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Review

Hydrogenotrophic denitrification of potable water: A review

K.A. Karanasios [a], I.A. Vasiliadou [a], S. Pavlou [b,c], D.V. Vayenas [a,*]

[a] Department of Environmental and Natural Resources Management, University of Ioannina, Seferi 2, 30100 Agrinio, Greece
[b] Department of Chemical Engineering, University of Patras, 26504 Patras, Greece
[c] Institute of Chemical Engineering and High Temperature Chemical Processes, 26504, Patras, Greece

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ABSTRACT

Several approaches of hydrogenotrophic denitrification of potable water as well as technical data and mathematical models that were developed for the process are reviewed. Most of the applications that were tested for hydrogenotrophic process achieved great efficiency, high denitrification rates, and operational simplicity. Moreover, this paper reviews the variety of reactor configurations that have been used for hydrogen gas generation and efficient hydrogen delivery. Microbial communities and species that participate in the denitrification process are also reported. The variation of nitrate concentration, pH, temperature, alkalinity, carbon and microbial acclimation was found to affect the denitrification rates. The main results regarding research progress on hydrogenotrophic denitrification are evaluated. Finally, the commonly used models and simulation approaches are discussed.

Contents

* Corresponding author. Tel.: +30 26410 74117; fax: +30 26410 74176.
E-mail address: dvagenas@cc.uoi.gr (D.V. Vayenas).

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1. Introduction

Worldwide an increase of nitrate concentrations observed in groundwater, as a result of the use of fertilizers, and the industrial wastewater, raises concerns due to the severe impacts on human health [1]. Research is carried out towards nitrate removal from water resources, whereas the most promising approach being studied is biological denitrification. Biological denitrification is considered to be the most economical strategy among other conventional techniques like physicochemical.

Denitrification is the respiratory process in which bacteria use nitrates or nitrites as terminal electron acceptors, while reduction of nitrates from contaminated water to nitrogen gas can occur [2]. In heterotrophic denitrification, organic carbon compounds can be used by denitrifiers as a source of biosynthetic carbon and electrons. Autotrophic denitrifiers utilize reduced inorganic compounds, such as sulfur, iron and hydrogen as electron sources and inorganic carbon for biosynthesis [3].

There is a considerable ongoing effort focused on hydrogenotrophic denitrification of drinking water, since it is a promising clean method with high efficiency. The main advantage of denitrification by hydrogen oxidation bacteria is the use of hydrogen gas as electron donor, which is harmless to humans and the inorganic carbon sources for substrate of bacteria which thereby removes any problems that are caused by residual organic carbon [4]. In addition, the growth rate of autotrophic denitrifying bacteria ensures low biomass build-up and limited operating problems. Thus, hydrogenotrophic bacteria have been successfully used for drinking water nitrate elimination to acceptable levels either in pure [4–7] or in mixed-cultures [8–15].

Hydrogenotrophic denitrification has been studied using suspended growth [16,17], fixed-bed [10,15,18] and fluidized-bed [4,8] reactors. Such experimental investigations suggest that great efficiencies with high denitrification rates can be established for long operating periods.

Operating conditions like the feed nitrate concentration [19,20] and the volumetric flow rate [21,22] appear to affect the process performance. Moreover, in an attempt to elucidate the factors controlling denitrification specified experiments have been conducted to assess the influence of hydrogen concentration [4,23], nutrient availability [6], pH [24,25], temperature [8,16] and microbial acclimation [26]. The variation of the oxidation–reduction potential and its effect on the denitrifying activity has been evaluated [14,27], as well.

An improved understanding of the factors controlling efficient hydrogen delivery is also important in the design of the in situ application of hydrogenotrophic denitrification, considering the low solubility of hydrogen gas and its possible accumulation in a closed head space, thus creating an explosive environment [28]. In such cases, investigators focus their attention on gas-permeable membranes [29,30] as microporous membranes [28], hollow-fiber membranes [5,14] and silicon tubes [31,32] in which gas mass transfer is successfully achieved and almost complete utilization of H_2 is possible. Finally, bio-electrochemical reactors (BER) in which hydrogen gas is produced by electrolysis of water has been experimentally investigated in an effort to minimize the cost of supplying the electron donor and the H_2 gas waste in the effluent [12,33–35].

Although, considerable effort has been made to improve designs for the efficient and economical removal of nitrate from water

Nomenclature

a_c	specific area of the cathode (cm^2/l)
C_{HNO_3}	nitric acid concentration (mg/l)
C_{NO_3}	nitrate nitrogen concentration (mg/l)
C_{NO_2}	nitrite nitrogen concentration (mg/l)
C_{H_2}	hydrogen concentration (mg/l)
C_{CO_2}	carbon dioxide concentration (mg/l)
$C_{NO_3^-f}$	molar concentration of nitrate (mol/l)
$C_{NO_2^-f}$	molar concentration of nitrite (mol/l)
D	diffusion coefficient (cm^2/h)
F_{NO_3}	switching function formulated: the observation that growth on nitrite occurs only at low NO_3 concentrations (mg/l)
F	Faraday's constant (C/mg)
$J_{NO_3^-}$	reduction nitrate rate (mg/cm^2 h)
$J_{NO_2^-P}$	production rate of nitrite (mg/cm^2 h)
$J_{NO_2^-R}$	reduction rate of nitrite (mg/cm^2 h)
k	maximum specific denitrification rate (mg/gVSS d)
k_d	decay rate constant (1/h)
k_{d1}	constant in growth rate expression (mg NO_2^-–N/mg NO_3^-–N)
k_{d2}	constant in growth rate expression (mg NO_3^-–N/mg NO_2^-–N)
K_{H_2I}	hydrogen saturation constant for nitrate (mg/l)
K_{H_2II}	hydrogen saturation constant for nitrite (mg/l)
K_i	nitrate inhibition constant (mg N/l)
K_m	nitrite inhibition constant (mg N/l)
K_{NH_2}	hydrogen saturation constant for nitrite (mg/l)
K_{NCO_2}	carbon dioxide saturation constant for nitrite (mg/l)
k_{NO_3}	specific NO_3 reduction rate (g N/gVSS d)
k_{NO_2}	specific NO_2 reduction rate (g N/gVSS d)
K_{NO_3}	saturation constant for nitrate (mg N/l)
K_{NO_2}	saturation constant for nitrite (mg N/l)
K_{SH_2}	hydrogen saturation constant for nitrate (mg/l)
K_{SCO_2}	carbon dioxide saturation constant for nitrate (mg/l)
m_{NO_3}	specific maintenance rate (mg NO_3^-–N/h mg biomass)
m_{NO_2}	specific maintenance rate (mg NO_2^-–N/h mg biomass)
n	stoichiometric ratio of hydrogen utilization to nitrate utilization (mg/mg)
p	potential (V)
R	universal gas constant (J/K/mg)
r_I	nitrate reaction rate (mg N/l h)
r_{II}	nitrite reaction rate (mg N/l h)
R_{NO_3}	biological nitrate utilization rate (mg/l d)
R_{H_2}	biological hydrogen utilization rate (mg/l d)
T	absolute temperature (K)
u_{mI}	maximum nitrate rate constant (mg N/l h)
u_{mII}	maximum nitrite rate constant (mg N/l h)
VSS	Volatile suspended solids concentration (mg/l)
X	cell mass concentration (mg/l)
X_e	effluent cell mass concentration (mg/l)

y_{NO_3}	growth yield coefficient on nitrate (mg biomass/mg NO_3^-–N)
y_{NO_2}	growth yield coefficient on nitrite (mg biomass/mg NO_2^-–N)
z	biofilm thikness (cm)
Z	charge number of ionic constituent (−)

Greek letters

θ	hydraulic retention time (h)
$\mu\left(C_{NO_3}\right)$	specific growth rate on nitrate (1/h)
$\mu\left(C_{NO_2}\right)$	specific growth rate on nitrite (1/h)
$\mu_{\max NO_3}$	maximum specific growth rate on nitrate (1/h)
$\mu_{\max NO_2}$	maximum specific growth rate on nitrite (1/h)
ϕ	biofilm porosity

by hydrogenotrophic denitrification [10,13,36–38], only few of the reported studies in the literature, use biological kinetic data [15–17,39,40] to facilitate the design and operation of biological nitrogen removal plants. Thus, the kinetics of this process has not been systematically investigated. Nevertheless, appropriate kinetic models have been developed for the dynamic characteristics of pure and mixed-cultures, such as zero order kinetic models [16], first or second order reactions [40], double Monod forms [8,39] and models of substitutable substrates [15,17].

The purpose of this paper is to critically review the hydrogenotrophic denitrification applications for nitrate elimination from polluted water. We summarized the traditional approaches and recent developments. The factors and mechanisms which influence the nitrate removal, the denitrification rate and the efficiency of the hydrogen-oxidizing populations are also reported extensively. The main equations and principles considered in mathematical models applied for describing the hydrogenotrophic denitrification process are presented.

2. Nitrate in water resources

Nitrate contamination of drinking water resources is a major concern, as it constitutes a threat to human health [2]. The potable water standard for nitrate recommended by the Council of European Communities [41] and the World Health Organization [42] is 11.3 mg NO_3^-–N/l, while for nitrite is 0.03 and 0.91 mg NO_2^-–N/l, respectively. The standard set by the United States Environmental Protection Agency [43] is the 10 mg NO_3^-–N/l and 1 mg NO_2^-–N/l.

2.1. Sources of nitrates

The increasingly growing of agricultural activities all over the world, make the use of fertilizers the main nitrate source of polluted water [34] and as a result about 22% of groundwater in agricultural land in Europe contains nitrate concentrations above the maximum permitted level [44]. Discharge from septic tanks and leaking sewers, atmospheric deposition and the spreading of sewage sludge and manure to land can all contribute, as well [45].

Contaminated land, such as abandoned industrial sites, are responsible for a significant amount of nitrogen in groundwater [45]. Nitrogen compounds are used extensively in industrial processes, like plastic treatment, household cleaning and pharmaceutical industry [46]. Industrial wastewaters from explosives, fertilizer [47], cellophane, and metals finishing industries [48] are reported to contain more than 1000 mg NO_3^-–N/l [2,48].

The emission of nitrogen to the atmosphere can be in its oxidized or reduced forms. These forms can be later carried in storm water and deposited resulting to groundwater pollution, however this nitrogen concentration is low and its contribution is negligible [45].

2.2. Harmful effects of nitrates

Nitrite nitrogen (NO_2^-–N) is known to be toxic for aquatic life [49], like for fish, benthic fauna, plants, and bacterioplankton [50]. A nitrate compound is not considered by itself a threat for animals or humans; however it can be converted to nitrite in the gastrointestinal tract. The nitrite reacts with the hemoglobin in blood and thus oxygen transfer to cells is inhibited. This phenomenon is called methaemoglobinemia or the blue baby syndrome [51].

In addition, receiving water containing high NO_3^-–N concentrations should be avoided since it is reported to increase the probability of non-Hodgkin's lymphoma and gastric cancer [52]. Also, scientific evidence shows that nitrate and nitrite are likely to cause mutagenesis and teratogenesis, miscarriage in pregnant women, coronary cardiac diseases, cancer of the ovaries and growth of hypertrophy of the thyroid [53].

