Experiments of aerobic granular systems inoculated with pure culture of denitrifying bacteria.

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Large amount of water resources is affected by nitrate pollution due to the agriculture development, among other activities, which contaminated the groundwater with synthetic fertilizers and organic matter (Galloway et al., 2008). Although the available food has increased due to these kind of products, other problems derived from agriculture are causing negative effects on the environment such as diffuse contamination of groundwater by nutrients. The results of the accumulation of these products in the land and water resource causes deterioration of ecosystems. In Europe, nitrate diffuse contamination in sensitive areas was an objective for the European Commissions of Nitrate Directive in 1991 (91/676/EC), followed by the 100 Water Framework Directive(2000/60/EC). This contamination could be a risk to the health of human population that ingest or are in contact with contaminated water.

Different technologies try to solve and avoid the consumption of contaminated groundwater. Among them, the ECOGRANULARWATER project incorporates an innovative treatment biotechnology based on granular aerobic systems, which eliminate a wide variety of contaminants present in wastewater such as nitrogen and phosphorus (Gonzalez-Martinez et al., 2018). The possibility of using strains of microorganisms that are capable of reducing nitrate and pesticides is an aim for scientific research related to this environmental problem, which the ECOGRANULARWATER project is currently developing.

The denitrifying bacterial strains are capable of reducing nitrate by generating nitrogen oxides or molecular nitrogen and, specifically, the species that contain in their genome the nosZ gene are able to transform nitrogen oxides (greenhouse gases) to molecular nitrogen, avoiding the emission of these harmful gases. The possible usefulness of these bacterial strains for the development of granular aerobic systems is a promise for the treatment of groundwater contaminated by nitrates due to the save energy and cost in comparison with other treatments such as reverse osmosis. For the realization of the LIFE ECOGRANULARWATER project of granular aerobic systems, we have used different bacterial strains with nosZ gene, in order to realize the complete denitrification of the nitrate, thus achieving a removal of this compound and at the same time without emitting greenhouse gases.

Specifically, for action A2 of this project, sequential bioreactors were constructed (Figure 1), based on 4 stages: aeration, settling of biomass, emptying of treated water and feeding with contaminated water. The volume of each of the bioreactors was 2.3 L, aerated at the bottom of reactor by fine bubble, with air flow rate of 4.5 L min -1. The hydraulic retention time was 12 hours. The feeding synthetic water, simulating the composition of an underground water contaminated by nitrates. The microorganisms that play the role of removing nitrate needed organic carbon, selecting methanol and sodium acetate. In this sense, different tests the concentration of organic matter was studied progressively reducing the carbon concentration during the operation until optimize the bioprocess. Nitrate removal ratio were studied based on the concentrations of organic matter and nitrates in different phases that are shown in Table 1.

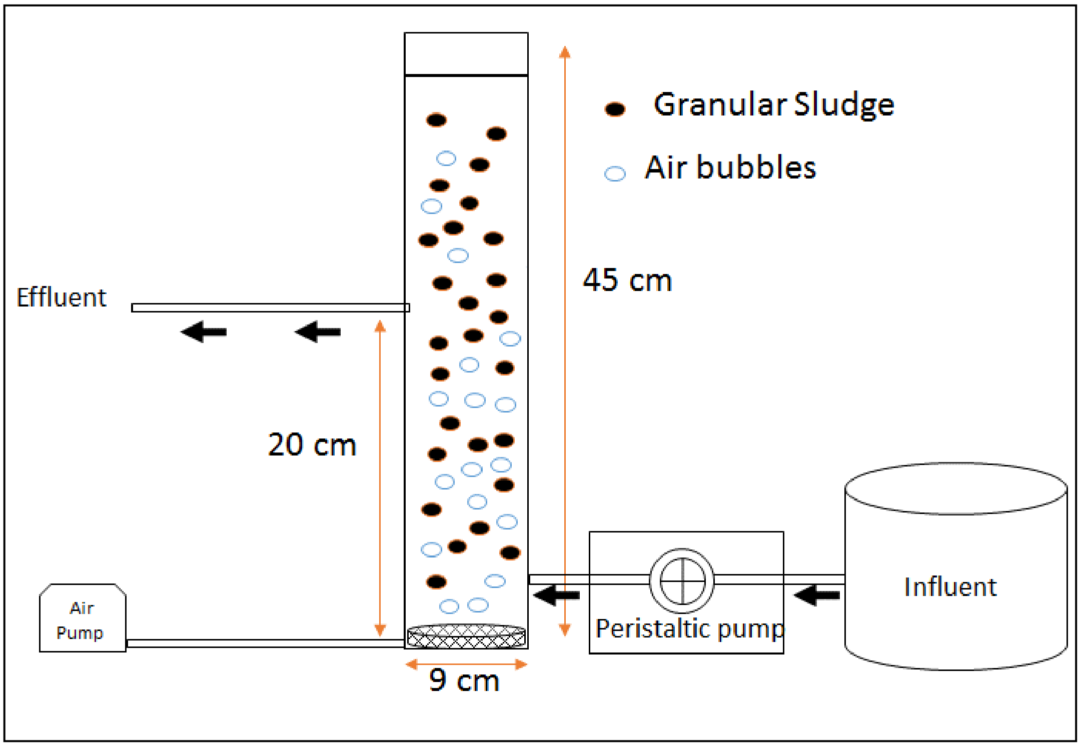

Figure 1.- Design of aerobic granular bioreactors.

