DEVELOPMENT OF EFFECTIVE SEQUENCE MULTI-BARRIER FOR NITRATE REMEDIATION IN GROUNDWATER SYSTEM: GEOCHEMICAL AND ANALYTICAL APPROACHES

MUNTAKA DAHIRU

FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
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MUNTAKA DAHIRU

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DEVELOPMENT OF EFFECTIVE SEQUENCE MULTI-BARRIER FOR NITRATE REMEDIATION IN GROUNDWATER SYSTEM: GEOCHEMICAL AND ANALYTICAL APPROACHES

ABSTRACT

Denitrification is a natural process of remediation, often exploited with modification to treat nitrate-polluted water in subsurface region. Carbon rich materials have been employed as electron donors in permeable reactive barriers (PRB), but their early depletion present serious challenge to the remediation technology. To find solution to these challenges, this study was conducted as a preliminary screening and selection of carbon sources for usage in simulated sequence multi barrier reactive media column. The study proposes and test a novel composition and effective packing structure of gate materials using wood chips, date seed, *Moringa oleifera*, sawdust, and paleo beach soil for denitrification. Our experimental findings lead to a series of trials in optimization for efficiency tests, which were followed by the addition of $CaCO_3$ to buffer medium and enhance the much-desired complete denitrification process. The results obtained from the field (Bachok) suggests multiple redox activity and simultaneous occurrences of heterotrophic and autotrophic denitrification at different soil strata. Evaluation of general variation pattern across the selected area in Kelantan revealed that the principle component analysis (PCA), hierarchical cluster analysis (HCA) and linear discriminant analysis (LDA) apportioned three parameters (SO_4^{2-}, Cl^- and conductivity) to the coastal sites and two parameters (Fe in the presence of NH_4^+ or Fe in the presence of NO_3^-) to in-land sites. The packing structure of the proposed sequence multi-barrier has dominant composition of date seed, wood chips, *Moringa oleifera* seed and activated carbon in the 1st, 2nd, 3rd and 4th compartments, respectively. The efficiency of the column setup was found to be dependent upon the ratio of the constituents, composition of the materials and the initial concentration of NO_3^- in the influent. The system shows removal efficiency of

96.4% and 98% of nitrite NO_2^- and NO_3^- and the attenuation of organic by-products (determined as total organic carbon) reached 53% within the first 46 cm height of the soil column. Surface characterization techniques of FESEM-EDX on the date seed and wood chips revealed a wider perforation in wood chips than in date seed, indicating preferential consumption of wood chips over date seed. Moreover, the results obtained from mesh bag technique revealed that the rate of decomposition within the date seed partitioning (endosperm and mannan-rich cell wall) and between date seed and wood chips differs with significant values, pointing to preferential consumption of one carbon source over the other and hence, fulfilling the need for delayed decomposition to ensure longer life span. It is also evident from the EDX that 30% reduction of carbon in wood chips is by far more than the 8.75% reduction of carbon in date seed for the same period. FTIR spectra revealed active redox conditions consisting of bond breakages/formation and addition and subtraction of functional groups that indicates more of oxidation process during decomposition. In summary, the process of decomposition entails multiple redox conditions at different site, depending on carbon of functional group, which is utilized by the bacteria.

Keywords: Multi barrier, Packing structure, Effective Carbon Source, Denitrification.

PEMBANGUNAN PELBAGAI-SEKATAN SECARA BERURUTAN YANG BERKESAN UNTUK REMIDIASI NITRAT DALAM SISTEM AIR BAWAH TANAH: PENDEKATAN GEOKIMIA DAN ANALISIS

ABSTRAK

Denitrifikasi adalah proses semulajadi pemulihan, sering dieksploitasi dengan pengubahsuaian untuk merawat air tercemar nitrat di rantau bawah permukaan. Bahan-bahan kaya karbon telah digunakan sebagai penderma elektron dalam halangan reaktif telap (PRB), tetapi pengurangan awal mereka kini memberi cabaran serius kepada teknologi remediasi. Untuk mencari penyelesaian kepada cabaran-cabaran ini, kajian ini dijalankan sebagai pemeriksaan awal dan pemilihan sumber karbon untuk kegunaan dalam simulasi media lajur pelbagai-sekatan berurutan yang reaktif. Kajian ini mencadangkan dan menguji komposisi novel dan struktur pembungkusan berkesan bahan pintu menggunakan serpihan kayu, biji kurma, biji *Moringa oleifera*, habuk papan, dan tanah pantai paleo untuk denitrifikasi. Penemuan percubaan kami membawa kepada satu siri ujian dalam pencapaian optimum untuk ujian kecekapan, yang diikuti dengan penambahan $CaCO_3$ untuk medium penampan dan meningkatkan proses denitrifikasi yang selengkap mungkin. Hasil yang diperolehi dari lapangan (Bachok) menunjukkan banyak aktiviti redoks dan kejadian serentak denitrifikasi heterotropik dan autotropik pada strata tanah yang berlainan. Penilaian pola variasi umum di seluruh kawasan terpilih di Kelantan mendedahkan bahawa analisis komponen prinsip (PCA), analisis kluster hierarki (HCA) dan analisis diskriminasi linear (LDA) membahagikan tiga parameter (SO_4^{2-}, Cl^-dan kekonduksian) ke tapak pantai dan dua parameter (besi (Fe) di hadapan dan NH_4^+ atau Fe di hadapan NO_3^-) ke tapak tanah. Struktur pembungkusan pelbagai-sekatan yang dicadangkan mempunyai komposisi dominan biji kurma, serpihan kayu, biji *Moringa oleifera* dan karbon diaktifkan pada petak 1, 2, 3 dan 4. Kecekapan persediaan lajur didapati bergantung kepada nisbah komponen, komposisi bahan dan kepekatan awal nitrat (NO_3^-) dalam influen. Sistem ini menunjukkan kecekapan penyingkiran 96.4% dan 98% bagi nitrit (NO_2^-) dan NO_3^-, dan pengecilan produk sampingan organik (ditentukan sebagai jumlah karbon organik) mencapai 53% dalam ketinggian pertama 46 cm tanah. Teknik pencirian permukaan FESEM-EDX pada biji kurma dan serpihan kayu mendedahkan rongga yang lebih luas dalam serpihan kayu daripada biji kurma, yang menunjukkan keutamaan penggunaan serpihan kayu berbanding biji kurma. Selain itu, hasil yang diperoleh daripada teknik "mesh bag" mendedahkan bahawa kadar penguraian dalam pembahagian biji kurma (endosperm dan dinding sel kaya dengan mannan) dan di antara biji kurma dan serpihan kayu berbeza secra signifikan, menunjuk kepada penggunaan keutamaan satu sumber karbon yang lain dan dengan itu, memenuhi keperluan untuk penguraian yang tertunda untuk memastikan jangka hayat yang lebih lama. Juga terbukti dari EDX bahawa pengurangan karbon 30% dalam serpihan kayu adalah jauh lebih tinggi daripada pengurangan karbon dalam biji kurma iaitu 8.75% bagi tempoh yang sama. Spektrum FTIR mendedahkan keadaan redoks aktif yang terdiri daripada pemutusan /pembentukan ikatan dan penambahan dan penyingkiran kumpulan berfungsi yang menunjukkan lebih banyak proses pengoksidaan semasa penguraian. Ringkasnya, proses penguraian melibatkan beberapa keadaan redoks di tapak yang berlainan, bergantung kepada kumpulan karbon yang berfungsi, yang digunakan oleh bakteria.

Kata kunci: Pelbagai-sekatan, Struktur pembungkusan, Sumber Karbon Berkesan, Denitrifikasi.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS AND ABBREVIATIONS

Symbol : Acronym

θ : Apex angle (Theta)

(1-A) : Recalcitrant portion of degradable substance

A : Labile portion of degradable substance

b : PRB thickness

K : Hydraulic gradient

$N_{1/2}$: number of half-life required

PRB : Permeable reactive barrier

SF : Safety factor

$t\_(res)$: Residence time

v : Velocity of groundwater

X : Proportion of degrading substance at a given time

X_o : Proportion of degrading substance at time zero (0)

Zi : Saturated thickness of the gate

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Study background

Anthropogenic impact on environmental deterioration is no longer a subject of debate as the evidences are conspicuously clear on daily basis. Environmental pollution brought about as a result of construction of dams, industries, research centers (Clarke & Barlow, 2003), application of fertilizers and by-products of organic compounds in farms as well as discharge of septic systems (Widory *et al.*, 2004) are but few examples. Air and soil pollution end up in surface water or groundwater pollution through rain/ sedimentation or seepage/percolation. However, not all water pollution ends up in air or soil pollution. To this end, water is one of the worst affected bodies that receives agents of pollution.

Being a precious commodity that supports all forms of life, water covers 70% of the earth surface, forming mounting of glaciers in the northern and southern hemisphere. Despite this overwhelming abundance however, percentage of clean water for human consumption is a fraction of the total amount of water and is getting depleted at unprecedented rate. It has been reported that human activities are depleting groundwater faster than the natural law of conservation can replenish (Rodell *et al.*, 2009). The human per capita consumption is increasing geometrically as it was recommended two decades ago that, the basic water need of human being should be 50 L per day (Gleick, 1996).

With the conspicuous pollution of surface water, humanity is fast mining groundwater for consumption. However, the threat to quality of groundwater forces humans to be critical of its utilization as its quality is often taken for granted. This is because, the knowledge of natural remediation of water through physical and biological means in the soil is so profound that even the scientists acknowledged the safety of drinking groundwater. The physical, biological and chemical attributes of soil is very significant as it makes the process of adsorption, filtration and bioremediation (biological) possible.

Amongst these, the biological process is the most important of all, as ranges of organic and inorganic substrate have been reported to be remediated *in-situ* using the biological process. Examples of these products includes remediation of organic matter (Thuriès *et al.*, 2001), chlorinated hydrocarbon (Ginn *et al.*, 2002), aromatic hydrocarbon (Kulkarni & Chaudhari, 2007; Samanta *et al.*, 2002), petroleum products (Mishra *et al.*, 2001) and nitrate (Murgulet & Tick, 2013b; Robertson *et al.*, 2000).

1.1.1 Nitrate

Nitrate is one of the major nutrients of concern in groundwater pollution as it poses a health risk of a condition known as methemoglobinemia (Fukada *et al.*, 2004; Jamaludin *et al.*, 2013). Researches indicates that, despite the multiple sources of nitrate input in groundwater, the heavy use of nitrogenous fertilizers in agriculture activities is the largest contributor of nitrate loading (Ruslan *et al*, 2004; Mahvi *et al.*, 2005; Tirado, 2007; Robertson, 2008; Suthar *et al.*, 2009; Zawawi *et al.*, 2010; Pu ***et al*.,** 2014). Report in Europe estimates the contribution of agriculture to nitrate loading in groundwater reach 55%. Depletion of nitrate is known as denitrification and the process is often associated with nitrification (formation of nitrate through oxidation), conversion of nitrate to nitrite (NO_2^-) and finally to N_2 gas (Equation 1.1).

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2 \qquad \textbf{(1.1)}$$

Therefore, denitrification is defined as a respiratory process in which bacteria use nitrates or nitrites as terminal electron acceptors, thereby reducing nitrates from contaminated water to nitrogen gas in the process (Karanasios *et al.*, 2010). This process is one of the many forms of natural remediation.

1.1.2 Remediation

Remediation efforts in contaminated sites is a comprehensive exercise that requires careful evaluation of the site and series of laboratory tests. The tests comprise of physical, chemical and biological analysis of either soil or groundwater, or both. Methods of analysis employed include tracer analysis, aquifer models and simulations. In-situ remediation techniques that are employed to treat groundwater include; pump and treat, sorption technique, injection and extraction wells, permeable reactive barrier (PRB) and permeable reactive interceptors. Amongst these techniques, the application of PRB receives wide acceptance due to its economic advantages. PRB are often employed to achieve a passive remediation goal, where less maintenance and monitoring is required. The bottom line in selecting any remediation alternative for attaining remediation goal lies on its ability to deliver effective remediation with less objectionable by-products and low cost of maintenance in the long term. In-situ remediation technology is a capital-intensive project that attracts attention around the world. This is because, the passive nature of the technology offset the initial burden of capital investment because, less monitoring is required. However, careful and thorough studies of the environment and reactive media is required before embarking onto the project. Since employment of PRB is site-dependent decision, the design of the structure is very important.

1.1.3 Design of remediating structure

The history of designing a remediating structure is marked with evolving conceptual modifications of the existing structure, in an operating remediation system. The modification is often born out of manifested defects of the structure, either at the laboratory scale or field application scenario. Developments evolves from pump and treat to reaction curtain to funnel and gate and then to permeable reactive interceptors. Several modifications such as trench and gate (Bowles, 1997) low capacity extraction-injection well (Hudak, 2014), non-pumped well (Hudak, 2016), injection wells (Piscopo *et al.*, 2016), vertical groundwater circulation well (Nyer & Palmer, 2006) and different packing structures that leads to parallel or series arrangement of the gates (Cath *et al.*, 2010; Streitelmeier *et al.*, 2001) were all made on the backbone of these structures. However, the problems of pollution swapping and mounding is still facing PRB technology. Pollution swapping is identified as the inadvertent release of a harmful product while remediating another product. It then becomes necessary to explore all avenues across scientific disciplines to resolve these two recalcitrant factors in the remediation technology.

One of the areas exploited for improving remediation strategy is geometric configuration of the remediating structure. Factors that are considered includes steady state flow (Kuusi *et al*,.2016), apex angle, orientation of the incident plume to the geometry of the structure, hydraulic gradient, ambient groundwater velocity, threshold capacity of the reactive media (half-life), rate of reaction, residence time in relation to the reaction rate (hence length of the gate), discharge rate and size of the gate.

In addition, amongst these are interdependent parameters that affects the degree of attenuation. While relative hydraulic conductivity is proportional to the rate of discharge through the gate, it is not directly proportional to reactive conductivities. This is due to the low surface area to mass ratio of larger grain, which causes low reactive conductivity (Starr & Cherry, 1994). It is therefore acceptable to assert that geometric configuration of a reactive barrier exerts significant influence on the success of its remediation capacity. Adjustments of some parameters (like hydraulic gradient and ambient groundwater flow) is difficult and, if achieved, they tend to offset the equilibrium status of the aquifer.

The design of PRB structure and packing order of the reactive materials has been receiving significant attention for three decades now. Two of the main structures employed in PRB are continuous wall, and funnel and gate structures (McGovern *et al.*, 2002; Obiri-Nyarko *et al.*, 2014). The mechanism of remediation is often considered at the design stage of the remediating structure. To this end, the most commonly employed mechanism-biological remediation-is extensively studied. This is because it has the advantages of utilizing the natural microorganism on site and the pollutants are degraded to minimum level. Many researchers worked on exploring the favorable conditions for the microbes during remediation. Hence, area for exploring good condition for biological remediation is extensively exploited.

Great deal of research and achievement has been performed and attained in the area of applying PRB for remediation purposes. However, the technology is facing several drawbacks, limiting its application for site-specific reasons. Site-specific parameters such as groundwater flow rate, mineral content, uniformity of packing structure (heterogeneity or homogeneity), pH variation pattern study (Zhen, 2010), dissolve oxygen content and availability of electron donors (such as organic carbon, Fe and Mn) are reported to have significant influence on the performance of reactive barrier (Hatzinger & Diebold, 2009). Most of these factors are governed by the type of remediation pathways employed. In biologically mediated remediation for instance, pH may be a strong factor or not, depending on the microorganism in the medium. Heterotrophs and autotrophs are known to influence the rate and pattern of any biological denitrification process.

1.1.3.1 Condition for Bioremediation

Like all forms of natural remediation, biological pathways exploit some existing conditions/ factors to remediate substances in a site. Conditions such as, the nature of the soil (Estavillo *et al.*, 2002; Parkin, 1990), the pH (Liu *et al.*, 2010; ŠImek & Cooper, 2002), the amount of oxygen and the presence of catalyst metals like as Cu (Rysgaard *et al.*, 2001) are identified. Other important factors include; presence of inorganic electron donors (such as Fe and Mn) amount and type of organic carbon (Webster & Goulding, 1989) and temperature (Huang *et al.*, 2015). The organic carbon source is of great importance because the rate of heterotrophic activity is determined by type and nature of the carbon source (Huang *et al.*, 2015). Amongst the carbon sources utilized with relative success are; saw dust, pine bark ethanol and cotton wool (Huang *et al*. 2015; Pan *et al*.,2013; Yang *et al*., 2012). On a general note, it has been established that, the easier the degradation rate of the carbon source is, the faster the denitrification rate (Martin *et al.*, 2009). However, one of the most challenging aspect in using carbon source is that they get depleted faster that the time required for remediation. Given a favorable condition for decomposition, carbon materials get easily decomposed and depleted, forcing replenishments and undermining the cost effectiveness of the remediation technology. This situation is often related to many factors upon which the soil type and its condition is one of the most important.

1.1.4 Soil impact on remediation

As mentioned above, the factors for biological remediation are directly linked with the type and condition of the soil and hence, considerable attention has been given to soil type and its content. As a matter of natural response of the eco-system to presence of foreign bodies (the pollutants), once pollution emerges, the development and adaptation of pollutant-degrading microorganism is ensured in the soil. That is why majority of field and laboratory researches on degradation rely on extracting the active microorganism from the soil of contaminated sites (Gibert *et al.*, 2008). This single factor gives the nature of the soil added advantage over most of the contributing factors. Hence, soil of the polluted sites is often utilized for any remediation analysis as the indigenous microorganism for the degradation of such pollutants are naturally developed within the soil.

In addition to chemical and biological requirements, denitrification potential of a soil in subsurface region is dependent upon secondary factors of which many, are physical parameters. The soil texture, the uniformity of the soil particle size, the pore volume of the soil medium, the packing structure and the extent of gradient are all related to total volume flowrate and groundwater flow. Ultimately, these parameters have direct impact on the residence time necessary for effective remediation. The bigger the particle size is, the higher the pore volume and the higher the groundwater flow, which shortens the residence time. Soil with homogenous distribution of particles has added advantage. To this end, riverbed soil has been utilized for laboratory aquifer studies and the result is encouraging (Jarvie *et al.*, 2005; McMahon & Böhlke, 1996; Pfenning & McMahon, 1997).

Related to the above soil property is the Paleo-sandy beach soil of coastal zones, which attracts research in many fields (Murgulet & Tick, 2013a). This represents the geochemical background of the medium within which the water is traversing through. Influence of sea on soil formation within the coastal zone has been documented across the globe (Behre, 2004; Enio *et al.*, 2011; Nyman *et al.*, 1993; Szczuciński, 2012)

However, research on nitrate attenuation carried in similar sites (coastal regions) with similar activities (farming) revealed different results, indicating the influence of hydro-geochemical matrix on the attenuation process. Nitrate concentration in riparian aquifer of North Carolina and coastal aquifer of Daweijia in China reached as high as 78 ppm and 521 ppm, respectively (Han *et al.*, 2015; Hunt *et al.*, 2004). Interestingly, nitrate concentration across the coastal aquifer of Kelantan in Malaysia is within the permissible limit (Fauziah *et al.*, 2014; Jamaludin *et al.*, 2013).

Across the continents, research on coastal plain are gaining attention because prediction of reasons for the occurrence of some environmental phenomenon helps in reconstructing historical models for evolutionary process. From the determination of residence time in pollutant transport (Han *et al.*, 2015) to simulation of sea water intrusion (Abd-Elhamid & Javadi, 2010) and contribution of isotopic tools for understanding groundwater flow (Edoulati *et al.*, 2013), different interpretation of the result leads to better understanding of the behavioral pattern of coastal aquifer systems. On one hand, the influence of sea water on the geological formation of the soil and hydro geochemical matrix due to transport of sediments by littoral drift and longshore current is evident in many literatures (Ishaq *et al.*, 2013; Kefu & Tegu, 2009; Koopmans, 1972; Raj *et al.*, 2007; Roslan *et al.*, 2010; Wang & Jiao, 2012). For instance, the discovery of pyrite bearing acid soil and reported interface of fresh and saltwater within coastal zones indicate inevitable influence of multiple factors on the quality of water and soil around sea (Roslan *et al.*, 2010; Samsudin *et al.*, 2008). This prompt multiple efforts of research in coastal regions around the world.

The coastal plain of northeastern part of peninsula Malaysia is an attractive area for research with a strategic geological significance (Heng *et al.*, 2006; Koopmans, 1972; Zakaria, 1975). The sedimentary settings of the plain was observed to have been largely influenced by transgressions (Wang & Jiao, 2012). Research conducted in the area includes performance of BRIS soil, sandy beach ridges, salinity mapping, geomorphology, water quality, hydro-geochemistry and hydro-geochemical studies (Hussin *et al.*, 2016; Ishaq *et al.*, 2013; Jamaludin *et al.*, 2013; Roslan *et al.*, 2010; Samsudin *et al.*, 2008; Sefie *et al.*, 2015; Zakaria, 1975).

A research conducted at Bachok agricultural rural areas reported that no violation of nitrate is detected in all the area (Jamaludin *et al.*, 2013). A year letter, Fauziah *et al.* (2014) reported same observation in a research on rural arears of Kelantan (where heavy agricultural activities are practiced). On a separate periodic exercise of screening groundwater through monitoring wells across the region, the Minerals and Geoscience Department of Malaysia (MGD) recorded a wide range of varying results that span across different geological matrix. The result indicated mild deviation of nitrate concentration from the maximum permissible limit in the area.

Despite the above-mentioned efforts however, it is observed that most of the research targets pattern recognition, source identification and geochemical evolution. Little attention is given to the questionable, but advantageous denitrification pattern in the area. Just as the study of the hydrological character of contaminated regions is necessary for the determination of source of contamination, so it is for the source of depletion. More so, it is observed that, one important but often-omitted factor in many in-situ attenuation-pattern research is the synergic contribution of hydro-geochemical formation on soil composition as well as soil formation to the attenuation process. It was established that delta formation in the area is affected by marine influence (Koopmans, 1972; Sefie *et al.*, 2015). More so, recent work have attributed high denitrification potential of a site to its proximity to redox line with high concentration of iron-embedded mineral (FeS in form of pyrite) and Mn (Roy *et al.*, 2017). In coastal plain of Kelantan however, despite the observed excellent attenuation capacity of NO_3^- in the area, no research work is reported on the dynamic parameters that might influence the observed sustainable attenuation capacity of the soil.

The above findings leave scientific community with challenges for finding the fate of the concentrated nitrate used in fertilizers for agricultural purposes. This is important because within the area, it is reported that Kelantan derives 35% of its water supply (100,000 m^3 day $^{-1}$) from groundwater (Idrisu *et al.*, (2014) whereas Terengganu (16,000 m^3 day^{-1}) and Perlis (6,000 m^3 day $^{-1}$) obtain substantial part of their water from ground too (Jamaluddin *et al*., 2013). While efforts are being made to curtail pollution effect and remediate water for domestic use at multibillion-dollar scale projects, natural form of remediation have been observed and reported with tremendous success. This serves as the motivating impetus for conducting the current research.

1.2 Statement of the problem

Heterotrophic activity is observed to be the most dominant process in denitrification as it has a higher rate of nitrate depletion and serve as a prelude for supporting other forms of denitrification by depleting oxygen. Because in-situ remediation requires excavation and placement of reactive media within a PRB in subsurface, the option is capital intensive. However, one limiting factor of heterotrophic denitrification is the rapid decline of the carbon source in gate material (Schipper *et al.*, 2010). This set back often forces the excavation of the structure for maintenance and hence, renders the system less cost effective. In relation to this, recent study indicates the need of conducting further studies that will ensure consistent, slow and stable release of carbon from cellulose-based solid substances (Huang *et al*., 2015). Subsequently, a similar paradigm shift of strategy is taken in remediating chlorocarbon compounds where control release materials are being studied (O'Connor *et al.*, 2018). Hence, safety features and long-term performance are desirable qualities of any PRB. In addition, pollution swapping often surfaces where the by-products or intermediary products from the decomposed reactive media generates other pollutants of concern. These problems have been featuring as recalcitrant factors defying various solutions and preventing the smooth application of the technology.

1.2.1 The current study approaches.

In an effort to simulate nitrate attenuation pattern and exploit it for industrial application, the present study explores three stage approach in which field evaluation, carbon source screening and application of soil (from the field) and carbon source is tested in a column experiment. The laboratory column experiment is designed to fit into the gates of a novel twin-funnel and gate structure to address the problem of early depletion of reactive media (longevity) and pollution swapping. Unlike most research in the field, where the role of soil is considered as simply supportive material for the remediation, this research pays special attention to the type and nature of the soil. Consequently, the soil is sand witched with the reactive media in the column to exploit its dual capacity of biological (autotrophic) and physical (filtration/adsorption) role in delivering effective remediation. For fast and steady supply of heterotrophic sources of electron, selected organic/inorganic substrates (date seed, *Moringa oleifera* seed and limestone) are applied at different position and in different proportion, representing sequence multi-barrier reactive media. The date seed was selected because of its concentrated carbon mass per weight (Streitelmeier *et al.*, 2001) and availability for useful application, rather than the environmental nuisance it represents now due to its resistivity to decomposition. *Moringa oleifera* was considered because of its anticoagulant properties dispenser (Ghebremichael *et al.*, 2005; Ndabigengesere & Narasiah, 1998). The methodology flow chart of the research is shown in Figure 1.

1.3 Research objectives

The objectives of this research are as follows

1. To investigate the factors responsible for the high degree attenuation of nitrate using Geochemical analysis in the area under study.

2. To identify and screen suitable carbon-based electron donors for utilization in denitrification process.
3. To develop effective and environmentally friendly gate composition and packing structure for nitrate remediation in a sequence multi-barrier (reactive media) system while achieving delayed remediation for attaining longer life span of the system.

This research is highly significant in that just a decade ago, the concept of sequential treatment using multi barrier was at the laboratory concept stage with no industrial scale transfer (Bike *et al*., 2007) . Therefore, research in the area is active with much needed input for industrial application.

1.4 Thesis design

This section gives a brief overview of the whole thesis, which is divided into five chapters:

Chapter one - This section gives a brief overview of the entire thesis in accordance with the sequence of conventional style. It presents a brief introduction of water and its significance, pollution and remediation strategies as well as the factors responsible for enhancing remediation. Significance of design in remediation alternative is highlighted and current problems facing the technology are enumerated. Consequently, inadequacies of gate materials and significance of packing structure are highlighted. The chapter also introduces the background of the observed denitrification phenomenon on a specific coastal site, which serves as the motivating factor for the present study. Finally, the chapter highlights the main objectives of the research.

Chapter two deals with the general literature review that are related to the objectives of this research. Literatures of previous and current research related to materials used for in-situ remediation, different types of remediation processes, influence of sea on the characteristics of paleo-sandy beach soils and configuration of gates in permeable reactive barriers are highlighted.

Chapter three outlines the account of methodology employed for screening, sampling, preparation and analysis of soil, water, carbon sources for denitrification and hypothetical design of packing structure in funnel and gate mode. The chapter also outlines the design and construction of the aquifer simulated column setup in line with packing structure of the gate. More so, the fundamental principles of the analytical techniques used and that of the characterization of fresh and used carbon sources are explained. The links between the chapters is reflected in the methodology chart, which indicate how each stage of the analysis is related to another as shown in Figure 1.1.

Chapter four Presents detailed explanation of the results obtained during the screening exercise of the carbon sources, geochemical analysis of water and soil samples, aquifer simulation using column analysis as well as the characterization of the materials used in the column.

Chapter five provides major synopsis of various findings and the conclusions of the findings followed by recommendation for future studies.

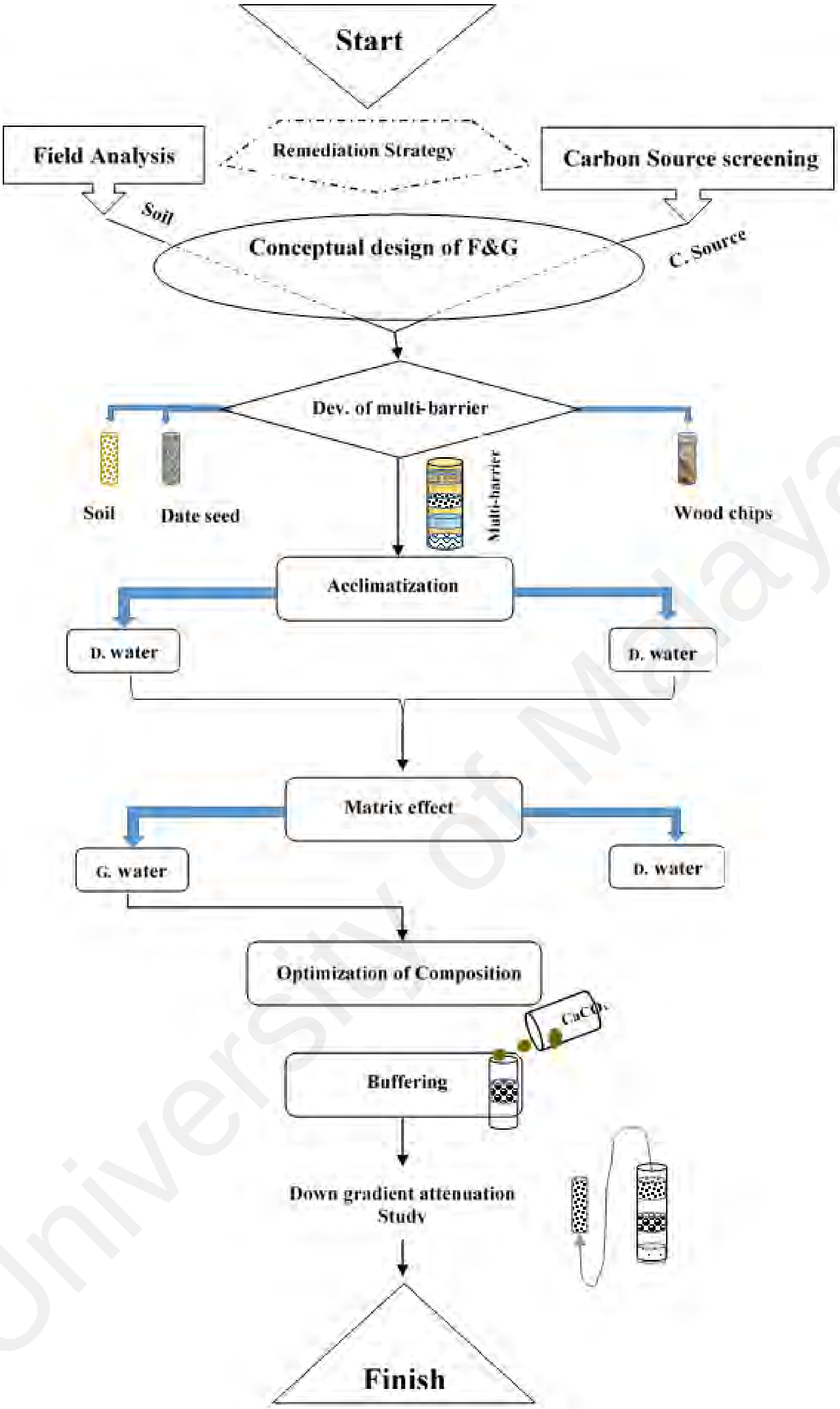

Figure 1.1: Methodology Flowchart

CHAPTER 2: LITERATURE REVIEW

This chapter deals with the literature review of previous and current research related to materials used for in-situ remediation, different types of remediation processes, influence of sea on the characteristics of paleo-sandy beach soils, degradation of organic substrate as it relates to remediation and configuration of gates in permeable reactive barriers. At the end of this chapter, synopsis of the application of gate materials in column analysis is given.

2.1 General introduction

The need for an all-round groundwater remediation technology has been on the rise for decades, largely because of the discoveries of synergistic effect and pollution swapping in the subsurface region (Huang *et al.*, 2015). However, certain natural and anthropogenic factors contribute to groundwater pollution. Therefore, water remediation technology continues to attract tremendous attention.

Groundwater remediation techniques require careful study as efforts in remediating selected pollutants can introduce other pollutants (Healy *et al.*, 2012). Though the reactive media and treatment mechanism are not mutually exclusive (Choi *et al.*, 2013), the cost/ benefit is the determining factor for selecting one technique over the other (Ibrahim *et al.*, 2015). In this regard, remediation techniques that uses natural and/or biodegradable substrates prove more advantageous. The use of natural and biodegradable substances for remediation has been subjected to multiple scientific scrutiny. This is necessary to ascertain the validity of the mechanism and, most importantly, the safety of using such natural products. This is because, while some natural and biodegradable products have toxic substances in them, some generates them as a by-product of their decomposition. Organic substances have been used as electron donors in remediating nutrients like nitrate, while nitrate is being used as nutrients for decomposing petroleum products and color-substituted organic compound (aliphatic and aromatic acids etc).

Research in this area revealed that order of placement of the carbon source (organic matter) in relation to other remediating substances that might be present has a significant influence on the efficiency of the process and general remediation goals. For any given remediation system, the optimum remediation goal is to have a successful remediation of the unwanted substance to the minimum level and generate less harmful substances to the environment.

Several carbon source materials, including synthetic compounds such as methanol, glucose, acetate (Pan *et al*.,2013; Yang *et al*., 2012) and plant-based, such as Pine bark, saw dust and leaf compost (Huang *et al*. 2015; Schipper, 2000; Robertson *et al*., 2000) have been utilized with relative success of excellent remediation capacity. Other elemental sources of electrons used in autotrophic processes include sulfur (Sahinkaya *et al*., 2011), iron (Son *et al.*, 2006; Yu *et al.*, 2006) and Ion sulfide & pyrite (Kong *et al.*, 2015). In most of the sources mentioned above, a setback of either pollution swapping, or early decline of the electron donor is experienced or ignored. This call for the need to screen the reactive materials for ascertaining the presence of the target analytes (for remediation), competing radicals or objectionable by-products of decomposition (Yang *et al*., 2012).

Research revealed that exploitation of natural order of remediation is economically effective means of remediating polluted sites. Recently, Rudolph *et al.* (2015) advocates for best management practice in monitoring the attenuation to avoid any structure that may be removed in the feature. The use of riverbed sand has been identified as an effective water treatment component that is investigated to be using both physical and biological process of remediation (Pfenning & McMahon, 1997). Recent studies shows that apart from the uniform distribution of soil particles of riverbed soil (which gives it excellent filtration and adsorption capacity), the mineral and organic content of the soil is of significance in the remediation process (Jarvie *et al.*, 2005). To this end, exploration of paleo beach soil for the same purpose is worth trying.

Another influential factor is the soil nature due to its history of formation. Sea littoral drift and Holocene occurrence exerts sea influence on the pedogenic pyrite sediments formation in some coastal soils (Enio *et al.*, 2011; Ishaq *et al.*, 2013). The influence of sea water on the geological formation of the soil and hydro geochemical matrix is evident in many literatures (Feher *et al.*, 2018; Ishaq *et al.*, 2013; Kefu & Tegu, 2009; Koopmans, 1972; Raj *et al.*, 2007; Roslan *et al.*, 2010; Wang & Jiao, 2012). For instance, the discovery of pyrite bearing acid soil and reported interface of fresh and saltwater (up to 6 km) within coastal zones indicate inevitable influence of multiple factors on the quality of water and soil around the sea (Roslan *et al.*, 2010; Samsudin *et al.*, 2008). These unique properties of coastal plain attracts a number of researches with significant findings. The development raises questions on the role of mineral and organic-rich soil in natural remediation process around coastal zones. Beach soil has been associated with energetics (McLachlan *et al.*, 1981) and in recent time is being used as a source of green enrgy in bloom box emerging energy technology (KR, 2010). Big companies like google and ebay are currently patronising the technology. Just as research is ongoing on the usability of paleo beach soil for exploiting energetics prospect, so it is worth expoloring for exploiting remediation prospect, especially remediation of nitrate.

