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Influence of carbon source on nitrate removal of contaminated groundwater in a denitrifying submerged filter

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Abstract

The effectiveness of three selected carbon sources (sucrose, ethanol and methanol) on submerged filters for the removal of nitrate from contaminated groundwater (100 mg NO_3^-/litre), was studied. Process yields, nitrite accumulation, biomass production and growth of denitrifying bacteria were compared. Process yields, represented as ratio C/N were 2.5, 1.08 and 1.1 for sucrose, ethanol and methanol assays, respectively, making sucrose the least efficient carbon source. Nitrite accumulation in treated groundwater was more notable for sucrose assays, reaching values of 5 mg NO_2^-/l. However, when ethanol or methanol were used as carbon sources, nitrite accumulation in treated water was practically zero during the experiments. On the other hand, a greater biomass production was observed in these assays with sucrose, causing clogging of the filter. Higher density of denitrifying bacteria in the biofilm, observed when ethanol and methanol were amended to the influent, suggested that these carbon sources increased the denitrification activity compared with the experiments performed with sucrose. Since methanol is toxic, ethanol is considered the most suitable carbon source out of the three tested, under the experimental conditions.

Keywords: Submerged filter; Biofilm; Groundwater; Nitrate; Carbon source

1. Introduction

Blue-baby syndrome (methaemoglobinemia) is related to nitrate ingestion [1]. This anion is transformed to nitrite, by nitrate-reducing bacteria in the intestine, which reacts with the

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haemoglobin in blood converting it into methaemoglobin, and as a consequence of this, oxygen is no longer carried to cells tissues, causing death. Furthermore, nitrosoamines are carcinogenic compounds that may be formed from nitrite in the stomach [2]. In view of these problems, the European Union promulgated specific regulations setting the maximum contaminating levels of nitrate and nitrite in drinking water at 50 and 0.1 mg/l, respectively [3].

Inorganic fertilizers containing nitrogen are commonly used to increase agricultural production and are the principal source of groundwater diffuse contamination by nitrate [4], which reaches the saturated zone because of its high solubility and percolates through soil. Water of these aquifers cannot be used for municipal water supply unless it is diluted with low nitrate-content water to reach acceptable levels, or treated to reduce nitrate concentration. Processes such as ion exchange, reverse osmosis, electrodialysis, distillation and activated carbon adsorption, have been employed to remove nitrate from drinking water supplies [5–7], but these processes are expensive and a brine of difficult management is generated. Nitrate removal can be achieved also by using biological processes such as biodenitrification, which is a potentially effective method of nitrate and nitrite reduction in water supplies. In recent years, intensive research has been conducted on the nitrate removal from groundwater and surface waters [8–10]. Biological denitrification has been proved to be one of the more advanced high-performance methods and the sole selective method for complete nitrate elimination [11].

Denitrification is the biological reduction of nitrate and nitrite to nitrous oxide and molecular nitrogen [12]. Its physiological significance is the generation of ATP through anoxic respiration, where nitrogenous oxides serve as the terminal electron acceptor in an electron transport chain. This process is carried out exclusively by bacteria of almost all major physiological and morphological groups of prokaryotes, being the Gram-negative aerobic heterotrophs most abundantly found in water and soil [13]. Under anoxic conditions, nitrogenous oxides are reduced and carbonaceous substrates are oxidized to carbon dioxide by these bacteria.

Different types of fixed film reactors, such as rotating biological contactor, moving-bed, fluid-bed and submerged filters, have been used from the beginning of the twentieth century for biological treatment of water and wastewater [14]. However, the technology based on submerged filters seems to have a better applicability for freshwater biological treatment [15]. In this process, the bacterial film grows on a fixed medium, through which the water passes.

