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Nitrification of the liquid phase of digestate can help with the reduction of nitrogen losses

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ABSTRACT

The effect of total ammonia nitrogen (TAN) transfer to nitrate via the nitrification process on the nitrogen loss during long-term storage of liquid phase of digestate (LPD) was verified. The treatments simulating summer (25 ±2 °C) and winter (10 ±0.5 °C) conditions were evaluated in addition to the influence of the stirring. The experiments proved that intensive nitrogen losses could be connected with the storage of raw LPD. Up to 87% of TAN was lost within 100 days and stirring led to a 96% increase in loss. Nitrogen loss observed during the storage of nitrified LPD did not exceed 6% even after 250 days where the stirring had no effect on the results. For raw and nitrified LPD, temperature had no significant influence on nitrogen loss.

1. Introduction

Digestate is produced as a by-product of the operation of biogas plants. In many cases, the digestate is separated into solid and liquid phases with the aim to optimise the use of nutrients contained in the digestate, to reduce the cost for transport of digestate, and to use solid fraction for specific purposes (Bauer et al., 2009; Rehl and Müller, 2011; Al Seadi et al., 2013). The liquid phase of digestate (LPD) contains relatively high concentrations of nitrogen mostly reaching from several hundreds of milligrammes up to several grams per litre. The nitrogen is present mainly in the form of total ammonia nitrogen (TAN; the sum of nitrogen present in NH_3 and NH_4^+). Also, the concentration of phosphorus, potassium and other nutrients is relatively high in digestate as well as in LPD (Kolář et al., 2008; Al Seadi et al., 2013; Risberg et al., 2017). At present, the LPD produced on agricultural biogas plants usually occurs on a relatively long-term basis. It is stored within lagoons in the areas of biogas plants, and consequently it is applied to the land where it serves as a promising source of nutrients for plants (Sigurnjak et al., 2017). The duration of the period of LPD storage is influenced by many factors including the size and availability of the soil acreage where the LPD will be applied. The legislation limits the application of digestate (as well as LPD) to the land in the winter where the amount of nitrogen that could be applied to soil during resting periods is also limited (European Commission, 1991). The mentioned factors strongly influence the duration of the storage period of liquid digestate as well as LPD. The percentage amount of volatile free ammonia (FA) within TAN increases with increasing pH value (Anthonisen et al., 1976; Whelan et al., 2010). Thus, the emission of ammonia during storage and application of LPD to soil (Nkoa, 2014; Möller, 2015; Tiwary et al., 2015; Nicholson et al., 2017) could be relatively intense owing to the slight alkaline pH value of the digestate (as well as the LPD), which reaches mostly 7.5–8.5 (Whelan et al., 2010). Storage tanks for LPD are frequently not covered, which represents another factor that increases

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the risk of nitrogen losses (Li et al., 2018). However, actual intensity of FA emission during the storage is very variable based on a range of factors. Real pH value of LPD during storage seems to be the most important of the reasons presented above. However, temperature also strongly influences the percentage amount of FA within TAN (Anthonisen et al., 1976; Whelan et al., 2010) determining the intensity of NH_3 losses. Having evaluated nitrogen losses from open storage tanks operated in field conditions, the representation of NH_3 increases with rising temperature causing a larger significance of NH_3 losses to be observed during the summer period (Sommer, 1997). Additionally, other environmental factors such as potential mixing of stored LPD caused by variable intensity of wind during the storage may influence the intensity of the loss due to the variable intensity of the contact between LPD and gas phase (Perazzolo et al., 2017). Nitrogen loss during LPD storage is problematic not only from the point of view of environmental protection, but also due to the decreasing amount of nutrients supplied to plants with LPD.

Nitrification induced in the LPD environment was considered a possible way of minimising nitrogen losses during the handling of LPD in the past (Svehla et al., 2017). Nitrification leads to the transformation of TAN into oxidised forms of nitrogen ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$), which should be stable from the point of view of potential volatilisation. Simultaneously, as a consequence of performing the nitrification process in the LPD environment, the pH may decrease from weakly basic values to ca. 5.5–7.0, or even lower under certain conditions (Botheju et al., 2010; Svehla et al., 2017). Thus, the volatilisation of FA could be prevented, even when certain parts of TAN are not converted into oxidised forms, as the consequence of the nitrification process. The representation of $N\text{-}NO_2^-$, which may have a toxic effect on plants (Court et al., 1962; Singh et al., 2007) within the products of LPD nitrification, could be minimised by rational control of the nitrifying reactor operation that treats the LPD (Svehla et al., 2017). However, possible risk of secondary nitrogen loss caused by denitrification occurring in the nitrified LPD environment has not yet been evaluated. During the long-term storage of nitrified LPD in non-aerated storage tanks, anoxic conditions suitable for the majority of denitrifying bacteria (Painter, 1970) are to be expected. Simultaneously, these bacteria typically belong to heterotrophic microorganisms and require the availability of organic substrate as the source of nutrients (Painter, 1970). The relatively high concentration of organic matter necessary for these bacteria in LPD additionally cannot be excluded, despite the material being exposed firstly to anaerobic degradation in the anaerobic digester and subsequently to aerobic degradation in the nitrification reactor. Svehla et al. (2017) found that the removal efficiency of organic matter in the nitrifying reactor treating LPD is relatively low, which indicates a limited content of easily degradable organic matter in raw LPD, and consequently, in nitrified LPD. Nevertheless, potential usability of organic compounds resting in nitrified LPD for denitrification, during long-term storage of nitrified LPD, remains questionable.

