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The inhibitory effects of heavy metals and organic compounds on the net maximum specific growth rate of the autotrophic biomass in activated sludge

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Abstract

A respirometry technique can be applied as an effective method to determine the net maximum specific growth rate of autotrophic biomass under both normal conditions and when inhibition occurs. The net maximum specific growth rate of uninhibited autotrophic biomass, expressed as $(\hat{\mu}_A - b_A)$, is approximately 0.8 per day [Proceeding of the International Congress on CHISA, Prague, 2002, p. 1].

Several heavy metals and organic compounds have inhibitory effects. Copper (Cu^{2+}) has stronger inhibitory effects than zinc (Zn^{2+}), and inhibits the nitrification process by 50% at 0.08 mg/l [$(\hat{\mu}_A - b_A) = 0.4$ per day], while the same concentration of Zn^{2+} establishes 12% inhibition only [$(\hat{\mu}_A - b_A) = 0.75$ per day]. Inhibition with Cu^{2+} starts at concentrations above 0.05 mg/l, while this is above 0.3 mg/l for Zn^{2+}. The inhibition of the nitrification process is complete at 1.2 mg/l for both Cu^{2+} and Zn^{2+}.

Among the selected organic compounds tested in the experiments, the degree of inhibition decreases as follows: chlorobenzene > trichloroethylene (TCE) > phenol > ethylbenzene. Chlorobenzene already inhibits the autotrophic biomass at 0.25 mg/l. The nitrification process is totally inhibited by adding 0.75 mg/l of chlorobenzene. TCE has a less inhibitory effect on the nitrification process and 50% inhibition is noticed at 0.75 mg/l TCE. The nitrification process is totally inhibited at 1 mg/l TCE. Phenol inhibits the nitrification for 50% at 3 mg/l. The inhibitory effect of phenol is almost constant in the range 4–10 mg/l and complete inhibition is reached at 50 mg/l. The inhibitory effect of ethylbenzene is 50% at 8 mg/l and the autotrophic biomass is totally inhibited at 50 mg/l.

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Experimental findings are compared with literature data, which generally and significantly overestimate the inhibition threshold concentrations.

Keywords: Respirometry; Nitrification; Autotrophic biomass; Oxygen uptake rate; Heavy metals; Organic compounds; Activated sludge

Nomenclature

b_A	specific decay rate of autotrophic biomass (per day)
K_{NH}	half saturation coefficient of ammonia (mg/l)
OUR	oxygen uptake rate (mg O_2/(l h))
OUR_A	OUR of autotrophic biomass (mg O_2/(l h))
$OUR_{A,max}$	maximum OUR of autotrophic biomass (mg O_2/(l h))
OURendo	the endogenous oxygen uptake rate (mg O_2/(l h))
OUR_H	OUR of heterotrophic biomass (mg O_2/(l h))
OUR total	total oxygen uptake rate (mg O_2/(l h))
r_0	reference nitrification rate
rg	nitrification rate
tg	time of growth (days)
X_A	concentration of autotrophic biomass (mg/l)
X_{A0}	initial concentration of autotrophic biomass (mg/l)
X_{Ag}	concentration of autotrophic biomass at tg (mg/l)
Y_A	overall yield coefficient of autotrophic biomass (g cell COD/g oxidized N)

Greek letters

μ_A	growth rate of autotrophic biomass (per day)
$\hat{\mu}_A$	maximum specific growth rate of autotrophic biomass (per day)
$\hat{\mu}_A - b_A$	net maximum specific growth rate of autotrophic biomass (per day)
$\hat{\mu}_H$	maximum specific growth rate of heterotrophic biomass (per day)

1. Introduction

The observations concerning the net maximum specific growth rate of autotrophic biomass have been reported by several authors for slightly different systems [1–6]. Some of the relevant findings without inhibition are given in Table 1.

Toxic effects of inhibitory compounds on the microbial growth in the activated sludge process have also been investigated by many researchers. A variety of parameters has been studied such as BOD, percent reduction of substrate conversion, respiration rate [7–9], rate of BOD reduction as indicated by the first and the zero order rate constants (k_1, k_2), dehydrogenase measurements [10], proton production [11], etc. These

Table 1
The comparison of different observations concerning $\hat{\mu}_A$ without inhibition at 20 °C

Authors	Observations, $\hat{\mu}_A$ (per day)
Anthoniou et al. [1]	0.12–0.97
Lesouef et al. [2]	0.57
Vanrolleghem and Verstraete [3]	0.77
Sözen and Orhon [4]	$(\hat{\mu}_A - b_A)$ for domestic sewage at 20 °C is 0.38, and 0.1 per day at 10 °C
Henze et al. [5]	1
Juliastuti et al. [6]	1.02

literature findings concerning inhibition in activated sludge [7–12] are summarized in Table 2.

