Go or no go for gel entrapped nitrifiers? A Belgian case study

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Abstract In the coming years, as stricter environmental requirements are imposed, many European Union wastewater treatment plants (WWTP) need to be expanded and/or upgraded. This requires considerable investments. Optimising the renovation recourses can lead to significant savings. The use of entrapped nitrifying bacteria for upgrading of WWTP towards nutrient removal may be benificial. Long term pilot tests were performed to evaluate a so-called pellet reactor. Differences in performance and microbiological composition of classical activated sludge and the pellet reactor were investigated. FISH analyses showed (i) absence of *Nitrobacter* cells and (ii) high abundance of *Nitrospira* in the pilot reactors. Two Belgian WWTP make use of fine bubble aeration and could – theoretically – easily be renovated towards nitrogen removal using encapsulated nitrifiers. Financial aspects are commented on.

Keywords Entrapped nitrifiers; fluorescence in situ hybridisation; nitrification; polyethylene glycol; renovation; WWTP

Introduction

In the coming years, as stricter environmental requirements are imposed, many European Union wastewater treatment plants (WWTP) need to be expanded and/or upgraded. This requires considerable investment costs. Optimising the renovation recourses lead to significant savings. The presented study gives a possible alternative to upgrade large WWTPs towards nutrient removal.

Natural immobilisation of bacteria by spontaneous attachment to inert support materials is widely used in wastewater treatment systems, in so-called biofilm processes. An alternative technique is artificial immobilisation by entrapment. Various techniques using microorganisms entrapped in gels are known and used for different industrial applications (Wijffels *et al*., 1996). A process using immobilised microorganisms entrapped in polyethylene glycol (PEG) pellets was developed (Tanaka *et al*., 1994 and Sumino *et al*., 1997) for wastewater treatment. This technology may be shown beneficial with regard to retrofit of non-nitrifying WWTP toward nutrient removal.

Autotrophic nitrifying bacteria have a low growth rate compared to heterotrophic bacteria, with which they have to compete for oxygen. In activated sludge plants, high organic loading and low sludge age in combination with low availability of oxygen and the slow nitrifiers growth rate, are reasons that nitrification often does not occur. A favourable condition for nitrification is an increased sludge age, which can be obtained by making use of e.g. nitrifiers entrapped in PEG pellets.

The single sludge pre-denitrification process shown in Figure 1 has a capacity of twice or more that of a conventional activated sludge process. It facilitates the reconstruction of the existing facilities of conventional activated sludge for nutrient removal. To evaluate both (1) treatment performance and (2) microbiology, the pellet reactor was tested at pilot-scale.

Methods

Pilot plants

The activated sludge process with entrapped nitrifiers was tested in parallel with a classical activated sludge treatment. The operational conditions of the tests are listed in Table 1.

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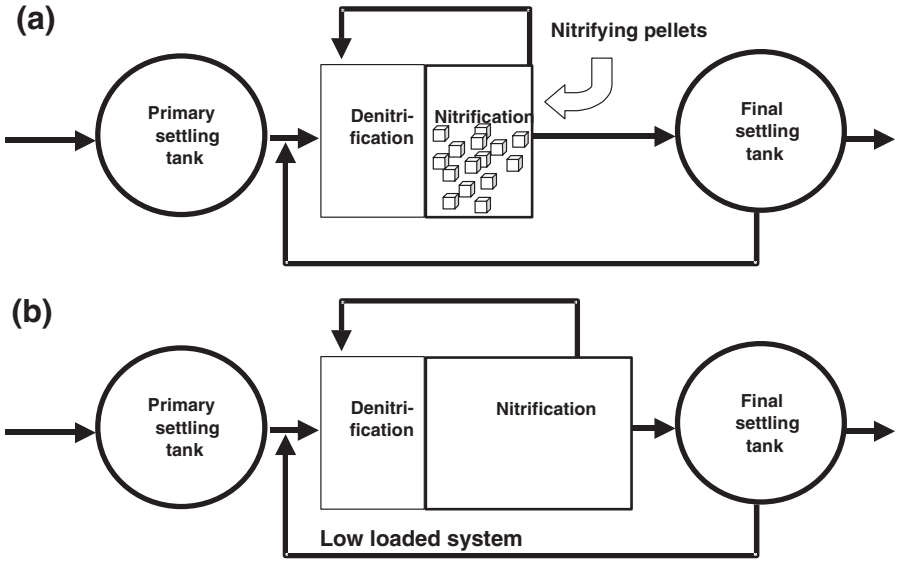

Figure 1 Schematic representation of nutrient removing installations: (a) a high loaded entrapped nitrifier pellet reactor, versus (b) a classical low loaded activated sludge process

