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ORIGINAL PAPER

Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor

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Abstract Partial nitrification to nitrite (nitritation) can be achieved in a continuous process without sludge retention by wash out of nitrite oxidising bacteria (NOB) while retaining ammonia oxidising bacteria (AOB), at elevated temperatures (the SHARON process) and, as demonstrated in this paper, also at low dissolved oxygen (DO) concentrations. Enriched AOB was attained at a low DO concentration (0.4 mg l^{-1}) and a dilution rate of 0.42 day^{-1} in a continuous process. A higher oxygen affinity of AOB compared to NOB seemed critical to achieving this. This was verified by determining the oxygen half saturation constant, K_o, with similar oxygen mass transfer resistances for enriched AOB and NOB as 0.033 ± 0.003 mg l^{-1} and 0.43 ± 0.08 mg l^{-1}, respectively. However, the extent of nitritation attained was found to be highly sensitive to process upsets.

Keywords Activated sludge · Continuous process · Dissolved oxygen concentration · Nitritation · Nitrite oxidising bacteria

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Introduction

Nitrogen removal within biological wastewater treatment plants via nitrite instead of the traditional nitrate has been previously recognised as economically beneficial (Turk and Mavinic 1986). However, the difficulty in utilising nitrogen removal via nitrite lies in retaining the ammonia oxidising bacteria (AOB) while eliminating the nitrite oxidising bacteria (NOB). The AOBs and NOBs catalyse the first (ammonia oxidation to nitrite; nitritation) and second (nitrite oxidation to nitrate, nitratation) steps of nitrification, respectively.

Nitritation processes for the treatment of high nitrogen concentration wastewaters (ammonium concentrations of >500 mg N l^{-1}) have been proposed in literature. A number of strategies has been suggested to achieve nitritation in these processes, including:

Wash out of NOBs

Selective wash out of NOBs from continuous flow reactors (such as a Chemostat) has been achieved at elevated temperatures (30–40°C), coupled with a dilution rate that is less than the growth rate of NOBs but greater than the AOB growth rate (about 1–0.5 $days^{-1}$) (SHARON process; Hellinga et al. 1998).

Batch processes

Within sequencing batch-type processes, some studies have reported sustained nitritation (Yoo et al.

1999; Fux et al. 2003; Lai et al. 2004; Peng et al. 2004). It has been demonstrated that terminating aeration prior to the completion of ammonium oxidation (i.e., ammonium remaining in the effluent) in a process with nitrite accumulation is a key factor leading to sustained nitritation (Yoo et al. 1999; Fux et al. 2003; Peng et al. 2004).

Low dissolved oxygen

Sustained nitritation with the use of low dissolved oxygen (DO) concentration has been observed in a variety of reactor configurations (Sliekers et al. 2005; Wyffels et al. 2004). However, no definitive explanation has been given for these observations. Instead, all reports quote AOBs selection over NOBs due to a hypothesised higher oxygen affinity of the AOBs compared to the NOBs (Hanaki et al. 1990; Laanbroek and Gerards 1993; Laanbroek et al. 1994; Bernet et al. 2001).

The aim of this paper is to attain conditions, which allow for wash out of NOBs in a continuous-flow reactor configuration using only the DO concentration as the selection factor. The concept is similar to that of the SHARON process where elevated temperature (>30°C) is used to achieve a situation where the growth rate of the AOBs exceeds that of the NOBs allowing for a selective wash out of NOBs. A corollary may be drawn with low DO concentration due to an apparent difference in oxygen affinities between AOBs and NOBs, which could be exploited to achieve a situation where the growth rate of AOBs exceeds that of NOBs, thus potentially allowing for selective wash-out of NOBs.

