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Are the current methods of remediation to reduce nitrate contamination in groundwater in the developing world effective?

A systematic review

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Abstract

Nitrate contamination of groundwater has become a growing problem, particularly in the developing world as a result of agricultural intensification and rapid urbanisation leading to leaching and run off. Large numbers of people depend on groundwater for drinking and ingestion of nitrate contaminated waters can lead to methaemoglobinemia and cancers. This review examines biological, chemical and physical methods currently employed to remove nitrate contamination from groundwater. Review of these methods is achieved by extensive literature searching and utilisation of inclusion criteria and quality assessments to obtain relevant results. The review found that all of the methods examined are effective. Effectiveness was measured against the WHO guideline of 50 mg/L nitrate concentration in groundwater. No one method was found to be more effective than any of the others. The operating parameters are variables that are known to affect the efficiency were also examined. It was found that these affect efficiency and also removal rate and the products formed. The review also discusses the biases associated with a systematic review, and the limitations involved.

1. Introduction

Approximately one third of the global population depend upon groundwater for drinking water (UNEP, 2002). Rising nitrate concentrations in groundwater have become a growing cause for concern as an exponentially increasing population requires food, to dispose of waste and treat water all of which contribute indirectly to rising nitrate concentrations in groundwater. Growing demands in the developing world for better water quality and stronger legislation for water safety enhances the need for nitrate remediation systems. The developing world has seen intensification in nitrate contamination of groundwater and rapidly growing populations, putting strains on drinking water resources (Gu *et al*., 2013).

A systematic review is a process of answering the research question by methodically searching databases and search engines to find studies that satisfy criteria chosen to ensure relevance of results. Systematic reviews aim to minimise bias in selecting studies and extracted data by the requirement of inclusion criteria. The quality of included studies is assessed to ensure high validity and multiple variations of search syntaxes undertaken to ensure reliability of results. The systematic review approach to this subject is used because of the minimised risk of bias and ensuring all literature is appropriate.

This study broadly examines literature that relates to removing nitrates from groundwater through a variety of processes in many locations. It provides a brief background on the topic to present rationale for this study. It also looks more in depth than the broad background literature review, into specific studies which are found by means of strategic searching. The method of this searching and selecting studies for inclusion will be covered extensively. Results of the findings are presented and analysed using a variety of methods, including descriptive statistics, analysis of variances, tables and graphs. The results are discussed for any significant findings and patterns. The limitations of this study are considered, supporting enhancements for further research. Each study that is included that is analysed individually with some cross-compared for those with similar variables, to add to understanding of results.

1.1. Background and literature review

Nitrates are anions that are highly soluble in water and are found naturally in low concentrations in water courses/bodies. Typically, industrial regions have greater concentrations of nitrates in waters than rural areas (WHO, 2011). However, many groundwater sources are contaminated with elevated concentrations of nitrate due to leaching and run-off from agricultural inputs. Ingestion of contaminated waters has health implications for humans and livestock, and may lead to algal blooms in water bodies. The elevated levels of nitrates in groundwater are largely attributable to the increased application of inorganic fertilisers used to increase crop yields. The other main causes of nitrates in groundwater are wastewaters containing biological waste and landfill leachates.

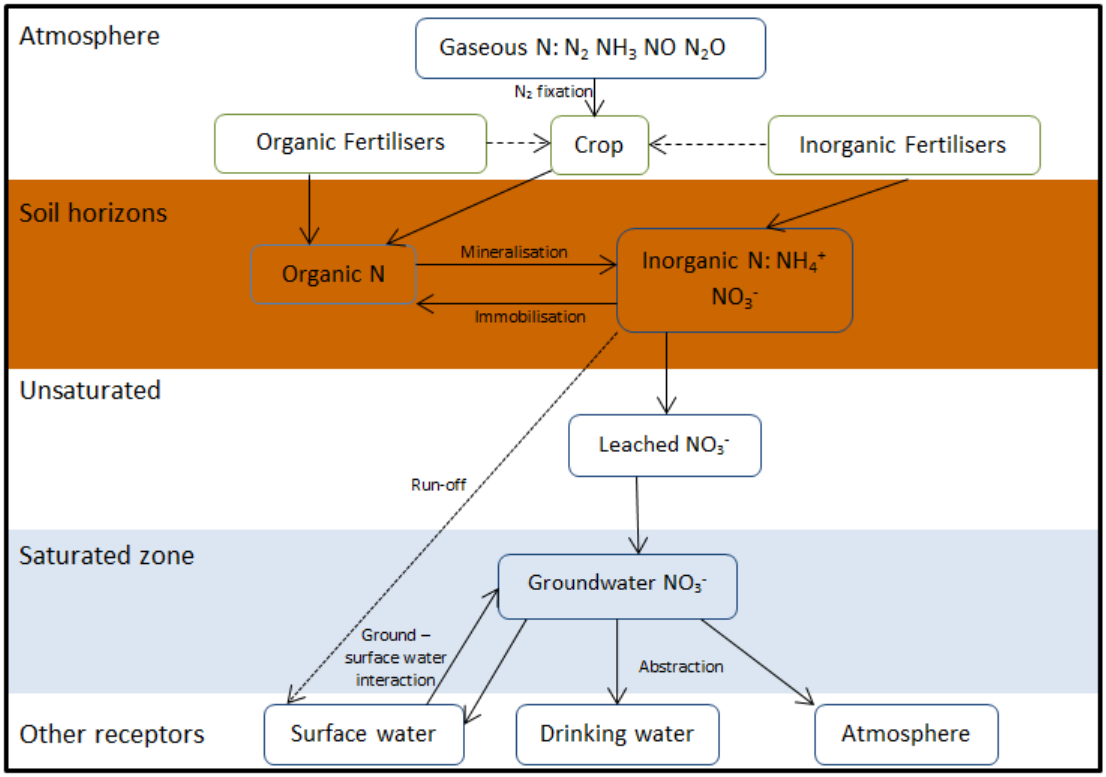

Figure 1: Movement of nitrates (Adapted from British Geological Society, 2011)

Figure 1 displays the movement and interaction of nitrates from application through to groundwater, surface water and drinking water. It shows the many interactions groundwater has with other bodies, importantly drinking water. Additionally, the movement of nitrates to surface water and wetlands can lead to eutrophication and subsequently, disrupt to ecosystems.

Groundwater is the largest accessible source of freshwater, with the exception of ice caps and glaciers. It is the water in the pore spaces of rocks, found under the top soil fractions (Environment Agency, 2014). The rock holding the water is an aquifer and they can be sizeable, such as The Great Artesian Basin in Australia, which has an area over 1.7 million square miles (Department of Environment and Resource Management, 2011). The underlying geology leads to differences in properties and consequently uses of groundwater which is why it is important to examine a range of different global locations. Groundwater, as shown in Figure 1, has interactions with surface water, and this is one point of discharge of groundwater. Groundwater is often recharged by rainfall, but this is variable with weather patterns and climate leading to vast global differences in recharge rates (Scanlon *et al.,* 2006). Dry regions which depend on groundwater for a main water source may abstract it faster than recharge rates, which can lead to increased nitrate concentrations in the groundwater.

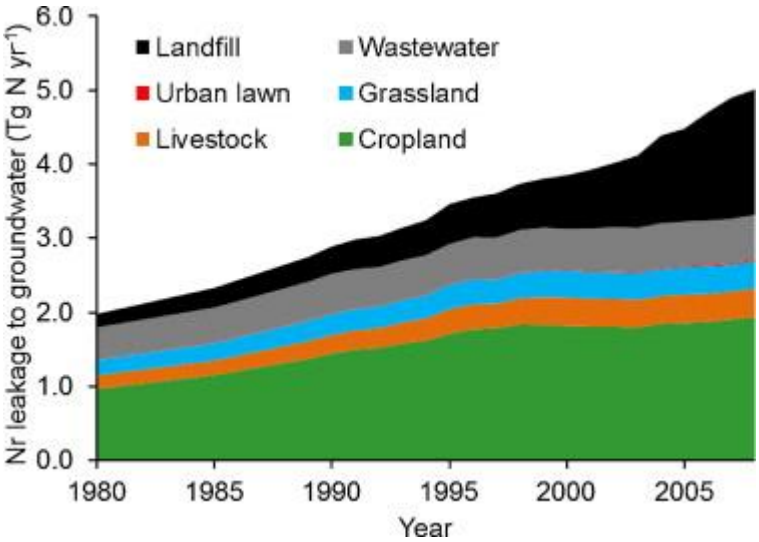

Figure 2: Sources of nitrates in groundwater in China. (Gu et al. 2013)

It has been suggested that agricultural inputs are the one of the greatest sources of nitrates into groundwater (Zhang *et al,* 1996). Zhang *et al.* (1996) state that nitrate fertiliser applications in China are almost equalling the rates of application in Western Europe, which has already seen a large increase in nitrate pollution of groundwater. Figure 2 displays the sources of nitrate into groundwater resources in China between 1980 and 2008. It shows an overall increase of nitrate leakage each year by two and a half times, and the largest input source is cropland. However, it shows landfill is rapidly increasing too and is reaching levels similar to that from cropland. Gu *et al.* (2013) agree that diffuse agricultural sources are the largest source of nitrate contamination, but point sources from landfill and waste waters are increasing. This Figure displays that there are many sources of nitrates into groundwater, and that whilst this only demonstrates China, all across the globe there are many and increasing sources of nitrate leakages.

There is a lot of literature available reporting the impact of nitrates on human and environmental health. Groundwater is often abstracted for human drinking water use but may also be used for irrigation and livestock drinking water. When ingested to high enough concentrations in humans there is the possibility of nitrate poisoning, which leads to methaemoglobinemia more commonly known as blue-baby syndrome (Bhatnagar and Sillanpää, 2011). This occurs when nitrate is reduced to nitrite in the gastrointestinal tract; the nitrite converts Fe^{2+} found in haemoglobin, which usually binds oxygen, to Fe^{3+} that cannot join with oxygen (O'Neill, 1985) leading to cyanosis. As well as causing a blue colouring of the skin, the lack of oxygen to the brain causes dizziness, headaches and in extreme cases coma and death. There are many processes currently used to remove nitrates from groundwater, aiming to minimise risk of health impacts.

Methaemoglobinemia occurs in adults and older children but infants younger than 6 months are particularly vulnerable as the microbial colonies in infant guts favour the pH for nitrate reduction (McDonald and Kay, 1988). In livestock such as cattle, nitrate poisoning is very quick and cattle may die within a day of consuming contaminated water (Bhatnagar and Sillanpää, 2011), which may have economic implications for cattle farmers. Additionally, amino acids in the human digestive tract can react with the nitrite that arises from nitrate ingestion creating nitrosamines which are carcinogenic (O'Neill, 1985).

