Simultaneous Ammonia removal and Methane Production from Chicken Manure under Dry Thermophilic Condition

[1]Fatma Abouelenien . [2]Nagham. Elsaidy and [2]Yutaka Nakashimada

[1*] Department of Hygiene and Preventive Medicine, Faculty of Vet Med, Kafer Elshikh University, Egypt
[2*] Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Kagamiyama 1-3-1, Higashi- Hiroshima
fabou2ga@yahoo.com (F. Abouelenien).

Abstract: In a trail to improve and reduce the cost of dry anaerobic fermentation of chicken manure with ammonia stripping through biogas recycling, raw chicken manure (RCM) is used as a sole substrate instead of using treated chicken manure (TCM) or mixture of TCM:RCM (1:1) used previously. Biogas produced was ranged from 23.5- 55 L kg-CM-1 with methane percentage of 70 to 85%, the amount which is 61 to 95% and 109 to 141% higher than that obtained from TCM and mixture of TCM and RCM (1:1). Ammonia removal reached 82.7% keeping the ammonia level in the reactor in most batches less than 3.6 g-N kg^{-1}. Acetate was less than 20 mmol kg^{-1} at the end of each batch. A maximum of 324 ml g-VS^{-1} of methane was obtained which is quiet higher than that obtained from any other previous study. Additionally RCM could be used as a substrate

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1. Introduction

Chicken manure CM has higher nitrogen content than cow manure, food waste, pig manure and waste active sludge (Qiao *et al.,* 2011). Dry anaerobic fermentation of CM under thermophilic condition often encounters very high concentrations of ammonia due to extensive hydrolysis of nitrogen compounds and fermentation of amino acids. The unionized ammonia content in the range of 560 – 568 mg L^{-1} caused a 50% inhibition of methanogenesis at pH 7.6 under thermophilic condition (Gallert and Winter, 1997). Sung and Liu (2003) observed that total ammonia-nitrogen (TAN) concentration of 4.92 and 5.77 g L^{-1} decreased the production of methane by 39% and 64%, respectively. Additionally they reported that 100% inhibition occurs in the range of 8-13 g L^{-1} depending on the condition of acclimatization and the pH of the system. Niu *et al.* (2013) reported that the biogas and COD conversion decreased to 0.3 L $g^{-1}VS_{in}$ and 205 at TAN (total ammonia nitrogen) 10,000 mg L^{-1} and was totally suppressed at 16,000 mg L^{-1}.

Few studies conducted on acclimation of methanogenic consortia to high ammonia levels, or raised ammonia tolerance, have proven a method for improving the process of anaerobic digestion and production of methane from chicken wastes (Demirci and Demirer, 2004; Abouelenien *et al.,* 2009b). In our previous study dry fermentation of CM (25% TS) under mesophilic condition was carried out, where methane was successfully produced 4.4 Lkg^{-1}CM, despite the presence of high level of ammonia of ca. 8 to 14g-N kg^{-1}CM, after an acclimation period of about 254 d (Abouelenien *et al.,* 2009b).

Further methods of treatment applied to different kinds of organic wastes for removal of ammonia. These methods include: chemical precipitation such as the magnesium ammonium phosphate (MAP) process (Demeestere *et al.,* 2001), and zeolite and clay process (Tada *et al.,* 2005). Nitrification/ denitrification process and anaerobic ammonia oxidization (Anammox) process were used as biological methods to reduce the inhibition by ammonia during anaerobic digestion (Dong and Tollner, 2003). Unfortunately, effectiveness of all the methods mentioned above relies on dilution of the manure to a total solid level of 0.5-3.0% (Chen *et al.,* 2008). However, the resulting increase in waste volume, that must be processed, makes this method economically unattractive (Callaghan *et al.,* 1999).

