



## AMMONIUM REMOVAL FROM CONCENTRATED WASTE STREAMS WITH THE ANAEROBIC AMMONIUM OXIDATION (ANAMMOX) PROCESS IN DIFFERENT REACTOR CONFIGURATIONS

MARC STROUS, ERIC VAN GERVEN, PING ZHENG\*, J. GIJS KUENEN<sup>®</sup>  
and MIKE S. M. JETTEN<sup>†</sup>

Kluyver Laboratory for Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands

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**Abstract**—Many concentrated wastewater streams produced in food and agro-industry are treated using sludge digestion. The effluent from sludge digestors frequently contains ammonium in high concentrations (up to  $2 \text{ kg m}^{-3}$ ). This ammonium-rich effluent is usually treated by a normal wastewater treatment plant (WWTP). When ammonium removal from this concentrated stream is considered, steam stripping or a combination of two biological processes, aerobic nitrification and anoxic denitrification, are the (costly) options. Recently, a novel process was discovered in which ammonium is converted to dinitrogen gas under anoxic conditions with nitrite as the electron acceptor. It has been named Anammox (anaerobic ammonium oxidation). The aim of this study was to demonstrate the feasibility of ammonium removal from sludge digestion effluents with the Anammox process. Using a synthetic wastewater, it was shown that a fixed-bed reactor and a fluidised-bed reactor were suitable reactor configurations. The effects of sludge digestion effluent on the Anammox process were investigated; during 150 days, 82% ammonium removal efficiency and 99% nitrite removal efficiency was achieved in a fluidised-bed reactor inoculated with Anammox sludge and fed with sludge digestion effluent from a domestic WWTP. The maximum nitrogen conversion capacity was  $0.7 \text{ kg NH}_4^+-\text{N m}^{-3}_{\text{reactor}} \text{ day}^{-1}$  and  $1.5 \text{ kg total N m}^{-3}_{\text{reactor}} \text{ day}^{-1}$ .  
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**Key words**—nitrogen removal, nitrite, nitrate, wastewater, fluidised bed, fixed bed

### INTRODUCTION

Anaerobic digestion is a common way to treat concentrated wastewaters or sludges (Walsh *et al.*, 1991). Concentrated waste streams are produced in many agricultural and food industries, such as the production of potato starch, the processing of fish meal and the production of citric acid. Piggery sludge and activated sludge produced in domestic wastewater treatment are also mainly digested anaerobically (Sandberg and Ahring, 1992; Abeilung and Seyfried, 1993; Gorecki *et al.*, 1993).

These concentrated waste streams contain up to  $2 \text{ kg NH}_4^+-\text{N m}^{-3}$ . Ammonium is not removed in the digestion process and therefore sludge digestion generally yields an ammonium-rich effluent, low in biodegradable COD. This effluent is usually fed

to a wastewater treatment plant (WWTP). Where nitrogen removal in the WWTP is troublesome, post-treatment of the sludge digestion effluent is often considered to remove the ammonium, thereby decreasing the nitrogen loading of the WWTP. Currently, steam stripping or biological nitrification/denitrification are well known options for post-treatment (Derycke and Pipyn, 1990; Walsh *et al.*, 1991).

The anaerobic ammonium oxidation process (Anammox) is a novel, promising, low-cost alternative to conventional denitrification systems (Graaf *et al.*, 1995; Mulder *et al.*, 1995). In the Anammox process, ammonium is converted to dinitrogen gas with nitrite as the electron acceptor (Graaf *et al.*, 1996). Since the Anammox process is autotrophic, there is no need for COD addition to support denitrification (Graaf *et al.*, 1996). Furthermore, if the Anammox process is combined with a preceding nitrification step, only part of the ammonium needs to be nitrified to nitrite while the Anammox process combines the remaining ammonium with this nitrite to yield dinitrogen gas. This will reduce oxygen demand in the nitrification reactor and lead to a

\*Present address: Zhejiang Agricultural University, Department of Environmental Science, Laboratory of Microbiology, Hangzhou 310029, China.

†Author to whom all correspondence should be addressed [Tel: +31-15-2781193, Fax: +31-15-2782355, e-mail: M.Jetten@STM.TU.Delft.NL].

second reduction in costs. The biomass yield of the Anammox process is very low; consequently, little sludge is produced. The low sludge production is the third factor that contributes to the substantially lower operation costs compared to conventional denitrification systems. However, the low biomass yield also demands an efficient sludge retention.

These characteristics make the Anammox process an interesting option to remove ammonium from concentrated, warm effluents from sludge digestion dewatering facilities.

The Anammox process was originally discovered in a denitrifying fluidised-bed reactor (Mulder *et al.*, 1995). The low biomass yield combined with the extremely low growth rate and complexity of the biofilm population responsible for the Anammox process initially slowed down research progress. However, it was demonstrated that the Anammox process had a biological nature, using inhibitor studies (Graaf *et al.*, 1995). Acetylene, phosphate and oxygen were shown to be strong inhibitors of Anammox activity (Jetten *et al.*, 1996). In an Anammox enrichment culture, the main product of anaerobic ammonium oxidation was  $N_2$ , but about 10% of the N-feed (nitrite and ammonium) was converted to  $NO_3^-$ . The overall nitrogen balance gave a ratio of  $NH_4^+$  conversion to  $NO_2^-$  conversion to  $NO_3^-$  production of  $1:(1.31 \pm 0.06):(0.22 \pm 0.02)$  (Graaf *et al.*, 1996).