In order to apply submerged filter biodenitrification to contaminated water like groundwater, a carbon source dosage is required besides pH, temperature and oxygen concentration controls. In this process, nitrate or nitrite can be removed from water by disassimilatory reduction transforming it into nitrogen gas (denitrification), or by assimilatory reduction incorporating it into protein and nucleic acids [16]. A carbon source is needed for both these processes. Complex and simple carbon compounds have been employed in wastewater nitrogen removal, and it is known that addition of simple carbon sources favours nitrogen removal [17]. In this context, the denitrification rate is strongly influenced not only by carbon content, but also by the quality of the electron donor. On the other hand, an elevated level of nitrite, the intermediate product of nitrate reduction during water denitrification, has been observed in bioreactors with fixed micro-organisms used in wastewater treatment and drinking water denitrification [18]. Oxygen level, pH, denitrifying micro-organisms

and mostly the type of available carbon source, influence nitrite accumulation [19]. This accumulation of nitrite in water supplies is extremely dangerous for public health.

Enhanced denitrification rates in these processes reduce the hydraulic retention time, allowing minimization of the plant size. With this aim, the influence of different carbon sources in groundwater nitrate removal with submerged filters was assayed, comparing its yields, nitrite accumulation, biomass production and growth of denitrifying bacteria in biofilm.

2. Experimental section

2.1. Pilot plant description

The pilot-scale plant used for these experiments consisted of a methacrylate cylindrical column (3.0 m high and 0.3 m diameter, Fig. 1) of the submerged biofilter type, operating with a upward flow of the groundwater to treat and an upward flow of air and rinsing water

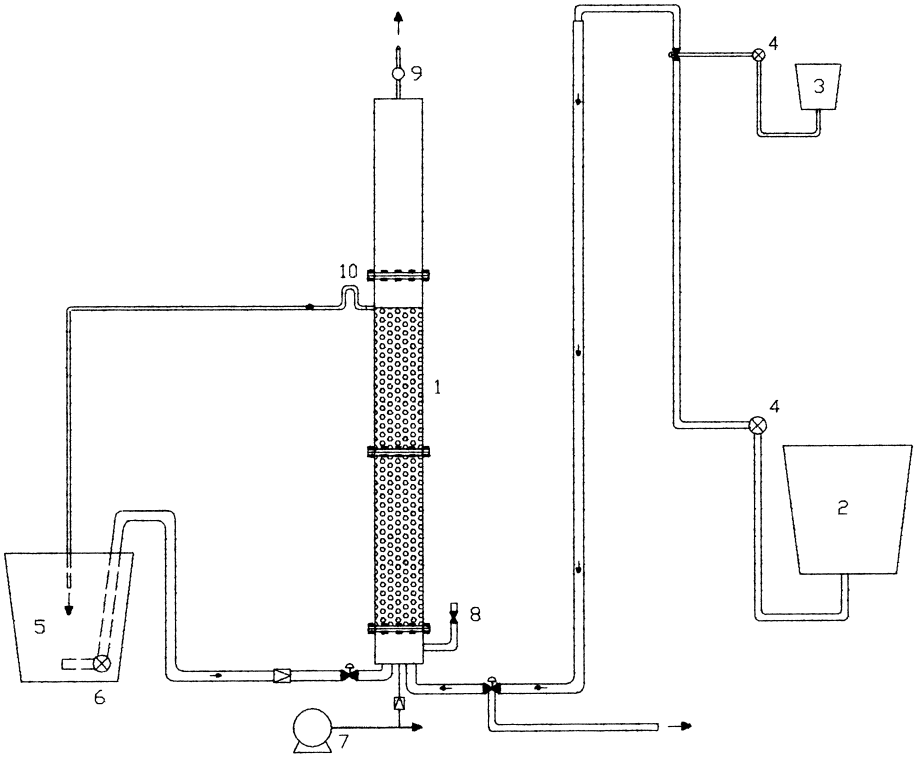


Fig. 1. The pilot-scale plant: (1) submerged filter ($V = 0.21\,m^3$); (2) influent tank ($V = 1.7\,m^3$); (3) carbon source tank ($V = 0.03\,m^3$); (4) piston pump; (5) effluent tank ($V = 0.5\,m^3$); (6) rinsing pump; (7) air compressor; (8) safety valve; (9) vent; and (10) U-bend.

for filter cleaning. The reactor was packed with clayey schists of 2–4 mm average size and 1.75 g/cm^3 density, up to 2.0 m high. A communicating-vessels system was employed for its operation.

