3. PROCESS CHEMISTRY AND BIOCHEMISTRY OF NITRIFICATION

3.1 Introduction

The purpose of this chapter is to present a review of the chemistry and biochemistry of nitrification. An understanding of this subject is important for an understanding of the factors affecting the performance, design and operation of nitrification.

Biological processes for the control of nitrogenous residuals in effluents can be classified in two broad areas: the production of an effluent where nitrogen (ammonia and organic nitrogen) is converted into nitrate nitrogen: and the reduction of nitrate into nitrogen gas.

In the first stage, nitrification is carried out by bacteria oxidizing ammonia into nitrate with the intermediate formation of nitrite. Nitrification must conform to existing water standards, where reduction of the residual demand on nitrogenous oxygen due to the presence of ammonia is necessary, or where reduction of ammonia is required to conform with existing standards.

The second stage, denitrification (for details see Chapter 4), is used following the nitrification when the total nitrogenous content of the effluent must be reduced.

These conversions are of great importance because ammonia is a highly toxic metabolic waste of aquatic organisms. Nitrite is somewhat less toxic than ammonia (as NH_3), although nitrite toxicity may occur at concentrations of less than 2.5 ppm for some species (Westin 1973). Nitrate is considered relatively non-toxic to most aquatic organisms. As an example, Knepp and Arkin (1985) showed that for channel catfish (a highly tolerant species) the LD_{50} value for ammonia was 37.5 ppm, but nitrate concentrations as high as 400 ppm did not affect feeding activities or growth rates.

3.2 Nitrification

The two principal genera of bacteria of importance in biological nitrification processes are Nitrosomonas and Nitrobacter. But Nitrosospira, Nitrosolobus and Nitrosovibrio are also nitrifying bacteria.

These groups are classified as autotrophic organisms. They are distinguished from heterotrophic bacteria in deriving energy from oxidation of inorganic nitrogen

compounds, rather than from the oxidation of organic compounds. These organisms are also special because carbon dioxide is used for the synthesis of biomass rather than organic carbon. Each group is limited to the oxidation of certain species of nitrogen compounds. Nitrosomonas, Nitrosospira, Nitrosolubus and Nitrosovibrio can all oxidize ammonia into nitrite, but cannot complete the oxidation into nitrate. On the other hand, Nitrobacter is limited to the oxidation of nitrite into nitrate. The apparent inability of these organic developments has been investigated and there is evidence that Nitrobacter can also utilize organic carbon as an energy source. This bacterial species is therefore classified as a facultative autotroph. As complete nitrification is a sequential reaction, treatment processes must be designed to provide an environment suitable for the growth of both groups of nitrifying bacteria.
In contrast to many heterotrophs, the growth of nitrifiers is very slow, and the yield of cells per unit of energy oxidized is low. Like other micro-organisms, nitrifiers can grow at their maximum growth rate when optimum environmental factors can be obtained in an environment without any toxic substances.

Two conditions, therefore, must be fulfilled in order to obtain nitrification in a treatment plant. First, the sludge age has to be sufficiently high to prevent the wash out of the slow-growing nitrifiers applying active sludge design systems. Second, the contact time between the bacterial mass and the ammonia must be long enough to oxidize the ammonia. Table 3.1 compares some characteristics of Nitrosomonas and Nitrobacter.

In Chapters 5 and 6, different plant designs will be outlined. The different mass balance equations for different nitrification plants will also be discussed, showing the relationship between the biomass content and the nitrification efficiency of these plants.

3.3 The Biochemical Pathway in the Nitrification Process

At the biochemical level the nitrification process is more complex than simply the sequential oxidation by Nitrosomonas of ammonia into nitrite, and the subsequent oxidation by Nitrobacter, of nitrite to nitrate. Various reaction intermediates and enzymes are involved in this processes. In soils, streams and treatment plants, conditions permitting the oxidation of ammonia and nitrite can be created by a variety of micro-organisms. Table 3.2 show some of the factors influencing the nitrification.

Table 3.1 Some characteristics of nitrifying bacteria and biological nitrification.

	Nitrosomonas	*Nitrobacter*
Morphology		
Cell shape	Ovoid to rod-shaped	Ovoid to rod-shaped
Cell size	1 x 1,5 μm	0,5 x 1,0 μm
Motile	may or may not be	may or may not be
Gram test	negative	negative
Cell weight	0.12-0.5 x 10^{-12} g	
Estimated generation time hours	8-36	12-59
Autotroph	Obligate	Facultative
Dissolved oxygen requirements to nitrify	Strict Aerobe	Strict Aerobe
Process	$NH_3 + 1,5\ O_2 \rightarrow NO_2^- + H_2O + H^+$	$NO_2^- + 0,5\ O_2 \rightarrow NO_3^-$
$\Delta G°$ kJ/mole NH_3-N	-271	-78
Maximum growth rate at 20 °C	0,5	0,8
Nitrogen oxidation rate mg N/g VSS at 20 °C	100	100
Yield constant mg vss/mg N	0,08	0,03
pH-optimum	7,8 → 9,2	8,5 → 9,2
Long-term temperature constant susp. culture, $°C^{-1}$	0,05	0,04
Long-term temperature constant att. culture, $°C^{-1}$	0,03	0,03
Temperature range for process °C	5°-35°	5°-35°
Reaction Kinetics used in literature	Monod, zero order first order	Monod, zero order first order
Saturation constant, mg N/liter	0,5	1,0
Saturation constant, mg O_2/liter	1,0	1,0

Table 3.2 Factors influencing the Nitrification Process and the section considering the this influence

Influence	Section
Temperature	3.8
Dissolved Oxygen	3.9
pH	3.10
Bacterial Population Dynamics	3.12
Inhibitors	3.13

3.4 The Energy and Synthesis Relationship

The overall stoichiometric reactions in the oxidation of ammonia into nitrate can be summed upas follows:

$$NH_4^+ + 1{,}5\ O_2 \Rightarrow 2\ H^+ + H_2O + NO_2^- \quad (3.1)$$

$$NO_2^- + 0{,}5\ O_2 \Rightarrow NO_3^- \quad (3.2)$$

Equations (3.1) and (3.2) serve as energy-yielding reactions for Nitrosomonas and Nitrobacter, respectively.

Equation (3.1) has been estimated by various investigators to yield a loss of free energy between 58 and 84 kcal per mole of ammonia.

Equation (3.2) has been estimated to release between 15.4 and 20.9 kcal per mole of nitrite. Thus, Nitrosomonas obtains more energy per mole of nitrogen oxidized than Nitrobacter.

The overall oxidation of ammonium is obtained by adding equations (3.1) and (3.2), providing equation (3.3).

$$NH_4^+ + 2\,O_2 \Rightarrow NO_3^- + 2\,H^+ + H_2O \quad (3.3)$$

Using the empirical formula $C_5H_7NO_2$ for the formation of biomass, the following reactions can be written to represent growth of the Nitrosomonas and Nitrobacter respectively:

$$15\,CO_2 + 13\,NH_4^+ \Rightarrow 10\,NO_2^- + 3\,C_5H_7NO_2 + 23\,H^+ + 4\,H_2O \quad (3.4)$$

$$5\,CO_2 + NH_4^+ + 10\,NO_2^- + 2\,H_2O \Rightarrow 10\,NO_3^- + C_5H_7NO_2 + H^+ \quad (3.5)$$

Although about 99 per cent of carbon dioxide in solution shown in equations (3.4) and (3.5) exists in the form of dissolved carbon dioxide, the carbonic acid-bicarbonate equilibrium system is as follows depending on the pH in the environment.

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \quad (3.6)$$

$$CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^- \quad (3.7)$$

The free acid produced in equations (3.1), (3.4) and (3.5) reacts to produce carbonic acid according to equations (3.6) and (3.7).

The equations for synthesis-oxidation using representative measurements of yields and oxygen consumption for Nitrosomonas and Nitrobacter are, according to Haug & McCarty (1972):

Nitrosomonas

$$55\ NH_4^+ + 76\ O_2 + 109\ HCO_3^- \Rightarrow C_5H_7NO_2 + 54\ NO_2^- + 57\ H_2O + 104\ H_2CO_3 \quad (3.8)$$

Nitrobacter

$$400\ NO_2^- + NH_4^+ + 4\ H_2CO_3 + HCO_3^- + 195\ O_2 \Rightarrow C_5H_7NO_2 + 3\ H_2O + 400\ NO_3^- \quad (3.9)$$

Equations (3.8) and (3.9) show that the oxidation of 100 mg NH_4^+ -N produces 14,6 mg of Nitrosomonas biomass and 2,0 mg of Nitrobacter biomass, respectively.

Adding equations (3.8) and (3.9) and simplifying, the overall synthesis and oxidation reaction for the conversion of ammonium into nitrate is:

$$NH_4^+ + 1{,}83\ O_2 + 1{,}98\ HCO_3^- \Rightarrow 0{,}021\ C_5H_7NO_2 + 1{,}041\ H_2O + 0{,}98\ NO_3^- + 1{,}88\ H_2CO_3 \quad (3.10)$$

The conversion of 100 mg/l of ammonia nitrogen to nitrate-nitrogen according to equation (3.10) therefore yields about 17 mg/l of total nitrifying biomass. This relatively low yield has some far reaching consequences in the design of nitrification treatment plants, as will be seen in later sections.

The oxygen consumption ratios in equation (3.10) are 3.22 mg O_2 per mg NH_4^+ -N oxidized and 1.11 mg O_2 per mg NO_2^- -N oxidized, respectively. This gives a total oxygen need of 4,32 mg O_2 per mg NH_4^+ -N oxidized to NO_3^- - N (Gujer and Jenkins 1974).

3.5 Kinetics of the Nitrification Process

The aim of this section and the following sections is to consider the number of environmental factors affecting the rate of growth and nitrification of a nitrifying biomass. A combined kinetic expression is proposed which accounts for the effect of ammonia concentration, temperature, pH, organic content, and dissolved oxygen concentration.

At several points, references are made to data obtained from various types of nitrification processes. One distinction that needs to be clearly understood in this Chapter is the difference between combined carbon oxidation-nitrification processes and the separate stage nitrification process (also called a tertiary nitrifying treatment process). The combined carbon oxidation-nitrification processes oxidize a high proportion of influent organics relative to the ammonia nitrogen content. This causes relatively low populations of nitrifiers to be present in the treatment plant relative to oxidizers of the total bacterial biomass.

Separate stage nitrification systems, on the other hand, have a relatively low organic load, relative to the ammonia load. As a result, higher proportions of nitrifiers are obtained.

A nitrifying activity test was proposed by Tomlinson *et al.* (1966) and later by Painter and Loveless (1981). The test is able to determine the activity of sludge to oxidize ammonia and ii is therefore suitable to determine the kinetics of the nitrification in activated sludge.

3.6 The Kinetic Expressions for the Nitrification Process

A review of the literature concerning the nitrification process shows diverse opinions regarding the reaction rate equation for the nitrification process. Several rate equations have been proposed. Each stems from different assumptions, and different results have therefore been obtained. A review of these equations is presented in Table 3.3.

Knowles, Downing and Barrett (1965) and Downing (1968), were among the first to attempt to quantify nitrifying bacteria in waste water treatment plants. They all used the Monod Model of population dynamics proposed by Monod in 1942, which is similar to the Michalis-Menten relationship for enzyme reactions.

Huang and Hopson (1974) reviewed four different reaction rate equations (see

Table 3.4) to determine the appropriate equation. From the initial ammonia-nitrogen concentration and the contact time studies, the nitrification process was shown to follow a zero-order reaction.

The Monod Model used to describe the kinetics of biological growth of either Nitrosomonas or Nitrobacter is the standard expression used in formulating the rate equation:

$$\mu = \mu_{max} \frac{S_N}{K_{S,n} + S_N} \qquad (3.11)$$

where μ = growth rate of micro-organisms, in day^{-1}.

μ_{max} = maximum growth rate of microorganisms, in day^{-1}.

$K_{s,n}$ = saturation constant = substrate concentration, mg/l, at half the maximum growth rate.

S_N = growth limiting substrate concentration, mg/l expressed as NH_4^+ - N.

When the reaction rate is independent of the substrate concentration, the reaction rate can be considered as a *zero order reaction*. This results from a high substrate concentration which leads to a maximum growth rate, indicating that no diffusional limitations exist.

When the reaction is directly proportional to the substrate concentration then the reaction can be considered as *first order* and the rate of reaction would be directly governed by the ambient ammonia concentration.

The saturation constant $K_{s,n}$ is temperature dependent, as will be discussed in section 3.8. As the maximum growth rate of Nitrobacter is considerably higher than the maximum growth rate of Nitrosomonas, and as the $K_{s,n}$ values for both organisms are less than 1 mg/l NH_4^+ -N at temperatures below 20° C, nitrite does not accumulate in large amounts in biological treatment systems under steady-state conditions.

Table 3.7 and Fig. 3.4 presents values for K_s for both nitrifying species as found under different environmental conditions.

Table 3.3 Summary of the different kinetic equations used in the literature to describe the nitrification process.

	Order		
Rate law	***Zero***	***First***	***Monod***
Integrated rate law	$\frac{ds}{dt} = - k$	$\frac{ds}{dt} = - k \cdot [N]$	$\frac{ds}{dt} = - \frac{\mu_{max}}{Y} \cdot \frac{N}{k_s + N} \cdot X_n$
Plot needed to give a straight line	[product] versus t	ln [N] versus t	$\frac{[1]}{[N]}$ versus $\frac{[1]}{[V]}$
Slope of the straight line	Slope = - k	Slope = - k	Slope = $\frac{k_m}{V_{max}}$
Half-life	$t_{½} = \frac{[N]_0}{2k}$	$t_{½} = \frac{0,693}{k}$	

Table 3.4 An overview of the kinetic rate equation used in different studies refered in the literature.

