Pergamon

Wat. Sci. Tech. Vol. 34, No. 1–2, pp. 91–99, 1996.

0273–1223/96 $15·00 + 0·00

PII: S0273-1223(96)00499-4

MICROBIAL ADAPTATION, PROCESS PERFORMANCE AND A SUGGESTED CONTROL STRATEGY IN A PRE-DENITRIFYING SYSTEM WITH ETHANOL DOSAGE

S. Hallin*, C.-F. Lindberg**, M. Pell*, E. Plaza*** and B. Carlsson**

** Department of Microbiology, Swedish University of Agricultural Sciences, Box 7025, S-750 07 Uppsala, Sweden*
*** Systems and Control Group, Uppsala University, Box 27, S-751 03 Uppsala, Sweden*
**** Department of Water Resources Engineering, Royal Institute of Technology, S-100 44 Stockholm, Sweden*

ABSTRACT

Biological nitrogen removal in activated sludge processes is dependent on sufficient supplies of easily metabolized carbon compounds for the denitrifying bacterial population. An external carbon source can increase denitrification rates and compensate for deficiencies in the influent C/N ratio. Plant performance and microbial adaptation were studied in a pre-denitrifying pilot-scale activated sludge plant with and without ethanol. Total nitrogen removal efficiency was 67 and 35% for the ethanol and reference line, respectively. The process responded rapidly to ethanol but one sludge age was necessary for full bacterial adaptation. An initial rapid increase suggests enzyme induction rather than alterations in bacterial species composition. Increased enzyme activity was explained by an increase in turn-over rate of biomass. Low effluent nitrate concentration was a result of the simultaneous use of influent COD and ethanol. Fluctuations in influent COD did not affect denitrification capacity with ethanol. Sludge settling properties were moderately better in the process without ethanol addition. An automatic control strategy for carbon dosage using feedforward from influent carbon and nitrate in the recirculated flow was simulated. Simulations with an adaptive linear quadratic controller demonstrated that the desired nitrate concentration at the end of the anoxic zone could be maintained despite relatively large disturbances. Copyright © 1996 IAWQ. Published by Elsevier Science Ltd.

KEYWORDS

Activated sludge; automatic control; carbon source; denitrification; nitrogen removal, wastewater.

INTRODUCTION

More stringent effluent requirements in combination with increasing loads and limiting basin volumes in many wastewater treatment plants call for more efficient processes. Successful biological nitrogen removal is dependent on many factors, including the supply of sufficient and suitable electron donors for the

denitrifying population. One strategy for achieving acceptable nitrogen removal rates in an activated sludge process lacking suitable electron donors and with poor COD/N ratio is to add an external carbon source. Possible carbon sources include methanol, ethanol, acetate, primary sludge and various industrial waste products.

In this paper we present experiences with ethanol as the external carbon source in a pre-denitrifying activated sludge process. Most reports concerning the effects of external carbon sources have dealt with post-denitrification processes. The choice of ethanol as the carbon source is an interesting alternative to the more commonly used methanol both with respect to economy and process flexibility. Pure methanol is now more expensive than pure ethanol in Sweden and ethanol can be obtained as an inexpensive waste product. Full scale experiments with ethanol added to a pre-denitrification process showed that the nitrogen removal efficiency was approximately proportional to the COD/N ratio for a particular carbon source and a specified anoxic zone fraction as long as nitrate was not limiting for denitrification (Plaza *et al.*, 1990). Full-scale experiments with methanol in a post-denitrification process demonstrated a lag period before an increase in both nitrogen removal and denitrification rates (Andersson *et al.*, 1995; Nyberg *et al.*, 1992). Methanol has been shown to select for a highly specialized denitrifying population consisting of facultative methylotrophs (Claus and Kutzner, 1985; Timmermans and Van Haute, 1983) but the literature is not consistent (Halmı and Eimhjellen, 1981; Christensson *et al.*, 1994). Ethanol, on the other hand, is converted by the bacterial cell to acetyl-SCoA, as is acetate, before entering the tricarboxylic acid cycle. Bacteria also utilize the same anaplerotic sequence when growing on ethanol as on acetate. Acetate might account for 5-10% of the total COD in sewage (Henze *et al.*, 1994). Suitable denitrifying populations with the appropriate enzymes for ethanol degradation should therefore already exist in activated sludge. Acetate is known as an easily degradable carbon compound for many bacteria. Isaacs *et al.* (1994) showed that nitrogen removal was enhanced immediately after acetate addition. Nitrogen removal also increased instantaneously when ethanol was used as an external carbon source in a post-denitrification process (Andersson *et al.*, 1995). It is important to adjust the external carbon dosage. A too high dosage is negative both for economic and process-efficiency reasons (Aspegren *et al.*, 1992). Due to carbon to nitrogen fluctuations in the influent, a constant dosage of external carbon does not allow optimum operation of a nitrogen removal system. If an external carbon source is used to compensate for deficiencies in the influent, during peak nitrogen loads or periods of low temperature, an immediate increase of the denitrification rate is desirable. Various control strategies for dosage of an external carbon source have been discussed by Hellström and Bosander (1990), Plaza (1990) and Vanrolleghem *et al.* (1993).

