

SEEDING OF NITRIFYING BACTERIA AS A WAY FOR NITRIFICATION IMPROVEMENT

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ABSTRACT

Seeding of nitrifying bacteria into the activated sludge process may decrease the necessary volume needs for a stable nitrification process. Both theoretical and experimental studies were carried out in order to evaluate the effects of seeding. A model was developed for prediction of the effects of seeding of nitrifying bacteria from a separate stage into the activated sludge process. Seeding technology is the most effective when used at an activated sludge process with a sludge age near or below the critical sludge age for nitrification at non-seeding conditions. The nitrifying bacteria in the seeding sludge have a positive effect on the nitrification process. Experimental studies have been performed at a specially built pilot plant with two separate lines of the activated sludge process located at the Uppsala municipal wastewater treatment plant. One line was supplied with supernatant from dewatering of digested sludge and the nitrification process provided an activated sludge with a high fraction of nitrifying bacteria, suitable for seeding. The other line was supplied with pre-precipitated wastewater and with the excess sludge from the line treating the supernatant. The experimental results showed that nitrification at sludge ages that would otherwise preclude nitrification could be obtained. Performance relationships for the system developed, based on laboratory and on-line measurements were studied and are presented in the paper. The studies indicate that seeding may be advantageous for improving process performance and stability; it provides also an interesting alternative in design and operation of the activated sludge process.

KEYWORDS

Activated sludge; bioaugmentation; digester supernatant; modelling; nitrification; pilot plant; seeding

INTRODUCTION

Nitrogen removal by use of biological nitrification and denitrification processes generates the need for an increased sludge age and increased volume requirements. Instead of building new volumes for nitrogen removal it might be more economical to modify operational modes. Methods which can be used are:

- partial nitrification of side-streams (supernatant) from dewatering of digested sludge, earlier studied in Sweden by Tendaj-Xavier et al., 1985; Björleinius, 1988, Mossakowska, 1994,
- feeding external nitrification bacteria into the activated sludge process, evaluated by Mathisen and Hultman, 1982; Finnson, 1994; Li and Hultman, 1997.

In Sweden many existing plants have been upgraded for nitrogen removal. A decrease of the nitrification efficiency during the snow thawing period with high flows and low temperatures has been often observed (Plaza et al., 1990). As a criterium for stable nitrification a temperature-compensated critical sludge age for nitrification bacteria is used. In order to obtain a high efficiency of the nitrification process during the winter time at low temperatures, the reactor volume need may be even three times higher than in the summer period. Seeding technology is one of the methods for reduction of the process volume requirements through the decreasing the critical sludge age. The excess activated sludge with a high fraction of nitrifying bacteria is seeded into another activated sludge tank to facilitate the nitrification process. Some other benefits of

seeding are: improved BOD removal, prevention of sludge bulking, and removal of specific pollutants by specially adapted bacteria (Babcock et al., 1992, Gerrard and Stephenson, 1990, and Stephenson and Stephenson, 1992, and Wilderer et al., 1991). Bioaugmentation of activated sludge for biological phosphorus removal has been studied by Belia and Smith (1997).

The effects of seeding nitrifying bacteria into the activated sludge process have also been studied based on theoretical models (Finsson, 1994, Lee, 1997, Li and Hultman, 1997, Rittman, 1996 and 1997, and Tendaj-Xavier, 1985) and experimental works (Daigger et al., 1993, Parker and Richards, 1994, Rittman and Whiteman, 1994, and Sinkjaer et al., 1996).

In order to evaluate the effects of seeding the experimental studies have been performed at a specially built pilot plant located at the Uppsala municipal wastewater treatment plant.

THEORY

In seeding technology of the activated sludge process it is necessary to consider both the net growth rate of the specific bacteria and the measured sludge age. The net growth rate of nitrifying bacteria, μ_N , may be written as (Rittman, 1996):

$$\mu_N = \frac{M_N - QX_{oN}}{X_N V_{aer}} \quad (1)$$

where M_N = amount of nitrifying bacteria per day from the activated sludge process in the excess sludge and in the effluent
 Q = influent flow rate
 X_{oN} = concentration of nitrifying bacteria in the influent to the activated sludge process
 X_N = concentration of nitrifying bacteria in the aerated zone of the activated sludge process
 V_{aer} = volume of the aerated zone

The measured sludge age, Θ_{daer} , calculated for the aerated zone of the activated sludge process is:

$$\Theta_{daer} = \frac{A}{M} \quad (2)$$

where A = sludge mass in the aeration zone
 M = sludge mass removed daily from the activated sludge process as the excess sludge and with the effluent

The fraction of nitrifying bacteria is assumed to be the same in the aerated zone as in the sludge from the activated sludge process (M). In this case the removed nitrogen concentration for an activated sludge process with complete mixing and stationary conditions may be written (Li and Hultman, 1997):

$$n_o - n = \frac{X_{oN}}{Y_n} \frac{\mu_N \Theta_{daer}}{1 - \mu_N \Theta_{daer}} \quad (3)$$

where n = effluent ammonium concentration
 n_o = influent ammonium concentration that can be nitrified
 X_{oN} = influent concentration of nitrifying bacteria
 Y_n = yield coefficient for nitrifying bacteria
 Θ_{daer} = aerobic sludge age
 μ_N = growth rate of nitrifying bacteria

For lower ammonium concentrations (<5 g N/m³) the Monod relationship may be applied:

$$\mu_N = \frac{\mu_{Nmax} n}{K_n + n} \quad (4)$$

where K_n = half saturation coefficient
 μ_{Nmax} = maximum growth rate of nitrifying bacteria