Both systems had an anoxic pre-denitrification zone followed by an aerated zone for nitrification and organic carbon removal. In the aeration basin, fine bubble air diffusers were installed to supply the required oxygen and to mix and agitate pellets and activated sludge. The pellets having typical dimension of $3 \times 3 \times 3$ mm, a 1.5 mm wedge wire screen was installed on the upper part of the aeration tank to prevent the washout of pellets. The mixed liquor passing through this wedge wire screen was recycled into the anoxic tank for denitrification with an internal recycling rate of three. At start-up the pellet-dosing ratio was 12 vol. % of the aerobic reactor volume. A pellet-dosing of 10–20 vol. % of the aerated volume is recommended in the literature (Chudoba and Ujioka, 1998). Analyses and photometry on-line measurements were made on influent and effluent streams. Dissolved oxygen, pH and dry solids concentration were registered on a continuous basis.

Microbial analyses

The microbial composition of the activated sludge in the pellet reactor versus the classical activated sludge system was investigated. Specific microbiological features and taxonomic classification of the observed protozoa were microscopically investigated.

Fluorescence *in situ* hybridisation (FISH) was performed to pinpoint and identify nitrifying bacteria. As a positive and respectively negative control, sludge of a (i) good nitrifying MBR-installation and (ii) a high loaded (not nitrifying) activated sludge system was taken. Samples were fixated in a 4% paraformaldehyde buffer (PBS), centrifuged, washed and re-suspended in PBS and ethanol (96%). Fixated cells were immobilised on the differ-

Table 1 Operational conditions of the pilot-scale investigation.

	Test 1		Test 2	
	Conventional	Pellet reactor	Conventional	Pellet reactor
Influent (m^3/h)	1	1–2	1	2
RAS (m^3/h)	3	3	3	4
Aerated zone (m^3)	7.7	6.4–4.3	7.7	4.3
Anoxic zone (m^3)	4.5	4.5	4.5	4.5
MLSS (g/l)	4	3–4	4	2.5–3
Sludge load (kg BOD/kg ds.d)	0.07	0.08–0.24	0.07	0.24

ent hybridisation zones of a slide with gelatine coating (0.01% $KCr(SO_4)_2$, 0.1% gelatine) and dehydrated with 3 minutes 50, 80 and 96% ethanol. *In situ* hybridisation was done by dosing 10 µl hybridisation buffer (35% formamide, 0.9 M NaCl, 0.01% SDS, 20 mM Tris/HCl, pH 7.2) and 1 µl of the tested oligonucleotide probes on the different hybridisation zones of the slide. The following oligonucleotide probes were tested:

1. EUB338 (5′-GCT GCC TCC CGT AGG AGT-3′) complementary for a 16S rRNA area of all bacteria (Amann *et al*., 1990),
2. S-*-BactV-338-a-A-18 (5′-GCT GCC ACC CGT AGG TGT-3′) complementary for an area of the 16S rRNA of *Verrucomicrobiales* (Daims *et al*., 2000),
3. S-*-BactP-338-a-A-18 (5′-GCA GCC ACC CGT AGG TGT-3′) complementary for a 16S rRNA area of *Planctomycetales* (Daims *et al*., 2000),
4. Nso1125 (5′-CGC CAT TGT ATT ACG TGT GA-3′) complementary for a 16S rRNA area of ammonia oxidising-*Proteobacteria* (Mobarry *et al*., 1996),
5. NEU (5′-CCC CTC TGC TGC ACT CTA-3′) complementary for a 16S rRNA area of halophylic members of *Nitrosomonas: Nitrosomonas europea*, *Nitrosomonas eutropha* (Wagner *et al*., 1995),
6. CTE (5′-TTC CAT CCC CCT CTG CCG-3′) competitor oligonucleotide for probe NEU, complementary for an area of the 16S rRNA of *Comamonas testosteroni*, *Brachymonas denitrificans*, *Rhodocyclus purpurens* and *Leptothrix discophora* (Wagner *et al*., 1995),
7. S-G-Ntspa-662-a-A-18 (5′-GGA ATT CCG CGC TCC TCT-3′) complementary for an area of the 16S rRNA of *Nitrospira* (Daims *et al*., 2000), and
8. S-G-Ntspa-662-Comp-a-A-18 (5′-GGA-ATT-CCG-CTC-TCC-TCT-3′) competitor oligonucleotide for probe S-G-Ntspa-662-a-A-18 (Daims *et al*., 2000).

Probes EUB338, S-*-BactV-338-a-A-18 en S-*-BactP-338-a-A-18 were labelled with 5(6)-carboxyfluorescein-N-hydroxysuccinimide ester (FLUOS). Probes Nso1125, NEU and S-G-Ntspa-662-a-A-18 were labelled with CY3 indocarbocyanine. The hybridisation was performed in a pre-heated isotonic equilibrated *Falcon* tube at a constant temperature of 46°C during 1.5 hour. Slides were washed in pre-heated buffer solution (1 M Tris/HCl, 5 M NaCl, 0.5 M EDTA pH 8.0, 0.01% SDS) during 10 minutes at 48°C. Slides were rinsed with chilled distilled water, dried and covered with *Vectashield* in order to temper fading of the auto-fluorescent signal. Fluorescence was detected with a Confocal Laser Scanning Microscope. Image analyses was performed with software KS400 (*AxioVision*).

