Possibilities of anaerobic biodegradation of nitrates in groundwater using the emulsified vegetable oil

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Abstract

This paper is a presentation of the bioremediation application as a treatment type of contaminated soils and groundwater. In addition to the method and mechanism of biodegradation, a possibility of biopromotion application is considered, i.e. stimulation of the biodegradation process by manipulating the conditions and factors that can significantly promote and accelerate the entire process. Bioremediation is a natural process that takes place spontaneously in natural conditions if all the factors are present in the soil and groundwater. However, this process is very slow in natural conditions. Knowing the mechanism of biodegradation and manipulating the subsurface conditions, we get the significantly faster and long-term results. Comparing to other methods and technologies, bioremediation is more cost-effective and naturally sustainable, i.e. it is in accordance with principles of sustainable development.

Introduction

Natural process of bioremediation is based on the principles of the electron donor and electron acceptor existence. Numerous different compounds can be an electron donor in the soil and groundwater: from organic matter in the soil to sodium lactate, molasses or vegetable oil, which are added to the soil in order to improve the process of bioremediation. In some cases, the contaminating matter itself can be an electron donor, as petrol carbohydrates are. Electron acceptor in aerobic conditions is oxygen, while in anaerobic conditions those are chlorinated solvents such as tetrachloroethane, PCB, TCE, etc.

Through biodegradation there are two processes taking place: fermentation and respiration. Fermentation is an anaerobic metabolism where degradation of natural molecules such as sugar glucose happens. Through this process a certain amount of energy (not all) is being released, therefore the products of fermentation contain chemical energy, in other words they are not totally oxidised, but they can not be further metabolised without the presence of oxygen or any other electron acceptor that is highly oxidised. In this way the compound that is an electron donor is being decomposed to get molecular hydrogen (H_2). Respiration is like active breathing, i.e. it is a process through which a number of biochemical oxidising processes take place, where organic matter, i.e. molecules are transferred in carbon-dioxide and water, while the chemical energy produced is used for cell processes. Henceforth, during fermentation the electron of hydrogen is being released and is further used to make cell

breathing possible, where electron acceptor, the substance that is (in our case) a contaminant, is decomposed and the energy necessary for work and cell reproduction is produced. Through this process the carbon-dioxide and water are released as side elements.

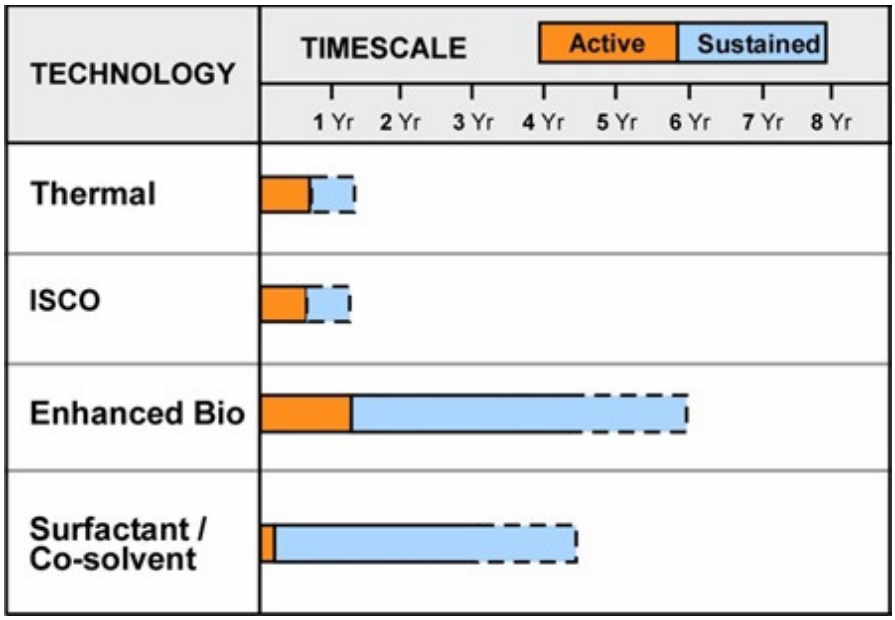
Cell breathing is a multi process consisting of a number of reactions where the compound, i.e. contaminating matter, gradually, through phases is being transferred to the final product and each of these steps and phases in decomposition is followed with energy production, which is used by microbe cells for growth and reproduction.

This process can take place totally spontaneously in natural conditions, if in the substrate exists an adequate consortium of bacteria needed for the existing contaminating substance decomposition, together with the source of carbon and conditionally saying “food” for microbes, i.e. a compound that will provide microbes with hydrogen necessary for cell respiration (breathing). This is a so-called self-refinement and it takes place without human interference. On the other hand, biopromotion or biostimulation considers the manipulation of natural conditions by adding the source of carbon to provide energy for reproduction or oxygen (aerobic conditions) or some other electron acceptor (anaerobic conditions).

In case that the adequate microorganisms for biodegradation of the existing contaminating matter are absent in the soil, it is possible to add microorganism to the soil and groundwater and it is then a bioaugumentation.