3. Nitrate removal methods

Several treatment processes including biological denitrification, ion exchange, chemical denitrification, reverse osmosis, electrodialysis and catalytic denitrification can remove nitrates from water with varying degrees of efficiency, cost and simplicity.

3.1. Physicochemical methods

Among the physical–chemical technologies considered for NO_3^-–N removal are ion exchange [54], reverse osmosis [55], catalysis [56] and electro-dialysis [57]. However, use of these processes is limited due to high capital and energy costs and the subsequent disposal problem of large volumes of waste brine [51]. The main disadvantages of the ion exchange procedure are the charging of the treatment water with chloride ions [58] and the additional operating cost caused from the disposal requirements. An electrodialysis system requires a supply of pressurized water, a membrane stack and a direct-current power source [59]. Catalytic denitrification in some cases produces ammonia and nitrite in the treated water and as a result an additional treatment is needed [60]. Generally, the pretreatment requirement, the production of soluble materials, suspended and colloidal particles and other contaminants, the generation of concentrated wastes, as well as the pH variations and chloride exposure, limit their applicability [59].

3.2. Biological denitrification

Biological denitrification is an alternative technology, which is carried out by facultative bacteria that can use NO_3^- as a terminal electron acceptor for respiration under anoxic conditions. Reduction of NO_3^- to nitrogen gas proceeds in a four-step process: microorganisms reduce NO_3^- to NO_2^-, nitric (NO), nitrous oxide (N_2O), and finally to nitrogen gas (N_2). In the right environment, specific microorganisms have the ability to adjust their metabolism in order to catalyze the above stages and as a result to reduce nitrates. In contrast to physicochemical methods, biological denitrification offers a treatment of nitrates without a need of post-treatment or production of by-products. Furthermore, due to the use of microbial cultures biological denitrification is considered a cost-effective and friendly to the environment method for nitrate removal. On the other hand, it seems to be a slower process with lower denitrification rates compared to physicochemical methods [2,58].

3.3. Autotrophic vs. heterotrophic denitrification

There are two types of biological denitrification, the autotrophic and the heterotrophic denitrification. Heterotrophic denitrification is a process that uses various carbon compounds as energy and electron sources such as, ethanol [61], methanol [62], acetate [63], or insoluble carbon source like wheat straw [64]. The main advantages of heterotrophic denitrification are the high denitrifying rates and treatment capacity [65]. Biological denitrification of drinking water with heterotrophic microorganisms has been widely investigated, due to its efficiency and high performance. However, the residual carbon sources from this process and the potential of bacterial contamination of treated water are the main disadvantages [66].

In autotrophic denitrification bacteria use hydrogen, iron or sulfur compounds as energy source and carbon dioxide or bicarbonate as carbon source. The groups of autotrophic denitrifiers are: hydrogen oxidation bacteria, reduced sulfur oxidation bacteria and ferrous oxidation bacteria [66,67].

In sulfur-autotrophic denitrification, several sulfur compounds such as sulfide, elemental sulfide, thiosulfate, tetrathionate and sulfite are used as electron donors by microorganisms [68]. Stoichiometric equations of denitrification with sulfide and thiosulfate as electron donors are [66]:

$$14NO_3^- + 5FeS_2 + 4H^+ \rightarrow 7N_2 + 10SO_4^{2-} + 5Fe^{2+} + 2H_2O \quad (1)$$

$$8NO_3^- + 5S_2O_3^{2-} + H_2O \rightarrow 4N_2 + 10SO_4^{2-} + 4H^+ \quad (2)$$

Autotrophic denitrification with elemental sulfur has been studied extensively [69–71] and its high denitrification efficiency compares well with that of heterotrophic denitrification. On the other hand, the low solubility of sulfur compounds, the production of sulfates [72] and the use of limestone for pH adjustment limit its applicability [70,71,73].

Denitrification with iron can take place under abiotic, biotic or both conditions. The biotic process by Fe^{2+} is known to reduce nitrate to nitrite autotrophically in reduced iron environments; the nitrite produced can then be reduced abiotically [3]. Stoichiometric equations of denitrification with iron as electron donor are [74]:

$$10Fe^{2+} + 2NO_3^- + 14H_2O \rightarrow N_2 + 10FeOOH + 18H^+ \quad (3)$$

$$15Fe^{2+} + NO_3^- + 13H_2O \rightarrow N_2 + 5FeOOH + 28H^+ \quad (4)$$

The main disadvantages are the small amount of oxygen required for microbial growth [74], the long start up period and the post-treatment necessity due to the formation of ammonium.

Autotrophic denitrification with hydrogen appears to have high selectivity for nitrate removal and the lack of a harmful by-product, in contrast to the use of sulfur, makes hydrogen a promising electron donor [4]. H_2 is an excellent autotrophic choice because of its clean nature and low biomass yield, as well as that it does not persist in the treated water and no further steps are required to remove either excess substrate or its derivatives [37]. In contrast to other electron donors hydrogen is less expensive per electron-equivalent delivered for contaminant reduction [37,75].

To conclude, advantages of hydrogenotrophic denitrification over heterotrophic denitrification include: (1) lower cell yield, (2) elimination of carryover of added organic electron donor to the product water, (3) the relatively low solubility of H_2, which makes it easy to remove from the product water by air stripping and (4) the fact that there is no need for post-treatment. The main disadvantage of this procedure is that an explosive atmosphere can be created within the treatment plant by the residuals hydrogen [31,37].

4. Hydrogenotrophic denitrification

4.1. Microbiology

Denitrifiers, which belong to a biochemically and taxonomically diverse group of facultative anaerobic bacteria [76], gain energy for synthesis and maintenance due to the transfer of electrons from donor to acceptor [66]. There have been a variety of studies characterizing the microbial ecology in hydrogenotrophic denitrification systems, where bacterial populations were isolated from mixed-cultures used by hydrogenotrophic denitrifying systems.

Most of the organisms reported as hydrogen-oxidizing denitrifiers belong to bacterial genera and specifically to the class of *Proteobacteria*. Thus, *Paracoccus denitrificans* that belongs to α subclass of *Proteobacteria* is one of the most intensively studied denitrifying microorganisms [11,77–79]. Populations of *Proteobacteria* [80,81] and especially of *β-Proteobacteria* [28,81], such as *Thauera sp.* and *Hydrogenophaga sp.* [1] and *Rhodocyclus* and *Hydrogenophaga* [82] were isolated from mixed microbial communities of hydrogenotrophic reactors. Bacterial communities within hydrogenotrophic denitrifying biofilms that belonged to the classes of *Flavobacteria* [81] and *Sphingobacteria* [80] have also been reported.

More specifically, bacteria belonging to the genera *Pseudomonas* [11,40], e.g. *Pseudomonas stutzeri* [79], were observed in many reactors where hydrogen gas was used to stimulate denitrification. Bacteria belonging to the genera *Acinetobacter* [11,83] have also been reported as dominating members in hydrogen-oxidizing microbial cultures. Members of *Acinetobacter sp.* cluster have also been shown to be able to partially reduce nitrate to nitrite under anoxic conditions although under high concentration of nitrites, their nitrate reductase would also be able to catalyze the reduction of nitrites [84]. Other species like *Aeromonas sp.* and *Shewanella putrefaciens* [11], *Ochrobactrum anthropi* and *Paracoccus panthotrophus* [79] and *Acidovorax sp.* strain Ic3 and *Paracoccus sp.* strain Ic1 [83] were reported to be denitrifying bacteria isolated from a H_2-dependent denitrification reactors.

Although, the above bacteria species have been isolated from reactors where hydrogenotrophic denitrification was carried out with mixed-cultures, pure cultures have also been successfully used. Chang et al. [4] used *Alcaligenes eutrophus* to evaluate denitrification in a fluidized-bed reactor. Lee and Rittmann [5] inoculated a denitrifying system for biofilm development with *Ralstonia eutropha* (formerly classified as *Alcaligenes eutrophus*), which is known to denitrify using hydrogen as electron donor. *Alcaligenes eutrophus* was also selected for hydrogenotrophic denitrification by Ho et al. [6]. Tiemeyer et al. [85] dealt with the identification of the growth kinetics of *Ralstonia eutropha* under hydrogenotrophic conditions. Finally, the ability of a purple non-sulfur photosynthetic bacterium *Rhodocyclus sp.* to remove nitrate autotrophically when grown in a fixed-film bioreactor was tested by Smith et al. [7].

To conclude, it was observed that the above investigations on hydrogenotrophic denitrification have involved a limited bacteria species, due to the fact that a hydrogenotrophic denitrifying environment is highly selective. In order to perform, organisms must have the capacity to utilize nitrate as nitrogen source, grow with inorganic carbon under anaerobic conditions, utilize H_2 as electron donor and use nitrate as terminal electron acceptor.

4.2. Stoichiometry

During denitrification, nitrate is reduced to gaseous nitrogen in accordance with the following general equation [86]:

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O \quad (5)$$

Table 1
Stoichiometric equations of hydrogenotrophic denitrification with various carbon substrates.

Stoichiometric reaction	Reference
$NO_3^- + 3.00H_2 + 0.22CO_2 + H^+ \rightarrow 0.48N_2 + 3.35H_2O + 0.04C_5H_7O_2NP_{0.2}$	[89]
$NO_3^- + 2.82H_2 + 0.139CO_2 + H^+ \rightarrow 0.486N_2 + 3.223H_2O + 0.0278C_5H_7O_2N$	[26]
$NO_3^- + 3.03H_2 + H^+ + 0.229H_2CO_3 \rightarrow 0.477N_2 + 3.6H_2O + 0.0458C_5H_7O_2N$	[24]
$0.33NO_3^- + H_2 + 0.34H^+ + 0.08CO_2 \rightarrow 0.16N_2 + 1.11H_2O + 0.015C_5H_7O_2N$	[28]
$NO_3^- + 3.03H_2 + H^+ + 0.229CO_2 \rightarrow 0.477N_2 + 3.37H_2O + 0.0458C_5H_7O_2N$	[37]

The stoichiometry of the reactions of denitrification with hydrogen as the electron donor is given in [37,87]:

$$\text{Nitrate reduction } NO_3^- + H_2 \rightarrow NO_2^- + H_2O \quad (6)$$

$$\text{Nitrite reduction } NO_2^- + H^+ + 0.5H_2 \rightarrow NO_{(g)} + H_2O \quad (7)$$

$$\text{Nitric oxide reduction } 2NO_{(g)} + H_2 \rightarrow N_2O_{(g)} + H_2O \quad (8)$$

$$\text{Nitrous oxide reduction } N_2O_{(g)} + H_2 \rightarrow N_{2(g)} + H_2O \quad (9)$$

Overall denitrification reaction from NO_3 to N_2

$$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_{2(g)} + 6H_2O \quad (10)$$

Here, each mole of NO_3^- reduced to N_2 gas consumes one acid equivalent (H^+). Therefore, 1 mg of NO_3^-–N would theoretically use 0.357 mg of hydrogen gas (Eq. (10)). The mass consumption ratio of hydrogen to nitrogen for nitrate reduction is 0.14 mg H_2/mg N (Eq. (6)), while the ratio for nitrite reduction is 0.21 mg H_2/mg N (Eqs. (7)–(9)). The equation shows that the pH will increase after the reaction, because 1 mole of H^+ is used when 1 mole of NO_2^- is converted to nitrogen gas (Eq. (7)). The second reaction produces base (or alkalinity) at a ratio of 1 base equivalent per N equivalent, or 3.57 mg as $CaCO_3$/mg N [24]. The release of alkalinity occurs when nitrite (NO_2) is reduced to nitric oxide (NO) (Eq. (7)). Increasing the alkalinity can increase the pH in the system, which might affect bacterial metabolism or cause precipitation of mineral deposits.

