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Treatment of anaerobic sludge digester effluents by the CANON process in an air pulsing SBR

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ABSTRACT

The CANON (Completely Autotrophic Nitrogen removal Over Nitrite) process was successfully developed in an air pulsing reactor type SBR fed with the supernatant from an anaerobic sludge digester and operated at moderately low temperatures (18–24 °C). The SBR was started up as a nitrifying reactor, lowering progressively the dissolved oxygen concentration until reaching partial nitrification. Afterwards, an inoculation with sludge containing Anammox biomass was carried out. Nitrogen volumetric removal rates of 0.25 g N L^{-1} d^{-1} due to Anammox activity were measured 35 d after inoculation even though the inoculum constituted only 8% (w/w) of the biomass present in the reactor and it was poorly enriched in Anammox bacteria. The maximal nitrogen removal rate was of 0.45 g N L^{-1} d^{-1}. By working at a dissolved oxygen concentration of 0.5 mg L^{-1} in the bulk liquid, nitrogen removal percentages up to 85% were achieved.

The reactor presented good biomass retention capacity allowing the accumulation of 4.5 g VSS L^{-1}. The biomass was composed by ammonia oxidizing bacteria (AOB) forming fluffy structures and granules with an average diameter of 1.6 mm. These granules were composed by Anammox bacteria located in internal anoxic layers surrounded by an external aerobic layer where AOB were placed.

1. Introduction

Supernatants of anaerobic sludge digesters are characterized by low COD/N ratios difficulting the conventional way of nitrogen removal via the nitrification–denitrification processes. From different researches, it is known that autotrophic ammonia removal was observed in several systems treating high ammonium and low organic carbon loads [1,2]. Around 15 years ago, the Anammox process was discovered as an alternative to remove ammonia from high nitrogen loaded wastewater in the absence of organic matter and oxygen. This process consists of the anaerobic oxidation of ammonia [3,4] using nitrite as electron acceptor according to the stoichiometry described by Strous et al. [5] according to Eq. (1).

$$NH_4^+ + 1.3NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2H_2O \quad (1)$$

The Anammox bacteria belong to the Plantomycetales group and operate in anoxic conditions. These bacteria have been detected in several wastewater treatment plants all around the world [6]; however, their slow growth rate and large doubling time of around 11 d [7] makes the start up of Anammox processes difficult.

A feasible treatment for digester supernatants comprises two processes: one aerobic, the partial nitrification, where 50% of ammonia is oxidized to nitrite and an anoxic one, the Anammox process, where ammonia and nitrite are converted to nitrogen gas producing a small amount of nitrate. The partial nitrification is achieved using different operational strategies:

(1) Increasing free ammonia concentration working at high pH values and limiting the growth of nitrite oxidizing bacteria (NOB) due to their higher sensitivity to free ammonia inhibition than ammonia oxidizing bacteria (AOB) [8].
(2) Decreasing the dissolved oxygen (DO) concentration due to the lower oxygen affinity of the NOB compared to AOB [9].
(3) Operating at temperatures above 25 °C since the maximum specific growth rate of the AOB will be higher than that of NOB at these conditions. This is the base of the SHARON process [10].

Partial nitrification and Anammox processes can be performed in two different units as the Sharon-Anammox combined system. The overall nitrogen removal in the Sharon-Anammox process, compared to the conventional nitrification–denitrification processes, requires less oxygen supply (1.9 kg O_2 (kg N)$^{-1}$ instead of

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4.6 kg O_2 (kg N)$^{-1}$), no presence of carbon source (no need of 2.6 kg BOD (kg N)$^{-1}$) and has a lower sludge production (0.08 instead of approximately 1.0 kg VSS (kg N)$^{-1}$) [11].

The combination of partial nitrification and Anammox processes can also be carried out in a single reactor [12,13] and has been given different names: Deammonification, OLAND (Oxygen-Limited Autotrophic Nitrification–Denitrification) or CANON (Completely Autotrophic Nitrogen removal Over Nitrite process). Under oxygen-limited conditions a co-culture of aerobic and anaerobic ammonium-oxidizing bacteria can be established as a co-culture in a single reactor. In this case, it is basic the control of the dissolved oxygen concentration in the liquid media not only due to the irreversible inhibition caused by DO concentrations up to 0.5% of air saturation on the Anammox bacteria [14] but also in order to achieve the appropriated operational conditions to obtain the required partial nitrification. In those systems, the growth of NOB (and subsequent nitrate production) is prevented due to their lower affinity for oxygen compared to AOB and for nitrite compared to Anammox bacteria [15].