Mechanism of bioremediation and its advantages

In comparison to other methods of remediation, bioremediation does not require construction of expensive infrastructural objects and treatment plants. It can be conducted *in situ* which also decreases the costs, such as for excavation and mobilization of the contaminated soil. Even in comparison to *in situ* technology this method is significantly cheaper *(Figure 1)*.



**Surfactants – similar to detergents, decrease the surface strain of the liquids and increase the mobility of chemicals. Co-solvents – such as alcohol, decrease solubility and mobility of different chemical contaminants. Such as chlorinated solvents and petrol carbohydrates.*

Figure 1. Duration and sustainability of bioremediation compared for several methods *(Ref. 1)*

It should be emphasised that the bioremediation system has extended results.

A diapason of contaminating matter that can be treated is very wide. Depending of the contaminating matter in the soil and if this matter is an electron donor or an acceptor, we determine what substrate is to be added i.e. injected into the soil to promote and accelerate the process of natural biodegradation (Figure 2).

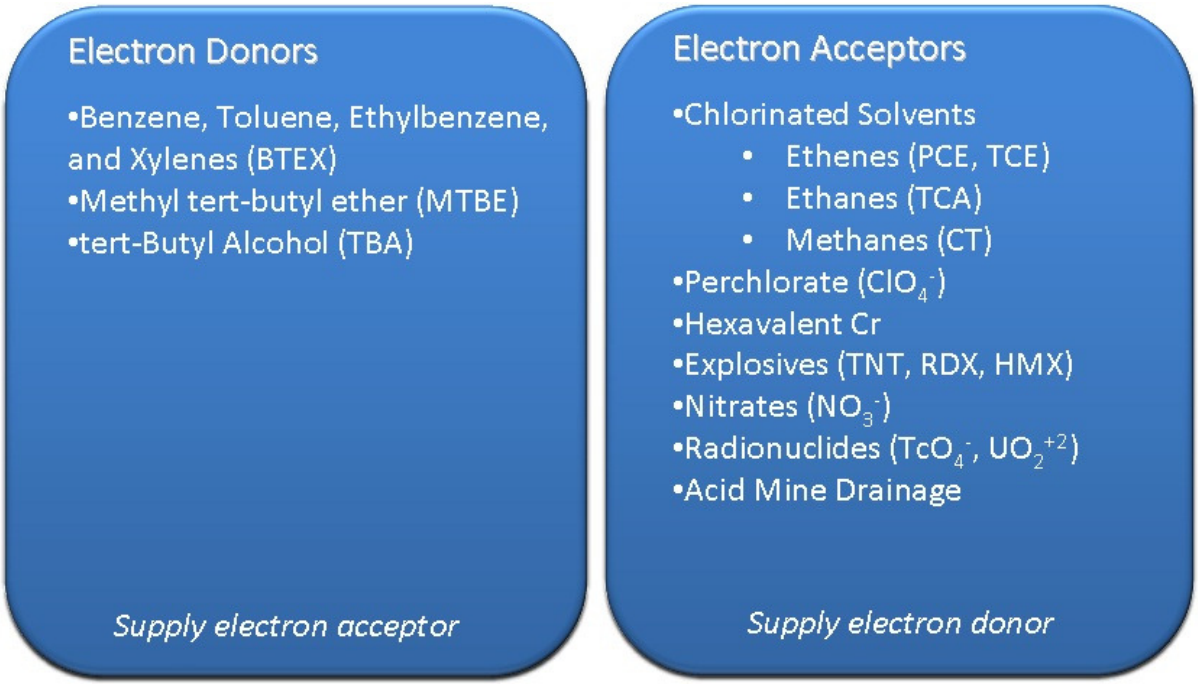


Figure 2. Contaminating matter as sources of electron acceptors and electron donors

Please note that Radionuclides and Acid mine drainage are redox sensitive

On the other hand the substrate used can be common vegetable oil, even edible oil, which means that natural environment will not be loaded with chemical, synthetic compounds. In order to optimize the solution, during the choice of the substrate for injection, more aspects should be taken into consideration to achieve better results with lower costs. Redox potential, biodegradation rate, duration, and system efficiency as well as the system optimization for injection, and then substrate distribution through the porous medium, are some of the basic factors.

