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Nitrification in a completely stirred tank reactor treating the liquid phase of digestate: The way towards rational use of nitrogen

Pavel Svehla, Helena Radechovska, Lukas Pacek, Pavel Michal *, Ales Hanc, Pavel Tlustos

Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamycka 129, Prague 165 21, Czech Republic

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ABSTRACT

The nitrification of the liquid phase of digestate (LPD) was conducted using a 5 L completely stirred tank reactor (CSTR) in two independent periods (P1 – without pH control; P2 – with pH control). The possibility of minimizing nitrogen losses during the application of LPD to the soil as well as during long-term storage or thermal thickening of LPD using nitrification was discussed. Moreover, the feasibility of applying the nitrification of LPD to the production of electron acceptors for biological desulfurization of biogas was assessed. Despite an extremely high average concentration of ammonia and COD in LPD reaching 2470 and 9080 mg/L, respectively, nitrification was confirmed immediately after the start-up of the CSTR. $N\text{-}NO_3^-$ concentration reached 250 mg/L only two days after the start of P1. On the other hand, P1 demonstrated that working without pH control is a risk because of the free nitrous acid (FNA) inhibition towards nitrite oxidizing bacteria (NOB) resulting in massive nitrite accumulation. Up to 30.9 mg/L of FNA was present in the reactor during P1, where the NOB started to be inhibited even at 0.15 mg/L of FNA. During P2, the control of pH at 7.0 resulted in nitrogen oxidation efficiency reaching 98.3 ± 1.5% and the presence of $N\text{-}NO_3^-$ among oxidized nitrogen 99.6 ± 0.4%. The representation of volatile free ammonia within total nitrogen was reduced more than 1000 times comparing with raw LPD under these conditions. Thus, optimum characteristics of the tested system from the point of view of minimizing the nitrogen losses as well as production of electron acceptors for the desulfurization of biogas were gained in this phase of reactor operation. Based on the results of the experiments, potential improvements and modifications of the tested system were suggested.

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1. Introduction

Digestate is produced as a by-product of anaerobic digestion of organic matter. A specific type of digestate is produced during the operation of biogas plants. In many cases, the digestate produced in biogas plants is separated into solid and liquid phases with the aim of optimizing the use of the nutrients contained in the digestate and to reduce the costs of transporting the digestate from the biogas plant to land (Al Seadi et al., 2013; Bauer et al., 2009; Rehl and Müller, 2011). The liquid phase of digestate (LPD) contains relatively high concentrations of total ammonia nitrogen (TAN; the sum of $N\text{-}NH_4^+$ and free ammonia - FA) reaching mostly several hundreds of milligrams or several grams per litre (e.g. Botheju et al., 2010; Xu et al., 2014; Zekker et al., 2015). Also the concentration of phosphorus, potassium and other nutrients is relatively high in LPD (Al Seadi et al., 2013; Kolář et al., 2008; Chiumenti et al., 2013). Presently, the LPD produced at agricultural biogas plants is usually stored in lagoons situated on the premises of the plants on a relatively long-term basis, and it is consequently applied to the soil, where it serves as a source of nutrients for plants in the growing season. However, the LPD contains a large amount of ballast water in which the concentration of nutrients is still limited. Simultaneously, legislation limits the amount of nitrogen that can be applied to soil in the EU states (European Commission, 1991). This fact reduces the possibility of utilizing LPD directly in situ. Therefore, the economic benefits of working with raw LPD may be decreased by the costs of transporting the LPD from the biogas plant to farms (Rehl and Müller, 2011). At the same time, the present-day approach to handling LPD causes intense emission of ammonia during storage and the application of the LPD (Whelan et al., 2010). These losses of nitrogen could be significant, not only from the perspective of environmental protection, but also simply because of the decrease in the amount of nutrients supplied to plants with LPD. Due to the reasons presented above, in some cases it is necessary to treat LPD with the aims of recovering the nutrients in concentrated form, contributing to environmental protection, or minimizing nitrogen loss. In order

* Corresponding author.
E-mail address: michalp@af.czu.cz (P. Michal).

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to treat the LPD, various physico-chemical or biological processes such as struvite precipitation (Münch and Barr, 2001; Song et al., 2011), stripping of ammonia (Guštin and Marinšek-Logar, 2011), sorption on zeolite, biochar or other materials (Cardoso et al., 2015; Kizito et al., 2015), incorporation of nutrients into the biomass of a specific algae (Prajapati et al., 2014; Tale et al., 2014), forward osmosis and subsequent reverse osmosis or other membrane processes (Al Seadi et al., 2013; Holloway et al., 2007), or thermal thickening by vacuum evaporation (Chiumenti et al., 2013) could be applied. Nevertheless, all the above mentioned methods require relatively high operational costs.

Nitrification in combination with a suitable additional process could also be advantageously used within LPD treatment aimed at the reasonable use of nitrogen. The nitrification represents biochemical oxidation of TAN to nitrite and thence to nitrate. Firstly, the TAN is converted to nitrite by ammonia oxidizing bacteria (AOB). Consequently, the nitrite is converted to nitrate using the activity of nitrite oxidizing bacteria (NOB). The genus *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira* and *Nitrosocystis* represent typical AOB, whereas *Nitrobacter*, *Nitrospira* or *Nitrocystis* belong to the important NOB genus (Daims et al., 2015; Painter, 1970). Moreover, the results presented by Daims et al. (2015) indicate that the genus *Nitrospira* may even be able to directly convert TAN to nitrate. During nitrification of extremely highly nitrogen-loaded water streams, such as LPD, the pH decreases as the result of the acidification of the water environment caused by H^+ production (Painter, 1970). At the same time, the representation of volatile FA within TAN falls significantly with decreasing pH value (Anthonisen et al., 1976). Therefore, the nitrification process induced in the environment of LPD could minimize the loss of nitrogen caused by the volatilization of FA during storage and application of LPD (Botheju et al., 2010). Additionally, the nitrate which could be, from the perspective of potential volatilization, considered as in "stabilized" nitrogen form, is introduced into the LPD. For the treatment of LPD using thermal thickening leading to minimizing the volume of LPD, the pH should be also decreased with the aim of minimizing volatilization of ammonia within this process (Chiumenti et al., 2013). Thus, nitrification could be applied with the aims of improving the pH of the LPD and introducing the nitrate into LPD before its thermal thickening (Botheju et al., 2010). Another potential use of nitrate (or nitrite) produced during the nitrification of LPD consists of their application as an electron acceptor for autotrophic denitrification in biogas cleaning by hydrogen sulphide scrubbing and bio-oxidation of captured sulphides (Doğan et al., 2012; Pokorna et al., 2015; Pokorna and Zabranska, 2015).