If formula (4) is inserted in formula (3) the following may be obtained:

$$n_o - n = \frac{X_{oN}}{Y_n} \frac{\Theta_{daer}}{\frac{K_n + n}{\mu_{Nmax} n} - \Theta_{daer}} \quad (5)$$

which may be arranged as:

$$n^2 - n \left(n_o - \frac{K_n + \frac{X_{oN} \mu_{Nmax} \Theta_{daer}}{Y_n}}{1 - \mu_{Nmax} \Theta_{daer}} \right) - \frac{K_n n_o}{1 - \mu_{Nmax} \Theta_{daer}} = 0 \quad (6)$$

and gives the solution:

$$n = \frac{a \pm \sqrt{a^2 - 4b}}{2} \quad (7)$$

where

$$a = n_o - \frac{K_n + \frac{X_{oN} \mu_{Nmax} \Theta_{daer}}{Y_n}}{1 - \mu_{Nmax} \Theta_{daer}} \quad (8)$$

$$b = - \frac{K_n n_o}{1 - \mu_{Nmax} \Theta_{daer}} \quad (9)$$

The positive sign before the square root in formula (7) is used when $\Theta_{daer} < 1/\mu_{Nmax}$ while the negative sign when $\Theta_{daer} > 1/\mu_{Nmax}$. For the special case of $K_n = 0$, formula (7) can be written as:

$$n = a = n_o - \frac{X_{oN}}{Y_n} \frac{\mu_{Nmax} \Theta_{daer}}{1 - \mu_{Nmax} \Theta_{daer}} \quad (10)$$

Formula (10) can directly be obtained by insertion of $\mu_N = \mu_{Nmax}$ in formula (3).

The seeding nitrifying bacteria are obtained from a separate nitrification step at the wastewater treatment

plant. The design and operational conditions in this step will influence the value of X_{oN}/Y_n and Θ_{daer} in formulas (8),(9) and (10).

The parameter M may be divided into two parts:

$$M = B + N \quad (11)$$

where B = measured mass of sludge per day from the activated sludge process without seeding
 N = additional mass of sludge per day from the activated sludge process due to supply of seeding sludge

The aerobic sludge age may then be written using formulas (2) and (11):

$$\Theta_{daer} = \frac{A}{B+N} \quad (12)$$

If no seeding occurs $Q_{daer} = 1/\mu_N$ and formula (4) may be written:

$$\Theta_{daer} = \frac{K_n + n}{\mu_{Nmax} n} \quad (13)$$

which may be solved for n:

$$n = \frac{K_n}{\mu_{Nmax} \Theta_{daer} - 1} \quad (0 < n < n_o) \quad (14)$$

Simulations of the effluent value of ammonium (n) as a function of the seeded amount of sludge with nitrification bacteria has been presented by Hultman et al. (1998). The simulations showed that seeding is always positive if the sludge age without seeding is below the critical age for nitrification. At sludge ages above the critical for nitrification without seeding a decrease of the nitrification efficiency may be observed especially if the fraction of nitrification bacteria in the seeded sludge is not high. It may be explained by the fact that seeding lowers the measured sludge age.

MATERIALS AND METHODS

Pilot plant study

A pilot plant, described earlier by Carlsson et al., (1997) and Trela et al., (1998), equipped with various on-line meters and with a specially designed novel computerized control supervision system (CCS) operated at the Kungsängen wastewater treatment plant, Uppsala. The plant consisted of two separate lines, of the activated sludge process P1 and P2, suitable for testing various operation strategies and modes. The volume of each activated sludge tank was 2.35 m³; each settling tank had a volume of 0.55 m³.

Experimental strategy

The studied system is presented in Fig. 1. Line 1 (P1) was continuously supplied with pre-sedimentated wastewater, pumped from the full-scale plant to an influent tank. Line 2 (P2) was fed with supernatant from dewatering of digested sludge as the influent. The supernatant water was pumped to P2 from a storage tank which was placed outdoors. Sodium hydrogen carbonate was added as alkalinity to keep the pH value at

about 7. The consumption of sodium hydrogen carbonate was about 4.2 kg per 1 kg of ammonia nitrogen oxidized.

While planning the experimental strategy computer simulations were carried out using the Danish computer program EFOR. The operational data obtained from the simulation was used in the performance of the experiment. The experiment was performed in three steps. During the first step, a steady state full nitrification of supernatant was obtained for Line 2. During the second step, the excess sludge from Line 2 with high fractions of nitrifying bacteria was seeded into Line 1. Meanwhile Line 1 was operated at a sludge age under the critical value. During the third step, the dynamic effect of seeding was investigated.

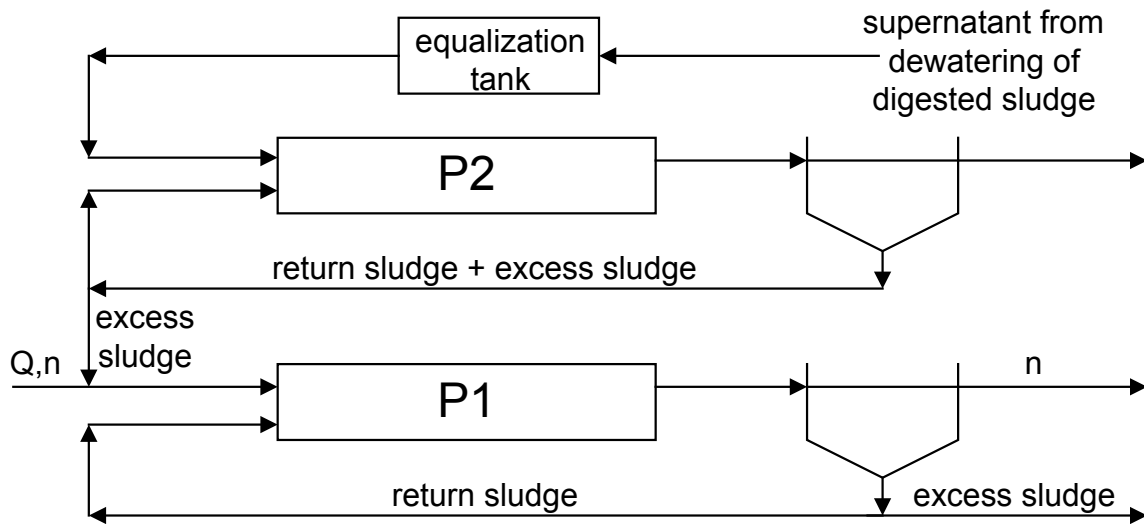


Fig. 1. Seeding strategy flowchart

Sampling procedure and analyses

Throughout the whole experiment 24-h composite samples were collected by vacuum samplers from the influent and effluent lines twice a week. For both lines the following analysis were performed: COD, filtered COD, Tot-N, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{+NO}_3\text{-N}$, HCO_3 and SS. Periodically an intensive sampling program was introduced with taking 24-h composite samples every day. Analytical procedures were performed according to the Swedish standards (SIS).

