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Nitrate Removal (NO_3^-) from Different Sources of Water by Laboratory-Scale Reactor Biofilters with Anaerobic Condition

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التخلص من النترات (NO_3^-) الملوث لمصادر مختلفة من المياه بواسطة وحدة معالجة معملية حيوية تحت الوسط اللاهوائي

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Abstract

The aim of this study was to identify the pollution of (NO_3^-) for some different water sources, whether surface or groundwater. The study included (3) water sources from lakes collected behind some dams or valleys. As well as the number of (13) sources of groundwater wells and the depths of these wells ranging from 10-200 m, and from concentrating the nitrates in the water. Designed a laboratory treatment unit for the purpose of bio-removal of nitrates, this unit operated under a Vertical Flow Biofilter (VFB), and a unit operating under the anaerobic system, with the use of support materials inside the unit (gravel and PVC). To grow bacteria and the form of Biofilm, the unit was operated under a Hydroelectric Retention Time (HRT) for 4 *days*. The samples were collected every four days from the treatment unit. The results showed that nitrate concentration is significantly higher in groundwater than in surface water, with a concentration ranging from 0.7 to 190 ppm. Finally, nitrate disposal after treatment reached (76.1%).

Keyword: *Biofilm, Biofilter, Nitrate, Anaerobic*.

الملخص

الهدف من هذا البحث كان هو التعرف على التلوث بالنترات (NO_3^-) لبعض مصادر المياه المختلفة سواء السطحية او الجوفية في منطقة زليتن، حيث شملت الدراسة عدد (3) مصادر لمياه بحيرات متجمعة خلف بعض سدود الوديان، وكذلك عدد (13) مصدرا من أبار المياه الجوفية وكانت المياه الجوفية تتراوح اعماقها من 10 الى 200 متراً، وتبعاً لنتائج تركيز النترات لهذه المياه تم تصميم وحدة معالجة معملية لغرض إجراء عملية الإزالة الحيوية للنترات وتعمل هذه الوحدة بنظام التدفق العمودي (Vertical Flow Biofilter, VFB)، وهى وحدة تعمل تحت الظروف اللاهوائية (Anaerobic) في وجود البكتريا مع استخدام مواد ساندة داخل الوحدة وهى الحصي و مادة (PVC) لغرض نمو البكتريا عليها وتكوين ما يعرف (Biofilm). تم تجميع العينات كل اربعة أيام بعد بدء عملية المعالجة، وكانت نتائج تركيز النترات في المياه السطحية والجوفية تتراوح من 0.7 إلى 190 جزء في المليون، وتواجدها في المياه الجوفية بنسبة اعلى عنه من المياه السطحية، وفى الغالب تتجاوز الحدود المسموح بها في مواصفات مياه الشرب. أما عن نتائج تركيز النترات بعد المعالجة فقد انخفضت ووصلت نسبة التخلص من النترات إلى (76.1%)، وقد تم تجميع الرواسب المتكونة على المادة الساندة داخل وحدة المعالجة والكشف فيها على بكتريا اختزال النيتروجين حيث تم الكشف على عدد من هذه البكتريا حيث تعمل الوحدة تحت معدل زمن احتفاظ هيدروليكي (Hydraulic Retention Time, HRT) قدره 4 أيام.

الكلمات الدالة: النترات، غشاء حيوي، مرشح حيوي، لاهوائي.

1. Introduction

The nitrogen cycle is the biogeochemical cycle by which nitrogen is converted into multiple chemical forms as it circulates among the atmosphere, soil and marine ecosystems. The conversion of nitrogen can be carried out through both biological and physical processes. Important processes in the nitrogen cycle include fixation, ammonification, nitrification, and denitrification. The majority of Earth's atmosphere (78%) is nitrogen, making it the largest source of nitrogen. From the above, nitrogen is changing element that substituted from one form to another in soil, plant, animal and even inside humans, as well as air and water. Nitrate ion (NO_3^-) is the form of oxidized nitrogen commonly found in aquatic systems (Al-Ghariani, 2002; and Rittmanm and McCarty, 2001). The most important processes associated with the elimination of nitrate are the process of reverse nitrification (Rittmanm and McCarty, 2001). A process that occurs mostly under the action of bacterial enzymes, which convert the oxidizing form of nitrogen (NO_3^-) into oxidized nitrogen gases, in order to reach the production of nitrogen as a gas (N_2), an anaerobic process, and mostly bacteria to do this process Gram–Negative, usually from the genus *Protreobacteria*, such as, *Thiobacillus*; *Paracoccus*, *Alcaligenes*, and *Pseudomonas*.