The nitrification of LPD has been successfully completed by several authors during the last few years (Botheju et al., 2010; Cydzik-Kwiatkowska et al., 2013; Magrí et al., 2013; Scaglione et al., 2013; Xu et al., 2014; Zhang et al., 2011). However, most of the available papers evaluate the biological processes ensuring ammonia removal from LPD without the ambition to reuse the nitrogen. In these cases, the nitrification (or more precisely its short-cut version – nitritation) represents one stage of LPD treatment by nitritation/denitritation or partial nitritation/anaerobic ammonium oxidation – anammox (Cydzik-Kwiatkowska et al., 2013; Magrí et al., 2013; Scaglione et al., 2013; Zhang et al., 2011). An extremely high concentration of TAN in LPD and resulting FA and/or free nitrous acid (FNA) or NO_2^- inhibition of AOB and NOB (Anthonisen et al., 1976; Buday et al., 1999) significantly complicates the start-up of traditional nitrification with nitrate as a dominant final product in the environment of LPD. NOB are generally much more sensitive towards FA/FNA/NO_2^- inhibition compared to AOB. Anthonisen et al. (1976) postulated that FA starts to inhibit NOB in a range of 0.1–1.0 mg/L, whereas more resistant NOB tolerate a concentration up to 10–150 mg/L. FNA seems to be an especially strong inhibitor of NOB activity where even 0.011 mg/L of N–HNO_2 corresponding with 0.037 mg/L of FNA may inhibit anabolic processes of NOB (Vadivelu et al., 2006a). On the other hand, even dissociated NO_2^- was presented as an NOB inhibitor inducing 50% inhibition at a concentration of 198 mg/L N-NO_2^- (Buday et al., 1999). Due to the risk of FA/FNA/NO_2^- inhibition, the necessity to dilute the LPD entering into the nitrification reactor to initial TAN concentration lower than 800 mg/L was reported by Xu et al. (2014). Moreover, nitrite accumulation was observed by these authors despite the abovementioned dilution of digestate. Botheju et al. (2010) achieved massive nitrate production without significant nitrite accumulation during digestate nitrification. However, it was necessary to pre-acclimate the inoculum for 20 days before the experiment. In addition, the hydraulic retention time (HRT) was extremely long (>33 days). Thus, the efficiency of the system operated by Botheju et al. (2010) was very low. The ability of NOB to produce nitrate efficiently, at high nitrogen loading rates (NLR), was not confirmed. Taking into consideration the fact, that AOB as well as NOB belong among aerobic bacteria, the availability of dissolved oxygen is essential from the point of view of a satisfactory course of nitrification applied to highly nitrogen-loaded water streams. Pacek et al. (2015) proved that the nitrification of highly nitrogen-loaded reject water (1450 ± 165 mg/L of TAN) producing nitrate as sole final product could be efficiently operated at a dissolved oxygen concentration of 3.0 mg/L in a completely stirred tank reactor (CSTR), whereas the decrease of dissolved oxygen to 0.7 mg/L leads to a massive accumulation of nitrite. On the contrary, when sequencing batch reactor (SBR) technology was applied, even a dissolved oxygen concentration reaching up to 7.5 mg/L was not able to prevent the accumulation of nitrite owing to significantly stronger FA and FNA inhibition pressure towards NOB in SBR compared to the CSTR (Svehla et al., 2014).

The aim of this paper is to define the optimal conditions for the nitrification of LPD based on the intended subsequent processing of nitrified LPD. Potential direct application to soil, long-term storage as well as thermal thickening of nitrified LPD is expected in this context. Moreover, the possibility of using the nitrified LPD as the source of electron acceptors for biogas cleaning will be also discussed. Attention was paid primarily to the acceleration of the treatment process start-up, to the control of final nitrification product (nitrate versus nitrite) representation and to the control of the nitrification process efficiency. With respect to minimization of nitrogen losses, the reduction of the proportion of nitrogen present in the form of volatile FA at least by 90% was required. With regard to a relatively high toxicity of nitrite to plants (Court et al., 1962; Singh et al., 2007), minimization of nitrite accumulation was required during the nitrification process for potential application of nitrified LPD to soil. The paper also aims to suggest suitable ways for subsequent research in this field.

2. Material and methods

2.1. Reactor set-up

The experiments were carried out in a 5 L laboratory-scale nitrification reactor with LPD as an influent. The reactor was aerated using a coarse bubble system. A peristaltic pump was used to transport the LPD into the laboratory model. The LPD was supplied continuously into the CSTR where the feeding rate of the peristaltic pump was dependent on the actual NLR applied. The reactor was operated according to the principle of CSTR using the biomass cultivated in the form of a suspension (activated sludge). The source of the inoculum for the start-up of the reactor is described in chapter 2.2. The reactor was combined with a sedimentation tank (1 L).

The sludge was continuously recirculated from the sedimentation tank to the reactor using the peristaltic pump. In accordance with Jenicek et al. (2004) and Svehla et al. (2014), with the aim of maximizing the sludge retention time (SRT) no excess sludge was withdrawn from the reactor. Only minor sludge losses were caused by its escaping with the effluent water. Thanks to this strategy, an SRT in the range of 10–35 days was maintained throughout the whole experiment. The actual value of SRT in the reactor was determined by the intensity of the escape of solid particles from the reactor which was dependent mainly on the actual value of HRT. The average biomass concentration in the reactor quantified as volatile suspended solids (VSS) reached 5.58 g/L. However, a certain portion of this matter was created by VSS present in raw LPD entering into the reactor (Table 2). All experiments were performed at laboratory temperatures (23 ± 2) °C with no oxygen limitation (the dissolved oxygen concentration varied within the range of 3.0 and 7.4 mg/L) with the aim of preventing the inhibiting effect of the low oxygen concentration on the activity of nitrifying bacteria (Pacek et al., 2015).

The experiment was divided into two independent phases. First, the reactor was operated without pH control for 30 days within Period 1 (P1). Consequently, Period 2 (P2), lasting for 90 days, was initiated. The value of the pH was controlled in this phase of the reactor operation with the aim of evaluating the possibility of improving the efficiency of the nitrification process as well as the representation of nitrate and nitrite between nitrification final products. During the first 60 days of P2, the pH was set at 7.0. Subsequently, the adjusted pH value was lowered to 6.5. An NaOH solution was dosed into the reactor using peristaltic pumps with the aim of improving the pH value. The GRYF sensor PCl 321 XB2 and GRYF MAGIC XBC measuring and controlling system (GRYF HB, Czech Republic) were used for the maintenance of the required pH value in the system. At the beginning of both periods (P1 as well as P2), the reactor was inoculated with nitrifying biomass (see below, chapter 2.2). The HRT and NLR was controlled during the experiment with the aim of quantifying approximately the productivity of the reactor. The overall volume of LPD treated during P1 and P2 corresponded to 14.5 and 60.2 L, respectively.

2.2. Start-up of the reactor

For each period of the reactor operation (P1 and P2), the reactor was inoculated with nitrifying activated sludge gained from the flow of recirculated sludge at the Prague central wastewater treatment plant. The concentration of this sludge was 10 g/L (expressed as total suspended solids - TSS). The method of inoculating the reactor was based on findings presented by Pacek et al. (2016). When starting the reactor operation, the whole reactor container was filled with an activated sludge used as an inoculum. Then, the LPD inflow was initiated. The characteristics of the inoculum are presented in Table 1.

2.3. LPD used for the experiments

The LPD used for this experiment originated from an agricultural biogas plant using pig slurry and grass silage as the main sources of the substrate for biogas production. Thermophilic conditions (55–57 °C) are applied in the anaerobic reactor of this biogas plant. The basic parameters of LPD used for this experiment are presented in Table 2.

Table 1
The characteristics of the inoculum.

