Micronutrient component changes in the biogas slurry treated by a pilot solar-heated anaerobic reactor

Z Y Yang1*, Y B Xu1, P F Li2, Y J Wang1, J Sun1 and Y P Zhang1

1 School of Environmental Science and Engineering, Guangdong University of Technology, Guangzhou 510006, China
2 Guangdong Industry Technical College, Guangzhou, 510300, China

*Corresponding author. E-mail: yangzy2003@126.com (Zuoyi Yang)

Abstract. A solar-heated anaerobic reactor system was applied to decompose livestock wastewater, in which cattle manure and chopped straw were mixed (CODCr 15,000~25,000 mg l⁻¹), the commercial microorganisms were added to ambient acidification (about 32°C) and the acclimated sludge was inoculated. Then, the experiments were carried out on wastewater anaerobic degradation and biogas production at 40~42°C, as fed every 10 days till stable running. The results showed that NH₃-N and PO₄³⁻ of the biogas slurry were 441 mg l⁻¹ and 65.0 mg l⁻¹ on the 35th day, respectively. The concentration of K was up to 350 mg l⁻¹ in the biogas slurry, rather higher than that of Mg and Fe, which indicated that the available K could contribute more in the agricultural irrigation. Total amino acids were up to 23.7 mg l⁻¹ after anaerobic digestion, in which Lys, Thr, Ala and Arg were prominent in the biogas slurry. These amino acids could be beneficial to seed soaking, feed adding and apply as foliar fertilizer. The major volatile organic compounds were detected in the biogas slurry, including toluene, m-cresol (up to 0.036% in the process of ambient acidification) and triethylsilane, which could be reduced to scarcely influence on agricultural application after anaerobic digestion.

1. Introduction
A large amount of concentrated fecaluria and wastewater discharged by livestock and poultry farms have become not only a new major source of pollution, but also a major cause of environmental pollutions in some cities and water source. Therefore, the farm wastewater should be either treated harmlessly or recycle use, and the biogas can be produced by anaerobic digestion to obtain clean energy [1]. Additionally, as a vice product of biogas fermentation, biogas slurry can be used rationally to provide a large number of high-quality or organic fertilizers or to feed for the modern agriculture.

The biogas slurry was shown to be beneficial to organic farming for fertilizing pastures and soil fertility and crop production [2, 3]. As an organic fertilizer rich in N, P, K and other elements, biogas slurry can enhance the availability and humification. Shahbaz et al [4] investigated the growth response and yield production of okra fertilized with various combinations of bioslurry and nitrogen fertilizer, the application of bioslurry alongside NPK fertilizers applied at reduced rates significantly, resulting in an increased N uptake by plants and improved N use efficiency. Li et al [5] evaluated soil organic matter, ammonia nitrogen, available phosphorus and available potassium of biogas fertilizer which can help China to realize low-carbon circular development of agriculture. Abubaker et al [6] studied the effectiveness of biogas slurry in combination with chemical fertilizers for the wheat growth and soil microbial activities.
The feed utilization rate was increased to shorten the feeding cycle by appending biogas slurry. Yu et al. [7] analyzed 17 kinds of amino acids including the contents of amino acids and the essential amino acids for pig growth, the total amino acids increased by 2.14%, 4.49% and 5.18% over control pigs, respectively. Zhang et al. [8] added the biogas slurries with the proportion of 1:0.5, 1:1 and 1:1.5 to the swine feeds for experiments, and the swine weights increased by 784.4 g, 843.3 g and 832.2 g respectively, in contrast with the control group increased by 748.9 g daily.

Biogas slurry has been used as the foliar fertilizer, the crops irrigation and the feed additive for a long history. However, the amino acids and the volatile components in biogas slurry were little studied and reported. In this research, the dynamic measurement was carried out on the contents of available nitrogen (NH$_3$-N), available phosphorus (PO$_4^{3-}$), K, Fe and Mg, amino acids as well as the volatile components during the anaerobic degradation of livestock wastewater, and the fertilizing and feeding values were analyzed. This study can contribute to the industrial breeding, the agricultural food security and the reasonable agricultural application of biogas slurry.