The objectives of this work were to study process performance with supplemental ethanol in a pre-denitrifying system and to follow adaptation of the denitrifying community to ethanol. The experiment was carried out in a pilot-scale plant with two parallel lines equipped with on-line nutrient meters. One line was operated with ethanol addition. Process performance was evaluated with data from on-line measurements, 24 hour composite samples, and grab samples. Batch tests were used to measure changes in potential denitrification rates with ethanol. Enumeration of different fractions of bacteria was done with culture dependent techniques. The paper also presents an automatic control strategy for carbon source dosage based on on-line meters for nitrate and total organic carbon (TOC). The suggested strategy was simulated using the IAWQ Activated Sludge Model No. 1.

MATERIALS AND METHODS

The pilot plant and the control and supervision system

At Kungsängsverket, Uppsala a pilot-scale plant consisting of two parallel lines was constructed and placed indoors (Fig. 1). Each line consisted of an activated sludge tank (2.35 m^3) with an anoxic zone fraction of 0.18 and a settling tank (0.55 m^3). Hose pumps were used to achieve well defined flows. The plant was equipped with on-line meters for nitrate, ammonia, dissolved oxygen (DO), pH, redox, suspended solids, and temperature.

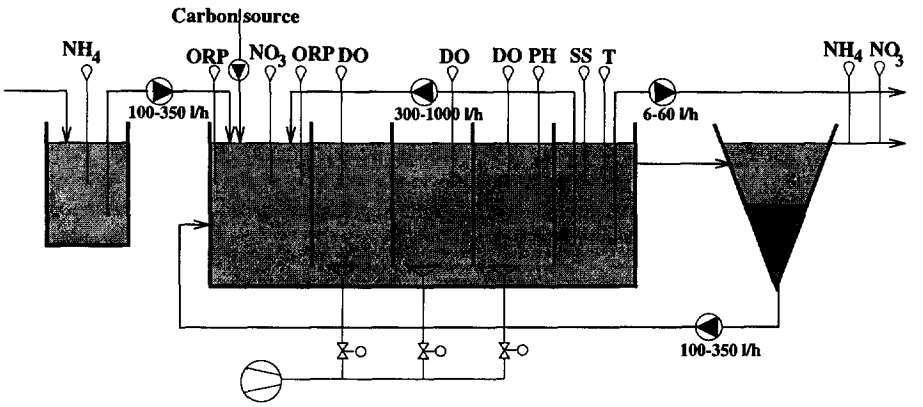

Figure 1. Layout of pilot-scale plant.

A prototype computerized control and supervision system (CSS) was designed and constructed. The user can, via a state of the art interface, control data acquisition, save and plot data, manage the system and respond to system alarms. Various sub-processes, including DO, are automatically controlled by the CSS. The CSS consists of a PC, equipped with AD, DA, and binary cards that communicates with the process via a switch cabinet. In total, the system uses 19 analog outputs, 18 analog inputs and 50 binary signals. Pump motors are controlled by the CSS via frequency converters. DO is controlled by PID controllers that regulate airflow rates via valves. Binary signals are mainly used for alarm handling and presenting status of the equipment, i.e. pumps operating in manual or automatic mode. Linux, a public domain UNIX dialect, was used as the operating system and extended for real time processes. The CSS is implemented in C++ and the graphic user interface was created with Motif (Latomaa, 1994). Data values from the process is collected and stored as average values over ten minute periods.

