Nitrogen loss characterization of stored human urine

Sukanta Rana

International Centre for Ecological Engineering, University of Kalyani, Kalyani–741235, West Bengal, India

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Corresponding Author:
Sukanta Rana
E–mail: sukantarana013@gmail.com

ABSTRACT

Human urine has a great fertilizer potential due to having high concentration of nutrients. The storage and application of human urine as fertilizer are the significant concerns in this respect. The objective of present study was to quantify the N loss from stored human urine under three storage conditions namely – small mouth open container, big mouth open container and aerated big mouth open container during month long investigation. The investigation was carried out for 39 days long at room temperature (28°C). The results revealed that 93.69% N loss occurred in 39 day from narrow mouth, 93.81% in 24 days from wide mouth and 94.93% in 9 days from wide mouth aerated condition. The rate of N loss in wide mouth aerated was 3 folds of the rate in wide mouth and 4.9 folds of the rate in narrow mouth. The % of N loss was proportionately linear to pH rise of urine in all three conditions. Storage of urine in narrow mouth container is the best option to minimize N loss. Furthermore, a comparative study is required on fertilizer efficiency of urine stored in open and closed conditions considering the role of associated microorganisms.

1. Introduction

Human urine has a great fertilizer potential (Jana et al. 2012). The Nitrogen (N), Phosphorus (P) and Potassium (K) ratio of human urine is 15:1:2 (Zsofia 2005). In fresh human urine, about 85% of N exists as urea and 5% of total ammonia [unionized ammonia (NH_3) and ionized ammonia (NH_4^+)] (Udert et al. 2006). The urea undergoes hydrolysis by microbes during the course of excretion through urinary passage. Microbial urease enzyme catalyzes the hydrolysis of urea into ammonium ions (NH_4^+) and bicarbonate ions (HCO_3^-) (Equation 1) and the urine becomes more alkaline by forming more HCO_3^- that raises the pH of the urine from 6.2 to 9.1 (Udert 2003). In alkaline condition (pH > 7) ammonium ions are converted into un-ionized ammonias ($NH_{3(dissolved)}$) which at normal temperature are transformed into ammonia gases ($NH_{3(gas)}$) and then volatilize from the upper surface of the solution (Equation 2).

$$CO(NH_2)_2 + H^+ + 2H_2O \xrightarrow[\text{Urease}]{} 2NH_4^+ + HCO_3^- \quad (1)\ \text{(Hydrolysis)}$$

$$NH_4^+ \xrightarrow[pH > 7.0]{25°C} NH_{3\,(dissolved)} + H^+ \quad (2)\ \text{(Conversion)}$$

$$NH_{3\,(dissolved)} \xrightarrow[pH > 7.0]{} NH_{3\,(gas)} \quad (3)\ \text{(Volatilization)}$$

Volatilization of NH_3 gas from alkaline urine causes loss of N (Equation 3). This is a great factor for stored urine than fresh urine. Even if the fresh urine is sprayed on land, N-loss also occurs. In stored urine, the total ammonia accounts for 90% when all urea molecules are hydrolyzed (Udert 2003). Udert et al. (2003) estimated that the concentration of NH_3 were 0.3 g N m^{-3} and 2700 g N m^{-3} in fresh and stored human urine respectively. The dissolved NH_3 gas escapes from

urine as bubbles, when the partial pressure of dissolved NH_3 gas in stored urine exceeds the pressure of ammonia gas in air. Therefore, stored human urine is more toxic than fresh urine.

This N loss is usually responsible for decreasing the fertilizer potential of urine. The N loss is more in stored urine than fresh urine. Hellström et al. (1999) showed that NH_3 loss was marginal in closed storage tanks but the concentration of NH_3 gas in the head space of storage tanks was disturbing. So working in urine storage tanks can be harmful. Besides, odour problem will occur if the tank is not closed properly. When stored urine is spread in field as fertilizer, the NH_3 loss would be high (Udert 2006). A study conducted by Stockholm Water Company revealed that application of stored urine caused NH_3 loss by 1 – 10% (Udert 2006). Apart from the NH_3 loss, the smell of stored urine may irritate the residential people near the field. In a study of Stockholm Water Company reported that NH_3 could be smelled for 24 hours after application in agricultural field (Johansson 2001).

