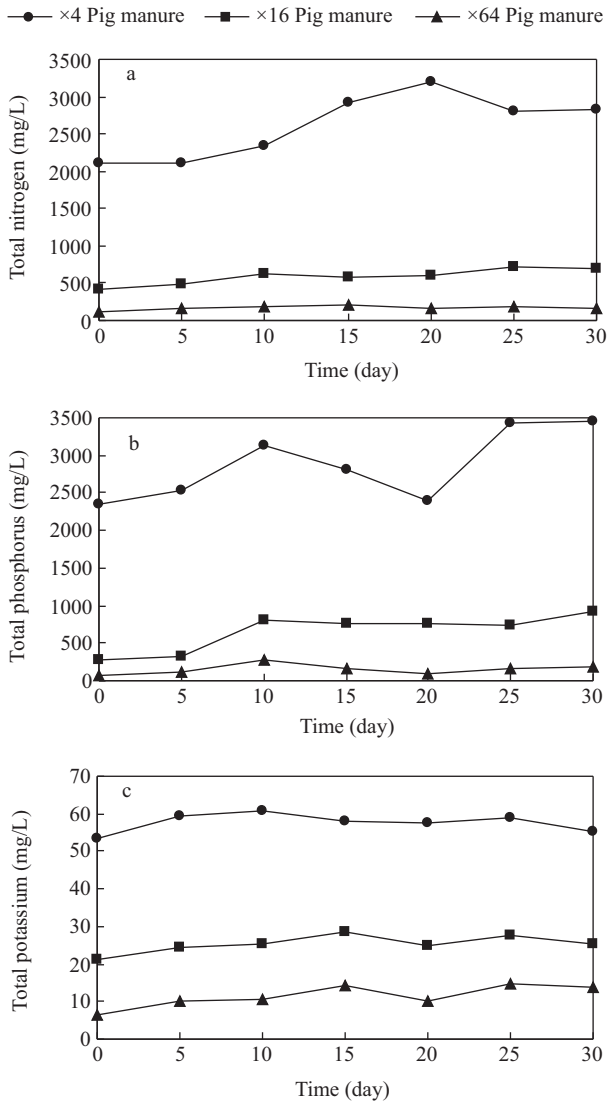

Fig. 4 Concentrations of total nitrogen (a), total phosphorus (b) and total potassium (c) in pig manure.

of swine wastewater of three different dilutions ranged from 2344 to 3136 mg/L (×4), from 280 to 800 mg/L (×16) and from 66 to 280 mg/L (×64). This might be the result of a "concentration effect" (Liu et al., 2008), and the reduction of total P concentrations thereafter, might be due to the fact that a lot of phosphorus was being consumed by microorganisms.

Nitrogen plays a major role in the growth of methanogens. Nitrogen-containing organic compounds generates amino acids after the mineralization and

hydrolyzation process. The amino acids are then absorbed by methanogens (Zhu et al., 2009). Therefore, the amount of nitrogen is directly proportional to the growth of methanogens. If 5% of removed COD was used to generate new cells, the demands for N and P would be 6 and 0.86 kg/1000 kg COD, respectively. The demand for P was about one-seventh of the demand for N during methanogen multiplication (Li, 1996). On the basis of the data of total N concentration, the nitrogen levels of all samples in this study were sufficient for the growth of methanogens. A similar conclusion for P can be made.

The analysis showed that total N and total P concentrations of pig manure were significantly affected by the acidification process. At an earlier stage of acidification, N concentration and P concentration significantly increased. However, the acidification process produced no significant effect on total potassium concentrations (Fig. 4c) compared to the N and P concentrations.

