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Biological denitrification in drinking water using *Glycyrrhiza glabra* and *Arunda donax* as the carbon source

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Abstract

In this research, two natural organic substances; liquorice (*Glycyrrhiza glabra*) and giant reed (*Arundo donax*) were investigated as a carbon source in the biological denitrification of drinking water. These materials act as a solid substrate and bio-film carrier. The experiments were carried out in batch, semi-batch and continuous processes. Complete denitrification was achieved with *G. glabra*. The removal efficiency of *A. donax* varied between 87 and 100% according to the type of process used. It was found that the nitrate removal rate of *G. glabra* was 6.96 mg/(L day) N-NO_3 and the nitrate removal rate of *A. donax* was 4.23 mg/(L day) N-NO_3. Results showed that these organic substrates could be used as an alternative carbon source for denitrification with complex process control and continuous monitoring. It should be noted that carbon source should be changed periodically for the continuation of the process.

Keywords: Biological denitrification; Carbon source drinking water; Giant reed *Arundo donax*; Liquorice; *Glycyrrhiza glabra*

1. Introduction

Biological denitrification is the reduction of nitrate to nitrogen gas, through a sequence of enzymatic reactions:

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

Each step is catalysed by an enzyme system. This respiratory process, in which nitrates or nitrites serve as terminal electron acceptors instead of oxygen is termed denitrification or dissimilatory nitrate reduction [1]. When electrons are transferred from the donor to the acceptor, the organisms gains energy which can be applied for the synthesis of a new cell mass and the maintenance of the existing cell mass. From the biochemical point of view, biological denitrification is an oxidation process in which the oxidation of organic substrate differs from respiration with molecular oxygen only in the final step, in which nitrite and/or nitrate-nitrogen serves as an electron acceptor. Since denitrification is a respiratory process, an oxidizable substrate or electron donor is needed as an energy source [2]. Many bacteria are capable of growing by reducing ionic nitrogenous oxides to gaseous products and this process requires an organic carbon source. Drinking water has a low carbon content therefore, various solid, liquid and gaseous carbon sources have been evaluated [3], such as ethanol [4–6], methanol [7,8], acetic acid [9], fatty acids [10], newspaper [11], wheat straw [12], unprocessed fibre cotton [13], sugar and sugar cane [14], water insoluble biodegradable polymers [15], synthetic polyester granules [16,17] and natural organic substrates, such as straw, bark of different trees, hydrolyzed birchwood, etc. [15,18].

The main objective of this research is to investigate the feasibility and efficiency of nitrate removal using *Glycyrrhiza glabra* and *Arundo donax*. Liquorice (*G. glabra*) and giant reed (*A. donax*) which also have a wide range of applications in various areas [19–24]. However, their use in biological denitrification processes is yet not known. Considering their relative low prices, they can become a good alternative.

2. Materials and methods

For the batch and semi-batch experiments, a laboratory-scale, fixed bed and mixing glass reactor, were connected to each other (Fig. 1). Recycle flow rate was regulated by a peristaltic pump and was directed to create an up-flow through the fixed bed column. The effluent from the fixed bed is recycled to the top of the mixing tank. The total water volume in the system was 15 L. Batch and semi-batch systems were observed using the same experimental set-up, however, batch processes were created by closing the effluent and influent valves.

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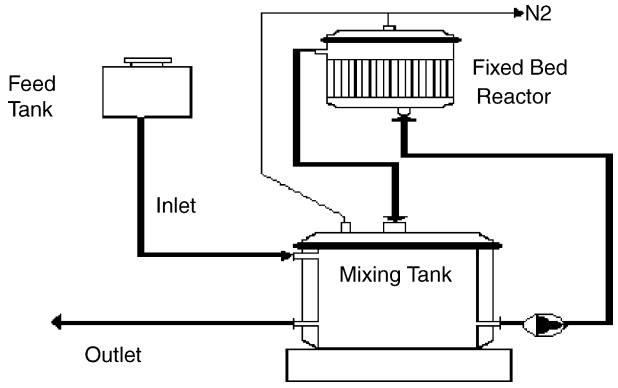


Fig. 1. Biological denitrification pilot plant reactor system.

For the continuous system experiments, a fixed bed (45 cm height, 4 cm inner diameter), vertical up-flow, glass columns were utilized. Using a cooling jacket around the mixing tank and columns, water temperature was kept constant at 20 °C. The flows were regulated by a peristaltic pump (Prominent Dosiertechnik).

Nitrate, nitrite, pH, O_2, UV absorbance, flow rate and temperature variations were measured daily. Semi-batch characteristics of the system and the continuous flow systems are shown in Tables 1a and 1b.