2.2 Nitrate and the environment

Nitrate is an oxidized form of nitrogen that is well distributed in many aqueous states. It is one of the most stable and reactive form of nitrogen element (WHO, 2003). Nitrogen is the most abundant element in the atmosphere (78% in form of molecular nitrogen N_2) recycling in a nitrogen fixation process on the earth surface. Its presence in animal tissue in form of amino acids further increases its distribution in land and groundwater, when the animals dies and decompose. One of the major decomposition products of animals as well as that of their west products (dungs, faeces etc.) is nitrate, which is soluble, colourless and tasteless anion in aqueous solutions. Anthropogenic input through fertilizer application and thinning systems bombards the environment with excess nitrate loading beyond the threshold of equilibrium concentration that would otherwise be remediated through natural process. Moreover, nitrification and nitrogen fixation add to the nitrogen supply (Levitus *et al.*, 1993).This is because while agriculture introduces inorganic form of nitrate, the plant residues and root system of thinned trees supplies a readily decomposable source of carbon and nitrogen for the soil microorganism (Thibodeau *et al.*, 2000).

The global distribution of nitrate in the subarctic and sub-antarctic waters is described to be increasing with depth and decreasing with distance (Levitus *et al.*, 1993). This implies that, if the sea water at the shore is highly polluted, the neighboring aquifers at the shorelines could be at the risk of pollution intrusion due to recharge activities. The heavy use of nitrogenous fertilizers in agriculture activities is observed to be the largest contribution of nitrate in groundwater (Korom, 1992; Mahvi *et al.*, 2005; Pu *et al.*, 2014; Robertson *et al.*, 2008; Ruslan Ismail, 2004; Suthar *et al.*, 2009; Tirado, 2007; Zawawi *et al.*, 2010) . In Europe, it is reported that agricultural input of nitrogen in groundwaters reaches 55% (Bouraoui & Grizzetti, 2014). For this reason, a close look at nitrogen circle is very important.

2.2.1 Nitrogen circle

Nitrogen in the atmosphere is fixed by a unicellular organism (Bacteria) known as prokaryotes, where they turn it to ammonia. Nitrogen is essential for our life and it features in many important macromolecules as primary nutrients in amino acids, chlorophyll, ATP and DNA. The nitrogen circle is completed when an organism dies (plant or animals), wherein bacteria decompose the organism and convert the amino acid to ammonia or to NO_3^-, NO_2^- and N_2 gas releasing the same nitrogen back into the atmosphere. For this reason, the study of nitrogen circle is very essential because, depending on the circumstances, the final and intermediate products in the process are interchangeable as shown in Figure 2.1

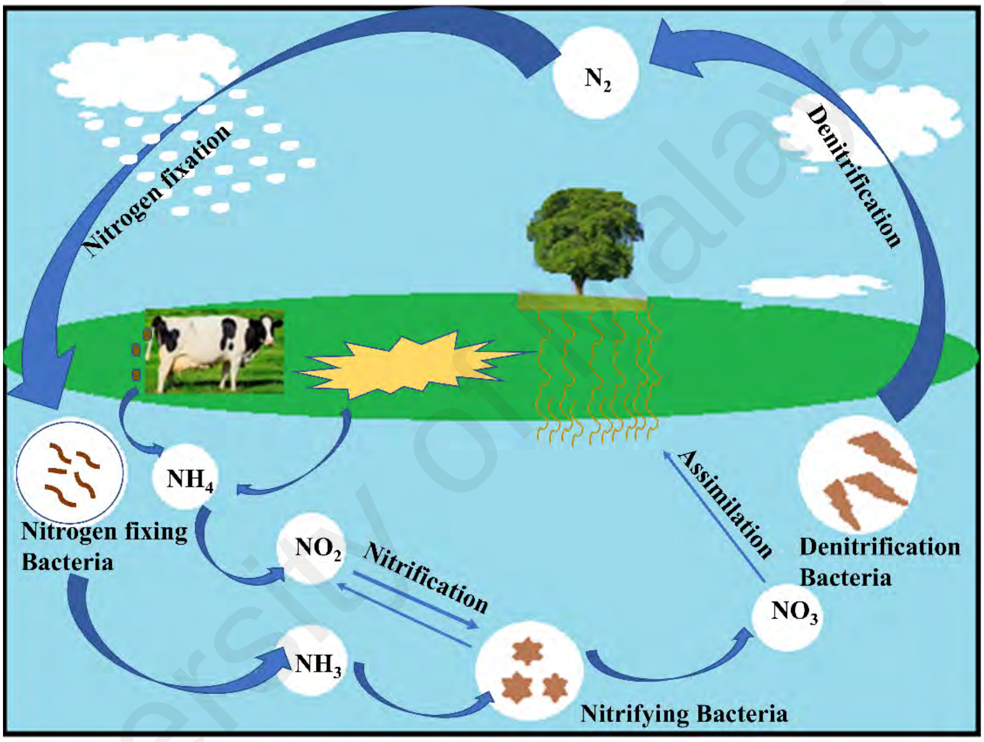

Figure 2.1: The nitrogen circle

2.2.2 Sources of nitrate

Nitrate is introduced into the environment through two major sources, the natural source and the anthropogenic source. The natural source is often exploited and amplified by human activities. The plant and animal west as well as their remains get decomposed to provide humus on top of soils and percolate to distribute soil fertility by supplying essential nutrients, nitrate inclusive. However, human activities pose threats to his life and upsets ecological balance. From household sewage to greenhouse production centres, fertilizers application to manure from intensive animal farming and utility building to animal feedlots, human input of nitrate in surface and groundwater is alarming. Human activities impacts on nitrate loading to the surface and groundwater with proposed position of barriers as suggested by Bednarek *et al.* (2014). This may be attributed to development of new technologies and exponential population outburst. Hence, anthropogenic sources are the most contributing factor to nitrate loading in the environment.

2.2.3 Effect of nitrate

The effect of excessive nitrate loading on human and environment is serious as it affects human health and offset the equilibrium in ecosystem. The effect of nitrate consumption manifest more in children, causing a medical condition known as methemoglobinemia (Wongsanit *et al.*, 2015). Methemoglobinemia is a state whereby an altered state of Haemoglobin, known as methaemoglobin is in excess in the blood. The altered state is brought about when ferrous ions (Fe^{2+}) of haem are oxidized to ferric state (Fe^{3+}) and hence, rendered unable to bind to oxygen (Ayebo *et al.*, 1997). Though, there has been arguments on the sole contribution of nitrate to causing methemoglobinemia, its synergic contribution with other predisposing factors has been established (Fewtrell, 2004).

The effect of excessive nitrate concentration on the environment is seen on surface water, where agricultural runoff carrying nitrate and phosphate residues (from the fertilizers) accumulates to encourage rapid growth of fungi in the water body. This process is called eutrophication. Eutrophication has a devastating effect on aquatic plants and animals as it blocks sunlight and oxygen from penetrating into the water, thereby causing death of aquatic plants and animals. Looking at the consequences of high nitrate concentration in the environment, it became necessary to device means for remediating polluted sites.

2.3 Remediation efforts

Several efforts have been made to mitigate anthropogenic groundwater pollution. The efforts become necessary because humanity is manning groundwater at an alarming rate. It has been estimated that more than two billion people depend directly on aquifers for drinking water (Thiruvenkatachari *et al.*, 2008). Therefore, water remediation technology continues to attract tremendous attention. The groundwater remediation techniques requires careful study as efforts in remediating selected pollutants can introduce other pollutants (Healy *et al.*, 2012). Though the reactive media and treatment mechanism are not mutually exclusive (Choi *et al.*, 2013), the cost/benefit margin of any remediation technique is the determining factor for selecting one technique over the other (Ibrahim *et al.*, 2015). In this regard, remediation techniques that uses natural and/or biodegradable substrates are more advantageous. Often, the system in which the remediation component is embedded within is called permeable reactive barrier (PRB). Therefore, permeable reactive barrier has been identified as the most widely utilized and fastest growing in-situ remediation technology(McMahon *et al.*, 1999; Xin *et al.*, 2013). The most important part of PRB is the reactive media- the permeable portion placed within the gate in which the water passes through. Consequent upon that, efforts in research on reactive media screening and selection are emphasized. This is often achieved through laboratory batch and column analysis, simulated to determine the pattern of reaction and the response of the material to real aquifer system.

2.3.1 Laboratory preliminary analysis

Typically, in-situ treatment system designs are obtained from the results of treatability or effectiveness studies involving both batch reaction tests and laboratory scale column experiments (Rael *et al.*, 1995) . In determining the suitability of any reactive material in subsurface remediation technique, it is necessary to thoroughly scrutinize the material's stability, reaction pattern and lifespan in real aquifer condition. The physical response to remediation pattern is often simulated after determining physical parameters such as pore volume, porosity and flow rate. Properties such as rate of reaction, adsorption-related parameters are measured using either batch analysis or column while retention time and rate of decomposition are measured using column analysis.

2.3.2 Batch analysis

As highlighted by McGovern *et al.* (2002) batch test is useful in obtaining measures of media relativities such as sorption pattern, degradation half-life, intrinsic chemical contents and capacity of the material. Different approaches are employed to achieve the above goals and longer half-life has been identified as one of the most desirable properties. This is because for cost effective technology, in-situ remediation is desired to stay underground with little or zero maintenance burden. The effort and investment put in for inserting a remediating structure underground would almost be replicated if the reactive media must be removed for treatment or exchange. Factors such as the physical geometry of the structure, steep gradients in pH, redox potentials and sharp permeability contrast has been reported to cause severe constraint on PRB longevity and mounding (Puls, 2006). For this reason, further test of aquifer simulation using column becomes essential.

2.3.3 Column analysis

Column study have been used by past and recent researchers to find out the effectiveness and response of proposed reactive material in a simulated aquifer condition. Remediation assessment of petroleum products, nitrate, chlorinated hydrocarbons, metals in acid sulfate terrain and assessment of long-term performance have been investigated with great deal of information (Gillham & O'Hannesin, 1994; Huang *et al.*, 2015).

Like every model, column analysis is run to mimic true aquifer situation except that most of it is conducted by packing reactive medium in a vertical column mixed with a defined ratio of aquifer material (soil). The contaminated water is then passed through the column adjusting the flow rate to simulate groundwater velocity. Depending on the packing structure and residence time, impact of the media on the quality of the water can be assessed. If the mechanism for treatment is precipitation or sorption, the life span of the media is limited by its ultimate capacity (McGovern *et al.*, 2002). If, however, the mechanism is biological, the life span of the media depends on its resistivity to fast microbial biodegradation (Yang *et al*., 2012).

2.3.4 Field application

After conducting the ex-situ analysis in laboratory, the next step is running a pilot test in the field before the final field application. A number of field applications of remediation efforts have been reported with varying ranges of success. Application of the technology for remediation on fields is a capital-intensive exercise that requires preparations and studies at different level. As indicated above, site characterization, study on the extent of lateral and transitional spread of the pollutants, interaction with the aquifer, advective dispersive characteristics of the plume are some of the essential factors for consideration. Amongst the successful field applications reported are : trench and gate system of oxidized lignite bio-filtration permeable reactive barrier for treatment of wide varieties of organic pollutants (Vesela *et al.*, 2006) and peat material as gate medium for treating aromatics using funnel and gate set-up (McGovern *et al.*, 2002).. The reactive media are often subjected to other tests such as characterization and durability study.

2.4 Surface characterization techniques

The use of surface characterization techniques to assess the nature of physical, biological or chemical reaction is a well-established scientific technique. The material under study (date seed and wood chip) has been exposed to FESEM-EDX and FTIR in a number of studies which include but not limited to; pyrolysis and combustion study of date seed (Sait *et al.*, 2012), adsorption isotherm and kinetic modelling of activated carbon derived from the date seed (Hameed *et al.*, 2009), chemical composition and lipid profiles of date seed (Besbes *et al.*, 2004) and study of chemical structure of soft and hard wood using FTIR (Pandey, 1999). Most of the analysis rallies around utilization of the material for adsorption studies (after activation) or finding the basic chemical composition of the materials. However, there has never been any report of utilizing date seed as carbon source for denitrification in PRB setting. Another area of intensive research on the target carbonaceous material is decomposition study.

2.5 Decomposition study

On account of ecological significance, majority of studies carried on wood materials are in decomposition and related topics. Research conducted in this area include those on moisture content of wood (Nyström & Dahlquist, 2004), modelling the decay of wood debris (Holeksa *et al.*, 2008), measurement of rate of decomposition of different woods (Coûteaux *et al.*, 1998; Karberg *et al.*, 2008) effects of nutrients addition on the net decomposition of litter (Li *et al.*, 2015) and changes of chemical composition of liters during decomposition (McTiernan *et al.*, 2003) . As for the utilization r carbon source in PRM there may not be a single most researched material than wood in the area. This is because the field is riffled with research on the suitability of wood substrate in PRB for heterotrophic denitrification. Publications highlighting the use of wood for PRB indicates many forms of wood such as wood chips (Christianson *et al.*, 2017; Fenton *et al.*, 2014; Greenan *et al.*, 2009; Phillips, 2009), saw dust (Della Rocca *et al.*, 2007; Robertson *et al.*, 2008; Schipper & Vojvodić-Vuković, 2001) and pine bark (Huang *et al.*, 2015). As it is the practice, wood materials are incorporated in the reactive media of PRB for remediation purposes. It is worth noting that the entire systems within which the structure is embedded is called permeable reactive barrier (PRB). Hence, it is important to review the concept of PRB.

2.6 Permeable reactive barrier (PRB)

PRB have been gaining attention because of their relative advantages in long term effect and mitigation of pollution swapping (Huang *et al.*, 2015) . Pollution swapping is seen in a situation where a precursor for remediating one pollutant or a by-product of the remediation turnout to be a source of other pollution (Ibrahim *et al.*, 2015). To this end, permeable reactive barrier have been identified as the most reigning and fastest growing in-situ remediation technology (McMahon *et al.*, 1999)

The term Permeable reactive barrier was first mentioned in 1985 (Guerin *et al.*, 2002) and is defined by Thiruvenkatachari *et al.* (2008) as "An emplacement of reactive media in the subsurface designed to intercept a contaminated plume, provide a flow path through the reactive media, and transform the contaminant(s) into environmentally acceptable forms to attain remediation concentration goals down-gradient of the barrier". The heart of any PRB set up is the reactive media -a distinct region placed within the PRB structure. In a funnel and get set up, the region of reactive media is referred to as the "gate". This is where all the mechanism for remediation takes place. PRB have been utilized for decades and are described as an effective remediation technology (Phillips, 2009), accepted, innovative, promising (Obiri-Nyarko *et al.*, 2014) and most successful in-situ remedial groundwater technology (Puls, 2006). This is because of their relative advantages, which includes; application for the treatment of wide variety of pollutants (Phillips, 2009), energy efficiency (Hudak, 2014), reduced operation and maintenance cost (Careghini *et* *al.*, 2013; McGovern *et al.*, 2002), reduced environmental impact (Higgins & Olson, 2009) and selective treatment ability (Liu *et al.*, 2013). Amongst the different approaches, such as chemical, biological and physical methods of remediation, biological method has been the most widely used (Lito *et al.*, 2012).

In exploiting biological pathways, the use of natural substrates in PRB gate for denitrification proves useful as bacteria in subsurface saturated zones obtains energy from both organic and inorganic sources. In addition, if the electron donor is a cellular carbon from organic origin, the process is called heterotrophic denitrification. If however, the electron donor is inorganic, as in Fe^{2+}, Mn^{2+} and sulfides, the process is called autotrophic. Examples of carbon sources used in field and laboratory scale are; methanol, glucose (Pan *et al*.,2013; Yang *et al*., 2012), soft wood, pine bark, sawdust and plant compose (Huang *et al*. 2015; Schipper, 2000; Robertson *et al*., 2000). In natural setting, both heterotrophic and autotrophic denitrification occurs simultaneously (Korom, 1992).

Biological denitrification is advantageous due to microbe-aided self-attenuating capacity and lower level of pollution swapping. Indigenous microorganisms are often utilized to break down the target pollutant while they feed on substrate within the environment for their metabolic process. Examples are bioremediation of halogenated compounds (Semprini *et al.*, 1992), petroleum compound (Gogoi *et al.*, 2003; Maila & Cloete, 2004) and nitrate (Della Rocca *et al.*, 2007; Liu *et al.*, 2014). The two most widely applicable in-situ configuration of remediation techniques for groundwater using permeable reactive barrier are ; continuous wall and funnel & gate design (Obiri-Nyarko *et al.*, 2014)

2.6.1 Funnel and gate

Funnel & gate is a physical structure designed to constrain plumes in-situ for remediation purposes. Hitherto 1995, it was deemed inapplicable to low conductivity sediments (Bowles *et al.*, 2000). This was widely viewed because of steep gradients in pH and redox potentials as well as sharp permeability contrast leading to serious constrain on PRB longevity and mounding (Puls, 2006). The mounding, defined as the overflow of remediating plume around, under or bypass the permeable reactive barrier was however, circumvented by the introduction of trenches along the line of the funnel (Bowles, 1997). Other related technology that are within the purview of permeable reactive barrier includes low capacity wells (Hudak, 2014), high resistant wells and permeable reactive interceptors (Huang *et al.*, 2015; Ibrahim *et al.*, 2015).

The definition of funnel and gate was given as, "the combination of cut off wall and in-situ reactor". Among the different kind of configuration given, consisting of V shaped, continuous (Thiruvenkatachari *et al.*, 2008), surrounded and rectangular geometry, the one with the widest possible angle (180^{o}) is envisaged to be the most advantageous (Starr & Cherry, 1994). This is because of the maximum angle it can cover to capture a wide dimension of the plume. Design and output of PRB are assessed based on how good they give a balance between capture zone and residence time. A typical example is seen in a work that introduced conceptual frame of equalization well. The most prominent geometric structure of funnel and gate in field studies are the V-shaped (Kacimov *et al.*, 2011) (Figure 2.2a.) and the flat-shaped (continuous wall)structures (Figure 2.2 b). North America prefer the use of continues wall while Europe prefer modified funnel and gate (Birke *et al.*, 2007a).

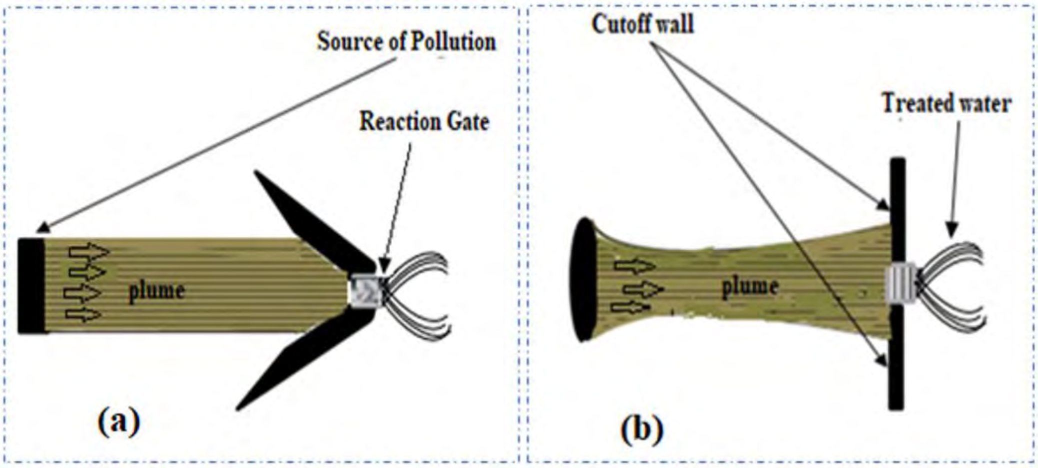

Figure 2.2: (a) V-shaped Funnel and gate **(b)** Flat-shaped funnel and gate

Lower cost of installation and maintenance are some of the advantages of funnel and gate over the reaction curtains (Higgins & Olson, 2009; Painter, 2004; Phillips, 2009; Thiruvenkatachari *et al.*, 2008). In contrast, some researchers accentuates on the required total length and operation time and hence, viewed funnel and gate setting as a less cost effective alternative than the reaction curtain PRB (Hudak, 2014). On the other hand, the arguments of North Americans on preferring continuous wall over funnel and gate is laid on continuous wall's lower sensitivity to design flow (Fenton *et al.*, 2014). This according to that point of view will have less risk in heterogeneous pollutant pathways and distribution. In either case, the need for striking a balance between the criteria of reconciling wider capture zone and enough residence time at the gate is critical (Painter, 2004; Starr & Cherry, 1994). Notably, interdependent parameters such as reaction rate and residence time vis-a-vis discharge rate and gate size determines the degree of attenuation. It has also been documented than a wider gate is the most desirable and effective in remediation. The followings are some of the influential features associated with funnel and gate.

2.6.1.1 Funnel apex angle

The effect of funnel apex angle and orientation to the regional gradient on discharge through the gate was shown to vary only when the funnel is not perpendicular to the regional hydraulic gradient. It was also deduced from simulation result that, to maximize discharge through the gate, an apex angle of about 180 +/- 50^o and setting the orientation of the funnel within 30^o perpendicular to the regional hydraulic gradient should be assured. Since the funnel and gate geometry and orientation to the regional gradient affect the size and shape of the capture zone, it is important that emphasis be given to the design vis a vis the orientation of the regional gradient. From the results of many studies, it can be deduced that the size and shape of the capture zone can be modified by using multiple gates.

2.6.1.2 Residence time

The effect of structural setting on the residence time was found to be related to the length of the gate. In addition to high reaction rate, lower reaction half-life and decreasing the discharge rate by constructing a longer gate are variable factor that can improve residence time efficiency. It should be noted that, having a longer gate is more desirable than decreasing the discharge rate as the latter has the effect of mounding. For degradation that proceeds by first order reaction, the retention time can be calculated

$N_{1/2} = [\text{In}\ (C_{\text{effluent}} / C_{\text{influent}})] / \text{In}\ (\ ½\ )$

Where $N_{1/2}$ is the number of half-life required.

Using Numerical steady-state groundwater flow simulations, sensitivity test on two basic geometry of the funnel and gate – U- Shaped and straight barrier- was conducted (Sedivy *et al.*, 1999). The finding revealed that the width of a capture zone is determined by the physical geometry and dimension of the barrier and is effectively independent on the aquifer transmissivity and the magnitude of the ambient hydraulic gradient. The study concluded that it is better to increase the width of the reaction medium than to increase the length of the funnel arm, as the former is more effective and less expensive.

The concept of multiple gates for large plume and multiple reactors for multi pollutant plumes is given considerable attention in recent researches. The multiple reactor setting seems to be the bedrock upon which the concept of permeable reactive interceptors (Fenton *et al.*, 2014) stems out from. The depth and length of any funnel & gate is a factor of the nature of the contaminants and their extent of distribution in the aquifer. The depth of a funnel for treating Dense Non-Aqueous Phase Liquid (DNAPL) for instance will be longer than the one for treating Light Non-Aqueous Phase Liquid (LNAPL) (Starr & Cherry, 1994).

The mechanism for the removal of contaminants is broadly categorized as either destructive biotic/destructive abiotic or non-destructive process (Obiri-Nyarko *et al.*, 2014). The most important portion of any PRB is the reactive media, where gate materials are packed in. A single barrier system treats only one pollutant, whereas a multi barrier system treats either multiple pollutants or single pollutants with its generated intermediary/by-products. The reactive materials have a special order of placement in the barrier system, which impact on the effectiveness of the remediating structures. For this reason, the pattern in which the reactive materials are arranged in barrier system of the gate is of paramount importance. In this study, multi barrier system will be given more attention to because of its significance in remediation.

2.6.2 Multi-barrier system.

This is a geometric and functional approach that tends to address recalcitrant pollutants in in-situ remediation process. The technology has been gaining recognition especially in Germany (Birke *et al.*, 2007a). The term have been featuring as proposed solution to envisaged problem associated with multiple pollutants plume (Madzin *et al.*, 2016;

Streitelmeier *et al.*, 2001) or pollution swapping (Huang *et al.*, 2015). Some researchers referred to it as multiple gates (Hudak, 2008). Multi-barrier system is broadly defined as a PRB system that consist of two or more treatment gates filled with the same or different reactive materials. The sequence of placement of the reactive media have been reported to exert significant influence on the efficiency of the remediation.

2.6.2.1 Placement pattern of reactive media - packing structure

The arrangement of materials in the gate has been a subject of discussion but it has been established that the order is determined by the type of remediation desired. This in turn will depend on the type and concentration of the pollutants. Where the barriers are separated, sequential removal of the contaminants occurs, and the structure is termed as "sequence multi barrier". However, if the barrier is single, but different type of reactive materials are mixed up within the barrier, the contaminants are expected to be simultaneously removed (Obiri-Nyarko *et al.*, 2014; Phillips, 2009). Figure 2.3 gives a representation of the sequence multi-barrier systems as depicted by Gibert *et al.* (2007).

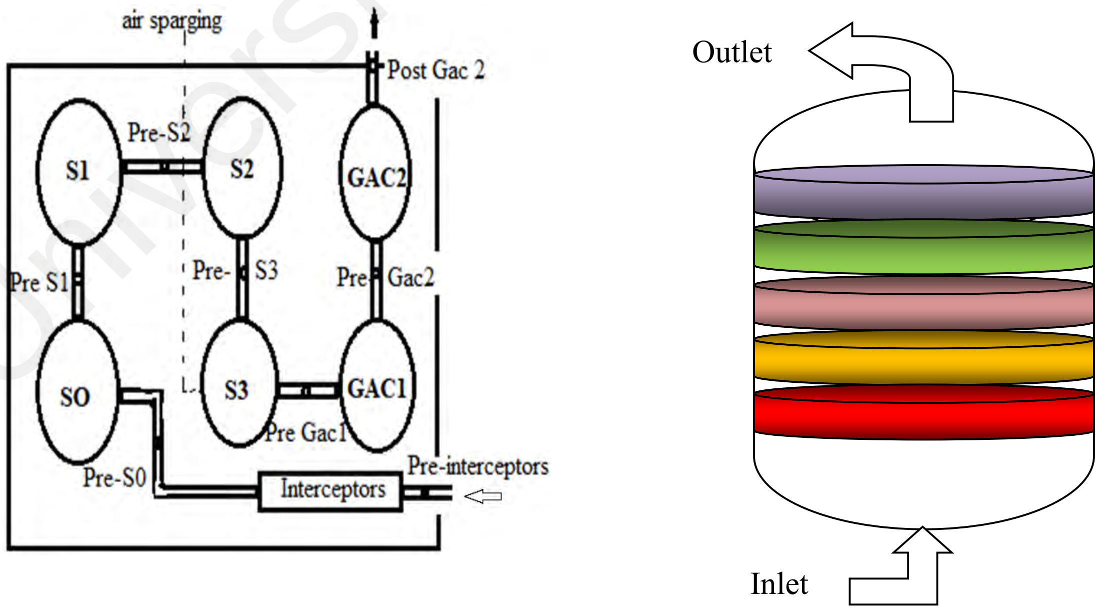

Figure 2.3 (a) Compartment sequence Multibarrier **(b)** Fused Sequence multibarrier

The fused sequence multi-barrier above may be considered as mixed multi treatment barrier- which is a barrier that has all the reactants in one compartment. However, the reactants have distinctive boundaries having a homogeneously cohesive action on the pollutants. In most cases, each boundaries of the reactants is aimed at remediating one or more components of the pollutants in the passing plume (Den Baars *et al.*, 2003; Streitelmeier *et al.*, 2001). However, in mixed-multi-barriers, the reactants are blended homogeneously in certain ratio and each reactant carries its function in a synergistic way, thereby putting off excessive load on one reactant at a time. The ratio is often varied with respect to the desired permeability, reactivity and adaptability of the reactants to the geochemical condition of the medium (Firdous & Devlin, 2015; Madzin *et al.*, 2016).

The sequential positioning of the reactive media in multi-barrier system is often dictated by the nature and evolutionary phase of the pollutants. Whereas specific affinity for a component within the pollutant is considered in positioning a section of the barrier in some instances, by-products of some remediation process and pollution swapping are considered for positioning specific barriers in other instances. The latter scenario is attested by Ibrahim *et al.* (2015) findings, wherein he deduced that placing pea gravel or zeolite after the woodchip would over burden the zeolite with the released NH_4^+ and dissolved reactive phosphorous (DRP) from the woodchip. Notwithstanding the observed intrusion, the research recommended placement of the woodchip cell first on account of the sustained concentration of dissolved oxygen carbon and denitrification potential in the subsequent cell, which was either zeolite or pea gravel (Ibrahim *et al.*, 2015).

The order of placing specific reactive barrier is evident from a conceptual work (Della Rocca *et al.*, 2007). This suggests putting the zero valent iron first in contact with the intended polluted water. The proposed order was predicated on an earlier work that proposes the action of the zero valent Iron (ZVI) first -indicating its strategic location- to reduce the dissolved oxygen in order to favor heterotrophic denitrification in the medium, and incur cathodic hydrogen for autotrophic denitrification (Della Rocca *et al.*, 2006). In the packing structure of that study, iron wool was placed before cotton for heterotrophic autotrophic denitrification.

In line with the prevailing effort of finding the best gate materials, a study was conducted using column test by varying the position and amount of the reactive media in form of mixtures of sand and spongy iron followed by mixtures of sand and pine bark (Liu *et al.*, 2014; Liu *et al.*, 2013). The finding revealed that as far as single contaminant (NO_3^-) remediation is concerned, the packing structure has a negligible effect on the performance of the remediation system. This suggests that when single pollutant is targeted, and effect of pollution swapping/by-products are generally ignored, the type of multi barrier (weather sequenced or mixed multi barrier) used is immaterial. However, in a further research that tends to improve the efficiency of the remediation by manipulating reaction conditions, sequential remediation steps that involved biological deoxygenation, heterotrophic denitrification, hydrogenotrophic (Autotrophic) denitrification and anaerobic iron corrosion yield contrary conclusion (Huang *et al.*, 2015) . The study proved that packing structure had a noticeable effect on denitrification. The processes were shown to be inter-connected as heterotrophic denitrification on organic substrate generates CO_2 when ZVI via Fe corrosion releases cathodic H_2 in which both (CO_2 and H_2), supports hydrogenotrophic denitrification.

2.6.2.2 Different kind of gate materials used in denitrification.

Amongst the materials that have been used in gate substrates for denitrification are granulated spongy iron and methanol (Huang *et al.*, 2012), wood chips of different sizes (Gibert *et al.*, 2008), soil and saw dust (Healy, 2016), card board (Greenan *et al.*, 2006) and compost, mulch or green waste (Della Roca, 2006). In line with the order of placing reactive material in the gate of permeable reactive barrier, a new concept of remediation known as Permeable reactive interceptors emerges.

2.7 Permeable reactive interceptors (PRI)

The concept of permeable reactive interceptors stems out of necessity for the need to curve pollution swapping. It has been developed by Fenton in 2014 (Ibrahim *et al.*, 2015), the concept tends to address the effects of inadvertent by-products of physiochemical and biological processes in remediation. This is achieved by "introducing new management strategy and additional remediation cells for specific contaminants in the form of solutes, particles or gases"(Fenton *et al.,* 2014). Structurally, it is described as a network of interconnected cells capable of removing specific contaminants from pollutant's body (Ibrahim *et al.*, 2015).

Though, there are not many published studies to verify the efficiency of PRI (Obiri-Nyarko *et al.*, 2014), the first attempt to test the concept at laboratory scale came with good result but unwanted emission of greenhouse gases (Ibrahim *et al.*, 2015). The research demonstrates the overall efficiency of the reactive media in PRI, emphasizing on their sequence for effective multipollutant remediation. Being the first laboratory scale study on PRI, the background knowledge of the decomposition products of the reactive media helped in making an informed decision on the sequential arrangement of the medias for simultaneous mitigation of NO_3^-, NH_4^+, dissolve organic nitrogen DON, dissolved reactive phosphorus DRP and dissolved unreactive phosphorus DUP. It can be inferred that the last media material in PRI should -in addition to mitigating the escaped pollutants- be able to screen the decomposition products of the preceding media materials. Here, the structural influence, in other words, the geometric pattern of arrangement is found to be very important in remediation.

2.8 The geometric contribution to remediation.

The term geometric aspect of PRB has been linked to the design of the structure as it relates to the sequential order of PRB structure in accordance with site specific factors such as hydrogeological/hydraulic conditions, size of the contaminant plume, site logistics and clean-up goals (Bürger *et al.*, 2007; Warner *et al.*, 1998). Therefore, the geometric aspect may be defined as the structural configuration of the PRB with respect to hydrogeological condition of the aquifer, dimension and bearing of the plume intended for remediation. While incorporating these multiple factors, it is important to consider the non-linearity of flow across the regions of the structure in the design. In this regard, amongst number of researches that highlights the significance of design in attaining remediation goals are Benner *et al.* (2001), who asserts that barrier design (geometry) is as significant as the barrier reaction media and mechanism. It is also acknowledged that effective remediation plan ends up at the design level (Careghini *et al.*, 2013) . More so, a study on the probability of plume bye-passing remediation structure reveals that geometry of PRB has a significant influence on its performance (Cirpka *et al.*, 2004)**.** PRB technology is active because its usage in management of complex contaminants in groundwater was stated to be at early stage of its application (Gibert *et al.*, 2007).

The geometric pattern of any remediation system may be guided by several factors among which functional and material composition are important. It is opined that the reactive media should have a higher hydraulic conductivity than the surrounding soil media (Choi *et al.*, 2013; Kacimov *et al.*, 2011). In supports of this assertion, a recent review states that in a PRB setting, it is important to make sure that the hydraulic permeability of the reactive media is at least twice that of the aquifer (Obiri-Nyarko *et al.*, 2014). The mathematical representation of the required PRB thickness $\boldsymbol{b}$ for the first order reaction is given in Equation 2.1.

$$b = v \times t\_(res\ ) \times SF \qquad \textbf{(2.1)}$$

Where v is the velocity of groundwater $t\_(res\ )$ is the residence time and SF is the safety factor. But the fact that velocity v is given as $v = KI/n\_p$ suggest that where we have high hydraulic gradient (K) and law porosity value of the medium (n_p), the velocity is going to be high.

2.9 An overall review on denitrification process

The simplest description of denitrification stands as the biological reduction of nitrate or nitrite to nitrogen in gaseous form. Several researches conducted on denitrification revealed that the nitrogen emitted through biological reduction of nitrate is in the form of nitrous oxide (N_2O) or molecular nitrogen (N_2). However, emission of nitric oxide (NO) is as well reported. Denitrification is known to take place under anaerobic condition and the bacteria utilizes nitrate as an oxidant for organic (Carbon based matter) or inorganic matter such as sulfide and iron minerals (Grau-Martínez *et al.*, 2017).The denitrifying bacteria that acts for the reduction of nitrate to gaseous forms of nitrogen are facultative anaerobes. These bacteria can survive in anaerobic and aerobic condition and they can use both oxygen and nitrate (or nitrite) as electron acceptors in the presence of oxidizable substrate.

Experimental evidences that suggest the bacteria utilizes nitrate only when there is no adequate supply of oxygen exists (Bremner & Shaw, 1957). Even in the soils where denitrification was reported to have occurred under seemingly aerobic condition, it was further discovered that the denitrification occurs at anaerobic sites within the soil strata (Broadbent *et al.*, 1965). Under heterotrophic denitrification process, the presence of readily decomposable organic matter is a critical factor. It was also found that the influence of organic materials on denitrification in soils varied with their resistance to decomposition (Bremner & Shaw, 1957). As echoed by recent researches, it is established that the effect of easily decomposable organic materials such as glucose is higher than the effect of complex organic materials like saw dust, wood chips and lignin (Pan *et al.*, 2013; Yang *et al.*, 2012). The findings also revealed that when the carbon source is allowed to decompose prior to their introduction as the energy source for denitrifying bacteria, the efficiency of the process is negatively affected. This according to the study is an indication that denitrification in soil is highly dictated by the availability of water soluble or decomposable organic matter (Burford & Bremner, 1975).

2.9.1 Forms of carbon in soil

Since denitrification occurs in the presence of decomposable organic matter, it is important to note that of the three types of carbon in soil viz: elemental carbon, inorganic carbon, and organic carbon, the latter (organic carbon) is the most relevant in denitrification. Lack of readily available organic carbon has been found to be the most common limiting factor for denitrification in subsurface region (Hunter, 2003). Organic carbon forms found in natural occurrence are derived from animal and plant decomposition. The sources of the plants as freshly deposited litters of leaves and tree branches decompose normally to finer particles like humus. Anthropogenic input on to the total organic carbon pull is considerable enough as human releases contaminants into the environment in tones. However, the total contribution of carbon to total carbon through contamination (measured in g or mg/kg) to the total organic carbon content (measured in % range) of the soil is close to negligible. Notable variation is only observed where point source pollution site is considered. An example of such is seen in sites with Wood fibers from pulp mill wastes, which are expected to have high anthropogenic input of organic carbon at that specific site (Schumacher, 2002).