In spite of these drawbacks, human urine must be stored to manage the annual massive production of urine (500 L per capita; Richert et al. 2010). A number of feasible techniques have been followed to minimize the NH_3 loss from stored urine, for example – acid addition (pH < 7.5) to inhibit ammonification of urea (Udert et al. 2006; Jones et al. 2007), nitrification of ammonia with addition of agricultural and horticultural soil (Pelczar et al. 1993), cooling (Jones et al. 2007) and application of urease inhibitors to delay the hydrolysis of urea by 2 – 10 weeks (Jones et al. 2007).

Container size and interaction between urine and air are also important factors for NH_3 loss from stored urine those have not been investigated so far. The present experiment was performed to characterize the N loss from human urine stored in three open containers over one month in three different conditions at room temperature.

2. Materials and methods

The N loss experiment was performed in three conditions – in narrow mouth (NM) glass bottle, in wide mouth (WM) glass beaker and in wide mouth glass beaker with motorized aeration (WMA), having three replicates. Human urine was collected from the water flush urinals of male students ageing 23 to 25 years of the department of Zoology, University of Kalyani and was stored instantly in a large plastic container (10 L). Collected urine was undiluted, since water flush pipes remained closed during collection of urine from urinals. Then the procured urine was poured into different experimental container within 1 to 2 hours of collection. The container had a small opening with tight fitting stopper to minimize the possibility of NH_3 loss.

The plastic container (Mark, Germany; 500 mL, Diameter 18 cm) were of high quality, leaching free and non light penetrating. Both the glass bottles and glass beakers (Borosil, Germany, 500 mL) were also of high quality and light penetrating. The diameter of the bottles and beakers were 5.73 cm and 8.91 cm respectively and their open surface areas were 25.77 cm^2 and 62.37 cm^2. The open mouth of each container was covered with nylon net to prevent any fall in of insect, moth, beetle or any unwanted material into the urine solution. An air pipe with terminal perforated bulb was dipped into one urine filled container and the pipe was connected to an electric air blower. The blower was switched on for five minutes ten minutes before the sampling. This experimental set up was kept in a closed room at room temperature (28 – 30°C) and with minimum aeration for more than one month. Samples were taken by separate injection syringe of 5 ml at every 24 hrs interval between 19:00 to 19:30 hrs a day.

The concentration of urea–N of the sample was quantified by Oxime method and total ammonia – N (NH_3–$N_{(dissolved)}$ + NH_4^+–N) by Indo–Phenol blue photometric method (APHA 1995). Dissolved NH_3-N was calculated following a standard chart (Chemistry Laboratory Methods Manual 2001). The pH of all the solution was measured by pH meter (Systronics, India, Model no – 335). Rate of N increase or loss was calculated by the following equation (Equation 4)

$$r = \frac{N_1 - N_2}{t} \quad (4)$$

r - rate, N_1 and N_2 – initial and final concentrations, t – time

Collected data of all parameters – concentration of Urea-N, Total ammonia-N, NH_3-N and NH_4^+-N were presented as arithmetic mean of three replicates (n = 3). The deviations of observed values from mean values were calculated as standard error of mean (± SE). The variances in the values of concentrations within same treatment and among three treatments were analyzed by the statistic one way ANOVA and paired t–test for comparing between treatments using SPSS 10.0 and the statistical acceptance of data was calculated at 5% level of significance i.e., $P < 0.05$.

3. Results

3.1. Total–N loss

The concentration of total–N (Urea–N + NH_3–N + NH_4^+–N) in urine sample of three conditions were reduced to 93.69 – 94.93% at 39 days of storage (Fig. 1) but this quantity of reduction took 24 days in WM and 9 days in WMA (Fig. 1). The average loss of N was 11.87% day^{-1} in WMA, 3.91% day^{-1} in WM and 2.40% day^{-1} in NM (Fig. 1).