2. Materials and methods

2.1. Materials and inoculants.
Cow manure (25% of moisture content) for experiments was taken from a dairy farm. Sugarcane leaves and straws were chopped to the length of about 5 cm, in order to prevent blocking. The commercial effective microorganisms (Foshan Jinkuizi Environmental Biotechnology Co., Ltd., China) were used for the acidification in this experiment. 200 l sludge (from the secondary sedimentation tank of Guangzhou Lijiao Sewage Plant Ltd., China) was mixed with 1,300 l livestock wastewater and 500 g urea, then acclimated for 10 days before inoculated to 5 m$^3$ anaerobic reactor.

2.2. Pilot system of solar-heated anaerobic biogas reactor.
The pilot system including 1 m$^3$ pretreatment tank and 5 m$^3$ solar-heated anaerobic reactor (40~42°C) were used for the two-step biogas fermentation. The devices were constructed with a solar water-heating system for the heating and automatic control by internal annular calandria. The reactor could be heated and insulated in the stage of biogas production, in order to improve the efficiency of livestock wastewater degradation and biogas production.

2.3. Feeding and sampling.
After pretreatment and acidification, the slurry (filling coefficient of 90%) in 5 m$^3$ reactor was inoculated with the acclimated sludge, 1 m$^3$ slurry was discharged every 10 days and added with the same volume of fresh wastewater. The experiment was conducted for 35 days and sampled every 3~5 days, the relevant parameter changes were determined. The biogas slurries for control were sampled from the anaerobic reactor tanks of a dairy farm and a pig farm.

2.4. Theoretical analysis.
The pH value, COD$_{Cr}$, TN, NH$_3$-N, TP, PO$_4^{3-}$ were detected with general methods, and the statistical analysis software SPSS10.0 was used for data processing. Analysis of trace elements (K, Mg, Fe): Varian SpectrAA240FS, minimum detection: 0.01 mg·l$^{-1}$, detection wavelength: 766.5 nm, slit: 1.0 nm, lamp current: 5 mA, temperature: 27°C, humidity: 66%. Analysis of free amino acids: Centrifugation (10000×g, 15 min) and ultrafiltration, Waters HPLC (USA): PICO. TAG amino acid analysis column, mobile phase: A. 0.02 mol·l$^{-1}$ of NaAC buffer with pH 7.2, containing 0.018% (V/V) triethylamine and 0.3% (V/V) THF, B. 0.02 mol·l$^{-1}$ of the mixture with NaAC (pH 7.2) buffer, acetonitrile and methanol in a proportion of 1: 2: 2, 38°C, 1 ml·min$^{-1}$, detection wavelength: 254 nm. VOCs were analyzed with GC/MS: 300ml biogas slurry filtrate was taken and the 100-fold concentrated sample was obtained as the method of Yu et al. [9], Capillary gas chromatography: column model: HP-5MS, specification: 30 m×250 μm×0.25 μm, the sampler was cleaned with solvent before and after sampling, carrier gas was He gas, Column Oven Temp.: 40°C, Injection Temp.: 250°C, Pressure: 49.5 kPa, Total Flow: 54.0 ml·min$^{-1}$, Column Flow: 1.00 ml·min$^{-1}$, Linear Velocity: 36.1
cm/sec, Purge Flow: 3.0 ml·min$^{-1}$, Split Ratio: 50.0. Experimental data processing was completed by Xcalibur software system.

3. Results and discussion

3.1. pH and CODCr.

The whole experiment process can be divided into two stages of ambient acidification and anaerobic fermentation at 40–42°C, and the specific operating conditions were shown in Table 1. The results showed that pH values were 7.0 in the experiment process and COD$_{Cr}$ decreased slowly in the pretreatment stage. However, COD$_{Cr}$ degraded rapidly after 1.5m$^3$ acclimated anaerobic sludge was added on the 10$^{th}$ day and then maintained at 40–42°C. After feed-in twice, COD$_{Cr}$ could drop to 2,267 mg·l$^{-1}$.

