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Heterotrophic denitrification of contaminated groundwater using arachis hypogaea. l shell as carbon source

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ABSTRACT/RESUME

Abstract: *In this research, agricultural waste, Arachis hypogaea.L shells (peanut shells) was investigated for use as carbon source and biofilm carrier to remove nitrate from groundwater in batch reactor laboratory. The feasibility of heterotrophic denitrification of a high nitrate concentration groundwater using a natural carbon source was studied, a series of batch tests was performed with synthetic groundwater to exanimate the effects of various environmental and operational factors such as temperature, pH, initial nitrate concentration and finally the mass of powder of Arachis hypogaea.L shells on the rate of heterotrophic denitrification. A range of physic chemical analysis was performed including: Infrared, granulometry, spectroscopy atomic absorption and Scanning Electron Microscope (SEM).Decreasing initial pH decreased denitrification; however, increasing pH had little effect on denitrification rates. Using Arachis hypogaea.L shells as organic substratum of varying size classes, we found that finer-graine showed higher rates of denitrification compared to large grains, likely due to increased surface area per volume of substratum.Our findings suggest that Arachis hypogaea.L shell play an important role in N removal because they gave a reduction percentage of 90%.*

I. Introduction

Nitrate is one of the most important and widespread of the numerous potential groundwater contaminants [1, 2]. Nitrate Nitrate pollution of water due to EPA[9]. EPA, Quality Criteria for Water, USA Environmental Protections Authority, Washington D.C., USA (1986).The World Health Organization has set a maximum contaminant level of 50 mg NO_3^-/L (11.29 mg NO_3^--N/L) [4], and China has proposed a limit of 10 mg NO_3^--N/L [5]. Introduction of high concentration of nitrogen and phosphorus in surface waters has a negative impact on water, mostly by stimulating its eutrophication [6,7,8].The global nitrogen (N) cycle has been modified by human activities that increase the pool of biologically available N, including fossil fuel combustion, fertilizer production, and watershed disturbance [9,10].The adverse environmental impacts associated with a very high nitrate concentration is undesirable owing to its extremely intensive agricultural activities has become a major environmental problem since 1970s[4] [3].
To reduce the health hazard, a nitrate standard of 50 mg/L nitrate- NO_3^- or 10 mg/L nitrate-N in drinking water has been set by WHO8 and US
toxicity to most aquatic species and human include also a strongly promotion of eutrophication [11].Possible health consequences of nitrate ingestion include methemoglobinemia, the blue-baby syndrome in infants under six months of age [12]. Biological denitrification, including autotrophic and heterotrophic denitrification, represents a promising approach to remediate nitrate-contaminated Groundwater [13, 14]. Many technologies have been developed to remove nitrate from drinking water, in order to respond to the constant increase of the nitrate pollution problem.). However, these technologies are still uneconomical and they have disadvantages such as producing more concentrated reject (reverse osmosis), increasing other ions in the outlet (ion exchange) and more

complex processes to be operated (electrodialysis) (Soares, 2000 [2]). The feasible methods for removing nitrate from water supply are: ion exchange, reverse osmosis, electrodialysis, catalytic denitrification, and biological denitrification. Of these methods, the biological denitrification is the most cost effective method [15]. The complete heterotrophic denitrification consists of sequential reductive reactions from NO_3^- to NO_2^- , nitric oxide (NO), N_2O and finally to nitrogen gas (N_2), carried out by heterotrophs [16,17,18,19,20].Biological denitrification has been considered as a suitable alternative process. In this process, heterotrophic denitrifiers utilize (added) organic substrates that serve as electron donors, and convert nitrate into nitrogen under anoxic conditions, achieving quite a high denitrifying rate and treatment capacity [21,22].Biological denitrification is a very important topic in the field of water pollution control [23]. External organic carbon sources such as methanol [24], ethanol [25]
glucose, glycerol, acetic acid, and lactic acid [26]), and starch [27] have been employed in heterotrophic denitrifying processes for water treatment [28, 29, 30].Heterotrophic denitrification, using an organic carbon source as the electron donor, is considered to be the most promising process. Biological heterotrophic denitrification has received cost-effective process [31]. For these reasons, the heterotrophic denitrification process has been widely studied in order to optimize the factors that could enhance the biological removal of nitrates from water. The type of treatment configuration (rotating vs. fixed-bed bioreactors) [32, 33]. As humans continue to dominate the global nitrogen cycle, denitrification plays an important role in the landscape by removing excess N inputs that may otherwise be available for uptake, transformation, and transport and, thus, impacts on receiving ecosystems (Galloway et al. 2004 [34,35,36].

