

Sustainable Removal of Nitrates from Wastewater using Membrane Bioreactors

Loris Pietrelli^a, Sergio Ferro^b, Andrea P. Reverberi^c, Marco Vocciante^{c,*}

^aDipartimento di Chimica, Università di Roma La Sapienza, Piazzale Aldo Moro, 5, 00185 Roma, Italy

^bEcas4 Australia Pty Ltd, 8/1 London Road, Mile End South, SA 5031, Australia

^cDCCI, Dipartimento di Chimica e Chimica Industriale, Università degli Studi di Genova, Via Dodecaneso 31, 16145 Genova, Italy

marco.vocciante@unige.it

The presence of nitrogen compounds in surface and groundwater is a serious concern. The treatment technologies currently used, based mainly on membrane and ion exchange processes, are expensive, complex and above all not very selective. On the other hand, biological processes are very efficient and selective towards nitrate, but the phases following denitrification, in which the treated water is separated from the biomass (potentially harmful to public health), are still very expensive.

In this work, an original solution is proposed that combines biological treatment with the use of membranes to keep the water to be treated separate from bacterial cultures, thus limiting or eliminating the subsequent disinfection stages. As a result, efficiencies of up to 98% are achieved depending on operating conditions, while keeping costs low.

1. Introduction

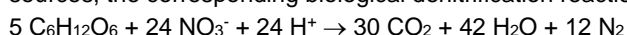
Contamination of groundwater with nitrates is becoming a serious problem in drinking water consumption.

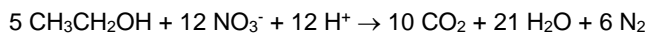
Among the numerous anthropogenic activities, possible sources of pollution, the most frequent cause of nitrate contamination is probably to be identified in the agricultural practices adopted. Nitric nitrogen is the form absorbed by plants, so its presence in irrigation waters is beneficial; however, nitrates are not retained by the colloids of the soil and end up reaching the deep layers of the soil, and hence the aquifers, rivers and lakes. As a result, the concentration of nitrates in most groundwater reserves is increasing and it seems inevitable that intense anthropogenic activity will further aggravate the problem (Bouchard et al., 1992), with consequent damaging effects on water bodies (Ferro, 2012).

Both changes in chemical conditions and biological processes can lead to the conversion of nitrates into other compounds. When found in surface and groundwater, nitrates are converted into ammonia and its salts, which are toxic to fish (Randall and Tsui, 2002) and a major cause of eutrophication (Smith et al., 1999).

Water containing nitrates has negative effects on human health, as bacteria in the gastrointestinal tract convert nitrates into nitrites, which are the cause of methemoglobinemia, or "blue baby" syndrome, and a potential cause of both gastric cancer and miscarriages (Lee et al., 1995). Further possible consequences of nitrates presence include the formation of carcinogenic compounds such as nitrosamines (Nawrocki and Andrzejewski, 2011).

Among the variety of approaches available, the problem of removing nitrates from water can be addressed by physicochemical methods, or biological processes. In particular, the biological reduction of nitrates to nitrogen is a process that occurs spontaneously in nature, and which can be usefully exploited; however, the reaction involves several steps ($\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$) and unfortunately most bacteria can only perform one or two of these. Therefore, the denitrifying microflora is actually made up of a group of complementary microorganisms capable of converting NO_3^- to N_2 in its entirety. Most denitrifying bacteria can use a wide range of carbon compounds (sugars, organic acids, amino acids) as a source of electrons, but bacterial activity is affected by the type of organic substance acting as a reducing agent. Taking glucose and ethanol as carbon sources, the corresponding biological denitrification reactions can be written as follows:





The common configuration of a denitrification process involves direct contact of the water to be treated with the denitrifying microorganisms, with the consequence that the residual organic substances can represent a problem from the point of view of water quality.