The N loss from all the three experimental conditions tended to follow an almost straight line (y = mx + c) with the storage time period (Fig. 1). In case of NM and WM, 50% N loss was achieved by 12 days period of storage showing almost similar trend of N loss from beginning in both cases. After 12 days of storage, the N loss in WM was higher than NM condition (Fig. 1). The exceptionally greater loss of N (83.43%) was observed in WMA within 4 days of storage period. The loss was logarithmic from the beginning and was reduced later on.

3.2. Urea–N loss

The Urea–N was lost by 98.25%, 98.40% and 98.70% in NM, WM and WMA respectively at the end of storage and relatively most remarkable loss (98.70%) was recorded in WMA at 9^{th} days of storage compared to 39.51% in NM and 39.74% in WM (Table 1).

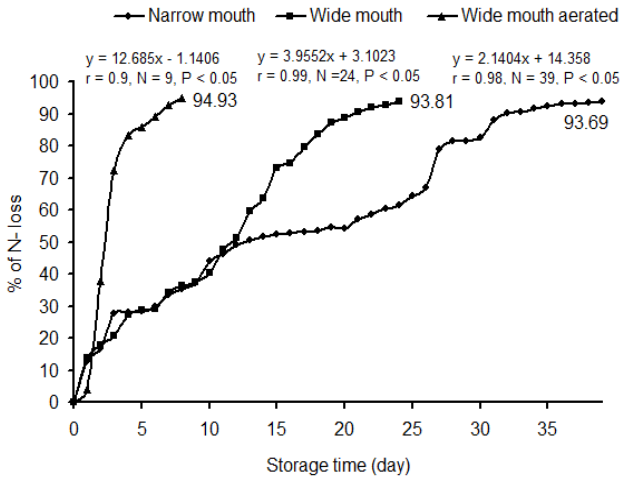

Fig. 1 Correlation between % of N loss and storage time of air contact human urine in narrow mouth, wide mouth and wide mouth aerated conditions. x and y – variables, r – correlation coefficient constant, N – number of observation and P – Probability value of correlation coefficient distribution

3.3. $NH_{3(dissolved)}$ –N increase

The concentration of NH_3-N during storage in NM, WM and WMA was increased by 509x – 514x of beginning (0.56 mg L^{-1}) (Table 1). The final concentration in WMA was greater than NM and WM by 1.04% and 0.52% respectively yet concentration variations in three conditions were statistically insignificant (P > 0.05) i.e., the final concentration values in three condition were same (Table 1).

The trend of increase in concentration of NH_3–N with the day of storage followed a positive linear path (y = 12.986x + 66.484 and y = 22.824x – 7.371) in NM and WM respectively but logarithmic path in WMA (y = 259.57Ln(x) – 7.3498) (Fig. 2). In case of NM and WM, the trend went parallel up to 16th days of storage after that the trend in WM was higher than NM (Fig. 2). Whereas, the trend in WMA was logarithmic and extensively higher up to 4th days of storage and was slow thereafter (Fig. 2).

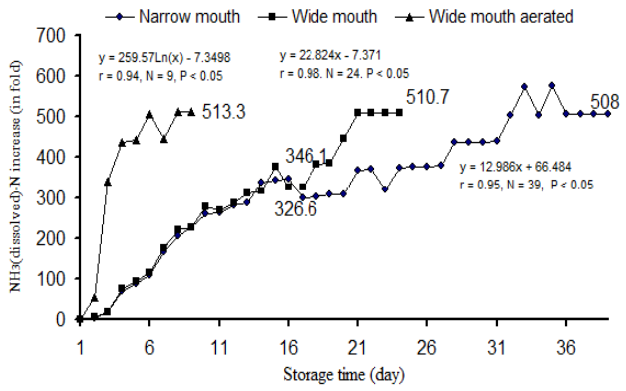

Fig. 2 Correlation between $NH_{3(dissolved)}$ –N increase and day of storage of human urine in narrow mouth, wide mouth and wide mouth aerated conditions. x and y – variables, r – correlation coefficient, N – number of sample, P – probability value

3.4. NH_4^+–N increase

The concentration of NH_4^+–N was magnified by 2x of initial value in all three treatments at the termination of the experiment (Table 1). Though the highest and lowest increase were recorded in WMA and NM treatments respectively yet the final concentrations of NH_4^+–N in all treatments were statistically same (P > 0.05).