Biological denitrification is a process often used to remove nitrates, and turns it into harmless nitrogen gas through stepwise reduction as follows:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

There are many reports of differing efficiencies of different carbon sources for biological denitrification. Frequently, liquid carbon substrates are deposited into the contaminated waters for the denitrifying bacteria to use as an energy source where nitrate is the terminal electron acceptor (Soares and Abeliovich, 1998). While organic carbon substances such as methanol and acetate are widely used, cheaper cellulosic materials are also an option (Volokita *et al.*, 1996). This includes using solid carbon substrates with options including recycling of waste materials such as newspaper. Methanol has been proposed as producing the highest denitrification results but this may be unsuitable for denitrifying drinking water, if the methanol is not controlled, as a result of possible methanol contamination (Shrimali and Singh, 2001). Elemental sulphur can also be used as an energy source for autotrophic biological denitrification because it is not toxic or expensive (Soares, 2002). Using microorganisms to remove nitrate biologically is temperature dependent (Bhatnagar and Sillanpää, 2011).

Biological denitrification is often favoured considerably due to the lower running costs on a large scale to other methods. Many have reported that the reducing bacteria used were obtained from sewage sludge (Hong *et al.,* 2012; Wang and Wang, 2012), reusing waste from another process. This is also a disadvantage because it means that the water has to be treated further before use (Bhatnagar and Sillanpää, 2011). An additional disadvantage is the potential creation of a strong greenhouse gas, nitrous oxide from the bacteria respiring aerobically (Wang and Wang, 2013).

A further method employed to remove nitrates from groundwater is ion exchange. Ion exchange processes use resins to exchange nitrate with either bicarbonate or chloride ions. However, this can lead to corrosive waste waters containing the nitrate and the exchanged ions (Reddy and Lin, 2000). Consequently, this waste brine needs treating which may lead to increased economic costs (Bhatnagar and Sillanpää, 2011). Canter (1996) reports that ion exchange is preferred by some for the removal of nitrates because of the lower financial cost compared to alternative removal processes but this is outdated and recent studies report that biodenitrification is the cheapest option.

Reverse osmosis is an additional intervention used to remove nitrates. This process involves increasing the pressure within a reverse osmosis cell and forcing the nitrate contaminated water through a semi-permeable membrane which is constructed to withstand these high pressures (Canter, 1996). Reverse osmosis is commonly used on groundwater that is abstracted for drinking. The disadvantage to using this process to purify drinking water is that it can also remove beneficial minerals such as calcium (Canter, 1996).

Nanofiltration is another method of removing nitrate from groundwater. This process was originally used in softening of water but has now been found to have properties that remove nitrate (Amouha *et al.,* 2011). Nanofiltration can be favoured as a nitrate removal mechanism due to the reliability of the membrane and the lack of additives required (Mahvi *et al.*, 2011). Nanofiltration is often used as a process for water that will be used as drinking water due the water softening properties this process provides. Nanofiltration is also used for removal of pesticides in groundwater which may coincide with agricultural areas of increased nitrate applications (Aslan and Türkman, 2005).

It is important to investigate nitrate contamination in developing countries because there is expanding agricultural industry growing cash crops, biofuel feedstock crops as well as food to sustain themselves. As a result of rapid development, agriculture has grown enormously with consequent increase in uses of nitrate heavy fertilisers. This raises the risk of run-off and leaching into watercourses. Many of these developing countries also lack the infrastructure for drinkable

water so groundwater is often abstracted for drinking. Around one third of the global population use groundwater as their main water supply (UNEP, 2002), with half of China's population depending on groundwater for their source of drinking water (Qian *et al.*, 2011). Those who use private wells may have increased risk of contamination from agricultural run-off and untreated sewage waste (WHO, 2011). Additionally, reduced access to healthcare makes these populations more vulnerable to the effects of methaemoglobinemia and the presence of pre-existing medical conditions increases the chance of the condition developing (McDonald and Kay, 1988).

The remediation of nitrate contamination in groundwater can be affected by a multitude of other environmental and physical variables. These include pH, temperature, flow rate, initial nitrate concentration and time. Some of the methods, such as biological denitrification are heavily dependent on the temperature and pH for high efficiency as the denitrifying bacteria have optimum conditions so as to not denature the enzymes. Flow rate and time can be a limiting factor for physical methods such as ion exchange or nanofiltration. These factors can also affect the by-products formed through nitrate removal.

Two countries of key interest in this literature review are India and China owing to the fact they have the two largest populations on the planet, with vulnerable people susceptible to the impacts of increased groundwater nitrate concentrations. Both have highly densely populated cities, and vast rural communities. Suthar *et al.* (2009) studied nitrates in India and argues that the impacts of nitrate contamination in the developing world are greatest in rural regions. However, Zhang *et al.* (1996) examined nitrates in groundwater in urban regions and found of the cities examined, found that more than half of the sampling locations had concentrations higher than the WHO guideline. This is supported by WHO (2011) that state industrial regions usually have greater nitrate concentrations in groundwaters.

Case study: India

India uses more groundwater as a water resource than any other country with its use for drinking water at 85% (World Bank, 2011). It is very useful to India's population due to the unpredictability of rain water in monsoon seasons (World Bank, 2011). The presence of groundwater in India is greatly varied as a result of the differing underlying geology (Bhawan, 2009). Fertiliser inputs are not the only cause of nitrate contamination of groundwater in India, cattle farming wastes have a large input into nitrate pollution of groundwater (Rao, 2006).

Case study: China

China is recognised as one of the most rapidly developing countries, with extreme growth in agriculture and nitrate fertiliser applications. A study by Gu *et al.* (2013) examined the nitrogen input as fertiliser and the nitrate measured in groundwater. It was found that higher concentrations of nitrate in groundwater are in areas where there is increased fertiliser applications. Huge population increases have caused vast expansions in urbanisation, which has led to reduced land available for agriculture. As a consequence, the intensity of agriculture, and the application of fertilisers has grown greatly to accommodate the growing need for food for the expanding population (Zhang *et al*., 1996). The North East of China was one of the first places to develop large scale agriculture which is why there is a high concentration of sampling points in this region (Gu *et al.,* 2013). This study from Gu *et al.,* (2013) examines the sources of nitrates in groundwater and found nitrate was present in 96% of groundwater samples tested.

To summarise, there is already a great extent of study of nitrates in groundwater. Many studies have already examined a variety of methods for removing nitrates from groundwater, but not as a whole across developing countries. For those examining biological denitrification, one point of

interest has been the effect of different carbon energy sources for the denitrifying bacteria. There's a lot of interest into the sources of nitrates in groundwater but the sources are of less relevance to this review. Much of the literature available for nitrates in groundwater has studied the nitrate pollution of groundwater in China, particularly Beijing, this study will diversify this and look at nitrates in groundwater with a more global focus.

2. Aims and objectives

The aim of this review is to examine the methods used to remove nitrates from groundwater. To achieve the aim, the first objective is the systematic searching of databases to explore the scope of literature available, and obtaining studies and extracting secondary data to use within this review. The second objective is to examine the results of the search for details of the methods commonly used for remediation of nitrate contamination. The next objective is to statistically analyse the results to consider if any one method is more effective than the others.

2.1. Primary question and outcomes

1. Are the current methods of remediation to reduce nitrate contamination in groundwater in the developing world effective?

The primary question will be achieved by reviewing pre-existing studies that have examined the current interventions used, and extracting data from the studies on the outcome concentration of nitrate post-intervention. This is accomplished by methodically searching databases to find relevant studies. The primary question aims to understand results in the developing world for which 18 developing countries were chosen to exclusively include studies from. The 18 developing countries are selected using information from The International Statistics Institute that used data on Gross National Income per capita from the World Bank. The countries are listed in Table 1, and displayed in Figure 3.

Table 1: Developing countries included in literature search

Algeria	Argentina	Bangladesh	Brazil	Chile	China
Ghana	India	Iran	Malaysia	Mexico	Nigeria
Pakistan	Philippines	Taiwan	Thailand	Tunisia	Turkey

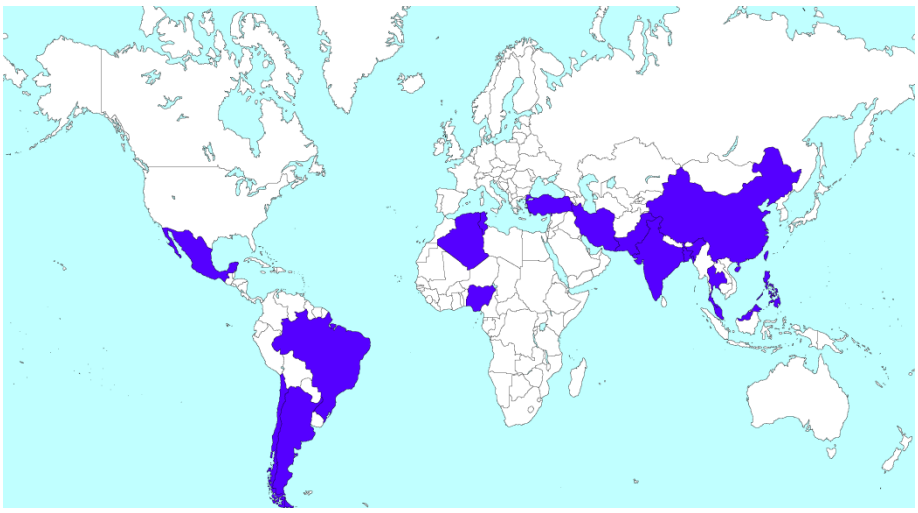

Figure 3: World map of developing countries included in study

The outcome is if the current interventions are effective. The results are tested by the using the WHO guideline of 50mg/L as a maximum permissible concentration of nitrate. This is a baseline for efficiency and the outcome concentration of nitrate measured against this.

2.2. Secondary questions and outcomes

1. Is one intervention greatly more effective than the others?

This is measured by the average concentrations of nitrate in groundwater after using the intervention, and the percentage decrease in concentrations. The averages for each method are compared against each other to discover if one is more effective than the others.

2. What effects do other variables have on the removal of nitrate?

These other variables are operating parameters and includes pH, flow rate, temperature, initial concentration and time. These are rate and efficiency limiting factors for the removal of nitrate from groundwater. The studies chosen for inclusion are also examined for study of these variables and results of these extracted and compared. These variables change with the different method as temperature for example is a very big limiting factor for the biological denitrification processes. These variables are not statistically compared as there are many combinations of nitrate removal methods and other variables examined. Instead, they are examined for similarities and consistencies, or contrasts, between results.

2.3. Variables

The independent variable in this study is the intervention method, the process used for removing nitrate. The dependent variable is the concentration of nitrate in the tested groundwater.

