New anaerobic process of nitrogen removal

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Water Science & Technology **Vol 54 No 8 pp 163–170** © IWA Publishing 2006

Abstract This paper reports on successful laboratory testing of a new nitrogen removal process called DEAMOX (DEnitrifying AMmonium OXidation) for the treatment of strong nitrogenous wastewater such as baker's yeast effluent. The concept of this process combines the recently discovered ANAMMOX (ANaerobic AMMonium OXidation) reaction with autotrophic denitrifying conditions using sulfide as an electron donor for the production of nitrite within an anaerobic biofilm. The achieved results with a nitrogen loading rate of higher than 1, 000 mg/L/d and nitrogen removal of around 90% look very promising because they exceed (by 9–18 times) the corresponding nitrogen removal rates of conventional activated sludge systems. The paper describes also some characteristics of DEAMOX sludge, as well as the preliminary results of its microbiological characterization.

Keywords ANAMMOX; autotrophic denitrification; DEAMOX; nitrogen removal

Introduction

Many industries strive to reduce the emission of nitrogen compounds (ammonia, nitrate, etc.) to surface and ground waters. The classical microbial process of nitrification combined with heterotrophic denitrification is the most widely used method for nitrogen control in wastewater treatment worldwide. Since the majority of are existing activated sludge systems are not designed for denitrification or are already overloaded with nitrogen, municipal wastewater treatment plants frequently place very strict ammonia limits for discharge of non-domestic effluents into their sewerage. In this situation, a dedicated nitrogen removal from strong nitrogenous wastewater (food industry effluents, sludge liquor, manure flushings, etc.) received primary attention during the past decade; and a number of new processes based on nitritation, anaerobic ammonia oxidation, de-ammonification, etc., have appeared in this domain of wastewater treatment (Kartal *et al.*, 2004). From a microbiological point of view, these processes are based mainly on the activity of chemolithoautotrophic bacteria (compared to conventional processes with heterotrophic denitrifiers); from a technological point of view, they are focused on saving energy and electron donor, i.e. cost-efficiency. The most pioneering process of this type is the so-called ANAMMOX, in which ammonia is oxidized to N_2 with nitrite serving as an electron acceptor under anaerobic conditions. To generate nitrite, a special process called SHARON was developed. However, the combination SHARON–ANAMMOX requires a very advanced and expensive process control on both stages, which may represent a burden for application as on-site treatment in conventional industries.

To overcome these limitations, a new process called DEAMOX (DEnitrifying AMmonium OXidation) was proposed recently (Mulder, 2004) to realize the anammox process under autotrophic denitrifying conditions. The principal flow diagram of this process, as well as the major biochemical reactions involved, are given in Figure 1.

doi: 10.2166/wst.2006.729

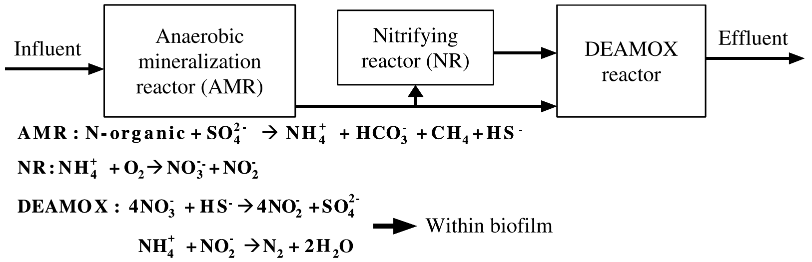

Figure 1 Flow diagram of the DEAMOX-process and major biochemical reactions involved

The essential distinguishing characteristics of this innovative process compared to the current anammox applications are the following: (a) nitrite is produced mainly from nitrate using sulfide as an electron donor; and (b) the DEAMOX-reactor is partially fed directly with anaerobic effluent from the pre-treatment (nitrogen mineralization) step; the distribution ratio of anaerobic/aerobic flows is determined by the composition of the wastewater, especially by the electron donor concentrations (sulfide, ammonia).

