Integrated fixed-film activated sludge ANITA™Mox process – a new perspective for advanced nitrogen removal

F. Veuillet, S. Lacroix, A. Bausseron, E. Gonidec, J. Ochoa, M. Christensson and R. Lemaire

ABSTRACT

ANITA™Mox is a Veolia process using moving-bed biofilm reactor (MBBR) technology tested and validated in full-scale for energy- and cost-effective autotrophic N-removal from sidestream effluent using anammox (ANaerobic AMMonium OXidation) bacteria. In order to increase the ANITA™Mox process performances under different operating conditions (e.g. mainstream and sidestream application), substrate transport and accessibility inside the biofilm must be enhanced. In this work, (i) two laboratory scale biofilm ANITA™Mox reactors were operated using different configurations (IFAS – integrated fixed-film activated sludge – and MBBR) and (ii) the distribution of the anammox (AnAOB) and ammonia-oxidizing bacteria (AOB) in the suspended sludge and the biofilm was characterized using molecular tools (qPCR). This study showed that in IFAS configuration, the ANITA™Mox process achieved very high N-removal rate (up to 8 $gN/m^2.d$), which was three to four times higher than that achieved in the pure MBBR mode. The high concentration of suspended solids (mixed liquor suspended solids (MLSS)) in the bulk obtained within the IFAS mode induces a very efficient bacterial distribution between the AOB and AnAOB population. AnAOB activity mainly occurs in the biofilm (96% of total AnAOB in the reactor), whereas nitritation by AOB mostly takes place in the suspended phase (93% of total AOB). This spatial distribution observed in the IFAS reactor results from a natural selection due to more easily substrate accessibility for AOB in the bulk (NH_4^+, O_2) creating higher nitrite concentration in the bulk liquid compare to pure MBBR mode. The efficient control of MLSS level in the IFAS reactor is a key parameter to enhance the nitrite production by AOB and increase the substrate availability in the AnAOB-enriched biofilm leading to higher N-removal rate. These promising results obtained at laboratory scale have been further confirmed in on-going full-scale IFAS ANITA™Mox trials opening new roads for the widespread application of a very compact and robust ANITA™Mox process for sidestream but also mainstream cost-effective N-removal.

Key words | Anammox, ANITA Mox, IFAS, MBBR, nitrogen removal, sidestream treatment

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INTRODUCTION

ANITA™Mox is a one-stage deammonification moving-bed biofilm reactor (MBBR) process developed for autotrophic N-removal (i.e. anammox: anaerobic ammonium oxidation) and recently implemented at full-scale in four plants in Europe (Sjölunda, Växjö – Sweden; Holbæk, Grindsted – Denmark) with two more in design in the USA (James River – VA, Durham – NC) for sidestream treatment of N-rich effluent (Christensson *et al.* 2013). Compared to conventional nitrification–denitrification systems, deammonification systems such as ANITA™Mox can reduce aeration requirement by 60% with no need of external carbon addition, making it a very cost- and energy-effective process. The high NH_4-removal rate achieved ($1.2\ kgN/m^3.d$) is explained by synergetic interaction in the biofilm carrier between anammox bacteria (AnAOB) (internal biofilm layers) and ammonia-oxidizing bacteria

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(AOB) (external layer). This microbial synergy is strongly influenced by transport limitations and substrate availability inside the biofilm, which are dependent on different environmental factors such as morphology, density and thickness of the biofilm, temperature, substrate concentration (NO_2^-, NH_4^+, O_2) and shear stress. Therefore, in order to improve the ANITA™Mox process performances under different operating conditions (e.g. mainstream and sidestream application), substrate transport must be enhanced. Several studies investigated the effect of combining suspended cultures and fixed biomass into one system called integrated fixed-film activated sludge (IFAS) for municipal (Ødegaard *et al.* 2000; Al-Sharekh & Hamoda 2001) and industrial (Wessman *et al.* 2004) wastewater treatment. More specifically, Paul *et al.* (2006) have reported that a clear spatial distribution of microbial population between floccular biomass (heterotrophs) and fixed biomass (nitrifiers) leads to higher chemical oxygen demand (COD) and N-removal performances.