2.9.2 The denitrification processes.

Several laboratory investigations have been conducted with the bid to understand the dynamic phenomenon in denitrification process. A technical protocol for air force center for engineering of 2008 revealed that, influential co-factors in the process of denitrification include dissolved oxygen (DO)and oxidation reduction potential (ORP). It further shows that background levels of DO and values of ORP are indicators of the natural redox conditions that must be lowered or optimized within the reactive zone to achieve efficient anaerobic degradation. Generally, it is envisaged that DO value of less than 0.5 mg/L and ORP levels less than 0 millivolts (mV - relative to a standard hydrogen electrode [SHE]) are appropriate values for stimulating anaerobic degradation processes. When high level of DO is experienced in aquifers, the situation can be overcome by supplying adequate amounts of substrate. Alternatively, other factors such as high rate of groundwater flow may play significant role in arresting the situation (Hatzinger & Diebold, 2009).

Recently, a research conducted by Huang *et al.*, (2015) dwells on the performance of a two-layer heterotrophic autotrophic denitrification (HAD) PRB for the remediation of NO_3–N contaminated groundwater in an aerobic environment. In the research, Iron, in form of spongy iron was observed to play two roles. First, via chemical reduction, Fe removes O_2 creating anaerobic environment and hence, supporting biological denitrification. Secondly, through anaerobic Iron corrosion, Fe forms cathodic hydrogen with the aid of hydrogenase enzyme and supports hydrogenotrophic denitrification (Equation 2.2)

$$Fe^0 + 2H_2O \longrightarrow Fe^{2+} + 2OH^- + H_2 \qquad \textbf{(2.2)}$$

Argumentatively, heterotrophic denitrifies utilizes organic carbon (as electron donor) to reduce NO_3-N generating CO_2 in the process and hence favoring hydrogenotrophic denitrification (Huang *et al.*, 2015). The following represent the equation for Autotrophic and Heterotrophic denitrification (equation 2.3 and equation 2.4)

(Autotrophic Denitrification)

$$5FeS_2 + 14\ NO_3^- + 4H^+ \longrightarrow 7N_2 + 10SO_4^{2-} + 5Fe^{2+} + 2H_2O \tag{2.3}$$

(Heterotrophic Denitrification)

$$\underset{\text{Organic Matter}}{CH_2O} + 4/5\ NO_3^- + 4/5\ H^+ \longrightarrow 2/5\ N_2 + CO2 + 7/5\ H_2O \tag{2.4}$$

Furthermore, in a separate study conducted for the selection of organic substrate to serve as a potential reactive material in-situ, Gibert *et al.* (2008) identified some specific environmental requirements of the denitrifying bacteria, which must be met before a successful remediation is achieved. These requirements include reducing condition (+300 Mv), pH (5.5- 8), presence of carbon source, temperature (25 – 35ºC), low level of oxygen (tolerate DO up to 4.5 mg dm^{-3}) and physical support for the bacterial attachment. In an attempt to simulate the aquifer at different flow rate, their work limits the maximum nitrate concentration to 50 mg dm^{-3} NO_3-N and soft wood was finally recommended. However, the study did not consider the consequences of longevity potential of the soft wood on the cost implication at the long run and no provision of arresting its decomposition by-product was made in case of pollution swapping. Denitrification can occur through physical or biological methods.

2.9.3 Biological method

Biological form of denitrification is one of the natural paths of remediation being exploited by humanity to clean up its domestic waste- which is more of nitrogen based. Unlike in surface water where the microbiological removal of nitrogen is a slow process due to presence of oxygen, in-situ denitrification is often in anaerobic condition and hence, not as slow as in surface water. The two major pathways of reducing nitrate are assimilatory and dissimilatory pathways. In assimilatory pathway, inorganic nitrate is converted to organic nitrogen form, in which the nitrate is reduced to nitrite, which in turn get transformed to ammonium by plant and microorganism. Dissimilatory nitrate reduction to ammonium (DNRA) is reported to occur when reduction of organic matter (fermentation) is not possible. In this respect, both DNRA and denitrification are dissimilatory reduction process because they supply energy to the cell for growth and maintenance (Korom, 1992; Tiedje, 1988). Equations 2.5-2.8 represents the assimilatory pathways of removing nitrate.

$$NO_3^- + 2e^- + 2H^+ \longrightarrow NO_2^- + H_2O \qquad \textbf{(2.5)}$$

$$NO_2^- + 2e^- + 2H^+ \longrightarrow NO + H_2O \qquad \textbf{(2.6)}$$

$$2NO + 2e^- + 2H+ \longrightarrow N_2O + H_2O \qquad \textbf{(2.7)}$$

$$N_2O + 2e^- + 2H^+ \longrightarrow N_{2\,(g)} + H_2O \qquad \textbf{(2.8)}$$

Biological denitrification is a mechanism whereby, in the absence of oxygen, nitrate is used as a terminal electron acceptor by denitrifying bacteria (Ghafari *et al.*, 2008). A group of microorganisms called facultative anaerobes carries out biological denitrification and by their definition, they can turn into aerobes or anaerobes. The following two distinct type of biological denitrification (heterotrophic and autotrophic) are driven from the function of the active microorganism involved in the process.

2.9.3.1 Heterotrophic denitrification

Heterotrophs are microorganisms that requires organic substrate for growth and other metabolic functions. Organic carbon from carbohydrate is very essential for providing energy to the microorganisms. They also utilize carbon that are obtained from inorganic sources. With a wide distribution of both organic and inorganic sources of carbon in the form of plant, animals and carbon-based minerals within the earth crust, heterotrophic denitrification is everyday phenomenon in the aquifer system.

$$5CH_2O + 4NO_3^- + 4H^+ \longrightarrow 2N_2 + 5CO_2 + 7H_2O \qquad \textbf{(2.9)}$$

The CH_2O represent a generic organic compound (Equation 2.9). As typical with any microbially -mediated reaction, certain conditions must be met before denitrification takes place. These conditions include The presence of electron donors (in form of carbon, which serves as a nutrient as well) reducing condition, suitable temperature and pH (ŠImek & Cooper, 2002)(at the range of 25-35ºC and 5.5 – 8.0), less presence of oxygen (Though up to 4.5 mg dm^{-3}) and physical support for bacterial attachment (Gibert *et al.*, 2008)

2.9.3.2 Autotrophic denitrification

Autotrophic denitrification occurs where carbon dioxide or bicarbonate are utilized as the inorganic carbon source, and iron, sulfur or hydrogen are utilized as electron sources for biosynthesis (Kuusi *et al.*, 2016). The process (autotrophic) is promising with comparative advantages over heterotrophic denitrification due to (i) absence of external carbon source(ii) relative low cost (iii) low biomass yield, and (iv) subsequent lowering of pollution swapping due to low biomass yield (Luo *et al.*, 2018). In both pathways, NO_3^- is first converted to NO_2^-, which is more toxic than NO_3^-. The maximum permissible concentration of NO_2^- in water is 0.46 ppm. An example of autotrophic denitrification is given in Equation 2.10.

$$5FeS_2 + 14\,NO_3^- + 4H^+ \longrightarrow 7N_2 + 10SO_4^{2-} + 5Fe^{2+} + 2H_2O \qquad \textbf{(2.10)}$$

However, in both heterotrophic and autotrophic denitrification, limited availability of electron donors serves as a limiting factor to the process and hence, external supply of electron donors (Carbon in the case of heterotrophic and Fe in the case of autotrophic) are resorted to (Grau-Martínez *et al.*, 2017).

2.9.4 Incomplete denitrification

Incomplete denitrification is a phenomenon that occurs when the necessary conditions for complete denitrification are not met or lost in the presence of denitrification. An example is that of intrusion of appreciable amount of oxygen in the middle of denitrification at anoxic level. This is bound to upset the equilibrium and favours the formation of different product apart from that of denitrification (Musvoto *et al.*, 1994). Incomplete denitrification leads to production of denitrification intermediates.

Denitrification intermediates include nitrous oxide (N_2O), nitrite (NO_2^-) and Ammonia. The potential of producing significant amount of nitrous oxide (N_2O) is associated with potent greenhouse gas characteristic (Dodla *et al.*, 2008). Incomplete denitrification has been observed in agricultural site, forest and coastal regions, but high C:N ratio have been observed to mitigate excess nitrous oxide production. A threshold value of 25 in C:N ratio have been reported, suggesting that high amount of carbon provide sufficient energy to push the reaction to completion (Hunt *et al.*, 2007).

Reduction of nitrate to ammonium is possible in the anaerobic condition through fermentative bacteria and the process is known as dissimilatory NO_3^- reduction to Ammonium (DNRA) (Grau-Martínez *et al.*, 2017). The limiting factor for complete denitrification is unavailability or inadequate electron acceptor. For this reason, external electron acceptor (in form of carbon substrate) is often added into the medium for complete reaction. Ammonium can be produced through the following nitrate reduction of zero valent iron (Equation 2.11) and organic substrate (Equation 2.12).

$$NO_3^{-} + 3Fe^0 + H_2O + 2H^+ \longrightarrow NH_4^+ + Fe_3O_4 \qquad \textbf{(2.11)}$$

$$2CH_2O_{(s)} + NO_3^{-} + 2H^+ \longrightarrow NH_4^+ + 2CO_{2(g)} + H_2O \qquad \textbf{(2.12)}$$

DNRA is therefore considered as counterproductive in denitrification and studies have shown that high organic carbon to nitrogen ration favours DNRA over denitrification (Gibert *et al.*, 2008). Therefore, from the information above, it can be deduced that while low C:N ratio ensures nitrous oxide formation, high C: N ratio ensures DNRA. Another process that oxidizes the produced ammonium under anaerobic condition to $N_{2(g)}$ is anaerobic ammonium oxidation (Anammox). The process involves the oxidation of ammonia utilizing nitrite as the electron acceptor (Equation 2.13). On the other hand, Anammox organism (*Planctomycete* Bacteria) utilizes CO_2 as carbon source and NO_2^{-} as electron donor to develop its cell material (Equation 2.14). Though nitrogen gas is the main product, about 10 % of the product is converted to NO_3^{-}:

$$NH_4^+ + NO_2^{-} \longrightarrow N_2 + 2H_2O \qquad \textbf{(2.13)}$$

$$CO_2 + 2NO_2^{-} + H_2O \longrightarrow CH_2O + 2NO_3^{-} \qquad \textbf{(2.14)}$$

Other forms of nitrogen removal technology that are not the focus of this research include SHARON (single reactor high activity ammonium removal over nitrite) and CANON (Completely autotrophic nitrogen removal over nitrite) process (Ahn, 2006)

2.9.4.1 Factors affecting biological denitrification

Several literatures indicate multiple factors that affects denitrification and, being a biologically mediated reaction, it is not surprising when more and more factors are being identified. This is because of the dynamic nature of the microbes and new active species that are being discovered. The followings are the identified factors that affects in-situ denitrification.

(a) *Types of denitrifying organism*

The type microorganism that acts on the substrates for denitrification define the mechanism and affects the rate and nature of the reaction. The availability of substrate upon which the microbes depends on also define the process and hence, the organism responsible for the denitrification. For example, bacteria that influences denitrification may be classified based on classes that synthesize a nitrite (NO_2^-) reductase that is solely dependent on haem-iron as a cofactor (e.g. *Paracoccus denitrificans*) or a Nitrate reductase, which is solely dependent on copper (Cu) as a cofactor (e.g. *Achromobacter xylosoxidans)* (Felgate *et al.*, 2012). Generally, all microorganism that partake in denitrification are called denitrifiers. Though denitrifiers are highly adaptive as they respond quickly to environmental drivers(Schipper *et al.*, 2010), the driver's fluctuation affects their performance and hence, denitrification potentials. Denitrifiers are mostly gram-negative bacteria belonging to classes of proteobacteria. Some gram-positive bacteria (such as bacillus) have also been known to partake in denitrification. Different responses to changes on environmental conditions by different strands of bacteria, as seen in salinization (Laverman *et al.*, 2007), is responsible for variation of denitrification potentials under the same condition. Several denitrifiers have been isolated from soil and they include those belonging to *Flavobacterium, Clostridium, Bacillus*, and *Novosphingobium.* Also, *Pseudomonas* were isolated from the hydrogenotrophic process. When sequence multi barrier system is well designed, the aerobic and anaerobic denitrifiers can co-habit augmenting each other's function in a symbiotic manner. This is seen where aerobic heterotrophs provide the anaerobic condition (CO_2) and hydrogen needed by heterotrophic and hydrogenotrophic denitrifiers (Equation 2.15 and 2.17) (Huang *et al.*, 2015; Karanasios *et al.*, 2010)

$$2C_6H_{10}O_2 + 15O_2^{-} \xrightarrow{\textit{Aerobic Heterotrophs}} 12CO_2 + 10H_2O \qquad \mathbf{(2.15)}$$

$$2\,NO_3^{-} + 5H_2 + 2H^{+} \xrightarrow{\textit{Hydrogenotrophic}} N_{2(g)} + 6H_2O \qquad \mathbf{(2.16)}$$

$$2C_6H_{10}O_2 + 6\,NO_3^{-} + 6H^{+} \xrightarrow[\textit{denitrifiers}]{\textit{Heterotrophic}} 6CO_2 + 10H_2O + 3N_2 \qquad \mathbf{(2.17)}$$

(b) ***Bioavailability and type of electron donor***

The bioavailability of electron donor is essential where nitrate is in excess. In the case of heterotrophic, the availability of degradable carbon source is the determining factor for successful remediation (Schipper *et al.*, 2010). However, in the absence of readily available organic substrate, other electron donors (inorganic) are scavenged for the metabolic function of the microbes. The process could then change from heterotrophic to autotrophic when inorganic sources of electrons (like Fe and Mn) are utilized. Where both are present, heterotrophic is preferred to autotrophic (Korom, 1992) and when the environment is challenged with a dramatic variation, a combine heterotrophic autotrophic denitrification (HAD) is ensured (Huang *et al.*, 2015; Su *et al.*, 2017). Bioavailability of organic carbon is ubiquitous across the earth ranging from plant and animal debris to marine-based deposits that forms the soil aggregates within the coastal plain. Leaf litters and remains of animal decomposed body forms the humus soil and with time, layers of soil envelop the humus part, pushing it below the ground level. Embedded within the earth crust are mineral ores that constitutes Fe and manganese in the form of pyrite (Roy *et al.*, 2017). Moreover , the soils around coastal zones, which was covered by the sea before the halocine, have been documented to be formed with inundated iron from the sea (Enio *et al.*, 2011; Roslan *et al.*, 2010)

(c) *Nature of carbon source (organic carbon)*

The type of carbon source can affect the microbial biomass as denitrifiers are not known for degrading complex polymeric carbon. That means they prepare using readily available and simple organic compounds (Schipper & Vojvodić-Vuković, 2001). Where soluble organic substrate is mixed with hard insoluble ones, the bacteria always degrade the soluble ones first before utilizing the insoluble ones (Jeris *et al.*, 1974; Yang *et al.*, 2012). More so, the size and physical texture of the carbon source have been reported to play significant role in the permeability of the reactive media (Robertson, 2010). The nature and size factor affect the rate of degradation as well as the quantum of the possible by-products of degradation as enumerated in the following sub-section.

(i) Rate of organic matter degradation (Biodegradation)

The fact that different carbon sources have different rate of degradation speaks volume of the nature of remediation. Fast degradable carbon source has the advantage of impacting high rate of denitrification (Martin *et al.*, 2009). This will ultimately shorten the period of treatment, require less longitudinal stretch of reactive media and hence, less cost implication for constructing the remediation structure. However, on the long run, the reactive media is susceptible to early depletion and hence, rejuvenation becomes inevitable. The initial advantage of cost effectiveness in construction is then overturned to disadvantage. This has been one of the rallying points of research in the area, with many studies racing to provide sustainable electron donors (Robertson, 2010). The anaerobic degradation of complex organic matter by fermentation defy stoichiometric relation as it proceeds in the absence of electron acceptors like nitrate and sulfate. The process is limited by the initial cleavage of complex polymeric carbon molecules, but not by the consumption of simple carbon in anaerobic respiration (Schipper & Vojvodić-Vuković, 2001).

(ii) Decomposition by-product

The fear of generating pollution swapping forces the attention of researchers to study decomposition by-product. This is because while organic matter decomposes for usage in remediation process, several organic by-products becomes labile and, depending on the reaction medium, inadvertent formation of more harmful product than the one being remediated is experienced (Ibrahim *et al.*, 2015; Yang *et al.*, 2012). A typical example is that of a study that revealed sulfate reduction and nitrite production as a by-product of incomplete denitrification in a denitrifying woodchip bio-reactor (Christianson *et al.*, 2017).

(d) *Availability of metal catalyst (Cu)*

Availability of copper has been strongly related to enhancement of complete denitrification process as the conversion of N_2O^- to N_2 in the final step is known to be catalysed by copper containing substrate (Kroneck *et al.*, 1988). A group of bacteria known as *Achromobacter xylosoxidans* are known to synthesize a nitrite (NO_2^-) reductase (Nir) that is dependent on Cu. But the most important step is the dependence of the process on a Cu containing nitrous oxide reductase (NosZ) for the conversion of N_2O^- to N_2 (Felgate *et al.*, 2012). Copper is also used in a Pb-imbedded catalyst form to denitrify drinking water through hydrogenation process as shown in equation 2.18 and 2.19

$$NO_3^- + H_2 \longrightarrow NO_2^- + H_2O \qquad \textbf{(2.18)}$$

$$2\,NO_2^- + 3H_2 \longrightarrow (NO, N_2O) \longrightarrow N_2 + 2H_2O + 2OH^- \qquad \textbf{(2.19)}$$

The process is only achieved by the copper formate ($Cu(HCO_2)_2$) decomposition at the surface of Pb particles, thereby releasing hydrogen and metallic copper (Matatov-Meytal & Sheintuch, 2005) as shown in Equation 2.20

$$Cu\,(HCO_2)_2 \longrightarrow Cu + 2CO_2 + H_2 \qquad \textbf{(2.20)}$$

Moreover, high alkalinity affects the availability of copper by decreasing its concentration and it has been reported that dosing of copper enhances the growth of ammonia oxidizing organism (Wagner *et al.*, 2018). The alkalinity is produced by the generation of OH^- during the process of denitrification (Su *et al.*, 2017).

(e) *Availabity of oxygen*

Though aerobic denitrification exist, in-situ denitrification is strictly anaerobic phenomenon and presence of oxygen strongly limit the process. As mentioned in incomplete denitrification process, the presence of oxygen in denitrification hampers the production of the final product, N_2. This is because the oxygen reacts with the intermediate product to reverse the reaction forming the initial reactant (NO_3^-) or form other undesirable product. The presence of oxygen above 4.5 mg dm^{-3} can also disrupt denitrification as it will preferentially be utilized as the electron acceptor (Gibert *et al.*, 2008; Scherer *et al.*, 2000). This consensus assertion had been reiterated by researchers across the spectrum, wherein it is confirmed that oxidants are consumed in order of decreasing energy production per mole organic carbon oxidized. Therefore, the sequence of exploitation starts from O_2, NO_3^-, Mn oxides,oxide ion and sulfate ion (Rysgaard *et al.*, 2001). Efforts in utilizing dual process of nitrification/denitrification led to manipulations of some operational conditions that may accumulate nitrite during the process. These operational conditions include DO, pH and temperature (Ruiz *et al.*, 2006). The result indicated that amongst the factors, control of the DO is the most effective means of nitrite accumulation, presenting a maximum of 65% at around 0.7 mg DO/L.

(f) *pH*

pH has a tremendous influence on the metabolic function and survival of microorganism. The optimum pH value range for biological activity is 6.5 to 9. However, depending on the type of organism, the optimum pH range for denitrification can vary.

The influence of pH variation on nitrification and denitrification have been extensively studied in both soil and water. While other researchers found the optimum range between 8 to 9 (Kim *et al.*, 2001) others found that there is no simple relation between denitrifying enzyme activity and pH (ŠImek & Cooper, 2002). In another research that tends to exploit nitrification and accumulation of nitrite, it was found that at pH lower than 6.45 and above 8.95, nitrification does not hold. Moreover, that pH range inhibits ammonia oxidizing as well as nitrite oxidizing bacteria (Ruiz *et al.*, 2003).

In another development, it has been shown that the total gaseous emission of denitrification intermediary products (N_2O, NO) and final product (N_2) to the atmosphere is less in acidic soil medium, suggesting less denitrification (ŠImek & Cooper, 2002). However, this observation was attributed to less organic matter in acidic soil rather than direct influence of pH on the process. Moreover, lower pH has been observed to raise the ratio of $N_2O:N_2$, suggesting the inhibition of growth or suppression of the organisms responsible for completing the reaction to its final product (N_2). Further research by Čuhel *et al.* (2010) revealed that pH has a significant influence on the nature of denitrification end product as the ratio of N_2O to ($NO + N_2$) increases with decrease of pH.

(g) *Temperature*

The fact that all living organisms have specific range of temperature within which their metabolic activities strive makes temperature important factor in denitrification. Temperature have been identified amongst the specific environmental factors that must be met for nitrification and denitrification to occur (Gibert *et al.*, 2008; Huang *et al.*, 2015; Ruiz *et al.*, 2006). The ranges have been reported to be within 25-35 ^{o}C across many regions (ŠImek & Cooper, 2002).

(h) ***Oxidation reduction potential (ORP)***

ORP is an important tool for gauging the condition of biological process in a system. It gives idea about the oxidative state, thereby differentiating different biological proses. Several studies investigate the use of ORP as an indicator for nitration/denitrification process. It has been compared with alkalinity (Li & Irvin, 2007) and correlated with pH (Chang & Hao, 1996; Peng *et al.*, 2002). However, inconsistencies of result obtained led to further research on other indicators. Another concern is that of the difficulty of its application in the practical operation of Sequencing Batch Reactor (SBR). Moreover, the accuracy of its measurement has been in question due to fouling in west water (Li & Irvin, 2007). The obtained values of ORP is positive for aerobic process and negative for anaerobic process.

(i) ***Aquifer properties***

As stated at the beginning of this chapter, several aquifer-based inter-related variables play significant role in the success of any in-situ remediation process. These variables include the hydraulic gradient (Virkutyte *et al.*, 2002), porosity of the media in relation to that of the aquifer particle size (Quintelas *et al.*, 2011), heterogeneity of the aquifer and hydraulic conductivity. These are properties that have direct bearing to aquifer properties which must be safeguarded for successful remediation (Christ *et al.*, 2004).

2.9.5 Physiochemical methods

These are combination of methods from pure physical methods to chemical method in conjunction with physical steps that are used for NO_3^- removal. The methods include catalysis and electrodialysis, reverse osmosis and ion exchange. Regardless of the promising results while using some of these methods, their cost implication has deprioritised them and hence little of the methods is heard of at industrial scale. A typical example is the overburdened requirement of charging the treatment with chloride ions in ion exchange procedure. In Catalytic denitrification, the disadvantage is the production of ammonia and nitrite, which requires additional treatment. Finally, the requirement for the supply of pressurized water, direct -current power source and membrane stack in electrodialysis makes the option too expensive for commercial purpose (Karanasios *et al.*, 2010). The followings are some of the applicable methods.

2.9.5.1 Adsorption

In-situ remediation of highly soluble substrate like NO_3^- are best handled with the passive technology that requires little or no attention. On this note alone, most of the experimental trials conducted to simulate NO_3^- adsorption may not fit into filed wok. This is because treatment of substrate using adsorption ends up being desorbed. Hence most of the work in this area is accentuated on treatment at surface. There are many researches on adsorption of NO_3^- onto specific or general adsorbents. Example is the comparative adsorption capacity of NO_3^- on to various materials (HCl activated Sepiolite and powdered activated carbon) (Öztürk & Bektaş, 2004). The kinetic studies applied on the adsorption study revealed that slag was not effective for nitrate removal. It was also found that the equilibrium time for the activated Sepiolite is much less than the remaining two materials. Interestingly, the research revealed a very low effective pH value (2) for the removal of NO_3^- and concluded that the pH does not have significant effect on the removal of NO_3^-. The isotherm studies undertaken to evaluate the compliance with the Langmuir and Freundlich models reveals that the adsorption follow second order rate kinetics(Öztürk & Bektaş, 2004).

Some researchers studied NO_3^- adsorption on agricultural residue (Orlando *et al.*, 2002), while some worked on its adsorption onto red mud (Cengeloglu *et al.*, 2006) . Synthetic adsorbents like chitosan hydrogen beads were also applied for the adsorption of NO_3^- and the result was promising (Chatterjee & Woo, 2009). Here also, the adsorption was observed to increase with the decrease of pH and the optimum temperature activity of 30°C was recorded. The kinetic result matches pseudo-second order rate equation. Below are the Pseduo first order (Equation 2.21) kinetics and second order kinetics(Equation 2.22) (Chatterjee & Woo, 2009).

$$\log(qe - qt) = logqe - (k1/2.303)t \qquad \textbf{(2.21)}$$

$$\frac{t}{qt} = \frac{1}{h} + (\frac{1}{qe}) \qquad \textbf{(2.22)}$$

Where "qt" and "qe" are the absorption capacity (mg g-1) of NO_3^- at time t and at equilibrium and "h" is the initial adsorption rate.

However, the kinetics of biological reduction of nitrate differs from the kinetic of nitrate adsorption. The mathematical models formulated by (Ghafari *et al.*, 2010) had two steps assumptions. The steps of nitrate reduction to nitrite followed by the nitrite reduction to nitrogen gas. Here, zero kinetics is applied to describe biological nitrate and nitrite removal rates (Hongwei *et al.*, 2009)

$$NO_3^- \longrightarrow NO_2^- \longrightarrow N_{2\,(g)}$$

$$dC_{NO3^-}/dt = K_{NO3^-}$$

$$dC_{NO2^-}/dt = K_{NO3^-} - K_{NO2^-}$$

Where C_{NO3^-} and C_{NO2^-} are the concentration of the nitrate and nitrite and = K_{NO3^-} and K_{NO2^-} are zero order constant.

2.9.5.2 Impact of structural mode on remediation (geometric impact)

The influence of geometric pattern of remediation structure is strong enough that it attracts attention right from the conceptual stage. This is because for any remediation effort, a structure that will contain the remediating media is essential, if not necessary.

For a passive remediation alternative to succeed, the condition of the remediating environment must be evaluated with respect to the obstructive influence of the foreign structure. In this respect, factors like hydraulic permeability (Obiri-Nyarko *et al.*, 2014), residence time (Han *et al.*, 2015)and reaction or degradation rate must be reconciled.

2.10 Summary

Remediation is a multi-faceted process, wherein many inter-related variables are considered for attaining desired remediation goal. In in-situ remediation process for instance, factors such as water table, gradient, flow velocity, aquifer homogeneity or heterogeneity, nature of the pollutants, geometric setting of the remediating structure, principle of the remediation involved, type of organism involved, and cost benefit status are factors to be considered before selecting one remediation alternative among others. These factors however, are interwoven and related to other physiochemical factors such as the level of dissolve oxygen, pH, temperature, availability of electron donors and nature of carbon source. A review of these factors is given and explanation on their relationship enumerated.

CHAPTER 3: METHODOLOGY

3.1 Introduction

In this chapter, methods and experimental procedures for the: (i) Sampling of water and soil in Bachok field, sample treatment and analysis for assessment of denitrification pattern- as reflected in objective one “1” (ii) Selection, screening, extraction procedures and analysis of carbon source materials to be used for aquifer simulation in column analysis- as reflected in objective two “2” (iii) design, arrangement and packing structure of the proposed reactive media and (iv) construction and operation of the column set up (in line with packing structure of the gate designed by Naftz and Davis III (1999)) in aquifer simulation studies for developing multibarrier reactive media- as reflected in objective 3.

3.2 Materials

These include the reagents used, tools and instrument used during the study.

3.2.1 Reagents

Concentrated nitric acid, hydrochloric acid, glucose, potassium hydrogen phthalate ($C_8H_5KO_4$), (TOC Standard) hydrofluoric acid, multi-element Ion chromatography anion standard Solution, 73426 nitrate reduction kit (reagent A (containing 0.8% sulfanilic acid in 30% acetic acid) and reagent B (N,N-dimethyl-α-naphthylamine in 30% acetic acid)) and Zn powder were obtained from Sigma Aldrich. KNO_3, H_2O_2, KOH, 0.5M $NaHCO_3$, while 0.5M $NaCO_3$ IC eluent concentrates were obtained from Fisher scientific.

3.2.2 Instruments

The instruments used include mobile field meters such as Thermoscientific ORION 329 meter and professional series YSI. Other instruments and equipment include; Polyethylene bottle, cold box, augur, Piezometer, sieve shaker (${}^{\text{Wfi}}$ wykeham Ferrante), Perkin Elmer AAS 400, Hot Plate, Dionex IC 1200, Ultrasonic machine, digital multi-use lab shaker, weighing balance, multiport peristaltic pump, SSM-5000A Shimadzu Total carbon Analyzer (TOC analyzer), PTFE syringe filters, microwave acid digestion system (model: Mars™ 6), Mettler Tolledo pH meter, Garmin GPS Meter, FESEM (Hitachi, model: SU8220) and FT-IR spectrometer (Perkin Elmer, model: spectrum 400).

3.3 Sampling and procedures

This section provides details on the site under study, sampling and procedures employed in the research. Detection parameters used to illustrate the potentials of denitrification in soil and water include nitrate and nitrite as the main interchangeable nitrogen specie in denitrification, Fe and Mn as inorganic electron donors in both water and soil. In addition, organic carbon and Cu are considered as organic electron source and catalyst for denitrification in soil, respectively.

3.3.1 The area

The area under study (Figure 3.1- with google attribution guidelines) is covered by 5°. 98′ to 6°.20′ N; between 102°.16′ -102°.34′ E. The area stretches from Bachok, Kelantan (north east) to Kampung Sungai Keladi (North) to Wakaf Baru (North west) extending to area bordering Pasukan Bomba Sukarela (South east). In order to explore the potential influence of sea on denitrification, the sampling sites were segmented into two categories according to their geographical locations i.e. - the coastal plain segment (< 10 km from the shore) and the inland segment (> 10 km from the shore)

Based on credible scientific reports, the influence of sea could reach several kilometres beyond the shoreline where paedogenic pyrite sediments were found several kilometers away from the shore (Enio *et al.*, 2011; Ishaq *et al.*, 2013). Moreover, this has also evidenced by the occurrence of ridges (up to 4 km away) Parallel to the shore (Roslan *et al.*, 2010) and interface of salt and fresh water several kilometers away from the shore (Samsudin *et al.*, 2008).

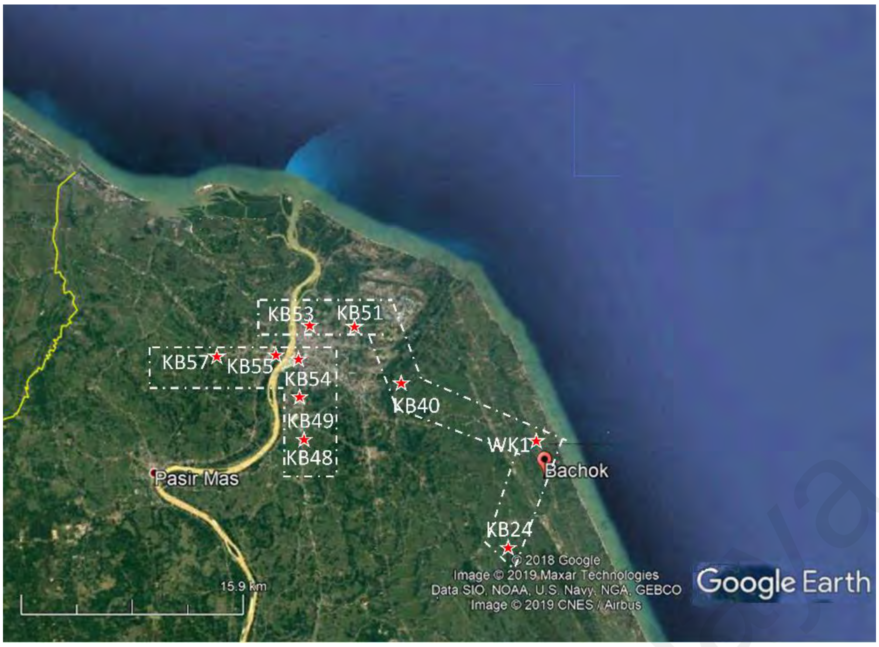

Figure 3.1: Sampling sites
Citation report graphic is derived from ©2018 Google image © 2019 Maxar Technologies SIO, NOAA, U.S. Navy, NGA, GEBCO.

3.3.2 Water and soil sampling

The soil samples were collected from Bachok at three sampling points (with their respective coordinates as;- N 06.08851: E 102.38123, N 06.08898: E 102.38110 and N06.088741: E102.381702) , all located less than 1.5 km from the shore. Briefly, the soils were sampled with the aid of augur at depth interval of 15 cm; and composite samples were blended from individual soil layers in order to mitigate the effect of heterogeneity (Enio *et al.*, 2011). Since the available information indicates that extended locations within the area are not threatened with nitrate pollution, a location known for tobacco plantation was chosen for the study. The three different sampling points were randomly chosen, and the coordinates of the exact locations were determined and recorded with the aid of Garmin GPS Meter.

Having known that soil distribution pattern in relation to its mineral composition is not homogeneous, the desire to study the soil while mitigating the effect of soil heterogeneity informed the researcher to constitute a composite soil samples by blending samples from layers with equal depth across the locations. This approach is advantageous in mitigating wide variation and is supported and implemented by several researchers (Dodla *et al.*, 2008; Hunt *et al.*, 2004; Sicardi *et al.*, 2004; Whalen *et al.*, 1990). More so, the practice is supported by the European soil sample guidelines (Theocharopoulos *et al.*, 2001) Samples taken at the first 15cm each from the three wells locations were blended to form composite L1, followed by the ones taken between 15-30 cm as composite L2 *etc*.

In accordance with Mansor *et al. (2012)* method, water samples were taken through a screened piezometer with the aid of pump. Water samples for the determination of anions (NO_3^-, SO_4^{2-}, Cl^-, PO_4^{3-} , and F^-) and cations (Fe, Mn, and Cu) were taken after bleeding the well with as much as the volume of the well. To mitigate effect of aquifer heterogeneity, samples of the three wells were mixed to form composite sample for a sampling period. The water for cations determination was sampled in a polyethylene bottle, acidified with HNO_3 to a pH less than 2 (Trang *et al.*, 2005) and kept in a cold box prior to the analysis.

3.3.3 Selection and treatment of carbon source materials

Three seeds were identified for trial. Two seeds (Date seed and Tamarind seed) were selected on account of their hard texture and condensed cellulose/mannan content as reflected in previous literatures. The third seed (*Moringa oleifera* seed) was selected because of its role in water purification process and soft carbon source, which can provide easy source of carbon at the onset of the reaction. A decision of splitting the seeds into three segments was deemed important because of the observation of excessive pigmentation in some seeds (Tamarind) and high amount of sulfate in others (*Moringa* *oleifera*) when extracts of the whole seed were subjected to analysis. The three segments were the outer seed coat, middle seed coat and inner layers (cotyledon).

3.3.4 Selection of column materials

The materials that are used in the column are the soil obtained from Bachok, the date seed obtained from Chowkit Arabian shop, *Moringa oleifera* seed obtained from Moringa tree at Serdang, saw dust/wood chips obtained from the university environment and pellets of limestone obtained from the Department of Geology, University of Malaya.

3.4 Experimental procedures

This section comprises of procedure for data collection, pre-processing, data analysis and general analysis for the parameters in the research.