The aim of this paper is to verify the positive effect of the transformation of TAN contained in LPD on oxidised forms of nitrogen via the nitrification process on nitrogen loss, during long-term storage of LPD in non-covered storage tanks under different conditions. The temperature effect was verified. Simultaneously, non-windy and windy conditions were simulated with the aim of evaluating the effect of gently mixing the storage tank volume that would be caused by the wind on the intensity of nitrogen loss. For this purpose, a set of kinetic laboratory tests, simulating the long-term storage of raw as well as nitrified LPD from an agricultural biogas plant, was performed. In this way, it was possible to compare the intensity of nitrogen loss, caused by the volatilisation of ammonia from raw LPD, with the intensity of nitrogen loss from nitrified LPD caused by the production of gaseous products of denitrification.

2. Material and methods

2.1. LPD used for the experiments

The experiments were performed using LPD obtained from an agricultural biogas plant using caw manure and slurry (ca. 70% of the raw matter weight of substrate for biogas production), maize silage (ca. 20%) and grass silage (ca. 10%) as the main sources of the substrate for biogas production operated under mesophilic conditions. Separation of digestate into solid and liquid phases was performed using screw press separator on this biogas plant. The sample of LPD collected (on a one time basis) was used for the experiments simulating the storage of raw LPD.

Nitrified LPD was gained by the exposure of raw LPD (gained on abovementioned biogas plant) to the nitrification process taking place in the 5 L continuously stirred tank reactor (CSTR) equipped by a sedimentation tank, operated under conditions presented by Svehla et al. (2017). The nitrifying biomass was cultivated in the form of a suspension (activated sludge) in this system. The sample of the nitrified LPD was collected from the effluent of the sedimentation tank. Initially, the nitrification CSTR was inoculated with nitrifying activated sludge gained from a municipal wastewater treatment plant. The entire volume of the reactor was filled by this sludge. Then, the LPD inflow was initiated where the LPD was dosed continuously to the reactor during the whole period of its operation. This start-up strategy enabled to keep nitrifying bacteria active despite the extremely high ammonia concentration in the raw LPD (Pacek et al., 2016). After the inoculation, the reactor was operated stable for 10 months. The sample of the nitrified LPD used for the experiment was collected during the last month of this period. The entire volume of the nitrified LPD gained during the collection period of the sample had been mixed together before the simulation of the nitrified LPD storage was initiated. The pH level in the nitrification CSTR system was controlled at 7.0 ± 0.1 by introducing NaOH (2.5 mol/L) solution. The nitrification reactor was operated at a nitrogen loading rate corresponding to 0.26 ± 0.05 kg/(m^3·d) and a hydraulic retention time corresponding to 21 ± 4 days. TAN conversion efficiency exceeded 99% during the whole period of the sample collection.

Table 1
Characteristics of raw and nitrified LPD.

Parameter (unit)	Raw LPD	Nitrified LPD
pH (–)	8.2 ± 0.0	6.9 ± 0.0
TAN (mg/L)	5240 ± 190	26 ± 2
$N\text{-}NO_3^-$ (mg/L)	0.0 ± 0.0	5070 ± 110
$N\text{-}NO_2^-$ (mg/L)	1.1 ± 0.1	2.9 + 0.2
N_{inorg} (mg/L)	5241 ± 190	5100 ± 110
N_{org} (mg/L)	350 ± 20	240 ± 10
N_{total} (mg/L)	5591 ± 200	5340 ± 120
suspended solids (mg/L)	5640 ± 350	1380 ± 110
COD_{total} (mg/L)	16400 ± 630	7840 ± 410
$COD_{soluble}$ (mg/L)	7540 ± 210	3630 ± 140

N_{inorg} - inorganic nitrogen concentration (the sum of TAN, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ concentrations.)
N_{org} - organic nitrogen concentration.
N_{total} - total nitrogen concentration.
COD_{total} – total chemical oxygen demand (original sample).
$COD_{soluble}$ - chemical oxygen demand of dissolved solids (centrifuged sample).

Table 2
Performance of the tests quantifying nitrogen losses.

Variant	type of LPD	temperature	stirring
R1	Raw LPD	25 ± 2 °C	No
R2	Raw LPD	25 ± 2 °C	Yes
R3	Raw LPD	10 ± 0.5 °C	No
R4	Raw LPD	10 ± 0.5 °C	Yes
N1	Nitrified LPD	25 ± 2 °C	No
N2	Nitrified LPD	25 ± 2 °C	Yes
N3	Nitrified LPD	10 ± 0.5 °C	No
N4	Nitrified LPD	10 ± 0.5 °C	Yes

Nitrification biomass was recirculated from the sedimentation tank back to the nitrification reactor where no excess biomass was intentionally withdrawn from the system with the aim of maximising the nitrification biomass retention. The only biomass losses were caused by its escaping with the effluent nitrified LPD in accordance with Svehla et al. (2017).

