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Enhanced Anaerobic Bioremediation Using Emulsified Vegetable Oils

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ABSTRACT

Large number of groundwater sources is contaminated with nitrates, this problem has put many of them out of function. The same situation is present all over the world, as well as in Serbia. The major cause of this problem is agricultural activity and fecal waste waters. By the infiltration of the atmospheric water and good hydraulic connection with groundwater, nitrates easily penetrate through geological media from the source of contamination to an aquifer. Nitrates are dissolved readily and do not adsorb to filtration media, so the propagation is very quick and the risk of the contamination is high. In aerobic conditions nitrates are non reactive and a very stable form of nitrogen, while in anaerobic conditions they are transformed by the biodegradation processes. In natural surroundings this process is very slow due to the absence of a carbon source (bacterial food source).

By injecting the emulsified vegetable oil in the aquifer we can create anaerobic conditions and stimulate biodenitrification. In this case vegetable oil is a carbon source and the process of biodegradation is much more efficient than in natural conditions. This technology is not only used for nitrate biodegradation, it gives good results in the remediation of chlorinated solvents, perchlorates, radionuclides, explosives, acid rock drainage and heavy metals.

According to data obtained from the laboratory this technology showed itself very successful. Based on the research there where undertaken numerous field tests (mainly in the USA) that also gave good results. In Serbia and its region there is a big problem of soil and groundwater contaminated with nitrates and the major reason why those locations remain like this is the lack of money. Particularly, a great effort has been made to motivate local municipalities and investors to do something about those problems, acquiring knowledge and coordinating with colleagues in order to start multidisciplinary projects with one main goal "Remediation of the contaminated water resources and their sustainable exploitation in the future".

KEYWORDS: bioremediation, emulsified vegetable oil, ground water, nitrates

INTRODUCTION

Nowadays nitrates are a big problem both in Serbia and worldwide. Their presence in groundwater is caused by different kinds of anthropogenic influences. Agricultural activity and faecal waste waters are major nitrate contamination sources.

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By the infiltration of atmospheric water and due to hydraulic connection with ground water, nitrates are easily transferred and filtered underground from the centre of contamination to the aquifer. Thereby, nitrates dissolve readily and do not adsorb in the porous environment which further facilitates their movement underground and increases the risk of contamination. In aerobic conditions, nitrates are highly stable and occur as a stable form of nitrogen, while in anaerobic conditions they are subjected to biodegradation, but this process is very slow and requires the presence of sufficient amounts of organic carbon.

Owing to their high solubility, nitrates easily migrate into groundwater and frequently threaten the very sources of groundwater used for the water supply of population. Even more common is the case of contamination of individual rural household water wells used for water supply. By the fertilization of arable land or positioning of septic tanks upstream of the water intake, some households may threaten their own groundwater aquifer.

The concentrations of nitrates above 40 mg/l may have detrimental effects on health – in the body, nitrates are reduced to nitrites, and a significantly reactive compound that penetrates the cell walls and enters the blood where it combines with haemoglobin whereby the transport of oxygen in the blood is blocked which can lead to death. It especially affects children younger than 6 years old and therefore the disease is called *Blue baby syndrome*.

The concentrations similar to those observed at the source Meadows (66-80 mg/l) in 1984 caused a serious illness of a child in Laurel, Nebraska, USA. In the year 1986, in South Dakota, a child died from drinking water with nitrate concentration of 100 mg/l. During the year 1982, the research was done and they drew the conclusion that in the Big Sioux Basin in South Dakota, there had been reported about 80 cases of poisoning by nitrates in the last 30 years. These are just some of the data from the United States Environmental Protection Agency.

Former studies have shown that by injecting of the emulsified vegetable oil into an aquifer, anaerobic conditions can be achieved and bionitrification enabled. Besides, in this way, there can also be added an artificial source of carbon which is necessary for this type of bioremediation and which often lacks in the natural environment and prevents the natural flow of the process. The application technology of natural emulsified oils in remediation proved to be very successful in the elimination of other contaminants such as chlorinated solvents, acid mine water and explosives as well.

By further research and work on the implementation of this method in different environmental conditions, and on as large number of examples as possible, effects of its application should be established and the method itself should be improved to enable its adaptation to different environmental requirements, thus its application in the future would be widely distributed and the method itself available and commercialized.

The main motive for its placement and broad application is certainly the price, which is reduced because the method does not require expensive infrastructure facilities, and it is also highly natural and environmentally sustainable, bearing in mind that for achieving the effect of anaerobic bionitrification it uses only emulsified vegetable oils, which can even be edible oil or soybean oil.

BACKGROUND

The research of literature data refers mainly to North American sources. Bionitrification has been known and proven in hundreds of examples in the research process of the aquifer natural attenuation. In his work, in 1995 Newell investigated 40 cases of aquifer pollution by petroleum hydrocarbons and drew the conclusion about the processes leading to their biodegradation. In all cases there occurred the consumption of electron acceptors including nitrates.

