DETERMINATION OF RATE PARAMETER FOR KINETICS OF NITRIFICATION

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Submitted 27 Juni 2005, reviewed 14 Juli 2005, and accepted 25 Agustus 2005

Abstract

The nitrification proces is the bottleneck step in the total nitrogen removal. The formation of nitrate is considered as the rate limiting step in the whole process and its kinetics determine the design of the nitrification reactor. Heavy metals (Zn^{2+} and Cu^{2+}) and different organic compounds are used as micropollutants. These kinetics were experimentally measured by respirometry. In line with the aim of the paper, the experimental investigation are conducted to develop design equations to describe kinetic rate relationships under optimum conditions, study the parameter influence such as pH and inhibition by reaction intermediates and inhibition by external pollutants. Results demonstrate that the maximum value of the specific growth rate of autotrophic biomass () is 1.02 day^{-1} at pH=7 and decreases at pH 7.5; inhibition occurs at substrate (NH_4^+) concentrations in excess of 15 mg N/l; inhibition occurs at increasing concentrations of NO^--N and Cu^{2+} has more pronounced inhibitory effect than Zn^{2+}. The inhibitory effect of organic compounds are listed as the Chlorobenzene > Trichloroethylene > Phenol > Ethylbenzene; the experimental oxygen uptake rate (OUR)-test results the autotrophic kinetic parameter values, which can be used in design equations.

Keywords: Respirometry, Autotrophic Biomass, Nitrification, Oxygen Uptake Rate

Abstrak

Proses nitrifikasi merupakan langkah penting pada penurunan kadar total nitrogen. Pembentukan nitrat dianggap sebagai tahap pembatas kecepatan reaksi pada keseluruhan proses dan kinetikanya menentukan perancangan dari bagian proses nitrifikasi. Logam berat (Zn^{2+} dan Cu^{2+}) dan berbagai jenis komponen organik digunakan sebagai mikropolutan. Kinetika ini secara eksperimental diukur menggunakan respirometer. Tujuan penelitian adalah mengembangkan persamaan perancangan yang menggambarkan hubungan laju kinetika pada kondisi optimum, studi pengaruh parameter seperti pH, inhibisi karena reaksi intermediat, dan inhibisi oleh polutan dari luar. Hasil penelitian ditunjukkan sebagai berikut: harga laju pertumbuhan biomasa autotrof maksimum spesifik adalah 1,02 hari^{-1} pada pH=7 dan menurun pada pH 7,5; inhibisi terjadi pada konsentrasi substrat (NH_4^+) lebih besar dari 15 mg N/l; inhibisi terjadi pada peningkatan konsentrasi NO^--N ; Cu^{2+} lebih dikenal sebagai penyebab inhibisi daripada Zn^{2+}. Efek inhibisi dari komponen organik di daftar mulai dari Chlorobenzene sampai Ethylbenzen . Tes OUR menghasilkan harga parameter kinetika yang dapat dipakai pada persamaan perencanaan lumpur aktif nitrifikasi.

Kata Kunci: Respirometer, Biomasa Autotrof, Nitrifikasi, Laju Kenaikan Oksigen

1. Introduction

Activated sludge nitrification/ denitrification is recognized as the most economical means of reducing the nitrogen content of wastewater. Nitrification is the most sensitive part in the nutrient removal of wastewaters. The growth rate of nitrifying biomass is much lower than its heterotrophic counterpart, and is strongly affected by on temperature, substrate concentration, oxygen content, pH, and the presence of inhibiting components.

The net maximum specific growth rate of the autotrophic biomass (can be determined using the oxygen uptake rate procedure. In the batch experiments involving autotrophic activity, the oxygen uptake rate (the OUR) is measured in a reactor containing both heterotrophic and autotrophic biomass before and after the inhibition of nitrifiers (Nowak and Svardal, 1993). Many authors have reported slightly different method for measuring OUR (Cech et al., 1985; Kappeler and Gujer, 1992; Kroiss et al., 1992; Nowak and Svardal, 1993). The OUR method discontinuously aerates the activated sludge and measures the gradient of dissolved oxygen concentration in this reactor represents the total OUR. A separate measurement to define the endogenous heterotrophic oxygen uptake rate (OUR_H) is taken into account to obtain the value of OUR_A,max . Allylthiourea (ATU) is added in this separate measurement to inhibit nitrification. The difference between both measurements is the OUR associated with nitrification.

Ammonia and nitrite, two intermediate products formed during the conversion of organic nitrogen. These compounds are inhibitory to Nitrosomonas and Nitrite-oxidizing bacteria within specific concentration ranges.

The aim of the paper are conducted to develop design equations to describe kinetic rate relationships under optimum conditions, study the parameter influence such as pH and inhibition by reaction intermediates and inhibition by external pollutants. To achieve these goals, the current experimental work focusses upon the nitrification process and is devided into 4 parts: (I) Measurement of the specific maximum growth rate of the autotrophic biomass using the Oxygen Uptake Rate to yield kinetic expressions under optimum condition. The test using respirometry makes a difference between autotrophic (including nitrifiers) and heterotrophic organisms; (II) The inhibitory effects of reaction intermediates NH_4^+, NH_3, NO_2^- are studied through defining the effect both of the concentration of ammonia-nitrogen or nitrite-nitrogen, and of the pH upon the net maximum specific growth rate of autotrophic biomass under optimum conditions using the respirometry procedure; (III) The inhibitory effects of various concentration of heavy metals and organic compounds on the net maximum specific growth rate of autotrophic biomass using the Oxygen Uptake Rate procedure; (IV) The use of ISO 9509-Test as alternative method to study the inhibition of nitrification by heavy metals and organic compounds. The test is based upon the rate of nitrification (against the rate of oxygen uptake in the OUR-test). The ISO 9509-test is applied to validate the OUR-test. The comparison of the present result with others concludes that the maximum growth rate is in the range of the literature data.

Table 1. The Comparison of Own and Literature Data Concerning The Maximum Growth Rate of The Autotrophic Biomass

Authors	Observations, $\hat{\mu}_A$ (day^{-1})
P. Anthoniou,[1990]	0.12 – 0.97
A. Lesouef et al., [1992]	0.57
P.A. Vanrolleghem et al., [1993]	0.77
O.Nowak et al., [1994]	1
M.Henze et al., [1999]	1

2. Fundamental

The nitrification is expressed as two-step process as described below and the result of oxidizing bacteria :

$NH_4^+ + 1.5\,O_2 \rightarrow 2\,H^+ + H_2O + No_2$ (1)

NH_4^+ - oxidizing bacteria degrade NH_4^+ to No_2

$NO_2 + 0.5\,O_2 \rightarrow NO_3$ (2)

NO_2 - oxidizing bacteria degrade No_2 to No_3

The maximum specific growth rate of the autotrophic biomass (μ_A) is the major kinetic parameter describing the growth and can be determined by the well-known respirometry technique, defining the oxygen uptake rate (OUR). Nitrification rate is influenced by several factors such as: substrate concentration, oxygen concentration, temperature, pH, mLSS, inhibitor compounds.