Table 1.-Operational stages of aerobic granular systems related to synthetic groundwater composition.

Stage	Sodium acetate (mgL^{-1})	Nitrate (mgL^{-1})	Time(d)
I	900	200	60
II	400	100	30
III	300	100	30

The experimentation stage I was mainly carried out for the start-up phase of the pilot plants, in order to establish the growth of the granular biomass. The nitrate removal ratio for each of the four strains were different, also granulation process occurred with obvious differences for each of the bacteria. Table 2 shows the properties that determine the granular biomass, which are the settling velocity and the mean size for the strains fed with sodium acetate as a carbon source.

Table 2.- Mean size and settling velocity of granular biomass for the bioreactors.

Biorreactor	Property	Day 30	Day 60	Day 90	Day 120
Strain I	Tamaño(mm)	1.79	3,47	3,94	4,02
	Vel (mh-1)	16,41	43,53	60,24	67,34
Strain II	Tamaño(mm)	-	4,56	5,67	-
	Vel (mh-1)	-	18,32	24,45	-
Strain III	Tamaño(mm)	-	4,65	8,37	-
	Vel (mh-1)	-	21,056	31,168	-
Strain IV	Tamaño(mm)	-	-	-	-
	Vel (mh-1)	-	-	-	-

Strain IV was discarded because the bacteria was not able to form a stable granular biomass, the presence of filamentous microorganisms hindered the operation process. The studies with strains II and III are still in experimentation. Figure 2 and Figure 3 reflect the granules formed after 90 days of operation in the bioreactors inoculated with Strain I and Strain III, respectively.

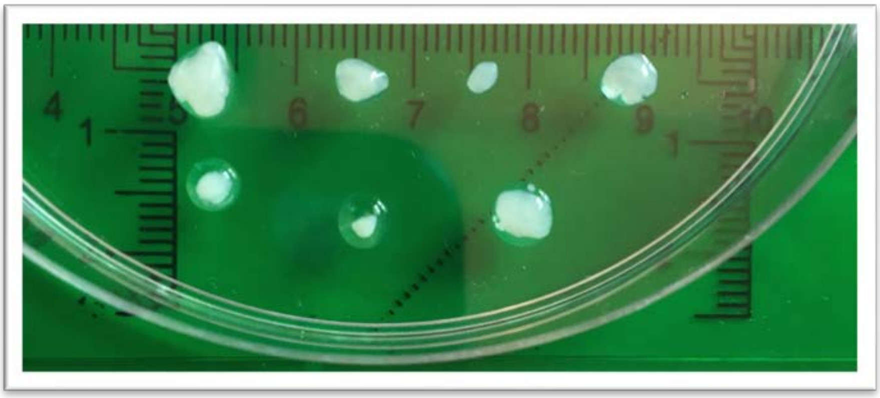

Figure 2.- Granules in the biorreactor inoculated with Strain I.

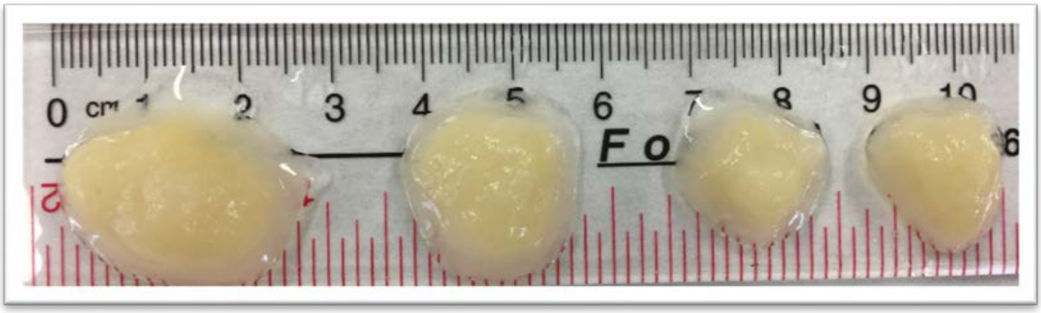

Figure 3.- Granules in the bioreactor inoculated with Strain III.

The results of nitrogen removal with sodium acetate as a carbon source for each of the strains selected due to their granulation capacity are shown in Table 3. At present, it is still in operation, showing the corresponding nitrogen elimination results on the 15th day of operation of this phase.

Table 3. Nitrogen removal for different strains during the operational time with sodium acetate as carbon source.

Biorreactor	**Period**	**Nitrogen removal (%)**
Strain I	**Stage I**	87%
	Stage II	92%
	Stage III	44%
Strain II	**Stage I**	81%
	Stage II	96%
	Stage III	-
Strain III	**Stage I**	87%
	Stage II	92%
	Stage III	-

In conclusion, aerobic granular systems inoculated with pure cultures of denitrifying bacteria are a potential nitrate removal technology for contaminated groundwater, due to its high nutrient removal capacity, granular properties, compact design and low energy required and cost saving. The action A2 is in experimentation phase without concluding. The minimum necessary limits of organic carbon have not been established to add to the incoming water so that the system has a good performance. On the other hand, the selection of organic carbon source has not been concluded when a global vision of the results in physical-chemical and economic terms is needed.

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