Microscopic and FISH observations were supported by measurements of nitrifying capacity (Chudoba and Pannier, 1994) of the different sludge types and of the pellets.

Results and discussion

Pilot plants

Influent concentrations for the raw wastewater are listed in Table 2. Both the conventional activated sludge and the pellet reactor easily met the BOD, COD and SS-consents during the length of the trials (Jonkers *et al.*, 2000). After eight weeks of operation the pellet reactor showed an unstable nitrification. Raising the dissolved oxygen concentration in the pellet reactor from 2 to 4 mg/l (Figure 2; day 112) cured the observed instability. Full nitrification was reached for the rest of the trials. Denitrification was sufficient to meet the TN-consent (Figure 2).

The classical activated sludge was operated at 4.0 g dry solids per litre. The pellet reactor on the other hand was operated at 3.5 g ds/l since higher sludge volume indexes were – based on previous experience – expected. Our experiments showed however no significant differences in SVI (Table 3).

Table 2 Raw influent characteristics during the trials

	BOD mg/l	COD mg/l	SS mg/l	Kj-N Mg/l	NH_4-N mg/l	NO_3-N Mg/l	TN mg/l	TP mg/l
Influent Average	113	289	138	21	29	0.1	28	4.9
STD	37	94	47	3	6	0.02	5	0.9
N*	73	95	89	89	91	97	95	78

* N = number of samples analysed

Microbial analyses

Microscopic investigation showed no large differences in the microbiological features between the activated sludge with or without presence of entrapped nitrifiers. However both *Zoogloea* fingered and amorphous colonies were solely detected in the presence of entrapped nitrifiers. *Zoogloea* is a floc-forming bacterium typically found in activated sludge when influent has high concentrations of readily metabolizable, soluble organics and operational conditions are nutrient deficient. This usually means a deficiency of nitrogen, phosphorus and/or trace nutrients such as iron (Wood *et al*., 1974; Novak *et al*., 1993).

Also composition of activated sludge in the pellet reactor differed in increased amounts of *Suctoria* and *Rotifera*. These organisms are typically more abundant in sludges characterized by low organic loading, high sludge age, and high DO in the aeration basin (Poole, 1984; Patterson, 1992). Both conditions of high sludge age and high DO are fulfilled in the pellet reactor.

FISH analyses showed absence of *Nitrobacter* cells and high abundance of the phylum *Nitrospira* in the pilot reactors. Due to the high density of fluorescent signals in the positive clusters, quantification of probe conferred signal intensity of single cells was not possible

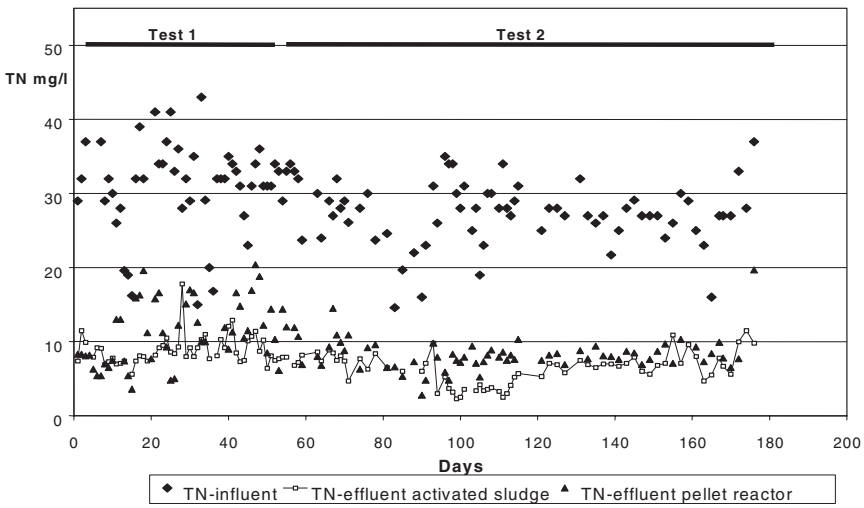

Figure 2 Total nitrogen (TN) concentration for the classical activated sludge versus the pellet reactor during the trials. The local TN-consent is 10 mg/l (yearly average)

Table 3 SVI for the classical activated sludge and the pellet reactor activated sludge during the tests

SVI (ml/g ds)		Average	STD	N* =
Classical activated sludge	Test 1	132	27	9
	Test 2	132	30	32
Pellet reactor activated sludge	Test 1	150	32	9
	Test 2	143	26	34

* N = number of samples analysed

by conventional epifluorescence microscopy (Figure 3). The analysis of ammonia-oxidizing populations in nitrifying activated sludge by conventional cultivation techniques or immonofluorescence often resulted in the identification of *Nitrosomonas* species as the numerically dominant representatives. FISH analysis indicates that the contribution of members of the genus *Nitrospira* in the nitrifying potential of sewage treatment plants has been underestimated (Brühl, 1999). This due to the inability of *in vitro* cultivation of *Nitrospira* as pure culture.