Plant design	*NH_4^+ range*	*Process*	*Kinetics application to describe system*	*References*
Lab exp.	2,5-67,3	Nitrification	0. order	Huang and Hopson (1974)
Lab exp.	1,6-52	Nitrification	0. order	Kiff (1972)
Lab exp.	6,0-60,0	Nitrification	0. order	Wild *et al.* (1971)
Lab exp.	-	Nitrification	Monod kinetics	Stratton and McCarty (1967)
Lab exp.	-	Nitrification	Monod kinetics	Downing and Hopwood (1964)
Lab exp.	-	Nitrification	Monod kinetics	Knowles *et al.* (1965)
Lab exp.	Up to 100 mg/l	Nitrification	Michealis-Menten	Charley *et al.* (1980)
Lab exp.	Up to 20 mg/l	Nitrite oxidation	1. order	Charley *et al.* (1980)
Lab exp.	100-1100 mg/l	Nitrification	0. order	Wong-Chong and Loehr (1975)
Lab exp.	0-8000 mg/day	Nitrification kinetic	Monod	Churchwell *et al.* (1980)
Lab exp.	-	Nitrification	0. order	Watanabe *et al.*(1980)
Trickling filter	-	Nitrification	1. order	Balkrishnan and Eckenfelder (1970)
Upflow submerged filter	-	Nitrification	Close to 1. order	Huang and McCarty (1972)
Trickling filter	-	Nitrification	0. order	Harkness (1966)
Trickling filter	-	Nitrification	1/2 order	Harremoes (1978)

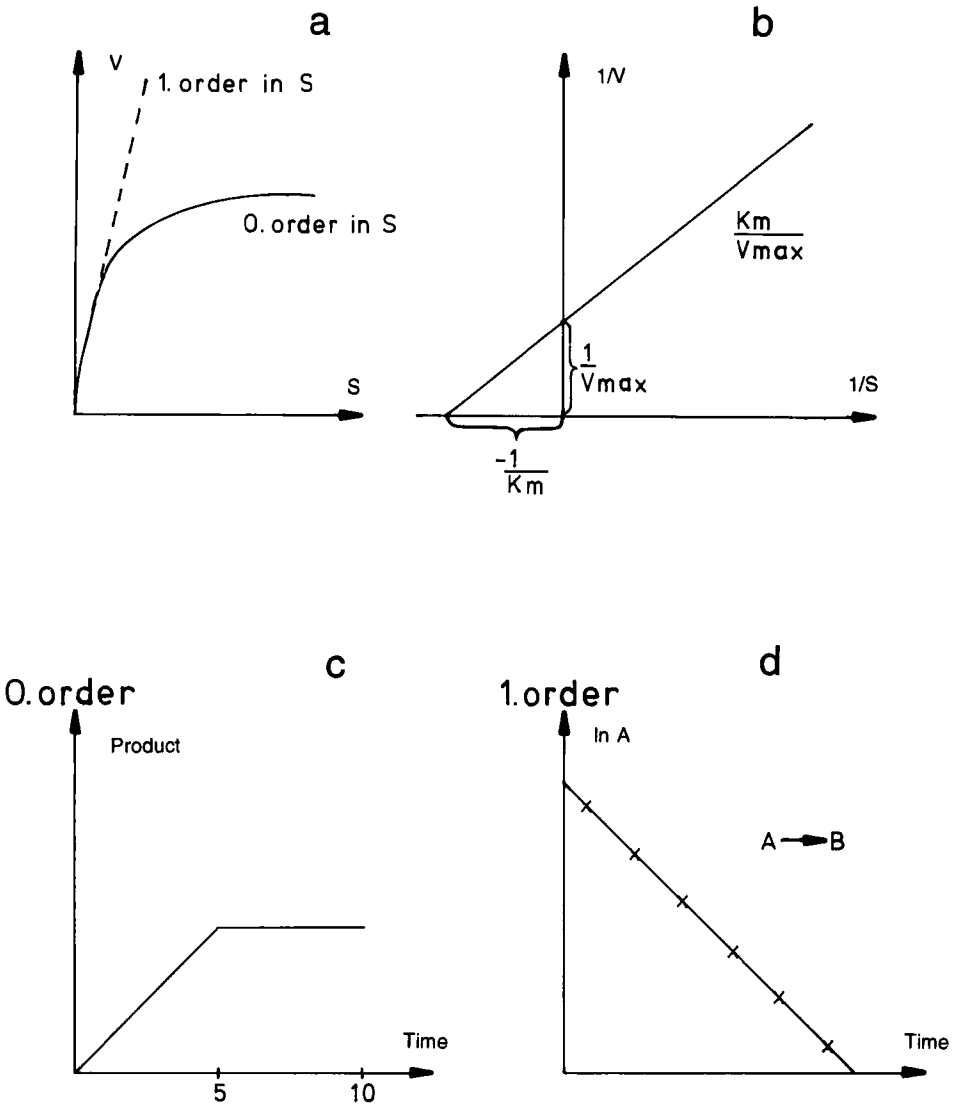

Figure 3.1 Graphical representations showing a) Monod kinetics; b) Tranformation of Michaelis-Menten Kinetics to the Lineweaver-Burk Plot; c) Zero Order kinetics and d) First Order kinetics.

Nitrosomonas and Nitrobacter are both sensitive to their own and each others substrate. Tables 3.5 and 3.6 show that wide ranges of ammonia and nitrite ion concentrations can be oxidized by the nitrifiers. Different conditions can account for the apparent discrepancies. Normal ammonia and nitrite ion concentrations in domestic waste waters are not in the inhibiting ranges. Substrate and product inhibition, however, are of significance in the treatment of industrial and agricultural wastes. Table 3.19 show the ammonium nitrogen and nitrate nitrogen concentration range for Nitrobacter inhibition as function of pH.

It would be desirable for the process of nitrification to be a reaction having zero-order kinetics at least to low concentrations (< 5 mg/l) as the rate would be constant and unaffected by the substrate concentration.

Mateles *et al.* (1965) showed that while the Monod Model for microbial growth was useful for steady-state cultures, its application in predicting the dynamic behaviour of chemostats has limitations.

3.7 Relationship Between Growth Rate and Oxidation Rate

The ammonia oxidation rate can be related to the Nitrosomonas growth rate, as follows:

$$\frac{dS_n}{dt} = -\frac{1}{Y_n} * \frac{dX_n}{dt} \qquad (3.12)$$

or in the differantiated form of Michaelis-Menten:

$$\frac{dS_n}{dt} = -\frac{\mu_{max}}{Y_n} * X_n * \frac{S_n}{k_{s,n} + S_n} \qquad (3.13)$$

Table 3.5 Effect of ammonia concentration on nitrification and nitrifying bacteria.

Concentration of Ammonia-nitrogen mg/l	*Effect/Observation*	*Condition of observation method of study*	*Reference*
2,5 - 110,0	Ammonia oxidation, a zero order reation	Film reactor; mixed culture	Huang (1973)
26,4 - 46,5 Up to 60	Ammonia oxidation, a zero order reaction. No inhibition.	Activated sludge lab. scale	Metcalf and Eddy (1973)
Up to 10	Rate of ammonia oxidation; a function of ammonia concentration (between first and second orders).	Submerged filter receiving pre-oxygenated feed.	Haug and McCarty (1972)
3,5	Michaelis constant for Nitrosomonas growth at 25 °C.	Pure culture; Warburg respirometer	Painter (1970)
1,0	Michaelis constant for Nitrosomonas growth at 20 °C.	Dropping-mercury electrode; pure culture	Loveless and Painter (1968)
0,063	Michaelis constant for growth of ammonia oxidizers at 23 °C.	Mixed continuous culture constant obtatined by computer fit of experimental data with assumed yield coefficient value.	Poduska and Andrews gwth (1975)
600	Oxidation possible	Poultry waste; Repeated nitrification on a batch scale.	Praksam *et al.*(1974)
100 - 1000	Ammonia oxidation, a zero order reaction	Lab. scale batch studies with mixed culture and mineral salt media.	Wong-Chong and Loehr (1975)
800	10,1% oxidation possible	Bench scale studies, activated sludge, synthetic waste derived from nitrified poultry waste.	Anthonisen (1974)

Table 3.6 Effect of nitrite concentration on nitrification and nitrifying bacteria.

Concentration of Nitrate-nitrogen mg/l	*Effect/Observation*	*Condition of observation method of study*	*Reference*
< 10	Limiting	Activated sludge; lab.scale	Tomlinson, Boon and Trotmann (1966)
140, 160, 280, 700 and 1400	Rate of oxidation may be described by first order rate equations; decrease in rate constant with increasing initial concentration explained by Michaelis-Menten kinetics.	Batch studies in a marine nitrifying filter system.	Srna and Baggaley (1975)
500	Nitrate toxic in the lag phase at all pH values; not so in the lag phase at alkaline pH.	Batch and pure culture of Nitrosomonas	Pokallus (1963)
1200	Ammonia oxidizers not completely inhibited	Mixed culture from an oxidation ditch; poultry waste: respirometric experiment.	Prakasam *et al.* (1974)
1400	Causes 40% inhibition of Nitrobacter activity	Measured by decrease in oxygen uptake by bacteria	Boon and Laudelout (1962)
4200	Complete inhibition of Nitrosomonas.		Painter (1970)

Table 3.7 Kinetic constants for nitrifying bacteria.

Organism	*Max spec. growth rate* μ_{max} d^{-1}	*Cellular yield* Y_{obs} *g VSS / g N*	*Ks* g/m^3	Ko_2 g/m^3	*Reference*
Nitrosomonas	0,46-1,86 (30°C)	0,06	10 (30°C)	0,5 (30°C)	Painter (1977)
			3,5 (25°C)		
			1,2 (20°C)	0,3 (20°C)	
			1,5 (18°C)		Marais and Ekema (1976)
	0,46-2,20	0,03-0,13	0,06-5,6 (15°-32°C)	0,3-1,3	Charley *et al.*(1980)
Nitrobacter	1,39 (32°C)	0,02	8 (32°C)	1,0 (30°C)	Painter (1977)
			5 (25 °C)	0,5 (32°C)	
				0,25 (18°C)	
	0,28-1,44	0,02-0,08	0,07-8,4 (15-32°C)	0,25-1,3	Sharma (1977)

where

μ_{max} = peak Nitrosomonas growth rate, day^{-1},

dSn/dt = peak ammonia oxidation rate, mg NH_4^+ - N oxidized /mg VSS/ day,

Y_n = nitrifying yield coefficient, mg Nitrosomonas grown (VSS) per mg NH_4^+ -N removed,

S_n = The substrate concentration, mg/l,

$K_{s,n}$ = Saturation constant, NH_4^+ -N in mg/l,

X_n = nitrifying mass cell concentration in mg/l,

If the substrate concentration S is much higher than K_s then equation (3.13) can be written as:

$$\frac{dS_n}{dt} = -\frac{\mu_{max}}{Y_n} * X_n \qquad (3.14)$$

In equations (3.13) and (3.14) only the effect of ammonia concentration is considered; in later sections, the effect of temperature, pH, organics and dissolved oxygen are also discussed.

If the temperature, pH, organics and dissolved oxygen concentration are unknown, equations (3.13) and (3.14) are proposed. But if the indicated parameters are known, equation (3.37) will be more precise to use.

The growth rate of organisms can be related to the design of activated sludge systems by noting the inverse relationship between solids retention time and growth rate of nitrifiers:

$$\phi_c = \frac{1}{\mu} \qquad (3.15)$$

where ϕ_c = solids retention time, days.

μ = growth rate of nitrifying organisms in day $^{-1}$.

The solids retention time can be calculated from systems operating data by dividing the inventory of microbial mass in the treatment system by the quantity of biological mass losted daily (EPA 1975).

3.8 The Influence of Temperature on the Nitrification Rate

The optimum temperature for the growth of nitrifying bacteria, according to the literature, is between 28° C and 36° C, although an optimum temperature of up to 42° C has been reported for Nitrobacter by Painter (1970). Growth constants of nitrifying bacteria are greatly affected by temperature (Table 3.9). Figure 3.2 shows that the nitrification rate is a function of temperatures between 5° and 35° C. The maximum growth rate occurs at approximately 30° C. Curve A, which was produced by Borchardt (1966) indicates that no sharp optimum temperature can be defined and that there is a plateau of maximum activity between 15° C and 35° C. Below 15° C however, the nitrification rate drops sharply, and is reduced by 50 per cent at 12° C. Wild *et al.* (1971) found (curve B) that an almost straight-line relationship exists between the nitrification rate and temperature. Similar temperature dependencies have been reported in single stage nitrification-denitrification schemes.

Data are also available on the effects of temperature on the oxidation of ammonia to nitrite by Nitrosomonas (curves E, F, G and H), and of nitrite to nitrate by Nitrobacter (curves C and D). Both species seem to be similarly influenced by temperature.

Randall and Buth (1970), however demonstrated that although both nitrite and nitrate formation were strongly inhibited at temperatures of 10° C or less, the inhibitory effect of lowered temperature was greater for Nitrobacter than for Nitrosomonas; this was evident from the nitrite build-up at low temperatures.

Barrit (1933) found that the thermal death point of a pure culture of Nitrosomonas was between 54° and 58° C. Almost no growth of nitrifying bacteria was found below 4° C.

Suspended growth cultures are more sensitive to temperature changes than biofilms (Murphy and Dawson 1972). The dependency on temperature of attached and

suspended growths is illustrated below (Fig 3.3).

Downing *et al.* (1964) presented results for the relationship between temperature and saturation concentration $K_{s,n}$ and temperature and maximum specific growth rate μ. Their results are presented in Fig. 3.4. As can be seen, both the maximum growth rate, μ and the saturation constants, K_s for Nitrosomonas and Nitrobacter are markedly affected by temperature. Further, the maximum growth rate for Nitrosomonas in activated sludge was found to be considerably less than for Nitrosomonas in a pure culture.

The literature suggests the following general relationship between the saturation constant $K_{s,n}$ and temperature t in °C.

$$K_{s,n} = 10^{0,051 * t - 1,158} \tag{3.16}$$

Reference: EPA 1975; Nitrosomonas in river water and activated sludge.

$$K_{s,n} = 10^{0,063 * t - 1,149} \tag{3.17}$$

Reference: EPA 1975; Nitrobacter in river water.

$$K_{s,n} = K_{20} * 1,15^{(T-20)} \tag{3.18}$$

Reference: Watanabe *et al.* 1980 applied to suspended culture of nitrifier at T °C.

