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# Effect of alkaline treatment pattern on anaerobic fermentation of swine manure

Lin Lin[a,b], Lei Wen[a], Si Chen[a], Xue Yang[c,d], Xiang Liu[a], Chunli Wan[a,*]

[a] Department of Environmental Science and Engineering, Fudan University, Handan Road, Shanghai 200433, China
[b] Department of Civil Engineering, Environmental Engineering Research Centre, The University of Hong Kong, Pokfulam Road, Hong Kong
[c] Postdoctoral Research Station of Civil Engineering, Tongji University, Siping Road, Shanghai 200092, China
[d] State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, Siping Road, Shanghai 200092, China

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ABSTRACT

Swine manure contains large amount of organic resource, turning to be a favorable substrate for volatile fatty acids (VFAs) production. Alkaline treatment has demonstrated an effective way to enhance VFAs production. To optimize the alkaline treatment pattern, swine manure fermentation under initial pH 10.0 and continued pH 10.0 conditions was carried out in batch experiments, to evaluate their efficiency on hydrolysis and acidogenesis. The results showed that compared with continued pH 10.0 adjustment, initial pH 10.0 pretreatment achieved similar VFAs production at 11939 mg-COD/l within shorter period. Kinetic analysis demonstrated the rate-limiting step for fermentation under alkaline adjustment was acidogenesis not hydrolysis. High throughput sequencing was applied to investigate microbial community, showing that new non-spore-forming Lachnospiraceae and Porphyromonadaceae enriched during initial pH 10.0 fermentation, while spore-forming Bacillaceae and Sporolactobacillaceae dominated at constant pH 10.0 condition. With lower chemical dosage and higher acidogenesis rate, initial pH 10.0 pretreatment is more favorable than continued pH 10.0 adjustment for swine manure fermentation.


## 1. Introduction

In China, the contribution of agricultural source to the chemical oxygen demand (COD) and nutrients (nitrogen and phosphorus) discharges into water has exceeded the industrial and domestic sources nationally [1]. Around 0.58 billion of swine manure is produced annually, amounting to one third of the total livestock wastes, which has become a serious challenge for rural environment protection [2].

Anaerobic fermentation (both hydrolysis and acidogenesis included) has been widely studied recently to convert organic wastes to soluble volatile fatty acids (VFAs), which can be utilized as the appropriate substrate for polyhydroxyalkanoates (PHA) production [3,4], as well as the supplemented carbon source for biological nutrient removal reactions [5]. As seen from literature (Table 1), alkaline pH treatment ranging pH 8 to pH 12 has been proven to be an effective way of accelerating hydrolysis and acidogenesis.

As high-strength organic waste, swine manure usually contains over 10,000 mg-COD/l [14–16]. However, limited attempts have been made to investigate the potential of using swine manure as the sole carbon feedstock to produce VFAs. Our previous study revealed that swine manure is a favorable substrate for anaerobic fermentation, and adjusting the initial pH to 10.0 produced 67% more VFAs under mesophilic condition [17]. Although most studies focused on continued pH adjustment during fermentation to maximize hydrolysis extent, the operation is inconvenient and costly owing to extended chemical addition. On the other hand, just by initial adjustment without further pH control, the hydrolysis enhancement might be incomplete due to the high buffering capacity in manure. Until now, no experiments have been conducted to study the fermentation performance at different patterns of alkaline treatment (i.e. initial and continued pH adjustment), so their relative efficiencies on hydrolysis and acidogenesis remain unclear.

The purpose of this study was to investigate the difference between the initial and continued treatment at pH 10.0 condition in swine manure fermentation process at mesophilic temperature. Organics release and VFAs production were studied, and kinetic tests were applied to better understand the rate-limiting step during the fermentation process under alkaline condition. Finally, the

* Corresponding author. Fax: +86 21 65643597.
*E-mail address:* hitwan@163.com (C. Wan).

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**Table 1**
Recent studies on anaerobic fermentation of organic wastes treated by alkaline pH adjustment.

| No. | Substrate | Best pH condition | | VFAs production | | Dominant bacteria | Reference |
|---|---|---|---|---|---|---|---|
| | | Value | Initial/continued | Yield | Enhancement[a](times) | | |
| 1 | Waste activated sludge | 10 | Continued | 250.4 mg VFAs/g VSS | 4.16 | NA | [6] |
| 2 | Waste activated sludge and rice | 8 | Continued | 520.1 mg COD/g VSS | 2.46 | *Clostridia*, *β-Proteobacteria*, *Bacteroidetes* | [7] |
| 3 | Waste activated sludge | 10 | Continued | 129.21 mg VFAs/g VS | 11.92 | *Anoxynatronum sibiricum* | [8] |
| 4 | Waste activated sludge | 10 | Initial | 134.2 mg VFAs/g VSS | 2.09 | Bacteroidia, $\epsilon$-proteobacteria, Clostridia | [9] |
| 5 | Excess sludge | 11 | Continued | 258.65 mg TOC/g VSS | 4.17 | NA | [3] |
| 6 | Excess sludge | 10 | Continued | 302.4 mg COD/g VSS | 423.1 | *Pseudomonas* sp. | [10] |
| 7 | Waste activated sludge from MBR | 11 | Continued | 219.7 mg COD/g VSS | 155 | NA | [11] |
| 8 | Rice straw | 10 | Initial | 0.27 g VFA/g DM[b] | NA | Ruminococcaceae, Bacteroidaceae, Porphyromonadaceae, Lachnospiraceae | [12] |
| 9 | Wetland plant litter | 12 | Continued | 127.22 mg VFAs/g DM | NA | NA | [13] |

[a] Compared with the control test without pH adjustment.
[b] DM means dry mass weight.

high throughput sequencing was adopted to disclose the microbial community responding to different alkaline treatment patterns.

