Total ammonia and N_2O emission characteristics from *Alcaligenes* sp. LS2T cultures and its application on laying hen manure associated with different pH conditions

Aldyon Restu Azkarahman, Yuny Erwanto, Lies Mira Yusiati, Widodo Widodo and Nanung Agus Fitriyanto*

Faculty of Animal Science,
Universitas Gadjah Mada,
Jl. Fauna, Daerah Istimewa Yogyakarta 55281, Indonesia
Email: dazkarahman@gmail.com
Email: yunyer@ugm.ac.id
Email: yusiati@yahoo.com
Email: widodohs@ugm.ac.id
Email: nanungagusfitriyanto@ugm.ac.id
*Corresponding author

Abstract: The main objective of this research was to investigate the *Alcaligenes* sp. LS2T characteristics in total ammonia and N_2O emission under different carbon sources and C/N ratios in the synthetic media. Observation of the strain application potential to suppress ammonia emission was also performed on laying hen manure with different initial pH conditions. The total ammonia concentration was observed by Nessler's reagent photometry method followed by Lide and Frederikse equation, while the N_2O emission was measured by gas chromatography. The result showed that the least total ammonia concentration (12.77 ± 2.61 ppm) was seen in acetate C/N 28 medium. The highest N_2O emission was seen in citrate C/N 14 medium, which emitted 377.13 ± 20.11 ppb N_2O. The least emitted ammonia in the manure media was seen in an acid medium (116.92 ± 8.34 ppm ammonia). The results showed the *Alcaligenes* sp. LS2T capabilities to remove ammonia under aerobic condition.

Keywords: *Alcaligenes* sp. LS2T; aerobic denitrification; total ammonia; N_2O emission; pH condition; laying hen manure.

Reference to this paper should be made as follows: Azkarahman, A.R., Erwanto, Y., Yusiati, L.M., Widodo, W. and Fitriyanto, N.A. (2021) 'Total ammonia and N_2O emission characteristics from *Alcaligenes* sp. LS2T cultures and its application on laying hen manure associated with different pH conditions', *Int. J. Environment and Waste Management*, Vol. 27, No. 1, pp.1–20.

Biographical notes: Aldyon Restu Azkarahman is a graduate student at the Faculty of Animal Science Universitas Gadjah Mada Yogyakarta. His research interests include animal waste processing, animal waste management and farm environment.

Yuny Erwanto is the Head of Animal Skins, By-products, and Animal Wastes Technology Department at the Faculty of Animal Science, Universitas Gadjah Mada. His research area is focused on animal by-product processing.

Lies Mira Yusiati is a Professor at the Animal Nutrition and Biochemistry Department in Faculty of Animal Science, Universitas Gadjah Mada. She specialises in animal microbiology, biochemistry and nutrition.

Widodo Widodo is a Lecturer at the Department of Animal Product Technology in Faculty of Animal Science and Department of Biotechnology in Graduate School, Universitas Gadjah Mada. He specialises in agricultural microbiology and biotechnology.

Nanung Agus Fitriyanto is a Lecturer at the Department of Animal Skins, By-products, and Animal Wastes Technology, Faculty of Animal Science, Universitas Gadjah Mada. His specialisations include environmental microbiology, bioremediation and animal wastes treatment.

1 Introduction

Animal waste has been known as the source of many problems, whether to the animal farm production or the environment due to the nitrogen compounds. In the poultry farm, NH_3 emissions from the manure are mainly due to high protein content in the chicken diets, which will be excreted as uric acid, ammonia, and urea (Goldstein and Skadhauge, 2000). Furthermore, uric acid and urea from the chicken manure will be converted into NH_3 and CO_2 through certain processes catalysed by uricase and urease enzyme (Bahl and Bahl, 2004). The NH_3 emission showed a negative effect on farm productivity, this would harm the farming finances. Research by Maliselo and Nkonde (2015) showed an adverse effect of NH_3 emission on poultry performances. Furthermore, the exposure of NH_3 at 70 mg/kg is known to decrease the relative weight of the spleen, serum globulin concentration, serum lysozyme and serum globulin of the broiler chicken (Wei et al., 2015).

Aside from its negative impact to the poultry performances, nitrogen runoffs were also known to cause a negative effect to the environment, one of the examples is the biotic disruption in Tondano Lake (Wantasen et al., 2012). According to O'mara (2011), animal agriculture contributes 8–10% of global greenhouse emission, while Maeda et al. (2013) explained that the contribution was mainly due to poor farm waste management. The high contribution was originated from the N_2O emission from the animal waste, regarding that N_2O is 200–300 times stronger than CO_2 to cause global warming, and has recently been deemed to be the foremost threat to the ozone layer of the twenty-first century (Ravishankara et al., 2009).

The problems associated with laying hen manure management then have given rise to the need for treatment technologies (Li et al., 2011). The intensive confinement system which mostly applied in the poultry farm has resulted in significant environmental challenges, such as an excess of NH_3 emission due to the massive volumes of laying hen manure excreted (Chen et al., 2017). Biological treatment is known as one of the approaches to reduce NH_3 emission from animal waste. A study on the consortium of *Pseudomonas* spp., *Candida* sp. LS3K and *Arthrobacter* sp. LM1KK as a microbial

treatment for poultry and livestock wastes showed the ability to suppress ammonia emission through an oxidation process (Pastawan et al., 2017). The other studies on *Alcaligenes spp*. showed the ability to remove the ammonium concentration on various wastes and media through nitrification and denitrification process (Gupta and Gupta, 2001; Joo et al., 2005; Liu et al., 2015). The concept of biological treatment on nitrogen removal was through the nitrogen cycle which resulted in safe N_2 gas. Thus, in-depth research to design a biological treatment to suppress ammonia emission with consideration to the consequences of the potent greenhouse gas emission is required (Maia et al., 2012).

In this study, we investigated the characteristic of *Alcaligenes* sp. LS2T, bacteria strain which previously isolated from a local poultry farm, and observed its total ammonia and N_2O gas emission in acetate and citrate medium. Furthermore, we also investigated the total ammonia emission on chicken layer manure at various pH conditions after inoculated by *Alcaligenes* sp. LS2T.

2 Materials and methods

2.1 Sample preparation

The research is a pilot-scale experimental study conducted in Laboratory of Leather, By-products and Waste, Technology, Faculty of Animal Science, Universitas Gadjah Mada, Indonesia; and N_2O measurement was analysed in the Agriculture Environment Research Institute, Pati, Indonesia. The *Alcaligenes sp*. LS2T was previously cultured and stocked in the agar media and stored at 4°C temperature before used for inoculation. The stock solution of *Alcaligenes* sp.LS2T strain was prepared by dissolving the following in 100 mL of distilled water: 1 g meat extract, 1 g bacteriological peptone, and 0.5 g NaCl. The stock solution was then adjusted at pH 7.2 and sterilised with an autoclave at 120°C 15 psi for 15 minutes. After reaching around room temperature, *Alcaligenes* sp. LS2T isolate from agar media was then added into the stock solution and incubated for 24 hours in a rotary shaker, after that the isolate was then inoculated into the synthetic and manure media.