Basic characteristics of raw and nitrified LPD used for the experiments can be seen in Table 1.

2.2. Performance of kinetic tests quantifying nitrogen losses

A set of simple kinetic tests was performed with the aim to compare the nitrogen losses during the storage of raw and nitrified LPD under different conditions. Four glass beakers were filled with 750 mL of raw LPD with the aim to monitor the intensity of ammonia volatilisation during the storage of raw LPD. The symbols R1–R4 were used for the description of particular beakers. Another four beakers marked as N1–N4 were filled with 750 mL of nitrified LPD in order to simulate the long-term storage of nitrified LPD. The intensity of nitrogen losses caused by denitrification was quantified in these variants.

Different temperature storage conditions (i.e. winter and summer) were simulated by incubating part of the beakers (R1, R2, N1 and N2) at laboratory temperature (25.0 ±2.0 °C), two of which (R2 and N2) were moderately and continuously stirred at 100 rpm (revolutions per minute) using a magnetic micro-stirrer (Velp Scientifica, Italy). This verified the behaviour of the sample under moderate wind conditions. The beakers R3, R4, N3 and N4 were stored in a thermostatic cabinet (Lovibond, Germany) at 10.0 ±0.5 °C, with R4 and N4 being constantly stirred at 100 rpm by a magnetic micro-stirrer (Velp Scientifica, Italy). The conditions applied in different variants of the tests to quantify nitrogen losses are summarised in Table 2. The kinetic tests were carried out for a period of 100 days (R1–R4, N3 and N4). Taking into consideration actual results of particular variants, the performance period of the tests for N1 and N2 was prolonged to 250 days.

The samples from each beaker were taken for analysis during the course of the experiment. Firstly, dissolved oxygen (DO) and temperature were measured. Then, the difference in volume caused by natural evaporation was compensated by the addition of demineralised water in each beaker. Subsequently, the volume of the beakers R1, R3, N1 and N3 were mixed for 1 min. Then, pH was measured and the samples from the beakers (R1–R4 as well as N1–N4) were taken for the performance of additional chemical analysis. Following that step, the level of remaining LPD was marked to quantify the volume reduction caused by natural evaporation until the following measurement. In the next step, the samples taken from the beakers were centrifuged at 9500 rpm (G-force corresponded to 12.007) for 12 min by means of a Rotina 420 centrifuge (Andreas Hettich GmbH & Co.KG, Germany). Finally, the TAN, $N\text{-}NO_2^-$, $N\text{-}NO_3^-$, N_{total} concentrations and the chemical oxygen demand (COD) were measured. Abovementioned analysis for the beakers marked as R1–R4 and N1–N4 (see above) was performed regularly and during the first five weeks, the samples were taken once per week. Consequently,

the intervals were prolonged. At all events, the frequency of the sampling was never longer than two weeks in the vessels operated for 100 days. The maximum interval for sampling N1 and N2 was 25 days in the period between days 100 and 250.

2.3. Analytical methods

The concentrations of TAN, N-NO_2^-, N-NO_3^-, COD (COD_{total} – original sample and $COD_{soluble}$ – centrifuged sample), and suspended solids were measured in accordance with the standard methods (APHA, 2005). The N_{total} concentration was determined using a HACH DR/4000 photometer (Hach Lange, Germany) by HACH method number 10071. Organic nitrogen (N_{org}) concentration was calculated as the difference between N_{total} concentration and inorganic nitrogen concentration (N_{inorg}, the sum of TAN, N-NO_2^- and N-NO_3^- concentrations).

DO concentration was measured employing a WTW Oxi 340i using the oxygen-sensor WTW Cellox 325 (WTW, Germany). Also, the temperature was monitored using this WTW system. The value of pH measurements was performed using a WTW pH 340i pH metre connected with pH-probe WTW SenTiX 21 (WTW, Germany).

2.4. Calculations

The percentage (%) amount of FA within TAN (R_{FA}) and the mg/L concentration of FA (c_{FA}) were calculated in accordance with Anthonisen et al. (1976) (Eqs. (1) and (2), respectively):

$$R_{FA}\ (\%) = \frac{10^{pH}}{\exp\left(\frac{6334}{273+T}\right)+10^{pH}} \tag{1}$$

$$c_{FA}\ (mg/L) = \frac{17}{14}.\ \frac{c\ (TAN)\ .\ 10^{pH}}{\exp\left(\frac{6334}{273+T}\right)+10^{pH}} \tag{2}$$

Taking into consideration the very slow changes of N_{inorg} concentration during the kinetic tests in N1–N4 variants and the limited accuracy of the N_{inorg} measurement, simple linear regression of the relationship between N_{inorg} concentration (dependent variables) and the storage time (independent variable) was performed using Excel 2016 with the aim to quantify N_{inorg} losses in these variants. The linear regression was evaluated based on Eq. (3):

$$c_{(R)}(N_{inorg}) = b.t + c_{(R0)}(N_{inorg}) \tag{3}$$

where $c_{(R)}$ (N_{inorg}) represents actual N_{inorg} concentration calculated in mg/L based on the regression of concrete time (t) during the test in days, b represents the guideline of the regression, and $c_{(R0)}$ (N_{inorg}) represents N_{inorg} concentration calculated (mg/L) based on the regression for day 0 (for the start of the experiment).

