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STUDY OF FACTORS AFFECTING SIMULTANEOUS NITRIFICATION AND DENITRIFICATION (SND)

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ABSTRACT

Experiments have been performed to gain an understanding of the conditions and processes governing the occurrence of SND in activated sludge systems. Sequencing batch reactors (SBRs) have been operated under controlled conditions using the wastewater from the first anaerobic pond in an abattoir wastewater treatment plant. Under specific circumstances, up to 95% of total nitrogen removal through SND has been found in the system. Carbon source and oxygen concentrations were found to be important process parameters. The addition of acetate as an external carbon source resulted in a significant increase of SND activity in the system. Stepwise change of DO concentration has also been observed in this study. Experiments to determine the effect of the floc size on SND have been performed in order to test the hypothesis that SND is a physical phenomenon, governed by the diffusion of oxygen into the activated sludge flocs. Initial results support this hypothesis but further experimental confirmation is still required. © 1999 IAWQ Published by Elsevier Science Ltd. All rights reserved

KEYWORDS

BNR; floc size distribution; Simultaneous Nitrification/Denitrification (SND); Sequencing Batch Reactor (SBR).

INTRODUCTION

Biological nutrient removal (BNR) is becoming increasingly common in both domestic and industrial wastewater treatment. While the removal of phosphorus can be achieved both chemically and biologically, nitrogen removal is almost exclusively done biologically. Total nitrogen removal in wastewater treatment plants is most commonly and most economically achieved in a two stage system. Ammonium is oxidised under aerobic conditions to nitrite and then nitrate (nitrification) which is subsequently reduced to nitrogen gas under anoxic conditions (denitrification). Therefore, two separate reactors (or sequences in intermittent systems) are required to provide the two different environmental conditions. However, recent studies have revealed that these two important steps can occur concurrently in the same reactor. This process has been termed Simultaneous Nitrification/Denitrification (SND).

SND is gaining increasing interest from engineers and scientists since it may have significant advantages compared to the conventional processes of separated nitrification and denitrification. If the rates of nitrification and denitrification are largely similar as in separated systems, the need for a separate denitrification tank or period (in intermittently operated systems) can be eliminated or at least the size of the tank can be reduced. This could help to simplify the overall process design dramatically.

At present, the SND phenomenon has not been well described or investigated. Therefore, the aim of the present study is to gain an understanding of the conditions and processes governing the occurrence of SND and to develop a mathematical model which could simulate the experimental data and help in the understanding of the SND mechanism.

BACKGROUND

From previous studies, it was found that three principal factors predominantly influence SND. These are carbon supply, oxygen concentrations and floc size.

Carbon supply

To accomplish denitrification in any process, organic carbon is found to be one of the most essential factors since it is needed as a carbon and energy source for the bacteria. Barnard (1992) found that in most domestic wastewaters, complete denitrification could be achieved with a TCOD:TKN ratio of 7. Generally, a minimum value of 9 is required for achieving both biological N and P removal (Goronszy, 1992). Isaacs and Henze (1994) proposed that 1.5-2.5 g COD/g P is used for the phosphate removal whereas the COD:N ratio for denitrification is in the range of 3.5-4.5 g COD/g N. This is close to the theoretical requirement for denitrification without COD loss due to aerobic processes.

Henze (1989) compared the denitrification rates using several organic components and found that the rate with domestic wastewater was about one third of the value obtained with acetic acid or methanol. In comparing acetate, methanol and glucose, Tam *et al.* (1992) and Gerber *et al.* (1986) found acetate to give the highest denitrification rates, followed by methanol and glucose in that order.

Dissolved oxygen

The control of dissolved oxygen (DO) present in the system is an important part in achieving a higher degree of SND. Denitrification can be best achieved with zero dissolved oxygen concentration. The effectiveness of the denitrification process decreases when oxygen concentrations are higher than 0.2 mg O_2/l. On the other hand, Painter (1977) indicated that the DO concentration for nitrification should be higher than 2 mg O_2/l otherwise DO can be the limiting factor. At the same time, a value of 0.2 mg O_2/l is considered as a critical value, at which nitrification does no longer occur (Bliss and Barnes, 1986). However, it has also been found that a DO concentration around 0.5 mg/l was suitable to achieve a nitrification rate equal to the denitrification rate which would therefore lead to complete SND (Münch. *et al.*, 1996).