Table 1. Wastewater characteristics

Parameter	Mean ($mg\ l^{-1}$)	SD* ($mg\ l^{-1}$)
COD	350	130
Tot-N	36.6	4.8
NH_4-N	25.2	3.6
NO_3-N+NO_2-N	0.3	0.2
Tot-P	4.1	2.4
PO_4-P	1.0	0.3
HCO_3	420	40
SS	70	14

*Standard deviation, n=17.

Experimental

The two experimental lines were operated as single sludge systems with pre-denitrification with and without ethanol addition. Both lines were operated with an influent flow of 220 $l\ h^{-1}$ and a return sludge flow of 220 $l\ h^{-1}$. Internal recirculation was 660 $l\ h^{-1}$. Excess sludge was pumped (8 $l\ h^{-1}$) directly from the last zone in the activated sludge basins. In order to obtain a stable nitrification process the pilot plant was operated with an aerobic sludge age of 11 days which is higher than the critical sludge age calculated according to Eklund *et al.* (1991). Ethanol corresponding to 100 mg COD l^{-1} was added continuously to one of the lines. During the experimental period the temperature was 14 C and the average mixed liquor suspended solids in the ethanol (E) and the reference (R) line were 2790 and 2560 mg l^{-1}, respectively. Pre-precipitated and pre-

settled wastewater was continuously pumped from the full-scale plant to an influent tank before it entered the activated sludge basins (Table 1). Twenty-four hour composite samples were collected from the influent and effluents twice a week for analysis of unfiltered COD and total N, NH_4-N, sum of NO_3 and NO_2-N, total P, filtered PO_4-P, alkalinity and suspended solids. Grab samples were collected from different parts of the basins once a week and analysed for the parameters listed above as well as mixed liquor suspended solids (MLSS) and volatile suspended solids (MLVSS). Sludge volume index (SVI) was determined once a week according to Standard Methods (APHA, 1985). Sludge quality index (SQI) was calculated according to Fitch and Kos (1976). SVI and stirred sludge volume index (SSVI), as described by White (1976), was measured on five separate occasions at the end of the experimental period. Denitrification rates were measured in sludge samples from the anoxic zones.

Denitrification assay and enumeration of bacteria

Potential denitrification rates were determined in triplicate at 15°C with the acetylene inhibition technique in sludge samples diluted (1:5) in 10 mM sodium phosphate buffer with pH 7.2, but otherwise treated as described by Hallin and Pell (1994). Initial concentrations of nitrogen, added as KNO_3, and ethanol were 28 and 184 mg l^{-1}, respectively.

The number of different fractions of bacteria were assessed after 49 days of continuous ethanol dosage. Sodium hexa-meta-phosphate (0.2% w/v) was added to the activated sludge samples before they were homogenized in a mixer. Dilution series (1:10) were made in 10 mM sodium phosphate buffer with pH 7.2. Aerobic heterotrophic bacteria were enumerated by spread plate count on nutrient agar (Oxoid) with five replicates incubated at 15°C for 2 weeks. The most-probable-number (MPN) technique presented by Allievi *et al.* (1987) based on acetylene inhibition was modified according to Hallin *et al.* (1996) and used to enumerate denitrifying bacteria. The total number of denitrifiers was enumerated in nutrient broth (Oxoid). The number of denitrifiers using ethanol was determined in a standard mineral medium containing 5 ml ethanol l^{-1} as the sole organic compound. Inoculated MPN tubes were incubated at 15°C for 19 days.