3. Methods

3.1. Search methods

Electronic searching was undertaken in Web of Science and Google Scholar. Hierarchal searching was the search strategy used with Web of Science. The first search was very broad and this was narrowed to generate relevant results by refining with further keywords until much smaller numbers of results were produced. Citation chasing has also been used as a strategy for finding resources, however this strategy was unreliable for finding studies within specified search limits.

The first search was ‘nitrate AND groundwater’ using the limits of English for language and the date range 2003-2013. These limits were used to reduce and refine the total number of relevant results produced. English was the default search language but was used as a limit as translating studies may lead to errors in understanding and interpretation of results. Each search was refined with further search terms including the ‘OR’ and ‘AND’ operators. Some refining was so categorised that the final results were not relevant to this study. An example of this was adding the search term ‘review’ to a search that had been refined 6 times previous since the original search, producing 0 results.

An example of the hierarchal search is:

'nitrate' AND 'groundwater' AND 'contamination' OR 'pollution' AND 'ion exchange' OR 'nanofiltration' OR 'denitrification' OR 'reverse osmosis'

These search terms were used to search 'topic', which includes the occurrence of these search terms in abstracts as this yielded more results than searching 'title'. This did however lead to more obscure results that weren't of interest in terms of this study.

This whole search process was undertaken on 3 separate occasions to reduce the likelihood that studies were missed. Searching in Google Scholar was not repeated as many times as a number of the results were duplications of the ones already located through Web of Science.

3.1.1. Search limits

Limits were used to narrow results, and ensure relevance. The limits included:

- Date – between 2003-2013
- Language – default of English
- Selected developing country

3.2. Criteria for considering studies

The results of each refined search are screened for titles containing at least three of the key words that were defined as the selection criteria. The key words that were looked for within the titles in the Web of Science searches are listed in Table 2. Studies that satisfied these selection criteria were imported into EndNote X2 saving the title, author and abstract information for later screening at the next stage. This resulted in 142 studies stored in EndNote X2 from systematic searching and from the scoping exercise.

Table 2: Key search terms

Nitrate	Groundwater	Removal	Reduction	Contamination	Synthetic
Pollution	Ion Exchange	Reverse Osmosis	Denitrification	Nanofiltration	

3.2.1. Types of interventions

A number of different removal methods are included in the review. The list of studies for inclusion was originally much smaller, but after scoping the literature it was found that there are a multitude of methods utilised and would gain a wider range of representative results from including these 'other' methods. Only interventions that mitigate the nitrate were examined. Interventions that prevent the nitrate leaching into the groundwater were not included.

3.3. Study selection

The syntax of searches and the search engine/database used along with the number of results produced was recorded to be able to trace results, both in subsequent searches and for repeat searches. The comprehensive list of searches performed in Web of Science can be viewed in Appendix 1. EndNote X2 was used to facilitate the searching of titles and abstracts in the screening stages to look for relevant studies. The studies found were sorted by date of search to make it easier to find which search the studies were included in.

The full selection process is seen in Figure 4. After importing the 142 possible studies into EndNote X2, 24 duplicated studies were removed before studies were selected for inclusion. Within EndNote X2, the term “groundwater” was searched for within all titles and abstracts. This search immediately excluded studies that were not about groundwater and led to the removal of a further 66 studies. The remaining studies were examined again for the appearance of the key words from Table 2

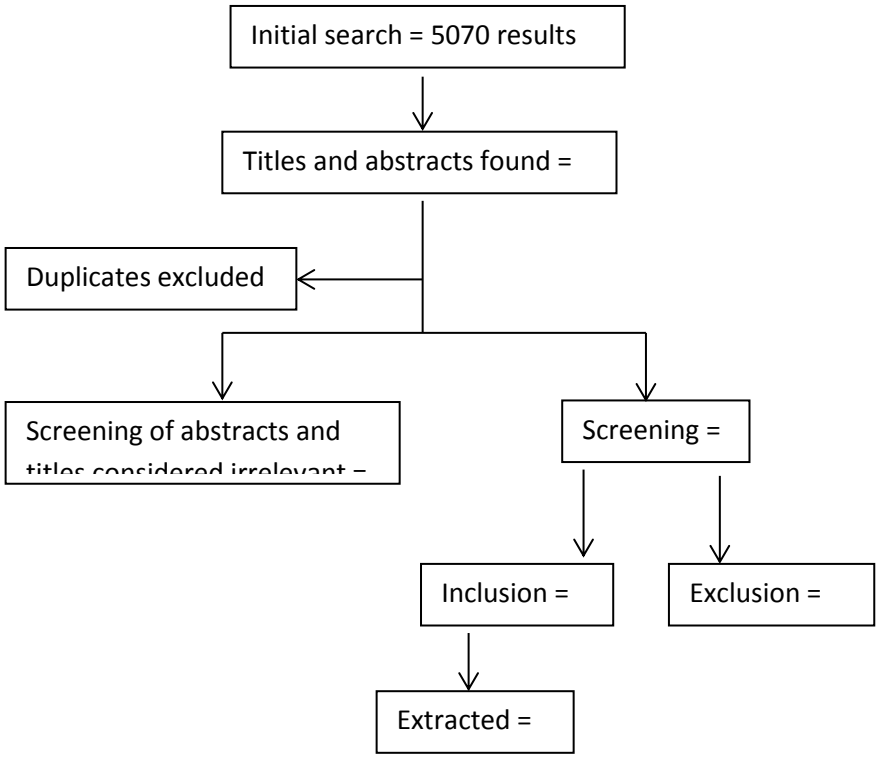

Figure 4: Method of inclusion

within their title and abstract removing a further 28 studies. 24 studies were selected for full examination of the study.

Of the studies examined in full, some did not satisfy the inclusion criteria. Inclusion criteria included 4 conditions of developing country, groundwater, listed method and outcome. Developing country was the first inclusion criteria. Some studies imported from the scoping exercise were not in one of the listed developing countries, leading to their omission. Even when searching using the limits in Web of Science, not all of the imported studies reported the location of the groundwater resource within the study, this eliminated some studies.

The second inclusion criteria was groundwater, as previously mentioned. Studies were removed at the first screening process for the lack of the keyword “groundwater” in the title or abstract. One limitation of using “groundwater” as a keyword is that many abstracts suggested “groundwater” as a possible medium for the application of removal technologies and the study did not examine groundwater. Synthetic groundwater is water with nitrate added to simulate nitrate contaminated groundwater. Additionally, many studies examined synthetic groundwater. Originally those examining synthetic groundwater were also omitted but this left very few studies so those testing synthetic groundwater were finally included as the number of studies included was too few to subject to statistical analysis.

The third inclusion criterion is listed method. There were many processes for removing nitrate from groundwater but to ensure a manageable number of studies this was originally limited to four. After

screening studies it was apparent this was unsuitable and more processes were needed to be included to ensure a representative sample of the processes employed to remove nitrates from groundwater. The original four methods were ion exchange, biological denitrification, nanofiltration and reverse osmosis. The number of processes finally included in the list for inclusion totalled 7.The final criteria for inclusion in the study is outcome. This was if the intervention used in the study produced results equal to or below the WHO guideline of 50mg/L. This was to ensure the intervention method removes the nitrate to the approved concentration. Further to these inclusion criteria, there were quality assessment criteria that were required for inclusion in this study.

3.4. Quality assessment

Table 3 shows the criteria and process for ranking studies that were included. The first three columns show three items that had to be present within the study for the quality to be valid. The study had to show evidence of a comparison or control, include the method of analysis used and the objectives of the study. As the table shows, if any one of these were missing the study as ranked not valid. The outcome column explains the presence of the outcome concentration of nitrate present in the study. This is not if the concentration was lower than 50mg/L, but if it was stated what the final concentration was. The quality and outcome together lead to the strong or weak ranking as seen in the final column. To be of strong quality the study had to be both valid and have an outcome stated. Ranking of studies was completed for further analysis to ensure the weak studies would not have significantly different results to the strong studies. Studies ranked very weak were not included in the final selection of studies.

Table 3: Quality ranking criteria

Comparison or control	Method of analysis	Objectives of study	Quality	Outcome (final nitrate concentration)	Rank
✔	✔	✔	Valid	✔	**Strong**
✔	✔	✕	Not valid	✔	**Weak**
✔	✕	✔	Not valid	✔	**Weak**
✕	✔	✔	Not valid	✔	**Weak**
✔	✔	✔	Valid	✕	**Weak**
More than one not present			Not valid	✕ / ✔	**Very weak**

Studies were also checked to see if they were peer reviewed. This was to minimise bias of the results stated in the studies. All studies included were peer reviewed however so this was not further analysed.

3.5. Data extraction and management

Data was extracted from selected studies and recorded in tables which can be seen in Appendix 4. The data extracted included the author and title/aims of study, information about the groundwater and the intervention used in the study. It also included the method of analysis of the intervention, the objectives of the study and any comparisons or controls used. The included studies were collated into one singular table, seen in Appendix 2 only listing the name of author and title, the method of removal, the location of the study, the amount of nitrate removed and the ranking (strong or weak) to easily manage data for running statistics and displaying visually.

A number of the studies expressed the nitrate concentration as NO_3-N (nitrate-nitrogen) consequently calculations to standardise all results to NO_3 were performed. The process of this calculation is seen in Table 4.

Table 4: Conversion of nitrate nitrogen to nitrate

	NO_3^- molecular weight	Example
Nitrogen	14	50mg/L NO_3^- N
Oxygen	48	
Total	62	50 x 4.42 = 221mg/L NO_3^-
Conversion factor	62/14 = 4.42	

As a result of inconsistencies of reporting of the outcome across all included studies, percentage of nitrate removed was calculated and used as the figure for most of the statistical comparisons. Equally, those which only included a percentage were worked out to the final concentration however this may not be accurate. It was also noted which studies used synthetic groundwater and which used genuine groundwater for later comparisons. Studies that did not include an outcome measurement of nitrate did not have data extracted but were still included in the screening stage. This is displayed in Figure 4 as 24 studies were included but only 19 extracted.

3.6. Data synthesis and analysis

19 studies were used and were grouped into a table as already mentioned to easily view and refer to all of the data. Minitab 17 was the statistical software used for analysis of results in this study. The following statistical manipulations were applied:

- Descriptive statistics for all variables
    - All methods
    - Each nitrate removal method individually
    - Strong and weak quality studies
    - Synthetic and natural groundwater
    - Each country included

Descriptive statistics includes means and standard deviations of the calculated percentage of nitrate removed.

