IRON AND MANGANESE REMOVAL FROM GROUNDWATER BY USING LIMESTONE FILTER WITH IRON-OXIDIZING BACTERIA

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ABSTRAK

Air bumi merupakan sumber penting yang dapat melengkap peningkatan permintaan untuk keperluan air bersih. Ia banyak terdapat di kebanyakan tempat, malangnya kerap hadir bersama besi dan mangan yang memerlukan proses rawatan lebih lanjut untuk memastikan kualiti air dapat diterima. Objektif utama kajian ini adalah untuk menilai prestasi rawatan air bumi dengan menggunakan penapis batu kapur dan batu kapur yang dibantu dengan bakteria pengoksidaan besi (IOB) yang terdapat di Loji Rawatan Air Kampung Chap, Kelantan. IOB telah digunakan di loji rawatan, dihidupkan pada permukaan penapis pasir. Batu kapur dengan saiz zarah 0.6-2.36 mm adalah hos alternatif kepada IOB dalam kajian ini. Batu kapur juga bertindak sebagai media penjerapan kerana kajian terdahulu menunjukkan keupayaannya dalam menyingkirkan logam berat. Sampel air bumi dari lubang jara USM dicirikan terlebih dahulu. Perbandingan prestasi rawatan dibuat berdasarkan lajur penapis pada kadar aliran 4.0 L/jam dan masa tahanan sebanyak 2.94 minit. Hasil kajian menunjukkan bahawa 2.0 jam waktu operasi adalah masa yang optimum untuk kedua-dua kaedah rawatan. Dengan masa operasi 2.0 jam, peratusan penyingkiran besi adalah 77.69% untuk penapis batu kapur sahaja dan 81.72% untuk batu kapur yang dibantu dengan IOB, manakala peratus penyingkiran mangan adalah 82.60% untuk penapis batu kapur sahaja dan 83.63% untuk kapur dibantu dengan IOB. Keseluruhannya, rawatan air bumi oleh kapur yang dibantu oleh IOB mempunyai prestasi yang lebih baik daripada penggunaan penapis kapur sahaja. Apabila masa operasi meningkat, prestasi air bawah tanah yang dirawat akan menjadi lebih baik. IOB dapat memangkinkan pengoksidaan bentuk besi (II) dan mangan (II) yang larut masing-masing kepada bentuk besi (III) dan mangan (IV) yang tidak larut. Dapat disimpulkan bahawa kehadiran IOB bermanfaat dalam meningkatkan penyingkiran besi dan mangan dalam air bumi.

ABSTRACT

Groundwater is an important resource that can complement the increasing demand for fresh water needs. It is abundant in many areas; unfortunately, it commonly presents with iron (Fe) and manganese (Mn) which requires further treatment process to ensure the water quality is acceptable. The main objective of this research was to evaluate the performance of the groundwater treatment using limestone filter alone and limestone assisted with iron-oxidizing bacteria (IOB) found at the Kampung Chap Water Treatment Plant, Kelantan. IOB has been used at the treatment plant, immobilized on the surface of the sand filter. Limestone with the particle size of range 0.6-2.36 mm was the host alternative to the IOB in this research. Limestone also acts as adsorption media as previous studies have indicated its capability in removing heavy metals. The groundwater sample from USM Borehole was first characterized. The comparisons of the treatment performances were made based on the filter column at a flow rate of 4.0 L/hour and retention time of 2.94 minutes. The result indicated that the 2.0 hours of operation time was the optimum running time for both treatment methods. With the 2.0 hours operation time, the percentage of Fe removal was 77.69% for limestone filter alone and 81.72% for limestone assisted with IOB, while the percentage of Mn removal was 82.60% for limestone filter alone and 83.63% for limestone assisted with IOB. Overall, the groundwater treatment by limestone assisted with IOB has better performance than the use of limestone filter alone. As the operation time increases, the performance of the treated groundwater is better. The IOB can catalyze the oxidation of soluble form of Fe (II) and Mn (II) to the insoluble form of Fe (III) and Mn (IV) respectively. It can be concluded that the presence of IOB is beneficial in improving the removal of Fe and Mn in the groundwater.

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CHAPTER 1

INTRODUCTION

1.1 Background

Groundwater is one of the potential natural resources for human consumption and is essential to the vitality of agriculture and industry. The declines in groundwater levels and other effects of pumping due to the large-scale of development of groundwater resources has led to concerns about the future availability of groundwater to meet the needs in domestic, agricultural, industrial, and environmental.

