Journal of Industrial Microbiology & Biotechnology (2000) 24, 161–166

www.nature.com/jim

ORIGINAL PAPERS

Measurement of potential activity of fixed nitrifying bacteria in biological filters used in drinking water production

A Kihn[1], P Laurent[2] and P Servais[1]

[1]*Ecologie des Systèmes Aquatiques, Université Libre de Bruxelles, Campus de la Plaine, CP 221, Boulevard du Triomphe, B-1050 Brussels, Belgium;* [2]*NSERC Industrial Chair on Drinking Water, Civil, Geological and Mining Engineering, Ecole Polytechnique of Montreal, CP 6079, succ Centre-Ville, Montreal, Quebec, Canada, H3C 3A7*

Nitrification during biological filtration is being used more and more in drinking water production to remove ammonia, which can be the source of several water quality problems during distribution. In this process, ammonia is converted into nitrite and then into nitrate by fixed autotrophic nitrifying bacteria. The purpose of this work was to develop a technique to estimate fixed nitrifying biomass (sum of ammonia- and nitrite-oxidizing populations). The quantification of autotrophic nitrifying biomass was determined by potential nitrifying activity measurement. The production of oxidized forms of inorganic nitrogen (nitrates and nitrites) was measured after an incubation of 2 cm^3 of colonized solid support in the presence of a 5-ml nitrifier medium containing 10 mg $N\text{-}NH_4$ L^{-1} for 30 min at 32°C. The production rate of oxidized nitrogen in optimal conditions was measured and converted into nitrifying biomass by using the maximum specific oxidizing activity. This technique was shown to be appropriate for conditions encountered in the biological filters used in drinking water production and sufficiently simple to be used for routine measurements. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 161–166.

Keywords: ammonia; nitrification; nitrifying biomass; drinking water production; biological filtration

Introduction

Removal of ammonia during drinking water production is required because its presence can be the source of several water quality problems during distribution such as bacterial regrowth [36,37], production of nitrites by incomplete oxidation of ammonia [19], and production of tastes and odors [21]. In drinking water production, both physicochemical and biological processes can be applied to remove ammonia. In the first group of processes, ion exchange and chemical oxidation are of primary concern. The most common method used is chlorination until break point. During this stage, the ammonia present in the water consumes high chlorine doses [16]. In addition, a high chlorination level usually induces, by reaction between organic matter and chlorine, the formation of disinfection by-products such as trihalomethanes (THM) [16], known to be potentially carcinogenic [12]. At present, biological processes, like biological filtration, seem to be more promising; the use of this process can avoid problems like biological instability in the distribution system and production of toxic by-products. In this process, ammonia is converted into nitrate by autotrophic nitrifying bacteria [10]. Biological nitrification is a two-step process in which sequential oxidation of ammonia into nitrite and nitrite into nitrate occurs. *Nitrosomonas* and *Nitrobacter* are the most common genera of bacteria, known respectively as ammonia oxidizers and nitrite oxidizers [41]. However, some authors [22,23] reported that nitrification in natural environments and biofilms is complex and they noted difficulty in identifying all the microorganisms capable of nitrification in such environments. In addition, even though several species of heterotrophic bacteria are able to produce nitrates and nitrites [17,39], their contribution to total nitrification seems to be insignificant in comparison to that of autotrophic processes.

To optimize biological transformation of ammonia in biological filters, it is necessary to identify the main factors controlling nitrification efficiency. Up to now, indirect measurement by monitoring ammonia concentration in the inflow, interstitial and outflow waters of the filters has been the most common technique used to investigate this efficiency [5,24]. The impacts of ammonia concentration, temperature, loading rate and time of operation have already been demonstrated [34]. However to go further in the investigation of nitrification in biological filters and to optimize this process, measurements of the autotrophic biomass fixed on the solid medium (sand, GAC, anthracite) used for filtration are required. The estimation of biomass could be useful to improve our knowledge of the nitrification process in order to model the functioning of biological filters. For example, it had been shown that the nitrifying biomass and its activity can be differently affected by filter backwashing ([31]; Andersson, Ecole Polytechnique of Montreal, personal communication). Moreover, estimation of fixed nitrifying biomass can lead to a better understanding of competitive effects between autotrophic and heterotrophic bacteria for the space and the oxygen in a mixed biofilm. In addition, parallel measurements of