Consequently, using the values of $c_{(R)}$ (N_{inorg}) for the start and end of the experiment, the loss of N_{inorg} in % during the experiment resulting from linear regression was quantified. The results of the calculations were used as alternative variants of the expression of loss, complementing the loss calculated directly from N_{inorg} concentration, which was measured at the start and end of the test. Also, the efficiency of organic matter degradation during the experiment, quantified in the % of COD removed, was calculated in the same way where actual value of COD was calculated in accordance with Eq. (4):

$$c_{(R)}(COD) = b.t + c_{(R0)}(COD) \tag{4}$$

where $c_{(R)}$ (COD) represents actual COD value in calculated mg/L based on the regression for concrete time (t) during the test in days, b represents the guideline of the regression, and $c_{(R0)}$ (COD) represents COD value in calculated mg/L based on the regression for day 0 (for the start of the experiment).

3. Results and discussion

The differences between N_{total} and N_{inorg} concentration, representing organic nitrogen concentration (N_{org}), remained stable in all tested variants simulating storage of raw as well as nitrified LPD (R1–R4; N1–N4) during the whole test. This indicated that no change in N_{org} concentration occurred in the beakers. The average N_{org} concentration reached 350 ± 30 and 240 ± 20 mg/L for raw and nitrified LPD, respectively, during the experiments.

3.1. Change in N_{inorg} concentration during the kinetic tests

N_{inorg}, representing the sum of TAN, N-NO_2^- and N-NO_3^- concentrations, was selected as the form of nitrogen serving for the comparison of nitrogen losses in different variants of the tests. All samples simulating the storage of raw LPD (R1–R4) were characterised by high initial concentrations of TAN, reaching 5240 ± 190 mg/L (Table 1). TAN represented at least 99.5% of N_{inorg} during the whole experiment in all variants. The average representation of TAN, N-NO_2^- and N-NO_3^- within N_{inorg} during the whole experiment for all tested variants can be seen in Table 3.

Table 3
The comparison of nitrogen losses and the average representation of different nitrogen forms on the concentration of N_{inorg} for particular variants.

Variant	Losses (%)	Losses — linear regression (%)	TAN (% of N_{inorg})	$N\text{-}NO_3^-$ (% of N_{inorg})	$N\text{-}NO_2^-$ (% of N_{inorg})
R1	86.83	–	99.95	0.00	0.05
R2	91.03	–	99.95	0.00	0.05
R3	87.41	–	99.94	0.00	0.06
R4	96.18	–	99.89	0.00	0.11
N1[a]	5.57/5.06	3.62/4.62	0.50/0.35	99.38/99.56	0.12/0.09
N2[a]	7.22/0.13	4.79/3.18	0.16/0.12	99.81/99.86	0.03/0,02
N3	7.02	5.80	0.37	99.59	0.04
N4	3.05	3.67	0.17	99.82	0.01

[a]Firstly, the value for the 100-day lasting period is presented; the second value characterises the period lasting for 250 days.

Very low concentrations of $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ that do not exceed 2 mg/L were detected in R1–R4 during the whole experiment period, where even zero $N\text{-}NO_3^-$ concentrations were detected. This observation excluded the nitrification process during LPD storage, which is in accordance with Patni and Jui (1991). No nitrification was registered despite DO concentration gradually increasing in stirred variants up to 2.5 and 9.5 mg/L in R2 and R4, respectively, which is suitable for this process (Painter, 1970). Inhibiting the activity of nitrifying bacteria at concentrations reaching 0.1–1.0 mg/L and 10–150 mg/L for ammonia oxidising bacteria and nitrite oxidising bacteria, respectively, the toxic effect of FA was described many years ago by Anthonisen et al. (1976). Later studies confirmed the negative influence of FA on the nitrification process (Vadivelu et al., 2006, 2007; Qian et al., 2017). Up to 2130 mg/L of FA was observed during our experiments where even the minimal values observed at the end of the experiment still reached several tens of mg/L.

All samples simulating the storage of raw LPD (R1–R4) followed similar trends in TAN as well as in N_{inorg} concentrations during the monitored period, in which rapid decrease was observed (Fig. 1). At the end of the period, the concentrations of N_{inorg} reached 690, 470, 660 and 200 mg/L for R1, R2, R3 and R4, respectively. Under such conditions, 87, 91, 87 and 96% of N_{inorg} was lost during the experiment in R1, R2, R3 and R4, respectively (Table 3). The average percentage loss of N_{inorg} per week reached 6.08, 6.37, 6.12, and 6.73% for R1, R2, R3 and R4, respectively, during 100 days of storage. These results are similar to Whelan et al. (2010) who reported significant reductions in TAN concentrations of approximately 7.6% per week during the storage of digestate.