II.Material and methods

To prepare an inoculum of denitrifying, the batch reactor assembly consisted of a 2 liter Erlenmeyer; it was installed over a magnetic-stirrer hot plate. The stopper on the top of the flask had three holes to provide for the following measurements: (a) a thermometer to measure the liquid temperature; a pH meter to measure pH and a pipette for taking samples of water. The digested sludge of Boumerdes Wastewater Treatment Plant was brought for seed. The bacteria culture was developed by adding 100 mL digested sludge, 1g Arachis hypogaea.L shells powder, and 50 mg/L $N0_3^-$-N in total liquid volume of 1000 mL of tap water in the reaction vessel of the batch reactor. The content of the batch reactor was mixed by the magnetic stirrer. Each day approximately 100 mL of reactor content was removed for analyses and equal volume of fresh feed was added. The sample analyses included measurement of pH, temperature, nitrate $N0_3^-$ and nitrite $N0_2^-$ concentrations. To improve the oxygen consumption 3.2 g of metallic iron was also added ; flasks were sealed by rubber plugs to maintain anoxic condition. They were analyzed for their nitrate concentration every 3 days. Arachis hypogaea.L shells were bought from a local city of Boumerdes, the same material was used in all experiments. They were washed with tap water before air drying (105°C). The material was ground to obtain an homogeneous powder.All experiments were conducted in light-tight
conditions by cover of aluminium paper. Medium solution was prepared daily by tap water supplemented with oligoelemnts Table 1. The pH was measured with a model HACH.The temperature was measured with a standard electrode (HACH). Standard methods were used to measure nitrate $N0_3^-$ and nitrite $N0_2^-$concentrations. $N0_3^-$ was analyzed using the UV- spectrophotometry method; $N0_2^-$-N was analyzed using spectrophotometric method with N-(l-naphthyl) ethylenediamine. A synthetic water medium was used to simulate the composition of nitrate contaminated groundwater, containing about 50 mg. L^{-1} of nitrogen and 10 mg. L^{-1} of phosphorus by adding KNO_3 and KH_2PO_4.The characteristics of groundwater used in the experiments are given in the table 1 .Several physico-chemical analyzes were carried out on the powder as Laser granulometry using **LA 950** sizer, The mineral composition was determined using atomic absorption spectroscopy (brand ANALYTIK JENA), Structural analysis using Fourier Transform Infrared Spectroscopy (FTIR) (brand JASCO IFTR-4100), The porosity of the powder used was determined by the scanning electron microscope (SEM) of the brand QUANTA 650.

Tableau 1. *Chemical composition of synthetic water [37].*

Ion	Concentration (mg/L)	Compound	Ion	Concentration (mg/L)	Compound
NO_3^-	50 - 130	KNO_3	Mg^+	2	$MgSO_4.7H_20$
Ca^{2+}	1,32	$CaCl_2.2H_2O$	Mn^+	0,023	$MnCl_2.4H_2O$
Co^{2+}	0,01	$CoCl_2.2H_2O$	Mo^{6+}	0,01	$(NH4)_6Mo_7O_{24}.4$

					H_2O
Cu^{2+}	0,02	$CuSO_4.5H_2O$	Zn^{2+}	0,1	$ZnSO_4.7H_2O$
Fe^{3+}	0,12	$FeCl_3$	Ni^{2+}	0,01	$NiSO_4.6H_2O$
PO_4^{3-}	10	KH_2PO_4	BO^{3-}	0,005	H_3BO_3

III.Results

For the carbon source used, an acclimation period of about two weeks was necessary to attain a constant value in nitrates concentration in the effluent. For batch testing, Arachis hypogaea. L shells were ground and sieved with a (0.5 mm) sieve to obtain a fine, homogeneous powder. The humidity level is obtained by mass difference between the material before and after drying in a drying stove. The water content is determined according to the following formula:

$$H\% = \frac{(M2 - M1)}{P} \times 100$$

$H\%$: Percentage of moisture.
$M1$: Crucible mass + powder before drying (g).
$M2$: Crucible mass + powder after drying (g).P: mass of the test portion (g).
The ash content was determinate buy incineration of 2 g of the Arachis hypogaea. L (peanut) shells powder which is placed in a crucible and then incinerated in a muffle furnace at about 550 ° C for 1 hour until a light gray or whitish color is obtained. The ash rate is expressed as a percentage of dry matter, three samples were incinerated simultaneously. The ash rate is calculated using the following formula:

$$MM\ \% = \frac{(W - T)}{T.S} \times\ 100$$

W: Weight of crucible and ash after calcination (g). T : Tare of crucible (g). $T.S$: Mass of the test sample (g). The percentage of organic matter is determined by the following equation MO%: The percentage of organic matter. Derived from Regulation 152/2009 / EC.