The objectives of this paper are (i) to demonstrate the advantages of the IFAS configuration for the ANITA™Mox process using comparative laboratory scale MBBR and IFAS reactors, (ii) to determine the distribution of the anammox and AOB between suspended sludge and biofilm-carrier using quantitative polymerase chain reaction (PCR) and (iii) to present the first full-scale IFAS ANITA™Mox results for sidestream treatment.

MATERIAL AND METHODS

Material

Experimental setup

Two 7 L laboratory scale reactors were operated in parallel to compare ANITA™Mox process performances under pure MBBR (R1) and IFAS (R2) configuration with a settling tank of 1.9 L for sludge recycling. The suspended sludge solids retention time (SRT) in IFAS (R2) was kept around 5 d (±2 d) through manual sludge extraction. Each reactor was equipped with sensors for measuring temperature, pH, dissolved oxygen (DO), NH_4^+-N and NO_3^--N. The temperature was controlled at 30 ± 0.5 °C via jackets of the double-walled reactors, the pH maintained between 7 and 8 by acid and base dosing, NH_4^+-N in the treated effluent controlled at 10–150 mgN/L and DO was kept in the range of 0.1–1 mgO_2/L. In both reactors aeration was supplied by diffusion through small holes in a plastic tube at an air flow rate of 0–5 L/min. In order to control the ammonia concentration in the outlet of the reactors and ensure the same effluent quality in both reactors (and therefore the same substrate diffusion limitation in the biofilm), a simple regulation based on the on-line NH_4^+-N sensor was used. If the NH_4^+-N level in the reactor was higher than the desired setpoint, the feeding pump was switched off until the NH_4^+-N level decreased below this setpoint.

Carrier media

K5 plastic carriers from AnoxKaldnes (protected surface area of 800 m^2/m^3) were used for biofilm growth in both reactors. The carrier filling degree was 43% in both reactors. This filling degree was chosen to guarantee a good mixing in the relatively narrow laboratory reactors but higher filling degree (up to 55%) can be used in full-scale ANITA™Mox with K5 carriers. As a result of this filling degree the total protected surface area in each reactor was 2.41 m^2.

Influent

Influent used in this study was reject water from a mesophilic anaerobic sludge digester collected in a wastewater treatment plant (WWTP) near Paris. The reject water was sampled once a week and stored for up to 72 hours at 4 °C. The mean reject water characteristics were: NH_4^+-N = 907 ± 200 mgN/L; COD = 414 ± 150 mg/L; biochemical oxygen demand = 29 ± 5 mg/L; total suspended solids (TSS) = 400 ± 200 mg/L; alkalinity = 4,200 ± 1,000 mg$CaCO_3$/L; pH = 8 ± 0.5. This sidestream effluent is very high in ammonia and low in COD with a ratio of COD/N = 0.45. However, the hard soluble COD represents on average 70% of the soluble COD, meaning that the soluble biodegradable COD to N ratio was around 0.025–0.03. PO_4^{3-} was very low in the reject water (<2 mgP/L) and KH_2PO_4 was added in the feed to be non-limiting with at least 2 mgPO_4^{3-}-P/L in the treated effluent.

Methods

Analytical methods

NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^{3-}-P, COD and soluble COD (sCOD) were measured using a colorimetric micro method with Dr Lange kits (Hach). Samples were filtered at 0.45 μm for soluble compounds analysis. Mixed liquor suspended solids (MLSS) and the volatile suspended solids were measured according to standard methods (AFNOR 2000, 2005).

Quantitative PCR

Quantification of AnAOB, AOB, NOB (nitrite-oxidizing bacteria) and total bacteria was performed with primers using Sso-Fast™ EVAGreen® (Bio-Rad, USA) containing nonspecific fluorophore EVAgreen. PCR was performed in 10 µl reaction mixtures with primers at a final concentration of 0.4 µM each. EVAGreen quantitative PCR (qPCR) was performed in triplicate on each serial dilution of plasmid solution and in duplicate on each DNA sample. Standard curves were generated by using dilution series of plasmid solutions ranging approximately from 1×10^8 to 1×10^2 copies per reaction. A plasmid containing a fragment of 16S RNA gene from *Escherichia coli* was used for quantification of total bacteria. A plasmid containing a partial sequence of *hzo* gene from *Brocadia anammoxidans* was used for quantification of AnAOB. A plasmid containing a partial *amoA* gene from *Nitrosomonas europaea* was used for AOB quantification. A plasmid containing a partial *nxrA* gene from *Nitrobacter* was used for NOB quantification. All plasmids were synthesized by Eurofins MWG Operon (Ebersberg, Germany). PCR reactions were run on a CFX 96 thermocycler (Bio-Rad, USA).