Correspondence: A Kihn, Groupe de Microbiologie des Milieux Aquatiques, Université Libre de Bruxelles, Campus de la Plaine, CP 221, Boulevard du Triomphe, B-1050 Brussels, Belgium. E-mail:akihn@ulb.ac.be
Received 28 July 1999; accepted 11 November 1999

nitrifying biomass and activity give information on the physiological state of the biofilm. The purpose of this work was therefore to develop a technique to measure fixed nitrifying biomass (ammonia- and nitrite-oxidizing populations), which would be appropriate for the conditions encountered in the biological filters used in drinking water production, on the one hand, and sufficiently simple and rapid to be used in routine measurement, on the other hand.

The standard procedure for estimating nitrifying bacterial abundance is the most probable number method (MPN) [4] but this technique considerably underestimates the abundance of such bacteria [6]. Moreover, as we were working with fixed nitrifying bacteria, the use of this technique needed a prior quantitative detachment of the cells, which was difficult to perform [27]. Another technique recently proposed is to estimate nitrifying biomass in the aquatic environment and in sediments by measuring potential nitrifying activity, which is considered proportional to the biomass [11]. Oxidation of ammonia to nitrate is accompanied by CO_2 incorporation for biomass synthesis. By measuring the difference between ^{14}C-CO_2 incorporation in two subsamples, one untreated and the other treated with a specific nitrification inhibitor, the carbon incorporation rate by nitrification was determined and the nitrogen oxidation rate calculated knowing the C incorporated/N oxidized yield [7,8,18,33,38]. The disadvantage of this technique for our purposes was the difficulty in performing routine experiments with radioactive compounds in water treatment plants and the required detachment of the cells from the support for measuring incorporated radioactivity.

Recently, the immunofluorescence technique [9] and methods based on genomic data using the polymerase chain reaction (PCR) [15] have been applied to the detection of *Nitrobacter* in aquatic environments. Newly developed fluorescent *in situ* hybridization (FISH) [2] has been applied by several authors for detection of nitrifying bacteria in activated sludge [30,40]. However, due to methodological (detection limit, availability of probes for all the nitrifying species, specificity of the probes, difficulty in detecting fluorescence on a solid support) and cost problems, these methods are not yet useful for routine application.

The method proposed in this work is based on a direct measurement of bacterial potential activity; it is based on the fact that the nitrification rate in potential (or optimal) conditions (temperature, ammonia concentration, pH, oxygen concentration) is proportional to the nitrifying bacterial biomass colonizing the solid support [6,11]. This approach is already used routinely for the estimation of fixed heterotrophic biomass [27]. In our case, the potential nitrifying activity was determined from the production rate of oxidized nitrogen (NO_2^- and NO_3^-). Tests conducted to determine the optimal conditions and the first application to full scale biological filters are presented.

Materials and methods

Origin of the samples

For the tests presented in this paper, samples of nitrifying bacteria fixed onto sand were collected in a pilot filter containing 50 cm^3 of filtration material. The pilot filter was fed with tap water at a temperature of $20 \pm 5°C$ and free of nitrites and chlorine but enriched with ammonia at a concentration of 2 mg $N\text{-}NH_4\ L^{-1}$. The filtration material was colonized by bacteria present in the tap water. The empty bed contact time in the columns was 10 min. Fixed biomass samples were collected after complete colonization of the sand by both ammonia-oxidizing and nitrite-oxidizing bacteria. At this moment, ammonia was completely oxidized into nitrate during filtration.

Samples from a full-scale activated carbon filter were collected in the St Rose treatment plant (Laval, Quebec, Canada). The ammonia concentration entering this second-stage filter ranged between 0.02 and 0.15 mg $N\text{-}NH_4\ L^{-1}$ and the empty bed contact time was 20–30 min. Water samples were drained from different depths in the filter and solid medium samples were removed from the filter using a core sampler.