Stirred specific volume index (SSVI) and sludge volume index (SVI) were performed to observe the sludge settling properties.

Batch tests

Potential nitrification rates were determined indirectly by oxygen utilization rate (OUR) tests. The activated sludge taken from the pilot plant was placed in a water bath and was continuously aerated (usually to a level of 6 to 8 $\text{mg O}_2/\text{l}$). In order to determine the nitrification rate two measurements of respiration rates were carried out.

Oxygen utilization rates for activated sludge with ammonia and with allylthiourea (ATU) were measured by an oxygen probe which was introduced into a 250 ml BOD bottle. The OUR values were calculated from the resulting oxygen utilization profiles. The net value of OUR calculated from the OUR value for activated sludge with ammonia minus the OUR value for activated sludge endogenous respiration was considered to be contributed to nitrification. Using a conversion factor of 4.33 $\text{g O}_2/\text{g N}$ a potential nitrification rate was estimated.

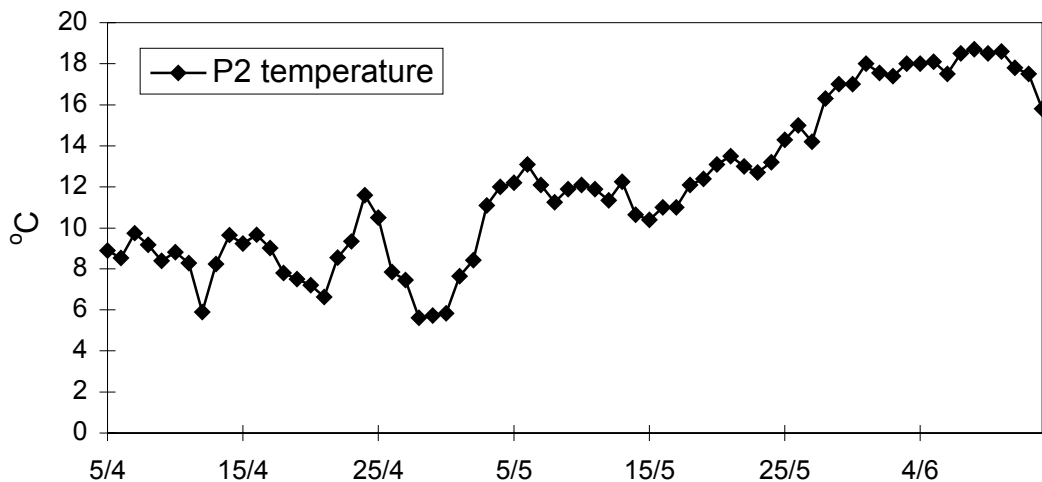
RESULTS

Experiments with nitrification of supernatant

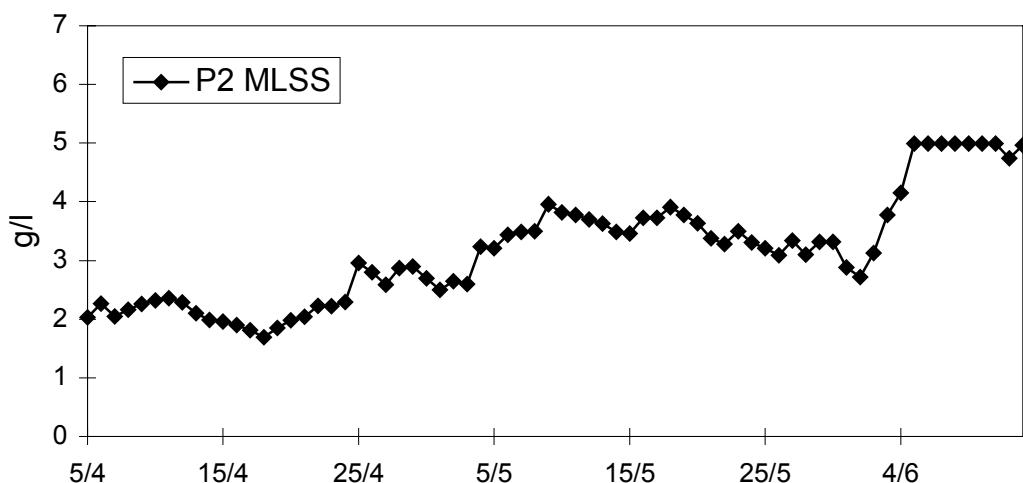
Operational performance. Experiments with nitrification of supernatant from dewatering of digested sludge were carried out in Line 2 of the pilot plant. The characteristics of the supernatant is presented in Table 1. The experiments started at an aerobic sludge age of about 6.7 days as suggested by the simulation. During the first 5 weeks it was difficult to get a stable nitrification process since a high SS concentration in the effluent from P2 was observed and the actual aerobic sludge age was only about 5 days. Also the temperature of the reject water was often lower than 10 °C (Fig. 2). In order to get a higher nitrification efficiency the aerobic sludge age was increased to about 8 days by reducing excess sludge flow rate to 5 l/h beginning from the 20th of April. An effect of substantial decreasing of ammonia concentration in the effluent by increasing of sludge age can be observed from Fig. 3. The average value of the nitrification efficiency obtained in Line 2 was 88 %.