Floc size

To test the hypotheses that SND is a physical phenomenon, experiments to determine the effect of the floc size on SND have been performed. The underlying physical explanation is that a substantial anoxic mass fraction exists in the center of the biomass flocs resulting from an oxygen diffusion limitation into the flocs. Typical floc sizes as measured in our experiments by a Malvern Mastersizer E are 50-110 μm, which is relatively large compared to 10-70 μm as indicated by Andreadakis (1993). Such large floc sizes could create an anoxic zone inside the flocs leading to denitrification in this area (Fig 1).

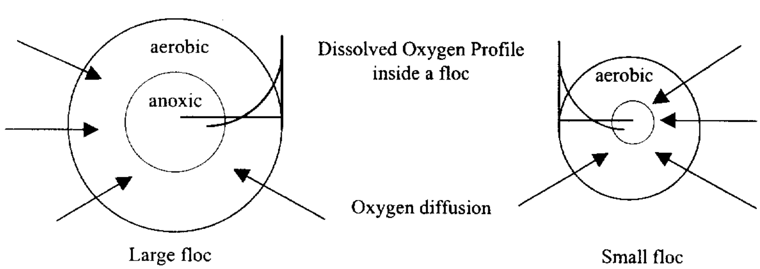

Figure 1. Influence of floc size on aerobic:anoxic zone ratio in schematic activated sludge floc.

MATERIALS AND METHODS

Reactor operating conditions

To investigate the SND phenomenon, sequencing batch reactors (SBRs) have been operated under controlled conditions similar to previous studies (e.g. Münch *et al*, 1996 or Subramaniam *et al.* 1994). To investigate the SND phenomenon, the sequences used for this study are fill (2 h), non-aerated reaction (0.5 h), aerated react (3 h), settle and draw (together 0.5 h). The maximum working volume of the reactors was 4.8 l. The feed wastewater was collected on a weekly basis from the effluent of the first anaerobic pond in an abattoir wastewater treatment system near Brisbane, Australia. The wastewater was stored in a cold room at 4°C. Typical SBR influent concentrations are shown in Table 1.

Table 1. Typical concentration of pretreated abattoir wastewater used as influent to SBRs

Parameter (mg/l)	Range	Average
NH_4-N	100-160	135
TKN	160-280	210
HPO_4-P	22-41	32
Total COD	860-3540	1960
Soluble COD	125-320	200

Biofilm growth in the feed lines and on the reactor walls was removed once a week. In each 6 hour cycle, the reactors were fed with 0.8 l of wastewater giving a hydraulic retention time (HRT) of 36 hours. Magnetic stirrers with 6 cm Teflon stirring bars, operating at approximately 200 rpm, were used to provide adequate mixing during the non-aerated reaction period. Additional mixing was provided during the aerated periods by the compressed air. Air diffusers located just above the magnetic stirrer bar were used to supply air to the reactor. The compressed air was passed through a solenoid valve with ON/OFF control based on the DO measurement in the reactor. Operation of influent pumps, magnetic stirrers and solenoid valves for aeration and decanting was controlled through a computer based control system using CYRANO and Mistic MMI software. Short, intermittent mixing (3 seconds each 1.5 minutes) was provided during the fill period to create contact between biomass and substrate without disturbing the liquid level above the sludge blanket. The reactor was operated with a sludge age of 15 days in a temperature controlled room at 18-22°C. DO level was controlled (details below), whereas pH was observed, but not controlled and remained between 7.0 and 8.0 throughout the cycle.