Parameter (unit)	Value
pH	7.4
TAN (mg/L)	12.0
N-NO_3^- (mg/L)	5.5
N-NO_2^- (mg/L)	0.0
TSS (mg/L)	10,000
VSS (mg/L)	8150

2.4. Analytical methods

Once a week, the TAN, N-NO_2^- and N-NO_3^- concentration in raw LPD used as the influent and in the effluent from the reactor were measured with the aim of evaluating the nitrification process. Simultaneously, chemical oxygen demand (COD) of centrifuged samples taken from the influent and the effluent was determined in order to evaluate the efficiency of the dissolved organic matter removal at the same intervals. A Rotina 420 centrifuge (Hettich GmbH & Co. KG, Germany) was used for centrifugation of samples where the speed of rotation corresponded to 9500 rpm and the G-force corresponded to 12.007. The samples were treated for 12 min using the centrifuge. Additionally, total nitrogen (N-tot) was measured at least once every two weeks in the influent as well as in the effluent. Alkalinity, TSS and VSS were monitored once every two weeks in the influent. VSS were also measured once a week in the reactor with the aim of quantifying the nitrifying biomass. The concentrations of TAN, N-NO_2^-, N-NO_3^-, COD, TSS, and VSS were measured in accordance with the standard methods (APHA, 2005). The N-tot 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-tot concentration and inorganic nitrogen concentration (the sum of TAN, N-NO_2^- and N-NO_3^- concentration). The alkalinity of the influent was determined by titration of the sample with hydrochloric acid (0.1 mol/L) up to pH 4.5. The results of alkalinity (Table 2) are presented in the number of mmol/L of strong monohydric acid needed to achieve a pH of 4.5 in the sample. The temperature, pH, and dissolved oxygen concentration in the reactor were gauged continually using GRYF sensors PCl 321 XB2 and KCl 12 XB4 (GRYF HB, Czech Republic). With the aim to describe more precisely the quality of nitrified LPD and to compare it with the quality of raw LPD, the concentration of other important nutrients (Ca, K, Mg, P and S) and selected risk elements (Cd, Pb, Hg, As, Cr, Cu, Mo, Ni, Zn) in the effluent from the reactor as well as in the influent was quantified on a one-time basis. The samples taken on day 32 of Period 2 were analysed for this purposes. Cd, Pb, As, Cr, Cu, Mo, Ni, Zn, Ca, K, Mg and S were determined according to Zakova et al. (2016) using inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent 720, Agilent Technologies Inc., USA) equipped with a two channel peristaltic pump, a Sturman-Masters spray chamber and a V-groove pneumatic nebulizer. By this way, so called pseudototal content of the elements expressing the amount of the elements extractable with aqua regia was analysed. The pseudototal content of Hg was measured according to Šípková et al. (2016) using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700x, Agilent Technologies Inc., USA), equipped with an autosampler ASX-500, a three-channel peristaltic pump and a MicroMist nebulizer. The determination of

Table 2
The composition of the LPD fed into the reactor.

Parameter (unit)	Value
pH	8.1 ± 0.1
Alkalinity (mmol/L)	97 ± 15
COD (mg/L)	9080 ± 1240
TAN (mg/L)	2470 ± 190
N-tot (mg/L)	2780 ± 230
TSS (mg/L)	3130 ± 370
VSS (mg/L)	2780 ± 320

Ca, K, Mg, P, S, Cd, Pb, Hg, As, Cr, Cu, Mo, Ni and Zn in the samples of influent and effluent was realized in 3 repetitions where average values and standard deviations were presented within the results.

2.5. Calculations

The concentrations of FA (C_{FA}) and FNA (C_{FNA}) in mg/L were calculated in accordance with Anthonisen et al. (1976) (Eqs. (1) and (2)):

$$c_{FA} = \frac{17}{14} \cdot \frac{c(TAN) \cdot 10pH}{\exp\left(\frac{6334}{273+T}\right) + 10pH} \quad (1)$$

$$c_{FNA} = \frac{46}{14} \cdot \frac{c(N\text{-}NO_2^-)}{\exp\left(\frac{-2300}{273+T}\right) \cdot 10pH} \quad (2)$$

where c(TAN) and $c(N\text{-}NO_2^-)$ represent the actual total concentrations of TAN and $N\text{-}NO_2^-$, respectively in mg/L, and T is the temperature in degrees centigrade.

The nitrogen oxidation efficiency (NOE) in% was calculated according to Eq. (3):

$$NOE\ (\%) = \frac{c(N\text{-}NO_2^-) + c(N\text{-}NO_3^-)}{c(N\text{-}NO_2^-) + c(N\text{-}NO_3^-) + c(TAN)} \cdot 100\% \quad (3)$$

where $c(N\text{-}NO_2^-)$, $c(N\text{-}NO_3^-)$ and c(TAN) represent the concentrations of $N\text{-}NO_2^-$, $N\text{-}NO_3^-$ and TAN in the effluent from the reactor.

The nitrite accumulation ratio (NiAR) in% was calculated according to Eq. (4):

$$NiAR\ (\%) = \frac{c(N\text{-}NO_2^-)}{c(N\text{-}NO_2^-) + c(N\text{-}NO_3^-)} \cdot 100\% \quad (4)$$

where $c(N\text{-}NO_2^-)$ and $c(N\text{-}NO_3^-)$ represent the concentrations of $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ in the effluent from the reactor.

3. Results and discussion

3.1. Experiments without pH control – Period 1

3.1.1. The initiation and the stability of the nitrification process

Despite extremely high concentrations of TAN and COD in raw LPD, the nitrification process with nitrate as the main final product was initiated immediately after the start of the experiment. An $N\text{-}NO_3^-$ concentration reaching 250 mg/L was observed in the effluent as soon as two days after the start of the experiment, where 1540 mg/L was measured on day 21 (Fig. 1A). These findings clearly confirmed high activity of AOB as well as NOB. Simultaneously with the increase of the $N\text{-}NO_3^-$ concentration, a gradual increase of the TAN concentration was observed in the effluent from the reactor during first 15 days of the experiment. Then, it was stabilised in the range of 1000–1250 mg/L (Fig. 1A). The results indicated that the TAN coming into the reactor with the LPD was partially converted to $N\text{-}NO_3^-$ and partially it remained in its original form. The $N\text{-}NO_2^-$ concentration did not exceed 50 mg/L during the first 21 days of the reactor operation. In accordance with the fact that very low concentrations of nitrogen forms were present in the reactor at the moment of the process initiation (Table 1), the concentrations of total nitrogen in the effluent and in the raw LPD were gradually equalized. During our experiment, no dilution of raw LPD entering into the reactor or long-term preacclimation of nitrifying bacteria mentioned by Xu et al. (2014) and Botheju et al. (2010), respectively, was needed for satisfactory initiation of the nitrification process with nitrate as the dominant final product observed during the first 21 days of the reactor operation. The biomass adapted to the conditions prevailing in the reactor naturally by a gradual increase of the nitrogen forms concentration resulting from the method of the reactor inoculation.

However, a gradual increase of $N\text{-}NO_2^-$ concentration was observed after day 21 which was accompanied by an $N\text{-}NO_3^-$ concentration decrease. While 47 mg/L of $N\text{-}NO_2^-$ was measured on day 21, on day 30, the concentration of $N\text{-}NO_2^-$ had increased to 1 022 mg/L (Fig. 1A).

3.1.2. The pH value and its influence on the inhibition of nitrification bacteria

The initial pH of the inoculum (measured on day 0) was 7.4 (Table 1). A subsequent decrease of the pH observed, despite the slightly alkaline characteristic of raw LPD (Table 2), confirmed the immediate start of the nitrification process (Painter, 1970). Due to this, the conditions achieved by Xu et al. (2014) using external acidification with the aim of supporting the start-up of the nitrification of the anaerobic digester effluent were attained naturally during our research. From day 2 until day 21, the value of pH ranged between 6.1 and 6.5 (Fig. 2A). Subsequently, a sudden decrease to 5.2 was registered between days 21 and 22. The reason for this significant change in the pH value during this phase of the reactor operation is not quite obvious. However, even significantly lower minimum pH values reaching 3–4 were observed by Botheju et al. (2010) during the nitrification of digestate at certain phases of the nitrification reactor's operation.