Table 1. Micronutrient Changes during the anaerobic cultivation.

| Days | Temperature /°C | pH       | COD$_{Cr}$/mg l$^{-1}$ | TN/mg l$^{-1}$ | TP/mg l$^{-1}$ | NH$_3$-N/mg l$^{-1}$ | PO$_4$-P/mg l$^{-1}$ | Remark |
|------|-----------------|----------|------------------------|---------------|---------------|----------------------|----------------------|--------|
| 0    | 32.0            | 6.1      | 16000±38               | 915±12        | 430±6         | 804±11               | 99±1.3               | 3 m$^3$ livestock wastewater in (Manure: straw=2: 1) and 1kg commercial microorganisms (dedicated to biogas) added. |
| 4    | 32.8            | 6.5      | 10333±16               | 1002±25       | 280±5         | 838±26               | 68±0.6               | 1.5 m$^3$ acclimated sludge in, maintaining at 40–42°C. |
| 7    | 31.8            | 6.6      | 10128±32               | 892±6         | 255±2         | 696±15               | 61±0.4               | 1 m$^3$ slurry out; and 1 m$^3$ livestock wastewater (COD$_{Cr}$ 22833 mg l$^{-1}$) in. |
| 10   | 41.5            | 6.8      | 7900±22                | 970±13        | 370±1         | 623±8                | 54±0.2               | 1 m$^3$ slurry out; and 1 m$^3$ livestock wastewater (COD$_{Cr}$ 20500 mg l$^{-1}$) in. |
| 13   | 40.2            | 7.2      | 6600±8                 | 735±6         | 205±1         | 516±4                | 45±0.8               |                                       |
| 18   | 41.5            | 7.1      | 2333±16                | 650±8         | 295±2         | 473±24               | 30±0.5               |                                       |
| 20   | 40.8            | 7.0      | 10676±31               | 101±10        | 290±3         | 759±32               | 10±0.9               |                                       |
| 26   | 41.0            | 6.9      | 5176±15                | 755±8         | 265±2         | 568±15               | 75±1.7               |                                       |
| 29   | 41.1            | 7.2      | 7524±26                | 845±3         | 385±2         | 655±16               | 97±1.1               |                                       |
| 32   | 40.7            | 7.3      | 5506±18                | 703±6         | 220±3         | 536±22               | 83±1.2               |                                       |
| 35   | 41.3            | 7.2      | 2267±16                | 632±4         | 217±2         | 441±7                | 65±0.9               |                                       |
|      | biogas slurries | from a dairy farm | 7.3                  | 1260±13       | 505±2         | 106±2                | 250±4                | 32±0.3             |
|      | biogas slurries | from a pig farm   | 7.3                  | 1805±12       | 488±5         | 125±1                | 327±6                | 50±0.8              |

The studies conducted by Feng et al.[10] showed that COD$_{Cr}$ in biogas slurry seasonally changed with farm water qualities, concentration, digestion time and so on. After anaerobic digestion at 40–42°C in this test, COD$_{Cr}$ was reduced by 85%–90% compared with raw water in livestock dung. COD$_{Cr}$ in biogas slurries from a dairy farm and a pig farm were above 1,000 mg·l$^{-1}$, which was not suitable for discharge directly, but could be applied to comprehensive utilization of agricultural irrigation or discharged after aerobic treating to satisfy the government standards.

3.2. TN/NH$_3$-N, TP/PO$_4$-P.

After anaerobic fermentation, the content of TN and NH$_3$-N were reduced in biogas slurry because of the consumption of microbial growth and metabolism. Additionally, the concentrations of NH$_3$-N and PO$_4$-P were enough in biogas slurry, which was beneficial to improve its value of agricultural irrigation [11]. NH$_3$-N and PO$_4$-P were higher in the biogas slurry from the pig farm and the biogas slurry in this experiment, both were more applicable to agricultural irrigation than the samples from the dairy farm.