Operational results for the reject water treatment plant are summarized in Table 2. Time variations for the temperature and sludge concentrations in the tank as well as ammonium nitrogen concentrations in the influent and the effluent are shown in Fig. 2. The influent flow varied from of 50 l/h to 100 l/h. The pilot plant was operated with the return sludge flow of 220 l/h and an internal recirculation rate of 660 l/h. The excess sludge in an amount of 5 l/h was pumped directly from the last zone of the activated sludge tank. During the seeding operation mode, the excess sludge was pumped into line 1.

a)



b)



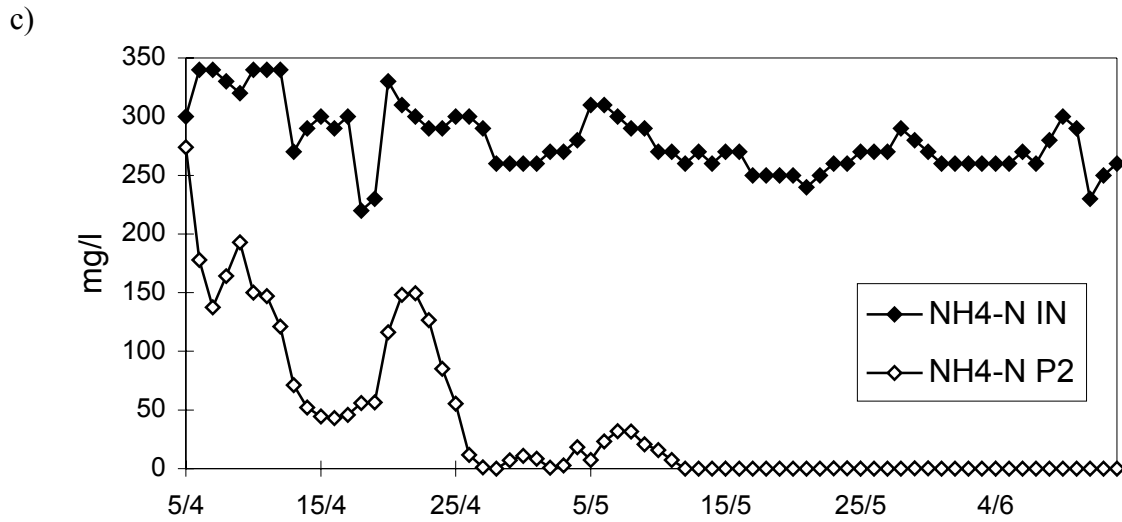


Fig. 2. Operational results - P2 (reject water line): a) temperature - on-line b) MLSS in the tank - on-line c) Ammonia nitrogen concentrations in the influent (analytical) and the effluent (on-line).

Table 1. Characteristics of the supernatant (March - July 1995)

Parameter	Concentration, g/m ³ Average	St. dev.
COD (total)	650	325
Tot-N	343	122
NH ₄ -N	290	60
Tot-P	15.5	3.6
PO ₄ -P	10	2.2
SS	345	88.6
HCO ₃	880	8.9*

Table 2. Summary of operational data

Parameter	Range
Aerobic sludge age, days	5 - 8.6
Temperature, °C	6 - 18.6
Reject water flow, l/h	50-100
MLSS, g/l	2-5
SVI, ml/g	66

* after addition of bicarbonate

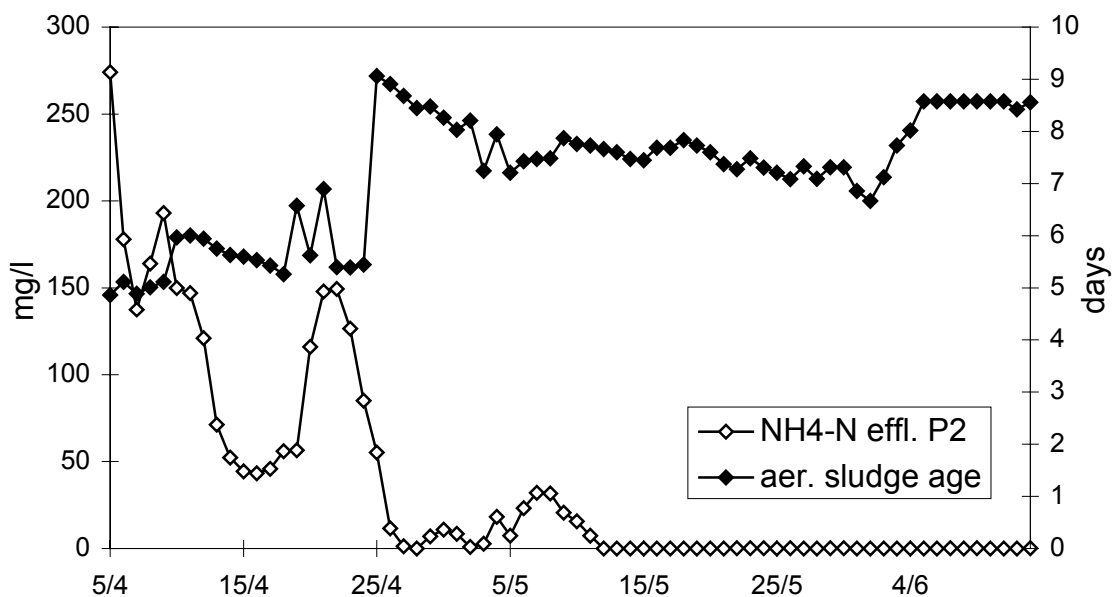


Fig. 3. Ammonia concentrations in the effluent and the aerobic sludge age - Line 2.