2.2. Experimental procedure

Influent to biofilter was groundwater from La Vega aquifer (Granada, Spain) with the following characteristics: Nitrate concentration, 50–70 mg/l; dissolved oxygen, 2.0–4.5 mg/l; phosphate concentration, 0.4–0.8 mg/l; sulphate concentration, 180–210 mg/l; and pH 7.0–7.5. Nitrate was supplemented by addition of an appropriate volume of a concentrated stock solution of $NaNO_3$ giving a final concentration of 100 mg/l. A stoichiometric quantity of sodium sulphite (Na_2SO_3) was added along with cobalt chloride ($CoCl_2$) to eliminate dissolved oxygen. The water was pumped at a 0.5-l/min flow rate using a piston pump. Water temperature in the system was in the range from 15 to 20°C. The system was operated under continuous organic carbon addition applying a range from 0 mg/l to the minimum concentration which could accomplish a total nitrogen removal of the water to treat. Each concentration was added for seven days, reaching steady-state conditions in three days. After this period of time the submerged biofilter was cleaned using an upward flow of air and rinsing water. A concentrated stock solution of carbon source was stored in a tank from which was pumped to influent pipe. Three carbon sources were selected for these experiments (sucrose, ethanol and methanol) on the basis of its widespread availability, easy handling and low specific cost. The submerged biofilter (fixed medium) was inoculated with an activated sludge amended of nitrate (3 g/l), which was recirculated for 7 days, after which the water to be treated was pumped in.

2.3. Sampling procedure

Every 24 h, water samples (200 ml) were collected from the inlet and the outlet of the column, obtaining three replicates for each carbon concentration assayed. Nitrate, nitrite, ammonium and carbon source concentration were routinely monitored in all samples.

The inert substrate was removed from the reactor through a special sampling port located along the column, using a cylindrical sampler. Samples (1 g) of the fixed medium were taken from three different heights (16, 64 and 123 cm) and were thoroughly mixed previous to the analysis described below.

2.4. Analytical determinations

Water samples were filtered through 0.45-μm membrane filters (Millipore HAWP). Nitrate and nitrite were measured by an ion chromatography system using conductivity detection (Dionex® DX-300). Separation and elution of the anions were carried out on an anion analytical column (Ionpac® AS14) using a carbonate/bicarbonate eluent and a sulphuric regenerant. Before measuring, the filtered samples were diluted to achieve nitrate and nitrite concentrations <10 mg/l. Ammonium concentrations were estimated using the phenol salt spectrophotometric screening method, according to *Standard Methods* [20].

Ethanol and methanol concentrations were measured in the effluent and influent by gaseous phase chromatography (Perkin–Elmer® Autosystem GC). Sucrose concentration was determined by the Roe and Papadopoulos method [21]. The pH and dissolved-oxygen level were measured continuously in the effluent using an pH-meter and an OXI 921 electrode (CRISON®), respectively.

For biomass production estimation, 1 g of mix clayey schists was introduced in sterile glass bottles with 100 ml of sterile saline solution (NaCl 0.9%). Biofilm was separated from inert substrate by sonication (5 min.), and suspended solids obtained were determined by vacuum filtration of the 100 ml of saline solution through a pre-weighed and pre-ignited fibreglass filter (0.45 μm), then dried for 24 h at 105°C. The dry-weight (mg/g substrate) was used as biomass estimation.

2.5. Quantification of denitrifying bacteria

The number of denitrifying bacteria were assayed by surface plate count on NSA medium (nitrate–sucrose–agar). The composition of NSA medium was as follows (per litre of distilled water): $NaNO_3$ 2.0 g, K_2HPO_4 1.0 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, KCI 0.5 g, $FeSO_4{\cdot}7H_2O$ 0.01 g, yeast extract 1.0 g, sucrose 30.0 g and agar-agar 20.0 g. Biofilm was separated from substrate as previously described for biomass production estimation, although samples were sonicated only for 1 min. The separated biofilm was homogenized using a magnetic stirrer at the maximum speed for 1 h. Dilution series (1:10) were made in sterile saline solution (NaCl 0.9%). From each dilution, 0.1 ml was spread on sterile Petri dishes with NSA medium (three replicates of each dilution) and incubated anaerobically (Anaerogen system, OXOID) at 28 ± 1°C for 2 weeks. Colony forming units (cfu) were counted on plates of the series featuring ≈10–100 cfu.