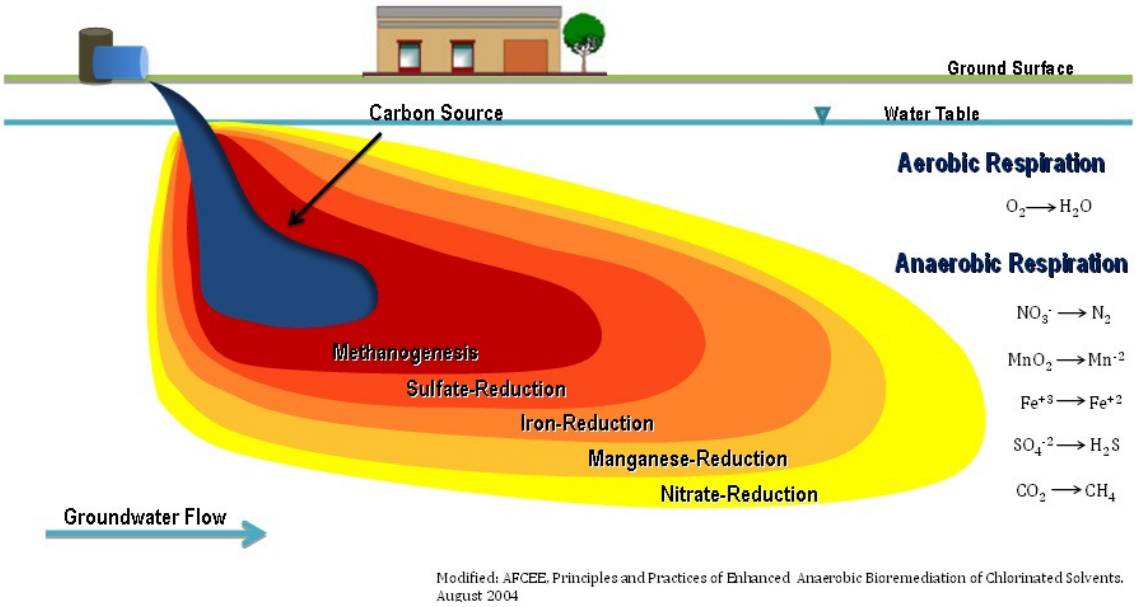


Figure 3. Sequence of the electron acceptor consumption with accordance to redox potential

With electron acceptors there is only one "consumption" sequence, according to redox potential. Oxygen is first to be consumed, then nitrate, iron, sulphates, chlorinated volatile organic compounds (PCE and TCE) and at the end carbon-dioxide (*Figure 3*)

Another important factor is hydrogen, i.e. how many molecules of hydrogen (H^+) are released through substrate injection into soil. It can be expressed through the amount of hydrogen released per kg of injected substrate H_2/kg. At *diagram 1* we see that soybean oil has the greatest value of hydrogen acquired per one kilogram of substrate.

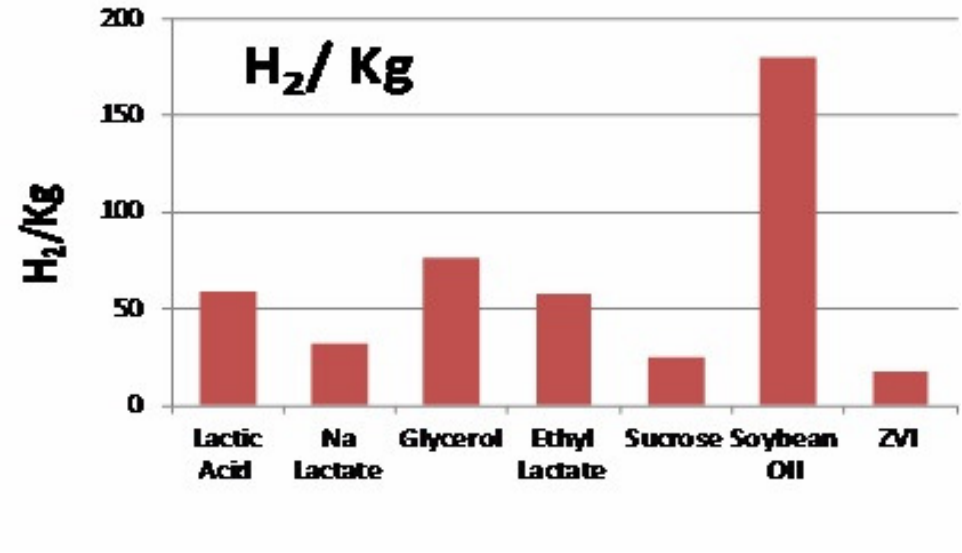


Diagram 1. Amount of acquired hydrogen per 1 kg of the injected substrate

The main factor influencing the time needed for biodegradation to initiate, and time taken for the process, i.e. the rate of substrate consumption, depends of the substrate structure and the time needed for microbes to dissolve the substrate. More complex the molecular structure,

more time needed for molecules. Diagram 2 displays for different substrates the time needed for biodegradation monitored over the gas emanation released through this process.