Stripping of ammonia from wastes in a liquid form was previously carried out and was useful for removal of ammonia, for example, from swine wastewater (Liao *et al.,* 1995), anaerobic digestion effluent (Lei *et al.,* 2007), and poultry litter leachate (Gengagni Rao *et al.,* 2008). However, very few studies on application of ammonia stripping from organic matter with high total solid content such as dehydrated waste activated sludge, have been reported (Nakashimada *et al.,* 2008). Previous studies have used ammonia stripping only for removal of the produced ammonia as a separate step (Liao *et al.,* 1995; Lei *et al.,* 2007; Gengagni Rao *et al.,* 2008; Nakashimada *et al.,* 2008; Abouelenien *et al.,* 2009a;

Niu *et al.,* 2013). In the previous study, we tried to improve the production of methane during dry anaerobic digestion of CM through a three-stage process, 1) ammonia fermentation of CM, 2) ammonia stripping to remove the accumulated ammonia (at 85^{o}C and pH 10) and, 3) methane fermentation of the ammonia stripped CM. As the result of this study, 104 ml g^{-1}VS of methane yield was obtained after stripping of ammonia twice. Further improvements were, however, needed to reduce the cost and the time, consumed by the multi step process of ammonia stripping during dry fermentation of CM.

In our previous paper (Abouelenien *et al.,* 2010), methane fermentation with ammonia stripping was evaluated. And we found that, Ammonia was successfully removed formation by means of recycle of biogas followed by gas washing in sulfuric acid to trap ammonia, when CM was anaerobically digested for 4 d under 55^{o}C and initial pH 8.5-9. By using this system, 80% of total nitrogen in CM was converted to ammonia and 82 % of the produced ammonia was removed.

A bench scale reactor equipped with the ammonia stripping unit for methane production from CM was developed and operated in repeated batch mode. At initial pH 8 and 55 ^{o}C, 195 ml g-VS $^{-1}$ and 157 ml g-VS $^{-1}$ of methane was successfully produced from the treated CM (TCM) and the mixture of TCM and raw CM (RCM) at 1:1, respectively. Using this system, ammonia concentration was maintained lower than 2 g-N kg-wet sludge^{-1} in the reactor. With the aim to improve the process and reduce its cost, 100% RCM were used as a sole substrate instead of TCM or TCM: RCM (1:1) used in our previous paper (Abouelenien *et al.,* 2010). Cost decreased by using RCM without pretreatment by stripping.

2. Materials and Methods

2.1. Chicken manure and seed sludge for methane production

Chicken manure from Hiroshima University chicken farm (cage layer system) was collected from deposits directly under chicken cages and had the following characteristics; total solids (TS), total organic carbon (TOC), total Kjeldahl nitrogen (TKN), total ammonia nitrogen (TAN), acetate, and pH, were 25% (w/w), 380 g-C kg-TS^{-1}, 87 g-N kg-TS $^{-1}$, 10.5g-N kg-TS $^{-1}$, 19 mmol kg^{-1} and 8.5, respectively. A sludge obtained after thermophilic anaerobic digestion of excess activated sludge was used as a seed sludge to initiate anaerobic digestion of CM. The sludge was collected from a Wastewater Treatment Center in Hiroshima, Japan. This sludge was anaerobically incubated at 55°C for 60 d in our laboratory to achieve complete consumption of the substrate. The seed sludge was characterized as 20% (w/w) of TS, 268 g-C kg-TS $^{-1}$ of TOC, 32 g-N kg-TS $^{-1}$ of TKN and 3.2g-N kg-TS $^{-1}$ of TAN.

2.2. Evaluation of an efficiency of spontaneous ammonia fermentation, ammonia stripping and methane production from 100% RCM in repeated batch culture in one bench scale reactor

In order to test the suitability for methane fermentation of RCM, repeated batch cultures were carried out at 55 ±2^{o}C by using the bench scale reactor illustrated in our previous paper (Abouelenien *et al.,* 2010). A bed weight of 3.2 kg of the seed sludge was used and the methane fermentation was initiated by adding 80 g of RCM. The biogas recycling to the bottom of the reactor was performed from the start of culture. When acetate concentration fell below 3 mmol kg^{-1} new substrate was added. The fermentation temperature was maintained at 55 ± 2 ^{o}C and the stirring velocity was 10 rpm. The gas volume and gas content was measured every day. Samples was drawn periodically and used for analysis of pH, ammonia, TKN an VFAs. Sulfuric acid samples were also collected to analyze the trapped ammonia.