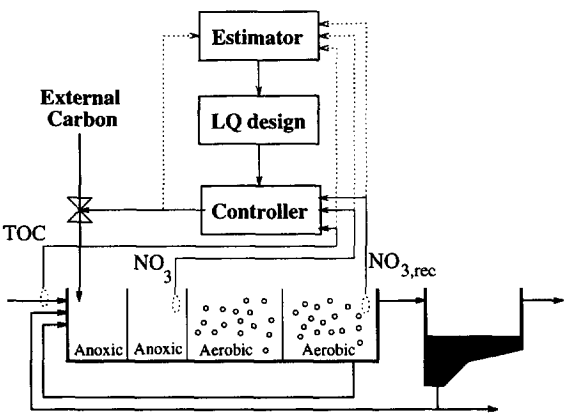

Figure 2. Block diagram of the control strategy for carbon dosage.

Simulation of a control strategy for carbon dosage

An automatic control strategy was developed and simulated. The IAWQ Activated Sludge Model No. 1 was used for simulating the process. The basic process model used in the simulation was taken from Wikström (1993). Maintenance of constant low nitrate concentration in the last anoxic zone was the control objective. The control signal, i.e. the flow rate of external carbon source, was treated as soluble substrate. The control strategy used feedforward from two measurable disturbances; influent substrate measured by a TOC meter, and nitrate concentration in the recirculated water measured with an on-line meter at the end of the last anoxic zone (Fig. 2). An indirect adaptive controller with a linear quadratic (LQ) design was used. In the

estimator block (Fig. 2) a linearized process model is recursively estimated. This model is used to calculate the control parameters in the LQ block. Lindberg (1995) describes this approach in more detail.

Analytical methods

Analytical methods of chemical and physical parameters were done according to Swedish standards (SIS). Nitrous oxide was analysed on a gas chromatograph equipped with a ^{63}Ni electron capture detector.

RESULTS AND DISCUSSION

Nitrogen removal efficiency was equivalent in both the ethanol and reference line before ethanol addition started (Fig. 3a). The capacity to denitrify with ethanol was also similar in the two lines (Fig. 3b). Dosage of ethanol increased the carbon to nitrogen ratio, expressed as mg COD/mg Tot-N, and the mean values during the period were 12.4 in the ethanol line and 9.6 in the reference line. The average values of total nitrogen reduction were 67 and 35% for the ethanol and reference line, respectively. Nitrification was almost complete with a nitrification efficiency of 99% throughout the period for both lines.

The effect of ethanol on nitrate removal and denitrification capacity with ethanol was rapid (Fig. 3b and 3d). While the nitrate content in the effluent stabilized immediately the capacity measured in batch tests continued to increase during the first 12 days (Fig. 3c). A period corresponding to about one sludge age was apparently necessary before the denitrifying bacteria in the sludge were fully adapted to ethanol. These findings are consistent with those reported by Andersson *et al.* (1995) from a post-denitrification system with ethanol addition. Rates measured in batch tests with only nitrate added were in the range 0.02-0.2 mg N g^{-1} VSS h^{-1}.