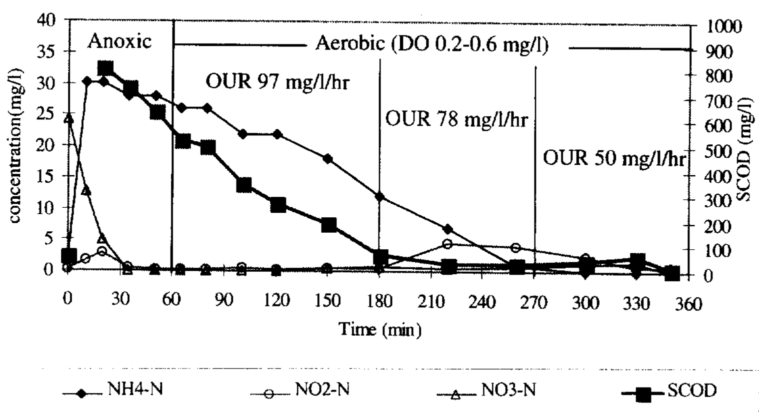

Figure 2. Cyclic study showing very high degree of SND, particularly while SCOD is available.

RESULTS AND DISCUSSION

Cyclic study

During these experiments, up to 95% of SND has been found in the system. Cyclic studies were performed in order to provide an insight into the nitrogen removal mechanism. The reactor was set up as a 6 hours batch experiment. 800 ml of feed was dumped into the reactor to achieve a total working volume of 4.8 l. The reaction sequences used for this cycle were anoxic (1 hour) and aerobic (5 hours). The influent ammonium concentration (190 mg/l) was considerably diluted to approximately 30 mg/l at the beginning of the cycle. A typical nitrogen profile through the reaction period in the cycle is shown in Fig 2.

During the anoxic period, sufficient substrate and absence of oxygen enabled complete removal of nitrate remaining from the last cycle (no SCOD addition) within the first 30 minutes. During the aerobic period, DO was controlled in the range of 0.2-0.6 mg/l (0.4 mg/l on average). This DO level was sufficient to achieve complete nitrification in the reactor with the ammonia levels reduced to effectively zero after 5 hours aeration period. During the first 2 hours of aeration, no nitrite or nitrate was produced, indicating that simultaneous nitrification and denitrification was perfectly achieved. In this period, most of the soluble COD was also removed which then lead to the temporary accumulation of nitrite until ammonium oxidation was completed.

It is surprising to find that nitrate did not occur in any significant quantity throughout the cycle. In fact, even after complete ammonium oxidation, the remaining nitrite was not further oxidised to nitrate but mostly denitrified leading to a very low oxidised nitrogen concentration at the end of the cycle. This could indicate that the nitrification process throughout the cycle is only producing nitrite which is subsequently denitrified to dinitrogen gas (N_2). If this short pathway is indeed utilised, the required (S)COD for the denitrification is reduced by approximately 40%. Further studies to confirm this process are required and currently underway.

The oxygen uptake rate (OUR) was calculated from the DO profile which was recorded during aeration time. Initially, a high OUR of 97 mg/l/h was found due to both nitrification and COD removal reactions proceeding. A clear reduction in OUR to 78 mg/l/h was noticed when the soluble, biodegradable COD in the system was completely removed. During this time, oxygen was predominantly used for the ammonium oxidation process. Following the elimination of ammonium after 4.5 hours, the OUR was again dramatically reduced to less than 50 mg/l/h which is due to slowly biodegradable COD removal and endogenous respiration.

Factors affecting SND

Three main factors influencing the SND process, ie. carbon source, DO concentration and floc size, have been further investigated in this study.

Experiment to determine SCOD effect

The ratio of TCOD:TKN of the influent in this study is between 5 and 20 which should be sufficient for the denitrification process. However, the results have shown poor removal efficiency in both phosphorus and nitrogen. This is likely due to the very low incoming soluble COD concentration and the limited biodegradability of the particulate COD in the anaerobically pretreated feed stream.

A long term experimental study of SCOD effect was performed. The dissolved oxygen was controlled in the range of 0.3-1.0 mg/l. Acetate was used as an external SCOD source. The graph in Figure 3 shows that for the first 10 days running without an external carbon source, the average nitrate level in the effluent was very high. On day 11, acetate was added to the feed and completely mixed with the original feed before pumping into the reactor. After some initial delay likely due to the acclimatisation and growth of the required microorganisms, the amount of nitrate dramatically decreased to less than 20% of the previous values. On day 20, acetate addition was stopped again leading to a gradual nitrate accumulation in the effluent.

