



Factors affecting Nitrogen Transformations and Related Nitrous Oxide Emissions from Aerobically Treated Piggery Slurry

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A laboratory treatment system was designed to study the fate of nitrogen during aerobic treatment of pig slurry. Different aeration strategies, and more particularly the influence of residence time and aeration level, were manipulated. A series of six experiments was carried out to determine the nitrogen mass balance, including measurement of the gaseous nitrogen forms particularly ammonia and nitrous oxide. Further nitrogen transformations were examined during the subsequent anaerobic storage of aerated pig slurry at 7, 21 and 60 days. Aeration level and carbon content of raw slurry were identified as the main factors influencing nitrogen transformation during treatment. A high aeration level (2–4 mg O₂/l) and/or low carbon content (biological oxygen demand of the raw slurry ≈ 2 g/kg) resulted in nitrite accumulation (up to 33% of the total nitrogen content of the raw slurry) while a low level of aeration (redox potential = 0 mV_{Ag/AgCl}) and high carbon content (biological oxygen demand of the raw slurry ≈ 16 g/kg) led to simultaneous nitrification and denitrification which removed 66% of the total nitrogen in the raw slurry. Nitrous oxide emissions were observed in all treatments and represent up to 30% of the total nitrogen content of the raw slurry. Both nitrification and denitrification appear to be sources of nitrous oxide during the treatment. Further nitrous oxide emissions were recorded during subsequent storage, especially when the biological oxygen demand to NO_x⁻-N ratio was lower than 1.3. However, during closed storage experiments, the nitrous oxide emitted was dissolved into the slurry and finally reduced to di-nitrogen after 60 days of storage.

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1. Introduction

In France, aerobic treatment of surplus slurry is seen as necessary in order to protect water courses from nitrate pollution.¹ Aeration systems have been designed to remove nitrogen from the slurry as di-nitrogen gas, via nitrification and denitrification.²

Several parameters are known to control the behaviour of nitrogen (*e.g.* the level of aeration and the residence time). Indeed, nitrification has been shown to occur with dissolved oxygen concentration of above 1–2% of saturation³ and with a residence time of more than three days.⁴ Denitrification can occur when the dissolved oxygen concentration is less than 10–15% of saturation.⁵ As a consequence, simultaneous nitrification and denitrification are possible with dissolved oxygen concentration between 1 and 10% of saturation,

especially with a redox potential between 0 and –200 mV (standard hydrogen probe). For residence times of less than three days, the removal of nitrogen is solely due to emissions of ammonia. This emission can represent up to 40% of total nitrogen of raw slurry⁶ and is largely influenced by the aeration rate and the temperature.⁷ During the aeration process, nitrous oxide (N₂O) can also be emitted. Nitrous oxide is an important greenhouse gas which may lead to global warming and climate change, and is also implicated in stratospheric ozone depletion.^{8–10} On a molecular basis, N₂O has a global warming potential about 250 times that carbon dioxide (CO₂). Nitrous oxide in the atmosphere accounts for about 6% of the direct radiative forcing of the long-lived greenhouse gases. Agriculture is presently estimated to contribute from 65 to 80% of the total anthropogenic N₂O,¹⁰ which represent approximately 2 Tg N₂O-N/yr.

Table 1

Characteristics of the slurry used in each experiment. Values are expressed on a fresh weight bases (standard deviation shown in parantheses)

Tests	Chemical oxygen demand, g/kg	Biological oxygen demand, g/kg	Total nitrogen, g N/kg	Total ammoniacal nitrogen, g N/kg	Total solids, g/kg	Total suspended solids, g/kg	Volatile suspended solids, g/kg	Volatile fatty acids, g/kg
1	32.9 (-)	9.0 (-)	3.47 (0.04)	2.35 (0.02)	30.1 (0.4)	-	-	4.55 (-)
2	-	-	3.57 (0.12)	2.33 (0.09)	33.2 (0.5)	19.7 (0.4)	11.1 (0.3)	2.34 (-)
3	21.4 (-)	6.6 (-)	3.43 (0.07)	2.54 (0.06)	24.4 (0.8)	11.9 (0.7)	7.0 (0.5)	7.6 (-)
4	26.6 (1.9)	7.6 (0.6)	3.44 (0.03)	2.35 (0.02)	29.2 (0.7)	17.3 (0.6)	10.3 (0.4)	1.8 (-)
5	35.9 (3.2)	16.4 (3.2)	4.28 (0.07)	3.15 (0.08)	32.6 (0.5)	20.1 (0.4)	11.7 (0.3)	3.35 (-)
6	19.0 (2.1)	1.8 (0.1)	2.88 (0.01)	1.99 (0.02)	25.8 (0.8)	17.8 (1.1)	9.3 (0.5)	0.13 (-)

Up to 13% of the total slurry N content can be lost as N₂O during the aeration process.^{7,11} However, the influence of the residence time and dissolved oxygen concentration, the source of the N₂O (*e.g.* nitrification or denitrification) and the related biochemical mechanisms involved remain poorly understood.