3.4.1 Field samples

These include soil and water samples taken from the field for evaluation of denitrification pattern in the field. The procedure for the analysis carried are standard procedures which comprises of;

3.4.1.1 Microbial test

To ascertain the presence of nitrate reducing bacteria in Bachok soil, Sigma Aldrich procedure of nitrate reduction test is employed (content Qty, 2013). In line with the procedure of the test, portions of the soil were inoculated and cultured for microbial growth on a nutrient agar. Colonies of the microbes were further inoculated and incubated at 37°C for 48 hours in a test tube. The test is conducted by adding 2-3 drops each of both reagent A (containing 0.8% sulfanilic acid in 30% acetic acid) and reagent B (N,N-dimethyl-α-naphthylamine in 30% acetic acid). Observation was then made for quick development of red colour, which indicates reduction of nitrate to nitrite. In the whole test, there was no notice conversion of pink to dark red colour development after adding the zinc powder, which would otherwise indicate negative result. In accordance with the principle of the test, observation of no colour change after the addition of Zn powder connotates positive result as complete denitrification is inferred by conversion of nitrate to nitrogen gas (N_2).

3.4.1.2 Soil digestion

The soil digestion was carried out with the aid of microwave, where 0.5 g of dry soil sample in 10 ml extract of HCl/HNO_3 in the ratio of (3:1). The procedure is applied in accordance with ISO Standard 1406 guidelines (Popescu *et al.*, 2013).

3.4.1.3 Soil texture

In order to predict the seepage pattern in the soil (from the surface to saturated level), a stratified soil texture analysis was conducted using mechanical sieving method (Gee & Bauder, 1986). The dried soil samples were screened to remove any root or plant materials, after which 10 g of each samples was transferred to the tray and agitated for about 10 min. The fraction of soil retained by each net was then weighed and recorded.

3.4.1.4 Anion and cation analysis

To determine the background anion concentration in the soil, the sonicated aqueous extract of the soil (obtained by applying 2 g in 20 ml of DI water) was filtered and subjected to IC analysis. Water samples were directly subjected to IC analysis after passing them through 0.45 μm ptfe syringe filters. The anions were determined using standard procedure of Dionex IC 1200 (Scientific, 2012). In accordance with manufacturer's guidelines, the IC was operated while allowing a long acquisition period aimed for stabilization. The eluents were prepared by transferring 8 ml of 0.5M $NaCO_3$ and 1 ml of 0.1M $NaHCO_3$ into 500 ml volumetric flask with DI water and top up to the mark. A serial dilution of certified reference material was made to a concentration of 0.2, 0.4 and 0.6 ppm, respectively. Injection of 1ml (DW), std1, std2 and std3 through 0.45µm filter was sequentially made at a program time of 15 min. The program time was limited to 15 min because of the retention time coverage of the anion of interest- NO_3^-, NO_2^- and SO_4^{2-}. The test for denitrification-associated cations in the soil viz -Fe, Mn and Cu was performed on the extracts wherein the resulting digested sample was measured using Perkin Elmer AAS 400 (Popescu *et al.*, 2013).

(i) Instrument validation technique

To ascertain the precession of the instrument using the same method of measurement, precession test of intra-day and interdayinterday were employed. A prepared concentration from the standard reference material (SRM) was chosen and consecutively ran through the IC three times to elucidate the interday variation. The same standard solution was run for three consecutive days to identify loss of sensitivity with time and precession of the machine for intraday assessment.

3.4.1.5 Organic matter and total organic content

The organic matter content of the soil was determined using loss on ignition method (Heiri *et al.*, 2001). The total organic carbon (TOC) in water was measured using standard procedure with SSM-5000A SHIMADZU total carbon analyzer while that in soil was obtained by using conversion factor of 2.5 from the value of organic matter (Nelson & Sommers, 1996).

3.4.2 Data collection

For the samples taken from the field, the datasets were derived from two sources: (1) the field and laboratory analyses of samples from three sampling sites at Bachok, and (2) records from periodic monitoring of wells distributed across the region by Mineral and Geoscience Department, Malaysia (MGD).The remaining data were generated from the laboratory analysis obtained through test and characterization of materials. The combined data is given in (Appendix A).

3.4.3 Data pre-processing

Only the data used for multivariate analysis was pre-processed. In accordance with the definition of outliers, in which the influence of a data changes the estimate of the parameter of their group by more than 10% (Ghosh & Vogt, 2012), five readings were observed to be critical outliers. Hence, the outliers values were normalized by group average (Troyanskaya *et al.*, 2001) and the obtained average values was applied in the calculation.

3.4.4 Statistical tools and data analysis

The hydro-geochemical dataset was processed with Microsoft® Excel 2010 and multivariate analyses i.e. principal component analysis (PCA), hierarchical cluster analysis (HCA), and linear discriminant analysis (LDA) were carried out by SAS® JMP Pro 12.

3.4.4.1 Principle component analysis (PCA)

PCA was performed to explore the latent pattern in the pre-processed dataset. In this study, the normalized data matrix was decomposed into a collection of scores and loadings which, respectively describe the relationships between samples and between the hydro-geochemical variables (Koki *et al.*, 2017). It has been established that physical, chemical or biological interpretation of data is made easy using PCA (Alberto *et al.*, 2001). The tool is also used for revealing seasonal pollution character in study sites (Zhang *et al.*, 2009b).

3.4.4.2 Hierarchical cluster analysis (HCA)

Two-way HCA was carried out to interpret the natural groupings corresponding to the hydro-geochemical variability. The dendrograms were constructed according to Ward's method based on the Euclidean distance (Idris *et al.*, 2017). The relevance of HCA is derived from the fact that it can be used for characterizing water quality and hydrogeochemical evolution (Hussin *et al.*, 2016).

3.4.4.3 Linear discriminant analysis (LDA)

Linear discriminant analysis (LDA) was attempted to enhance the separation between the predefined classes using Mahalanobis distance.

3.4.5 Extraction procedure in seed materials

In order obtain the background concentration of the ions in the seeds, 2 g of the seed was transferred into 20 ml of water at ambient temperature. The ultrasonic machine was set at operating frequency of 40 kHz for 30 min. A duplicate samples of the seeds was subjected to digital shaker agitated at 570 rpm for 30 min (Baharvand *et al.*, 2014). Physical observations of the extracts in the two methods and pH measurements were made and recorded. Data from the Ion Chromatography and water adsorption potential were used to determine the most suitable method of extraction and seed amongst the seeds.

3.4.5.1 Nitrate adsorption capacity

Nitrate adsorption capacity of date seed and tamarind seed was measured by introducing 25 ml of 100 ppm KNO_3 solution into 50 ml flask containing 25 ml of water and 2 g of the seed. The extraction procedure was then followed before the absorption measurement using IC.

3.4.5.2 Impact of pH adjustment on the adsorption

To measure the effect of variation of pH on adsorption capacity, 0.5M HCl and 0.5 M KOH was introduced into two separate date seed extraction media at the ratio of 1:20 (v/v). The resultant solution was then taken to IC for analysis

3.4.6 Geometric modification using trigonometric means

The procedure under this section is purely mathematical. This is because the section is hypothetical generation of geometric configuration of mathematically optimized funnel and gate design. The result is therefore mathematically generated using trigonometric principle.

3.4.7 Column tests and materials

A multi-approach to heterotrophic autotrophic denitrification (HAD) is taken, wherein combination of organic and inorganic electron sources are incorporated into the reactive media. A method of parallel packing structure in series column arrangement is employed. The following are the procedures taken for treatment and formation of the column.

3.4.7.1 Preliminary treatment of the gate material

The obtained hard seeds (date and tamarind seed) were crushed to irregular shapes in sizes ranging from 3 mm to 5 mm. After washing to remove impurities, the seeds were blended with the soil analyzed. The soil composite was applied to provide optimum permeability, Fe-based mineral (for autotrophic denitrification) and serve as a supporting medium for all the material throughout the column. Percentage composition of the materials were gravimetrically measured after considering the density of each material.

3.4.7.2 Experimental design and column setup

The packing structure of the reactive media in the column is designed to simulate the cross-sectional area of the novel funnel and gate design. Since the remediated water would get back to the aquifer after treatment, a short column is introduced to simulate the immediate pristine aquifer region after exiting from the structure (i.e down the gradient). Hence, two opaque PVC columns of 4.9 cm internal diameter each, with the first having 100 cm and second 50 cm height were assembled. The first column is set to simulate the reactive media while the second one simulates the attenuation pattern at the immediate aquifer region after the PRB. The effluent of column 1 is made to be the influent of column 2 and the influent water in both columns were administered in an up-flow mode with the aid of multiport peristaltic pump (Figure 3.2). In addition, a separate column serving as the control column was packed with soil only and run. The first column was categorized into four different compartment whose composition were varied to attain effective remediation. Each compartment is separated with pea gravel and diaphragm of thin cloth material to reduce mixing between the layers and facilitate sampling.

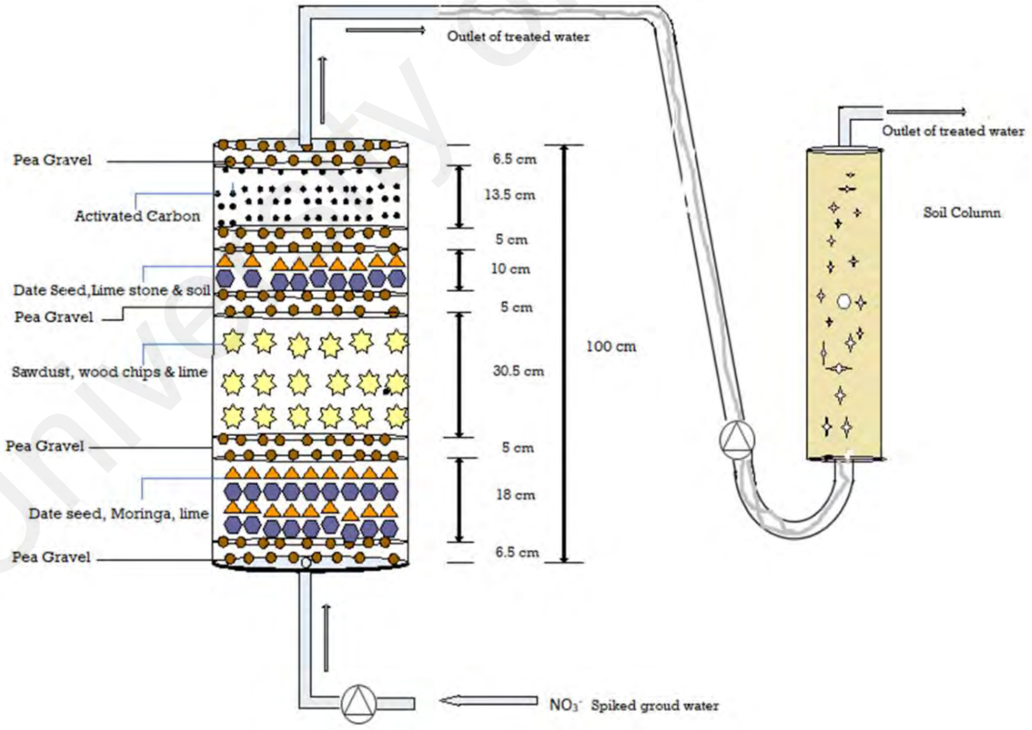

Figure 3.2 : Column Design and assembly

Figure 3.4 shows that from bottom up, the first compartment (A) consist of 18 cm long mixed layer packed with *Moringa oleifera*, soil, date seed, and limestone in the ratio of 1.0: 115.4:50.5 and 24.3, respectively. The second layer (B) is 30.5 cm long, and consists of mixture of soil, wood chips, saw dust and limestone in the ration of 32:3:1: and 5.2, respectively. The third (C) and fourth (D) compartments constitutes 35% of the total column height. With the third layer (10 cm long) consisting of a mixture of soil, date seed and limestone at 8.3:1.7:1 ratio, and the fourth (13.5 cm long) constituting of activated carbon and soil in ratio 1: 1.6, respectively. The pore volume and porosity were measured to be 693 cm^{-3} and 43.6 %, respectively. From bottom up, five sampling points were punctured along the column height at 3.25 cm (influent), 27 cm (1st layer), 62.5cm (2nd layer), 77.5 cm (3rd layer) and 96.75 cm (4th layer).

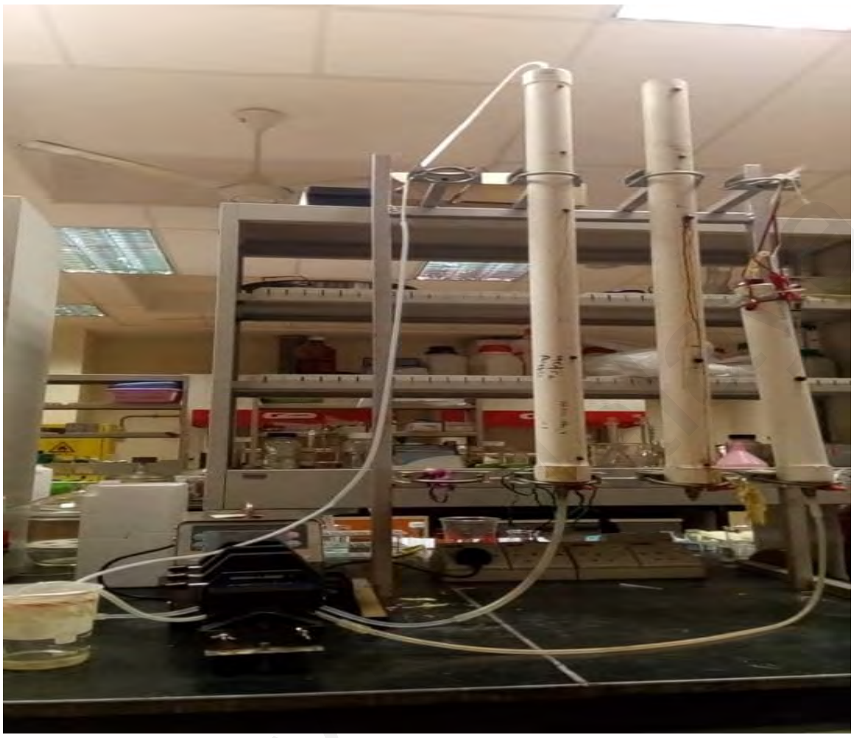

Figure 3.3: Assembly of the Column

The column was initially loaded with distilled water to flush out adsorbed materials, attain acclimatization and determine pore volume, porosity and leachable solutes. After the effluent was tested to attain relative equilibrium of anions, the system was loaded with nitrate spiked distilled water and run for three weeks before loading nitrate-spiked groundwater (Feleke *et al.*, 1998). In both column, water samples were abstracted from the influent port, four of the intermediate sampling ports (P_1 to P_4) and effluents port (P_5) for analysis.

3.4.7.3 Alignment of the column packing structure with the PRB design

The design of the PRB in which the sequence multi barrier reactive media is expected to align with is a funnel and gate system design. Most research dwell on either the funnel structure or the gate fitting or gate structure alone. Unlike most of researches, this study attempts to align the packing structure of the gate with the funnel configuration. This is done with the purpose of maximizing the remediation potential for attaining desired remediation goal. To this end, the packing structure had the first layer representing funnel arm's gate (A), followed by the second layer representing the wood chips parked cavity(B) and the third (C) and fourth (D) layers representing the lower central gates at the tail end of the funnel (Figure 3.4 (R.) and (S)). During the column analysis, the order is reversed since the influent was loaded from the bottom up direction (Figure 3.4 Q).

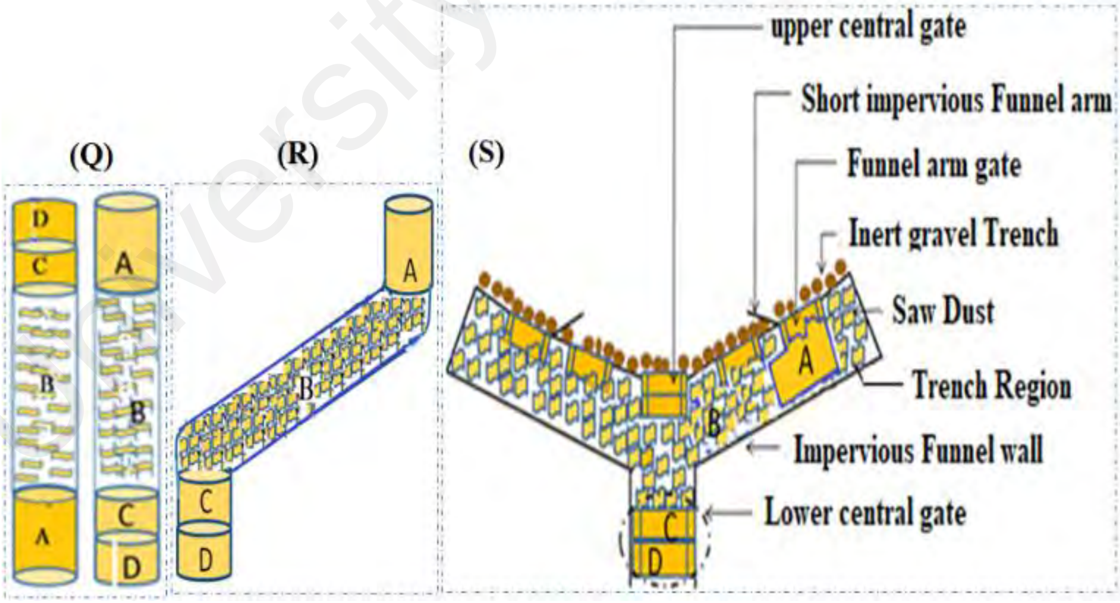

Figure 3.4 Order of the sequence multi-barrier reactive media in the funnel & gate. (Q) the column packing structure (R) the alignment of the column packing structure on to the geometry of funnel and gate, and (S) The funnel and gate

3.4.7.4 Analytical techniques employed in column analysis

The fact that microbially-aided changes is dynamic necessitates a fast method of determining the parameters. Dynamic parameters such as PO_4^{3-}, Cl^-, SO_4^{2-}, $NO_3^-$and NO_2^- were measured using Ion Chromatography Dionex method while temperature and pH were measured with the aid of temperature measuring Mettler Tolledo pH meter, respectively. The instrument's (IC machine) method validation was conducted by running intraday and interday analysis. Total organic carbon (TOC) was measured using SSM-5000A Shimadzu Total carbon Analyzer (TOC analyzer). Volumetric and gravimetric method was employed to measure the pore volume and porosity of the packed column. Differences in the morphology and functional group appearance between fresh hard carbon sources (date seed & wood chips) and used ones are explored by interpreting FESEM-EDX and FTIR result to predict longevity potential and degradation pattern. Analytical weighing balance, ovum and desiccator were used for the gravimetric determination of mass loss in litter bag technique.

3.4.7.5 The rate of decomposition

Mesh bag technique was employed with adjustment on tagging and distribution (Wider & Lang, 1982). Date seeds and woodchips were dried at 60ºC for 48 hours before usage. In each set, efforts were made to cut date seeds into two equal halves (by weight) as close as possible. A combination of date seeds that gives total mass of 1 gram was chosen for degradation study while the endosperm content of its complementary half was carefully scratched off, leaving the hard cover of the seed alone. In either case, each portion was weighed and recorded. The weights of the wood chips were also measured to be at the boarder of 1 g. The same treatment was applied on controlled samples, which were introduced in a column packed with only soil but fed with un-spiked groundwater. This was to assess the influence of the nutrient on the degradation pattern of the carbonaceous solids.

The weighed scratched and intact seeds as well as pieces of wood chips were individually wrapped in a separate permeable mesh litter bag and placed in two chambers of the column (2nd chamber for the wood chips and third chamber for the date seed) and labelled. Each one of the one equal half was brought out at a time interval (end of every month) and weighed again after screening (removal of soil) and oven drying at 70ºC for 24 hours (Wider & Lang, 1982). In accordance with Fisher (2004) technique, the result was expressed as percent of dry mass loss from each sample, which is expressed as the [(Initial – Final)/Initial) * 100]. The rate of decomposition was determined using single exponential model and double exponential model (Kurz-Besson *et al.*, 2005; Mackensen *et al.*, 2003).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

The results and discussions of this research work is grouped into four sections; field study, carbon source study, design and mathematical simulation study as well as aquifer simulation (column set up) study.

The first section (field study) deals with the discussion on the results obtained from field the analysis, wherein using three different chemometric techniques, the researcher proposes to evaluate the variation pattern and identify the most influential parameters in determining the temporal and spatial hydro-geochemical changes. Concurrently, the method reduces data dimensionality while predicting sources. Special emphasis is given to the proximity of the sampling point to South China Sea. This, as observed by researchers has influence on the quality of groundwater (Alberto *et al.*, 2001; Sefie *et al.*, 2015).

The second section of the discussion dwells on screening three seeds (*Moringa oleifera* seed, Tamarind seed and date seed) to assess their suitability for usage as carbon source in PRB. The task was achieved through investigation of the materials for the presence of the target analyte (NO_3^-), and/or any objectionable radical using Ion chromatography. Being handy in aqueous region extraction, Ion chromatography (IC) provides faster approach for screening of the materials.

The third section provides discussion on theoretical and mathematical justification of proposing double-sided trench, alternate gates and twin-funnel and gate design. Discussion on hypothetical construction of the proposed structure for possible exploration in in-situ remediation technology is presented. The trenches owed their significance from previous work, where voids were filled up with inert and porous materials (Bürger *et al.*, 2007). The alternate gates were considered for attainment of equilibrium between the incoming and outgoing flow without compromising residence time while achieving optimum composite capture zones (Careghini *et al.*, 2013; Starr & Cherry, 1994). Finally, the twin funnel was proposed to give room for a combination of parallel and series gate arrangement in a typical heterotrophic autotrophic remediation structure set-up. The model is designed through exploration of trench and gate (Bowles, 1997), multiple gates (Burger & Finkel, 2005; Hudak, 2008), heterotrophic autotrophic approach (Della Rocca *et al.*, 2007; Zhao *et al.*, 2011; Zhao *et al.*, 2012) and permeable reactive interceptors (PRI) concept (Fenton *et al.*, 2014).

4.2 Research findings from the field work

The selection of the sampling location is governed by the fundamental quests of finding the influence of the soil formation/necessary condition on denitrification potential (Roy *et al.*, 2017). The following are the research findings from the field study.

4.2.1 Common geological/environmental factors

The common environmental factors shared by the coastal sampling locations are; relative proximity to sea, agricultural activities in or around the site, high temperate condition, heavy precipitation and presence of sea ridges at different level (Roslan *et al.*, 2010). The soil formation pattern is envisaged to be the same, but the period of formation differs and hence, the record of different soil matrix (Raj *et al.*, 2007). Consequently, the uniformity of soil formation pattern ensures a relatively ubiquitous distribution of common mineral across the area and hence, similar behavioral pattern is expected. Literature indicates that the Bachok area falls under Metang Gelugor member of Gula Formation, which is a Holocene marine unit (Hussin *et al.*, 2014; Suntharalingam, 1983). In such coastal sites, the soil is associated with shallow marine deposition, consisting of dead particulate organic materials known as detritus (Biddanda & Pomeroy, 1988; Kefu

& Tegu, 2009). This makes the soil easy target for heterotrophic activity (Ibrahim *et al.*, 2015; Korom, 1992).

The in-land sampling sites share the same characteristics of heavy precipitation and temperate condition with the coastal sites but falls under geological setting of mixed Pleistocene and Holocene continental deposit. More so, the dominant source of carbon in the soil is plant fossils and no influence of sea littoral drift on the soil formation has been recorded (Ishaq *et al.*, 2013). It can then be deduced that there is a marked difference in the matrix of organic content between the coastal site and inland sites.

4.2.2 Nitrate reducing bacteria/soil matrix

The qualitative test of nitrate reducing bacteria gave positive result before and after adding zinc powder, indicating the presence of partial and complete reducing microbial species in the soil, respectively. This is because while some tubes developed red colour, some did not change colour even after the addition of Zn powder (content Qty, 2013; Zomorodian *et al.*, 2016). This indicates multiple activities at different stages within the soil, and, as explained in section 4.2.6, many factors can be responsible for such variation.

The results of soil texture analysis are shown in Table 4.1 indicating that the horizontal distribution of soil particle size in the paleo beach site consist of 87% of 69-212 μm soil size, which is categorizes under fine sand (Gee & Or, 2002) (Figure 4.1). However, going by the textural triangle for soil using US Dept. of Agriculture (USDA), the soil having a cumulative % of 3.43 clay, 50.6 very fine sand and 28.6 fine sand is sandy loam. This mix attributes are envisaged to give it excellent filtration property as soil particles with fine and uniform diameter dominates the matrix.

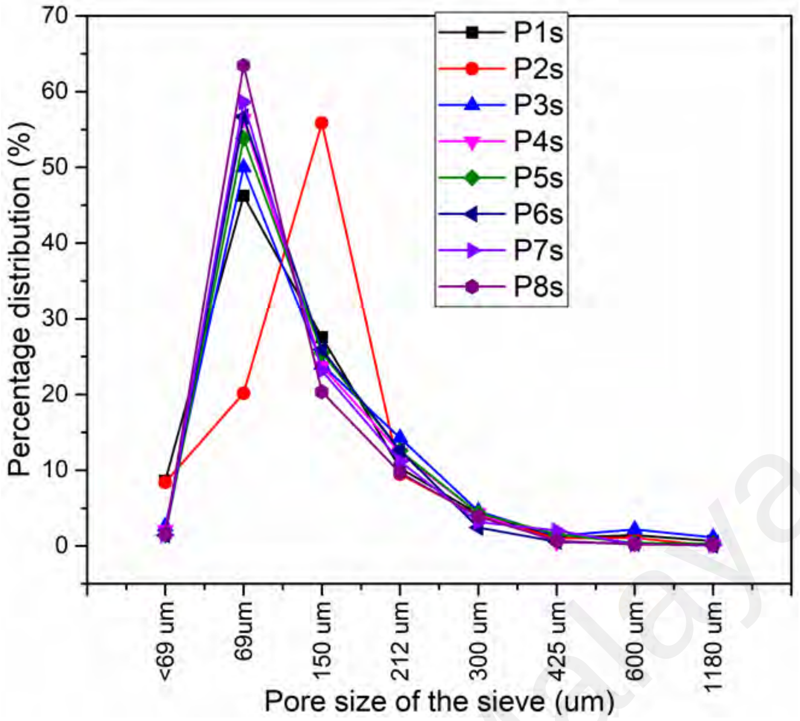

Figure 4.1: Soil texture distribution across layers with depth

Table 4.1: Percentage distribution of Soil texture (International Soil Science Society)

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			Very fine Sand (0.045-0.1 mm)			Fine sand (0.1-0.25)		
SP	<69 µm	69 µm	150 µm	212 µm	300 µm	425 µm	600 µm	1180 µm
P1s	8.5832	46.245	28	10	4.201	1.063	1.404	0.642
P2s	8.3917	20.174	55.9	9.5	3.971	1.04	1.08	0
P3s	2.5923	49.967	23.9	14	4.592	1.326	2.184	1.129
P4s	2.0787	56.366	23.7	12	4.358	0.505	0.397	0.224
P5s	1.4666	53.842	25.5	13	4.36	1.54	0.385	0.315
P6s	1.4069	56.698	25.9	13	2.432	0.576	0.255	0.044
P7s	1.4753	58.607	23.2	11	3.173	2.037	0.241	0.138
P8s	1.5185	63.469	20.4	9.7	3.886	0.689	0.203	0.203

4.2.3 Vertical distribution of denitrification parameters in Bachok soil

Figure 4.2 demonstrates the plot for the distribution of denitrification-relevant parameters across the soil layers taken. While nitrate, sulfate and TOC values are high on the topsoil (P1), the concentration of Fe and Mn are relatively low (Table 4.2). This is well expected due to agricultural practice in the area, which ensures fertilizer application (Kaown *et al.*, 2009) and humus found on top soil (Azlan *et al.*, 2012; Haynes & Naidu, 1998; Roslan *et al.*, 2010; Zanella *et al.*, 2011).

Figure 4.2 shows that as the Fe concentration begins to rise, the nitrate concentration declines. However, both began dropping to the lowest level within layers 3-4. The attenuation of Fe across the layers here is attributed to the mineral distribution in the crust within the soil rather than reductive process in denitrification.

Table 4.2: Distribution of denitrification-related parameters across soil layers

Sampling Point	**Mn (mg/kg)**	**Fe (mg/kg)**	**NO_3^- (ppm)**	**SO_4^{2-} (ppm)**	**TOC (%)**
P1s	25.80	319.00	10.55	47.99	2.12
P2s	57.40	1333.00	*9.63*	29.37	1.47
P3s	51.60	1129.00	*2.04*	16.70	0.69
P4s	2.60	146.70	*1.31*	1.19	0.36
P5s	0.10	13.70	*1.19*	4.87	0.52
P6s	119.00	1332.00	*9.22*	5.67	0.62
P7s	105.60	1351.00	*0.42*	3.47	0.37
>1m	100.70	1361.00	*0.94*	9.10	0.47

This is because if autotrophic denitrification were taken place, the Fe would remain high (Rysgaard *et al.*, 2001). More so, the top soil is rich in oxygen, which is preferentially utilized over inorganic electron donors like Fe and Mn (Scherer *et al.*, 2000). The nitrogen transformation process from NO_3^- to either NO_2^- , N_2 or ammonium through denitrification, fixation. ammonification and nitrification-is another factor of consideration (Westermann & Ahring, 1987). However, both NO_3^- and Fe shoot up at layer 5 to 6, only for NO_3^- to decline from layer seven onward, leaving the Fe concentration high. This suggests autotrophic denitrification activity especially that the TOC value is low at the point.

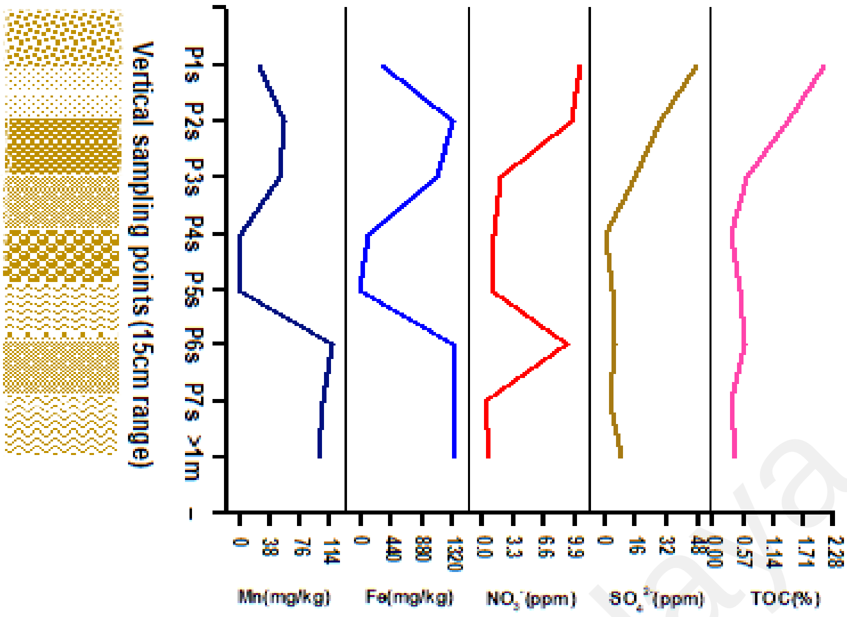

Figure 4.2: Vertical distribution of key players in denitrification across soil layers

Since contaminant reduction at the iron surface stimulates microbial growth and reductive bio-transformations through the production of water-derived H_2 (Da Silva *et al.*, 2007), it is hypothesized that the more closer the pollutants are to the vadoose zone, the more likely it is that autotrophic denitrification will occur. Two possible scenarios can be deduced from the pattern of the above results;

1. A combination of heterotrophic denitrification (to a lesser extent) and NO_3^-interconversion to ammonium from P2-P5, causing the sharp decline of NO_3^- concentration within the layers. The attenuation pattern of sulfate decline is similar to that of TOC (Figure 4.2), suggesting a possible indication of its competition with NO_3^- for degrading the organic matter (Canfield *et al.*, 1993) and hence, paving way for possible conversion of NO_3^- to other forms of nitrogen (Westermann & Ahring, 1987). The possible occurrence of heterotrophic denitrification is attributed to presence of long-aged marine deposition at coastal plains, including detritus (Biddanda & Pomeroy, 1988).

2. Autotrophic denitrification from P6 and above because of sharp drop of NO_3^- concentration, sustained high Fe and Mn concentration and low level of TOC. The re-emergence of NO_3^- at P6 is attributed to nitrification within the medium as nitrate is more stable amongst the nitrogen species (Kurtén *et al.*, 2008; Sebranek & Bacus, 2007; Siu & Henshall, 1998)

It is logical to assume that at P1- P5, the geochemical condition is not favorable to the facultative microorganism for anaerobic reduction and hence their activity is witness at P6 and above. The sampling layer (P6 and above) has high moisture, which encourages Fe corrosion for hydrogenotrophic denitrification (Autotrophic). This prediction is supported by the average ORP values of 485 mV for the three wells, providing favorable condition for the microbes (Suslow, 2004). So, a natural combination of heterotrophic denitrification (through the utilization of organic detritus) and autotrophic denitrification (through the anaerobic corrosion of Fe-bearing mineral in the soil) is hereby deduced to have occurred and hence responsible for the observed high nitrate attenuation in the area. This conclusion is supported by finding of higher denitrification on pyrite embedded soil (Zhang *et al.*, 2009a).

4.2.4 Hydro-geochemical variation of the sampling sites

Figure 4.3 shows the bi-plot obtained from the PCA of all the data, regardless of season and location. The two components (PC1 and PC2) explained 50.7 % of the variance in the data set. Despite clear clustering pattern, the scores seem dispersed towards the direction of PC1 or PC2 depending on their hydro-geochemical characteristics that possibly related to their spatial variation i.e. coastal and inland zones. The loading details indicates that conductivity, SO_4^{2-}, Cl^- have considerable weights on PC1; Fe and NH_4^+ on PC2; pH and NO_3^- on PC3. According to the mapping between the scores and loadings, Cl^-, SO_4^{2-} and conductivity are likely to be associated with samples from coastal zones; while Fe and NH_4^+ with inland samples. The former is supported by number of researches around the coastal zones (Hayashi, 2004; Jamrah *et al.*, 2008; Sefie *et al.*, 2015) while the latter is in tandem with researches carried in the area (Ahmad *et al.*, 1996; Dasar *et al.*, 2009). However, irregular trend was observed for the borderline sample that originated from the regions between coastal and inland i.e. sampling well no-53, with dominance in conductivity and SO_4^{2-} particularly during the dry season. The location of the well within a build-up area and industrial complexes suggests anthropogenic impact. Subsequently, elevated concentration of NO_3^- at Well W54 is attributed to anthropogenic inputs for the same reason. The separation of the region in Figure 4.3 is sharp enough so much so that two distinct regions of coastal and inland zones with distributions of loaded parameters are identified and hence, conveniently separated with a line drawn diagonally across the distribution.

Table 4.3 demonstrates how component 1 is associated with positive weighing of conductivity, sulfate and chloride. Component 2 majors in positive weighing of Fe and NH_4^+ while PC3 has pH, NH_4^+ and NO_3^-.