The decrease in the pH value discovered in our reactor on day 21 is a positive development with respect to a potential minimization of nitrogen loss during storage, application or thermal thickening of nitrified LPD (Botheju et al., 2010; Chiumenti et al., 2013). On the other hand, it resulted in a significant increase of FNA concentration despite the fact that a relatively low $N\text{-}NO_2^-$ concentration reaching 47 mg/L was actually measured in the reactor. The FNA concentration reached 0.15 mg/L at this time (Fig. 2B). At the same time, even 0.011 mg/L of $N\text{–}HNO_2$ corresponding with 0.037 mg/L of FNA may inhibit anabolic processes of NOB (Vadivelu et al., 2006a). This fact probably induced the massive accumulation

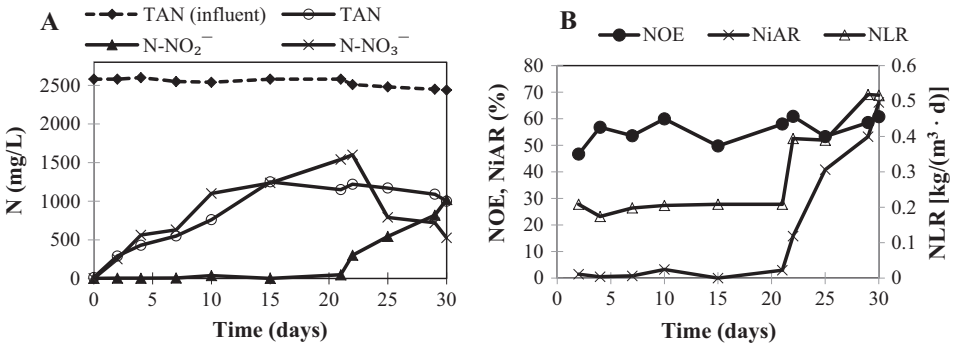

Fig. 1. Results of Period 1 – TAN, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ concentrations in the effluent compared to TAN concentration in the influent (A); NLR, NOE and NiAR (B).

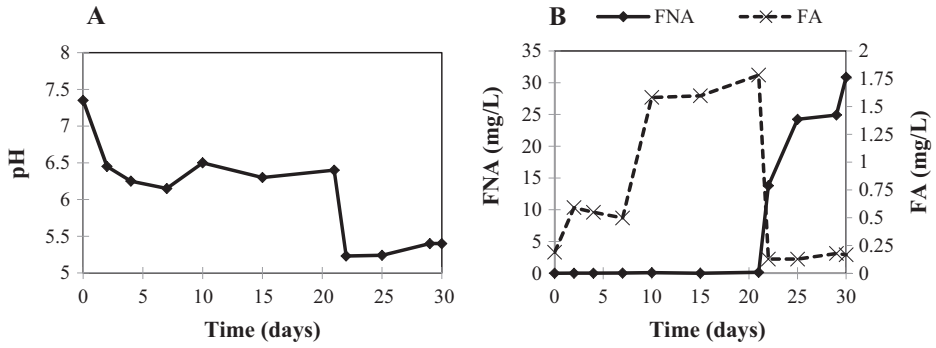

Fig. 2. Results of Period 1 – pH value (A); FA and FNA concentration (B).

of N-NO_2^- after day 21 of the reactor operation (300 mg/L measured on day 22, Fig. 1A). Thus, the biomass was exposed to an extremely high concentration of FNA reaching 13.8 mg/L on this day (Fig. 2B). Under such conditions even the inhibition limit 0.2–2.8 mg/L of FNA for NOB presented by Anthonisen et al. (1976) was exceeded several times. The subsequent reactor operation was characterised by further increases of N-NO_2^- and also FNA concentration (Fig. 1A and 2B). The maximum FNA concentration (30.9 mg/L) was achieved on day 30. Under such high FNA concentration, the restoration of NOB activity could not be expected (Anthonisen et al., 1976; Vadivelu et al., 2006a) and even AOB activity could have been endangered (Vadivelu et al., 2006b). Therefore, the experiment was interrupted and subsequent tests were performed with the pH control.

A maximum FA concentration reaching 1.6–1.8 mg/L was achieved between days 10 and 21 during Period 1. Despite the inhibiting level for NOB in the range of 0.1–1.0 mg/L (Anthonisen et al., 1976) being exceeded, no massive N-NO_2^- accumulation was observed until day 21 which indicates that FNA was a much more important NOB inhibitor compared to FA during this period of reactor operation.

3.1.3. Nitrogen oxidation efficiency, nitrite accumulation ratio and nitrogen oxidation rate

During all of Period 1 the nitrogen oxidation efficiency (NOE) values ranged between 47 and 61% (Fig. 1B). The limitation of achievable NOE is caused by the reduced alkalinity of the treated LPD which is insufficient for the compensation of H^+ production and from it we observed the resulting pH decrease (Painter, 1970; Jenicek et al., 2004). The achieved NOE is similar to the results of the experiment performed under comparable conditions with reject water (Jenicek et al., 2004). Nitrite accumulation ratio (NiAR) did not exceed 4% until day 21 (Fig. 1B). However, after day 21 it increased gradually up to 66% as the result of massive NOB inhibition. Thus, the conditions applied during Period 1 do not seem to be suitable for the requirement to minimize NiAR value.

The NLR was kept around 0.2 kg/(m^3 d) during the first 21 days of the reactor operation. Subsequently, it was gradually increased to 0.52 kg/(m^3 d) (Fig. 1B). Taking into consideration the values of NOE achieved in the reactor, the nitrogen oxidation rate and specific nitrogen oxidation rate reached up to 0.31 kg/(m^3 d) and 2.31 mg/(gVSS·d), respectively during this period of the reactor operation.

3.2. Experiments with pH control – Period 2

3.2.1. The initiation and the stability of the nitrification process

Similarly, as in the case of the experiments performed without the pH control, TAN was converted to N-NO_3^- immediately after the beginning of the experiment (Fig. 3A). No massive N-NO_2^- accumulation was observed during any of Period 2. The N-NO_2^- concentration did not exceed 30 mg/L. The TAN concentration in the effluent did not exceed 60 mg/L until day 47. A temporary increase of the TAN concentration to 400 mg/L registered on day 55 seems to be the result of exceeding the optimum LPD feeding rate when the NLR reached 0.64 kg/(m^3 d) and the nitrifying bacteria were not able to nitrify all the TAN incoming into the reactor (Fig. 4A). Therefore, the NLR was intentionally reduced on this day. As a result of this change in feeding strategy, the TAN concentration in the effluent decreased again to 60 mg/L on day 61. The subsequent long-term increase of TAN concentration observed after day 68 was caused by the change of the pH control strategy (the adjusted value was changed from 7.0 to 6.5). Nitrifying bacteria prefer slightly alkaline pH value reaching 7–9 (Painter, 1970) whereas the value of 6.5 applied during the days 61–90 is outside this interval. For this reason, the TAN concentration increased up to 780 mg/L in this phase of the reactor operation (Fig. 3A). This finding is in accordance with Jenicek et al. (2004) who observed massive accumulation of TAN (almost 40% of the TAN measured in the influent) in the reactor treating reject water when the pH was not controlled. On the contrary, more than 99% of TAN was successfully oxidized by these authors when a pH control ensuring a value falling in the abovementioned optimal range for nitrifying bacteria was applied.