Materials and methods

Nitritation/ammonia oxidising bacteria reactor

The operation of the nitritation reactor, which was also used as a source of AOBs, is described in more depth in subsequent sections. Briefly, the reactor was a 12 l working volume continuous-flow reactor which was inoculated with mixed liquor from a local domestic wastewater treatment plant (Brisbane, Australia). The temperature of the reactor was not controlled, but remained between 19 and 23°C. The DO concentration (measured by YSI model 5739, Yellow Springs, Ohio, USA) was controlled via a programmable logic controller to concentrations specified in subsequent sections, as was the pH (measured by Ionode IJ44, TPS, Australia) to a value of 7.6 by dosing of sodium carbonate solution. Aeration was provided to the base of the reactor through an air stone. The dilution rate was controlled to values specified in subsequent sections. The feed consisted of the following (per litre): $(NH_4)_2CO_3$, 3,700 mg; K_2HPO_4, 38 mg; $CaCl_2{\cdot}2H_2O$, 3.8 mg; $MgCl_2{\cdot}6H_2O$, 6.3 mg; $FeSO_4{\cdot}7H_2O$, 2.5 mg and trace nutrients, 0.17 mg. The total inorganic nitrogen concentration in the reactor was approximately 1,000 mg N l^{-1}, which was comprised of different fractions of ammonium, nitrite or nitrate, depending on the extent of nitrification or nitritation attained, as discussed in subsequent sections. A batch of trace nutrients was made as a stock and consisted of $NaMoO_4{\cdot}2H_2O$, 0.1 g/g; $CuSO_4{\cdot}5H_2O$, 0.05 g/g; $ZnSO_4{\cdot}7H_2O$, 0.1 g/g; $MnCl_2{\cdot}4H_2O$, 0.2 g/g; $CoCl_2{\cdot}6H_2O$, 0.05 g/g; H_3BO_4, 0.1 g/g and KCl, 0.4 g/g.

Feed was provided by a peristaltic pump (SEKO Italia PR4, Italy), which operated in an on/off fashion to approximate continuous operation due to very short feed and non-feed times (about 1 min feed to 3 min non-feed). Mixing was provided at 100 rpm by an overhead stirrer (IKA RW20.n, IKA Works Asia, Malaysia).

Fluorescence in situ hybridisation and microscopy

The fraction of AOB in the total bacterial population was determined by fluorescence in situ hybridisation (FISH) (Manz et al. 1992) using NEU, a probe that identifies AOBs belonging to the *Nitrosomonas* genera, (Wagner et al. 1995) labelled with sulfoindocyanine dye Cy3 and compared against the general bacterial probe, EUBmix (Daims et al. 1999) labelled with fluorocrome Cy5. Other AOB probes were also tested, but these did not bind to any bacteria present in this culture. Similarly, the qualitative identification of NOBs was assessed for the presence of the NOB genera *Nitrobacter* using Nit3 (Wagner et al. 1996) and/or NOB phylum *Nitrospira* using Ntspa662 (Daims et al. 2001). The Nit3 and Ntspa662 probes were labelled with Cy3 while EUBmix was labelled with fluorescein isothiocyanate (FITC). FISH preparations were viewed with a Zeiss LSM 510 Meta

Confocal microscope with a 63× Plan-Apochromat oil immersion lens. For quantification of the AOB, the method presented in Hall et al. (2003) was used to determine the relative abundance of *Nitrosomonas* bacteria as the mean percent ratio of the countable pixel area of NEU signal to EUBmix.

Floc size determination and sonication

The AOB volumetric floc size distribution was determined using the Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK) as per the methods used in Blackburne et al. (2007). The Malvern Mastersizer 2000 returns a volume fraction for each of the 100 size bands between 0.01 μm and 10,000 μm, with a $\log_{10}$ interval of 0.06. Reduction of the AOB volumetric floc size distribution was achieved by sonication as described in Blackburne et al. (2007) to reduce oxygen mass transfer resistance.

Mass transfer resistance estimation

The quantification of oxygen mass transfer resistance for a given culture was done using a model developed by Gapes (2003) and coded in MATLAB V 6.5 (Mathworks, Natick, Massachusetts, USA). The model equations depict both internal and external oxygen mass transfer through a spherical floc under steady or pseudo-steady state. Mass transfer in the model is represented by Fick's Law. The recommended parameters from Gapes (2003) (e.g., diffusivity, density, viscosity, etc.), with exception of the porosity, and input of case specific oxygen uptake Monod kinetics was used in the model. The chosen porosity value was most sensitive to the degree of oxygen mass transfer resistance determined. Gapes (2003) recommended a default porosity value of 0.99, but noted that the porosity of biomass is typically between 0.95 and 0.99. Hence, the oxygen mass transfer resistance was determined for the range of these porosity values.