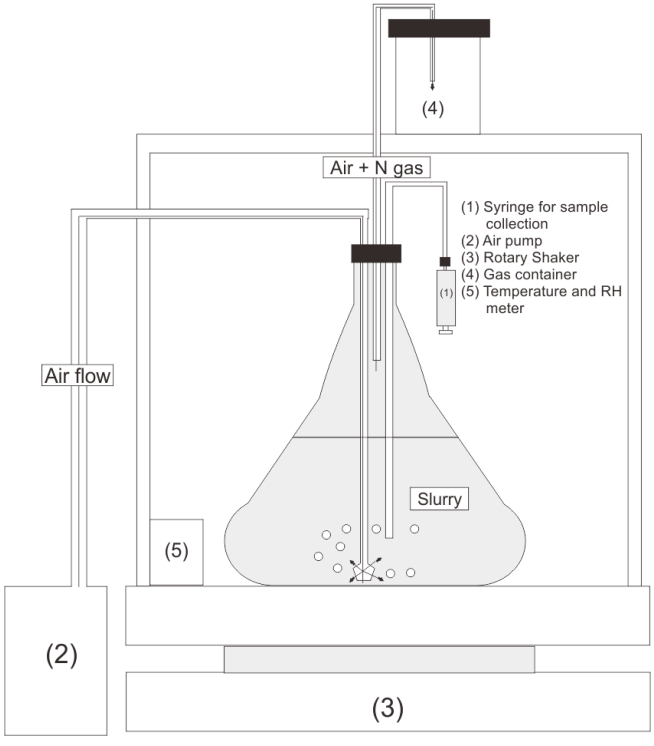
The synthetic media were prepared by dissolving the following in 1 L of distilled water: $(NH_4)_2SO_4$ 0.472 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, K_2HPO_4 0.2 g, NaCl 0.12 g, $MnSO_4 \cdot 4H_2O$ 0.01 g, and $FeSO_4$ 0.01 g. Sodium acetate and trisodium citrate were used as sole carbon source separately in the media to further observe the effect of the different carbon source to the nitrogen removal, abbreviated as acetate medium and citrate medium respectively. In order to elucidate the influence of carbon to nitrogen (C/N) molar ratio on the performance of *Alcaligenes* sp. LS2T, the amount of ammonium in both media were fixed at 130 ppm while the amount of carbon was adjusted to the appropriate C/N ratios, which were 7, 14, 21 and 28.

Laying hen manure was collected from the Faculty of Animal Science, Universitas Gadjah Mada farm during the laying period. The slurry was then made by diluting fresh laying hen chicken manure with distilled water in 1:10 ratio and divided into control (without pH conditioning); acid (pH 5); neutral (pH 7); and base (pH 9) medium. The pH fixation was done by using NaOH and tris-HCl as a buffer.

2.2 Inoculation and sample collection

The synthetic and manure media were added with a stock solution of *Alcaligenes* sp.LS2T strain as much as 1% of the media volume, except for the control medium which was not added with the stock solution. The bacteria density in the stock solution was measured with standard Total Plate Count and showed that it contained 1.35×10^7 CFU/mL of *Alcaligenes* sp. LS2T. The inoculated media were then aerated and placed on a rotary shaker and continuously shaken at 90 rpm for 96 hours. One mL of synthetic media was taken every 12 hours for ammonium measurement while the gas samples were collected every 24 hours and kept in a gas-tight tube for N_2O gas measurement. For the manure media, 200 mL of 0.02N Boric acid was used to trap ammonia and then taken as much as 1 mL every 12 hours for ammonia measurement. The schematic representation of the laboratory set up can be seen in Figure 1.

Figure 1 Schematic representation of the laboratory set up



2.3 Total ammonia, N_2O and pH measurement

The collected liquid samples from synthetic and manure media was centrifuged at 14,000 g, and the obtained supernatant was used for the ammonium analysis by following Nessler's reagent photometry method (American Public Health Association, 1998) using

spectrophotometer (Shimadzu, Japan). Sample as much as 1 mL were added with 1 mL of Nessler reagent and homogenised by vortex for 10 minutes. The brown color dispersion in the solution then observed with a spectrophotometer at 425 nm, and the ammonium concentration can be measured by calculating the result with the standard curve equation. The ammonium concentration then can be used for total ammonia emission calculation through polynomial regression from Lide and Frederikse (1993) by the following equation:

$$[NH_3] = \frac{\left[NH_3 + NH_4^+\right]}{1 + \frac{H^3}{K_a}} = \frac{\left[NH_3 + NH_4^+\right]}{1 + 10^{pK_a - pH}} \quad (1)$$

where $[NH_3]$ is the free ammonia concentration, $[NH_3 + NH_4^+]$ is the total ammonia concentration, $[H^+]$ is the hydrogen ion concentration, and K_a is the acid ionisation constant for ammonia, pK_a can be expressed as function temperature of T by the following equation:

$$pK_a = 4 \times 10^{-8} \times T^3 + 9 \times 10^{-5} + T^2 - 0.0356 \times T + 10.072 \quad (2)$$

The collected gas samples were measured for the N_2O concentration by following the Indonesian Agricultural Environment Research Institute (2007) using gas chromatography (Shimadzu, Japan). The pH changes in all media were measured using pH meter (HANNA instruments, Italy) every 12 hours for 96 hours.

2.4 Statistical analysis

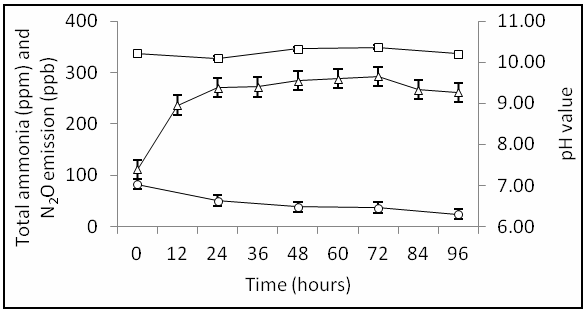
In this experiment, all of the treatments were performed in triplicate, and the obtained data were analysed by using split-plot ANOVA following the procedures of Gomez and Gomez (1984), and the mean differences between treatments were tested by least significant differences (LSD) tests.

3 Results

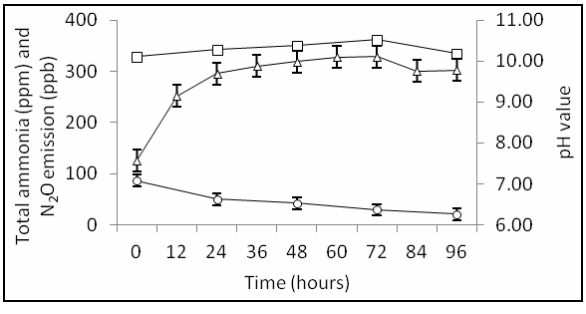
3.1 Total ammonia, N_2O emission, and pH change on acetate and citrate medium

The observation of *Alcaligenes* sp. LS2T performances regarding the total ammonia, N_2O emission and pH changes in acetate and citrate medium with different C/N ratios can be seen in Figures 2 and 3. In the acetate medium, the least of total ammonia concentration can be seen at the C/N 28, which contained 12.77 ± 2.61 ppm of total ammonia after 96 hours of incubation. However, measurement in the citrate medium showed relatively higher total ammonia removal; with the least total ammonia concentration (39.25±13.66 ppm) after 96 hours of incubation could be seen at C/N 7.