3. Analytical methods

Fermentation sample (ca. 0.3 g wet weight) was withdrawn into a 2-ml plastic tube, and suspended with 1.2 ml deionized water. The suspension was centrifuged at 3,000 rpm for 10 min at 4°C, and the clear supernatant was used for measurement of pH, ammonia, and volatile fatty acids (VFAs). VFAs were measured using a High Performance Liquid Chromatograph (Shimadzu, Kyoto, Japan) equipped with Aminex HPX-87H Column, 300mm x 7.8mm (Bio-Rad, Tokyo, Japan). The column temperature was 65 °C. The flow rate was 0.8 ml min $^{-1}$ for 0.005 M H_2SO_4 solution. Ammonia was measured by using a commercially available ammonia testing kit (Wako Ltd. Osaka, Japan). TOC was determined by a TOC analyzer (TOC-5000, Shimadzu). TS, VS, TKN, and pH were measured in accordance with the standard methods (APHA, 1998). Gas production was measured periodically by displacement of saturated aqueous NaCl in a graduated cylinder. The composition of CH_4, H_2, and CO_2 was determined by a gas chromatograph (GC-8A, Shimadzu) with a thermal conductivity detector equipped with a glass column (2m x 3 mm) packed with unibeads C 60/80 (Shimadzu) at 140 °C. Argon was used as the carrier gas at a pressure of 100 kPa.

4. Results and discussion

4.1 Methane fermentation RCM in a bench scale reactor with ammonia stripping by biogas recycle and bubbling

The possibility of methane production with ammonia stripping by biogas recycle was tested using same bench scale reactor used in our previous work (Abouelenien *et al.,* 2010). The substrate used was 100% RCM and the operational conditions are described in materials and methods. The culture profile of RCM is shown in Fig. 1-1. Methane was successfully produced in the range of 23.5– 55 L kg-CM^{-1} with a percentage of 70 to 85% of biogas content. Ammonia removal reached 82.7% keeping the ammonia level in the reactor in most batches less than 3.6 g-N kg^{-1}. Acetate was kept at low concentrations less than 20mmol kg-1. At the end of each batch except the 3rd batch it reach 34.6mmol kg-1. The pH was kept within the range of 8.4- 8.8.

