

THE EFFECT OF DISSOLVED OXYGEN CONCENTRATION ON NITRIFICATION

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Abstract—The effect of dissolved oxygen concentration on the rate of nitrification has been investigated by a number of researchers using both pure and mixed cultures, and cultures found in wastewater treatment systems. The maximum growth rate of both nitrification reactions are reported to be affected by dissolved oxygen concentration over the range of 0.3 mg l^{-1} to as much as 4.0 mg l^{-1} . In some instances, it has been reported that a dissolved oxygen concentration in excess of 4.0 mg l^{-1} is required to achieve maximum nitrification rates, while other investigators have found that only 0.5 to 1.0 mg l^{-1} is required.

It has been proposed that several factors are responsible for the wide range of reported nitrification rates with varying dissolved oxygen concentrations. Among these factors are the effects of oxygen diffusion in flocs, variation between measured results due to steady-state and dynamic measuring techniques, and double-substrate limited kinetics. This paper reviews the nitrification literature with respect to the effects of dissolved oxygen concentration, and shows that double-substrate limiting kinetics could account for the variation in the reported results.

INTRODUCTION

Nitrification has long been recognized as a biological means of ammonia removal from soils, wastewaters, and rivers and lakes. Nitrification in wastewater treatment is increasing in importance due to more stringent effluent requirements, and need for nutrient removal. Nitrification-denitrification appears to be one of the more promising methods of nitrogen removal. The subject of biological nitrogen removal has been investigated by and continues to be of interest to microbiologists, engineers, and biochemists. Undoubtedly, the nitrifiers' unusual chemoautotrophic metabolism and slow growth rates are partially responsible for scientific and engineering interest, which has produced several comprehensive nitrification review papers by Wallace & Nicholas (1969), Aleem (1970), Painter (1970), Focht & Chang (1975), and Sharma & Ahlert (1977).

The objective of this paper is to review the reported effects of dissolved oxygen (DO) concentration on the rate of nitrification rate, and to evaluate the mechanisms which could produce the variability in the reported findings.

LITERATURE REVIEW

The major early work to elucidate nitrification kinetics in natural waters and wastewater treatment systems was performed by Downing *et al.* (1963, 1964) at the Water Pollution Laboratory, Stevenage, England. Their work showed that the single most important factor to achieve nitrification in wastewater treatment plants is the organism growth rate, which can be related to the mean cell resi-

dence time (MCRT). Since then, investigators in the United States and Canada, such as Balakrishnan and Eckenfelder (1969), Wild *et al.* (1971), Haug & McCarty (1972), Gujer & Jenkins (1974), Poduska & Andrews (1975), Srinath *et al.* (1976), Murphy *et al.* (1977), and Hockenbury *et al.* (1977) have continued to elucidate the mechanism of nitrification processes in wastewater treatment. It is clear that the organism growth rate for the nitrifiers is dependent on inorganic substrate concentration, temperature, pH, inhibitory and toxic materials, and dissolved oxygen concentration. The importance of these factors has been demonstrated in previous work; however, the reported effects of DO concentration on maximum growth rate and process dynamics are varied, and not well understood.

Pure culture investigation

The effects of DO concentration on pure nitrifying cultures have been noted. Butt & Lees (1960), using Warburg techniques reported on the inhibitory effects of low DO concentration on pure cultures of *Nitrobacter winogradskyi*. Boon & Laudelout (1962) in similar experiments reported growth inhibition of *Nitrobacter winogradskyi* at various temperatures between 20 and 35°C. They determined that at approx. 1.0 mg l^{-1} DO concentration, the relative growth rate of *Nitrobacter winogradskyi*, as compared to growth rates at saturation DO concentration was reduced by 21, 20, 30, and 42% at 20°, 23.7°, 29°, and 35°C, respectively. Their data indicate that the half-saturation coefficient (K_s) for Monod-type (1949) growth rate kinetics, as shown in equation (1) would be approx. 0.5 mg DO l^{-1} .

$$\mu = \frac{\mu_s S}{S + K_s} \quad (1)$$

where

- μ_s = maximum specific growth rate (hr^{-1})
- S = limiting substrate concentration (mg DO l^{-1})
- K_s = half-saturation substrate coefficient (mg DO l^{-1})
- μ = growth rate (hr^{-1}).