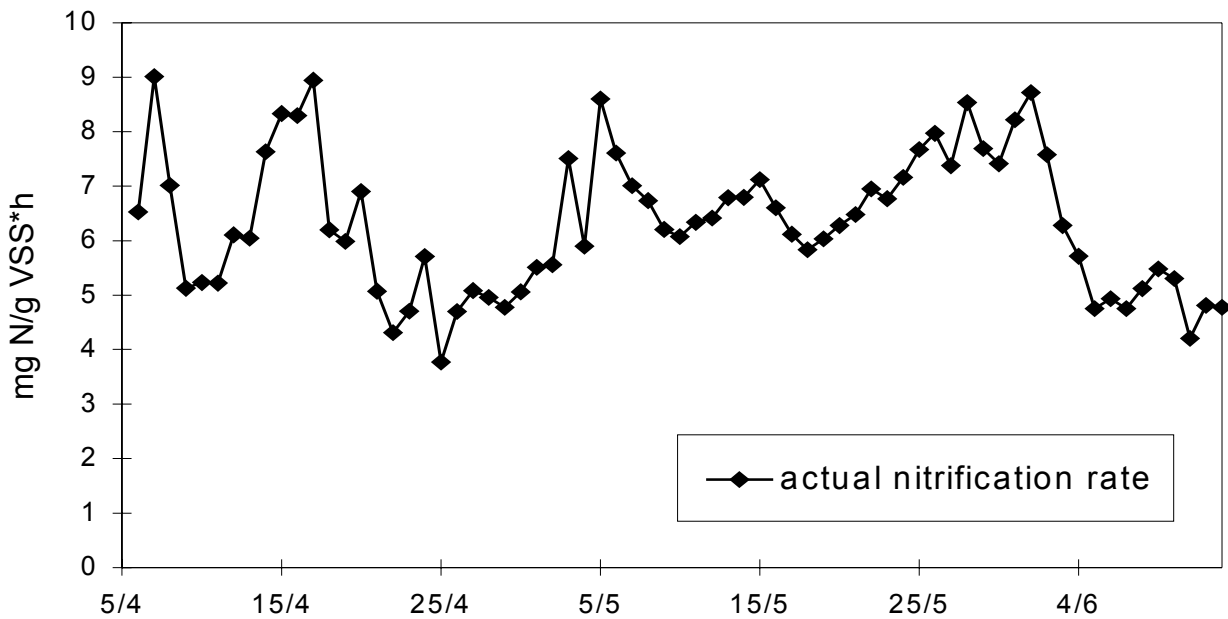


Fig. 4. Actual nitrification rates - Line 2.

Nitrification process. The variability in actual nitrification rate is presented in Fig. 4. The mean value of 6.8 mg N/g VSS*h was obtained for the period analysed. No inhibition effect of the influent on the nitrifying bacteria was observed during this period. The potential nitrification rate was measured during the period between April and June. The potential nitrification rate was about 8.3 mg N/g VSS*h for the sludge taken from Line 2. Laboratory tests showed that the ratio between nitrification rates of the sludge from Line 1 and Line 2 was about 3 when full nitrification was obtained in Line 2. The fraction of nitrifying bacteria in the sludge treating the municipal wastewater is about 3%. Using the factor 3, as obtained from tests, the fraction of nitrifying bacteria in the investigated sludge was estimated as about 9%.

Experiments with seeding of nitrifying bacteria