$N\text{-}NO_3^-$ was the dominant chemical form of N_{inorg}, which represented at least 99.2% of N_{inorg} during the experiment performed with nitrified LPD in all tested variants (N1–N4). TAN and $N\text{-}NO_2^-$ concentration did not exceed 40 and 13 mg/L, respectively, during the whole operational period in all tested variants, as it was not significant from the perspective of total N_{inorg} concentration. Taking into consideration initial N_{inorg} concentration reaching 5100 ± 110 mg/L, the concentration of N_{inorg} was relatively stable during the whole operational period in all tested variants reaching 4670–5330 mg/L with no clearly visible trends in the concentration among treatments (Fig. 1). If we compare N_{inorg} concentration at the beginning of the test with the concentration measured after 100 days, we will find a loss of 5.57, 7.22, 7.02 and 3.05% for N1, N2, N3 and N4, respectively (Table 3). Slow changes are typical for the variants used with nitrified LPD (N1–N4), with no significant differences between particular variants. Furthermore, the operational period of selected variants (N1 and N2) was prolonged from 100 to 250 days. Comparing levels gained after 100 days of storage with values measured after 250 days in N1 and N2, suggests that even lower losses (5.1 and 0.1%, respectively), compared with the situation after 100 days, will be observed. The possibility that this phenomenon was caused by gradual release of TAN (produced as a consequence of N_{org} mineralisation) seems to be excluded by the observation that N_{org} concentration was practically constant during the experiment (see above). Rather, it seems to be caused by limited precision of the determination of particular nitrogen forms, which was amplified by dilution of samples characterised by their high concentration during these experiments (APHA, 2005). Therefore, the evaluation of the N_{inorg} losses, based on the slope of linear regression for the relationship between N_{inorg} concentrations and storage time (where the values for the start and end of the test resulted from the regression were compared), was used with a simple comparison of the concentration at the start and at the end of the experiment for N1–N4 variants. The parameters of linear regression for particular variants are presented in Table 4.

Using this method of loss quantification, 3.62, 4.79, 5.80 and 3.67% of N_{inorg} was lost for N1, N2, N3 and N4, after 100 days (Table 3). After 250 days, the losses quantified using the linear regression reached 4.62 and 3.18% in N1 and N2, respectively. Even using the linear regression for N2, a more significant loss was found after 100 days (compared with 250 days), clearly demonstrating that it is practically impossible to exactly quantify the intensity of loss under given conditions. For all experiments, the maximum loss calculated by different methods was 7%, which could be considered a very positive finding. Liquid stirring in the beakers, as well as the temperature applied, showed no significant effect on the intensity of nitrogen losses during the simulation of long-term storage of nitrified LPD.

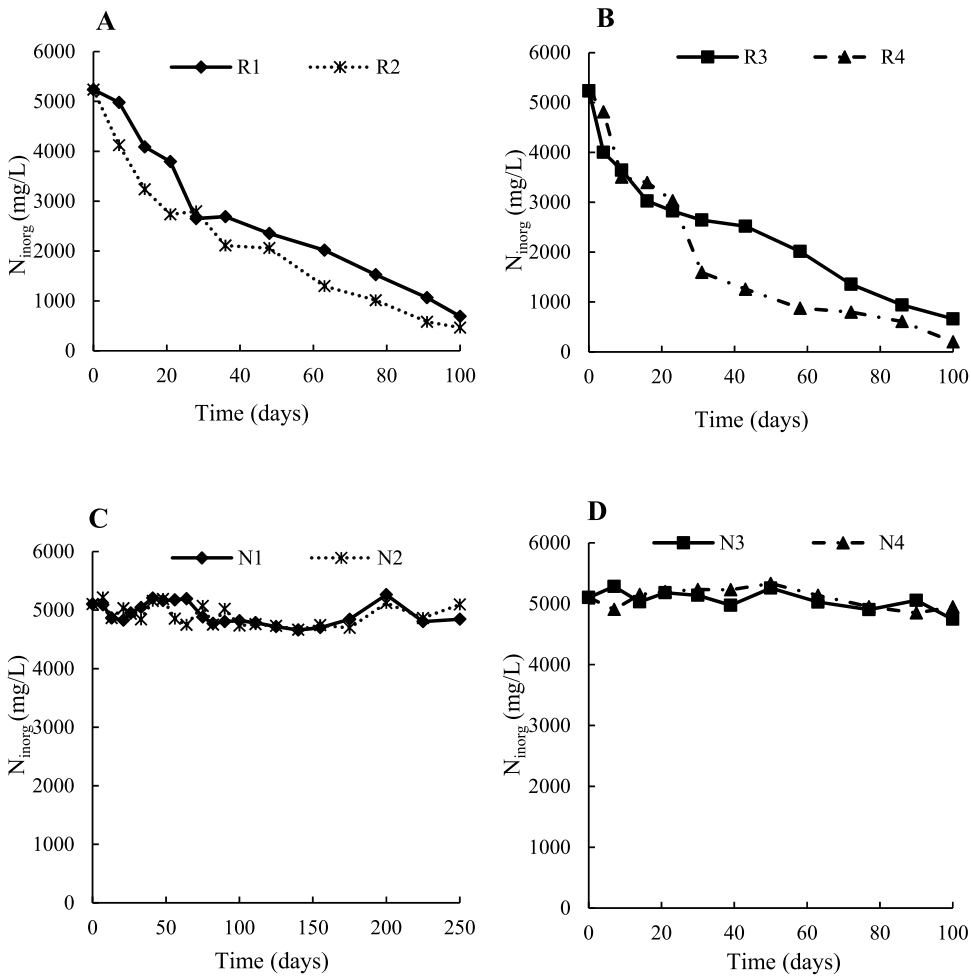

Fig. 1. Concentration of N_{inorg} during kinetic tests (A: R1 and R2; B: R3 and R4; C: N1 and N2; D: N3 and N4).