- Comparison and significance of remaining nitrate concentrations compared with WHO guideline
- Comparison and significance of all methods to see if one is more effective than others

The remaining concentration of nitrate in groundwater was compared against the WHO guideline of 50mg/L as a baseline to measure efficiency. A significant difference between the average remaining nitrate and the baseline qualified the efficiency of all methods. By looking for comparisons between methods, this expressed if any one method was more efficient than the others. An ANOVA test was run on the data with the following hypotheses:

- Significant differences between averaged results and the WHO guideline
    - H_1 There is a significant difference between averaged results and the WHO guideline

- H_0 There is no significant difference between averaged results and the WHO guideline

Significant differences between strong and weak ranked studies were examined to ensure the quality of all included studies does not differ. This was achieved by undertaking a one way ANOVA with a P-Value of 0.05. The same test was also used for synthetic and natural groundwater to ensure there are no differences by including studies that examined synthetic groundwater. Usually, ANOVA is testing for a significant difference between variables, however in this case significant differences were not desirable as this was a test of homogeneity.

- Significant differences between strong and weak studies
  - H_1 There is a significant difference between strong and weak studies
  - H_0 There is no significant difference between strong and weak studies
- Significant differences between synthetic and natural groundwater
  - H_1 There is a significant difference between synthetic and natural groundwater
  - H_0 There is no significant difference between synthetic and natural groundwater

The data was also input to Microsoft Excel to create simple graphs for visual display of the data. Where studies did not give a final concentration of nitrate, only a percentage, all were calculated into a percentage for standardisation. Those with the percentage and no final concentration in mg/L were also calculated back from the percentage but this is not reliable hence percentages being used for statistics.

4. Results and discussion

4.1. Summary of all results

24 studies are included after the screening stage, with 19 studies which have data extracted for examination within this review. Full details of results including author, title, removal process, objectives, location, comparisons and outcomes can be found in Appendix 4. This comprises both the included and excluded studies. The excluded studies totals 5 and they are ranked very weak in the assessment of their quality, hence their exclusion. Results for included studies are split into different categories for assessment, shown in Table 5. The Table shows the number of results in each category that was examined.

The table shows that the modal removal method is biological denitrification and the least common removal method is nanofiltration, and there are no results studying reverse osmosis, which was originally outlined as a key method to be examined. Both ion exchange and nanofiltration have too few numbers of results to be statistically meaningful. The modal group for study location is China, followed by India. There are only 4 countries included in this review. There are an odd number of studies, and as such the split between strong/weak quality studies and synthetic/natural groundwater is not even. This means that the choice of statistical tests was narrowed as the groups were not even.

Biological denitrification may likely be the most common removal method in the developing world as it is relatively cheap, and has a high efficiency, compared with some of the other processes.

Presented in Table 5 is the mean and standard deviation for all variables. From this it can be seen in the ‘n’ column some variables have a small number of results so these will be less reliable.

Table 5: Summary of means and standard deviation for different categories

Category	n	Mean %	Standard Deviation
All	19	90.73	11.56
Biological denitrification	12	96.63	4.36
Ion exchange	2	95.75	0.495
Nanofiltration	1	62.2	-
Other	4	77.93	9.32
Strong	9	88.41	15.77
Weak	10	92.92	5.97
Synthetic groundwater	9	94.24	8.92
Groundwater	10	87.67	13.19
China	11	94.57	8.32
Iran	2	78.00	23.5
India	4	84.73	13.31
Turkey	2	93.55	3.61

4.2. Primary outcome results

4.2.1. All methods

To summarise all methods, no one method is more effective than any of the others. However, there is not enough evidence to reasonably draw this conclusion. Of the 19 studies, 12 examined biological denitrification, and the 7 other studies split across ion exchange, nanofiltration and 'other' methods, which is not enough results to draw conclusions about each individual method. As a whole, all of the methods are significantly different to the WHO 50mg/L guideline meaning it can be said that all of the methods examined in this review are effective. Evidence of this is shown in Figure 7. Although this review makes comparisons to many other studies, this is only for the methods reviewed within this review, for the set limits. If the limits were to be wider, different results would be found, possibly changing the efficiency results.

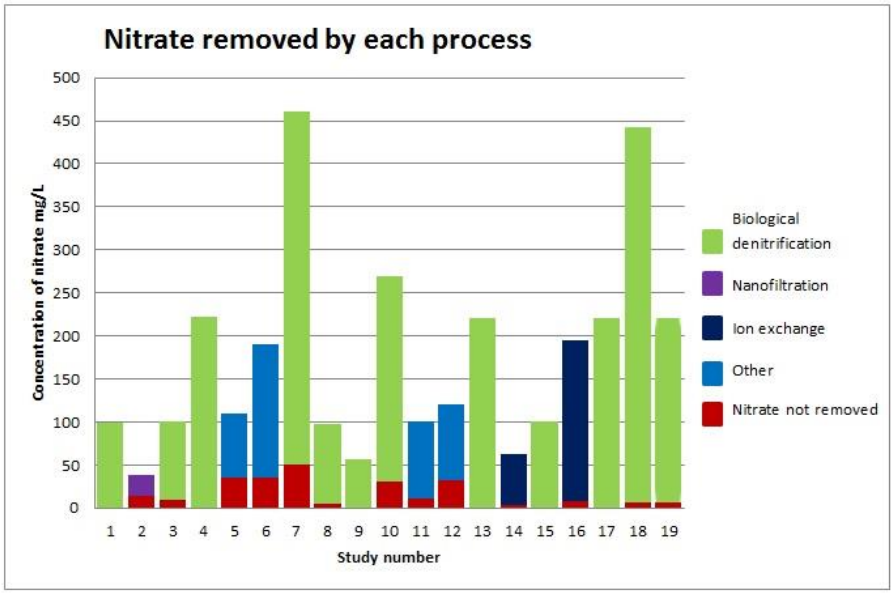

Figure 5: Graph showing overall concentration of nitrate and nitrate leftover

Figure 5 displays all of the studies graphically with the starting concentrations of nitrate, and the nitrate remaining in the groundwater if it was not removed by the process. This visualises the modal group of studies of biological denitrification. It also illustrates that all of the studies had an outcome concentration of nitrate at 50mg/L or less. The starting concentration of nitrate in groundwater is very variable, with two studies examining groundwater with a starting concentration greater than 400mg/L. This gives reason to the use of percentage calculation for efficiency, because the studies used a wide range of synthetic and groundwater resources thus leading to the differing initial concentrations. On the other hand, groundwater resources do have different nitrate concentrations, even at different points of abstraction so comparing the remaining concentration to the WHO guideline is necessary.

4.2.2. Comparison with the WHO guideline

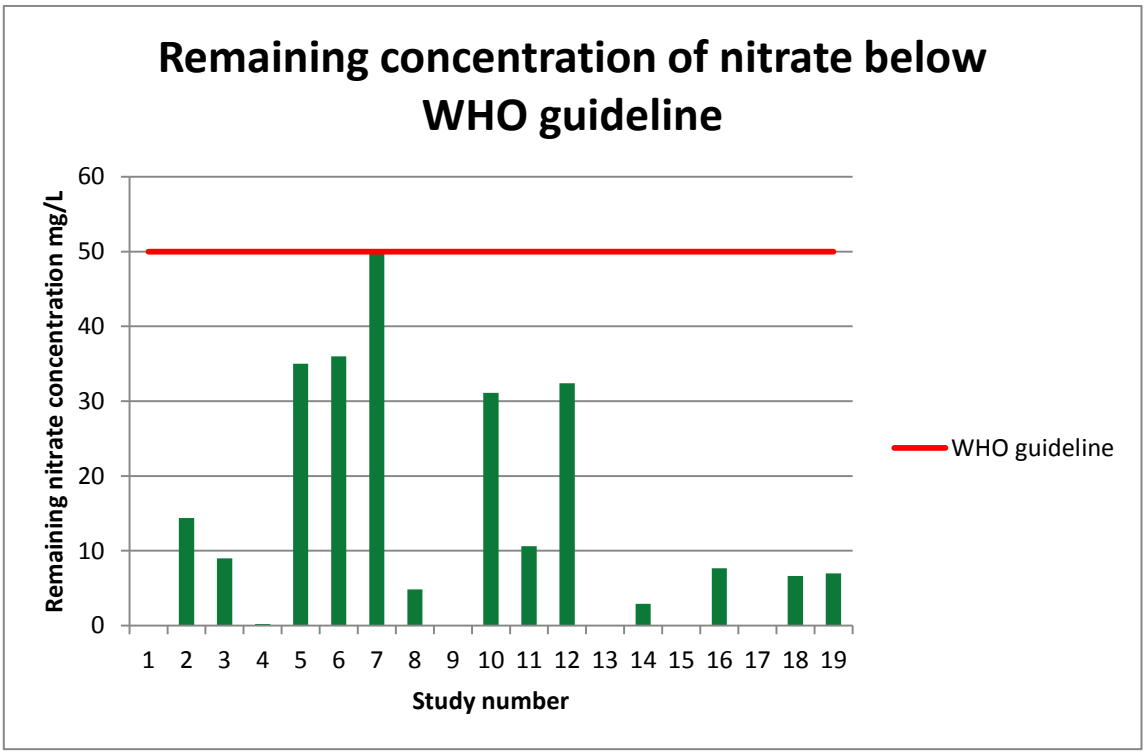

Figure 6: Nitrate below WHO guideline in each study

Figure 6 presents the remaining concentration of nitrate for each study, this can also be termed the concentration of nitrate in the effluent. It shows that all of the studies are below the guidelines, except for number 7, which meets this guideline. A number of the included studies entirely removed the nitrate from the groundwater. This Figure only shows the remaining concentration of nitrate in the groundwater, which cannot be compared against each other for efficiency because all of the studies started with different concentrations. The WHO guideline of 50mg/L is used as a baseline for efficiency. This is the limit set as safe by the WHO and so by comparing the results of this study to this baseline, efficiency and safety for human health can be implied.

Additionally, there is no significant correlation between lower effluent concentration and higher efficiency. As the correlation is not significant, it can be inferred there is no relationship between the efficiency and remaining nitrate concentration.

Comparing the studies to the WHO guideline ensures the results are fair across the globe. As this is the guideline for health, it is regarded as the baseline for minimal harm to health. Each of the

included countries has their own guideline for nitrates in drinking water, but also has different limits for groundwater that is not used for drinking.