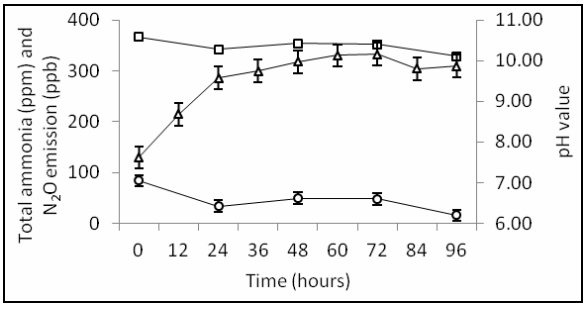
Figure 2 Total ammonia concentration (circle), N_2O emission (square) and pH value (triangle) of *Alcaligenes* sp. LS2T on acetate medium with different C/N ratios, (a) C/N 7 (b) C/N 14 (c) C/N 21 (d) C/N 28



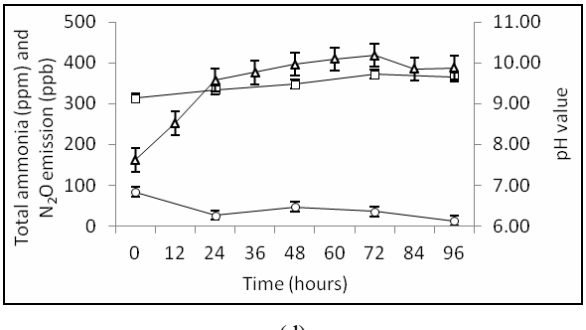
(a)



(b)

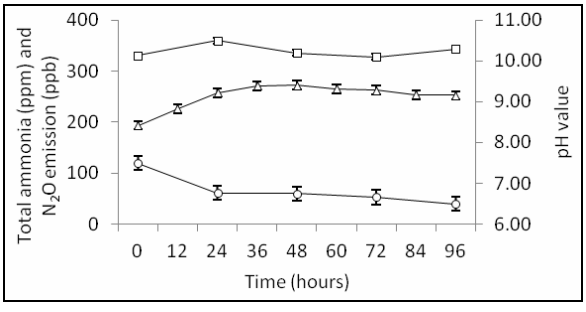


(c)

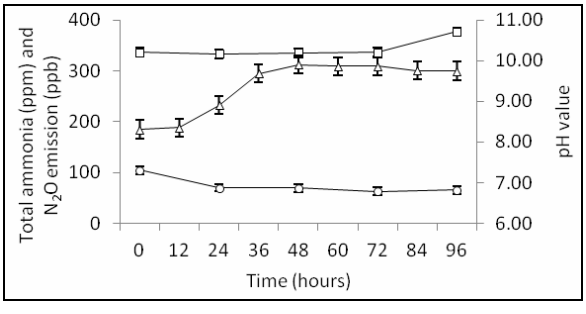


(d)

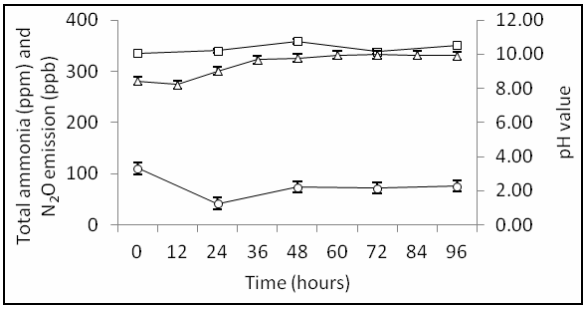
Figure 3 Total ammonia concentration (circle), N_2O emission (square) and pH value (triangle) of *Alcaligenes* sp. LS2T on citrate medium with different C/N ratios, (a) C/N 7 (b) C/N 14 (c) C/N 21 (d) C/N 28



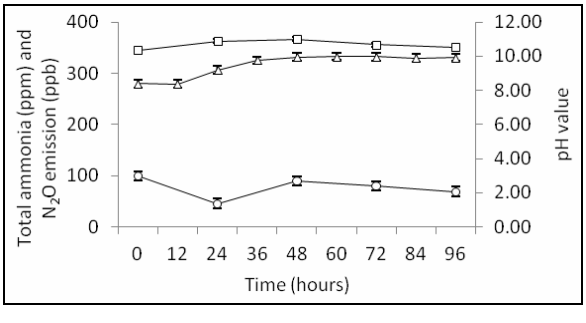
(a)



(b)



(c)



(d)

Furthermore, the statistical analysis for both acetate and citrate media showed a highly significant difference ($P < 0.01$) on total ammonia concentration and can be seen in Table 1 for acetate medium and Table 2 for citrate medium.

Table 1 Total ammonia concentration on acetate medium after *Alcaligenes* sp. LS2T inoculation

Time (h)	*Total ammonia concentration (ppm)*			
	C/N 7	*C/N 14*	*C/N 21*	*C/N 28*
0	82.16 ± 7.52^{g}	86.16 ± 5.57^{g}	84.02 ± 3.92^{g}	83.57 ± 4.04^{g}
24	50.48 ± 0.96^{f}	49.59 ± 11.89^{f}	33.82 ± 7.84^{c}	26.52 ± 4.10^{a}
48	37.89 ± 5.90^{d}	41.86 ± 6.88^{e}	49.22 ± 7.78^{f}	47.11 ± 0.45^{f}
72	36.78 ± 2.81^{a}	29.32 ± 0.00^{b}	47.65 ± 15.52^{f}	35.89 ± 0.00^{c}
96	24.16 ± 2.79^{a}	20.33 ± 3.46^{a}	15.57 ± 7.47^{a}	12.78 ± 2.61^{a}

Note: ^{a,b,c,d,e,f,g}Different superscripts show a highly significant difference ($P < 0.01$).

Table 2 Total ammonia concentration on citrate medium after Alcaligenes sp. LS2T inoculation

Time (h)	*Total ammonia concentration (ppm)*			
	C/N 7	*C/N 14*	*C/N 21*	*C/N 28*
0	119.33 ± 42.66^{i}	104.85 ± 32.73^{g}	110.06 ± 30.83^{h}	99.16 ± 26.71^{g}
24	60.95 ± 3.44^{b}	70.01 ± 17.03^{d}	41.75 ± 15.43^{a}	45.14 ± 4.35^{a}
48	59.97 ± 33.01^{b}	69.52 ± 0.00^{c}	73.61 ± 17.18^{d}	86.58 ± 17.13^{f}
72	52.50 ± 37.33^{a}	62.96 ± 0.05^{c}	71.26 ± 8.52^{d}	79.76 ± 27.42^{e}
96	39.25 ± 13.66^{a}	65.82 ± 1.54^{c}	75.06 ± 5.06^{d}	68.67 ± 35.56^{c}

Note: ^{a,b,c,d,e,f,g,h,i}Different superscripts show a highly significant difference ($P < 0.01$).

Measurement of N_2O on the acetate and citrate media showed that the highest N_2O emission in all measurement was found at the citrate medium with C/N 14 at 96 hours of incubation, producing 377.13 ± 20.11 ppb of N_2O gas. However, statistical analysis showed no significant difference ($P > 0.05$), indicating that C/N ratio of 7; 14; 21; and 28 whether on acetate or citrate medium and time of incubation gave no significant effect to the N_2O emission. Furthermore, the least emitted N_2O after 96-hours of incubation was shown in the acetate C/N 21 medium, emitting 329.00 ± 31.45 ppb N_2O or converting 0.25% of total nitrogen input. The statistical analysis of N_2O gas emission of *Alcaligenes* sp. LS2T on acetate and citrate medium can be seen in Table 3 and Table 4 respectively.

Other studies have shown a strong correlation between pH value to the nitrification process in which ammonia/ammonium was utilised by bacteria (Curtin et al., 1998; Paul and Clark, 1996; Strayer et al., 1981), indicating that the pH measurement could help elucidate the activity of the bacteria in the media. In this research, pH values were measured every 12 hours for 96 hours. The result of pH measurement showed a pH increase in both acetate and citrate media at every C/N ratio applied over time. The highest pH was achieved in acetate C/N 28 medium after 72-hours incubation, which reached 10.18. Even though the pH value experienced a decline in some media, the overall pH value during 96-hours incubation showed an increase compared to the initial pH, which ranging from 9.1–9.93 at the end of incubation. The understanding of the pH conditions on acetate and citrate media provide a broader knowledge of the application of

the bacteria as its ability to thrive and increase the pH of the media. Research done by Shen et al. (2008) showed that the *amoA* gene from ammonia oxidising bacteria were found until pH 8.6. Furthermore, pH was also known for its association with nitrification (Kemmitt et al., 2006) and denitrification process (Simek and Cooper, 2002) in which nitrogen gasses were produced.