The DEAMOX process configuration has several major advantages, which are summarized below: (a) no complex process control is required for the production of nitrite; (b) the denitrifying conditions in the DEAMOX reactor will enhance the growth of granules stimulating the development of the anammox process; and (c) the absence of high nitrite levels, which may be toxic, reactive and results in the unwanted emission of NO_x-gases, which are greenhouse gases. This paper reports about lab testing of the DEAMOX process for the treatment of baker's yeast effluent (strong nitrogenous wastewater). Some microbiological characteristics of the DEAMOX sludge are also presented.

Materials and methods

Wastewater

Baker's yeast wastewater was simulated by tap water dilution of the cultivation medium from the first yeast separation process (CM-1S), representing the major and the strongest stream from yeast factories. The variation of some characteristics of CM-1S taken from a Moscow baker's yeast factory during 2004–2005 and used in this study are presented below (mg/L, except pH): total COD–17,900–31,100; soluble COD–15,000–26,600; total N–993–1,651; total P–13–78; sulfate–682–3,028; pH–4.0–5.7.

Anaerobic mineralization reactor

The laboratory UASB reactor (rectangular cross-section 31.7 cm^2, height 80 cm, total working volume 2.53 L, no recycle) made from transparent plastic was kept in the thermostat (under 35 ± 1 °C) and seeded with granular sludge from the full-scale EGSB reactor treating brewery wastewater (Efes-Moscow).

Nitrifying reactors

In the beginning, the biofilter (rectangular cross-section 31.9 cm^2, height 75 cm; total working volume 1.9 L) made from transparent plastics and packed by 0.5–2 cm fraction of road metal was used for this purpose. However, due to unwanted nitrogen losses (see below), this biofilter was replaced in March 2005 by a laboratory activated sludge reactor (rectangular cross-section 283 cm^2, height 12 cm; total working volume 3.42 L) with internal settler. Both reactors, working under the ambient temperature of the laboratory (20 ± 3 °C), were fed by anaerobic effluents from the UASB reactor (~50% flow) under a continuous supply of air. Electronic sensors were inserted in the middle of both reactors for online monitoring of soluble oxygen, the concentration of which was kept as high as possible (to achieve full nitrification of ammonia to nitrate) during all experimental periods, except those where lower concentrations of soluble oxygen were maintained to stimulate nitrite production. The nitrifying sludge from a Moscow sewage treatment plant was used as a seed. The excess of sludge was periodically withdrawn from the both reactors.

DEAMOX-reactors

Two laboratory UASB-like reactors made from transparent plastic and equipped with effluent recycle (to ensure adequate mixing) were used. The bigger one (rectangular cross-section 33.1 cm^2, height 7.7 cm, total working volume 2.53 L) was applied during start-up of the DEAMOX process, whereas the smaller one (rectangular cross-section 21.1 cm^2, height 46 cm, total working volume 0.97 L) during application of the highest loadings (close to the end of testing). Anaerobic effluents were fed in the bottom of the DEAMOX reactors, while nitrified effluents were fed to the recirculation line of these reactors. The reactors were kept in the thermostat (under 35 ± 1 °C). Since the ANAMMOX sludge was not available in our laboratory before the start of testing, the bigger DEAMOX reactor was seeded with a mixture of anaerobic granular sludge from the full-scale EGSB reactor treating brewery wastewater (Efes-Moscow) and the mature denitrifying activated sludge from a pilot installation at Kur'yanovskaya sewage treatment plant (Moscow). It was hoped that the ANAMMOX bacteria could survive in such environments and would further develop under the imposing of selection pressure in this reactor. For this, this reactor was fed initially for 6 months with synthetic medium containing ammonia and nitrite, as described by Imajo *et al.* (2004). When the ANAMMOX activity became noticeable, the reactor was switched on to the testing of the DEAMOX process. The smaller DEAMOX reactor was seeded by the sludge from the bigger reactor when the DEAMOX process was well established in the latter.

Sludge characterization

Assessment of sludge specific activities (methanogenic, denitrifying, nitrifying, and ANAMMOX) was performed as described by Imajo *et al.* (2004).

