A Review on In-Situ Denitrification Technology for Consideration in Jaffna Peninsula Aquifer Remediation

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Abstract The groundwater nitrate levels in the Jaffna peninsula of Sri Lanka are well above the World Health Organization limit of 10 mg/L as N and recent studies point to the high use of chemical fertilizers and the close proximity of septic systems to drinking water wells as probable causes. Since aquifers in the peninsula are primarily porous, and shallow karstic Miocene limestone, they provide high levels of infiltration. If the current situation continues unabated, the public may suffer the harmful effects of nitrate toxicity. This paper discusses in-situ bioremediation processes, along with other possible mitigation measures, to remove nitrate and improve the quality of the drinking water. Five in-situ denitrification projects conducted in the Northern USA and Canada are presented, using carbon sources such as ethanol, methanol, and acetate. Treatment was achieved by a) injecting carbon and phosphorus or b) infiltrating treated water with excess carbon and phosphorus into groundwater. Nitrate-nitrogen concentrations as high as 60 mg/L have been reduced to below the limit of 10 mg/L with no ill effects. Pump-and-treat methods are conventional techniques and comparatively high-cost solutions. Furthermore, greener solutions such as controlling inorganic fertilizer addition and implementing long-term protective measures are inexpensive, but the minimal threat continues to exist. In addition, sustainable solutions such as banning agrochemicals, switching to organic farming, and establishing groundwater source protection zones have no negative impacts on the environment, but they are highly expensive to implement. In addition, restorative methods such as in-situ bioremediation and carbon farming, cultural or reconciliatory practices such as mulching seaweeds as organic fertilizer and using organic Neem-based pesticides, and regenerative solutions such as agroforestry or permaculture (includes intercropping with symbiotic nitrogen fixing crops) and holistic farming are less expensive and highly resilient or systemically vital methods suggested by this review.

Keywords: Bioremediation, denitrification, nitrate toxicity, pump and treat, groundwater quality

INTRODUCTION

Groundwater is the sole source of water in the Jaffna peninsula - Sri Lanka, where it is used for drinking, agriculture, and other activities. However, residents are threatened by increased levels of nitrate and nitrite in water, which are often above the World Health Organization’s (WHO) safe upper limit of 10 mg/L of NO3-N and 1 mg/L of NO2-N. According to Vithanage et al. (2014), NO3-N values were 0-35 mg/L in Chunnakam, where 44 wells were sampled in January, April, July and October. Thirty-eight percent of the samples exceeded 10 mg/L (Figures 1 and 2). In a previous study, the highest value of 17.5 mg/L was recorded at Kondavil beneath cropland (Jeyaruba & Thushyanthy 2009). Nitrate toxicity in drinking water may cause illnesses such as methemoglobinemia (blue baby syndrome), thyroid effects, neurodevelopmental effects, and gastric cancer (Health Canada, 2013). Biopsy specimens revealed that among the nine provinces in Sri Lanka the Northern Province showed the highest occurrence of malignant tumors (184 per 100,000 people) (Jeevaratnam et al. 2018). Two main reasons cited are 1) increased usage of chemical fertilizer and 2) leaching from toilet pits and septic tanks. Further, a study in the Chunnakam area revealed significant coliform contamination along with elevated nitrogen levels (Jeevaratnam et al. 2018). Farmers in the peninsula widely use chemical fertilizers as demonstrated by a report published by the International Water Management Institute (Mikunthan et al. 2013⁹), and the nitrate/nitrite levels in the area of cultivation is about 35 mg/L as N and 38% of the sampled farm wells exceeded the WHO limit of 10 mg/L (Mikunthan et al. 2013⁹). Even if the recommended distance of 25ft between septic...
tanks and drinking water wells is maintained, wastewater may still leach into the vadose zone and then into the silt layer directly above the aquifer. This is evident from elevated levels of NO$_3^-$ N, which is approximately 12.1 mg/L throughout the year in home gardens and public areas, where agriculture has not been recently practiced. However, in certain other areas of the domesticated and public wells, nitrate-nitrogen level is below 10 mg/L (Vithanage et al. 2014). This report evaluates the possibility of using in-situ bioremediation to enhance natural denitrification and the effects of such remediation on the quality of drinking water.