Table 4.3: Eigen vectors of the general data distribution in the area

	Prin1	**Prin2**	**Prin3**
pH	**0.01679**	0.19269	0.64392
Con	0.57705	**0.07191**	**0.04894**
Fe	0.19114	0.63882	**-0.00530**
Mn	0.14847	0.30277	**-0.08416**
NH_4^+	**0.00236**	0.51624	-0.54262
Cl^-	0.50953	-0.31468	**-0.12094**
SO_4^{2-}	0.54040	-0.19867	**-0.14847**
NO_3^-	0.23768	0.23010	0.49473

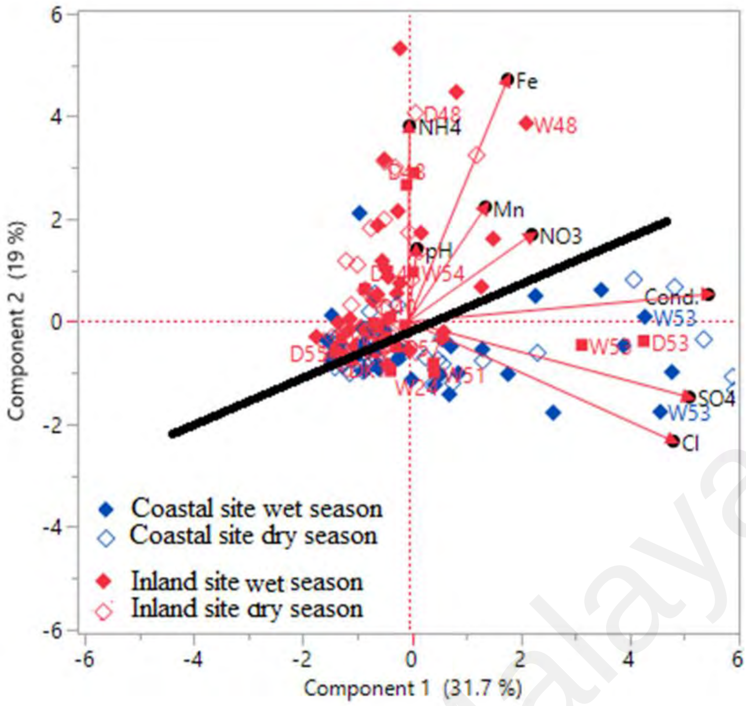

Figure 4.3: Bi-plot for the general variation

The PCA and bi-plot identified two important factors. First, the five significant parameters in monitoring groundwater quality variation within and outside the coastal plain. Second, three significant parameters are considered in the coastal zones (Cl^-, SO_4^{2-} and conductivity) while two (Fe and NO_3^- or NH_4^+) assigned to inland zones. The pH tends to be a strong factor in PC3 but its insignificant Eigen vector in PC1 and PC2 shrinks its loading and hence, not considered. Sampling well W48 at the up gradient position is influenced by high loading of Fe in wet season, indicating the dissolution impact of the season on the iron -rich site (Ishaq *et al.*, 2013; Kamal & Hashim, 2014; Roslan *et al.*, 2010).

The canonical plot (Figure. 4.4) of linear discriminant analysis (LDA) shows the same pattern of apportionment with PCA. The step forward analysis reveals four parameters in order of decreasing significance as $Cl^- < SO_4^{2-}$ <Fe and <conductivity, respectively. This classification supports the decision of disregarding pH in PC3 as discussed in PCA.

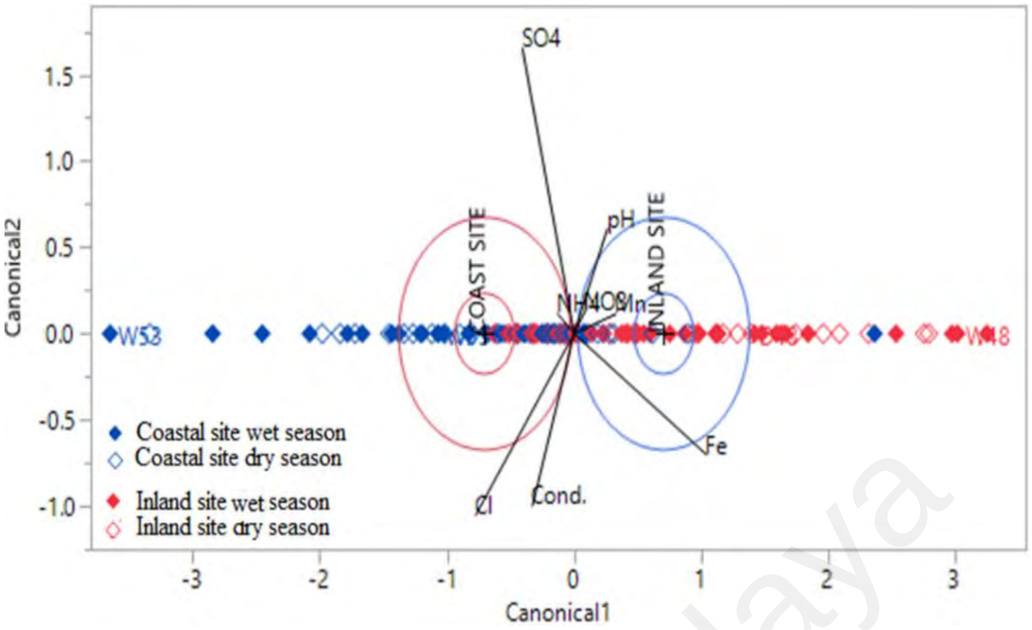

Figure 4.4: Canonical plot for the distribution of parameters across coastal plain and inland site

The two-way HCA dendogram (Figure 4.5) reveals three significant hydro chemical facies (clusters). The first one is intersecting cluster, comprising of wet season for coastal sample WK1, W24 and inland sample W54. The intersect may be justified by high chloride and Fe in sampling well W54 (as indicated in the bi-plot). The second cluster consist of mixed season of dominated in-land upstream wells (W/D48) and downstream well (W57), while the third cluster consist of a downstream mixed season of coastal wells that are situated at the borderline and within industrial zone. This division shows less significant influence of season and affirm the influence of location and activity-induced variation within the site. Hence, the results of PCA, DA and HCA (cluster (b) and (c) are in concordance on classifying the parameters based on the location and identifying the most influential in each location.

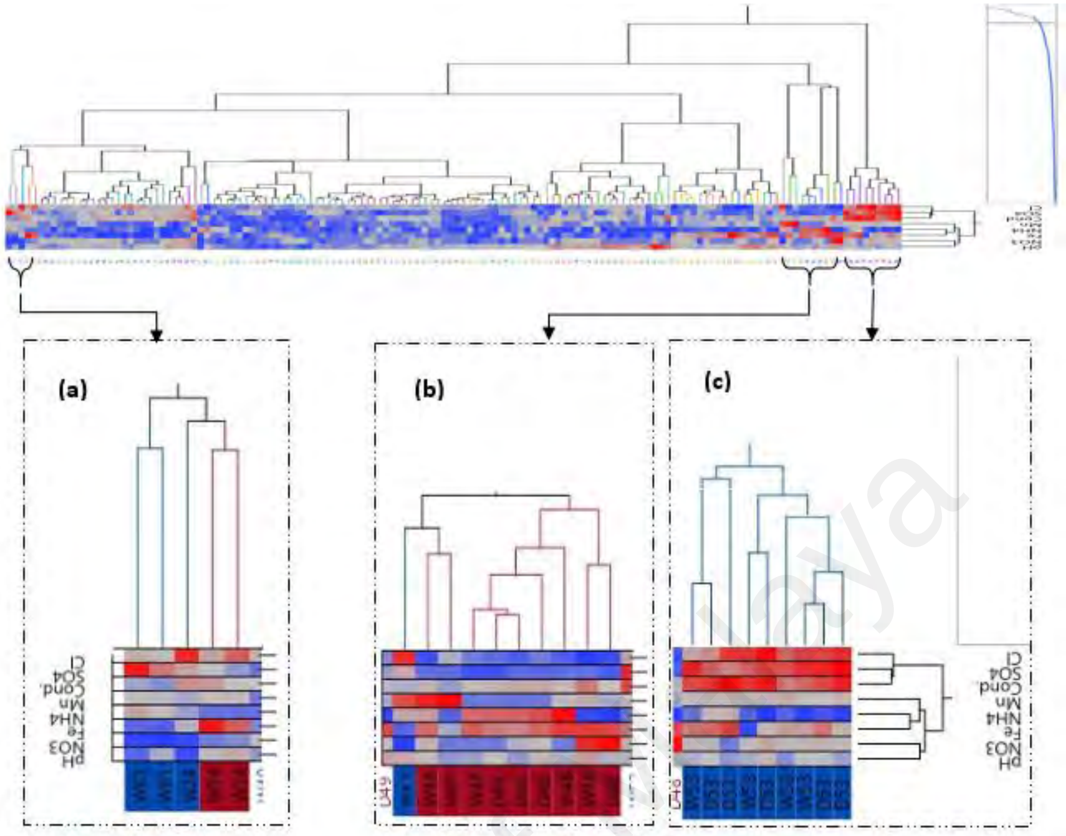

Figure 4.5: Two-way HCA dendogram for the entire sample. (a) intersect cluster (b) upstream inland-dominated cluster (c) borderline coastal cluster

4.2.5 Seasonal (temporal) variation

The seasonal phase was formed by pairing data of different season (wet and dry) obtained from the same site (*i.e* either coastal plain site or inland site). The following chemometric tools are used to gauge the variation pattern.

4.2.5.1 Principle component analysis (PCA) – [seasonal]

Figure 4.6(a) and (b) shows the bi-plot of the variables in inland and coastal sites across the seasons, respectively. Though neither of the pattern of distribution allows for a sharp dividing line between the seasons, conductivity features as a significant factor in each site. Within in-land site, the eigen vectors revealed high loading of Fe in PC1, suggesting the abundance of Fe-bearing minerals in the area. This trend is reported by (Kamal and Hashim (2014); Roslan *et al.* (2010); Tosi *et al.* (2009)) . Also, the significant loading of ammonium in PC1 shows the presence of ammonium-bearing substrates (fertilizers)in the area and, for the same reason, NO_3^- becomes significant parameter in PC3.

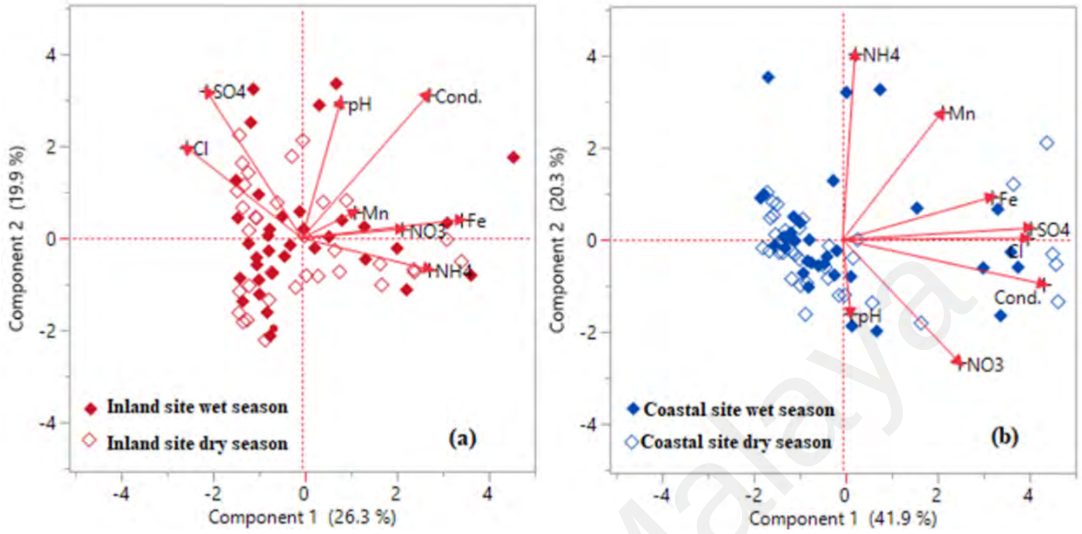

Figure 4.6: Bi-plot for dry and wet season data in (a) In-land (b) Coastal sites

The mineral dissolution process (Hussin *et al.*, 2016; Kortatsi, 2006) in wet season and groundwater recharge withdrawal in dry season is expected to induce redox activities/surge of pH and hence,the significant loading of pH in PC2. The influence of sea water inrusion (in inland site) is less probable due to insignificant loading of chloride.

In the coastal site however, the PC separation in the bi-plot is high, accounting for 62.8% of the total variance as against 42.2% of the in-land site. However, the replication of PC1 significant parameters in the coastal plot and bi-plot of combined seasons suggest that coastal parameters have stronger influence over that of the in land site. Of importance is the significant positive loading of Mn and NH_4 alongside negative loading of NO_3^- in PC2. This entailes depreciative signal of nitrate and possible participation of Mn in autotrophic denitrification (Roy *et al.*, 2017; Rysgaard *et al.*, 2001).

As observed in the general variation, the hydrochemical signature of coastal sites points more to sampling well 53 (D53 &W53) majouring in both Cl^-, SO_4^{2-}, conductivity Fe and NO_3^- . This mixed attribute of coastal (in the wet season)and in-land (in the dry season) features is attributted to its boarderline position, down gradient location and clossiness of the sampling point to both river and industrial site. Ammonium loading in wet season of sampling well WK1 is attributed to debris of marine origin in the soil (Hedges & Oades, 1997; Sweeney & Kaplan, 1980) and heavy agricultural practice in the area. In in-land sampling site however, dry and wet season of sampling well 48 dominates the loading of NH_4^+, Fe and NO_3^- where as well sample W49 and W54 took charge of SO_4^{2-} domination in wet season. It is suggested that the perculier location of sample 48 at the upgradient position expose it to distinguished state of low dilution-influence from the river and hence, it gives true representation of mineral and spertial influence in the area. The loading of chloride observed in dry season of sample D57 is attributed to recharge withdrawal and excessive evaporation (Han *et al.*, 2015).

Finally, the fact that the plot is not distinct enough to demarcate the separation of parameters on seasonal basis shows that the distinction of parameters on seasonal basis is not significant enough to be relied upon for explaining the behavioral pattern of the area**.**

4.2.5.2 Hierarchical cluster analysis (HCA) – [seasonal variation]

Reference to the reasons stated in PCA variation, Figure 4.7(a) shows how well sample D48 exhibit a unique feature that segregates it from the rest of the sampling points. The same projection can be applied to dry season sample D57, which tends to share its charesterestic with samples close to river (D49, D54 and D55) and those far away from it (D48). This behaviour may be explain by the groundwater flow around the area, which was maped-out by Islami *et al.* (2012) to be flowing from east to north west, heading towards Kelantan river. In this way, sampling well D48 is up gradient (hence exhibiting unique characteristics) while D57- being at the down gradient - share its characteristics.

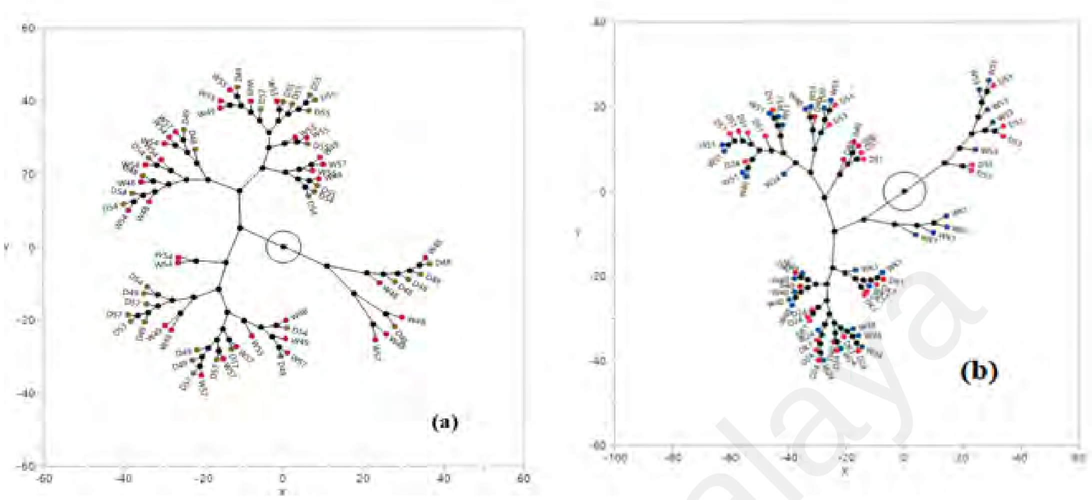

Figure 4.7: Constellation plot of dry and wet season (a) In-land sampling sites
(b) Coastal sampling sites

Sampling well D57 (the furthest away from water recharge) appeared only once at the upper constallation branch, indicating how furthest samples away from water source differ in their properties based on the seasonal variation. Hence, the overlaping manifestation of results in sampling points between the season is postulated to be justified by uniform hydrological condition, high precipitation and common geological formation of the area. This assumption is supported by PCA values of the most influencial parameters in the in-land site as abundant distribution of Fe reflect the common geological influence on the region.

In contrast to the in-land site, the right upper branch of the constellation plot in the coastal site (Figure. 4.7 b) consist of wet and dry season of sampling well 53 (W53 & D53). It can be deduced that the influence of proximity of sample well 53 to the river and its location within industrial zone overshadows the anticipated temporal variation. The grouping of WK1, D24 and D40 is corroborated by better PCA separation of the coastal site explaining up to 62.2% of the total variation.

4.2.5.3 Discriminant analysis (DA) - [Seasonal variation]

As shown in Figure 4.8, the conspicuous intersect of the two seasons in canonical plot justifies the narrow discriminant factors and explain why neither step backward nor forward analysis yield any significant factor of discrimination in coastal sites. In inland site however, Mn is the only significant factor of discrimination. This may be as a result of the observed lower pH record of the dry season, which encourages dissolution of minerals in the soil to precipitate active Mn for Mn-induced denitrification (Chao, 1972). The denitrification indicator is supported by high loading of Mn and Fe (Rysgaard *et al.*, 2001) in the wet season of the in-land site than its corresponding dry season.

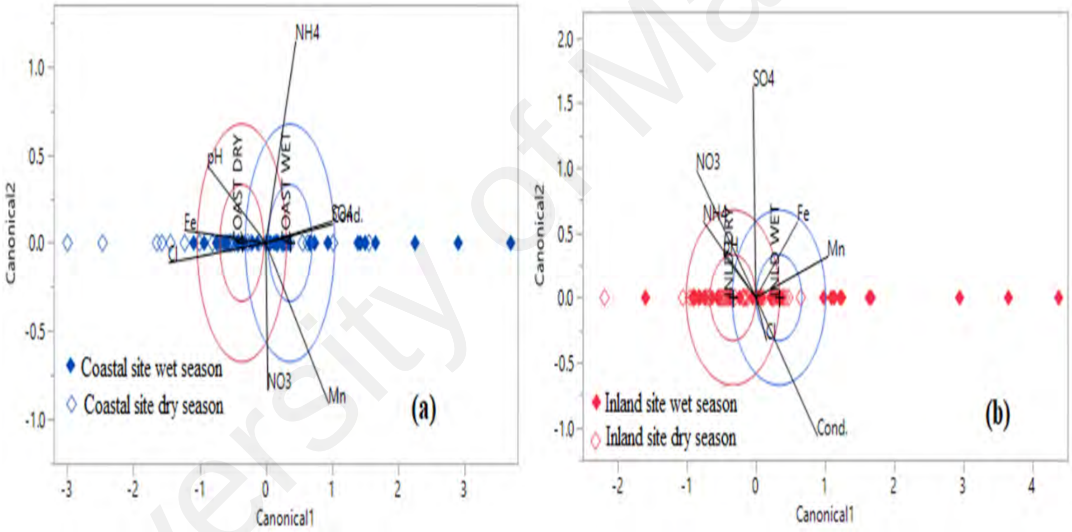

Figure 4.8: Canonical plot for seasonal variation. (a) dry and wet season for inland (b) Dry and wet season for coastal sampling sites

The discriminant analysis of the in-land sites supports PC distribution of the site. However, in contrast to in-land sampling sites, the coastal samples reveal less loading and more centralized classification of NO_3^- and NH_4^+ tilting towards the wet season. This may be due to more agricultural practice that ensure heavy application of fertilizers. Hence, the extent of overlap of wet season distribution into the dry season qualified NO_3^- in PC1 of wet season.

4.2.6 Spatial-based variation.

The pairing pattern of data in spatial–based variation ensures that any observed variation is caused by the hydrogeological characteristic of the site or anthropogenic influence. Formed by pairing data of different site (coastal plain site and inland site) obtained from the same season (either wet or dry season), the following are the chemometric tools used for gauging the variations.

4.2.6.1 Principle component analysis (PCA) – spatial

Figure 4.9 shows the bi-plot of in-land and coastal sites in wet and dry seasons. The separation of the distribution based on the location is so distinct that a sharp dividing line is drawn between the locations in both seasons. PC's extracted with Eigen values >1 explained up to 48.7 % and 56.4% of the total variance in wet and dry season, respectively.

The analysis of the hydro chemical characterization of the two location-based variation in wet season indicates three influential factors for predicting behaviors of groundwater in the area. These factors are; direction of the groundwater flow, soil composition (mineral content) and water recharge source.

Considering the bearing of the groundwater flow, it is logical to observe that upstream sampling point 48 is loaded with only one of the most abundant mineral resources in the area, which is Fe. In the same vain, both the downstream sampling points 54 and 53 measures in conductivity loading with sampling well 53 having dominance in SO_4^{2-} (being at the proximity to both rivers, build area and industries) while sampling well 54 is loaded with NH_4^+ and Mn – indicating the influence of its strategic downstream location and proximity to farming activities). Sampling well 55 that shares less loading of NO_3^- with wells WKI and W24 is attributed to recharge and dilution influence of the river, which aligns with the groundwater flow.

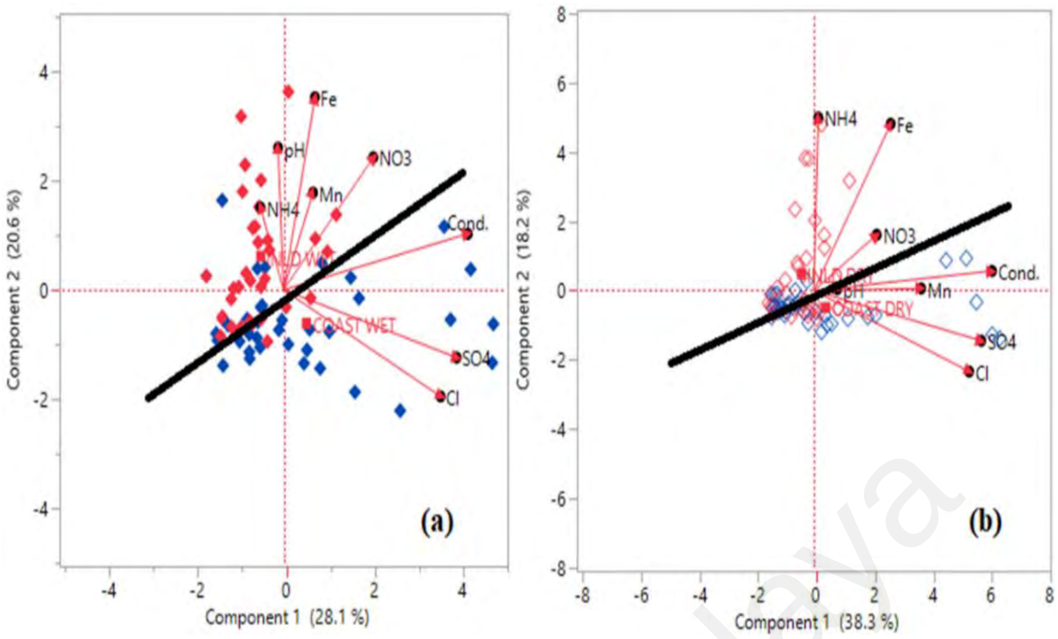

Figure 4.9: Bi-plot for distribution of data in both coastal and inland site (a) wet season (b) dry season

It is pertinent to observe that the domineering influence of sea-associated parameters (conductivity, chloride and sulfate) prevails in PC1 whenever a coastal sampling is involved. It can also be deduced from the dry season that, with the influence of fertilizers in a less diluted condition, the precedence of NH_4^+ significance (in PC 2) over that of NO_3^- (in PC4) is an indication of delayed conversion process. In wet season however, NO_3^- fluctuation manifests in PC2 and PC3 (with negative sign) while NH_4^+ is loaded in PC3. This suggests prompt conversion and denitrification process as the condition is more favorable in wet season.

4.2.6.2 Hierarchical cluster analysis (HCA) –spatial

The right-hand branch of Figure 4.10 (a) consists of down gradient coastal sampling sites of W53 interspersed with up-gradient sample W48, W57 and WKI. As explained by the hydrological signature of the site, well 53 share high loading of conductivity and SO_4^{2-} with well 48. The commonality amongst the two may not be unconnected with pattern of groundwater flow and similar agricultural activities in both locations. Hence, spatial influence could be the unifying factor for this grouping.

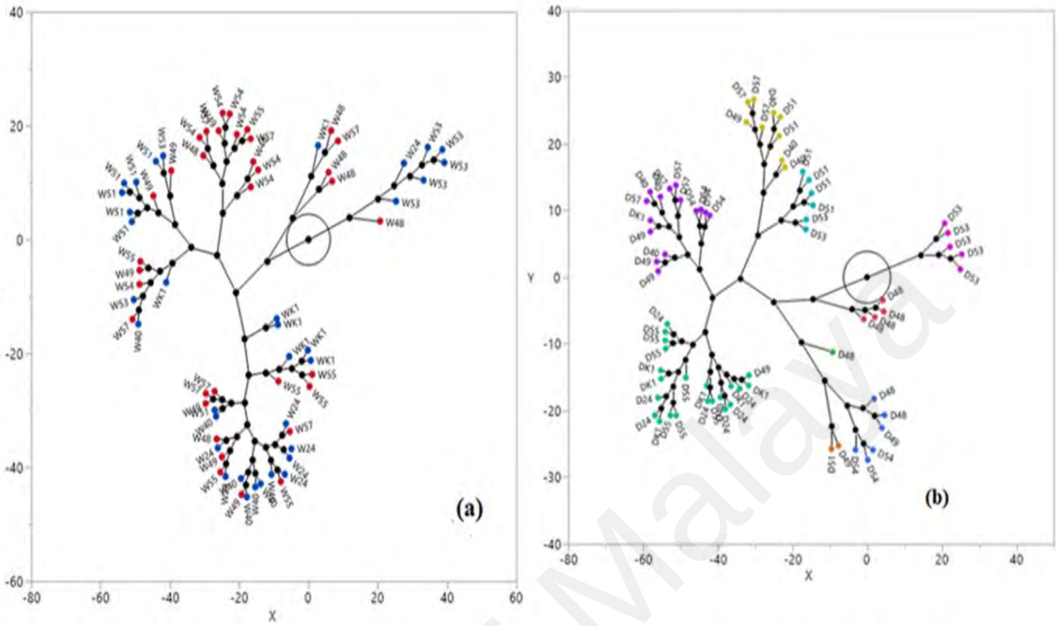

Figure 4.10: Location-based constellation plot . (a) Wet season of coastal and inland (b) Dry season of coastal and inland sites

Lower branch is riffled with coastal–based sampling site (having few in-land sampling sites) while the right-hand side of the upper left canonical branch is dominated with in-land sampling sites. Within the branch of the later however, are more of the coastal sampling wells W53, W51 and W40, which are closer to the in-land sampling sites than the coastal plain. This, as suggested by Roslan *et al.* (2010), indicates the strength of the hypothesis of proximity influence of the sea to the sampling site.

The constellation plot of the dry season in coastal and in-land sites [Figure 4.10 (b)] indicate a sheer similarity in behavior between sample D53 of the coastal site and sample D48 of the in-land site. This may be attributed to groundwater flow pattern and proximity to rivers.

4.2.6.3 Discriminant analysis-spatial

In contrast to season-based discriminant analysis, the location-based D.A differs in majoring on Fe, Mn, NO_3^- and conductivity for coastal dry season, coastal wet season, in-land dry season and inland wet season samples, respectively. The relative apportionment of NO_3^- and conductivity in the inland site shows less attenuation capacity of the nitrogen-based compound in the site. The influence of dilution factor, soil nature and geological formation of the coastal sites tends to support natural attenuation of NO_3^- more than that in the in-land site. More so, the differential manifestation of Fe in dry season and Mn in wet season of the coastal site supports the presence of pyrite formed by sea water inundation (Enio *et al.*, 2011; Roslan *et al.*, 2010) and dissolution influence of wet season. The step forward analysis of the wet season canonical plot revealed parameters in the order of reducing significance from Cl^-, Fe to SO_4^{2-} whereas that of the dry season gave Cl^- and Fe, respectively. This signifies the dominant active specie in wet season is a combination of sea influence and dissolution of mineral substances in the soil. Moreover, the sampling location within which these parameters are loaded suggest that the impact of human activities around the sampling points add to the account of making SO_4^{2-} and Cl^- significant. In dry season however, recharge withdrawal and significant evaporation concentrate residual species (Kortatsi, 2006) thereby making them more prominent.

4.3 Carbon source screening for nitrate remediation

This section presents results obtained from screening the different carbonaceous materials screened for possible application in reactive media for the purpose of denitrification.

4.3.1 Results and discussion

The results of the analysis conducted shows a reliable repeatability and precision of the ion Chromatography (IC). In order to ascertain the precession of the IC machine, instrument validation techniques were employed. Here, the IC spectra of the three measurements (Figure 4.11 and Figure 4.12) for both intra and interday option is superimposed on one another and the Figure shows a very close relation with mild variation on some peak signals. The variation is more conspicuous on interday analysis. Considering the sensitivity of the instrument to the main analyte (NO_3^-) the result (Table 4.4) shows a standard deviation of 0.005954 and 0.01716 as well as average percentage recovery of 97.95% and 95.22% for the intraday and interday, respectively.

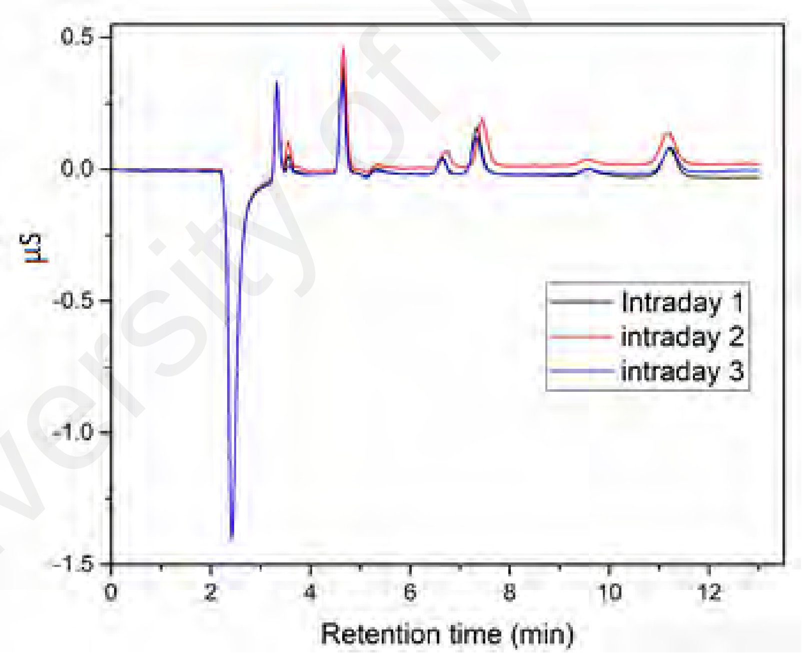

Figure 4.11: IC spectra for intraday instrument validation technique

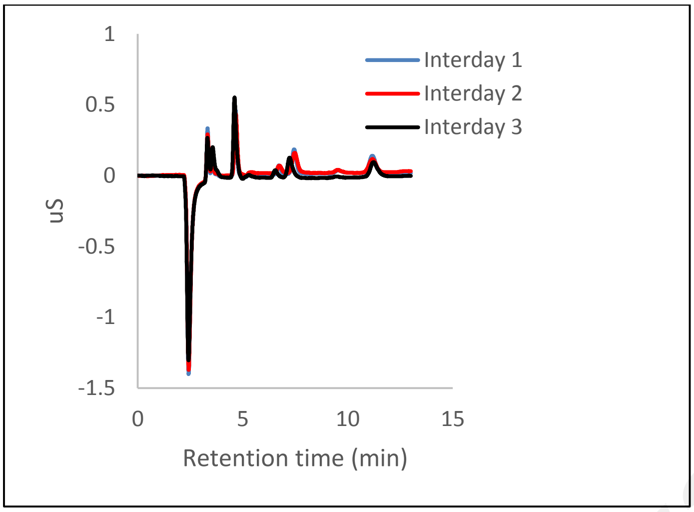

Figure 4.12**:** IC spectra for interday instrument validation technique

Table 4.4: Nitrate concentration (ppm) for intraday and interday

	Intraday	Interday
1st run	0.5542	0.551
2nd run	0.5673	0.5863
3rd run	0.5663	0.6032
RSD (%)	1.7	4.6
Average recovery(%)	97	95

4.3.2 Physical properties of the seed's fractions and extracts

The observed physical properties of the Tamarind seed revealed a more pigmented outer fraction and extracts of the seed cover with less cohesive texture in the cotyledon. The pH values of the Tamarind extract fractions were 8.87, 8.27 and 6.78 for outer, middle and inner extracts, respectively. The date seed showed more uniform distribution of hard texture across the layers and had a pH distribution of 6.2, 5.4 and 5.7 for the three fractions, respectively. The Moringa seed had more layers with the outer one being lighter, and the cotyledon dampened with moisture and air sacs. The pH of Moringa seed were 8.96, 9.2 and 6.69 for the three fractions, respectively. A noticeable odor was perceived in Moringa seed extracts, which intensified to rotten egg smell akin to hydrogen sulfide after two days.

The water absorption potential measured indicated that the date seed had the least propensity to water absorption followed by Tamarind and Moringa seed. However, due hydrophobic nature of Tamarind seed coat, the differential margin of its water adsorption with that of date seed is marginal. The Moringa seed however, tends to lose its texture with time, slowly dissolving away in the aqueous solution.

4.3.3 Effect of extraction method towards anion analysis

The results of the IC ran for the two extraction methods indicated that the ultrasonic-aided extraction had better extraction results than the agitation-aided method. This is due to the recovery of higher concentration of the analyte (NO_3^-) in the ultrasonic aided extraction than in the agitation-aided method. A difference margin of 8-12% between the two extraction methods was consistent, proving the efficacy of ultrasonic -aided extraction over the agitation aided extraction.

Adsorption capacity of the date seed and Tamarind seed for NO_3^- solution was found to be within (15%) only, indicating a poor adsorption potential in the absence of activation. Since the stoichiometric value of NO_3^- concentration in the flask was 50 ppm, it would be logical to determine the contribution of adsorption potential of the raw seeds at the threshold limit value of the analyte (45 ppm). The result, however, shows that the margin of adsorption between the date seed and tamarind seed was negligible and the spectral distribution of anions indicated more objectionable products in Tamarind and Moringa seed than in date seed (Figure 4.13).

4.3.4 Evaluation of the anion distributions across the seed layers

The anion distribution across the seed layers shows a remarkable variation that justifies the separation of layers for proper assessment and evaluation before decision making. It was observed that though, Tamarind (Figure 4.13(c)) has low nitrate concentration of 0.124 ppm, it has high sulfate content of 26.95 ppm. Depending upon the concentration and condition, chloride (eluted at retention time of 4.80 min) is reported to either be insignificant in influencing denitrification (Lancaster *et al.*, 2016) or advantageous (Azam & Müller, 2003). Despite this, the Tamarind seed is considered inappropriate for the purpose due to possible competition with the oxygen bearing anions (Scherer *et al.*, 2000). The chromatogram of Tamarind is also full of multiple untagged peaks at 3.19, 3.80 and 4.52 min retention time (falling within the range of 3-6 min), indicating possible presence of organic acids in the medium (Krol, 2000).

The result in Figure 4.13(b) indicates that *Moringa oleifera* has low nitrate (0.122 ppm), moderate chloride (3.723 ppm) across the layers and medium sulfate (2.927 ppm) on the cover. Considering the assertion of Pu *et al.* (2014) , sulfur containing rocks are useful in heterotrophic denitrification. On this note, it is recommended that, the cotyledon of the seed can be exploited for prompt heterotrophic denitrification. More so, the selection of Moringa seed is supported by its documented role in water treatment as anticoagulant, antimicrobial and ion exchange dispenser (Ghebremichael *et al.*, 2005; Ndabigengesere & Narasiah, 1998). The chromatogram in Figure 4.13 (a) shows the chromatogram of date seed with clear less objectionable anion content. More so, the observed low water absorbtion capacity of the seed is expected to ensure slow decomposition process and hence, longer life span.

Moreover, the high carbohydrate percentage (72%) recorded in dates (Nehdi *et al.*, 2010) is of great advantage. This is because as the carbon mass is condensed in the seed, the microorganism will be assured of a slow but steady supply of the electron source and this means, longer life span of the system (Streitelmeier *et al.*, 2001).