Under autotrophic growth conditions, carbon dioxide or bicarbonate are used as a carbon source for microbial cell synthesis. Stoichiometric equations of hydrogenotrophic denitrification with various carbon sources that have been reported in the literature are listed in Table 1. In addition, the stoichiometry for bacteria cell synthesis with nitrate as nitrogen source and inorganic carbon is as follows [26,88]:

$$0.04NO_3^- + 0.18CO_2 + 1.04H^+ + e^- \rightarrow 0.04C_5H_7O_2N + 0.39H_2O \quad (11)$$

where electrons in the above reaction are supplied by hydrogen.

Based on the equations in Table 1, the cell yield takes values of 0.22 g cells/g NO_3^-–N [26] and 0.37 g cells/g NO_3^-–N [24,28,37] which are lower than the 0.60–0.90 g cells/g NO_3^-–N typically reported for heterotrophic denitrification [13]. The equation given by Ghafari et al. [26] (Table 1) shows that hydrogenotrophic denitrification is carried out using 2.82 mol H_2 and 0.14 mol CO_2 per mol nitrate. Namely, according to the equations in Table 1 the hydrogen theoretical demand is reported to be from 0.40 mg H_2/mg NO_3^-–N [26] to 0.43 mg H_2/mg NO_3^-–N [24,28,37]. Moreover, the process requires 0.44 mg CO_2 per mg NO_3^-–N (C:N = 0.12) [26] to 0.76 mg CO_2 per mg NO_3^-–N (C:N = 0.21) [28] and 1.01 mg H_2CO_3 per mg NO_3^-–N (C:N = 0.20) [24]. Although, it is observed that low amounts of nutrients are required for the process, aiming to acclimatize and cultivate denitrifiers, higher doses of carbon and electron donor should be applied to provide abundance of supply and prevent any possible deficiency.

4.3. Factors controlling denitrification

4.3.1. Nitrate concentration

The effects of nitrate concentration on various hydrogenotrophic systems have not been systematically investigated; however, it seems that the findings vary. Chang et al. [4] reported that the reactor performance at high nitrate concentration was not inhibited, while the bacteria were able to handle the high nitrate nitrogen loadings. Park et al. [19] varied the initial nitrate concentration in a range from 20 to 492 mg NO_3^-–N/l in order to investigate the nitrate reduction rate. Their data show that the nitrate removal rate increased as the initial nitrate loading increased, while nitrite accumulation was observed. Similar results were observed by Park et al. [1] with the initial nitrate concentration ranging from 20 to 150 mg NO_3^-–N/l.

Zhou et al. [20] observed that at initial nitrate concentrations of the order of 10 mg NO_3^-–N/l, complete removal was achieved, while at higher nitrate concentrations above 30 mg NO_3^-–N/l an inhibition appeared to take place in the denitrification process. More intense nitrite accumulation and higher peak concentration occurred by the presence of high initial nitrate concentration. Moreover, Vasiliadou et al. [17] found that the rate of hydrogenotrophic denitrification was inhibited at high nitrate concentrations (above 40 mg NO_3^-–N/l), while the nitrite concentration remained at very low values.

4.3.2. pH

The hydrogenotrophic denitrification process is positively related to pH, with an optimum value in the range of 7.6–8.6 [16,24,25,90,91]. However, due to the different hydrogenotrophic cultures used and to the variability of operating conditions, many researchers [8,20,92] indicate that the optimum pH is about 7.5–7.6, whereas denitrification is inhibited or nitrite accumulation is observed above this value.

An increase of the pH value above 8.6 can cause nitrite accumulation and a significant decrease in the nitrate removal rate [8,24,93]. Moreover, low pH values like 7 [93] or below [25] can also inhibit the denitrification reaction. At pH below 7 the decomposition of carbonate ions and carbon dioxide stripping, can strongly affect the hydrogenotrophic denitrification process [16]. Hydrogenotrophic denitrification at pH as low as 5.4 has been shown to be feasible, with carbon dioxide being injected to a fixed-film reactor [10], although low denitrification rates were observed. As a result pH adjustment or carbon supplies were considered to be necessary during denitrification process.

In order to avoid pH rise and to increase denitrification efficiency phosphate buffers were used by many researchers [14,15,92,94]. The pH in experiments reported by Lee and Rittmann [5,37] was held nearly constant between 7.0 and 7.2 by a strong phosphate buffer. Thus, the limited pH increase is attributed to the fact that the biological reactors are well buffered [40].

It must be noted that application of high phosphate buffer concentration in a biofilm reactor can lead to a decrease in the denitrification rate, due to the mineral precipitation which leads to changes in biofilm density. On the other hand, introducing carbon

dioxide and avoiding any additional chemical, allows pH control without the above risks [26,32,95].

Ho et al. [6] demonstrated that nitrate could be reduced effectively with no nitrite accumulation when carbon dioxide was applied, while the pH of the bioreactor remained at about 7. However, when bicarbonate was supplied to the biofilm, nitrite accumulated critically, since the formation of alkalinity raised the pH of the bioreactor to 9.5. On the other hand, Ghafari et al. [26] showed that carbon dioxide gas manipulates the pH and drops it to the acidic range of 5.5–6, while bicarbonate as carbon source provides a buffered environment which helps pH control. Finally, Jha and Bose [96] demonstrated a different method where pyrite was effective in controlling pH, with no detrimental effect on the denitrification process by consuming the hydroxide ions produced.

4.3.3. Temperature

The optimum temperature for denitrification is between 25 and 35 °C, while due to the bacteria capacity to survive in extreme environmental conditions, denitrification processes can occur in the range 2–50 °C [97]. Most of the temperature values applied in studies on hydrogenotrophic denitrification varied between 10 and 30 °C. The lower temperatures were chosen based on the average temperature of groundwater [10,16], while higher temperatures were used to allow the growth and good performance of the hydrogenotrophic cultures [15,28,98].

Experimental evidence suggests that temperature affects the denitrification process by affecting bacteria behavior. Kurt et al. [8] demonstrated that reaction rates in a fluidized-bed reactor were doubled for every 10 °C increase in temperature (according to Arrhenius rate law). A maximum for the denitrification rate was found at 42 °C, although denitrification was observed at temperatures below 10 °C. Another study reported by Rezania et al. [16], showed that the denitrification rate increased as temperature increased from 12 to 25 °C. Finally, Zhou et al. [20] suggested that the suitable temperature range was 30–35 °C, since increasing the temperature from 25 to 35 °C nitrate removal also increased, while at 25 °C high nitrite accumulation was observed. A further increase above 35 °C led to lower nitrate removal rates.

4.3.4. Hardness – alkalinity

Hardness and alkalinity are known to have a negative impact on denitrification process. Dries et al. [36] studied the effect of hardness on the denitrification process. They used fixed-bed reactors to treat different types of polluted water: 'hard' water with 317.5–375 mg $CaCO_3$/l and 'soft' water with 145–165 mg $CaCO_3$/l. It was observed that after a period of few weeks 'hard' water treatment stopped due to the precipitation of $CaCO_3$ which created operating problem such as clogging of pores. As a conclusion, the denitrification rate was inhibited by high concentration of $CaCO_3$, since no problem occurred with the 'soft' water treatment.

Using the stoichiometric equation of Lee and Rittmann [24] (Table 1) it is realized that one equivalent of alkalinity is produced per mol of NO_3^- which reduced to N_2. Alkalinity added by denitrification can be removed through precipitation of $CaCO_{3(s)}$. Except from $CaCO_{3(s)}$ precipitation, biomass synthesis can also remove carbonate from solution, while precipitation plays the most important role. The net change in alkalinity is negative for systems with a high carbonate buffer and high pH, as the alkalinity removal by precipitation is more prominent [24].

An increase in alkalinity can increase the pH of a system, which might affect bacterial metabolism or cause precipitation of mineral deposits. Production of alkalinity may have a greater impact in a biofilm than in a well-mixed liquid reactor, because precipitation of mineral solids during the denitrification process might limit the mass transfer and decrease biomass activity [24,99,100].

4.3.5. Oxidation–reduction potential

Rezania et al. [16] reported that at oxidation–reduction potential or ORP below −250 mV, hydrogen can be consumed by several bacteria such as methanogenic, sulfate-reducing, or homoacetogenic. In contrast, at higher ORP, namely, above −50 mV, under anoxic conditions, the activity of sulfate-reducing and methanogens is limited by the presence of nitrate.

Islam and Suidan [101] using a bio-electrochemical reactor noticed that hydrogen and nitrate concentration affected the ORP. Sakakibara and Nakayama [27] observed a variation in ORP levels in a bio-electrochemical reactor in which at the cathode zone the ORP dropped below −400 mV and at the anode zone increased. This was caused by the H_2 and O_2 formation creating highly reducing and oxidizing zones at the cathode and anode, respectively. Mo et al. [14] observed that full denitrification was achieved when the oxidation–reduction potential (ORP) was stable between −230 and −120 mV. They also observed that the ORP increased when the nitrate loading rates increased, resulting to incomplete denitrification with residual of nitrates in the reactor.

Sakakibara et al. [33] reported that the ORP decreased when the hydraulic retention time increased in a biofilm electrode reactor. Generally, when hydrogen concentration increases the ORP decreases [101], while an increase of nitrate concentration leads to an increase of ORP [19].

4.4. Hydrogen concentration

Chang et al. [4] reported that the critical limit for dissolved hydrogen concentration appeared to be 0.2 mg/l. Incomplete denitrification occurred when the dissolved hydrogen concentration fell below 0.2 mg/l, during which the nitrite concentration increased. Nitrite and nitrate reductases were inhibited at a hydrogen concentration lower than 0.2 and 0.1 mg/l, respectively, as nitrite reductase is more sensitive than nitrate reductase. Nevertheless, high liquid-phase hydrogen concentrations between 1.1 and 1.4 mg H_2/l have been reported by many researchers [8,15,36]. Karanasios et al. [23] reported that complete nitrate nitrogen removal was achieved with hydrogen concentrations varying from 0.4 to 0.8 mg/l.

Celmer et al. [95] studied the possibility of controlling the process rates, as well as biofilm parameters by supplying limited amounts of electron donor (hydrogen) in a membrane biofilm reactor for autotrophic denitrification of wastewater. They demonstrated that limitation of the hydrogen availability inhibited not only the removal rate but also growth of the biofilm. However, limiting the hydrogen supply proved to be efficient in controlling the biofilm growth and consequently the performance of the fiber membrane biofilm.