In Malaysia, groundwater is also an important resource that can complement the increasing demand for fresh water needs. The usage of groundwater in many countries is limited because the usage is limited to the shallow unconfined aquifers by using dug wells (Ang, 1994). Deep tube wells in coarse sand aquifers have started to be developed in Malaysia in the past 30 years for the water supply of coastal towns such as Kota Bharu, Kelantan (Sofner, 1989).

Based on the population census published by the Department of Statistics Malaysia (2010), Jajahan Bachok has a population of 126,350 people. The population in Mukim Kg. Chap, Kelantan is about 2,451 people. The treatment of the groundwater in this locality is carried out by pumping the well with the pH adjustment. Then, the raw groundwater went through the coagulation and flocculation process to coagulate the suspended solids and sent to the sedimentation tank. Last, the effluent was treated by the sand filter with locally available ammonia-oxidizing bacteria (AOB) and the treated water is pumped to the elevated reservoir for supply.

At present, development is progressing in Jajahan Bachok such as the construction of housing estates, institutional premises, business centres, trading premises and also in agriculture. These developments have resulted in increases in the demand for public water supply. Currently, the production capacities in the treatment plants in the district are not able to meet the growing demand. The water supply scenario is worse during the drought season due to the limited availability of suitable water resources. Shortages also occur in the delivery of good quality potable water. These occurrences have seriously affected the socio and economic development activities in the district.

1.2 Problem Statement

As the demand for water usage increased, the surface water is not able to withstand the high demand of the people. Thus, groundwater is the most concern and the best alternatives for drinking water purposes and daily usages. Due to the natural geological factors, almost all of the groundwater has the problem especially the presence of iron (Fe) and manganese (Mn) which requires further treatment to reach the acceptable range of recommended raw water quality and drinking water standard based on the Engineering Services Division, Ministry of Health Malaysia (2010).

The presence of Fe and Mn in drinking water and water supplies can cause many problems, such as reddish colour and odour. In general, Fe exists in the soluble form of Fe (II) and is converted to the insoluble form of Fe (III) before the removal of Fe in the water purification processes (Cho, 2005), while Mn exists in the soluble form of Mn (II) and is converted to the insoluble form of Mn (IV). Due to the process of rain filtering through the soil, rocks, and minerals, the presence of Fe and Mn is very common in the groundwater. Throughout its descent, the rainwater will collect the Fe and Mn from these sources and deposits in the groundwater.

Thus, this research is carried out to determine the Fe and Mn removal efficiencies from the groundwater by using the two different treatment methods, which are limestone filter alone and limestone assisted with iron-oxidizing bacteria (IOB) found at the groundwater sample of Kampung Chap Water Treatment Plant, Kelantan. It has been used at the treatment plant, immobilized on the surface of the sand filter. Limestone with the particle size of the range 0.6-2.36 mm is the host alternative to the IOB in this research. Limestone also acted as adsorption media as previous studies have indicated its capability in removing heavy metals. IOB is expected to contribute to the oxidation reaction of the soluble form of Fe (II) and Mn (II) to the insoluble form of Fe (III) and Mn (IV), respectively.

1.3 Objectives

The objectives of the research include:

a. To culture the iron-oxidizing bacteria (IOB) from Kampung Chap Water Treatment Plant, Kelantan.
b. To identify the iron (Fe) and manganese (Mn) removal efficiency by limestone filter, with and without the presence of iron-oxidizing bacteria (IOB) at different operation times.
c. To determine the adsorption bonding (ionic, covalent, chemical) on the treatment process in (b).

1.4 Scope of Work

The main project scope is to determine the removal performances of different treatment methods, which are limestone filter alone and limestone assisted with IOB in removing the Fe and Mn. The adsorption bonding which is ionic, covalent and chemical bond of the reaction of limestone filter alone and limestone assisted with the IOB will be examined. Before the determination of the adsorption bonding, the cultivation of IOB will be carried out, and then cultured and duplicated on the agar plate. The performance of the Fe and Mn removal from the groundwater by two treatment methods will be analyzed and discussed based on the bonding testing of Zeta potential, Scanning Electron Microscopy (SEM) and X-ray Powder Diffraction (XRD).

1.5 Dissertation Outline

The thesis is categorized into five chapters which are introduction, literature review, methodology, results and discussion and conclusions.