Table 3 N_2O emission of *Alcaligenes* sp. LS2T on acetate medium

Time (h)	*N_2O emission (ppb)*			
	C/N 7^{ns}	*C/N 14*^{ns}	*C/N 21*^{ns}	*C/N 28*^{ns}
0	337.70 ± 25.73	329.13 ± 3.91	366.63 ± 22.76	313.17 ± 16.67
24	327.40 ± 19.28	342.60 ± 14.94	342.20 ± 6.16	333.77 ± 6.31
48	346.57 ± 10.36	350.43 ± 16.96	353.73 ± 27.29	347.33 ± 16.66
72	348.73 ± 8.17	362.20 ± 44.32	352.50 ± 21.60	372.10 ± 10.57
96	336.23 ± 28.20	334.77 ± 41.74	329.00 ± 31.45	366.03 ± 18.27

Note: ^{ns}Superscript shows no significant difference ($P > 0.05$).

Table 4 N_2O emission of *Alcaligenes* sp. LS2T on citrate medium

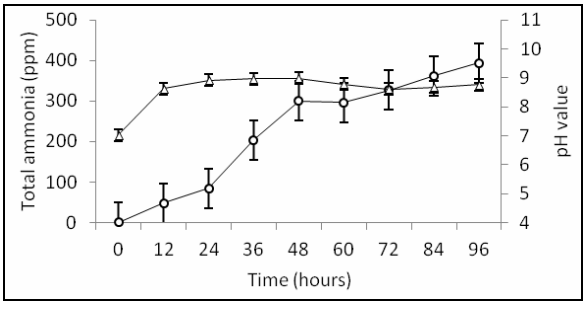
Time (h)	*N_2O emission (ppb)*			
	C/N 7^{ns}	*C/N 14*^{ns}	*C/N 21*^{ns}	*C/N 28*^{ns}
0	330.20 ± 117.87	336.40 ± 19.54	335.30 ± 29.17	345.13 ± 20.00
24	360.13 ± 15.15	333.30 ± 46.81	340.00 ± 42.18	363.13 ± 10.80
48	335.43 ± 54.79	335.43 ± 23.78	358.53 ± 18.12	366.60 ± 13.22
72	327.50 ± 27.18	336.70 ± 54.38	338.80 ± 31.48	356.30 ± 42.99
96	342.97 ± 25.87	377.13 ± 20.11	351.40 ± 26.80	350.67 ± 59.83

Note: ^{ns}Superscript shows no significant difference ($P > 0.05$).

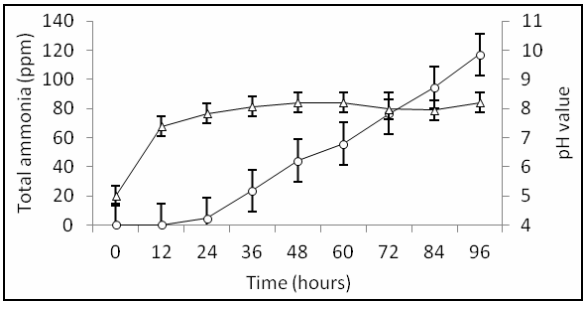
3.2 Total ammonia emission and pH changes in manure medium inoculated with Alcaligenes sp. LS2T

At the first measurement, all media showed no difference in the ammonia emission. However, the total ammonia emission on each media began to show highly significant differences ($P < 0.01$) after 12 hours of incubation based on the result of the LSD test (Table 5). The least emitted ammonia was shown at the acid medium with 116.92 ± 8.34 ppm after 96 hours of incubation; followed by a neutral medium with 298.91 ± 23.78 ppm; control medium with 394.11 ± 114.20 ppm and the highest total ammonia emission was on base medium with 437.34 ± 104.77 ppm after 96 hours of incubation. The results of this study also showed the strain abilities to suppress ammonia emission on a wide range of pH condition. The statistical analysis of total ammonia emission (Table 5) also revealed that the control medium began to emit higher ammonia other media after 24 hours of incubation, indicates that the addition of *Alcaligenes* sp.LS2T inoculum addition would show ammonia suppression after 24 hours of incubation. The change of total ammonia emission and pH changes in manure medium with different initial pH condition can be seen in Figure 4.

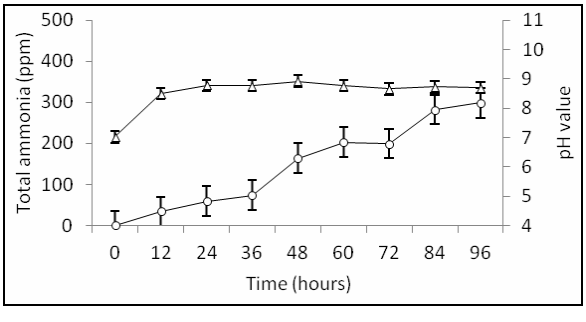
Figure 4 Total ammonia emission (circle) and pH value (triangle) of *Alcaligenes* sp. LS2T on manure media with different initial pH condition, (a) pH control (b) pH 5/acid medium, (c) pH 7/neutral medium (d) pH 9/base medium



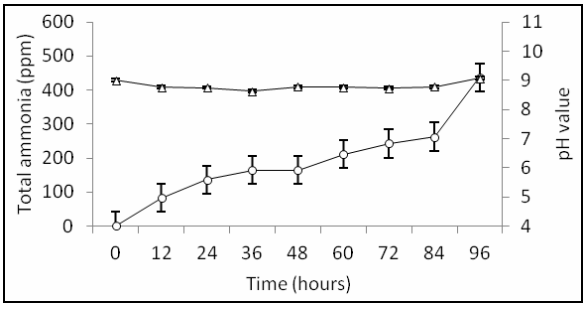
(a)



(b)



(c)



(d)

Table 5 Total ammonia emission on manure media after inoculated by *Alcaligenes* sp. LS2T

Media	*Total ammonia emission (ppm)*								
	0-h	*12-h*	*24-h*	*36-h*	*48-h*	*60-h*	*72-h*	*84-h*	*96-h*
Control	0.00 ± 0.00^{a}	48.28 ± 26.46^{a}	84.15 ± 44.46^{d}	306.25 ± 117.68^{j}	203.45 ± 93.42^{o}	296.42 ± 94.92^{m}	$3,226.46 \pm 132.36^{l}$	361.91 ± 69.46^{o}	394.11 ± 114.20^{p}
Acid	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	4.49 ± 0.44^{a}	23.53 ± 2.17^{a}	44.26 ± 24.00^{a}	55.85 ± 13.28^{b}	76.43 ± 18.41^{c}	94.57 ± 121.23^{e}	116.92 ± 8.34^{f}
Neutral	0.00 ± 0.00^{a}	34.01 ± 2.45^{a}	58.97 ± 20.53^{b}	73.99 ± 34.17^{c}	163.65 ± 38.94^{h}	202.72 ± 83.76^{h}	199.42 ± 31.44^{i}	282.34 ± 39.14^{n}	298.91 ± 23.78^{o}
Base	0.00 ± 0.00^{a}	82.24 ± 7.17^{d}	136.52 ± 63.60^{g}	164.40 ± 99.85^{h}	164.25 ± 78.99^{h}	211.48 ± 121.82^{h}	$242.55 \pm 0,77^{k}$	262.82 ± 100.84^{k}	437.34 ± 104.77^{p}

Note: ^{a,b,c,d,e,f,g,h,ij,k,l,m,n,o,p}Different superscripts show a highly significant difference ($P < 0.01$).

This study showed that differences in each initial pH condition of laying hen manure showed no correlation to the initial ammonia emission. Furthermore, all media showed the increase of pH on each measurement, reaching 8.76 on control medium; 8.2 on the acid medium; and 8.7 on neutral medium, while base medium showed the least pH increase which was reaching to 9.07 (Figure 4) after 96 hours.