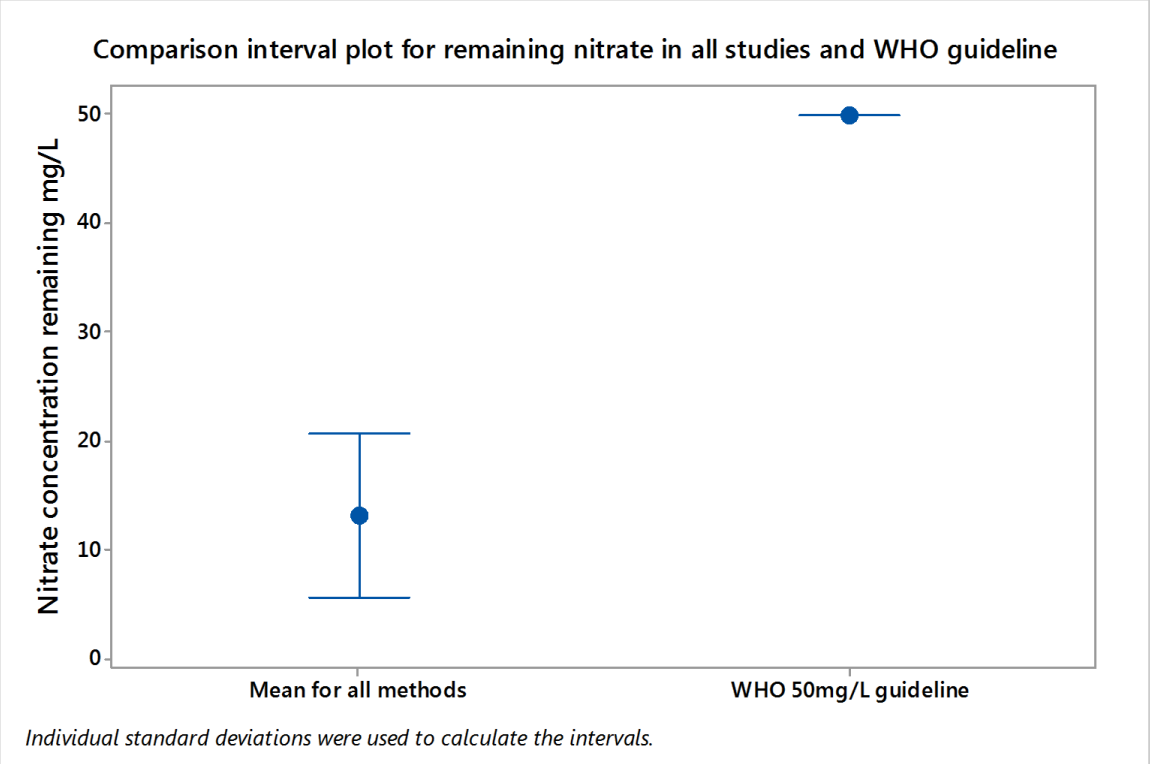

Figure 7: Comparison of all studies and WHO guideline

Figure 7 shows the comparison of the mean effluent nitrate concentration with the WHO guideline as the baseline for all methods. It shows no overlap from the mean of all the methods and as such there is a significant difference between the guideline and the results from the included studies. The p-value is 0.00 qualifying the rejection of the null hypothesis that there is no difference. From this it can be said that all of the studies included in this review, as a whole, are effective at removing nitrate from groundwater to below the guideline. However, this uses the average effluent concentration for all of the methods, compared against one guideline figure.

4.2.3. Difference between different methods

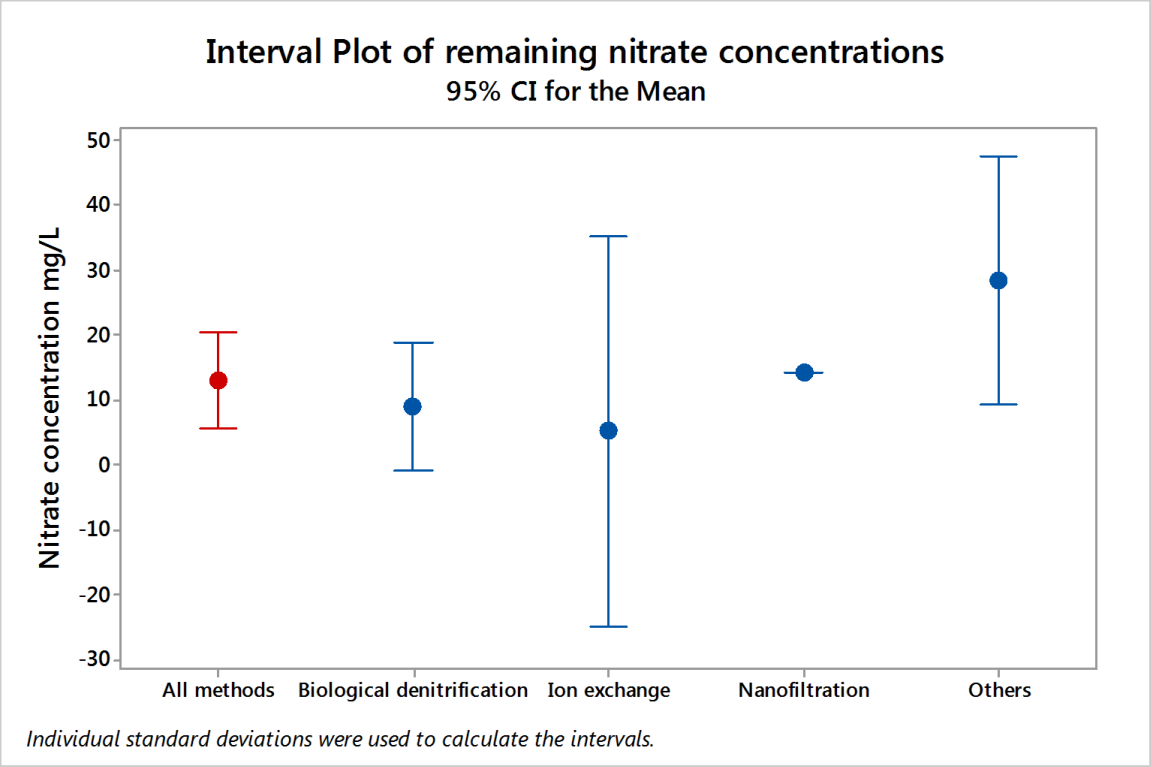

Figure 8: Nitrate concentration remaining post-method with confidence intervals

Table 6: Remaining nitrate concentration

	All	Biological denitrification	Ion exchange	Nanofiltration	Others
Mean remaining concentration	13.04mg/L	9.07mg/L	5.28mg/L	14.4mg/L	28.50mg/L

Figure 8 demonstrates the overlap between all of the methods included in this study, for the remaining nitrate concentration in the groundwater after the removal process. It shows there are no significant differences; no one method is more effective than the others. However, the results are not reliable for nanofiltration or ion exchange because there are not enough individual results to meaningfully compare these two methods. Table 6 shows the average concentration of nitrate that was remaining in the groundwater effluent of these methods. From the table it can be seen that the overall average concentration of nitrate in effluent is much lower than the WHO guideline, which as discussed could point towards the efficiency of these results.

The 95% confidence intervals for each method overlap. However, the confidence intervals show negative values for remaining concentration of nitrate, which is not possible. The concentration of nitrate in the effluent from the group 'other' methods is much higher, over double the concentration of the average for all the methods.

Each method will be discussed individually.

4.2.4. Biological denitrification

Over 60% of the studies included for this review examined biological denitrification. The average efficiency is high at over 96%. This could be due to a variety of reasons but one is the bacteria undertaking the denitrification are highly efficient, they catalyse the breakdown using nitrate as the terminal electron acceptor. Biological denitrification does not rely on physical machinery, or chemical reactions, although these aspects are still involved in the reactors used for biological denitrification.

The advantages of biological denitrification are that there is no brine waste water that comes with some of the other methods of nitrate removal. This method is also considered environmentally sound as there are no waste chemicals (Karanasios *et al.,* 2010), and the bacteria are naturally occurring organisms, although it should be noted they may not be naturally occurring at the site of denitrification. This method of denitrification of groundwater is cheaper than some of the other options available with overall operating costs estimated at $0.40/m^3 (UNEP, 1998). One of the included studies in this review Wang and Wang (2012), supports this as it is stated that on a large scale, it is the most economic process to remove nitrate.

A consideration not in favour of biological denitrification is the possible build-up of nitrite and ammonia through the step-wise reduction process. These are not desirable compounds to be in the effluent if its use is drinking water. As already mentioned, there is no brine waste but the inputs of carbon energy source and numbers of bacteria, need to be controlled to prevent contamination of effluent. This could lead to problems should the water be used for drinking (Bhatnagar and Sillanpää, 2011).

Autotrophic denitrification uses bacteria that are chemoautotrophs, they use inorganic sources of energy. The advantages to this is that there aren't carbon substances left in the water post-treatment which can minimise concern (Till *et al.,* 1998). Autotrophic denitrification may require adjustment of pH when sulphur is used as the energy source because of the acidic nature of sulphur in water (Karanasios *et al*., 2010). This was seen in one of the included studies that used autotrophic denitrification. Wan *et al.* (2009) used autotrophic bacteria and the addition of limestone to control the pH. Within this study, comparisons to other heterotrophic biological denitrification methods were observed and it was found that this autotrophic method produced a higher nitrate removal efficiency with a shorter reaction time.

A second study included in the review examined a method using autotrophic bacteria that were coupled with heterotrophic and also made comparisons to other methods. Zhao *et al.* (2011) reported that joint autotrophic and heterotrophic denitrification produced higher results than just autotrophic denitrification. Whilst these are not truly conflicting findings, they do not run in complete concordance with each other, which may be a need for further research.

Although only two studies examined autotrophic denitrification, many of the other included studies made reference to autotrophic denitrification and its advantages. Two studies in agreement of one of the advantages are Zhao *et al.* (2011) and Huang *et al*. (2012). Both concur that there is little biological pollution of the effluent water as there is no requirement for sewage sludge containing bacteria. This is an advantage of autotrophic over heterotrophic. However, heterotrophic bacteria have a high efficiency and the potential for many carbon sources to be used (Karanasios *et al*., 2010).

The majority of the studies identify a number of different carbon sources used as the energy supply for the bacteria. Common sources are methanol, ethanol, acetic acid and solid carbon sources. The majority of the included studies made reference to the use of liquid carbon sources, however only three studies used methanol for the carbon source, and six used solid or non-conventional carbon sources such as sugar cane, reed stalks, rice stalks, cellulose and wheat straw (Wang and Wang, 2012; Wang and Wang 2013; Ayyasamy *et al.,* 20007; Aslan and Türkman, 2005; Qian *et al*., 2011;

Rajakumar *et al.,* 2008).

Solid carbon sources are an advantageous alternative to liquid carbon energy sources for bacteria. The advantage to using solid carbon sources as they can act as a permeable reactive barrier (Rocca *et al*., 2005). A permeable reactive barrier can be used to prevent further spread of contamination in groundwater; it removes the nitrate as it passes over but still allows the water to move through an aquifer (Wilkin, 2012). This is an advantage to the developing world because the intense fertiliser use and leaching may be difficult to control, but using a permeable reactive barrier is a cheap option of pollution control. A readily available carbon source is cellulose (Rocca *et al*., 2005). This is one of the most abundant resources available and is renewable. This further supports the favourability of solid carbon sources. As this is a renewable resource, the use of cellulose will not cause environmental damage through its use in denitrification. As mentioned, some of the studies utilised cellulosic materials for the carbon source for bacteria.