2.5 Analysis of the microbial community

2.5.1 DGGE analysis of the dynamics of pig manure microbial community

The diversity and bacterial dynamic shifts of pig manure during the acidification process were studied by PCR-DGGE (Fig. 5). A total of 33 bands (Bands 1–33) were retrieved from the DGGE gel and several ribotypes were found. The strains identified by genetic relationship represented by the identified DGGE bands were Prevotellaceae bacterium (Band 5, 99%) (the value indicats similarity between the sequence of the band and the closely related reference sequence); *Bacteroides* sp. (Band 6, 96%); *Aquaspirillum* sp. (Band 8, 98%); *Botryosphaeria dothidea* (Band 9, 97%); Uncultured bacterium (Band 10, 96%); Uncultured bacterium (Band 11, 98%); *Acinetobacter lwoffii* (Band 12, 98%); *Acinetobacter lwoffii* (Band 13, 99%); Uncultured bacterium (Band 14, 96%); Uncultured bacterium (Band 15, 97%); Uncultured bacterium (Band 16, 98%); *Botryosphaeria dothidea* (Band 17, 96%); *Alcaligenes* sp. (Band 18, 98%); Uncultured *Pseudomonas* (Band 19, 95%); *Comamonas* sp. (Band 20, 96%); Uncultured bacterium (Band 22, 95%); Uncultured bacterium (Band 23, 99%); Uncultured bacterium (Band 24, 98%); Uncultured bacterium (Band 26, 89%); Uncultured *Hydrogenophaga* (Band 27, 96%); and *Botryosphaeria dothidea* (Band 28, 95%).

The diversity of the bacteria was greatest on day 0. As time progressed, the dominant bacteria in pig manure of different concentrations were distinct. The diversity of the ×4 manure dilution was the lowest, and the dominant bands were 9, 12 and 13, identified as belonging to *B. dothidea* (Band 9) and *A. lwoffii* (Bands 12 and 13). New strong bands (20, 16 and 22) appeared at day 10. They remained constant for the rest of the monitoring period and were identified as *Comamonas* sp. and two uncultured bacteria. According to the previous report, *B. dothidea* is well recognized as a serious pathogen of various woody plants (Smith et al., 1996), and *A. lwoffii* has the ability to induce gastritis (Rathinavelu et al., 2003). Therefore, after the acidification, the species of pathogenic bacteria are not reduced, probably because the low pH is the optimum growth pH for *B. dothidea* and *A. lwoffii*.

The dominant bands of the ×16 manure dilution were Bands 17, 18, 20, 14, 16, and 24, which were related to