The trend in rise in the concentration of NH_4–N followed a polynomial way in NM and WM treatments (Fig. 3). The trends in both cases were parallel up to 19th day of storage (Fig. 3). Thereafter, the trend in WM attained a stable state (2.04x) after small declination. This stability was also reflected in case of NM (2.03x) and WMP (2.05x) at the termination of the storage (Fig. 3). In case of WMA, the trend was logarithmic from the beginning up to 4th day of storage and the rise was declined thereafter (Fig. 3).

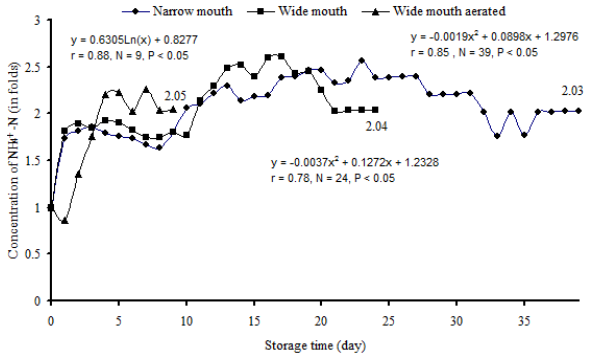

Fig. 3 Correlation between concentration of NH_4^+–N and day of storage of human urine in narrow mouth, wide mouth and wide mouth aerated conditions. y and x – variables, r– correlation coefficient, N – number of sample, P - Probability value

3.5. pH rise and N loss

Percentage of N loss was increased linearly with the rise of pH values in all three conditions (Fig. 4). The degree of the co relationship was more than 80% (r > 0.8) that was statistically significant (P < 0.05). The loss was greater at the pH value more than 8.8 in case of NM and WM whereas, in case of WMA, the loss appeared higher at the pH more than 7.2.

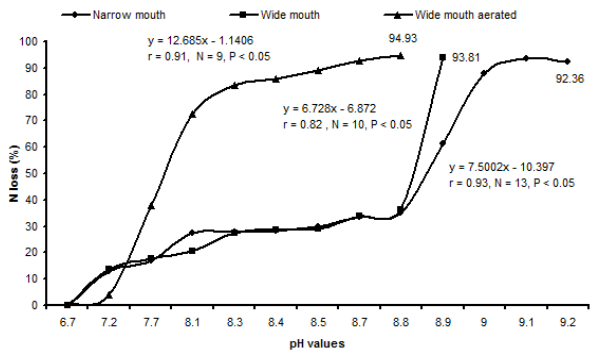

Fig. 4 Correlation between % of N loss and pH during storage of human urine in narrow mouth, wide mouth and wide mouth aerated conditions. y & x – variables, r – correlation coefficient, N – number of sample and P – Probability value

3.6. Open surface area, aeration and N loss

The rate of N loss was higher in WM (3.91% day^{-1} 62.37 cm^{-2}) than NM (2.40% day^{-1} 25.77 cm^{-2}) by 62.9% (Fig. 1) though the open surface area of WM was 58.7% greater than NM. Aeration solely accelerated the N loss (11.87% day^{-1}; Fig. 1) by 3 fold of WM treatment.

4. Discussion

The principal nitrogenous component in human urine was urea. The concentration of urea in urine used in the present study was 13.11 g L^{-1} (Table 1) that was within the normal range (12 – 24 g L^{-1}; Berne et al. 2004). This concentration in NM, WM and WMA treatments was reduced gradually in 39 days period of exposure (Table 1) due to the conversion of urea [$CO(NH_2)_2$] into $NH_{3(gas)}$ at room temperature (28 - 30°C) (Equation 1, 2 and 3) that escaped in air from the exposed superficial surface of urine.