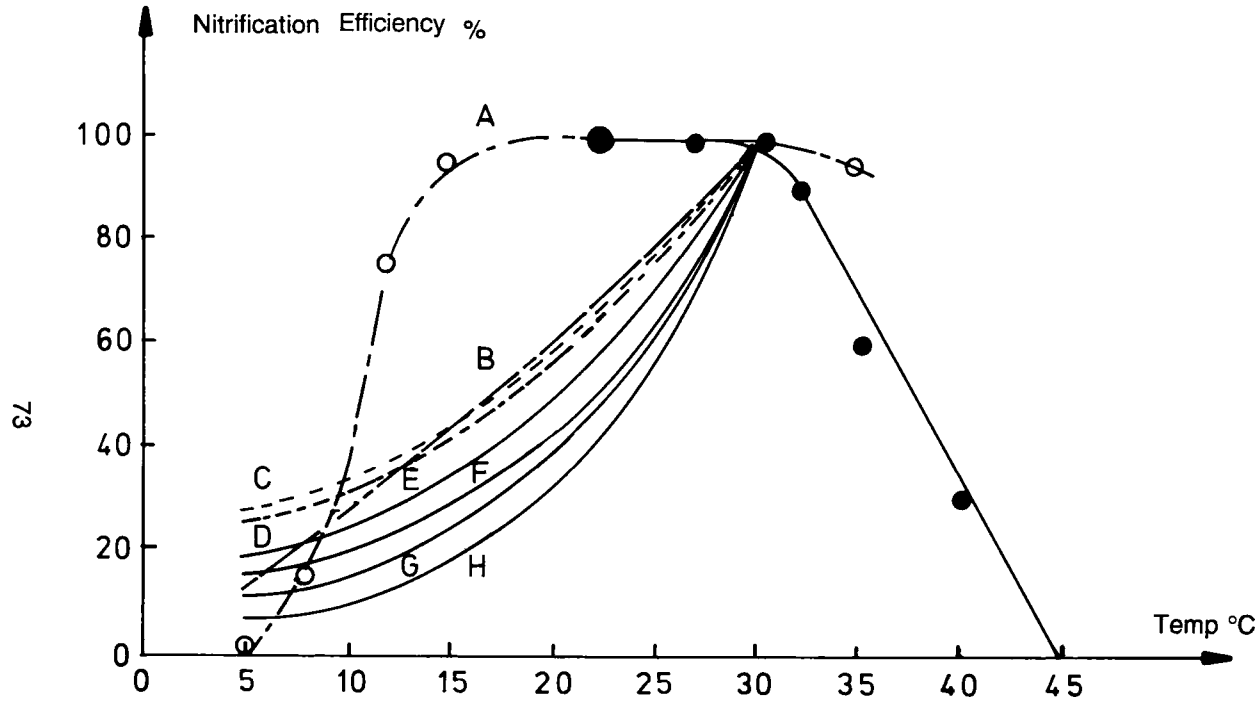

Fig. 3.2 The influence of temperature on the nitrification process, presented in the text as A to H, (Source: EPA 1975).

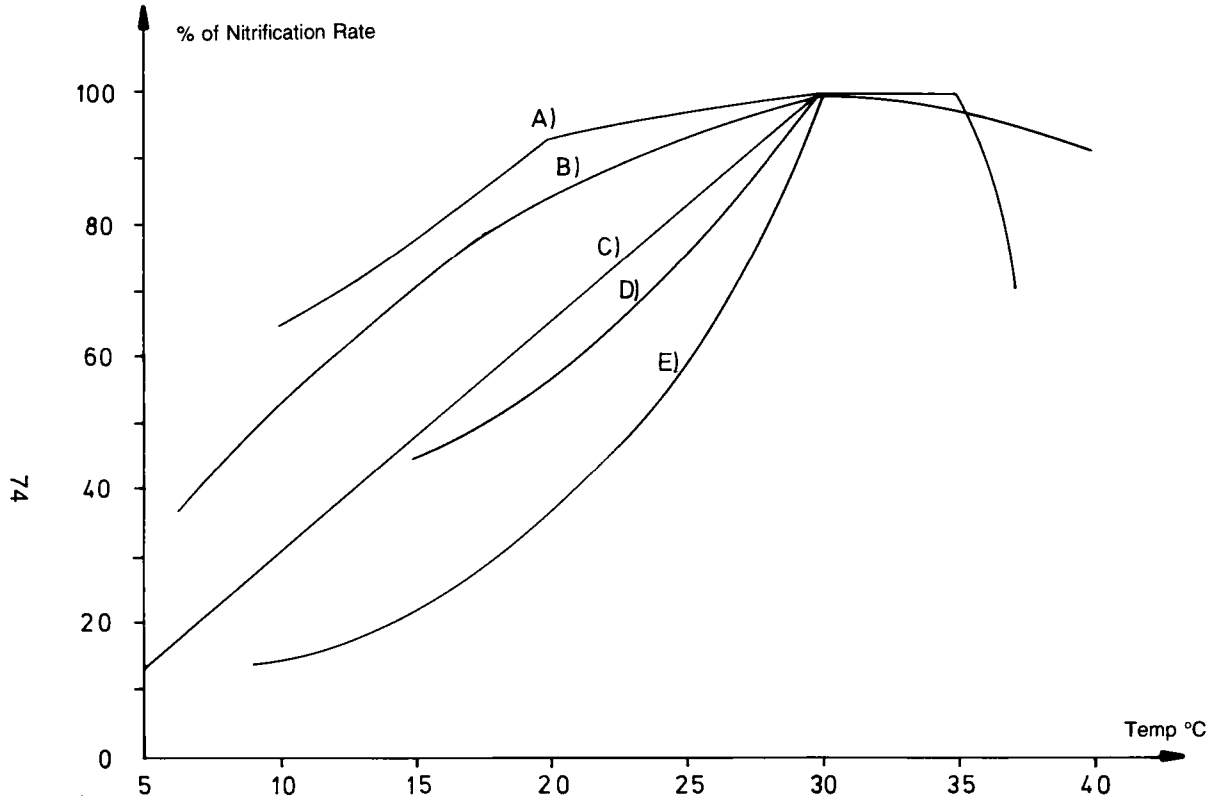

Fig 3.3 Comparison on the effect of the temperature on suspended growth and attached growth nitrification systems. A) to D) are attached growth systems and E) is a suspended growth system (Source EPA 1975).

Table 3.8 The influence of temperature on the nitrification process.

Temperature C	*Degree of inhibition %*	*Circumstances of obsevation*	*Reference*
15^0-35^0	0	Nitrification in activated sludge	Borchardt (1966)
13^0	25		
12^0	50		
5^0	90		
30^0	0	Nitrification in activated sludge	Wild *et al.* (1971)
27^0	10		
17^0	50		
26^0	0		Sutton *et al.* (1974)
7^0	21		
5^0	53		
30^0	0	Nitrobacter in river water	Stratton and McCarty (1967)
15^0	60		
5^0	75		
30^0	0	Nitrobacter in estuary water	Knowles *et al.*(1965)
15^0	62		
5^0	77		
30^0	0	Nitrosomonas in pure culture	Buswell *et al.*(1954)
15^0	70		
5^0	83		
30^0	0	Nitrosomonas in river water	Stratton and McCarty (1967)
15^0	75		
5^0	85		
30^0	0	Nitrosomonas in estuary water	Knowles *et al.*(1965)
15^0	80		
5^0	90		
30^0	0	Nitrosomonas in activated sludge	Downing (1968)
15^0	85		
5^0	93		

Knowles *et al.* (1965) proposed the following two relationships between temperature and the saturation constant for Nitrosomonas and Nitrobacter, following the Arrhenius law:

$$K_{s,Nitrosomonas} = 0,405 * e^{0,118(T-15)} \quad (3.19)$$

$$K_{s,Nitrobacter} = 0,405 * e^{0,146(T-15)} \quad (3.20)$$

Neufeld *et al.* (1986) showed that the nitrification rate followed Michaelis-Menten Kinetics and proposed the following relationship between K_M and the temperature. K_M was found to decrease in the temperature range of 22-30 °C in accordance with the equation:

$$\log(K_M) = 1,53-0,032(T) \quad (3.21)$$

and at temperatures > 30 °C K_M was found to follow the expression:

$$\log(K_M) = -1,88+0,082(T) \quad (3.22)$$

The relationships between the effect of temperature t in °C and the maximum growth rate μ_{max} in d^{-1} for nitrifying organisms:

$$\mu_{max} = 0,47 * e^{0,098(t-15)} \quad (3.23)$$

Reference: EPA (1975); Nitrosomonas in river water and pure culture.

$$\mu_{max}=0,18*e^{0,116(t-15)} \qquad (3.24)$$

Reference: EPA (1975); Nitrosomonas in activated sludge.

$$\frac{1}{\mu_{max}} = 6,5*0,914^{(t-20)} \qquad (3.25)$$

Reference: Faup, G.M *et al.* (1982); Nitrosomonas in a UFBR (upflow fluid bed reactor). Temperature, t, between 9 °C and 20 °C.

$$\mu_{max} = 0,79*e^{0,69(t-15)} \qquad (3.26)$$

Reference: EPA (1975); Nitrobacter in river water.

Table 3.9 Temperature dependence of the maximum growth rates of nitrifiers.

T °C	μ_{max} d^{-1}	K_s (g N / m^3)
5	0.18	0.13
10	0.29	0.23
15	0.47	0.40
20	0.77	0.73
25	1.25	1.30

Source Knowles *et al.* (1965)

The literature shows that the relations obtained between the temperature and $K_{s,n}$ and the temperature and μ_{max} are dependent upon the environment and test circumstances.

Somewhat differing temperature effects have been found for attached growth systems and suspended growth systems.

Comparing the suspended-growth and attached-growth nitrification data, one can conclude that attached-growth systems have an advantage in withstanding low temperatures (below 15°C) without significant reduction in nitrification rates. Measurements of nitrification rates for suspended-growth systems, however, are not normally made on the same basis as those made on attached-growth systems. In suspended-growth systems, rates are expressed on a per-unit-of-biomass basis (MLVSS is used). Precise measurements of biomass are normally not possible in attached-growth systems so other parameters are used, such as reaction rate per unit surface or volume.

Attached-growth systems can also compensate for colder temperature conditions by the biofilm growth growing thicker. If rates could be expressed on a unit biomass basis for both system types, the variation in reaction rates with temperature might thus be more similar.

Shammas (1986) showed that the effect of temperature on nitrogen kinetics fitted the popular modified Arrhenius relationship.

$$K_{s,n} = K_{20} * e^{b*(t-20)} \qquad (3.27)$$

where

$K_{s,n}$ = maximum growth rate at temperature t (d^{-1}).

K_{20} = maximum rate constant at 20 °C

b = temperature coefficient

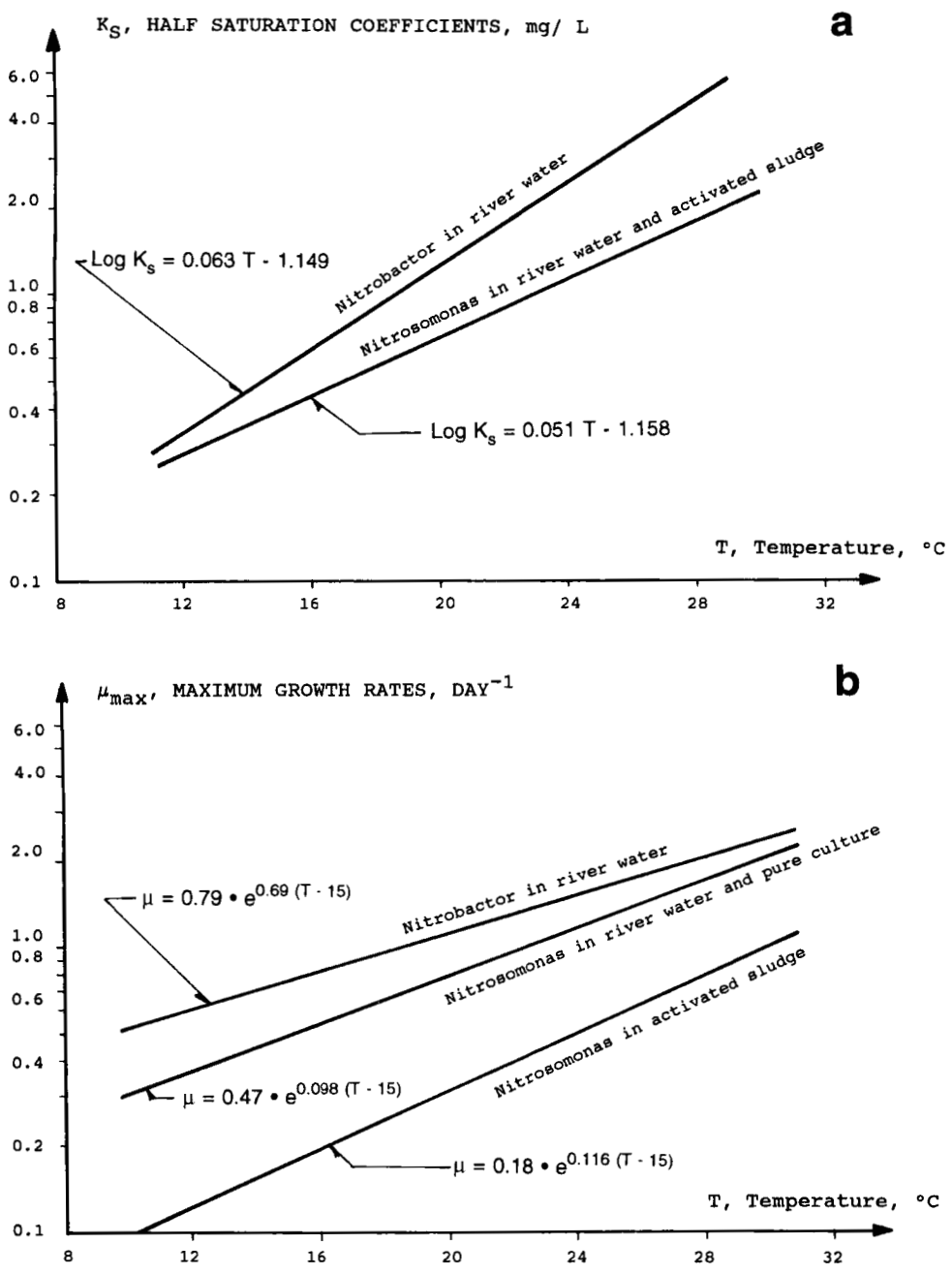

Figure 3.4 The influence of temperature on a) $K_{s,n}$ and b) μ_{max} for the nitrification process, (EPA 1975).

Shammas (1986) also showed that b varies with the bacterial concentration calculated as MLVSS. Different values of b is shown in Table 3.10.

$$b = 0,00044 * X^{0,69} \qquad (3.28)$$

where

X = MLVSS concentration in mg/l.
and b is constant with respect to pH.

The same authors reported that values of the nitrification rate constant $K_{s,n}$ ranged from 0,0085 d^{-1} at 4 °C and pH = 7 to 0,175 d^{-1} at 33 °C.

The temperature relationship to maximum specific growth by an exponential expression has been described by several authors (Zanoni 1969; Andersen and Poulsen (1976); Jenkins (1969) and McHarness *et al.* (1975)):

$$\mu = \mu_{m,ref} * A^{(t-t_{ref})} \qquad (3.29)$$

where:

μ_m and $\mu_{m,ref}$ are the maximum specific constants at temperature t and t_{ref} (0°C) respectively, and A is a constant for a specific temperature range referred to as the "temperature coefficient".

All studies mentioned in Table 3.11 were conducted under steady-state conditions, obtained with long-term temperature conditions.

Only very few studies were conducted with rapid temperature changes, and then only under marine conditions.

Table 3.10 Values of b with comparable values from different literature sources. The highest coefficient for b for ammonia oxidation in an activated sludge medium was reported by Downing *et al.* (1968).