3. Results

High correlation coefficients were obtained between nitrogen removal and sucrose, methanol and ethanol concentrations in the influent, these being: $r_s = 0.993$; $r_m = 0.991$; $r_e = 0.981$, respectively. Fig. 2 illustrates the linear regressions established for each carbon source assayed and the different behaviors they showed. In sucrose experiments, a slope of 0.13 was obtained. This value was the lowest of the three compounds tested, thus the smallest yield in nitrogen removal was observed with sucrose. A lower concentration of carbonaceous compounds was required for equal yields in nitrogen removal when alcohols were used, mainly for ethanol. The slopes obtained were 0.42 for ethanol and 0.38 for methanol.

As expected, the C/N in the influent needed to remove completely the 100 mg/l of nitrate of the effluent varied according to the nature of the carbonaceous compounds assayed, being the maximum value obtained for sucrose ($C/N = 2.5$) and the minimum for alcohols ethanol and methanol ($C/N = 1.08$ and $C/N = 1.1$, respectively).

The nitrate concentration in the effluent went down immediately when increasing the dosage of carbon source in all tests made, as reflected in Fig. 3 . However, nitrite concentration in the effluent experimented an initial increase as the carbon source concentration

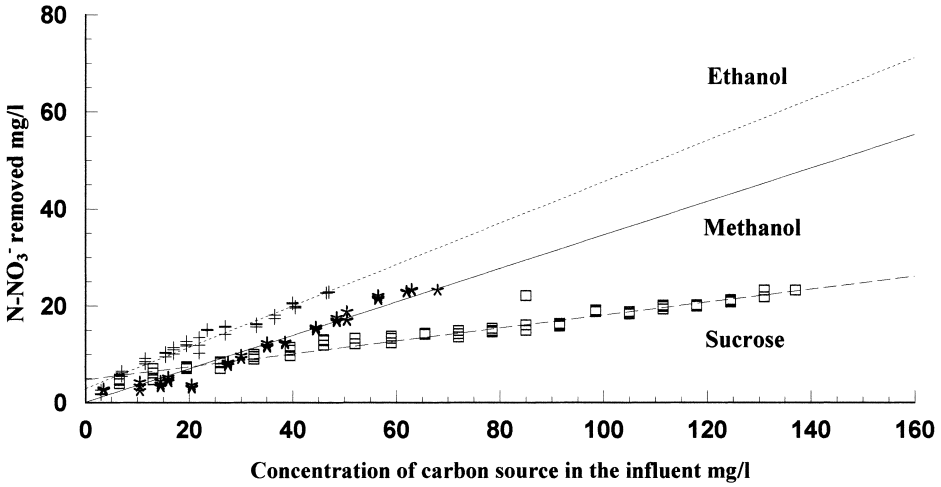
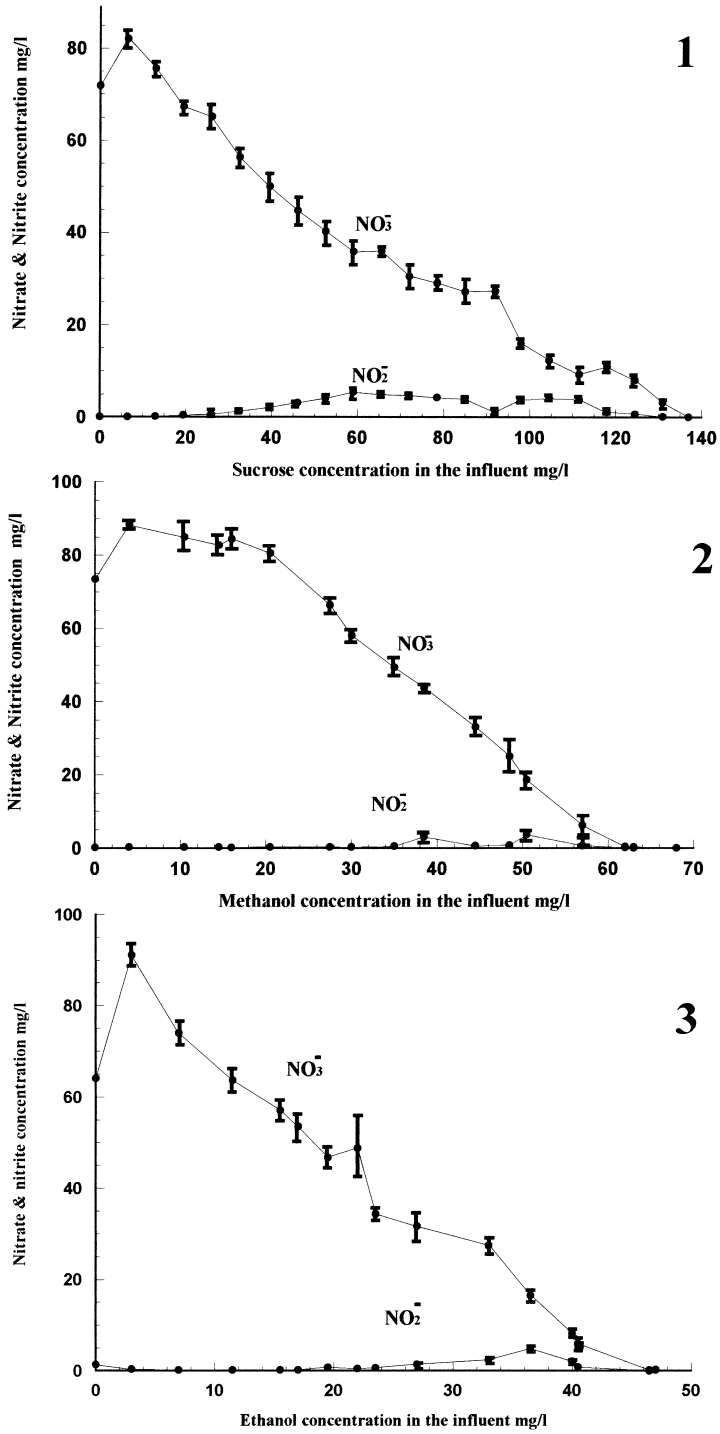