The sum of TAN, N-NO_3^- and N-NO_2^- concentrations in the effluent was higher compared to the concentration of TAN in the influent during some measurements. The reason for this phenomenon is that a certain portion of pure water contained originally in the raw LPD evaporated during the retention of the LPD in the nitrification reactor. In this way, the LPD was thickened to a certain extent and the concentration of various chemical compounds, including nitrogen forms, increased. At relatively constant conditions (mainly temperature and aeration intensity) applied during this research, the volume of water which evaporated each day was almost constant. However, if the HRT is extended significantly, the percentage of the losses of water through evaporation (related to volume of raw LPD) increases significantly. Therefore, the phenomenon of thickening was observed mainly during the last 30 days of Period 2 (Fig. 3A) characterised by long HRT reaching 13–14 days on a long-term basis (Fig. 4A). Also the transformation of part of organic nitrogen into inorganic forms in the nitrification reactor may to a certain extent support the increase of the sum of TAN, N-NO_3^- and N-NO_2^- concentrations. However, this phenomenon seems not to be as significant from this point of view (see chapter 3.3.4). Certain losses of water caused by evaporation and the resulting thickening of LPD must also be expected in a full scale system, where the intensity of this process will be determined by local conditions including actual HRT.

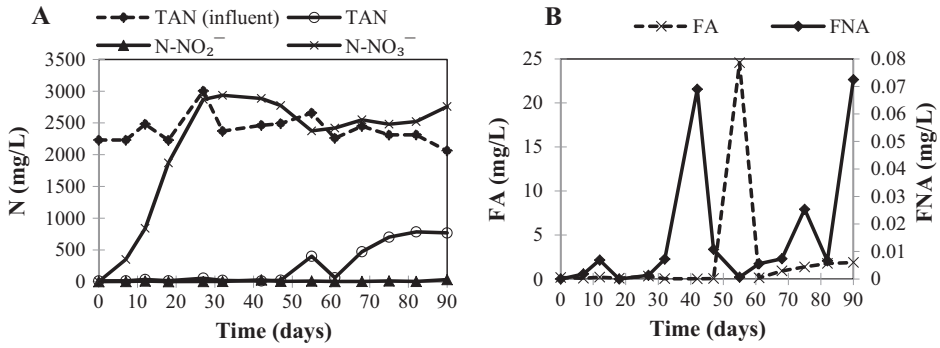

Fig. 3. Results of Period 2 – TAN, $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ concentrations in the effluent compared to TAN concentration in the influent (A); FA and FNA concentration (B).

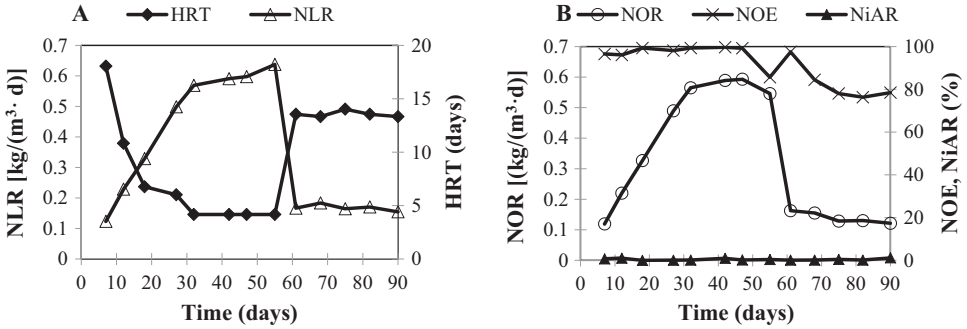

Fig. 4. Results of Period 2 – HRT and NLR (A); NOE, NOR and NiAR (B).

3.2.2. Inhibiting factors affecting nitrification activity

Due to the pH control, the pH value reached 7.00 ± 0.05 and 6.50 ± 0.05 during first 60 days and during the rest of Period 2, respectively with the exception of day 55 when the pH increased to 8.0 on a short-term basis. Under such conditions, the biomass inhibition induced by FA and/or FNA was significantly weaker during Period 2 compared to Period 1. FA and FNA concentration did not exceed 1.88 and 0.07 mg/L, respectively, with the exception of day 55 (Fig. 3B). On day 55, the FA concentration increased to 24.6 mg/L as a consequence of a pH increase to 8.0. Exceeding the optimum LPD feeding rate by TAN at the maximum applied NLR reaching 0.64 kg/(m^3 d) (Fig. 4A) seems to be responsible for this temporary pH deviation. In this phase, the intensity of acidification during the LPD nitrification (Painter, 1970) was restricted by the limited potency of nitrification process resulting in a pH increase observed on day 55. The exceeding of the optimum LPD feeding rate by TAN on day 55 is discussed more precisely in chapter 3.2.3. In any event, FA inhibition limits for NOB were significantly exceeded on day 55 (Anthonisen et al., 1976). However, no massive nitrite accumulation (indicating a strong limitation in NOB activity) was observed even in this phase of the reactor operation (Fig. 3A). Taking into consideration these results, the resistant culture able to adapt to relatively high FA concentrations reached on a short-term basis in the reactor was probably developed in the reactor within its long-term operation in accordance with Turk and Mavinic (1989).

3.2.3. Nitrogen oxidation efficiency, nitrite accumulation ratio and nitrogen oxidation rate

Keeping the pH value at 7.0 enabled the reactor to achieve an NOE reaching 98.3 ± 1.5% during the first 47 days of the observation (Fig. 4B). Despite the decrease of the adjusted pH value to 6.5 after day 60, NOE reaching at least 75% was achieved through the end of the experiment. These results confirmed the positive effect of pH control on NOE during nitrification of highly nitrogen-loaded water streams observed during the nitrification (or more precisely nitritation) of reject water by Jenicek et al. (2004).

The NLR was intentionally gradually increased during the first 55 days of the experiment from 0.12 to 0.64 kg/(m^3 d) (Fig. 4A). While an NLR of 0.60 kg/(m^3 d) on day 47 produced an NOE of 99% (Fig. 4B), the increase of NLR to 0.64 on day 55 induced the decrease of NOE to 86% which clearly demonstrated an exceedance of the optimum LPD feeding rate through the presence of TAN in the effluent in this phase of the experiment. According to this, the approximate maximum NLR enabling the complete transformation of TAN to nitrate seems to be a value of 0.60 kg/(m^3 d) under the conditions applied during Period 2. Consequently, the NLR was reduced radically to 0.15–0.18 kg/(m^3 d) for the rest of Period 2 with the aim of stabilizing the process. This led to the recovery of excellent NOE values which reached 97% on day 61. Thus, after having temporarily exceeded the optimum LPD feeding rate, the reversibility of high NOE was proved. The decrease of NOE observed during the rest of Period 2 (Fig. 4B) was caused by the change of the pH control strategy (the adjusted value was changed from 7.0 to 6.5) in accordance with Jenicek et al. (2004).

Considering the actual NOE, the maximum nitrogen oxidation rate reaching 0.59 kg/(m^3 d) was registered on day 47 (Fig. 4B) exceeding many times the maximum values achieved by Botheju et al. (2010). The specific nitrogen oxidation rate reached 4.41 mg/(gVSS d) on this day. However, taking into consideration the radical decrease of NLR performed to stabilize the process, such high values of the nitrogen removal rate were not re-established during the rest of Period 2. Thus, the ability of the system to ensure such high nitrogen oxidation rate levels on a long-term basis should be verified within additional research (see chapter 3.4.1). The productivity of the reactor treating the LPD seems to be significantly lower compared to the systems treating reject water. In the

research by Jenicek et al. (2004), the system treating reject water using the nitritation/denitritation process was able to nitrify ammonia satisfactorily even at NLR levels exceeding 1.5 kg/(m^3 d) under comparable conditions. The chemical composition of the LPD (especially extremely high COD concentrations exceeding values usual for reject water multiple times) seems to be responsible for this observation. NiAR did not exceed 2% during all of Period 2, where the change of the pH control strategy made on day 61 (the target pH value changed from 7.0 to 6.5) did not have any measurable effect on this parameter (Fig. 4B).