This work presents a biological process of nitric nitrogen removal based on the use of biological membrane reactors; the membrane is permeable to nitrates and divides the reactor into two physically distinct environments: the water to be treated circulates on one side, while the bacterial film grows on the other. The latter needs nutrients and a carbon source to carry out the process. In this way, the water to be treated never comes into direct contact with either the denitrifying biomass or the carbon source which, leaving no residues in the water, can also be of poor quality and low cost. Extensive research has previously been carried out by other authors (McCleaf and Schroeder, 1995), who have achieved good results using flat membranes; in this research, the use of a hollow fiber membrane was considered, able to guarantee a high exchange surface while maintaining a small size for the reactors and components. The aim of the research is to provide a technology capable of treating polluted groundwater by minimizing the problems of microbial contamination of the water itself, providing a solution in line with the adoption of "green methods" (Reverberi et al., 2017) and allowing the replacement of processes and materials with eco-friendly alternatives (Reverberi et al., 2018).

2. Materials and Methods

The flow-through experimental setup, schematically described in Figure 1, was developed and tested on a laboratory scale. The system was mainly composed of: (i) a hollow fiber module (cut-off from 2,000 to 100,000D); (ii) a suspended growth denitrifying culture bioreactor; (iii) a storage tank for nitrate-contaminated water; (iv) two recirculation pumps.

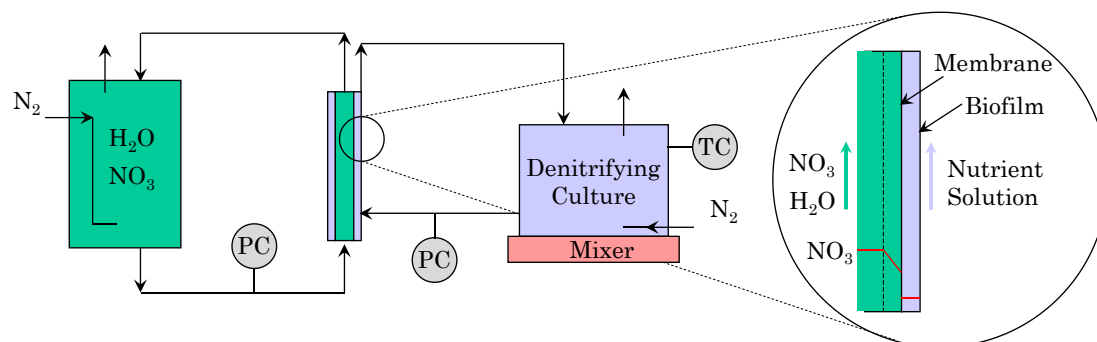


Figure 1: Membrane biofilm denitrification system

The water containing the nitrates was circulated to the inner side of the fibers, while the biomass and nutrients (phosphates, glucose) were supplied from the outside. Nitrates are removed from contaminated water by molecular diffusion through the fibers, which allows them to reach and be metabolized by denitrifying microorganisms. In this way, the water remains completely separated from the biomass and nutrients, and it is possible to maintain the necessary anoxic conditions by blowing in gaseous nitrogen. The phenomenon of molecular diffusion is governed exclusively by the concentration gradient: nitrates diffuse through the membrane, and since they are continuously consumed by the biological film present on the outer side of the membrane, this guarantees the maintenance of the maximum concentration gradient. At the same time, the biofilm limits the counter-diffusion of nutrients.

To maintain the aforementioned anoxic conditions, the water tank ($V = 30\text{L}$) was kept under nitrogen flow. For the recirculation of the solutions, two peristaltic pumps with variable flow rates between 60 and 6,000 mL/h were exploited, mostly used at very low speeds.

For the execution of the experimental tests, a commercial hollow fiber module (Amicon, Millipore) was used, made of polysulfone, a polymer that has sufficient resistance to temperature (max 75 °C), and an excellent tolerance to pH variations (within the values from 1.5 to 13). Commercial modules contain membranes with an average pore diameter between 10 and 200 Angstroms, corresponding to a molecular weight cut-off (MWCO) between 1,000 and 500,000 Dalton. The fiber bundle is enclosed in a plastic container (housing) which has the task of keeping it compact and limiting the volume of the external area of the module. The main physical characteristics of the modules considered in this work are shown in Table 1.

Table 1: Main characteristics of the hollow fiber membranes considered.