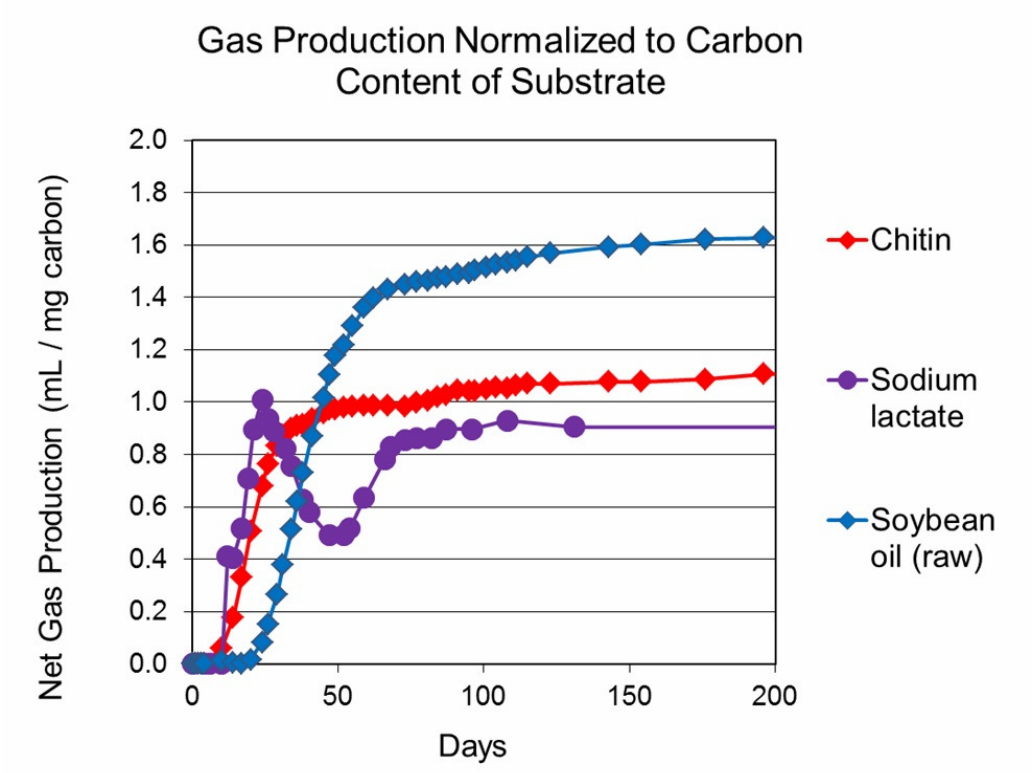


Diagram 2. Time needed for biodegradation monitored over the gas emanation released through this process comparing different substrates (Modified *Ref 11*)

With soybean oil the longest time is needed for process to initiate, but it is also noticeable that effects are equable and extended in comparison with, for example lactate, where the methane emanation is noticed momentarily because it is fermented quickly and easily, but it is as well noticeable that the process of biodegradation quickly decrease with intensity, then it stagnates for a certain period of time and not coming back in the more productive phases.

Therefore, it can be concluded that for the process of biodegradation more convenient is soya oil where the gas production is advancing with smaller rate, but equally and continues in the very long period of time. The rate and the degree of degradation are very important for durability, i.e. the sustainable process of biodegradation. With soluble substrates the process is very quick, fermentation and hydrogen release take place immediately where the reduction conditions are formed, and therefore the durability of the process is not provided. Faster fermentation into hydrogen and acetate comes to place together with production of the large but short-term supplies of electron donor and pH value decreases. Caused by the all aforementioned, the substrate is quickly consumed which requires frequent injections.

With soya oil caused by the more complex structure, there is an additional step that substrate undertakes in order to release hydrogen (first it ferments in fat acids) and that is a very slow process of fermentation, and then the poly- fat acids are being decomposed to hydrogen and

acetates, slowly as well, which additionally prolongs the procedure: providing the equable and extended process.

Substrate can as well be the combination of several substrates (soluble substrate and emulsion), it can contain additions such as vitamins and in that way the process is additionally improved because the microorganisms beside the common source of electron donors require – fats, protein and vitamins for cell respiration and enzymes production.

With any substrate, especially when the emulsion is in concern, its structure is important for injection and then distribution through the porous medium and groundwater. Emulsion usually contains highly varied and irregularly distributed droplets, differently grouped, which can make the injection harder and lead to the pores and intergranular space clogging, or harden the circulation of groundwater and block their hydraulic relation. For this reason it is suggested to use a special stabilised emulsions, specially prepared, which are containing the smallest and equable droplets of oil *(figure 4)*.