Table 4
Parameters of linear regression for the relationship between N_{inorg} concentration and the storage time calculated in accordance with Eq. (3).

Variant	b	$c_{(R0)}$ (N_{inorg})
N1 (100 days)	−1.8386	5079
N1 (250 days)	−0.9277	5018
N2 (100 days)	−2,4312	5079
N2 (250 days)	−0.6327	4976
N3 (100 days)	−3.016	5198
N4 (100 days)	−1.8985	5179

3.2. Mechanism of nitrogen losses

Based on the raw LPD's pH reaching 8.2 at the start of the experiment, a significant portion of TAN was present in the form of volatile unionised FA in R1–R4. Therefore, only the volatilisation of FA seems to be responsible for the loss of N_{inorg} during the experiment in R1–R4. Because higher temperature increases the representation of volatile FA within TAN (R_{FA}) at constant pH (Anthonisen et al., 1976; Patni and Jui, 1991; Koirala et al., 2013);), significantly higher R_{FA} (reaching 7.7%) was calculated based on Eq. (1) for R1 and R2 compared with R3 and R4, in which the R_{FA} of 3.3% was found at the beginning of the experiment period. In accordance with Li et al. (2018), the value of pH gradually increased up to 9.0–9.3 (Fig. 2) during the experiment, which was caused mainly by the emissions of CO_2 (Hafner et al., 2013). Subsequent pH decrease, observed in particular variants around day 40–80, could mainly be explained by the significant reduction of basic ammonia content, and eventually by the effect of volatile acid production taking place within the degradation of organic matter (Blanes-Vidal et al., 2009; Perazzolo et al., 2015, 2017). For all experiments, the R_{FA}increased up to 47.1, 60.6,

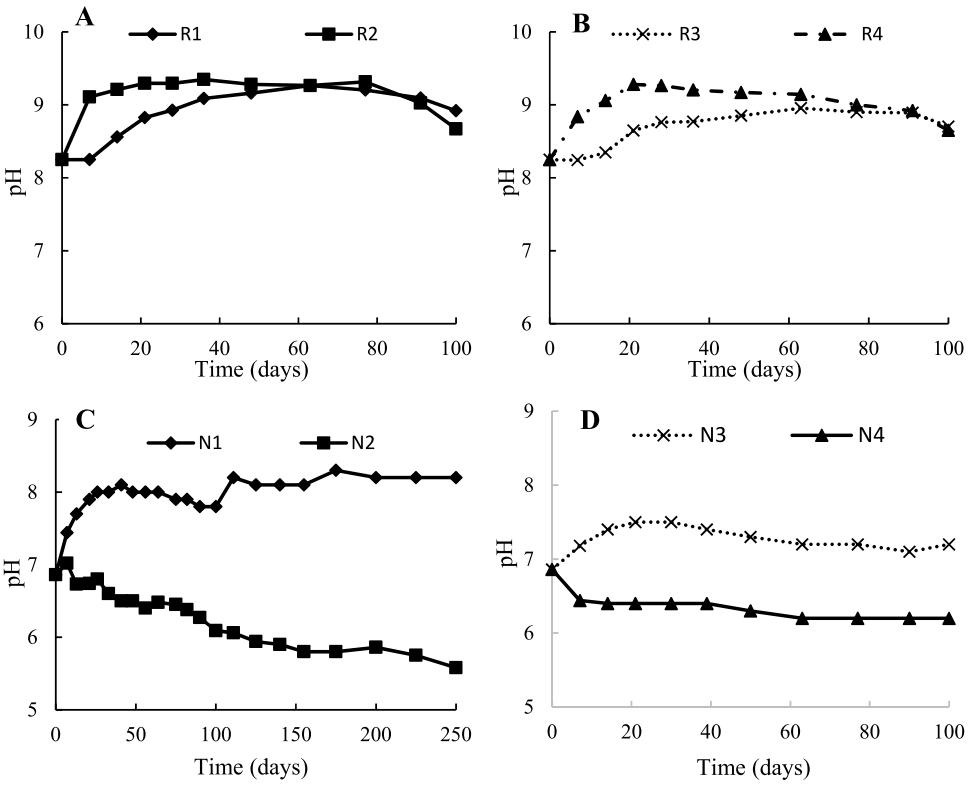

Fig. 2. The changes in pH values during the kinetic tests (A: R1 and R2; B: R3 and R4; C: N1 and N2; D: N3 and N4).