## 2. Materials and methods

### 2.1. Experimental set-up

The raw swine manure used in this study was obtained from a pig farm located in Kunshan, Jiangsu Province, China. Its characteristics are shown in Table 2. Before added into reactors, the raw manure was diluted by distilled water to 4.8% of total solids based on our preliminary experiments.

The batch experiments were carried out in a series of 1000 ml reactors, which had a liquid volume of 600 ml. The diluted swine manure was firstly adjusted to pH 10.0 by adding 5 M sodium hydroxide (NaOH), and then divided equally into four reactors. For group P1, pH of the substrate was uncontrolled during fermentation. For group P2, the pH was regularly adjusted to 10.0 every two days via 5 M NaOH. Each group had two identical reactors. Before fermentation, nitrogen gas purged headspace air off and reactors were immediately sealed with rubber stoppers with two holes, one for pH online monitoring and another for sampling and pH adjustment. The reactors were then incubated at 35 ± 1 °C while shaking at 80 rpm (rotations per minute). Fermentation tests were carried out for 24 day.

### 2.2. Analytical methods

#### 2.2.1. Chemical analysis

pH was measured by pH meter (Multi 340i-WTW, Germany). Samples from reactors were immediately centrifuged at 10,000 rpm for 10 min and the supernatant was obtained for the determination of soluble chemical oxygen demand (SCOD), volatile fatty acids (VFAs), total ammonium nitrogen (TAN), orthophosphate phosphorus ($PO_4^{3-}$-P) in accordance with Standard Methods [18]. The soluble protein and carbohydrate were measured using Lowry–Folin and Phenol–Sulfuric methods, with bovine serum albumin (BSA) and glucose as standard, respectively [19,20]. VFAs including acetic acid (HAc), propionic acid (HPr), *n*-butyric acid (*n*-HBu), iso-butyric acid (iso-HBu), *n*-valeric acid (*n*-HVa) and iso-valeric acid (iso-HVa) were quantified by using gas chromatograph (7890A, Agilent, USA) fitted with HP-FFAP (30 m × 0.25 mm × 0.25 μm) capillary column and FID detection. The sample injection volume was 1.0 μl. The carrier gas was nitrogen at the flux of 25 ml/min. The injection port and the detector were maintained at 200 and 250 °C, respectively. The oven of GC began at 80 °C at first 2 min, then increased at a rate of 10 °C/min to 180 °C, and finally held at 200 °C for an additional 2 min. The experimental data were expressed as the average of triplicate tests.

The statistical analysis was carried out using OriginPro version 8.0 software. Paired Sample *t*-test was used to evaluate the effect of pH adjustment pattern on the cumulative SCOD and VFAs, and statistically significant differences were set with $P$ value <0.05.

#### 2.2.2. High throughput sequencing

Total genomic DNA (100 μL) from the sample sediment was extracted using Mo–Bio Isolation Kit (Mobio Laboratories, Inc., USA) based on the manufacturer's manual and was then verified by 1% agarose gels. After amplification by PCR, the product was sequenced by the ROCHE Emulsion-PCR technology and by Ion Torrent PGM (Life Technology, American), as described previously by Wan et al. [21].

**Table 2**
Characteristic of the raw swine manure used in the experiment.

| Parameter | Unit | Value |
|---|---|---|
| TS (total solids) | (%) | 13.6 ± 0.2 |
| VS/TS (volatile solids/total solids) | (%) | 72.6 ± 0.3 |
| pH | – | 7.13 ± 0.05 |
| SCOD (soluble chemical oxygen demand) | mg/l | 18900 ± 500 |
| Soluble proteins | mg/l | 4200 ± 100 |
| Soluble carbohydrates | mg/l | 1520 ± 74 |
| $PO_4^{3-}$-P | mg/l | 183 ± 12 |
| TAN (total ammonium nitrogen) | mg/l | 2400 ± 80 |

### 2.3. Kinetic analysis

According to previous study, hydrolysis can be considered as a first-order process with respect to the concentration of degradable particulate organic matter as presented in Eq. (1) [22–24].

$$\gamma_h = \frac{dC_{pt}}{dt} = -K_h C_{pt} \quad (1)$$

in which $\gamma_h$, $C_{pt}$ and $K_h$ are particulate organics releasing rate (mg/l d), particulate organics concentration (mg/l), and first-order