So far, respirometry techniques have frequently been used for the assessment of toxic effects of substances [9]. The toxicity results are often expressed in terms of IC_{50}, the concentration that produces 50% inhibition.

Many kinds of inhibitory compounds are present in wastewater. Some organic compounds and also heavy metals fall within this category. Metals have several effects on the microbial growth either as trace elements or as inhibitor. IC_{50} of Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+} were the same when measured via the INT-dehydrogenase and through the oxygen uptake rate [10]. In most cases, the inhibition by aromatic hydrocarbons exhibits a non-competitive pattern as illustrated by Hyman et al. [13]. Keener and Arp [14] concluded

Table 2
Several observations of inhibition in activated sludge

Authors	Observations
Nowak and Svardal [7]	No nitrification occurs at any sludge age, if the "inhibited" maximum autotrophic specific growth rate is smaller than b_A.
Nowak et al. [8]	The actual maximum autotrophic growth rate can be evaluated from operational data and OUR-values. Half saturation coefficients and decay rates can be estimated by respirometry.
Kong et al. [9]	$\hat{\mu}_A$ decreases faster than $\hat{\mu}_H$ under inhibition of CN^- and 3,5-DCP than the C-oxidation, Cu^{2+} exhibits the contrary effect.
Anderson et al. [10]	IC_{50} of Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+}measured via the INT-dehydrogenase were same as measured by the oxygen uptake rate. The INT-dehydrogenase is less sensitive to Ni^{2+} than oxygen uptake.
Gernaey et al. [11]	Increasing CN^-, 3,5-DCP concentrations resulted in a decrease of the maximum nitrification capacity $((\mu_A \times X_A)/Y_A)$ and an increase of the K_{NH} value. K_{NH} value tended to increase with increasing Cu^{2+}. The CN^- concentration of 0.5 mg/l is reported to give 50% or more inhibition of nitrification and the observed inhibitory effect of 3,5-DCP is comparable to the results of respirometric nitrification inhibition. Nitrification speeds up when phenol is almost completely degraded.
Larson and Schaeffer [12]	Glucose uptake by activated sludge is rapid, concentration-dependent, specific and totally dependent on the presence of a metabolically active sludge. In the absence of test chemicals more than 80% of glucose is removed after 15 min.

that for 15 hydrocarbons and halogenated hydrocarbons non-competitive inhibition was the most common for *Nitrosomonas europae*, and phenol showed 90% inhibition at a concentration of 4.7 mg/l. The physiological state of the culture has a large influence on the threshold concentrations for nitrification [15,16] and 100% inhibition of nitrification was expected at 3.5 mg/l of phenol.

The nitrifying micro organisms are more susceptible to heavy metal inhibition than the micro organisms responsible for the oxidation of carbonaceous material [17]. The effects of heavy metals on the activated sludge process have been reviewed by Anderson et al. [10].

Several factors that influence the degree of inhibition are the pH, the concentration of inhibitor, the species present, the suspended solids concentration, the sludge age, the solubility of the inhibitor and the concentration of other cations and molecules present. The optimal nitrification rate is achieved at a pH in the range 7.0–8.2 and is an increasing function of temperature less than 30 °C [15]. The concentration of suspended solid (SS) or of volatile suspended solids (VSS) has been generally used as an indicator of the heterotrophic or autotrophic biomass concentration in pure cultures or in activated sludge system [18]. Many researchers [19,20] have investigated inhibition by heavy metals as function of biomass and substrate concentrations. Shock loads have also been demonstrated to produce a greater effect on unacclimated than acclimated sludge [21,22].