Potential nitrifying activity

Potential nitrifying activity was determined by incubating duplicate samples of colonized filtration material in a nitrifying medium [29] (2 g L^{-1} NaCl, 0.05 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.5 g L^{-1} K_2HPO_4, 0.017 g L^{-1} $NaHCO_3$, 2 ml L^{-1} of a chelated metals solution [13]), adjusted to pH 8.0. This pH value was in the optimal range (7.0–8.2) for the nitrification process mentioned in a literature review [3]. We verified that the pH value did not significantly decrease during the incubation. The medium was enriched with ammonia chloride (see the section 'routine procedure' in Results and discussion). Nitrate and nitrite concentrations were measured after incubation by a colorimetric method [25] to measure the production of oxidized ammonia and thus the nitrification activity of fixed ammonia- and nitrite-oxidizing populations. The colorimetric method consists in measuring nitrites before and after a reduction of nitrates by shaking the medium on cadmium. In all our experiments, no nitrite was detected before reduction on cadmium.

Ammonia

NH_4 was measured in duplicate samples using the indophenol colorimetric method [1]. This method is fairly accurate ($\pm 3\ \mu g\ N\text{-}NH_4\ L^{-1}$) at low ammonia concentrations (detection limit 5 $\mu g\ N\text{-}NH_4\ L^{-1}$).

Results and discussion

Determination of the optimal conditions for nitrifying activity

Nitrifying activity of fixed ammonia- and nitrite-oxidizing populations was first measured at various temperatures (10–35°C). Duplicate samples were incubated in a nitrifying medium containing 10 mg $N\text{-}NH_4Cl\ L^{-1}$ (Figure 1). The optimal temperature was determined by a Gauss function, which classically fits the effect of the temperature on microbial process [28]. The optimal temperature was $32.0 \pm 2.4°C$. This value was in the optimal temperature range (between 25°C and 35°C) mentioned in a literature review for the nitrification process [20].

To determine the saturating ammonia concentration, duplicate samples of sand containing fixed nitrifying bac-

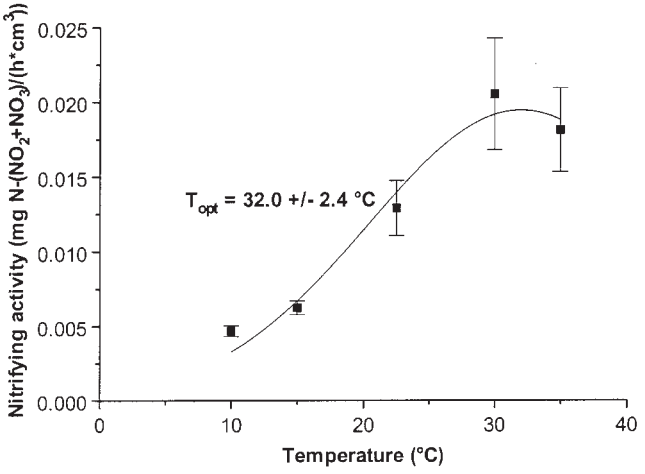

Figure 1 Effect of temperature on nitrifying activity of a mixed bacterial population fixed on sand originated from a laboratory pilot filter.

teria were incubated at various added ammonia concentrations (0–10 mg N-NH_4 L^{-1} added) at 32°C (Figure 2). A half-saturation constant (K_m) of 2.2 ± 0.4 mg N-NH_4 L^{-1} was determined. Copp and Murphy [14] mentioned, in their literature review, half-saturation constants for mixed nitrifying populations between 0.06 and 5.6 mg N L^{-1}. To be sure to reach a saturating concentration of ammonia and thus a maximal nitrification rate, an initial concentration of 10 mg N-NH_4 L^{-1} was chosen for routine measurement of the potential nitrifying activity. This concentration was five times the half saturation constant determined experimentally.