Temperature coefficient b	***Condition***	***Reference***
	Activated sludge	
	ammonia to nitrate pH 7,0 to 8,3	
0,028	MLVSS = 430 mg/l t = 4 °C to 33 °C	Shammas *et al.* (1986)
0,059	MLVSS = 1200 mg/l t = 4 °C to 25 °C	"
0,121	MLVSS = 3200 mg/l t = 4 °C to 25 °C	"
	Pure culture	
0,073	Ammonia to nitrite	Buswell *et al.* (1954)
	Thames estuary water	
0,095	Ammonia to nitrite	Knowles *et al.* (1965)
0,059	Nitrite to nitrate	"
	River water	
0,084	Ammonia to nitrite	Stratton *et al.* (1967)
0,056	Nitrite to nitrate	"
	Activated sludge	
0,120	Ammonia to nitrite	Downing *et al.* (1968)
	Single stage activated sludge	
0,075	Nitrification	Sutton *et al.* (1978)

From Shammas (1986).

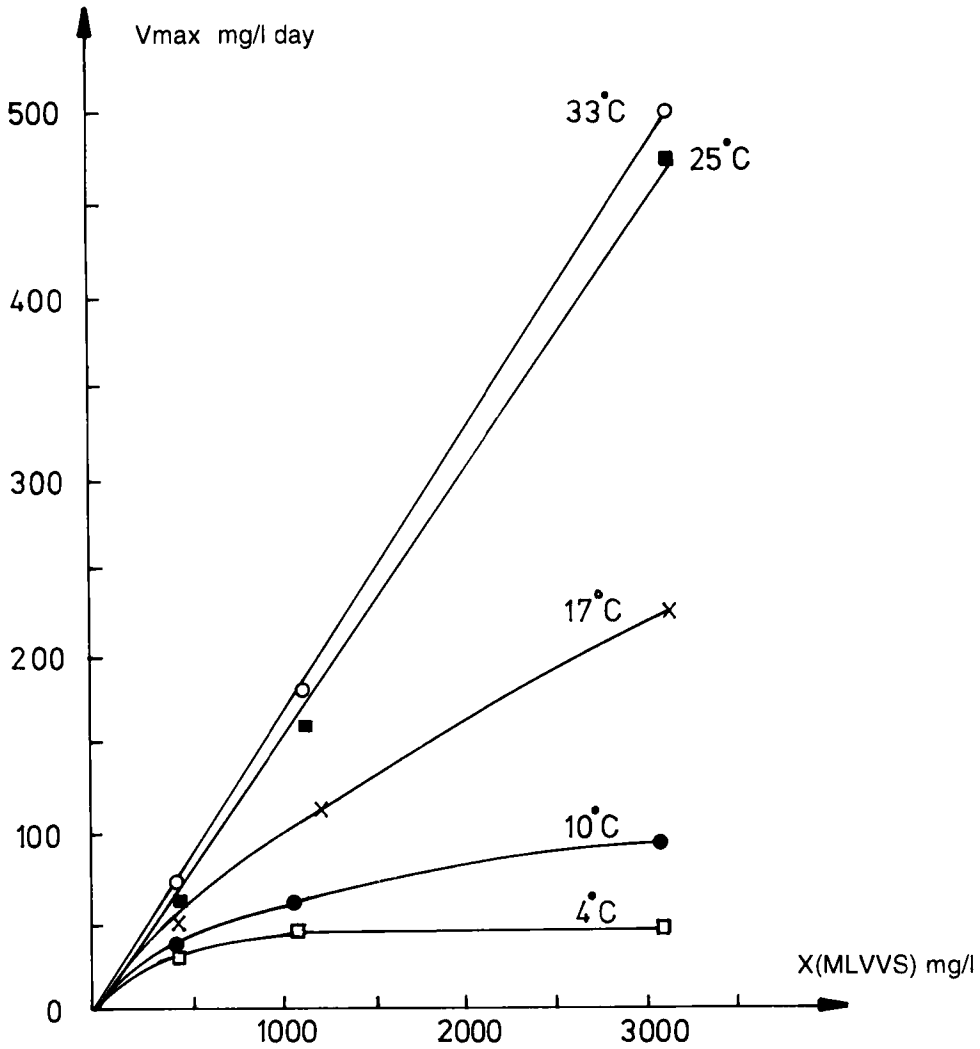

Figure 3.5 Variation of maximum nitrification velocity with MLVSS concentration at different temperatures. (From Shammas 1986).

Table 3.11 Temperature coefficient for nitrifying bacteria.

Process	*Range*	T_{ref}	*A*	μ_m,*ref*	*Reference*
Nitrogenous phase in BOD bottle analysis	10-22° C	20° C	1.097	0,12	Zanoni (1969)
Nitrification in suspended culture	5-20° C	20° C	1,12	-	Andersen and Poulsen (1976)
Nitrifying in treatment process	10-30° C	12° C	1,07	0,5	McCarty (1976)
" "	5-10° C	10° C	1,19	0,25	" "
Nitrosomonas in fill and draw pilot plant activated sludge	8-20° C	-	1,12	1,18	Jenkins (1969)
Nitrosomona in water from Thames estuary	8-30° C	15° C	1,099	0,47	Knowles *et.al.*, from (1965)
Nitrobacter in water from Thames estuary	8-30° C	15° C	1,058	0,79	" "
Nitrosomonas in activated sludge	10-25° C	15° C	1,123	0,18	Downing & Hopwood (1964)
Nitrosomonas in pure culture	10-25° C	15° C	1,103	0,47	" "
Attached separate culture	5-25° C	-	1,08	-	McHarness *et et al.* (1975)

Partly from Ohgaki and Wantawin (1990).

3.9 The Influence of Dissolved Oxygen on the Nitrification Rate

In engineering calculations, an aeration requirement of 4,6 mg O_2 per mg NH_4^+ -N is just sufficient to be used for the nitrification process.

In almost all treatment systems, oxygen is also required to oxidize other materials than ammonia present in the waste water. This, therefore, often raises the total oxygen demand in a nitrifying plant.

Results from a number of studies on the effect of dissolved oxygen concentrations on the nitrification efficiency are summarized in Table 3.12. Most studies were conducted on suspended-growth systems. In the case of attached growth systems, the oxygen availability to the nitrifying biofilm can be affected by many parameters.

The concentration of dissolved oxygen (DO) has a significant effect on the rates of nitrifier growth and nitrification in biological waste treatment systems. The Monod relationship has been used to model the effect of dissolved oxygen, considering oxygen to be a growth limiting substrate, as follows:

$$\mu = \mu_{max} * \frac{DO}{K O_2, n + DO} \qquad (3.30)$$

where: DO = dissolved oxygen, mg/l and

$K O_2,n$ = half-saturation constant for oxygen, mg/l, in the nitrification process.

While the general effect of DO on kinetics is firmly established, further study is needed to determine the factors affecting the value of $K O_2,n$. All of the various estimates are from systems where combined carbon oxidation-nitrification is practiced, and no measurements have been made on separate stage nitrification systems. $K O_2,n$ values for separate stage nitrification systems may very well be different from those for combined carbon oxidation-nitrification systems. Most often the operating DO is 2.0 mg/l or less, in studies (see Table 3.12), therefore a value of $K O_2,n$ of approximately 1,3 mg/l, will give a nitrification (or nitrifier) growth rate (equation 3.30) of about 60 % of the peak rate, following Downing *et al.* (1978).

Table 3.12 The influence of dissolved oxygen on the nitrification process.

Dissolved oxygen concentration mg/l	*Observation*	*Circumstance/Method of observation*	*Reference*
0,5		Dropping-mercury method used to measure oxygen uptake	Painter and Jones (1963)
Below 2	Limiting for Nitrosomonas growth (*)	10-1 batches; water from Thames; determination made from a model	Knowles, Downing & Barrett (1965)
Below 4	Limiting for Nitrobacter growth		
2,4,8	Degree of nitrate about 10% lower at 2 mg/l	Small-scale plant	British Ministry of Technology (1965)
Below 3	Limiting	Activated sludge	Downing & Knowles (1966)
0,08	Critical	Pure culture of *Nitrosocystis oceanus*	Gunderson (1966)
> 7,5	Inhibiting		
Below 1-1,5	Limiting for growth	Activated sludge	Wuhrmann (1964)
> 0,1	Nitrification	Pure culture of *Nitrosocystis oceanus*	Carlucci & McNally (1969)
0,5-0,7	Critical (**)	Activated sludge	Downing and Knowles (1966)
Saturation	Limiting	Batch tests with activated sludge	Kiff (1972)
1	Limiting	Pilot plant; activated sludge	Metcalf & Eddy (1973)
0,6-0,7	Limiting	Percolating filter receiving sea water marine nitrifiers	Forster (1974)
Up to 60	No inhibition no increase in rate of ammonia oxidation	Submerged filter receiving pre-oxygenated waste water	Haug & McCarty (1972)

(*) Rate of nitrification is the concentration below this value.
(**) Minimum concentration necessary for nitrification to occur.

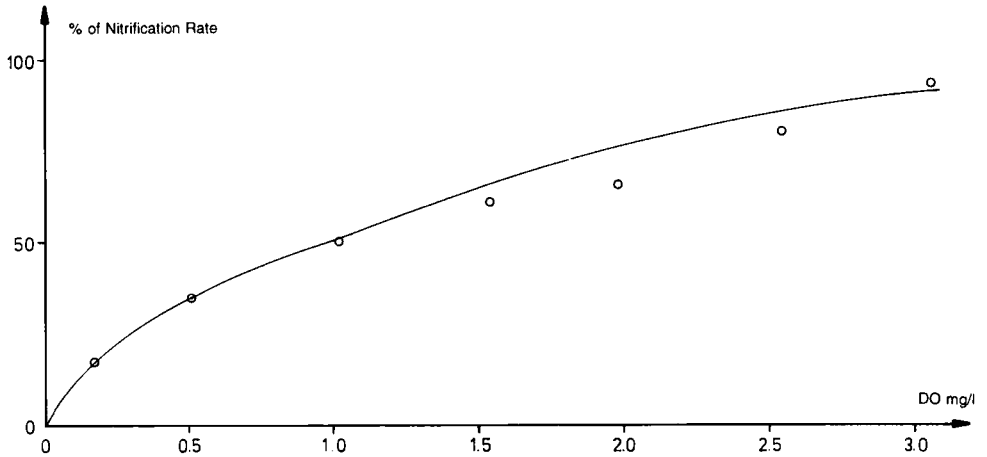

Figure 3.6 The influence of dissolved oxygen on the nitrification rate.

Most mathematical models for biological growth take into account only one substrate, such as the Monod model, since experimental studies are usually performed with all other nutrients in excess. But Stenstrøm and Poduska (1980) used a double substrate-limiting kinetic expression to describe the combined effect of dissolved oxygen and ammonia-nitrogen on the growth rate, as shown in the following equation. The equation is a modified form of the Monod single substrate model.

$$\mu = \mu_{max} \left(\frac{S_N}{K_{S,N}+S_N} \right) * \left(\frac{DO}{K O_{2,n}+DO} \right) - K_d \qquad (3.31)$$

where

μ = Specific growth rate (d^{-1})

μ_{max} = Maximum specific growth rate (d^{-1})

S_N = Ammonia concentration

DO = Dissolved oxygen concentration

$K_{S,N}$ = Half saturation constant for ammonia nitrogen

$K_{O,2}$ = Half saturation constant for dissolved oxygen

K_d = decay or maintenance coefficient (d^{-1})

The double substrate-limiting kinetics is interesting, because substrate diffusion through biofilms will result in the limitation of either the electron donors or the electron acceptors in the biochemical reaction.

Typical values of the half saturation constant $K_{O,2}$ are shown in Table 3.7
It would appear, looking at Table 3.7 that the activity of Nitrobacter is suppressed under low dissolved oxygen concentrations more than that of Nitrosomonas.
Painter (1977) noted that the presence of organic matter can directly inhibit nitrifiers by virtue of heterotrophs oxidizing the compounds and successfully competing for the available dissolved oxygen, if this is kept at a fairly low concentration, as the $K_{S,O}$ for heterotrophs is generally lower than that for nitrifiers.

3.10 The Influence of pH on the Nitrification Rate

In the literature, the optimum pH value for the nitrification process varies between 8 and 9. Figure 3.7 summarizes investigations of pH effects on the nitrification rate. Usually the nitrification rate decreases, as the pH decreases. By measuring the nitrification rates Meyerhof (1916) found the pH optimum for Nitrosomonas to be between 8,5 and 8,8, and for Nitrobacter to be 8,3 to 9,3.

Hofman *et al.* (1973) made similar investigations, and found for both organisms an optimum pH of 8.3, and that the nitrification rate fell almost to zero at pH 9,6. They also found that nitrification proceeded with considerable speed until the pH was as low as 6,5. Hofman *et al.* (1973) further reported that the optimum pH for nitrite oxidation by Nitrobacter was 7,7 and not 8,8 as found by Mayerhof (1915). Wild *et al.* (1964) suggested the optimum pH for nitrification to be 8,4 and that 90 per cent of the maximum nitrification rate occurs between pH 7,8 and 8,9. Less than 50 per cent of the optimum rate occurs outside the range of pH 7,0 to 9,8. Painter (1972) reported that the point at which the rate of nitrification decreased was between pH 6,3 and 6,7, and that between pH 5 and 5,5, nitrification ceased.

Anthonisen (1974) suggested the following mechanism by which pH affects the rate of nitrification. His hypothesis is based on the fact that the ammonia/ammonium and nitrite/nitrous acid equilibria depend on pH. Both "free ammonia" NH_3 and "free nitrous acid" HNO_2 inhibit the nitrifying organisms. When the intracellular pH of a nitrifying organism is lower than the pH of the extracellular environment, free ammonia will penetrate the cell membrane, and inhibit the bacteria.

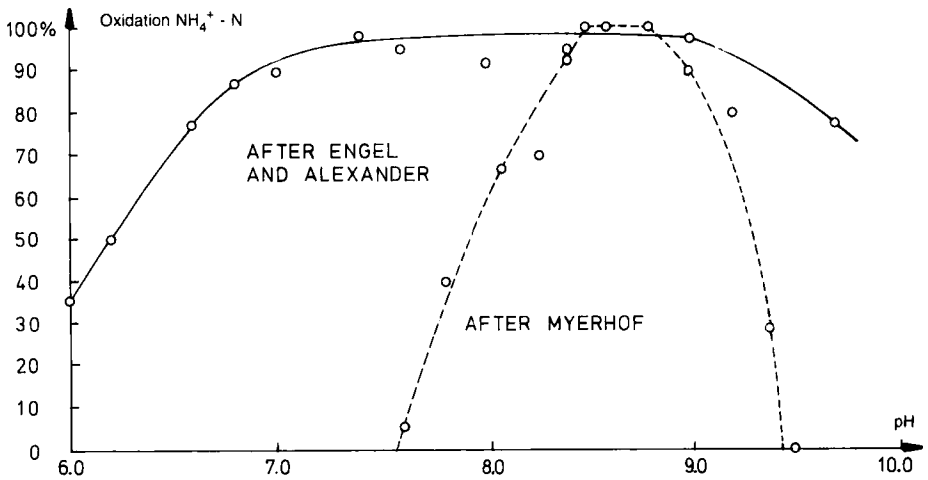

Figure 3.7 The influence of pH on the nitrification process.