Operational conditions

IFAS and MBBR were started with pre-colonized K5 carriers coming from a full-scale ANITA™Mox plant in Sjölunda, Sweden. Colonized media were collected and stored in effluent at 4 °C before introduction into the reactors. To prevent unwanted biofilm formation on the reactor wall surfaces, the walls were manually cleaned two times a week. A summary of the experiments is provided in Table 1.

Table 1 | Operational conditions

Stage (d)	Reactor	DO (mg/L)	Temperature °C	pH	NH_4^+-N_{out} (mgN/L)
I 0–47	R_1: MBBR R_2: MBBR	0.8–1	30	7.5 ± 3	150 ± 50
II 48–177	R_1: MBBR R_2: IFAS	0.4–0.8 0.1–0.2	30	7.5 ± 3	150 ± 50
III 178–212	R_1:MBBR R_2: IFAS	0.8 0.1–0.2	30	7.5 ± 3	70 ± 10
IV 213–330	R_1: MBBR R_2: IFAS	0.6 0.1–0.2	30	7.5 ± 3	30 ± 5
V 331–388	R_1: MBBR R_2: IFAS	0.6 0.1–0.25	30	7.5 ± 3	10 ± 2

Full-scale IFAS demonstration

The full-scale demonstration of the IFAS configuration was conducted in the full-scale MBBR ANITA™Mox plant at Sjölunda WWTP (550,000 people equivalent), Malmo, Sweden. The full-scale plant consists of four separate 50 m^3 cylindrical tanks. The main design and operating parameters and the reject water composition were detailed by Christensson *et al.* (2013).

One of the four MBBR tanks filled at 50% with K5 carriers was converted to an IFAS reactor by installing a conical clarifier for sludge retention (February 2013, Day 960). The clarifier had to be fitted inside the existing MBBR due to space constraint on site. The clarifier has a surface area of 5 m^2 and a total volume of 7 m^3. Compared to typical operation for MBBR ANITA™Mox, the IFAS reactor was operated at lower DO (0.2–0.6 mgO_2/L versus 1.0–1.3 mgO_2/L) and at higher MLSS level (2–4 g/L versus 0.02–0.2 g/L).

RESULTS AND DISCUSSION

The results for comparison of the MBBR and IFAS reactors (R1 and R2) are presented with emphasis on (i) ammonia, nitrite and TSS influence on N-removal efficiencies, (ii) biofilm morphology and (iii) the AnAOB and AOB population distribution between suspended and fixed biomass.

Nitrogen removal performances

Figure 1 shows the evolution of the N-removal rate in the MBBR (R1) and IFAS (R2) reactors and the MLSS in the IFAS during the entire experiment period. During Stage I, N-removal in both reactors increased from 1.2 ± 0.1 gN/m².d to 2.0 ± 0.2 gN/m².d due to some loss of activity during the carrier transportation and the initial reseeding procedure. Steady-state performances in both reactors operated as pure MBBR ANITA™Mox were obtained after 50 days using a DO around 0.8 mgO_2/L. On Day 48, R2 was switched to IFAS mode while R1 remained in pure MBBR mode with no sludge recycling. In Stage II, N-removal rate for R1 remained around 1.9 ± 0.3 gN/m².d (Figure 1) while, for R2 (IFAS), the positive effect of the IFAS configuration is very clear with N-removal rate increasing from 2 to 6–8 gN/m².d between Day 56 and 84 with MLSS level reaching up to 3 g/L with the use the clarifier to recycle part of the sludge. These very good