Schoberl & Engel (1964) evaluated the effect of DO concentration by observing DO uptake rates as a function of DO concentration. They found that the growth rate for *Nitrosomonas* was independent of the DO concentration above 1.0 mg l^{-1} , and that for *Nitrobacter*, growth rate was independent above 2.0 mg DO l^{-1} . Loveless and Painter (1968) report a Monod saturation coefficient of 0.3 mg l^{-1} for a culture of *Nitrosomonas europaea* that was isolated from activated sludge. Peeters *et al.* (1969) using respirometric methods, reported half saturation coefficients of 0.25 and 1.84 mg l^{-1} for *Nitrosomonas europaea* and *Nitrobacter winogradskyi*, respectively. Laudelout *et al.* (1974) used half saturation coefficients of 0.5 and 2.0 mg l^{-1} for *Nitrosomonas* and *Nitrobacter* in their dynamic model for nitrification.

Wastewater treatment plant investigations

Investigations of nitrification in laboratory environments and wastewater treatment systems have also produced a variety of findings. Among the earliest work to quantify nitrification in wastewater treatment processes was that performed by A. L. Downing and Associates at the Water Pollution Lab at Stevenage. Their earlier work reports some interesting effects of DO concentrations with respect to nitrification. Downing & Scragg (1958) reported a "fall-off" of respiration rate in nitrifying activated sludge plants when the DO concentration fell below 0.3 mg l^{-1} . Although they did not attempt to quantify their findings, they speculated that one possible reason was a decrease in nitrification rate. Downing and Boon (1963) found a similar decrease in respiration rate when conducting aeration experiments in 5000 l pilot aeration tanks. Downing, Painter & Knowles (1964) reported that DO concen-

trations between 0.3 and 1.0 mg l^{-1} are required for nitrification and that nitrification ceases entirely below 0.2 mg l^{-1} . Knowles *et al.* (1965) in a paper from the same laboratory, reported kinetic coefficients and developed a mathematical model for nitrification. They investigated nitrification kinetics using Thames river water in 10 l completely-mixed reactors, and reported that DO concentration affected the growth rate of *Nitrosomonas* very little above 2 mg l^{-1} , and that *Nitrobacter* was more sensitive to DO concentration, showing reduced growth rate at DO concentrations below 4 mg l^{-1} . This work contrasts with the observations of Bragstad & Bradney (1937) who reported that 0.5 mg l^{-1} DO concentration is sufficiently low to prevent nitrification. Wuhrman (1963) noted the effect of DO concentration on nitrification in three identically operated activated sludge plants. The three plants were operated at 1, 4, and 7 mg l^{-1} DO concentration in a series of three experiments with varying organic loading rates. At the highest loading rate, only the plant operating at 7 mg l^{-1} nitrified. At the lowest loading rate, all three plants nitrified. These results demonstrate the importance of both the DO concentration and the MCRT for nitrification.

Stankewich (1972) stated that the Monod saturation coefficient for DO in pure oxygen activated sludge plants is 0.43 mg l^{-1} , but showed no data of experimental conditions. Murphy (1974) reported that the rate of nitrification in wastewater treatment increases as the DO concentration is increased to 7 or 8 mg l^{-1} . Nagel and Haworth (1969) report a half saturation coefficient of 2.0 mg l^{-1} for nitrification in activated sludge plants.