Operational performance. The nitrifying bacteria produced in Line 2 were seeded to an activated sludge process fed with pre-precipitated wastewater (Line 1). Experiments with seeding of nitrification bacteria were carried out in Line 1 of the pilot plant. Characteristics of the pre-precipitated wastewater is presented in (Table 3).

Table 3. Characteristics of the pre-precipitated wastewater (March - July)

Parameter	Concentration, g/m ³ Average	St. dev.
COD	190	86.3
CODf	100	26.5
Tot-N	26.3	7.2
NH ₄ -N	20	3.7
NO ₂ -N + NO ₃ -N	0.35	0.39
Tot-P	3	1.5
PO ₄ -P	0.76	0.53
SS	80	24.9
HCO ₃	400	37.4

The seeding of the activated sludge from Line 2 to Line 1 started on May 15. In the seeding experiments, nitrifying bacteria were obtained from the pilot plant line with nitrification of supernatant from dewatering of digested sludge. All excess sludge from this line was supplied to the line where the seeding effects were studied except for 3 July - 10 July, when seeding was stopped. Line 1 was operated at the flow rate of 220 l/h, which corresponds to the hydraulic retention time in the aeration tank of 11 h.

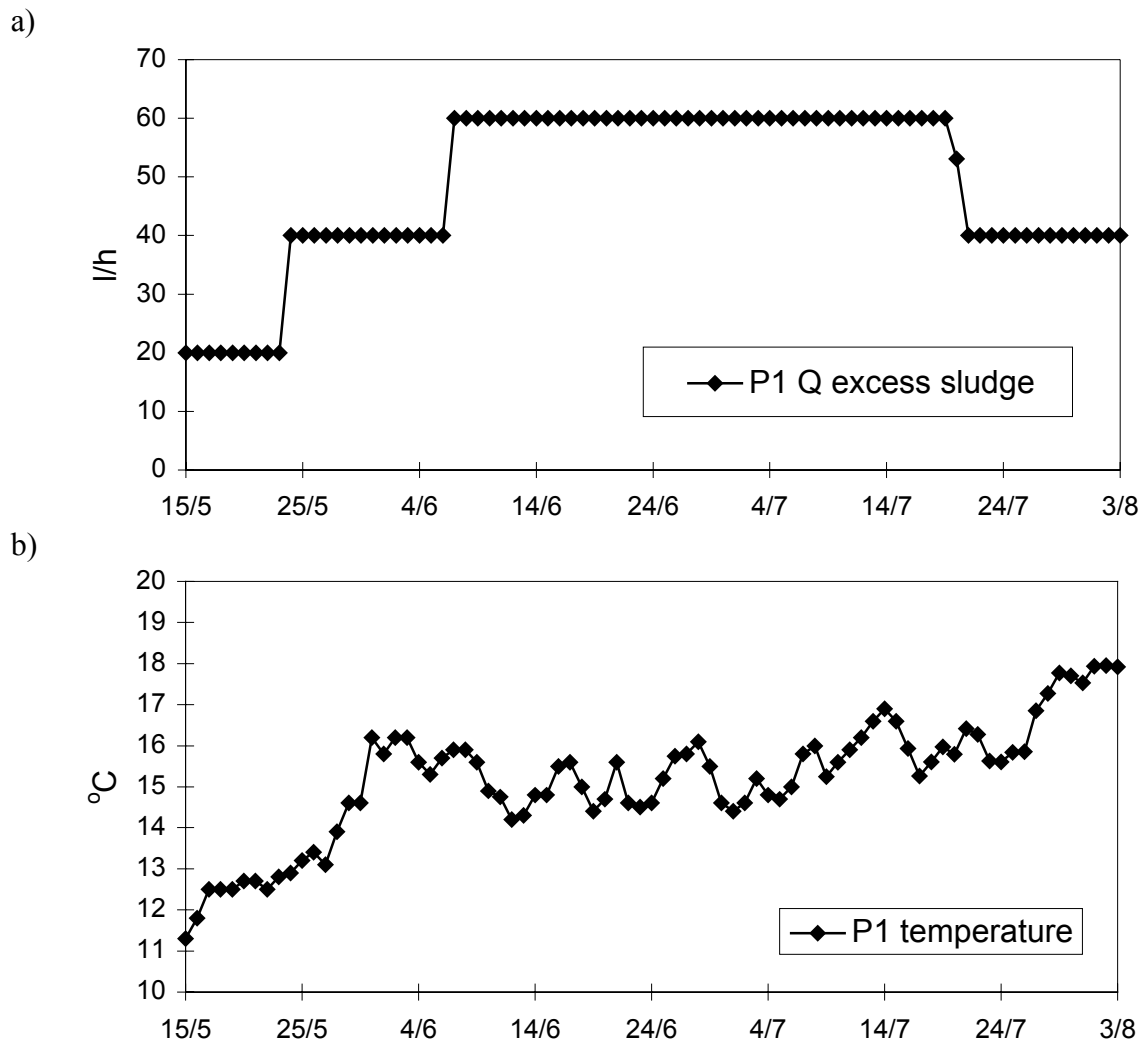
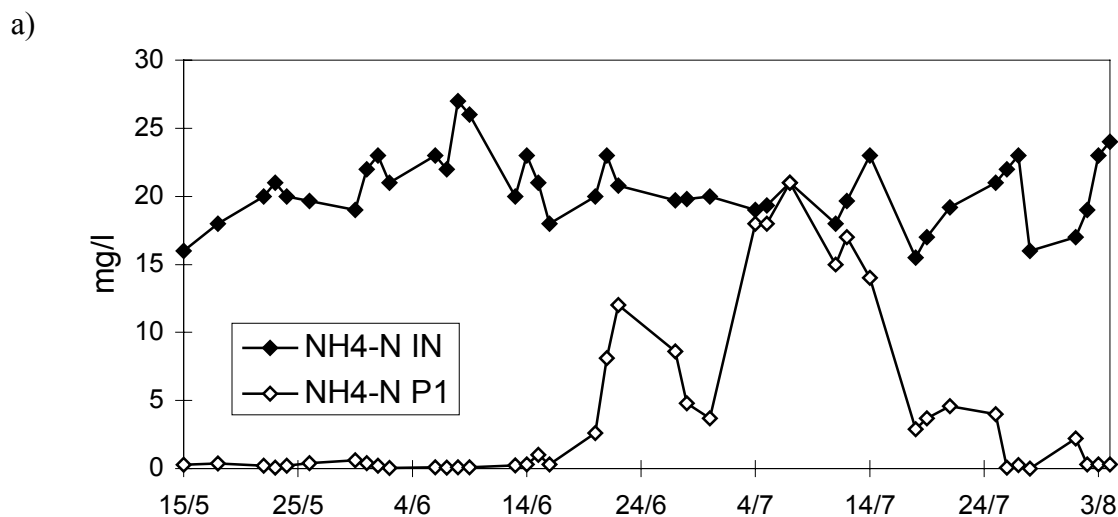