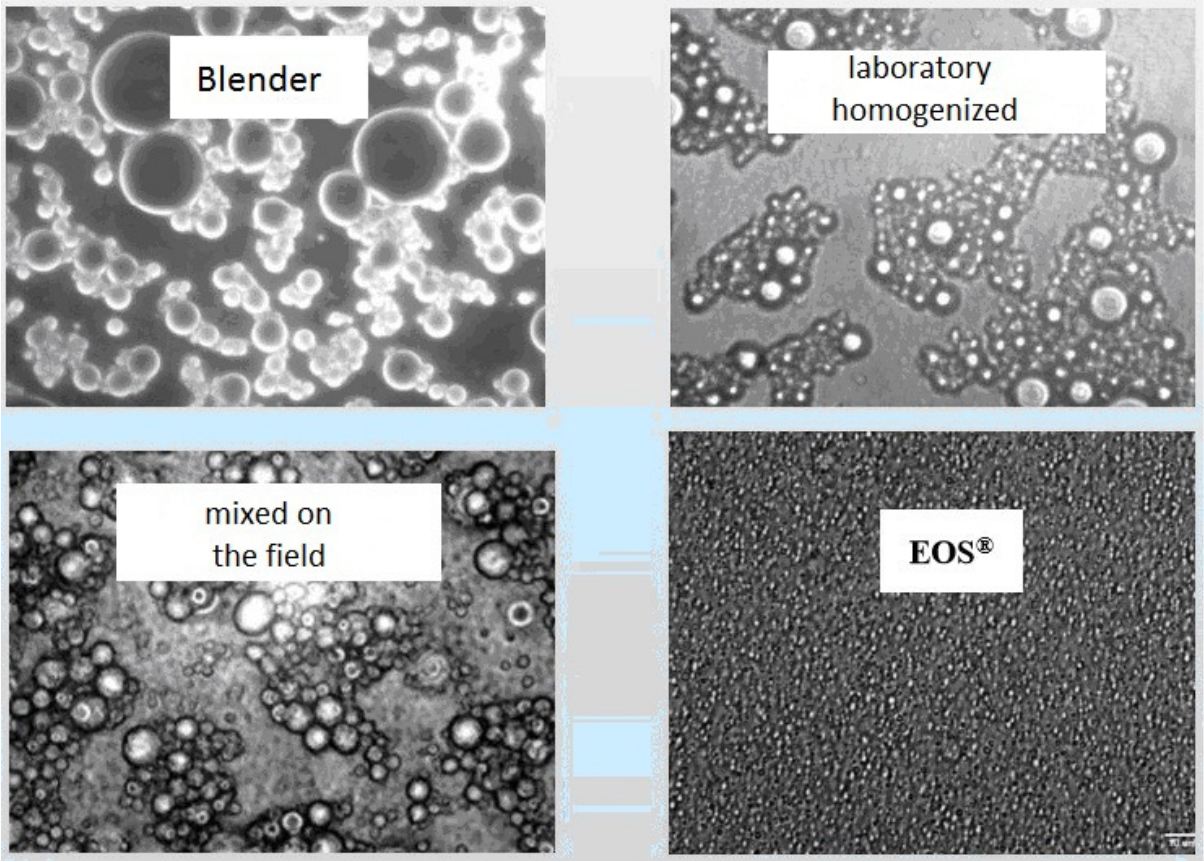


Figure 4. Structure of the emulsion in accordance to the preparation procedure (with kindness of EOS Remediation, LLC.)

The distribution of substrate during the injection in soil certainly depends of the lithology of the terrain. In sandy-gravel formation during the injection of about 100 ml of vegetable oil emulsion blended with water, the expansion and influence radius of one meter is expected.

Droplets of emulsion have negative charge; therefore they are sorbed to the positive particles of the soil. For this reason in the clay soil the expansion radius of up to only 0.3 m is expected for the equal amount of the injected substrate. The main reason for this is the smaller porosity and greater positive charge of the clay soil particles.

The emulsion injection into the porous medium can be done in a number of ways. The possibilities are numerous and at the first place they depend of the terrain itself, hydraulic relation of groundwaters, extension of the contaminated body, existing objects for monitoring and exploitation etc. Injection wells can be temporary, and in some cases existing objects can be used, injection can be direct, the wells position also varies with accordance to the terrain conditions and bioremediation requirements.

In some cases systems with recirculation are constructed, where the water is filtrated through the barrier then is being pumped out and put back to repeat the procedure several times. The injection points number and density requirement depends on the actual case therefore in some cases the treatment requires more biobarriers at the certain distance towards groundwater flow or focused action in the contaminant source itself.

Up-to-date investigations in the test site of the Požarevac source "Ključ"

The problem of nitrate contamination of groundwater is present and much extended in our country as well.

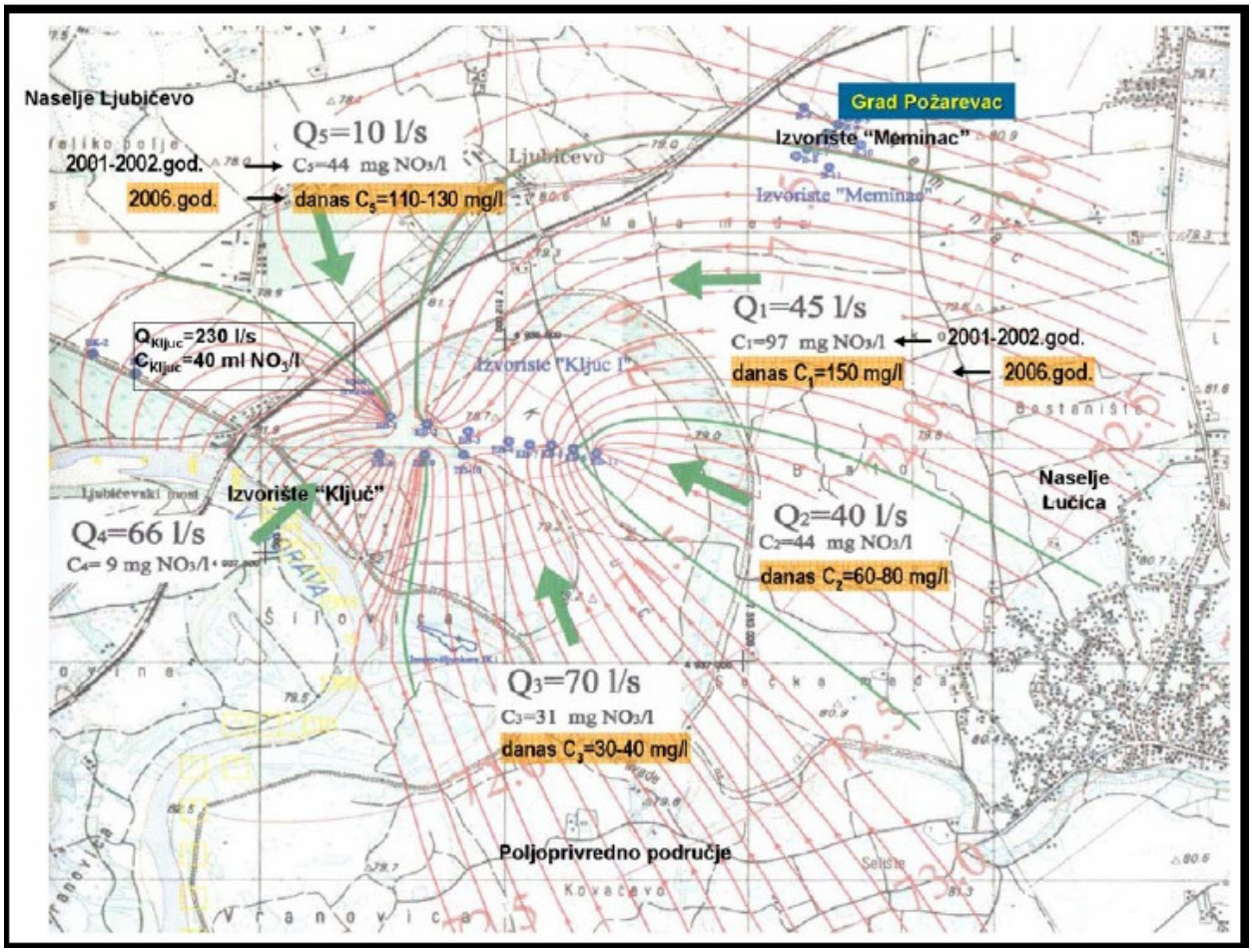
Požarevac its public water supply is founding on the groundwater extraction at the source Ključ from the gravel waterbearing formation of alluvium of Velika Morava. Captured groundwater after being chlorinated is distributed to the consumers without any additional treatment.