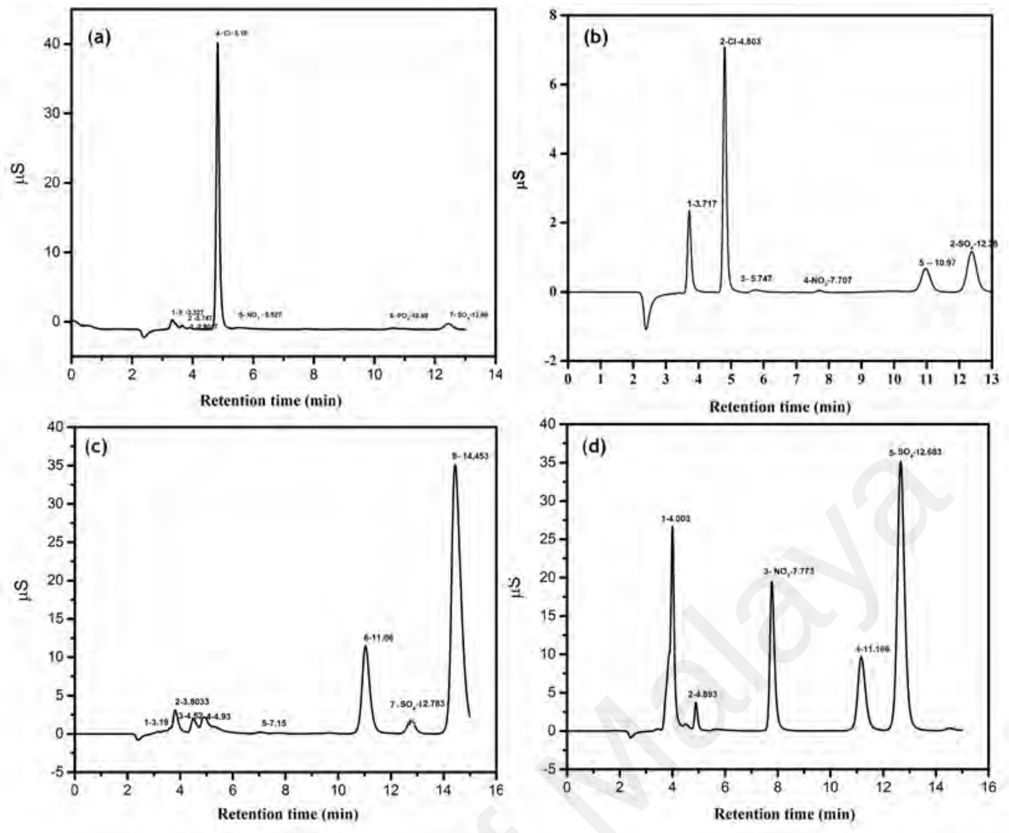

Figure 4.13: Chromatogram for (a) Date seed (b)Moringa seed (c)Tamarind seed (d)Adsorption of NO_3^- on Tamarind

When date seed was subjected to nitrate adsorption analysis (Figure 4.14 (a)), the result shows that like Tamarind, the adsorption capacity of the date seed at ambient condition is not significant (within 15%). However, the detection of nitrite marks the signal indicating the possibility of denitrifying bacteria releasing the intermediary product (NO_2^-) in the process. Parameters such as temperature, different solvents (such as organic solvents) are not considered in the extraction process because in-situ remediation operates at ambient condition and in aqueous medium.

Figure 4.14 (b and c) shows the effect of pH variation on the aqueous extraction and two possible explanation are proposed for the observations. In Figure 4.14 (b), the lower pH generated by HCl seems to shift the retention time backward, eluting chloride earlier. Secondly the lower pH suppresses the release of chlorides within the seed and generate species within the phosphate detection region. On the other hand, the extraction under basic medium (KOH) in Figure 4.14 (c) seems to support the release of chloride while the water deep signal is prominent. The peak for phosphate eluted at 11.04 min is also conspicuous. However, both acid and base extraction did not show significant improvement of nitrate extraction from the seed. This finding agrees with Aziz *et al.* (2004) findings, where pH is found to have no significant influence on the adsorption of ammoniacal nitrogen on the media. In contrast to the above-mentioned research however, this work is on decomposition rather than adsorption. More so, significant variation of pH is not in the interest of the denitrifying microbes as natural aquifer condition is expected to be maintained in real time pristine condition during the remediation process.

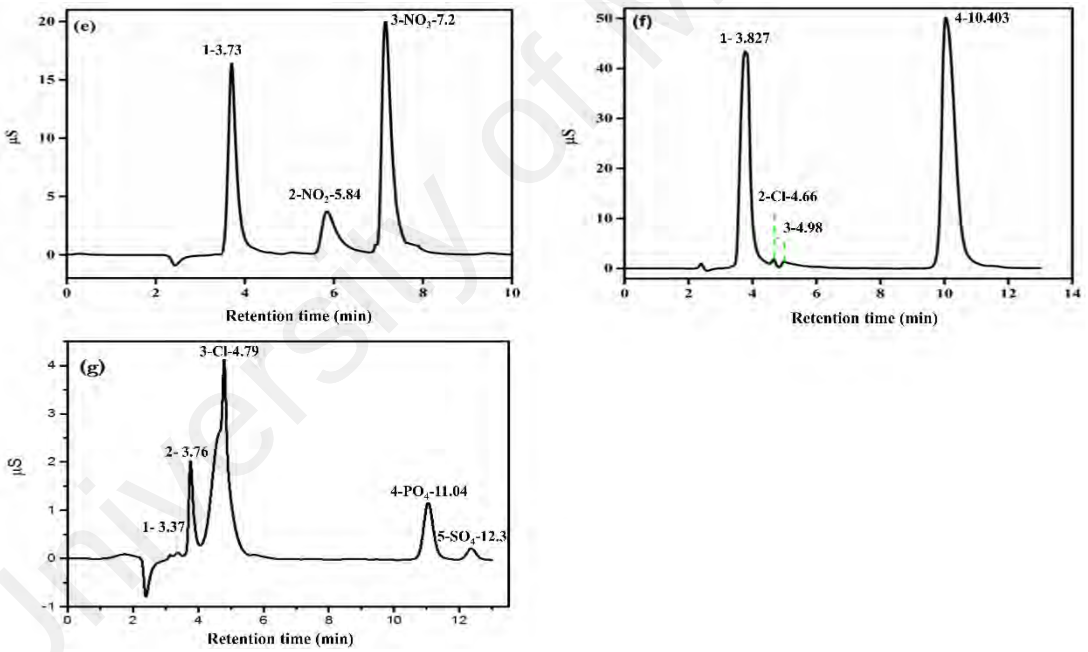

Figure 4.14: Ion chromatogram of date seed. (a) Adsorption of NO_3^- (b) HCl treated date seed extract (c) KOH treated date extract

While considering the parameters for selection of any organic substrate in denitrification process, oxygen bearing radicals, chloride, organic acids and water absorbance capacity of the substrate (Figure 4.15) are important factors. On this note, date seed is observed to have the lowest values of all the parameters (except for high chlorides) and hence recommended for consideration.

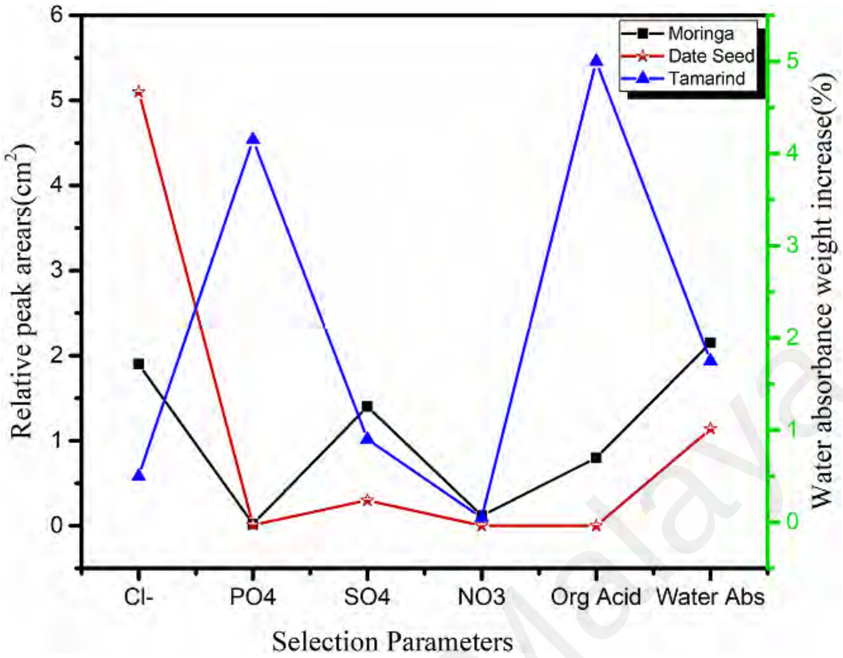

Figure 4.15: Distribution of the selection parameters in the organic substrate

4.4 Trigonometric means of modifying funnel and gate geometry

In this section, the efforts of modifying funnel and gate geometry in such a way that the gate packing structure would be arranged is discussed as follows.

4.4.1 Discussion on the theoretical background of the hypothesis

Since funnel and gate was introduced to circumvent cost implication of continuous wall (Faisal *et al.*) by constructing cut off walls from both sides of the reactor region (Grube, 1992) and thicker homogeneous barrier was found to be useful in ensuring less variable flow (Benner *et al.*, 2001). Painter (2004) found a gap to work on PRB design that can provide optimum equilibrium values between capture zone and residence time. The area witnessed many input in this direction, where the proposed utilization and, or application of multiple gates (Burger & Finkel, 2005; Obiri-Nyarko *et al.*, 2014), velocity equalization wall (Christodoulatos *et al.*, 1996; Klammler *et al.*, 2014), small increase in the width of the treatmentzone (Sedivy *et al.*, 1999) and introduction of down-gradient pump well (Bilbrey & Shafer, 2001), trench and gate (Bowles *et al*., 200) were all aimed at controlling this conflicting requirements. Other important factors include width of the gate, apex angle and orientation to the regional gradient and residence time.

4.4.2 Hypothetical construction

If we consider apex angle of 150°, the resultant gradient angle incident on the funnel arm (angle Q) would be 105° (Figure 4.16). Here, the advantages of maximum capture zone, optimum composite capture zone, reduced pressure on the central gate and gradient stability for attaining residence time in all the gates would be obtained. Going by trigonometric rules, the alternate angles (ϕɪ and δ^{I}), opposite angles (ϕɪ and ϕ) and corresponding angles (δ^{I} and ϕ) are equal. This indicates that the slide at which the incident plume strikes the funnel arm is influenced by angle δ. Angle δ is inversely proportional to apex angle and the highest apex angle of 180° corresponds to zero (0 °) angle δ.

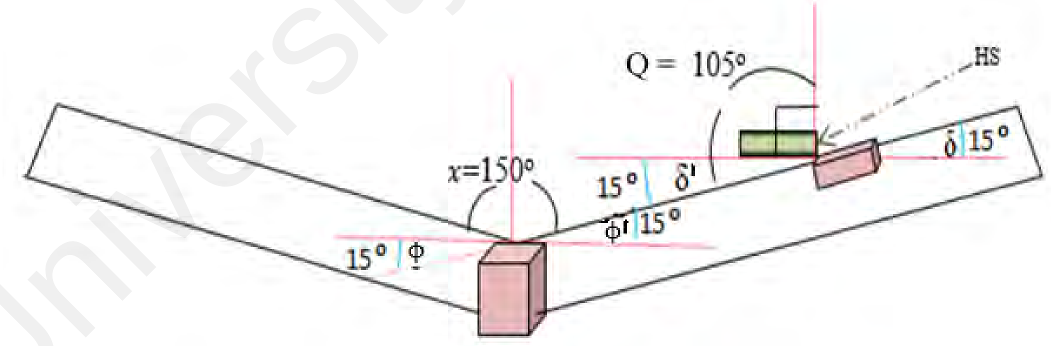

Figure 4.16: Schematic illustration of geometric impact of Apex angle

Trigonometrically, the relationship between the apex angle$x$, gradient angle Q and slope angle δ (Figure 4.16) is represented in Equation 4.1

$$Q = 90 + \left(\frac{180-x}{2}\right) \tag{4.1}$$

To achieve significant retardation of the plume under the influence of angle δ, two geometric presentations are of horizontal (Figure 4.16) and inclined (Figure 4.17) short structure are proposed. The inclined short structure is made parallel to the complementary arm of the funnel. This will form a sub-funnel (l) on the funnel arm's gate with an apex angle of 150^{o}, (Region of RGF in Figure 4.17). Unlike the baffled structures in Hudak (2011), which tends to diverge and stagnate the flow lingering the contaminants, the short structure here is embedded in the funnel structure. These proposals serves as improvement strategy of utilizing baffles because prior to this submission, baffles are considered counterproductive (Hudak, 2011).

The arrangement in this structure is similar to what Bürger *et al.* (2007) termed as superior features arrangement. By deduction from McMahon *et al.* (1999) finding, the discharge through the sub-funnel would create a "sub- composite capture zone" within the bigger funnel capture zone. More so, considering the recommendation of Sedivy *et al.* (1999), the possibility of extending the length of the funnel arm's gate through the sawdust cavity (left arm of the funnel in Figure 4.18) can compensate for the inadequacies of high hydraulic conductivity.

The complete design of the proposed funnel and gate structure (Figure 4.18) is envisaged to have four advantages as: (1) three stages of independent reactive media (funnel arm's gates/central gate, sawdust and lower central gate) (2) dual trench (3) twin funnel and (4) two sub-funnels. For these unique features, the structure is here by termed as "Double-sided trench/twin funnel and gate" set up.

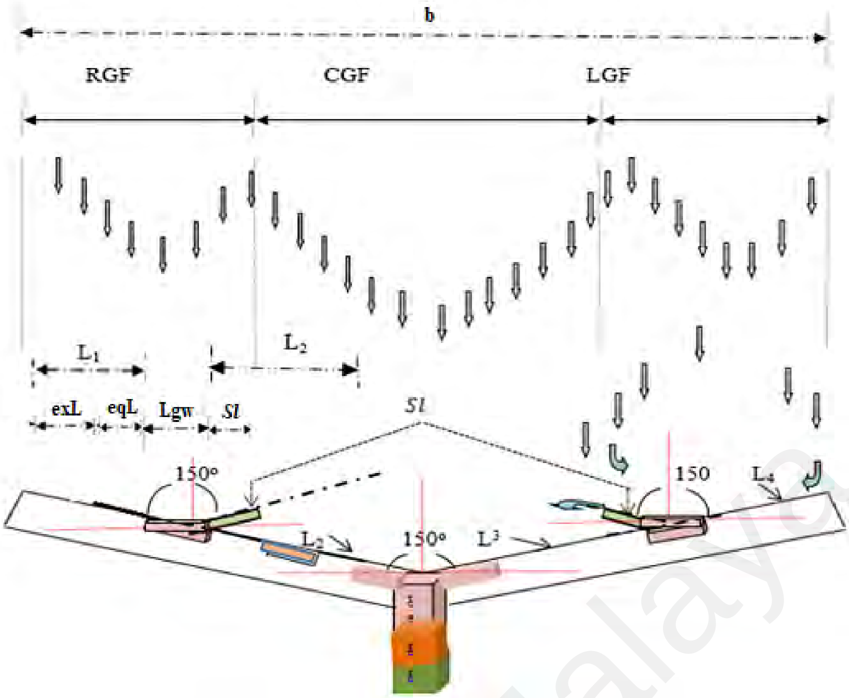

Figure 4.17: Multiple gates with inclined sub-funnel arm

KEY: RGF= Right get flux; **CGF** = Central gate flux; **LGF** = Left gate flux; *Sl* = Sub funnel length; **exL** = Extended length (beyond symmetric dimension); **eqL** = Equivalent length (with Sl necessary for the formation of symmetric funnel); **Lgw** = left gate width;

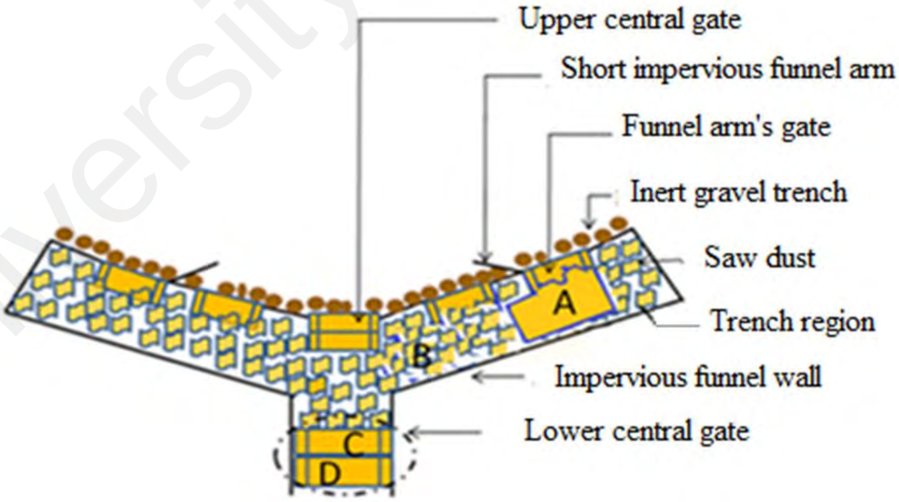

Figure 4.18: Proposed Double-sided Trench/Twin funnel and gate

Interaction of the structure with the plume could be complex as different capture zones are expected to interact across fields of gate's influences amidst dynamic orientation of groundwater flow. Considering the simulation pattern in symmetric and asymmetric funnel (Starr & Cherry, 1994), Figure 4.19. depicts how plume approaching the funnel at 90° perpendicular to the gate's orientation and 30° variation from left and right (60° and 120°) is expected to be controlled by the gates at both symmetric and asymmetric funnel sites.

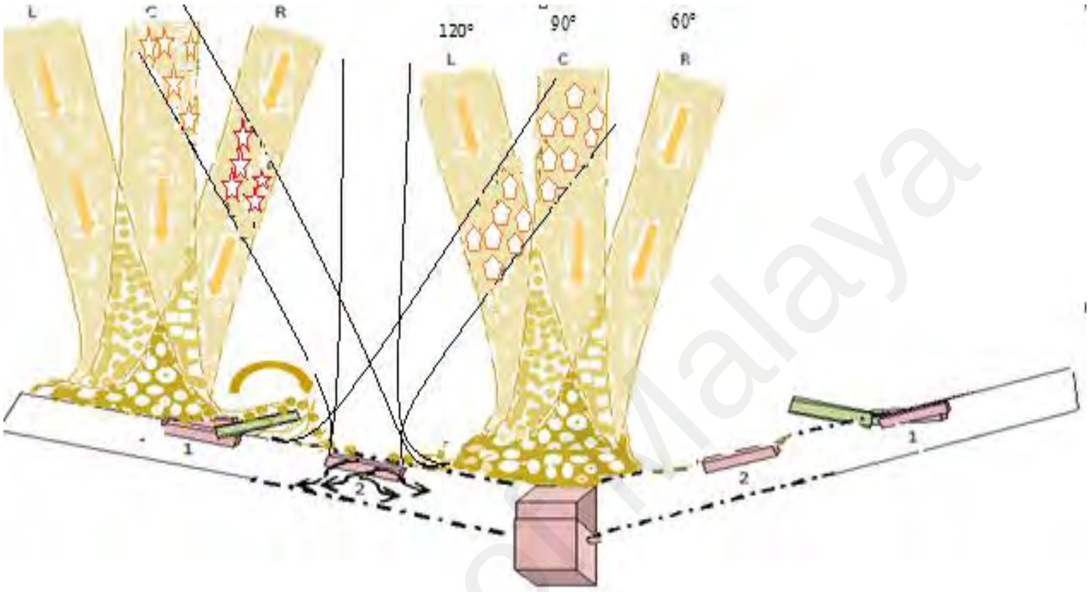

Figure 4.19: Effect of regional hydraulic gradient orientation to capture zone.

The star-shadded and pentagon-shaded sections at the left and right sides represents the intersect of the gate's capture zones. The round-shaded and square-shaded sections represents where the two orientations (60° and 120°) intersects with the perpendicular (90°) orientation while the sphere-shaded section represents the composite capture zone

4.4.3 The significance and application of none-linear flow

In order to detarmine the conformity of the structure with the provision of continuity eqation in laminar flow, the total volumetric flow (**$q_{i.f}$**) before entering the structure and after passing through must be compared.

$$q_{i.f} = \left(\frac{A_{i.f}\,.G_{i.f}}{\mu .\ l_{if}}\right)(P_i - P_f) \tag{4.2}$$

Where $P_i - P_f$ stands for a pressure at the initial and final stage of the measurement, μ is the viscosity of the fluid, l_{if} is the length, $A_{i.f}$ is the cross-sectional area and $G_{i.f}$ is the cross sectional shape coefficient, respectively. Hence, for a given shape, say square tube, G is constant (0.562) (Xiong *et al.*, 2016).

It also followes that flow rate "Q", is defined as:

$$Q = KA(\frac{hL}{L}) \tag{4.3}$$

Taking equation (4.2) and equation (4.3), the followings are obtained

$$\left(\frac{A_{i.f}\,.G_{i.f}}{\mu.\;l_{if}}\right)(\boldsymbol{P_i} - \boldsymbol{P_f}) = KA(\frac{hL}{L}) \tag{4.4}$$

This shows that $(\boldsymbol{P_i} - \boldsymbol{P_f})$ is equivalent to h_L and since G and K are constants, the only difference is density (μ). If we consider the density of pristine water as "1", the pressure can be substituted with "h_L", which is the difference in the hydraulic gradient between one stratum to another. However, it is worth noting that even on a horizontal plain, variation of porosity across strata will bring about variation of hydraulic gradient. To this effect, the current study considers the assumption of variating the porosity as a mean of offsetting the values of hydraulic gradient (h_L). This value is hereby considered as the determinant variable, represented here as (*P*). The value may take the form of pressure or hydraulic gradient, of which both are affected by varying the porosity between strata. By toggling around with values of porosity across the strata, assigned values of determinant variables (*P*) that are proportional to the differences between one stratum to another can be substituted in Equation (4.2) to determine the total volumetric flow rate in a given dimension of a stratum. Hence, the application of non-linear flow in this scenario is advantageous in groundwater remediation structures.

It is therefore possible to determine the total volume flow rate at each stratum. Discrete packets of squared portions, numbered 1 – 4, are considered in aquifer medium (Figure 4.20). Moreover, the variables (*P*) are assigned in line with field requirement PRB design, as literatures indicates that it is essential to make sure that the groundwater velocity at the gate is near the range of 10 times the velocity of water in the aquifer (McMahon *et al.*, 1999).

Therefore, using Equation 4.2 and assuming twofold layers of four squared packs for the area (due to possible sizes of plume) stretching 3.5m (*l*) along the direction of the groundwater flow to the funnel, the total volumetric flowrate at that portion of the aquifer is calculated as follows

$$\boldsymbol{q_{i.f} = \left(\frac{A_{i.f}\,.G_{i.f}}{\mu.\;l_{if}}\right)\left(P_i - P_f\right) * \ \mathrm{DPT}} \qquad \textbf{(4.5)}$$

Where μ is given a value of “1” and determinant variable (P) is considered to have a value of “1” for this aquifer strata because it is the ground zero variant. However, since eight discreet square packets are considered in the aquifer, a new factor termed number of discrete packet variable (DPT) is introduced (Equation 4.5**).**

$$\boldsymbol{q_{i.f} = \left(\frac{12.5 * 0.56}{1 * 3.5}\right)(1)\ * \mathrm{DPT}}$$

Hence, $\boldsymbol{q_{i.f} = \left(\frac{12.5 * 0.562}{1 * 3.5}\right)(1)\ * 8 = 15.74}$

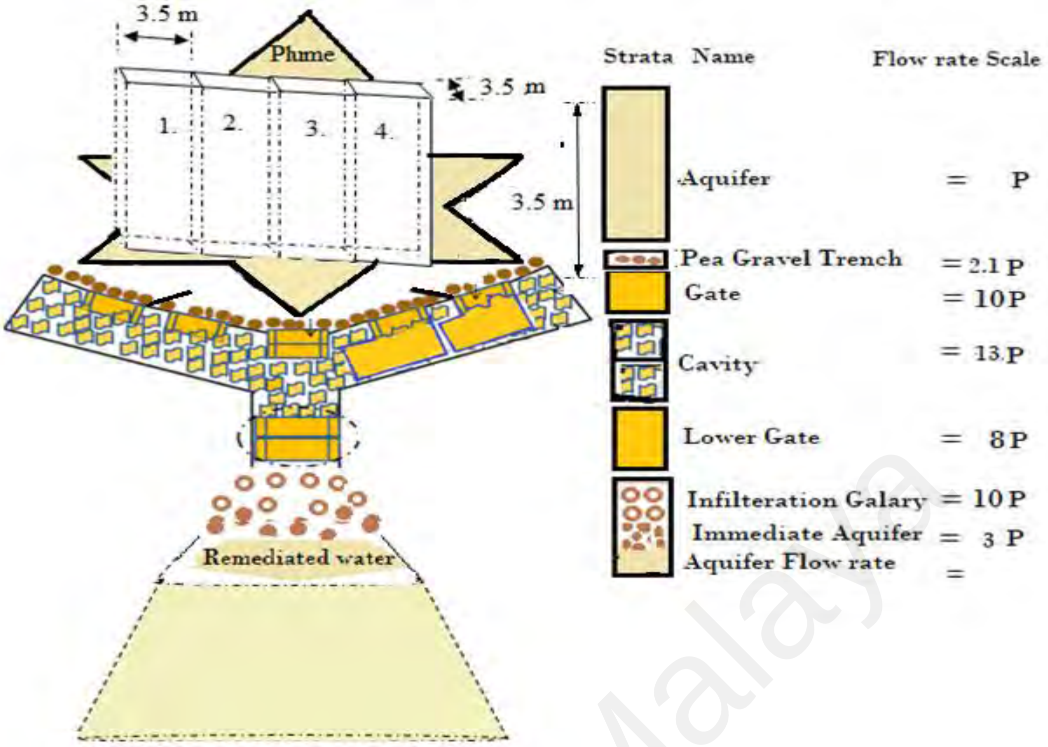

Figure 4.20: Pro and post-funnel & gate remediation stages in different strata

Table 4.5 indicates how different combinations of varying (*P*) and DPT helps in determining the total volumetric flowrate at different sizes within strata and at the same time sustain steady flow without mounding. It is clear from the table that the variation pattern of total volume flow rate between the strata presents a positive non-linear flow that would allow for smooth operation of the remediating structure.

Table 4.5: Distribution of volumetric flowrate across different strata with varying DPT and P

S/N	Strata name	Sides of Square(m)	Area (m^2)	Constant	Length (m)	DPT (Units)	*P* (Units)	T.Volumetric Flow (L^3T^{-1})
1	Aquifer	$(3.5)^2$	12.25	0.562	3.5	8	1	15.74
2	Gravel	$(3.5)^2$	12.25	0.562	3.5	6	2.1	24.78
3	Gate	$(3.5)^2$	12.25	0.562	3.5	5	10	98.35
4	Cavity	$(3.8)^2$	14.44	0.562	4	6	13.05	158.88
5	Lower Gate	$(6)^2$	36.0	0.562	3.5	1	8	46.24
6	Infiltration Gallery	$(3.5)^2$	12.25	0.562	5	4	10	55.07
7	Immediate Aquifer	$(3.5)^2$	12.25	0.562	3.5	5	3	29.51
8	Aquifer	$(3.5)^2$	12.25	0.562	3.5	9	1	17.70

4.4.4 Application to hypothetical case

Considering the geometry in Figure 4.17, the cross-sectional breadth (b) represents the safety zone within which plume has the highest tendency of passing through the funnel. However, adjustment of the funnel gate's width and hydraulic conductivity is necessary for the attainment of equilibrium. The equilibrium in the law of conservation of energy and continuity equation seeks to ensure that product of cross-sectional area and velocity of a moving fluid within a capture zone is equal to the product of the cross-sectional area and hydraulic conductivity (advective velocity) in the gates. To determine the required width of the gate and length of the funnel arm when the hydraulic conductivity of the gate is made to be 10 times that of the aquifer (McMahon *et al.*, 1999), relative factors of cross-sectional surface area (on plain axis) and velocity can be equated. If the initial velocity of water in the aquifer is taken as V_o and that in the gate is V, then

$$V = 10\,V_0 \qquad \textbf{(4.6)}$$

For simplification, left arm sub-funnel region of RGF (Figure 4.17) will be considered.

$$RGF * \; V_0 = Lgw * V \qquad \textbf{(4.7)}$$

But $V = 10V_0$ and $RGF > Lgw$ with value x because $(RGF = Lgw + x)$

Where $x = L_1 + Sl$ for asymmetric funnel and 2L for symmetric funnel arm

$$[Lgw + (L_1 + Sl)] * V_0 = Lgw * 10V_0 \qquad \textbf{(4.8)}$$

$$L_1 = 9Lgw - Sl \qquad \textbf{(4.9)}$$

If a gate width (Lgw) of 3.5 m and short funnel arm (Sl) length of 3.2 m is considered in a hypothetical case, the length of funnel arm (L_1) per surface area of a gate can be determined by applying equation 4.8,

$$L_1 = (9 * 3.5) - 3.2\ ,$$

$$L_1 \ \ = 28.3\, m$$

It then follows that the aspect ratio (defined as the ratio of gate's width to that of the cut-off wall width) of the sub-funnel section would be (3.5) : (28.3 +3.2). = 9. At 180^o orientation, the width of the sub-funnel at the influence of capture zone (RGF) can be deduced by simple addition as (L_1 +Lgw+ Sl = 28.3+3.5+3.2) = 35 m.

However, since the apex angle is 150^o, the width would be W_{funnel} =W_{gate} +2L $\sin(\frac{theta}{2})$

where $2L$ represents $(L_1 + Sl)$ in this asymmetric setting.

W_{funnel} = [3.5 m + (28.3+3.2)] m sin $(\frac{150}{2})$. Hence,

W_{funnel} = 30.426 m

This is the effective width of the funnel within the influence of the flow channel when the incident plume is perpendicular (90^o) to the orientation of the gate. However, since reduction in the width of the flow channel intercepted by the sub-funnel due to incident angle ($\boldsymbol{\varphi}$) of less than 90^o is given as $\boldsymbol{W_\varphi = w_\perp\ \sin(\varphi)}$**,** **where** $w_\perp$ is the width of the flow channel when the incident angle is perpendicular to the barrier gate (Sedivy *et al.*, 1999) **,** it follows that the effective funnel width when the incident angle is 60^o would be $W_\varphi = 30.426\, m *\ \sin(60)$ = 26.35 m

The above relation is only applicable to symmetric funnel. Geometrically, the effective width of a symmetric sub-funnel would be the sum of the short arm (*Sl*), gate width (Lgw) and equivalent length of the short arm (eql) as shown in Figure 4.17. By extrapolation, capture zone of the sub-funnel can be deduced from the graph of (Sedivy *et al.*, 1999) as indicated below.

The ideal symmetric funnel would have a width of $W_{funnel} = W_{gate} + 2L\ \mathbf{sin}(\frac{theta}{2})$

$W_{funnel} = 3.5\text{ m} + 2(3.2)\sin 75$ where $W_{funnel} = 9.5819$ m. The aspect ratio for this part would only be $(\frac{9.5819}{3.5} = 2.7)$ and by extrapolation from Figure 4.21 the ratio of capture zone to gate is 2.1, giving just 7.525 m (3.5*2.1) as the capture zone.

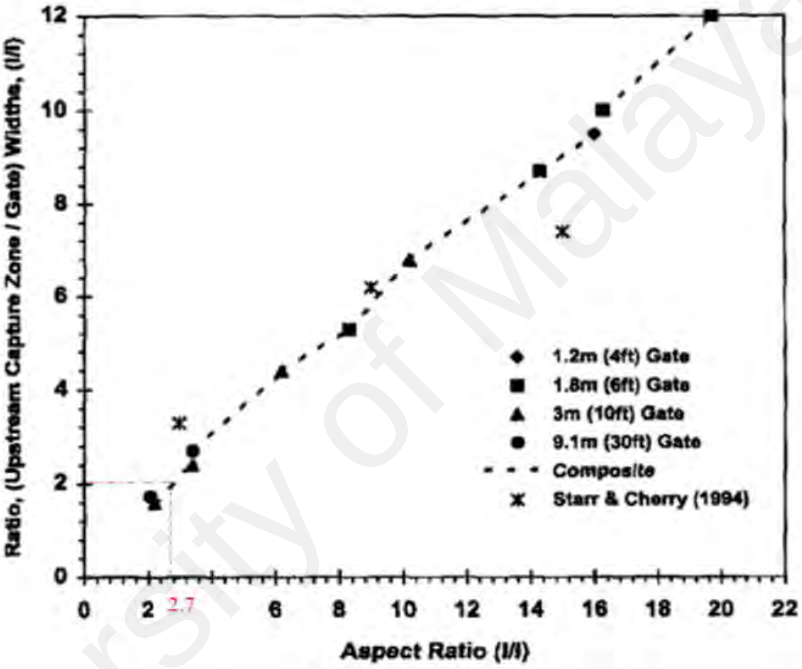

Figure 4.21: Relative capture zone trend for straight funnel& gate configuration (Reproduced with permission from WILEY)

Two inferences are deducible from the above result.

i) The symmetric funnel width calculated as 9.5819 m would have been equal to or greater than the capture zone when the orientation of the gate is perpendicular to the regional hydraulic gradient. The extrapolated capture zone width, with a value of 7.525 m would be the minimum capture zone regardless of the tendencies for fluctuation of groundwater flow and orientation. This may be considered as the composite capture zone.

ii) The asymmetric nature of the complete sub-funnel expands the capture zone, accommodate more volume of water and ensure efficient distribution of the water across the gates.

Literature indicates that, to satisfy the above requirement, *Sl* must be equal to eqL and exL is recommended to be at least 3.0 times eqL (Sedivy *et al.*, 1999) . It then follows that, instead of calculated 28 m, exL length can be shortened to 12.8 m and still serve the purpose of maximizing the capture zone. Consequently, if the two impervious portions of L_2 (Figure 4.17) is assumed to be 6 m each, the total length of a funnel arm would be 31.8 m. Going by this, the funnel and gate effective width (considering 150^o apex angle) of 66.9 m would give aspect ratio (here defined as the ratio of effective width to gates width) of 3.52 (19:66.9). By extrapolation from Figure 4.21, ratio of capture zone to gate is 2.45, which gives a total capture zone of 46.5 m. With this estimates, 10 m extra arm from both sides of the funnel arm will offset the uncertainties associated with aquifer heterogeneity, change of orientation of groundwater flow and accommodate any factor of safety. Other advantages include cost effectiveness of multiple gates (Hudak, 2011; Kovar & Hrkal, 2003). The following section indicates the potential of geometric modification for attaining higher level of enhanced capture zone.

4.4.4.1 Length of the gate

The appropriate length of gate that can assure enough residence time can be deduced from the assumptions generated at the beginning of the chapter. Considering the gate's width of 3.5 m, we assume a gate porosity of 0.43, saturated thickness of 2.93 m, residence time of 28 hours and aquifer-based advective groundwater velocity of 0.274 m/day (derived from average value of 100m/year by Mackay *et al.* (1985)). With these values, the distance that would be covered by the water within the residence time of 28 hrs in the aquifer would be 0.27m + 1/6(0.274 m) = 0.319 m/28 hours. Since the porosity of gate is expected to allow for tenfold flow rate compared to the value of the aquifer, the distance that would be covered within the residence time would be 3.19 m. This would be the effective length of the gate.

Equation 4.10 (McMahon *et al.*, 1999) is used to find out the volumetric flux through the gate.

$$Q = \sum_{i=1}^{5} Wi.Zi.Vi.n \qquad \textbf{(4.10)}$$

Where Q is the volumetric flux through the gates, i is the number of gates (5), Wi is the width of the gates (3.5m *4 + 5 m) =19m, Zi is the saturated thickness of the gate (2.93 m), Vi is the groundwater velocity at the (central) gate (2.74 m/d) and “n” is the porosity of the gate (0.43). However, considering the temporal variations between the gates (due to changes in hydraulic heads) and effect of apex angle on the orientation of the side gates to the hydraulic gradient, the groundwater velocity at the funnel arm’s gates (gate 1, 2, 4 and 5) are taken to be 15% less than that at the central gate. Substituting the values in equation 4.10 gives a Q value of 68.0404 m^3/28 hrs. To determine the approximate capture zone that will equal this volumetric value, continuity equation is applied as follows.