Both nitrification and denitrification could be a possible source of nitrous oxide. During denitrification, dissolved oxygen, low pH or low carbon content will lead to incomplete denitrification and an increase in the N₂O emission.^{12,13} During nitrification, according to Yoshida and Alexander,¹⁴ N₂O is generated during nitrification from an intermediate ahead of nitrite and emission is promoted by high ammonium concentration, while Zheng *et al.*¹⁵ found that the production of N₂O is favoured by lower oxygen concentrations and shorter residence times and can be due to nitrite accumulation. Goreau *et al.*¹⁶ observed that *Nitrosomonas* was responsible for N₂O emission rather than *Nitrobacter* and spoke about the production of N₂O both during oxidation of hydroxylamine and as a by-product of the reduction of nitrite.

In this study, the effect of the aeration level, residence time and slurry composition on nitrogen transformation was investigated during continuous aeration of raw pig slurry in a laboratory treatment system. The aim was to determine the influence of these parameters on nitrogen behaviour, and more particularly on N₂O emission, during the aeration process and also during the following anaerobic storage.

2. Methods and procedures

2.1. The slurry

Pig slurry was collected from an experimental farm in Brittany (Caulnes Agricultural School, France). The

experiments were carried out on the liquid fraction of handled, screened (0.63 mm) slurry (to remove coarse material and stones). The liquid fraction used in all tests is called "raw slurry" in the following subsections. The raw slurry composition varied between experiments but was fairly constant through the duration of each. Its mean composition is given in Table 1.

2.2. Laboratory treatment system

The laboratory treatment system (*Fig. 1*) consisted of a 10 l glass reactor (1) (5 l working volume), a feed vessel of 5 l (2) and a discharge vessel of 5 l (3). The reactor was designed for continuous feeding with one peristaltic pump (4) and semi-continuous discharging (every 4 h) with a second peristaltic pump (5). Residence time was controlled by fixing the rate of feeding of raw slurry. The slurry was mixed in the reactor by a magnetic stirrer (6) and a flow rate of slurry recirculated from the bottom to the top of the vessel at a flow rate approximately of 0.3 m³/h by means of a third peristaltic pump (7). This flow of slurry resulted in mixing, aeration and foam control. Dissolved oxygen, pH and redox potential (8) were continuously monitored in the flow of slurry and recorded on a data logger (9). This data logger could be programmed with a set point (for dissolved oxygen or redox potential, as appropriate) to enable two solenoid valves (10) to switch and allow the entry of air (11) or di-nitrogen gas (12) into the system in order to control the aeration level.

The injection of air or di-nitrogen gas resulted in a constant gaseous flow rate which carried out the gas produced during anaerobic step as well as during aerobic step. The gas flow rate was controlled with two gas