Resumption of acetate addition on day 32 led to an immediate reduction of nitrate concentration again. This time, no acclimatisation time was required because significant numbers of denitrifying organisms were still present in the sludge. This experiment confirms that the SCOD is critically important in this wastewater to achieve complete denitrification.

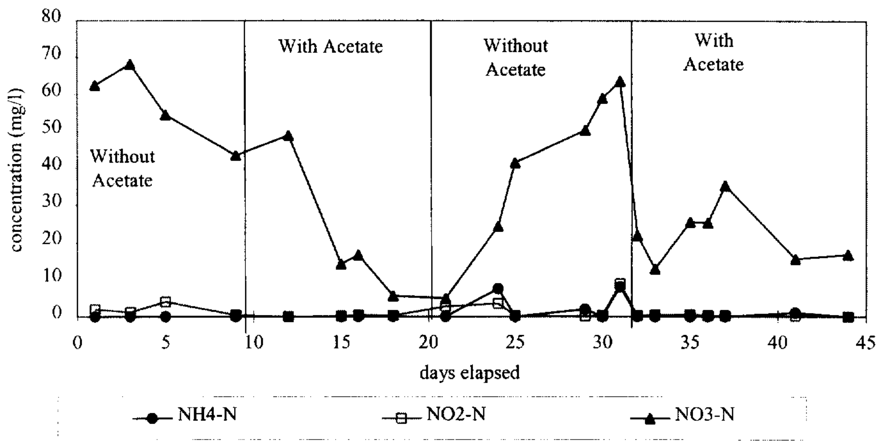

Figure 3. SBR effluent concentration during SCOD effect experiment

Experiment to determine floc size effect

Based on the hypothesis of oxygen diffusion limitation as the cause for SND (see Fig. 1), the effectiveness of SND should be dependent on the floc size and floc size distribution. To investigate the effect of changing the floc size distribution on SND, a detailed analysis of two subsequent cycles was performed. Both cycles were operated in the usual way but the activated sludge flocs were physically broken up between the two cycles. The first cycle was operated with the usual floc size (median 80μm), whereas the second cycle was operated with smaller flocs (median 40-50μm). To reduce the floc size, the entire sludge from the reactor was subjected to high speed blending (24,000 rpm) for 5 minutes. Floc size measurements were performed during the first cycle, directly after blending and throughout the second cycle to determine any reflocculation effects. The initial feed concentration of these 2 cycles was 140 mg NH_4-N /l, 1450 mg TCOD/l, 204 mg SCOD/l. The dissolved oxygen during the aerobic period was controlled in the range of 0.3-2.5 mg/l in both cycles.

Figure 4 shows the floc size distribution of those two subsequent cycles. The distribution of the floc sizes showed a very consistent pattern. It should be noticed that the number of large flocs was dramatically decreased by this blending technique. During the 3 hours aeration time, only a slight increase in floc size was observed due to reflocculation.

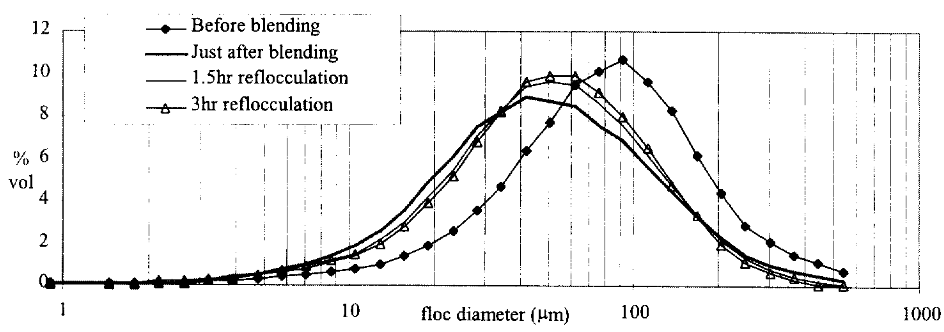

Figure 4. Floc size distributions prior to and following dissociation by high speed blending.