$W_{(a)} * Z_{(a)} * V_{(a)} * n_{(a)} = \sum_{i=1}^{5} Wi.Zi.Vi.n$ where (a) stands for the respective values of dimension and properties of the plume in the aquifer. Considering the frontline gravel trench, subsequent obstruction of the cut-off walls and the potential volume of water that can be released through the gate, it is safer to consider the accommodation of volume of water that would be displaced by two and a half (2.5) times the residence time in the cross-sectional area of the aquifer under consideration.

$$Wi_{(a)}\ m * 2.93\ \cancel{m} * (\mathbf{2.5}*0.319)\ \frac{\cancel{m}}{\cancel{28hrs}} * 0.33 = 68.040\ \frac{\cancel{m3}}{\cancel{28hrs}}$$

$$\mathrm{Wi}_{(a)} = \frac{68.040m3}{0.7711m2} = 88.23 \text{ m}$$

This signifies that, a funnel with the above feature can have a capture zone of 88.23 m and hence, plume as wide as that are treatable using the structure. So, if the residence time is not violated, the size of capture zone may be enhanced many times fold by toggling with the hydraulic conductivity of the gates in relation to that of the aquifer.

4.5 Development of effective sequence multi-barrier reactive media

Here, the sequential steps for the development the reactive media is given below.

4.5.1 Reactive media (materials) used and rational

As explained in section 4.3, the carbonaceous materials used as reactive media were screened and selected, out of which date seed was found to be the most preferable. In this section, the application of the chosen materials will be put to test and the justification of using them in combined form as indicated in this column analysis is here by explained as follows.

4.5.1.1 The date Seed

Since literature indicates the need for finding a material with continuous but steady supply of electron donors (carbon sources) for longer life span of the system, the date seed was chosen. The choice of date seed was dictated by its property of having the lowest rate of water absorption capacity and least objectionable organic extracts (Dahiru *et al.*, 2017). In addition, the thick walled endosperm of the seed consist of over 90% mannan and cellulose, serving as a storage for carbohydrate (DeMason *et al.*, 1985). More so, it has been reported that some date seed excavated in 1965 were measured to date back before the common era (BCE)-signifying their ability to resist decomposition for more than 2000 years (Sallon *et al.*, 2008).

4.5.1.2 *Moringa oleifera* seed

As indicated in the preceding section, *Moringa oleifera* was chosen due to its historical antecedents in water treatment and sulphur content. The participation of sulfate reducing bacteria in microbially induced corrosion in aqueous subsurface region and the susceptibility of undissociated proton (in H_2S) for reacting more rapidly with iron-derived electrons than the hydrogen in water is well documented. Therefore, occurrence of hydrogenotopic denitrification is highly likely (Enning *et al.*, 2012). This is because as observed in the preliminary study of this research, hydrogen sulfide generation is inferred in dissolved *Moringa oleifera* seed. More so, literature indicates the presence of metal sulfide in form of iron sulfide in the paleo beach soil used (Enio *et al.*, 2011; Roslan *et al.*, 2010). In coastal region, the H_2S might as well be produced from respiratory reduction of sulfate from seawater with organic nutrients. The undissociated proton in H_2S can then react with iron-derived electrons (Equation 4.11) (Enning *et al.*, 2012) .

$$Fe + H_2S \longrightarrow FeS + H_{2(g)} \qquad \textbf{(4.11)}$$

4.5.1.3 Wood chips

Serving as the principle carbon provider, wood chips have been utilized for denitrification purposes. With the saw dust embedded within the mixture, woodchip is expected to be the second source of the most labile carbon as the decomposition proceeds.

4.5.1.4 Auxiliary carbon sources

These are circumstantial carbon sources whose presence in the soil is ascertained but not quantified or were added due to exigencies of the experiments. The carbon sources considered in order of ease of decomposition and specific substrate of interest includes humus (in the soil), sawdust and limestone.

4.5.1.5 Activated carbon

The last material used is an obtained commercial grade activated carbon tested to be effective in removing excessive organic by-products. Its presence is sought to be essential because of the possible generation of chlorinated hydrocarbon. As such, activated carbon serves as a safety filter for adsorption of organic degradation by-products.

4.5.2 Column analysis

The results of the column analysis, surface characterization and decomposition of the materials provides light on the efficacy of the gate material and idea on the decomposition pattern of the carbonaceous materials used in the experiment. Discussion on experimental developments that leads to adjustments of column composition due to evaluation of the attenuation process is emphasized.

4.5.2.1 The column experiments

In pursuance to the observed denitrification potential of the soil used in this experiment, the soil is envisaged to provide the required population of the denitrifying community to the system and hence, is distributed across the column (Ibrahim *et al.*, 2015; Jamaludin *et al.*, 2013). The column experiment is divided into three phases, each phase necessitated by the observed frailties of tested column material composition and condition. The first phase after acclimatization (hydrating the column with distilled water and assessing the leachable nutrients from the media) consists of observed period of nitrate spiked distilled water. This is followed by the second phase, which involved nitrate-spiked groundwater to evaluate the impact of matrix effect. Adjustment of column composition and concentration of nitrate in the influent failed to address the observed inadequacies of this setup, which is the elevated nitrite concentration. This situation led to introduction of lime in the system, leading to phase three of the experiment._The followings are the detailed description of the phases.

(A) Phase (I)- Distilled water

When a nitrate-spiked distilled water was run, the pattern of nitrate and nitrite attenuation in the column indicates a rapid response of nitrite buildup attaining its peak at P_2, where the nitrate concentration drops sharply reaching its lowest peak at P_3 (Figure 4.22 a). This sudden response may be explained by the presence of nutrient (NO_3^-) for the microbes to degrade the carbon material and incomplete denitrification process, which tends to attain some level of relative stability after the second layer at P_3.

As the test continues in the second run (Figure 4.22 b), a clear shift of nitrite peak is observed at P_3 suggesting the acclimatization of the microbial community and active engagement of the sawdust chamber, which contains more readily decomposable substrate than date seed. A noticeable elution of phosphate at P_5 indicates a possible release of leachable phosphate from media 3 and 4 within the column.

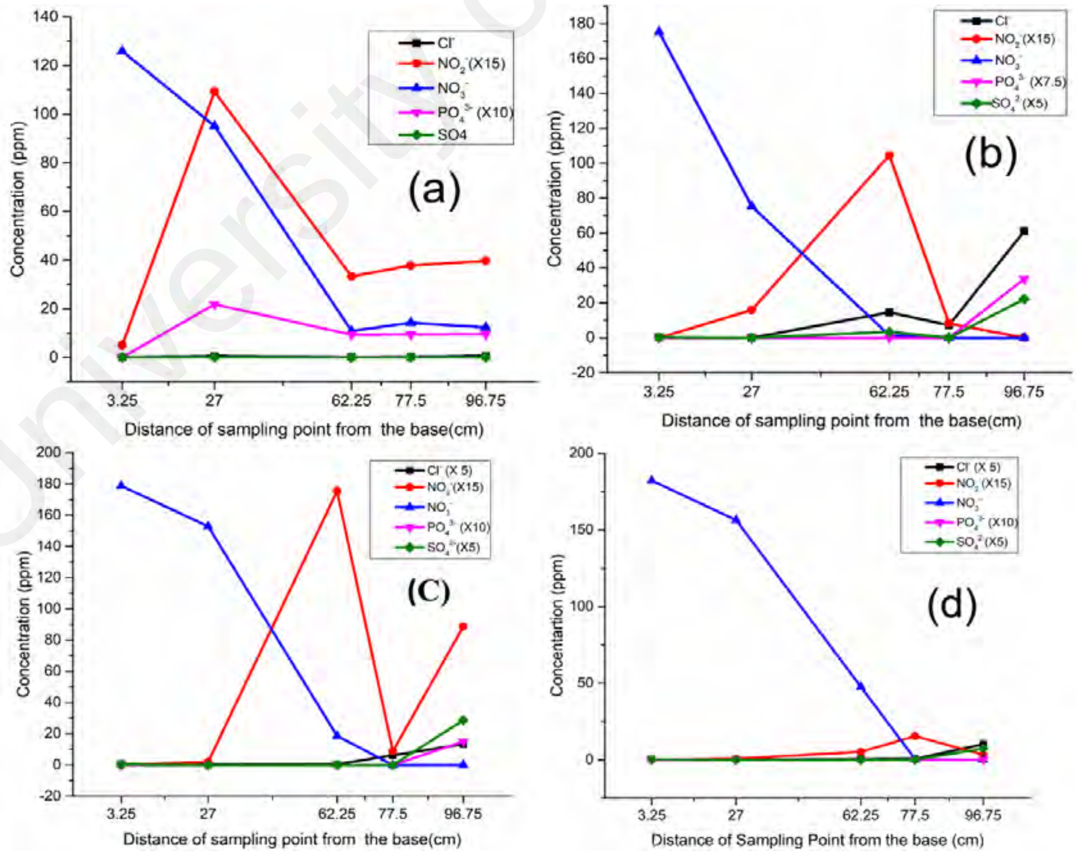

Figure 4.22: Response of NO_3^- spiked distilled water (a)1st run (b)2nd run (c)3rd run (d)4th run

The same trend is observed in the third run except that while the nitrate was completely depleted, the nitrite began to buildup up to the fifth sampling point (Figure 4.22 c). This development points to the possibility of oxygen infusion, or dissimilatory nitrate reduction to ammonia (Van Rijn *et al.*, 2006) and/or, incomplete denitrification due to shorter retention time. This informed the decision of lowering the flow rate from 3.15 ml/min to 2.5 ml/min to increase the residence time.

With the adjustment of the flow rate however, a further shift of nitrite peak is noticed in P_4 instead of P_3 in the previous run (Figure 4.22 d). This development further suggests a more robust metabolic activities because the nitrate keeps declining (terminating at P_4) while little amount of nitrite is produced. It is encouraging to observe that from a maximum concentration of 182 ppm (32 mg L^{-1} greater than three times the maximum permissible limit of 50 mg L^{-1}), both the nitrate and nitrite were successfully remediated to 0.0 and 0.6 mg L^{-}1, respectively. In concordance with previous research, the analysis using distilled water as the vector of nutrients (NO_3^-) then revealed the influence of the flow rate (Gillham & O'Hannesin, 1994), nature of the carbon source (Gomez *et al.*, 2000) and timing of the process on the attenuation pattern.

(B) Phase II- Well water

Considering the fact that very high concentration of nitrate was recorded in some natural sites, the study considers increasing the concentration of the nitrate in the groundwater influent set. The attenuation pattern of the parameters in ground (well) water with the highest nitrate concentration of 230 ppm in the influent shows similar trend with that of the distilled water, wherein the fastest decline of nitrate concentration is observed between P_2 and P_3 (Saw dust chamber). However, while nitrate was declining, a surge of nitrite concentration was observed from P_3 to P_5 (Figure 4.23 A). Another notable phenomenon is the decrease in inverse relationship between nitrate and nitrite from P_1 to P_3 (unlike in the previous phase). This manifestation is attributed to the matrix effect of the natural water as it is reported to support more of redox process in the presence of natural concentration of species like O_2, H_2S, Fe^{2+}, CH_4, SO_4^{2-} etc (Appelo & Postma, 2004) With this, the microbial community are bound to find the groundwater more adaptable for their metabolic activities than distilled water and hence, favorable condition for complete denitrification.

The situation, however, tends to be different in the third compartment, where a different carbon source and slight change of pH is encountered by the microbes. As highlighted in previous research, it may be deduced that requirement for the adjustment of their metabolic process to acclimatize with the new environment resulted to incomplete denitrification and hence high nitrite production (Baggs *et al.*, 2010; Glass & Silverstein, 1998) . This observation is in line with several researches that indicates the occurrence of the phenomena due to culture under stressed condition (Tiedje, 1988).

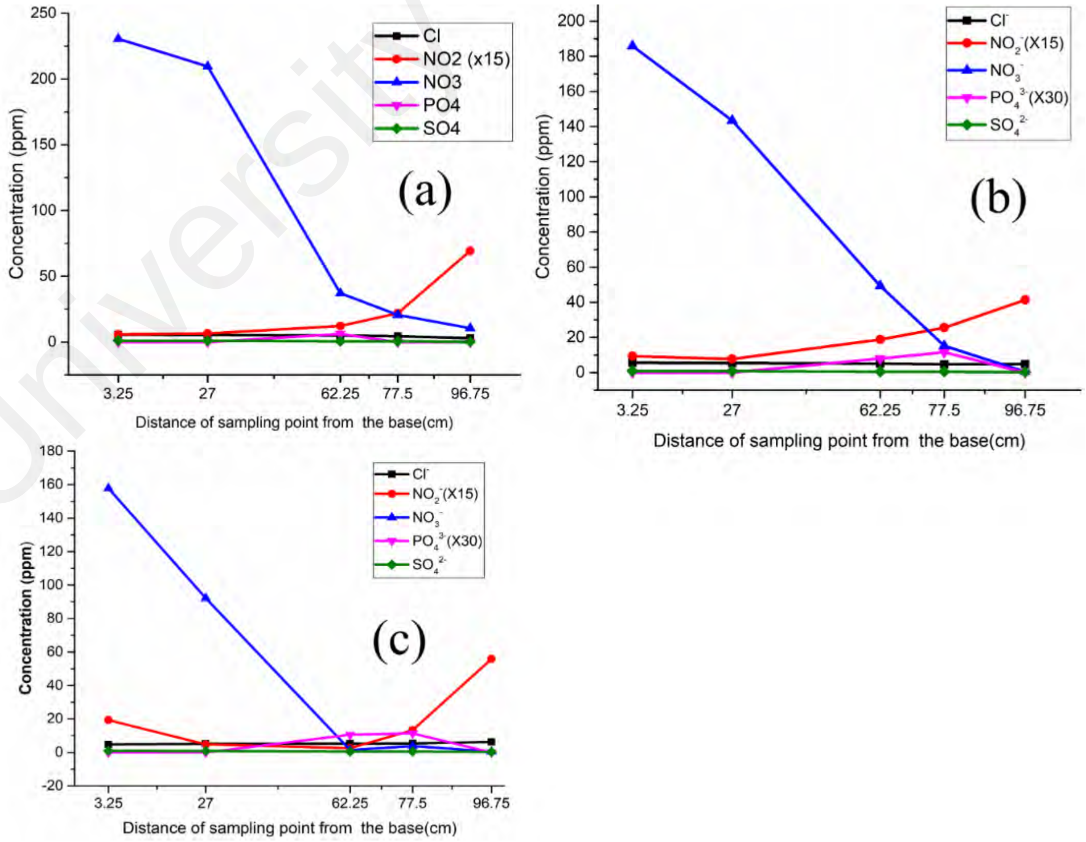

Figure 4.23: Response of NO_3^-spiked Well water. (a) 1st run (b)2nd run (c)3rd run

The possible second factor that might be responsible for this phenomenon is the high concentration of the nitrate in the influent (230 ppm). For this hypothesis, the influent concentration was reduced in the second run to 185 ppm (Figure 4.23 B.). Another observation is the release of leachable organic phosphate from the third compartment, suggesting the possible influence of the sample matrix on the media. A curious inverse relation of chloride with nitrite suggest a favorable condition for eluting adsorbed chloride on the activated carbon and this is corroborated by the observed decrease of pH around the media and hence, a slowed denitrification process (Ruiz *et al.*, 2006).

Little adjustment is observed when the influent concentration was reduced to a favorable range in the first phase (185 ppm) because, the nitrite concentration still rises at P_5. Throughout the sulfate attenuation pattern, a decline with distance of sampling point is observed. This is attributed to possible sulfate reduction utilized for decomposition of organic matter. This inference is supported by constant trend of sulfate observed from P_3 to P_4, where nitrate was declining.

The situation does not change in the third run (where the influent concentration was further brought down to 157 ppm) because, unlike in the first phase of distilled water, the nitrite keeps elevating after the final exit (P_5). Like the previous run, phosphate emerges at P_3 and P_4, affirming the release of dissolved organic phosphate from the wood media (Figure 4.23 C).

Considering the surge of leachable products in the column, adjustment was made to lower down the composition of carbon-based constituents across the compartments with 15%. The concentration of the nitrate was then raised to 196 to observe the trend at that C:N ratio. The result indicates a sharp decline of nitrate concentration with accompanying surge of nitrite production (Figure 4.24). Like the previous runs, the nitrite value indicates build up at P5, signaling the need for further modification.

Although, the concentration of nitrate and nitrite falls within the maximum permissible limit, the elevating concentration of nitrite near the threshold limit value of 3 mg L^{-1} presents uncomfortable possibilities with undesirable signal to remediation goal. This is because of the possibility of concentration buildup that can exceed the maximum permissible limit of nitrite or regeneration of nitrate upon oxidation down the gradient.

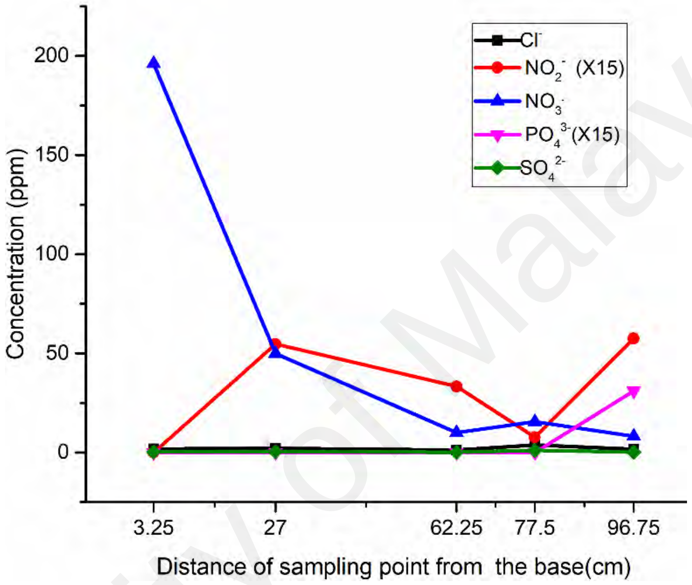

Figure 4.24: First run of nitrite- spiked well water in new column

(C.) Phase (III) new column

Two factors were then considered, and adjustment was made to the column composition. Lime ($CaCO_3$) was introduced to regulate the pH as a buffering medium to alkalinity (Koenig & Liu, 2002) and provide dissolved inorganic carbon in form of CO_2 for sulfur related autotrophic or hydrogenotopic denitrification (Huang *et al.*, 2015; Zhang & Lampe, 1999). The buffering action is expected to address the effect of pH fluctuation from one media to the other, which may upset the stability of the microbial community and affect their performance. This led to phase III of the experiment with the formation of a fresh lime-embedded composition. The 1st compartment of the column consists of soil, date seed, *Moringa oleifera* seed and lime stone in the ratio of 115.4:

50.5: 1:24.3 while the 2nd compartment constitute of soil, wood chips, saw dust and lime stone in the ratio of 32:3:1:5.2, respectively. The third compartment represents the final stage of carbon source with a constituent of soil, date seed and limestone in the ratio 8.3:1.7:1, while the 4th compartment had soil and activated carbon in the ratio of 1:1.6, respectively.

The first run of the modified column revealed a skewed pattern of both nitrate and nitrite, with nitrate gaining fresh momentum in the sawdust chamber and nitrite rising much higher in the last compartment (Figure 4.25 A). The former is attributed to leachable nitrate in the second compartment while the latter is attributed to possible incomplete denitrification or elution of adsorbed nitrite from the activated carbon in the previous run.

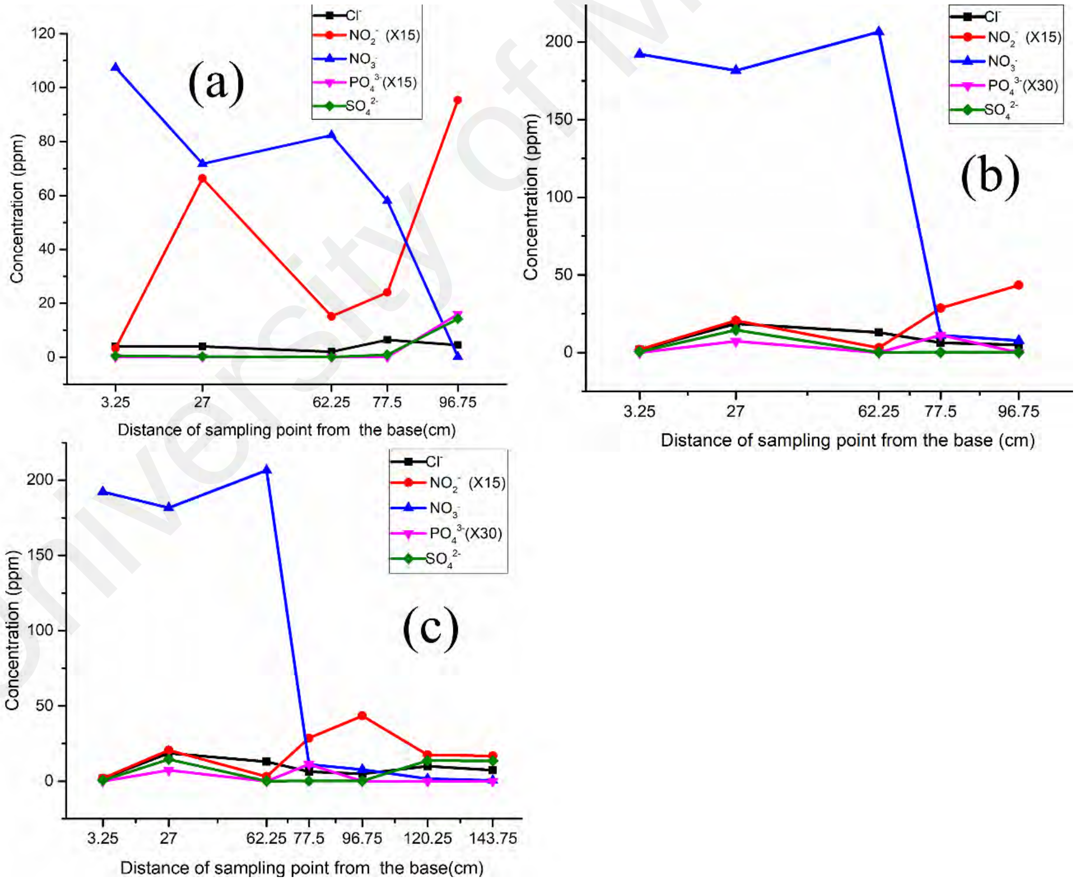

Figure 4.25: Nitrite spiked Well water in new colum. (A) 2nd (B) 3rd (C) 4th run

is was also observed that PO_4^{3-} and SO_4^{2-} signals were not detected until at P_5, suggesting their possible suppression or depletion by the limestone influence in the preceding compartments. Their detection at the last sampling point can also suggest that a catalyzed elution occurs due to pH adjustment by the lime.

However, the influent concentration was raised to 196 ppm at the second run and continuous flow of the influent through the column revealed a much lower concentration of SO_4^{2-} at P_4 and P_5, justifying the assumption of its elution from the activated carbon (Figure 4.25 B). Another observation is that of elevated concentration of SO_4^{2-} at P_2, which is attributed to leaching from *Moringa oleifera* seed. The recurrence of elevated concentration of nitrate at P_3 and its subsequent drop at P_4 confirms the leaching property of the sawdust chamber. Nitrate attenuation matches nitrite on inverse scale but as the time passes by, the amount of nitrite generated at the exit reduced to less than 50% of the ones generated at the previous test, dropping the concentration from 6.36 to 2.89 ppm.

Further test on determining the impact of the packing structure on the attenuation pattern of the parameters down the gradient attests to the efficiency of the gate design at short distance. This is because when the effluent of the above assembly was made to be the influent of a whole soil column, concentration of nitrite and nitrate were reduced to 98% and 60 %, respectively (Figure 4.25 C). This excellent attenuation capacity of the soil is however, higher than what is observed when a nitrate spiked sample is passed through a soil column, suggesting that attenuation capacity of soils are improved when the process of the remediation began at the influent plume.

Having observed the descending trend of nitrite concentration within the gate and down the gradient, another run of influent with lower nitrate concentration of 103 ppm was tested to determine if the assembly can be applied without considering further attenuation down the gradient. The result revealed a reduced concentration of nitrate to 5.81 ppm and nitrite to 0.37 ppm (Figure 4.26). This final test confirms that the setup can be relied up on for remediating nitrate concentrated plume that is twice the threshold limit value of 50 mg/L without considering further treatment down the gradient. Also, the results obtained throughout the test indicates that leaching of organic by-products and other inorganic radicals is dependent upon degradation process taking place, which progresses with time. Irregular detection of SO_4^{2-} and PO_4^{3-} at the 2nd and 4th sampling point is a good proof to this observation as the content of the compartments therein contain such analytes. In contrast to the excellent remediation response of the working column, the control column appeared to get supersaturated with the continuous supply of NO_3^- , reducing only 24-34% of the initial concentration.

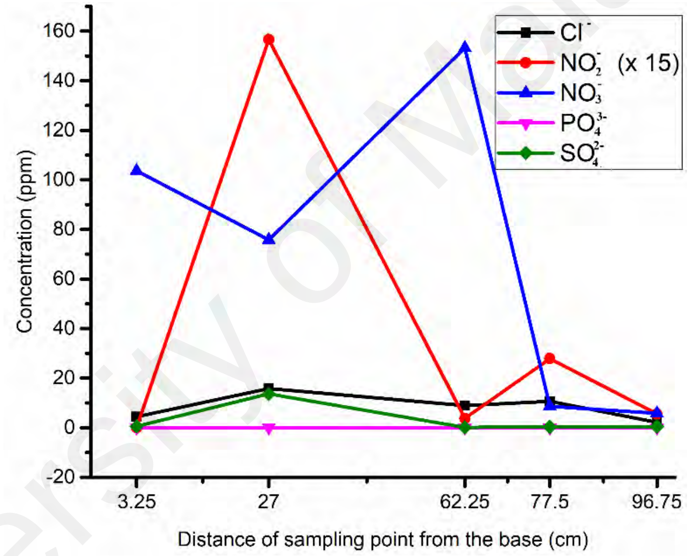

Figure 4.26: Third run of nitrite spiked Well water in modified column.

4.5.2.2 Effect of carbon source composition.

It is observed that total organic by-product's reducing capacity of the system is more achieved through down gradient attenuation. Starting with the initial TOC value of 3.55 ppm in the influent (at P_1) and a maximum total organic carbon concentration of 40.35 ppm at P_3, a carbon buildup of 18.77 ppm was recorded at the soil column exit. This accounts for 11th fold carbon input by the materials and 53% reduction of the carbon by the system. To this end, it is safe to assert that unlike similar remediation alternatives, this laboratory scale composition of sequence multi barrier reactive media has the advantage of remediating 94.4% of the target pollutant (NO_3^-) within the system, 99.20% of the target pollutant down gradient of the system and 96.4% of its lethal by-product -NO_2^- (Figure 4.27).

The relatively low attenuation capacity of organic substituent within the setup is attributed to lower flow rate, various carbonaceous matrix (labile and recalcitrant) and shorter distance (as compared to aquifer). The lower flow rate tends to dissolve more organic substances out of which the labile is mostly utilized by the denitrifying bacteria, generating incomplete denitrification with concomitant liberation of nitrite. With the exhaustion of nitrate, the rate of degradation slows down and since denitrification is a major source of organic matter removal (Ruiz *et al.*, 2003), this causes dissolved complex organic components to take longer time before complete degradation (Gomez *et al.*, 2000). Further observation of the effluent behavior in the simulated pristine aquifer condition (in a soil tube) revealed a gradual but effective downward attenuation trend of the organic constituents generated by the media at the gate.

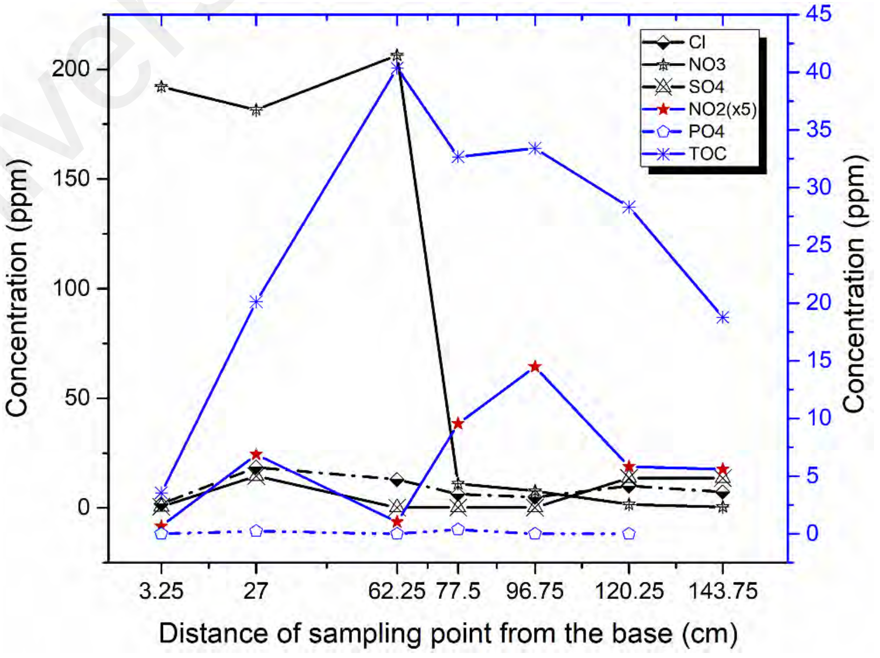

Figure 4.27: Pattern of TOC distribution across the sampling point of the column.

The concern for the accumulation of biomass due to release of heavy organic products is allied by the presence of indigenous decomposers within the gate system and down the gradient. This is manifested in the level of reduction of the total organic carbon within a short distance in the soil tube column (from 33.41 ppm in P5 to 28.32 ppm at ½ soil column and 18.77 ppm at the final exit of soil column (Figure 4.27). Going by the rate of reduction within the half-length and full-length of the soil column, it can be deduced that the treatment would be more effective in real aquifer system due to the effect of advective dispersive properties (Gelhar *et al.*, 1992) . It is worth noting that, generation and release of organic matter, begins at the first compartment and intensified at the second compartment due to the presence of readily dissolved organic substrate (sawdust). Thereafter, the declining concentration of the organic matter tallies with the reduced concentration of nitrate after the third compartment. This is because of microbial utilization of the nutrient (nitrate) for the degradation of the organic matter in the anaerobic medium.

4.5.3 Surface characterization techniques

Surface characterization technics is employed to assist in assessing longevity potential of the gate's materials. To achieve that, isolated fragments of date seed and wood chips were subjected to two surface characterization techniques (FESEM-EDX and FTIR) before and after subjecting them to degradation process in the column.

4.5.3.1 FESEM-EDX result

The result obtained from the Field Emission Scanning Microscopy/Energy Dispersive X-ray Analysis (FESEM-EDX analysis) revealed a clear visual indication of preferential decomposition amongst the materials. This is because while the date seed relatively maintained its surface texture after the column analysis and was only perforated with few cracks and tiny pores Figure 4.28 (b-c), the wood chips was deformed with wider openings across the surface Figure 4.29 (b-c). The distortion is conspicuous in both 10 μm and 5 μm magnifications. Fragments of weakening surface is also evident on the images of wood chips after column experiment, depicting image of cracks associated with the crushing activity rather than decomposition (Figure 4.28 d). This may not be unassociated with the composition of date seed, which has been reported to consists of hemicelluloses (23%), lignin (15%) and cellulose (57%) (Danish *et al.*, 2014). It is also observed that medium magnification exposes more of the elements for mapping in EDX.

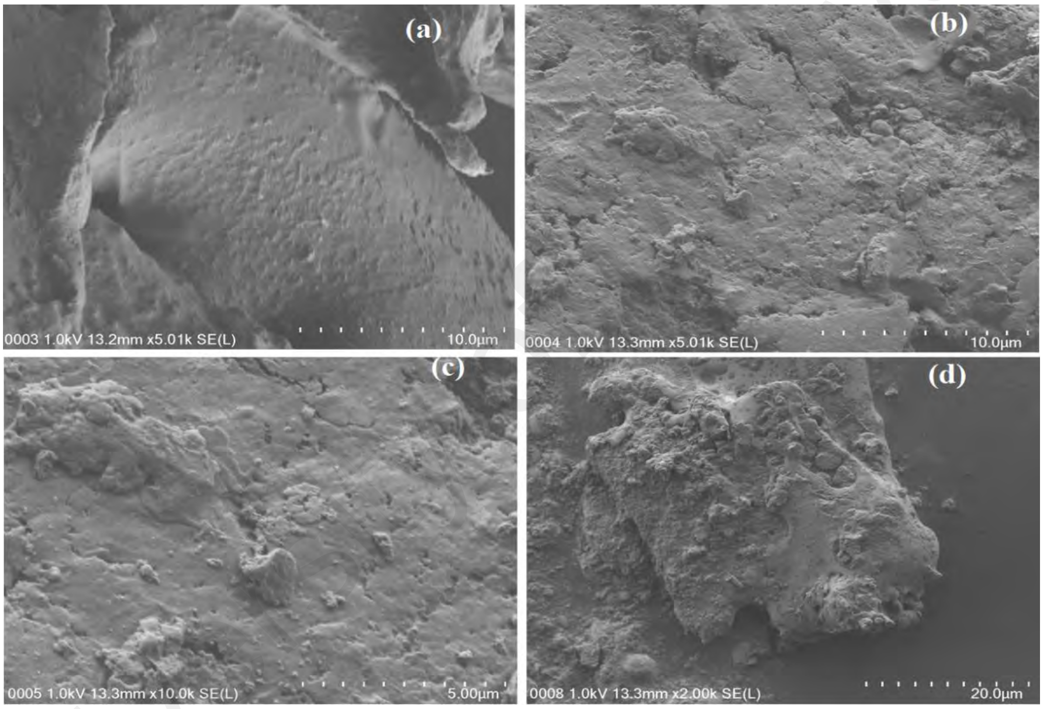

Figure 4.28: FESEM image for date seed. (a) 10 μm fresh seed (b) 10 μm used seed (c) 5 μm used seed (d) 20 μm used seed

The elemental mapping of the fresh wood chips gave the highest weight % distribution of carbon at 61.12% followed by oxygen and nitrogen at 31.79% and 7.09%, respectively. After subjecting the wood chips to column test however, new elements emerged with a concomitant decrease of weight % distribution of carbon to 42.96% and rise of nitrogen and oxygen to 7.58% and 33.36%, respectively (Table 4.6).