**Geography of the selected area**

Limestone bedrock in Jaffna has a high permeability due to sedimentary deposition and fractures (Chilton et al. 2006). This can produce groundwater velocities exceeding 100 m/d. Soil thinness is another cause of rapid water movement via fissures. Natural groundwater flow rates are only 0.0001-0.1 m/d in the matrix, but up to 1000 m/d in karst fissures characterized by cavities and tunnels. Limestone deposits are prevalent in the Jaffna peninsula, and there are over 100,000 wells. Around 17,860 of these are used primarily for irrigation, with the remainder being domestic wells (Punthakey & Gamage 2006). In places where groundwater usage is high, the nitrate concentrations exceed 10 mg/L as NO$_3^-$ N (Vithanage et al. 2014).

**Fig 1** Geo-location of Nitrate-nitrogen in the study area; Source: (Vithanage et al. 2014).
MATERIALS AND METHODS

The qualitative content analysis methodology (Fig 3) was applied to the raw data collected to satisfy the objective, which suggests in-situ remediation as the plausible solution to groundwater nitrate contamination in the Jaffna peninsula. Links between each environmental problem as cause-and-effect relationships were supported by real-world evidence from the literature. Regenerative and sustainable solutions were identified as link cutters and bridge links (later explained in Fig 5).

Fig 2 Spatial and temporal variation of Nitrate-N in Jaffna aquifers based on usage of wells; a) January and April; b) July and October. D: Domestic wells, D + H: Domestic wells serving home gardens, P: Public wells, and F: farm or agricultural wells (agro-wells). Source: (Vithanage et al. 2014) with permission

Fig 3 Qualitative content analysis methodology Adapted from (Adu 2017)
RESULTS

Chemical fertilizers

Chemical fertilizers consist of inorganic salts of nitrogen, phosphorus, potassium, and sulphur. Nitrogen is present in the form of ammonium and nitrate (Table 1), both of which are highly soluble and mobile and can rapidly leach through soil. Since they are relatively inexpensive, easy to apply, and can be used immediately, chemical fertilizers have become the primary source of nitrogen to increase crop yields. Traditional organic fertilizers such as cattle manure and compost continue to lose market share.

Climatic factors such as rainfall play a vital role in leaching minerals into groundwater. Since Jaffna lies in a tropical zone with a monsoonal climate, nitrate leaching increases during the rainy season. According to Lawrence & Kumppnarachi (1986), the aquifer nitrate-nitrogen concentrations beneath paddy and horticulture cultivation areas increased from an average of 10-25 mg/L in the dry season to more than 40 mg/L during the rainy season. The high level of chemical fertilizer leaching by percolation is the main cause. In Sri Lanka’s tropical climate with little seasonal variation, farmers are able to grow several crops per year. Unless farmers reduce the use of chemical fertilizers, any remediation method may not be adequate to improve groundwater quality sufficiently. Land use and its vegetation cover have a high influence on nitrate leaching, with permanent grassland having a lower leaching rate than ploughed or arable grassland (Appleyard & Schmoll 2006). According to Vithanage et al. (2014), estimated nitrate loading was 15 times higher in fertilizer-applied agricultural areas than in domestic and irrigational areas. Thus, the use of artificial irrigation mechanisms such as tilling and keeping land flow can accelerate nitrate leaching by ninefold from pasture land and twofold from cropped land (Juergens-Gschwind 1989). Urea, Ammonium sulphate, and Diammonium phosphate are the most widely used nitrogen fertilizers in the Jaffna peninsula.

Table 1 Percentage of N in common inorganic fertilizers or fertilizer formulations

<table>
<thead>
<tr>
<th>Common name</th>
<th>Chemical formula</th>
<th>N%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium nitrate</td>
<td>NH₄NO₃</td>
<td>34</td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td>(NH₄)₂SO₄</td>
<td>20.6</td>
</tr>
<tr>
<td>Ammonium nitrate-urea</td>
<td>NH₄NO₃ + (NH₂)₂CO</td>
<td>32</td>
</tr>
<tr>
<td>Aqua ammonia</td>
<td>NH₂OH</td>
<td>20</td>
</tr>
<tr>
<td>Urea</td>
<td>(NH₂)₂CO</td>
<td>46</td>
</tr>
<tr>
<td>Superphosphate</td>
<td>Ca(H₂PO₄)₂</td>
<td>0</td>
</tr>
<tr>
<td>Monoammonium phosphate</td>
<td>NH₄H₂PO₄</td>
<td>13</td>
</tr>
<tr>
<td>Diammonium phosphate</td>
<td>(NH₄)₂HPO₄</td>
<td>18</td>
</tr>
<tr>
<td>Urea-ammonium phosphate</td>
<td>(NH₂)₂CO + (NH₄)₂HPO₄</td>
<td>28</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>KCl</td>
<td>0</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>K₂PO₄</td>
<td>0</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>KNO₃</td>
<td>13</td>
</tr>
<tr>
<td>Potassium sulphate</td>
<td>K₂SO₄</td>
<td>0</td>
</tr>
</tbody>
</table>