The overall volumetric denitrification performance r_{DV} in mg/(L h) N-NO_3 of a denitrification reactor is given by the equation:

$$r_{DV} = Q_D \times (c_0 - c_E)/V_D \quad (1)$$

Table 1a
Semi-batch system characteristics

	Organic substance	
	Liquorice	Giant reed
Initial fixed bed mass (g)	670	230
Shape of granules	Cylindrical	Annulus
Percentage of substrate consumed	11.86	10.13
Average surface area of the organic substance (m^2/m^3)	78.76	23.37
Fixed bed height (m)	0.15	0.15
Fixed bed (L)	2.0	2.0
Water volume in system (L)	15	15
Average influent (L/day)	8.22	10.0
Recirculation flow rate (L/h)	32.5	32.5
Temperature (°C)	17.9–18.6	17.3–0
pH	7.02–7.3	6.99–7.17

Table 1b
Continuous system properties

	Organic substance	
	Liquorice	Giant reed
Shape of granules	Cylindrical	Annulus
Initial fixed bed mass (kg)	76×10^{-3}	144×10^{-3}
Percentage of substrate consumed	28	26
Average surface area of the organic substance (m^2/m^3)	961	1178
Fixed bed height (m)	0.45	0.45
Fixed bed (L)	0.450	0.70
Average influent (L/day)	0.5–1–2	1–2–1
HRT (h)	21.36/10.68/5.34	18/9/18
Temperature (°C)	20–4	21–24
pH	6.9–7.53	7.0–8.22

where c_0 is the initial nitrate concentration (mg/L) N-NO_3) and c_E is the minimum effluent nitrate concentration. V_D is the denitrification rector volume (L) and Q_D is the flow rate (L/h).

2.1. Influent water and the analytical methods

In this study, the synthetic standard water medium in the reaction vessels was based upon the medium in DIN V 54900-2 or ISO 14851 [25], with the addition of $NaNO_3$ (100 mg/L N-NO_3^-), 0.5% inoculum and a trace element solution (per L: $ZnSO_4{\cdot}7H_20$ (0.2 mg), $MnCl_2{\cdot}4H_20$ (0.06 mg), H_3BO_4 (0.6 mg), $CoCl_2{\cdot}6H_20$ (0.6 mg), $CuCl_2{\cdot}6H_2O$ (0.02 mg), $NiCl_2{\cdot}6H_2O$ (0.04 mg) and $Na_2MoO_4{\cdot}2H_2O$ (0.06 mg).

In order to observe the performance of carbon sources respectively high nitrate concentration (100 mg/L N-NO_3^-) were used. During the duration of the study, each new experiment was inoculated with microorganisms taken from the last experiment. To establish anoxic conditions in the reactor, the media was swept by nitrogen gas. The experiments were done in total darkness; the systems were covered with aluminium paper to prevent light penetration.

Analytical grade chemicals were used without additional purification. All samples were filtered through a 0.45 μm membrane filter before analysis and were tested within 1 h of collecting. To measure the system efficiency with water samples, NO_3 and NO_2 values were measured based on photometric methods (Nanocolor 300 D). Nitrate and nitrite were measured at wavelengths of 517 and 525 nm, respectively.

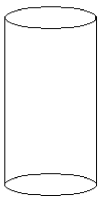
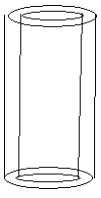
2.2. Natural organic substances

The liquorice (*G. glabra*) and giant reed (*A. donax*) were bought from a local company and the same material was used in all experiments. These natural materials were preserved at room temperature (20 °C) and kept in a container free from moisture.

Liquorice (*G. glabra*) root extract is a dark-nearly black color, has a slight odor and a lingering sweet taste. This extract is commonly consumed and sold in liquid and concentrated forms. Before the experiments, the liquid was extracted from the *G. glabra* in order to prevent the dense color. Extraction was carried out with water to liquorice root mass ratio of 40:1 at 50 °C. The mixture was shaken continuously for 100 min then filtered and dried at room temperature.

The cylindrical shape liquorice and annulus shape giant reed were cut into equal lengths (Table 2). Before placement into the reactors, the diameter, height and thickness (for giant reed) of each piece were measured to calculate the surface areas. Initial and final dry masses of the substances were measured to determine the percentage loss of usable organic material during the experimental period.

Table 2
Measured properties of natural organic substances

	Liquorice	Giant reed
Shape		
Color	Brown	Yellow
o.d. (m) $\times 10^{-2}$	1.05	1.43
i.d. (m) $\times 10^{-2}$		0.96
Thickness (m) $\times 10^{-2}$		0.24
Length (m) $\times 10^{-2}$	1.5	1.46
Surface area (m^2) $\times 10^{-4}$	10.58	15.56
Volume (m^3) $\times 10^{-6}$	1.10	1.32
Density (kg/m^3)	1.09	0.96
C, H, N (%)	45, 6, 1.4	45, 6, 1.4