3.3. Amino acids.

Table 2 showed the amino acid changes in the digestion of pilot devices. In the initially-prepared untreated wastewater, total amino acids (TAA) were only 4.7 mg l$^{-1}$, and Arg, Thr, His, Val, Phe, Lys were not detected. In the acidification stage, TAA were 43.0 mg l$^{-1}$ on the 4$^{th}$ day, and His was the maximum at 7.7 mg l$^{-1}$, due to the decomposition of protein in the wastewater accelerated by the microbial proteases. TAA were measured at 16.6 mg·l$^{-1}$ on the 10$^{th}$ day, and Thr was 5.4 mg·l$^{-1}$. TAA
in the biogas slurry increased slightly after the anaerobic sludge was acclimated. When the system was running stable, Arg, Thr, Lys and Ala concentrations were higher in the biogas slurry. In the acidification stage, a large number of free amino acids were generated due to the microbial proteases, and some amino acids were used by cell growth, resulting in the reduction of the concentration. However, free amino acids were slightly increased because of the microbial role in the stage of anaerobic digestion.

Table 2. Changes of the amino acids during the anaerobic cultivation (mg·l⁻¹)

| Amino acids | Days       | biogas slurry from a dairy farm | biogas slurry from a pig farm |
|-------------|------------|--------------------------------|------------------------------|
|             | 0          | 4                               | 10                           | 18                           | 35                           |                                |
| Asp         | 0.6        | 2.8                             | 1.7                          | /**                        | 1.0                          | 0.1                           | 0.2                           |
| Glu         | 0.2        | /                               | 0.3                          | /                          | 0.8                          | 0.1                           | 0.1                           |
| Ser         | /          | 4.9                             | 2.0                          | 3.0                        | /                            | 0.7                           | 0.6                           |
| Gly         | /          | 2.7                             | 0.4                          | 1.8                        | 0.6                          | 0.8                           | 0.4                           |
| His         | /          | 7.1                             | 0.7                          | 2.2                        | 1.7                          | /                             | /                             |
| Arg         | /          | 6.2                             | /                            | 1.4                        | 3.8                          | 2.5                           | 2.1                           |
| Thr         | /          | 4.3                             | 5.4                          | 2.5                        | 4.4                          | 1.5                           | /                             |
| Ala         | /          | 1.8                             | 0.0                          | 3.3                        | 3.1                          | 1.6                           | /                             |
| Pro         | /          | 0.9                             | /                            | 3.4                        | /                            | /                             | /                             |
| Tyr         | 0.4        | 0.3                             | 0.3                          | 1.1                        | 1.0                          | 0.9                           | 0.4                           |
| Val         | 0.1        | 4.7                             | 2.0                          | 1.8                        | 0.3                          | 1.8                           | /                             |
| Met         | 0.1        | /                               | /                            | 0.4                        | /                            | 1.4                           | 0.2                           |
| Cys         | 0.4        | 0.4                             | 0.4                          | 0.0                        | 1.3                          | /                             | /                             |
| Ile         | 1.1        | 0.2                             | 0.8                          | 1.9                        | 0.9                          | 2.6                           | 1.3                           |
| Leu         | 0.8        | 0.3                             | 0.2                          | 0.4                        | 0.9                          | 1.1                           | 0.5                           |
| Phe         | 1.1        | 5.9                             | 1.1                          | 3.3                        | 1.3                          | 1.0                           | 0.3                           |
| Lys         | /          | 1.3                             | /                            | 2.9                        | 2.2                          | 2.2                           | 1.4                           |
| Total       | 4.7        | 43.0                            | 16.6                         | 21.7                       | 23.7                         | 7.4                           | 18.4                          |

**a **"/"** means not detected.

Shen et al [12] detected relatively larger content of Cys, Ser, Gly, Thr and Lys in TAA (15.09 mg·l⁻¹) of the swine biogas slurries. TAA in our 35th day test sample and the pig farm sample reached 23.7 mg·l⁻¹ and 18.4 mg·l⁻¹, 2-3 times higher than the dairy farm’s sample. The types of amino acids from the dairy farm sample were less abundant than those from the pig farm, which might be due to its lower nitrogen content in manure waste. Arg, Thr, Lys were relatively higher in the biogas slurry, in which Thr and Lys were taken for the essential amino acids in livestock feed. TAA in the biogas slurry may contribute little to the breeding value, but some amino acids could be the nutrients to stimulate seed growth and promote embryonic cell division in biogas-slurry seed soaking process.