In the present study, the potential of the Anammox process for ammonium removal from sludge digestion effluents was explored. In the first part of the study, two reactor types were compared: a fluidised-bed reactor and a fixed-bed reactor. A defined synthetic wastewater was used, because the Anammox population had been cultured successfully on this medium in the past. In the second part of this study, one reactor type was selected to demonstrate that the process was also capable of ammonium removal from the effluent of the anaerobic sludge digestion of the Dokhaven WWTP (Rotterdam, The Netherlands).

## MATERIALS AND METHODS

### Sludge inoculum

Sludge from a 27-litre denitrifying (and anaerobic ammonium oxidising) fluidised-bed reactor (Mulder *et al.*, 1995) was used as the source for biomass. This 27-litre fluidised bed was fed with the effluent from a methanogenic reactor that was operated with wastewater from a bakers yeast production plant. This water contained the following ( $mg\ litre^{-1}$ ): COD 550–750, TOC 165–190,  $SO_4^{2-}$  25–150 and  $S^{2-}$  90–130. A  $75\text{-g}\ litre^{-1}$   $NaNO_3$  solution was separately supplied.

### Synthetic wastewater and sludge digestion effluent

The composition of the synthetic wastewater and the sludge digestion effluent used in this study is shown in Table 1. The sludge digestion effluent was obtained from the activated sludge digestion facility of the Dokhaven WWTP.

### Determination of the pH and temperature optima

In two series of bottles with butyl-rubber stoppers, 8 ml sludge digestion effluent was diluted 10 times with a  $500\text{-mg}\ litre^{-1}$   $KHCO_3$  buffer to a final concentration of  $100\text{-mg}\ NH_4^+\text{-N}\ litre^{-1}$ ; nitrite was added to  $100\text{-mg}\ NO_2^-\text{-N}\ litre^{-1}$ . The bottles were inoculated with 2 ml homogenised sludge ( $21.2\text{-mg}\ VSS\ litre^{-1}$ ). They were made anoxic by flushing them with an argon/ $CO_2$  (95/5%) mixture. In the first series of bottles the pH was adjusted to values between 6 and 10 and the bottles were incubated at  $34^\circ C$ . In the second series the pH was 7.8 and the bottles were incubated at different temperatures. Samples were taken every 2 days and analysed for ammonium and nitrite.

### Operation of the fluidised bed

The Anammox fluidised bed was maintained in a 2.5-litre (height 70 cm, diameter 7 cm, liquid volume 2.25 litre) glass column reactor with a water jacket. The temperature was  $36^\circ C$ . The pH was controlled at pH 8 using a  $1\text{-M}\ H_2SO_4$  solution. Anoxic liquid from the top of the reactor was recirculated to boost the flow to approximately  $47\text{-litres}\ h^{-1}$  in order to keep the bed fluidised at a superficial liquid velocity of  $12\text{-m}\ h^{-1}$ . All tubing and connectors were of butyl rubber, noreprene or polyvinylchloride to limit oxygen diffusion into the system. For the same reason, the settler at the top of the reactor was flushed with argon. The hydraulic retention time with synthetic medium was 22–42 h. The hydraulic retention time with sludge digestion effluent was 3.5 h to 11 days. A synthetic  $0.38\text{--}1.43\text{-kg}\ NO_2^-\text{-N}\ m^{-3}$  nitrite solution was supplied together with the sludge digestion effluent. Nitrite served as the electron acceptor. Sand particles were the carrier material. To start up the fluidised bed, the reactor was filled with 0.5 kg

Table 1. Composition of the synthetic wastewater and sludge digestion effluent used in this study

Synthetic wastewater ( $kg\ m^{-3}$ )		Sludge digestion effluent ( $kg\ m^{-3}$ )	
$KHCO_3$	2.5	$HCO_3^-$	4.1
$NaH_2PO_4$	0.05	Total-P	0.027
$CaCl_2 \cdot 2H_2O$	0.3		
$MgSO_4 \cdot 7H_2O$	0.2		
$FeSO_4$	0.00625		
EDTA	0.00625		
Trace elements Solution <sup>a</sup>		1.25 ml $litre^{-1}$	
$(NH_4)_2SO_4$	0.07–0.84	$NH_4^+\text{-N}$	1.1–2.1
$NaNO_2$	0.07–0.84	Kjeldahl-N	1.1–2.1
		Suspended solids	0.056
		BOD	0.023
		COD	0.081
pH	7.7–8.2	pH	7.7–8.2

<sup>a</sup>Described by Graaf *et al.* (1996).

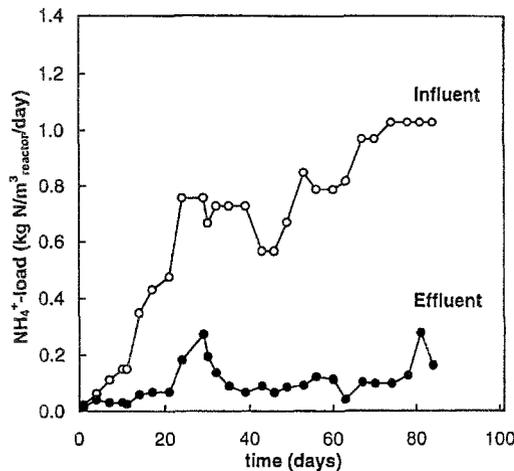


Fig. 1. Operation of a fluidised-bed reactor with synthetic wastewater. In 100 days, the load could be increased to 1 kg  $\text{NH}_4^+\text{-N m}^{-3} \text{ day}^{-1}$ , using 1 kg  $\text{NO}_2^-\text{-N m}^{-3} \text{ day}^{-1}$ : influent ammonium load (○), effluent ammonium load (●).