The pH condition on each media also showed different changes. Control medium showed the continuous pH increase from 0 hours to 48 hours of incubation, then decreased during 60 to 72 hours of incubation, and bounced back until 96 hours of incubation, while the highest pH was reached on 48 hours of incubation. The similar trend also showed in the neutral medium.

However, acid medium showed continuous pH increase until reached its highest pH at 8.19 after 68 hours of incubation, then went down after 72 hours and 84 hours of incubation, but went back to 8.19 after 96 hours of incubation. Moreover, base medium showed a different trend than other media, which fell from the initial pH value and continuously losing its pH until 36 hours of incubation, then bounced back and reached 9.06 after 96 hours of incubation. Other experiments also showed that higher initial pH value in ammonia solution led to an increase in ammonia removal rates as well (Katehis et al., 1998).

4 Discussion

Research on nitrogen removal in waste products through microbial approach has been continuously performed, whether it is for domestic/municipal wastewater treatment (Zhou et al., 2016) or industrial wastewater treatment (Zuo et al., 2017). Since then, enormous amounts of microbial strains have been isolated from various environments and then observed on its unique characteristics. The understanding of the bacterial characteristics will provide information on its potential for field application, noting that factors such as dissolved oxygen concentration (Patureau et al., 2000), carbon and nitrogen (C/N) load ratio (Huang and Tseng, 2001; Kim et al., 2008), temperature (Joo et al., 2005; Lukow and Diekmann, 1997; Robertson and Kuenen, 1988), pH (Čuhel et al., 2010; Čuhel and Šimek, 2011), salinity (Ji et al., 2018) and other substances like mineral (Kiskira et al., 2017; Pan et al., 2018) were known to affect the nitrogen removal. In addition, among various factors which affect the bacterial capabilities to remove nitrogen load, the C/N ratio has drawn the attention as it directly affects the bacterial community and nitrogen removal as well (Heylen et al., 2006; Joo et al., 2005). It is also added by the varying C/N ratio condition in the environment, which can be as low as 2.8 (Li, 2016) or even up to 21 (Chiumenti, 2015), and both would require different treatment for efficient nitrogen load removal.

In this study, we observe the total ammonia removal characteristics of *Alcaligenes* sp. LS2T and the emitted N_2O through the process by adding the bacteria inoculum into the synthetic media and determine the total ammonia concentration and N_2O over 96 hours of incubation. Furthermore, its capabilities to suppress ammonia emission were also observed on the hen manure associated with different pH conditions. The synthetic media were divided into acetate and citrate medium based on the respective carbon source in the medium, and each further divided into four groups of different C/N ratio (7, 14, 21 and 28) in order to better understand the effect of different carbon sources and C/N ratios to

the bacteria performance in removing nitrogen, while the manure media were divided into acid, neutral, and base condition.

The different C/N ratio of synthetic media was prompted from the varying C/N ratio of poultry waste, which ranged from 5.8 to 14.7 (Silva et al., 2009) depending to the other organic matters in the mixture like straw or sawdust. However, a wider observation of the C/N ratio would further elucidate the strain potentials to remove nitrogen in various wastewater C/N ratio condition. Another research which has observed the bacterial nitrogen removal in the media with C/N ratio up to 28 was done by Liu et al. (2015) on the heterotrophic nitrification and aerobic denitrification of *Alcaligenes faecalis* C16. In addition, different carbon sources are also known to affect bacterial nitrification and denitrification performance. Research by Joo et al. (2005), showed that different carbon sources affect the nitrification and denitrification of the bacteria, in which, their study showed that glucose, sucrose, fructose, and methanol as a carbon source in the medium was not utilised by the heterotrophic nitrifier-aerobic denitrifier *Alcaligenes faecalis* no. 4, and the nitrogen removal only occurred when acetate or citrate was used as the carbon source. Moreover, research by Liu et al. (2015) reported that citrate was more preferred by *Alcaligenes faecalis* C16 over acetate as carbon source in terms of the cell growth, in which will affect the strain to perform heterotrophic nitrification and aerobic denitrification, allegedly caused by the different carbon's affinity to be directly involved in citric acid cycle.

Our previous study on *Alcaligenes* sp. LS2T (Azkarahman et al., 2017) showed the strain ability to utilise ammonium as a nitrogen source for the metabolism which produced nitrite and nitrate through the process. In this study, we further analyse its total ammonia concentration and N_2O emission as it showed a potential application for nitrogen removal from poultry waste. The decreasing total ammonia concentration in this research described the strain characteristics in performing heterotrophic nitrification. Moreover, the reduction in total ammonia concentration and N_2O gas emission on the acetate and citrate media indicate the strain ability to perform aerobic denitrification process. Allen (2009) and Prosser (2005) stated that the oxidation of NH_3 or NH_4^+ into nitrite (NO_2^-) and nitrate (NO_3^-) could further undergone denitrification process which produced nitrogen gasses like N_2O or N_2. Other studies on *Alcaligenes* spp. also showed the strain ability to perform heterotrophic nitrification and aerobic denitrification simultaneously (Joo et al., 2005; Liu et al., 2015; Zhao et al., 2012).

From the results, it can be seen that *Alcaligenes* sp. LS2T was able to perform aerobic nitrification and denitrification in the media even at the C/N ratio as high as 28. Research by Akunna et al. (1993), Ramakrishnan and Gupta (2008) showed that the carbon availability and C/N ratio in the medium could affect the denitrification process, in which will affect the nitrate reduction pathway and resulted in different denitrifying performance. Research done by Joo et al. (2005) showed that the heterotrophic nitrification and aerobic denitrification of *Alcaligenes faecalis* No. 4 was optimum at the medium with C/N ratio 10. The capability of *Alcaligenes sp*. LS2T strain to thrive and remove nitrogen content in C/N 28 medium thus propose its possibility for nitrogen removal treatment in high C/N ratio wastewater. The overall total ammonia concentration in this study was lower at the acetate medium over citrate medium, while the lowest ammonia concentration was achieved in acetate C/N 28 medium (±82.5% of total ammonia removed). A similar condition was seen in another heterotrophic nitrifier-aerobic denitrifier, *Alcaligenes faecalis* C16, which showed higher total nitrogen

removal in acetate medium over citrate medium, in which the highest nitrogen removal was achieved in acetate C/N14 medium followed by C/N 28 even though C16 showed higher total nitrogen removal capabilities (Liu et al., 2015). However, a different condition was shown by *Alcaligenes faecalis* No.4, as the strain preferred acetate as the carbon source and completely removed the ammonium at C/N 10 and 20 (Joo et al., 2005). Moreover, the *Alcaligenes faecalis* No.4 could not remove the ammonium content completely in C/N 5 medium, both in acetate and citrate as a carbon source. The condition combined with C16 and this study were thus in accordance to Heylen et al. (2006) which showed that the optimum nitrogen removal by denitrifying bacteria will be favourable for treating high C/N wastewater up to 25.

It is well known that the quantification of bacteria would further elucidate the bacterial condition during the whole nitrification and denitrification process, yet, due to limitations, the *Alcaligenes* sp.LS2T density during 96 hours of incubation was not measured except for the initial addition, which was 1.35×10^7 CFU/ml. However, in our previous study, the growth phase of *Alcaligenes* sp.LS2T in similar synthetic media was observed. The OD_{600} measurement showed that the strain is known to reach the stationary phase after 21 and 18 hours incubation in acetate and citrate medium respectively (Azkarahman et al., 2017). An in situ observation of aerobic denitrifiers' density measurement was done by Zhou et al. (2019) and showed that the aerobic denitrifiers in Zhoucun reservoir were increased from $0.71 \pm 0.22 \times 10^2$ CFU/ml to $8.64 \pm 2.08 \times 10^3$ CFU/ml along with total nitrogen removal. In addition, research by Pastawan et al. (2017) also showed a similar result, in which a consortium of *Pseudomonas* sp. LS3K, *Candida* sp. LS3T, and *Arthrobacter* sp. LM1KK was increased over time along with ammonia reduction for 96-hours incubation. Moreover, research by Foglar et al. (2005) showed that the bacterial biomass from activated sludges was increased along with nitrate removal, increased from 1.8×10^9 to 2.1×10^9 after 3 hours of denitrification in the synthetic media. Based on previous observation and other research on denitrifiers bacteria density, the *Alcaligenes* sp.LS2T density in this study is also expected to be increased along with the nitrogen removal in the media. Nevertheless, direct measurement should be performed to confirm the assumption.