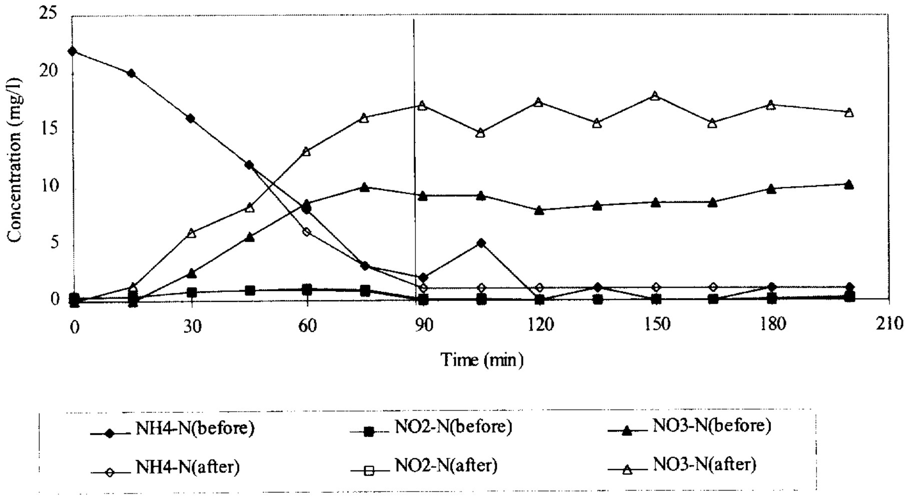

Figure 5. SND behaviour of the cycle before and after blending.

The concentrations of nitrogen compounds in the two cycles before and after blending are shown in Fig 5. In both cycles, the ammonium oxidation rate was identical, confirming that the blending procedure did not affect the viability or performance of the biomass. Interestingly, no increase in nitrification rate was observed due to the decreased floc size. This indicates that nitrification was not oxygen diffusion limited in the initial cycle. In both cycles, the ammonia is completely oxidised. After the first 90 minutes of aeration, no further reaction occurred. The remaining COD is likely non-biodegradable and therefore, together with the relatively high DO level, limiting the denitrification process.

Comparing the oxidised nitrogen compounds it can be seen that the first cycle operating with a median floc size of 80 μm can achieve 52% SND, whereas the SND activity decreased to only 21% after the floc size was reduced. This result certainly supports the hypothesis that a physical phenomenon is responsible for SND, in particular the oxygen diffusion limitation. It has to be considered that a reduction of the median floc size from 80 μm to 40 μm represents a major reduction of the available internal floc volume where denitrification could occur. Furthermore, the significant reduction in flocs larger than 100 μm further compounded this effect.

Not only the floc size influences the proportion of anoxic volume inside the floc but the DO concentration in the liquid phase plays an important role as well. Therefore, the effect of dissolved oxygen on SND efficiency was investigated.

Dissolved oxygen experiment

The reactor was set up as a 5 hours batch experiment. In order to eliminate nitrite and nitrate left over from the previous cycle, an anoxic period was introduced during the first 30 minutes of the react time. DO control points were set sequentially to 0.1-0.2, 0.3-0.4 and 0.6-0.7 mg/l. At each level, DO was maintained in the particular range for 90 minutes. The measured average DO concentrations in each step were 0.3, 0.5 and 0.8 mg/l, respectively. The abattoir wastewater was used as basic feed but the SCOD content was increased by addition of sodium acetate. This was to ensure sufficient supply of all important components other than oxygen to determine the effect of DO on the SND performance. After addition, the feed concentrations were 2340 mg TCOD/l, 2150 mg SCOD/l, 170 mg NH_4-N/l and 30 mg HPO_4-P/l. 800 ml of feed was dumped into the reactor to achieve a total working volume of 4.8 l. The reactor was operated with 7600 mg MLSS/l and 6200 mg MLVSS/l. Additional chemicals, as shown in Table 2, were added at the beginning of each DO step change to eliminate any possible limitation of these compounds during the experiment.