As the same as heterotrophic bacteria, the specific growth rate of Nitrosomonas and Nitrobacter with NH_4^+-N and NO_2^--N as growth limiting substrate is illustrated as Monod equation

$$\mu = \frac{\mu_m S}{K_S + S} \quad (3)$$

If S is ammonium concentration, the growth rate of bacteria is written below

$$\mu_N = \mu_{N,max} \frac{[NH_4^+ - N]}{K_S + [NH_4^+ - N]} \quad (4)$$

Relationship between Nitrification rate and the growth rate is given as

$$q_N = \frac{\mu_N}{Y_N} = q_{N,max} \frac{[NH_4^+ - N]}{K_N + [NH_4^+ - N]} \quad (5)$$

DO (dissolved oxygen) has an important effect on the nitrification as written by Monod equation

$$\mu_N = \mu_{N,max} \frac{DO}{K_{O_2} + DO} \quad (6)$$

pH influences nitrification with the optimum pH as 7.0-8.5.

$$\mu_N = \mu_{NS,max} [1 - 0.833(7.2 - pH)] \quad (7)$$

The influence of temperature on the growth rate of autotrophic nitrification bacteria such as

$$\mu_{NT} = \mu_{N15} \exp[K(T-15)] \quad (8)$$

From all of the factors, empirical equations are given as

$$\mu_{NS} = \mu_{NS,max} \left(\frac{[NH_4^+ - N]}{0.4e^{0.118(t-15)} + [NH_4^+ - N]} \right) \left(\frac{[O_2]}{[O_2]} e^{0.095(T-15)} \right) x\, [1 - 0.833(7.2 - pH)] \quad (9)$$

3. Methodology

A jacketed batch reactor of 2 liter volume is filled with mixed activated sludge from a municipal wastewater treatment plant at a constant temperature of 20°C. A temperature controlling water bath surrounding the wall and the bottom of the reactor keeps the temperature constant. The reactor is equipped with a Dissolved Oxygen probe, temperature gauge, pH controller, injection of substrate, magnetic stirrer, and porous aerator. Base (0.25 M NaOH) or acid (0.25 M HCl) is used to control the pH. The range of pH investigated in the experiments was 6.5-8.5.The determination of the respiration rate was done by aerating the activated sludge mixture for dissolved oxygen levels in the wastewater between 2 and 6 mg/L. The dissolved oxygen concentration decreases when biomass oxidizes substrate and due to its metabolism. The gradient of dissolved oxygen determines the OUR. The biomass concentration is approximately 2 g/L in all experiments. The experimental set-up is shown in Figure1.

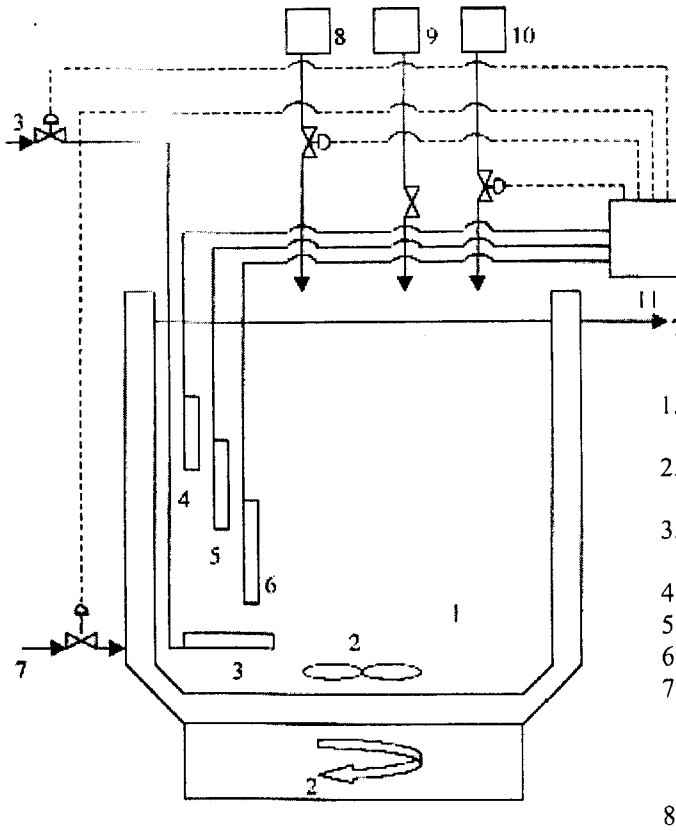
The experimental procedure consists of two phases, with a first phase of discontinuous aeration of the mixture of sludge and water overnight to reach the endogenous conditions. This phase allows the heterotrophic biomass to utilize the organic carbon in the wastewater. The respiration rate in this phase is called the endogenous OUR (OURendo). Feeding nitrogen-substrate starts the second phase. This condition was defined by the calculation of the OUR at the first feeding, called $OUR_A(0)$. The example of an activity measurement is shown in Figure 2.

The second phase involved the growth phase of the autotrophic biomass. The respiration rate in this phase is determined by subtracting the OURendo of the total respiration rate OUR(t). Calculation of the maximum specific growth rate of the autotrophic biomass is shown in the equation (10).

$$\ln \frac{OUR_A(t)}{OUR_A(0)} = \ln \frac{OUR(t) - OUR(endo)}{OUR(0) - OUR(endo)} = (\hat{\mu}_A - b_A) \quad (10)$$

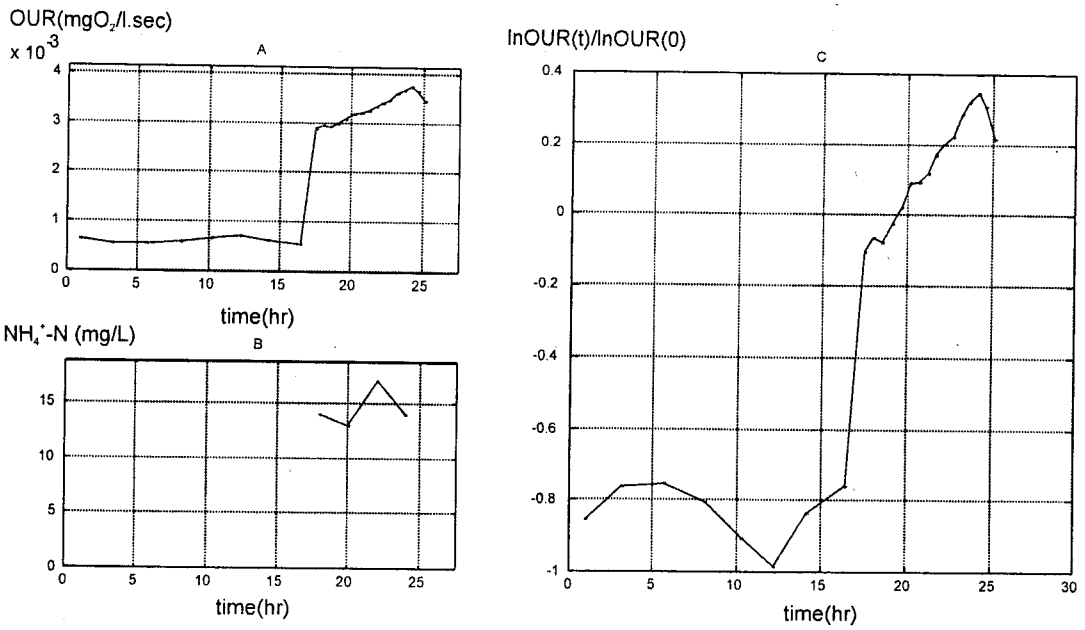
The relationship of OUR(t), OUR(0) and t can be represented in Figure 3.