Respirometric and titrimetric measurements

The Titration and Off-Gas Analyser (TOGA, Pratt et al. 2003) was used for determination of the oxygen substrate uptake kinetics by the same method as outlined in Blackburne et al. (2007) except the pH value was kept at 7.80 as this is within the optimum pH range for AOBs quoted elsewhere (Painter and Loveless 1983; Grunditz and Dalhammar 2001). Briefly, the method involved using the TOGA as a respirometer for numerous batch experiments of about 30–40 min duration, each at various DO concentrations, and all with substrate in excess. A Monod curve was fitted through the experimental data and this allowed for determination of kinetic parameters such as the maximum reaction rate and/or half saturation constant. Concentrated ammonium feed was regularly injected to maintain the substrate concentration well above the substrate Monod half saturation constant, K_s, value as confirmed by offline sampling.

Analytical methods

MLSS/VSS were determined using Whatman GF/A filters and in accordance with Standard Methods (APHA 1992). Ammonium, nitrite and nitrate were measured with a flow injection analyser (FIA) (Lachat QuikChem 8000, Milwaukee, WI, USA).

Results and discussion

Establishing a nitritation culture

To select for a nitritation culture a nitrifying continuous reactor was operated at a variety of DO concentration and dilution rate combinations, which are denoted as different operational phases shown in Table 1.

It was only in phase F that sustained nitritation was attained, as shown in Fig. 1.

Initially, to establish an enriched nitrification culture, the dilution rate was set to 0.2 $days^{-1}$ and the DO concentration was 4 mg l^{-1} resulting in full nitrification to nitrate (Phase A). To induce nitritation, which was hypothesised to occur at low SRT and DO concentrations, the DO was lowered to 0.25 mg l^{-1}. However, this seemed to be too low as evidenced by the sudden increase of ammonium in the effluent (Phase B). Hence, the DO was increased to 1 mg l^{-1} (Phase C) to recover nitrification.

In a second attempt to establish nitritation at a low DO concentration, the DO was lowered in a step-wise fashion. First, the DO was lowered to 0.5 mg l^{-1} (Phase D) which showed no rapid increase in

Table 1 List of reactor phases with corresponding DO concentration and dilution rate

Phase	DO concentration (mg l^{-1})	Dilution rate ($days^{-1}$)	Loading rate ($mgNH_4$–N l^{-1} d^{-1})
A	4	0.2	200
B	0.25	0.2	200
C	1	0.2	200
D	0.5	0.2	200
E	0.2	0.2	200
F	0.4	0.42	420

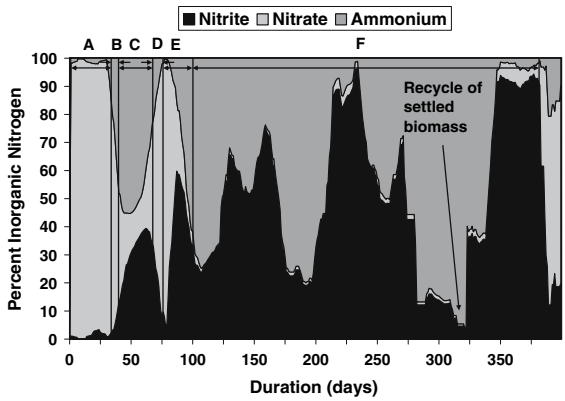

Fig. 1 Percent of inorganic nitrogen species as fractions of the total inorganic nitrogen in the effluent from the continuous reactor without sludge retention. Letters represent different operating phases (see Table 1) to achieve either nitrification or nitritation

ammonium concentration as was observed in Phase B, but also produced no increase in nitrite concentration. Hence, the DO was lowered further to 0.2 mg l^{-1} (Phase E). While Phase E was effective in achieving nitritation as evidenced by the removal of nitrate from the effluent, the ammonium concentration seemed to increase similarly to what was observed in Phase B. Furthermore, the very low DO concentration was difficult to accurately control due to limitations in the physical reactor configuration. Therefore, the dilution rate was raised to 0.42 $days^{-1}$ so that the DO concentration could be increased slightly to 0.4 mg l^{-1} (Phase F). This combination seemed effective in sustaining nitritation and resulted in an immediate increase of ammonium conversion.