14.7, and 27.5% for R1, R2, R3 and R4, respectively. Decreasing R_{FA} and temperature caused lower values for R3 and R4, compared with R1 and R2. Despite this, Perazzolo et al. (2017) described decrease of pH during the first month of storage, which was consequently changed to increase during the resting period of digestate or LPD storage. However, an increase was observed during the first phase of our research as well as during the research performed by Li et al. (2018). This could be explained by variable significance of the acidifying effects on the environment of stored material (volatile acid production, the decrease of the content of basic ammonia) where the effects of increasing pH (volatile acid consumption, CO_2 emission) could show also variable intensity in particular phases of the storage period based on actual conditions prevailing in stored material. For example, the degree of the stability of organic matter after anaerobic digestion seems to be very important from the point of view of potential volatile acids transformation.

However, the differences in R_{FA} for the variants operated at different temperature values had no significant effect on final N_{inorg} loss intensity (Fig. 1, Table 3). This finding is in contrast with Li et al. (2018) who observed a significantly stronger decrease in TAN concentration during the 60-day lasting simulation of the manure digestate storage under laboratory conditions at 30 °C, compared with the situation at 15 °C. Furthermore, Perazzolo et al. (2017) reported limited N losses of approximately 2%–9% during the winter storage of LPD in field conditions (90 days), versus 32%–35% N losses observed during the summer storage of the same LPD in the same period. Similarly, Sommer (1997) found that almost no NH_3 losses were observed around 0 °C, while 30% of TAN was lost during the summer period during one year with monitoring of the storage tank losses operated in full scale conditions. The fact that different concrete temperature values were applied during our research, where very high R_{FA} was reached even for lower temperatures (10 ±0.5 °C), seems to be responsible for significantly lower temperature effects on TAN losses, compared with other authors.

Generally, a very high portion of TAN was lost in R1–R4 during the storage. This observation is in accordance with Li et al. (2018) who reported the decrease of TAN concentration from 4.5 g/L to ca. 0.5 g/L during 60-day lasting storage that was simulated under laboratory conditions at 30 °C. A significantly higher portion of nitrogen losses founded during our research in R1–R4, compared to the 2%–35% loss during 90 days reported by Perazzolo et al. (2017), may be attributable to the fact that the samples used during our research were stored at higher maximum pH values reaching up to 9.3 (Fig. 2). Contrary, during the research performed by Perazzolo et al. (2017) in pilot plant conditions, the pH value did not exceed 8.5. Additionally, the significantly lower intensity of nitrogen loss could generally be expected under pilot plant (as well as under full-scale) conditions compared with laboratory conditions, for example, due to possible development of the crust at the stored material surface in summer and a very dilute surface layer of storage material in winter. This has previously been described for the storage of slurry (Misselbrook et al., 2005; Blanes-Vidal et al., 2009; Perazzolo et al.,

2017), which is not possible to simulate under lab-scale conditions. Also, the ratio between the area of the surface of stored material and the depth of the storage tank seems to be important from the point of view of FA volatilisation due to the variable intensity of the contact between the LPD with the atmosphere, which may partially cause the differing intensities of nitrogen loss observed by different authors.

The comparison of variants R1 and R3 with R2 and R4, respectively, indicates that the stirring of storage tank volume (caused, for example, by the wind) may slightly increase the intensity of nitrogen loss, which is in accordance with Perazzolo et al. (2017).

Converting $N\text{-}NO_3^-$ (and/or $N\text{-}NO_2^-$) to gaseous products such as N_2 or N_2O (Painter, 1970) by denitrification seems to be the only potential explanation of the above-presented decrease in N_{inorg} concentration in N1–N4. However, heterotrophic denitrification microorganisms strictly require readily biodegradable carbon sources. Characterised by barely decomposable organic matter content, the raw LPD does not contain a sufficient amount of suitable carbon sources required to achieve nitrate reduction (Möller and Müller, 2012). After the treatment of raw LPD in the nitrification reactor, the concentration of organic matter is even lower (Table 1). No decrease of COD value observed during the experiment in N1–N4 (see below) confirms this assumption. In addition, satisfactory denitrification in the water environment only proceeds in the absence of oxygen (Painter, 1970). During our experiment, DO concentration reached up to 9.5 mg/L, especially in the stirred variant performed at 10 ±0.5 °C (N4). Under such conditions, endogenous denitrification performed by heterotrophic biomass observed frequently during wastewater treatment (Bernat et al., 2008) was neither able to reduce measurable $N\text{-}NO_3^-$ concentration during the simulation of the nitrified LPD storage. At the same time, the decrease of COD_{total} from 16400 mg/L measured in the raw LPD to 7840 mg/L observed in the nitrified LPD (Table 1) indicates a significant activity of the heterotrophic biomass in the nitrification reactor. The fact that the majority of active heterotrophic biomass was separated in the sedimentation tank of the nitrifying system and recirculated back to the reactor where it was not transferred to the effluent from the sedimentation tank seems to be responsible for these findings. In accordance with this assumption, suspended solids concentration of the nitrified LPD taken from the sedimentation tank of the nitrification system was even significantly lower than suspended solids concentration in the raw LPD entering the nitrification reactor (Table 1). As a result of the above presented facts, the intensity of denitrification was extremely low resulting in a very slow decrease of N_{inorg} concentration during the tests.

