Desalination 274 (2011) 44–53

Contents lists available at ScienceDirect

Desalination

journal homepage: www.elsevier.com/locate/desal

Potential of the bacterial strain *Acidovorax avenae* subsp. *avenae* LMG 17238 and macro algae *Gracilaria verrucosa* for denitrification

O.O. Nalcaci [a], N. Böke [b], B. Ovez [b,*]

[a] Karlsruhe Institute of Technology (KIT), Engler-Bunte-Institut, Division of Combustion Technology, 76131 Karlsruhe, Germany
[b] Ege University, Engineering Faculty, Chemical Engineering Department, 35100 Izmir, Turkey

ARTICLE INFO

Article history:
Received 18 October 2010
Received in revised form 25 January 2011
Accepted 25 January 2011
Available online 18 February 2011

Keywords:
Biological denitrification
Acidovorax avenae subsp. *avenae*
PCL
Gracilaria verrucosa
Monod model

ABSTRACT

In this biological nitrate removal study, the performance of the bacterial strain *Acidovorax avenae* subsp. *avenae* LMG 17238 using different carbon sources such as ethanol, methanol, sodium acetate, glucose and poly (ε-caprolactone) was investigated. Additionally the parameters such as an increase of nitrate concentration, carbon source amount (C/N) and dilution of a synthetic medium were studied. In laboratory conditions LMG 17238 and a mixed bacterial culture (soil suspension, wastewater treatment and macro algae *Gracilaria verrucosa*) have been immobilized as a comparative study to determine their efficiency in the biological denitrification of drinking water. For the continuous system as a carbon source, *G. verrucosa* was studied in fixed-bed columns. Various variables such as hydraulic retention time (HRT), mass amount of the substrate, and the initial nitrate concentration were investigated. A comparative study was achieved by using biodegradable poly(ε-caprolactone) as a carbon source using a mixed bacterial culture (LMG 17238 and *G. verrucosa*) in a fixed-bed column. Also Monod type equations were used to model the denitrification kinetics. The study proves that, LMG 17238 can be successfully used with different carbon sources. *G. verrucosa* can be considered as a promising alternative to poly(ε-caprolactone) with a maximum denitrification rate of 13.83 and 0.94 mg NO_3^-N/L d respectively.

1. Introduction

Excessive application of fertilizers and other nitrogen compounds in various industries, e.g. agricultural, pharmaceutical, and dairy or food, contributes to nitrogen pollution [13].

Regulations for drinking water are required in order to limit human risks and environmental pollution. The maximum admissible concentration limit of 10 mg/L as NO_3^-–N (nitrate–nitrogen) set by the U.S. Environmental Protection Agency or 50 mg/L as NO_3^- (nitrate) set by the World Health Organization and the European Economic Community.

Concern has led to the development of a number of techniques, such as ion-exchange, reverse osmosis, electrodialysis, and biological denitrification for lowering nitrate concentration to acceptable levels. Lower efficiency, waste brine disposal, high capital and operating costs are the major disadvantages of these methods except for denitrification [4,24,27,47,55]. Among various available methods (physical, chemical, physico-chemical and biological) for lowering nitrate concentration to acceptable levels, biological removal (denitrification) is considered to be the most economically sound and feasible on a large scale [42].

The majority of biological denitrification which is the reduction of nitrate to nitrogen gas relies on heterotrophic bacteria that require an organic carbon source. Many bacteria are capable of growing, by reducing ionic nitrogenous oxides to gaseous products. The bacteria use nitrate for respiration, converting it to nitrogen gas through a sequence of enzymatic reactions [11,39,40]:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO(g) \rightarrow N_2O(g) \rightarrow N_2(g) \quad (1)$$

An essential requirement for the reduction of nitrate is the presence of a carbon source that can be oxidized as an electron donor. Because drinking water has a low carbon content, additional solid, liquid or gaseous carbon sources have been evaluated for biological denitrification [39,40].

Due to the application of fertilizers and pesticides in agricultural methods, the combined occurrence of nitrate and pesticide in groundwater is frequently encountered [6]. As a consequence, the development of processes that allow the simultaneous elimination of both kinds of contaminants seems advantageous. Such a simultaneous elimination seems possible in the framework of a biological denitrification process by using a solid carbon source. As a consequence, a prospective denitrification process has been developed [8,36]. It uses the biodegradable synthetic polymer poly(ε-caprolactone) (PCL) as a substrate for both denitrification and as an electron donor. During the simultaneous denitrification and pesticide removal, the substrate acts as an adsorbent for the pesticides, which are then biologically degraded in a co-metabolism under anoxic condition.

* Corresponding author.
E-mail address: bikem.ovez@ege.edu.tr (B. Ovez).

0011-9164/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.desal.2011.01.067

It has been generally accepted that the design of a commercial-scale reactor, which is the heart of a plant, cannot be accomplished by a purely theoretical approach alone. To start with, at least laboratory data on the reactions involved must be available which should be followed by mathematical modeling in order to achieve a satisfactory scale-up procedure.

Biological reactor systems are extremely complex and the models describing them can be very complicated. Variables such as the bacterial types (enrichment culture or mixed culture), hydrodynamic characteristics (flow regime) and growth environments (limiting-substrate bulk concentration, pH, and temperature) must be taken into account [23]. For that reason, various models have been investigated as Michaelis–Menten kinetics [56], Grau second-order, Haldane model [3] and the Contois equation [21] in order to describe the process kinetics. However, the Monod type kinetic model is the most reliable and widely used model to describe the processes [15–17,51,57].

This study aimed to investigate the parameters affecting the biological denitrification process described and to model the removal kinetics of nitrate from drinking water.