Lee and Rittmann [37] reported that the most important factor in controlling denitrification efficiency is hydrogen pressure. They noted that 100% nitrate removal was achieved in a hollow-fiber membrane biofilm reactor when hydrogen pressure increased from 0.45 to 0.56 atm. Rezania et al. [102] reported that dissolved hydrogen concentration in a submerged membrane bioreactor ranged between 0.2 and 0.55 mg/l, while complete denitrification was achieved even when low hydrogen concentrations (0.001 mg/l) were observed at the effluent. Haugen et al. [40] observed a decrease of hydrogen concentration from 0.1–0.2 to 0.0004 mg/l when biological activity increased, in a membrane reactor. During this decrease nitrite accumulation occurred.

4.5. Carbon source

As mentioned above (Section 4.2) the theoretical carbon demand for complete hydrogenotrophic denitrification is 0.20 mg C (in the form of bicarbonate) (Table 1) [24] and 0.12–0.21 mg C (in

the form of carbon dioxide gas) (Table 1) [26,28] per mg NO_3^-–N converted to nitrogen gas. These mass ratios are low enough however higher ratios were used by researchers to ensure that carbon was not rate-limiting during the process of culture acclimation.

The bicarbonate–carbon to nitrate–nitrogen ratio used by Mansell and Schroeder [28] and Visvanathan et al. [22] in order to enrich their hydrogen-oxidizing cultures was 2:1 to ensure that carbon was not a limiting nutrient based on the stoichiometry (C:N = 0.21). It must be noted that high ratio of C/N may lead to nitrite accumulation or extra production of nitrous other than nitrogen gas [103]. In contrast, a low C/N ratio leads to incomplete denitrification [104,105].

Ghafari et al. [26] studied the acclimation of autohydrogenotrophic denitrifying bacteria by using two inorganic carbon sources (CO_2 and bicarbonate) and hydrogen gas as electron donor. They observed that bicarbonate as the only carbon source showed a faster adaptation, while the use of carbon dioxide resulted in longer acclimation period.

Usually, after the cultivation of microorganisms, the investigators try to find the optimum operating condition with regard to carbon supplies. Ghafari et al. [26] observed that bicarbonate is more appropriate for a faster growth and adaption, however, a combination of bicarbonate and carbon dioxide has the ability to develop enough denitrification capacity. In addition, Ghafari et al. [91] reported that the optimum bicarbonate concentration from a range 20 to 2000 mg/l was 1100 mg $NaHCO_3$/l for an initial nitrate concentration of 20 mg NO_3^-–N/l, providing a mass ratio of 7.85 mg C/mg NO_3^-–N. However, experiments conducted by Karanasios et al. [23] showed that completed nitrate and nitrite removal was achieved with a mass ratio of only 0.504 mg C/mg NO_3^-–N, while dissolved carbon dioxide concentration ranged from 0.6 to 1.1 g/l.

Ho et al. [6] varied the carbon dioxide concentration as hydrogen and carbon dioxide flowed together into a lumem side of a gas-permeable silicone tube. The maximum rate of nitrogen removal occurred when the carbon dioxide ranged from 20% to 50% of the total gas volume sparged in the reactor.

5. Trialed reactor technologies

Due to the low biomass yield of hydrogenotrophic denitrifiers, most research conducted on hydrogenotrophic denitrification has been with attached growth systems. Attached growth systems, have lower space requirements and especially lower capital and operating costs compared to suspended biomass reactors. Researchers used this technology like fixed- and fluidized-bed reactors, membranes and biofilm electrode reactors providing a support surface area for biofilm growth (high biomass concentration), thus allowing the possibility of maintaining bacteria at high hydraulic and nitrate loadings.

A number of configurations and operating conditions have been tried by many researchers in an effort to achieve high performances of hydrogenotrophic denitrification and reduce operating problems. Advantages and drawbacks of traditional and new technologies, as well as the concerns regarding the use of hydrogenotrophic denitrification are also analysed in detail in the following sections.

5.1. Fixed-bed reactors

The support media is considered to be the main parameter for the design of a packed-bed reactor. Characteristics of support media such as shape, size or material type have great influence on the performance of the system. Size and shape determine the porosity and the specific surface area, respectively. The specific surface area concerns the available surface for bacteria growth and porosity determines biofilm thickness and pore clogging. As a result, reactor performance and efficiency are mainly determined by the support media.

Dries et al. [36] used a dual-column reactor, which was comprised of a down flow fixed-bed for the first column and an upflow column for the second bed, to study the performance of hydrogenotrophic denitrification. The H_2 was supplied to the reactor by direct bubbling of H_2 gas in the down flow column. Three types of polyurethane sponge matrixes were used as the biofilm carrier. For water containing 15 mg NO_3^-–N/l, removal rates of 0.25 kg NO_3^-–N/m^3 d were reached at 21 °C. Gros et al. [10] constructed a full-scale biological drinking water denitrification plant of nine reactors packed with polypropylene carrier. The nitrate removal was 0.25 kg NO_3^-–N/m^3 d with initial nitrate nitrogen concentration of 17 mg/l.

Park et al. [1] used glass beads as support media to treat different initial concentrations in the range of 20–150 mg NO_3^-–N/l, while the highest nitrate removal rate achieved was 0.225 kg N/m^3 d. A cheap and effective installation using silicic gravel as support media was proposed by Vasiliadou et al. [15]. The size of the support media was found to drastically affect denitrification efficiency. Using a triple-column reactor, high nitrate concentrations up to 340 mg NO_3^-–N/l were treated giving a denitrification rate of 6.2 kg N/m^3 d. Grommen et al. [21] reported a low rate of 0.036 kg N/m^3 d using ceramic cylinders, while longer hydraulic retention time was needed to achieve complete denitrification.

Most of the studies reported here used the conventional method for hydrogen supply, namely, external tank for H_2 gas absorption and H_2 sparged directly in the bioreactor. On the other hand, alternative methods for hydrogen diffusion have been proposed. Haugen et al. [40] in order to determine the technical feasibility of in situ hydrogenotrophic denitrification developed a flow-through reactor packed with aquarium rocks with H_2 fed of silicone hollow-fiber membranes. Complete denitrification of 16.34 mg NO_3^-–N/l was achieved with a velocity of 0.3 m/d.

Lu et al. [106] used a tank in which hydrogen was diffused via gas-permeable membrane and water was hydrogenated. Afterwards, the hydrogenated water was introduced in the fixed-bed reactor. Szekeres et al. [38,79] use an alternative mode. The hydrogen was produced in an electrolysis cell and subsequently was introduced in a fixed-bed reactor. Hydrogen production, generated from anoxic corrosion of metallic iron was tested by Sunger and Bose [107]. The hydrogenated water from the hydrogen generation system was mixed with nitrate solution in the mixer bottle and introduced in the fixed-bed reactor. Grommen et al. [21] generated hydrogen gas with a two-compartment electrolytic cell containing two plain perforated nickel electrodes, while hydrogen was supplied through the top of the reactor. Vagheei et al. [108] produced in situ hydrogen and carbon dioxide by the electrolysis of methanol. Fixed-bed reactors were used in which gas entered from the bottom of the reactor. Finally, the performance of a triple packed-bed reactor with hydrogen produced from electrolysis of water and electric power provided by a solar cell was investigated by Karanasios et al. [23]. The use of inexpensive support media as well as the use of systems for cheap hydrogen production can make the hydrogenotrophic denitrification economically viable for potable water treatment.

Operating conditions and apparatus information of several studies in autohydrogenotrophic denitrification using fixed-bed reactors are listed in Table 2. The limitations associated with the use of fixed-bed attached growth systems are the difficulty in biofilm control, the limited mass transfer and the decreasing biomass activity due to thick biofilm formation [14]. Experimental data showed that the use of the appropriate support media is of crucial importance for hydrogenotrophic denitrification, since it determines the extent of biofilm development as well as pore clogging. In addition,

the operating conditions (nitrate nitrogen concentration, volumetric flow rate) combined with a well constructed configuration can enhance bioreactor performance.

A comparison between fixed-bed and suspended growth reactors shows clearly that attached growth systems achieve higher denitrification rates (Table 2). Specifically, Vasiliadou et al. [15] achieved the highest denitrification rate compared to other fixed-bed reactor processes (1.53–6.2 kg NO_3^-–N/m^3 d). However in a previous study [17] by using the same mixed culture in a suspended growth reactor, the denitrification rate was as low as 0.076 kg NO_3^-–N/m^3 d. Other researchers using suspended growth reactors in a sequencing batch mode (Table 2) have also reported very low denitrification rates in contrast to fixed- and fluidized-bed reactors [16,26,90,91].

5.2. Fluidized-bed reactors

The use of a fluidized-bed reactor may solve the problems of packed-bed reactors, such as clogging and channeling, which may threaten its stable operation of the reactor. However, although there are several studies on hydrogenotrophic denitrification reported in the literature, only few of them have been conducted with fluidized-bed reactors. Different materials, like spherical beads or sand, in various sizes have been used to investigate denitrification in this type of reactor (Table 2).

Kurt et al. [8] studied autotrophic denitrification in a cone-shaped fluidized sand-bed reactor using a mixed culture. H_2 was transferred to the reactor using a bubbling-absorption tank in the recycle line. Batch experiments in this study exhibited nitrite accumulation, but continuous experiments resulted in complete N removal. For complete denitrification of water containing 25 mg NO_3^-–N/l, a residence time of 4.5 h was required, while a nitrate elimination rate of 0.13 kg NO_3^-–N/m^3 d was achieved.

Another similar configuration was used by Chang et al. [4] who studied the immobilized bacteria species *Alcaligenes eutrophus*, in a polyacrylamide and alginate copolymer to evaluate denitrification in continuous and batch mode. The maximum rate was 0.6–0.7 kg N/m^3 d and nitrite accumulation was affected by the phosphate concentration. Komori and Sakakibara [98] used a fluidized-bed reactor equipped with a solid-polymer-electrolyte membrane electrode (SPEME) for the efficient production and dissolution of hydrogen, using polyvinylalcohol (PVA) porous cubes as a biofilm carrier. Denitrification rate up to 2.16 kg N/m^3 d, was achieved.

Despite the fact that high denitrification rates are achieved in fluidized-bed reactors (Table 2) in order to ensure fluidization of the bed the upflow velocities must be high resulting in a very short retention time. This may lead to insufficient nitrate elimination [66]. For that reason recirculation of effluent is often used [8] making the process performance more complicated and difficult to control [66].

5.3. Membrane biofilm reactors

To date, a variety of reactor configurations have been used for efficient hydrogen delivery. Many of the reviewed systems have the same H_2 provision scheme (gas sparging) either in a separated hydrogen saturation tank [8,10,106] or directly to the reactor [15,36]. The main limitation of hydrogen-driven denitrification is the low solubility of hydrogen gas resulting in low-mass transfer rate and possible accumulation of hydrogen gas in a closed head space thus creating an explosive environment [28].