Two factors determined the incubation time required to measure the oxidized nitrogen formation rate: the substrate had to remain at a saturating concentration of ammonia throughout the incubation and the quantity of oxidized nitrogen produced had to be detectable. When a 2-cm^3 sand sample was incubated in 5 ml of nitrifier medium enriched by 10 mg N-NH_4 L^{-1} at 32°C, the formation of nitrate was linear for about 60 min, then the nitrifying activity decreased until the substrate was completely oxidized (Figure 3). An incubation period of 30 min seemed reasonable for our experimental conditions. As this incubation time was lower than the minimum generation time of the nitrifying bacteria (t_d = 15–70 h) [14], the production of oxidized nitrogen measured was due only to the biomass present at the sampling time.

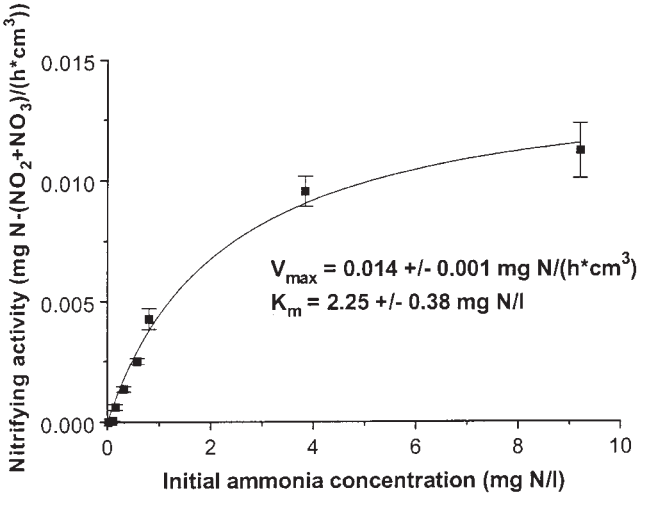

Figure 2 Relationship between nitrifying activity and initial concentration of ammonia in the incubation medium at 32°C. Determination of the half saturation constant for a mixed nitrifying bacterial population fixed on sand originated from a laboratory pilot filter.

Nitrification is a two-step aerobic chemoautotrophic process, which consumes important quantities of oxygen. The theoretical value of oxygen consumption for complete nitrification is 4.27 mg O_2 mg^{-1} N-NH_4. To oxidize ammonia completely into nitrate, a minimum concentration of 4 mg O_2 L^{-1} in the incubation system is necessary to avoid a decrease in effectiveness of the transformation and thus formation of nitrite [26]. In order to ensure saturation of oxygen, samples were aerated during the incubation period by air bubbling using an aquarium pump.

Tests showed that without suitable washing of the solid sample, the final concentration of nitrate was higher than the initial concentration of non-oxidized nitrogen. As a matter of fact, the solid material and the interstitial liquid could trap nitrite and nitrate as well as ammonia, and release these compounds during incubation. The colonized filtration material was therefore washed by the nitrifier medium without ammonia to avoid interference. As it was impossible to eliminate all traces of oxidized nitrogen, nitrate and nitrite were measured at the beginning of the incubation (t_0) and these values were subtracted from the values measured at the end of incubation to obtain the concentrations produced during incubation.

Routine procedure

From the tests described above, the routine procedure proposed to measure potential activity of nitrifying bacteria fixed on the solid support was as follows: three duplicate samples of 2-cm^3 colonized material were washed several times with the nitrifier medium without ammonia. A duplicate sample was then incubated at 32°C for 0, 15 or 30 min in 5 ml of nitrifier medium [29] containing 0.037 g L^{-1}

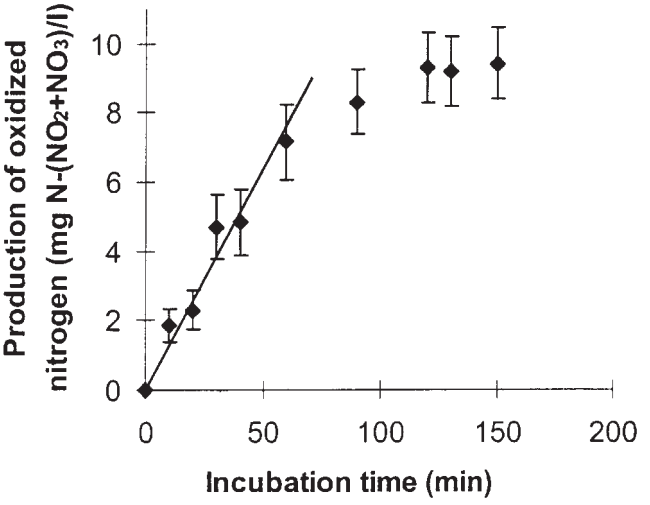

Figure 3 Determination of the optimal incubation time for a 2-cm^3 sand sample incubated at 32°C in a medium containing 10 mg N-NH_4 L^{-1}.