While there was considerable variability in the ammonium conversion achieved during Phase F, nitrite was consistently the dominant oxidised nitrogen form observed. However, not all the oxidised nitrogen was in the form of nitrite with a small percentage of nitrate being present throughout Phase F, possibly caused by NOBs growing in biofilms on the walls and other surfaces of the reactor.

The nitritation variability is attributable to the process being quite difficult to keep stable (defined by a constant ammonium conversion). Some of the identified process upsets were:

- The sudden drop in nitritation activity after day 160 was due to an accumulation of ammonium and carbonate (as ammonium carbonate was fed to the reactor) and hence a subsequent increase in pH, lasting maximally 2 days. This condition generated free ammonia concentrations of up to 20 $mgNH_3$ l^{-1} which Anthonisen et al. (1976) suggested would be inhibitory to both AOBs and NOBs. It took the culture about 50 days to recover. No alterations to the reactor operation were implemented to assist in this regard.
- From about day 240 to day 320, the process performance deteriorated due to a number of operational problems and errors such as insufficient sodium carbonate or feed supply. Only limited monitoring of the reactor operation was conducted during this period. Hence, the full understanding of the reasons for this instability cannot be determined due to the limited operational input during this period.

The biomass from the effluent was recycled to the reactor for about a week from day 320 to minimise the time to re-establish the nitritation culture. It is likely, given the return of the culture on day 200 onwards, that the culture would have eventually returned to a high ammonium conversion even without the biomass recycle.

Despite the sensitivity and variability of the process, there were periods where high sustained nitritation was attained (up to 90%). In the first period of high conversion, between days 200 and 250, ammonium conversion to nitrite of 80–90% was retained for about eight sludge ages. In the second period of high conversion, between days 340 to 380, a nitrite fraction of about 90% in the effluent was retained for 18 sludge ages. Both of these periods could be considered at steady state (typically accepted to be attained after three sludge ages). The

nitritation capacity during these periods reached a maximum of 0.38 kgN m^{-3} day^{-1}, which is high considering the low DO concentration.

Although the reactor ammonium concentrations were often high enough, given the operating pH of 7.6, to generate free ammonia levels that might be inhibitory to NOBs (Anthonisen et al. 1976), it is unlikely that this was the major factor in establishing nitritation. The long-term operation of the reactor would suggest likely acclimatisation of the NOBs to free ammonia (Turk and Mavinic 1989). Conversely, the high nitrite concentrations during the periods of high conversion did not result in free nitrous acid concentrations that are inhibitory to NOBs (Blackburne et al. 2007). Hence, it is hypothesised that the low DO concentration coupled with the high dilution rate lead to the observed washout of NOBs.

Investigation and verification of the oxygen affinity theory

To investigate the hypothesised cause for the observed nitritation, previous suggestions that the oxygen affinity of AOBs exceeds that of NOBs need to be investigated. The oxygen affinity is represented by the oxygen Monod half saturation constant, K_o, values. However, the K_o values for NOBs and AOBs reported in literature vary greatly as shown in Table 2.

The floc size distribution, which was not reported in the cited studies, has been suggested to largely affect oxygen mass transfer rates even in floccular biomass aggregates (Mueller et al. 1966; Bakti and Dick 1992; Beccari et al. 1992; Gapes 2003). Due to the oxygen mass transfer resistance, the DO concentration would not be uniform for bacteria inside different sized floccular aggregates. Hence, different specific oxygen uptake rates would be attained for different floc sizes at the same bulk DO concentration. As a consequence, the apparent K_o value will be affected by the oxygen mass transfer kinetics and the variation in K_o values shown in Table 2 may be at least in part due to these culture specific effects. Particularly the high K_o values are likely affected by these mass transfer limitations and should not be considered as "true" biological characteristics of the AOB or NOB.