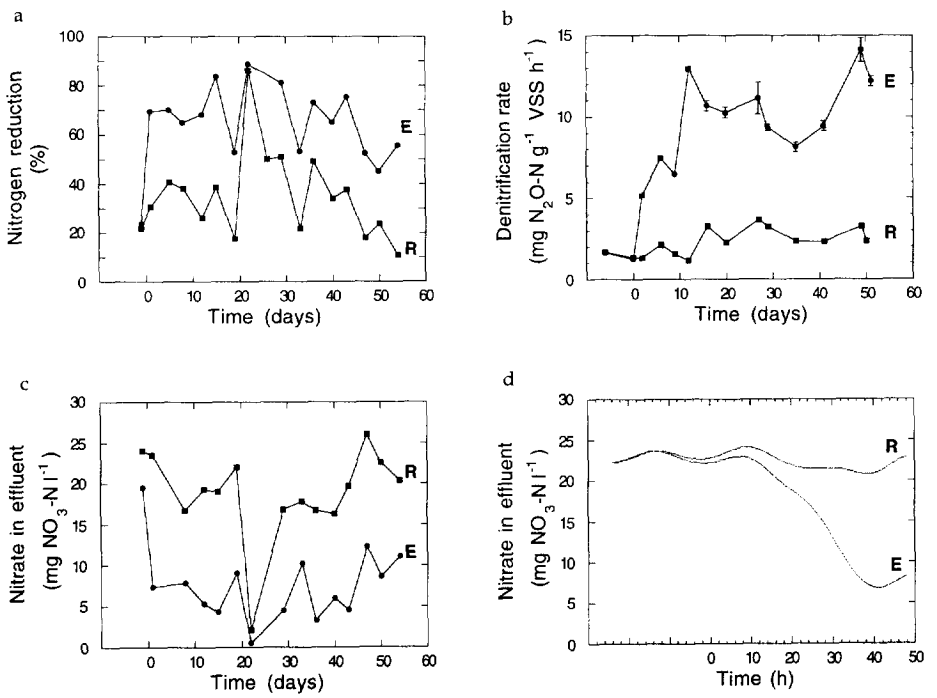

Figure 3. Response to ethanol addition in the ethanol (E) and reference (R) line: Nitrogen removal efficiency calculated from 24 hour composite samples of total influent and effluent nitrogen (a). Denitrification capacity with ethanol (mean ± SD, n = 3) (b). Effluent nitrate measured as 24 hour composite samples (c). Low-pass filtered values from on-line measurements of effluent nitrate during the first 48 hours of ethanol addition (d).

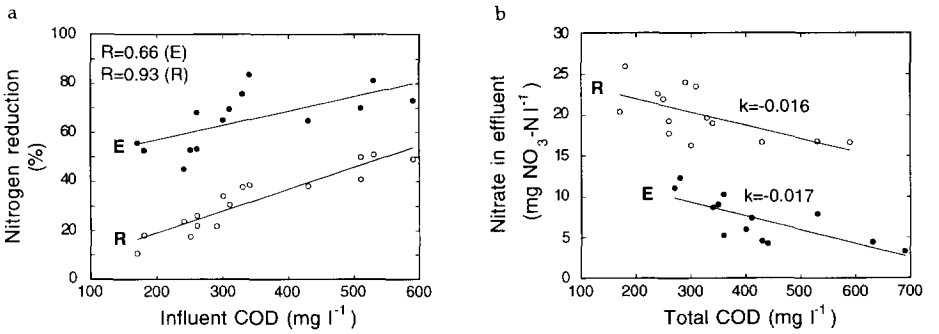

Figure 4. Total nitrogen removal efficiency versus COD content in influent wastewater in the ethanol (E) and reference (R) line (a) and nitrate in effluent versus total influent COD in the ethanol (E) and reference (R) line (b). Values from day 22 are excluded.

Influent COD affected nitrogen removal efficiency and nitrate concentration in the effluent in both lines (Fig. 4a and 4b). Still, nitrogen reduction had a stronger correlation to influent COD in the reference line than in the ethanol line. Although the bacteria were adapted to ethanol, organic matter in the influent was apparently used simultaneously with ethanol for denitrification. This can be concluded because effluent nitrate concentrations were lowered by ethanol addition while the impact of influent COD in the wastewater was similar on effluent nitrate in both lines as indicated by the slopes in Fig. 4b. Ethanol adapted bacteria also seemed to keep the appropriate enzymes regardless of fluctuations in influent substrate since the capacity to denitrify with ethanol was unaffected by discharges of COD to the wastewater treatment plant (Fig. 3b). Nitrogen removal was high and nitrate concentration in the effluent was low on day 22 (Fig. 3a and 3c). The high COD/N ratio in the influent that day may have caused denitrification to occur not only in the anoxic zones but also in other parts of the system. There was a major impact on the whole wastewater treatment plant as well.