Alluvium thickness is 15-20 m. Waterpermeable horizon is made of gravel with coefficient of filtration 5x10-3 m/s and it is 6 to 11 m thick, where 7-8 m is waterbearing nowadays. The aquifer is nowadays unconfined. Water table is at the depth of about 6-8 m from the terrain surface. Aquifer recharges from the direction of the river Velika Morava and from the direction of the background where the settlements are located.

For a long period of time water quality from the source "Ključ" is being endangered with contaminated groundwater from the background with high nitrate content that is increasing with every day. Initially nitrates have intruded the central part of the source in 2000-2001. Investigations conducted in 2001-2002 determined that nitrate in the groundwater mostly originated from the communally unordered settlements of Lučica, Prugovo, Poljana and Ljubičevo.

In the surroundings of the source nowadays in the groundwater are registered concentrations in range of 100 to up to 160 mg of NO_3/l.

The source Ključ is operating as an infiltration source with 14 production wells and 6 injection wells (*figure 5*). Infiltration system represents actually the protection system. Injecting the water into the infiltration basins the hydraulic barrier is formed that blocks the inflow of contaminated groundwater with nitrates from the surroundings in the zone of the production wells. The water for injection is supplied with extracted groundwater in the Velika Morava banks (9 pipe wells distanced about 1 km upstream from Ključ).



Slika 5. Hydrodynamic flow model 2002, share of groundwater from the surroundings with different nitrate content in a total water balance of the source Ključ compared with recorded nitrate content (*Ref 8*)

However, beside the system of protection by groundwater sampling at the piezometers in the immediate surroundings of the source it was determined that nitrate content is drastically above the proscribed value.

By sampling on 9.7.2013. it was determined that nitrate content in the wider zone of the source ranges from 45.38 to 107.05 mg/l NO_3. With further analysis of the results and flow model of the wider zone of the source it was determined that maximal concentrations occur within the zone of the old river bed, representing the direction of the groundwater flow. This characteristic of the terrain could be prospective to make easier the design of the biobarrier

that would be installed perpendicularly to the old river flow. In this way the nitrate inflow from the background towards the source would be "cut".

The highest nitrate concentration was recorded in the piezometer Pb-12, which is located at only few hundred meters of the source fence. In October 2013 the piezometer Pb-12' was constructed 15 m away from Pb-12 *(Figure 6)*. This piezometer was made for purpose of soil and groundwater sampling for chemical and biochemical analyses but it is positioned in the way that it could be used for pilot test, provided in the further investigation phase. The wells are of the equal depth, with the screens at the same depth while the position and diameter are adjusted for the easier approach to the object and sampling.

Figure 6. Groundwater and soil sampling during the construction of piezometer Pb-12', *in situ* measurement and piezometer rinsing (*photo Jelena Zarić - September 2013*)

Table 1 Parameters measured at Pb-12 and Pb-12'

	X	Y	Z	WT (m)	T (˚C)	Conductivity (µS/cm)	pH	Eh (mV)
Pb-12	7512520	4938620	80	7,89	14	1155	8,10	150,1
Pb-12'	7512527	4938642	87	8,28	12,9	1267	7,31	169,1

12.10.2013

	T air (˚C)	T water (˚C)	pH	Dissolved O_2 (mg/l)	NH_4^+ (mg/l)	NO_2 (mg N/l)	NO_3 (mg/l)
Pb-12	0	10,3	8,8	5,94	0,13	<0,01	188
Pb-12'	0	10,5	7.89	5,73	0,15	<0,01	186

28.11.2013.