Ionized ammonia NH_4^+, on the other hand, will remain in the extracellular environment. Similarly, when intracellular pH is higher than that of the extracellular environment, free nitrous acid penetrates the cell, not the nitrite ions. Anthonisen proposed, therefore, that the ability of ammonia and nitrous acid to penetrate the nitrifying organisms was one of the reasons why the nitrification process is less affected at pH values between 8 and 9.

Equation (3.3) shows that H^+ is produced by the oxidation of ammonia and carbon dioxide. When the biomass synthesis is neglected, it can be calculated that 7,14 mg of alkalinity, as $CaCO_3$, is destroyed per mg of ammonia nitrogen oxidized. Experimentally determined ratios are presented in Table 3.13. A ratio of 7,1 mg alkalinity (as $CaCO_3$) destroyed per mg of ammonia nitrogen oxidized may be used theoretically in plant design.

As the nitrification process reduces the HCO_3^- level and increases the H_2CO_3 level, it is obvious that the pH would tend to be decreased. This effect is mediated by stripping of carbon dioxide from the liquid by aeration, and the pH is therefore often raised. If the carbon dioxide is not stripped from the liquid, the pH may be depressed to as low as 6,0. Haug *et al.* (1974) calculated that to maintain the pH greater than 6,0 the alkalinity of the waste water must be 10 times higher than the amount of ammonium nitrified.

It is important to distinguish between long-term and short-term pH effects on the environment where the nitrification process is to occur.

There is a great difference in the effects that can be observed in the nitrification process, if pH varies over short (hours, days) or long periods (months, years). Most investigations referred to in this text have been on a short-term basis. Investigations of long-term effects have not been described in the literature.

Table 3.13. Alkalinity destruction ratios in experimental studies

System	***X mg alkalinity destroyed mg NH_4^+ -N oxidized***	***Reference***
Suspended growth	6,4	Mulbager *et al.* (1971)
Suspended growth	6,1	Horstkotte *et al.*(1973)
Suspended growth	7,1	Newton *et al.* (1973)
Attached growth	6,5	Gasser *et al.* (1974)
Attched growth	6,3 to 7,4	Osborn *et al.* (1965)
Attached growth	7,3	Haug *et al.* (1972)

as $CaCO_3$, the theoretical value is 7.1
From EPA (1975).

The hydrogen ion concentration (pH) has been found to have a strong effect on the rate of nitrification. There is a wide range in reported pH optima; the almost universal finding is that, as the pH moves into the acid range, the rate of ammonia oxidation declines. This has been found to be true for both unacclimatized and acclimatized cultures, although acclimation tends to moderate pH effects.

Downing *et al.* (1966) showed that the effect of pH on nitrification for pH values less than 7,2 can be estimated from the following relationship:

$$\mu = \mu_{max} \ (1 - 0{,}833 \ (7{,}2 - pH)) \qquad (3.32)$$

This expression was developed for combined carbon oxidation-nitrification systems, but its application to separate stage nitrification systems would appear useful. For pH levels between 7,2 and 8,0 the rate is assumed constant.

Table 3.14 Effect of pH on the nitrification.

pH	*Degree of inhibition %*	*Circumstances of observation*	*Reference*
8,5 - 8,8 7,9 9,3	0 50 50	Pure culture of Nitrosomonas	Mayerhof (1917)
6,7 - 8,0 5,5 9,2	0 100 100	Pure culture, test-tube scale	Barritt (1933)
8,0 - 8,5	0	Pure culture of Nitrosomonas	Buswell *et al.* (1954)
8,3 - 8,6	0	Pure culture of Nitrosomonas	Lees (1954)
7,2 - 8,2	0	Pure culture of Nitrobacter	Lees (1954)
7,2 - 8,2 6,2	0 50	Pure culture of Nitrosomonas	Engel & Alexander (1958)
9,6	50	Batch Culture	Engel & Alexander (1958)
7,3 - 8,4	0	Pure culture of Nitrobacter	Boon & Landelout (1962)
7,5 - 8,0	0	Pure culture of Nitrosomonas isolated from activated sludge.	Loveless & Painter (1968)
7,0 - 8,0 6,1 5,5 - 6,0	0 50 (*)	Submerged filter, mixed but predominantly nitrifying bacteria.	Haug & McCarty (1972)
4,9 - 7,2	(+)	Mixed culture; lab. scale	Praksam & Loehr (1972)
8,4 - 8,5	0	Two-stage, activated sludge pilot plant.	Rimer & Woodward (1972)

Table 3.14 (continued)

8,0 - 8.8	0	Batch activated	Medcalf & Eddy (1973)
7,1	50	sludge; lab. study	
9.8	50		
8,0	0	Percolating filter	Forster (1974)
5,9	50	lab. scale mixed population.	
7,45	0	Marine nitrifying filter system; batch studies	Sma & Baggaley (1975)
7,8	0	Simultaneous nitrification and denitrification attached growth UFBR.	Halling-Sørensen & Hjuler (1992)

(*):Adaptation in 10 days, the rate of ammonia oxidation becomes the same as that at pH 7-8,5.
(+): pH not controlled, nitrification occured at pH 4.9; no improvement between pH 5 and 11.

Because of the effect of pH on the nitrification rate (see Fig. 3.8), it is especially important that there be sufficient alkalinity in the waste water to balance the acid produced by nitrification. Addition of alkalinity to the waste water may be necessary.

Boon and Laudelout (1962) developed a kinetic expression for the effect of pH on the nitrite oxidation by *Nitrobacter winogradskyi*. They suggested that inhibition of high nitrite concentration results from non-competitive inhibition of nitrous acid, while at pH over 7 there is a competitive inhibition of the adsorption of nitrite on the enzyme sites by OH^- -ions.

The rate equations for pH below 7 and pH above 7 are shown separately in equations (3.33) and (3.34) respectively.

$$\mu = \frac{\mu m * S}{(Ks+S)\,(1+(S[H+]/Ka*Ki))} \tag{3.33}$$

$$\mu = \frac{\mu m * S}{Ks(1+(Kb/[H+]))+S} \tag{3.34}$$

where:

S = nitrite concentration.
K_a = equilibrium constant of nitrous acid and nitrite ion dissociation.
K_i = dissociation constant of the enzyme-nitrous acid complex.
K_b = basic acid-base dissociation constant of the active enzyme site.

The total rate equation for pH effects was thus determined by combining equations (3.33) and (3.34) as in equation (3.35).

$$\mu = \frac{\mu m * S}{(Ks(1+(Kb/[H+]))+S)(1+(S[H+]/Ka*Ki))} \qquad (3.35)$$

Results showed that K_b and K_i were 0,004 and 8,2 μM of NO_2^-, respectively.

Suzuki *et al.* (1974), using the Lineweaver-Burke plot, in the study of the pH effect on the oxidation of ammonia by *Nitrosomonas europaea*, found that the value of the Monod saturation ammonia constant decreased when pH increased. This means that having pH as the parameter, the plot shows competitive inhibition.

As Nitrosomonas and Nitrobacter are both sensitive to their own substrates of unionized ammonia and nitrite, and the unionized-ionized nitrogen equilibria depend on pH, it follows that the pH value is an important factor.

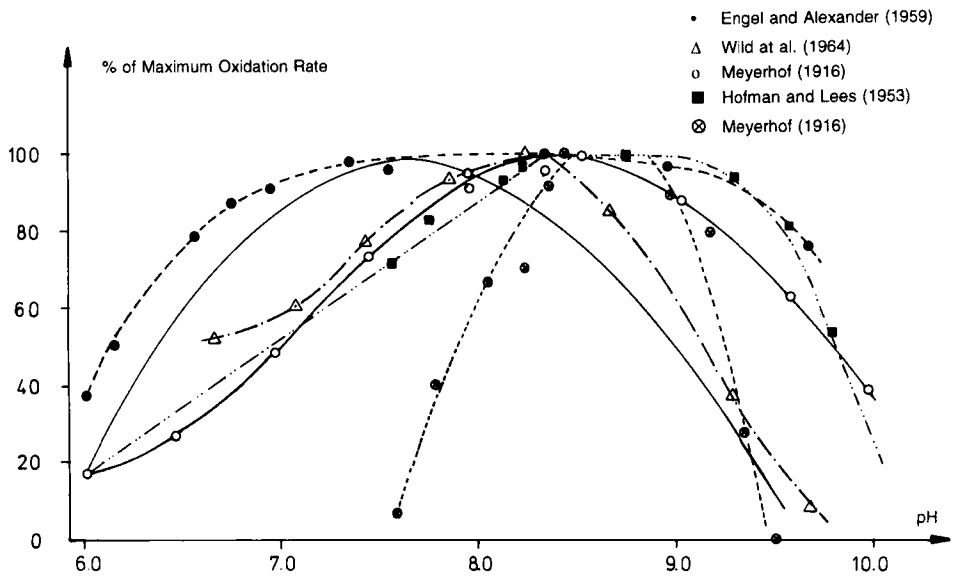

Figure 3.8 The influence of pH on the nitrification rate. A summary of different results found in the literature. Source: Shammas (1986).

3.11 A Kinetic Expression Combining Several Limiting Factors of the Nitrification Process

In previous sections, the effects of ammonia level, temperature, pH, and dissolved oxygen on the nitrification rate have been presented. In all practical systems, these parameters influence the nitrification rate simultaneously. Chen (1970) showed

that the combined effect of several limiting factors on biological growth can be introduced as a product of a Monod-type expression.

Taking this approach for nitrification, the combined kinetic expression for nitrifier growth would take the following form (EPA 1975):

$$\mu = \mu_{max} * \frac{S}{(K_s+S)} * \frac{DO}{(Ko_2+DO)} * (1-0,833\,(7,2-pH))$$

(3.36)

where: μ = maximum nitrifier growth rate at temperature T and pH less than 7,2.

Using specific values for temperature, pH, ammonia and oxygen, from Tables shown in the EPA (1975), the following expression results for pH less than 7,2 for Nitrosomonas and is valid for temperatures between 8 °C and 30 °C:

$$\mu = 0,47 * (e^{0,098*(t-15)}) * (1-0,833\,(7,2-pH)) * \frac{Sn}{10^{0,051*t^{-1,1}}} * \frac{DO}{DO+1,3}$$

(3.37)

In equation (3.36) the first term in brackets allows for the effect of temperature. The second term in brackets considers the effect of pH. For pH less than 7,2 the second quantity in brackets is taken to be unity. The third term in brackets is the Monod expression for the effect of the ammonia nitrogen concentration. Similarly, the fourth term in brackets accounts for the effect of DO on the nitrification rate.

Equation (3.37) has been adopted for illustrative use. When other reliable data become available, equation (3.37) can be modified to suit particular circumstances.

If the ammonia removal rate is defined as in equation (3.36), then equation (3.38) can be written as follows:

$$\frac{dS_n}{dt} = -\frac{\mu_{max}}{Y_n} * X_n * \frac{S_n}{K_{s,n}+S_n} * \frac{DO}{Ko_2+DO} * (1-0,833\,(7,2-pH))$$

(3.38)

The biggest problem in the analysis of rate data for microbial nitrifying bacteria, with or without heterotrophic bacteria, is the estimation of nitrifier concentration for determination of the specific growth rate μ, the yield coefficient Y_n and the saturation constant $K_{s,n}$.

3.12 Bacterial Population Dynamics Applied in the Nitrification Process

The kinetics of the growth of nitrifiers have been discussed in the previous sections. In all practical applications in waste water treatment, nitrifier growth takes place in waste treatment processes, where other types of biological growth occur. In no case are there opportunities for pure cultures to develop.

This fact has significant implications in process design for nitrification.

In combined carbon oxidation-nitrification systems as well as in separate stage nitrification systems, there is sufficient organic matter in the waste water to enable the growth of heterotrophic bacteria. In this situation, the yield of heterotrophic bacteria growth is greater than the yield of the autotrophic nitrifying bacteria. Because of this dominance of the culture, there is the danger that the growth rate of the heterotrophic organisms will be established at a value exceeding the maximum possible growth rate of the nitrifying organisms. When this occurs, the slower growing nitrifiers will gradually diminish in proportion to the total population, and be washed out of the system.

Because waste water is a mixed culture system, a knowledge of the mutual relationship between nitrifying and heterotrophic bacteria is very important in the construction of nitrifying waste water plants.

Painter (1977) showed that the maximum specific growth of nitrifying bacteria, determined in the treatment process, is significantly different from that observed in a pure culture.

The reasons for this difference may be explained as follows:

1) Domination of heterotrophic bacteria which suppress nitrifying growth, because growth conditions, i.e the COD/N ratio, in the treatment plant enable the growth of heterotrophic bacteria prior to nitrifying bacteria.

2) Because the half saturation constant $K_{S,O}$ for heterotrophs is generally lower than that for nitrifiers, heterotrophs will generally compete with the nitrifiers for the available dissolved oxygen.

3) The toxic constituents of waste water may inhibit nitrification.

4) Fluctuation or limitation of nutrients.

5) A genuine difference between isolated strains and those effecting nitrification in the treatment process.

Especially 1) is an important factor in the construction of nitrifying waste water systems. Stover *et al.* (1976) have presented experimental results showing the effects of different COD/N ratios on nitrification, in both the activated sludge process and in the UFBR, system in both cases applied using non-toxic synthetic media.

The competition for nitrogen by heterotrophs, or inhibition, interferes with the removal of ammonia and reduces the production of nitrate under the conditions of a high COD/N loading. Applying a high COD/N loading also favours the development of a heterotrophic bacteria population and producing a lower nitrifying population.

Christensen and Harremoes (1978) have explanied how it is to be expected that nitrification in the attached growth treatment process, under a high organic carbon loading will not occur in the upper part of the trickling filter, nor on the first disks of a rotating disk unit.

It may be assumed that in the upper layer, the nitrifying population will lose in the competition with the heterotrophic bacteria, and carbonaceous matter only will be removed. In the lower part of the trickling filter and at the last disk unit, the ammonium-N loading is now high, compared with the organic loading, and, therefore the heterotrophic bacteria will be suppressed by the nitrifying bacteria. Nitrification will consequently occur there.