Most of the research on toxicity of inhibitors has been directed towards the minimum concentration that yields inhibition, towards establishing the maximum concentration which can be tolerated, or towards the removal of toxic substances by the primary and the secondary sludges [23,24]. The biochemical literature describes the effects of inhibitors for the single, pure enzyme systems. These equations are necessarily valid only under these experimental conditions [25,26] and can not be extended to more complex systems of wastewater treatment where various microorganisms can exist, producing many enzymes inhibited in different ways by the toxic compounds. The observed effect is the total influence of the toxic compounds on the biological system rather than the inhibition of a single enzyme. The effect of heavy metals on the microbial growth in wastewater has already been investigated by Tyagi [27]. The inhibitory effects were described in terms of the variation of the specific growth rate of microorganisms. According to investigation of Vismara [28], the concentration range of heavy metals and organic compounds which inhibit nitrification are Zn^{2+} (0.08–0.5 mg/l); Cu^{2+} (0.005–0.5 mg/l) and phenol (4–10 mg/l). Wood et al. [29] has reported that the nitrification process is not inhibited by 165 mg/l of chlorobenzene. Additional quantitative limits are given in Baeyens et al. [30]. The objective of our experiments is to establish the effects of different inhibitors (heavy metals and organic compounds) on the net maximum specific growth rate of the autotrophic biomass by oxygen uptake rate measurements.

2. Experimental set-up and procedure

2.1. Experimental conditions

The experimental work is carried out in a 2 l jacketed batch reactor which is filled with wastewater collected from a municipal wastewater treatment plant at 2 g/l mixed activated

sludge (Fig. 1). This experiment takes place at a constant 20 °C. The reactor is equipped with a dissolved oxygen probe, temperature gauge, pH controller, injection of substrate, magnetic stirrer, and porous aerator. The experiment is carried out at a constant pH of 7.0 using 0.25 M NaOH or 0.25 M HCl as pH-controlling agents.

2.2. Principles of measurements

This wastewater contains a mixed population of biomass of mainly heterotrophic and autotrophic nature. The maximum specific growth rate of the autotrophic biomass is calculated through the measurement of the oxygen uptake rate, i.e. the respiration rate. The respiration rate in this experimental procedure consists of two phases with a first phase of discontinuous aeration whereby the concentration of O_2 is allowed to vary from 2 to 6 mg/l. The air feed is stopped when the O_2 level reaches 6 mg/l, whereas it is automatically started again at 2 mg/l. The discontinuous aeration of the mixture of sludge and water is performed overnight to reach the endogenous condition. This phase provides the utilization of organic carbon by heterotrophic biomass in the wastewater. The respiration rate at the end of this phase is considered to be the endogenous oxygen uptake rate (OURendo).

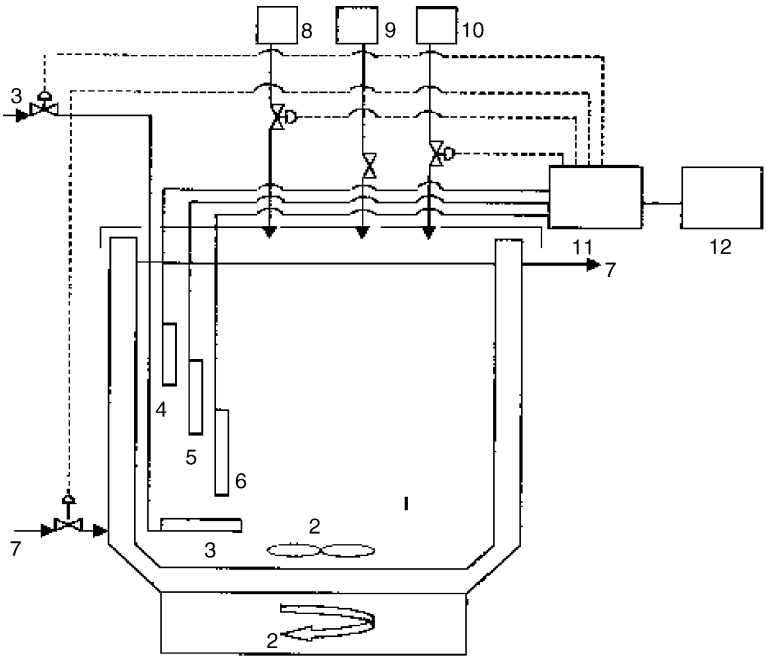
The second phase is started by substrate addition (NH_4Cl) and discontinuous aeration. This phase provides the oxidation of ammonia-nitrogen as performed by autotrophic biomass. The respiration rate at the starting point of substrate addition defines OUR(0) as the beginning of the nitrification process. Inhibitors are added together with the substrate. The substrate concentration (NH_4^+–N) is kept constant at 15 mg/l by adding appropriate quantities of NH_4Cl. The concentration of inhibitors is varied in different tests.