Results and discussion

Performance of UASB reactor

Taking into account the auxiliary role of this reactor, only the most important data on its functioning throughout an entire period of testing of the DEAMOX process are presented in Figure 2. In short, this mineralization reactor demonstrated a very stable performance

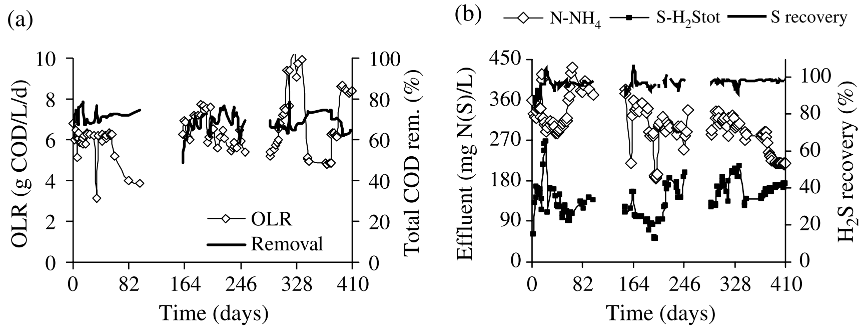

Figure 2 Performance of UASB reactor: (a) OLR and total COD removal; (b) effluent ammonia and sulfide concentrations

in the range of the organic loading rates (OLR) applied (3–10 g COD$_{tot}$/L/d), giving the average total COD removal of 70 ± 5% (Figure 2a) and almost complete conversion of sulfate into sulfide (97 ± 3%, Figure 2b) by the accompanying process of biological sulfate reduction. Generally, the reactor produced effluents with ammonia and sulfide concentrations of 302 ± 50 mg N/L and 144 ± 39 mg S/L, respectively (Figure 2b). However, during the first 215 days of testing, the S/N ratio in this effluent was lower than the theoretically required one (0.57 mg S/mg N under 50:50 split of anaerobic effluent between the NR and the DEAMOX reactor) to fulfil the denitrification requirements for the DEAMOX process (see the corresponding equation in Figure 1). In addition, some losses of sulfide occurred due to its oxidation, with occasional oxygen penetrations frequently happening in the lab-scale reactors. Further, to ensure an availability of electron donor (sulfide) for denitrification in the DEAMOX reactor, the raw wastewater with a higher sulfate/N ratio was fed to the UASB reactor starting from day 226; however, precautions were taken in order to prevent an inhibition of the latter reactor by high sulfide concentrations.

Performance of nitrifying reactors

Biofilter (days 0–253). The performance data of this nitrifying reactor are presented in Figure 3. Besides nitrification, some additional total COD removal (33 ± 12%, data not shown) mainly due to complete oxidation of sulfide (data not shown) occurred there. From Figure 3a, it is seen that ammonia removal in this reactor was incomplete, accounting for 82 ± 14% (on average). This was related mainly to a limitation of soluble oxygen concentrations performed by purposely during days of 13–27, 53–73 and 165–195. The idea was to enhance the nitrite production in this system. Indeed, during those periods, elevated concentrations of the latter (up to 83 mg N/L) were observed; however, the overall efficiency of ammonia removal dropped (Figure 3). After restoration of adequate aeration (days 28–52, 74–105, 196 onwards), the efficiency of ammonia removal usually exceeded 90%, approaching 100% at the end of the experiments with the nitrifying biofilter when more frequent withdrawal of excess sludge (once per 7–10 days) was executed. Though the problem of nearly complete nitrification was solved, the other feature of this reactor was unfavourable for the subsequent DEAMOX reactor (see below). Namely, the quite significant losses of nitrogen (39 ± 9%, on average, Figure 3b) occurred inside the biofilter, resulting in effluents with reduced NO$_x$–concentrations –37 ± 30 and 101 ± 52 mg N/L for nitrite and nitrate, respectively (Figure 3b). The possible reasons for such phenomenon may include–stripping of ammonia, denitrification of nitrite and nitrate by sulfide and other BOD (e.g. from decay of biomass), and development of