Fig. 2. Linear regression between concentration of carbonaceous compounds in the influent and nitrogen removal with different carbon sources: (□) sucrose; (∗) methanol; and (+) ethanol.

in the influent was risen, although at high concentrations of the three compounds tested nitrite completely disappeared, as happened with nitrate concentration. The maximum nitrite concentration in the treated water varied depending on the carbon source used. If a concentration of 59 mg/l of sucrose was added to the influent, a maximum average of 5.5 ± 0.5 mg/l was detected in nitrite concentration. For sucrose dosages that brought in a yield among 45–85% of nitrogen removal, these high nitrite concentrations in treated water were maintained. Experiments with ethanol and methanol showed lesser nitrite concentrations in the effluent. Ammonium in treated water was never detected, regardless of the type of carbon source or the concentration assayed.

Linear regressions between the C consumed by the process (mg/l) and biofilm growth (mg biofilm dry-weight by g medium) for each one of carbon sources assayed, are represented in Fig. 4. As observed for nitrogen removal, high correlation coefficients were obtained between consumed concentration of carbonaceous compounds and biomass production estimated ($r_s = 0.979$, $r_m = 0.994$ and $r_e = 0.985$ for sucrose, methanol and ethanol, respectively). The slope of the plots varied according to the carbon source used. Thus, when sucrose was used, the greatest growth of biofilm was noticed ($m_s = 1.14$), being this lower for alcohols ethanol ($m = 0.757$) and methanol ($m = 0.662$). The highest values in biomass were observed when 100 mg/l of nitrate of effluent were completely removed, being similar for the alcohols (27.23 ± 0.66 mg/g of substrate for ethanol and 26.72 ± 0.25 mg/g of substrate for methanol), whereas with sucrose the values were triplicated (73.2 ± 4.56 mg/g of substrate). The intercepts of the linear regressions were 10.38, 10.33 and 10.3 for sucrose,

Fig. 3. Residual nitrate and nitrite concentration in contaminated (100 mg/l NO_3^-) groundwater treated in a denitrifying submerged filter amended with different carbon sources: (1) methanol; (2) sucrose; and (3) ethanol. Bars represent ± S.E. of five samples.