The concentration of NH_4^+-substrate was varied in a first series of experiments, within the range of 5 to 40 mg/L NH_4^+-N. The optimum amount of substrate concentration was 15 mg/L NH_4^+-N. A second series of tests studied the inhibition by NO_2^--N. The substrate NH_4^+-N concentration was kept at its optimum of 15 mg/L and $NaNO_2$ was added as inhibitor of nitrification. The concentration of Sodium Nitrite used as inhibitor was between 5 and 50 mg/L. The concentrations of NH_4^+-N and NO_2^--N in the reactor were monitored every 2 hours and analysed by spectrophotometer, NH_4^+-N and NO_2^--N were added in appropriate quantities to keep these concentrations constant.



1. Batch reactor with cover
2. Magnetic Stirrer and its drive
3. Compressed air and porous aeration pad
4. Dissolved O_2 Probe
5. Thermocouple
6. pH-meter
7. Cooling/heating Jacket with H_2O-connection to thermostatic bath
8. Acid dosage (HCl)
9. Substrate (NH_4Cl)
10. Base dosage (NaOH)
11. Controller and indicator

Figure 1. Experimental Set-up



A: Oxygen Uptake Rate vs Time; B: NH_4^+-N vs Time;
C: ln OUR (t)/ln OUR (0) vs Time

Figure 2. The Example of An Activity Measurement

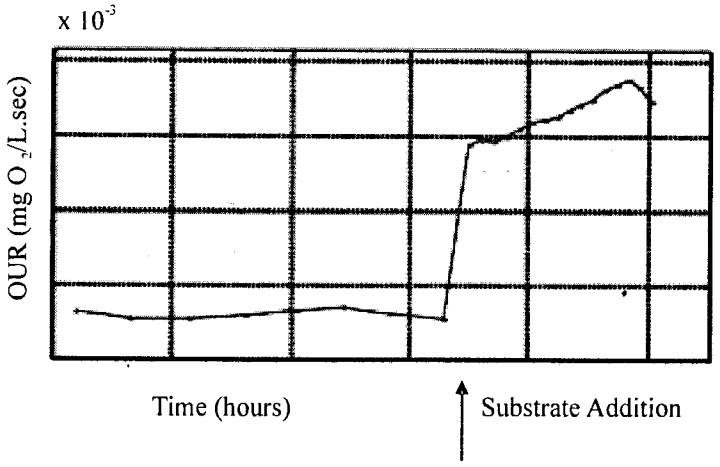


Figure 3. The Curve of OUR vs. Time

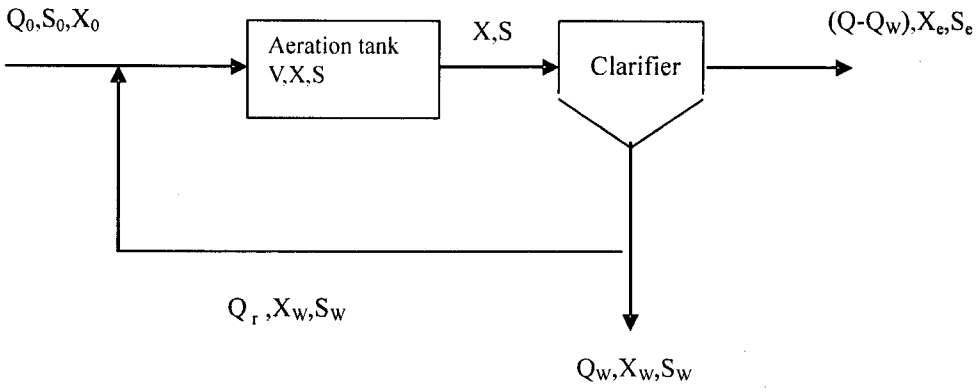


Figure 4. Activated Sludge System

In general, the activated sludge system is shown in Figure 4. The experimental OUR-test results define the autotrophic kinetic parameter values, which can be used in design equations of activated sludge nitrification described below. These design equation are based upon a material balance for the biomass and the substrate, and upon the Monod expression for the specific growth of biomass under limited substrate concentration. The resulting equation defines the required reactor volume for given characteristics of the influent, effluent and operational mode:

$$V = \frac{Q_0}{\frac{\mu_{max} S}{K_S + S}} \frac{Y}{X}(S_0 - S) = \frac{Q_0}{\mu} \frac{Y}{X}(S_0 - S) \quad (11)$$

Provided sufficient ammonium and oxygen are supplied, Equation (2) defines the aeration tank volume for nitrification:

$$\mu \approx \mu_{A,max}$$

$$V = \frac{Q_W X_W}{(\mu_{A,max} - b_A) X_A} \quad (12)$$

$$V = \frac{Q_0}{\mu_{A,max}} \frac{Y}{X_A}(S_0 - S) \quad (13)$$

using the linear regression of the non-competitive inhibition from Kroiss (1992), the value of kI (inhibition correction factor) and k_i (inhibition constant) can be defined for any inhibitor. The extra aeration volume become :

$$V = \frac{Q_0}{\frac{\mu_{A,max}}{(1 + I/k_i)}} \frac{Y}{X_A}(S_0 - S) \quad (14)$$

4. Results and Discussion

The maximum specific growth rate of the autotrophic biomass (μ_A) was calculated equation (1). The endogenous OUR was determined from the first phase, which was indicated by a constant OUR. Addition of ammonium chloride to the reactor immediately results in an increasing respiration rate. By continuously feeding ammonium chloride at a constant rate, the OUR increase is due to the growth of the autotrophic biomass. The net maximum specific growth rate of the autotrophic biomass is indicated as (and is determined by the slope of the second phase.

At pH=7.0, the average maximum value of was 1.02 day^{-1} for an average b_A-value of 0.15 day^{-1} as the specific decay rate of autotrophic biomass. The average results of the experimental investigations are illustrated in Table 2.

Table 2. Illustration of Average Experimental Results

$\hat{\mu}_A$ (day^{-1})								
pH at NH_4^+-N (15mg/l)			NH_4^+-N (mg/l) at pH=7.0			NO_2^--N (mg/l) at pH=7.0		
6.5	7.0	8.5	5	15	40	8	15	40
0.82 (24%)	1.0 (0%)	0.61 (47%)	0.50 (60%)	1.0 (0%)	0.20 (94%)	0.91 (13%)	0.78 (28%)	0.32 (81%)

Note: Values between brackets indicate the measured % inhibition

The effects of the ammonia-N concentration, the nitrite-N concentration and the pH upon the net maximum specific growth rate (i.e.) are shown in Figure 5 to Figure 7.