The essence of the process of petroleum hydrocarbon biodegradation is that the bacteria obtain energy for cell maintenance and production of the new catalytic transfer of electrons from the electron donors to the electron acceptors.

This results in the oxidation of the electron donor and reduction of the electron acceptor. The electron donors are organic compounds susceptible to biodegradation and the electron acceptors are elements or compounds in a relatively oxidation state and include oxygen (O_2), nitrate (NO_3^-), iron (Fe^{3+}), sulphate (SO_4) and carbon dioxide (CO_2). Dissolved oxygen is used first as the electron acceptor, and after it is consumed, anaerobic conditions arise. The electron acceptors are consumed in the following order: nitrate (NO_3^-), iron (Fe^{3+}), sulphate (SO_4) and finally carbon dioxide (CO_2).

A specially detailed description of nitrate reduction caused by micro organisms is given in *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water*, of Wiedemeier [14] where the overlapping of pollutant bodies, BTEX, and reduced nitrate concentration can be clearly seen. This was also seen in the API paper [11] where about 600 pollutant bodies of petroleum hydrocarbons all over the USA were analyzed. Results of this considerable research are clear, if an aquifer is polluted by nitrates, the addition of petroleum hydrocarbons in the process of bionitrification will result in the concentration reduction and the disappearance of nitrates used as electron acceptors in the metabolism of bacteria. Certainly, this is not the practice as petroleum hydrocarbons are also pollutants, but the process itself suggests that it is possible to inject an environmentally friendly organic compound into an aquifer which will serve as an electron donor in the process of biodegradation. In practice related to the remediation of petroleum hydrocarbons, water rich in nitrates is frequently added as it facilitates biodegradation.

There are numerous explorations in which it has been proved that vegetable oils can be used as a substrate for bionitrification. They were conducted in laboratory conditions under varied systems. The majority of work dealing with this topic has been done by Hunter (1994-2001) who is said to be a pioneer in the research and application of vegetable oils for denitrification. As it can be concluded from the years of paper publishing, these explorations are relatively new. Hunter did the test on the column with the dimensions of 1.1x2.0x0.085m filled with sand (from a local aquifer) and at whose enter the sand was coated by vegetable oil. Water with nitrates of 88 mg/l by the capacity of 144 l/day was pumped through the column. The capacity of vegetable oil for bionitrification and reduction of nitrate concentration below the detection level was shown clearly.

The implementation of this technology in the field has been conditioned by a number of cases of pollution in aquifers owing to the filtration of chlorinated solvents and perchlorates. They are highly ubiquitous pollutants. Considerable investments have been used for the research of remediation of these pollutions. As it has already been mentioned vegetable oil causes

anaerobic conditions in aquifers accelerating the growth of a specific group of micro organisms which uses chlorinated solvents and perchlorates in addition to nitrates as electron acceptors.

The Strategic Environmental Research and Development Program (SERDP) has financed several projects related to the injecting of vegetable oil into an aquifer in order to create anaerobic conditions but target pollutants have been chlorinated solvents and perchlorates. Over 100 cases of field injecting have been conducted, being efficient in causing of anaerobic conditions in the aquifer and removing of chlorinated ethens. Despite the fact that nitrates have not been the target group, the reduction of nitrates occurred in all cases. As an example a study from Maryland, USA can be taken where an injection curtain of 100 boreholes in the overall distance of 20 m has been made in which vegetable oil has been injected, and where, in addition to perchlorate, nitrate has been reduced as well.

In the year 2006 the first field application of this technology with the direct aim of nitrate biodenitrification occurred in Belgium, in the town of Bassevelde where after injecting 150kg of vegetable oil, the concentration of nitrates of about 320 mg/l in three months was reduced to 50 mg/l, and in 6 months' time nitrates disappeared completely. Another example of the application of vegetable oil emulsion in nitrate and perchlorate remediation used in explosive production is in California. The work on remediation began in the year 2000 and a large number of tests have been done so far.

These results are completely expected as regards similar occurrences in aquifers that were the subject of considerably higher number of pieces of research related to petroleum hydrocarbons, chlorinated ethanes and perchlorates.

BIODENITRIFICATION AS REMEDIATION METHOD OF AQUIFER POLLUTED BY NITRATES

The population of bacteria in an aquifer responsible for biodegradation requires a carbon source, an electron donor, an electron acceptor, nutrients, the adequate temperature range (10-40° C) and pH values (pH = 6-8). Organic carbon is frequently used as an electronic donor. Biodegradation is facilitated by manipulating with these conditions. It must be mentioned that it has been shown on a large number of examples that denitrifying bacteria already exist in aquifers and there is no need to inject them into an aquifer. The lack of an electron donor, i.e. a carbon source limits microbiologic denitrification in most cases. In natural conditions this process is highly slow and can't reduce high concentrations of nitrates in groundwater.