Nitrous oxide (N_2O) is a nitrogen gas which could be produced whether by the reduction of NO_3^- through unfinished denitrification process and slightly through anaerobic ammonium oxidation (anammox) process. The production of N_2O gas from acetate and citrate media further describe the *Alcaligenes sp.* LS2T contribution to the nitrogen cycle allegedly through aerobic denitrification process, as in our previous study (Azkarahman et al., 2017) nitrite and nitrate were also produced. However, the possibility of *Alcaligenes sp*. LS2T to produce N_2O through anammox process is yet to be known. The strain ability to perform denitrification under aerobic condition gave special notation to the strain as denitrifying bacteria usually thrive in anoxic conditions, where nitrates were used as electron acceptors during the respiratory process in the place of the oxygen (Rossi et al., 2015). The effects of the C/N ratio on denitrification have been extensively investigated in a wide range of ratios for different carbon sources (Dhamole et al., 2015; Mohan et al., 2016a; Mohan et al., 2016b). In the process of denitrification, the nature of the carbon source and its availability in terms of the C/N ratio impact both the nitrate removal pathway and the carbon utilisation pattern, which resulted in different removal performances (Akunna et al., 1993; Ramakrishnan and Gupta, 2008). However, the result of this study showed that the C/N ratio gave no significant differences to the denitrifying ability.

Research by Joo et al. (2005) showed that about 5% of the removed ammonium in the medium was denitrified into N_2O gas. In this study, the least N_2O emission was shown at acetate C/N 21 medium which converted only 0.25% of the total nitrogen input into N_2O gas. The amount of N_2O emission in this study is also lower compared to the aerobic denitrification of *Alcaligenes faecalis* TUD, which converted 9% of used nitrogen into N_2O with 76% air saturation (Otte et al., 1996). Moreover, the denitrification process is also known to be affected by the pH condition (Simek and Cooper, 2002). A study by Bai et al. (2017) showed the denitrification rates were only significantly and negatively correlated with soil pH (ranging from 7.06 to 9.11), implies that higher pH values (at least higher than 8.59) could prohibit the denitrification processes. However, this study showed that the aerobic denitrification ability from *Alcaligenes sp.* LS2T gave no significant differences on the N_2O production at every hour of measurement, even though the pH value were increased along 96 hours of inoculation.

In this study, we also inoculate *Alcaligenes* sp. LS2T into manure with different initial pH conditions to further determine strain ability when applied to reduce ammonia emission from waste. The absence of manure sterilisation was aimed to understand the direct strain inoculation to the manure with different pH value on the application level. A lot of studies have been done to reduce the ammonia emission from animal waste like piggery wastewater (Joo et al., 2006), poultry manure (Posmanik et al., 2014) or dairy wastewater (Neerackal et al., 2016) by using microbial treatment. The 4-days measurement used in this research was considered enough to reveal the possible differences in ammonia emission. According to earlier studies, ammonia emission is at its highest rate on the day of application and declines sharply in the following few days (Yang et al., 2003). In addition, Meisinger et al. (2001) reported that losses of ammonia through emission are very rapid during the first 6–12 hours, while Rostami et al. (2015) stated that the 4-day estimations of the NH_3 emission from animal wastes are enough while estimation from chemical fertilisers will require longer time.

The low ammonia emission in acid medium allegedly caused by the combined effect of strain capabilities, another biomass occurred in the manure and the pH condition. The similar result also showed with Joo et al. (2005), Liu et al. (2015) and Zhao et al. (2012), where less ammonia emission was shown on the neutral medium compared to the control medium which has same pH condition. Ammonia is usually correlated with the N availability and pH, which tend to have effects on both the bacteria and archaea. Increasing soil pH positively affects ammonia oxidising bacteria (Nicol et al., 2008). Research by Li (2000) showed that lower pH leads to a lower proportion of aqueous ammonia and therefore decreases ammonia emission. Gay and Knowlton (2005) described that the acid condition of media would yield lower ammonia concentration as most of the component would be ionised into ammonium. Huijsmans et al. (2003) reported similar results, in which the increase of pH could raise ammonia emission of 10 times.

On the ammonia removal from pig slurry, research by Bonmati and Flotats (2003) also showed that the lower initial pH condition leads to higher ammonia removal. The correlation of pH and ammonia emission showed a unique relationship as bacteria grow better on the base pH condition, while acid condition could lower the emitted ammonia. This study showed that the acid medium released significantly less ammonia emission than any observed media, which indicates that lower pH yields less ammonia emission. Furthermore, the neutral medium was able to release less ammonia emission compared to

the control medium but, showed that the effect of ammonia oxidation by bacteria could also be seen without initially acidify the media. However, the high total ammonia emission in base medium showed the limitation for bacteria in suppressing ammonia.

5 Conclusions

In this research, *Alcaligenes sp.* LS2T showed its capabilities to remove nitrogen content from the acetate and citrate media and showed that the least total ammonia concentration was shown in acetate C/N 28 medium, contained 12.77 ± 2.61 ppm of total ammonia concentration in the medium after 96 hours of incubation. The N_2O emissions from the acetate and citrate media also indicate the strain ability to perform aerobic denitrification. However, no significant difference ($P < 0.05$) was found in N_2O emission, while the highest N_2O emission (377.13 ± 20.11 ppb) was found in citrate medium with C/N ratio 14.

The inoculation of *Alcaligenes* sp. LS2T on laying hen manure with different initial pH condition was also done to understand strain potential to suppress ammonia emission. The inoculation was able to reduce the ammonia emission on acid to neutral pH conditions. The reducing effect was also known to be highly correlated with the pH condition. The acid medium showed the lowest ammonia emission, while the pH of the medium also seen to increase continuously through the time of incubation.

References

Akunna, J.C., Bizeau, C. and Moletta, R. (1993) 'Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: glucose, glycerol, acetic acid, lactic acid and methanol', *Water Resource*, Vol. 27, No. 2, pp.1303–1312.

Allen, J. (2009) *Ammonia Oxidation Potential and Microbial Diversity in Sediments from Experimental Bench-Scale Oxygen-Activated Nitrification Wetlands*, Washington State University, Washington.

American Public Health Association (1998) *Standard Methods for the Examination of Water and Wastewater*, 20th ed., APHA, Washington.

Azkarahman, A.R., Erwanto, Y., Hadisaputro, W., Yusiati, L.M. and Fitriyanto, N.A. (2017) 'Characteristics of *Alcaligenes* sp. LS2T heterotrophic and aerobic ammonium removal for potential livestock's wastewater treatment', in Isnansetyo, A. and Nuringtyas, T. (Eds.): *Proceeding of the 1st International Conference on Tropical Agriculture*, pp.337–344, Springer, Cham.

Bahl, A. and Bahl, B.S. (2004) *Advanced Organic Chemistry*, S. Chand & Company, New Delhi.

Bai, J., Wang, X., Jia, J., Zhang, G., Wang, Y. and Zhang, S. (2017) 'Denitrification of soil nitrogen in coastal and inland salt marshes with different flooding frequencies', *Phys. Chem. Earth*, Vol. 97, pp.31–36.

Bonmati, A. and Flotats, X. (2003) 'Air stripping of ammonia from pig slurry: characterization and feasibility as pre- or post-treatment to mesophilic anaerobic digestion', *Waste Management*, Vol. 23, No. 3, pp.261–272.