Membrane type	Cut-off (MW)	Surface (m ²)	Length (cm)	Internal fiber (mm)	Thickness (μm)	Number of fibers
H5P2-43	2,000	0.45	63.8	1.1	200	235
H1P3-20	3,000	0.06	20.3	0.5	120	250
H1P30-20	30,000	0.06	20.3	0.5	120	250
H1P100-20	100,000	0.06	20.3	0.5	120	250

Nitrate standards were prepared using potassium nitrate (analytical grade) and deionized water. The nitrates and phosphate concentrations were measured using a Dionex DX-500 Ion-chromatograph. The concentrations of Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) were measured according to standard ASTM methods (gravimetrically, by preliminary filtration, followed by drying in an oven at 105 °C and subsequent combustion at 550 °C, in order to cause the destruction of any organic substance present). Total Organic Carbon (TOC) content was measured using a Dohrmann DC-190 High Temperature TOC Analyzer (Rosemount Analytical Inc.), while pH, conductivity and dissolved oxygen measurements were performed with the aid of a single instrument (MultiLine P4, WTW), capable of analyzing and recording data with variable time intervals.

2.1 Hydraulic characterization tests

The permeability tests were carried out by circulating deionized water inside the hollow fibers with variable flow and pressure, to evaluate the quantity of water that permeates from the inside to the outside of the fiber according to the module and the operating conditions adopted. The tests were conducted by varying the influent flow rate in a decreasing way between 522 and 160 mL/h, setting the external recirculation flow rate first to 170 mL/h and subsequently to 240 mL/h, and evaluating the flow rate of the permeate overflowing from the external circuit. With each change in flow rate, it took approximately 20 minutes for the flows to stabilize before measurement. The measurement of the quantity permeated to the outside was carried out by measuring the variation in the volumes entering and leaving the external compartment, using calibrated tanks.

Diffusion tests were also carried out to evaluate the diffusivity characteristics of the various membranes used against the various compounds involved, namely nitrates, the carbon source and phosphates. The tests were carried out by circulating the solution with the nitrates inside the fibers and the nutrient solution outside the fibers, alternately replacing one of the two solutions with deionized water to avoid the triggering of unwanted denitrification reactions. To evaluate the diffusion effect only, limiting the permeation as much as possible, low flow rates were maintained, in the range 200 ÷ 400 mL/h, similar to the values estimated for use in continuous operation, evaluable between 100 and 600 mL/h. Indicating with A and B respectively the internal and external compartments of the fibers, the experimental conditions can be summarized as follows:

1. Diffusion of nitrates: solution containing nitrates (A) and deionized water (B); $V_A=V_B=1000$ mL; $P_A=P_B=0.04$ kg/cm²; $Q_A=330-450$ mL/h; $Q_B=200-350$ mL/h.
2. Diffusion of glucose and phosphates: deionized water (A) and solution of glucose and phosphates (B); $V_A=V_B=1000$ mL; $P_A=0.02$ kg/cm²; $P_B=0.03$ kg/cm²; $Q_A=300-310$ mL/h; $Q_B=380-430$ mL/h.

The diffusion test lasted seven hours, with hourly sampling; at each sampling all pressure values were recorded: inlet (external and internal) and outlet (external and internal); the values of flow rates and concentrations (external and internal) were also measured. Diffusion coefficients calculated using Fick's equation were used to compare the diffusion of the different compounds.

2.2 Activity tests of the denitrifying biomass

The denitrifying cultures were extracted from an activated sludge reactor of a municipal wastewater treatment plant. The selection of the denitrifying biomass to be inoculated inside the fiber bioreactor was carried out according to the procedure proposed by Reising & Schroeder (1996), by administering glucose and potassium nitrate (3 g of glucose per g of NO₃-N) to the biomass kept in a shaken and sealed flask. The culture was buffered to approximately neutral pH by adding 17.4 g/L of KH₂PO₄ and 21.4 g/L of K₂HPO₄ per g/L of NO₃-N fed. Every three days, the culture was allowed to settle, half the liquid volume was removed, and the flask was refilled with water, glucose, potassium nitrate and phosphate buffer.