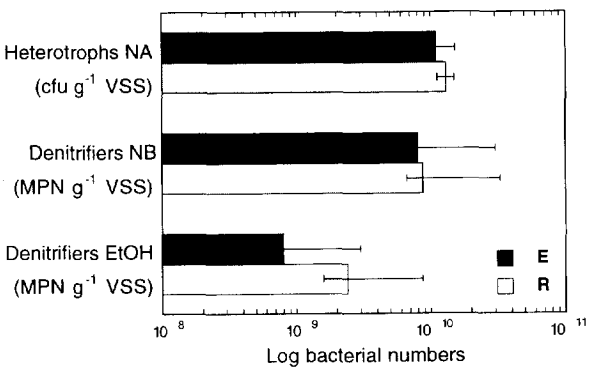

Figure 5. Bacterial numbers in the ethanol (E) and reference (R) line (cfu as mean ± standard deviation, n = 5 and MPN with 95% confidence interval).

The initial rapid increases in both nitrogen removal and capacity to utilize ethanol suggest enzyme induction by bacteria present in the sludge rather than alterations in bacterial species composition. The increase in enzyme activity was probably followed by bacterial growth. However, the numbers of different fractions of bacteria assessed after 49 days of ethanol dosage in the ethanol line did not differ significantly from the reference line (Fig. 5). Laboratory studies with acetate as an external carbon source demonstrated that adaptation was mainly due to an increase in activity per bacterium (Hallin *et al.*, 1996). We assume therefore that there was an increase in bacterial growth which in turn increased the feed for the protozoa. The turn-

over rate of biomass has thus increased and this is seen as an increased enzymatic activity, i.e. a higher potential denitrification rate.

The observed sludge yield was 0.22 and 0.24 mg VSS mg^{-1} COD for the ethanol and reference line, respectively. This is in agreement with values reported by other workers studying pre-denitrification systems (Plaza, 1990; Henze *et al.*, 1992). Christensson *et al.* (1994) reported a growth yield of 0.28 mg SS mg^{-1} COD in batch cultivation studies of denitrifying bacteria with ethanol as carbon source. Similar sludge yields in the two lines showed that ethanol dosage did not lead to greater sludge production. Sludge settling properties measured on five occasions at the end of the experimental period showed that the reference line had somewhat better settling properties (Table 2). Average values for SVI determined once a week during the whole period, however, were 261 ± 54 and 206 ± 63 mg l^{-1} in the ethanol and reference line, respectively. Some sporadic sludge loss from the settling tank in the ethanol line was observed.

Table 2. Sludge settling properties (mean ± standard deviation, n = 5)

Line	SVI (mg l^{-1})	SSVI (mg l^{-1})	SQI (mg l^{-1})
Ethanol	329 ± 37	105 ± 5	185 ± 57
Reference	182 ± 46	77 ± 4	142 ± 19