Adapted from ("U.S. EPA", 2000) and (Ceylon Fertilizer Company Ltd. 2014).

All man-made environmental problems are interconnected as causes and effects (Sivaramanan 2021). For instance, deforestation causes water scarcity, and water scarcity causes land degradation and desertification. Deforestation also pave way for air pollution, which leads to global warming and acid rain. Intensive farming causes water pollution through agrochemicals and subsequently, eutrophication, leading to biodiversity loss.

Intensive farming as a keystone environmental problem

Intensive agriculture is one of the largest contributors to man-made climate change and accounts for around 12% of total emissions and a quarter of greenhouse gas emissions (Smith et al. 2007). Animal husbandry accounts for 37% of
mehane emissions and 65% of nitrous oxide emissions (Watson 2020). Also, runoff from farms causes eutrophication. Poor living conditions in industrial farms cause animal diseases, and animals are said to be subjected to cruel handling. Agrochemicals such as pesticides, fungicides, herbicides, and chemical fertilizers lead to toxic effects on waterways and the atmosphere and affect non-targeted biota such as insects, birds, and other animals. Agrochemicals also have an impact on soil microflora and fauna and cause soil salinization and desertification. High concentrations of nitrate in groundwater from chemical fertilizers cause methemoglobinemia (blue baby syndrome). Nitrate and phosphate effluents from excessive chemical fertilizers lead to eutrophication, which results in algal blooms that clog the fish gills and increase the biological oxygen demand. According to the World Wide Fund for Nature, intensive palm oil agriculture causes deforestation in Indonesia and affects orangutan habitats (WWF 2023). Intensive farming, including slash-and-burn techniques, causes severe biodiversity loss and poses a threat to indigenous people. It has been stated in a report published by the US Government Accountability Office that, in the United States the number of factory farms increased by 230% between 1982 and 2002 (Mittal 2008). Besides, according to the website of the Food and Agriculture Organization (FAO), the global pesticide usage skyrocketed by 81% in the period between 1990 and 2017 (FAO 2019) as cited in (Boedeker et al. 2020). Antibiotics and growth hormones used in animal farming also affect humans (Bridgeman 2020).

Overexploitation of natural resources, desertification, deforestation, biodiversity loss, animal slaughtering and cruelty, agrochemicals, solid waste and sewage, eutrophication, groundwater contamination, dam construction, water pollution-water scarcity, wetlands or draining of wetlands, and hazardous waste from toxic pesticide chemicals are all consequences of intensive farming or poses a significant dependency on intensive farming (Fig 4).

**Evidence 1: Intensive farming causes agrochemical pollution**

According to Feuerbacher et al. (2018), Bhutan’s large-scale conversion to 100% organic agriculture by desolating the agrochemical methods resulted in 24% lower yields than conventional yields. The study also depicted a considerable reduction in Bhutan’s GDP, substantial welfare losses, particularly for non-agricultural households, and adverse effects on food security caused by the chemical fertilizer ban. However, the reduction in agricultural yield was largely compensated by imported foods from India, but, ironically, the country’s cereal self-sufficiency was weakened. Though soil P and K levels remained unchanged, soil nitrogen levels have gone down by -22.4% because the nitrogen release from animal manure had been too slow. However, the study also suggested that to overcome these pitfalls, Bhutan should improve the management of fertilizer application, crop protection, and the integration of livestock to obtain a better yield as part of truly holistic, organic farming. Thus, Bhutan’s present agriculture policy and its implementation revealed that the absence of intensive farming brought the use of agrochemicals to a halt. Thus, it is clear that and also based on the definition, intensive farming is a keystone man-made environmental problem. However, increasing food demand as a result of an increasing population may question Bhutan’s 100% organic policy in the future, and it may further increase food insecurity and poverty in the country. Thus, the population explosion acts as the precursor to many major man-made environmental problems.