Fig. 5. Operational results - Line 1: a) excess sludge b)temperature - on-line.

The return sludge flow was 220 l/h and the internal recirculation rate was 660 l/h. The excess sludge flow was 20 l/h, 40 l/h and 60 l/h with the corresponding aerobic sludge ages 2.6, 1.4 and 0.9 days, respectively. The excess sludge flow varied according to a pattern presented in Fig. 5a. Aeration basin temperatures ranged from 11.3 to 17.9 °C and averaged 14.6 °C (Fig. 5b). Fig. 6 summarizes the biological treatment efficiency obtained at the pilot plant - Line 1 - operated with continuous seeding of nitrifiers from a reject treatment plant.



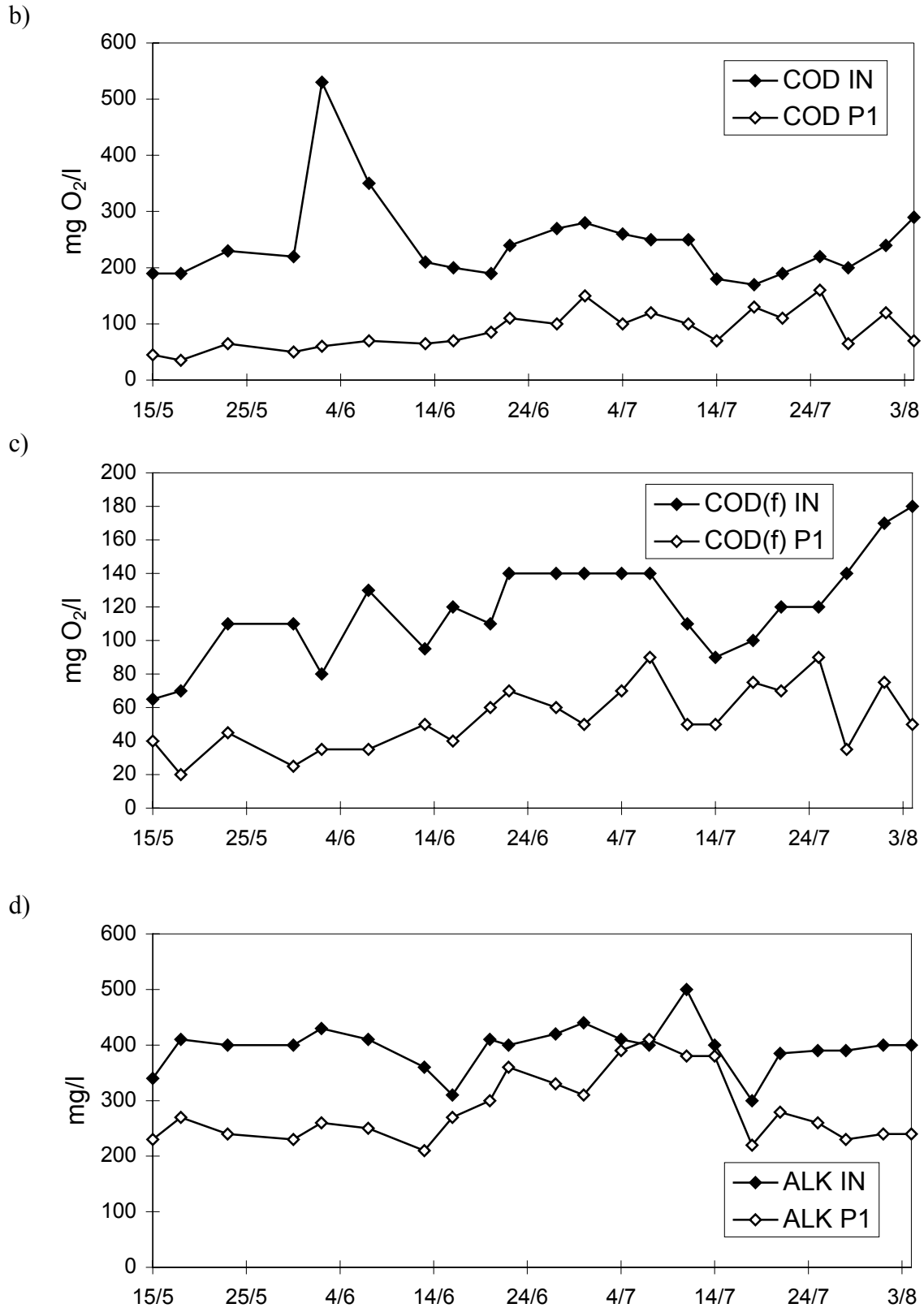


Fig. 6. Treatment efficiency - Line 1: a) ammonia nitrogen b) COD c) CODfiltrated d) alkalinity (Influent and effluent concentrations measured as 24-h composite samples).

Fig. 7 shows the time series of the measured aerobic sludge age during the time of study. By comparison of these values with the ammonia concentrations in the effluent, it can be observed that nitrification can be maintained at lower aerobic sludge age than it would be possible if seeding did not occur. It can be seen that a high removal efficiency could be obtained for nitrification in the seeded line for sludge ages down to about 0.5 days at a temperature of 17 °C. There was no seeding of nitrification bacteria between July 3rd and 10th

which explains the high peak value of ammonium during that period. The experiments showed that nitrifying bacteria from the line for treatment of supernatant retained their activity after they had been transferred to the line treating pre-precipitated wastewater.

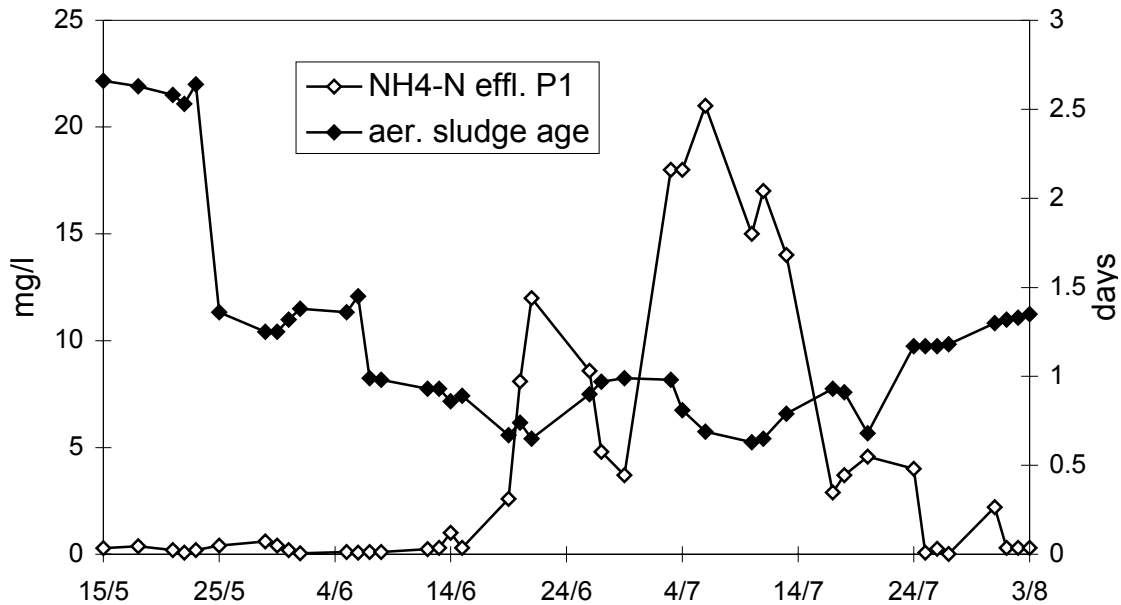


Fig. 7. Aerobic sludge age and effluent ammonium nitrogen concentrations as a function of time.

The very large effect of seeding of sludge with nitrifying bacteria on the required sludge age depends on the large amount of seeded sludge. In practice a much lower amount of seeded sludge can be used.

Sludge properties.

Observations of sludge settling properties, which were determined twice a week during the whole period of the study showed that Line 2 had better settling properties. (Fig. 8). Average values for SVI were 120 ml/g (st.dev. 71 ml/g) and 66 ml/g (st.dev. 19 ml/g) in the wastewater (Line 1) and the supernatant line (Line 2), respectively. The measured mean value of SSVI from Line 2 was 45 ml/g. A good settling property of the sludge treating reject water was also reported from others studies (Mossakowska, 1994). Bubble forming in the aerated tank and sludge floating in the sedimentation tank were observed.

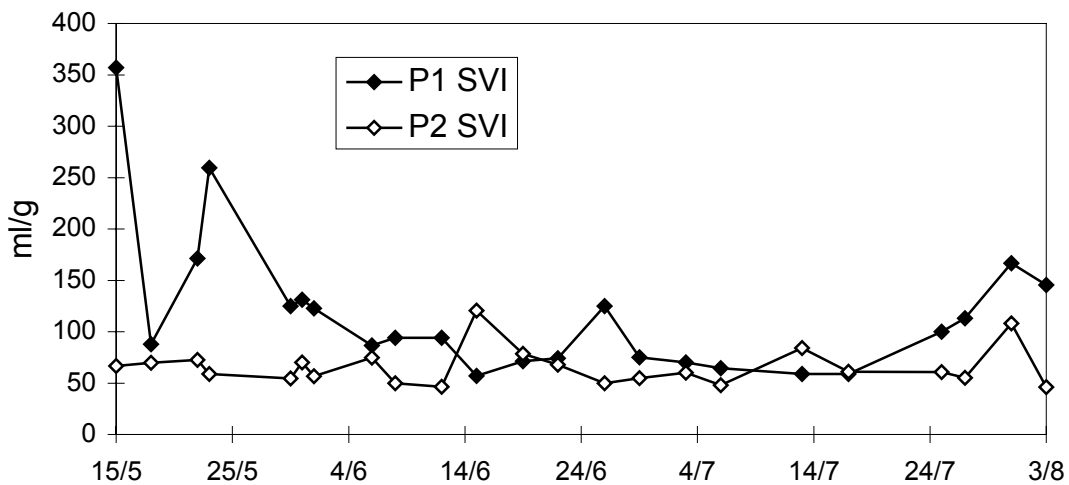


Fig. 8. Sludge settling properties - Line 1 and Line 2.