3.3. Chemical composition of nitrified LPD

Considering the fact, that the application of nitrified LPD to soil (directly or after subsequent thermal thickening) is one of the most important expected applications of this material (see above), the chemical composition of the effluent from the reactor represents very important aspect of the treatment process. From this point of view, the nutrition of agricultural crops, potential toxicity of nitrified LPD towards crops as well as environment protection were also emphasised during this research together with minimization of nitrogen losses and other aspects mentioned above.

3.3.1. Nitrogen forms

Total nitrogen (N-tot) present in the LPD could be divided into TAN, N-NO_3^-, N-NO_2^- and organic nitrogen (N-org) forms. Nitrification of LPD results in significant changes of the representation of these forms. By this way, the risk of nitrogen losses, the agricultural value of LPD as well as potential environmental risks connected with its application to soil could be changed significantly after the nitrification process. The proportion of TAN, N-NO_3^-, N-NO_2^- and organic nitrogen (N-org) in N-tot in the effluent during selected phases of reactor operation were compared with the values characteristic for raw LPD serving as the influent for the nitrification reactor during this research (Table 3). Taking into consideration variable concentrations of N-tot in raw LPD (chapter 3.3.5), percentage expression was used. Additionally, the proportion of nitrogen present in the form of FA (N-FA) was evaluated with the aim to compare the risk of nitrogen losses during the storage and during the application of LPD to soil. Taking into consideration that N-FA represents part of TAN, it is not included in the sum of nitrogen forms creating N-tot.

Considering actual conditions prevailing in the reactor, the data gathered during Period 1 were divided into two separated phases in Table 3. Firstly, the phase ending on day 21 was evaluated. Then, the period starting on day 22 which was characterised by massive nitrite accumulation was separately assessed. Similarly, Period 2 was divided into parts lasting for days 1–47 and 68–90, respectively, taking into consideration the different pH values applied during these phases (see chapter 2.1). Taking into account unstable conditions between days 47 and 67 of Period 2 (see Figs. 3 and 4), this phase of the reactor operation was not included in Table 3.

As can be seen in Table 3, the nitrification process induced in the environment of LPD led to a radical decrease of the proportion of N-FA compared to raw LPD in which it represented 8.1% of N-tot. The lowest proportion of N-FA (0.007% on average) was definitely achieved on days 1–47 during Period 2 proving the conditions applied during this phase (with pH control value of 7.0 almost all the TAN was converted to nitrate) to be optimal from the point of view of minimization of nitrogen losses during the handling of LPD. In this case, the proportion of N-FA was decreased even more than 1000 times. Although several times higher proportions of N-FA (0.033% on average) were observed on days 68–90, during Period 2 (pH control of 6.5), these results seem to be still very positive. A lower pH did not enable a more significant increase of the proportion of N-FA although the actual TAN concentration increased rapidly in this phase compared to days 1–47 (Fig. 3A). Similarly, a low pH (Fig. 2A) resulted in low proportion of N-FA (0.05% on average) even on days 1–21 of Period 1, although TAN concentration reached up to 1250 mg/L in this phase of the reactor operation (Fig. 1A).

TAN, as well as N-NO_3^-, represent suitable forms of nitrogen from the point of view of plant nutrition. N-NO_3^- which is introduced into the LPD within the described technology is characteristic with higher mobility which seems to be more suitable compared to TAN from the point of view fast transport into the plants (Pilbeam, 2015). It is beneficial mainly from the point of view of the application of nitrified LPD during growing season.

With the aim to prevent eutrophication of natural water resources, the legislation limits the total amount of nitrogen that can be applied to soil with LPD as well as with other fertilizers while concrete values are adjusted to different regional conditions (e.g. European Commission, 1991) depending on the local extent of the risk of the contamination of natural water by nitrogen compounds. So, the dosages of nitrified LPD to the soil must meet the standards valid for the locality where it will be applied. The amount of specific chemical nitrogen forms (TAN, N-NO_3^- and N-NO_2^-) applied to land is usually not limited strictly within the legislation.

However, the application of nitrified LPD during non-growing season represents slightly higher environmental risk compared to the application of raw LPD by the reason of higher mobility of N-NO_3^- (Pilbeam, 2015) than TAN and its potential faster transfer into natural water resources.

N-NO_2^- represents an even significantly larger environmental risk compared to N-NO_3^-. It is toxic to plants where its concentration in soil water exceeding 0.9 mg/L was identified as the limit of the toxicity (Court et al., 1962; Singh et al., 2007). Thus, if we would like to apply nitrified LPD to soil (directly, after long-term storage or after thermal thickening), the presence of nitrite in the effluent should be minimized with the aim to prevent the exceeding of this toxic level. Taking into consideration very variable conditions prevailing at different localities on which nitrified LPD could be applied, it is very complicated to suggest objectively a maximum amount of nitrite which could be applied to soil without the risk of temporary exceeding this value after the application of nitrified LPD to soil at the locality. The variability of the physico-chemical properties of the soil (mainly the soil type and the humidity), the intensity of precipitations, the method of the soil management (particularly the depth of potential ploughing after the application of nitrified LPD) and many other factors are very important from this point of view. Taking into the consideration

Table 3
The proportion of the various nitrogen forms in the influent and effluent.

Nitrogen form (%)	Influent	Period 1 (days 1–21)	Period 1 (days 22–30)	Period 2 (days 1–47)	Period 2 (days 68–90)
TAN	88.1 ± 2.2	41.7 ± 4.8	37.1 ± 4.5	1.52 ± 1.31	18.48 ± 3.15
N-FA	8.1 ± 1.0	0.05 ± 0.02	1.49 ± 3.01	0.007 ± 0.007	0.033 ± 0.008
N-NO_3^-	1.20 ± 0.20	48.4 ± 4.1	22.9 ± 11.6	87.4 ± 1.5	70.5 ± 3.3
N-NO_2^-	0.08 ± 0.04	0.77 ± 0.73	32.0 ± 12.8	0.37 ± 0.38	0.31 ± 0.34
N-org	10.60 ± 1.30	8.50 ± 1.05	8.82 ± 0.63	8.20 ± 0.95	8.35 ± 1.30

these factors, maximum safe dose of $N\text{-}NO_2^-$ could be estimated in each concrete case. For example, if the LPD will be applied in the amount of 5 m^3/ha (corresponding with 5 L/m^2), seepage of the liquid to a depth of 0.2 m and the humidity of the soil corresponding to 30% will be expected, nitrite contained in 0.5 L of nitrified LPD will penetrate the soil characteristics by a total volume of ca. 200 L in which ca. 60 L of soil water will be present. Thus, if the concentration of $N\text{-}NO_2^-$ in nitrified LPD reaches 100 mg/L, 50 mg of $N\text{-}NO_2^-$ will be diluted into ca. 60 L resulting in an $N\text{-}NO_2^-$ concentration in soil water slightly higher than 0.8 mg/L. Thus, the concentration limit mentioned by Court et al. (1962) and Singh et al. (2007) will still not be exceeded. Naturally, this is only one concrete example, actual concentration of $N\text{-}NO_2^-$ in soil water will change significantly based on local conditions (see above). Additionally, the above presented calculation is very approximate, being only illustrative.

Positively, the nitrite represents a relatively unstable nitrogen form which could be relatively easily biochemically oxidized to nitrate within nitrification or reduced within denitrification (Painter, 1970). Taking into consideration the above presented discussion, it seems to be reasonable to perform additional research focused on the study of the dynamic of the migration and biochemical transformations of $N\text{-}NO_2^-$ in the soil after the application of nitrified LPD.