NH_4Cl (10 mg N-NH_4 L^{-1}). While it was incubated, the medium was oxygenated by air, which was purified by passing it through a sulphochromic solution. After incubation, the supernatant was filtered through a 0.2-μm pore size filter to stop the biological reactions by removing the bacteria and then frozen until analysis. Nitrate and nitrite concentrations were measured before and after incubation by a colorimetric method [25] to determine the biological oxidation of ammonia. The experimental design is shown in Figure 4.

In the routine procedure, the concentrations of nitrate and nitrite were measured in each sample containing 2-cm^3 filtration media, after an incubation period of 0, 15 or 30 min in 5 ml nitrifier medium in potential conditions. The results were then plotted against time and the slope (α) was determined. Potential nitrifying activity (PNA) was calculated as follows, considering the volume of nitrifier medium, the incubation time and the volume of samples used in our procedure:

$$PNA\ (\text{mg N/(h} * \text{cm}^3)) = \alpha\ (\text{mg N/(min} * 1)) * \frac{5}{1000} * 60 * \frac{1}{2}$$

The accuracy of the method was determined on 160 samples. For each of them, the potential nitrifying activity was measured by incubating a set of three duplicate subsamples. Samples were collected from pilot and full-scale filters (sand and granular activated filters) fed with different ammonia concentrations ranging from 0 to 2 mg N L^{-1}. The standard error was calculated on the slope determined when the production of nitrates and nitrites was plotted against time. The average standard error on potential nitrifying activity, according to these tests was 15%. This percentage includes the errors in incubation time, sampling volume of the solid support, the heterogeneity of the fixed biomass on the solid support and the measurement of the oxidized nitrogen after incubation.

Two examples of the measurement of potential nitrifying activity of fixed nitrifying biomass showing the accuracy of the method are shown in Figure 5. The samples were collected from a lab-scale pilot filter filled with sand. A duplicate of each sample was incubated according to the routine procedure and the results shown were the average value of the nitrate concentration measured in the duplicates. No nitrites were detected after incubation showing that both nitrifying populations (ammonia- and nitrite-oxidizing bacteria) were present.

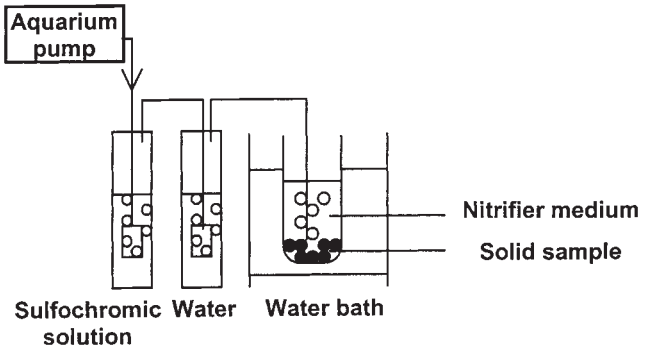

Figure 4 Experimental design used for measurement of the potential nitrifying activity: the solid sample supported fixed bacterial biomass (2 cm^3); the nitrifier medium contained 10 mg N-NH_4 L^{-1} and the water bath was heated to 32°C.