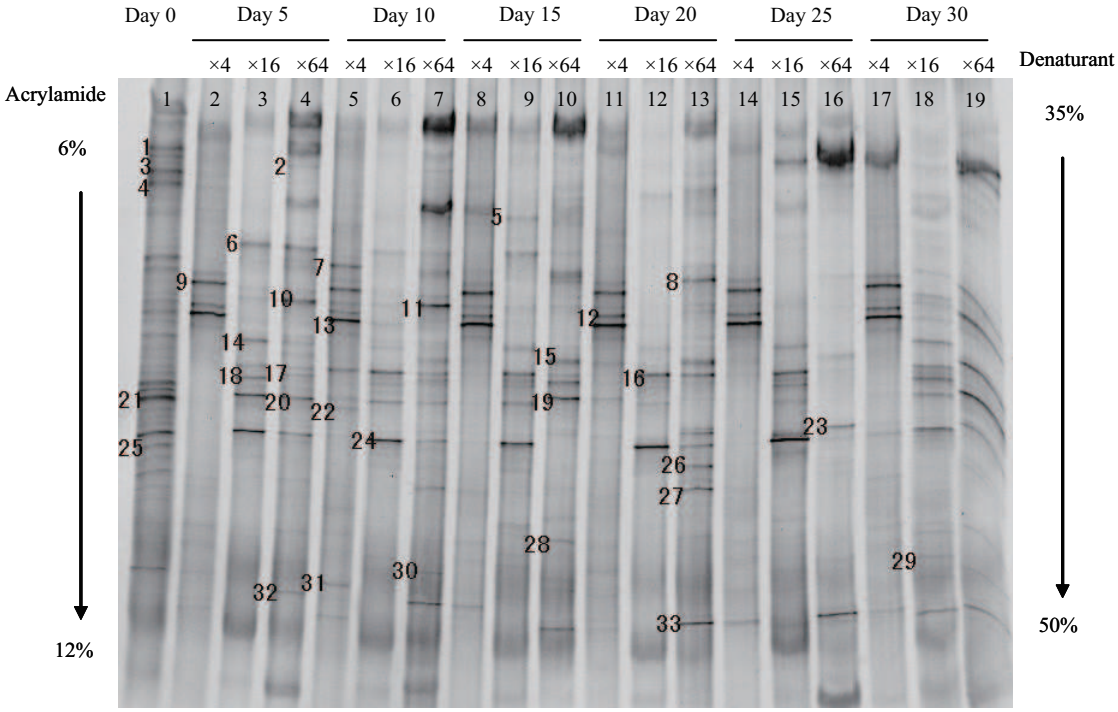

Fig. 5 DGGE profiles of bacterial dynamic shifts during the acidification process. The long arrows represent the direction of the denaturants (35%–50%) and the polyacrylamide (6%–12%) gradients. The bands that migrated to a similar position in the gel were presumed to have similar sequences. Lane 1 is the sample on day 0; lane 2, 5, 8, 11, 14 and 17 are the samples of pig manure diluted 4 times (on day 5, 10, 15, 20, 25 and 30 respectively); lane 3, 6, 9, 12, 15 and 18 are the samples of pig manure diluted 16 times (on day 5, 10, 15, 20, 25 and 30 respectively); lane 4, 7, 10, 13, 16 and 19 are the samples of pig manure diluted 64 times (on day 5, 10, 15, 20, 25 and 30 respectively).

B. dothidea, *Alcaligenes* sp., *Comamonas* sp., and three uncultured bacteria. A new weak Band 5, closely related to Prevotellaceae bacterium, appeared at day 15, and was not detectable at day 20. *Bacteroides* sp. (Band 6) was only detected as a very strong band from day 15 onwards. *Alcaligenes* sp. can cause pneumonia (Pereira et al., 2007), and *Bacteroides fragilis* are both key commensals and important human pathogens (Sears, 2001). Therefore, the species of pathogenic bacteria of the ×16 dilution would be reduced after twenty days of acidification.

In the ×64 manure dilution, the dominant microbial groups were *Aquaspirillum* sp. (Band 8), five uncultured bacteria (Band 10, 11, 15, 16 and 24), and uncultured *Hydrogenophaga* (Band 27), and they could be commonly detected in all DGGE lanes of the ×64 dilution. However, other dominant bands (Band 6, 17, 20, 24 and 27) became weaker and weaker, disappeared during the course of the following days, and corresponded to *Bacteroides*, *B. dothidea*, *Comamonas*, uncultured bacterium and uncultured *Hydrogenophaga*, respectively. Two new strong bands suddenly appeared at day 15. They disappeared thereafter and were identified as uncultured *Pseudomonas* (Band 19) and *B. dothidea* (Band 28). Therefore, the species of pathogenic bacteria of the ×64 manure dilution would be reduced after about three weeks of acidification.

After the acidification process, the species of pathogenic bacteria would be reduced in the ×16 and ×64 manure dilutions. This is in agreement with previous reports showing