The applicability of the CANON process has already been demonstrated for the treatment of anaerobic digester supernatant. Full-scale plants are operating in Strass (AT), Hattingen (DE) or Glanerland (CH) [16,17].

The obtaining of the microaerobic conditions for the CANON process can be achieved in different kind of systems like SBR, gas-lift, etc. [12] and the air pulsing flow reactor. The use of pulsing air flow can be advantageous compared to the continuous mode due to the reduction of the aeration costs and better control of the required low dissolved oxygen concentrations. From previous works the use of pulsing flow patterns has provided beneficial effects on the operation of anaerobic systems improving the biomass aggregation [18,19] or in fermenting aerobic systems [20]. When biofilms and granular systems are used to treat wastewaters, external mass transfer resistance uses to be the limiting step. In this sense, pulsing reactors could be a suitable technology to improve mass transfer [18].

The aim of this work relies on the development of the CANON process in an air pulsing SBR under microaerobic conditions and at room temperature. The suitability of this reactor will be analyzed according to the start-up duration to achieve significant CANON capacity, the reached maximal nitrogen removal capacity and the stability of the process operation.

2. Materials and methods

2.1. Reactor description

A SBR with a working volume of 1.5 L was used. Dimensions of the unit were: height of 465 mm and inner diameter of 85 mm, the height to diameter ratio being 5.5. The exchange volume was fixed at 50%. A set of two peristaltic pumps was used to introduce the feeding solution (on top of the reactor) and to discharge the effluent (at medium height in the column reactor), respectively. A programmable logic controller Siemens model S7-224CPU controlled the actuations of the pumps and valves, and regulated the different periods of the operational cycle. Air was supplied by an air pulsing device operated at a constant frequency of 0.09 s^{-1}, meaning that air is supplied in repeated cycles of 1 s of flow and 10 s without flow. The function of the air pulses consists of providing a good mixture to the liquid media and supplying the required DO concentration for the activity of the biomass.

2.2. Operational conditions

The reactor was operated at room temperature (18–24 °C) and the pH value ranged around 7.7 ± 0.2, both without control. The DO concentration ranged between 0.2 and 4.0 mg L^{-1} along the operational period. The DO concentration in the system was regulated by changing the air volume injected in each pulse and keeping the frequency of pulsation constant. The air volume was adjusted varying the aeration flow that ranged between 1.0 and 3.5 L min^{-1} during the experiment. The net air consumption can be obtained by multiplying the aeration flow time the pulsation frequency and this value ranged from 120 to 425 L d^{-1}.

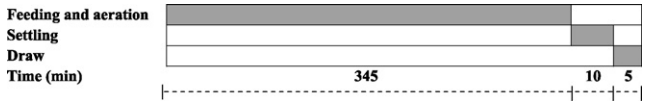

Fig. 1. Distribution of the operational cycles.

The SBR was operated in cycles of 6 h distributed according to the scheme described in Fig. 1. The hydraulic retention time was fixed at 0.5 d with a feeding velocity of 2.2 mL min^{-1}. It is important to point out that the SBR operates in semicontinuous mode and that the feeding and aeration phases take place simultaneously during 95% of the cycle time.

The SBR was firstly fed with a synthetic autotrophic media and then with the effluent of an anaerobic sludge digester of the WWTP of Lugo (Spain). The synthetic media was composed by 0.018 g KH_2PO_4 L^{-1}, 0.047 g K_2HPO_4 L^{-1}, 0.012 g $MgSO_4$ L^{-1}, 0.005 g KCl L^{-1}, 2 g $NaHCO_3$ L^{-1}, 0.15 g NH_4^+-N L^{-1} as ammonium chloride and trace elements. The industrial wastewater was diluted with tap water 1:1 to reach concentrations of ammonium that ranged from 0.15 to 0.35 g NH_4^+-N L^{-1}.

The reactor was operated in five different operational stages to test the effect of DO concentrations on the reactor performance (Table 1). Regarding the cycle operation the DO concentration remained practically constant during the aeration period; its value decreased to values close to zero during the 15 min corresponding to the settling and withdrawal phases.