The conversion of urea began with the hydrolysis of urea at room temperature but this reaction did not take place chemically because hydrolysis of urea molecules requires high activation energy (Ea = 35.3 KJ mol^{-1}; Fidaleo and Lavecchia 2003) that could not be provided to urea molecules at room temperature (28 – 30°C). So, this hydrolytic reaction had been catalyzed by enzyme molecules. Literature showed that human body could not be the source of the enzyme rather bacteria could secret the enzymes and during the passage of urine through normal urinary tract, urine could not be contaminated with urease secreting bacteria. Therefore, urea hydrolysis did not begin in urinary tract but began in stored urine outside of the body where it was contaminated by bacteria like *Proteus vulgaris*, *Ureaplasma urealyticum*, *Nocardia*, *Cryptococcus* sp. (fungus), *Helicobactor pylori*, *Brucella* sp., *Klebsiella* sp., *Morganella* sp., *Providential* sp., cellulose decomposing bacteria etc. (Pelczar et al. 1993) which could grow and propagate in urine medium.

The pH range in three treatments (6.7 – 9.1, Table 1) was conducive to urease molecules for catalyzing urea hydrolytic reaction. Literature showed that urea hydrolysis occurred within the pH range of > 1 to > 14 (Warner 1942) and at pH above 7, $NH_{3(dissolved)}$ inhibits urease activity (Havlin et al. 1999).

In these three treatments, the pH value of urine rose from 6.7 to 9.2 because of the conversion of urea into bicarbonate ions (HCO_3^-) and $NH_{3\,(dissolved)}$ (Equation 1 and 2).

The concentration of N in stored urine of NM, WM and WMA treatments was lost by 93.69 – 94.93% in 39 days period storage due to volatilization of $NH_{3(gas)}$ into air (Jones et al. 2007). This loss was initiated when the partial pressure of $NH_{3(gas)}$ was higher in urine than overlying air (Mendham et al. 2000) and this pressure depended directly upon the microbial urea hydrolysis at alkaline condition (pH > 7) (Table 1).

Slow escaping of $NH_{3(gas)}$ from urine characterized a straight line equation ($y = 12.986x + 66.484$ and $y = 22.824x – 7.371$) with the period of storage in NM and WM respectively whereas, aeration accelerated the $NH_{3(gas)}$ and N loss that characterized a logarithmic path ($y = 259.57\ln(x) – 7.3498$) in WMA (Fig. 1).

In NM, the slower rate of N loss (2.40% day^{-1}) was due to exposed small surface area of the container (25.77 cm^2) coupled with urease activity inhibition by $NH_{3(dissolved)}$ molecules within the stored urine that decelerated the rate of urea hydrolysis. On the contrary, the higher rate in WM (3.91% day^{-1}) was caused of greater exposed surface area (62.37 cm^2) and the loss was magnified in WMA (3 – 4.9 folds) due to aeration which drove away dissolved $NH_{3(gas)}$ of urine into air that eliminated the chances of catalytic inhibition of urease.

Amplification of NH_3-N in NM, WM and WMA treatments was recorded due to ammonification of urea (Equation 1, 2 & 3) and that $NH_{3(dissolved)}$ and $NH_{3(gas)}$ were accumulated in urine because NH_3 had a great affinity to dissolve in water by 702 volume in one volume of water (Lee 1996). Rapid volatilization of $NH_{3(gas)}$ from urine accelerated the rate of urea hydrolysis and ammonia formation that followed a logarithmic path in WMA (Fig. 2) but in case of NM and WM, slow release of $NH_{3(gas)}$ inhibited urea hydrolysis and NH_3 formation that followed a straight line with the period of storage (Fig. 2).

5. Conclusions

The following conclusions can be drawn from the present study:

- Aeration increases N loss from stored urine.
- N loss is proportional to the open surface area of stored urine.
- Storage of human urine in narrow mouth open container is the best option to prevent N loss.
- Application of urine stored in narrow mouth container is best within 1-6 days of storage.
- A comparative study is further required on fertilizer efficiency of urine stored in open and closed conditions including the role of associated microorganisms.

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Table 1 Concentration of urea-N, total NH_3-N, NH_3-N, NH_4^+-N and pH of human urine stored in narrow mouth, wide mouth and wide mouth aerated conditions at normal temperature (28°C). Values presented here as the arithmetic mean with standard error of mean (± SE) of three samples (n = 3). Different superscripts within column (a, b, c & d) and among different treatments (x & y) denote statistically significant values at 5% level of significance ($P < 0.05$) and like superscripts among treatments (x & x) denote statistically non significant value ($P > 0.05$).