Ion nitrates in must be at specific rates, does not cause harm to the health of all living organisms especially human. Previous study conducted on ground water on the quaternary reservoir in Jafara district, Libya found that nearly half of the wells surveyed were nitrate concentrations of more than 45 *ppm*, which exceeded the limits allowed in the specifications of Libyan drinking water (Belaid, 1999). According to the specifications of the World Health Organization or Libyan specifications, Nitrates (NO_3^-), the drinking water should not exceed 45 *ppm* is equal 10 *ppm* (NO_3^--N) (WHO, 2011; and LNCSM, 2009). In addition, microorganisms have been used by (Bourgues and Hart, 2001), especially bacteria, in the formation of thin films (biofilms) in wetlands, after being configured to dispose of nitrogen from industrial waste water and was found to play a major role in this process. In a nitrate disposal study using a Fiber Based Biofilm (FBB) treatment unit and using industrial waste water, the removal efficiency was close to 99% and the pH value was close to 7. Therefore, this study works to remove nitrates above the rates specified in the national or international standards.

2. Materials and Methods

2.1. Area of Study

This study was conducted in the city of Zliten, a 120 *km* east of Tripoli. The study was based on the collection of water samples from different sources, including three water lakes dams as well as a number of thirteen groundwater wells of different depths. Samples of these sources were collected and nitrate concentration (NO_3^-) was measured. Water with higher nitrate

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(NO_3^-) concentration was selected, and treatment of water in a laboratory treatment unit designed for this experiment.

2.2. Reactor Design

The reactor was made from PVC material as a cylinder shape form, with a volume 48.3 *liters* as shown in Figure (1). Several different layers of support material were placed inside the unit for bacterial growth. The top layer is filled with a plastic material granulated from (PVC) at thickness equal to 300 *mm*. The bottom layer was composed of gravels, is divided into two layers, the size of the gravels in terms of granular diameter in the upper layer was less than 7.5 *mm*, and thickness of this layer is 75 *mm*. In addition, the bottom layer of the gravels in terms granular diameter is 22-15 *mm*, and thickness of this layer is 75 *mm*. As well as, all these materials above have been washed before use treated. Moreover, layer of water was 100 *mm* thick above the materials inside the unit.

2.3. Preparation of Bacterial *Inocula*

Bacteria were prepared without focusing on a specific species and were incubated under anaerobic medium at room temperature in a plastic container. The source of bacteria was water sediments from the lakes of dams studied with a quantity of water wells (1 *kg* sediments: 6 *liters* of water contaminated with nitrates). The bacteria were supplied with carbon in the form of glucose at a rate of 360 *mg/l*. In addition, with continuous stirring of this mixture, the incubation continued for 12 *days*.

2.4. Experimental Procedure

At the start of the treatment, the bacteria were left in the container, which was prepared (for one day). To separate the sediments and obtain a liquid suspension, take the suspended liquid and inject in the proposed treatment unit from the input point as shown in Figure (1) was collected from the outlet and recycled within the unit again for ten days to fix the bacteria on the materials inside the treatment unit and creating a good growth from Bio-films. After that, water containing nitrates (190 *mg/l*) was fed. Whereas the highest value of nitrates recorded by the study well as shown in (Tables 1 and 2). Finally, samples of water were collected from the input and outlet every four days, nitrates and pH were measured. Also at the end of the experiment, thin Bio-films were collected and some strains of bacteria were evaluated. Moreover, the carbon element was added during the treatment of water within the processing unit (Reactor), carbon was added to nitrate ($C:NO_3^-$) as (1:0.5).