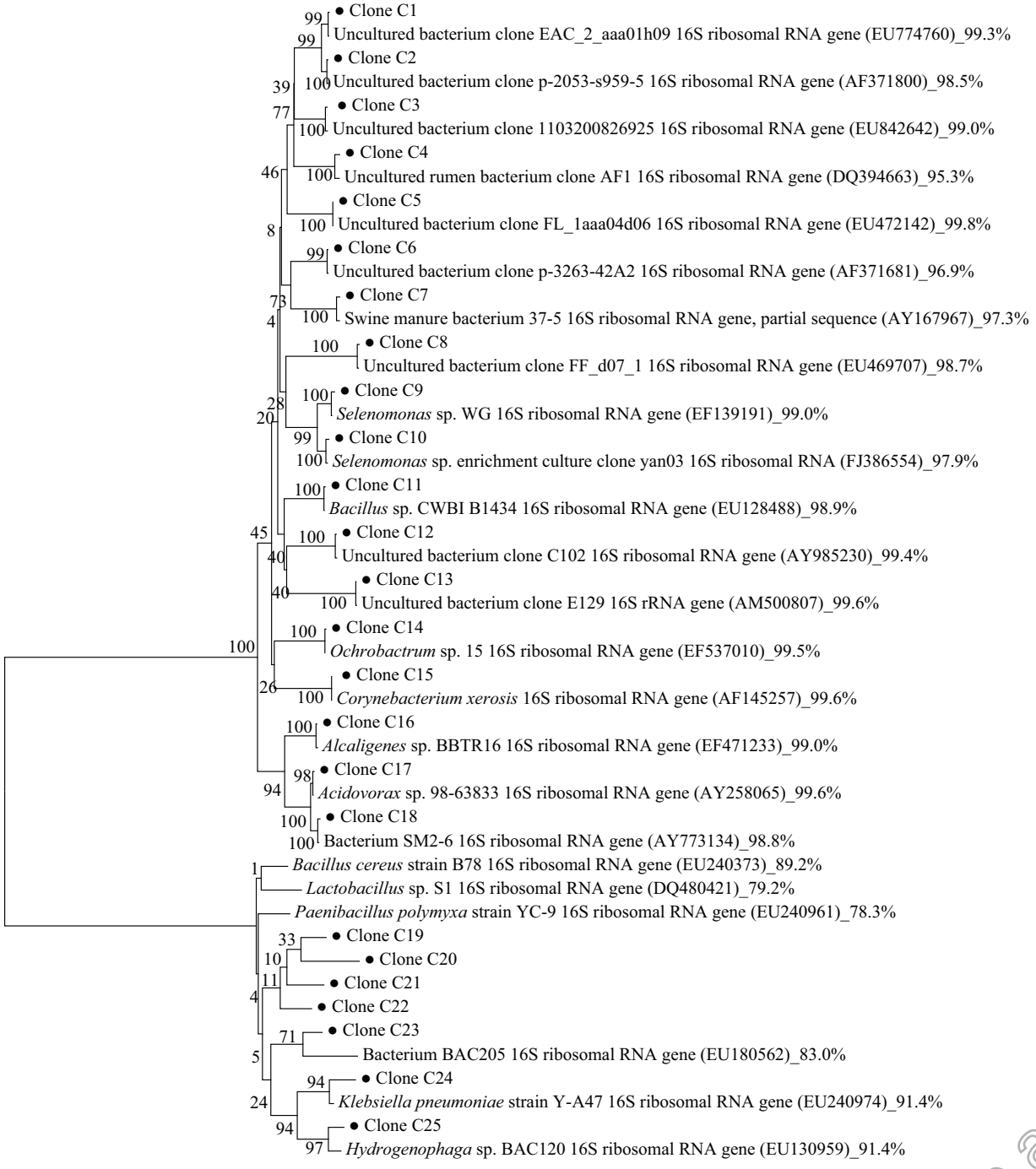

Fig. 6 continued

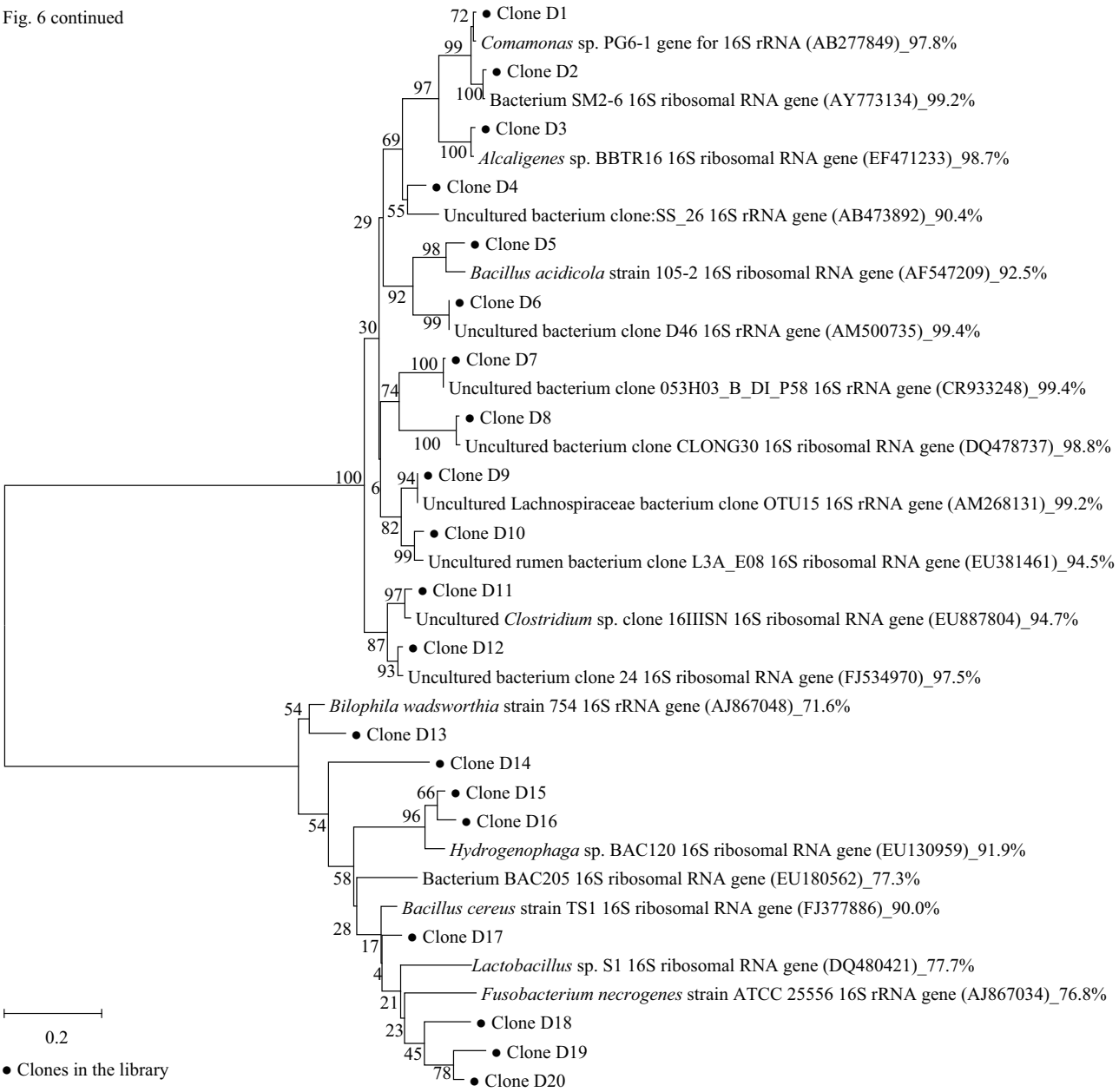

Fig. 6 Phylogenetic trees showing the relationship between representative clones in sample C (initial fecal sample) and sample D (acidified fecal sample) and reference organisms. Reference sequences derived from the GenBank database are shown with their accession number in parentheses, and the similarity values between the clones and the closely related reference sequences are also shown after the underline (_). The trees were constructed by the neighbor-joining method. Bootstrap values were based on 1000 replications shown at branch points and both of bars represent 20% sequence divergence.

that the number of pathogenic bacteria decreases rapidly and exponentially with time during pig slurry storage, but only in the low dose of pig slurry (Munch et al., 1987; Monroy et al., 2009). Although acidification has been shown to reduce the number of human pathogenic microorganisms in pig slurry, little is known about the mechanisms involved and factors that affect the intensity of this effect.

The DGGE profile obtained for pig slurry samples indicated that the diversity of ×4 manure dilution was the lowest, while higher diversity was observed in the ×16 and ×64 dilutions. This is probably because the low pH was not the optimum growing environment for the majority of bacteria. The dominant microbial communities in pig manures at three different concentrations were quite distinct from each other. This may be due to the significant difference of pH or nutrients. Further work is needed to investigate this cause.

2.5.2 Analysis of 16S rRNA gene clone library and phylogenesis

Because DGGE analysis showed that the diversity of the ×16 manure dilution is the highest after acidification, the acidifying fecal sample was further analyzed at day 30. The composition diversity of microorganisms and bacteria dynamic shifts before and after acidification were investigated by construction of a 16S rRNA gene clone library. A phylogenetic tree based on complete 16S rRNA

gene sequences of clones is shown in Fig. 6. To select unique clones prior to sequence analysis, colony-PCR was applied to 96 clones, and the resulting amplification products were screened by DGGE analysis. The clones with different melting positions were selected for sequence analysis. Twenty five different clones were identified in initial fecal samples, while 20 different clones were identified in acidifying fecal samples, thus showing that the species diversity is reduced after acidification. This result correlates with the results obtained from the DGGE profile.

The closest relative species corresponding to clones that disappeared after acidification were *Acidovorax* sp. (clone C17), *Ochrobactrum* sp. (clone C14), Swine manure bacterium (clone C7), *Klebsiella pneumoniae* (clone C24), *Selenomonas* sp. (clone C9), *Corynebacterium xerosis* (clone C15).