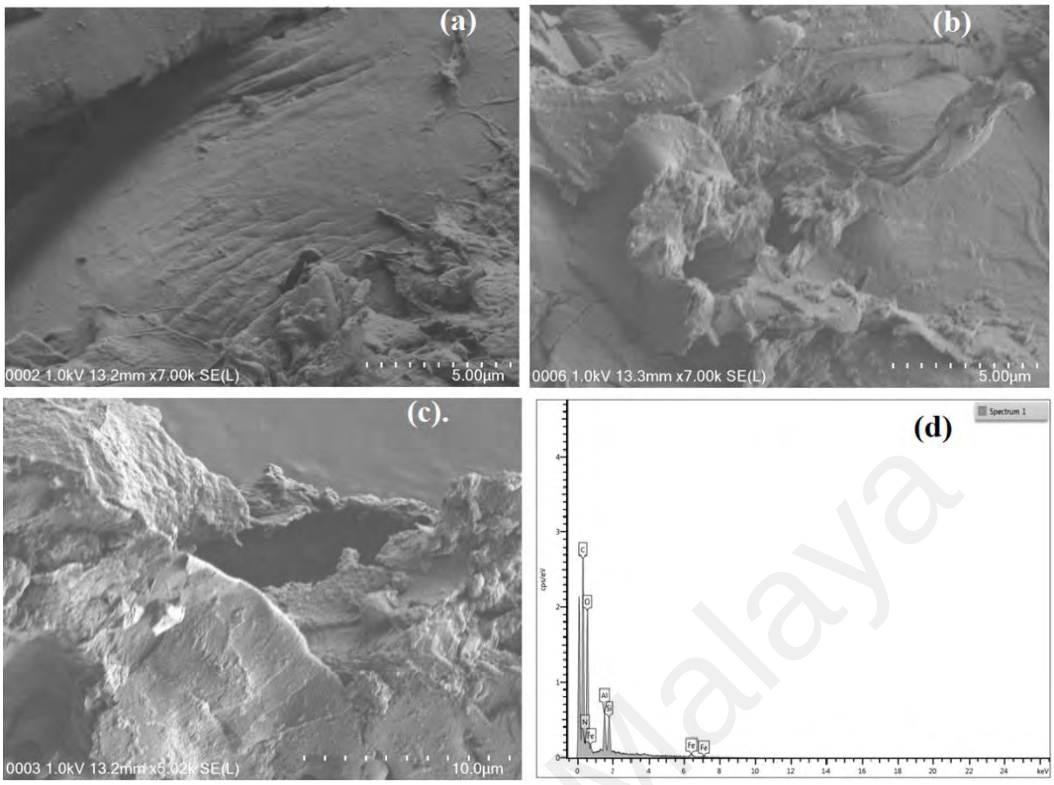

Figure 4.29: FESEM image for wood chips. (a) 5 µm fresh wood chips (b) 5 µm used wood chips(c) 10 µm used wood chips

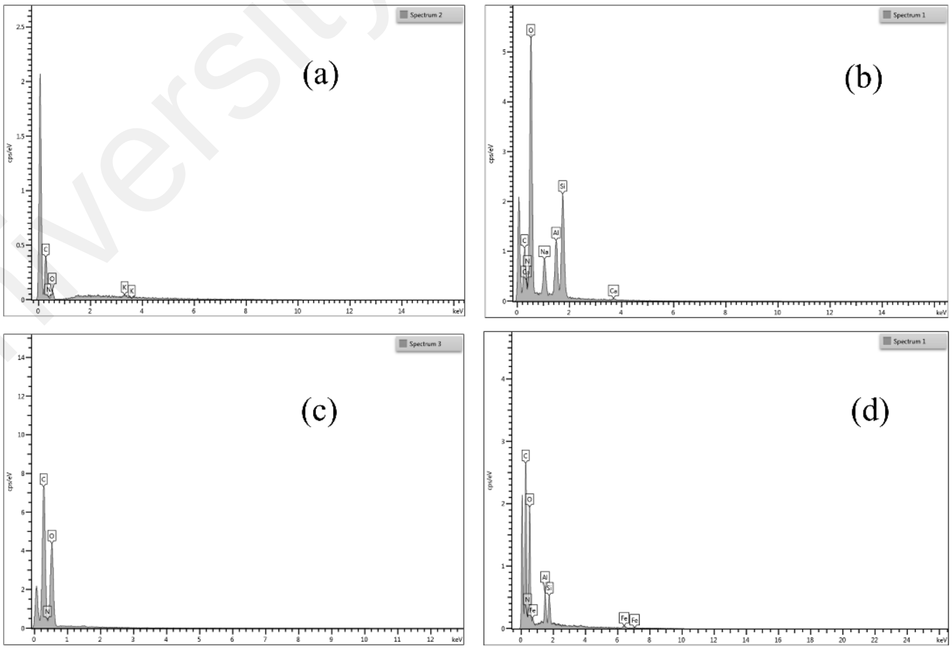

Figure 4.30:: Elemental mapping of wood chips by EDX. (a) fresh date seed 10 µm (b) used date seed 20 µm (c) fresh wood chips (d) used wood chips 25 µm

Table 4.6: EDX Elemental mapping for fresh and used wood chips at10 µm

S/N	Element	Wood Chips Before (10 µm) Weight %	Wood Chips After (10 µm) Weight %
1	C	61.12	42.96
2	N	7.09	7.58
3	O	31.79	33.36
4	Al	–	3.85
5	Si	–	4.13
6	Fe	–	8.12
	Total	100.00	100.00

While the decline of mass of the carbon (30% depletion) is understandable on account of dissolution and decomposition, the rise of nitrogen may indicate possible leaching or adsorbed portion of the nitrate spiked influent (due to the possibility of mineralization) or both. The post-column emerging elements of Al, Si and Fe revealed a percentage composition of 3.85, 4.13 and 8.12 %, respectively. Here, while a possible leaching of Aluminum is envisaged, much of the Fe and all of the Si are certainly derived from the soil.In contrast to the wood chips, elemental mapping of fresh date seed by EDX revealed four elements with values in descending order. These elements are; carbon, oxygen, potassium and nitrogen, with weight % distribution of 74.36%, 20% and 0.27%, respectively (Table 4.7).

Table 4.7: EDX Elemental mapping for fresh and used date seed at 10 µm

S/N	Element	Fresh date seed (10 µm) Weight %	Used date Seed (10 µm) Weight %	Used date Seed (25 µm) Weight %
1	C	74.36	67.85	–
2	N	0.27	9.79	–
3	O	20.0	22.36	–
4	K	5.37	–	–
5	Na	–	–	4.53
6	Al	–	–	6.83
7	Si	–	–	13.82
8	Ca	–	–	0.57
	Total	100.00	100.00	

After the column test, the weight % values changed to 67.85%, 22.36% and 9.79 % for carbon, oxygen and nitrogen, respectively. This reaffirms the depletion trend of carbon (with a value of just 8.75% reduction at the given period) and appreciation of oxygen and nitrogen as experienced in the wood chips.

Upon expanding the area of magnification to 25 μm, interesting revelation of additional elements was made, in which Ca (though in low percentage) and Na are of interest (Table 4.7). The detection of additional elements not found in the fresh seed becomes important because as the literature indicates, Ca and Na amongst the elements identified in date seed (Devshony *et al.*, 1992). However, the change in loading of the ions as opposed to the ones in literature is attributed to the influence of column-materials, as many substances are embedded. For example, in contrast to the percentage of Fe recorded in this study (0.07 % in used wood chip), lower percentage was reported in a decaying wood by previous research (Enoki *et al.*, 1997). As for the date seed, only 0.0023% was reported by Besbes *et al.* (2004). The emergence of these elements after the column test also implies that some of them were leached or sequestrated as the decomposition progresses (Devshony *et al.*, 1992).

Finally, it can be deduced from the FESEM surface morphology of wood chips and date seed that the wood chips are more susceptible to biological decomposition than the date seed. It is also evident from the EDX result that 30% reduction of carbon atom in wood chips is by far more than the 8.75 % reduction of carbon in date seed for the same period. In both cases, nitrogen and oxygen appreciate significantly, indicating the effect of NO_3^- spiked influent water and the redox environment that envelops degradation process.

4.5.3.2 Fourier-transform infrared spectroscopy (FTIR)

The same samples of date seed and wood chips, which was obtained before and after column test, were subjected to FTIR analysis to observe the changes in chemical functional groups formation and removal. Distortions in the intensities of the absorption occur in the region 2250-750 cm^{-1}. In line with previous research finding, the bands observed at 2932 cm^{-1} and 2865 cm^{-1} assigned to the asymmetric and symmetric vibration of CH_2 and CH_3 as well as the band at 1650 cm^{-1} assigned to carbonyl stretching vibration of amide due to double bond stretching vibration do not change after decomposition (Özcan *et al.*, 2005).. It is hereby inferred that unlike in the wood chips, this stability denotes resistance to decomposition. However, in fresh date sample, a manifestation of protruding band peak at 2073 cm^{-1} assigned to (N=C=S) absorption due to isothiocyanate stretching is missing in the decomposed date seed. Likewise, the band peak assigned to carbonyl C=O absorption (1711 cm^{-1}) due to carboxylic acid stretching is missing in the fresh seed (Figure 4.31). This indicates a redox situation consisting of bond formation and breakage in the process of decomposition.

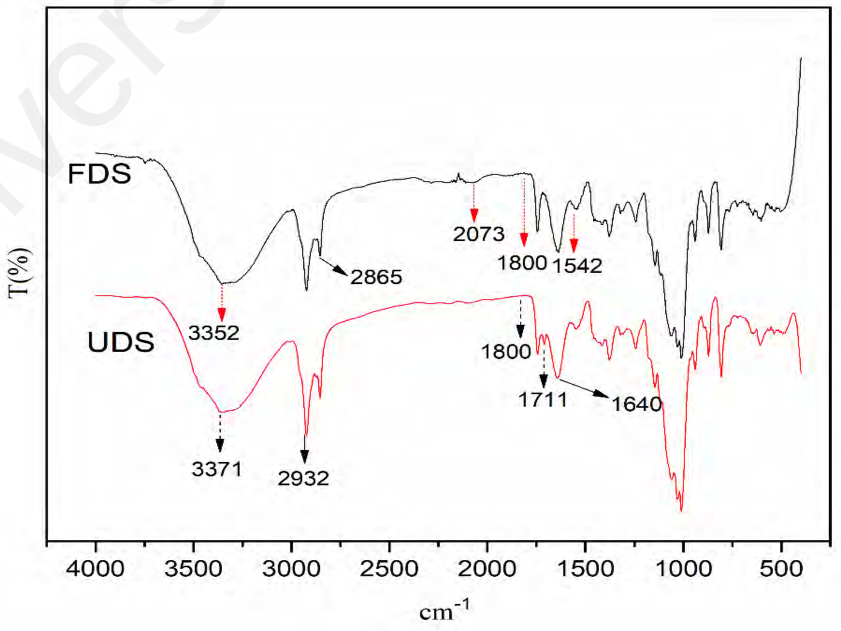

Figure 4.31: FTIR spectra of fresh date seed (FDS) and used date seed (UDS)

Also, in line with previous research finding on date seed, all the samples manifest weak bands at 1800, indicating the presence of aromatic compound (Sait *et al.*, 2012). Moreover, the strong and broad band for –OH absorption of alcohol stretching (3550-3200 cm^{-1}) is present in both fresh and decomposed seed. In addition to the carboxylic acid group identified in the decomposed date seed, this could be the reason why decomposed products always record higher oxygen content in EDX result than the fresh products.

The wood chips spectra manifest distortions of intensities that occurs in the region 2115-1250 cm^{-1} (Figure 4.32). A region wider than what was obtained by Pandey (1999) using transmission and drift methods of FTIR. However, the manifestation of C-H stretching absorption around 2900 cm^{-1} obtained by the above research coincides more with the fresh wood than with the used wood in this study. This may be attributed to differences in decomposition period between the studies or methodology applied in the infrared analysis. The distribution of the spectra indicates a significant difference of absorption band between the fresh wood and decomposed wood in alkyne stretching of C≡C absorption (falling within the range of 2140-2100 cm^{-1}). The weak signals at stretching range of monosubstituted alkyne at 2114.89 cm^{-1} in decomposed wood attest to that.

The other signals observed are of the same skeleton, but different functional group. Here, signal at 1727.86 cm^{-1} denoting the carbonyl C=O absorption of aliphatic ketone stretching is observed in decomposed wood chips while signals at 1731.64 cm^{-1} for C=O absorption of β-unsaturated ester stretching is observed in fresh wood chips. In the same vain the aromatic skeletal vibration band at 1609 cm^{-1} in the fresh wood chip diminishes in the used wood chip, indicating possible ring opening reaction during the decomposition.

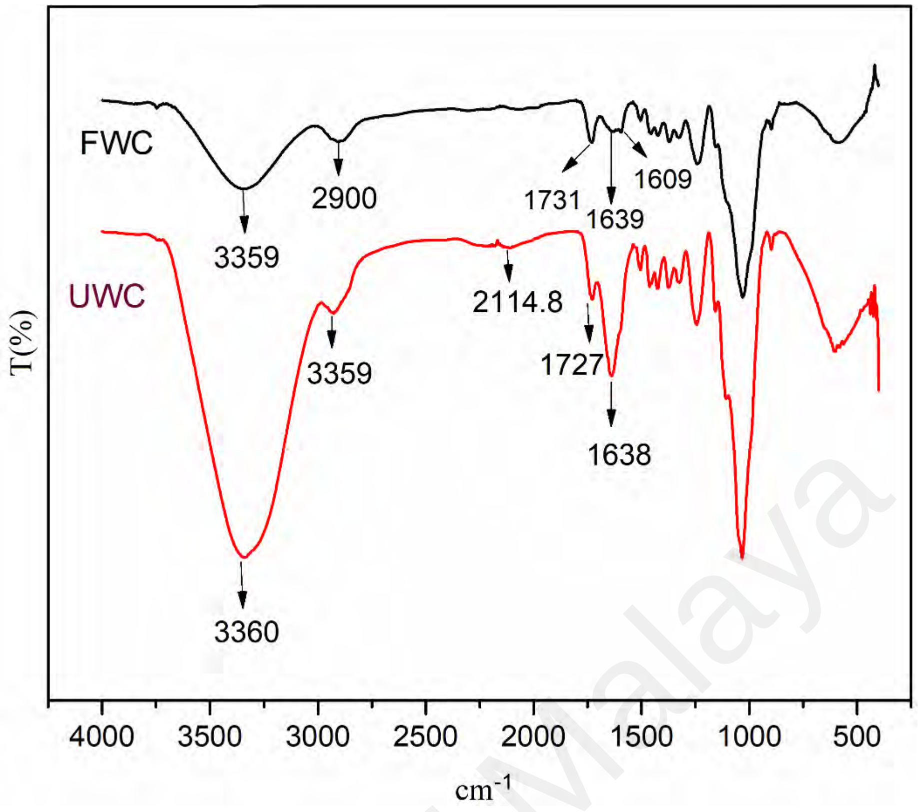

Figure 4.32: FTIR spectra of fresh wood chips (FWC) and used wood chips (UWC)

However, the intensity of the band at 1638 cm^{-1} (C=C absorption due to alkene stretching) in used wood chips is higher than signal at fresh wood chips, supporting the argument of promoting bond breakage in decomposition process. These changes indicate the occurrence of reduction at that particular site. Finally, the strong and broad band for –OH absorption of alcohol stretching (3550-3200 cm^{-1}) is present in both samples but more prominent in decomposed wood chips than fresh woodchips. In summary, the process of decomposition entails multiple redox condition at different site and, depending on which bond or functional group is affected in the process, different transformations are evident in this study.

4.5.4 Rate of decomposition- The kinetic study

The commonly used model of single-exponential model (Equation 4.12) is considered first in this study and the relative decomposition rate, which is expressed in terms of percentage of fraction is applied. In line with previous findings (Ximenes *et al.*, 2008), readings obtained from the observation of the decomposition process revealed that the initial rate of decomposition for both date seed and wood chips was very slow, indicating the preliminary setting of acclimatization period. Also, inverse relation pattern between the date seed and wood chip began on the 7th month but the rate of degradation is faster in wood chips than in date seed.

$$X = X_o \, e^{-kt} \qquad \textbf{(4.12)}$$

While the decomposition of wood chips (Figure 4.34) indicates a possible pattern of steady rate at the 11th month, that of the date seed appears slower and inconsistent. In the latter, the observation is attributed to varying decomposition rate between the endosperm and hard wall cover of the date seed as shown in Figure 4.33. From the values of R^2 in both date seed and wood chips, it can be deduced that exponential model would fits the decay pattern of the materials. Except for the interpolated degradation pattern of the date endosperm (Xe) with R^2 value of 0.8805, both date seed, date hard cover and wood chips have good R^2 values of 0.9073 and 0.9148 and 0.9403, respectively. While the k values depict the degree of rate of degradation, the distribution pattern speaks volume of the differences in their degradation potential. The degradation pattern of the endosperm (interpolated values) dictates the degradation pattern of the entire seed and hence, similar distribution is observed between the date seen (Xd) and endosperm (Xe) (Figure 4.33).

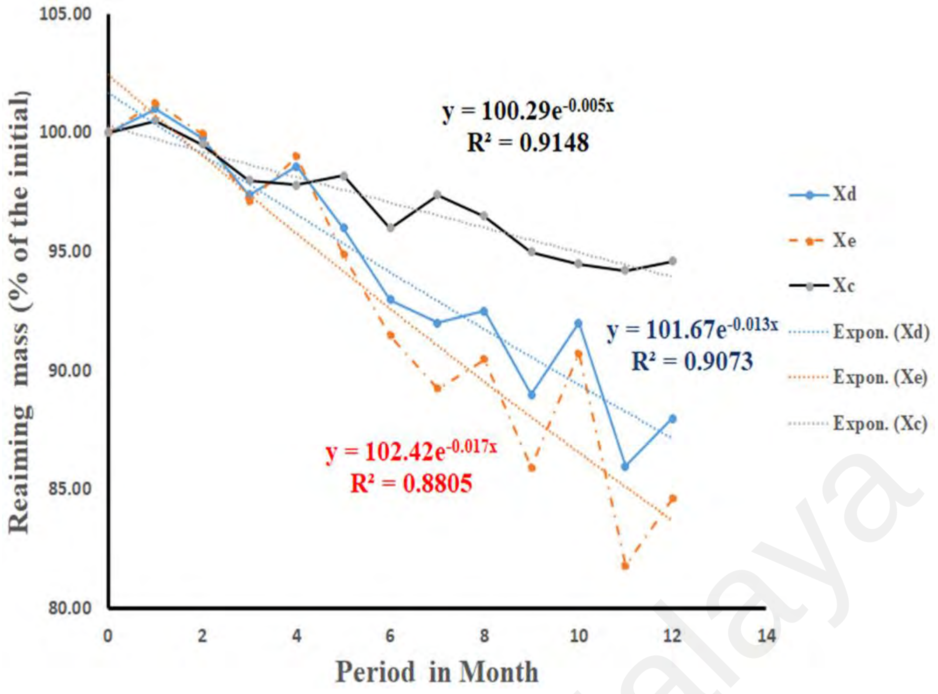

Figure 4.33: Rate of degradation of date seed. Xd is the entire date, Xc is the cover wall of the seed and Xe is the deduced degradation pattern of the endosperm of the seed

z

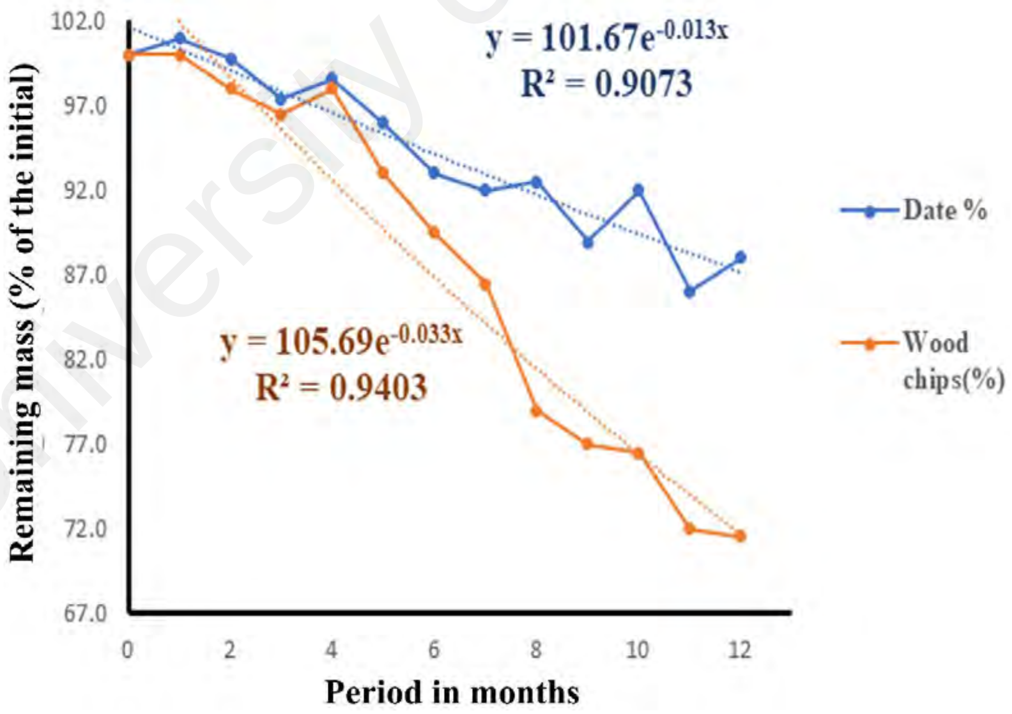

Figure 4.34: Comparative rate of date seed and wood chips in the same column

The initial surge in mass of the date seed observed in the first month may be attributed to binding capacity of the soft endosperm surface to dissolved organic debris from sawdust within the column. It is pertinent to observe that sharp decline of date seed plot began at the fourth month of the column operation and at that moment, the degradation rate of the date cover began to lose momentum. The same observation is made from 6th month through the 8th month, where every slight loss of mass on the date seed is accompanied with slower degradation process of the date cover and vice versa. In the same vain, from the 7th month upward, wherever the date seed cover decline in mass, the percentage mass loss of the interpolated endosperm is observed to be closer to that of the entire date seed and vice versa. This may be inferred to be the period in which the date cover starts to exert impact on the degradation rate of the entire seed and hence be factored into the rate determining substrate. This differential influence of the date cover on the degradation potential might be the reason why the date cover tends to indicate a stabilising pattern from the 9th month upward while the date seed is yet to show any sign of stability one year after the beginning of the experiment. This clearly indicates the presence of recalcitrant component within the seed, compelling the need for considering double exponential model (Equation 4.13) in expressing the absolute decay rate of the seed.

$$X = Ae^{-k_1 t} + (1-A)\, e^{-k_2 t} \qquad \textbf{(4.13)}$$

Where “X” stands for the total mass remained after a time “t” and “A” is the labile portion of the material under review. In determining X through the above exponential model, the values of the results obtained at the seventh month was substituted in both equation 4.12 and 4.13 With values of Xo as 1.11 g, $k_1 = 0.01$, $k_2 = 0.005$, $t = 0.5833$ (7/12) and A= 0.66 g, substitution of the values in the equations gives the following results.

Single exponential $X = X_o e^{-kt}$

$X = 1.1035$

Double exponential function $X = Ae^{-k_1 t} + (1-A)\, e^{-k_2 t}$

$X = 1.099$

With a differential margin of 8.05% and 7.64% deviation from the experimental value of 1.02 for single exponential and double exponential result, it can be deduced that double exponential model fits more than the single exponential model as it is closer to the experimental value. Moreover, judging from the value of rate constant alone, comparative analogy of differential life span can be drawn between wood chips and date seed. Using the rate constant to determine the half-life $t_{(0.5)}$, it follows that

$t_{(0.5)} = -\ln(0.5)/k = (0.693)/k$

Given the respective values of k for date seed and woodchips, their half-life would be

$t_{(0.5)} = 0.693/0.013 = 53$ month (about 4.5 years) for date seed and

$0.693/0.033 = 21$ month (about 2 years) for wood chips.

This spell a lifetime of 1 gram of date seed stretching to 9 years and 1 g of the wood used to four (4) years. The life time obtained here is similar to the low density woods reported by (Mackensen *et al.* (2003)). Depending on the quantity and quality of the carbonaceous material used, the lifetime can be several times multiple of what is obtained here. The advantage of using date seed is seen in utilizing the slow degradation rate of the cover wall, which contains mannan. Since the above half-life was calculated based on exhausting 95% of the material, the 5% that would remain after 9 years would be the cover wall. Going by its “k” value, the half-life of the cover would be 138.6 months, which is equivalent to 12 years. The lifetime of the date seed can then be deduced to be

24 years under such weathering condition. With this, it can be deduced that the date seed would spend as much as six times the period a wood chip would last before it is completely degraded. Though the process can be slow in date seed, but constant supply of carbon is assured for longer period, which serves the most desirable need of the in-situ remediation technology of the day.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

The research focused on exploiting natural phenomenon and utilizing different type of carbon sources presenting preferential affinity to degrading microorganism for decomposition. Consequently, the researcher designs a novel funnel and gate setup of which the packing structure of the sequential multi-barrier reactive media takes the pattern of the gate's geometry. The present chapter, therefore, summarizes the previously presented results and conclusions of the various chapters of this thesis, following the objectives set at the beginning of the thesis. More so, the chapter highlights some limitations of the present work and gives some recommendations for future studies.

5.1 Conclusions

In line with the outlined objectives of this study stated in chapter one, the key experimental findings from chapters are summarized under the stated objectives as follows:

Consequently, the first objective seeks to investigate the factors responsible for the high degree attenuation of nitrate using geochemical analysis in the area under study (Bachok, Kelantan) and is addressed through the following findings:

The data obtained from physiochemical analysis conducted on soil and groundwater samples taken from established low nitrate agricultural site of Bachok using three different chemometric techniques were paired with a long-term hydro chemical data record of the wider area to evaluate the general variation pattern and identify the most influential parameters in determining the temporal and spatial hydro-geochemical variations.

The principle companent analysis run on the combined data, gave a general variation with eigen vectors of PC1 majoring in conductivity, chloride and sulfate. The Biplot drawn separates the two seasons (wet and dry seasons) sucessifully, apportioning the related parameters to coastal sampling sites. This result is corroborated by both the two-way HCA and canonical plot of discriminant analysis with a step foreward analysis revealing four parameters in order of decreasing significance as Cl^{-}, SO_4^{2-} Fe and conductivity, respectively.

Temporal analysis revealed that Fe is the most significant parameter in both locations, signifying its ubiquitous distribution in the area and hence supporting autotrophic denitrification where carbon substrates may be in limited supply.

Spatial-based analysis proved more significant with clear separation of all parameters and higher eigen vectors, suggesting that seasonal variation have little significance in denitrification potential of the area as compared to soil constituents and anthropogenic inpact

Also, it was revealed that the dominant factors in wet season is a combination of sea influence and dissolution of mineral substances in the soil. In dry season however, recharge withdrawal and evaporative concentration of solutes makes them more prominent. Factor of mineral content also plays role in dry season due to recharge from rivers

Hinging on the aforementioned it is concluded that anthropogenic inpact contributes immensely to water quality status around the area. These are attributed to industrial and agricultural input, which proved to be significant factors. It also follows that the natural attenuation capcity of the soil is enhanced by the abundance presence of reducing mineral such as iron ores and manganese. The iron is believed to have been formed by the inundating seawaves deposited along the coastal plain during the halocene period. The soil is therefore identified as a rare soil with tremendous denitrification capacity. It is recommended that further analysis be carried out to detarmine the distribution of these elements across the coastal and inland plain. The recommendation for the utilization of this soil as denitrification agent in areas detarmined with low denitrification potential is desirable and hence, this research continued with testing the soil as a grafting agent at laboartory scale. In line with this recommendation, another section (section 4.5) targets the utilization of the soil for that purpose.

The second objective was to identify and screen suitable carbon-based electron donors for utilization in denitrification process. This objective seeks to explore means of providing a cost effective and long-term source of carbon, utilized in reactive media of PRB for denitrification purposes. The present thesis reports easier approach towards screening carbon sources bearing in mind their leachable content and by-products. Three seeds (date seed, *Moringa oleifera* seed and Tamarind seed were screened using two extraction methods viz agitation-aided aqueous extraction and ultrasonic aided aqueous extraction. Comparative measure of the two methods reveals that the latter is more effective than the former. Tamarind seed was discarded because of objectionable output after extraction. It is also observed that while using IC for identifying anions, lower pH shifts the retention time backward and generates detectable ion species within the region of phosphates, while higher pH exposes and enhance the water deep and chloride peaks. With the exception of NO_3^-, the result signifies the influence of pH on the dissolution or leaching potential of the solvent (water) on the seed.

Finally, date seed and *Moringa oleifera* seed (in small quantity) were selected in which date seed is identified for its unique quality of high percentage of carbohydrate, slow degradation process and less objectionable by-products. It is recommended that further research be conducted to determine composition of the degradation by-products of date seed to avert pollution swapping. The identification of effective soil (Section 4.2) and selection of suitable carbon source for denitrification in this section led to the formation sequence multibarrier system. The effort of combining the two was made for attaining long term remediation through testing effective composition materials assembled in a fashion that that they aligned with the geometry of a novel funnel and gate design. This leads to the third objective.

The third objective is to develop effective and environmentally friendly gate material for nitrate remediation in a sequence multi-barrier system while achieving delayed remediation for attaining longer life span of the system. The above objective is treated and achieved through dual approach of (1) conceptual design of funnel and gate and (2) Aquifer simulation via laboratory column study. The combined approach makes it possible for the research to consider a novel design of twin funnel and gate, in which the packing structure of the gate is made to align with the geometric configuration of a funnel.

The novel design of the funnel and gate termed as "double-sided trench/twin funnel and gate" set up is made with the view of exploiting geometric pattern of the structure to mitigate mounding and pollution swapping. Application to hypothetical case revealed an improved accommodation of wider capture zone, higher flexibility of gate configuration and better control of damning effect. In addition, the novel structure gives room for placing reactive material in parallel and series order, paving way for the possibility of mounting permeable reactive interceptors. The concept was then put to test through laboratory column analysis, simulating the aquifer response to a combination of soil in objective 1 and selected carbon sources in objective 2. This objective then serves as the confluence point where objective one (1) and objective two (2) are put to test

The findings from laboratory column analysis revealed a clear variation of response to denitrification between groundwater and distilled water, stressing the influence of matrix effect. The efficiency of the column setup is found to be dependent upon the ratio, composition, flow rate and the initial concentration of NO_3^- in the influent. The composition of materials in the four compartments were dynamically adjusted to attain optimum ratio that can yield excellent remediation of the primary pollutant and generate less objectionable by-products. The zones are in the ratio of 115.4: 50.5: 1: 24.3 for soil, date seed, *Moringa oleifera* seed and lime stone ; 32:3:1:5.2 for soil, wood chips, saw dust, lime stone ; 8.3:1.7:1 for soil, date seed, Lime stone and 1: 1.6 for soil and activated carbon in the 1st , 2nd 3rd and fourth compartment, respectively. While the soil plays a significant role in providing the required denitrifiers, the Introduction of limestone into the 1st 2nd and 3rd compartments was found to be useful in providing buffering conditions necessary for stabilizing the microorganisms.

In attempt to estimate the susceptibility of the carbon sources to degradation, surface characterization techniques were employed on the gate materials. The result indicates different response of materials exposed to the same condition. The differences are exhibited in terms of manifestation of morphological variation between different materials (date seed and wood chips) and same material (date seed or wood chips), before and after the decomposition process. Differences in images of surface deformation and sizes of holes and cracks were exploited in FESEM and the results indicates that wood chips are more susceptible to decomposition than date seed. The EDX results corroborates with the FESEM and sequestration of embedded minerals is observed. The FTIR analysis of used wood chips indicates number of reduction changes, attesting to the susceptibility of the wood for consumption by the microbes, and hence, more prone to decomposition. It can therefore be concluded that in addition to the successful remediation capacity of the sequence multi-barrier reactive barrier, the physical and morphological analysis obtained from the surface characterization techniques revealed a preferential consumption of one carbon source over another. This preferential consumption causes delay in the degradation process (of date seed in preference to sawdust and woodchips) and hence increase the lifespan of the gate materials.

Finally, the result of decomposition study on the two materials (date seed and wood chips) goes in tandem with the above-mentioned findings. This is evident from the measured rates of decomposition as the respective half-life of 2, 4.5 and 12 years for 1 g of wood chips, date seed and 0.37 g of date cover were respectively deduced. Though the rate of decay model for both materials fits into double exponential model, the absolute lifetime of the date seed was deduced to be 24 years, ensuring steady supply of carbon for longer period and serving the most desirable need of the in-situ remediation technology. With as much as six times longer life span than the wood chips, date seed has the potential to replace the preferred wood chip in remediation industry.

It is recommended that more efforts be put into biodegradable seeds with higher carbon content for utilization in PRB gate materials. This is because the high lignin content seeds are lying down as environmental liability consuming energy and resources for their disposal. It is also recommended that the present composition and order of placement be utilized on field PRB system for a long-term treatment of pollutants. In view of the uncertainties associated with the conceptual designed that is tested mathematically, is recommended that further research on uncertainty in design be conducted to enhance the optimization of the remediation design.

5.2 Contribution

In a summarized form, the followings are the visible contribution of this research work in the area

i. It is pertinent to note that even though number of researches are conducted around the coastal plain of Kelantan region, no research seeks to find out the hydro-geochemical factors responsible for excellent nitrate attenuation across the area. This is important observation that forms the leading enquiry for the development of this research.

ii. A remediation potential of Paleo-beach soil of the area is identified and the development has the potential of opening doors for further research that may lead to the utilization of the iron-rich soil as a grafting agent in filling within permeable reactive barrier meant for denitrification.

iii. Finding in this research highlight the fact that due to the active roles of mineral content in the soil and anthropogenic influence around the area, influence due to spatial variations is more prominent in the area and hence, the need to curtail anthropogenic input to prevent pollution build-up is essential.

iv. Effect of pH variation on a fast method of screening carbon source to determine leachable anions in aqueous solution is reported after determining the most suitable extraction method.

v. A lignin-based seed with high content of mannan is explored for possible utilization as a carbon source in PRB reactive media and the material shows a promising trait with prospect of long-term usage and hence, longer lifespan of the remediation system.

vi. A novel design of funnel and gate is forwarded with the aid of trigonometric application and the design is mathematically tested to possess wide capture zone with embedded safety factor against mounding.

vii. Using the design order of the funnel and gate in (vi) above, a column assembly with a packing structure is developed with varying composition of materials in (ii) and (iv) above. The column order proved to be effective with 96.4% NO_2^- removal efficiency and the attenuation of organic by-products is recorded to reach 53% within the first 46 cm of the simulated down gradient column.

viii. Surface characterization techniques using FESEM and FTIR revealed images that indicate preferential degradation of wood chips over date seed and more of reduction process on the materials after the column test, respectively. This indicates delayed decomposition process, which will ensure higher lifespan of the gate materials. In the light of the above, the degradation analysis corroborates the above conclusion as different carbonaceous materials were calculated to have wide difference of half-life. Date seed was found to have the longest half-life, tripling that of the wood chips.

5.3 Limitation of the study

Due to time constraints and financial hurdles, this study is limited to aquifer simulation using column study. An alternative means of conducting the study is through rectangular aquifer model, which would fit as well for modelling the funnel and gate design into the aquifer. In such a practical scale, the prospect of averting mounding by the structure is more prone to be determined compared to the mathematical and/or computer assisted simulation programmes. Also, no measurements of absolute leachable anions on the materials is made, and hence the extraction is limited to the timing and condition employed in the method.

5.4 Recommendations for future work

The contribution of the present research work on this vibrant area of remediation has been itemized above. However, the limitation highlights the shortcomings of the research that need further study. The followings are some suggestion for further research.

It is then recommended that the proposed funnel and gate be constructed using 3-D printing technique to avert any variation of angle measurement. It is also recommended that a rectangular aquifer model box be employed for measuring the efficacy of the proposed twin funnel and gate and potassium bromide (KBr) be used as the conservative tracer agent for measurement of damning effect. This is because the flat form aquifer condition is more complex than the column type aquifer condition and hence closer to measurement of plume's cross-sectional area.

Looking at the different distribution of leachable metals across the surface of the same material in EDX result, it is recommended that a thorough and prolonged leachable ion analysis be carried out on the carbonaceous materials before utilization for any remediation purposes. This is because, even though the dissolution entropy of the experiment is much higher than the one in natural environment, the prolonged contact in the latter can present different matrix.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

a) List of publications

1. **Dahiru, M**., Kartini, A. B. N., & Yusoff, I. (2019). Development of effective sequence multi-barrier reactive media for nitrate remediation in groundwater systems. *RSC Advances*, *9*(27), 15437-15447.

b) List of Papers Presented

1. **Dahiru, M**., Kartini, A. B. N., & Yusoff, I. (2017). Carbon source screening for nitrate remediation in permeable reactive barrier: Ion chromatography technique. Paper presented at the 5th International Science Post graduate conference proceedings, Johor, Malaysia.
2. **Dahiru, M**., Kartini, A. B. N., & Yusoff, I. (2017). Design, Construction and Application of Portable Water Treatment Model from Locally Available Materials. Paper presented at the 6TH International Conference of Young Chemist (ICYC2017) conference proceedings, Penang, Malaysia.