The influence of the NH_4^+-N concentration on the net maximum specific growth rate is shown in Figure 5. The optimum value of was obtained at 15 mg/L NH_4^+-N. Higher or lower concentrations of substrate reduce the -value. This finding confirms earlier work by Nowak et al. (1994). The decreasing value of above 15 mg/L NH_4^+-N is caused by free ammonia inhibition: high concentrations of free ammonia inhibit the growth rate of both *Nitrosomonas* and nitrite-oxidizing bacteria, while lower concentrations of free ammonia, considered as limitation of biomass substrate, inhibit *Nitrosomonas* to a lesser extent than nitrite-oxidizing bacteria. The maximum addition of ammonia-nitrogen used in the experiments was 50 mg/L NH_4^+-N since higher concentrations lead to total inhibition.

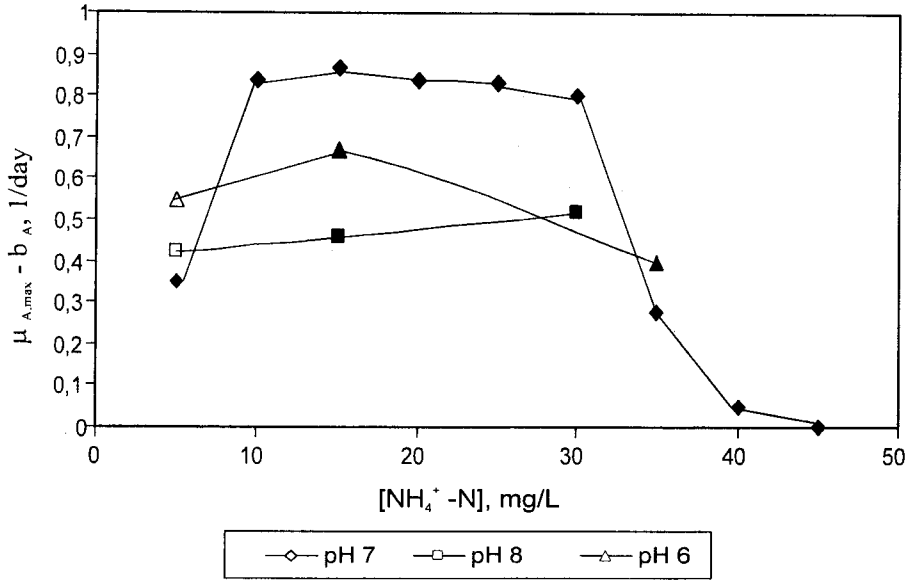


Figure 5. The Effect of The NH_4^+-N Concentration on The Net Maximum Specific Growth Rate of Autotrophic Biomass: (A-bA) vs. [NH_4^+-N]

To assess the effect of pH on the maximum specific growth rate of the autotrophic biomass (μ_A) at optimum ammonia-nitrogen concentration and the range of pH investigated. The value of (μ_A-b_A) decreases at a pH below 7.0. At pH 7.0, (μ_A-b_A) was 0.87 day $^{-1}$; at a pH 6.5 the value of (μ_A-b_A) was 0.67 day $^{-1}$. There was a slight decrease of (μ_A-b_A) between pH 7 to 8.0. The value of (μ_A-b_A) became 0.74 at pH 8.0. The value of (μ_A-b_A) was low at pH higher than 8.0.

Especially at pH 8.5, that the value of (μ_A-b_A) was 0.46 day $^{-1}$ only. The effect of the pH on the net maximum specific growth rate of the autotrophic biomass is shown in Figure 6 When the pH increases, the concentration of the non-ionized ammonia will increase. High concentration of non-ionized ammonia will inhibit the growth rate of both *Nitrosomonas* and nitrite-oxidizing bacteria. At lower concentration non-ionized ammonia will be less inhibitory to *Nitrosomonas* than to nitrite-oxidizing bacteria.

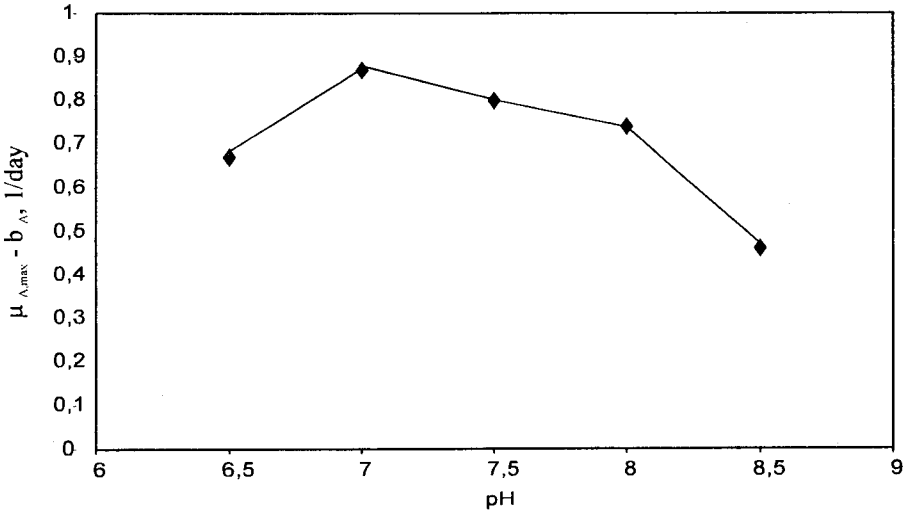


Figure 6. The Effect of The pH on The Net Maximum Specific Growth Rate of Autotrophic Biomass at Optimum NH_4^+-N Concentration (15 mg/L): (A bA) vs. PH

The effect of the NO_2-N concentration with respect to the net maximum specific growth rate is shown in Figure 7. The net maximum specific growth rate of the autotrophic biomass decreases with increasing NO_2^--N concentration. At pH 7.0, the concentration of 8 mg NO_2^--N /L results in 0.76 day^{-1} ($-b_A$), whereas 0.63 day^{-1} was obtained at 15 mg/L NO_2^--N. The value of ($-b_A$) decreases at a NO_2-N concentration higher than 15 mg/L. ($-b_A$) reaches 0.17 day^{-1} at 40 mg/L NO_2^--N. The effect of the pH is also indicated: ($-b_A$) decreases at pH below 7.0 and also at pH 8.5.