**Evidence 2: Intensive farming causes water pollution and scarcity**

According to FAO (2011) as cited in Water for Sustainable Food and Agriculture: A report produced for the G20 Presidency of Germany (2017) “Agriculture accounts for 70% of total freshwater withdrawals on average worldwide, thus, agriculture is the largest water user in the world and these amounts can reach as much as 95% in some developing countries” (FAO 2017). However, intensive farming plays a significant role in water pollution through nutrient loading, pesticides, and weedicides (FAO 2017).

**Evidence 3: Intensive farming causes deforestation**

According to the report published by Wageningen University and Research, ‘Agriculture is the direct driver for worldwide deforestation’ (Wageningen University and Research Centre 2012). Globally, 80% of deforestation is due to intensive farming or intensive agriculture.
High energy solution vs. Low energy solution

In the above scenarios, it is evident that intensive farming is a keystone environmental problem, and seeking high-energy sustainable solutions often comes at a huge cost. Thus, the only viable option is to seek low energy (low cost) restorative, reconciliatory, and regenerative solutions.
According to Bill Reed, "When compared with a (high energy) sustainable system, (low energy) regenerative systems have higher efficiency, lower cost, reduced generation of waste, faster time to market, result in a variety of products and benefits, and are the only way towards the realization of the exponential value of the social, ecological, financial, and human qualities of the project, community, and ecosystem" (YERT 2010). Wahl (2016) reports a regenerative human culture is healthy, resilient, and adaptable.

Low energy solutions should be considered the highest priority when handling keystone environmental problems. The interconnected nature of man-made environmental issues should be studied using the concept maps. The co-evolving (adaptable) feature of the proposed regenerative development should be tested prior to implementation (with respect to other environmental parameters). In contrast to regenerative solutions, other low energy solutions such as restorative solutions require continuous human maintenance (human dominance), however, this feature does not available in both reconciliatory (cultural or aboriginal practices) and regenerative solutions, where human participation is considered as equal to other sentient beings in the ecosystem.

Since all man-made environmental problems are linked as causes and effects, establishing high energy solutions (conventional, green and sustainable) is often affected by human adaptability factors, such as economic, political, and social. This can be overcome by low energy solutions (restorative, reconciliatory and regenerative). This is because low energy solutions have high systemic vitality (resilience). Figure 5 depicts different kinds of available solutions for the issue.

**Pits and septic tanks**

Pits and septic tanks are widely used to treat and disperse municipal waste in Jaffna. Pit latrines include simple pits, ventilated improved pits, pour-flush latrines, raised pit latrines, and composting latrines. Twin-pit latrines have benefits such as ease of construction and improved treatment, which can produce a decomposed, odourless product that is relatively easy to handle when the pits are emptied. Twin-pit systems are often dug to shallower depths, which reduces the chance of effluent reaching the aquifer. It is also essential to ensure a two-meter layer of sandy or loamy soil below the base of the pit. Septic tanks require frequent additions of water to maintain the water seal (solids deposited on the bottom where scum forms a crust on the surface). Effluent may be filtered by soil microorganisms after it is dispersed by a shallowly buried permeable pipe or a parallel series of pipes. Each septic tank chamber should be followed by a soak-away pit with a maximum depth of 6.5m (21 ft) per U.S. Development Control Zone Policy (Chilton et al. 1991); (Howard, et al. 20066).

According to the Manual of Septic Tank Practice (1965), septic tanks should be at least four feet (1.2m) away from the underlying water column of the aquifer. Carbonaceous contents may be completely degraded. However, organic nitrogen and ammonia are primarily oxidized to nitrate before leaching into the groundwater aquifer system (Howard, et al. 20066).