A key genera identified by two studies found to be highly efficient is *Pseudomonas* spp. (Ayyasamy *et al.*, 2007; Rajakumar *et al.,* 2008). This is supported by Karanasios *et al*. (2010), not included within this review. These species of bacteria can be favoured as they grow rapidly, increasing the speed of denitrification (Carlson and Ingraham, 1983). However, the species of bacteria used for denitrification was not reported on within many of the included studies. This could be an opportunity for development of further study, is to see which species have greatest efficiency for different environmental conditions.

The large number of included studies examining biological denitrification could show that this could be a suitable method for the developing world. It is cheaper than many other methods, especially on a large scale. Using solid carbon sources can not only remove the nitrates, but prevent further spread of the contamination.

4.2.5. Ion exchange

Two studies included tested ion exchange (Samatya *et al.,* 2006; Hekmatzedah *et al*., 2012). Ion exchange had a high efficiency, with a low standard deviation, meaning the results obtained are similar. Two results is not enough to be able to say if this is an efficient method, but the two included can be said to be highly efficient. The high efficiency of 95.75% parallels the results found by Chabani *et al.* (2006) and Bae *et al*. (2002), not included in this review, where the efficiency was found to be 96% for both studies. Ion exchange is an attractive method for removing nitrates from groundwater as the exchange resins can have a long life time, minimising maintenance costs (Kim and Benjamin, 2004).

The two studies do not agree on the order of anions removed from the groundwater. It is important to understand the order of anions removed because this can affect the efficiency of nitrate removal. Samatya *et al*. (2006) states that the order for removal of ions through ion exchange is $NO_3^- > SO_4^{2-} > Cl^- > HCO_3^-$ but this contrasts what Hekmatzedah *et al*. (2012) reports. Hekmatzedah *et al*. (2012) states that the sulphate is removed first, which alters the efficiency of nitrate removal. This might be argued to be a drawback of using ion exchange as a method of remediation of nitrate contaminated groundwater. If the groundwater is also contaminated by sulphates, then ion exchange may not be the most suitable option for removing nitrates as the sulphates are removed first (Bae *et al*., 2002).

However, the resin used by Samatya *et al*. (2006) has been developed to be nitrate specific, to overcome this problem with sulphates. This is supported by Boumediene and Achour (2004), which was not included in this review, but found that these resins are an effective method of removing nitrate ions from groundwater. The included results show that this is a highly efficient method of nitrate removal, but more research is required for the application in the developing world.

4.2.6. Nanofiltration

The study by Mahvi *et al.* (2011) included in this review examining nanofiltration also examined many other factors that affect the efficiency. The efficiency in this study was the lowest efficiency of all studies included in this review. This can was accounted for by the association of other ions in the water tested, which affected the pressure of the membrane, and which ions were adsorbed onto the membrane surface.

Although there was only one study using nanofiltration, the result gained is an agreement with results in other studies that were not included in this review. Amouha *et al.* (2011), found that nanofiltration removes nitrate from groundwater with approximately 60% efficiency, very similar to the 62% efficiency found in this study by Mahvi *et al.* The disadvantage of nanofiltration is that it may not be the most efficient method available for removing nitrate. Further study of nanofiltration is required to explicitly state the efficiency at nitrate removal.

4.2.7. Other methods

There were four ‘other’ methods included in this review that were not laid out as the original selection of methods. The four include uptake by aquatic plants, catalytic reduction, hydrogen reduction and electro-reduction. All of these methods were situated in either Turkey or Iran. These methods had a much lower average efficiency than biological denitrification but the ‘other’ methods were not significantly different to any of the other methods.

Uptake by aquatic plants had a result of approximately 60% efficiency. This method may not have as high efficiency as some of the other methods because it is very dependent on other environmental conditions in order for the plants to grow (Piña-Ochoa and Álvarez-Cobelas, 2006). This method may be unsuitable to some developing countries with harsh arid environments, such as Iran included in this study, as it requires wetlands.

Nitrate reduction by these ‘other’ methods which are not biological can have advantages including no need to add anything to the groundwater for the reaction to occur. Additionally there is reduced interference with the other compounds in the groundwater maximising efficiency (Deganello *et al*., 2000). These advantages may be attractive to those suffering nitrate contamination of groundwater in the developing world because it simplifies the process, which in turn reduces the cost of remediation by not having to add anything prior, or remove anything after.

A key point of interest in these reduction methods is the products in the effluent. The catalytic reduction examined by Chen *et al.* (2003) found that ammonia built up in the effluent but was dependent on the hydrogen concentrations in the reactors. Electrolytic reduction studied by Prasad *et al*. (2005) found that the pH had an effect on the production of ammonia; that at neutral pH, less ammonia was formed. These two findings are in agreement with each other that the control of hydrogen is important to control the products post-reduction. Liu *et al.* (2012) also noted the production of ammonia through the hydrogen reduction method studied.

4.3. Examining for bias

4.3.1. Comparisons in quality of studies

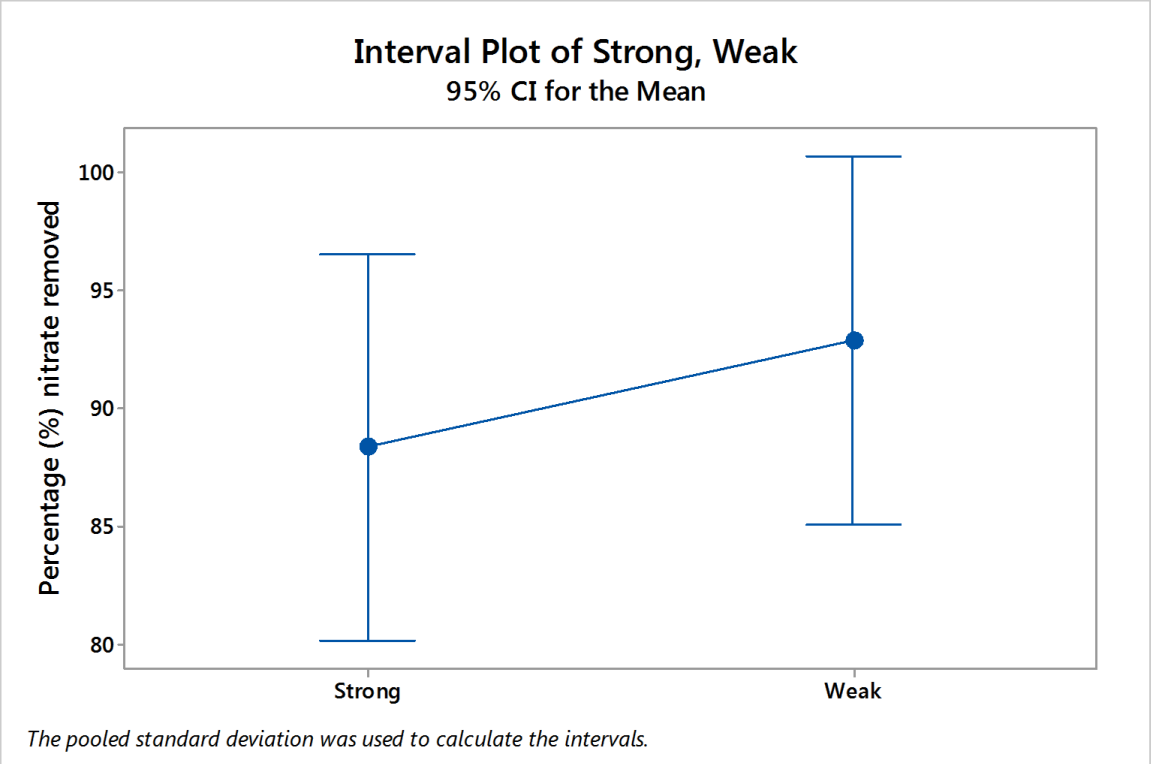

Figure 9: Significance between strong and weak studies

The comparison between the efficiency of strong and weak quality studies is seen in Figure 9. The P-Value between these two variables is 0.412 resulting in the accepting of the null hypothesis; there is no significant difference between strong and weak ranked studies. This could be because the efficiency may not be changed by the inclusion or not of objectives, method of analysis, comparisons or controls, or the outcome. This comparison is to check for homogeneity, not to look for differences. Results are required to be homogenous to minimise bias, and improve reliability and precision of results.

It is necessary to discuss the possibilities of bias within this study, and within the included studies to ensure results are reliable, and do not show evidence that is more appealing to satisfy the primary or secondary questions. The validity of all included studies is assessed by the outlined criteria, ensuring the inclusion of required quality categories. This is to ensure homogenous quality of results included, and only those ranked very weak are excluded. Five studies that are included from screening were unsuitable for extraction due to them being ranked very weak because they do not include an outcome concentration of nitrate, so cannot be compared to the other studies.

All of the included studies are peer reviewed articles to minimise likelihood of publication bias of the own study results. This is also the purpose of the selection criteria for the inclusion of studies, to minimise bias within this review. However, the limits of this study could be seen as bias. The time constraints of 2003-2013 were chosen to keep results relevant, but this could be argued that it is a long time in terms of development of a nation and so could be too long to provide relevant results. It could also be disputed that for developing countries, this is too short of a range to produce high

quality, reliable results. Whilst these arguments could be reasoned, the limits allow for further study outside of these limits.

The limit of language also biases studies published in English, which may have reduced the number of studies included from the outlined developing countries. If results that were not English were included there would arguably be more results. Although, English leads the language of publication so most studies are likely to be published in English (Ferguson *et al*., 2011). The countries chosen to include studies, are a selection aiming to be representative of the developing world, and were elected from a given list and a literature search to minimise bias. However, as a result of the included studies only representing four developing countries, this could be argued to be biased towards these countries, especially China as this is where most of the studies included are located. But this risk of bias is minimised by following the limits outlined for study location. These limits are not measured for bias though, unlike the quality of the study, which is found to be consistent.

As a consequence of differences to the protocol, examples of bias are observed. Initially, four methods were outlined for inclusion however it appeared that this was too narrow, therefore the study expanded to include 'other' methods if the study also satisfied the other inclusion criteria. These became labelled 'others' so to still identify them separate to the included methods. However, even though this bias of including methods not originally outlined, Figure 9 shows the confidence interval for the group 'others', and it is overlaps the range of the average of all of the methods.