In addition, on day 125, the reactor was inoculated with 0.16 g of volatile suspended solids (VSS) of biomass from another reactor (representing the 8% (w/w) of the total biomass amount present in the reactor and a concentration of 0.1 g VSS L^{-1}) with an Anammox activity of 0.05 g N g VSS^{-1} d^{-1} at 25 °C.

2.3. Analytical methods

The pH and the concentrations of DO, ammonium, VSS were determined according to the Standard Methods [21]. Nitrite and nitrate concentrations were determined by capillary electrophoresis [22]. Concentrations of total organic carbon (TOC) and total inorganic carbon (TIC) were measured with a Shimadzu analyser (TOC-5000). The morphology and size distribution of the granules were measured regularly using an image analysis procedure [23] with a stereomicroscope (Stemi 2000-C, Zeiss) incorporating a digital camera (Coolsnap, Roper Sicientific Photometrics). For the digital image analysis the programme Image Pro Plus was used.

Bacterial populations were identified by the Fluorescence in Situ Hybridization (FISH) technique. Biomass samples from the reactor were collected, disrupted and fixed according to the procedure

Table 1
Operational stages conditions.

Stages	Days	DO (mg L^{-1})	Observations
I	0–54	3.1 ± 1.1	Synthetic feeding
II	55–79	2.4 ± 0.8	
III	80–124	2.4–0.5	Day 95: Feeding change to supernatant
IV	125–179	0.5 ± 0.1	Day 125: Anammox inoculation
V	180–350	0.3 ± 0.2	Day 180: System destabilization

Table 2
Targeted organisms and the corresponding formamide (F) percentages for the used oligonucleotide probes.

Probe	Probe sequence (5′ → 3′)	% F	Targeted organisms	Ref.
PLA46	GAC TTG CAT GCC TAA TCC	30	Planctomycetales	[25]
Amx820	AAA ACC CCT CTA CTT AGT GCC C	40	Anaerobic ammonium-oxidizing bacteria *Candidatus* "Brocardia anammoxidans" and *Candidatus* "Kuenenia stuttgartiensis"	[26]
Alf1b	CGT TCG YTC TGA GCC AG	20	Alphaproteobacteria, some Deltaproteobacteria, Spirochaetes	[27]
Gam42a	GCC TTC CCA CAT CGT TT	35*	Gammaproteobacteria	[27]
NSO190	CGA TCC CCT GCT TTT CTC C	55	Betaproteobacterial ammonia-oxidizing bacteria	[28]
NEU653	CCC CTC TGC TGC ACT CTA	40*	Most of the halophilic and halotolerant *Nitrosomonas* spp.	[29]
Ntspa712	CGC CTT CGC CAC CGG CCT TCC	50*	Most members of the phylum *Nitrospirae*	[30]
Nit3	CCT GTG CTC CAT GCT CCG	40*	*Nitrobacter* spp.	[31]

* Used with an equimolar amount of corresponding unlabelled competitor oligonucleotide probe.

described by Amann et al. [24] with 4% paraformaldehyde solution. Hybridization was performed at 46 °C for 90 min adjusting formamide concentrations at the percentages shown in Table 2. The used probes for in situ hybridization were 5′ labelled with the fluorochromes fluorescein isothiocyanate (FITC) and Cy3. Fluorescence signals were recorded with an acquisition system (Coolsnap, Roper Sicientific Photometrics) coupled to an Axioskop 2 epifluorescence microscope (Zeiss, Germany).

2.4. Calculations

2.4.1. Nitrogen removal rates

Ammonia and nitrite oxidation rates (AOR and NOR, respectively) and nitrogen removal rate by Anammox bacteria (ANR) were estimated based on nitrogen balances and stoichiometry according to Eqs. (2)–(5). Due to the low C/N ratio of anaerobic digester supernatants, no heterotrophic denitrification was considered, assuming that the removal of nitrogen is only due to Anammox reaction.

$$\Delta N = (NH_4^+ - N_{inf}) - ((NH_4^+ - N_{eff}) + (NO_2^- - N_{eff}) + (NO_3^- - N_{eff})) \quad (2)$$

$$AOR = \frac{(NH_4^+ - N_{inf}) - (NH_4^+ - N_{eff}) - (\Delta N/2.04)}{HRT} \quad (3)$$

$$NOR = \frac{(NO_3^- - N_{eff}) - (0.26 \cdot \Delta N/2.04)}{HRT} \quad (4)$$

$$ANR = \frac{\Delta N}{HRT} \quad (5)$$

Being NH_4^+-N_{inf} the ammonium concentration in the influent (mg N L^{-1}); NH_4^+-N_{eff}, NO_2^--N_{eff}, NO_3^--N_{eff} the ammonium, nitrite and nitrate concentrations in the effluent (mg N L^{-1}), respectively. AOR, NOR and ANR are expressed in mg N L^{-1} d^{-1}.