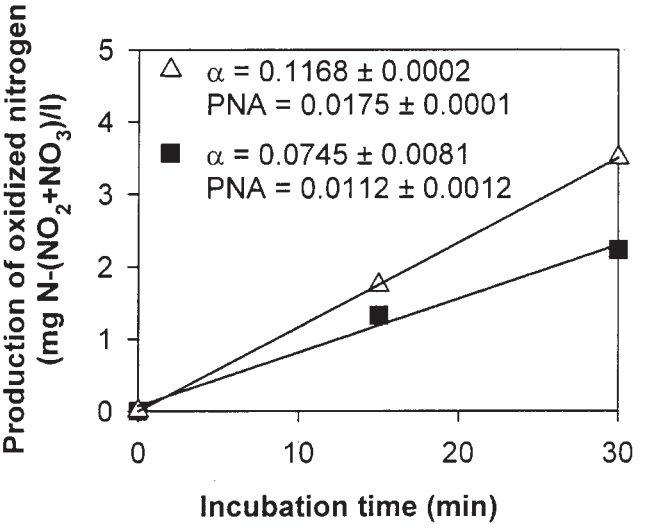

Figure 5 Two examples of the determination of potential nitrifying activity for a mixed nitrifying bacterial population fixed on sand (samples originated from a laboratory pilot filter).

As nitrification activity measured in potential conditions is proportional to nitrifying biomass, the approximate amount of nitrifying biomass (sum of ammonia- and nitrite-oxidizing biomass) can be estimated by using the maximum specific activity of ammonium- and nitrite-oxidizing bacteria according to the following equation [6,11]:

$$B = \frac{PNA}{AS_{sp_1}} + \frac{PNA}{AS_{sp_2}}$$

where B is the sum of the ammonia oxidizing biomass and the nitrite oxidizing biomass (μg C cm^{-3} support); PNA is the potential nitrifying activity [mg N/(h * cm^3 support)]; AS_{sp_1}, the maximum specific ammonia oxidizing activity [mg N/(μg C * h)]; AS_{sp_2}, the maximum specific nitrite oxidizing activity [mg N/(μg C * h)].

For the calculation of fixed nitrifying biomass, the maximum specific oxidization activities recently proposed by Brion and Billen [11] were used: respectively $AS_{sp_1} = 0.7 \times 10^{-3}$ mg N/(μg C * h) and $AS_{sp_2} = 2.9 \times 10^{-3}$ mg N/(μg C * h).

Application on full-scale filters

As an example of application of the proposed procedure, the vertical distribution of nitrifying biomass in a second-stage activated carbon filter at the St Rose treatment plant (Laval, Quebec, Canada) was determined. The potential nitrifying activity and ammonia concentration were measured for samples collected at different depths in the filter (Figure 6). The nitrifying biomass decreased with increasing depth, as the substrate concentration in the water decreased. However, nitrifying biomass persisted in the lower part of the filter, even though the ammonia had been

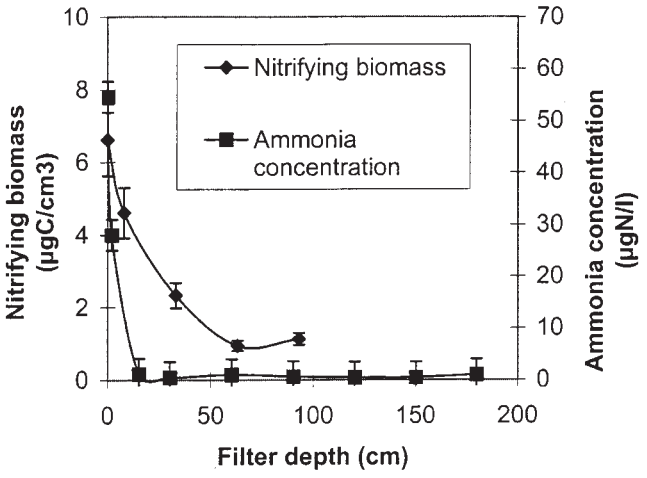

Figure 6 Distribution of the autotrophic nitrifying biomass and the ammonia concentration as a function of depth in a downflow second-stage biological activated carbon filter (drinking water production plant of St Rose, Laval, Quebec, Canada).

completely oxidized in the first 25 cm of the filter. Similar profiles of bacterial distribution have already been measured for heterotrophic biomass fixed on filtration material [32,35].