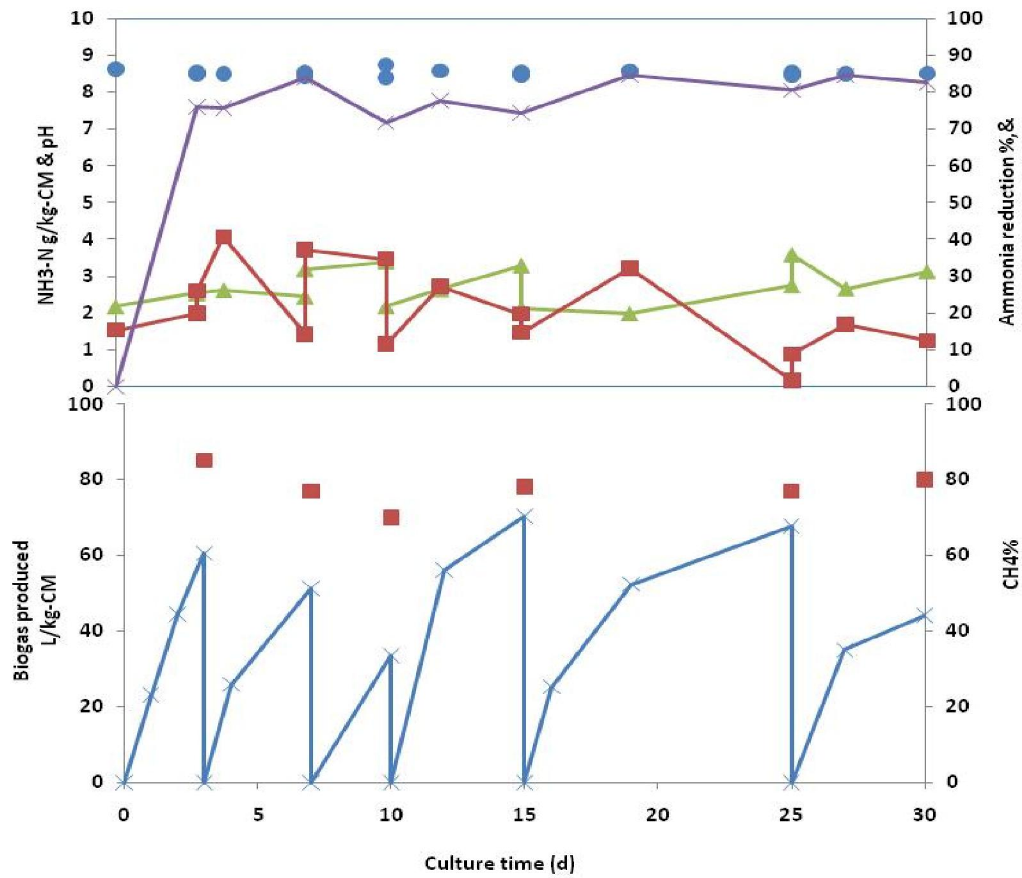

Fig. 1 Ammonia fermentation coupled to ammonia stripping and methane fermentation in repeated batch culture. Seed sludge was inoculated to 100% raw chicken manure (RCM). Symbols: a) *closed triangle,* ammonia concentration in the reactor; *closed square,* acetate; *asterisk*, ammonia reduction, %; *closed circle*, pH; . b) *closed square*, methane % of biogas; *astrisk*, biogas produced.

Table 1 illustrated the Comparison between the current results and that obtained in our previous paper (Abouelenien *et al.,* 2010). It was found that biogas production was increased about 61 to 95% and 109 to 141% from using 100% RCM as a substrate than using 100% TCM or using 50% TCM mixed with 50% RCM substrates (Abouelenien *et al.,* 2010). Additionally it was found that percentage of methane gas (70 – 85%) that produced from the current substrate (100% RCM) was slightly higher than that obtained from the other two substrates (Abouelenien *et al.,* 2010). As RCM substrate has higher organic matter and higher VS content than TCM or mixture of TCM and RCM (50%:50%) substrates, and VS content is the part which actually converted by bacteria so it resulted in higher methane production than other substrates.

Ammonia removal percentage was increased by 4% than that obtained from 100% TCM, and by 6% than that obtained from mixture of 50% RCM and 50% TCM (Table 1). The current reactor ammonia concentration was higher than that obtained from the two other substrates this since, 100% RCM substrate contain higher amount of organic nitrogen than TCM which subjected to partial ammonia fermentation and stripping and same case when using 50% RCM and 50% TCM. Current acetate concentration was lower by 9% and 44% than that obtained when using 100% TCM and 50% RCM and 50% TCM; this result explained the higher production of methane with RCM than with others (Table 1).

In spite of Ammonia concentration (lower than 3.6 g-N kg^{-1}) in the reactor was slightly higher than the level reported in our pervious study (Abouelenien *et al.,* 2010), methane production was higher. This result may resulted from the significant difference in inhibiting ammonia concentration which attributed to the differences, in substrates and inocula, environmental conditions (temperature and pH) and acclimation periods (Hashimoto, 1986; Angelidaki and Ahring, 1994; Abouelenien *et al.,* 2009). Using TS above 5% and under thermophilic condition is a conditions helps to intensify this inhibition (Bujoczek *et al.,* 2000; Magbanua *et al.,* 2001; Abouelenien *et al.,* 2009a,b). The system we used allows ammonia to be stripped at once, keeping ammonia (less than 3.6) under low level in spite of using RCM as a substrate instead of TCM or TCM: raw CM (1:1). This low level of ammonia accumulation allowed production of methane, which was higher than any obtained by others and even obtained in our previous studies.