$$MO\ \% = 100 - MM$$

% MM: the ash rate (mineral matter). Water content is a quality criterion used primarily to estimate the humidity level of peanut shells and provides information on the stability of the product against the risk of alteration (deterioration) during storage. The humidity level of peanut shells is 5.55% (Table 1), so they are poor in water and rich in dry matter, which represents more than 94% in peanut shells powder. According to the results (Table 2), the peanut shells powder has 6.30% ash rate, so it is poor in mineral matter relative to 94.7% organic matter which is used as a source of energy and carbon for Growth and development of denitrifying bacteria. According to previous studies, the peanut shells have a high Cellulose content of 69.42% [38]. They degrade more easily. However, peanut shells show better initial carbon availability than woody substrates, suggesting better degradability, provided the nutritional composition of the medium is sufficiently rich [39]. For the content of mineral elements in peanut shells determined by atomic absorption spectroscopy Table 3 summarizes the mineral composition of peanut shells. The presence of nutrients in considerable quantities is noted in relation to the requirements of denitrifying bacteria. These elements are Mg^{2+}, Ca^{2+}, Zn^{2+} and Fe^{2+}. Analysis of the results shown in Table 3 reveals a very important point that needs to be studied

Table 1. *Humidity level of peanut Shells powder*

H% Medium	H1	H2	H3
5.55	5.45	5.54	5.65

Table 2.*Ash rate of peanut shells powder*

MM% Medium	MM3%	MM2%	MM1%
6.30	6.54	6.91	5.46

Since peanut shells are rich in trace elements, it is possible to envisage the elimination of these elements from the feed solution and to see the possibility of the denitrifying bacteria to develop in an important way by consuming the minerals naturally present in the organic support. Consequently, this last will constitute in addition to the source of carbon, the bacterial support, and an important source of trace elements.

Table 3.*Mineral content of the peanut shell powder*

Element	Cd	Fe	Pb	Ca	Mg	Zn
Concentration (mg/L)	1,10	92,43	244,7	0,9642	0,07627	0,01492

Table 4. *Granulometry laser of the peanut shell powder*

Sample	Width of particle size range (µm)	Percentile			Ø Median (µm)	span
		Fine particles 10%	Median particles 50%	Large particles 90%		
Peanut shells	7.69-262,37	<28.92	<381.74	<1212.03	526.95	3.09

At the end of the laser granulometric analysis, it can be concluded that the homogeneous peanut shell sample analyzed has a particle size range which varies between 7.69 µm and 262.37 µm with a median size of 381.74 µm. This homogeneity allows a good dispersion of the peanut shells in the solution to be treated and therefore a homogeneous adhesion of the denitrifying microorganisms and therefore a good removal of the nitrates. Third, fine particles provide more surface area for bacterial colonization per volume of substratum than do larger particles [40, [41].

Results of FTIR analysis of peanut shells before and after treatment with NaOH are shown in Figure 1. These spectra show absorbance bands characteristic of different chemical bonds such as: The bond (OH) at about 3467.94 cm -1, the (CH) bond at the wavelength of 3079.91 cm^{-1}, the (C≡C) bond at the wavelength of 2362.35 Cm-1, the bond (C = C) at 1644.85 cm^{-1}, and the (-NH) bond at 1410.19 cm^{-1}, and the bond (C = O) at 1107.85 cm^{-1} [42]. According to Song et al. The decrease in the intensity of the spectroscopic bands reflects the rupture of the bonds by the action of NaOH

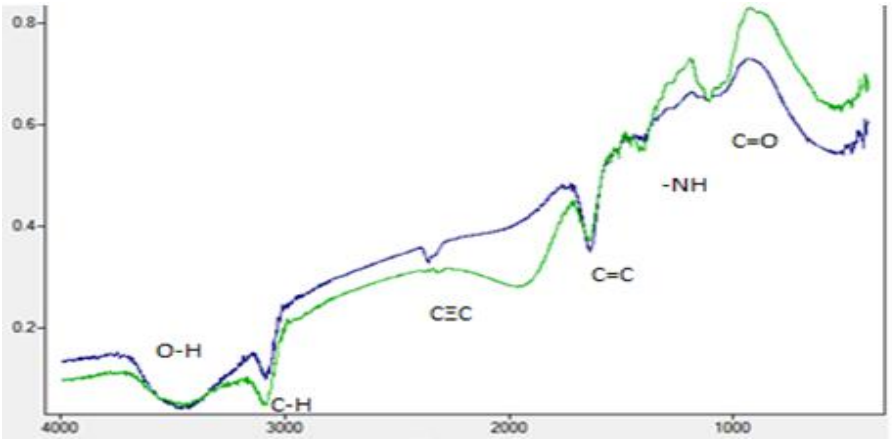

Figure 1. *Comparison between infrared spectra of peanut shell powder before and after treatment*

The various images of peanut shells obtained using a scanning electron microscope (SEM) are shown in figure 2 For native peanut shells, we note the presence of very large pores that promote the adhesion of microorganisms. On the other hand, the development of the biofilm in Figure V6 clearly

shows the peanut shells which served as a support for denitrifying bacteria. The photographs show the different states of the porosity, it is of various shapes and diameters constituted essentially of micropores and mesopores. This porosity allows the bacterial cells to attach firmly to the immersed surface in an aqueous environment. The growth of fixed cells involves the multiplication of cells and the production of polymers (exopolysaccarides). The latter extend from the cell and form a matrix; the set of cells and attached polymers is called biofilm [43].A biofilm is defined as a bacterial population enclosed in an adherent matrix. In nature, microorganisms usually attach to solid surfaces (especially to liquid–solid interfaces). After attachment, they form microcolonies, usually produce extracellular polymeric substances (EPS), entrap debris and other species of cells, and form biofilms. In clinical and industrial settings, bacteria are found predominantly in biofilms and not as planktonic cells, such as those typically studied in the laboratory [44].