Many researchers have demonstrated effective hydrogenotrophic denitrification with gas-permeable membranes, which were used to enhance the efficiency of hydrogen delivery and limit explosion risks through the bubble-less introduction of hydrogen [13,37]. The investigators focus their attention on gas-permeable membranes because they can act as both the hydrogen diffuser and the biofilm carrier. Thus, membrane selection is a critical factor for the performance of this technology. Gas-permeable membranes are mainly composite membrane (e.g., sandwich structure) [5], polypropylene [13], polysulfone [92], platinum cured silastic [110] and silicone coated ferro-nickel slag [31]. Membrane biofilm reactors minimize the cost of supplying electron donor, because almost 100% utilization of H_2 is possible. Furthermore, the retention time is minimized due to the counter-current diffusion which allows high fluxes of nitrate and H_2.

The most common type of membrane which used is hollow-fiber membrane due to the fact that it has lower space requirements than other types of membranes and can achieve high performances. Several studies have been carried out with different materials of hollow-fiber membranes (Table 3). Ergas and Reuss [13] operated a polypropylene hollow-fiber membrane bioreactor, to study the performance of hydrogenotrophic denitrification of contaminated drinking water. Denitrification rates of up to 2.49 g N/m^2 d (0.77 kg NO_3^-–N/m^3 d) were achieved with an influent NO_3^- concentration of 145 mg NO_3^-–N/l and a hydraulic residence time of 4.1 h. Lee and Rittmann [5,37] used a polyethylene/polyurethane hollow-fiber membrane achieving removal rates of 1.27–2.07 and 0.63–1.6 g N/m^2 d, respectively. Zhang et al. [82] reported a high rate of 1.5 g N/m^2 d by using a polyvinyl chloride hollow-fiber membrane. Mo et al. [14] and Rezania et al. [93] used a different microporous hollow-fiber membrane (Celgard), and achieved high denitrification rates of 2.87 and 14.2 g N/m^2 d, respectively. Smith et al. [110] reported a rate of 4.4 g N/m^2 d by using a platinum cured silastic hollow-fiber membrane. Shin et al. [111] used a hollow-fiber membrane reactor with multi-layered composite fiber and attained a removal rate of up to 1.72 g N/m^2 d.

Hollow-fiber membranes are typically employed as gas-permeable membranes, although silicon tubes have been tested as well [6,31]. Hydrogen flows through the lumen and diffuses into the bulk liquid through the membrane walls. Ho et al. [6] used such a membrane of silicone achieving a high denitrification rate of 1.6–5.4 g N/m^2 d. In another study reported by Sahu et al. [80] the membrane was gas-permeable microporous hydrophobic with its lumen side was coated with perfluoropolymer. A removal rate of 0.22–5.88 g N/m^2 d was achieved (Table 3).

Membranes offer high specific surface area and nitrate removal efficiencies, but they have high cost, due to the operating cost and the cost of membrane cleaning because of clogging. For instance, the precipitation of mineral solids during the denitrification process might have a long-term negative impact on the operation of a hollow-fiber membrane bioreactor, which increases its operating cost [24]. The operating cost of membranes is a major problem as a consequence of the energy consumption for the operation, while the cost for replacement of the membranes due to the fouling represents another cost of the process. The cleaning of membranes can be done in two ways: physical and chemical, with the chemical cleaning having the additional cost from the use of chemicals.

In addition, there is a dependency of hydrogen diffusion and biofilm growth in a permeable performance of membrane. The two processes interact with each other, leading to poor stability of the denitrification system and difficulty of biomass control. As a result, the transfer of hydrogen to the bulk liquid is impeded decreasing the zone of influence around the membranes [112].

In an effort to enhance the performance of the denitrification process in a fiber membrane biofilm reactor Celmer et al. [95] applied limited amounts of hydrogen in order to control the parameter named biofilm. They observed that biofilm density was a more important factor for the process operation than the biofilm thickness. In another study Celmer et al. [113] tried to estimate the

Table 2
Operating conditions of fluidized-, fixed-bed and suspended growth reactors.

Reactor system/operation	Working volume (m^3)	Carbon source	T (°C)	HRT (hrs)	Carrier	Influent concentration (mg NO_3^-–N/l)	Denitrification rate NO_3^-–N (kg/m^3 d)	Reference
Suspended growth/sequencing batch	3.5×10^{-3}	$NaHCO_3$	12–25	3.5–1.3	–	20	0.11–0.37	[16]
Suspended growth/sequencing batch	2.5×10^{-3}	CO_2 and $NaHCO_3$	25 ± 5	3–11	–	20–50	0.16–0.11	[26]
Suspended growth/sequencing batch	4×10^{-3}	$NaHCO_3$	25 ± 5	4.5	–	20 as NO_2^-–N	0.12	[90]
Suspended growth/sequencing batch	4×10^{-3}	$NaHCO_3$	25 ± 5	4.5	–	20	0.11	[91]
Suspended growth/draw-fill and batch	2×10^{-3}	CO_2	30 ± 1	25 and 25–170	–	80 and 7–200	0.076 and 0.007–0.028	[17]
Suspended growth/batch	1.2×10^{-3}	CO_2	30	14–26	–	168–329	0.28–0.3	[85]
Fixed-bed/continuous	N/A[a]	CO_2	10	1	Polypropylene carrier	17	0.25	[10]
Fixed-bed/continuous	4.2×10^{-3}	Carbonic acid	12–20	1.42–5.11	Polyurethane carrier (d:0.56–2.19 mm) SSA[b]: 20.57–4.88 cm^2/cm^3	15–50	0.25–0.2	[36]
Fixed-bed/continuous	N/A	CO_2	N/A	N/A	Lamellar reticulated polyurethane	16–18	N/A	[11]
Fixed-bed/continuous	0.27×10^{-3}	$NaHCO_3$	25–27	1	Granulated activated carbon (d: 0.85–1.70 mm)	21–27	0.25	[38,79]
Fixed-bed/continuous	7×10^{-3} (total)	HCO_3^-	20	97.6	Aquarium rocks (d: 0.3–1.0 cm)	16.4	0.004	[40]
Fixed-bed/continuous	0.45×10^{-3}	CO_2	18–23	2	Pea gravel (d: 2–4 mm)	28	0.343	[7]
Fixed-bed/batch	0.9×10^{-3}	$NaHCO_3$	30	16	Glass beads (d: 5 mm)	150	0.225	[1]
Fixed-bed/batch	6.5×10^{-3}	$NaHCO_3$	24 ± 1	12	Hollow ceramic cylinders (d: 1 cm) and polyurethane sponges	20	0.036	[21]
Fixed-bed/draw fill and continuous	0.250×10^{-3} and 0.75×10^{-3}	CO_2	27 ± 2	0.16–1.25	Silicic gravel (d: 1.75–4 mm) SSA: 32.07–14.16 cm^2/cm^3	10–340	1.53–6.2	[15]
Fixed-bed/continuous	0.187×10^{-3}	N/A	N/A	374	Sand (d: 1–2 mm)	N/A	0.027	[107]
Fixed-bed/sequencing batch	4.71×10^{-3}	CO_2	27 ± 3	3	Hollow cylindrical media, total surface area: 1.46 m^2	43	0.342	[106]
Fixed-bed/continuous	4.1×10^{-3}	CO_2	18–23	2–5	Light expanded clay aggregates (d: 3–5 mm)	27	0.3387	[108]
Fixed-bed/continuous	0.75×10^{-3}	CO_2	26 ± 1	1.25	Silicic gravel (d: 1.75–4 mm) SSA: 32.07–14.16 cm^2/cm^3	100	2	[23]
Fixed-bed/continuous	2.5×10^{-3}	$NaHCO_3$	23 ± 1	2	Polyurethane sponge (side: 1.2 cm)	22	2.419	[109]
Fluidized-bed/continuous	0.72×10^{-3}	CO_2	30	4.5	Sand (d: 0.2–0.3 mm)	25	0.13	[8]
Fluidized-bed/batch and continuous	0.8×10^{-3}	$NaHCO_3$	30	0.88	Polyacrylamide-alginate copolymer spherical beads (d: 3–5 mm)	22–25	0.6–0.7	[4]

[a] N/A: Not Available,.
[b] SSA: Specific surface area.

Table 3
Operating conditions and denitrification rates of Membrane biofilm reactors.

Process	Type/material	Working volume (m^3)	Carbon source	Surface area (cm^2)	HRT (h)	Pore size (μm)	Gas flow	Influent concentration (mg NO_3^-–N/l)	Denitrification rate (kg N/m^3 d)	Denitrification rate (g N/m^2 d)	Ref.
Continuous	Hollow-fiber/polyethylene and polyurethane	0.42×10^{-3}	$NaHCO_3$	750	0.7	N/A	0.31–0.42 (H_2:atm)	10–12.5	0.228–0.37	1.27–2.07	[5]
Continuous	Tube/silicone	1.5×10^{-3}	CO_2	588.75	8.33	N/A	20 ml H_2/min 0–20 ml CO_2/min	120	0.063–0.211	1.6–5.4	[6]
Batch	Hollow-fiber/polypropylene potted in polysulfone fittings	1.2×10^{-3}	CO_2	3700	4.1	0.05	28 (H_2:kPa)	145	0.77	2.49	[13]
Continuous	Hollow-fiber/polyethylene and polyurethane	0.42×10^{-3}	$NaHCO_3$	750	0.7	N/A	0.2–0.45 (H_2:atm)	5–15	0.23–0.505	0.63–1.6	[37]
Continuous	Membrane/polytetrafluoroethylene	0.02×10^{-3}	HCO_3^-	N/A	N/A	0.02	N/A	20–40	N/A	2.7–5.3	[28]
Continuous	Hollow-fiber/Celgard® X30–240 microporous and ZeeWeed®-1	7×10^{-3}	$NaHCO_3$	55.6	9–12	0.04	N/A	12–72	0.024–0.192	1.76–2.87	[14]
Sequencing batch	Hollow-fiber/Celgard® and ZeeWeed®-1	8.1×10^{-3}	$NaHCO_3$	56 and 470	9–12 48–81.6	0.04	0.28–0.55 (H_2:atm)	330	0.56–0.046	8.2–14.2	[93]
Continuous	Hollow-fiber/polysulfone	0.075×10^{-3}	$NaHCO_3$	1300	6	N/A	N/A	50–150	0.83–2.48	0.48–1.43	[92]
Continuous[a]	Hollow-fiber/ZeeWeed®-1	5.6×10^{-3}	N/A[a]	940	3	0.04	120 (H_2:psi)	33	0.14	8.34	[102]
Continuous	Membrane/matrix of poly (dimethylsiloxane, silicone)	0.35×10^{-3}	CO_2	163 SSA: 47 m^2/m^3	7.78–14.6	N/A	20–50 (H_2:kPa) 50 (CO_2:kPa)	100	0.164–0.306	3.53–6.58	[31]
Continuous[a]	Membrane	3×10^{-3}	CO_2	N/A	4–5	N/A	18 ml H_2/min 1.5 ml CO_2/min	15–25	N/A	0.50–0.59	[95]
Continuous	Hollow-fiber/platinum cured silastic	2.2×10^{-3}	$KHCO_3$	582	24.48	N/A	0.60 (H_2:psi)	10–30	0.12	4.4	[110]
Continuous	Hollow-fiber/Zeeweed-1 (by Zenov Env. Inc.)	5.6×10^{-3}	$NaHCO_3$	940	3	0.04	120 (H_2:psi)	25	0.11	6.55	[114]
Continuous[a]	Membrane/polypropylene fibers	3×10^{-3}	N/A[a]	N/A	4	N/A	10 ml H_2/min	20	N/A	0.93–1.20	[113]
Continuous	Hollow-fiber/polyethylene	6.5×10^{-3}	$NaHCO_3$	8143	6–10	N/A	N/A	50	0.118–0.22	0.95–1.72	[111]
Continuous[b]	Hollow-fiber/polyethelene	1.25×10^{-3}	$NaHCO_3/CO_2$	4200	2–9	0.1	0.4–0.5 (H_2:bar)	50	0.104–0.380	0.309–1.13	[22]
Continuous	Hollow-fiber/polyvinyl chloride	0.045×10^{-3}	$NaHCO_3$	124	0.625	0.01	0.04 (H_2:MPa)	10	0.414	1.50	[82]
Continuous	Hollow-fiber/non porous	1.6×10^{-3}	N/A	1140	7–18	N/A	2.5 (H_2:psi)	30	0.0434–0.0598	0.61–0.84	[94]
Continuous	Tubular membrane/perfluoropolymer coating	0.07×10^{-3}	$NaHCO_3/CO_2$	2800	1.5–6.7	N/A	10 ml H_2/min	40–50	0.88–23.52	0.22–5.88	[80]