For the denitrification tests, the inoculation phase of the bioreactor took place as follows: once the denitrifying biomass was introduced into the external compartment, the recirculation circuit was activated, ensuring a continuous supply of nitrates, phosphates and organic substance. During this phase the internal line was kept deactivated. The biomass used was fed daily with constant quantities of carbon, nitrates and phosphates, dosed to obtain the following initial concentrations: N-NO₃⁻ = 200 mg/L, P-PO₄³⁻ = 10 mg/L, and TOC = 200 mg/L.

The duration of the inoculation phase was 24 hours, time considered sufficient (based on previous tests) for the colonization of the fibers but such as to limit the clogging of the recirculation circuit (problem encountered for longer times).

After the inoculation phase (always performed in the same way), various test cycles were then conducted (not discussed in this work) to determine the effects on the process efficiency of parameters such as: (i) concentration of nitrate in the internal compartment; (ii) concentration of the carbon source in the external compartment; (iii) residence time of the water contaminated by nitrates inside the fibers; (iv) pressure regime in the external compartment. In these tests, excessive pressure differences on the two sides of the membranes were avoided to limit the permeation from one compartment to the other and therefore the overlap of the two phenomena, and the diffusive transport mechanism of the nitrate was favored by high concentration gradients close to the membranes. The final setup for the experimental tests is summarized in Table 2.

Table 2: Composition of solutions used in denitrification tests.

	Compartment	Q (L/h)	N-NO ₃ ⁻ (mg/L)	P-PO ₄ ³⁻ (mg/L)	C (mg/L)
Contaminated water	A	0.2, 0.4, 0.6	110	0	0
Nutrient solution	B	1.8	0	10-15	110-160

The recirculation rate of the nutrient solution (1.8 L/h) was chosen to avoid excessive clogging by the biomass in the external part of the module (problem highlighted in previous tests, not discussed in this work). The biomass inside the reactor was continuously recirculated, similarly to what is expected at the plant level; the addition of nutrients was carried out directly in the recirculation, delivering the aforementioned quantities every 8h by means of a timed dosage. With these operating conditions, the process worked with both adherent and suspended biomass, but the compromise of a partial permeation towards the interior had to be accepted.

All solutions, even those used to simulate groundwater, were prepared using tap water with the following average characteristics: NO₃⁻, NO₂⁻, C, PO₄³⁻ = 0 mg/L, SO₄²⁻ = 1.6 mg/L, Cl⁻ = 29.3 mg/L, temperature 18 °C, pH 7.15, conductivity 350 µS/cm, dissolved oxygen 5.6 mg/L; the presence of further mineral salts is possible but has not been evaluated.

3. Results

The results of the tests described in the previous section are shown below. For reasons of brevity, the complete data are shown only for the H5P2-43 module which proved to be the most performing, while for the other modules only the general performance and the differences in behavior compared to H5P2-43 will be highlighted.

3.1 Hydraulic characterization tests

Preliminary tests (not shown) made it possible to highlight that the permeate flow strongly depends on the water pressure, which is why all the tests were conducted with low pressures, in an attempt to limit the gradient between the inside and outside of the membrane. The results of the permeation test, reported in Table 3 for the H5P2-43 module, show that the permeation varies according to the flow rate; in particular, the permeate flow rate decreases as the external flow rate increases (from 170 to 240 mL/h), while it decreases in absolute value (but increases in percentage) as the internal flow rate decreases (from 522 to 160 mL/h). In Table 3 and thereafter, A and B respectively indicate the internal and external compartments; IN and OUT indicate the values at the inlet and at the outlet of the module, respectively.

Table 3: Permeate flow rate as the internal flow rate to the fibers varies, with two different external flow rates.