The activity of the autotrophic biomass is illustrated in Fig. 3 as the slope of the nitrification phase. When ammonia is degraded in non-limiting conditions of oxygen, alkalinity and substrate, the nitrification rate is solely a function of the yield, the growth rate and biomass concentration. From substrate consumption rate equations at time zero, and time tg, the growth rate of the autotrophic biomass can be calculated:

$$\ln\left(\frac{\mathrm{rg}}{r_0}\right) = (\mu_\mathrm{A} - b_\mathrm{A}) \tag{1}$$

Toxic substances were added simultaneously with NH_4OH to study the inhibitory effects of common chemicals (Fig. 2). Two heavy metals are used as inhibitors: zinc ($ZnSO_4{\cdot}7H_2O$) and copper ($CuSO_4{\cdot}5H_2O$). The concentrations of Zn^{2+} and Cu^{2+} measured in the activated sludge mixed liquor solution, are 0.3–1.2 and 0.1–0.5 mg/l, respectively. Ethylbenzene, chlorobenzene, trichloroethylene (TCE) and phenol are chosen as the organic compound inhibitors. The organic compound inhibitor concentrations are 10–50, 0.25–0.75, 0.5–1, and 4–50 mg/l, respectively. The concentration of NH_4^+–N in the reactor was monitored every 2 h, analyzed by spectrophotometer and adjusted to 15 mg/l.

Again the oxygen uptake rate is measured from the slope of the O_2 versus time function. This OUR-value is defined as OUR(t). The net respiration rate in the second phase is determined by subtracting the OURendo of the total respiration rate, and the maximum specific growth rate of autotrophic biomass can be determined indirectly by calculating the slope of the nitrification process [6]. The calculation of the net maximum specific growth



1. Batch reactor with cover (less than 30 ml of air present between cover and water)
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 (pH, T, O_2)
12. Computer (data logger)

Fig. 1. Experimental set-up. 1: Match reactor with cover (less than 30 ml of air present between cover and water); 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 (pH, T, O_2); 12: computer (data logger).

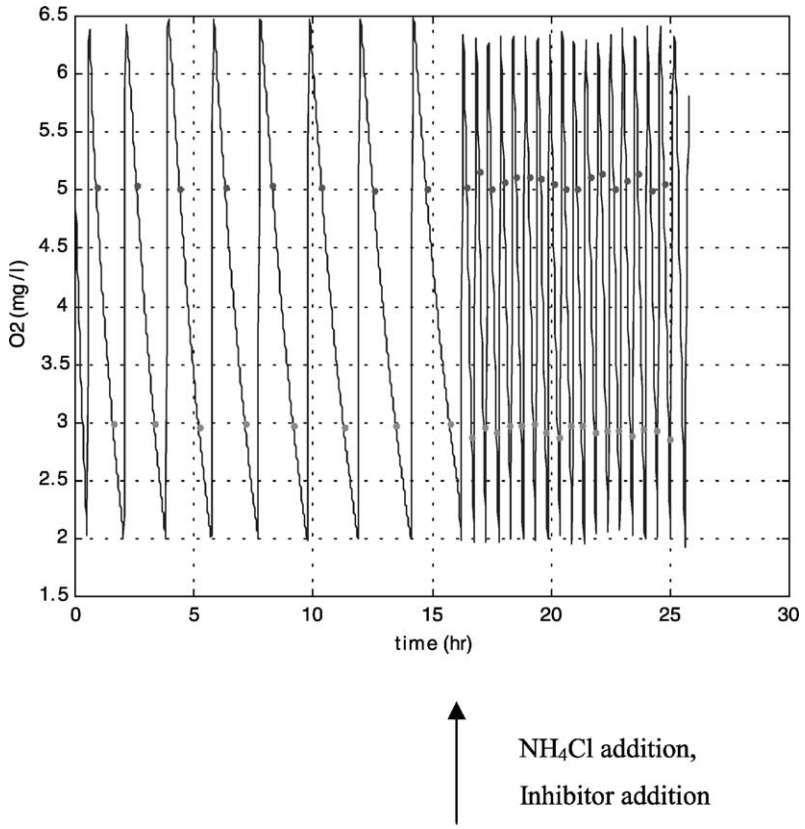


Fig. 2. The aeration cycle. Dissolved oxygen vs. time.