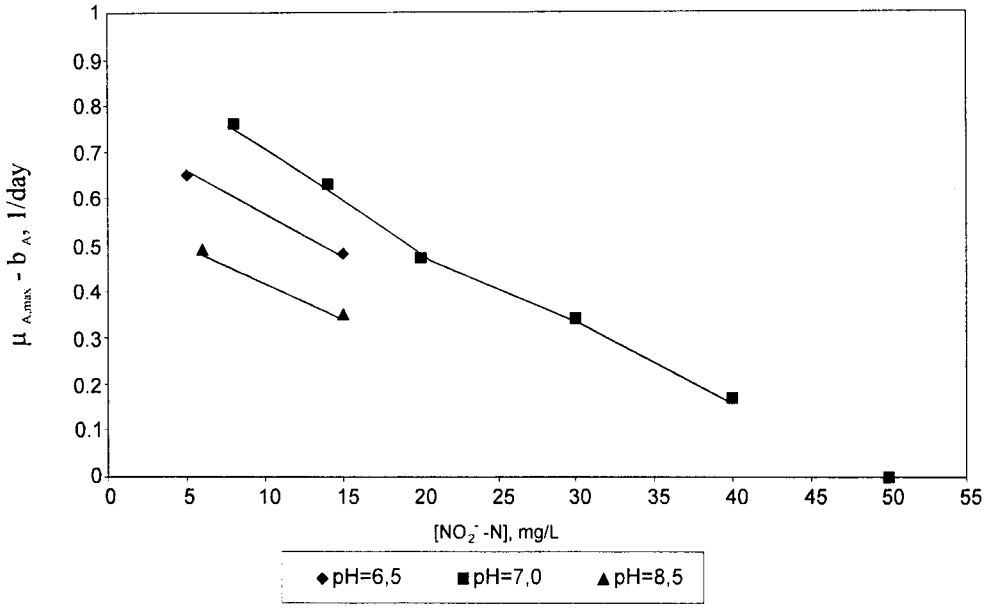


Figure 7. The Effect of The NO_2^--N Concentration on The Net Maximum Specific Growth Rate of Autotrophic Biomass: ($\mu A-bA$) vs. [NO_2^--N]

Two heavy metals were used as inhibitors: Zinc ($ZnSO_4.7H_2O$) and Copper ($CuSO_4.5H_2O$). The concentrations of Zn^{2+} and Cu^{2+} measured in the activated sludge mixed liquor solution, are 0.3 to 1.2 mg/L and 0.1 to 0.5 mg/L, respectively. Ethylbenzene, Chlorobenzene, Trichloroethylene and Phenol were chosen as the organic compound inhibitors. The organic compound inhibitor concentrations are 10 to 50 mg/L, 0.25 to 0.75 mg/L, 0.5 to 1 mg/L, and 4 to 50 mg/L respectively.

The presence of Cu^{2+} in wastewater inhibits the net maximum specific growth rate of autotrophic biomass to a larger extent than Zn^{2+} as illustrated in Figure 8. The value of ($\mu_A - b_A$) reaches 0.4 d^{-1} at 0.1 mg/L Cu^{2+}. The inhibition percentage at this concentration is 54 %, and reaches 82 % at 0.5 mg/L Cu^{2+}, whereas 65 % inhibition is found only at 0.5 mg/L Zn^{2+}. The autotrophic biomass is hardly inhibited by Cu^{2+} at concentrations below 0.05 mg/L, where the value of ($\mu_A - b_A$) is close to 0.90 d^{-1}.

The net maximum specific growth rate of the autotrophic biomass is reduced by the organic compounds used in the experiments. The inhibition effect depends on the type and concentration of organic compound. Higher concentrations increase inhibition. Trichloroethylene and Chlorobenzene are considered in the first group, whereas Ethylbenzene and Phenol are considered in the second group.

The autotrophic biomass is nearly totally inhibited at 1 mg/L Trichloroethylene, with a value of ($\mu_A - b_A$) equal to 0.22 d^{-1}, and 50 % inhibition is reached at 0.75 mg/L Trichloroethylene as illustrated in Figure 9.

The inhibitory effect of Chlorobenzene is more pronounced than for Trichloroethylene as shown in Figure 9. Adding 0.5 mg/L Chlorobenzene causes the net maximum specific growth rate to drop to 0.31 d^{-1}, with a 64 % inhibition. Over 80 % is achieved by adding more than 0.75 mg/L Trichloroethylene.

Ethylbenzene has a lower inhibitory effect on the nitrification process than Trichloroethylene or Chlorobenzene: adding 10 mg/L Ethylbenzene in the wastewater, as shown in Figure (10), yields 0.39 d^{-1} as the value of ($\mu_A - b_A$) corresponding with 55 % inhibition. The same value of ($\mu_A - b_A$) is reached at 0.75 mg/L Trichloroethylene or 0.25 mg/L Chlorobenzene.

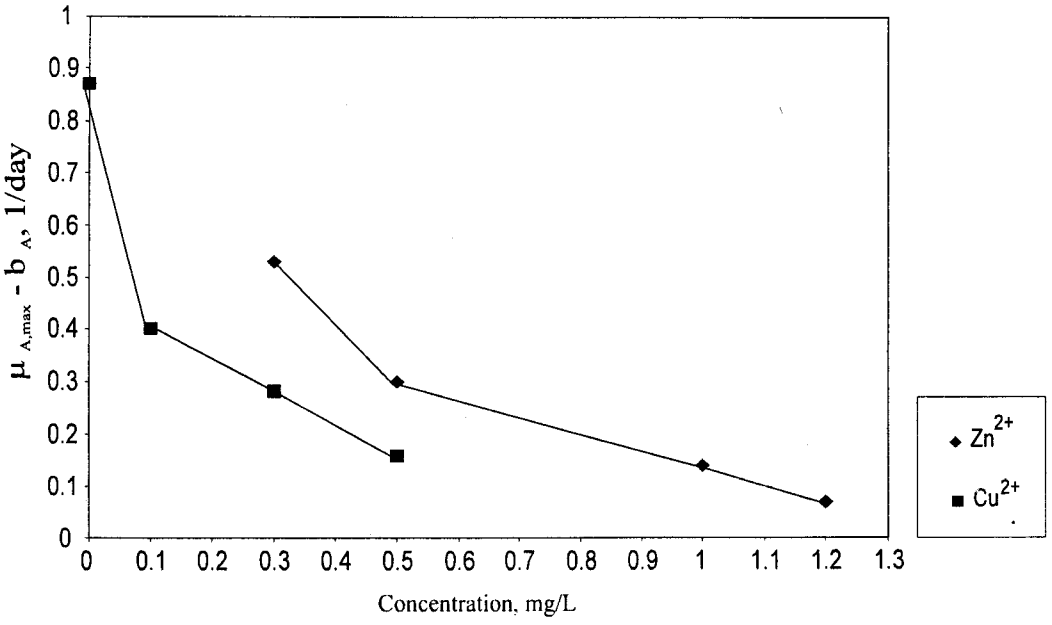


Figure 8. The Effect of Heavy Metal Concentrations on The Net Maximum Specific Growth Rate of The Autotrophic Biomass as (μ_A,max-b_A)