P _{IN} (g/cm ²)		P _{OUT} (g/cm ²)		T _c (°C)	Q _A (mL/h)	Q _B =170 mL/h		Q _B =240 mL/h	
A	B	A	B			Permeate	%	Permeate	%
70	60	10	5	13.1	522	30	5.7	22	4.2
70	60	10	0	16.3	420	28	6.7	19	4.5
70	55	10	0	19.5	350	26.5	7.6	18	5.1
70	55	10	0	25.3	270	24	8.9	16	5.9
70	55	10	0	29.7	230	22	9.6	13	5.7
70	55	10	0	42.8	160	20	12.5	12.5	7.8

From the measured permeability data (Table 3 values for H5P2-43, data not shown for the remaining modules), the permeability constant of the various fibers was calculated using Darcy's law; the values obtained are summarized in Table 4.

Table 4: Comparison between the permeability of the various modules tested.

	H5P2-43	H1P3-20	H1P30-20	H1P100-20
K (cm ² / kg h)	8.43	2.00	39.57	4.49
S (cm ²)	4500	600	600	600
K/S (1 / kg h)	0.0187	0.033	0.659	0.074
ratio of K/S	1	1.8	35.2	4.0

The significantly high permeability observed for the H1P30-20 module and the resulting process problems that would arise led to the exclusion of this module from subsequent experiments.

The results of the nitrate diffusion across the H5P2-43 membrane are shown in Table 5.

Table 5: Nitrate diffusion tests through the H5P2-43 membrane ($P_{IN} = 20 \text{ g/cm}^2$; $P_{OUT} = 0 \text{ g/cm}^2$).

Time (h)	NO ₃ ⁻ (mg/L)		Q _{OUT} (L/h)	
	A	B	A	B
0	65.5	0	0	0
1	49.8	22.6	0.33	0.35
2	35.4	29.2	0.36	0.36
3	31.9	33.5	0.45	0.26
4	31.4	33.8	0.42	0.27
5	30.6	31.7	0.35	0.31
6	30.6	31.6	0.42	0.23
7	28.4	30.8	0.45	0.2

The temporal trend shows that the nitrate concentrations inside and outside the fibers are practically the same after just two hours of recirculation. At the third hour, the concentration on the outside of the fibers was slightly higher than on the inside. This phenomenon is attributable to a permeation effect which, despite the modest pressures involved, cannot be eliminated. For the evaluation of the diffusion coefficient, the concentration values evaluated after one hour were used. In fact, at this stage, the internal-external gradient is close to that actually found during the continuous tests and the presence of the permeation does not cause excessive disturbance. Diffusion tests with the carbon source (glucose) and with the phosphates were performed with similar operating procedures. The diffusivity data for the three species involved and for the three investigated membranes (as anticipated, the H1P30-20 membrane has not been further investigated, due to its anomalous permeability) are shown in Table 6.

Table 6: Comparison between the diffusivity of the various modules tested.

Compounds	D (cm ² /s)		
	H5P2-43	H1P3-20	H1P100-20
Nitrates	2.6×10^{-7}	5.81×10^{-6}	1.35×10^{-5}
Carbon	1.94×10^{-7}	4.35×10^{-6}	1.49×10^{-5}
Phosphates	2.23×10^{-7}	6.56×10^{-6}	6.56×10^{-6}

Similarly to what emerged with the permeation values, the diffusion values also decrease as the membrane cut-off decreases, demonstrating the importance of a careful choice with regard to the characteristics at the molecular level of the fibers used.

The data in Table 6 show how better permeation limitations can be achieved using the H5P2-43 membrane, even if this results in a more limited diffusion of nitrates (disadvantage) and nutrients (advantage).

Overall, this outcome led to a preference for the H5P2-43 module for the later stages of the research. In fact, despite being characterized by a lower diffusivity than the other modules, it allows to minimize the phenomenon of counter-diffusion of the organic substance towards the inside of the fibres (and consequently minimize the contamination of the treated water); the diffusion coefficient with respect to nitrates (higher than that of nutrients) is in any case such as to allow the correct carrying out of the process. Ultimately, it was considered that the molecular cut-off at 2000 Dalton guaranteed the best compromise between the diffusion of nitrates and the counter diffusion of nutrients.

3.2 Denitrification test under continuous conditions

Table 7 presents a summary of the main results obtained during the last test cycle, characterized by different values of the feed rate and, therefore, of the time spent by the contaminated water inside the fibers.