Storage time (day)	Narrow mouth					Wide mouth					Wide mouth aerated				
	pH	Urea - N ($g L^{-1}$)	Total NH_3 -N ($mg L^{-1}$)	$NH_{3(dissolved)}$ -N ($mg L^{-1}$)	NH_4^+ - N ($mg L^{-1}$)	pH	Urea - N ($g L^{-1}$)	Total NH_3 -N ($mg L^{-1}$)	$NH_{3(dissolved)}$ -N ($mg L^{-1}$)	NH_4^+ - N ($mg L^{-1}$)	pH	Urea - N ($g L^{-1}$)	Total NH_3 -N ($mg L^{-1}$)	$NH_{3(dissolved)}$ -N ($mg L^{-1}$)	NH_4^+ - N ($mg L^{-1}$)
0	6.7	13.11^{ax} ± 0.53	159.57^{ax} ± 1.79	0.56^{ax} ± 0.02	159.01^{ax} ± 1.69	6.7	13.11^{ax} ± 0.55	159.57^{ax} ± 1.79	0.56^{ax} ± 0.02	159.01^{ax} ± 2.03	6.7	13.11^{ax} ± 0.22	159.57^{ax} ± 1.79	0.56^{ax} ± 0.02	159.01^{ax} ± 1.82
1	7.2	11.30 ± 0.57	280.11 ± 1.62	3.08 ± 0.08	277.03 ± 1.60	7.2	11.15 ± 0.52	291.23 ± 1.87	3.20 ± 0.01	288.03 ± 2.97	8.5	12.56 ± 0.20	166.43 ± 1.45	30.29 ± 0.07	136.14 ± 0.81
2	7.7	10.73 ± 0.47	297.36 ± 1.71	10.11 ± 0.41	287.25 ± 1.62	7.7	10.58 ± 0.48	311.56 ± 1.90	10.59 ± 0.32	300.97 ± 2.83	9.1	7.83 ± 0.02	407.38 ± 2.02	191.06 ± 1.11	216.32 ± 1.57
3	8.1	9.3 ± 0.42	321.58 ± 2.23	26.11 ± 0.74	295.47 ± 1.79	8.1	10.22 ± 0.42	319.62 ± 1.91	25.89 ± 1.17	293.73 ± 2.88	9.2	3.52 ± 0.01	592.17 ± 2.37	312.07 ± 2.18	280.10 ± 1.78
4	8.3	9.24 ± 0.41	324.46 ± 2.02	39.91 ± 0.81	284.55 ± 2.18	8.3	9.31 ± 0.37	348.17 ± 1.97	42.82 ± 2.13	305.35 ± 2.92	9.0	1.60 ± 0.01	598.11 ± 2.63	246.42 ± 1.86	351.69 ± 2.53
5	8.4	9.19 ± 0.42	328.82 ± 2.00	49.32 ± 0.88	279.5 ± 2.03	8.4	9.12 ± 0.31	355.09 ± 2.02	53.26 ± 2.74	301.83 ± 2.90	9.0	1.27 ± 0.01	604.23 ± 3.24	248.94 ± 2.01	355.29 ± 2.69
6	8.5	8.96 ± 0.32	336.89 ± 2.11	61.31 ± 1.23	275.58 ± 1.97	8.5	9.07 ± 0.33	356.31 ± 1.99	64.84 ± 3.03	291.47 ± 2.76	9.1	0.83 ± 0.01	608.09 ± 3.44	285.19 ± 2.33	322.90 ± 2.08
7	8.7	8.47 ± 0.31	359.87 ± 2.57	93.56 ± 2.37	266.31 ± 2.01	8.7	8.38 ± 0.30	376.43 ± 2.11	97.87 ± 3.32	278.56 ± 2.71	9.0	0.37 ± 0.00	610.43 ± 3.07	251.49 ± 1.92	358.94 ± 2.66