In situ biodenitrification is based on the principle of organic substrate injecting (or the formation of a bioreactive wall) into an aquifer which facilitates the growth of bacteria, produces anaerobic conditions and by that causes "consumption" of nitrates and other acceptors. In addition to nitrates, there are chlorinated ethane, ethane, perchlorates, TNT, RDX, radionuclides, and acid mine water.

Substances injected into an aquifer can be water soluble, a solid substrat, viscous fluid substances and vegetable oils in the form of NAPL and emulsions.

In current research, emulsified vegetable oils show most potential for simple and economic causing of anaerobic conditions in aquifers where biodegradation of nitrates and other above mentioned compounds occur.

Vegetable oils are mixed intensively with water and with the addition of specific matters keeping the emulsion stable, form the emulsion consisting of tiny drops (about 1 μm , of the order of bacteria). After injecting, the emulsion stays in a porous environment and forms a bioreactive zone rich in organic hydrocarbon. By groundwater movement through this zone biodenitrification is facilitated for vegetable oil with time releases the source of carbon for bacteria. This zone is stationary and lasts several years after one injecting. Essentially, all vegetable (edible) oils are fermented into hydrogen and acetate owing to the activity of micro organisms and when vegetable oil is selected primary factors that should be taken into account are: cost, availability, viscosity and the melting point. Soy bean oil is inexpensive and easily available thus it is frequently used for biodenitrification.

The applicability of the method depends on a number of factors beginning with environmental conditions all to the existing consortium of bacteria present in the soil. Results which will be achieved depend on all these factors. Each case requires a specific solution and the adjustment of the method to the existing conditions. In order to develop and implement the method in existing conditions as good as possible, it is necessary, prior to in situ biodenitrification, to carry out a number of analyses and do tests both in the laboratory and in the field. The application of this method of biodenitrification is proposed for implementation at the Ključ source. This case has great local significance as regards that the water supply source of the town of Požarevac (Serbia) is in question being threatened constantly by penetration of groundwater from the back contaminated by nitrates owing to the illegal building of informal settlements with the unsolved sewer network. The problem is even more complex as the moving away of the informal settlements and displacement from this terrain is practically impossible. The construction of communal network requires means which the local government can't provide easily, and on the other side there are no other alternatives for water supply, except the existing groundwater source. The other solution could be to move the source away beyond the impact of the settlement area, but this solution is highly expensive. In this particular case, the application of a bio reactive barrier could be a transitional solution until the means for the sewer network for the questionable settlements at the back of the source have been provided.

Prior to initiating such a large scale project, it is necessary to study in details the hydraulic connection of groundwater, atmospheric and surface waters as well as the concentration and migration of nitrates in groundwater and the geologic medium. The first phase of bench tests is underway. That implies tests on water and soil samples taken from the terrain which will be subjected to bioremediation by the application of soy bean emulsion. If the method proves to be successful, the second phase proposes a pilot test in the field by which the evaluation not only of the method successfulness in situ but of the entire project dimensionalization will be enabled. On the basis of that, necessary amounts of vegetable oil for injecting will be determined, the velocity of its consuming by denitrification, as well as the spatial position of the barrier in accordance with before studied hydrodynamic conditions and setting and lithology of the terrain. The research of this kind can last for years but it is essential that local governments and scientific institutions put their heads together, pool their sources, knowledge and invest in projects of this kind, for only in this way a solution and the commercialization and implementation of this method on localities contaminated by nitrates worldwide can be achieved.

This method was already confirmed in laboratories as the successful one in 1980-es and was applied in the case of chlorinated solvents (especially in America) where it was tested and confirmed on a large number of cases. In all these cases, the reduction of nitrate concentration was noticed as an accompanying phenomenon to the biodegradation of chlorinated solvents. All this is in favour of additional testing and adjusting of this method to nitrate biodegradation.

CONCLUSION

The problem of increased nitrate concentration in source groundwater for water supply is a significant problem nowadays. Bionitrification by adding a carbon source, in this case vegetable oils, in spite of the fact that it has been known for a longer period has not been applied widely yet. Rich practice in biodegradation of chlorinated solvents points out that this method is a highly successful one, as well as particular cases from the practice of removing nitrates out of groundwater. By further work on the development of this technology all aspects of its application in field conditions should be considered and all that in order to advance the technology itself and its practical application.

The growing problem of source contamination by nitrates caused by anthropogenic impact should not be neglected. In Serbia, numerous sources are threatened to be closed if an adequate solution is not found. The communal network that has not been constructed yet and unregulated dewatering of arable land which is fertilized, are a big problem and the most frequent cause of increased concentration of nitrate in groundwater. Certainly, in perspective, contamination sources should be removed bearing in mind that it is mainly continuous, not accidental contamination. Simultaneously with solving of these problems (by constructing of the infrastructure and communal network) it is necessary to work on the development of bioremediation methods and their implementation.

Results obtained at the test polygon of the Ključ source are a precondition for further work on solving of the Požarevac source nitrate problem. At the same time they are an essential step in the development of bioremediation in these areas.

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