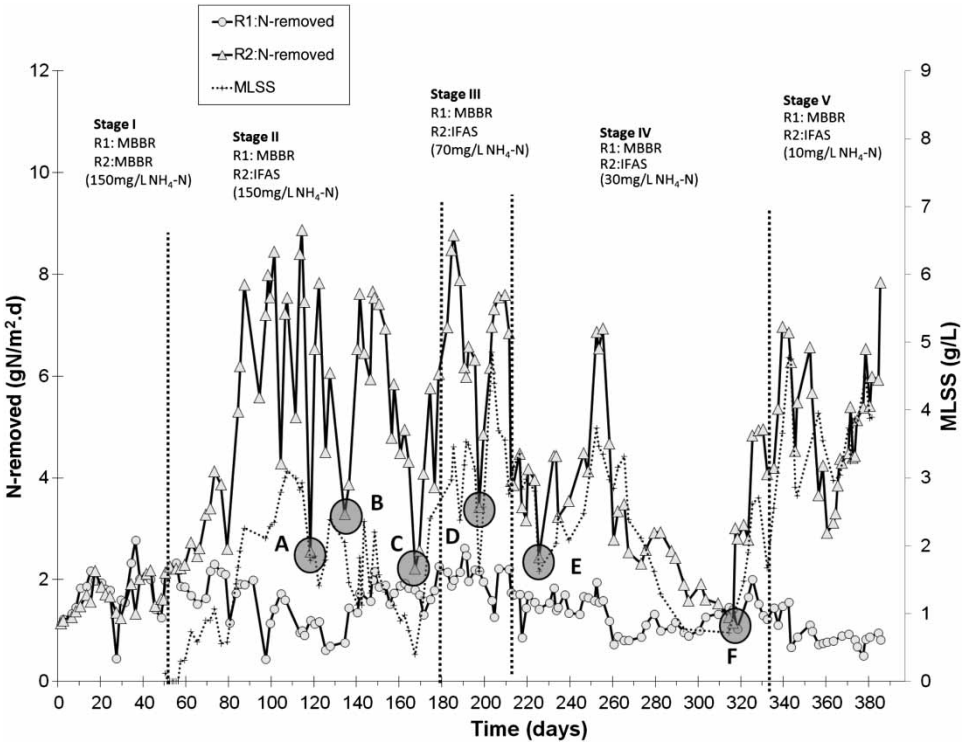

Figure 1 | Comparison of N-removal rate in MBBR (R1) and IFAS (R2) reactors and MLSS in IFAS (R2). Points A, B, C, D, E, F are identified as perturbation periods in IFAS mode.

performances remained constant for R2 during the stabilized period between Day 86 and 122.

To evaluate the effect of MLSS on the N-removal rate in the IFAS configuration, trials under lower MLSS level were performed (points A, B, C in Figure 1). The decrease of the suspended sludge concentration immediately impacted the N-removal rates in IFAS, but they quickly recovered to reach 6–8 gN/m².d again when MLSS was increased to its previous level (2–3 g/L).

From Day 178 (Stage III) the NH_4^+ outlet concentration was controlled at 70 mgNH_4^+-N/L in both reactors. The N-removal rates in IFAS stayed constant during Stage III at 8 gN/m².d with MLSS increasing slightly to 2–3 g/L. The robustness of the process was confirmed once again when MLSS was suddenly decreased from 3 to 1.5 g/L before reaching 3 g/L again a few days after (point D on Figure 1), with N-removal quickly recovering to 8 gN/m².d. N-removal for R1 (MBBR) was rather constant at 2 gN/m².d during Stage III.