Chen, W., Liao, X., Wu, Y., Liang, J.B., Mi, J., Huang, J., Zhang, H., Wu, Y., Qiao, Z., Li, X. and Wang, Y. (2017) 'Effects of different types of biochar on methane and ammonia mitigation during layer manure composting', *Waste Management*, Vol. 61, pp.506–515.

Chiumenti, A. (2015) 'Complete nitrification-denitrification of swine manure in a full-scale, non-conventional composting system', *Waste Management*, Vol. 46, pp.577–587.

Čuhel, J. and Šimek, M. (2011) 'Proximal and distal control by pH of denitrification rate in a pasture soil', *Agriculture, Ecosystem, and Environment*, Vol. 141, Nos. 1–2, pp.230–233.

Čuhel, J., Šimek, M., Laughlin, R.J., Bru, D., Cheneby, D., Watson, C.J. and Philippot, L. (2010) 'Insights into the effect of soil pH on N_2O and N_2 emissions and denitrifier community size and activity', *Applied Environmental Microbiology*, Vol. 76, pp.1870–1878.

Curtin, D., Campbell, C.Z. and Jalil, A. (1998) 'Effects of acidity on mineralization: pH dependence of organic matter mineralization in weakly acidic soils', *Soil Biol Biochem*, Vol. 30, No. 1, pp.57–64.

Dhamole, P.B., Nair, R.R., D'Souza, S.F., Pandit, A.B. and Lele, S.S. (2015) 'Denitrification of high strength nitrate waste from a nuclear industry using acclimatized biomass in a pilot scale reactor', *Appl. Biochem. Biotechnol.*, Vol. 175, No. 2, pp.748–756.

Foglar, L., Briški, F., Sipos, L. and Vuković, M. (2005) 'High nitrate removal from synthetic wastewater with the mixed bacterial culture', *Bioresource Technology*, Vol. 96, No. 8, pp.879–888.

Gay, S.W. and Knowlton, K.F. (2005) *Ammonia Emissions and Animal Agriculture*, Virginia Cooperative Extension Fact Sheet publication.

Goldstein, D.L. and Skadhauge, E. (2000) 'Renal and extrarenal regulation of body fluid composition', in Whittow, G.C. (Eds.): *Sturkies Avian Physiology*, pp.265–297, Academic Press, San Diego, California.

Gomez, K.A. and Gomez, A.A. (1984) *Statistical Procedures for Agricultural Research*, Wiley-Interscience Publication, New York.

Gupta, A.B. and Gupta, S.K. (2001) 'Simultaneous carbon and nitrogen removal from high strength domestic wastewater in an aerobic RBC biofilm', *Water Res.*, Vol. 35, No. 7, pp.1714–1722.

Heylen, K., Vanparys, B., Wittebolle, L., Verstraete, W., Boon, N. and Vos, P.D. (2006) 'Cultivation of denitrifying bacteria: optimization of isolation conditions and diversity study', *Applied and Environmental Microbiology*, Vol. 72, No. 4, pp.2637–2643.

Huang, H.K. and Tseng, S.K. (2001) 'Nitrate reduction by *Citrobacter diversus* under aerobic environment', *Applied Microbiology and Biotechnology*, Vol. 55, No. 1, pp.90–94.

Huijsmans, J.F.M., Hol, J.M.G. and Vermeulen, G.D. (2003) 'Effect of application method, manure characteristics, weather and field conditions on ammonia volatilization from manure applied to arable land', *Atmospheric Env.*, Vol. 37, No. 26, pp.3669–3680.

Indonesian Agricultural Environment Research Institute (2007) *N_2O Sample Collection and Analysis*, IAERI, Pati.

Ji, J., Peng, Y., Wang, B., Mai, W., Li X., Zhang, Q. and Wang, S. (2018) 'Effects of salinity build-up on the performance and microbial community of partial-denitrification granular sludge with high nitrite accumulation', *Chemosphere*, Vol. 209, pp.53–60.

Joo, H.S., Hirai, M. and Shoda, M. (2005) 'Characteristics of ammonium removal by heterotrophic nitrification-aerobic denitrification by *Alcaligenes faecalis* No. 4', *J. Biosci. Bioeng.*, Vol. 100, No. 2, pp.184–191.

Joo, H.S., Hirai, M. and Shoda, M. (2006) 'Piggery wastewater treatment using *Alcaligenes faecalis* strain no.4 with heterotrophic nitrification and aerobic denitrification', *Water Res.*, Vol. 40, No. 16, pp.3029–3036.

Katehis, D., Diyamandoglu, V. and Fillos, J. (1998) 'Stripping and recovery of ammonia from concentrate of anaerobically digested biosolids at elevated temperature', *Water Environ. Res.*, Vol. 70, No. 2, pp.231–240.

Kemmitt, S.J., Wright, D., Goulding, K.W.T. and Jones, D.L. (2006) 'pH regulation of carbon and nitrogen dynamics in two agricultural soils', *Soil Biol. Biochem.*, Vol. 38, No. 5, pp.898–911.

Kim, M., Jeong, S., Yoon, S.J., Cho, S.J., Kim, Y.H., Kim, M.J., Ryu, E.Y. and Lee, S. (2008) 'Aerobic denitrification of *Pseudomonas putida* AD-21 at different C/N ratios', *Journal of Bioscience and Bioengineering*, Vol. 106, No. 4, pp.498–502.

Kiskira, K., Papirio S., van Hullebusch, E.D. and Esposito, G. (2017) 'Fe(II)-mediated autotrophic denitrification: a new bioprocess for iron bioprecipitation/biorecovery and simultaneous treatment of nitrate-containing wastewater', *International Biodeterioration & Biodegradation*, Vol. 119, pp.631–648.

Li, C.S. (2000) 'Modeling trace gas emissions from agricultural ecosystems', *Nutrient Cycling in Agroecosystems*, Vol. 58, Nos. 1–3, pp.259–279.

Li, J.T., Zhong, X.L., Wang, F. and Zhao, Q.G. (2011) 'Effect of poultry litter and livestock manure on soil physical and biological indicators in a rice-wheat rotation system', *Plant Soil Environment*, Vol. 57, No. 8, pp.351–356.

Li, K. (2016) *A Study of Low C/N Ratio Waste Water Treatment Using Fluidized Bed Bioreactor*, Master's thesis [online] https://ir.lib.uwo.ca/etd/3523/ (accessed 4 January 2019).

Lide, D.R. and Frederikse, H.P.R. (1993) *CRC Handbook of Chemistry and Physics: A Ready-Reference Book of Chemical and Physical Data*, 74th ed., CRC Press, London.

Liu, Y., Wang, Y., Li, Y., An, H. and Lv, Y. (2015) 'Nitrogen removal characteristics of heterotrophic nitrification-aerobic denitrification by *Alcaligenes faecalis* C16', *Chin. J. Chem. Eng.*, Vol. 23, No. 5, pp.827–834.

Lukow, T. and Diekmann, H. (1997) 'Aerobic denitrification by a newly isolated heterotrophic bacterium strain TL1', *Biotechnology Letters*, Vol. 19, No. 11, pp.1157–1159.

Maeda, K., Toyoda, S., Hanajima, D. and Yoshida, N. (2013) 'Denitrifiers in the surface zone are primarily responsible for the nitrous oxide emission of dairy manure compost', *J. Hazard Mater.*, Vols. 248–249, pp.329–336.

Maia, G.D.N., Day, V.G.B., Gates, R.S. and Taraba, J.L. (2012) 'Ammonia biofiltration and nitrous oxide generation during the start-up of gas-phase compost biofilters', *Atmos. Environ.*, Vol. 46, pp.659–664.