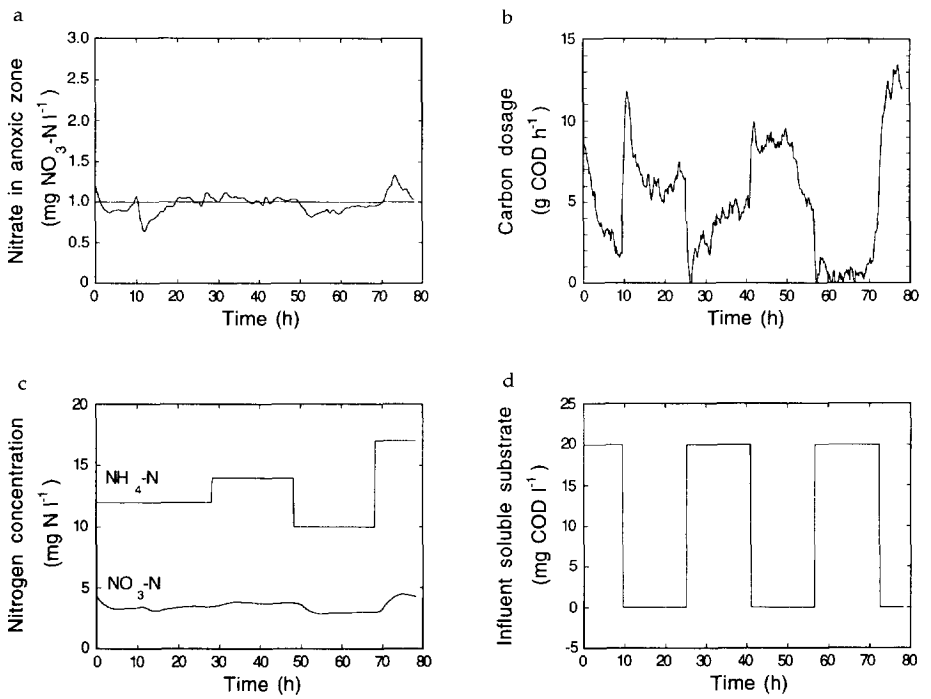

Figure 6. Simulation of an indirect adaptive controller with feedforward to control carbon dosage in a pre-denitrifying plant. Set point and actual nitrate level in the anoxic zone (a). Dosage of external carbon (b). Ammonium concentration in the influent and nitrate concentration in the recirculated flow (c). Influent soluble substrate. Flow rate = 220 l h^{-1} (d).

The fast response to ethanol on nitrogen removal (Fig. 3) allows implementation of an automatic control strategy. It may then be possible to compensate for variations in influent COD as observed in Fig. 4. A simulation of the indirect adaptive controller using LQ design and feedforward is shown in Fig. 6 a-d. The

set point for nitrate concentration at the end of the anoxic zone was 1 mg l^{-1}. Despite relatively large disturbances in influent soluble substrate concentrations, the controller maintained the desired nitrate concentration in the anoxic zone reasonably well, see also Lindberg (1995). This strategy is currently being tested in the pilot-scale plant. Andersson *et al.* (1995) concluded from EFOR simulations that automatic control did not save more carbon than constant dosage. Use of feedforward control, however, makes it possible to obtain tight control of the nitrate concentration without using an excessively high gain in the feedback loop. It is simple to constrain the controller so that a minimum dosage of external carbon is guaranteed. The suggested strategy requires reliable sensors which may need frequent maintenance. On-line respirometric measurements may be used instead of TOC meters to determine biologically available carbon. The possibility of saving external carbon during periods of low loads while still maintaining effluent standards during periods of high load makes the suggested strategy attractive.

CONCLUSIONS

Ethanol can be used to support the denitrifying bacteria in a pre-denitrifying system even during short periods of carbon deficiency. Despite the rapid (30-40 h) response to ethanol on effluent nitrate concentration the bacteria were not fully adapted until 12 days later. The bacterial population used influent COD simultaneously with ethanol for denitrification. We propose that ethanol addition caused enzyme induction rather than alterations in species composition. These conclusions are important in the successful application of an automatic control strategy. Simulation of the suggested control strategy, using the IAWQ model No. 1, showed promising results where a low nitrate level in the anoxic zone could be maintained in spite of large disturbances.

ACKNOWLEDGEMENT

We thank the staff, and especially Jan Söderlund and Bert Alvén, at the Kungsängen wastewater treatment plant. We are also grateful to Serena Hasselblad and Bengt Hultman for valuable discussions. Financial support for this work was provided by The National Board for Industrial and Technical Development (contract 93-04185P) as well as Uppsala Municipal Services.

REFERENCES

Allievi, L., Catelani, D., Ferrari, A. and Treccani, V. (1987). A method for counting denitrifiers by N_2O detection. *J. Microbiol. Meth.* **7**, 67-72.

Andersson, B., Nyberg, U. and Aspegren, H. (1995). Methanol and ethanol as carbon sources for denitrification. Nordic seminar: Nitrogen Removal from Municipal Wastewater, Helsinki University, Otaniemi, Espoo, Finland, 25-25 January.

APHA, AWWA and WPCF (1985). *Standard Methods for the Examination of Water and Wastewater*, 16th ed. American Public Health Association, Washington DC.

Aspegren, H., Nyberg, U., Jansen, J. la Cour and Andersson, B. (1992). Model and sensor based optimization of nitrogen removal at the Klagshamn plant. *Wat. Sci. Tech.*, **26**(5-6), 1315-1323.

Christensson, M., Lie, E. and Welander, T. (1994). A comparison between ethanol and methanol as carbon sources for denitrification. *Wat. Sci. Tech.*, **30**(6), 83-90.