[a] Wastewater treatment plant effluent.
[b] Aquaculture wastewater.

impact of shearing stress by nitrogen sparging and different levels of reactor mixing, on the biofilm structure. Biofilm thickness was reduced by increasing levels of mixing and shearing stress. Experimental data accordingly indicated that denitrification rate improved when biofilm density increased as a result of increase in the shearing force and decrease in biofilm thickness.

5.4. Bio-electrochemical denitrification

Researchers have recently proposed a bio-electrochemical reactor (BER) in which autotrophic denitrification is stimulated with the passing of electric current. Biofilm electrode reactors consist of a couple of electrodes [12], in which denitrifying bacteria are cultured on the cathode surface. In a BER, the following reactions take place:

$$0.5O_2 + 2e^- + H_2O \rightarrow 2OH^- \quad (12)$$

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (13)$$

$$2.5C + 5H_2O \rightarrow 2.5CO_2 + 10H^+ + 10e^- \quad (14)$$

After dissolved oxygen is completely utilized (Eq. (12)), hydrogen gas is produced on the surface of the cathode by electrolysis of water (Eq. (13)) and autotrophic denitrifying microorganisms are directly immobilized on this electrode. The process is highly selective for the reduction of nitrate to nitrogen gas with simultaneous neutralization by carbon dioxide (Eq. (14)) at the anode [34,35].

Such a reactor configuration which proposed by Sakakibara and Kuroda [12], addresses effective hydrogen delivery and has been used by many researchers (Table 4) [19,27,35,101,115]. Although, different electrode materials have been reported in the literature, they did not report to affect denitrification efficiency. Thus, an anode electrode can be composed of amorphous carbon [116], titanium coated with platinum [35] or modified β-PbO_2 [20], while cathode electrodes can consist of carbon [101,117], graphite felt [19] and stainless pipe [33].

Islam and Suidan's [101] long-term study showed a stable nitrate removal rate at 0.8 g NO_3^-–N/m^2 d of electrode surface. However, the liquid retention time (10–13 h) was high, mainly due to low specific surface area (42 m^2/m^3). Wang and Qu [73] using a BER with an electrode reaction area of 321 cm^2, achieved a higher denitrification rate (0.381 kg NO_3^-–N/m^3 d) than Park et al. [81] (0.077–1.68 kg NO_3^-–N/m^3 d) who used a surface area of only 105 cm^2, indicating that this is an important factor which affect the performance of the process.

The advantage of this process is the easy operation and maintenance; however, the denitrification rates are low. Thus, longer hydraulic retention time (HRT = 10 h to several days) is needed to achieve complete denitrification [12,101,117–119].

Sakakibara and Nakayama [27] proposed a multi-electrode system which showed great potential, since the HRT was reduced to about 2 h. This superior performance was attributed to the large effective surface area and the formation of a low ORP zone in the multi-cathode region. However, the denitrification rate was still lower than that those in cases of external feeding of hydrogen gas, where H_2 was dissolved in a pressurized hydrogen saturator or supplied directly in the biofilm reactor (Table 2). It must be noted that, the main drawback of biofilm electrode reactors is the gradual scale formation on the surface of the cathode, suppressing hydrogen production, which causes a dramatic decrease in the denitrification rate [115].

Another concern regarding the use of BERs is that excess biomass leaves the process, and calls for an additional treatment. Since suspended solids escape from a BER, it is necessary to incorporate a solid/liquid separator into the process. A multi-cathode BER combined with microfiltration (MF) was proposed by Prosnansky et al. [35]. The multi-cathode electrodes were composed of multiple-granular activated carbons (GACs). Since some suspended solids were escaping from the BER, a MF membrane with plate modules and a pore size of 0.2 μm was placed after the BER. Microfiltration was chosen by Prosnansky et al. [35] for this goal because of production of high-quality water and simple operation. Experimental results demonstrated that it was possible to operate the multi-cathode BER with high denitrification rates and HRT as low as 20 min. The denitrification rate was enhanced compared with previous studies of the BER.

The bio-electrochemical reactor might be a solution to the problem of high cost of the hydrogen supplies needed during the hydrogenotrophic denitrification. However, the low nitrate removal rates, the longer hydraulic retention times and the escaping biomass as main disadvantages, limit its applicability.

5.5. Effluent water quality

Waters' quality at the effluent of a denitrifying reactor is an important factor for effective denitrification. More specifically, the concentration of organic carbon plays a significant role in the denitrification process and in the quality of the treated water. An increase of the total organic carbon (TOC) across the length of the reactor is expected due to the production of soluble microbial products by the microbial reactions [22,122]. Lee and Rittmann [5] observed an increase of dissolved organic carbon (DOC) from 1.4 mg/l in the influent to 2.3 mg/l in the effluent of the bioreactor. An increase of 1.7 mg DOC/l due to the detachment of biomass from biofilm, was reported by Zhang et al. [82]. Ergas and Reuss [13] reported that TOC increased by 20–25 mg/l from the influent to the effluent of the reactor due to the sloughing of biomass. Haugen et al. [40] observed a small increase of the TOC about 0.5 mg/l. This low-effluent TOC resulted from biomass transport through the material which served as biomass carrier and filter. Schnobrich et al. [32] also noticed that the aquifer material seems to be quite effective in TOC removal from the water.

Mo et al. [14] suggest that an additional treatment step is required, as the DOC in the effluent was about 8 mg/l. The same observation was made by Rezania et al. [102] in a system of wastewater treatment. The produced water met all drinking water guidelines [123], e.g. total coliforms, except for color and organic carbon (17 mg COD/l). To reduce the organic carbon and color of the effluent, post-treatment is required. Experimental data of Rezania et al. [114] showed that the TOC was similar to that of the feed water (6 mg/l), however no volatile suspended solids were observed in the effluent. When nitrate-contaminated water contains low levels of organic carbon, low-effluent DOC can be expected. Generally, color, DOC and suspended solids can be reduced by post-treatment technologies as granular activated carbon [102], microfiltration membranes [35] or by the own support material itself of the bioreactor [32,40].

5.6. Industrial scale applications

In an effort to make the hydrogenotrophic denitrification economically viable and effective for potable water treatment experimental experience was applied, in order to design and operate industrial scale applications. However field studies are very limited due to the difficulties of the possibility of an explosive environment by accumulation of hydrogen and the high cost of the hydrogen supplies needed.

Ginocchio [124] in Switzerland used hydrogen as electron donor for in situ denitrification in which contaminated water was withdrawn from the aquifer, add hydrogen, carbon dioxide and phosphate to it and then was reinjected back into the aquifer. Gros et al. [10] demonstrated the performance of the

Table 4
Operating conditions and denitrification rates of biofilm electrode reactors (BER).

Process	Material	Working volume (m^3)	Carbon source	Temp (°C)	HRT (h)	Electrode surface area (cm^2)	Electric current (mA)	Influent concentration (mg NO_3^-–N/l)	Denitrification rate (kg N/m^3 d)	Denitrification rate (g N/m^2 d)	Ref.
Batch	Cathode: carbon	2.4×10^{-3}	CO_2	25–30	N/A	Cathode: 520	0–40	10	0.1968	0.38	[12]
Continuous	Anode: carbon cathode: stainless	0.205×10^{-3}	CO_2	25	9	Anode: 160 cathode: 251	2.5	15	0.038	N/A	[33]
Batch	N/A	2.4×10^{-3}	CO_2	25–30	N/A	Cathode: 520	5–40	140–420	N/A	0.28–1.93	[39]
Continuous	Anode amorphous carbon cathode: stainless	0.205×10^{-3}	CO_2	25	10–50	Cathode: 251	1–10	20–24	0.01–0.045 0.048_{max}	N/A	[116]
Continuous	Anode amorphous carbon cathode: stainless	0.205×10^{-3}	CO_2	20–30	10	Cathode: 251	5	20	0.06	2.39	[117]
Continuous	Anode and cathode: carbon	N/A	$NaHCO_3$	N/A	10–13	Cathode: 42 (m^2/m^3)	20	20	0.035	0.8	[101]
Continuous	2 anodes: Pt metal coated 8 cathodes: metal	36×10^{-3}	$NaHCO_3$	25 ± 3	2–6	Cathode: 1096	80–960	13.8–20.8	0.12	N/A	[27]
Continuous	Anode amorphous carbon cathode: stainless	0.2×10^{-3}	CO_2	N/A	10	Anode: 160 cathode: 251	0–10	24	0.0576	0.470	[34]
Continuous	Anode: titanium coated with platinum cathode: five electrodes with granular activated carbon	0.6×10^{-3}	CO_2	N/A	0.33	Anode: 150 cathode: 750	300	15	0.393	3.15	[35]
Continuous	Anode: carbon cathode: stainless steal	0.52×10^{-3}	CO_2	30	1.9–5	Cathode: 321	3–16	30	0.381	0.43	[73]
Batch	Anode dimensionally stable cathode: graphite felt	1×10^{-3}	$NaHCO_3$	30	N/A	Cathode: 105	200	20–492	0.077–1.68	1.7	[19,81]
Continuous	Anode: modified β-PbO_2 cathode: activated carbon fiber	N/A	CO_2	25–40	2.4–6	Cathode: 500	15	10–50	N/A	2.22	[20]
Continuous	Anode: stainless steel mesh cathode: granular palm shell activated carbon	0.8×10^{-3}	$NaHCO_3/CO_2$	N/A	6–36	N/A	0–20	20	0.013–0.08	N/A	[120]
Batch[a]	Anode: ploutinized titanium rod cathode: ploutinized titanium rod	3×10^{-3}	CO_2	24 ± 1	48	N/A	10–80	27–44.15	N/A	N/A	[121]

[a] Significance of 'Aquaculture wastewater'.

first commercial-scale biological drinking water denitrification plant utilizing hydrogen at Rasseln near Monchengladbach, Germany. Named the Denitropur process, this plant consisted of nine upflow, fixed-bed denitrification reactors in a series and packed with Mellapack, which is a mixing element (made of polypropylene) with a three-dimensional corrugated structure. The raw water (groundwater) was saturated with hydrogen under overpressure and enriched with phosphate and carbon dioxide. After denitrification, the water was aerated and filtered on a two-layer filter. Disinfection was ensured by means of UV radiation. The 50 m^3/h facility eliminated nitrate from 17 to less than 1 mg NO_3^-–N/l within a residence time of water in the reactors of about 1 h. The nitrate removal rate was 0.250 kg NO_3^-–N/m^3 d.