8	8.8	8.23 ± 0.31	374.88 ± 2.73	115.08 ± 2.54	259.8 ± 2.00	8.8	8.03 ± 0.27	401.37 ± 2.38	123.22 ± 3.56	278.15 ± 2.73	9.1	0.26 ± 0.00	612.06 ± 3.12	287.06 ± 2.31	325.00 ± 2.43
9	8.8	7.93^{bx} ± 0.22	412.12^{bx} ± 2.97	126.52^{bx} ± 2.82	285.60^{bx} ± 2.12	8.8	7.90^{bx} ± 0.22	412.51^{bx} ± 2.47	126.64^{bx} ± 3.21	285.87^{bx} ± 2.71	9.1	0.17^{by} ± 0.00	614.18^{by} ± 3.05	288.05^{by} ± 2.26	326.13^{by} ± 2.57
10	8.8	6.97 ± 0.23	473.62 ± 2.99	145.40 ± 2.43	328.22 ± 2.34	8.9	7.44 ± 0.21	438.06 ± 2.33	156.82 ± 3.57	281.24 ± 2.73	-	-	-	-	-
11	8.8	6.67 ± 0.20	481.30 ± 3.12	147.75 ± 2.66	333.55 ± 2.53	8.8	6.48 ± 0.17	491.49 ± 2.62	150.88 ± 3.21	340.61 ± 2.83	-	-	-	-	-
12	8.8	6.23 ± 0.21	511.67 ± 3.22	157.08 ± 2.89	354.59 ± 2.67	8.8	5.95 ± 0.17	527.23 ± 2.98	161.85 ± 3.22	365.38 ± 3.11	-	-	-	-	-
13	8.8	6.03 ± 0.20	527.21 ± 3.32	161.85 ± 2.57	365.36 ± 2.62	8.8	4.80 ± 0.13	569.04 ± 4.22	174.69 ± 3.48	394.35 ± 3.16	-	-	-	-	-
14	8.9	5.91 ± 0.21	530.28 ± 3.17	189.84 ± 2.91	340.44 ± 2.71	8.8	4.23 ± 0.11	577.38 ± 4.67	177.25^{c} ± 3.72	400.13 ± 3.23	-	-	-	-	-
15	8.9	5.77 ± 0.20	540.66 ± 3.19	193.55 ± 2.97	347.11 ± 2.57	8.9	3.0 ± 0.11	590.82 ± 4.39	211.51 ± 3.87	379.31 ± 3.27	-	-	-	-	-
16	8.9	5.71 ± 0.19	543.08 ± 4.11	194.42 ± 2.88	348.66 ± 2.59	8.8	2.79 ± 0.10	597.66 ± 4.57	183.48 ± 3.91	414.18 ± 3.24	-	-	-	-	-
17	8.8	5.68 ± 0.17	546.72 ± 4.03	167.84 ± 2.13	378.88 ± 2.74	8.8	2.11 ± 0.10	598.13 ± 4.42	183.62 ± 3.86	414.51 ± 3.13	-	-	-	-	-
18	8.8	5.62 ± 0.17	551.35 ± 4.23	169.26 ± 2.33	382.09 ± 2.72	8.9	1.57 ± 0.07	601.07 ± 4.52	215.18 ± 3.97	385.89 ± 3.22	-	-	-	-	-
19	8.8	5.49 ± 0.17	563.63 ± 4.72	173.03 ± 2.18	390.60 ± 2.79	8.9	1.10 ± 0.04	606.11 ± 4.61	216.98 ± 3.88	389.13 ± 3.19	-	-	-	-	-
20	8.8	5.53 ± 0.13	564.07 ± 4.51	173.16 ± 2.21	390.91 ± 2.83	9.0	0.88 ± 0.04	607.20 ± 4.55	250.17 ± 4.11	357.03 ± 2.97	-	-	-	-	-