At all events, the conditions applied on days 22–30 of Period 1 seem to be very unsuitable from the point of view the presence of nitrite in the effluent. $N\text{-}NO_2^-$ represented 32.0% of N-tot on average at actual concentrations reaching up to 1022 mg/L in this phase of the reactor operation (Table 3, Fig 1A). On the contrary, values not higher than 0.4% on average at $N\text{-}NO_2^-$ concentrations below 30 mg/L were observed during the whole of Period 2 seeming to be safe from the point of view the toxicity of nitrite to plants even regardless of actual conditions.

The proportion of N-org decreased very poorly in the effluent from the reactor compared to the influent (Table 3) which is in accordance with the discussion presented in chapter 3.3.4.

3.3.2. Other nutrients

During this research the influence of the nitrification of LPD on the content of other basic nutrients (Ca, K, Mg, P and S) was also evaluated on a one-time basis. For this purpose, the samples of the raw LPD serving as the influent for the nitrification reactor and the effluent taken on day 32 of Period 2 were analysed. The results proved that the biological treatment of LPD in the nitrification reactor did not result in significant changes of the concentration of any particular nutrients (Table 4).

In accordance with Al Seadi et al. (2013), Kolář et al. (2008) or Chiumenti et al. (2013), a relatively high content of particular nutrients (especially K, S, P and Ca) was observed in dry matter (DM) of raw as well as nitrified LPD. Taking into consideration the liquid nature of LPD, the concentration of monitored nutrients expressed in mg/L is presented in Table 4. Thus, the concentration of nutrients in nitrified LPD could be easily compared to the concentrations of the different nitrogen forms presented in chapters 3.1.1 and 3.2.1.

3.3.3. Heavy metals and other risk elements

Actual legislation related to the application of digestate, LPD and separated solid phase of digestate to soil strictly defines the concentration limits of various heavy metals and other risk elements which should not be exceeded in the case of agricultural use of these materials. The samples of the influent and the effluent of the nitrification reactors taken on day 32 of Period 2 were analysed for this purpose. The results were compared with the legislation limits valid in various countries which are presented in mg of risk elements contained in 1 kg of DM (Table 5). Taking into consideration that the LPD used for this study was collected at a biogas plant operated in the Czech Republic where the application of LPD to soil in this country is also expected, the limits valid for the Czech Republic were primarily discussed. Also the limits valid for other selected countries (UK and Germany) are presented in Table 5. Additionally, a non-binding proposal for the EU aiming to unify the requirements for European countries is mentioned in this table.

The results proved that the treatment of LPD in the nitrification reactor did not result in any significant changes in the concentration of evaluated risk elements. The samples of raw LPD (influent) as well as nitrified LPD (effluent) were within the limits defined by the legislation valid in various countries. Taking into consideration the data presented in Table 5, the potential subsequent thermal thickening (Chiumenti et al., 2013) leading to the reduction of the volume several times (and simultaneously adequately increasing the concentration of risk elements) should not lead to the exceeding of these limits.

3.3.4. Organic matter

A very low COD removal efficiency reaching 22.6% on average was recorded in the experiments. These results indicate poor biological degradability of organic compounds contained in the LPD under the conditions applied. In accordance with this, N-org were not converted in larger extent into inorganic forms of nitrogen (Table 3). The fact that the biologically degradable organic matter present in the substrate for biogas production was decomposed already in the anaerobic digester before the LPD was originated seems to be responsible for these findings. Taking into consideration the facts presented above, a similar structure of organic compounds present in the nitrified LPD compared to raw LPD could be expected.

3.3.5. Variability of the properties of nitrified LPD

The results related to the chemical composition of nitrified LPD cannot be generalized for potential treatment of LPD originated under different conditions. The particular parameters of nitrified LPD could fluctuate significantly according to local conditions prevailing on the given biogas plant. Mainly the representation of different materials in the substrate for biogas production and the technological arrangement of anaerobic digestion will dramatically influence the properties of raw LPD including N-tot concentration, the content of other nutrients as well as the potential presence of risk elements or other contaminants. On the other hand, the data presented in Table 3 seem to be useful from the point of view of primary prediction of the representation of particular nitrogen forms under conditions analogical with this research. In accor-

Table 4
The content of the nutrients in the influent and effluent.

	Influent (mg/kg DM)	Influent (mg/L)	Effluent (mg/kg DM)	Effluent (mg/L)
Ca	6590 ± 380	264 ± 15	6450 ± 410	258 ± 16
K	103,000 ± 4000	3730 ± 150	89,300 ± 7200	3570 ± 290
Mg	461 ± 123	18.4 ± 4.9	510 ± 150	20.4 ± 6.0
P	6720 ± 240	269 ± 9	6120 ± 230	245 ± 9
S	9970 ± 520	399 ± 21	10100.0 ± 600	403 ± 22

Table 5
The content of the selected risk elements in the influent and effluent.

	Influent (mg/kg DM)	Effluent (mg/kg DM)	Czech Republic[a]	UK[b]	Germany[c]	EU proposal[d]
Cd	0.13 ± 0.06	0.11 ± 0.04	2	1.5	1.5	1.5
Pb	2.54 ± 1.24	2.47 ± 1.54	100	200	150	120
Hg	0.08 ± 0.006	0.09 ± 0.01	1.0	1.0	1.0	1.0
As	1.95 ± 0.39	2.02 ± 0.73	20	–	–	20
Cr	3.70 ± 1.63	4.18 ± 3.17	100	100	100	100
Cu	39.9 ± 18.9	34.9 ± 11.9	250	200	100	250
Mo	1.62 ± 0.65	1.46 ± 0.48	20	–	–	–
Ni	7.72 ± 3.26	7.17 ± 2.98	50	50	50	50
Zn	101 ± 58	88 ± 42	1200	400	400	1200

[a] Ministry of Agriculture of the Czech Republic (2014).
[b] British Standards Institution (2010).
[c] Siebert (2008).
[d] Saveyn and Eder (2014).

dance with Saveyn and Eder (2014) and Tampio et al. (2016) who quantified risk elements in digestate originated on agricultural biogas plants, the content of heavy metals in nitrified LPD produced within our experiments fulfilled the requirements defined by the legislation (see chapter 3.3.3). On the other hand, the presence of such elements in the substrate for biogas production will result in higher concentrations in nitrified LPD (Al Seadi et al., 2013; Kupper et al., 2014). Similarly, potential risk of the presence of specific persistent organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) or polychlorinated biphenyls (PCBs) must be expected in the case of the contamination of the substrate (Al Seadi and Lukehurst, 2012).

3.4. Application of the results

In accordance with Botheju et al. (2010), it seems to be feasible to "stabilize" nitrogen contained in raw LPD using the nitrification process thanks to the pH decrease and nitrate production. Simultaneously, the nitrification of LPD in the system described within this paper seems to be applicable as the pre-treatment of LPD ensuring the minimization of NH_3 emission during subsequent thermal thickening (Chiumenti et al., 2013). This research also confirmed the possibility efficiently producing oxidized nitrogen forms usable as acceptors of electrons for autotrophic denitrification applied within biogas cleaning by hydrogen sulphide scrubbing and bio-oxidation of captured sulphides (Doğan et al., 2012; Pokorna et al., 2015; Pokorna and Zabranska, 2015). Our results proved that the oxidized nitrogen could be produced directly in the biogas plant using LPD originated as a by-product of the biogas plant operation. On the other hand, the above presented experiments have some limitations and the results produce further topics for subsequent research. Despite the fact that the possibility to successfully initiate and operate the nitrification process in the environment of LPD was proved within this research, stable conditions were not achieved for any extended period of reactor operation. This is caused by the fact that different parameters such as NLR and pH were changed significantly during the experiment with the aim to identify quickly basic properties of the system and to find out the reaction of the biomass to these changes. The text situated below aims to summarize the possibilities for the utilization of results for practical application of the tested system and particularly for the verification and improvements of its characteristics.