The solutions for the groundwater contamination could be classified as conventional, greener, sustainable, restorative, and regenerative techniques (Fig 5). According to Sivaramanan & Kotagama, (2022) banning agrochemicals was a sustainable solution adopted by the Sri Lankan government in May 2021. A year later, in May 2022, the country faced a severe economic crisis, social unrest, and political instability. This is because Sri Lanka is an agricultural country, and the ban on chemical fertilizers severely affected its crop production and export market. As a consequence, citizens suffered from a fuel crisis, increased prices of all essential goods, a medical drug shortage, a food shortage, etc. Island-wide protests ended in attacks on the government representatives (ministers); finally, the President resigned and a new government was inaugurated under the new presidency. Thus, sustainable solutions are either high-energy solutions or they are expensive (YERT 2010) (Fig 6), and because they are highly expensive, even affluent nations may not be able to afford them (chemical fertilizer ban). As a developing country with vast foreign debt, the Sri Lankan economy collapsed completely because of the effects of the fertilizer ban.
Fig 5 Possible solutions to agrochemicals, which is caused by intensive farming

Black circle: problem under concern, Blue circle: cause, Red circle: effect. The double-lined circle: keystone environmental problem, while the single-lined circle: environmental problem. Dotted circle: a problem that will be mitigated once the keystone environmental problem is resolved, Black arrow: cause-effect link for which solutions are given, Blue arrow: cause-effect link

N.B.: Each problem in the circles is connected to many other problems based on cause-and-effect links, which are not shown here.

(The author developed the above diagram after consulting with Bill Reed, Principal of Regenesis Group Inc., 20 Woodland St. Arlington, MA 02476, USA.)
Accordingly, banning agrochemicals is a sustainable solution, but it is relatively expensive, whereas restorative, reconciliatory, and regenerative solutions are cheaper, healthier, more adaptable, and have higher resilience.

**Fig 6** Restorative vs. other designs (including sustainable design) [With permission from Bill Reed]

**In-situ bioremediation process (restorative method)**

According to Perpetuo *et al.* (2010), “Bioremediation is a process that uses microorganisms or their enzymes to promote degradation and/or removal of contaminants from the environment”. In-situ bioremediation is a process whereby indigenous microorganisms are used to improve the biochemical nature of soil as well as groundwater. It has become a widely used technology due to its low cost, adaptability to different site-specific environmental conditions, and efficacy when properly implemented. The rate of natural bio-attenuation by indigenous denitrifying bacteria is stimulated through the controlled addition of a carbon source such as ethanol. Ethanol is non-toxic in low concentrations; its high-water solubility and high chemical oxygen demand make it a suitable organic substrate. When aerobic bacteria utilize ethanol to consume available oxygen, the resulting anoxic environment is favourable for denitrifying bacteria. Unless truly anaerobic conditions are reached through the depletion of all nitrate, and similar electron acceptors, the generation of toxic hydrogen sulphide is avoided.

A comparison study on the denitrification rates conducted by the Department of Railroad, Civil and Environmental Engineering, WooSong University, Daejeon, Korea revealed that denitrification rates for organic substrates in the order of fumarate > hydrogen > formate/lactate >
ethanol > propionate > methanol > acetate. When fumarates were used as a substrate, the rate of denitrification was 0.66mmol/day, while the conversion rate from nitrate to nitrogen gas and other by-products was 87%. A microcosm test required 42 mg of fumarates to remove 30 mg of NO$_3^-$–N/L (Seong-Wook et al. 2007).

Factors considered in the conceptual site model (CSM)

Aquifer type and lithology are important because the magnitude and distribution of hydraulic conductivities affect the ability to deliver amendments to the subsurface. A baseline characterization of the microbial community is desirable to ensure the presence of the correct indigenous microbes. If bioaugmentation is required (which is unlikely), added microorganisms must be grown without harming the indigenous flora.

Land use and risk: This is an evaluation of past, current, and planned future land use, as well as ecological value. In addition, it further evaluates the potential human health and ecological risks, establishes protective cleanup criteria, and evaluates acceptable control measures.

Geological settings: It is also essential to understand the geological settings of the land, as bedrock limestone has a significant impact on the direction of plume migration through fractures, faults, and porous sponge-like cavities and tunnels. If porosity is low, it may be difficult to treat the target area by delivering amendments due to poor connectivity. Porosity will influence application features such as an injection well’s radius of influence, the total number of injection wells, and the need for multiple screened intervals. Effective porosity would be initially measured by measuring the velocity of groundwater flow in a tracer test.