During Stage IV, NH_4^+-N outlet concentration was further reduced to 30 mgNH_4^+-N/L to verify if the IFAS reactor could maintain a high N-removal rate under lower NH_4^+-N concentration in the reactor bulk liquid. N-removal initially decreased to 4 gN/m².d with MLSS slightly below 3 g/L but, when higher MLSS level was maintained in R2 between Day 245 and 260, N-removal of 7 gN/m².d could still be achieved. After Day 260, an unexpected change in the reject water composition was observed (sudden increase of salinity and possible toxicity likely due to on-going upgrade work on the WWTP digesters) which perturbed the operation of both reactors for 50 days. The suspended sludge in R2 (mostly constituted of AOB, see later) started to deflocculate and to be washed out of the clarifier as shown by the clear drop of MLSS in R2 down to 0.8 g/L, resulting in a much lower N-removal of 1.3 gN/m².d (point F in Figure 1). For R1 (MBBR), N-removal also decreased from 2 gN/m².d before this pollution event to 0.7 gN/m².d. The main composition of the collected reject water (i.e. NH_4^+, COD, MLSS) did not change during this period but the overall salinity did. The composition of reject water collected on site went back to normal after Day 314 (point F in Figure 1) and MLSS in R2 quickly increased back to 2.6 g/L at the end of Stage IV due to better floc formation and improved sludge separation in the clarifier. As a result, N-removal rate quickly increased to 5 gN/m².d in 12 days, and reached back to 7 gN/m².d after 30 days when MLSS reached 4–5 gMLSS/L. It further demonstrates that the IFAS process is very robust and resilient even after this unexpected event which lasted 50 days.

In Stage V, NH_4 outlet concentration was finally decreased to 10 mgNH_4^+-N/L. For R1, the N-removal

dropped to 1 gN/m^2.d, likely due to substrate (i.e. NH_4) diffusion limitation in the biofilm, which impacted the AnAOB activity. N-removal rate in R2 was proportional to MLSS concentration in the reactor. The low NH_4^+-N concentration in the bulk liquid resulted in slightly lower N-removal rate (i.e. 4–6 gN/m^2.d) but the effect of NH_4 diffusion limitation into the biofilm was less visible than for pure MBBR operation. The NH_4^+ bulk liquid concentration under which N-removal rate is impacted due to substrate diffusion limitation in the biofilm is related to the boundary layer thickness between the liquid and biofilm phases, which is dependent on the reactor hydrodynamics condition.

Effect of nitrite concentration in the bulk liquid

Figure 2 shows the relation between NO_2^--N level measured in the bulk liquid and the N-removal rate observed in each reactor during the entire experiment. N-removal in R1 was higher when NO_2^--N level was above 2 mgNO_2^--N/L but the NO_2^--N level rarely reached more than 4 mgNO_2^--N/L in the bulk liquid (Figure 2(a)). This relation between NO_2^--N level and N-removal rate is also observed for R2 with a very clear correlation between the two parameters up to 25 mgNO_2^--N/L (Figure 2(b)). The more MLSS in R2 (IFAS), the more AOB nitritation activity was obtained, leading to higher NO_2^- level in the bulk liquid and therefore better diffusion into the deep layer of the biofilm to reach the AnAOB.

It clearly demonstrates that one-stage deammonification biofilm processes are often limited by the AOB activity and the mass transfer limitation of substrate (i.e. NO_2^-) towards the AnAOB. Operating the ANITA™Mox in IFAS mode can therefore improve the process performance by enhancing the AOB activity and reducing the mass transfer limitation of NO_2^--N into the biofilm.

Repartition of AnAOB and AOB in biofilm and liquid phase

Table 2 shows the calculated repartition of AnAOB and AOB in the biofilm and liquid phase for each reactor on Day 147. In IFAS mode, AnAOB activity mainly occurs in the biofilm (96% of total AnAOB in the reactor), whereas nitritation by AOB mostly takes place in the suspended phase (93% of total AOB). This spatial distribution observed in the IFAS reactor results from a natural selection due to more easily substrate accessibility for AOB in the bulk (NH_4^+, O_2) creating higher nitrite concentration in the bulk liquid compared to pure MBBR mode.

NOB were always detected in both R1 and R2 but their activity was not impacting the overall deammonification process, as indicated by the measured ratio of NO_3^--N produced/NH_4^+-N removed that was always lower that 11% (stoichiometric ratio for deammonification). This ratio was close to 6% in R2 (IFAS) reactor during the test period, meaning that some heterotrophic denitrification took place using the small amount of COD available in the reject water together with hydrolysed particulate organics. For both reactors, the low DO strategy applied was very successful in washing-out NOB from the system while maintaining high AOB activity. For the IFAS reactor, the additional control of the suspended sludge SRT at only 5 days could also have participated in reducing NOB activity in the suspended sludge.