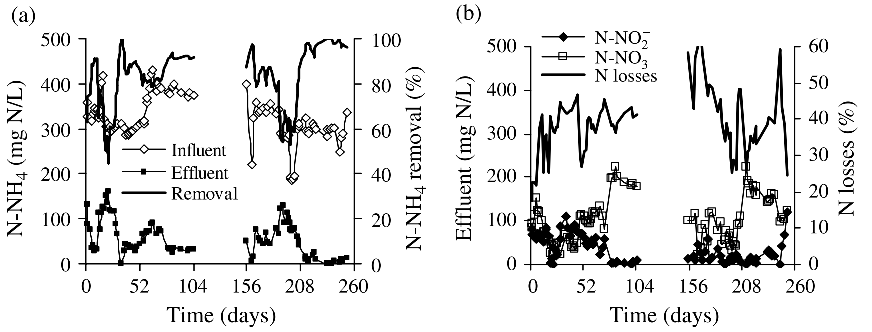

Figure 3 Performance of nitrifying biofilter: (a) influent and effluent ammonia concentrations as well as its removal; (b) effluent nitrate and nitrite concentrations as well as nitrogen losses

an ANAMMOX reaction inside the biofilm. Since the main focus of this study was testing of the DEAMOX process, we did not investigate this unwanted phenomenon in detail and decided to replace the biofilter by a more predictable nitrifying system–conventional activated sludge reactor.

Airtank (days 287–410). Compared with the biofilter, even higher total COD removal (44 ± 7%) was observed in the airtank, accompanied by complete oxidation of sulfide (data not shown). The ammonia removal in this reactor was complete (Figure 4a) with a prevalent production of nitrate (192 ± 54 mg N/L) over nitrite (51 ± 47 mg N/L, Figure 4b). The initial rise in nitrite concentrations (days 288–312, Figure 4b) was due to non-homogeneous supply of air in the airtank. After fixing this problem, the effluent nitrite concentrations were 3–7 times less compared to the nitrate ones (Figure 4b). The losses of nitrogen (10 ± 5%, Figure 4b) were significantly lower compared to the biofilter (Figure 3b), resulting in more suitable influent for the subsequent DEAMOX reactor.

Performance of DEAMOX reactors

Start-up with biofilter as a nitrifying reactor (days 0–105). It should be noted that in spite of 6 months pre-feeding of the ‘DEAMOX’ sludge with nitrite/ammonia synthetic media, its specific ANAMMOX activity remained quite low (0.87 ± 0.03 mg $N\text{-}NH_4$/g VSS/d) upon commencing testing of the DEAMOX process; therefore, it required a prolonged start-up period. Though methanogenic activity of this sludge was not detectable, the reactor removed 32 ± 8% of total COD (on average, data not shown) during the start-up period, mainly due to autotrophic denitrification with sulfide. The latter was present as traces in the reactor effluent (data not shown). The data dealt with nitrogen species for this stage are generalized in Figure 5. The nitrogen loading rate (NLR) was maintained around 210 mg N/L/d, with some increase until 270 mg N/L/d by the end of the start-up period (Figure 4d). After initial sporadic behaviour (during the first 3 weeks), the ammonia removal steadily improved until it reached 60% by the end of the start-up period (Figure 5a), accompanied by an almost complete absence of nitrite in the effluent (Figure 5b). On the contrary, nitrate removal decreased throughout that period (Figure 5c) as a result of development of the ANAMMOX reaction producing 0.26 mol nitrate per mol of ammonia consumed (Kartal *et al.*, 2004). It should be noted that although the influent nitrite concentrations were negligible during days 80–105 (Figure 5b), the reactor improved even ammonia removal (Figure 5a), manifesting establishment of the DEAMOX process (Figure 1) in this reactor. Total nitrogen removal was rather stable during the start-up period, accounting for 51 ± 10% (on average, Figure 5d). This was mainly related to a shortage of sulfide (data not shown) for