Conclusions

A technique for the estimation of autotrophic nitrifying biomass fixed on a solid material in biological filters is proposed. This method is appropriate for the conditions encountered in the biological filters used in drinking water production, with ammonia inlet concentrations up to 2 mg N L^{-1}, and easy to use for routine measurement. Nitrifying biomass can thus be traced along biological filters. The technique is helpful for identifying the factors controlling nitrification efficiency in biological filters used in drinking water production in order to optimize the biological removal of ammonia. The estimation of fixed nitrifying biomass can be used to improve the modeling of biological filters and can lead to a better understanding of competitive effects with the heterotrophic biomass for the space and the oxygen in a mixed biofilm.

Acknowledgements

The present study was supported by Pica Company (Paris, France). Anne Kihn is a doctoral research fellow of the Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture (Brussels, Belgium).

References

1 AFNOR. 1990. Ammonia dosage by indophenol colorimetric method, #T90–015. In: Recueil de Normes Françaises. Méthode d'Essai. 4th edition (Agence Française de Normalisation, ed), Paris.
2 Amann RI, W Ludwig and KH Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59: 143–169.
3 Antoniou P, J Hamilton, B Koopman, R Jain, B Holloway, G Lyberatos and S A Svoronos. 1990. Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. Wat Res 24: 97–101.
4 APHA. 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition. American Public Health Association, Washington DC.
5 Bablon G, C Ventresque and R Ben Aïm. 1987. Evolution des techniques de filtration rapide: le bicouche sable—charbon actif. TSM-L'Eau 82: 153–160.
6 Belser L W and E L Mays. 1982. Use of nitrifier activity measurements to estimate the efficiency of viable nitrifier count in soils and sediments. Appl Environ Microbiol 43: 945–948.
7 Berounsky VM and SW Nixon. 1993. Rates of nitrification along an estuarine gradient in Narrangaset Bay. Estuaries 16: 718–730.
8 Billen G. 1976. Evaluation of nitrifying activity in sediments by dark ^{14}C-bicarbonate incorporation. Wat Res 10: 51–57.
9 Bonnet C, B Volat, R Bardin, V Degranges and B Montuelle. 1997. Use of immunofluorescence technique for studying a *Nitrobacter* population from wastewater treatment plant following discharge in river sediments: first experimental data. Wat Res 31: 661–664.
10 Bouwer EJ and PB Crowe. 1988. Biological processes in drinking water treatment. JAWWA 80: 82–93.
11 Brion N and G Billen. 1998. Une réévaluation de la méthode de mesure de l'activité nitrifiante autotrophe par la méthode d'incorporation de bicarbonate marqué au ^{14}C et son application pour estimer des biomasses de bactéries nitrifiantes. Rev Sci Eau 11: 283–302.
12 Cantor KP, R Hoover, P Hartge, TJ Mason and D Verman. 1990. Bladder cancer, tap water consumption and drinking water source. In: Water Chlorination, vol 6 (Jolley RL, ed), pp 411–419, Ann Arbor Science Publishers/The Butterworth Group, Ann Arbor, MI.
13 Carlucci AF and JDH Strickland. 1968. The isolation, purification and some kinetic studies of marine nitrifying bacteria. J Exp Mar Biol Ecol 2: 156–166.
14 Copp JB and KL Murphy. 1995. Estimation of active nitrifying biomass in activated sludge. Wat Res 29: 1855–1862.
15 Degrange V and R Bardin. 1995. Detection and counting of *Nitrobacter* in soil by PCR. Appl Environ Microbiol 61: 2093–2098.
16 Doré M. 1989. Chimie des Oxydants et Traitement des Eaux (Tec Doc, ed), Lavoisier, Paris.
17 Focht DD and W Verstraete. 1977. Biochemical ecology of nitrification and denitrification. Adv Microb Ecol 1: 135–214.
18 Hall GH. 1982. Apparent and measured rates of nitrification in the hypolimnion of a mesotrophic lake. Appl Environ Microbiol 43: 542–547.
19 Hasley C and H Leclerc. 1993. Traitements biologiques de l'eau. In: Microbiologie des Eaux d'Alimentation (Tec Doc, ed), pp 305–372, Lavoisier, Paris.
20 Helder W and RTP De Vries. 1983. Estuarine nitrite maxima and nitrifying bacteria (Ems-Dollard estuary). Neth J Sea Res 17: 1–18.
21 Houel N, A Deguin, JP Moulinot, Y Richard, M Rizet and J Sibony. 1982. Elimination des formes azotées dans les eaux alimentaires. TSM-L'Eau 6: 292–300.
22 Hovanec TA and EF DeLong. 1996. Comparative analysis of nitrifying bacteria associated with freshwater and marine aquaria. Appl Environ Microbiol 62: 2888–2896.
23 Hovanec TA, LT Taylor, A Blakis and EF Delong. 1998. *Nitrospira*-like bacteria associated with nitrite oxidation in freshwater aquaria. Appl Environ Microbiol 64: 258–264.
24 Janda V and J Rudovsky. 1994. Removal of ammonia from drinking water by biological nitrification. J Water SRT-Aqua 43: 120–125.
25 Jones MN. 1984. Nitrate reduction by shaking with cadmium, alternative to cadmium columns. Wat Res 18: 643–646.
26 Knowles G, AL Downing and MJ Barret. 1965. Determination of kinetic constants for nitrifying bacteria in mixed culture with the aid of an electronic computer. J Gen Microbiol 38: 263–278.
27 Laurent P and P Servais. 1995. Fixed bacterial biomass estimated by potential exoproteolytic activity. Can J Microbiol 41: 749–752.
28 Lehman JT, DD Botkin and CE Likens. 1975. The assumptions and estimates of a computer model of phytoplankton populations dynamics. Limnol Oceanogr 20: 343–364.
29 Matulewich VA, PF Strom and MS Finstein. 1975. Length of incubation for enumerating nitrifying bacteria present in various environments. Appl Microbiol 29: 265–268.
30 Mobarry BK, M Wagner, V Urbain, BE Rittmann and D Stahl. 1996. Phylogenic probes for analyzing abundance and spatial organization of nitrifying bacteria. Appl Environ Microbiol 62: 2156–2162.
31 Niquette P, M Prévost, RG Maclean, D Thibault, J Coallier, R Desjard-