DISCUSSION

Seeding of sludge from a separate nitrification stage may be an advantageous technology in implementation of nitrogen removal at new plants or in upgrading existing plants. The system needs a separate reactor for culturing the specialised microorganisms used for bioaugmentation. The produced amount of nitrifying bacteria must be added continuously. Many studies showed that ammonia rich supernatant may be oxidised by nitrifying bacteria to nitrate in a separate nitrification step (Mossakowska, 1994; Mossakowska et al., 1997 and Tendaj-Xavier, 1985). It can be operated in a reliable way at temperatures around 20 °C throughout the whole winter. Special conditioning methods (i.e. heat treatment or the addition of acids or bases) can improve the release of substances from the sludge and thereby increase the ammonium concentration of the supernatant from dewatering of sludge. In this way a larger amount of nitrifying bacteria can be produced in the separate nitrification step. Conditioning techniques can be used before or after the digestion. The nitrifying bacteria produced in the separate step are then supplied to the activated sludge process. The example of such system with possibilities for increased ammonia release through special conditioning methods is presented in Fig. 1.

The model described in this paper shows that it is possible to calculate in a simple way the effects of seeding. Assumptions in the model may, however, show that modifications may be necessary of the model. An important assumption is that the seeded nitrifying bacteria will maintain its activity in the activated sludge processes. Activity losses due to changed environmental conditions and grazing of protozoes on the bacteria may reduce the positive seeding effects.

The model has been developed for stationary conditions and with a continuous seeding. It may also be possible to use a separate nitrification stage for seeding at special occasions such as starting up again the nitrification process after it has been inhibited by industrial discharges or other process disturbances.

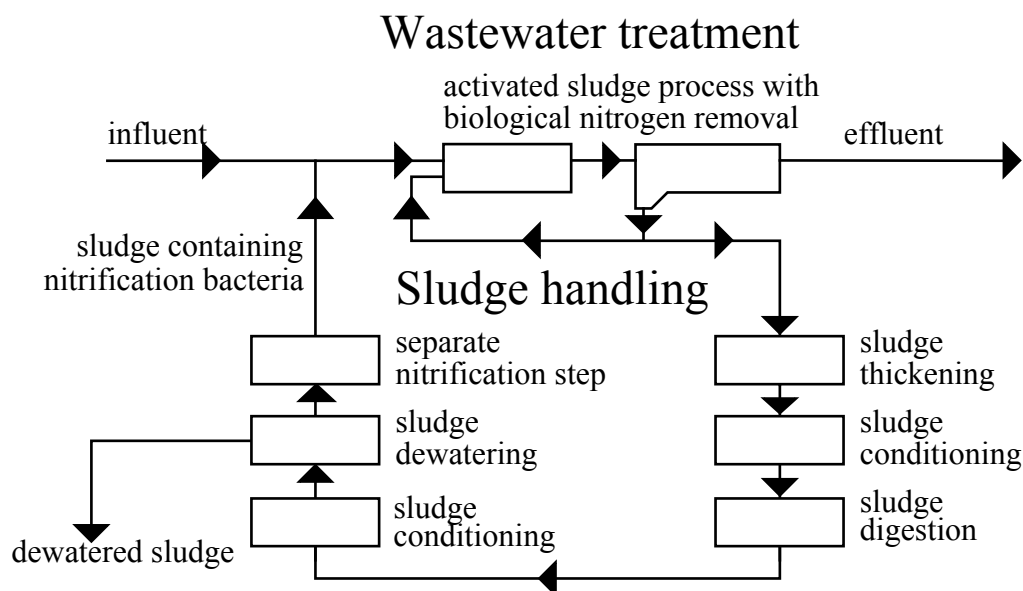


Fig. 9. System for wastewater treatment and sludge handling with a separate step for nitrification of supernatant.

The simulations completed with the model and presented by Hultman et al. (1998) show that it is important to operate the separate stage in such a way that the fraction of nitrifying bacteria remains high in the seeding sludge. Therefore, the separation efficiency must be high in the dewatering step. Otherwise the seeding sludge will decrease the sludge age to such an extent that the nitrification efficiency significantly decreases in the activated sludge process.

CONCLUSIONS

1. The seeding effect of nitrifiers grown in the activated sludge tank supplied with reject water and passed into the activated sludge tank supplied with primary settled wastewater, allows to nitrify at sludge ages that would otherwise preclude nitrification.
2. The minimum value of the sludge age that may be used for treatment of supernatant in order to secure a stable and efficient nitrification process at 15 °C is 5 days.
3. A simple model has been developed for prediction of the effects of seeding of nitrifying bacteria from a separate stage into the activated sludge process.
4. Seeding of nitrifying bacteria has a positive effect on nitrification if the activated sludge process operates near or below the critical sludge age for nitrification at non-seeding conditions. Above the critical sludge age, seeding has a low effect and may even slightly decrease the nitrification efficiency due to that seeding causes a decrease of the sludge age.
5. The seeding effects are highly dependent on the fraction of nitrifying bacteria in the seeding sludge and the amount of seeding sludge. The fraction of nitrifiers should be as high as possible. Application of special conditioning techniques result in the increase of the amount of the solubilised ammonium in the sludge if compared with the conventional methods. The increased amount of ammonium enhances the growth of nitrifying bacteria and thereby the seeding effects is significantly higher.

ACKNOWLEDGEMENT

The authors would like to thank Shulan Xu for his excellent pilot plant experiment management and Jan Söderlund and Bert Alvé, at Kungsängen wastewater treatment plant, for their contribution to the experimental part. This study has been financially supported by Uppsala Municipal Services and by NUTEK (Swedish National Board for Industrial and Technical Development) within the STAMP (Control and Operation of Wastewater Treatment Plants) project.

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