Although Bujoczek *et al.* (2000) carried out fermentation of CM with 21.7 to 5% of TS content, they failed to produce any methane production from fresh CM (21.7% TS) without dilution during 120 days at 35°C. Magbanua *et al.* (2001) also conducted anaerobic batch tests using hog and poultry wastes in various proportions, and produced a very low amount of methane not exceeding 0.9 ml g-VS $^{-1}$, which was obtained after 99 days of fermentation with 17.4 % TS and 14.6% VS.

Additionally we obtained higher methane production than that obtained by Chen *et al.* (2012) who obtained 107.25 ml g^{-1} TS of biogas (76.92% methane) was produced from co-digestion of CM with spartina alterniflora residues (SAR) at 35 °C with intial TS % of 8. Ahn *et al.* (2010) studied the performance of anaerobic digestion of poultry manure-switch grass mixture under 15% TS and thermophilic conditions (55°C) resulted in very poor methane yield of 2 ml g^{-1}VS after 62 d digestion. They attributed this poor methane yield to VFA accumulation and pH drop.

In our previous studies, we obtained about 31 ml g-VS $^{-1}$ of methane production from CM after 254 days with acclimatization to high ammonia level (Abouelenien e *et al.,* 2009b) and 104 ml g^{-1}VS by using twice ammonia fermentation, and twice ammonia stripping, and then methane fermentation (Abouelenien *et al.,* 2009a). Abouelenien *et al.,* 2010 obtained 195 ml g-VS $^{-1}$ of methane from TCM and, 157 ml g-VS $^{-1}$ from TCM: raw CM (1:1). In this current study maximum methane production was 324 ml g-VS $^{-1}$ which is quiet higher than that obtained from any other previous study especially in this study we used 100% RCM as a substrate under thermophilic condition.

Table 1 The culture profile of different substrates, 100% TCM (treated chicken manure), 100% RCM (raw chicken manure), and mixture of TCM and RCM (1:1)

Substrate	100% TCM*	RCM: TCM (50%:50%)*	100% RCM
Biogas production range (L kg-CM^{-1})	14.6 - 28.2	7 - 22.8	23.5 - 55
Methane production%	67 – 80 %	55 – 74 %	70 – 85 %
Ammonia removal%	79 %	77 %	82.7 %
Reactor Ammonia concentration (g-N kg^{-1})	Less than 2	Less than 2	Less than 3.6
Acetate concentration (mmol kg^{-1})	Less than 22	Less than 36	Less than 20
pH range	8.1 – 8.7	8.5 – 8.9	8.4 – 8.8

*Source : Abouelenien *et al.,* 2010.

5- Conclusions

Using RCM as a substrate without pretreatment resulted in the production of 23.5-55 L kg-CM^{-1} biogas with a methane percentage of 70 to 85% of biogas content. Ammonia removal reached 82.7% keeping the ammonia level in the reactor in most batches less than 3.6 g-N kg^{-1}. Acetate was kept at low concentrations less than 20 mmol kg-1 at the end of each batch.

Using 100% RCM as a substrate resulted in 61 to 95% and 109 to 141% increase in biogas production than using 100% TCM or using 50% TCM mixed with 50% RCM substrates (Abouelenien *et al.,* 2010).

In this current study maximum methane production was 324 ml g-VS $^{-1}$ which is quiet higher than that obtained from any other previous study.

The step of pretreatment of CM by partial ammonia stripping (TCM) is no longer required as using RCM directly by this system gives higher methane production than using TCM as long as the system able to keep ammonia under inhibitory level. These results will solve the ecologic and economic problems associated with CM dilution with water or its pre- treatment.

Corresponding author
Fatma Abouelenien
Department of Hygiene and Preventive Medicine,
Faculty of Vet Med, Kafer Elshikh University, Egypt
fabou2ga@yahoo.com

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