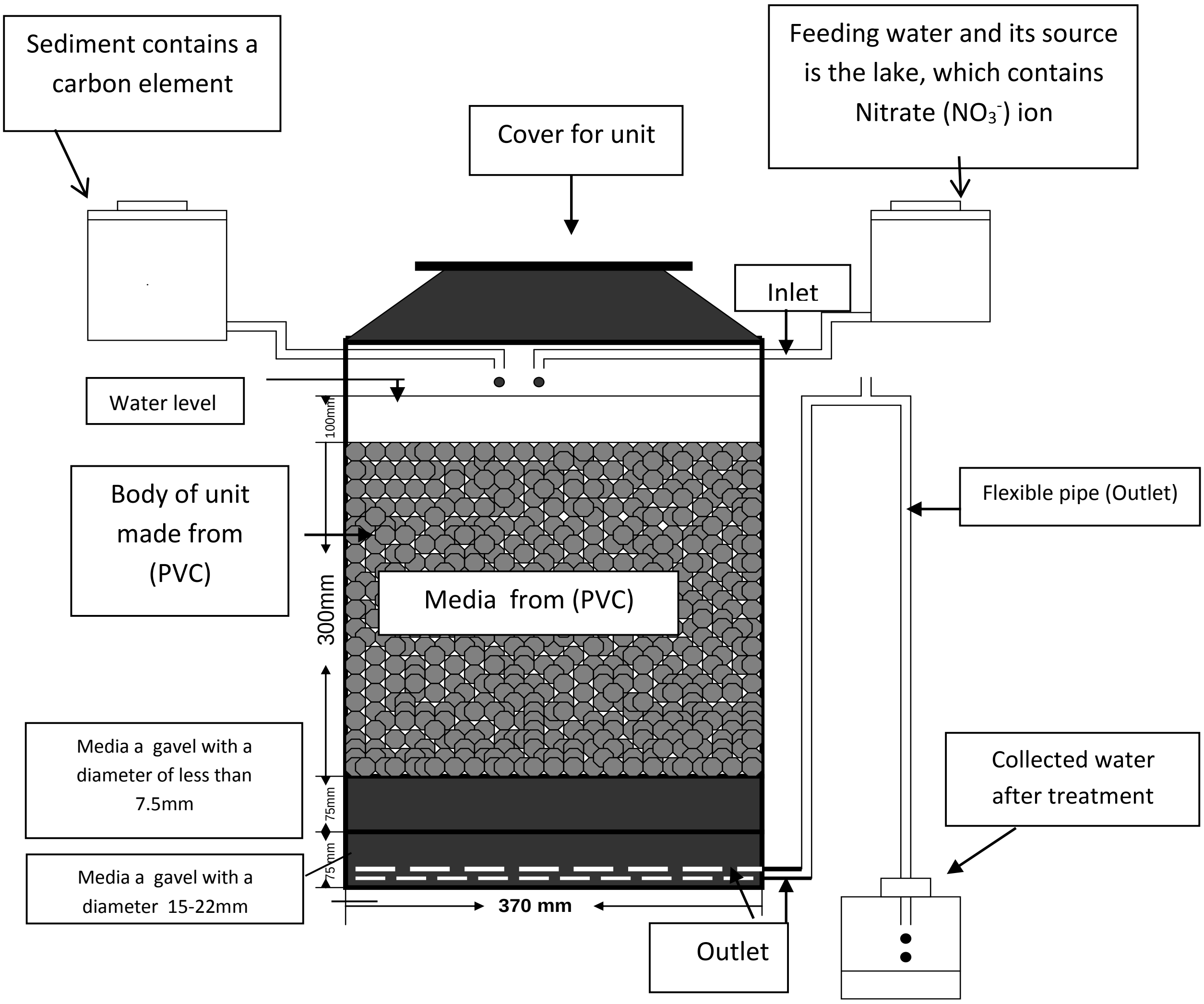

Figure 1. Schematic diagram of the cross-section of Processing unit (Reactor).

2.5. Analytical Techniques

The collected water samples were measured as follow:

- pH by pH-Meter (Jeneway 3310).
- Nitrate (NO_3^-), was measured by using spectroscope analysis techniques at scientific apparatus (Jeneway, 6305 UV), was used Ultraviolet Spectrophotometer Screening method at a wavelength of 220 *nm* to 275 *nm* (Eaton *et al.*, 2005).
- Total organic carbon (TOC), was measured by device Spectrophotometer. It works at 20±5 ^oC and moisture ratio 60±15% (Eaton *et al.*, 2005).
- Sulphate Reducing Bacteria (SRB) by SRB–BARTTM), and was incubated at temperature 15-25 ^oC (Droycon Bioconcepts Inc., 2002).

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- Bacteria (Pseudomonas aerugino) by Pseudomonas Broth Ampules, and incubated at temperature 37 ^oC (Abdalhafida and Al-Mobark, 1992).

Table 1. The wells and their depths, in addition to the concentration of nitrates (NO_3^-) and the degree of pH for feed water in the study.