2.4.2. Observed yield

The biomass production during an interval of time was estimated using the observed yield that can be obtained according to Eq. (6) as:

$$Y_{obs} = \frac{Y}{1 + b \cdot SRT} \quad (6)$$

Being Y the stoichiometric yield (g VSS (g N)$^{-1}$) and b the decay rate constant (d^{-1}), these values for AOB, NOB and Anammox bacteria were taken from Hao et al. [32].

3. Results

3.1. Removal of nitrogen compounds

The SBR reactor was operated for 400 d (data not shown) previously to the 350 operation days of the present work. A synthetic media was used as feeding media in order to better control the operation of the pulsing reactor since no information is available about nitrification in aggregates formed in this kind of reactors. The efficiency of the reactor was estimated by the determination of the concentrations of ammonium, nitrite and nitrate in the liquid samples collected at the end of the operational cycle. These concentrations were representative of the whole cycle, due to the length of the feeding period as it can be stated from performed cycle measurements. During the first 54 d (Step I), 0.25 g N L^{-1} d^{-1} of ammonia were oxidized to nitrate operating the reactor at a mean DO concentration in the liquid media of 3.1 mg L^{-1} (Fig. 2). From this point and in order to achieve partial nitrification in the system, the DO concentration was diminished by stepwise decrease in the air flow supplied in each pulse. From days 55 to day 79 the mean DO concentration was fixed at 2.4 mg L^{-1}. Under these conditions, no complete nitrification was achieved and nitrite accumulated in the liquid media due to DO limitation of NOB which present lower oxygen affinities for oxygen than AOB. From day 80 onwards the DO concentration was gradually decreased reaching a value of 0.5 g L^{-1} at the end of Stage III. During this Stage III, on day 95 of operation, the synthetic media was substituted by the supernatant of an anaerobic digester in order to prove that the system was able to operate in stable conditions treating this effluent. At the end of the Stage III, partial nitrification with 1:1 molar ratio of NH_4^+/NO_2^- was achieved while nitrate was absent due to NOB oxygen limitation. On day 125 of operation (Stage IV), once suitable conditions to grow Anammox bacteria (low DO concentration and equal concentrations of ammonium and nitrite) were achieved, 100 mL of sludge containing Anammox bacteria were inoculated to the reactor. One month after inoculation, significant decrease of ammonium and nitrite concentrations together with the slight increase of nitrate concentration and disappearance of nitrogen from the balance were measured in the reactor indicating the occurrence of an incipient Anammox activity. At this point it was considered that the nitrogen removal via the CANON process was established. To favour the nitrogen removal the nitrite concentration in the liquid media

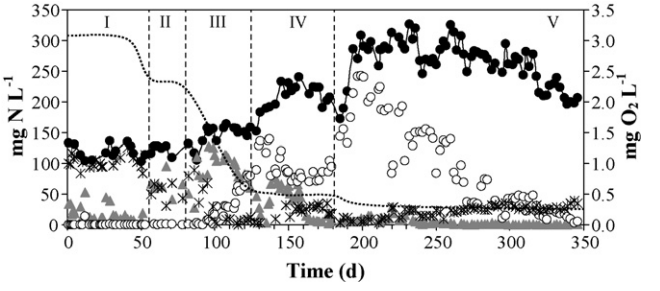

Fig. 2. Concentrations of nitrogen compounds in the influent mg NH_4^+-N·L^{-1} (●), and in the effluent mg NH_4^+-N·L^{-1} (○), mg NO_2^--N·L^{-1} (▲), mg NO_3^--N·L^{-1} (✕), and DO concentration (···).

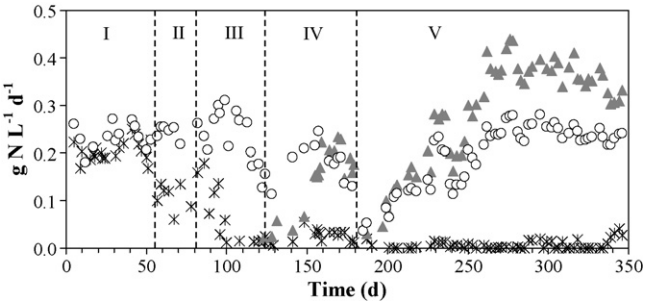

Fig. 3. Evolution of the ammonia and nitrite oxidation rates, AOR (○), and NOR (✳), respectively and nitrogen removal rate by Anammox bacteria (ANR) (▲).