Table 2 K_o values for ammonia and nitrite oxidising bacteria

Reference	K_o (mg l^{-1})	pH value	Temperature (°C)
Ammonia oxidising bacteria			
Hanaki et al. (1990)	0.32	7.3–7.8	25
Laanbroek and Gerards (1993)	0.04–0.48	7.5	25
Laanbroek et al. (1994)	0.22–0.56	7.5	25
Nitrite oxidising bacteria			
Laanbroek and Gerards (1993)	0.7–5.3	7.5	25
Laanbroek et al. (1994)	0.17–4.33	7.5	25

Small flocs (less than 40 μm) have been suggested to essentially eliminate mass transfer resistances at all bulk liquid DO concentrations (Beccari et al. 1992). Indeed, this was verified again in another study (Blackburne et al. 2007), where the K_o value for three different floc sizes distributions of a *Nitrobacter* enrichment were determined. The results from this study indicated that for median volumetric floc sizes of less than 63 μm, mass transfer resistance seemed to be reduced to be no longer rate limiting as evidenced by the statistically insignificant differences in K_o values compared to those obtained with smaller floc sizes. The smallest median volumetric floc size tested was 44 μm and the corresponding K_o value was 0.43 ± 0.08 mg l^{-1}. This value is probably close to the true bacterial K_o value and corresponds with the lower K_o values for NOB shown in Table 2.

For verification of the oxygen affinity hypothesis, direct comparison between the oxygen uptake rate against DO concentration for the nitritation culture (AOB) and the enriched NOB culture (*Nitrobacter* culture from Blackburne et al. 2007) is desired. This is possible given the following conditions:

1. Assumption of similar oxygen mass transfer resistance for both the AOB and NOB cultures.
2. Similar degree of enrichment for both AOB and NOB cultures allowing for direct comparison of the oxidation rate (measured as oxygen uptake rate, OUR).

The first condition is met for similar floc size distributions of both AOB and NOB cultures assuming the remaining floccular characteristics (density, porosity, etc.) and the experiment-specific oxygen diffusivity are constant. These assumptions are justified given the very similar nature of both biomass populations and the near identical experimental

conditions (temperature, pH, media composition, etc.). The second condition was validated by quantitative FISH analysis, which resulted in 82% and 73% enrichment for the AOB and NOB cultures, respectively.

The AOB kinetics was determined using the same method as for the NOB samples in Blackburne et al. (2007). Briefly, the method involved sonication of the AOB enrichment to the desired floc size (median volumetric size of 49 μm) and determination of the average OUR values from batch experiments at various DO concentrations. The kinetics of both the AOBs and NOBs are plotted as Fig. 2.

Based on the data shown in Fig. 2 the K_o value for the AOBs was found to be 0.033 ± 0.003 mg l^{-1} while the K_o value for the NOBs was 0.43 ± 0.08 mg l^{-1}. The K_o values and associated 95% confidence intervals were determined using least sum of squares and the asymptotic confidence interval algorithms available in the GraphPad V 4.00 Prism software. These results do indeed confirm the hypothesis that the AOBs have a significantly higher affinity for oxygen than the NOBs. The difference in terms of the K_o value is more than an order of magnitude and both values are near the lower end of the literature reports for AOBs and NOBs. This further supports the notion that many of the reported values are "apparent" affinity constants that are largely influenced by oxygen mass transfer limitations.

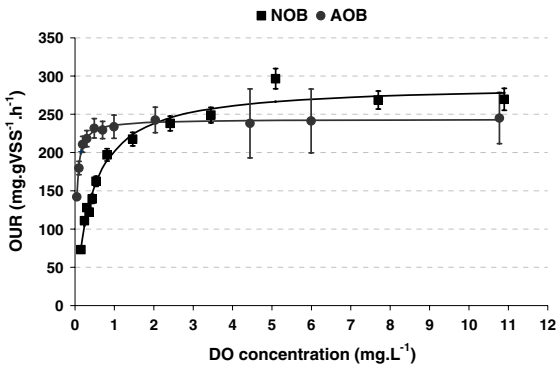

Fig. 2 Oxygen uptake kinetics for two enrichment cultures of AOB (median volumetric floc size of 49 μm) and NOB (median volumetric floc size of 44 μm). The error bars represent the 95% confidence interval of the mean value at each measurement point