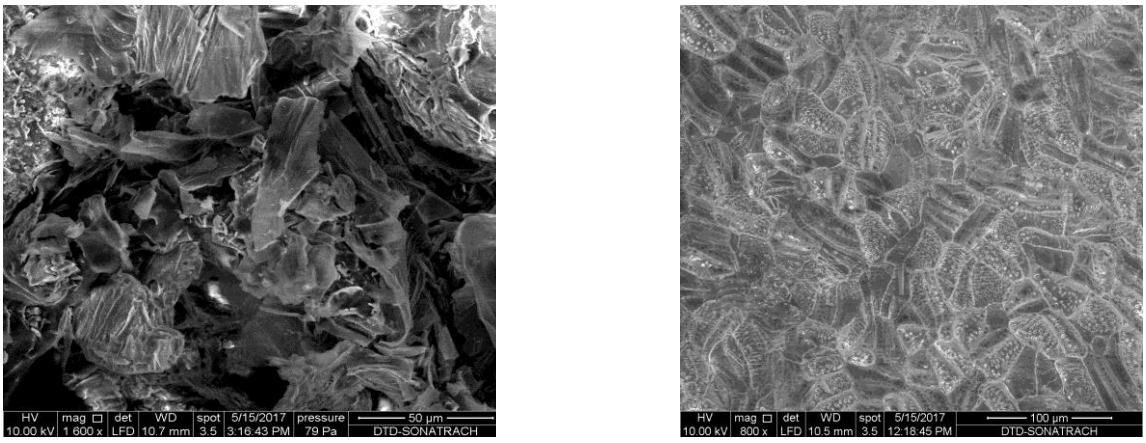

Figure 2. *structural of the peanut shell powder showing with SEM and biofilm-forming, bacterioplankton*

According to Figure 3, it is noted that when the initial nitrate concentration is increased from 50 mg / l to 200 mg / l, the denitrification rate is unstable and the value of 100 mg / l gives the best denitrification rate and for this we took this value for the rest of the experiments.

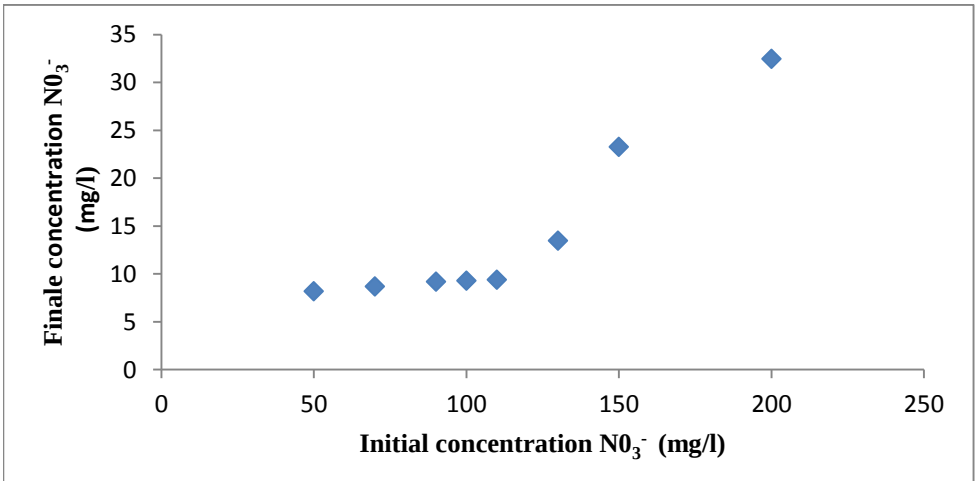

Figure 3*. Graphical presentation of final nitrate concentrations with respect to initial nitrate concentrations*

The variation of temperature directly influences the bacteria latency time. The more the temperature is raised, the more the latency time decreases. According to Figure 4, it is noted that the concentrations are decreased with all temperatures. Increasing the temperature from 5 ° C to 60 ° C; The denitrification rate increases to 25 ° C. and then decreases because the high temperatures (40 and 60 ° C.) have a negative influence on the development of the bacteria. For the low temperature (5 ° C), which gives us a decrease in the denitrification rate, we can deduce that the ideal temperature for denitrification is 25°C.