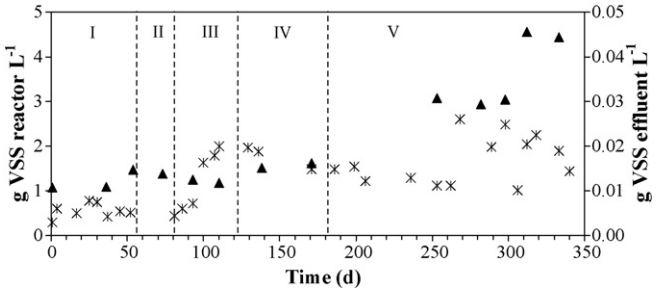

Fig. 4. VSS concentration in the reactor (▲) and VSS concentration in the effluent (✳).

was maintained close to zero, in order to avoid nitrite inhibition of Anammox bacteria, while the volume of air injected in each pulse was slightly increased to augment the ammonia oxidation to nitrite. The increase of air volume pulsed did not revert on an increase of DO concentration in the liquid media because of its fast consumption by the ammonia oxidizing bacteria. At the end of stage IV, a stable ANR of 0.25 g N L^{-1} d^{-1} was achieved.

On day 180 (Stage V), the feeding pump did not work properly and provoked the destabilization of the system due to ammonium limitation which caused DO increase and subsequent nitrite peak that reached 50 mg N L^{-1}. After 20 d the previous operational conditions were restored in the reactor and the nitrogen removal capacity increased continuously until the end of the experiment. From days 295 to 350, a stable operational period for the CANON process was obtained with an average ANR of 0.36 g N^{-1} L^{-1} d^{-1} (Fig. 3) and nitrogen removal percentage of 77%. The assumption made in the balances (Eqs. (3)–(5)) not considering heterotrophic denitrification was justified by the low TOC removal of only 82 mg TOC L^{-1} d^{-1} which do not justified the amount of nitrogen removed from the reactor. Once nitrogen removal percentages close to the theoretical maximum value of 89% (according to Eq. (1)) were reached, no further study of the maximal ANR reachable in this system was performed. The obtained nitrogen removal efficiency demonstrates that the growth of NOB is avoided due to nitrite and oxygen limitations.

3.2. Evolution of biomass

During the two first stages, the biomass concentration in the reactor was of 1.5 g VSS L^{-1} and solids concentration in the effluent was of 5 mg VSS L^{-1} which allowed working at a solids retention time (SRT) of 150 d (Fig. 4). Solids concentration in the effluent increased up to 20 mg VSS L^{-1} during stage III whereas the biomass concentration in the reactor remained constant, reducing the SRT down to 40 d. This concentration in the effluent remained constant until the end of the operation and due to the increase of biomass concentration up to 4.5 g VSS L^{-1} (stage V) the initial value of SRT was restored.

Biomass production during stage V was calculated taking into account the nitrogen consumption and the observed yield coefficients under these operational conditions (Eq. (6)). The estimated amounts of biomass produced corresponding to AOB, NOB and Anammox bacteria were 1.27; 0.01 and 3.64 g VSS, respectively for the whole stage, which involves a retention capacity of 87% of generated biomass. A high biomass retention capacity is a key factor for a fast start-up of bioreactors working with slow growing biomass such as ammonium oxidizing and Anammox bacteria.

A significant change in size, colour and aspect of the biomass was observed from stage I to stage V (Fig. 5). During the two first stages, the biomass was composed by granules with a mean diameter of 0.8 mm. After the decrease in the DO concentration, AOB formed fluffy structures in order to overcome diffusion limitation into the granules. As the ANR was increasing after the inoculation, an increase in the average diameter of the granules was observed reaching values around 1.6 mm, doubling the initial size, with some granules reaching 3.6 mm of diameter. This increase could be attributed to the development of granular Anammox biomass during this period.

Therefore, according to Nielsen et al. [33], two different biomass types are expected to perform the CANON process, a fluffy structure performing mainly partial nitrification and granules where a thin layer of AOB consumes oxygen protecting and providing nitrite to Anammox bacteria responsible of the autotrophic nitrogen removal.