During Test 1 of the pilot trials the pellet reactor showed an unstable nitrification (first 50 days) (Table 4). This observed instability was cured by raising the dissolved oxygen (DO) set point in the aerobic reactor from 2 to 4 mg/l. This DO- rise clearly had a positive effect on the nitrification capacity and stability in the pellets reactor. Full nitrification was reached for the rest of the trials. Similar findings were reported by Sumino *et al*. (1997) who reported a limiting oxygen concentration to gel immobilized nitrifying pellets of 3 mg/l.

Pellet reactor for WWTP renovation

Many of the older Belgian sewage treatment plants are being renovated to achieve nutrient removal. WWTP Hasselt and WWTP Harelbeke make use of fine bubble aeration and could – theoreticaly – easily be renovated towards nitrogen removal using encapsulated nitrifiers. In total these installations treat 245,000 PE and a retrofit cost of 16 million Euro (classical expansion of the current activated sludge installation) was estimated. Part of this cost – 5.8 million Euro – is due to the necessary expansion of the aeration basins concerned.

For the WWTP renovations concerned a retrofit to a nutrient removing pellet reactor was investigated. To make a possible financial benefit with encapsulated nitrifiers compared to classical activated sludge expansion, the maximum investment cost for the pellets was calculated. This investment cost should be lower than 0.7 Euro per litre pellets (filling ratio 20 volume %) to have a break even. However in the calculation of this price indication

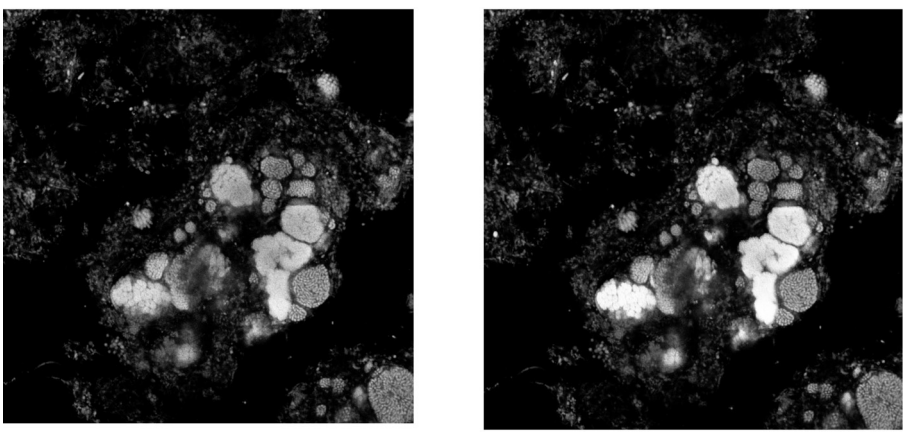

Figure 3 FISH-hybridisation of (a) all bacteria and (b) *Nitrospira* spp. (yellow) of pellet reactor activated sludge

Table 4 Nitrification capacity of the pellet reactor activated sludge and pellets during the tests

Nitrification capacity		Average	STD	N* =
Pellet reactor activated sludge	Test 1	3.6	0.4	2
(mg NH_4^+-N/g ds.h)	Test 2	4.5	1.1	7
Entrapped nitrifier pellets	Test 1	40	24	5
(mg NH_4^+-N/l pellets.h)	Test 2	130	72	10

* N = number of samples analysed

no additional costs for the installation of pellet screens, surplus aeration capacity or pellet consumption (through wear) was included.

Conclusion

Addition of entrapped nitrifiers to non-nitrifying activated sludge systems can create a shift in microbiological activated sludge composition. The relative importance of the presence of nitrifying pellets was studied. The pilot research shows that the incorporation of the pellets in non-nitrifying activated sludge can result in full nitrification and sufficient denitrification to comply with European WWTP consents.

Only minor microscopic differences between conventional activated sludge and pellet reactor activated sludge were detected. FISH analyses showed absence of *Nitrobacter* cells and high abundance of *Nitrospira* in the investigated reactor.

For the renovation towards nutrient removal of two WWTP, several scenarios – including retrofit with entrapped nitrifiers – were investigated. First cost estimates show a maximum competitive pellet cost of 0.7 Euro for the regarded cases.

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