Table 2. Chemicals added in DO batch experiment

Average DO (mg/l)	$CH_3COONa.3H_2O$	$NaNO_2$	$NaNO_3$	NH_4HCO_3 (136.5 mg/l)
0.3	4g	0.2 g	0.4 g	3 ml
0.5	4g	0.2 g	0.4 g	2 ml
0.8	4g	0.2 g	0.4 g	3 ml

This experiment was performed to investigate the maximum rate of nitrification and denitrification at the different DO levels. Figure 6 shows the results for the entire run. In the first period, running with DO 0.3 mg/l, denitrification of NOx-N (ie. NO_3-N + NO_2-N) was completed within less than 30 minutes. Furthermore, all products from the nitrification process were also converted to nitrogen gas. However, only limited nitrification was observed. As expected, at the higher DO concentrations, a significant increase in the nitrification rate was noticed. At the same time, nitrite and nitrate levels became more detectable with reduced denitrification rates.

Figure 7 shows the rates of nitrification and denitrification at the various DO levels. The nitrification rate increased strongly with the increase in average DO in this range. This result is in line with many previous findings that the DO directly influences ammonia oxidation process. Theoretically, the denitrification rate should be increasingly inhibited by higher DO concentrations particularly in the range used in this experiment. However, the graph shows the opposite result with an increasing denitrification rate at increasing DO concentrations. This behaviour can be explained in that the rate of denitrification at 0.3 mg/l DO was limited by the rate of nitrification (initial reduction of NO_x not considered). The lack of NO_x simply limited the denitrification activities in the system. This is obvious from Fig. 7 since the rate of nitrification equals the denitrification rate. Therefore, 100% SND degree was achieved but at a reduced nitrification rate compared to the higher DO levels.

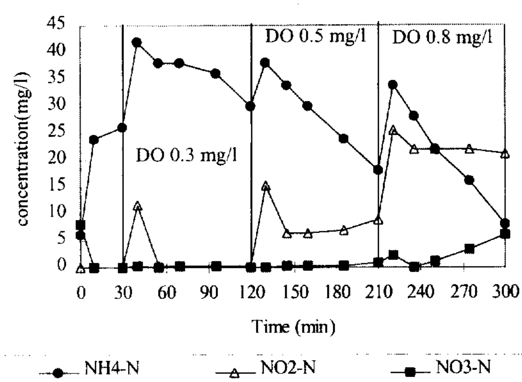

Figure 6. Stepwise DO testing.

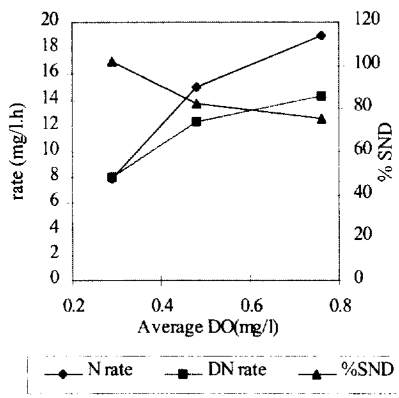

Figure 7. The rate of nitrification and denitrification in different DO concentration.

SND effectiveness at the higher DO concentrations still reached approximately 80%. Interestingly, the denitrification rate still increased even up to 0.8 mg/l DO. The increase in nitrification rate, together with the higher concentration of nitrite present in the system is likely responsible for this further increase of the denitrification rate.

This result partially explains the relationship between the DO concentration and SND activities. Again, it points towards an oxygen diffusion limitation as a possible cause for SND. However, the interactions are quite complex as seen from Fig. 7 and depend on a number of other factors as previously discussed. Most notably, the floc size distribution seems to have a direct effect on the SND performance, indicating an oxygen diffusion limitation. To incorporate all these effects and determine the relative importance under specific operating conditions, a mathematical model seems most suitable. This could also be used to identify optimal operating parameters for SND.