Recently, Chaplin et al. [30] developed a technology to stimulate autotrophic denitrification using gas-permeable membranes in order to supply hydrogen in groundwater. The study took place in Becker, Minnesota where there were high levels of NO_3^- (23 mg N/l). Membranes installed in groundwater wells were successful in delivering H_2 to the groundwater over the 2-year operating period. They observed that the depth of groundwater (13.7 m) caused reoxygenation of water during recirculation and as a result this technology is not suitable for use at deep sites.

6. Denitrification kinetic models

Mathematical models of hydrogenotrophic denitrification generally consider denitrification as a two-step process occurring by the consecutive reduction of nitrates to nitrites and then to nitrogen gas. The most commonly used approach to describe the behavior denitrifying bacteria in the presence of nitrate is the dependence of the bacterial activity on nitrate/nitrite with a Monod type expression. Mathematical models used in the literature to describe hydrogenotrophic denitrification are listed in Table 5. According to these models types, nitrate and nitrite reduction rates are dependent on their concentrations, on biomass concentration, as well as on dissolved hydrogen concentration.

Specifically, Kurt et al. [8] studied autotrophic denitrification kinetics considering denitrification as a two-step process. The kinetics was expressed in a double Monod form and NO_3, NO_2, and H_2 were assumed to be the limiting substrates (Table 5). A steady-state mathematical model for an electrochemically activated denitrifying biofilm was developed by Sakakibara et al. [39]. A double Monod mathematical model was used, as well, to describe the rates of nitrate and hydrogen utilization with kinetic parameters taken from the literature. Park et al. [19] used a Monod type expression to describe the dependence of the nitrate reduction rate on nitrate concentration.

As shown in Table 5 Tiemeyer et al. [85] used Monod expressions with nitrite inhibition and switching function for bacteria growth on nitrite. The specific growth rate was assumed to be the sum of the specific growth rates with nitrate and nitrite as limiting substrates. It was assumed that increasing nitrite concentration inhibits the total growth rate. Visvanathan et al. [22] also used a Monod equation to describe nitrate and biomass effluent variation of a membrane denitrification system. Finally, a double Monod expression was employed by Lu et al. [106] to describe the two-step hydrogenotrophic denitrification process, and the saturation constants of nitrate, nitrite and hydrogen were determined by batch experiments.

The kinetics of the hydrogenotrophic denitrification process was extensively studied in batch experiments by Vasiliadou et al. [17]. The growth kinetics could be very well described by using expressions for double nutrient limitation (nitrate, nitrite). Thus, a model of substitutable substrates with inhibition from nitrate was proposed as listed in Table 5. Nitrate inhibition was modeled by an Andrews-type expression. In a subsequent study, the growth kinetics of pure cultures of hydrogen-oxidizing denitrifying bacteria used by Vasiliadou et al. [83] included nitrite inhibition expressions and consumption of nitrates and nitrites for cell maintenance requirements in the form of maintenance rates (Table 5).

The kinetics of hydrogen-oxidizing denitrifying bacteria has been also examined [77,78]. Haring and Conrad [77] determined the kinetics of H_2 oxidation of a denitrifying species. Pseudo-first-order rate constants were determined from the logarithmic decrease of H_2. The kinetics for hydrogen uptake during denitrification was determined by Smith et al. [78] for nine isolated hydrogen-oxidizing denitrifiers. Experimental data indicate that consumption of hydrogen followed Monod kinetics rather than a first-order transfer of hydrogen. Finally, Tiemeyer et al. [85] presented a kinetic study on autohydrogenotrophic growth of *Ralstonia eutropha*.

Values of several kinetic parameters that are reported in the literature are listed in Table 6. The maximum specific growth rates for nitrate and nitrite that are listed in Table 6 vary between 0.0023 [22] to 0.155 [83] (1/h) and 0.00813 [85] to 0.917 [15] (1/h), respectively. The difference in the parameter values between different studies is due to the different conditions and different hydrogenotrophic culture used. Although, high values of saturation constants have been reported, [17,19,23], very low values appeared as well. Saturation constants of 0.18 and 0.16 mg N/l were reported for nitrate and nitrite, respectively [8]. The values of nitrate saturation constants determined by Visvanathan et al. [22] and Lu et al. [106] were 0.0001 and 2.09 mg N/l, respectively. In another study, the reported hydrogen saturation constant ranged from 0.0009 to 0.0066 mg H_2/l [78]. With such low saturation constants, many researchers assumed that the kinetics of nitrate and nitrite reduction are independent of nitrate, nitrite, and hydrogen concentrations. For example, Rezania et al. [16] considered a zero order kinetic model to describe the hydrogenotrophic denitrification based on the assumption that the saturation constants of nitrate, nitrite, and hydrogen are so low that their influences on denitrification could be neglected.

A zero order type kinetic model was also proposed for NO_3^- and NO_2^- reduction by Lu and Gu [25]. Haugen et al. [40] performed kinetic experiments in batch mixed-cultures from soil. They estimated pseudo-first- and second-order rate constants for NO_3 and NO_2 reduction and concluded that these constants were drastically affected by the number of microorganisms present in the soil-derived enrichment culture. Ghafari et al. [91] developed a zero order kinetic model, where kinetic constants were estimated for different hydrogen supplies.

In contrast to traditional approaches for description of the denitrification process, some simplified or empirical expressions have been proposed. A kinetic expression that takes into account the sequential reduction of nitrate and inhibition of the N_2O reduction step by toxic pesticide was developed by Feleke and Sakakibara [34] and used to evaluate the process performance of a BER. A simplified mathematical model with nitrate molecular diffusion through a microporous membrane into the denitrifying culture was proposed by Mansell and Schroeder [28]. A second order polynomial model was generated by Ghafari et al. [120] in order to obtain the sufficient electric current and hydraulic retention time in a BER. The same model was used in a subsequent study [90] to describe nitrite reduction rates in relation to pH values and sodium bicarbonate dosage.

It must be noted that certain approaches may have inherent weaknesses that should be addressed. As shown in Table 5 many of the models proposed for hydrogenotrophic denitrification did not include biomass concentration, because it was assumed biomass concentration to be constant during the process of nitrate or nitrite elimination. As a result, the influence of biomass growth and activ-

Table 5
Kinetic models for hydrogenotrophic denitrification for nitrate and nitrite elimination.