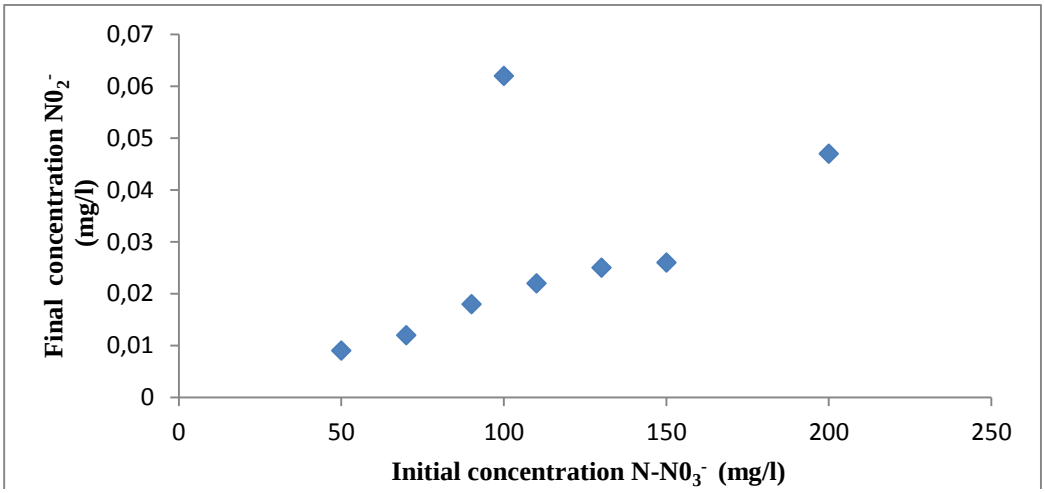

Figure 4. *Graphical presentation of final nitrite concentrations with respect to initial nitrate concentrations*

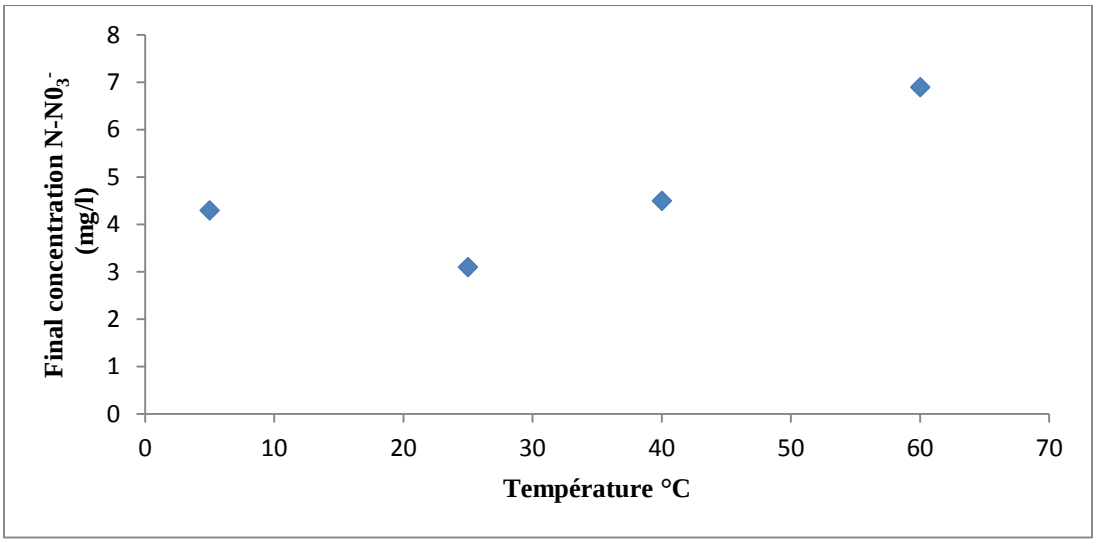

Figure 5.*Graphical presentation of final nitrate concentrations with respect to temperature*

As shown in Figure 6, nitrate reduction increases in the neutral medium (7.16) and decreases in the other two acid media (4.60) and base (9.08), hence it is deduced that denitrification is more efficient in the medium neutral.The maximum denitrification rate is generally in the neutral zone of pH. In the literature Yang et al. [45] measured an optimum pH of 7.1. Folgar and Vukovie (2003) found a pH of 7.4 and for Likiardopol et al., The optimum pH was 7 to 7.5 [45]. For our study pH 7.16 the denitrification rate is higher than 90%. This phenomenon is explained by the fact that several enzymes possess weak basic or acid groups which are involved in the catalytic reactions.