(310 ml) sand (diameter 0.3–0.6 mm) and inoculated with 1.2 litres sludge.

#### Operation of the fixed bed

The Anammox fixed bed was maintained in a 2-litre (height 60 cm, diameter 5.5 cm, liquid volume 1.4 litres) glass column reactor with a water jacket. The reactor was filled up to a volume of 1.2 litres with sintered glass beads (Syrax glass, Schott, diameter 3–5 mm). The temperature was 36°C. The pH was controlled at pH 8 using a 1 M  $\text{H}_2\text{SO}_4$  solution. The influent (containing up to 0.84 kg  $\text{NH}_4^+\text{-N m}^{-3}$  and up to 0.84 kg  $\text{NO}_2^-\text{-N m}^{-3}$ ) was supplied at the bottom of the reactor. Anoxic liquid from the top of the reactor was recirculated (boosting the flow to approximately 30 litres  $\text{h}^{-1}$ ) to prevent high nitrite concentrations at the bottom of the reactor. All tubing and connectors were of butyl rubber, noreprene or polyvinylchloride to limit oxygen diffusion into the system. The reactor was inoculated with effluent from the fluidised-bed reactor. The hydraulic retention time was 6–23 h.

#### Analytical procedures

Nitrate, nitrite and ammonium were determined as previously described (Graaf *et al.*, 1996). Dry weight was determined after drying the sample at 65°C for at least 24 h. Subsequently the sample was ashed in a furnace (700°C) for 1 h; the amount of sand in the dried sample could be determined. The dry weight minus the ashed weight is termed "volatile suspended solids" (VSS).

## RESULTS AND DISCUSSION

### Fluidised-bed reactor

A fluidised-bed reactor was inoculated with Anammox sludge and maintained for 100 days. During this period, the anaerobic ammonium oxidising population was enriched and the nitrogen load could be increased from 0.02 to 1.0 kg  $\text{NH}_4^+\text{-N m}^{-3} \text{ reactor day}^{-1}$  by increasing the influent flowrate from 0.2 to 1.2  $\text{m}^3 \text{ m}^{-3} \text{ reactor day}^{-1}$  and the ammonium concentration from 0.1 to 0.84 kg  $\text{NH}_4^+\text{-N m}^{-3}$  (Fig. 1). Nitrite as the electron acceptor was supplied together with ammonium and was always completely (>99%) converted (Table 2). The ammonium removal rate was 0.8 kg  $\text{NH}_4^+\text{-N m}^{-3} \text{ reactor day}^{-1}$ . The specific ammonium removal rate was 0.08 kg  $\text{NH}_4^+\text{-N (kg VSS)}^{-1} \text{ day}^{-1}$ , and the combined ammonium and nitrite removal rate was 0.18 kg  $\text{N (kg VSS)}^{-1} \text{ day}^{-1}$ .

After 100 days, the colour of the sludge granules had changed from brownish to red, and electron microscopy revealed that the same organism as described by Graaf *et al.* (1996) (a morphologically irregular, coccoid, gram-negative bacterium) had become the dominant organism. The expansion of the fluidised bed indicated that new sludge granules had grown.