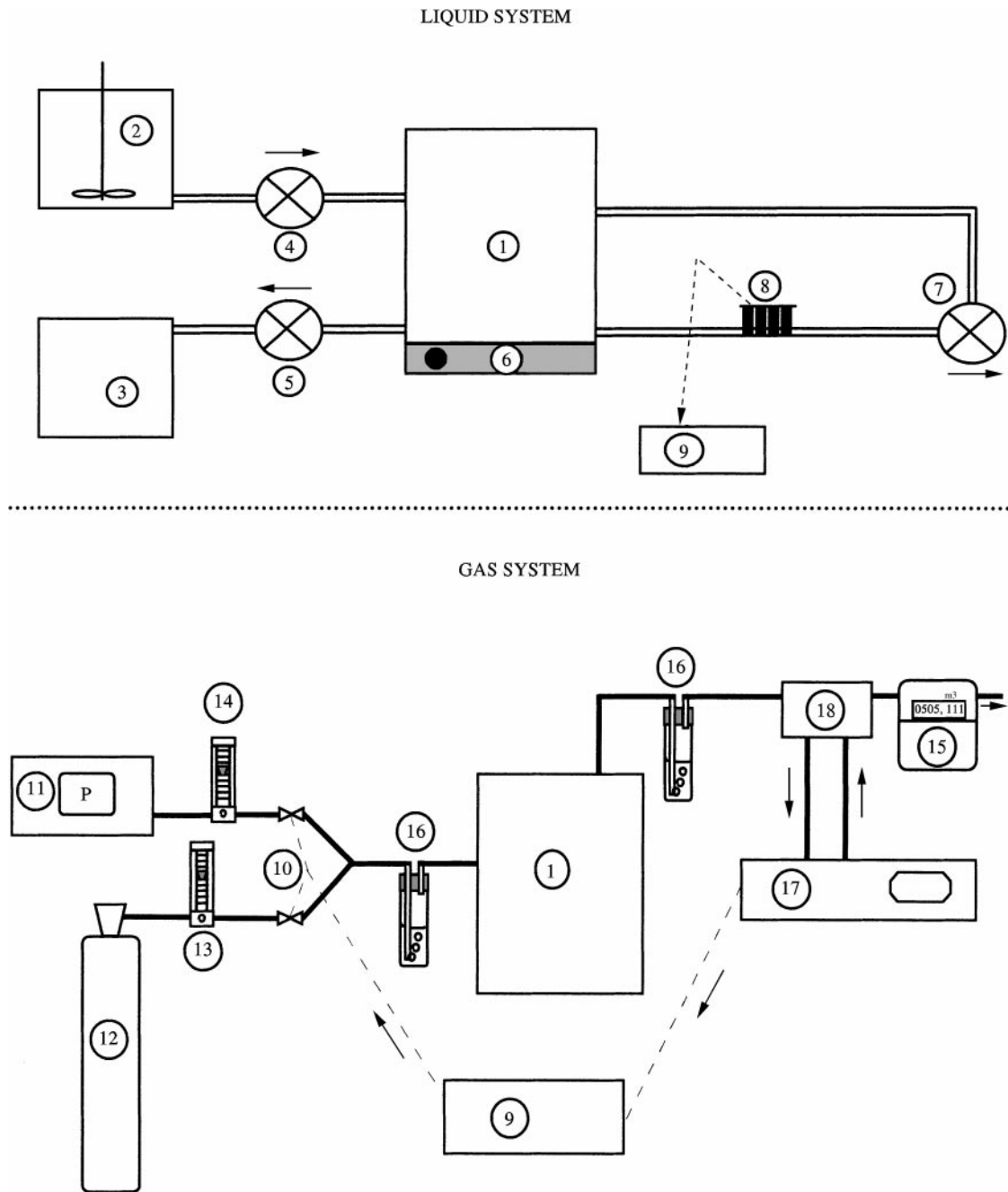


Fig. 1. Laboratory treatment system showing: aeration reactor (1), feed slurry vessel (2), discharged slurry vessel (3), feed slurry peristaltic pump (4), discharged slurry peristaltic pump (5), magnetic stirrer (6), recirculation peristaltic pump (7), pH, redox potential and dissolved oxygen sensor (8), data logger (9), solenoid valves (10), air pump (11), di-nitrogen gas compressor (12), gas flow meter (13 and 14), gas meter (15), acid trap for ammonia (16), infrared analyser (17), and buffer vessel (18)

flowmeters (13, 14) and the exhaust gas was quantified by a gas meter (15). At the reactor exits, gas was drawn through absorption flasks (16) containing 50 ml of sulphuric acid (0.2 M) for ammonia trapping and further determination of the acid traps. The exhausted gas was continuously monitored for carbon dioxide, nitrous ox-

ide and nitric oxide (17) determination (except trials 1-3 when samples of gases were taken by syringe daily from the headspace of the reactor for gas determination by chromatography).

In each experiment, analyses started after an elapsed time equal to three residence times. Representative

Table 2
Treatment regimes (standard deviation shown in parantheses)

Regime	Test	Residence time, day	O ₂ , mg/l	Redox, mV Ag/AgCl
Dissolved oxygen control	1	4.0	2.0 (0.15)	–
	2	4.2	4.0 (0.3)	–
	3	6.2	4.0 (0.2)	–
	4	6.6	3.8 (0.3)	–
Redox potential control	5	4.6	–	0 (15)
	6	5.1	–	– 48 (4)

samples of raw slurry and treated slurry were then taken daily throughout a two-week monitoring period and analysed for total solids (TS), total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), volatile fatty acids (VFA), total nitrogen (TN), total ammoniacal nitrogen (TAN), nitrate and nitrite. The values obtained were averaged over the monitoring period and the values indicated in this paper are the mean values of 15–20 individual analyses for TS, TSS, VSS, TN, TAN, nitrate and nitrite, three individual analyses for COD and BOD and one analysis for VFA. All input and output slurry quantities were recorded daily in order to establish mass balances during the monitoring period.

A total of six tests were carried out; these are summarized in Table 2. During the experiments, two main parameters were manipulated: residence time and aeration level. A short residence time of 4 days was chosen in order to allow nitrification. A longer residence time, 7 days, was used to evaluate its influence on nitrogen transformation. In the same way, different aeration level were evaluated. High dissolved oxygen concentrations, 2 mg O₂/l, and very high concentrations, 4 mg O₂/l, were used. Low aeration level experiments were performed with redox potential control.

2.3. Storage experiment

After each aeration treatment (except test 1), treated slurry (100 g) was stored in closed 250 ml flasks for two months (three replicates). The slurry in these flasks was analysed for TN, TAN, nitrate and nitrite after 7, 21, 60 days of storage. Nitrous oxide emission was determined by sampling the headspace gas by syringe followed by gas chromatography analysis.

A complementary experiment was undertaken to check the effect of carbon supply on denitrification efficiency during storage of aerated slurry used in test 6.

Aerated slurry (100 g) was stored with and without an external carbon source added (glucose, 8 g C/l) for 7 days (three replicates for each treatment). The slurry was analysed for nitrate and nitrite at the start and after 7 days of storage.