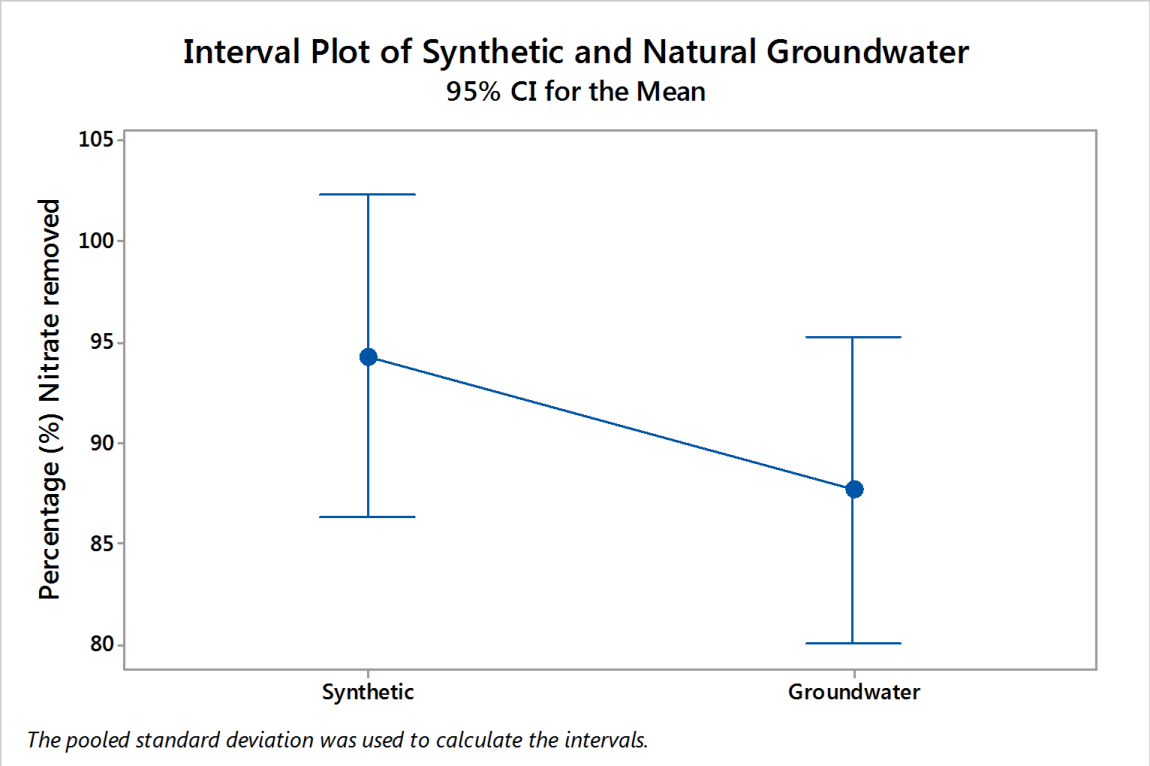

Figure 10: Significance between synthetic and natural groundwater

Comparisons between the results obtained from using synthetic groundwater or natural groundwater are shown in Figure 10. The P-Value for this comparison is 0.226 meaning the accepting of the null hypothesis as there is no significant difference. The interval plot shows a considerable overlap between the confidence intervals for both variables. Again, the purpose of comparing the significant differences was not to look for distances but to ensure consistency in results. Synthetic does show a

higher efficiency, but there is only a small difference between the efficiency of the two, and the mean of all methods (Table 5) is similar to both results for synthetic and groundwater.

Examining the difference between synthetic and genuine groundwater is also a measure of bias. Synthetic groundwater could be argued to lead to higher efficiency results, because the nitrate solution may have been augmented to provide better results. However, as all of the studies included are peer reviewed, this reduces the likelihood of this occurring.

4.3.2. Results by country

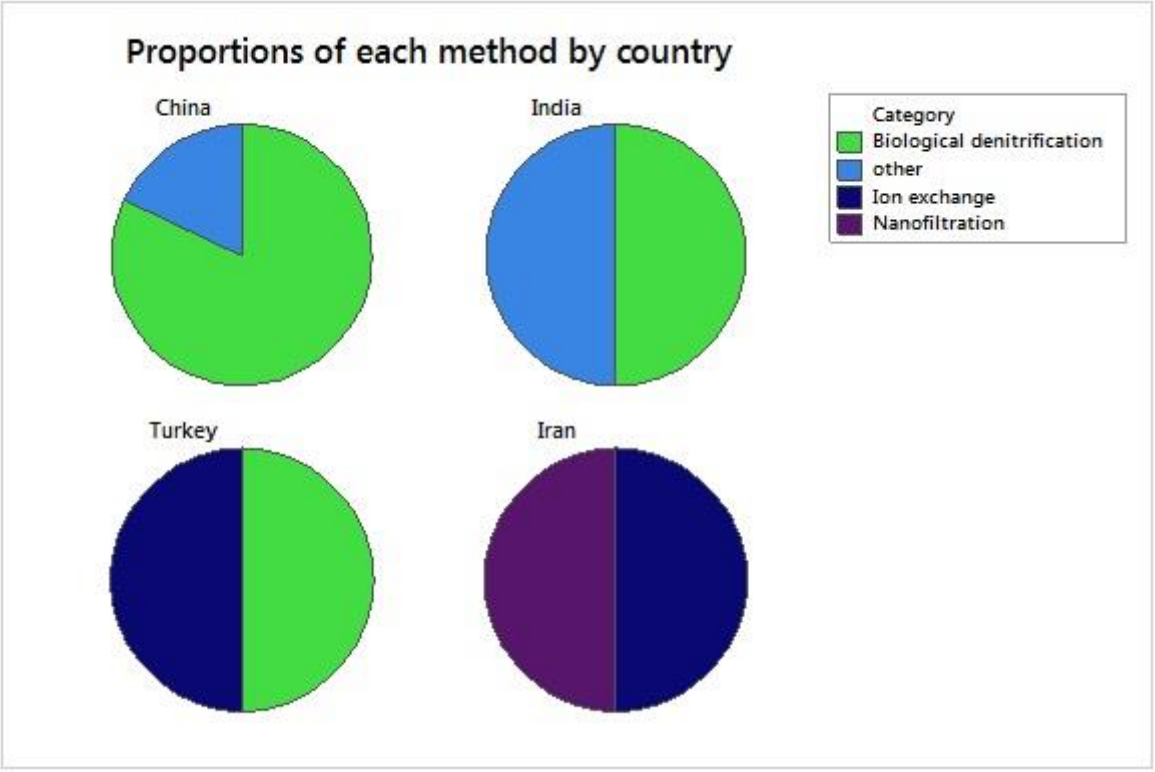

Figure 11: Proportion of each method in each country

Shown in Figure 11 is the proportion of each removal method for each of the included countries. It displays the large proportion of studies in China observing biological denitrification, and no studies examining ion exchange or nanofiltration in China. India, Turkey and Iran have a 50% divide in all of the methods studied with biological denitrification assessed in India and Turkey, and ion exchange only studied in Turkey and Iran.

Although all of the results for every country are compared against the WHO guideline, each country has its own guideline. However, using the country's own guideline could lead to bias in the results as guidelines are different and there are different numbers of results for each country. Additionally, this difference in country guidelines has different significance in different regions, as groundwater uses are different for different aquifers, even within a country. It should be noted that the guideline is for drinking water. If the groundwater resource is not used for drinking then nitrate pollution is not as much of an issue. Conversely, the guidelines provide a good baseline for quality, regardless of use of the groundwater.

Firstly, of all of the included countries, the majority are located in China, with 8 of the 11 in China investigated in Beijing. Beijing has a massive population with water shortages and surrounding

agricultural inputs of nitrate. Many of the studies in Beijing utilised sewage sludge for the source of anaerobic bacteria for biological denitrification. As Beijing has such a huge population the need for sewage treatment is large, but also the need for drinking water is great. This is a key area of interest for nitrate contamination as a result of the intense agriculture and rapid urbanisation led to increases in concentrations of nitrate in groundwater. With the rapid urbanisation, the population has grown vastly, increasing the need for drinking water, which is commonly abstracted from groundwater (Zhang *et al*., 1996).

It could be argued that this is not a representative sample of developing countries. The original list used by the World Bank included 130 countries, of which 18 were selected, spanning the globe to ensure coverage of a representative sample. As a result of the rigorous literature searching and screening stages, only these 4 countries are included. All of the countries are in Asia, which could argued to be bias. Whilst this is a small sample of the developing world, it still shows a selection of countries considered developing. Additionally, included are China and India, which have extremely large populations and have seen large increases in development. Furthermore, all of the studies included are within the other limits of language and date. To get a wider spread of results globally results outside of these other limits could be included, but this may lead to bias. This would be an example selection bias, by choosing results to fit country and disregarding other selection criteria.

4.4. Secondary outcome results – operating parameters

Not all included studies had evidence of other variables included within the study. The results are tabulated in Appendix 3**.** Those studies which did provide evidence will be discussed in the following sections.

4.4.1. pH

Table 7: pH findings across included studies

No.	Author and year	Method	Findings
6	Prasad *et al*., 2005	Electro-reduction	Highest efficiency at pH 2 and pH 8
9	Wang *et al.,* 2009	Biological denitrification	Denitrification effect excellent at neutral and alkalescent pH
12	Liu *et al.,* 2012	Hydrogen reduction	Higher nitrate reduction rate obtained at acidic over neutral conditions
15	Rajakumar *et al.,* 2008	Biological denitrification	The maximum of nitrate was reduced from 100 to 0.61mg/L (99.4%) in pH 7

Four studies examined the effect of pH on the removal of nitrate shown in Table 7. Studies 9, 12 and 15 state that higher nitrate reduction rate occurs at a neutral pH. Study 6 states pH 8 had the highest efficiency, which is in accordance with study 9 stating higher denitrification occurring at ‘alkalescent’ conditions.

Two of the methods using reduction noted the importance of controlling the pH because this is a significant limiting factor for product build up. The product prone to building up in these methods was ammonia which is a result of the hydrogen ions in the water (the pH) react with the nitrogen from the nitrate, producing ammonia. Consequently, the pH has to be controlled for these methods. This was also the case for the biological denitrification method studied in Rajakumar *et al.* (2008) which found that ammonia build up was a result of influent pH. When studying biological

denitrification, the pH of the groundwater can denature the enzyme that the bacteria use to reduce the nitrate.

4.4.2. Flow rate

Table 8: Flow rate in included studies

No.	Author and year	Method	Findings
2	Mahvi *et al.,* 2011	Nanofiltration	Flow rate 0.4mg/L = 75.7% nitrate removal 0.8mg/L = 69.3%
14	Hekmatzadeh *et al*., 2012	Ion exchange	The breakthrough time generally occurred faster with higher flow rates

The flow rate results are shown in Table 8. Only 2 of the 19 studies made reference to flow rate but there are contrasting results between these two. Study 2 found that nitrate removal efficiency was lower at a higher flow rate, whilst study 14 found that a higher flow rate led to faster removal time. However, these two studies are comparing different removal methods so cannot be meaningfully compared. Additionally, study 2 is referring to efficiency of removal whereas study 14 is making reference to reaction time.