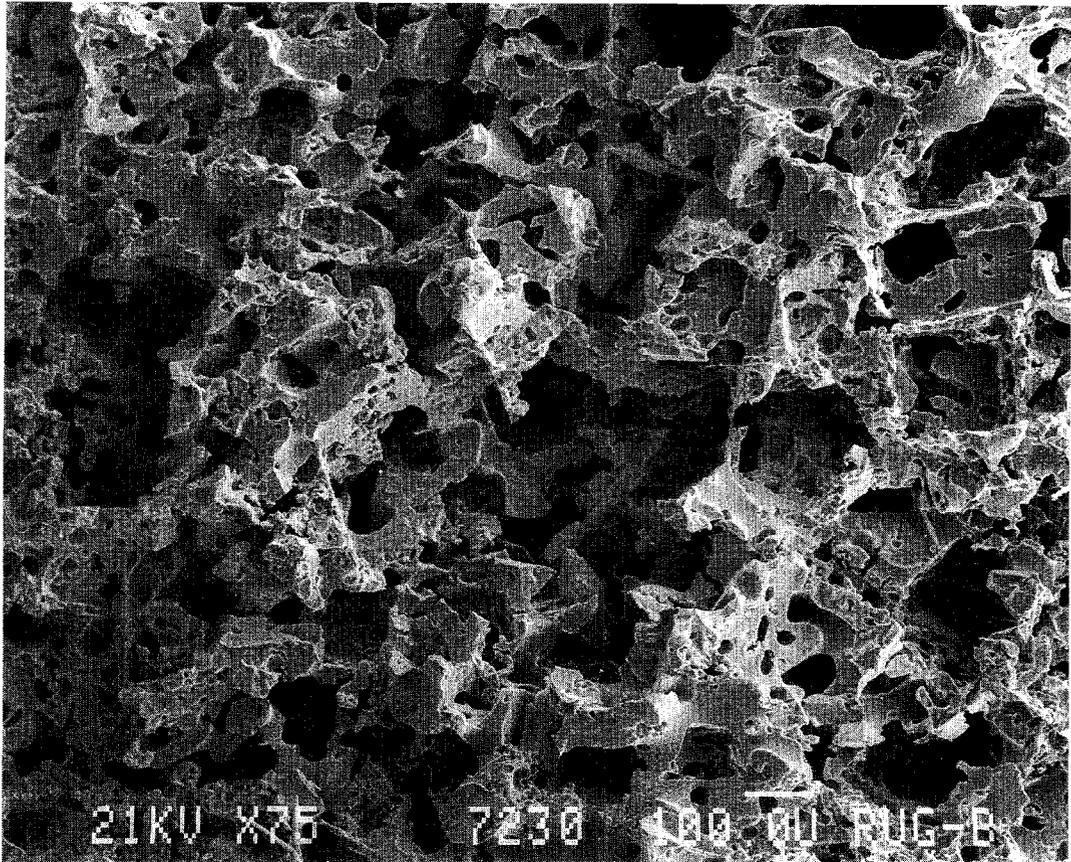
Although operation of this fluidised bed was stable, in a few cases it was not. In two out of 15 attempts, the sludge inoculum deteriorated after an initial increase in activity, resulting in almost complete wash-out of the sludge and a loss of activity (results now shown). The deterioration of the sludge granules in two cases could have been related to a microbial population shift. The inoculum granules were taken from a sulphide-oxidising, denitrifying fluidised bed fed with effluent from a methanogenic reactor. The mineral medium used in this experiment contained nothing but ammonium and nitrite. After inoculation, the anaerobic ammonium oxidising population grew, but a large part of the other populations were starved. This could lead to a momentary increase in concentrations of organic nutrients and biofilm space, causing the transient growth of a heterotrophic, denitrifying population. When growth and starvation processes of the different populations

Table 2. Ammonium removal from synthetic wastewater and sludge digestion effluent in a fluidised-bed and fixed-bed reactor fed with synthetic wastewater and a fluidised-bed reactor fed with sludge digestion effluent

Parameter	Fluidised bed (synthetic wastewater)	Fixed bed (synthetic wastewater)	Fluidised bed (sludge digestion effluent)
Duration of experiment (days)	84	115	150
Influent $\text{NH}_4^+$ concentration (kg $\text{NH}_4^+\text{-N m}^{-3}$ )	0.07–0.84	0.07–0.84	1.1–2.1
Effluent $\text{NH}_4^+$ concentration (kg $\text{NH}_4^+\text{-N m}^{-3}$ )	0.02–0.28	0.00–0.13	0.00–0.56
Influent $\text{NO}_2^-$ concentration (kg $\text{NO}_2^-\text{-N m}^{-3}$ )	0.07–0.84	0.07–0.84	0.07–0.84
Effluent $\text{NO}_2^-$ concentration (kg $\text{NO}_2^-\text{-N m}^{-3}$ )	0.00	0.00	0.00
Ammonium removal (%)	84	88	82
Nitrite removal (%)	99	99	99
Total nitrogen removal (kg total-N $\text{m}^{-3} \text{ reactor day}^{-1}$ )	1.8	1.1	1.5
Specific total nitrogen removal (kg total-N (kg-VSS) $^{-1} \text{ day}^{-1}$ )	0.18	— <sup>a</sup>	0.15

<sup>a</sup>The amount of biomass could not be determined in the fixed-bed reactor.

(A)



(B)