Table 2 Results of microbiological analyses of the groundwater and soil samples from Pb-12 and Pb-12'

Sample	Code	TC	YM	Coli	Anaer	Denitrif	Amonif	Sulph red
1	PB-12	$1,25 \times 10^2$	2.5×10^2	9×10^2	2×10^3	45	$1,4 \times 10^3$	<5
2	PB-12'	3×10^3	1.1×10^4	1.1×10^3	5×10^4	$1,4 \times 10^3$	$1,4 \times 10^3$	<5
3	Soil sampled from the depth 12-12.8m	3×10^6	5×10^4	$2,5 \times 10^5$	$1,4 \times 10^7$	$1,4 \times 10^4$	$1,4 \times 10^4$	<10
4	Soil sampled from the depth 13.3-13.6m	5×10^5	9×10^4	1×10^5	5×10^6	$1,4 \times 10^4$	$1,4 \times 10^4$	<10
5	Soil sampled from the depth 14-14.3m	5×10^5	4×10^4	5×10^3	7×10^6	$1,4 \times 10^4$	$1,4 \times 10^4$	<10
6	Soil sampled from the depth 9-11m	3×10^5	1×10^5	5×10^4	7×10^6	$1,4 \times 10^4$	$1,4 \times 10^4$	<10

TC= Total count of bacteria, growth on nutritious agar plate at 28° C, 2-3 days

YM = Total count of yiest and mould, growth at malt agar plate at 28 °C, 4 days

Coli = Total count of coliform, growth at endo agar plate at 28 °C, 4 days

Anaer = Total count of anaerobic bacteria, growth at nutritious agar plate with 0,5 % of glucose, 28 °C, 3 days

Denitrif – Denitrifiers, gas production at Hilty substrate, 28 °C, 4 days

Amonif = Growth at peptone and production of ammonium, substrate for ammonifiers , 28 °C, 4 days

SulphRed = microbes that reduce sulphates, Calcium-lactate substrate, 28 °C, 7 days

Laboratory Experiment

Upon completion of chemical and physical analyses a test in the laboratory was conducted. Test was conducted on groundwater samples from Pb-12'. Standard method of determination of denitrifying bacteria was applied, where beside the standard sample with citrate as the

carbon source, another was established with added vegetable oil emulsion (Figure 7). In particular case the emulsion of soya oil was used made by the American company EOS Remediation (www.eosremediation.com).