A few models have been developed involving the competition between heterotrophic and nitrifying bacteria (Harremoes,1982; Wanner and Gujer 1984). All of these models, developed recently, have predicted that the fraction of nitrifiers in relation to the heterotrophic population is greater in the inner layer (near the surface of the media) than in the outer layer of biofilm.

There are many types of competition between two or more microbial populations. Competition occurs when the component populations are restricted in either their growth rates or their final population sizes, as a result of a common dependence on an external factor.

Competition can occur in either a closed culture, where growth is ultimately limited by the availability of a particular growth resource, or in an open culture (as a waste water plant), where growth is continuously limited. In open culture systems, as in a waste water plant, it is inevitable that those populations which are the least competitive, are eliminated from the growth environment. In this case the saturation constant $K_{s,n}$, usually becomes the most important factor determining the outcome of competitive growth.

Figure 3.9 shows different systems with competition between organisms A and B. Organism B is initially a minor population compared to A.
The dilution rate of organisms, D is used to predict the washout of organisms from a system plant.

Theoretically, if the growth rate $\mu > D$, then ds/dt (the substrate removal per unit of time) is negative and the growth limiting substrate concentration decreases. The biomass concentration is increasing under this condition.

If the growth rate $\mu < D$, then ds/dt is positive and the growth-limiting substrate concentration increases, and the biomass concentration decreases.

Finally, if $\mu = D$, then $ds/dt = 0$, and the growth limiting substrate concentration reaches a constant, steady-state value at the same time as the biomass concentration.

There are two basic cases to consider in assessing whether or not the growth of population B is more or less competitive than that of the established population A, where neither of the two organisms are limited by the substrate.

For the new population B to succeed in becomming greater than population A, dX_B/dt from the Monod equation (3.11) has to be positive. This can be achieved, if $\mu_B > D$, and pertains if either $\mu_{max,B}$ (the maximum growth rate for organism B) > $\mu_{max,A}$ (Fig. 3.9a) or $K_{s,B} < K_{s,A}$ (Fig. 3.9b). It must be noted, however, that it is the combined effect of these which is important, in determining whether or not organism B is more competitive than organism A. Fig. 3.9c illustrates the situation in which $\mu_{max,B} > \mu_{max,A}$, but $K_{s,B} > K_{s,A}$. For this pair of organisms, at any growth-limiting concentration, organism B is the more competitive, sustaining a higher growth rate than organism A at all substrate concentrations.

Initially, the growth rate of organism B is determined by the steady-state conditions established by organism A ; that is at a dilution rate D, the growth limiting substrate concentration s_A. Gradually, as the proportion of the two populations begins to change in favour of population B, s begins to decrease and tend towards s_A (see

Fig. 3.9a and 3.9b) which is the growth-limiting substrate concentration, which supports a growth rate of $\mu_B = D$. At this substrate concentration dS_A/dt must be negative, and accordingly population A is unable to grow at the imposed dilution rate and must continue to be washed out of the culture vessel.

The opposite situation is that population B does not replace population A, if $\mu_B < D$ and so dX_B/dt is negative, a situation which results if either $\mu_{max,B} < \mu_{max,A}$ (Fig. 3.9d) or $K_{s,B} > K_{s,A}$ (Fig. 3.9e).

Table 3.15 Comparison of parameters of heterotrophs and autotrophs (nitrifier) determining bacterial population dynamics (Fruhen *et al.* 1991).

Parameter	Symbol	Value
Heterotrophic bacteria maximum growth rate, d^{-1}	$\mu_{H,max}$	4,0
Heterotrophic bacteria decay coefficient, d^{-1}	b_H	0,15
Heterotrophic yield coefficient, g/g^{-1}	Y_H	0,57
Autotrophic bacteria maximum growth rate, d^{-1}	$\mu_{N,max}$	0,83
Autotrophic bacteria decay coefficient, d^{-1}	b_N	0,05
Autotrophic yield coefficient, g/g^{-1}	Y_N	0,24

The parameters presented in Table 3.15 show that both μ_{max} and K_S for the heterotrophic population favour heterotrophic growth. Supplying a treatment plant with both heterotrophs and nitrifier (autotrophic bacteria), it is therefore important to stock the plant with a high nitrifying biomass X_n, so the nitrifying population initially dominates the plant. A combination of high nitrifier and a limitation of heterotrophic substrate may be necessary.

To establish condition for a consistent nitrification it is therefore important that the specific nitrifier growth μ_n is higher than the maximum heterotrophic growth μ_h, assuming pH and DO do not limit the growth of the nitrifier.
This can be expressed in the following terms:

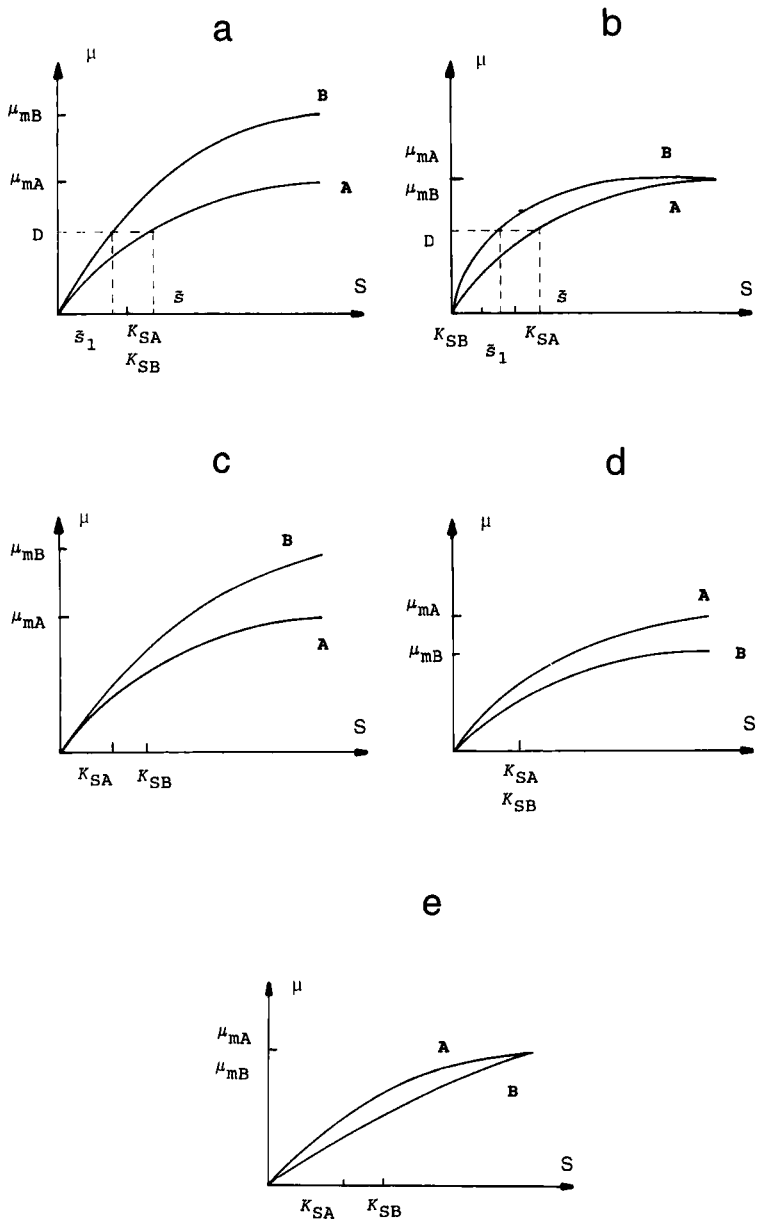

Fig 3.9 The various possible Monod relationships between two organisms, A and B, used to predict the outcome of free competition between them under conditions of growth limited by the substrate. After Slater and Bull (1978).

$$\mu_n > \mu_h \tag{3.39}$$

where: μ_n = maximum growth rate of the nitrifying population.

μ_h = growth rate of the herterotrophic population.

Reduced DO or pH can act to depress the growth rate of the peak nitrifier $\mu_{max,n}$ and cause a wash out situation. A new growth rate μ_{obs} will then be the peak nitrifier growth rate. The Monod Equation for this special condition is presented in EPA 1975:

$$\mu_{obs} = \mu_{max,n} * \frac{DO}{K_{s,o} + DO} * (1 - 0,833(7,2 - pH)) \tag{3.40}$$

where: μ_{obs} = maximum possible nitrifier growth rate under environmental conditions of T, pH, DO and S>> K_s.

To "correct" the calculations for the competition between the nitrifier and the heterotrophic bacteria in the application of biological treatment, Lawrence and McCarty (1968) introduced the concept of a safety factor (SF). A conservative safety factor is recommended to minimize process variation caused by pH extremes, low DO, fluctuation of substrate, and toxicants.

The growth rate can be expressed in reciprocal form in terms of a solid retention time.

$$\mu = \frac{1}{\phi_c} \tag{3.41}$$

where ϕ_c = solids retention time in days.

$$\phi_c = \frac{1}{\mu} = \left[\frac{DOUBLINGTIME}{\ln 2}\right] \tag{3.42}$$

Equation (3.42) is useful from the standpoint of process design.

The safety factor was defined as the ratio of the minimum retention time for solids. The safety factor can also be related to the nitrifier growth rate.

$$SF = \frac{\phi_c}{\phi_{obs}} = \frac{\mu_{obs}}{\mu} \tag{3.43}$$

where ϕ_{obs} = the minimum retention time for solids in days for nitrification at a given pH, T and DO.

EPA 1975 proposes that the safety factor should equal or exceed the ratio of peak load expected in the suspended growth nitrification system.

Today the safety factor approach is rarely used in the literature, but it is absolutely necessary to use some form of safety factor in designing biological nitrification plants, because the knowledge of the risk of introducing more species of bacteria into the same system is still very limited.

Today, therefore, too many treatment plants still show too many differences in their efficiency of nitrogen removal.

3.13 Effect of Inhibitors on Nitrification

Nitrifiers are slow-growing organisms and they are accordingly particularly susceptible to toxicants. Certain heavy metals and organic compounds are toxic to nitrifiers. The presence of toxic compounds causes a change in the environmental conditions for the nitrifying population, and they are therefore, a threat to any nitrification plant.

Tomlinson *et al.* (1966), however showed that nitrifiers are capable of adapting to almost any toxic substances, when the toxic compound is consistently present at a concentration higher than the concentration of the toxic compound that would cause sludge discharge of the plant. Most toxic compounds in municipal systems stem from industrial dumps or urban storm water inflow.

The possibility of a toxic inhibition must be recognized in every design of nitrification systems. Either implementation of source control programs or inclusion of toxicity removal processes upstream may be required, particularly in cases where significant industrial discharges are tributary to the collection system.

It is therefore important to understand the difference between long-term and short-term toxic inhibition. Figure 3.10 shows the difference in nitrification efficiency, applying a long-term or a short-term inhibition with a toxic substance. This difference is brought about because nitrifying bacteria are capable of developing adaptation to most toxic compunds especially during a long-term contact.

Any inhibition of the nitrification process results in a decrease in the maximum specific reaction rate of the nitrifying organisms. A change in the maximum specific reaction rate can be compensated for by a longer solid retention time in a waste water plant. If we suppose that for a specific plant an SRT (solids retention time) of 8 days were required for an efficient nitrification and carbonaceous removal in a single process; and if, after the plant was built, a new waste flow containing an inhibitory compound were added; and if the maximum specific reaction rate of the nitrifying organisms was reduced by 40%, it would be necessary to increase the SRT to 8 days/0,40 = 12 days. Such a large increase in SRT might not be possible without extensive plant modifications, and when carried out, it might harm the heterotrophic population.

Today, unfortunately only very little is known about the influence of different groups of toxic substances on nitrifiers. Almost nothing is known about the consequen-

ces, when two or more toxic substances are present at the same time. It is, therefore difficult to predict how a toxic compound or a number of toxic compounds will change the biomass concentration in a plant. Investigators should in future study this field carefully, because it would be of benefit to and facilitate the daily maintenance of any type of nitrifying plant.

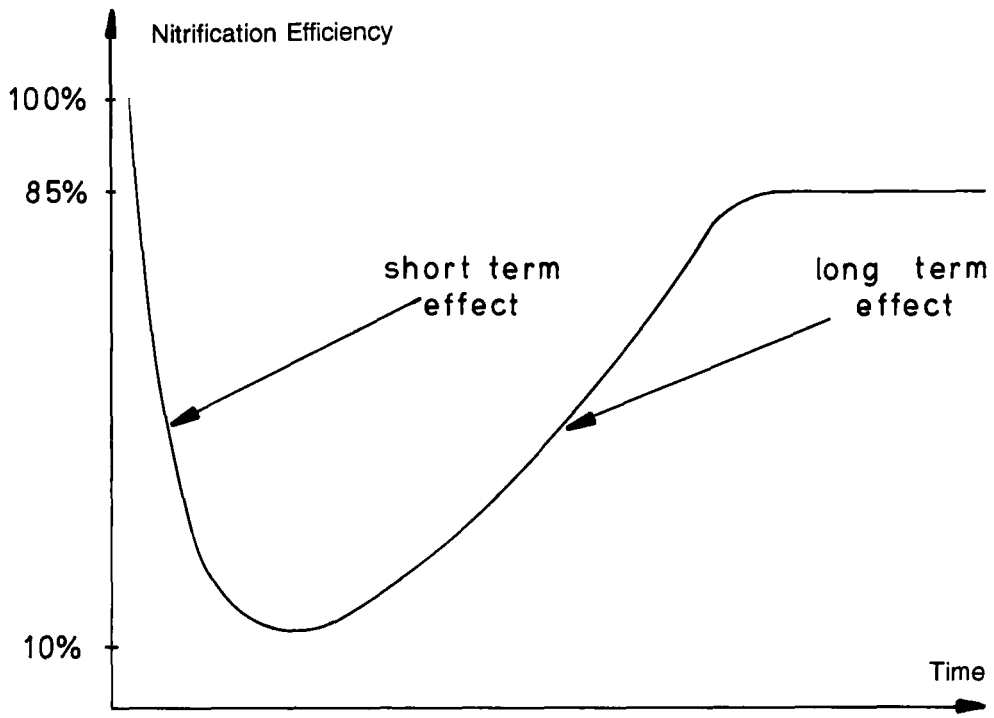

Fig 3.10 Differences in nitrifying efficiency, comparing long- and short-term effects of a toxic substance.

The reduction of maximum specific growth rates which results from the effect of environmental parameters on enzyme reactions can be expressed by different models of enzyme inhibition.