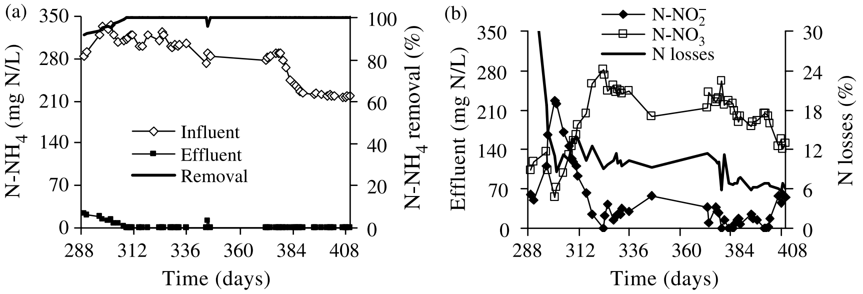

Figure 4 Performance of nitrifying airtank: (a) influent and effluent ammonia concentrations as well as its removal; (b) effluent nitrate and nitrite concentrations as well as nitrogen losses

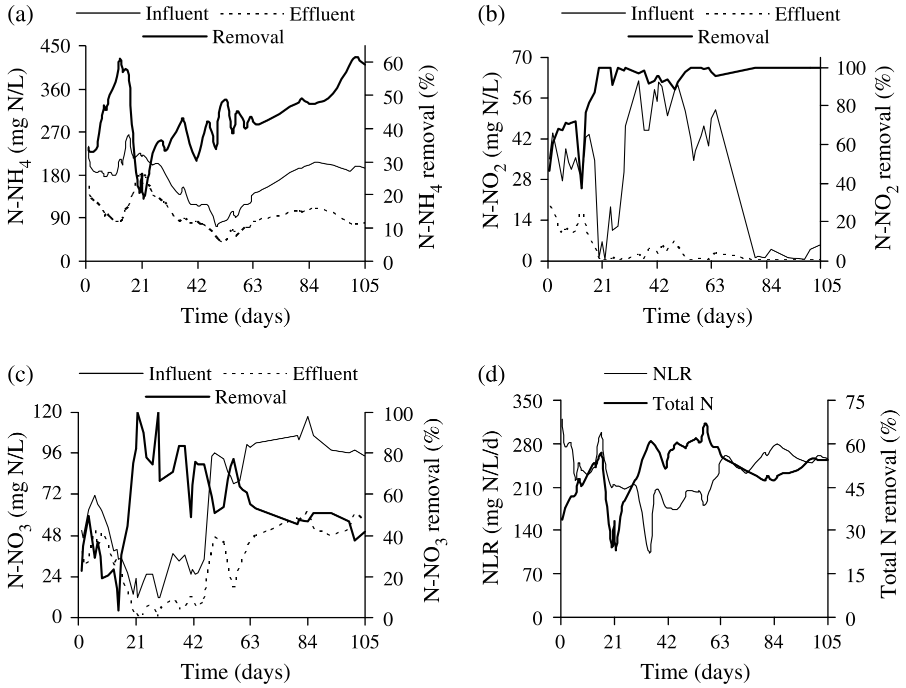

Figure 5 Influent and effluent concentrations and removal of ammonia (a), nitrite (b), and nitrate (c), as well as NLR and total nitrogen removal (d) of the DEAMOX reactor fed by nitrifying effluent from the biofilter (start-up period, 50:50 distribution of the UASB effluent flow between the biofilter and the DEAMOX reactor)

denitrification of nitrate into nitrite. This problem was solved during the next period of experimental testing.

Process maturation with biofilter as a nitrifying reactor (days 149–253). Compared with the start-up period, the DEAMOX reactor improved the total COD removal to 42 ± 10% (on average, data not shown), but sulfide was always present in the effluent (as traces, data not shown), ensuring denitrifying conditions in this reactor. The NLR was maintained around 250 mg N/L/d (Figure 6d). Owing to high losses of nitrogen (40–60%, days 149–207) in the biofilter (Figure 3b), the DEAMOX reactor suffered from a shortage of NOx-species (Figure 6b–c), resulting in insufficient ammonia removal (Figure 6a, days 149–207). As a consequence, the total nitrogen removal was even lower (days 149–207, Figure 6d) than during the start-up period (Figure 5d). To mitigate this unwanted phenomenon (losses of NOx-species in the biofilter), the latter was thoroughly washed, excessive biofilm and other SS were removed on day 208. This gave an improvement of ammonia and total nitrogen removals up to 62 and 67%, respectively (on average, Figure 6a, 6d, days 209–220). However, nitrate appeared in the effluent during this period (Figure 6c), indicating a deficiency of sulfide for denitrification (the average influent S-H_2S/N-NO_3 ratio for the DEAMOX reactor was only 0.41 mg S/mg N (data not shown), i.e. 72% of the theoretical ratio (0.57)). To overcome this limitation, another batch of raw wastewater with more balanced N/S ratio was fed to the system during days 221–253. The better S-H_2S/N-NO_3 ratio (0.70 mg S/mg N, on average) immediately led to substantial improvements in performance of the DEAMOX reactor–the ammonia and total nitrogen removals approached 80 and 90%, respectively, by the end of this period (Figure 6a–d). The determination of specific ANAMMOX activity of the DEAMOX