Table 1 Contd.

Storage time (day)	Narrow mouth pH	Urea - N ($g L^{-1}$)	Total NH_3 -N ($mg L^{-1}$)	$NH_{3(dissolved)}$ -N ($mg L^{-1}$)	NH_4^+ - N ($mg L^{-1}$)	Wide mouth pH	Urea - N ($g L^{-1}$)	Total NH_3 -N ($mg L^{-1}$)	$NH_{3(dissolved)}$ -N ($mg L^{-1}$)	NH_4^+ - N ($mg L^{-1}$)	Wide mouth aerated pH	Urea - N ($g L^{-1}$)	Total NH_3 -N ($mg L^{-1}$)	$NH_{3(dissolved)}$ -N ($mg L^{-1}$)	NH_4^+ - N ($mg L^{-1}$)
21	8.9	5.14 ± 0.14	576.33 ± 4.77	206.32 ± 2.12	370.01 ± 2.80	9.1	0.65 ± 0.04	608.76 ± 4.49	285.51 ± 4.21	323.25 ± 2.84	-	-	-	-	-
22	8.9	4.91 ± 0.11	581.21 ± 4.68	208.07 ± 1.93	373.14 ± 2.69	9.1	0.45 ± 0.03	609.34 ± 4.52	285.78 ± 4.16	323.56 ± 2.81	-	-	-	-	-
23	8.8	4.68 ± 0.12	587.37 ± 4.82	180.32 ± 1.82	407.05 ± 3.11	9.1	0.34 ± 0.01	609.89 ± 4.67	286.04 ± 4.23	323.85 ± 2.77	-	-	-	-	-
24	8.9	4.54^{cx} ± 0.11	589.09^{cx} ± 4.90	210.89^{cx} ± 2.07	378.20^{cx} ± 2.78	9.1	0.21^{cy} ± 0.01	611.02^{cy} ± 4.66	286.56^{cy} ± 4.05	324.24^{cy} ± 2.73	-	-	-	-	-
25	8.9	4.14 ± 0.14	590.65 ± 4.91	211.45 ± 2.09	379.20 ± 2.71	-	-	-	-	-	-	-	-	-	-
26	8.9	3.82 ± 0.11	593.02 ± 4.90	212.30 ± 2.17	380.72 ± 2.66	-	-	-	-	-	-	-	-	-	-
27	8.9	2.19 ± 0.11	595.46 ± 4.97	213.17 ± 2.12	382.29 ± 2.77	-	-	-	-	-	-	-	-	-	-
28	9.0	1.88 ± 0.08	596.73 ± 4.99	245.85 ± 2.27	350.88 ± 2.69	-	-	-	-	-	-	-	-	-	-
29	9.0	1.85 ± 0.08	596.89 ± 5.12	245.91 ± 2.14	350.98 ± 2.61	-	-	-	-	-	-	-	-	-	-
30	9.0	1.70 ± 0.06	597.07 ± 5.22	245.99 ± 2.19	351.08 ± 2.81	-	-	-	-	-	-	-	-	-	-
31	9.0	0.98 ±0.07	599.02 ± 5.38	246.79 ± 2.34	352.23 ± 2.62	-	-	-	-	-	-	-	-	-	-
32	9.1	0.70 ± 0.01	601.10 ± 5.57	281.91 ± 2.52	320.19 ± 2.33	-	-	-	-	-	-	-	-	-	-
33	9.2	0.65 ± 0.02	601.43 ± 5.77	321.76 ± 2.43	279.67 ± 2.01	-	-	-	-	-	-	-	-	-	-
34	9.1	0.53 ± 0.02	603.22 ± 5.80	282.91 ± 2.62	320.31 ± 2.11	-	-	-	-	-	-	-	-	-	-
35	9.2	0.41 ± 0.01	604.05 ± 5.81	323.16 ± 2.71	280.89 ± 2.01	-	-	-	-	-	-	-	-	-	-
36	9.1	0.32 ± 0.01	605.11 ± 5.89	283.79 ± 2.66	321.32 ± 2.77	-	-	-	-	-	-	-	-	-	-
37	9.1	0.29 ± 0.01	606.03 ± 5.90	284.22 ± 2.74	321.81 ± 2.70	-	-	-	-	-	-	-	-	-	-
38	9.1	0.27 ± 0.00	606.45 ± 5.91	284.42 ± 2.69	322.03 ± 2.66	-	-	-	-	-	-	-	-	-	-
39	9.1	0.23^{d} ± 0.00	607.86^{d} ± 5.90	285.08^{d} ± 2.72	322.78^{d} ± 2.69	-	-	-	-	-	-	-	-	-	-

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