rate of the autotrophic biomass, $\hat{\mu}_A - b_A$, is shown in the equation below:

$$\ln\frac{\mathrm{OUR_A}(t)}{\mathrm{OUR_A}(0)} = \ln\frac{\mathrm{OUR}(t) - \mathrm{OURendo}}{\mathrm{OUR}(0) - \mathrm{OURendo}} = (\hat{\mu}_A - b_A)\, t \tag{2}$$

Measurements were made over a period in excess of 5 h. Experiments were repeated three times under identical conditions, and average values of $(\hat{\mu}_A - b_A)$ were determined (Fig. 3). Deviations of results and average values were less than 6%.

Using literature values of the decay coefficient, b_A, the maximum specific growth rate of the autotrophic biomass can be calculated.

Indeed, the autotrophic decay rate, b_A (20 °C) was determined as 0.2 per day under aerobic conditions, and 0.1 per day under anoxic condition [7]. The IAWPRC task force on activated sludge modeling [5] reported values of b_A between 0.05 and 0.15 per day. Lesouef et al. [2] measured a value of the maximum specific growth rate of the autotrophic biomass (20 °C) as 0.57 per day and a specific decay rate of 0.13 per day. Martinage and Paul [31] indicate a value of 0.15 per day. An average value is used in this study as 0.15 per day.

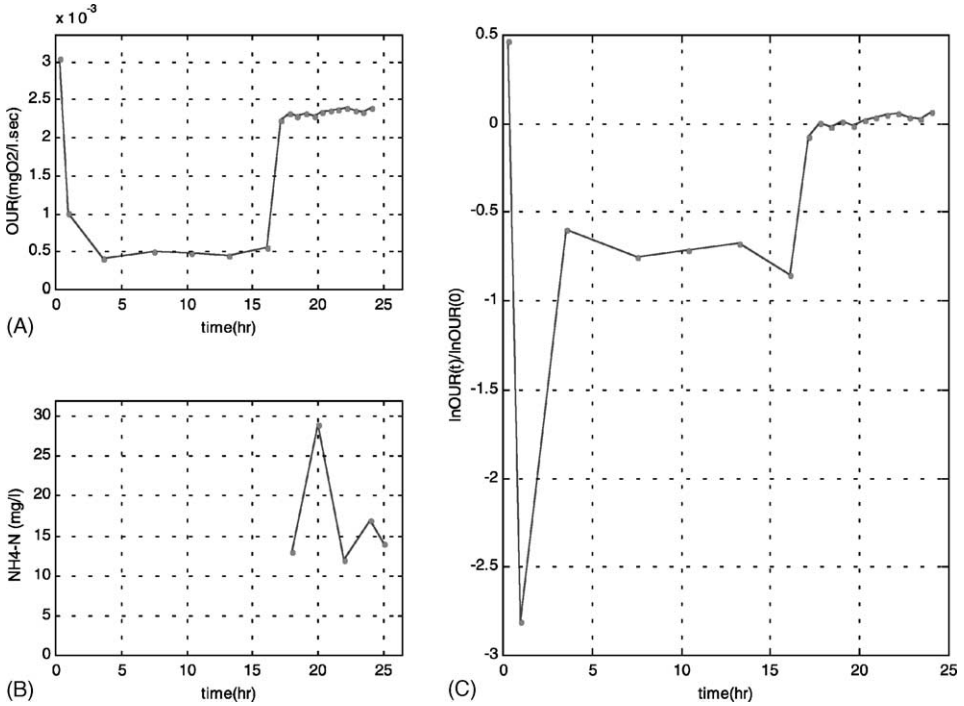


Fig. 3. The activity of biomass: (A) oxygen uptake rate vs. time; (B) NH_4^+–N vs. time; (C) ln OUR(t)/ln OUR(0) vs. time.