Application of the difference in oxygen affinity to attain nitritation

Since the enriched NOB culture was identified as *Nitrobacter* (from Blackburne et al. 2007), and the dominant NOB identified during Phase A of the reactor operation was also identified as *Nitrobacter*, the kinetics determined with the enriched *Nitrobacter* culture are assumed to be identical to that of the in-situ *Nitrobacter* culture. The AOB culture present in this continuous-flow reactor was identified as *Nitrosomonas* and oxygen uptake kinetics were determined directly for this culture as described above. Using the kinetics developed from the data shown in Fig. 2, a more directed investigation of the DO-controlled nitritation is now possible. The maximum growth rates with 95% *t*-distribution confidence intervals of both the *Nitrosomonas* culture (sampled from Phase F of the reactor operation) and the *Nitrobacter* culture were determined by a novel growth rate method from Blackburne et al. (in press). The maximum growth rate value for *Nitrosomonas* was 0.54 ± 0.09 day^{-1} and for *Nitrobacter* it was 0.67 ± 0.03 day^{-1}. The K_o values and maximum growth rate values for both *Nitrosomonas* and *Nitrobacter* were used to derive their growth rate versus DO relationship as shown in Fig. 3.

The alphabetical letters marked in Fig. 3 represent the different phases of the reactor operation (Phases G –K are described subsequently). The DO concen-

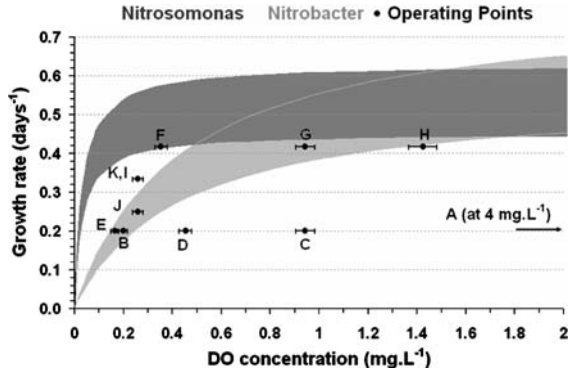

Fig. 3 Growth rate of *Nitrosomonas* and *Nitrobacter* against DO concentration. The 95% confidence intervals in both the growth rate and K_o values of each culture create a confidence bound which is marked by the shaded regions. The operating points of the process are marked with letters. The DO concentration at each operating point is adjusted for mass transfer resistance from the bulk liquor DO concentration

trations for each operating point have been adjusted to lower values than the bulk DO concentrations by quantification of the oxygen mass transfer resistance using the model from Gapes (2003). The model was applied to a floc size distribution, which consisted of a spread of different floc sizes, each with a different oxygen mass transfer resistance. The approach was to calculate the oxygen mass transfer for all the different floc sizes and to find the total mass transfer resistance on a number-weighted basis.

As an example, considering a 100 μm floc, Gapes' model determines the DO concentration profile through a radii of the floc as shown in Fig. 4. The oxygen mass transfer resistance for this floc is the difference between the bulk liquor DO concentration (0.4 mg l^{-1}, in this case) and the model determined DO concentration through the floc radius. The mass transfer resistance in this case lowers the number-weighted average DO in the floc by 42% relative to the bulk DO concentration.

The number-weighted average oxygen mass transfer resistance for the different floc sizes with corresponding cumulative number floc size distribution measured during Phase F is shown in Fig. 5.

Figure 5 shows that while the oxygen mass transfer resistance approaches 100% as the floc sizes reach 200 μm (for the porosity = 0.95 case), the number of large flocs (>100 μm) within the distribution is very small compared to the number of flocs at smaller floc sizes. Therefore, on a number-weighted basis, the contribution to the overall mass transfer resistance by larger flocs with large mass transfer resistances is minor compared to smaller flocs. Nevertheless, the overall mass transfer resistance determined for the

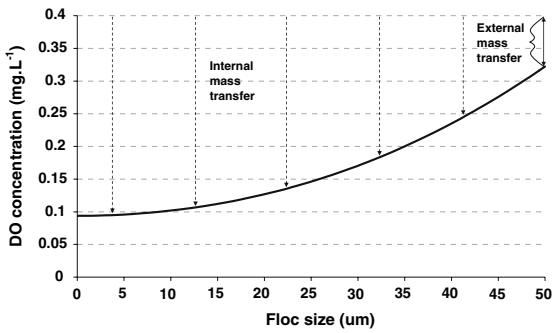

Fig. 4 Example DO concentration profile through a 100-μm floc determined from the model (bulk liquor DO concentration = 0.4 mg l^{-1} and porosity = 0.99)