Table 7: Summary of the results obtained from the denitrification tests under continuous conditions with the H5P2-43 module.

Q _A (mL/h)	Duration (h)	Contact time (min)	NO ₃ ⁻ removal (%)	Denitrification rate (g N-NO ₃ ⁻ / m ² d)	C specific consumption (g C / g N-NO ₃ ⁻)	P specific consumption (g P / g N-NO ₃ ⁻)
200	170	35	98.2	1.71	4.87	0.09
400	170	18	73.1	2.35	7.79	0.22
600	65	12	53.3	2.6	8.64	0.31

From the data of Table 7 it is possible to observe that a longer contact time (lower internal flow rate) determines increasing percentage yields of nitrate removal, but lower specific denitrification rates.

Unlike the previous experimental phases, the choice not to remove the suspended biomass from the external compartment allowed to improve the biological removal yields of nitrates. In fact, the presence of suspended biomass allows to remove those nitrates which, not having been degraded by the biomass attached to the membrane, manage to reach the external compartment; it is important to note that a zero concentration of nitrates on the outside allows to maximize the diffusion of nitrates from the inside to the outside.

In a subsequent phase, a specific experimentation will try to investigate the use of higher concentrations of carbon and phosphorus, looking for the optimal conditions to reduce contact times without penalizing the nitrate removal efficiency and at the same time avoiding the contamination of the treated water.

4. Conclusions

The extensive experimentation conducted confirmed the possibility of obtaining satisfactory yields of biological nitrate removal by using hollow fibres as a support for the denitrifying biofilm and as a separator element between the water to be treated and the nutrient solution necessary for bacterial activity.

It has been observed that the use of membranes with reduced cut-off provides greater guarantees against the contamination of the water to be treated by nutrients and that controlled acclimatization methods and times can significantly improve the yield of the entire process. From the point of view of the denitrification process, the tests carried out under continuous conditions have shown removal yields up to 98%, which vary with the flow rate. Specifically, the nitrate abatements ranged from 1.71 to 2.6 gN-NO₃⁻ / m² d.

This nitrate treatment technology can be particularly economical when applied to the treatment of contaminated water streams on a large scale (e.g. potable water from contaminated aquifers). Research will continue by taking the process to a larger scale and using methanol instead of glucose as a carbon source. Furthermore, it will be necessary to check whether the denitrifying activity remains stable over time.

References

- Bouchard D.C., Williams M.K., Surampalli, R.Y., 1992, Nitrate contamination of groundwaters: sources of potential public health effects, *Journal of American Water Works Association*, 84, 85–90.
- Ferro S., 2012, Removal of nitrates from highly contaminated industrial wastewater, *Chim. Ind.*, 94(2), 100–110.
- Lee Y.W., Dahab M.F., Bogardi I., 1995, Nitrate-Risk Assessment Using Fuzzy-Set Approach, *Journal of Environmental Engineering*, 121, 245.
- McCleaf P.R., Schroeder E.D., 1995, Denitrification Using a Membrane Immobilized Biofilm, *Journal of American Water Works Association*, 87, 77–86.
- Nawrocki J., Andrzejewski P., 2011, Nitrosamines and water, *Journal of Hazardous Materials*, 189, 1–18.
- Randall D.J., Tsui T.K.N., 2002, Ammonia toxicity in fish, *Marine Pollution Bulletin*, 45, 17–23.
- Reising A.R., Schroeder E.D., 1996, Denitrification Incorporating Microporous Membranes, *Journal of Environmental Engineering*, 122, 599.
- Reverberi A.P., Vocciante M., Lunghi E., Pietrelli L., Fabiano B., 2017, New trends in the synthesis of nanoparticles by green methods, *Chemical Engineering Transactions*, 61, 667–672.
- Reverberi A.P., Varbanov P.S., Lauciello S., Salerno M., Fabiano B., 2018, An eco-friendly process for zerovalent bismuth nanoparticles synthesis, *Journal of Cleaner Production*, 198, 37–45.
- Smith V.H., Tilman G.D., Nekola J.C., 1999, Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems, *Environmental Pollution*, 100, 179–196.