Model type	Mathematical model		Reactor	Reference
	Nitrate reduction	Nitrite reduction		
Zero order	$\frac{dC_{NO_3}}{dt} = -k_{NO_3} \cdot X$	$\frac{dC_{NO_2}}{dt} = (k_{NO_3} - k_{NO_2}) \cdot X$	Suspended growth	[16,91]
Monod	$\frac{dC_{NO_3}}{dt} = -\frac{u_{mI} \cdot C_{NO_3}}{C_{NO_3} + K_{NO_3}}$		BER	[19]
Monod	$\frac{dX}{dt} = \left(\frac{\mu_{max\,NO_3} \cdot C_{NO_3}}{K_{NO_3} + C_{NO_3}}\right) \cdot X - k_d \cdot X - \frac{1}{\theta} \cdot X_e$		Membrane	[22]
Double Monod	$r_I = \frac{u_{mI} \cdot C_{NO_3} \cdot C_{H_2}}{(C_{NO_3} + K_{NO_3})(C_{H_2} + K_{H_2I})}$	$r_{II} = \frac{u_{mI} \cdot C_{NO_3} \cdot C_{H_2}}{(C_{NO_3} + K_{NO_3})(C_{H_2} + K_{H_2I})} - \frac{u_{mII} \cdot C_{NO_2} \cdot C_{H_2}}{(C_{NO_2} + K_{NO_2})(C_{H_2} + K_{H_2II})}$	Fluidized-bed, suspended growth with hollow cylindrical media	[8,106]
Double Monod	$\frac{Z_{NO_3} - FD_{NO_3}}{RT} \frac{d}{dz} \cdot (C_{NO_3} \frac{dp}{dz}) + D_{NO_3} \cdot \frac{d^2 C_{NO_3}}{dz^2} + D_{HNO_3} \cdot \frac{d^2 C_{HNO_3}}{dz^2} - R_{NO_3} = 0$ $R_{NO_3} = \frac{1}{n} \cdot R_{H_2} = \frac{k \cdot X \cdot C_{NO_3} \cdot C_{H_2}}{(C_{NO_3} + K_{NO_3})(C_{H_2} + K_{H_2I})}$		BER	[39]
Monod with switching function for nitrite growth	$\frac{dC_{NO_3}}{dt} = -\frac{1}{y_{NO_3}} \cdot \frac{\mu_{max\,NO_3} \cdot C_{NO_3} \cdot X}{K_{NO_3} + C_{NO_3}}$	$\frac{dC_{NO_2}}{dt} = \frac{1}{y_{NO_3}} \cdot \frac{\mu_{max\,NO_3} \cdot C_{NO_3} \cdot X}{K_{NO_3} + C_{NO_3}} - \frac{1}{y_{NO_2}} \cdot \frac{\mu_{max\,NO_2} \cdot C_{NO_2} \cdot X}{K_{NO_2} + C_{NO_2}} \cdot \frac{F_{NO_3}}{F_{NO_3} + C_{NO_3}}$	Suspended growth	[85]
Substitutable substrates with nitrate inhibition	$\frac{dC_{NO_3}}{dt} = -\frac{1}{y_{NO_3}} \cdot \frac{\mu_{max\,NO_3} \cdot C_{NO_3} \cdot X}{K_{NO_3} + C_{NO_3} + k_{d2} \cdot C_{NO_2} + \frac{C_{NO_3}^2}{K_i}}$	$\frac{dC_{NO_2}}{dt} = \frac{1}{y_{NO_3}} \cdot \frac{\mu_{max\,NO_3} \cdot C_{NO_3} \cdot X}{K_{NO_3} + C_{NO_3} + k_{d2} \cdot C_{NO_2} + \frac{C_{NO_3}^2}{K_i}} - \frac{1}{y_{NO_2}} \cdot \frac{\mu_{max\,NO_2} \cdot C_{NO_2} \cdot X}{K_{NO_2} + C_{NO_2} + k_{d1} \cdot C_{NO_3}}$	Suspended growth	[17]
Substitutable substrates with nitrate inhibition	$-D_{NO_3} \cdot \phi \cdot \frac{\partial^2 C_{NO_3}}{\partial z^2} + \phi \cdot \frac{\partial C_{NO_3}}{\partial t} + \frac{1}{y_{NO_3}} \cdot \mu(C_{NO_3}) \cdot X = 0$ $\mu\left(C_{NO_3}\right) = \frac{\mu_{max\,NO_3} \cdot C_{NO_3}}{K_{NO_3} + C_{NO_3} + k_{d2} \cdot C_{NO_2} + \frac{C_{NO_3}^2}{K_i}}$	$-D_{NO_2} \cdot \phi \cdot \frac{\partial^2 C_{NO_2}}{\partial z^2} + \phi \cdot \frac{\partial C_{NO_2}}{\partial t} + \frac{1}{y_{NO_2}} \cdot \mu(C_{NO_2}) \cdot X - \frac{1}{y_{NO_3}} \cdot \mu(C_{NO_3}) \cdot X = 0$ $\mu\left(C_{NO_2}\right) = \frac{\mu_{max\,NO_2} \cdot C_{NO_2}}{K_{NO_2} + C_{NO_2} + k_{d1} \cdot C_{NO_3}}$	Fixed-bed	[15]
Substitutable substrates with nitrate inhibition and Double Monod	$-D_{NO_3} \cdot \phi \cdot \frac{\partial^2 C_{NO_3}}{\partial z^2} + \phi \cdot \frac{\partial C_{NO_3}}{\partial t} + \frac{1}{y_{NO_3}} \cdot \mu(C_{NO_3}) \cdot X = 0$ $\mu(C_{NO_3}) = \frac{\mu_{max\,NO_3} \cdot C_{NO_3}}{K_{NO_3} + C_{NO_3} + k_{d2} \cdot C_{NO_2} + \frac{C_{NO_3}^2}{K_i}} \cdot \frac{C_{H_2}}{K_{SH_2} + C_{H_2}} \cdot \frac{C_{CO_2}}{K_{SCO_2} + C_{CO_2}}$	$-D_{NO_2} \cdot \phi \cdot \frac{\partial^2 C_{NO_2}}{\partial z^2} + \phi \cdot \frac{\partial C_{NO_2}}{\partial t} + \frac{1}{y_{NO_2}} \cdot \mu(C_{NO_2}) \cdot X - \frac{1}{y_{NO_3}} \cdot \mu(C_{NO_3}) \cdot X = 0$ $\mu(C_{NO_2}) = \frac{\mu_{max\,NO_2} \cdot C_{NO_2}}{K_{NO_2} + C_{NO_2} + k_{d1} \cdot C_{NO_3}} \cdot \frac{C_{H_2}}{K_{NH_2} + C_{H_2}} \cdot \frac{C_{CO_2}}{K_{NCO_2} + C_{CO_2}}$	Fixed-bed	[23]
Substitutable substrates with nitrate and nitrite inhibition	$\frac{dC_{NO_3}}{dt} = -\frac{1}{y_{NO_3}} \cdot \frac{\mu_{max\,NO_3} \cdot C_{NO_3} \cdot X}{K_{NO_3} + C_{NO_3} + k_{d2} \cdot C_{NO_2} + \frac{C_{NO_3}^2}{K_i}} - \frac{m_{NO_3} \cdot C_{NO_3} \cdot X}{K_{NO_3} + C_{NO_3} + k_{d2} \cdot C_{NO_2} + \frac{C_{NO_3}^2}{K_i}}$	$\frac{dC_{NO_2}}{dt} = \frac{1}{y_{NO_3}} \cdot \frac{\mu_{max\,NO_3} \cdot C_{NO_3} \cdot X}{K_{NO_3} + C_{NO_3} + k_{d2} \cdot C_{NO_2} + \frac{C_{NO_3}^2}{K_i}} - \frac{1}{y_{NO_2}} \cdot \frac{\mu_{max\,NO_2} \cdot C_{NO_2} \cdot X}{K_{NO_2} + C_{NO_2} + k_{d1} \cdot C_{NO_3} + \frac{C_{NO_2}^2}{K_m}} - \frac{m_{NO_2} \cdot C_{NO_2} \cdot X}{K_{NO_2} + C_{NO_2} + k_{d1} \cdot C_{NO_3} + \frac{C_{NO_2}^2}{K_m}}$	Suspended growth	[83]
Completely mixed flow reactor model	$\frac{C_{NO_3^-f} - C_{NO_3}}{\theta} - a_c \cdot J_{NO_3^-} = 0$	$\frac{C_{NO_2^-f} - C_{NO_2^-}}{\theta} + a_c \cdot J_{NO_2P} - a_c \cdot J_{NO_2R} = 0$	BER	[34]

Table 6
Values and units for saturation constants, maximum specific growth rates, growth yield coefficients and zero order kinetic constants for the models used in hydrogenotrophic denitrification simulation.

K_{NO_3} mg N/l	K_{NO_2} mg N/l	K_{H_2} l mg H_2/l	u_{ml} mg N/l h	u_{mll} mg N/l h	$\mu_{max\,NO_3}$ 1/h	$\mu_{max\,NO_2}$ 1/h	y_{NO_3} mg biomass/mgNO_3^-–N	y_{NO_2} mg biomass/mgNO_2^-–N	k_{NO_3} g N/gVSS d	k_{NO_2} g N/gVSS d	Reference
									0.21–0.74	0.25–1.70	[16]
		0.0009–0.0066							0.037–0.051[a]	0.013[a]	[40]
									0.33–0.60	0.37–0.45	[25]
									0.623–0.710	0.707–0.836	[91]
											[78]
28.63	4.79				0.0485	0.55	0.4207	0.084			[17]
0.5–8.82	0.778–28.45				0.0876–0.155	0.455–0.868	0.719–1.077	0.0047–1.467			[83]
37.8	42.98				0.0212	0.00813	2.055	1.497			[85]
2.09	1.55	0.059	11.06 and 14.12	3.07 and 4.86							[106]
0.18	0.16	<0.002									[8]
317.39			434.78								[19]
0.0001					0.0023		0.345				[22]
9.1	39.1				0.0115	0.917	0.132	0.00806			[15]
8.3	38.4	25.3			0.152	0.834	0.128	1.06×10^{-3}			[23]

[a] First-order degradation coefficient (1/h).

ity on the rates of nitrate and nitrite reduction was not considered [8,19,106] and this could lead to significantly erroneous predictions. On the other hand, some growth kinetics were described by using expressions dependent only on constant biomass concentration with no influence from nutrients concentration [16].

Another limitation of the majority of modeling approaches of attached growth processes is that the equations for the nutrient concentrations in biofilm reactors do not include nitrate and nitrite diffusion from the bulk liquid to the biofilm. For example, Kurt et al. [8] made the assumption that biofilm diffusion effects do not influence the kinetics of all substrates. However, the steady-state mathematical model which was developed by Sakakibara et al. [39] expressed the flux of species in an electrochemically-activated biofilm under the electric field by diffusion terms. The values of nitrate, hydrogen and carbon dioxide diffusion coefficients were reported to be 0.0683, 0.2104 and 0.0691 (cm^2/h), respectively. Lee and Rittmann [37] developed a model with mass balances for nitrate and nitrite in the biofilm and an expression for hydrogen transfer rate from the hollow-fiber membrane into the biofilm. Also, Vasiliadou et al. [15] by using specific growth expressions based on those proposed by Vasiliadou et al. [17] represented nitrate and nitrite diffusion, from the bulk liquid to the biofilm, with diffusion terms in the mass balance-equations. Thus, the nitrate and nitrite were assumed to be consumed only inside the biofilm. They also showed that for the simulation of the denitrification process in a fixed-bed biofilm reactor the computed values of the kinetic parameters were different from those of a suspended growth system [17], due to the changes in the bacterial activity during fixation (Table 6). Finally, a mathematical model was developed by Karanasios et al. [23] using diffusion and growth kinetic expressions for four-nutrient limitation (nitrate, nitrite, hydrogen and carbon dioxide) with inhibition by nitrate. Hydrogen and carbon dioxide were considered as complementary nutrients together with nitrate or nitrite, while their influence was modelled by Monod expressions.

In conclusion, a mathematical model must be reliable and simple, so that it can be easily used for the design of the appropriate reactor configurations for the hydrogenotrophic denitrification of potable water. Mathematical models must be able to predict concentration variations of the basic nutrients, as nitrate, nitrite, hydrogen and carbon source, as well as the biomass growth. Finally, for the satisfactory description of the hydrogenotrophic denitrification process, reduction rates of nitrate and nitrite should be dependent on their concentrations as well as the concentration of biomass and dissolved hydrogen and carbon concentration.

7. Conclusions

Several methods of treatment have been applied in the past and results showed that biological denitrification is more beneficial than physicochemical methods. Hydrogenotrophic denitrification appears to have advantages in regard to the use of other electron donors in autotrophic and heterotrophic denitrification.

In this survey were examined in detail the factors that affect the hydrogenotrophic denitrification process. The main conclusions are:

- The effect of fed NO_3^-–N concentration varies. Nitrate concentrations up to 492 mg NO_3^-–N/l were reported to increase denitrification rate. In contrast, other researchers found that denitrification was inhibited for nitrate concentrations above 30 mg NO_3^-–N/l.
- The optimum pH for hydrogenotrophic denitrification ranges from 7.6 to 8.6. The pH rise can lead to nitrite accumulation and to decrease of the nitrate removal rate.

- The most suitable temperature range is 25–35 °C, however higher values as 42 °C were reported as well.
- Alkalinity introduced by denitrification and water hardness affected bacterial metabolism, caused precipitation of mineral deposits and created operating problem.
- Carbon and hydrogen concentrations were generally reported to be higher than the theoretical demands.

Furthermore, trialed reactor technologies for denitrification were presented. The analysis of the critical points of each configuration showed that fixed-bed and membrane biofilm reactors achieve high performances. Nevertheless, each one of the developed technologies can be used in relation to the characteristics of the water supplied for treatment and the economics of the process.

Based on the studies, a number of different mathematical approaches have been proposed to model the hydrogenotrophic denitrification process in suspended or attached growth reactors. Several of these modeling approaches have inherent weaknesses which are often overlooked by their users. However, some mathematical models that were developed and applied were able to describe all the main processes in a hydrogenotrophic denitrifying application, such as the consumption of nitrates, nitrites, carbon and hydrogen and biomass build-up.

Even though significant progress has been made so far in the study of hydrogenotrophic denitrification, further research is needed. An area that requires further study in view of cost minimization and high efficiency is the appropriate reactor design, including the selection of the best material for attached growth reactors, i.e., the one with the highest specific surface area, and the appropriate gas diffusion into the bioreactor, which will overcome the limitation of low solubility and the danger of explosion from hydrogen.

In addition, hydrogen production appears to be a significant economical factor for the viability of denitrification. Producing hydrogen with energy provided from renewable energy resources is a technology of the future and several in situ methods could be applied to reduce the cost and make the hydrogenotrophic denitrification economically viable for potable water treatment.

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