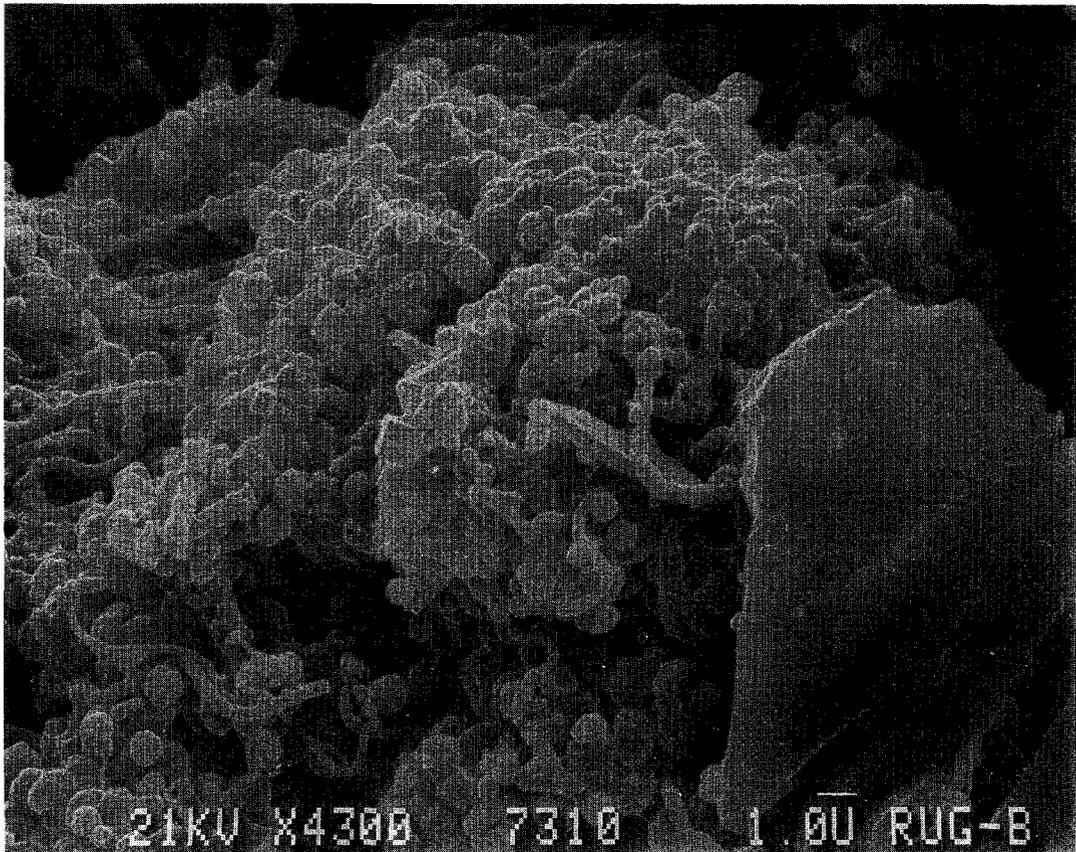


Fig. 2. Scanning electron micrographs of sintered glass bead surface: (A) before inoculation; (B) after 60 days of operation the surface is colonised by morphologically different bacteria. Electron microscopy was carried out by the Department of Electron Microscopy of the Groningen State University, The Netherlands.

were not well attuned, sludge deterioration might result.

#### Fixed-bed reactor

Because in a few cases the sludge granules were not stable and sludge retention is essential for the Anammox process, a fixed-bed reactor was selected as an alternative to the fluidised-bed reactor. Syran glass beads were used as the carrier material because these beads have a high surface area (Fig. 2A). A recycle was applied to dilute the influent, because high nitrite concentrations could be toxic to the Anammox process (Graaf *et al.*, 1996). It was expected that the bed would not clog because the yield of the Anammox process is low ( $0.07 \text{ kg VSS} (\text{kg NH}_4^+-\text{N})^{-1}$ ) (Graaf *et al.*, 1996).

The fixed-bed reactor was inoculated with sludge from a fluidised-bed reactor and maintained for 120 days. Initially, the load could be increased rapidly. The glass surface was almost completely covered by biofilms consisting of several types of bacteria (Fig. 2B). However, after 60 days of operation, the fixed bed became unstable due to a combination of clogging and intense gas production of the Anammox process (Fig. 3). Gas bubbles remained trapped in the bed and clusters of grown-together beads were lifted up and obstructed the recycle, leading to periods with toxic nitrite concentrations in the lower part of the bed and to a reduced activity. Still, the average ammonium removal efficiency was 88% (Table 2).

In spite of these problems, the fixed-bed experiment demonstrated that the Anammox process was supported by a fixed-bed reactor. The entrapment of gas bubbles and clogging of the bed were problems likely to be restricted to the laboratory-scale reactor. When an open type of packing material is selected

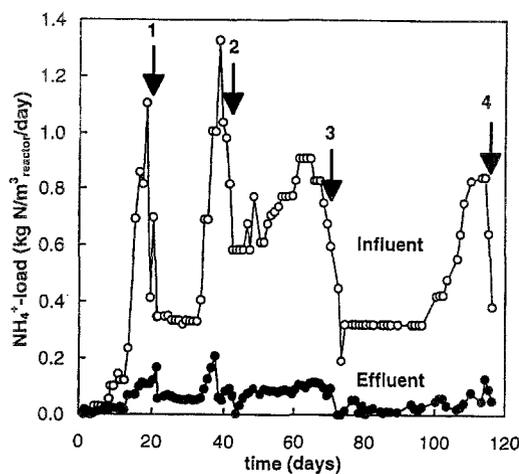


Fig. 3. Operation of a fixed-bed reactor with synthetic wastewater. Operation was sometimes unstable (arrows 1, 2, 3) due to clogging of the reactor. On one occasion (arrow 4), reactor performance decreased after increase of ammonium and nitrite concentrations in the influent. Influent ammonium load (○), effluent ammonium load (●).

(such as Raschig rings) these problems may be avoided.

Because of the problems on laboratory-scale with the fixed bed, the fluidised bed was selected as the reactor type for a feasibility study with sludge digestion effluent on a laboratory-scale.

#### Sludge digestion effluent

In the second part of this study, the Anammox process was operated on sludge digestion effluent. Preliminary batch experiments indicated that the sludge inoculum was not inhibited by the sludge digestion effluent (results not shown). It was shown in batch experiments that the pH and temperature optima of the sludge inoculum matched the pH and temperature of the sludge digestion effluent ( $30^\circ\text{C}$  and pH 7.7–8.2) (Fig. 4).