Maliselo, P.S. and Nkonde, G.K. (2015) 'Ammonia production in poultry houses and its effect on the growth of *Gallus gallus domestica* (broiler chickens): a case study of a small scale poultry house in Riverside, Kitwe, Zambia', *Int. J. Sci. Technol. Res.*, Vol. 4, pp.141–145.

Meisinger, J.J., Lefcourt, A.M. and Thompson, R.B. (2001) 'Construction and validation of small mobile wind tunnels for studying ammonia volatilization', *Appl. Eng. Agric.*, Vol. 17, No. 3, pp.375–381.

Mohan, T.V.K., Nancharaiah, Y.V., Venugopalan, V.P. and Sai, P.M.S. (2016a) 'Effect of C/N ratio on denitrification of high-strength nitrate wastewater in anoxic granular sludge sequencing batch reactors', *Ecol. Eng.*, Vol. 91, pp.441–448.

Mohan, T.V.K., Renu, K., Nancharaiah, Y.V., Sai, P.M.S. and Venugopalan, V.P. (2016b) 'Nitrate removal from high strength nitrate-bearing wastes in granular sludge sequencing batch reactors', *J. Biosci. Bioeng.*, Vol. 121, No. 2, pp.191–195.

Neerackal, G.M., Ndegwa, P.M., Joo, H.S., Wang, X., Frear, C.S., Harrison, J.H. and Beutel, M.W. (2016) 'Potential application of *Alcaligenes faecalis* strain No.4 in mitigating ammonia emissions from dairy wastewater', *Biores. Technol.*, Vol. 206, pp.36–42.

Nicol, G.W., Leininger, S., Schleper, C. and Prosser, J.I. (2008) 'The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria', *Environ. Microbiol.*, Vol. 10, No. 11, pp.2966–2978.

O'Mara, F.P. (2011) 'The significance of livestock as a contributor to global greenhouse gas emissions today and in the near future', *Anim. Feed Sci. Technol.*, Vols. 166–167, pp.7–15.

Otte, S., Grobben, N.G., Robertson, L.A., Jetten, M.S.M. and Kuenen, J.G. (1996) 'Nitrous oxide production by *Alcaligenes faecalis* under transient and dynamic aerobic and anaerobic conditions', *Applied and Environmental Microbiology*, Vol. 62, No. 7, pp.2421–2426.

Pan, J., Wei, C., Fu, B., Ma, J., Preis, S., Wu, H. and Zhu, S. (2018) 'Simultaneous nitrite and ammonium production in an autotrophic partial denitrification and ammonification of wastewaters containing thiocyanate', *Bioresource Technology*, Vol. 252, pp.20–27.

Pastawan, V., Erwanto, Y., Yusiati, L.M., Jamhari, Hayakawa, T., Nakagawa, T. and Fitriyanto, N.A. (2017) 'Ability of indigenous microbial consortium in the process of ammonia oxidation of livestock waste', *Asian J. Anim. Sci.*, Vol. 11, No. 2, pp.74–81.

Patureau, D., Bernet, N., Delgenes, J.P. and Moletta, R. (2000) 'Effect of dissolved oxygen and carbon-nitrogen loads on denitrification by an aerobic consortium', *Applied Microbiology and Biotechnology*, Vol. 54, No. 4, pp.535–542.

Paul, E.A. and Clark, F.E. (1996) *Soil Microbiology and Biochemistry*, Academic Press, San Diego.

Posmanik, R., Amit, G. and Nejidat, A. (2014) 'Effect of high ammonia loads emitted from poultry-manure digestion on nitrification activity and nitrifier-community structure in a compost biofilter', *Ecol. Eng.*, Vol. 62, pp.140–147.

Prosser, J.I. (2005) 'Nitrogen in soils/nitrification', in Hillel, D. (Eds.): *Encyclopedia of Soils Environment*, pp.31–39, Elsevier, New York.

Ramakrishnan, A. and Gupta, S.K. (2008) 'Effect of COD/NO_3^--N ratio on the performance of a hybrid UASB reactor treating phenolic wastewater', *Desalination*, Vol. 232, Nos. 1–3, pp.128–138.

Ravishankara, A.R., Daniel, J.S. and Portmann, R.W. (2009) 'Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century', *Science*, Vol. 326, No. 5949, pp.123–125.

Robertson, L.A. and Kuenen, J.G. (1988) 'Heterotrophic nitrification in *Thiosphaera pantotropha*: oxygen uptake and enzyme studies', *Microbiology*, Vol. 134, No. 4, pp.857–863.

Rossi, F., Motta, O., Matrella, S., Proto, A. and Vigliotta, G. (2015) 'Nitrate removal from wastewater through biological denitrification with OGA 24 in a batch reactor', *Water*, Vol. 7, No. 1, pp.51–62.

Rostami, M., Monaco, S., Sacco, D., Grignani, C. and Dinuccio, E. (2015) 'Comparison of ammonia emissions from animal wastes and chemical fertilizers after application in the soil', *Int. J. Recycl. Org. Waste Agric.*, Vol. 4, No. 2, pp.127–134.

Shen, J.P., Zhang, L.M., Zhu, Y.G., Zhang, J.B. and He, J.Z. (2008) 'Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam', *Environ. Microbiol.*, Vol. 10, No. 6, pp.1601–1611.

Silva, M.E., Lemos, L.T., Cunha-Queda, A.C. and Nunes, O.C. (2009) 'Co-composting of poultry manure with low quantities of carbon-rich materials', *Waste Manage Res*., Vol. 27, No. 2, pp.119–128.

Simek, M. and Cooper, J.E. (2002) 'The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years', *Eur. J. Soil Sci.*, Vol. 53, No. 3, pp.345–354.

Strayer, R.F., Lin, C.J. and Alexander, M. (1981) 'Effect of simulated acid rain on nitrification and nitrogen mineralization in forest soils', *J. Environ. Qual*., Vol. 10, No. 4, pp.547–551.

Wantasen, S., Sudarmadji, E., Sugiharto, and Suprayogi, S. (2012) 'The impact of nitrogen transformation on the biotic environment in the Lake Tondano North Sulawesi', *Jurnal Manusia dan Lingkungan*, Vol. 19, No. 2, pp.143–149.

Wei, F.X., Hu, X.F., Xu, B., Zhang, M.H., Li S.Y., Sun, Q.Y. and Lin, P. (2015) 'Ammonia concentration and relative humidity in poultry houses affect the immune response of broilers', *Genet. Mol. Res.*, Vol. 14, No. 2, pp.3160–3169.

Yang, Z., Niimi, H., Kanda, K. and Suga, Y. (2003) 'Measurement of ammonia volatilization from a field, in upland Japan, spread with cattle slurry', *Environ. Pollut.*, Vol. 121, No. 3, pp.463–467.

Zhao, B., An, Q., He, Y.L. and Guo, J.S. (2012) 'N_2O and N_2 production during heterotrophic nitrification by *Alcaligenes faecalis* strain NR', *Bioresour. Technol.*, Vol. 116, pp.379–385.

Zhou, H., Li, X., Chu, Z. and Zhang, J. (2016) 'Effect of temperature downshifts on a bench-scale hybrid A/O system: process performance and microbial community dynamics', *Chemosphere*, Vol. 153, pp.500–507.

Zhou, S., Zhang, Y., Huang, T., Liu, Y., Fang, K. and Zhang, C. (2019) 'Microbial aerobic denitrification dominates nitrogen losses from reservoir ecosystem in the spring of Zhoucun reservoir', *Science of the Total Environment*, Vol. 651, No. 1, pp.998–1010.

Zuo, K., Chang, J., Liu, F., Zhang, X., Liang, P. and Huang, X. (2017) 'Enhanced organics removal and partial desalination of high strength industrial wastewater with a multi-stage microbial desalination cell', *Desalination*, Vol. 423, pp.104–110.