Groundwater flow velocity: A key parameter affecting contaminant transport as groundwater flows from a high hydraulic head to a low hydraulic head. High groundwater flow may require frequent additions of carbon to maintain anoxic conditions. The direction of groundwater flow is another key parameter to be considered before injecting supplements (if not, the injection goes to waste without circulation or establishing a permissible reactive barrier).

Aquifer diffusion potential: This factor is less important in karstic aquifers because the flow in karstic aquifers is vertical and the flow path is usually through the predefined network. However, if heterogenous layers are present, zones of high permeability facilitate quicker remediation, while low diffusion zones mean longer remediation times. If the concentration gradient between high and low diffusion zones is reversed, contaminants may diffuse back to a more permeable zone.

pH and buffering capacity: Aquifers in Jaffna are slightly alkaline, with a pH range of 6.93–9.36 (Hidayathulla & Karunaratna 2013). As denitrifying microorganisms prefer a slightly alkaline pH (Foht 1974), this environment is preferable for in-situ bioremediation. Buffering capacity could be measured during bench testing. However, since the overall nitrate concentrations to be remediated are relatively low (compared to the levels we use in the bench test), only small changes in pH are anticipated.

Electrical conductivity: Ranges between 99.3 and 8,820 μS/cm in the soil in Jaffna (Hidayathulla & Karunaratna 2013).

Oxygen reduction potential (ORP) describes the tendency of the aquifer solution to accept electrons. Denitrifying conditions need an ORP of -100 mV to 100 mV (Dabkowski 2006). It is essential to monitor bacterial growth frequently in order to avoid reduced circulation or a plugged formation. Temperatures in Jaffna are typically 28-32°C and remain relatively static in that tropical climate. It is also required to have a clear understanding of available nutrients and growth inhibitors. A lack of major or minor nutrients or even trace elements may inhibit microbial growth. Many practitioners provide vitamin B12 as a supplement at bioremediation sites. Biostimulation can be achieved by adding electron donors, while bioaugmentation can also be provided when indigenous organisms are insufficient to accomplish denitrification.

To determine the quantity and the method of amendment delivery, information on the lateral extent, depth, and thickness of the contaminated zone is required. Mass flux must be determined by estimating the contaminant concentration, groundwater flow velocity, and plume attenuation rate (if any). More accurate targeting of the area will eventually reduce the cost and facilitates effective remediation.

During the pilot-scale application of amendments (supplements), biostimulation can be done in three different ways. Figures 7, 8, and 9 depict direct injection, circulation, and permeable reactive barrier.
Fig 7 Direct injection

Fig 8 Circulation
**Fig 9** Permeable reactive barrier
Adapted from configurations (U.S. EPA 2000), as mentioned in (U.S. EPA 2013)

**In-situ denitrification projects in groundwater**

Five in-situ denitrification projects held in North America over the past decade are given below:

1. Nitrate removal in Colorado
2. Nitrate and perchlorate remediation in California
3. Nitrogen removal in Montana
4. Nitrate and sulphate removal in Washington State
5. Nitrate removal in Canada

The first project was implemented in the South Platte River alluvial aquifer in Julesburg, Colorado, USA (population 1,300), with drinking water obtained from three wells drilled into the alluvial aquifer. Heterotrophic denitrification was carried out from January 29th to September 9th, 1996, with a groundwater depth of 12ft (3.7 m) and a saturated thickness of about 23ft or 7.0 m (McMohan et al. 1998). The injection process began by pumping raw water at a rate of 110–120 gallons per minute (g.p.m) or 25-27 m³/hr for the first five days. The injection rate was slightly reduced while holding the same pressure and the injection rate then decreased to 105 g.p.m (24 m³/hr) and the pressure was increased. Ethanol concentrations were increased from zero to 40 mg/L over the first 45 days, which built up biomass in and around the well. After a certain period of time, the biomass was removed with commercially available chemicals and 75 days after pre-treatment, additional chemical treatment was required. Concentrations of dissolved oxygen in the aquifer decreased from 5.6 mg/L to a mean value of 3.4 mg/L (a 40% reduction). Nitrate levels were reduced from 24.5 to 20.6 mg/L (a 16% reduction), as ethanol was used by bacteria to consume both oxygen and nitrate. Following ethanol addition, the colony count of heterotrophic bacteria increased from 101 colony-forming units (CFU) per ml of water up to 610 CFU/ml.