A limited mathematical model for the diffusion limitation process has already been developed (Bakti and Dick, 1992). However, this model does not incorporate the dynamic behaviour in the system and does not include all reactor conditions that significantly influence the occurrence of SND. Therefore, a dynamic model considering all activities in both solid and liquid phase in the reactor is now being developed based results from this and other studies.

CONCLUSIONS

From this present and previous studies into SND, three main factors affecting the performance have been identified. These are (S)COD source, dissolved oxygen content and floc size and the following conclusions can be made in this regard.

1. The addition of readily biodegradable COD source resulted in a significant increase of SND activity in the system. This indicates that the soluble COD fraction is strongly affecting SND performance. This is consistent with the higher rates of denitrification found in anoxic reactors with high soluble COD fractions.
2. SND activities seemed to be increased in reactors with larger activated sludge floc sizes.
3. Increased dissolved oxygen concentrations in the reactor bulk liquid negatively affect SND. However, the relationship is not directly obvious with denitrification and nitrification rates increasing at DO levels up to 0.8 mg/l.

All conclusions are in accord and even support the hypothesis that SND is caused by an oxygen diffusion limitation into the flocs thereby generating anoxic conditions around the centre of the flocs. Based on these findings, a dynamic reactor model incorporating intracellular floc behaviour is being developed to achieve a better understanding of the SND behaviour based on the diffusion hypothesis.

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REFERENCES

Andreadakis, A. D. (1993). Physical and chemical properties of activated sludge flocs., *Wat. Res.*, **27**, 1707-1714.

Bakti, N. A. K. and Dick, R. I. (1992). A model for a nitrifying suspended-growth reactor incorporating intraparticle diffusional limitation. *Wat. Res.*, **26**(12), 1681-1690.

Barnard, J. L. (1992). *Design and retrofit of wastewater treatment plants for biological nutrient removal.* Technomic Publishing, Pennsylvania.

Bliss, P. and Barnes, D. (1986). Modelling nitrification in plant scale activated sludge. *Wat. Sci. Tech.*, **18**(6), 139-148.

Gerber, A., Mostert, E. S., Winter, C. T. and de Villiers, R. H. (1986). The effect of acetate and other short-chain carbon compounds on the kinetics of biological nutrient removal. *Wat. SA,* **12**, 7-12.

Goronszy, M. C. (1992). In: Course Notes on "Intermittently operated activated sludge plants". Department of Chemical Engineering, University of Queensland, Australia.

Henze, M. (1989). Carbon sources for denitrification of wastewater. Seminar on nutrients Removal from Municipal Wastewater, 4-6 September 1989. Tampere University, Tampere, Finland.

Isaacs, S. H. and Henze, M. (1994). Controlled carbon source addition to an alternating nitrification-denitrification wastewater treatment process including biological P removal, *Wat. Res.*, **29**, 77-89.

Münch, E. v., Lant, P. A. and Keller, J. (1996). Simultaneous nitrification and denitrification in bench-scale sequencing batch reactors. *Wat. Res.*, **30**(2), 277-284.

Painter, H. A. (1977). Microbial transformations of inorganic nitrogen. *Prog. Wat. Tech.*, **8**(4/5), 3-29.

Subramaniam, K., Greenfield, P. F., Ho, K. M., Johns, M. R. and Keller, J. (1994). Efficient biological nutrient removal in high strength wastewater using combined anaerobic-sequencing batch reactor treatment. *Wat. Sci. Tech.*, **30**(6), 319-321.

Subramaniam, K., Keller, J., Ho, K. M., Johns, M. R. and Greenfield, P. F. (1994). Effect of Pretreatment on the nutrient removal efficiency in high strength wastewater using SBR technology, Proceedings of the second Australia conference on biological nutrient removal from wastewater, Albury, NSW, Australia, 217-226.

Tam, N. F. Y., Wong,Y. S. and Leung, G. (1992). Significance of external carbon sources on simultaneous removal of nutrients from wastewater. *Wat. Sci. Tech.*, **26**(5-6), 1047-1055.