2.4. Chemical analyses

Total ammoniacal nitrogen was analysed by steam distillation using MgO followed by back titration of the boric acid distillates using sulphuric acid (0.1 M). Nitrate plus nitrite were determined by steam distillation using MgO and Devarda's alloy. Nitrate was determined by the same method with a prior removal of nitrite using sulphamic acid.¹⁷ Nitrite was then calculated by difference between these two values. Samples were digested using the Kjeldahl procedure for raw slurry (or Olesen modified procedure¹⁸ for treated slurry) and distilled with NaOH (30%) to determine the total nitrogen (TN). The remaining analyses, TS, TSS, VSS, COD, BOD, VFA followed standard methods.¹⁹

All nitrogen analyses were made within 1 h of sampling. For the other analyses, samples were kept at 4°C (storage < 2 days) or frozen (storage > 2 days). Concentration of CO₂, N₂O and NO in the airflow were continuously recorded by non-dispersion infrared analyzer for aeration sequences (except for tests 1–3). Determination of N₂O in the headspace of the storage vessels and aeration sequences 1–3 was made by gas chromatography (HP 5890 series II fitted with an electron capture detector operating at 300°C). The column was operated at 60°C with a backflush system.

3. Results

3.1. Overall aeration performances

Slurry temperature was recorded daily in the reactor and varied between 20 and 28°C for the six tests. Ambient temperature during the measurements was similar to the laboratory temperature, *i.e.* 20°C. For each test, the mean compositions of aerated slurries are shown in Table 3. The reduction of COD and TS varied between 28–42% and 4–19% respectively. Reductions in BOD were high and were very similar for all experiments (76–88%) except test 6 (52%); removal of VFAs was nearly complete in all tests ranging from 82 to 100%.

3.2. Nitrogen transformations during aeration

The fate of aerated slurry nitrogen, expressed as the percentage of the total nitrogen content of raw slurry, is

Table 3
Average composition of treated slurry (standard deviation shown in parantheses)

Test	Chemical oxygen demand, g/kg	Biological oxygen demand, g/kg	Total nitrogen, g N/kg	Total ammoniacal nitrogen, g N/kg	Nitrate, g N/kg	Nitrite, g N/kg	Total solids, g/kg	Total suspended solids, g/kg	Volatile suspended solids, g/kg	Volatile fatty acids, g/kg
1	19.1 (-)	2.0 (-)	2.80 (0.08)	0.58 (0.04)	0.04 (0.02)	1.09 (0.03)	27.1 (0.7)	-	-	0
2	-	-	2.84 (0.10)	0.43 (0.07)	0.08 (0.01)	1.17 (0.02)	30.6 (0.4)	19.1 (1.2)	13.6 (0.8)	0
3	-	1.4 (-)	2.60 (0.09)	0.64 (0.05)	0.19 (0.01)	1.03 (0.04)	19.9 (0.7)	9.5 (0.8)	6.6 (0.6)	0
4	17.2 (3.9)	0.9 (0.3)	2.91 (0.08)	0.73 (0.04)	0.28 (0.01)	1.02 (0.05)	25.1 (0.7)	17.0 (0.9)	10.5 (0.6)	0.32
5	24.3 (1.6)	4.0 (0.1)	1.46 (0.02)	0.14 (0.02)	0 (0.01)	0.02 (0.01)	28.1 (0.9)	19.3 (0.6)	13.4 (0.4)	0
6	13.7 (2.8)	0.9 (0.4)	2.43 (0.08)	0.76 (0.03)	0 (0.02)	0.90 (0.02)	24.8 (1.0)	17.4 (1.0)	9.1 (0.5)	0

presented in Fig. 2. Organic nitrogen content in the aerated slurry represented between 81 and 115% of the organic nitrogen content in the raw slurry. Organic nitrogen was estimated by calculating the difference between three individual analyses ($N\text{-org.} = TN - TAN - NO_x^-$ where NO_x^- are nitrogen oxides) and thus the combined error may be relatively large. However, these results seem to indicate few exchange between organic and mineral nitrogen content during aerobic treatment.

3.2.1. High aeration level

A large part of the initial TAN was oxidized (69–81%). This oxidation led to a large accumulation of nitrite: concentrations as high as 1.17 g N- NO_2^- /kg of slurry were found in the aerobic reactor, which represents

30–33% of the TN content of the raw slurry. Depending on aerating conditions, the nitrate pool varied from 1 to 8% of the TN of the raw slurry. The higher values were related to high oxygen supplied, long residence time and high dissolved oxygen concentration.

Despite the high level of aeration in tests 1–4, we measured nitrogen removal of 15–24% of the TN of the raw slurry. This removal of TN was mainly due to nitrous oxide emissions: 7.4–31.2% of the total nitrogen of the raw slurry was measured as N_2O in the gas. In spite of the high ammonium concentration in the reactor, no ammonia volatilization was observed. However, in test 4, we detected small quantities of NO which represented nearly 1% of the total nitrogen content of raw slurry. For these tests (1–4), mass balance including nitrous oxide and ammonia emissions varied between 93 and 107% of the nitrogen content of the raw slurry. These results seem to indicate a production of di-nitrogen very low and probably nil.

3.2.2. Low aeration level

Under low aeration conditions, an oxidation of 95% of the TAN was observed (e.g. test 5). The simultaneous denitrification resulted in 66% removal of the total nitrogen content of the raw slurry. Nitrous oxide was emitted and represented 37% of the total nitrogen removed. No ammonia and nitric oxide emissions were measured in this experiment.

In test 6, in spite of a lower aeration rate than in test 5, denitrification did not occur. Indeed, an incomplete oxidation of ammonium (62%) and a nitrite accumulation (up to 0.9 g N- NO_2^- /kg of slurry) were observed. The low removal of nitrogen (18%) was mainly due to emissions of nitrous oxide and nitric oxide.