In these batch experiments, the maximum activity of the sludge ( $0.014 \text{ kg NH}_4^+-\text{N} (\text{kg VSS})^{-1} \text{ day}^{-1}$ ) was five times lower than the activity in the fluidised-bed and fixed-bed reactors. This was not caused by the use of sludge digestion effluent but was also found with mineral medium by Graaf *et al.* (1995, 1996). The reason for the low activity in batch

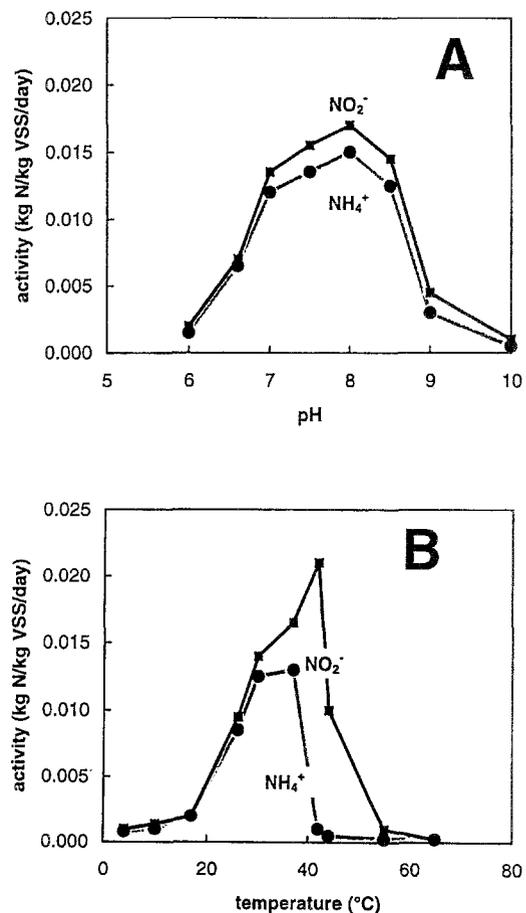


Fig. 4. Anammox activity in sludge digestion effluent as a function of pH (A) and temperature (B): ammonium (●), nitrite (■).

experiments remains unclear. In batch experiments, the sludge granules were homogenised and mass transport limitation was much less important compared to the fluidised-bed and fixed-bed reactors. Therefore, a higher activity would be expected, unless the sludge was inhibited by the high nitrite concentrations. The inhibition kinetics are currently under investigation.

The results shown in Fig. 4(B) indicate that, with increasing temperatures, ammonium and nitrite conversion were uncoupled. The electron donor for the uncoupled nitrite conversion might be a storage product or the biomass itself. It remains unclear which population is responsible for the ongoing nitrite conversion; it could have been the anaerobic ammonium oxidising community itself, or, alternatively, a denitrifying population, that used biomass (lysed by the increasing temperature) as the electron donor.

Next, a fluidised-bed reactor was inoculated with Anammox sludge and run on sludge digestion effluent for 150 days. Nitrite as the electron acceptor was supplied together with the sludge digestion effluent and was always completely (>99%) converted. During the first 100 days, the ammonium load could not be increased (Fig. 5A). Apparently, the sludge had to adjust to the sludge digestion effluent. After this lag phase the load could be increased as with the synthetic wastewater (Fig. 5B). In 30 days the load was increased to  $1.2 \text{ kg NH}_4^+\text{-N m}^{-3} \text{ reactor}^{-1} \text{ day}^{-1}$ . Since the removal efficiency decreased from 95 to 56%, it was obvious that the sludge growth did not keep up with the increase in load. In this period, the specific activity of the sludge increased to  $0.07 \text{ kg NH}_4^+\text{-N (kg VSS)}^{-1} \text{ day}^{-1}$  ( $0.15 \text{ kg total-N (kg VSS)}^{-1} \text{ day}^{-1}$ ).

In the last 20 days, the load was reduced and increased for a second time. No increase in reactor performance and sludge specific activity was observed. After this period, the fluidised-bed reactor still converted  $0.7 \text{ kg NH}_4^+\text{-N m}^{-3} \text{ reactor}^{-1} \text{ day}^{-1}$ . This indicated that during this period little or no sludge growth occurred. Based on the Anammox yield value ( $0.07 \text{ kg VSS (kg NH}_4^+\text{-N)}^{-1}$ ) found by Graaf *et al.* (1996), and the amount of ammonium and nitrite that was converted ( $12 \text{ kg NH}_4^+\text{-N}$  and  $14.4 \text{ kg NO}_2^-\text{-N}$ ), it was expected that after this 20-day period the amount of sludge present in the fluidised bed would be capable of removing  $1 \text{ kg NH}_4^+\text{-N m}^{-3} \text{ reactor}^{-1} \text{ day}^{-1}$ , while  $0.7 \text{ kg NH}_4^+\text{-N m}^{-3} \text{ reactor}^{-1} \text{ day}^{-1}$  was found experimentally.