No. of source	Depth of well (*m*)	Concentration of Nitrate (*mg/l*)	pH
1	14	190	7.62
2	10	101.6	7.77
3	25	31.65	7.3
4	70	0.7	7.47
5	88	23	7.5
6	180	11	7.34
7	184	19	7.31
8	200	18	7.34
9	25	28	7.3
10	30	70	7.32
11	25	85	7.41
12	30	60	7.32
13	40	90	7.33

Table 2. Nitrate concentration (NO_3^-) and pH in water dam lakes

No. of source (Lake of Dam)	Concentration of Nitrate (*mg/l*)	pH
1	3.20	7.8
2	1.23	7.13
3	1.37	7.5

3. Results and Discussion

3.1. Concentration of (NO_3^-) in Groundwater and Lake of Dams

Shows the high concentration of nitrates in the number of six wells (It is the wells number 1, 2, 10, 11, 12, and 13) from the thirteen wells studied, these six wells had a greater concentration compared to the specifications of drinking water, whether local or international (WHO, 2011; and LNCSM, 2009). The number of two wells (wells 3 and 9) approaches from specifications was mentioned above but less than them, the rest of the wells have a much lower level than the above was mentioned specifications. As for dam lakes, the concentration of nitrates is much lower compared to local or international drinking water specifications was mentioned above in Tables (1 and 2). These results are consistent with the results of the study conducted by Alia and Salman (2014), that included five underground sources in Syria, (an

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area with large population activity) in their study, the sources contaminated nitrates were above the international standards for drinking water.

3.2. pH in Groundwater and Lake of Dams

The pH degree was approaching the neutral for all sources of water, whether for wells (underground water) or lake dams, (Shown in Tables 1 and 2) this is before treatment in the proposed processing unit. After then, Table (3) shows the pH values after treatment in unit (Reactor) continued at the same rate near the neutral. All these results match the specifications of drinking water, whether Libyan or international mentioned above. Also, agrees with research results (Rafida and Ben Aisha, 2015) that are based on the treatment of rainwater by sulphate reducing bacteria (SRB).

Table 3. Concentration of nitrates (NO_3^-) and pH in the water after treatment within the treatment unit.

Samples*	**Concentration of nitrates after treatment (*mg/l*)**	**pH after treatment**
Sample 1	142.5	7.50
Sample 2	140	7.28
Sample 3	110	7.45
Sample 4	37.5	7.66
Sample 5	24.5	7.76
Sample 6	2.4	7.69
Sample 7	2.33	7.65
Sample 8	5.3	7.71

* Collection of samples was every four days starting from the processing unit.

3.3. Concentration (NO_3^-) in Water After Treatment

The nitrate decreased in water treatment within the processing unit (Reactor). This decline was gradual over time, while increasing the effectiveness of the treatment process as shown in Table (3) and Figure (2). On this, after twenty days of treatment the concentration of nitrates in the treated water has become within the specifications, whether International or Libyan standard mentioned above. Also, that after the same period that the effectiveness of the disposal of nitrates was (2.33 *ppm*), and with nitrates continue to decrease in treated water. At the end of the experiment the efficiency of the treatment unit was (76.1%). Final, this means that the growth and development of microorganisms within thin bio-films has increased with the passage of processing time. The results of this study are consistent with the results of the study conducted by (Bourgues and Hart, 2001), on the water of the wetland under the anaerobic medium by thin bio-films. These bio-films are actively involved in the process of reducing nitrates and converting into different nitrogen gases.

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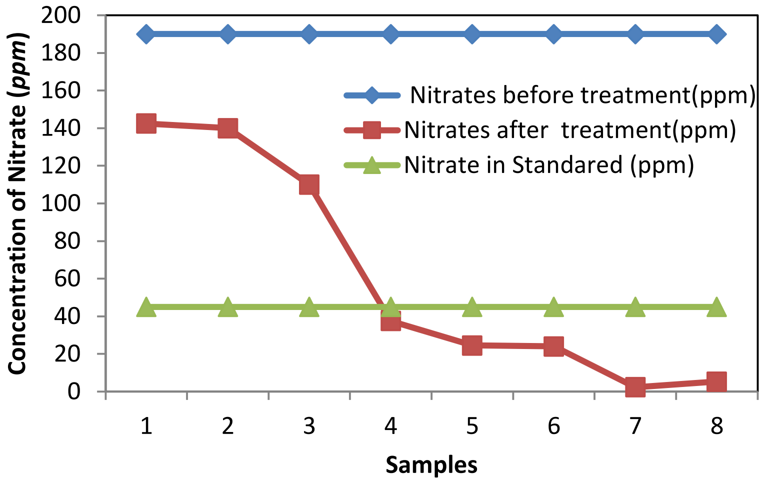

Figure 2. Concentration of nitrates in water before and after treatment within the treatment unit as well as in Libyan and international specifications.