1
Nitrate & Nitrite concentration mg/l
NO3-
NO2-
Sucrose concentration in the influent mg/l
2
Nitrate & Nitrite concentration mg/l
NO3-
NO2-
Methanol concentration in the influent mg/l
3
Nitrate & nitrite concentration mg/l
NO3-
NO2-
Ethanol concentration in the influent mg/l

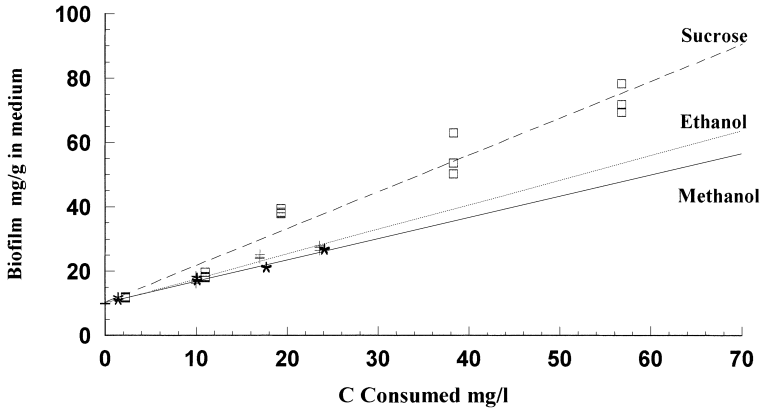


Fig. 4. Linear regression between biofilm growth and C/N ratio for (□) sucrose, (∗) methanol and (+) ethanol assays.

ethanol and methanol, respectively. These values correspond to milligrams of biofilm dry-weight obtained after inoculation with activated sludge.

Denitrifying bacteria (active biomass) grew in conformity with the increase of C consumed by the process. Both parameters were highly correlated in all tests performed ($r_s = 0.978$, $r_m = 0.970$, $r_e = 0.975$ for sucrose, methanol and ethanol, respectively), perfectly fitting into a linear regression model (Fig. 5). The maximum averages of biofilm denitrifying bacteria were again achieved when the carbon source concentration in water to be treated gave a 100% yield of nitrogen removal. These values were similar the for three carbon sources assayed: $((3.16\pm0.2)\times10^8$ for sucrose, $(3.13\pm0.15)\times10^8$ for ethanol and $(2.910\pm$

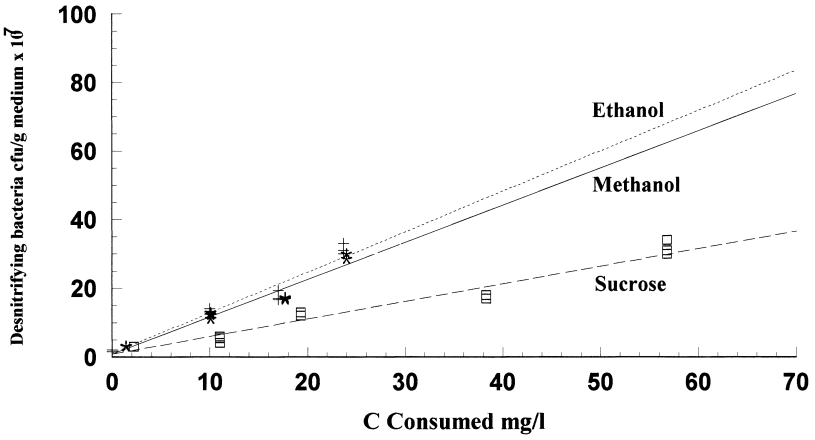


Fig. 5. Linear regression between denitrifying bacteria growth in biofilm and C/N ratio for (□) sucrose, (∗), methanol and (+) ethanol assays.

$0.27) \times 10^8$ for methanol). Nevertheless, due to the different need for carbon source in each case, the slope of the plots varied, these values being similar for ethanol ($m_e = 1.18 \times 10^7$) and methanol ($m_m = 1.081 \times 10^7$), but lower for sucrose ($m_s = 0.51 \times 10^7$). The intercepts of the linear regressions were1.08×10^7, 0.88×10^7 and 0.84×10^7 for ethanol, methanol and sucrose, respectively. These values correspond to denitrifying bacteria in biofilm after inoculation.