Other sites have used similar methods, as cited in Janda et al. 1988; Mercado et al. 1988; Hamon & Fustec 1991 and Hiscock et al. 1991. Carbon sources such as acetate, corn oil, ethanol, and sucrose were used and data were collected from one pumping well, three monitoring wells, and nine injection wells (McMohan et al. 1998).

The second case study was conducted at Alpha Explosives, an explosives manufacturing and distribution business near Lincoln, California,
Nitrate and perchlorate have been used in explosives manufacturing at the site and are found in groundwater at concentrations above state water quality objectives. In-situ biological treatment at the Alpha site appears promising for remediation of nitrate and perchlorate (Reinsel & Thompson 2013). Over a six-year period, periodic reagent addition to injection wells in two source areas removed up to 48% and 75% of the nitrate and 61% and 82% of the perchlorate, respectively. Nitrate and perchlorate concentrations were as high as 1,400 mg/L and 1,300 ug/L, respectively, prior to treatment. A schematic of direct injection using ethanol and phosphate is shown in Fig 10. Sodium acetate is now the preferred carbon source at Alpha Explosives.

In the third case study, methanol was added to mine water to increase the removal of total nitrogen (nitrate and ammonia) at the Stillwater Mine near Nye, Montana, USA. Methanol addition to existing bioreactors for mine water treatment was increased to provide additional carbon for in-situ denitrification in groundwater (Reinsel 2006). Treated water was percolated into groundwater and monitored in downgradient wells (Fig 11).

The Stillwater test significantly reduced nitrate concentrations in groundwater. A “stretch goal” of 1 mg/L of total nitrogen in groundwater was met, which is below the background level. This treatment method has the potential to substantially reduce nitrogen concentrations with minimal capital equipment or minimal modification to existing treatment facilities.

In the fourth in-situ case study, nitrate and sulphate were removed from contaminated groundwater near Republic, Washington, USA. The biological treatment completely removed nitrate from Key Mine water (Reinsel 2010), with initial concentrations as high as 30 mg/L. With the addition of excess carbon (methanol), it is believed that in-situ treatment reduces groundwater concentrations even further. A schematic of the treatment process is shown in Fig 12.

![Figure 10: Pilot-scale biological treatment at Alpha Explosives](image-url)
In a Canadian pilot-scale project, a network of injection and extraction wells was constructed. This resulted in a rapid decline of nitrate from 40–60 mg/L to less than 10 mg/L in just 2–4 months (NRC-CNRC, 2004).

According to a feasibility study report evaluating the barriers to the application of in-situ bioremediation method in South Africa, in towns such as Marydale, Leliefontein, Revielo, and Rietfontein in the Northern Cape, and rural areas of the Northwest and Limpopo provinces, clogging issues were expected after the injection due to the high concentration of ions such as iron and manganese in the groundwater. Furthermore, higher multiplication of iron bacteria was also another factor; thus, in-situ oxygenation treatments such as the Vyredox method were suggested. As per the report, switching to an ex-
situ plant may increase the cost by seven times, and therefore, the permeable reactive barrier method was suggested as the cheapest solution for rural settings (Foundation for Water Research, 2004).

**Biofilm Electrode Reactors**

In modern days, higher nitrate removal performance has been achieved by cooperation of heterotrophic and hydrogen autotrophic denitrification under low carbon or nitrogen conditions (Zhai et al. 2022). A study on the performance of both heterotrophic and electro-autotrophic denitrification processes revealed that the combination of both solid phase heterotrophic denitrification and electrochemical hydrogen autotrophic denitrification shows satisfactory performance in groundwater denitrification (Yao et al. 2022).