The results found for flow rate across these included studies are contradictory, but there are only two studies that made reference to flow rate, this is not enough to draw conclusions. Examining the effect of flow rate across different methods could be an option for further study.

4.4.3. Temperature

Table 9: Temperature across included studies

No.	Author and year	Method	Findings
1	Huang *et al.*, 2012	Biological denitrification	Denitrification rate at 27.5°C was 1.36 times higher than at 15°C
3	Aslan and Turkman, 2005	Biological denitrification	Almost complete removal of 100mg/L nitrate at 31°C
15	Rajakumar *et al.,* 2008	Biological denitrification	At 30°C, about 90% of reduction was noticed at 24h and attained 99.4% at 48h
17	Wang and Wang, 2012	Biological denitrification	100% efficiency at 25°C reduced to 40% efficiency at 12°C

Temperature of the nitrate removal reactions in included studies is shown in Table 9**.** A range of temperatures between 25°C and 31°C have found to yield best denitrification results. Study 1 and 17 compared a higher temperature to a lower temperature and results are in agreement that the higher temperature leads to a higher denitrification rate and efficiency. All of these methods are biological denitrification.

The enzymes used by the bacteria to catalyse the nitrate reduction process can be temperature dependent, resulting in the possibility of denaturing the active site of the enzyme. The results found in this study, agree with results in literature not included for this study. Temperature increases lead to higher removal rates of nitrate nitrogen (Amatya *et al*., 2009). Through the stepwise reduction of nitrate, the enzymes reducing the nitrate are not as adversely affected by temperature as those reducing the nitrite (Huang *et al*., 2012).

The optimum temperature range identified in this study, 25-31°C, is similar to the optimum temperature range reported Karanasios *et al*. (2010) of 25-35°C. Denitrification has been noted at temperatures below 10°C and higher than 35°C, but at these temperatures the rate of denitrification may not be as fast as it could be (Karanasios *et al.,* 2010).

It is important to understand the impact of temperature on denitrifying rates because climate and temperature of the groundwater resource cannot be easily controlled, and so becomes a rate limiting factor for biological denitrification. Additionally, the groundwater temperature averagely is lower than the optimum temperature for denitrifying bacteria (Huang *et al*., 2012). This is an important consideration for developing countries looking to use biological denitrification as a process to remove nitrate from groundwater because additional spending and energy would be required to heat water, within the reactor used for biological denitrification, to optimum temperatures.

4.4.4. Initial concentration

Table 10: Initial concentration of nitrate in included studies

No.	Author and year	Method	Findings
5	Ayyasamy *et al.,* 2009	Aquatic plants	This suggests the optimum initial nitrate concentration in the medium was 300mg/L
11	Chen *et al.,* 2003	Catalytic reduction	Higher initial concentration linear relationship with higher removal rate
12	Liu *et al.,* 2012	Hydrogen Reduction	Nitrate removal rate promotes with increasing nitrate concentration
14	Hekmatzadeh *et al*., 2012	Ion exchange	That initial nitrate concentration has a negligible effect on the total adsorption capacity

Four different methods were found to include report of the effect of initial concentration on the removal of nitrate from groundwater. Three of the four studies found that a higher initial starting concentration of nitrate increases the removal rate of nitrate. Study 14 found that there is not much of an effect of initial nitrate concentration. However, because there are four different methods examined in Table 10, initial concentration may impact the efficiency differently for each method.

A higher nitrate concentration was found to lead to higher likelihood of removal in Liu *et al.* (2012) as there are more chances of a reaction to remove the nitrate because there are more ions in the groundwater. However, as a result of many different sources of nitrate pollution, the initial concentration of groundwater nitrate may be difficult to control. The effect of initial concentration is easier to measure in those studies which used synthetic groundwater, as the concentration was augmented.

4.4.5. Time

Table 11: Included studies examining time

No.	Author and year	Method	Findings
12	Liu *et al.,* 2012	Hydrogen Reduction	Nitrate removal rate increases with increasing reaction time
17	Wang and Wang, 2012	Biological denitrification	Removal efficiency increased gradually with running time

Table 11 presents two studies that examined time. They both are in concordance with each other that increasing the time of the process, increases the efficiency. It would be expected that with a longer reaction time, more nitrate can be removed or reduced. However, to be an economically sustainable method for developing countries, the time taken to remove nitrates should be fairly short. If the method is left running for a longer period of time it may lead to increased operating costs.

All of these operating parameters examined can affect the efficiency, the rate of denitrification and the products formed. Ammonia production needs to be controlled because of the potential harm if may cause if it remains in the effluent for drinking water. The pH and the temperature are significant operating parameters that control the ammonia product build up, across many methods. The results found in this review show that time and initial concentration can increase the efficiency, which would be expected as the higher concentration of nitrate ions and the more time provided for the method to remove/reduce ions, the likelihood increases that more ions will be removed. These factors are important considerations, especially in the context of developing countries, as they can lead to economic and environmental implications if not controlled when removing nitrates from groundwater.

4.5. Excluded studies and missing data

There are 5 studies that were included after screening that have not had data extracted for final inclusion in this review, a brief overview of these can be seen in Table 12.

Table 12: Studies not included for data extraction

Author and year	Method	Location	Reason for exclusion
Zhang *et al*., 2006	Biological denitrification	China	No information given on concentration of nitrate in groundwater
Zhou *et al*., 2007	Biological denitrification	China	No starting concentration of nitrate given, just the percentage removed
Lacasa *et al.,* 2011	Electrocoagulation	Spain	Not in a listed developing country
Sierra-Alvarez *et al.,* 2007	Biological denitrification	Not given	Unknown location for study
Lin *et al*., 2008	Biological denitrification	Taiwan	No beginning concentration of nitrate given

The information in the table about studies not included for extraction shows that most of the methods for nitrate removal are biological denitrification. Had these results been included, the spread of methods would further distort in favour of biological denitrification. The main reason for not including the data from these studies is that there was not full information given about the concentration of nitrate in groundwater. The exclusion of these results is evidence of selection bias, as it was actively chosen to exclude these studies from extraction. However, they are excluded because they do not satisfy the inclusion criteria set out in the protocol.

The second reason for not having data extracted is the location of the study. One of the studies was located in Spain, which was not one of the listed developing countries. The other study that is not included as a result of location did not state the location so it does not satisfy the criteria for developing country.

Within some of the included studies, some has missing data. Some did not state a final

concentration of nitrate after the use of the removal method. They instead, stated the percentage removed. From this the remaining concentration of nitrate has been calculated, but this is unreliable and may be a cause of bias. This could be potential to further this study to exclude this results that did not state explicitly the final concentration.

5. Differences between protocol and review

The protocol is available in Appendix 5.

One of the main differences between the protocol and the review is the difference between the secondary question set out in the protocol and the secondary questions included in this review. In the protocol, the secondary question aimed to look at what criteria it is that make these methods effective. Whilst this is not hugely different from the secondary question within this study, there were two secondary questions within the review. The questions aim to answer what the effects of the other variables included in these studies are and look for differences in effectiveness between methods. It was changed because the scoping exercise undertaken in the protocol was not wide enough to fully appreciate the depth of material available. The original secondary question did not account for the specific criteria that determined effectiveness.

The other main variances are the differences in searching; included methods and limits.

Originally, the protocol laid out four methods for review but upon undertaking the full literature search, it was found that there were more than four methods that should be included. They are included because they are also current methods. The four methods laid out were initially chosen to ensure appropriate results but the other search limits restricted the searches too much, and so the other methods needed including.

As a result of the broad search limits in the protocol, the limits had to be restricted. The scoping exercise outlined in the protocol used the limits 1980 to 2013, which was narrowed to 2003 to 2013. This change occurred as a result of when starting the final literature searches using the limit beginning from 1980 leading to large numbers of irrelevant results.

Again, these differences have occurred as a result of the scoping exercise being too broad and not going deep enough into the available literature. This has also become a limitation of this study. Background knowledge on the topic is required to effectively search literature for relevant results.

6. Conclusion

The following conclusions can be drawn from this review:

- The results obtained from all methods, can be said to be efficient as they are significantly different from the WHO guideline
- Bias of included studies, and within the review cannot be avoided
- pH, temperature, flow rate, initial concentration and time are other variables that can be rate limiting, effect the efficiency and alter the products formed
- There are not enough results to cover the developing world
- The quality of the results was homogeneous

Examples of bias were witnessed, but they were minimised to reduce alterations to results. The most frequent example of bias is the differences to the protocol, with the inclusion of other methods not outlined in the protocol being one of the most important differences. Many of the included studies also examined the other variables that can affect the efficiency of nitrate removal as they are

rate limiting factors. The biggest rate limiting factors identified across these studies are initial concentration and flow rate. Only four countries were included, which cannot be representative of the developing world. The small number of results is possibly a result of the search limits. However, the quality of the results included was found to be homogenous, but the quality of the results may not affect the efficiency of the nitrate removed.

Primary question: Are the current methods of remediation to reduce nitrate contamination in groundwater in the developing world effective?

The results obtained from the reviewed studies indicated that the methods used to remove nitrates from groundwater are effective, when compared to the WHO 50mg/L guideline when used as a baseline. However, this review has a limited scope and would need to be broadened to show a wider representation of the developing world. It would also need to be further broadened to include more methods to represent 'current methods'.

Secondary questions: What effects do other variables have on the removal of nitrate? Is one intervention greatly more effective than the others?

Both of the secondary questions have been discussed, and it was found that the other variables may affect the rate, efficiency and the by-products. No one intervention was found to be more effective than the others., but there weren't enough results to reasonably draw a conclusion.

6.1. Review limitations and further study

One of the limitations of this review is the appreciation of available material. To be able to effectively search for relevant literature, one has to have some prior knowledge to what is available. This is almost a circular process. A further limitation is the biases witnessed within this review. To reduce these biases, the review could be repeated with changes to search limits and selection criteria. Moreover, the use of secondary literature is a limitation. Not all of the studies were published with the same standards of quality. For example, some of the studies did not include their objectives and some made no comparisons to other studies. This is a limitation because it leads to variations in quality of results.

For further study, the other variables that affect rate, efficiency and by-products formed, could be examined to further depth. They could be the focus of the review, rather than the secondary question. Alternatively, China could be examined on its own instead of developing countries. Most of the literature used in this study examined China; the other limits could be expanded to include more results studying groundwater exclusively in China. Equally, the other limits could be broadened to include more results from the outlined developing countries to give more of a representation of the developing world.

To advance this study further, considerations to the three pillars of sustainability; economic, social and environmental, might be researched and become one of the secondary questions. This would be an important consideration for the developing world as there are increasing pressures to develop sustainably. Furthermore, research into populations explicitly effected by increasing nitrate concentrations in groundwater could be examined.

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