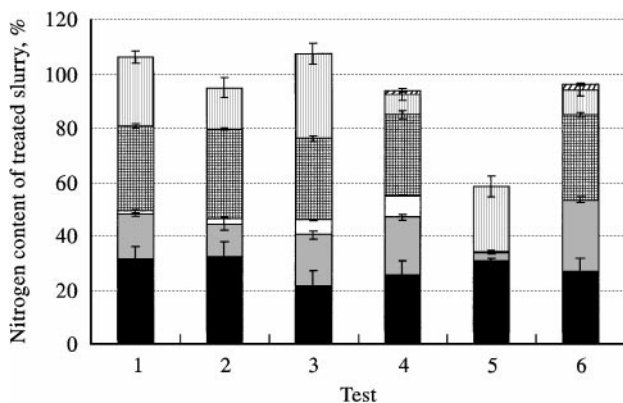


Fig. 2. The fate of nitrogen during aeration of pig slurry expressed as the percentage of the total nitrogen of the raw slurry: ■, organic nitrogen; ■, ammonium; □, nitrate; ▨, nitrite; ▤, nitrous oxide gas; ▥, nitric oxide gas; vertical line indicates \pm standard deviation

3.3. Nitrogen transformation during storage of aerated slurry

Nitrogen transformation were monitored during the storage period following five of the aerobic treatments (tests 2–6). No significant changes in the organic nitrogen content were observed after one week of storage (Fig. 3). However, after two months of storage, between 9.7 and 38.3% of organic nitrogen was mineralized to NH_4^+ . This mineralization led, in test 5, to an increase in the TAN content from 170 mg N/kg of slurry after aeration to 500 mg N/kg of slurry after 2 months of storage.

Figure 4 shows the rate of denitrification after 7, 21 and 60 days of storage. This rate was largely influenced by the carbon availability (expressed as the BOD to NO_x^- -N ratio) in the treated slurry. A low BOD to NO_x^- -N ratio (< 1.4) led to incomplete denitrification after 7 days of storage (NO_x^- -N removal varied between 14 and 77%). Nevertheless, in all cases, total denitrification was obtained after 60 days of storage (> 90%).

Analysis of the flask headspace gases after 7, 21 and 60 days of storage showed significant emissions of N_2O (Fig. 5). After 7 days, a BOD to NO_x^- -N ratio of greater than 1.3 led to a N_2O production of less than 9% of total nitrogen losses, whilst a ratio between 0.7 and 1.1, resulted in N_2O emissions representing between 27 and 70% of the total losses. The N_2O concentration found in the headspace of the flasks decreased with time so that after 60 days, N_2O concentrations were close to zero.

4. Discussion

4.1. Degradation of carbonaceous compounds

The percentage removal of carbonaceous compounds during the aerobic treatment of these slurries are consistent with results from other workers^{5,20,21} except for test

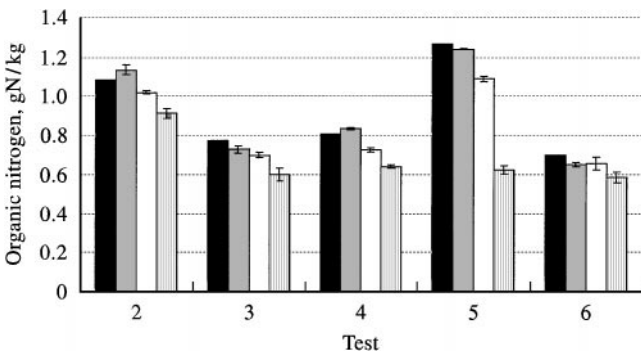


Fig. 3. The fate of organic nitrogen of aerated slurry during storage after: ■, 0 days of storage; ▒, 7 days; □, 21 days and ▨, 60 days; vertical line indicates ± standard deviation

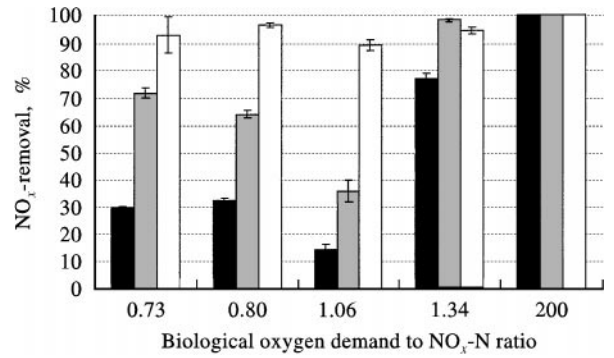


Fig. 4. Percentage of denitrification during storage of treated pig slurry after: ■, 7 days of storage; ▒, 21 days and □, 60 days; vertical line indicates ± standard deviation; NO_x^- , nitrogen oxides

6 where the differences could be explained by the low concentration of carbonaceous compounds in the raw slurry used in this experiment. As indicated by Smith and Evans,⁵ the carbon content reduction was unaffected by dissolved oxygen concentration in the studied range.