Other investigations

The effect of DO on nitrification in soils has also been

Table 1. Summary of the effects of DO on nitrification

Organism	Dissolved Oxygen Parameter		Conditions and remarks	Reference
	$K_s(\text{DO})$ mg l^{-1}	DO mg l^{-1}		
<i>Nitrosomonas</i>	0.3		20°C, Pure culture	Loveless & Painter (1968)
	0.25		Pure culture	Peeters <i>et al.</i> (1969)
		1.0	Zero-order ¹ , pure culture	Schoberl & Engel (1964)
		2.0	Zero-order	Knowles <i>et al.</i> (1965)
<i>Nitrobacter</i>	1.84, 2.46		Pure culture, by respirometric	Peters <i>et al.</i> (1969) and Laudelout
	0.83		amperometric and micro	<i>et al.</i> (1976)
	0.34, 0.48,		calorimetric technique	
	0.72		at 20°, 29°, 35°C, respectively.	
		2.0	Calculated from Boon & Laudelout	
		4.0	(1962)	
<i>Nitrosocystic Oceanous Nitrifiers, generally</i>		2.0	Zero-order	Schoberl & Engel (1964)
		4.0	Zero-order	Knowles <i>et al.</i> (1965)
		7.5	Zero-order	Gundersen (1966)
		0.5-0.7	Inhibitory	Forster (1974)
		1.0-1.5	Proper operation in ASP ²	Jenkins (1969), Balakrishnan & Eckenfelder (1969), Wild <i>et al.</i> (1971), Wuhrman (1964-8)
	0.8		In soil	Calculated by Shah & Coulman (1978)
		0.5	Inhibitory ³ in ASP	Bragstad & Bradney (1937)
		0.3	Inhibition ⁴ in ASP	Downing & Scragg (1958)
		0.3	Inhibition ⁴ in ASP	Downing & Boon (1963)
		0.2	Inhibitory in ASP	Downing <i>et al.</i> (1964)
	0.3-1.0	Proper operation in ASP	Downing <i>et al.</i> (1964)	
	4.0	Maximum rate of nitrification	Wuhrman (1963)	
	0.42	In pure oxygen ASP	Stankewich (1972)	
	2.0	In ASP	Nagel & Haworth (1969)	

¹ Zero-order—MIN DO concentration for zero-order kinetics.

² ASP—Activated sludge process.

³ Inhibitory—no reaction.

⁴ Inhibition—reaction depends upon concentration.

investigated. Ames & Bartholomew (1951) found that the half saturation coefficient was at an oxygen partial pressure corresponding to fluid DO concentration of 0.8 mg l^{-1} . It is not known to the writers if this value is meaningful in wastewater treatment, although Shah & Coulman (1978) support their mixed culture model of nitrification-denitrification with the results of Ames & Bartholomew (1951).

Nitrification has also been studied in marine environments. Gundersen (1966) reported that the optimum dissolved oxygen concentration occurred when sea-water was in equilibrium with approx. 120 mm partial oxygen pressure, corresponding to roughly 3.5 to 4 mg l^{-1} DO. Forster (1974) found that DO in excess of 0.6 to 0.7 mg l^{-1} was required for nitrification in sea-water. Since the nitrifying species formed in marine environments may be different than those found in wastewater treatment plants, extrapolation of Gundersen's and Forster's results to treatment plants may not be possible.

The effects of extremely high DO concentration have received less attention than low DO concentrations. Okun (1949) and Haug & McCarty (1972) investigated high DO concentration and report no adverse effect for DO concentrations of 33 and 60 mg l^{-1} respectively.

A summary of the reported effects of DO concentration on the rate of nitrification is shown in Table 1. Based on the information available in the literature, it appears that the most probable range of DO concentration needed to reliably achieve nitrification is between 0.5 and 2.0 mg l^{-1} .

DISCUSSION

From the literature review, it is apparent that the DO concentration requirements for nitrification are not well defined and that a broad range of DO half saturation coefficients have been reported. There are several factors which could produce the variability in the reported effects of DO concentration on nitrification. In activated sludge plants, the nitrifying organisms are mixed in large flocs and the DO concentration within the flocs may be considerably less than the bulk fluid concentration. This phenomena was originally investigated by Wuhrman (1963) and has more recently been studied by Scaccia & Lee (1977), and Kossen (1978). The concentration of DO in the bio-floc is dependent upon a number of factors. Most prominent among these are bulk DO concentration, size and shapes of the flocs, bulk mixing, turbulence and intensity, and the oxygen uptake rates of both heterotrophic nitrifying organisms in the floc. Therefore, it would be expected that the results of pure culture investigations could be different from the results of investigations using mixed or activated sludge cultures. The growth rate dependence of the nitrifying bacteria on inorganic nitrogen substrates and the intensity of mixing have been discussed by Poduska (1973). Based on work by Lilly & Sharp (1968) on enzyme kinetics in continuous flow stirred reactors, it was shown that the half-saturation coefficients was inversely proportional to mixing intensity and mathematically related to the diffusion boundary layer thickness surrounding the enzyme particles. Powell (1967) suggested that diffusion of substrates to cells can reduce the availability of those substrates at low concentrations. He mathematically demonstrated

that the effect of diffusion would be to increase the apparent half-saturation coefficient. Therefore, the variations reported for the oxygen half-saturation coefficient for nitrification could in part be due to differences in the intensity of mixing in each study.