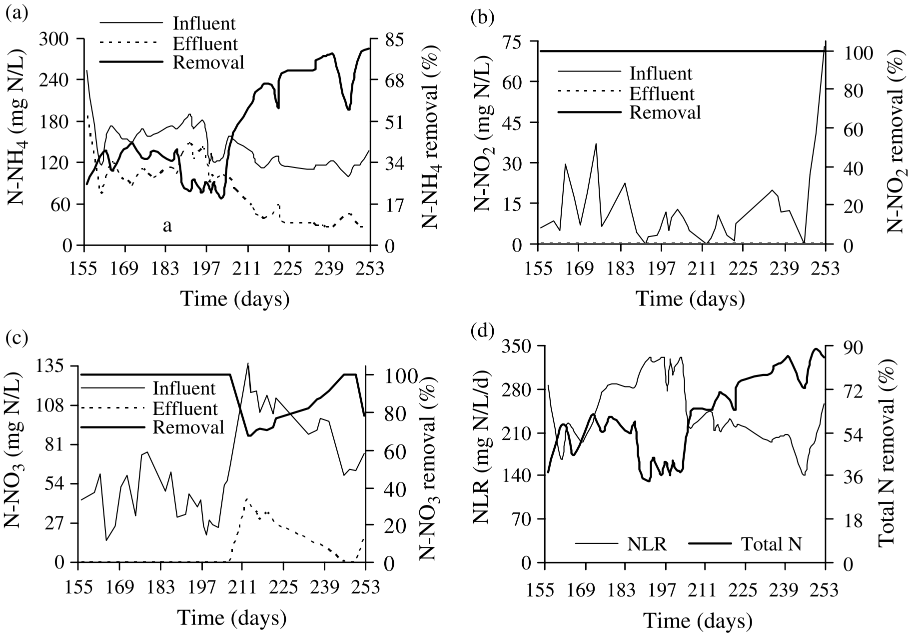

Figure 6 Influent and effluent concentrations and removal of ammonia (a), nitrite (b), and nitrate (c), as well as NLR and total nitrogen removal (d) of the DEAMOX reactor fed by nitrifying effluent from the biofilter (maturation period, 59:41 distribution of the UASB effluent flow between the biofilter and the DEAMOX reactor)

sludge performed at day 220 showed its increase up to 10.0 ± 0.4 mg N-NH$_4$/g VSS/d (i.e.$>$11-fold increase compared with the start). The latter shows the significant development of ANAMMOX bacteria. However, owing to imminent drawback the biofilter dealt with losses of NOx-species (Figure 3b), further process optimization was performed with the nitrifying airtank.