Claus, G. and Kutzner, H. J. (1985). Denitrification of nitrate and nitric acid with methanol as carbon source. *Appl. Microbiol. Biotechnol.*, **22**, 378-381.

Fitch, B. and Kos, P. (1976). Towards a more meaningful index of sludge quality. *J. Wat. Pollut. Control Fed.*, **48**, 1979-1987.

Eklund, L., Hellström, B. G., Hultman, B., Lind, J. E. and Nordström, B. (1991). Swedish full-scale experiments on modified operational modes in removal of nutrients. *Wat. Sci. Tech.*, **24**(7), 97-102.

Hallin, S. and Pell, M. (1994). Acetylene inhibition for measuring denitrification rates in activated sludge. *Wat. Sci. Tech.*, **30**(6), 161-167.

Hallin, S., Rothman, M. and Pell, M. (1996). Adaptation of denitrifying bacteria to acetate and methanol in activated sludge. *Wat. Res.*, **30**, 1445-1450.

Halmø, G. and Eimhjellen, K. (1981). Low temperature removal of nitrate by bacterial denitrification. *Wat. Res.*, **15**, 989-998.

Hellström, B. G. and Bosander, J. (1990). Styrstrategier för optimering av extern kolkälla vid fördenitrifikation. (Control strategies for optimization of external carbon in pre-denitrification). SYVAB, Himmerfjärdsverket, S-147 92 Grödinge, Sweden. (In Swedish)

Henze, M., Harremoës, P., Jansen, J. la Cour and Arvin, E. (1992). *Spildevandsrensning. Biologisk og kemisk (Wastewater treatment. Biological and chemical)*, 2nd ed. Polyteknisk forlag, Lyngby. (In Danish).

Henze, M., Kristensen, G. H. and Strube, R. (1994). Rate-capacity characterization of wastewater for nutrient removal processes. *Wat. Sci. Tech.*, **29**(7), 101-107.

Isaacs, S. H., Henze, M., Søeberg, H. and Kümmel, M. (1994). External carbon source addition as a means to control an activated sludge nutrient removal process. *Wat. Res.*, **28**(3), 511-520.

Latomaa, J. (1994). *A control and supervision system for an activated sludge process*. Master thesis. (Uppsala University, Systems and Control Group, Report UPTEC 94077E). Uppsala.

Lindberg, C.-F. (1995). *Control of Wastewater Treatment Plants*. Licentiate thesis. (Uppsala University, Systems and Control Group, Report UPTEC 95071R). Uppsala.

Nyberg, U., Aspegren, H., Andersson, B. and Jansen, J. la Cour (1992). Full-scale application of nitrogen removal with methanol as carbon source. *Wat. Sci. Tech.*, **26**(5-6), 1077-1086.

Plaza, E. (1990). *Biological nitrogen removal from municipal wastewater under conditions of organic carbon l imitations*. PhD thesis. (Royal Institute of Technology, TRITA-VAT-1905). Stockholm.

Plaza, E., Hultman, B. and Trela, J. (1990). Effect of easily degradable carbon sources on nitrogen removal efficiency. *Wat. Sci. Tech.*, **22**(7-8), 281-282.

SIS - Swedish Standards Institution, Box 3295, S-103 66 Stockholm, Sweden.

Timmermans, P. and Van Haute, A. (1983). Denitrification with methanol. Fundamental study of the growth and denitrification capacity of *Hyphomicrobium* sp. *Wat. Res.*, **17**(10), 1249-1255.

Vanrolleghem, P. A., Vermeersch, L. K., Dochain, D. and Vansteenkiste, G. C. (1993). Modelling of a non-linear distributed parameter bioreactor: Optimisation of a nutrient removal process. In: *Modelling and Simulation*, A. Pavé (Ed.). SCS, San Diego. pp. 563-567.

White, M. J. D. (1976). Design and control of secondary settlement tanks. *Wat. Pollut. Control*, **75**, 459- 467.

Wikström, A. (1993). Simnon models for activated sludge systems. Technical report. (Lund Institute of Technology, Department of Industrial Electrical Engineering and Automation). Lund.