A second possible source of variability in reported results is the dynamic or steady-state nature of the measuring techniques for half-saturation coefficients. Several of the values for half saturation coefficients reported in Table 1 were calculated from batch tests using dissolved oxygen exhaustion rates. Other values were calculated from steady state operation at several values of liquid phase dissolved concentrations. Very little is known about the dynamic behavior of nitrification under oxygen limited conditions. Only one dynamic model (Laudelout *et al.*, 1974) includes the effects of oxygen limiting kinetics. The results of this model appear to confirm the possibility of differences between dynamic and steady-state measuring techniques to determine needed DO concentrations.

A third possible source of variability in the results reported in Table 1 is the likelihood of double substrate limiting kinetics. Normally, microbiological cultures are thought to be limited by only one substrate. Most mathematical models for biological growth account for only one substrate, as shown previously in equation (1) since experimental studies are usually performed with all other nutrients in excess. Ryder & Sinclair (1972) and Sykes (1973) have recently investigated the possibilities of multiple, but not simultaneous substrate limiting kinetics. Both investigators show that at one operating point or condition, substrate A limiting, while at another operating point or condition substrate B may be limiting. Both authors propose to determine maximum growth rate by evaluating the microbial yields associated with growth rates, and then selecting the expression which is limiting. They do not consider a case where two substrates can be simultaneously limiting.

Bader *et al.* (1975) and Bader (1978) have shown that the analysis proposed by Ryder & Sinclair (1972) and Sykes (1973) is not general and have discussed simultaneous multiple substrate limiting kinetics, using growth rate contours, similar to that shown in Fig. 1. The growth rate contours in Fig. 1 were calculated from a double Monod function, with endogenous or decay coefficient as follows:

$$\mu = \hat{\mu} \left[\frac{S_1}{K_{S_1} + S_1} \cdot \frac{S_2}{K_{S_2} + S_2} \right] - K_D, \quad (2)$$

where

- μ = net specific growth rate (T^{-1})
- $\hat{\mu}$ = maximum specific growth rate (T^{-1})
- S_1 = substrate 1 concentration
- S_2 = substrate 2 concentration
- K_{S_1} = half-saturation coefficient for substrate 1
- K_{S_2} = half-saturation coefficient for substrate 2
- K_D = decay or maintenance coefficient (T^{-1}).

To illustrate the effects of double substrate limiting

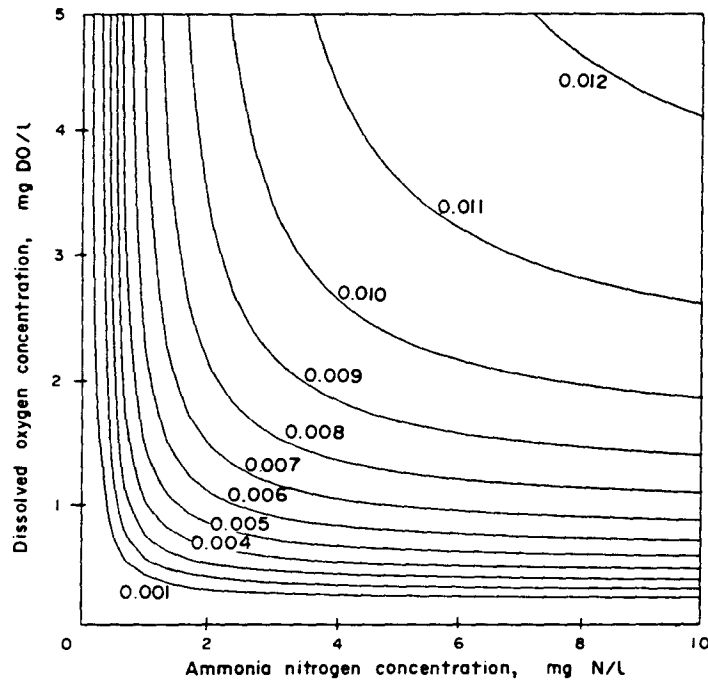


Fig. 1. Net growth rate contours for nitrification: ammonia nitrogen vs dissolved oxygen concentration (numbers on curve indicate growth rate, μ , hr^{-1}).