In all the tests, the carbon source concentration in treated water was analyzed (Fig. 6). When methanol was used, with C/N ratio in influent above 0.25, the concentration of this compound was always around 1.0 mg/l, and went up considerably when applying carbon source dosages for which maximum yield was obtained. With sucrose and, especially, ethanol experiments, the highest carbon source concentrations were detected in treated water, during the maximum yield phases (Fig. 6).

4. Discussion

Mateju et al. [22] defined the theoretical stoichiometric equations for the denitrification with ethanol as carbon source. This equation estimates that a C/N ratio of 0.71 is necessary for complete nitrate reduction to molecular nitrogen. Similar chemical equations were described by Drtil et al. [23] and Cheng and Lin [24] for denitrification with sucrose and methanol. Thus, the theoretical C/N ratios for sucrose and methanol were established as 1.07 and 0.71, respectively. Our studies suggested C/N ratios for denitrification with ethanol, sucrose and methanol of 1.08, 2.5 and 1.1, respectively. These higher consumptions of carbon source showed that heterotrophic denitrification process were not the only ones taking place in the biofilm. Due to biofilm increase found for all the tests done, we believe that assimilatory processes occurred. Therefore, part of the nitrate present in water to be treated was removed by assimilatory reduction, which caused biomass growth in the biological reactor. The high biomass concentration in fixed-film systems is correlated with clogging, which is one of the main problems in submerged filters [25] and forces to wash the reactor. This was especially necessary when sucrose instead of alcohols was the carbon source used.

The consumption of alcohols (methanol and ethanol) remained much lower than the ones of sucrose for the same nitrate removal level. These observations, obtained from our experiments, are similar to the estimations derived from the chemical equations described by Mateju et al. [22], Drtil et al. [23] and Cheng and Lin [24].

The biofilm denitrifying bacteria showed an increase as the concentration of carbon source in the influent rose, this more significant in the case of alcohols than with sucrose. Constantin and Fick [26] indicated that bacterial growth could be most favored when ethanol was used as carbon source, because the ethanol catabolism allows formation of $NADH_2$, an energy source for the microorganisms. This could be the reason of the greater denitrifying bacterial growth found when alcohols were used as carbon source, while with sucrose, which is more difficult to metabolize, this parameter was lower.

If the data for denitrifying bacterial growth were associated with biofilm increase data, a higher bacterial density in the biofilm was developed with alcohols as carbon source when compared to biofilm developed with sucrose. Samrakandi et al. [27] observed that a great