An enzyme inhibitor is a compound which acts to reduce the rate of an enzymatically catalysed reaction by binding with either the free enzyme E and/or with

the enzyme-substrate complex ES as shown in Table 3.16. Types of enzyme inhibition can be classified (following Grady and Lim 1980) into five groups for reversible inhibitors. Reversible inhibitors are inhibitors where the activity of the enzyme returns to normal, when the inhibitor is removed.

1. Competitive inhibition.
An inhibitor which is classed as competitive competes for the same active sites as the substrate.

2. Uncompetitive inhibition.
An uncompetitive inhibitor binds with the enzyme-substrate complex to form an inactive enzyme substrate-inhibitor complex which cannot undergo further reaction to yield the product.

3. Non-competitive inhibition.
A non-competitive inhibitor can combine with both free enzyme and the enzyme substrate complex.

4. Substrate inhibition.
When their concentrations are very high, some substrates will bind with the enzyme substrate complex as well as with the free enzyme.

5. Product inhibition.
The product may bind with the enzyme substrate complex, forming an unreactive enzyme substrate product complex, ESP.

The mechanisms and inhibition-model of these different types are shown in Table 3.16 and Fig. 3.11. The figures show the inhibition models for competitive, uncompetitive and non-competitive inhibition.

Transforming the Michaelis-Menten expressions into one of the linear equations, i.e. Lineweaver-Burke, makes it easier to quantify the various parameters that are affected by the inhibitior. A specific μ_{max} and $K_{s,n}$ can therefore easily be distinguished for each condition and type of inhibitor.

Krittiya (1984) used the Lineweaver-Burke plot to estimate the effect of sodium

ion on the nitrite oxidizing bacteria, as shown in Fig. 3.12. Results showed that the sodium ion inhibiton on the nitrite oxidizing process was categorized as a non-competitive type and the inhibition constant $K_{inhib.}$ was 2,0 g/l as Na^+.

Visut (1985) made similar experiments with sodium inhibition on ammonium oxidizing bacteria and proposed the following expression for the inhibitory effect of sodium ion on oxidizing bacteria:

$$\mu = \frac{\mu_{max} \cdot S}{(K_s + S)\ (1 + I/K_{inhib})} - K_d \qquad (3.44)$$

where

μ = specific growth rate, d^{-1}
S_n = ammonium concentration mg/l as N
I = inhibitor concentration g/l as Na^+
μ_{max} = maximum specific growth rate
$K_{s,n}$ = saturation constant
$K_{inhib.}$ = inhibition constant
K_d = decay rate, h^{-1}

Visut (1985) found the following experimental values:
μ_{max} = 0,0313 h^{-1}, $K_{s,n}$ = 11,6 / 13,5 mg/l as N, $K_{inhib.}$ = 6,64 mg/l as Na^+
and $K_d = 3{,}1 \cdot 10^{-3}$ h^{-1}.

Hockenbury and Grady (1977); Beg *et al.* (1982) ; Akai *et al.* (1983) and Hassan *et al.* (1988) have all used the rate expression for enzyme inhibition in their studies of effects of inhibitors in the nitrification process.

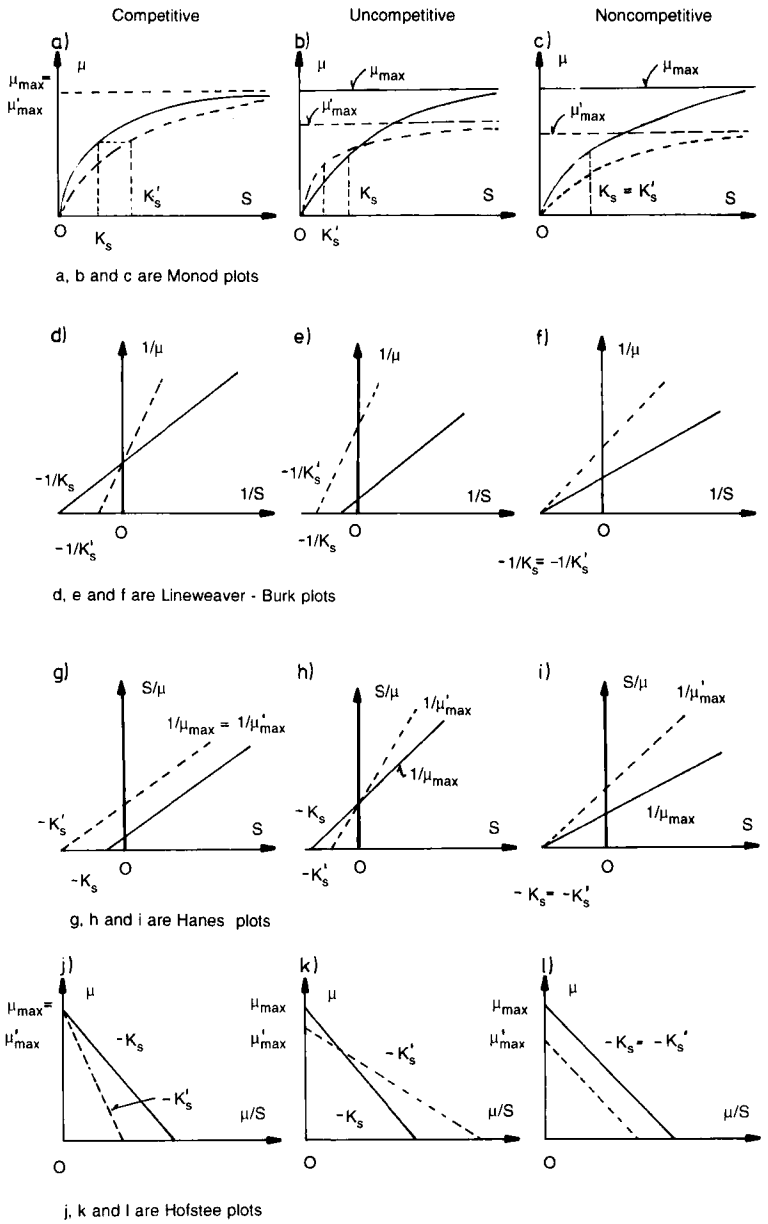

Fig 3.11 Typical plots for identifying the types of enzyme inhibition. The solid cu represent the uninhibited cases, the dashed curves the inhibited cases. (Ohgaki and Wanttawin 1990).

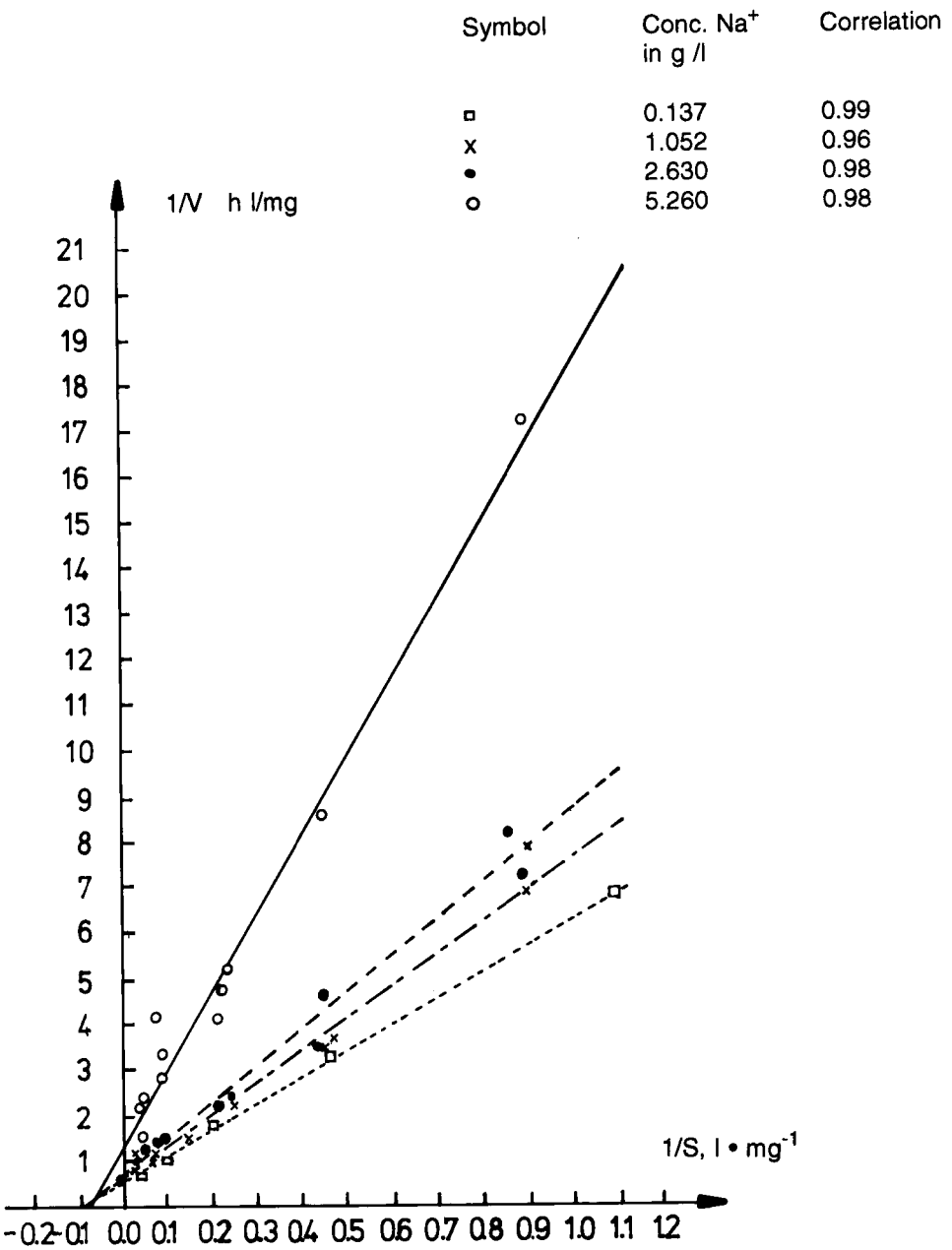

Fig 3.12 The Lineweaver-Burke plot for identifying the type of inhibition of sodium ion concentration for nitrite oxidizing bacteria (Krittiya 1984).

Hassan *et al.* (1988) evaluated the performance of a packed-bed biological reactor in the presence of inhibitors, following either complete or partial modes of competitive, non-competitive, mixed or uncompetitive inhibition. For all types of inhibition, it was found that an increase in the inlet substrate concentration reduces the steady-state conversion in the reactor. The increase in the value of the parameter I/K_i, which indicates the specific action of the inhibitor, increases the conversion for the partially competitive and non-competitive inhibition mode, while it reduces that for product inhibition.

Substances inhibitory to nitrifying bacteria or nitrification.

Some research has been carried out by microbiologists on the effect of specific organic and inorganic compounds on pure cultures of Nitrifiers. Table 3.17 show the results presented by Blum and Speece (1991) for nitrosomonas toxicity due to organic compounds for IC_{50} concentration of less than 20 mg/l.

More compounds have been found to be inhibitory to ammonia oxidation by Nitrosomonas species than to nitrite oxidation by Nitrobacter species. No explanation for this has so far been given in the literature.

Most inhibitory compounds in a waste water treatment plant are present in the range of mg/l and even some in the range of µg/l, and may, therefore, be difficult to detect analytically when they are present in waste water.

Only a few studies have been made on nitrification inhibition in activated sludge; the most complete one was made by Tomlinson *et al.* (1966). Five of the compounds included in the list are among the compounds most used by industry. Two of these, chloroform and phenol, are general inhibitors of bacterial metabolism.

Most of the very potent inhibitors in the nitrification process are sulphur-containing compounds; they can act as metal-chelating compounds, and thus inhibit enzymes requiring metals for activation (Dixon *et al.* 1964; Downing *et al.* 1964).

No reports have been found on inhibition of ammonia oxidation induced by aliphatic or aromatic amines. Hockenbury and Grady (1977) pointed out that the inhibitory effect of nitrogen-containing compounds was caused by competition with ammonia for the active site on an enzyme, although no supporting evidence has been given in the literature. Likewise, compounds, similar in structure to nitrite, have been hypothesized to be inhibitory because of their competitive effects, although only few

Table 3.16 Different types of inhibition models.

Types of inhibition	Mechanism	Rate expression	Michaelis-Menten form
Competitive	$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$ $E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI$	$\mu = \frac{\mu_m S}{(K_s(1+I/k_I)+S)}$ where $K_I = k_3/k_{-3}$	$\mu'_m = \mu_m$ $K'_s = K_s(1+I/K_I)$
Un-competitive	$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$ $E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI$	$\mu = \frac{\mu_m S}{(K_s + S(1+I/k_I))}$ where $K_I = k_3/k_{-3}$	$\mu'_m = \frac{\mu_m}{1 + I/K_I}$ $K'_s = \frac{K_s}{1+I/K_I}$
Non-competitive	$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$ $E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI$ $ES + I \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} ESI$	$\mu = \frac{\mu_m S}{(K_s + S)(1+I/k_I))}$ where $K_I = k_3/k_{-3} + k_4/k_{-4}$	$\mu'_m = \frac{\mu_m}{1 + I/K_I}$ $K'_s = K_s$
Substrate	$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$ $ES + S \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} SES$	$\mu = \frac{k_2 E_o S}{(K_c + S + S^2/K'_c)}$ $\mu_m = \frac{k_2 E_o}{(1 + 2(K_c/K'_c)^{0.5})}$ where K_c & K'_c are the dissociation constants for ES and SES respectively	
Product *	$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$ $ES + P \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} ESP$	$\mu = \frac{\mu_m S}{(K_s + S(1+P/k_P))}$ where $K_P = k_3/k_{-3}$	$\mu'_m = \frac{\mu_m}{1 + P/K_P}$ $K'_s = \frac{K_s}{1+P/K_P}$

where $\mu_m = k_2 E_o$ (E_o = initial enzyme concentration), $K_s = (k_{-1} + k_2)/k_1$

* This is the simplest mechanism, other mechanisms could be hypothesized which would lead to alternative rate expressions.

data have been presented by Hockenbury and Grady (1977).

An investigation conducted by Hockenbury and Grady (1977) concluded that p-Nitrobenzaldehyde, p-nitroaniline and n-methylaniline were all inhibitors of nitrite oxidation by Nitrobacter species when present in a concentration of at least 100 mg/l. Dodecylamine, aniline and n-methylaniline were potent inhibitors of ammonia oxidation by Nitrosomonas species, causing 50% inhibition at concentrations of less than 1 mg/l. Aniline, ethylenediamine, hexamethylenediamine and monoethaniolamine are commonly used organic substances, known to inhibit ammonia oxidation by Nitrosomonas species. Ammonia exerts substrate inhibition on its own oxidation, and the inhibition of ammonia oxidation by aniline, dodecylamine and ethylenediamine is niether competitive nor non-competitive. Hockenbury and Grady (1977) proposed that it is related to substrate inhibition. The inhibitory effect of aniline, dodecylaniline and ethylenediamine increases as the concentration of ammonia nitrogen in the medium is increased. The results presented by Hockenburg and Grady (1977) are shown in Table 3.18. The results are divided into two levels, compound concentrations yielding 50 and 75 % inhibition of the nitrifying culture.