ins and P Lafrance. 1998. Backwashing first-stage sand-BAC filters. JAWWA 90: 86–97.
32 Niquette P, M Prévost, P Servais, JF Beaudet, J Coallier and P Lafrance. 1998. Shutdown of BAC filters: effects on water quality. JAWWA 90: 53–61.
33 Owens NJP. 1986. Estuarine nitrification: a naturally occurring fluidized bed reaction?. Estua Coast Shelf Sci 22: 31–44.
34 Rittmann BE and VL Snoeyink. 1984. Achieving biological stable drinking water. JAWWA 76: 106–114.
35 Servais P, G Billen and P Bouillot. 1991. Activité biologique dans un filtre à charbon actif en grains. Rev Sci Eau 4: 43–58.
36 Servais P, G Billen, P Laurent, Y Levi and G Randon. 1992. Studies of BDOC and bacterial dynamics in the drinking water distribution system of the Northern Parisian suburbs. Rev Sci Eau 5: 69–89.
37 Servais P, P Laurent and G Randon. 1995. Comparison of the bacterial dynamics in various French distribution systems. J Water SRT-Aqua 44: 10–17.
38 Somville M. 1978. A method for the measurement of nitrification rates in water. Wat Res 12: 843–848.
39 Verstraete W and M Alexander. 1973. Heterotrophic nitrification in samples of natural ecosystems. Envir Sci Tech 7: 39–42.
40 Wagner M, G Rath, HP Koops, J Flood and R Amann. 1996. In-situ analysis of nitrifying bacteria in sewage treatment plants. Wat Sci Tech 34: 237–244.
41 Watson SW, E Bock, H Harms, H-P Koops and AB Hooper. 1989. Nitrifying bacteria. In: Bergey's Manual of Systematic Bacteriology, vol 3 (Staley MP, Bryant N, Pfenning N, Holt JG, eds), pp 1808–1834, The Williams & Wilkins Co, Baltimore.