3.4. Trace element K, Fe, Mg:

In Figure.1, K and Mg changed greatly during acidification, reaching the maximum concentrations of 530 mg·l⁻¹ and 118 mg·l⁻¹ on the 4th day, while reduced to 280 mg·l⁻¹ and 7 mg·l⁻¹ on the 10th day, respectively. In the digestion process at 40~42°C, the contents of Fe and Mg first increased and then reduced in the initial period after the acclimated sludge was inoculated. When the reactor was running stably after twice feeding, K remained at 300~450 mg·l⁻¹, while Fe and Mg remained at 10 mg·l⁻¹. The concentration of K was higher than those of Fe and Mg in the whole experiment process. Figure.2 showed that the concentration of K was prominent in each sample, just like the other researcher’s result [10, 12].
In this experiment, the concentration of K was especially prominent, which could increase the effect of available potassium in agricultural irrigation. Gupta et al\(^{(13)}\) analyzed the losses of N, P, K, Zn, Cu, Mn, Fe and other elements in the biogas slurry treatment process. GAN et al\(^{(14)}\) studied the application of biogas slurry to promote the growth of tabebuia, it could improve the soil’s physical and chemical properties, increase the organic matters as well as N, K and trace elements in soil. Therefore, taking into account the losses in the anaerobic process, the content of K was rather higher than those of Fe and Mg, indicating that when applied to agricultural irrigation and foliar fertilizer, available K has greater values than Fe and Mg in biogas slurry.

3.5. Volatile organic compounds.

Three volatile compounds of toluene, m-cresol and triethylsilane were detected in biogas slurry extracts. Figure 3 showed the changes of three major volatile components of the wastewater in the pilot process, in which the m-cresol concentration changed significantly. In the stage of ambient acidification (i.e., before the 10\(^{th}\) day), the concentration of m-cresol increased rapidly and reached the maximum of 0.036%. After inoculated with the acclimated sludge, the concentration of m-cresol was reduced in the fed-batch stage, and only a trace amount of concentration was detected in the discharged biogas slurry after running stably.

In Figure 4, Peak 1, 2, 3, 4 indicated the characteristics of ether solvent, but Peak 5, 6 and 7 indicates triethylsilane, toluene and m-cresol, respectively. The triethylsilane was detected in the different samples. However, no toluene was detected in the biogas slurry of this experiment device, while it had been detected in the biogas slurries from the pig farm and the dairy farm. Trimethylsilylmethanol was showed distinctly in the biogas slurry sample’s VOCs \(^{(12)}\). Rasi et al\(^{(15)}\) detected a trace amount of organic silicon compounds in the biogas slurry generated in landfill leachate. Xu et al\(^{(16)}\) detected three new 29 carbon skeletons pentacyclic triterpenoids and s-equol from biogas slurry, and the molecular structures were elucidated by the extensive spectroscopic data.
analysis. Benzene-ring substances might be generated in the process of cellulose degradation. Particularly, the m-cresol was detected clearly in the ambient acidification, but disappeared after the anaerobic digestion.

![GC chromatograms of different biogas slurries.](image)

**Figure 4.** GC chromatograms of different biogas slurries.

4. Conclusions
Our laboratory experiment suggests that multiple trace element nutrients were contained in the biogas slurry after anaerobic digestion at 40–42°C in the pilot solar-heated anaerobic reactor: In addition to NH₃-N and PO₄³⁻, the available K was prominent than Fe and Mg. Amino acids of Lys, Thr, Ala and Arg were higher in the biogas slurry. The residues of benzene-ring substances were low at the end of anaerobic digestion. It suggested that the anaerobic digestion technology at medium temperature could
not only reduce the treatment time of livestock wastewater, but also effectively improve biogas slurry’s value of seed soaking, irrigation and other agricultural application.