Chapter 1: Introduction – this chapter shows the big picture of this research which includes the overall background of Fe and Mn removal from the groundwater by using limestone filter alone and the limestone assisted with IOB, problem statements, the scope of work and objectives of this research.

Chapter 2: Literature review – this chapter gives the detailed explanation to the technical terms, findings, studies, and topics related to the research with references to the research papers that published earlier.

Chapter 3: Methodology – this chapter describes the methodology of the research to obtain the expected outcome and the ways to achieve the objectives of this research.

Chapter 4: Results and discussion – this chapter describes the analysis and discussion of the results obtained from the experimental procedure.

Chapter 5: Conclusion and recommendations – this chapter showed a short of conclusions, limitation of works and recommendation to cover the findings of this research.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

Groundwater is one of the most important resources to complement the human needs for drinking purposes, usages in agricultural, industrial, and etc. Due to the increasing population of human, the demand of water required is also increased. Groundwater has been extracted to complement the increasing demand, but it is not readily suitable for human consumption due to the presence of high level of iron (Fe) and manganese (Mn) which requires further treatment.

In Kelantan, groundwater is the main source of water since the surface water is not enough to complement the water demand needs. Based on the previous research and testing, the groundwater in Kelantan needed further treatment due to the presence of heavy metals such as Fe and Mn. The presence of Fe and Mn affect several aesthetic and operational problems including bad taste, discolouration, staining, and deposition in distribution systems leading to aftergrowth and incidences of high turbidity (Sharma et al., 2005).

Limestone is an alkaline agent with the ability to neutralize, or partially neutralize strong acids. The neutralization process occurs when strong acids, in intimate contact with limestone chips, react with calcium carbonate ($CaCO_3$), which is the primary constituent of limestone to form water, carbon dioxide (CO_2) and calcium salts. Limestone creates a high pH which promotes Fe and Mn removal. Limestone acts as an adsorption medium for Fe (II) and Mn (II) ions which the ions will be adsorbed onto the catalytic surface of the limestone. Subsequently, in the presence of oxygen, the adsorbed soluble form of Fe (II) and Mn (II) are oxidized forming insoluble form of Fe

(III) and Mn (IV) ions respectively, which will further react to form Fe (III) oxide and Mn (IV) oxide which are insoluble in the water.

2.2 Groundwater

Groundwater is any water that lies in the aquifers beneath the land surface (Oskin, 2018). While some of the water that falls as the precipitation is channelled into the streams or lakes, and some is used by the plants or evaporates back into the atmosphere, most of it seeps underground. Figure 2.1 shows the world water distribution.

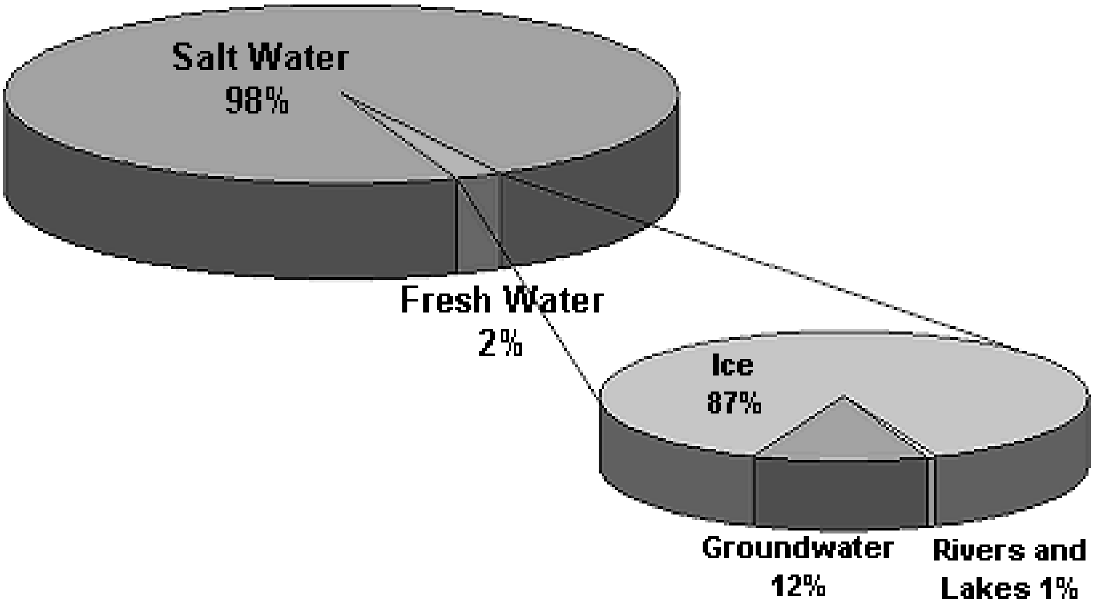

Figure 2.1: World Water Distribution (Gleick, 1996)