Further process optimization with airtank as a nitrifying reactor (days 287–410). The generalized data dealing with nitrogen species removal for this stage are presented in Figure 7. Although the NLR was steadily increased during this until the values above 1000 mg N/L/d (Figure 7d), the ammonia, nitrite and nitrate removals were fairly stable, accounting for 73 ± 6, 97 ± 6 and 91 ± 8% respectively (Figure 7a–c). As a result, the average total nitrogen removal accounted for 82 ± 5% (Figure 7d). More precise analysis of the data obtained showed that values around 90% of total nitrogen removal in the DEAMOX reactor are achievable under the influent N-NO$_x$/N-NH$_4$ ratio higher than 1.2 (stoichiometry of the ANAMMOX reaction) and the influent S-H$_2$S/N-NO$_3$ ratio higher than 0.57 mg S/mg N (stoichiometry for denitrification of nitrate to nitrite). The highest nitrogen removal rate (NRR) achieved was 890 mg N/L/d, i.e. in 9–18 times exceeding the corresponding NLR of activated sludge systems. This was due to the fact that the specific ANAMMOX activity of the sludge determined at the end of testing was further increased up to 17.5 ± 0.6 mg N-NH$_4$/g VSS/d (i.e. $>$ 20-fold increase compared to the start). Significant changes of the bacterial morphology of the DEAMOX sludge were also observed under electron microscopy investigation, showing a prevalence of ANAMMOX-like bacteria at the end of testing (data not shown). Preliminary results of PCR detection of ANAMMOX bacteria from the DEAMOX reactor showed that there was only amplification with the general primer of the genus *Planctomycetales* (which ANAMMOX bacteria belong to) but amplification was absent with primers of the

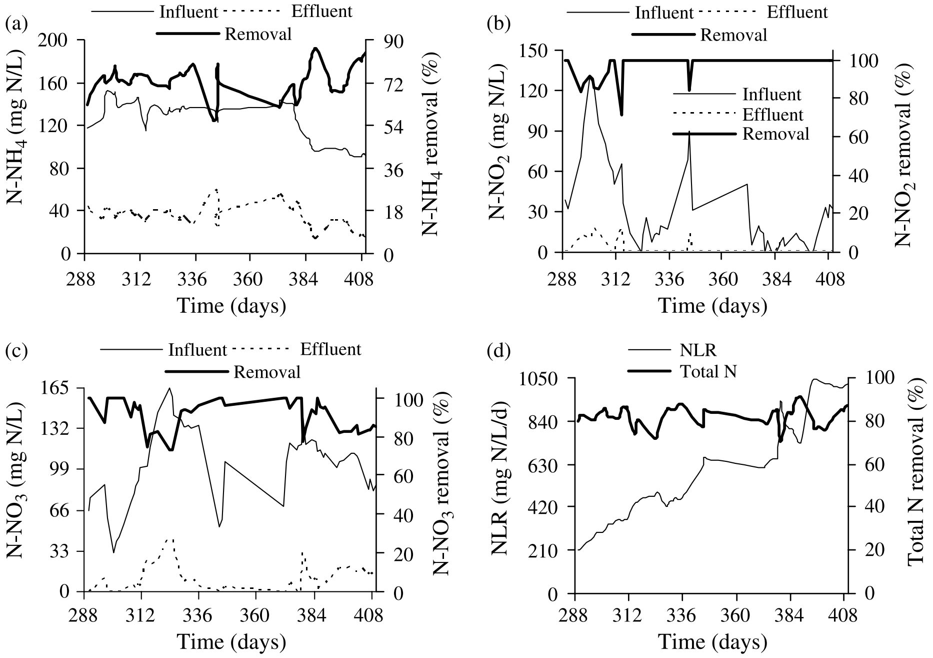

Figure 7 Influent and effluent concentrations and removal of ammonia (a), nitrite (b), and nitrate (c), as well as NLR and total nitrogen removal (d) of the DEAMOX reactor fed by nitrifying effluent from the airtank (optimization period, 55:45 distribution of the UASB effluent flow between the airtank and the DEAMOX reactor)

most-studied ANAMMOX species: *Brocadia anammoxidans* and *Kuenenia stuttgartiensis*. Thus, the species flourishing in the DEAMOX sludge are different from the latter.

Conclusions

Thus, the results described above undoubtedly indicate a successful realization of the DEAMOX concept in the chosen experimental setup. The imposing of autotrophic denitrifying conditions on the weak ANAMMOX sludge led to a gradual development of the ANAMMOX activity in the DEAMOX reactor, which was finally able to accommodate NLR higher than 1000 mg/L/d, with total nitrogen removal of around 90%. The observed NRR of around 900 mg N/L/d look very promising because they exceed (by 9–18 times) the corresponding NRR of conventional activated sludge systems.

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