Apparently, the fluidised-bed reactor could not accommodate more active anaerobic ammonium oxidising biomass and the remainder was washed out. Thus, the maximum ammonium removal capacity of the fluidised-bed reactor with sludge digestion effluent was  $0.7 \text{ kg NH}_4^+\text{-N m}^{-3} \text{ reactor}^{-1} \text{ day}^{-1}$  ( $1.5 \text{ kg total-N m}^{-3} \text{ reactor}^{-1} \text{ day}^{-1}$ ). This might be explained as follows. The sludge digestion effluent contained a lot of suspended solids ( $0.056 \text{ kg m}^{-3}$ ) and organic

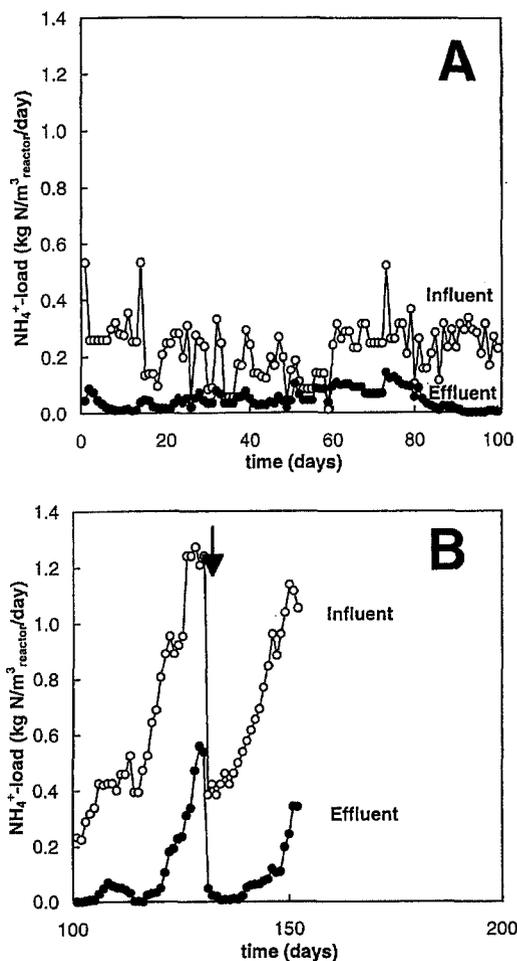


Fig. 5. Operation of a fluidised-bed reactor with sludge digestion effluent. After a lag phase of 100 days (A), the ammonium load could be increased to  $1.2 \text{ kg NH}_4^+\text{-N m}^{-3} \text{ reactor}^{-1} \text{ day}^{-1}$ . (B) After 30 days the ammonium removal efficiency had decreased to 56% (arrow) and the load was reduced and increased for a second time. Influent ammonium load (○), effluent ammonium load (●).

substrate for heterotrophic microbial populations ( $0.023 \text{ kg BOD m}^{-3}$ ). The amount of ammonium present in the sludge digestion effluent would yield  $0.077\text{--}0.16 \text{ kg VSS m}^{-3}$  of anaerobic ammonium oxidising micro-organisms. Entrapment of suspended solids and growth of heterotrophs could lead to less biofilm space available for the desired population and a lower specific ammonium removal capacity for sludge digestion effluent.

The results of the fluidised-bed reactor run with sludge digestion effluent are summarised in Table 2.

#### Process evaluation

In this study the potential of the Anammox process for nitrogen removal was investigated. In Tables 3 and 4 this potential is compared to nitrogen removal in an activated sludge reactor and nitrogen removal by an air-lift reactor. In Table 3 the experimentally obtained values for two important kinetic parameters

Table 3. Two kinetic parameters of the Anammox process in a fluidised-bed reactor compared to aerobic nitrification in an activated sludge reactor and in a gas-lift reactor

Parameter	Anammox fluidised bed	Nitrification and denitrification in an activated sludge reactor	Nitrification in a gas-lift reactor
Sludge activity (kg total-N (kg-VSS) <sup>-1</sup> day <sup>-1</sup> )	0.15 (34°C)	0.012 (20°C) <sup>a</sup>	0.42 (30°C) <sup>b</sup>
Reactor capacity (kg total-N m <sup>-3</sup> reactor day <sup>-1</sup> )	1.5 (34°C)	0.005–0.05 (20°C)	4.6 (30°C) <sup>b</sup>

<sup>a</sup>Value obtained from Weremeus Buning (pers. comm.).

<sup>b</sup>Values obtained from Tijhuis *et al.* (1994).

(sludge specific activity and reactor capacity) are compared. From this table it can be concluded that the Anammox process is capable of compact nitrogen removal. The achieved nitrogen loading rate was only three times lower than the high rates in a nitrifying air-lift reactor achieved by Tijhuis *et al.* (1994).

In Table 4, three important stoichiometric parameters are compared. Since nitrogen removal is not accomplished by one of these processes alone, but can only be achieved by combining nitrification and denitrification or by combining partial nitrification to nitrite and the Anammox process (Jetten *et al.*, 1996), Table 4 shows the parameters for the combined processes. The stoichiometry of the activated sludge process and the air-lift reactor are the same, because in both of these processes ammonium is removed via nitrification/denitrification.

It is shown that the combination of partial nitrification and Anammox would require less than 50% of the oxygen compared to nitrification/denitrification. Furthermore, application of the Anammox process would make unnecessary the addition of extra organic electron donors such as methanol and would lead to a lower sludge production. Addition of extra electron donors would be required in the other two options to sustain denitrification, since sludge digestion effluents contain little biodegradable COD.

The results presented in Table 2 indicate that ammonium removal from sludge digestion effluent using the Anammox process can be achieved. When this ammonium removal, obtained with the Anammox process, is compared to ammonium removal with conventional processes (as is done in Table 3), it appears that the Anammox process may be an interesting alternative to conventional nitrification/denitrification systems. Therefore, this study indicates that ammonium removal from sludge digestion effluents using the Anammox process is a

feasible option where post-treatment of the sludge digestion effluent is desired.