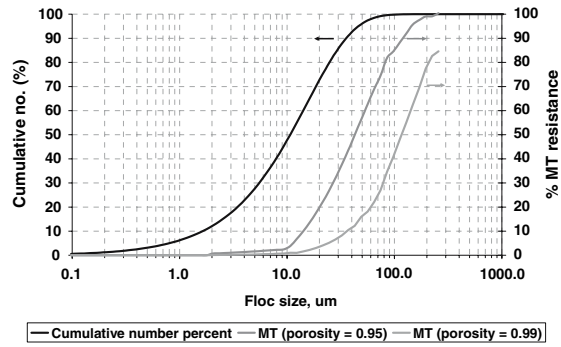

Fig. 5 Cumulative number percent for the floc size distribution during Phase F and corresponding oxygen mass transfer resistance for the porosity values tested

distribution from Fig. 5, amounted to a decrease in bulk liquor DO concentration from 0.4 mg l^{-1} to an average floccular DO concentration between 0.34 and 0.38 mg l^{-1}, for a porosity between 0.95 and 0.99, respectively. Hence, the operating DO concentrations shown in Fig. 3 correspond to the actual average DO concentration observed by bacteria in the biomass with mass transfer resistance accounted for. Therefore, since the growth rate curves for both *Nitrosomonas* and *Nitrobacter* represent growth rate kinetics for cultures with minimal mass transfer resistance and the bioreactor culture has been accounted for mass transfer resistance, a direct comparison is possible.

Figure 3 shows the operating points during Phases A, C and D are all at dilution rates well below the growth rate of *Nitrobacter* and therefore should result in nitrate as the dominant form of oxidised nitrogen, which is indeed confirmed by the results shown in Fig. 1. The operating points B and E appear within the 95% confidence interval of the *Nitrobacter* growth rate curve and hence may lead to wash out of *Nitrobacter*, which did seem to occur as shown by the increasing effluent nitrite concentrations during these phases (Fig. 1).

During Phase F, the process was operated at such a DO and dilution rate combination that the NOBs would be washed out since the required operating dilution rate was well above the growth rate for NOBs under these conditions (Fig. 3). However, it should also be noted that these conditions are also directly in the 95% confidence interval of the *Nitrosomonas* growth curve and therefore the AOBs were also close to being washed out during this phase. This is a likely explanation for the high

sensitivity of the nitritation process to even minor variations in operating conditions, which was causing the variable performance observed during Phase F (Fig. 1).

To further investigate the use of the oxygen affinity concept coupled with a low SRT in removing or retaining *Nitrobacter* populations in the reactor, the reactor continued to be operated but under the conditions listed in Table 3 and indicated by the letters G–K in Fig. 3.

The results of these operational phases are shown in Fig. 6. Initially, during phase G, full nitrification to nitrate was intended to be achieved by operating at a dilution rate of 0.42 days^{-1} and DO concentration of 1 mg l^{-1}. A clear shift to full nitrification was observed for nearly 50 days, but nitritation seemed to return just after day 400. This nitritation could possibly have been caused by the marginal operation of the reactor at point G, which is within the 95% confidence interval for *Nitrobacter* (Fig. 3) and the small free ammonia accumulation from the decreased ammonium conversion at about day 415–420. To assist in the nitrification recovery, acid was added to the feed in an attempt to lower the pH of the reactor and therefore reduce the free ammonia presence. The cause of the sudden decrease in ammonium conversion, as observed at about day 400 and also at day 430, cannot be clearly explained but may have been influenced by the dilution rate being close to the growth rate of *Nitrosomonas*.