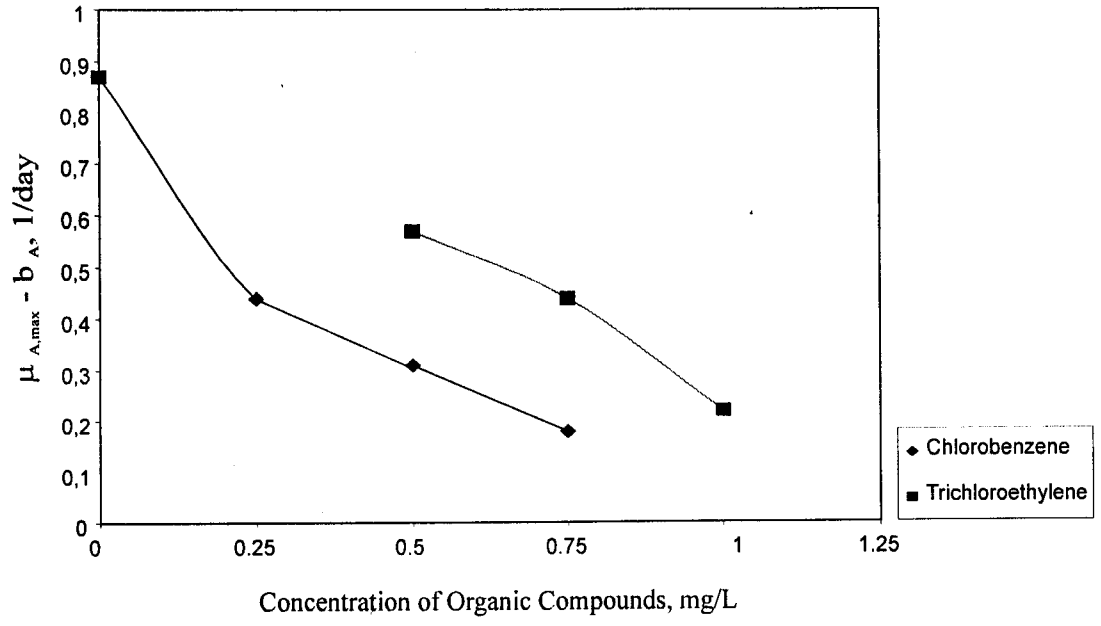


Figure 9. The Effect of Organic Concentrations on The Net Maximum Specific Growth Rate of The Autotrophic Biomass: (μ_A,max-b_A) vs. Concentration of Organic Compounds (for lower concentrations : <10mg/L)

The inhibitory effect of Phenol is more pronounced than Ethylbenzene as presented in Figure 10. The reduction percentage of the net maximum specific growth rate of the autotrophic biomass is 62 % at 4 mg/L. The inhibitory effect increases slowly in the range 4 to 10 mg/L Phenol, to reach 78 % at 25 mg/L. Above 25 mg/L Phenol, inhibition again increases faster than 25mg/L.

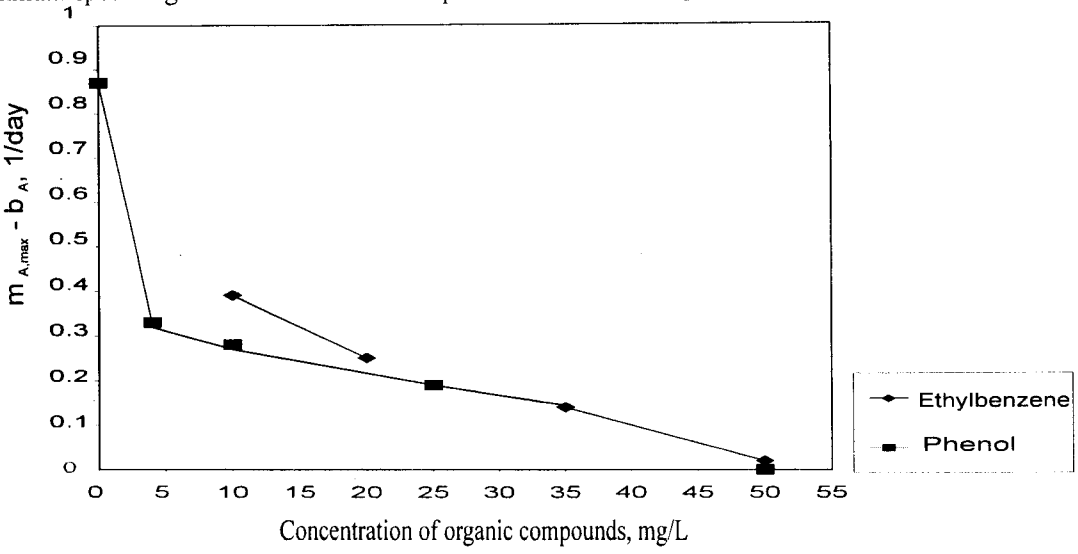


Figure 10. The Effect of Organic Compound Concentrations on The Net Maximum Specific Growth Rate of The Autotrophic Biomass : (A,max-bA) vs. Concentration of Organic Compounds (for higher concentrations : > 10 mg/L)

The experiments are performed in 2 isothermal, closed and well-mixed reactors. One reactor as reference of the noninhibited system, and one with the inhibitor to be tested.

According to the standard procedure of ISO 9509-test each reactor is filled with 400 mL of activated sludge collected from a municipal wastewater treatment plant at 3-3.5 g/L mLSS, 80 mL of substrate fluid (solution of $NaHCO_3$ and $(NH_4)_2SO_4$), 20 mL of basic food (solution of $(NH_4)_2HPO_4$, NaCl, $CaCl_2.2H_2O$, $MgSO_4.7H_2O$ and $FeCl_3.6H_2O$) and 400 mL of either distilled water (reference) or of the sample to be tested, possibly diluted to 400 mL with distilled water (test reactor). The concentrations of nitrite and nitrate are first measured at t=0 hours by spectrophotometer. These concentrations are again measured after set times (e.g. 4 hours). The test is based upon the rate of nitrification.

All heavy metals and organic compounds used in the experiments have inhibitory effects on the nitrification process and results are expressed as degree of inhibition (%). The results are moreover classified according to the value of IC50, IC20 and NOEC (less than 10 % inhibition). The degree of inhibition is defined as follows:

$$\%\,\text{Inhibition} = \frac{C_r - C_i}{C_r} \times 100 \qquad (15)$$

The degree of inhibition can also be defined by the equation from Kroiss et al.(1992)

$$\%\,\text{Inhibition} = \left(1 - \left(\frac{1}{1 + I^{kI}/k_i}\right)\right) \times 100 \qquad (16)$$

The extra aeration reactor volume is calculated from Equation (14), provided sufficient ammonium and oxygen are supplied. Experimental results and literature data are given in Table 3 and are shown in Figure 11 for an influent flow rate of 100 m^3/hr at different influent nitrogen-concentrations. The aeration reactor volume is calculated for the optimum conditions from the experimental results, with $\mu_{A,max}$ =1.02 day^{-1}; b_A= 0.15 day^{-1}; pH=7.0. The yield coefficient (Y) for both calculations is taken from literature data (Baeyens et al., 1999) as average 0.08 (g Cells per g NH_4^+-N oxidized).