Acknowledgements
Authors appreciate the financial support from Science and Technology Project of Guangdong Province, China (No. 2014A020216038, 2016B020240003), and the Youth Fund of Guangdong University of Technology (14QNZD006).

References
[1] Wang X J, Lu X G, Yang G H, Feng Y Z, Ren G X and Han X H 2016 Development process and probable future transformations of rural biogas in China. *Renew. Sust. Energ. Rev.* **55** 703-712.
[2] Koszel M and Lorencowic E 2015 Agricultural use of biogas digestate as a replacement fertilizers. *Agriculture and Agricultural Science Procedia*. 7 119-124.
[3] Bougnom B P, Niederkofler C, Knapp B A, Stimpfl E and Insam H 2012 Residues from renewable energy production: Their value for fertilizing pastures. *Biomass. Bioenerg.* **39** 290-295.
[4] Shahbaz M, Akhtar M J, Ahmed W and Wakeel A 2014 Integrated effect of different N-fertilizer rates and bioslurry application on growth and N-use efficiency of okra (Hibiscus esculentus L.). *Turk. J. Agric. For.* **38** (3) 311-19.
[5] Li J S, Duan N, Guo S, Shao L, Lin C, Wang J H, Hou J, Hou Y, Meng J and Han M Y 2012 Renewable resource for agricultural ecosystem in China: Ecological benefit for biogas by-product for planting. *Ecol. Inform.* **12** 01-110.
[6] Abubaker J, Risberg K and Pell M 2012 Biogas residues as fertilisers – Effects on wheat growth and soil microbial activities. *Appl. Energ.* **99** 126-134.
[7] Yu D B, Zeng G K, Zhang W D, Song H C, Hu X J and Duan Z S 2006 Effect of biogas digestive liquid for feeding pigs. *Renewable Energy Resources*. **5** 25-28, 31. (in Chinese)
[8] Zhang H B, Li J R and Shao K W 2009 Safety and effect of biogas slurry in swine feed. *Acta Ecologiae Animalis Domastici*. 3 68-72. (in Chinese)
[9] Yu A N, Sun B G and Hu W B 2008 Top note compounds of Chinese traditional bacteria-fermented soybean. *Nat. Prod. Res.* **22** (17) 1552-1559.
[10] Feng Z Q, Lin C S, Yuan X F, Cui Z J and Li H L. 2009 The law of biogas slurry changes to fertilizer in the process of high density pig manure treated by anaerobic reactor. *Hunan Agric. Sci.* **11** 145-14. (in Chinese)
[11] Federolf C P, Westerschulte M, Olfs H W and Trautz D 2015 Optimizing nitrogen and phosphor use efficiencies from liquid manure by slurry injection to reduce environmental pollution. *Procedia Environ. Sci.* **29** 227-228.
[12] Shen Q L, Shan S D, Zhou J J and Wang Z R 2014 Determination and analysis of compositions in biogas slurry produced by swine manure digestion. *China Biogas* **32** (3) 83-86. (in Chinese)
[13] Gupta A P, Gupta V K, Antil R S and Nath J 1985 Possible losses of plant nutrients from biogas slurry. *Agricultural Wastes*. **12** (4) 317-320.
[14] Gan F D, Wei S Q, Qin W N, Zeng G Y, Li J H and Jiang H B 2011 Effect of biogas slurry on tube bean quality and soil fertility. *China Biogas* **29** (1), 59-60. (in Chinese)
[15] Rasi S, Veijanen A and Rintala J 2007 Trace compounds of biogas from different biogas production plants. *Energy*. **32** 1375-1380.
[16] Xu J F, Wu H B, Liu D C, Sha L, Wu W H, Fan H, Song Y S and Zhu H G 2015 Three new 29 carbon skeletons pentacyclic triterpenoids and s-equol from biogas slurry. *B. Korean. Chem. Soc.* **36** (12) 2862-2868.