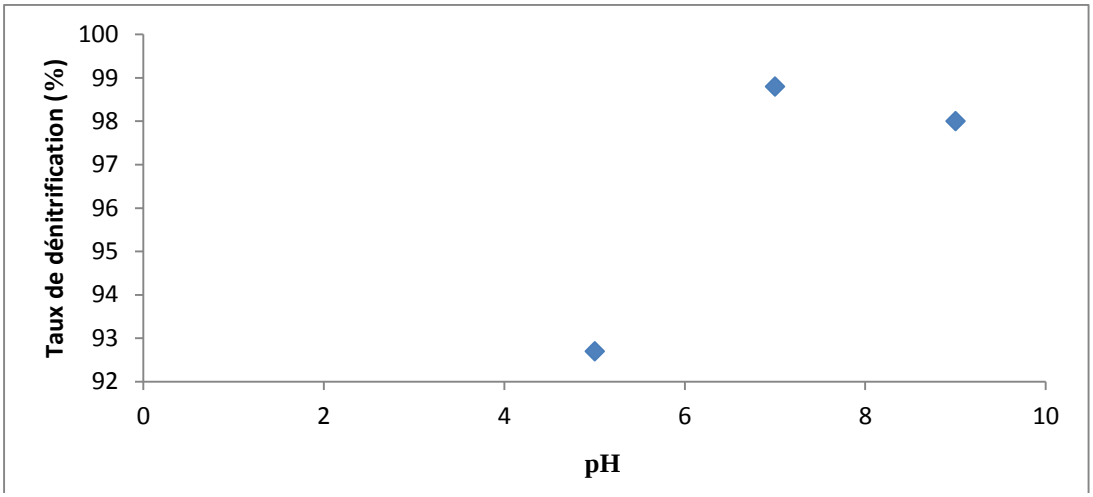

Figure 6. *Graphical presentation of final nitrate concentrations with respect to pH*

Figure 4 shows that by increasing the initial nitrate concentration from 50 to 200 mg / l, the concentration of nitrites is unstable. The apparition of the nitrites is bound to the disappearance of the nitrates. It decreases to the concentration 50 mg / l and increases to 100 mg / l, then decreases again to the concentration of 150 mg / l and then increases to the concentration of 200 mg / l.The pH has an important influence on the control of biological denitrification. Denitrification can take place in the pH range 4– 11, but most of the bacteria are slightly basophilic, with a pH optimum range of 7.5–8.5. Almost the same denitrification performance was achieved. pH in the range of 6.5–8 did not significantly affect biological denitrification [37].

Also for pH variation and temperature the concentration of nitrites is unstable; For the pH there is a better decrease in the neutral zone (Figure 6), and with respect to temperature, nitrites were absent at 25 ° C (Figure 4).The instability of nitrite concentration due to enzyme activity reductase.Denitrification is found to be effective in temperatures of (5 to 60 ° C) and in pH zones (4.60, 7.16, 9.08), but it will be much better under normal conditions (neutral pH and ambient temperature).

IV.Conclusion

Based on the results of an extensive literature search, and detailed experimental program with a batch reactor, it is concluded that the heterotrophic denitrification is a viable process for nitrate removal from groundwater supply sources. Under this research program many specific conclusions have been drawn. The experimental results concerning peanut shells showed that they could potentially remediate ground water with high level of nitrate. The effective parameters such as temperature, pH, and initial concentration of nitrates on whole system performance were investigated. The heterotrophic denitrification using a natural carbon source (peanut shells) showed good nitrate removal capacity, with a daily removal of around 95% of nitrates. No significant nitrite accumulation occurred in the denitrified water. The process is affected by temperature decreasing the performance so optimal operational temperature was obtained around 25°C.The optimum pH range for autotrophic denitrification using peanut shells is pH 7, decreasing initial pH decreased denitrification activity; however, increasing pH had little effect on denitrification. The results suggest that peanut shells are effective in ground water treatment as the sole chemical and physical substrate for the denitrifying microorganism. Moreover, peanut shells are a safer substrate when compared with traditional, liquid carbon sources. The natural organic substrate described here not only avoids the use of expensive carbon sources (e.g., ethanol, acetic acid, poly-3-hydroxybutyrate) but also offers an alternative way to reuse agriculture waste. Other benefits of using peanut shells included high denitrification rate, easy operation they are recommended as an economical

and effective external carbon source for nitrate removal.