The difference between calculated and literature volume is about 30 %. This difference is due to the fact that literature previously used higher values for $\mu_{A,max}$ (up to 1.33 day^{-1}).

Table 3. Summary of Experimental Results Using Added Inhibitor to Municipal Wastewater

Compounds	IC50 (mg/L) OUR-Test	Concentration (mg/L, ISO-Test		
		IC50	IC20	NOEC
Zn^{2+}	0.5	0.35	0.16	0.1
Cu^{2+}	0.1	0.09	0.04	0.025
Chlorobenzene	0.25	0.27	0.1	0.05
Trichhloroethylene	0.75	0.65	0.21	0.15
Ethylbenzene	10	8.5	3.5	1.4
Phenol	4	2.6	1	0.5

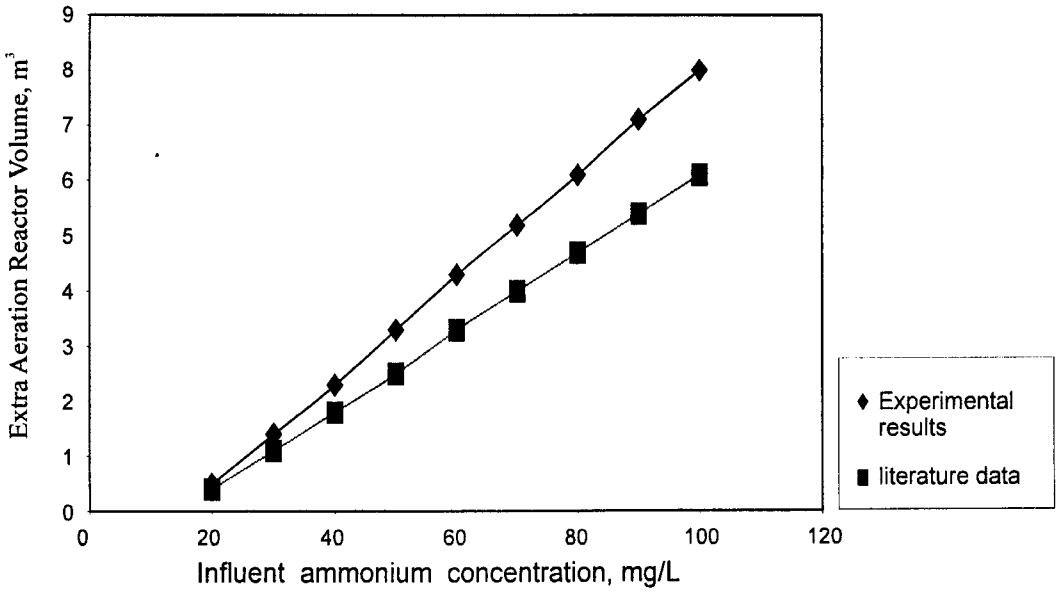


Figure 11. The Influence of Influent Ammonium-nitrogen Concentration on The Extra Aeration Reactor Volume for Nitrification (Influent flow rate: 10 mg/L)

The experimental results of the inhibitory effect of nitrite-nitrogen are shown in Figure 12. The extra aeration volume is bigger than non-inhibited one. The design calculation is based on the influent flow rate of 100 m^3/hour at different nitrite-N concentration and at the optimum condition. The yield coefficient (Y) for *Nitrobacter* activity is taken from literature data (Baeyens et al., 1999) as average 0.05 (g Cells per g NH_4^+-N oxidized). The calculation is based on the influent substrate concentration of 50 mg NH_4^+-N/l. The effluent substrate concentration remains constant at 15 mg/L.

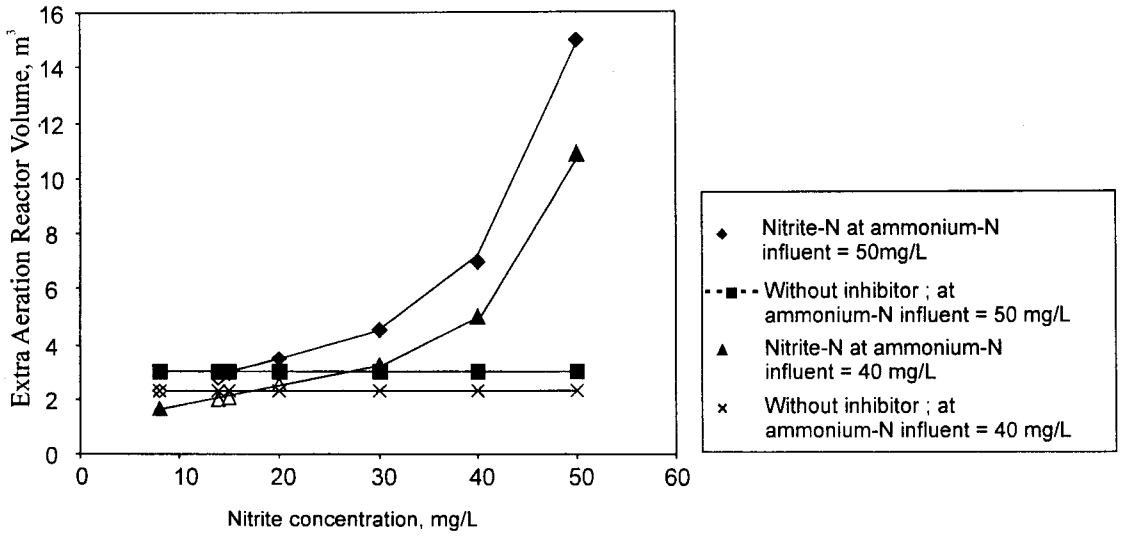
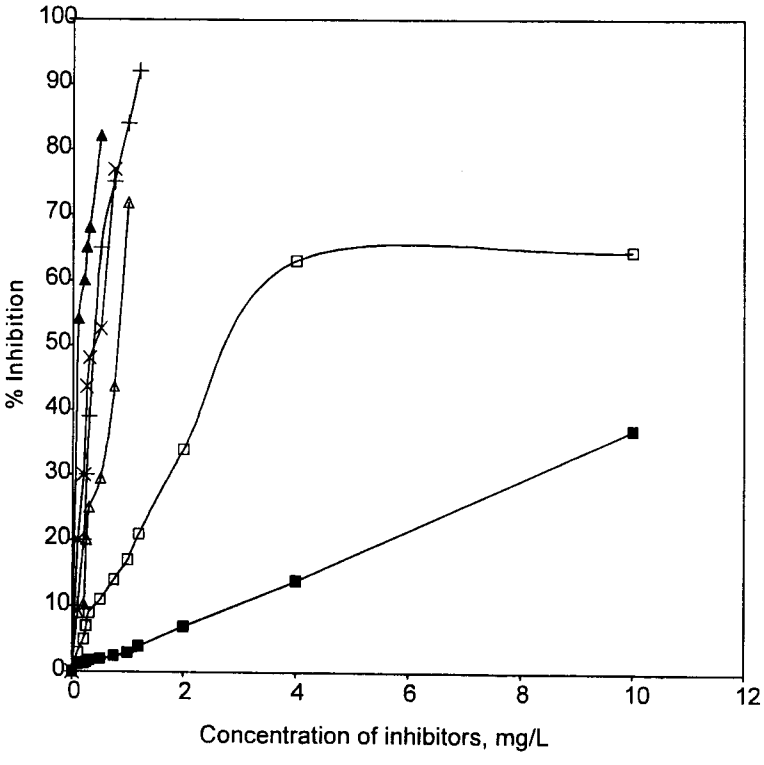