$$\mu = \hat{\mu} \left[\frac{S}{K_S + S} \cdot \frac{DO}{K_{SDO} + DO} \right] - K_D$$

$$\hat{\mu} = 0.02 \text{ hr}^{-1}, \quad K_{SDO} = 0.5 \text{ mg DO l}^{-1},$$

$$K_S = 1.0 \text{ mg NH}_4^+ \text{-N/l}, \quad K_D = 0.005 \text{ hr}^{-1}.$$

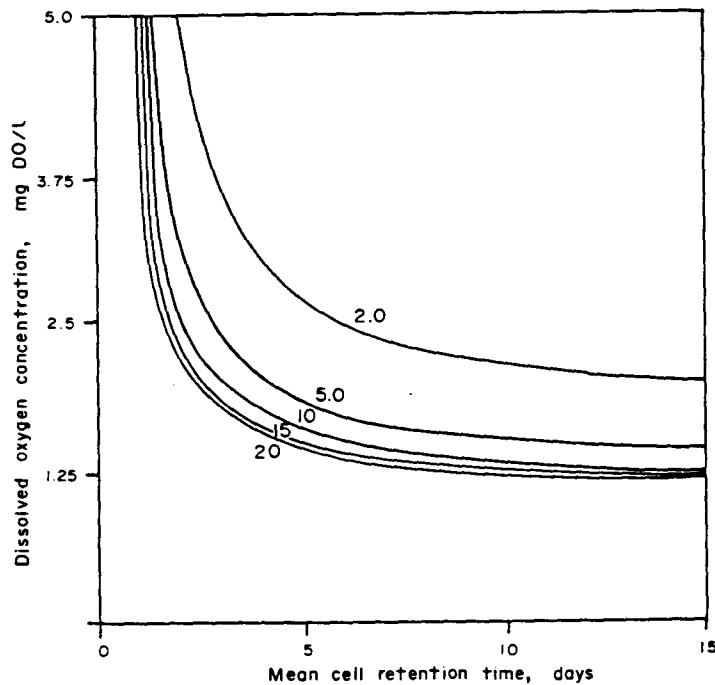


Fig. 2. Effluent ammonia nitrogen contours: cell retention time vs dissolved oxygen (numbers on curves indicate effluent ammonia nitrogen concentration).

$$\hat{\mu} = 0.02 \text{ hr}^{-1}, \quad K_{SDO} = 0.5 \text{ mg DO l}^{-1},$$

$$K_S = 1.0 \text{ mg NH}_4^+ \text{-N/l}, \quad K_D = 0.005 \text{ hr}^{-1}.$$

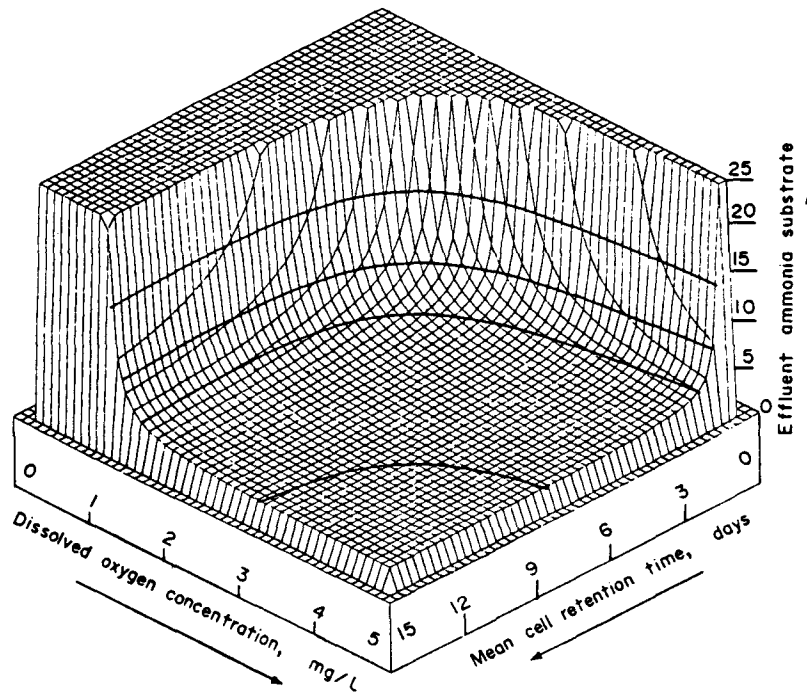


Fig. 3. Ammonia nitrogen substrate perspective diagram.