The start up of the Anammox process on full-scale is still a great challenge, because (1) biomass yield is low, (2) adaptation of the sludge granules to sludge digestion effluent takes a long time (100 days) and (3) little sludge is available for inoculation. But once the process is operated on full-scale, enough sludge will be available to facilitate start-up of new installations.

In this study a synthetic nitrite solution was supplied to the Anammox process, whereas in practice this nitrite would be obtained by nitrifying part of the ammonium in a preceding partial nitrification step. Before the partial nitrification/Anammox combination can be applied, two important questions should be answered: (1) how would wastewater ammonium be converted partially to nitrite (Loosdrecht and Jetten, 1996); and (2) what would be the impact of the preceding nitrification step on the Anammox process?

## CONCLUSIONS

1. A fixed-bed reactor and a fluidised-bed reactor seem suitable reactor configurations for the Anammox process.

2. The Anammox process shows potential for ammonium removal from sludge digestion effluent.

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Table 4. Three stoichiometric parameters of combined partial nitrification/Anammox compared to combined nitrification/denitrification in an activated sludge reactor and in a gas-lift reactor

Parameter	Combined partial nitrification and Anammox	Nitrification and denitrification in an activated sludge reactor	Nitrification in a gas-lift reactor combined with denitrification
Oxygen consumption <sup>a</sup> (kg O <sub>2</sub> (kg NH <sub>4</sub> <sup>+</sup> -N) <sup>-1</sup> )	1.9	3.4–5	3.4–5
Denitrification BOD consumption <sup>a</sup> (kg BOD (kg NH <sub>4</sub> <sup>+</sup> -N) <sup>-1</sup> )	0	> 1.7	> 1.7
Sludge production (kg VSS (kg NH <sub>4</sub> <sup>+</sup> -N) <sup>-1</sup> )	0.08 <sup>b</sup>	1 <sup>c</sup>	1 <sup>c</sup>

<sup>a</sup>Follows from reaction stoichiometry.

<sup>b</sup>Values obtained from Graaf *et al.* (1996) and Tijhuis *et al.* (1994).

<sup>c</sup>Values obtained from Wiesmann (1994).

## REFERENCES

- Abeilung U. and Seyfried C. F. (1993) Anaerobic-aerobic treatment of potato starch wastewater. *Wat. Sci. Technol.* **28**, 165-176.
- Derycke D. and Pipyn P. (1990) Anaerobic digestion, ammonia stripping/recovery and (de)nitrification of a citric acid factory effluent. *Med. Fac. Landbouwwet. Rijksuniv. Gent* **55**, 1481-1483.
- Gorecki J., Bortone G. and Tilche A. (1993) Anaerobic treatment of the centrifuged solid fraction of piggery waste-water in an inclined plug flow reactor. *Wat. Sci. Technol.* **28**, 107-114.
- Graaf A. A. van de, Mulder A., Bruijn P. de, Jetten M. S. M., Robertson L. A. and Kuenen J. G. (1995) Anaerobic ammonium oxidation is a biologically mediated process. *Appl. Environ. Microbiol.* **61**, 1246-1250.
- Graaf A. A. van de, Bruijn P., Robertson L. A., Jetten M. S. M. and Kuenen J. G. (1996) Autotrophic growth of anaerobic, ammonium-oxidising microorganisms in a fluidized bed reactor. *Microbiology (UK)* **142**, 2187-2196.
- Jetten M. S. M., Logemann S., Muyzer G., Vries S. de, Loosdrecht M. C. M., Robertson L. A. and Kuenen J. G. (1997) Novel principles in the microbial conversion of nitrogen compounds. *Antonie van Leeuwenhoek* **41**, 75-93.
- Loosdrecht M. C. M. van and Jetten M. S. M. (1996) De RWZI (AWZI) van de toekomst; een combinatie van het ANAMMOX-proces en het SHARON-proces. In *25 jaar toegepast onderzoek waterbeheer* (Edited by Klapwijk S. P.), pp. 155-172. STOWA, Utrecht, The Netherlands. ISBN 90-74476-59-7.
- Mulder A., Graaf A. A. van de, Robertson L. A. and Kuenen J. G. (1995) Anaerobic ammonium oxidation discovered in a denitrifying fluidised bed reactor. *FEMS Microbiol. Ecol.* **16**, 177-184.
- Sandberg M. and Ahring B. K. (1992) Anaerobic treatment of fish meal process waste-water in a UASB reactor at high pH. *Appl. Microbiol. Biotechnol.* **36**, 800-804.
- Tijhuis L., Rekswinkel H. G., Loosdrecht M. C. M. and Heijnen J. J. (1994) Dynamics of population and biofilm structure in the biofilm airlift suspension reactor for carbon and nitrogen removal. *Wat. Sci. Technol.* **29**, 377-384.
- Walsh J. L., Valentine G. E. and Ross C. C. (1991) Agricultural wastes. *Res. J. Wat. Pollut. Control Fed.* **63**, 452-459.
- Wiesmann U. (1994) Biological nitrogen removal from wastewater. *Adv. Biochem. Engng Biotechnol.* **51**, 113-153.