K. pneumoniae is a major cause of nosocomial infections and may also cause widespread outbreaks, particularly when multi-resistant (Legakis et al., 1995). *C. xerosis* is a rare cause of endocarditis, mainly affecting immunocompromised patients and those with predisposing cardiovascular lesions (Pessanha et al., 2003). And *Selenomonas* can cause serious human diseases including bacteraemia (Pomeroy et al., 1987).

Another pathogenic bacterium, which could still be detected after acidification, was closely related to *Bacillus cereus*. *B. cereus* has at least one gene or component involved in human diarrhoeal disease, while emetic toxin was related to only one *B. cereus* strain (Rosenquist et al., 2005).

Taken together, our clone results clearly demonstrate that the species diversity of pathogenic bacteria is greatly reduced after acidification. This also correlates to the results obtained by DGGE profile.

3 Conclusions

Based on the experimental results, several conclusions could be made. (1) The SCOD and VFAs of pig manure increased sharply during the two weeks of acidification. Therefore, the pig manure after two weeks of acidification was more suitable to be treated by anaerobic wastewater treatment systems. (2) The acidification process had a significant impact on total nitrogen and total phosphorus concentrations of pig manure, while it had no significant effect on total potassium concentration. The concentrations of total N and total P in liquid pig manure increased at the very beginning, and then dropped, but the N and P levels of all samples in this study were sufficient for the growth of methanogens. (3) Based on the analysis of DGGE and 16S rRNA gene clone library, after the acidification process, the species of pathogenic bacteria were reduced in the ×16 and ×64 dilutions. Therefore, the acidified fecal material has a reduced pollutant effect on the environment and contains fewer human pathogens.

Acknowledgments

This work was supported by the National Key Technology Research and Development Program of China during the 11th Five-Year Plan Period (No. 2008BADC4B01, 2008BADC4B17) and the National Water Pollution Control and Management Special Project of China (No. 2008ZX07209-003).

References

Chen J, Liu H, Li X F, Hua Z Z, 2008. Protocols of Environmental Microbiology. Chemical Industry Press, Beijing. 175–177.

Fukumori F, Kudo T, Sashihara N, Nagata Y, Ito K, Horikoshi K, 1989. The third cellulase of alkalophilic *Bacillus* sp. strain N-4: evolutionary relationships within the cel gene family. *Gene*, 76: 289–298.

Haruta S, Cui Z, Huang Z, Li M, Ishii M, Igarashi Y, 2002. Construction of a stable microbial community with high cellulose-degradation ability. *Apply Microbiology Biotechnology*, 59: 529–534.

Haruta S, Kondo M, Nakamura K, Chaunjit C, Aiba H, Ishii M et al., 2004. Succession of a microbial community during stable operation of a semi-continuous garbage-decomposing system. *Journal of Bioscience and Bioengineering*, 98(1): 20–27.

Gao L J, Wang X F, Yang H Y, Li X M, Cui Z J, 2007. Construction and composition diversity of a lactic acid bacterial community SFC-2. *Environmental Science*, 28(5): 1088–1094.

Guo P, Wang X F, Zhu W B, Yang H Y, Cheng X, Cui Z J, 2008. Degradation of corn stalk by the composite microbial system of MC1. *Journal of Environmental Sciences*, 20(1): 109–114.

Legakis N J, Tzouvelekis L S, Hatzoudis G, Tzelepi E, Gourkou A, Pitt T L et al., 1995. *Klebsiella pneumoniae* infections in Greek hospitals. Dissemination of plasmids encoding an SHV-5 type β-lactamase. *Journal of Hospital Infection*, 31(3): 177–187.

Li Y X, 1996. The inorganic nutritional requirements of methanogens during the anaerobic digestion. *China Biogas*, 14(1): 1–5.

Liu D Y, Xu J G, Yuan L, Li S Q, 2008. Composting of pig manure under low temperature conditions's dynamic change of nutrient. *Journal of Northeast Agricultural University*, 39(11): 32–35.

Mallin M A, Burkholder J M, Mciver M R, Shank G C, Glasgow H B, Touchette B W et al., 1997. Comparative effects of poultry and swine waste lagoon spills on the quality of receiving streamwaters. *Journal of Environmental Quality*, 26: 1622–1631.

