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A kinetic study of hydrogenotrophic denitrification

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Abstract

The progress of hydrogenotrophic denitrification in batch assays with a mixed culture was examined. The aim was to study the performance of the culture for hydrogenotrophic denitrification of drinking water at various NO_3^- concentrations. The kinetics of the process were studied in batch experiments. The rates of the process were found to be inhibited at high nitrate concentrations. The nitrite concentration remained at very low values throughout the process but it still affected biomass growth. A kinetic model was developed and its kinetic parameters were determined from the batch experiments. The growth kinetics could be very well described by using expressions for double nutrient limitation (nitrate, nitrite) with inhibition from nitrate. The proposed model is capable of describing accurately enough cellular growth, nitrate and nitrite utilization and utilization rates under anoxic conditions in the presence of various concentrations of nitrate in the range of 7 to about 200 mg N/l.

Keywords: Hydrogenotrophic denitrification; Mixed cultures; Bioreactors; Kinetic parameters; Batch processing; Modelling

1. Introduction

Removing nitrate (NO_3^-) and nitrite (NO_2^-) from drinking water has gained great attention in recent years because of the human health risks induced from ingestion of nitrate-contaminated groundwater and surface water. A significant fraction of groundwater currently used for municipal water supplies exceeds the European Community standards (50 mg NO_3^-/l and 0.5 mg NO_2^-/l) [1]. Denitrification is the biological process that reduces NO_3^- to NO_2^-, to nitric oxide (NO), to nitrous oxide (N_2O) and finally to nitrogen gas (N_2) [2,3]:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Denitrification is carried out by facultative bacteria that can use NO_3^- as a terminal electron acceptor for respiration under anoxic conditions. Since drinking water always has a very low concentration of biodegradable organic materials, reduction of nitrate (or nitrite) requires addition of an electron donor substrate. There are many organic (heterotrophic denitrification) and a few inorganic (autotrophic denitrification) possible electron donors [4].

Biological denitrification of drinking water with heterotrophic microorganisms has been widely reported [5–9], but the residual carbon sources from these processes cause many problems in drinking water treatment [10].

Both hydrogen gas and various reduced-sulphur compounds can be used as alternative electron donors for autotrophic denitrification because they are less harmless to human health over heterotrophic systems, and no further steps are needed to remove either excess substrate or its derivatives [10–12]. The low cost of these inorganic substrates and low formation of biomass are important advantages, although their application can also present some limitations [13].

Elemental sulphur as an electron donor for autotrophic denitrification systems has been studied extensively [12,14,15], and the high denitrification efficiency can be compared with that of the heterotrophic denitrification in the nitrate-contaminated water treatment. However, the production of sulphates [13], and the use of limestone for pH adjustment limit its applicability [14–16].

H_2 is an excellent autotrophic choice because of its clean nature, low biomass yield and relatively low cost, as well as because it does not persist in the treated water to create biological instability and no further steps are required to

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remove either excess substrate or its derivatives [2]. Previous studies on autohydrogenotrophic denitrification of drinking water are limited [2,10,11,17–23], since H_2 forms flammable and explosive mixtures with air, is poorly soluble in water (1.6 mg/l at 20 °C) [20] and results in lower denitrification rates compared to heterotrophic denitrification [19]. These properties have limited its use in water treatment. Kurt et al. [11] studied autotrophic denitrification in a cone-shaped fluidized sand-bed reactor using a mixed culture and concluded that for complete denitrification of water containing 25 mg NO_3^-–N/l, a residence time of 4.5 h was required; it achieved a nitrate elimination rate of 552 g NO_3^-–N/(m^3 day). Dries et al. [18] used a dual-column reactor, which comprised of a downflow fixed bed for the first column and an upflow bed for the second column, to study the performance of autohydrogenotrophic denitrification. For water containing 15 mg NO_3^-–N/l, removal rates of 500 g NO_3^-–N/(m^3 day) were reached at 20 °C. Gross et al. [17] demonstrated the performance of the first commercial-scale biological drinking water denitrification plant utilizing hydrogen. 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 50 m^3/h facility reduced nitrate from 17 to less than 1 mg/l of NO_3^-–N with a residence time of water in the reactors of about 1 h. The nitrate removal rate was 250 g NO_3^-–N/(m^3 day). More recently, Ergas and Reuss [19] operated a hollow fibre membrane bioreactor (HFMB), to study the performance of hydrogenotrophic denitrification of contaminated drinking water. Denitrification rates of up to 770 g NO_3^-–N/(m^3 day) were achieved with an influent NO_3^- concentration of 145 mg NO_3^-–N/l and a hydraulic residence time of 4.1 h.