In the event of inhibition, the calculated degree of inhibition (%) is defined as follows:

$$\left[1 - \frac{(\hat{\mu}_A - b_A)_{\text{inhibited}}}{(\hat{\mu}_A - b_A)_{\text{non-inhibited}}}\right] \times 100 \tag{3}$$

3. Results and discussion

3.1. Heavy metals inhibitory effect

Fig. 4 illustrates that the net maximum specific growth rate of the autotrophic biomass decreases as the concentration of heavy metals increases. The growth rate of the autotrophic biomass is 92% inhibited at 1.2 mg/l Zn^{2+}. The net maximum specific growth rate is severely reduced by Zn^{2+} at concentrations above 0.3 mg/l. Eysenbach [32] reported 0.08 mg/l Zn^{2+} as the minimum inhibition threshold value, and 0.08–0.5 mg/l Zn^{2+} as the range of inhibition levels.

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 shown in Fig. 4. The value of $(\hat{\mu}_A - b_A)$ reaches 0.4 per day at 0.1 mg/l Cu^{2+}. The inhibition 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

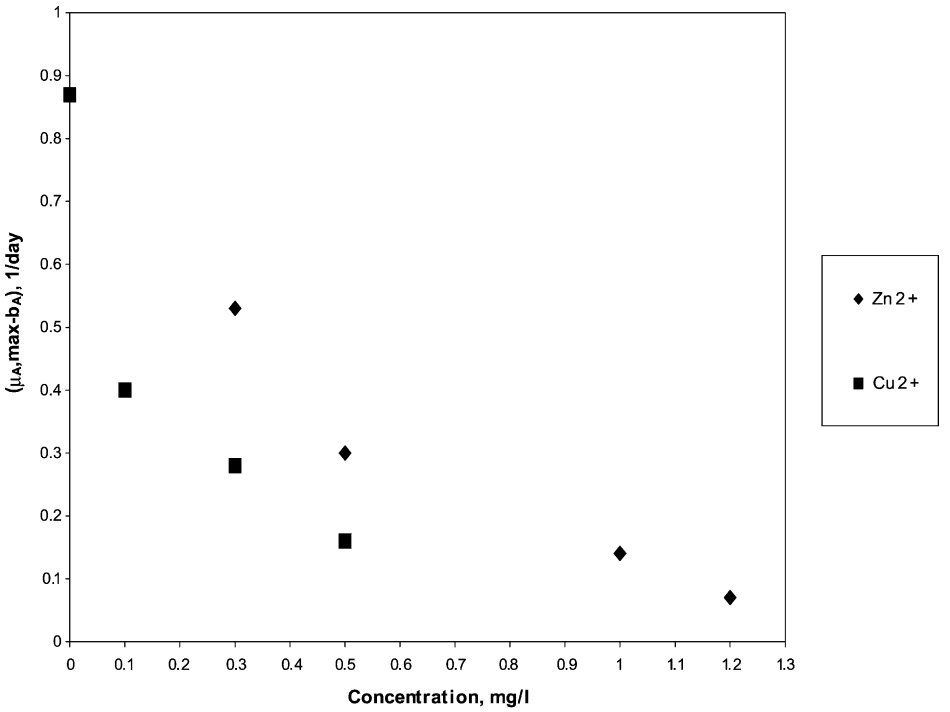


Fig. 4. The effect of heavy metal concentrations on the net maximum specific growth rate of the autotrophic biomass as ($\mu_{A,max}$–b_A).

autotrophic biomass is hardly inhibited by Cu^{2+} at concentrations below 0.05 mg/l, where the value of ($\hat{\mu}_A - b_A$) is close to 0.90 per day. Madoni et al. [33], reported that nitrified bacteria are less inhibited than non-nitrified bacteria, because as little as 0.2 mg/l of Cu^{2+} is used as essential micronutrients for their metabolism. Cenci and Morozzi [34] reported a value of IC_{50} for Cu^{2+} inhibition as 40 mg/l and for 16 mg/l for Zn^{2+}. These values largely exceed experimental findings of the present research.

3.2. Inhibition by organic compounds

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 a first group, whereas ethylbenzene and phenol are considered in a second group.