V. References

1. Soares, MI.M.; Brenner, A.; Yevzori, A.; Messalem R.; Leroux, Y.; Abeliovich, A. Denitrification of ground-water: Pilot-plant testing of cotton-packed bioreactor and post-microfiltration, *Water Science and Technology 42*(1-2) (2000) 353-359.
2. Morteza, M.; Shamsuddin, S.; Kumars, E. Removal Techniques of Nitrate from Water, *Asian Journal of Chemistry* 26 (2014) 7881-7886.
3. WHO., Guidelines for Drinking-water Quality Incorporating First and Second Addenda, Volume 1, Recommendations, 3rd ed. *World Health Organization Geneva* (2008).
4. NHFPC. Standards for Drinking Water Quality, 1st ed. National Health and Family Planning Commission of the PRC, Beijing (2006).
5. Alonso Fernández, J.R.; García Nieto, P.J.; Díaz Mu˜niz, C.; Álvarez Antón, J.C. Modeling eutrophication and risk prevention in a reservoir in the Northwest of Spain by using multivariate adaptive regression splines analysis, *Ecological Engineering* 68 (2014) 80-89.
6. Kiedrzy´nska, E.; Kiedrzy´nski, M.; Urbaniak, M.; Magnuszewski, A.; Skłodowski, M.; Wyrwicka, A.; Zalewski, M. Point sources of nutrient pollution in the low land river catchment in the context of the Baltic Sea eutrophication. *Ecological Eng*ineering 70 (2014) 337-348.
7. Kłodowskaa, J.; Rodziewicza, W.; Janczukowicza, A.; Cydzik-Kwiatkowskab, K. Effect of citric acid on the efficiency of the removal of nitrogen and phosphorus compounds during simultaneous heterotrophic-autotrophic denitrification (HAD) and electro coagulation, *Ecological Engineering* 95 (2016) 30-35.
8. Vitousek, P.M.; Aber, J.D.; Howarth, R.W.; Likens, G.E.; Matson, PA.; Schindler, D.W.; Schlesinger, WH.; Turnan, D.G. Human alteration of the global nitrogen cycle: sources and consequences, *Ecological Application* 7 (2007) 737-750.
9. Inwood, S.E.; Tank, J.T.; Bernot, M.J. Factors Controlling Sediment Denitrification in Midwestern Streams of Varying Land Use, *Microbial Ecolog* 53(2) (2007) 247-258.
10. Angar, Y.; Kebbouche-Gana, S.; Djellali, N.E. Comparison of biological nitrate reduction Effectiveness of Two strains Isolated from Activated sludge. *Asian Journal of Chemistry* 28(2) (2016) 463-466.
11. Winneberger, J.H. Nitrogen, Public Health and the Environment. Ann Arbor Science Publishers, Inc. *Annal Arbor* Michigan. (1982) 5.
12. Pu, J.Y.; Feng, C.P.; Liu, Y.; Li, R., Kong, Z.; Chen, N.; Tong, S.; Hao, C.B.; Liu, Y. Pyrite-based autotrophic denitrification for remediation of nitrate contaminated groundwater, *Bioresource Technology* 25 (2014) 117–123.
13. Qiaochong, H.; Chuanping, F.; Tong, P.; Nan, C.; Qili, H.; Chunbo, H. Denitrification of synthetic nitrate-contaminated groundwater combined with rice washing drainage treatment. *Ecological Engineering* 95 (2016) 152-159.
14. Shih-hui P. Autotrophic denitrification of groundwater in a granular sulfur-packed up-flow reactor. Presented to the Faculty of the Graduate School of The University of Texas at Arlington in Partial Fulfillment (2007).
15. Pan, Y.; Ni, B.J.; Bond, P.L.; Ye, L.; Yuan, Z. Electron competition among nitrogen oxides reduction during methanol-utilizing denitrification in wastewater treatment. *Water Res*earch 47(10) (2013) 3273-3281.
16. Desloover, J.; Vlaeminck, S.E.; Clauwaert, P.; Verstraete, W.; Boon, N. Strategies to mitigate N_2O emissions from biological nitrogen removal systems, *Current Opinion in Biotechnol*ogy 23(3) (2012) 474-482.
17. Wunderlin, P.; Mohn, J.; Joss, A.; Emmenegger, L.; Siegrist, H. Mechanisms of N_2O production in biological wastewater treatment under nitrifying and denitrifying conditions, *Water Res*earch 46(4) (2012) 1027-1037**.**
18. Law, Y.; Ye, L.; Pan, Y.; Yuan, Z. Nitrous oxide emissions from wastewater treatment processes, *Philosophical Transactions Royal Soc*iety 367 (2012) 1265-1277.
19. Qilin, W.; Guangming, J.; Liu, Y; Maite, P.; Zhiguo, Y. Heterotrophic denitrification plays an important role in N_2O production from nitritation reactors treating anaerobic sludge digestion liquor, *Water research* 62 (2014) 202 -210.
20. Wen, J.P.; Pan, L.; Du, L.P.; Mao, G.Z. The denitrification treatment of low C/N ratio nitrate-nitrogen wastewater in a gas-liquid-solid fluidized bed bioreactor, *Chemical Enineering J*ournal 94(2) (2003) 155-159.
21. Rui, L.; Chuanping, F.; Weiwu, H.; Beidou, X.; Nan, C.; Baowei, Z.; Ying, L.; Chunbo, H.; Jiaoyang, P. Woodchip-sulfur based heterotrophic and autotrophic denitrification (WSHAD) process for nitrate contaminated water remediation. *Water Research* 89 (2016)171-179.
22. Wang, J.L.; Yang, N. Partial nitrification under limited dissolved oxygen conditions. *Process Biochemistry* 39(10) (2004) 1223-1229.
23. Wang, Q.H.; Feng, C.P.; Zhao, Y.X.; Hao, C.B. Denitrification of nitrate contaminated groundwater with a fiber-based biofilm reactor. *Bioresource Technol*ogy 100 (2009) 2223-2227.
24. Gomez, M.A.; Galvez, J.M.; Hontoria, E.; Gonzalez-Lopez, J. Influence of ethanol concentration on biofilm bacterial composition from a denitrifying submerged filter used for contaminated groundwater, *Bioscience Bioeng*ineering 95(3) (2003) 245-251.
25. Akunna, J.C.; Bizeau, C.; Moletta, R. Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: glucose, glycerol, acetic acid, lactic acid and methanol. *Water Res*earch 27 (1993) 1303-1312.
26. Kim, Y.S.; Nakano, K.; Lee, T.J.; Kanchanatawee, S.; Matsumura, M. On-site nitrate removal of groundwater by an immobilized psychrophilic denitrifier using soluble starch as a carbon source,*Journal of Bioscience Bioengineering* 93(3) (2002) 303-308.
27. Yingxin, Z.; Baogang, Z.; Chuanping, F.; Fangyuan, H.; Peng, Z.; Zhenya, Z.; Yingnan, Y.; Norio, S. Behavior of autotrophic denitrification and heterotrophic denitrification in an intensified biofilm-electrode reactor for nitrate-contaminated drinking water treatment, *Journal of Bioresource Technology* 107 (2012) 159-165.
28. Liu, Q.J.; Hu, X.; Wang, J.L. Performance characteristics of nitrogen removal in SBR by aerobic granules. *Chinese Journal of Chemistry Engineering* **13(5)** (2005) 669-672.
29. Chen, Z.Q.; Wen, Q.X.;Wang, J.L.; Li, F. Simultaneous removal of carbon and nitrogen from municipal-type synthetic wastewater using net-like rotating biological contactor (NRBC). *Process Biochemistry* 41(12) (2006) 2468-2472.
30. Liu, L.H.; Koenig, A. Use of limestone for pH control in autotrophic denitrification: batch experiments. *Process Biocheistry* 37 (2002) 885-893.
31. Mohseni-Bandpi, A.; Elliott, D.J.; Zazouli, M.A. Biological nitrate removal processes from drinking water supply-a review. *Journal of Environmental Health Science and Eng*ineering 11 (2013) 35-45.