Figure 12. The Effect of Nitrite-nitrogen on The Extra Aeration Volume at Flow Rate of Influent = 100 m3/hour; NH_4^+-N Effluent = 15 mg/L

The effect of inhibitors being present results in different inhibition constant (k_i), which are shown in Figure 13. The %-inhibition can be predicted, using the Kroiss-equation (16) introducing an inhibition correction factor k_I, with experimental values as given in Figure 13.



Inhibitor	k_i
Zn 2+	1.12
Cu 2+	1.0
Trichloroethylene	0.57
Chlorobenzene	0.26
Phenol	4.2
Ethylbenzene	32.2

The effect of inhibitors being present in the wastewater results in the value of the extra aeration reactor volume, see equation (13) and (14). Higher inhibitory effects result in higher value of the extra aeration reactor volume as illustrated in Figure 14.

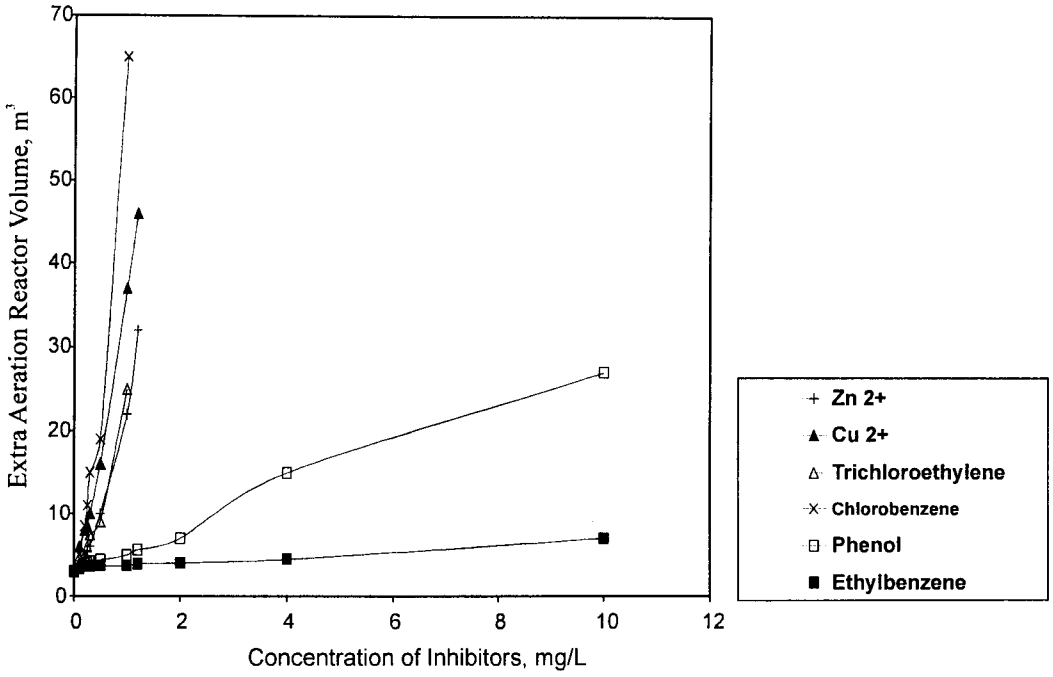


Figure 14. Effect of Different Inhibitors on The Extra Aeration Volume of Nitrification Process

4. Conclusion

The maximum specific growth rate of autotrophic biomass can be determined by the Oxygen Uptake Rate respirometry procedure. The nitrification process is started after the addition of substrate and when the heterotrophic biomass has reached the endogenous respiration condition. The endogenous phase was defined by a constant oxygen uptake rate.

In these experiments, the maximum value of the maximum specific growth rate of the autotrophic biomass (μ_A) is reached at pH 7.0 or 0.87 day^{-1}as net value. The addition of ammonium-nitrogen (NH_4^+-N) affects the maximum specific growth rate of the autotrophic biomass until it reaches the optimum concentration of ammonium-nitrogen. The optimum concentration of ammonium-nitrogen was 15 mg/L at pH 7.0. There is a decrease of μ_A at higher or lower pH. The addition of ammonium-nitrogen above 15 mg/L causes inhibition. At lower concentrations, there was a slight inhibition.

The net maximum specific growth rate of the autotrophic biomass decreases with increasing No_2^--N concentration. The addition of No_2^--N values are measured at the optimum concentration of NH_4^+-N substrate.

The experimental OUR-test results define the autotrophic kinetic parameter values which can be used in design equations of activated sludge nitrification. The possible influence of nitrification inhibition can be added to the design equation as a safety factor.

Notations

b_A = specific decay rate of autotrophic biomass, d^{-1}

C_r = the difference in concentration (mg/L) of nitrate and nitrite at t=0 hour and t = t hours for the reference sample (i.e. without inhibitor being added)

C_t = the difference in concentration (mg/L) of nitrate and nitrite at t=0 h our and t = t hours for the test sample, with inhibitor

I = Inhibitor concentration (mg/L or mL/L)

$\hat{\mu}_A$, $\mu_{A,max}$ = maximum specific growth rate of autotrophic biomass (d^{-1})
$\mu_{N,max}$ = maximum specific growth rate of autotrophic biomass (d^{-1})
k_i = inhibition constant (mg/L ormL/L)
kI = Inhibition correction factor
K_N = substrate (N) half-saturation constant (mg/L)
K_S = substrate half-saturation constant (mg/L)
OUR = oxygen uptake rate (mg O_2 /L.h)
OUR_A = OUR of autotrophic biomass (mg O_2 / L.h)
OUR_H = OUR of heterotrophic biomass (mg O_2 /L.h)
OUR_{total} = total OUR (mg O_2 / L.h)
Q_0, Q_W = influent, effluent flow rate, m^3/d
Q_r = recycle flow rate, m^3/d
S_0, S = substrate concentration in the influent and in the reactor , kg/m^3
T = temperature (° K)
V = reactor volume, m^3
X, X_0 = biomass concentration in reactor, in influent, kg/m^3
X_e, X_W = biomass concentration in effluent, in excess sludge (kg/m^3)
Y = the yield coefficient of biomass (g cell COD per g oxidized N)

Acknowledgements

The authors thank AQUAFIN (Belgium) for making the experimental set-up available. Sludge was obtained from their sewage treatment plant of Huldenberg.

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