3.2.1. The inhibitory effect of trichloroethylene

As shown in Fig. 5, the inhibitory effect of adding trichloroethylene is evident, with constantly decreasing values of ($\hat{\mu}_A - b_A$). The autotrophic biomass is nearly totally inhibited at 1 mg/l trichloroethylene, with a value of ($\hat{\mu}_A - b_A$) equal to 0.22 per day, and

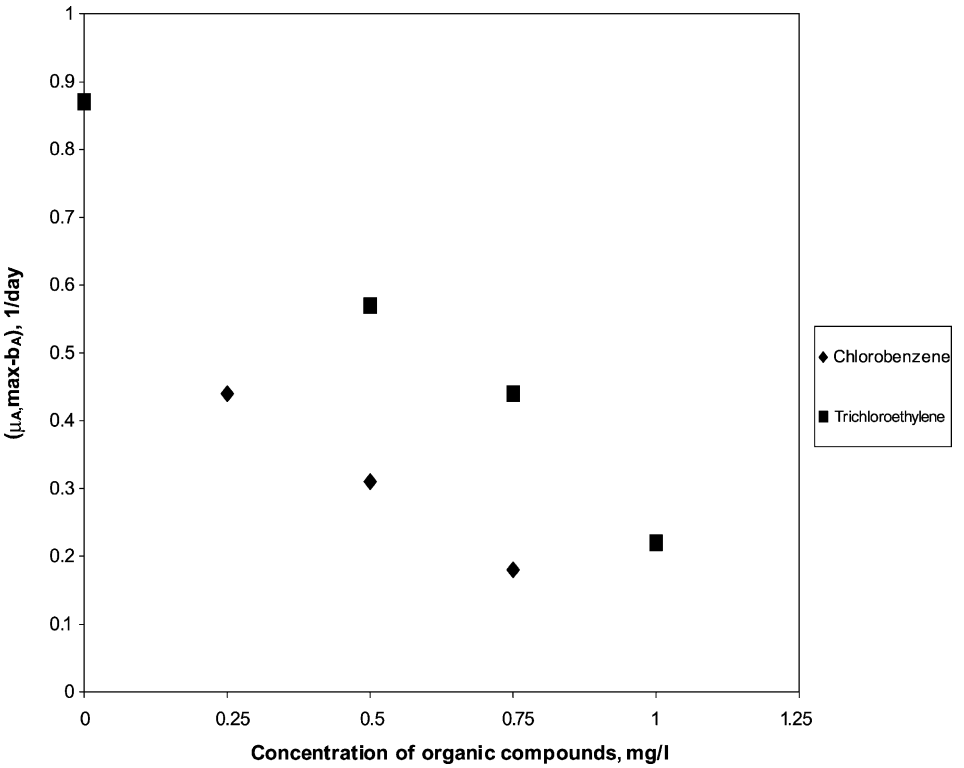


Fig. 5. The effect of organic concentrations on the net maximum specific growth rate of the autotrophic biomass ($\mu_{A,max}$–b_A) vs. concentration of organic compounds (for lower concentrations: <10 mg/l).

50% inhibition is reached at 0.75 mg/l trichloroethylene. Blum and Speece [35] reported 0.81 mg/l trichloroethylene as the 50% inhibition level of *Nitrosomonas*. This value is in line with the results of our experiments.

3.2.2. The inhibitory effect of chlorobenzene

The inhibitory effect of chlorobenzene is more pronounced than for trichloroethylene, as shown in Fig. 5. Adding 0.5 mg/l chlorobenzene causes the net maximum specific growth rate to drop to 0.31 per day, with a 64% inhibition. Over 80% is achieved by adding more than 0.75 mg/l trichloroethylene. The net maximum specific growth rate of autotrophic biomass at this concentration is 0.18 per day only. Blum and Speece [35] reported that 50% inhibition of *Nitrosomonas* is reached by adding 0.71 mg/l chlorobenzene. In comparison to this value, the inhibition of the autotrophic biomass of our experiment is higher than these reported results.