Monroy F, Aira M, Dominguez J, 2009. Reduction of total coliform numbers during vermicomposting is caused by short-term direct effects of earthworms on microorganisms and depends on the dose of application of pig slurry. *Science of the Total Environment*, 407: 5411–5416.

Munch B, Larsen H E, Aalbaedk B, 1987. Experimental studies on the survival of pathogenic and indicator bacteria in aerated and non-aerated cattle and pig slurry. *Biological Wastes*, 22: 49–65.

Muyzer G, Waal E C D, Uitterlinden A G, 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, 59: 695–700.

Pedro M S, Haruta S, Hazaka M, Shimada R, Yoshida C, Hiura K et al., 2001. Denaturing gradient gel electrophoresis anal-

yses of microbial community from field-scale composter. *Journal of Bioscience and Bioengineering*, 91(2): 159–165.

Pereira G H, Muller P R, Levin A S, 2007. Salvage treatment of pneumonia and initial treatment of tracheobronchitis caused by multidrug-resistant Gram-negative bacilli with inhaled polymyxin B. *Diagnostic Microbiology and Infectious Disease*, 58(2): 235–240.

Pessanha B, Farb A, Lwin T, Lloyd B, Virmani R, 2003. Infectious endocarditis due to *Corynebacterium xerosis*. *Cardiovascular Pathology*, 12: 98–101.

Peu P, Brugère H, Pourcher A M, Kérourédan M, Godon J J, Delgenès J P et al., 2006. Dynamics of a pig slurry microbial community during anaerobic storage and management. *Applied and Environmental Microbiology*, 72(5): 3578–3585.

Pomeroy C, Shanholtzer C J, Peterson L R, 1987. *Selenomonas* bacteraemia – Case report and review of the literature. *Journal of Infection*, 15(3): 237–242.

Randazzo C L, Torriani S, Akkermans A D L, de Vos W M, Vaughan E E, 2002. Diversity, dynamics, and activity of bacterial communities during production of an artisanal sicilian cheese as evaluated by 16S rRNA analysis. *Applied and Environmental Microbiology*, 68: 1882–1892.

Rathinavelu S, Zavros Y, Merchant J L, 2003. *Acinetobacter lwoffii* infection and gastritis. *Microbes and Infection*, 5: 651–657.

Ren N Q, Wang A J, 2004. Principle and Application of Anaerobic Biotechnology. Chemical Industry Press, Beijing. 20–21.

Rosenquist H, Smidt L, Andersen S R, Jensen G B, Wilcks A, 2005. Occurrence and significance of *Bacillus cereus* and *Bacillus thuringiensis* in ready-to-eat food. *FEMS Microbiology Letters*, 250(1): 129–136.

Sears C L, 2001. The toxins of *Bacteroides fragilis*. *Toxicon*, 39: 1737–1746.

Smith H, Wingfield M J, Petrini O, 1996. *Botryosphaeria dothidea* endophytic in eucalyptus grandis and eucalyptus nitens in South Africa. *Forest Ecology and Management*, 89: 189–195.

Song M, Shin S G, Hwang S, 2010. Methanogenic population dynamics assessed by real-time quantitative PCR in sludge granule in upflow anaerobic sludge blanket treating swine wastewater. *Bioresource Technology*, 101: S23–S28.

Wang X F, Haruta S, Wang P, Ishii M, Igarashi Y, Cui Z J, 2006. Diversity of a stable enrichment culture which is useful for silage inoculant and its succession in alfalfa silage. *FEMS Microbiology Ecology*, 57: 106–115.

Wei R, Jin Z Y, 1997. Determination of potassium and sodium in drinking water by flame atomic absorption spectrometry. *Journal of Environment and Health*, 14(6): 270–271.

Zeng S C, Chen B G, Jiang C A, Wu Q T, 2007. Impact of fertilization on chestnut growth, N and P concentrations in runoff water on degraded slope land in South China. *Journal of Environmental Sciences*, 19(7): 827–833.

Zhu H, Qu F, Zhu L H, 1993. Isolation of genomic DNAs from plant, fungi and bacteria using benzyl chloride. *Nucleic Acids Research*, 21: 5278–5280.

Zhu Z Q, Cheng G W, Liang B, 2009. Experimental study on methane fermentation potential about organic waste in agriculture. *Agricultural Machinery Research*, 2: 150–152.