Based on Figure 2.1, groundwater is the 2nd largest available reservoir of fresh water. The majority of the fresh water is locked away as the ice in the polar ice caps, continental ice sheets, and glaciers. Surface waters such as rivers and lakes only account for less than 1% of the worlds fresh water reserves whereas groundwater accounts for 12% of the worlds freshwater resources (Oskin, 2018).

Groundwater is one of the most important natural resources which can provide an alternative for drinking water supply. It is essential to the vitality of agriculture and industry. The declines in the groundwater levels and other effects of pumping due to the large-scale of development of groundwater resources has led to concerns about the future availability of groundwater to meet the needs in the domestic, agricultural industry, and environmental. Groundwater has been identified as a new water source to meet the needs of the future generation. It is mainly used for drinking water supply in many countries in the world (Akbar et al., 2015b).

In Malaysia, groundwater is also an important resource that can complement the increasing demand for fresh water for various uses (Mohamed et al., 2009). Although the groundwater has been used for many centuries, the usage is still limited due to the shallow unconfined aquifers by using dug wells (Ang, 1994). In Malaysia, deep tubewells in coarse sand aquifers have started to be developed in the past 30 years for the water supply of coastal towns such as Kota Bharu, Kelantan (Sofner, 1989).

The geological nature of the soil determines the chemical composition of the groundwater. Water is constantly in contact with the ground in which it stagnates or circulates, so equilibrium develops between the composition of the soil and that of the water. For example, water that circulates in a sandy or granitic substratum is acidic and has a few minerals; water that circulates in limestone contains bicarbonates alkalinity (Lenntech, 2019b). Table 2.1 shows the characteristics of groundwater based on the major analysis parameters.

Table 2.1: Characteristics of Surface Water and Groundwater based on the Major Analysis Parameters (Lenntech, 2019b)

Characteristic	**Surface Water**	**Groundwater**
Temperature	Varies with season	Relatively constants
Turbidity, SS	Level variable, sometimes high	Ow or nil (except in karst soil)
Colour	Due mainly to SS (clays, algae) except in very soft or acidic waters (humic acids)	Due above all to dissolved solids
Mineral content	Varies wih soil, rainfall, effluents, etc.	Largely constant, generally appreciably higher than in surface water from the same area
Divalent Fe and Mn in solution	Usually none, except at the bottom of lakes and ponds in the process of eutrophication	Usually present
Aggressive CO_2	Usually none	Often present
Dissolved O_2	Often near saturation level, absent in very polluted water	Usually none
H_2S	Usually none	Often present
NH_4	Found only in polluted water	Often found
Nitrates	Level generally low	Level sometimes high
Silica	Usually moderate proportions	Level often high
Mineral and organic micro-pollutants	Ca be present but liable to disappear rapidly once the source is removed	Usually, none but any accidental pollution lasts a very long time
Living organisms	Bacteria, viruses, plankton	Iron bacteria frequently found
Chlorinated solvents	Rarely present	Often present
Eutrophic nature	Often. Increased by high temperatures	None

Some of the most typical characteristics of groundwater are weak turbidity, constant temperature, and chemical composition and the almost overall absence of oxygen. Circulating groundwater can have extreme variation in the composition with the appearance of pollutants and various contaminants. Furthermore, groundwater is often very pure microbiologically.

2.3 Heavy Metals

There are a lot of heavy metals found in water and wastewater. Heavy metals such as Nickel (Ni), Cadmium (Cd), Copper (Cu), Zinc (Zn), Lead (Pb) and etc are the common waste released from the industrial sector that uses metals in their operation; electroplating, petrochemical industry and etc. Besides heavy metals, there are a few metals that can be found in water and wastewater such as Calcium (Ca), Magnesium (Mg), Tin (Sn), Sodium (Na), Silicon (Si) and etc. So, it is important to know the concentration of each metal before it is released to the environment.