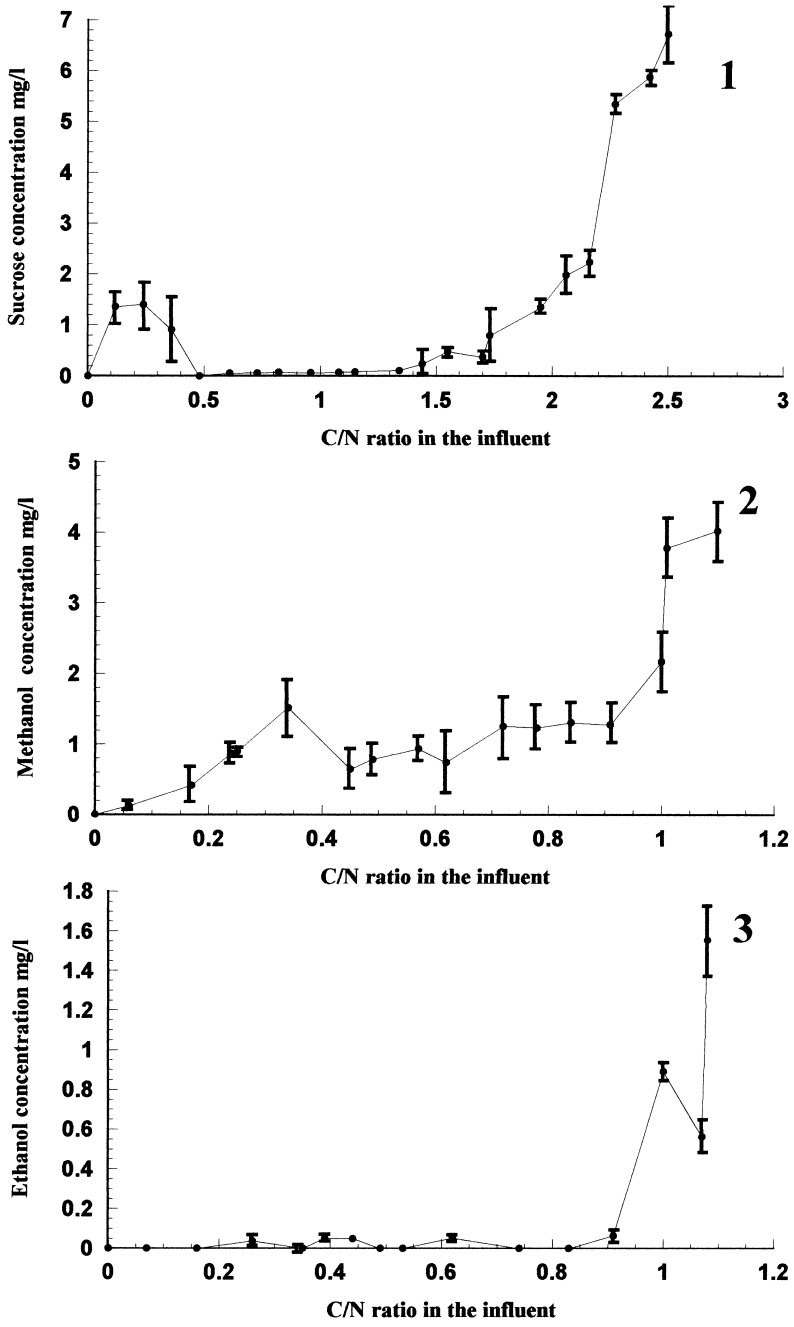


Fig. 6. Residual carbonaceous compounds concentration in treated water for each C/N ratio assayed with (1) methanol, (2) sucrose and (3) ethanol as carbon source. Bars represent ± S.E. of five samples.

quantity of exopolysaccharide was produced in biofilms when sucrose was used as carbon source. Because of this, thick biofilms with low bacterial density were produced. In thick biofilms, a decrease of the metabolically active biomass with depth was noticed, and this affected nutrient transformation and diffusion processes [28]. If this is considered together with the lower bacterial density obtained in biofilms when sucrose was used as carbon source, the lowest yield in nitrate removal was justified.

Different mechanisms have been found to be responsible for nitrite accumulation, like repression of the nitrite reductasa synthesis in the presence of oxygen [29] or inhibition of the enzymatic activity by pH [30]. According to McCarty et al. [31], another factor influencing the amount of residual nitrite may be the choice of the carbon source. This agrees with the results observed in our experiments, where a higher nitrite concentration was detected in effluents when sucrose was used as carbon source.

Obviously, nitrite accumulation in treated water never occurred when 100% of nitrogen was removed. However, only the amount of nitrate necessary to carry out with legislative demands was eliminated, in order to economically optimize the process. For these lower yields, nitrite concentration in the effluent was observed only occasionally when alcohols were used, whereas with sucrose a high nitrite concentration was observed constantly. This forced us to treat the effluent again until the nitrite concentration in treated water was under 0.1 mg/l [3]. Nitrites can be easily removed from outlet water by the addition of oxidant reagents also used for disinfection; however, further studies must be achieved in order to avoid nitrite production.

5. Conclusions

From the set of our experiments, it is deduced that alcohols (ethanol and methanol) are more suitable as carbon sources than sucrose for a fixed-film system, in order to eliminate nitrate from contaminated groundwater. There were no big differences between these two compounds either with regard to nitrite concentrations in the effluent or in the characteristics of bacterial density in the biofilm. However, the yield of the process was better when ethanol is used. On the other hand, it is worth noting that, for all carbon sources assayed, certain residual concentrations of carbonaceous compounds in the effluent were detected, mainly in points of maximum yield in nitrogen elimination. This can be problematic if the nature of the carbon source used affects human health, as is the case of methanol. Thus, it could be suggested that, of the three carbon sources tested, ethanol seems to be the most suitable for use with submerged filters for the treatment of groundwater contaminated with nitrate.

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