4.2. Organic nitrogen transformations

The conservation of the organic nitrogen during aerobic treatment indicate that the likely mineralization of the organic nitrogen is approximately equal to the concurrent immobilization of ammonium.²²

As observed in a previous experiment during anaerobic storage of raw pig slurry,²³ a decay of organic nitrogen fraction was measured concurrently with the increase in the ammoniacal pool during the storage of the aerated pig slurry (2 months). This result indicates that, even if a large part of ammoniacal nitrogen is removed during aerobic treatment, the aerated slurry is not fully free from nitrogen and it may still supply mineral

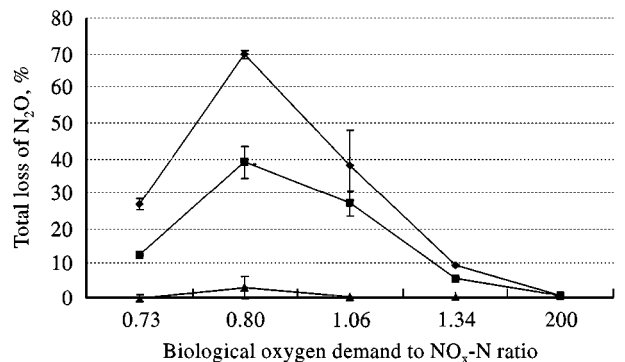


Fig. 5. Total loss of N_2O expressed as the percentage of nitrogen oxides (NO_x^-) removed during storage of treated pig slurry after: ◆, 7 days of storage; ■, 21 days and ▲, 60 days; vertical line indicates ± standard deviation

nitrogen at the time of landspreading, particularly following a long storage period.

4.3. Ammoniacal nitrogen transformations

4.3.1. Nitrification

In all tests, as expected from previous knowledge,^{3,4,6} a level of aeration above 1–2% of oxygen saturation and residence times greater than three days enabled nitrification to occur. However, depending on the conditions, an oxidation of the ammonium from 62 to 95% was observed. A residence time of 4 days and a dissolved oxygen concentration of 2 mg O₂/l (test 1) led to an incomplete oxidation of ammonium and to a nitrite accumulation. The increase of dissolved oxygen concentration to 4 mg O₂/l (test 2) and an increase of residence time to 6–7 days (tests 3 and 4) did not result in complete nitrification. According to these results, the residence time or oxygen supplied are not the sole factors limiting ammonium and nitrite oxidation. The differential toxicities of “free ammonia” and “free nitrous acid” are often described as parameters that inhibit nitrification and are responsible for nitrite accumulation. Indeed, *nitrobacter* (bacteria responsible for the oxidation of ammonium to nitrite) are more susceptible to environmental stress than *nitrosomonas* (bacteria responsible for the oxidation of nitrite to nitrate). Anthonisen *et al.*²⁴ indicated that an ammonia (NH₃) concentration of 0.1–1.0 mg N/l and a nitrous acid (HNO₂) concentration of 0.2–2.8 mg N/l inhibit *nitrobacter* while the corresponding NH₃ concentration inhibiting *nitrosomonas* is comprised between 10 and 150 mg N/l.

The amount of NH₃ and HNO₂ calculated from pH, TAN and nitrite concentration in the reactor by using Eqns (1) and (2), respectively, are presented in Table 4.

$$NH_3 = \frac{TAN}{(1 + 10^{pK_{a_1} - pH})} \quad (1)$$

$$HNO_2 = \frac{NO_2^- 10^{pK_{a_2} - pH}}{(1 + 10^{pK_{a_2} - pH})} \quad (2)$$

where the value of the exponent pK_{a₁} is 9.25 and that of pK_{a₂} is 3.3.²⁴

In test 5, although the calculated concentration of NH₃ (7.08 mg N/kg) was higher than in tests 1–4, a full oxidation of ammonium was obtained (except a small part indicated as a non biodegradable pool by Evans and Smith⁶). This result indicates that, as previously observed by Blouin *et al.*,²⁵ *Nitrosomonas* may be active in high NH₃ concentrations. According to these results, *Nitrosomonas* inhibition seems to be due to HNO₂ rather than NH₃. Also, a full oxidation of ammonium was obtained only when nitrite were removed.

Table 4
Calculated NH₃ and HNO₂ concentrations in the reactor expressed as mg N/kg

Test	1	2	3	4	5	6
HNO ₂ , mg N/kg	0.29	0.69	0.5	0.38	0	0.18
NH ₃ , mg N/kg	2.71	0.93	1.63	2.48	7.08	4.75

4.3.2. Denitrification

Full oxidation of ammonium and high nitrogen removal could be obtained with nitrite reduction by denitrification. As indicated by others workers,³ simultaneous nitrification and denitrification can occur with low dissolved oxygen concentration (0.1–0.2 mg O₂/l). In test 5, these conditions led to a pH close to neutrality, avoiding nitrite accumulation and, in consequence, preventing *Nitrosomonas* inhibition. However, in this test, NH₃ concentration calculated from Eq. (1) (7.08 mg N/kg) was greater than concentrations found in tests 1–4 and *Nitrobacter* were probably inhibited. Consequently, nitrite was probably directly denitrified without oxidation to nitrate.²⁶