3.3. Characterisation of biomass populations

In order to identify the main bacteria populations present in the reactor the FISH technique was applied to biomass samples collected on day 262 (Fig. 6). By the application of FISH probes, bacteria belonging to the genus *Nitrosomonas* were identified as the dominant population in the samples accounting for the 85–65% of the total biomass according to a rough calculation. Anammox bacteria gave positive results of both PLA46 and Amx820 probes, indicating the presence of *Candidatus* Brocadia anammoxidans and/or *Candidatus* Kuenenia stuttgartiensis. The coexistence of ammonia

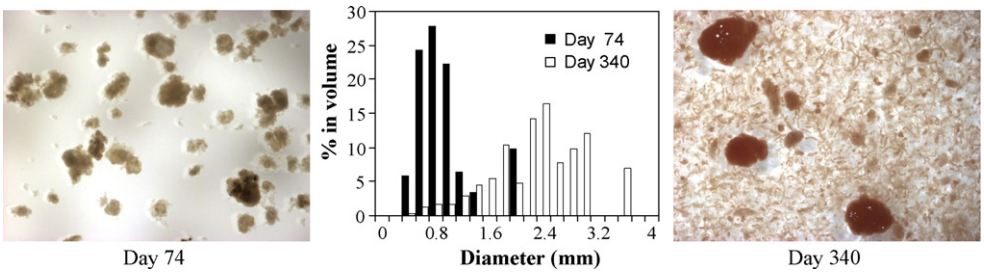

Fig. 5. Pictures of the biomass taken at 0.65× and size distribution by volume on days 74 and 340. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Table 3
Comparison of autotrophic nitrogen removal in one stage reactors.

Reactor[a]	Feeding	Volume (L)	T (°C)	ANR (g N L^{-1} d^{-1})	N removal (%)	DO (mg L^{-1})	Ref
Airlift	Synthetic	1.8	–	1.5	42	0.5	[34]
MBBR	Sludge liquor	50	30	0.36[b]	60	1.8	[35]
	Sludge liquor	319,000	–	0.25–0.35	70	<1	[17]
	Sludge liquor	2100	25	0.4	62	1.9	[36]
RBC	Synthetic	44	29	1.05	89	0.6	[37]
	Synthetic	50	30	1.80	88	0.3	[38]
	Sludge liquor	50	14	0.42	42	1.0	[38]
MABR	Synthetic	4	35	0.77	89	0.5	[39]
UGBR	Sludge liquor	10	30	0.06	76	0.6	[40]
SBR	Sludge liquor	500,000	28	0.7	86	0.3	[16]
	Sludge liquor	1.5	21	0.36	78	0.5	This study

[a] MBBR: Moving Bed Biofilm Reactor, RBC: Rotating Biological Contactor, MABR: Membrane Aerated Biofilm Reactor, UGBR: Upflow Granular Bed Reactor.
[b] Estimated from published data.

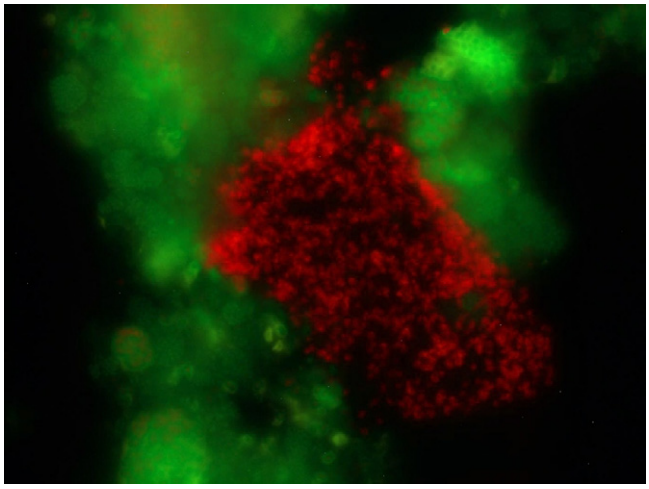

Fig. 6. FISH analysis of biomass from CANON reactor with NEU653 (green) and AMX820 (red) probes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

oxidizing and Anammox bacteria was well-supported by the results obtained with CANON process inside the pulsing SBR,

Positive results were obtained with probes Alf1b and Gam42a, indicating the presence of some α- and γ-Proteobacteria whereas no positive results were obtained when the probes Nit3 and Nitspa712 indicating the absence of NOB.