Despite reported studies on hydrogenotrophic denitrification in the literature, kinetics of this process were not systematically investigated. Kurt et al. [11] studied autotrophic denitrification kinetics considering denitrification as a two-step process occurring by the consecutive reduction of nitrates to nitrites and then to nitrogen gas. The kinetics were expressed in a double Michaelis–Menten (Monod) form and NO_3^-, NO_2^- and H_2 were assumed to be the limiting substrates. A steady-state mathematical model for an electrochemical-activated denitrifying biofilm was developed by Sakakibara et al. [24]. A double Monod expression was used to describe the rates of nitrate and hydrogen utilization, using kinetic parameters reported in the literature. Haugen et al. [22] suggested a first- or second-order reaction for nitrate and nitrite reduction for hydrogenotrophic denitrification. Kinetics of heterotrophic denitrification, are usually assumed to be described by Monod expressions [25–29]. The kinetics of sulphur-based autotrophic denitrification could be very well described by using a half-order reaction [15,30].

In this work, we examined the progress of hydrogenotrophic denitrification in batch assays with a mixed culture. Our aim was to study the nitrate elimination rate and the efficiency of the culture for hydrogenotrophic denitrification of drinking water at various NO_3^- concentrations. An inhibitory effect of nitrate on the bacteria efficiency and a reduction of nitrogen removal rate were assessed. In addition the objective of this work was to develop a mathematical model capable of describing the hydrogenotrophic denitrification process of drinking water; namely, the nitrate and nitrite reduction and cell growth, as affected by the nitrate inhibition on biomass efficiency. The proposed model is a simple one and is able to predict the biomass growth and the consumption rates of nitrate and nitrite at various concentrations of nitrate. Kinetic experiments were used for the estimation of the model parameters.

2. Materials and methods

2.1. Cultivation of microorganisms

All test runs were inoculated with mixed culture of denitrifying bacteria. These bacteria were enriched from sludge taken from the wastewater treatment plant of Agrinio city, Greece. Enrichment cultures were incubated under anoxic conditions using growth medium. The growth medium containing tap water, KNO_3 (0.722 g/l), KH_2PO_4 (3.39 g/l), Na_2HPO_4 (3.53 g/l) and initially glucose (0.5 g/l) as an organic carbon source. After 24 h of heterotrophic denitrification, the reactors receiving glucose were switched to H_2 as an electron donor and the batch reactors were sparged with a gas mixture of CO_2 and H_2. Nitrate, nitrite and biomass concentrations were measured in all reactors on a daily basis. As soon as nitrate degradation was complete, 20 ml of fresh synthetically produced contaminated water, containing tap water, KNO_3 (0.722 g/l), KH_2PO_4 (3.39 g/l) and Na_2HPO_4 (3.53 g/l), was added to the reactors. The evaporation, which occurs through the gases outlet valve, was controlled by adding tap water.

2.2. Experimental system

All batch experiments were performed in 3 l closed sealed flasks with working volume of 2 l. The experimental system used in the denitrification tests consisted of three reactors and is shown in Fig. 1. The suspension medium was continuously stirred at a constant rate of 600 rpm during all the runs. The system was maintained at a temperature of 29–31 °C. The synthetically produced contaminated water used in all batch experiments was composed of tap water, KNO_3 (0.051–1.44 g/l) as the contaminant, KH_2PO_4 (3.39 g/l) and Na_2HPO_4 (3.53 g/l). Carbon dioxide was used as carbon source and hydrogen as electron donor.

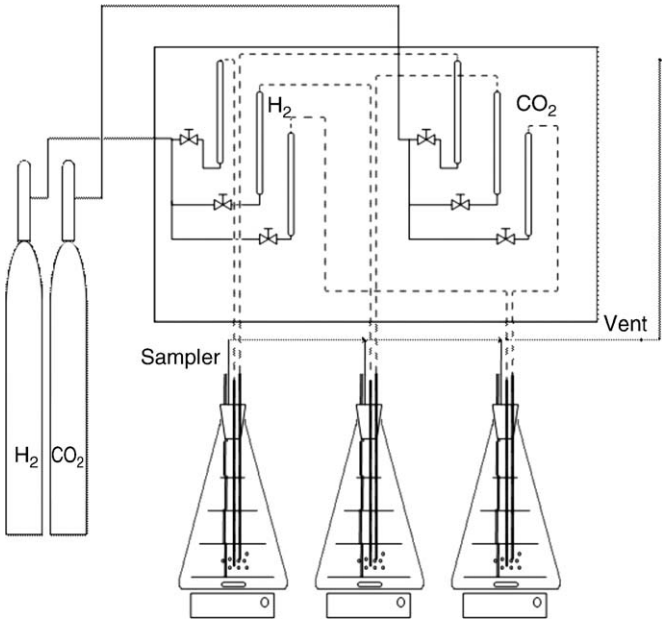

Fig. 1. Schematic diagram of the experimental apparatus.