2. Materials and methods

2.1. Batch reactor system

The batch denitrification experiments were carried out 500 ml glass reaction vessels (anoxic Eudiometer) with a water medium (250 ml) in each reactor (Fig. 1). The carbon sources were placed in the reactor as a substrate. The reaction vessels were connected via gas-tight tubes to a graduated gas-collecting glass tube [38]. There is a small outlet at the bottom of the glass reaction vessel for taking water samples. Nitrogen stripping was used to eliminate the initial oxygen and further access was prevented with the use of gas-tight tubes and fittings. The gas collection vessel is connected to an expansion tank that can be moved up and down to equalize the levels of the barrier solution (aqueous solution of 20% NaCl and 0.5% citric acid). In the reaction vessel, connected to the lid over the liquid surface, there is a container that is filled with soda-lime to adsorb CO_2.

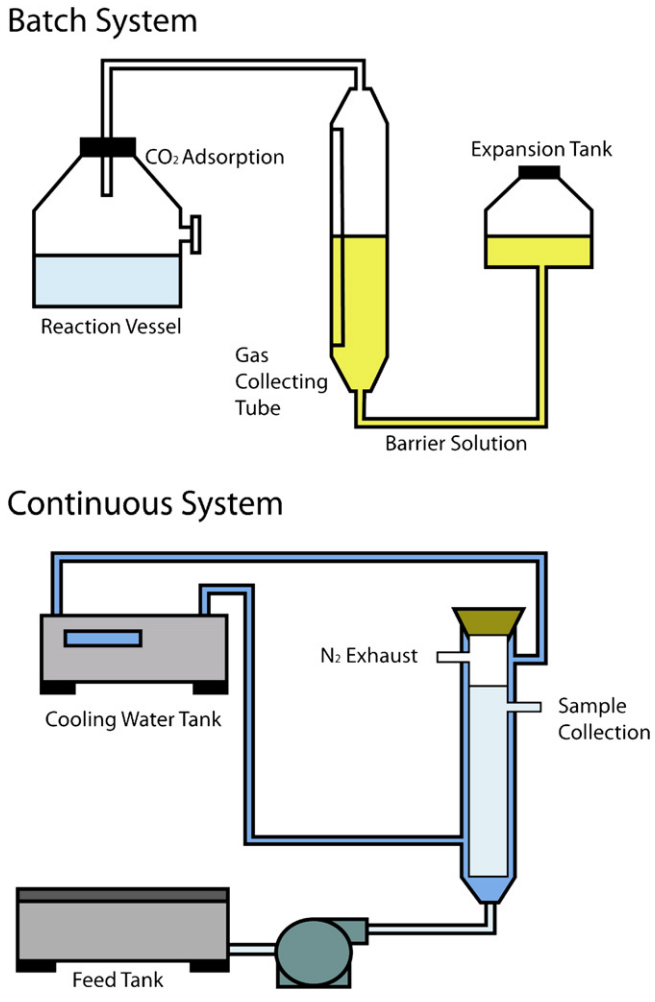

Fig. 1. Anoxic biological denitrification experimental setup.

The tests were performed in total darkness for achieving the condition suitable for natural denitrification. Denitrification occurs mainly in the dark, because the metabolic activity of the aquatic macrophyte is dependent on light; in light, high oxygen concentrations and in dark, low oxygen concentrations are induced [38,41]. Experiments were carried out at a constant temperature of 20 °C.

The volume of N_2 gas (ml) in the collecting tube, atmospheric pressure and temperature were recorded daily. At the end of the experiments, the nitrate and nitrite concentrations of the water media in the reactor were measured. The denitrification medium sample was tested with a further development of Lowry's method for total protein determination [49].

In the test results of different organic substances, the N_2 gas volumes were converted to standard conditions (1013.25 mbar, 273.15 K).

$$V_{sample} = ((P_t - P_{wt})^*(V_t + V_{RVST})^*273.15)) / (T_t^*1013.25)$$

The produced N_2 gas volume (equal concentration in water solved) is therefore:

$$NV(\mathrm{mg}/\mathrm{Lg}_{Carbon\ Source}) = (28.014/22.41^*VR)^*\left(V_{sample} - V_{Blank}\right)/E$$

where;

V_{sample}	Amount of produced gas at the time t, in ml
P_t	Atmospheric pressure at the time t of reading in mbar
P_{wt}	Water vapor pressure at the time t of reading at given temperature T_t in mbar.
T_t	Temperature at the time t of reading in K.
V_t	Amount of gas at the time t, read at the gas collecting tube, in ml.
V_{RVST}	Amount of gas in reaction vessel at the start in ml.
E	Weighted portion of natural organic substance in g
VR	Solution volume in reaction vessel in L.

The produced volume of N_2 can be derived from the reaction equation of denitrification which depends on used carbon sources:

$$C_cH_hO_oN_nP_pS_s + aNO_3^- \rightarrow cCO_2 + (w-a)H_2O + OH^- + (n+a)/2 + pH_3PO_4 + sH_2SO_4$$

a	4/5 c + 1/5 o + p + 6/5 s
w	2/5 c + 3/5 h − 1/5 o − p − 2/5 s.

The theoretical produced N_2 volume and N_2 (%) are therefore:

$$Th\ N\ V\ (\mathrm{mL}) = \frac{E \times \mathrm{MolVol}}{MG(CS)} \times \frac{(n+a)}{2}$$

$$D(N_2) = \frac{\left(V_{Sample} - V_{Blank}\right) \times 100}{Th\ N\ V}$$

MG (CS) molecular weight of carbon source in mg/mmol
MG (N) molar volume at standard conditions = 22.41 ml/mmol
V_{Medium} 0.250 L