**Other possible treatment or mitigation technologies**

a. **Pump and treat** (conventional method)

Many technologies are currently used for nitrate removal from groundwater pumped to an above-ground treatment system, as opposed to in-situ treatment. In ion exchange, water is passed through a bed of synthetic resin where the anions, including nitrate, are removed. Membrane separation methods such as reverse osmosis and electrodialysis can also be used. However, membrane technologies are typically more expensive than ion exchange Kapoor & Viraghaven (1997) as cited in Reddy & Lin (2000).

b. **Long-term protective measures** (greener solutions)

The primary measure ensuring long-term protection from nitrate leaching from the soil is intercropping with cover crops that enhance nitrogen ion assimilation which also depends on crop rotation. Limiting tillage frequency where nitrate leaching is high is also an important consideration.

c. **Controlling nitrate pollution from agriculture** (greener solutions)

Lenzburg, Switzerland, and surrounding municipalities draw drinking water from the same area of groundwater recharge. Part of the area is used for agriculture. Due to high fertilizer applications, nitrate concentrations had reached 15 mg/L in the 1960s and had risen to 40 mg/L in the 1980s. The regional Nitrate Committee encouraged the intercropping of high-nitrate-consuming varieties by providing a subsidy of 400 Swiss francs per hectare to farmers. Subsequently, nitrate concentrations in soil decreased to 25–30 mg NO₃/L in 2003 (WHO 2004) and (Howard et al. 2006). In Jaffna, cultivation of plants such as tobacco consumes high inorganic fertilizer application. It has been proven that the groundwater NO₃ as N exceeds 27.65 mg/L at the Innuvil–Jaffna tobacco farm, whereas it is considerably lower in other vegetable farms (Sivaramanan & Piyadasa 2016). Thus, banning tobacco cultivation or finding other organic fertilizer sources such as compost and vermicomposting could solve the issue.

**d. Groundwater source protection zones** (sustainable solution)

This strategy aims to control activities that cause nitrate leaching, such as 1) inorganic fertilizers and 2) pit latrines. The first problem (inorganic fertilizers) can be prevented by maintaining clearly defined boundaries where inorganic farming is not allowed. According to UK Environment Agency (2009), the factors considered here include a) distance from drinking water wells; b) drawdown (the extent that pumping lowers an unconfined aquifer); c) time of travel (the maximum time taken by nitrate to reach the water column), d) assimilative capacity (the degree to which natural attenuation or denitrification occurs in the subsurface); and e) flow boundaries (demarcation of recharge regions or other hydrological factors that control groundwater flow). Such nitrate-vulnerable zones are declared in Scotland, Indonesia, and Ireland. Activities leading to nitrate leaching are prohibited within the protection zones (UK Environment Agency 2009). For the second problem (pit latrines), a 34 m distance should be maintained between septic tanks and groundwater sources (Ngasala et al. 2021).

**CONCLUSION**

Nitrate-nitrogen concentrations in Jaffna's drinking water are often well above the World Health Organization limit of 10 mg/L. This is due to the high use of chemical fertilizers and the close proximity of septic systems to drinking water wells. Aquifers in Jaffna typically have a
high permeability (Karstic aquifers), which increases nitrate leaching but also lends itself well to the addition of amendments such as carbon. Direct injection or circulation would be the best addition method for soluble carbon sources. Aquifer type, site geology, land use, permeability, groundwater flow velocity, and chemistry are all important factors in in-situ bioremediation. In-situ biological (heterotrophic) denitrification through the addition of carbon sources such as ethanol or methanol has been demonstrated in many U.S. applications. In the Jaffna peninsula, flow in karstic aquifers is vertical and the flow path is usually through a predefined network often accompanied by sinkholes, sinking streams and springs on the limestone bedrock. Based on the geographical distribution there are four major aquifer systems are found such as Chunnakam (Valikamam), Thenmaratchi, Vadamaratchi and Kayts. In addition, two main soil types—Calcic yellow latosols and Calcic yellow latosols—are found here. In terms of climate, Jaffna falls under dry zone, where it solely depends on seasonal rain for water intake. Thus, biological denitrification using heterotrophic bacteria is ideal. In addition to heterotrophic denitrification, modern Biofilm Electrode Reactors (BER) can also be considered due to their higher number of satisfactory performances all over the world. In-situ denitrification techniques and carbon farming practices are restorative solutions or more positive solutions, as they are more beneficial to the ecosystem and sentient beings, but both require continuous human maintenance. Restorative solutions are comparatively less expensive and ecologically more productive than sustainable solutions (net zero impact to the ecosystem and sentient beings) such as agrochemical bans, switching to organic, and establishing groundwater source protection zones and greener solutions (solutions with minimal or reduced environmental hazards) such as controlling inorganic fertilizer addition, and implementing long-term protective measures (cover crops, intercropping, crop rotation, reducing tillage, and banning crops requiring high fertilizer input).

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