3.4.1. Suggestions for objective identification of maximum achievable nitrogen oxidation rate

During future experiments, it will be necessary to prevent simultaneous changes of several parameters within short periods of reactor operation. It seems to be necessary operate the reactor at a controlled and stable value of pH during long-term operation of the reactor. Based on the results of Period 2, the value of pH reaching 7.0 seems to be optimal from this point of view. Under such conditions, the NLR should be increased step by step from the values applied at the start of Period 1 and Period 2 (ca. 0.2 kg/(m^3 d)) to values approaching 0.60 kg/(m^3 d) which enabled the complete transformation of TAN to nitrate during Period 2. Progressive application of NLR values reaching 0.20, 0.30, 0.40, 0.50 and 0.60 kg/(m^3 d) is recommended where after each change of the NLR value, it will be necessary to operate the reactor under constant conditions for a period lasting for at least one month with the aim of stabilizing the system. Each observation indicating a disruption of the stability of the nitrification process (especially uncontrolled pH increases similar to the pH deviation during day 55 of Period 2) should immediately result in the reduction of the NLR to the level applied during the previous step of the gradual increase of NLR. Finally, the reactor should be operated for a period lasting at least three months under the maximum NLR level ensuring satisfactory operation of the system. By this way, the maximum achievable NOR of the tested system could be objectively quantified.

All the above mentioned experiments were performed using the LPD originated from one concrete biogas plant. However, the chemical composition (N-tot and TAN concentration, pH, COD value as well as other parameters) of LPD produced at different biogas plants could differ significantly because of the variable structure of the substrate used for biogas production as well as the variable technological conditions applied for anaerobic digestion (Al Seadi et al., 2013). Thus, the inhibition effect of FA and/or FNA could differ significantly during the treatment of LPD originating at different biogas plants. Consequently, the maximum achievable NOR and other basic characteristics of the system could be affected by this fact. Similarly, the maximum achievable NOR could be significantly influenced by the content of organic matter quantified by the COD value of each concrete sample of LPD. Therefore, during the start-up of the nitrification reactor treating the LPD, as well as during its regular operation, the actual conditions prevailing in the reactor must be strictly taken into the account.

3.4.2. Prevention of nitrite production during nitrification

The presence of nitrite in nitrified LPD which is planned to be applied to soil should be minimized (see chapter 3.3.1). Simultaneously, considering the oxidation stage of nitrogen in nitrite (III) and nitrate (V), the effectivity of nitrite used as an electron acceptor for biogas desulfurization is 1.67 times lower compared to nitrate which makes nitrate the preferred nitrification final product in this case.

The results of Period 2 clearly demonstrated that the NiAR as well as N-NO_2^- concentrations in the effluent from the nitrification reactor could be maintained at very low levels, preventing a toxic

effect to plants using a pH control of 7.00 ± 0.05 as well as 6.50 ± 0.05. Taking into consideration FNA and/or nitrite's strong inhibition effect towards NOB (Anthonisen et al., 1976; Buday et al., 1999), it seems to be necessary to prevent any temporary restriction of NOB activity inducing increases of $N\text{-}NO_2^-$ concentration in the nitrification reactor. Such situations seem to be highly risky from the point of view of secondary long-term inhibition of NOB as in the case of the operation of the reactor on days 22–30 of Period 1 (Fig. 1A) where significant changes of pH and NLR resulting in the limitation of NOB activity were characteristic for this phase of the reactor operation. Also temporary restriction of dissolved oxygen availability may result in massive long-term $N\text{-}NO_2^-$ accumulation (Pacek et al., 2015).

3.4.3. Maximization of nitrogen oxidation efficiency

Based on the results presented in chapters 3.1.3 and 3.2.3, the maximization of NOE and minimization of TAN concentration in the effluent could be achieved by a pH control at a level of 7.0. Using this method, up to 100% of the TAN was oxidized to nitrate where the TAN concentration did not exceed 60 mg/L. This is beneficial for the minimizing of ammonia emissions during the direct application of nitrified LPD to soil as well as during its long-term storage or thermal thickening (see chapter 3.3.1). Simultaneously, it is useful for subsequent autotrophic denitrification applied to biogas cleaning, because the maximum amount of oxidized nitrogen usable as an acceptor of electrons is produced.

On the other hand, a pH control of a lower value may represent a successful strategy from the point of view of minimization of nitrogen losses. Within this research, the representation of N-FA was dramatically reduced at pH value 6.5 although the actual concentration of TAN increased significantly compared to the operation of the reactor at a pH value reaching 7.0 (see chapter 3.3.1). Therefore, additional research aimed at the evaluation of the potential possibility of decreasing the cost for alkali supplying thanks to the operation of the reactor at lower pH values seems to be reasonable.

With the aim to prevent the thickening of LPD in the nitrification reactor taking place as the consequence of evaporation of water (see chapter 3.2.1), it seems to be reasonable to compensate the evaporation by the addition of an adequate amount of distilled water. For this purpose, continual monitoring of the intensity of the flow of the influent raw LPD as well as effluent nitrified LPD will be necessary. By this way, the volume of distilled water required per day will be determined as the difference of the volume of raw LPD incoming into the reactor per day and the volume of nitrified LPD escaping the reactor daily. This improvement of the system will result in the enhancement of the objectivity of the evaluation of its basic characteristic including NOE.

3.4.4. Potential problems resulting from the application of the nitrification of LPD on biogas plants

Regarding the long-term storage of nitrified LPD, the potential occurrence of heterotrophic denitrification using the rest of the organic matter contained in nitrified LPD as a substrate and from it a resulting loss of nitrogen in the form of dinitrogen gas cannot be excluded. In addition, the extent of the potential risk of alkalization of LPD as a consequence of the denitrification process (Painter, 1970) and subsequent increase in the intensity of NH_3 emission is also questionable. On the other hand, the biological degradability of the organic compounds contained in raw LPD seems to be very poor (see chapter 3.3.4). Additionally, the majority of the degradable organic matter will be decomposed in the nitrification reactor where extremely low concentration of biologically degradable COD could be expected in nitrified LPD. In any event, these aspects should be verified in the future.

The operation of the biological reactor applying nitrification for LPD treatment is essentially connected to energy consumption, particularly, for supplying the air into the reactor. Thus, the economic aspects connected to the energetic demands must be evaluated for each concrete biogas plant individually.

4. Conclusions

Nitrification could be advantageously used with the aim of minimizing nitrogen losses during the storage and/or the application of LPD as well as for the pre-treatment before thermal thickening of LPD. It also could be applied to the production of electron acceptors for the biological desulfurization of biogas. Despite the extremely high concentration of TAN and COD in LPD used for the experiment, the nitrification process with $N\text{-}NO_3^-$ as a final product began immediately after the start of the reactor operation. The pH control of 7.0 enabled the reactor to convert practically all TAN to nitrate decreasing the representation of volatile N-FA more than 1000 times compared to raw LPD, which is optimal for minimizing the nitrogen losses during storage application or thermal thickening of the nitrified LPD. Simultaneously, the high efficiency of the conversion of TAN to $N\text{-}NO_3^-$ is beneficial for the production of electron acceptors for the biological desulfurization of biogas. Uncontrolled decreases of pH to values lower than ca. 5.5, similar to other sudden changes of environmental conditions prevailing in the nitrification reactor seem to be a high risk with respect to potential FNA inhibition towards nitrifying bacteria resulting in nitrite accumulation in the effluent from the reactor. Although very promising results were gained during the experiments, additional research is recommended with the aim of verifying and improving the basic characteristics of the tested system taking into the consideration variable chemical composition of raw LPD.

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