3.4. Comparison of the air pulsing SBR with other CANON reactors

The maximal nitrogen removal rate of 0.45 g N L^{-1} d^{-1} achieved in the present was in the range of 0.06–1.5 g N L^{-1} d^{-1} reported for different Deammonification/OLAND/CANON systems summarized in Table 3.

Several strategies have been tested to start-up and optimize the performance of reactors where autotrophic nitrogen removal takes place. Among them, two can be pointed out: (1) to inoculate an Anammox reactor with nitrifying biomass and to supply air to maintain microaerobic conditions [34,41] or (2) to operate a nitrifying reactor under oxygen-limited conditions to obtain the desired ammonia to nitrite molar ratio inside the system and then inoculate Anammox biomass [38,39].

As represented in Table 4, the second strategy seems to be more suitable because an important decrease of the Anammox activity is observed when the first strategy is applied [34]. The inoculation of Anammox enriched biomass in a nitrifying reactor accelerates the start-up process and allows registering important increases in the ANR after one or two months instead of several months or even years without inoculation. The full-scale example is very illustrative since in Strass, 2.5 years were necessary to start-up the plant in front of the 55 d registered in Glanerland once an enriched inoculum was available.

Gong et al. [39] multiplied by 16 the ANR in 40 d whereas in our study this increase was three times lower due to the lower temperature applied. Most of the CANON systems reported in literature were operated at temperatures around 30 °C. It must be taken into account that, according to the activation energy of the Anammox process, the activity at 24 °C would be only 40% of that at 35 °C. The feasibility of Anammox systems at temperatures around 20 °C was already reported by Isaka et al. [42], Dosta et al. [43] in Anammox reactors and Pynaert et al. [38] in one stage systems.

From these results, it is inferred that the air pulsing SBR could be a suitable technology to carry out autotrophic nitrogen removal at moderately low temperatures. The good retention capacity of the reactor and the low VSS concentration in the effluent will minimize the size or even the need of a posterior settler. The air pulsing flow would reduce the requirements of aeration with a consequent decrease of aeration costs and would allow an easy control of the dissolved oxygen level by changing the pulsing frequency or the amount of air pulsed. Finally, the high H/D ratio of the reactor will

Table 4
Comparison of autotrophic nitrogen removal start up.

Background	Inoculation	Initial ANR (g N L^{-1} d^{-1})	Final ANR (g N L^{-1} d^{-1})	Start-up time	Ref
Anammox reactor	Nitrifying sludge 0.2 g VSS L^{-1}	8.9	1.5	0 d	[34]
Nitrifying granules	Anammox 0.12 g VSS L^{-1}	0.05	0.25	35 d	This study
Nitrifying Biofilm	Anammox 1L	0.04	0.63	40 d	[39]
Glanerland (400 m^3) Nitritation/denitritation	Anammox from Strass WWTP 1.25 g VSS L^{-1}	0.15	0.63	55 d	[16]
Nitrifying Biofilm	Anaerobic granular sludge 10 g L^{-1}	~0	0.4	100 d	[38]
Nitrifying/denitrifying activated sludge	–	~0	0.36	1 year	[35]
Strass (500m^3): Nitritation/denitritation	Stepwise enrichment 4 L–0.3 m^3–2.4 m^3–500 m^3	~0	0.7	2.5 years	[16]

reduce the surface needed and makes this technology promising under an economical point of view. Nevertheless, more studies are necessary to study the maximal ANR that can be reached and the feasibility of a pulsing device at an industrial scale.

3.5. Conclusions

A CANON reactor was operated at room temperature (18–24 °C) by regulating the DO concentration of the liquid bulk. Significant nitrogen removal rates around 0.25 g N L^{-1} d^{-1} were measured 35 d after inoculation of Anammox biomass. The maximum nitrogen removal rate obtained was of 0.45 g N L^{-1} d^{-1} treating the supernatant from an anaerobic sludge digester.

The strategy of inoculating Anammox biomass in a reactor running partial nitrification allows a quick start-up of the CANON process. A high nitrifying activity in the reactor before the inoculation protects the Anammox bacteria from oxygen and provides them enough nitrite during the start-up.

The operation of the air pulsing SBR allows simple and robust regulation of the DO concentration of the liquid bulk for the stable operation of the CANON process.

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