$$\begin{aligned}\hat{\mu} &= 0.02 \text{ hr}^{-1}, \\ K_S &= 1.0 \text{ mg l}^{-1},\end{aligned}$$

$$\begin{aligned}K_{SDO} &= 0.5 \text{ mg l}^{-1}, \\ K_D &= 0.005 \text{ hr}^{-1}.\end{aligned}$$

kinetics, the net growth rate has been calculated and plotted as a contour graph shown in Fig. 1. A value of 0.5 mg DO l^{-1} was selected for the DO half saturation coefficient. This value was picked as representative of the range of coefficients shown in Table 1. It is interesting to note that Laudelout *et al.* (1974, 1976) have also used a value of 0.5 mg l^{-1} in their work. The analysis shown in Fig. 1 is also applicable for other values of half saturation coefficients. The figure shows regions where the growth rate can be limited by both substrates, and regions where only one substrate is limiting. Most activated sludge plants which nitrify successfully are capable of producing effluents with less than one or two mg l^{-1} of ammonia nitrogen. The results shown in Fig. 1 indicate that the only way to achieve this effluent nitrogen concentration at a net growth rate of 0.005 hr^{-1} (8.3 days mean cell retention time), is to operate at a DO concentration in excess of 2.5 mg l^{-1} .

It has been well established that successful activated sludge nitrification must be accomplished at mean cell retention times (MCRT) greater than the wash-out MCRT of the nitrifying organisms. It is useful to observe ammonia nitrogen substrate contours as a function of DO concentration and MCRT. A contour graph of effluent ammonia nitrogen concentration as a function of MCRT and DO concentration is shown in Fig. 2 and a perspective diagram is shown in Fig. 3. From these two figures, the hypothetical effects of DO concentration upon nitrification at any given MCRT are clearly demonstrated. At low MCRT, it is possible to nitrify as efficiently as at

higher MCRT, although the required DO concentration is much greater.

From Fig. 2, it becomes obvious why numerous investigators have reported different values of DO concentration required for nitrification. For example, it is possible to explain why Wuhrman (1963) observed poor to zero nitrification at 4.0 mg l^{-1} DO concentration and excellent nitrification at 7.0 mg l^{-1} DO concentration. Wuhrman does not report MCRT for this observation, but he reports the hydraulic loading rate and mixed-liquor solids concentration, which can be used to estimate a food-to-mass ratio or MCRT. The estimated MCRT for this observation is low and borders upon washout. Therefore, it would be expected that a high DO concentration was needed for Wuhrman's observation as Fig. 2 shows.

It has been argued by Ryder & Sinclair (1972) and Sykes (1973) that stoichiometric considerations will make only one of multiple substrates limiting. Undoubtedly, for many cases they are correct, but for the case of nitrification in wastewater treatment plants, the DO concentration can be independent of MCRT, or the concentration of other substrates. This situation exists because many treatment plants are provided with variable aeration rates. The treatment plant operators can maintain constant DO concentration over a range of growth rates or substrate loading rates. As treatment plants are modernized and energy becomes more expensive, more treatment plants will install real-time DO control systems. The results of a recent survey by Genthe *et al.* (1978) indicated that more automatic DO control systems are

being installed, and concluded that the trend will continue. Moreover, this survey shows that DO control can result in improvements in process efficiency. It also appears from work performed by Sezgin *et al.* (1978), and Palm *et al.* (1978) that DO control may be used to improve process performance with respect to sludge bulking. It must be concluded that many more plants will incorporate DO control, and that the necessity of nitrifying under oxygen limited conditions will increase.

SUMMARY AND CONCLUSIONS

From the previous discussion, it is obvious that there exists no clearly defined DO concentration for optimum nitrification. It appears that at higher MCRT, nitrification can be achieved at DO concentrations in the range of 0.5 to 1.0 mg l⁻¹, and at lower MCRT higher DO concentrations are needed. Based on the analysis of this study, the lowest DO concentration at which nitrification can occur appears to be approximately 0.3 mg l⁻¹.

There appear to be several mechanisms which may explain the wide distribution of reported half saturation coefficients and inhibitory DO concentrations. One possible reason is the effect of oxygen diffusion with the flocs. A second mechanism is double substrate limiting kinetics, which can explain the wide variation of reported results.

It is clear that further experimental work is needed to quantify the effects of DO concentration on nitrification. Particular emphasis must be placed upon the steady-state and dynamic effects of DO concentration.

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