Dynamic model of spatial bacterial distribution in the MBBR and IFAS mode

The bacterial population distribution and specific role for both pure MBBR and IFAS deammonification configurations are schematically represented in Figure 3.

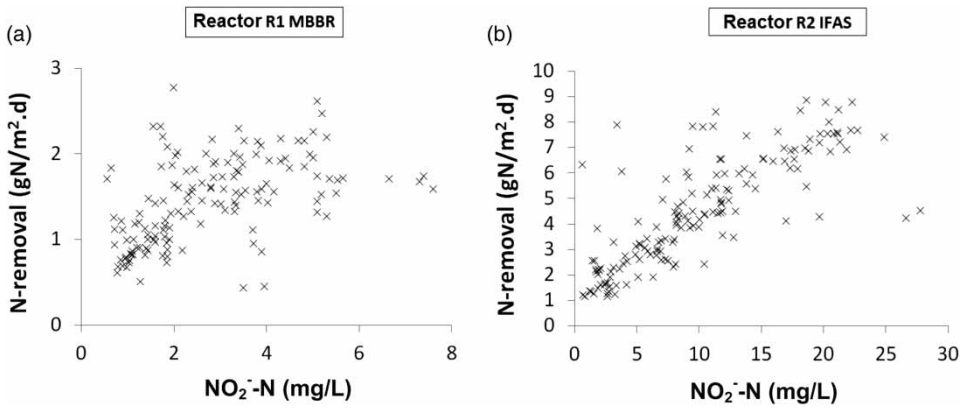

Figure 2 | Effect of nitrite concentration on N-removal rate under (a) MBBR and (b) IFAS modes.

Table 2 | Repartition of AnAOB, AOB and total bacteria in biofilm and liquid phase for each reactor on Day 147

Reactor	AnAOB		AOB		Total bacteria	
	Liquid	Biofilm	Liquid	Biofilm	Liquid	Biofilm
R1 (MBBR) (%)	1 ± 1	99 ± 1	1 ± 1	99 ± 1	8 ± 2	92 ± 2
R2 (IFAS) (%)	4 ± 1	96 ± 1	93 ± 2	7 ± 2	48 ± 2	52 ± 2

In the MBBR, the high proportion of AnAOB and AOB in the biofilm on the carrier and lower microbial growth in the bulk are the result of two mechanisms. Firstly, the low SRT of the suspended biomass (SRT = hydraulic retention time = 20–24 h) limits the autotrophic biomass growth in the suspended phase due to continuous wash-out. This condition promotes the growth of autotrophic bacteria (AnAOB and AOB) in the biofilm. Secondly, the differences of maximum specific growth rates between AnAOB (0.04–0.08 d^{-1}) and AOB (0.8–1.0 d^{-1}) can also explain the spatial distribution in the biofilm. Typically in the ANITA™Mox pure MBBR mode, the AOB are located in the outer layers of the biofilm to access oxygen, while AnAOB are located in the inner anoxic layer of the biofilm. This way the AnAOB are protected from oxygen, which is consumed in the external layers by AOB. Excess NH_4^+-N in the bulk liquid and NO_2^--N produced by AOB in the biofilm are transported to deeper layers of the biofilm by diffusion and by convection in the biofilm voids and micro-channels.

In the IFAS configuration, higher SRT for suspended biomass (around 5 days) enhances the AOB growth as flocculated biomass. Substrate diffusion limitation in flocs is less apparent than in biofilms which are thicker and denser. This leads to better substrate accessibility for AOB (oxygen, NH_4) in the suspended solids meaning that AOB that pre-existed on the outer layer of the biofilm gradually disappear from the biofilm due to a lack of oxygen (i.e. DO in bulk liquid is lower in IFAS than MBBR), which is now mostly consumed by AOB in the liquid phase. The biofilm is therefore almost exclusively composed of AnAOB with a very fine top layer of oxygen scavengers like AOB or normal heterotrophs. The larger AOB population in the IFAS configuration improves the overall flux of NO_2 produced for AnAOB but also the residual concentration in the bulk, improving the diffusion of the NO_2 through the basal layer of the biofilm where AnAOB are located and therefore increasing their active fraction in the biofilm. In IFAS and MBBR configurations, the global amount of AnAOB was similar, but the improvement of nitrite flux and production in IFAS mode led to a N-removal rate near the maximum values (i.e. 8 $gN/m^2.d$) obtained during fully anoxic AnAOB activity batch test with non-limiting substrate levels (i.e. NH_4^+ and NO_2^-).