The differences in pH trends between stirred variants of nitrified LPD storage (N2 and N4) and the variants without stirring (N1 and N3) reached more than 2.5 units, being the variants without stirring characteristics of higher values in some phases of the experiment (Fig. 2). Such differences may be induced by varying intensity of particular biochemical and/or physical–chemical processes taking place in the system such as nitrification of resting TAN, gentle denitrification, transformation of volatile fatty acids, stripping of CO_2, etc. (Painter, 1970; Blanes-Vidal et al., 2009; Hafner et al., 2013). However, specific research is necessary for potential confirmation of these hypotheses. For all events, the increase of pH up to 8.3 or 7.5 during the storage of nitrified LPD registered in N1 and N3, respectively, may represent high risk of the increase of N_{inorg} loss in certain cases. The findings of Svehla et al. (2017) indicate that in some cases, it may be problematic to reach complete transformation of TAN in a nitrification reactor treating LPD, which may lead to a high content of resting TAN in nitrified LPD. Subsequently, it may result in relatively intensive nitrogen losses caused by the volatilisation of FA after intensive pH increase.

3.3. Degradation of organic matter during LPD storage

During the 100-day experiment, $COD_{soluble}$ decreased by 14.8; 28.2; 7.6 and 18.2% for R1, R2, R3 and R4, respectively (calculated using the values for the start and end of the experiment from linear regression in accordance with Eq. (4)), indicating that a certain portion of organic matter decomposed during the simulation of raw LPD storage. COD_{total} decreased by a maximum of 12%. COD removal efficiency was relatively low, probably due to the limited portion of easily biodegradable organic compounds contained in LPD (see above). In accordance with Perazzolo et al. (2017), under continuous stirring during raw LPD storage, the initial anaerobic conditions were altered and changed into aerobic in R2 and R4 where up to 2.5 and 9.5 mg/L of DO was measured in R2 and R4, respectively. This supports aerobic degradation of organic matter resulting in higher COD removal efficiency, compared to R1 and R3 in which DO concentration did not exceed 0.1 mg/L.

On the other hand, no measurable decrease in organic matter quantified by COD was observed during the simulation of nitrified LPD storage performed within N1–N4, despite the prolonged operational period of N1 and N2 to 250 days. In some cases even slight increase of COD concentration was observed, which was probably caused by limited accuracy of COD determination. The removal of a significant part of resting biodegradable organic matter already present in nitrification reactor treating LPD (Table 1) seems to be responsible for this observation. Thus, nitrified LPD could be considered a biochemically stable matter from the point of view of potential biological degradation of organic matter during storage.

3.4. Applicability of the results and suggestions for subsequent research

Based on the results presented above, it is clear that the intensity of N_{inorg} loss during the storage of nitrified LPD could be many times lower compared with the losses resulting from raw LPD storage. For example, the comparison of R1 and N1 variants leads to the conclusion that N1 losses were 16–24 times lower than R1. Similarly, nitrogen losses typical for

N1–N4 are many times lower compared with other authors who quantified the losses during raw digestate storage in different conditions, especially at summer temperatures (Sommer, 1997; Perazzolo et al., 2017; Li et al., 2018). Thus, the results indicate that the nitrification could represent a suitable method for minimisation of nitrogen losses, which could be used as an alternative for covering the storage tanks or the acidification of digestate suggested, for example, by Perazzolo et al. (2016). Simultaneously, the reduction of nitrogen losses during the application of LPD to soil could be minimised in the case of nitrified LPD where even 40% of nitrogen could be lost after the application of raw digestate (Nicholson et al., 2017). However, future research, focused on nitrified LPD storage under full scale or at least pilot plant conditions, is needed to obtain more accurate results that will objectively simulate real situations. For such an experiment, a pilot plant for nitrification of LPD should be installed. Simultaneously, long-term operation of pilot plant should enable to estimate energy demand, investment and operational costs related to potential installation of the system for LPD nitrification in full scale conditions.

Also different factors influencing N_{org} concentration during the LPD nitrification and during the nitrified LPD storage should be studied intensively in the future. During our research, no significant changes of N_{org} concentration were observed as the consequence of the nitrification of LPD as well as the consequence of the storage of raw/nitrified LPD. At the same time, mineralisation of N_{org} and/or incorporation of N_{inorg} into the living biomass may cause significant changes to the value of this parameter. Chemical composition of each concrete source of LPD seems to be very important in this respect.

Based on the results of this research, the possibility to separate effectively active nitrification and heterotrophic biomass in the sedimentation tank of the nitrifying CSTR system treating the LPD should also be verified within special future experiments with the aim to predict potential influence of this biomass on the course of the nitrified LPD storage based on local conditions prevailing on a concrete biogas plant.

Experimental verification of fertilising properties of the nitrified LPD and their comparison with the fertilising properties of the raw LPD is also recommended with the aim to prove the reasonability of the installation of a nitrification technology for the LPD treatment.

4. Conclusions

The experiments proved that the intensity of nitrogen losses during LPD storage could be effectively minimised by the initiation of the nitrification process in the LPD environment. Produced nitrate was very stable under given conditions. Additionally, no significant influence of the temperature and potential stirring of stored nitrified LPD was observed. Thus, the treatment of LPD, consisting of biological nitrification of TAN, seems to be promising and a relatively simple alternative for the future where subsequent research focused on the verification of the results in pilot plant and/or full scale conditions is recommended.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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