32. Konstantinos, A.; Karanasios, IA.; Vasiliadou, A.G.; Tekerlekopoulou, C.S.; Akratos, S.P .; Dimitrios, V. Effect of C/N ratio and support material on heterotrophic denitrification of potable water in bio-filters using sugar as carbon source, *International Biodeterioration & Biodegradation* (2016) 62-73.
33. Galloway, J. N. Nitrogen cycles: past, present, and future. *Biogeochemistr* 70 (2004) 153-226.
34. Schlesinger, W. H. On the fate of anthropogenic nitrogen. Proceedings of the *National Academy of Sciences (USA)* **106** (2009) 203-208.
35. Michelle L. McCrackin1 and James J. Elser. Atmospheric nitrogen deposition influences denitrification and nitrous oxide production in lakes, *Ecology* 91(2) (2010) 528-539.
36. Ovez, B. Batch biological denitrification using Arundo donax, Glycyrrhiza glabra, and Gracilaria verrucosa as carbon source. *Process Biochemistry* 41 (2006) 1289-1295.
37. Boudergues, R; Calvet, H . Note sur la digestiblité des coques d'arachides utilisées en alimentation animale ; Revue D'élevage et de Médecine Vétérinaire Des Pays Tropicaux 23 ; (2000).
38. Misra, R.V. ; Roy, R.N. ; Hiraoka, H. Méthodes de compostage au niveau de l'exploitation agricole. Organisation des Nations Unies pour l'Alimentation et l'Agriculture. Rome (2005).
39. Pattinson, S. N.; garcia-ruiz, R.; Whitton, B. A. Spatial and seasonal variation in denitrification in the Swale-Ouse system, a river continuum. *Science of the Total Environment* 210 (1998) 289-305.
40. Inwood, S.E.; Tank, J.L.; Bernot, M.J. Factors controlling sediment denitrification in Midwestern streams of varying land use, *Microbial Ecology* 53 (2007) 247– 258.
41. Salem, Z.; Lebik, H.; Cherafa, W.K.; Allia. K. Valorisation of olive pits using biological denitrification. *Desalination* 204 (2007) 72-78.
42. Filloux, A.; valkex, I. Biofilm set up and organization of bacterial community. *Medicine Science Paris* 19 (2003) 77-83.
43. Kawarai, T.; Furukawa, S.; Narisawa, N.; Hagiwara, C.; Ogihara, H.; Makari, Y. Biofilm formation by Escherichia coli in hypertonic sucrose media. *Journal of Bioscience and Bioengineerin* 107(6) (2009) 630-635.
44. Yang, X.; Grailer, J.J.; Rowland, I.J, Javadi, A.; Hurley, S.A.; Matson, VZ, Steeber, D.A; Gong, S. Multifunctional stable and pH-responsive polymer vesicles formed by heterofunctional triblock copolymer for targeted anticancer drug delivery and ultrasensitive MR imaging. ACS Nano., 23(11).
45. Canter, L.W. Nitrates in Groundwater. Boca Raton: CRC Press (1997).

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