Even if a low aeration level is maintained in the reactor, denitrification is not always observed. Indeed, an aeration level lower in test 6 than in test 5 did not result in nitrite removal, suggesting that a low aeration level during treatment is not sufficient on its own to lead to denitrification. Differences observed between tests 5 and 6 seem to be due, mainly, to the raw slurry composition used in each experiment. The raw slurry used in trial 6 had a very low COD and BOD (19.0 and 1.8 g O₂/kg respectively) whilst the slurry used in test 5 had a COD and BOD of 35.9 and 16.4 g O₂/kg, respectively. This led to a BOD in the reactor equal to 4 g O₂/kg for test 5 and 0.9 for test 6. The low carbon content in test 6 may have inhibited denitrification. The complementary experiment undertaken to check the effect of carbon supply on denitrification efficiency during storage of aerated slurry used in test 6 (when nitrite accumulation was observed) showed a complete denitrification (96 ± 1%) when glucose was added and an incomplete denitrification (43.5 ± 2%) without external carbon supply. Hence, the low carbon content directly limited the nitrite removal.

4.4. Nitrous oxide emissions

Mass balance found in tests 1–4 and 6 demonstrate a low nitrogen removal, mainly as N₂O, and a production of di-nitrogen close to zero. These results seem to indicate that, during these tests, denitrification was

completely inhibited and nitrification was probably the main source of N_2O . Ammonium concentration, residence time or oxygen concentration did not significantly influence N_2O production. The production of N_2O was probably due to nitrite accumulation (0.9–1.2 g N/kg) observed in these tests.^{15,16} Although there was no nitrite accumulation in test 5, N_2O emissions were measured. If it is assumed that N_2O emissions were due to nitrite accumulation during nitrification, results found in test 5 (nitrous oxide emissions without nitrite accumulation) indicated that denitrification is also a source of N_2O . Both nitrification and denitrification seem clearly to be sources of N_2O . However, further experiments should be carried out in order to confirm these hypothesis.

Aerobic treatment of pig slurry led in some cases to high concentrations of nitrite. Nitrogen transformation could then continue during storage. Bernet *et al.*¹² indicated that a total organic carbon to NO_x^- -N ratio of 3.4:1 for non-treated slurry resulted in denitrification without N_2O production. In the same way, this work showed that a biological oxygen demand to NO_x^- -N ratio of lower than 1.34:1 did not lead to denitrification without N_2O production.

The flask used for storage experiments were closed and the emitted gas (as nitrous oxide) was in contact with the slurry during storage. The decrease of the N_2O concentration in the headspace of the flasks with time indicate that N_2O was dissolved again and finally reduce to di-nitrogen (N_2) during storage. It would appear that a long period of contact between the N_2O gas and the slurry, and the production of available carbon by anaerobic digestion leads to complete denitrification of N_2O .

5. Further work

The use of ^{15}N -labelled nitrogen would be an appropriate method to confirm that both nitrification and denitrification are sources of N_2O during aerobic treatment of pig slurry. In the same way, ^{15}N labelled nitrogen would allow to highlight the probably concurrent immobilization and mineralization of nitrogen during aeration.²³

In this study, all conditions tested during continuous aerobic treatment lead to N_2O emissions. Also, other aeration strategies would be studied, as intermittent aeration or aeration using two separate tanks (aerobic tank for nitrification and anaerobic tank for denitrification) where conditions seems more appropriate for the two biochemical processes (nitrification and denitrification). Indeed, this aeration strategies could avoid nitrite accumulation during nitrification and nitrous oxide reductase inhibition by dissolved oxygen or low carbon content during denitrification.

6. Conclusions

1. Nitrogen transformations during continuous aerobic treatment of pig slurry depend largely on aeration level and raw slurry composition. Inhibition of denitrification by high levels of aeration or low carbon content led systematically (for the range of residences time tested) to incomplete ammonium oxidation, nitrite accumulation and a low nitrogen removal. A full oxidation of ammonium (95%) and high nitrogen removal (66%) could be obtained using simultaneous nitrification and denitrification promoted by a low aeration level and raw slurry with a sufficient carbon content.
2. The results found in this study confirm the production of nitrous oxide during continuous aerobic treatment of pig slurry. Both nitrification and denitrification seem to be sources of N_2O . Accumulation of nitrite promotes N_2O emissions during nitrification while oxygen dissolved lead to incomplete denitrification and N_2O emissions.
3. Nitrogen transformations continue during storage of aerated slurry. Indeed, mineralization of the organic nitrogen, denitrification of oxidized nitrogen forms and N_2O emissions were observed during the storage of aerated slurry. According to these results, it seems important to include the subsequent anaerobic storage of aerated slurry in the treatment evaluation.