Table 3 List of reactor phases with corresponding DO concentration and dilution rate

Phase	DO concentration (mg l^{-1})	Dilution rate (day^{-1})	Loading rate (mgNH_4–N L^{-1} d^{-1})
G	1	0.42	420
H	1.5	0.42	420
I	0.3	0.33	330
J	0.3	0.25	250
K	0.3	0.33	330

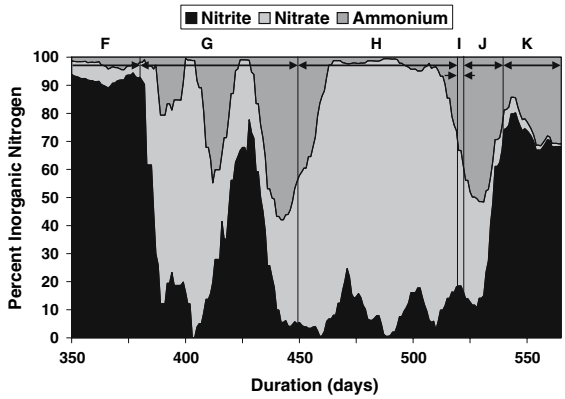

Fig. 6 Percent of inorganic nitrogen species of total inorganic nitrogen in the effluent from a continuous reactor without sludge retention. Letters correspond to different phases (Table 3) to achieve either nitrification or nitritation

To stabilise the ammonium conversion, the DO concentration was increased to 1.5 mg l^{-1} while the dilution rate remained at 0.42 days^{-1} (Phase H), which resulted in near complete nitrification to nitrate. During Phase H, there were peaks of nitrite observed for which no clear explanation could be determined at the time. At the end of Phase H, the ammonium conversion suddenly decreased for no apparent reason. This may be due to the operating point still being close to the growth rate of the AOBs and even a small temperature drop could reduce the maximum growth rate sufficiently to cause a temporary wash-out of the AOBs. For example, a drop in temperature of 1°C, i.e., from 22 to 21°C, would result in a 10% drop in the maximum AOB growth rate (given a relationship of $1.1^{(T-21)}$ from Melcer et al. 2003).

To establish nitritation again, the operating conditions in Phase I were changed to a DO concentration of 0.3 mg l^{-1} and a dilution rate of 0.33 days. However, the ammonium conversion continued to decrease during this short phase and hence it was decided to lower the dilution rate to 0.25 days to improve ammonium conversion (Phase J). During this phase, the DO concentration remained at 0.3 mg l^{-1} to allow the bacteria to adapt to the low DO concentration as a sudden reduction of the DO concentration was previously found to lead to substantial ammonium accumulation (Phase B). Once the ammonium conversion was improving, the operating conditions were returned to the same conditions as Phase I, now termed Phase K. This strategy was effective in establishing stable nitrite formation with about 70% ammonium conversion. A further improvement of the ammonium conversion could likely be achieved over extended periods, as was experienced during Phase F (Fig. 1). Nevertheless, the nitritation rate achieved during this phase was

around 0.25 kg N m^{-3} day^{-1} with very little nitrate being present during this phase.

The different phases of operation, A through to K, demonstrate that nitritation can be attained by operating at low DO concentrations and high dilution rates. Furthermore, altering DO and dilution rate combinations to switch between nitritation and full nitrification seemed successful. However, through all the phases it was difficult to maintain stable nitritation. Given the very low biomass retention, even small operational changes, such as leaving the reactor without feed for 2 days, can lead quickly to a drastic change in performance. This suggests that the process is quite sensitive to operational conditions. This low robustness is the major limitation of the process and optimal operating conditions would need to be further investigated to improve the stability of the process performance.

Conclusions

The key conclusions from this work are as follows:

1. The K_o values of enriched AOB (identified as *Nitrosomonas*) and NOB (identified as *Nitrobacter*) cultures with negligible oxygen mass transfer resistances were found to be significantly different with values of 0.033 ± 0.003 mg l^{-1} and 0.43 ± 0.08 mg l^{-1}, respectively. This supports the previously suggested difference in oxygen affinity of AOBs compared to NOBs.
2. It was shown that a continuous process without sludge retention can be operated at a low DO concentration and a corresponding dilution rate such that *Nitrobacter* is washed out but *Nitrosomonas* is retained. The most stable operation would probably be at a dilution rate of 0.33 $days^{-1}$ and a DO concentration of 0.3 mg l^{-1} resulting in a nitritation capacity of 0.25 kg N m^{-3} day^{-1} but only about 70% conversion of ammonium to nitrite.
3. While the process successfully achieved nitritation, it was prone to instability and is very sensitive to process disruptions. In practice, this process may need to be coupled with another nitritation selection factor reported in literature (e.g., elevated temperature, free ammonia or free nitrous acid inhibition, etc.).

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