Table 3.19 show the ammonium nitrogen and nitrate nitrogen concentration range for nitrobacter inhibition as function of pH at 20°C. The results are in accordance with knowledge of the ionisation of both ammonium nitrogen and nitrate nitrogen.

Neufeld *et al.* (1986) presented different equations for the inhibition of phenolic compounds on the nitrification and discussed the influence of free cyanide and complexed cyanide compounds on the nitrification kinetic.

Figure 3.13 shows that even small amounts of free cyanide in solution inhibit the biological rate of nitrification. The relationship of the maximum reaction rate V_{max} and the free cyanide concentration was found to follow the equation:

$$\log(V_{max}) = 0,079 - 3,0[CN^-] \qquad (3.45)$$

where $[CN^-]$ is the free cyanide concentration in mg/l at pH = 8,0. It is important to know the actual pH in the waste water environment and correct the $[CN^-]$ to pH = 8 using the proposed equation.

Table 3.17 Inhibitory effect of organic compounds with an IC_{50} value of less 20 mg/l, on pure cultures of Nitrifiers.

Organic Compound	*IC_{50} Concentration mg/L*
4-Aminophenol	0,07
3-Chlorophenol	0,20
2-Aminophenol	0,27
2-Bromophenol	0,35
2,3-Dichlorophenol	0,42
2,3,6-Trichlorophenol	0,42
1,3-Dichloropropene	0,48
5-Chloro-1-pentyne	0,59
2,3-Dichlorophenol	0,61
1,3-Dichloropropene	0,67
Chlorobenzene	0,71
4-Chlorophenol	0,73
2,4-Dichlorophenol	0,79
Trichloroethylene	0,81
4-Bromophenol	0,83
1,1-Dichloroethane	0,91
2,3,5,6-Tetrachlorophenol	1,30
1,1,2,2-Tetrachloroethane	1,40
1,1,2-Trichloroethene	1,90
2,2,2-Trichloroethanol	2,00
4-Nitrophenol	2,60
2-Chlorophenol	2,70
3,5-Dichlorophenol	3,00
2,3,5-Trichlorophenol	3,90
2,4,6-Tribromophenol	7,70
Resorcinol	7,80
2,4,6-Trichlorophenol	7,90
Pentachloroethane	7,90
2,6-Dichlorophenol	8,10
1,1,1,2-Tetrachloroethane	8,70
1,2,4,5-Tetrachlorobenzene	9,80
2-Nitrophenol	11,00
Benzene	13,00
1,5-Dichloropenthane	13,00
1,2,3,4-Tetrachlorobenzene	20,00

Source: Blum and Speece (1991)

Table 3.18 Inhibitoriy effect of organic and inorganic compounds in pure Nitrobacter culture on the nitrification process.

Compound	*Concentration (mg/l) at approximately 75% inhibition*
Acetone[+]	2 000
Allyl alcohol	19,5
Allyl chloride	180
Allyl isothiocyanate	1,9
Benzothiazole disulfide	38
Carbon disulfide[+]	35
Chloroform[+]	18
o-Cresol	12,8
Di-allyl ether	100
Dicyanidiamide	250
Diguanide	50
2,4-Dinitrophenol	460
Dithio-oxamide	1,1
Ethanol[+]	2 400
Guanidine carbonate	16,5
Hydrazine	58
8-Hydroxyquinoline	72,5
Mercaptobenzothiazole	3,0
Methylamine hydrochloride	1 550
Methyl isothiocyanate	0,8
Methyl thiuronium sulfate	6,5
Phenol[+]	5,6
Potassium thiocyanate	300
Skatole	7
Sodium dimethyl dithiocarbamate	13,6
Sodium methyl dithiocarbamate	0,9
Tetramethyl thiuram disulfide	30
Thioacetamide	0,53
Thiourea	0,076
Trimethylamine	118

[+]In the list of industrially significant chemicals.

Table 3.18 (continued)

Compound	*Concentration (mg/l) at approximately 50% inhibition*
Dodecylamine	< 1
Aniline	< 1
n-Methylaniline	< 1
Ethylenediamine	15
Napthylethylenediamine-di-HCl	23
2,2 Bipyridine	23
p-Nitroaniline	31
p-Aminopropiophenone	43
Benzidine-di-HCl	45
p-Phenylazoaniline	72
Hexamethylene diamine	85
p-Nitrobenzaldehyde	87
Triethylamine	127
Ninhydrin	> 100
Benzocaine	> 100
Dimethylgloxime	140
Benzylamine	> 100
Tannic acid	> 150
Monoethanolamine	> 200

Source: Hockenburg and Grady (1977)

Compound	*Inhibition Concentrations*
Phenol	100 mg/l
Vitamins:	
Riboflavin	50 mg/l
Thiamine	5 mg/l
Amino acids:	
L-Lysine	
L-Threonine	
L-Histidine	
L-Valine	
L-Arginine	
L-Methionine	4 mg/l
2-Chloro-6-trichloromethyl-pyridine	10 mg/l
Diethyldithiocarbamate	10^{-5} M
Methyl Blue	10^{-4} M
Tannin	10^{-6} M
Tannin derivatives	10^{-8} M

Source: Shrama and Ahlert (1976)

Table 3.19 Ammonium Nitrogen and Nitrate Nitrogen Concentration Range for *Nitrobacter* Inhibition as function of pH (T = 20 ^{0} C).

pH	NH_4^+ - N *Range, mg/l*	NO_2^- - N *Range, mg/l*
6,0	210 - 2100	30 - 330
6,5	70 - 700	88 - 1050
7,0	20 - 210	260 - 3320
7,5	7 - 70	
8,0	2 - 20	

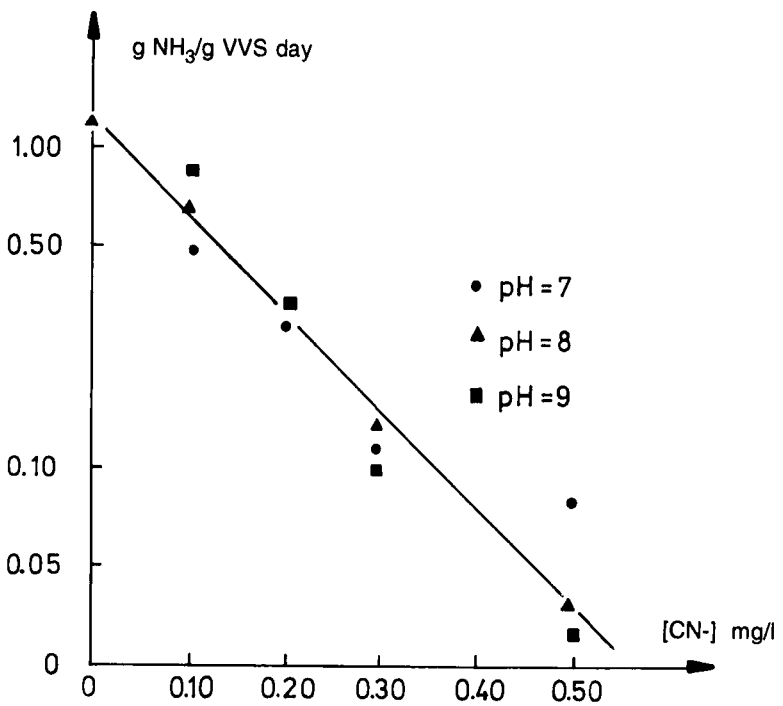

Fig 3.13 The influence of [CN^-] on the nitrification rate. After Neufeld *et al.* (1986).

Complexed cyanide was also found to cause a decrease in the maximum reaction rate for nitrification processes in accordance with the following equation:

$$\log(V_{max}) = 1,24 - 0,63(\log[CN]) \tag{3.46}$$

where [CN] is the complexed cyanide concentration in mg/l.

Using thiocyanate, Fig. 3.14 shows that a plot of V_{max} versus the thiocyanate concentration yield a constant reaction rate up to a thiocyanate level of about 236 mg/l. Above this value the reaction rate declined according to the following equation:

$$\log(V_{max}) = 1,91 - 0,77\log[SCN] \tag{3.47}$$

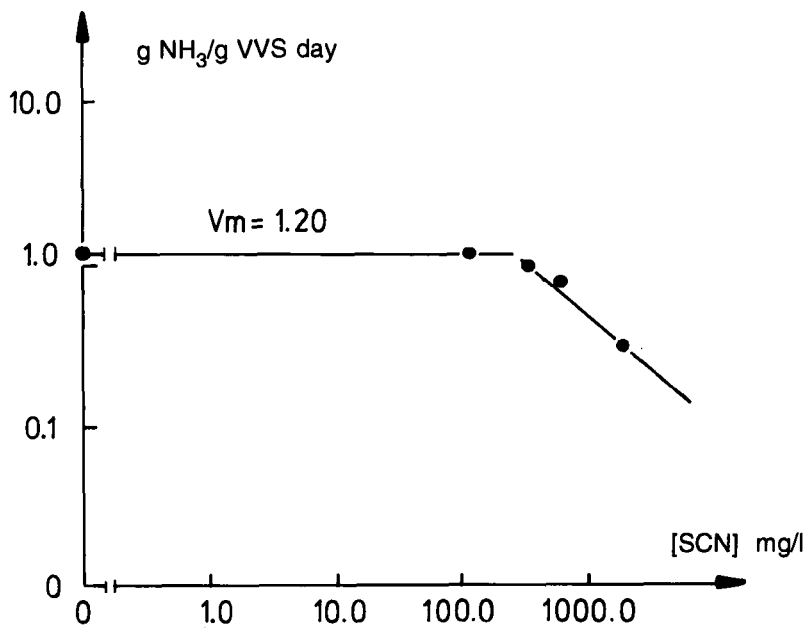

Fig 3.14 The influence of thiocyanate on the nitrification rate. After Neufeld *et al.* (1986).

Beg and Atiqullah (1983) conducted experiments with a fixed film reactor and showed that As^{3+}, Cr^{6+} and F^- were reversible non-competitive inhibitors, having inhibitor constants of 305, 65,3 and 1276 mg/l, respectively. Also, interaction between the three inhibitors showed that it did not affect the zero-order kinetic of nitrification with respect to the NH_4^+ - N substrate concentration.

When the concentration of a strong inhibitor was kept constant, and that of a weaker one was varied, two phenomena were observed (Beg and Atiqullah 1983). Firstly, for a shock dose at lower concentrations of the stronger inhibitors such as chromium and arsenic, the degree of inhibition was increased with the increase in the concentration of the weakest inhibitor, fluoride. This tendency was more pronounced at lower concentrations of the weaker inhibitor than at higher concentrations. Secondly, at higher concentrations of the stronger inhibitors, chromium (> 40 mg/l) and arsenic (> 300 mg/l), the degree of inhibition initially decreased to a minimum value, and then increased with the increase in the concentration of the weaker inhibitor.
Beg and Atiqullah developed the following rate expression for the shock load of As^{3+}, Cr^{6+} and F^-, in pairs:

$$\frac{dS}{dt} = V_{max}\left(1 - \sum k(I_2)\,(I)\right) \tag{3.48}$$

where; I is the concentration of the stronger inhibitor I_2, that of the weaker one in the pair.

Table 3.20 show a list of inorganic compounds that lead to inhibition of the nitrification process. It is important to remember that the inhibition of inorganic compounds is dependent on the actual pH in the environment, because it is often the free inorganic compound, for instance copper ion, that inhibits the nitrification process. As an example, the free copper ion concentration increases with decreasing pH.

Table 3.20 Inorganic compounds that lead to inhibition of the nitrification process.

Compound	*Concentration mg/l*	*Type of Plant*	*References*
CN^-	Toxic in all conc.	Coke plant waste water	Neufeld *et al.* (1986)
$Fe(CN^-)_6^{3-}$	80	"	"
SCN^-	236	"	"
As^{3+}	305	Fixed bed reactor	Beg and Atiqullah (1983)
Cr^{6+}	65,3	"	"
F^-	1267	"	"
Ag^+	5	Plastic media trickling filter	USPHS (1965)
Zn^{2+}	-	-	USPHS (1965)
Cu^+	-	-	USPHS (1965)
Ni^+	-	-	USPHS (1965)
Hg^+	-	-	USPHS (1965)
S^{2-}	Toxic in all conc.	Fixed bed pilot pilot plant.	Hjuler (1992) (unpublished)

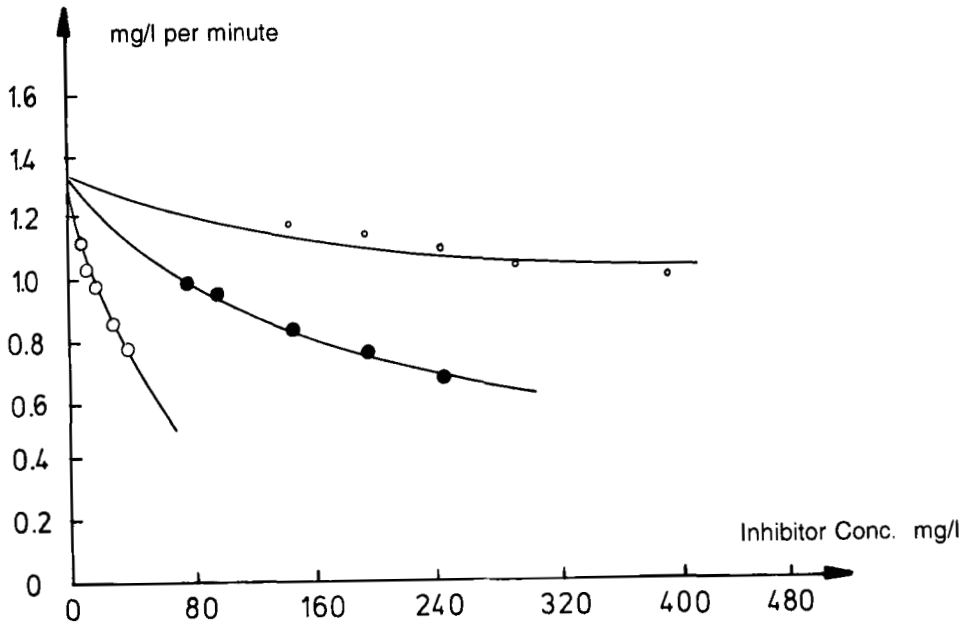

Figure 3.15 Effects of As^{3+}, Cr^{6+} and F^- on the nitrification rate. After Beg and Hassan (1987).