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References

- 1 **EEC/91/676**, Protection of waters against pollution caused by nitrates from agricultural sources. O.J. N L 375, 31.12. 1991 p.1
- 2 **Goriaux-Perrais M I; Carpiér H** Le traitement des lisiers par voie biologique. Suivi de cinq filières en Bretagne (The biological treatment of slurry. A survey of five process options in Brittany). Congrès GRUTTEE, 19–20 mars 1997, 21pp
- 3 **Burton C H** A review of the strategies in the aerobic treatment of pig slurry: purpose, theory and method. Journal of Agricultural Engineering Research, 1992, **53**, 249–272
- 4 **Evans M R; Smith M P W; Deans E A; Svoboda I F; Thacker FE** Nitrogen and aerobic treatment of slurry. Agricultural Wastes, 1986, **15**, 205–213
- 5 **Smith M P W; Evans M R** The effects of low dissolved oxygen tension during the aerobic treatment of piggery slurry in completely mixed reactors. Journal of Applied Bacteriology, 1982, **53**, 117–126

- ⁶ **Evans M R; Smith M P W** Treatment of farm animal wastes. *Journal of Applied Bacteriology Symposium Supplement*, 1986, 27S–41S
- ⁷ **Burton C H; Sneath R W; Farrent J W** The effect of continuous aerobic treatment on the fate of the nitrogen in piggery slurry. In: *Nitrogen Flow in Pig Production and Environmental Consequences. Symposium Proceedings* (Versteegen M V A; Den Hartog L A; van Kempen G J M; Metz J H M eds), Wageningen, 8–11 June 1993, EAAP publ. no. 69, 404–409
- ⁸ **Crutzen P J** Atmospheric chemical processes for the oxides of nitrogen including nitrous oxide. In: *Denitrification, Nitrification and Atmospheric Nitrous Oxide* (Delwiche C C ed.), pp. 17–45. New York: Wiley
- ⁹ **Kaplan W A; Wofsky S C** The biogeochemistry of nitrous oxide: a review. *Advances in Aquatic Microbiology*, 1985, 3, 181–206
- ¹⁰ **IPCC**, *Climate Change* (Houghton J T; Jenkins G J; Ephraums J J; Ephraums J J; Varney S K eds), Cambridge: Cambridge University Press, 1990
- ¹¹ **Willers H C; Derikx P J L; Ten Have P J W; Vijn T K** Emission of ammonia and nitrous oxide from aerobic treatment of veal calf slurry. *Journal of Agricultural Engineering Research*, 1996, 63, 345–352
- ¹² **Bernet N; Delgenes N; Moletta R** Denitrification by anaerobic sludge in piggery wastewater. *Environmental Technology*, 1996, 17, 293–300
- ¹³ **Hanaki K; Hong Z; Matsuo T** Production of nitrous oxide gas during denitrification of wastewater. *Water Science and Technology*, 1992, 26, 1027–1036
- ¹⁴ **Yoshida T; Alexander M** Nitrous oxide formation by *Nitrosomonas europaea* and heterotrophic microorganisms. *Soil Science Society of America Proceedings*, 1970, 34, 880–882
- ¹⁵ **Zheng H; Hanaki K; Matsuo T** Production of nitrous oxide gas during nitrification of wastewater. *Water Science and Technology*, 1994, 30, 133–141
- ¹⁶ **Goreau T G; Kaplan W A; Wofsky S C; McElroy M B; Valois F W; Watson S W** Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. *Applied and Environmental Microbiology*, 1980, 40, 526–532
- ¹⁷ **Bremner J M; Keeney D R** Steam distillation methods for determination of ammonium, nitrate and nitrite. *Analytica Chimica Acta*, 1965, 32, 485–495.
- ¹⁸ **Dimmock S J; Martinez J** Nitrite plus nitrate recoveries in piggery slurry by direct distillation and modified Kjeldahl methods. *Bioresource Technology*, 1994, 48, 21–24
- ¹⁹ **APHA, AWWA, WEF** *Standard Methods for the Examination of Water and Wastewater*, 18th edn. (Greenberg A E; Clesceri L S; Eaton A D eds), Washington, DC, USA, 1992
- ²⁰ **Evans M R; Deans E A; Hissett R; Smith M P W; Svoboda I F; Thacker F E** The effect of temperature and residence time on aerobic treatment of piggery slurry; degradation of carbonaceous compounds. *Agricultural Wastes*, 1983, 5, 25–36
- ²¹ **Williams A G; Shaw M; Selviah C M; Cumby R J** The oxygen requirements for deodorizing pig slurry by aerobic treatment. *Journal of Agricultural Engineering Research*, 1989, 43, 291–311
- ²² **Oleszkiewicz J A** Nitrogen transformations in an aerated lagoon treating piggery wastes. *Agricultural Wastes*, 1986, 16, 171–181
- ²³ **Béline F; Martinez J; Marol C; Guiraud G** Nitrogen transformations during anaerobically stored ^{15}N -labelled pig slurry. *Bioresource Technology*, 1998, 64, 83–88
- ²⁴ **Anthonisen AC; Loehr R C; Prakasam T B S; Srinath E G** Inhibition of nitrification by ammonia and nitrous acid. *Journal of Water Pollution Control Federation*, 1976, 48, 835–852
- ²⁵ **Blouin M; Bisaillon J G; Beaudet R; Ishaque M** Nitrification of swine waste. *Canadian Journal of Microbiology*, 1990, 36, 273–278
- ²⁶ **Turk O; Mavinic D S** Preliminary assessment of a shortcut in nitrogen removal from wastewater. *Canadian Journal of Civil Engineering*, 1986, 13, 600–605