3.2.3. The inhibitory effect of ethylbenzene

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 Fig. 6,

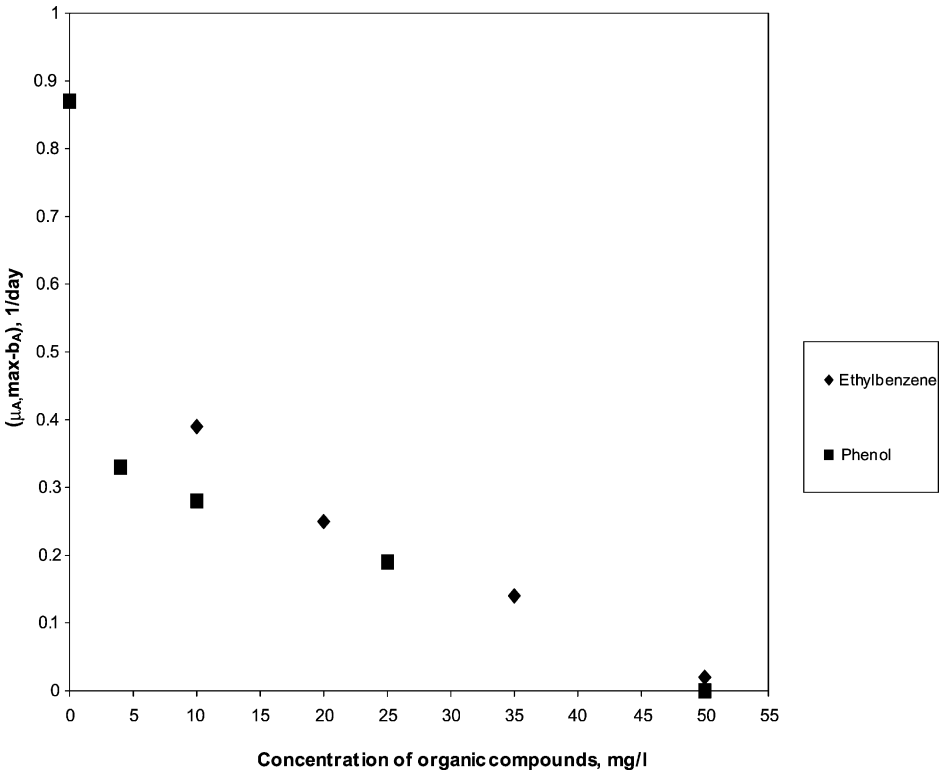


Fig. 6. The effect of organic compound concentrations on the net maximum specific growth rate of the autotrophic biomass ($\mu_{A,max}$–b_A) vs. concentration of organic compounds (for higher concentrations: >10 mg/l).

yields 0.39 per day as the value of $(\hat{\mu}_A - b_A)$ corresponding with 55% inhibition. The same value of $(\hat{\mu}_A - b_A)$ is reached at 0.75 mg/l trichloroethylene or 0.25 mg/l chlorobenzene. The net maximum specific growth rate of the autotrophic biomass decreases further as the concentration of ethylbenzene increases and inhibition is complete at 50 mg/l ethylbenzene. Blum and Speece [35] reported a 50% inhibition of the *Nitrosomonas* activity at 96 mg/l ethylbenzene. In our experiments, this inhibition is reached by adding approximately 10 mg/l ethylbenzene only.

3.2.4. The inhibitory effect of phenol

The inhibitory effect of phenol is more pronounced than ethylbenzene as presented in Fig. 6. The reduction 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 4–10 mg/l phenol, to reach 78% at 25 mg/l. Above 25 mg/l phenol, inhibition again increases faster. Dyreborg and Arvin [36] reported 75% inhibition by adding 5.6 mg/l phenol. Blum and Speece [35] defined 20 mg/l phenol as the concentration that yields 50% inhibition of the *Nitrosomonas*, and 4–10 mg/l phenol has been reported as the minimum threshold value by Eysenbach [32]. The highest concentration of phenol in our experiment is 50 mg/l, when the autotrophic

Table 3
Summary of experimental results of 50% inhibition (IC_{50})

Compounds	Concentration (mg/l)
Zn^{2+}	0.35
Cu^{2+}	0.08
Chlorobenzene	25
Trichloroethylene	0.75
Ethylbenzene	8
Phenol	3

biomass is totally inhibited. Whereas Dyreborg values correspond with our findings [32,35] exceed the experimental results.

The present experiments defined concentrations of inhibitors which produce 50% inhibition as illustrated in Table 3.

4. Conclusion

Respirometry was applied as an effective method to determine the maximum specific growth rate of autotrophic biomass under inhibitory conditions. Heavy metals such as zinc and copper do inhibit the autotrophic biomass at different concentrations. Copper has a stronger inhibitory effect than zinc, with IC_{50}-values of 0.08 mg/l for Cu^{2+}, and 0.35 mg/l for Zn^{2+}, respectively.

The autotrophic biomass is also inhibited by several organic compounds such as trichloroethylene and chlorobenzene to a larger extent than ethylbenzene and phenol. IC_{50}-values are summarized in Table 3.

The discussion of the experimental results also summarizes literature threshold values of inhibition, which are in general significantly higher than the experimental limits of the present research.

These literature data would hence tend to underestimate the occurring degree of inhibition at a given concentration of inhibitor.

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