3.4. Ratio Consumption of C:NO_3^-

As mentioned previously that, the addition of carbon to nitrate was distributed at ratio (C:NO_3^- 1:0.5). This was during the treatment of water within the processing unit (Reactor). After then, the water out of the treatment the consumption ratio of carbon to nitrate was distributed (C:NO_3^- 1:0.7). This is evidence of the growth and functioning of living organisms within the processing unit as shown in Tables (3 and 4) and Figure (3). Note that carbon measurement was in the form of total organic carbon (TOC). This is consistent with the research (Sigunga *et al.*, 2002), nitrate was removed from groundwater by microorganisms and some plants with organic carbon dissolved with water. Addition of carbon was close to the presence of nitrates. Also, the consumption ratio was close to carbon and nitrate. In another search by (Rafida and Ben Aisha, 2015) used Sulphate reducing bacteria (SRB) removal the lead element from the lake water dam inside the processing unit, with carbon element addition Where carbon consumption was found to be sulphate at ratio (1:0.5).

Table 4. Total Organic Carbon (TOC) concentration before and after treatment

Samples*	Total organic carbon in water before treatment (*mg/l*)	Total organic carbon in water after treatment (*mg/l*)
Sample 1	325	200
Sample 2	325	50
Sample 3	325	45

* Collection of samples was every four days starting from the processing unit.

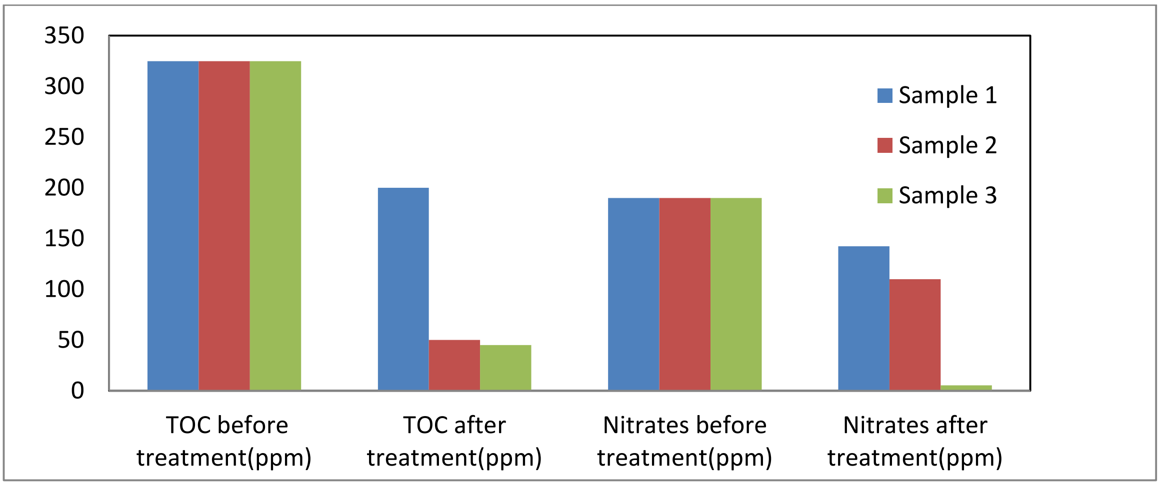

Figure 3. Concentration of Total Organic Carbon (TOC) and nitrates in feed and treated water (*ppm*).

3.5. Types of Bacteria in Effective Biofilms

Bio-films were collected at the end of the experiment, which was involved in the removal of nitrates from the water. The number of Sulphate Reducing Bacteria (SRB) and *Pseudomonas aeruginosa* were estimated, the results were (1.8×10^4 *cell/ml*) and (4.0×10^2 *cell/ml*) respectively for the previously mentioned bacterium species. The results of this research are consistent with the results of several previous research, such as research (Gomez *et al*., 2003), five types of bacteria have been isolated which have the ability to reduce nitrates. It was a precipitation wastewater treatment unit, and the dominant bacteria are Sulphate reducing bacteria (SRB).

4. Conclusion

The study showed the presence of nitrate contamination in the groundwater studied, and the values of pollution exceed the limits allowed in the specifications mentioned above. Accordingly, a special treatment unit has been designed for the growth of microbial organisms especially bacteria on materials from (PVC), growth of bacteria for the purpose of removing nitrates (NO_3^-). Also, the percentage of removal of contaminated from (NO_3^-) water to about (76.1%). Final, the treated water within the proposed treatment unit is within the limits allowed in the standards of drinking water mentioned above.

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