Full-scale demonstration IFAS

Figure 4 presents the preliminary results obtained in the ANITA™Mox full-scale reactor at Sjölunda WWTP before and after the installation of the clarifier. Average temperature in the reactor during this test was about 30 ± 3 °C. The clarifier was installed on Day 960 and a stable performance was achieved after Day 985. During the stable period, average ammonia removal efficiency was 95% and average total nitrogen (TN) removal efficiency was 85% while the ratio of nitrate produced to ammonium removed (NO_3^--N prod/NH_4^+-N rem) was around 8.5% (Figure 4(b)).

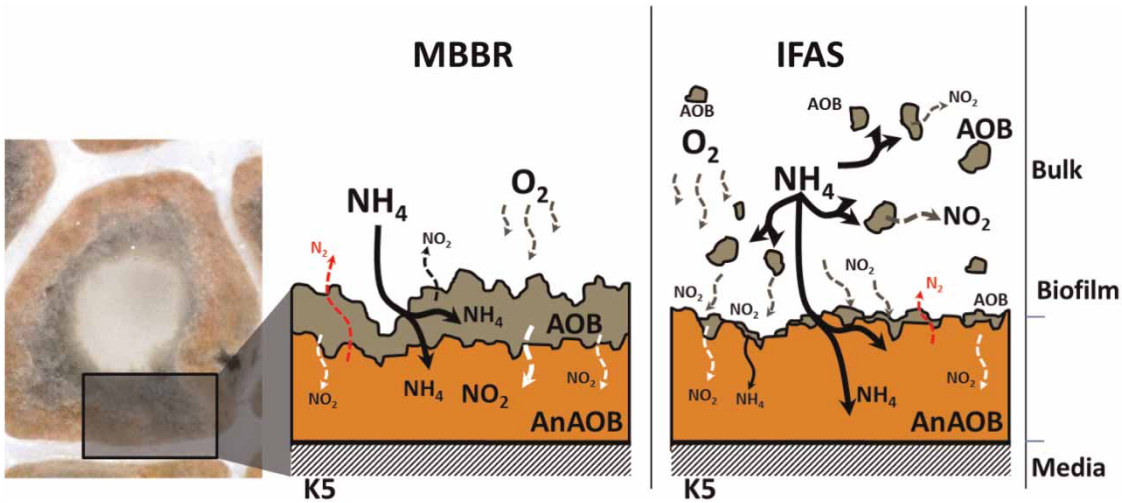

Figure 3 | Dynamic model of spatial distribution of bacterial populations involved in N-removal and biofilm structure in pure MBBR versus IFAS ANITA™Mox configurations.