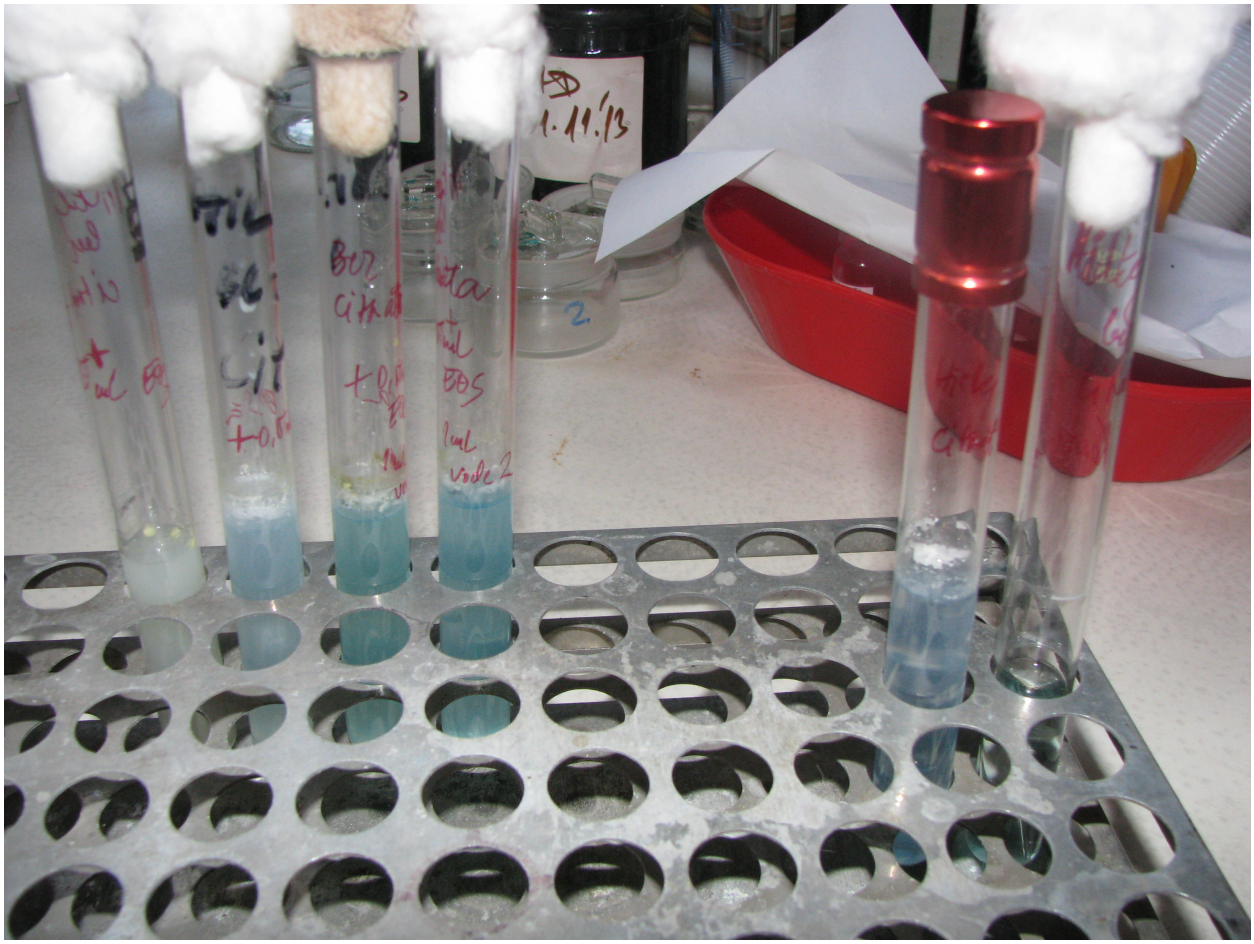


Figure 7 Laboratory experiment of biodenitrification, quantitative procedure by nitrogen production monitoring *(photo Jelena Zarić- January 2014*)

Experiment was established on 20.12.2013. and samples were not additionally cultured with microbes isolated in the soil samples. The entire process of denitrification was made by denitrifying bacteria present in the soil samples (this should be emphasised, considering the fact that in the soil they are present in much higher amount *table 2*). On 23.12.2013 the activity in the test tube with the citrus substrate was detected. The effects of denitrification were monitored through the gas nitrogen emanation that was collected in the Durham tubes submerged into each sample. On 27.12.2013. the activity in the test tube with emulsion was detected. In this way it was proved that the vegetable oil emulsion can be the carbon source necessary for the denitrification process and without additionally cultured microbes but only with the help of microbes present in the groundwater.

This experiment also proved that in the case of soya oil emulsion application more time for the process to begin is needed in comparison to the sample with citrate substrate. This behaviour of the samples is expected for the reasons explained in the first chapter of the paper

where it was spoken about the advantages of the substrate for bioremediation according to compound complexity it their molecular structure that influences directly the speed and duration of biodegradation.

A following step is the application and testing of the method *in situ* i.e. in the field considering that all up-to-date analyses have proved this method as a successful one.

Denitrification by applying the emulsified vegetable oil (EOS)

This technology was described in details in the text above. Its major advantages are as follows:

- ✓ A possibility of using the existing objects for substrate emulsion injection and bioremediation effects monitoring. This is very important because with other technologies construction of infrastructure represent the major investment in the entire project.
- ✓ Long-term solutions, after one injection substrate has an effect in the following several years (up to 7), with no need for additional injection. Although for the first results more time is needed (3 to 6 months, sometimes up to a year) this method gives good and what is the most important, long term results.
- ✓ There is no consumable equipment such as filters, no need for the construction of the supporting infrastructure or objects that need continual control and supervision. Upon the injection completion it is sufficient to monitor the bioremediation effects with the monitoring system which includes groundwater sampling (in some cases and soil), not more frequent than 2 to 4 times a year.

References

1. Adamson et. al. *2011. Sustained Treatment: Implications for Treatment Timescales Associated With Source-Depletion Technologies, Remediation Journal 43.*

2. Air Force Center for Environmental Excellence Brooks City-Base, Texas, Naval Facilities Engineering Service Center Port Hueneme, California And Environmental Security Technology Certification Program Arlington, Virginia, *Principles and practices of enhanced anaerobic bioremediation of chlorinated solvents*, August 2004.

3. Bradley, P.M., *Microbial Degradation of Chloroethenes in Groundwater Systems,* Hydrogeology Journal. Vol. 8 (p 104-111), 2000.

4. David Christopher Bertolacci, Master Thesis *In situ bioremediation of perchlorate in soil and groundwater*, Faculty of California State University, San Bernardino, December 2009.

5. Hunter, W. J., and Follett, R. F. *Bioremediation of high nitrate aquifer water using vegetable oil,* Abstracts of the Annual Meeting of the American Society for Microbiology, 1994.

6. Hunter, W.J., Follet R.F., Cary G.W., *Use of vegetable oil to remove nitrate from flowing groundwater*, Trans, ASAF 40, 345-353, 1997.

7. Hunter, W.J., *Use of vegetable oil in a pilot scale denitrifying barrier*, Journal of Contaminant Hydrology 53, 119-131, 2001.

8. Institut za vodoprivredu "Jaroslav Černi", *Generalni razvojni plan vodosnabdevanja opštine Požarevac - generalni projekat*, Decembar 2007.

9. Kaluđerović D., *Mogućnost remedijacije izdani zagađene nitratima metodom in situ biodenitrifikacije*, Vodoprivreda (vol. 40, br. 4-6, str. 227-232), 2008.
10. Kaluđerović D., *Prirodno prečišćavanje izdani*, Doktorska disertacija, 2008.
11. *Webinar: Principles and Practices of Bioremediation*, Brad Elkins, MS, PG, EOS Remediation, LLC, Raleigh, NC