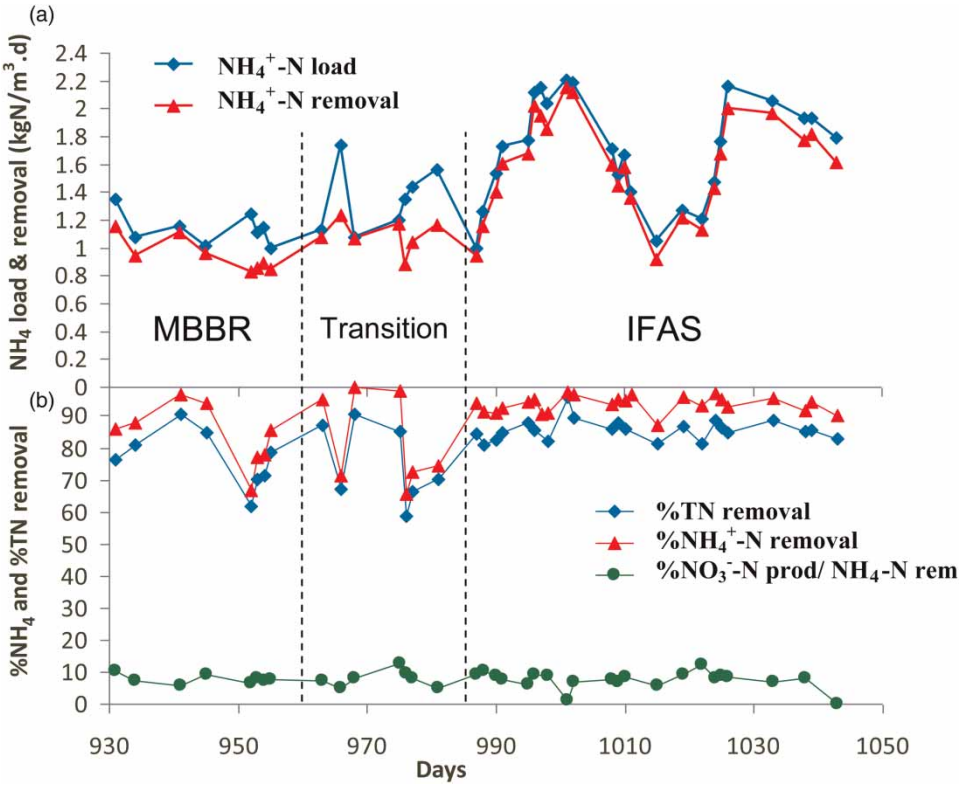

Figure 4 | Full-scale performance before and after switching to IFAS configuration: (a) NH_4^+-N loading and removal rates; (b) NH_4^+-N and TN removal efficiencies and % of NO_3^--N produced.

NH_4^+-N loading and removal rates increased sharply after switching to IFAS mode, reaching 2.2 kgN/m^3.d (Figure 4(a)) before dropping back to 1.2 kgN/m^3.d due to a shortage of reject water supply and some foaming issue in the reactor with the built-in clarifier, leading to difficulties in controlling the MLSS level in the tank. After modifying the operation of the IFAS system, NH_4^+-N loading and removal rates quickly reached back to 2.2 kgN/m^3.d.

Despite the MLSS variation, nitritation in the suspended sludge was enhanced at bulk DO concentration between 0.2 and 0.5 mg/L. Compared to the pure MBBR operation, higher nitrite level was observed (4–8 mgNO_2^--N/L) with the IFAS configuration. As indicated by the low ratio of NO_3^--N prod/NH_4^+-N rem measured in the reactor (<10%), the low DO condition applied in the IFAS reactor was sufficient to repress the NOB growth in the suspended sludge even with higher nitrite level. Due to the improvements of both nitritation and anammox activities with the IFAS mode, NH_4^+ removal rate has doubled compared to the pure MBBR configuration and is still expected to increase with the optimization of the settler operation.

CONCLUSION

- In the IFAS configuration at laboratory scale, the ANITA™Mox process reached a very high N-removal rate of up to 8 gN/m².d. These performances are three to four times higher than those achieved in pure MBBR mode with similar high TN and NH_4^+ removal efficiency (90% and 95% respectively).
- The IFAS mode induces a spatial differentiation of the N-removal microbial population: anammox are mainly located in the biofilm (96% of total AnAOB), whereas AOB are mostly located in the suspended phase (93% of total AOB).
- The efficient control of MLSS in the IFAS reactor is a key parameter to enhance the nitrite production by AOB and increase the substrate availability in the AnAOB-enriched biofilm, leading to higher N-removal rate.
- N-removal performance quickly recovered after perturbation periods, confirming the robustness of the IFAS configuration.
- IFAS ANITA™Mox is currently being tested at full-scale on a sidestream at Sjölunda WWTP with preliminary

N-removal rate up to 2.2 $kgN/m^3_{react}.d$ achieved so far and 3 $kgN/m^3_{react}.d$ expected after optimisation of the system.

- The easy physical separation between AOB-rich suspended sludge and AnAOB-rich biofilm carriers through the use of a non-clogging sieve is a clear advantage to secure the retention of AnAOB in the system.
- The very promising results obtained with the IFAS ANITA™Mox process for sidestream treatment open new attractive solutions for mainstream N-removal application due to the easy retrofit of the IFAS process into activated sludge systems with existing clarifiers.

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