Heavy metals are a class of metallic elements which are abundant in earth’s crust. Heavy metal contamination has been a serious concern throughout the world. Humans may require trace amounts of heavy metals. Unfortunately, heavy metals can be dangerous at high levels. Heavy metals accumulation at higher levels can result even in death. Heavy metals toxins contribute to a variety of adverse health effects (Romeo et al., 2004). Global environmental changes have dramatically increased the overall environmental load of heavy metals (Lee et al., 2005). Nowadays, heavy metals are abundant in our air, soil, and even drinking water.

In this research, Fe and Mn acted as the main parameters. Based on Table 2.2, the acceptable value for recommended raw water quality is 1.0 mg/L for Fe and 0.2 mg/L for Mn; while for maximum acceptable value for drinking water standard is 0.3 mg/L for Fe and 0.1 mg/L for Mn. Table 2.2 below shows the drinking water quality standard that retrieved from the Engineering Services Division, Ministry of Health Malaysia.

Table 2.2: Drinking Water Quality Standard (MOH, 2010)

Parameter	**Group**	**Recommended Raw Water Quality**	**Drinking Water Quality Standards**
		Acceptable Value (mg/litre (unless otherwise stated))	**Maximum Acceptable Value (mg/litre (unless otherwise stated))**
Total Coliform	1	5000 MPN/100 mL	0 in 100 mL
E. coli	1	5000 MPN/100 mL	0 in 100 mL
Turbidity	1	1000 NTU	5 NTU
Colour	1	300 TCU	15 TCU
pH	1	5.5 – 9.0	6.5 – 9.0
Free Residual Chlorine	1	-	0.2 – 5.0
Combined Chlorine	1	-	Not Less Than 1.0
Temperature	1	-	-
Clostridium perfringens (including spores)	1	-	Absent
Coliform bacteria	1	-	-
Colony count 22º	1	-	-
Conductivity	1	-	-
Enterococci	1	-	-
Odour	1	-	-
Taste	1	-	-
Oxidisability	1	-	-
Total Dissolved Solids	2	1500	1000
Chlorine	2	250	250
Ammonia	2	1.5	1.5
Nitrate	2	10	10
Ferum/Iron	2	1.0	0.3
Fluoride	2	1.5	0.4 – 0.6
Hardness	2	500	500
Aluminium	2	-	0.2
Manganese	2	0.2	0.1
Chemical Oxygen Demand	2	10	-

Fe and Mn are present in many water supply sources. Alone or in combination with the other, Fe and Mn may cause serious impairment of water quality. They are natural constituents of the Earth's crust and found in both surface and groundwater. The concentrations of these elements in groundwater are influenced by the geological structure of the soil and rocks formation, the hydrological conditions of the area, the physical and chemical make-up of the surrounding rocks and soil, and the presence of microorganisms (Kassim, 1994).

There are two methods to determine metals which are wet chemistry method and secondly equipment method. Normally, the wet chemistry method involved the titration process. While in analyzing metals using the equipment, DR 2000 Spectrophotometer is used to analyze metals in a short time.

2.3.1 Iron (Fe)

Fe is one of the most abundant metals in the Earth's crust which it occurs naturally in water in the soluble form as the ferrous Fe (bivalent Fe in dissolved form as Fe (II) or $Fe(OH)^+$) or in complexed form as the ferric Fe (trivalent Fe in precipitate form as Fe (III) or $Fe(OH)_3$) or in the bacteria form, too (Ghosh et al., 2008). World Health Organization has set a guideline value of 0.3 mg/L for Fe (WHO, 1984). Figure 2.2 shows the Fe element that naturally occurred in Earth's crust.

Figure 2.2: Iron (Wahab, 2019)

The presence of Fe in drinking water and water supplies can cause certain problems and also may cause health issues. Although there are no health-based guidelines for the concentration of Fe in drinking water, the presence of Fe is undesirable as it causes several aesthetic and operational problems (Sharma et al., 2005). Figure 2.3 shows the reddish colouration of water due to the presence of Fe.

Figure 2.3: Reddish Coloration of Water due to the Presence of Iron (Bluey, 2019)

The presence of Fe in groundwater does not affect much to human health. Although it is less harmful, the continuous intake of these types of water will give healthy problems such as iron poisoning, dizziness, low blood pressure and a fast or weak pulse, headache, fever, shortness of breath and fluid in the lungs, greyish or bluish colour in the skin, jaundice (yellowing of the skin due to liver damage) and seizures (Judith Marcin, 2017).

2.3.2 Manganese (Mn)

Mn is one of the most abundant metals in Earth's crust, usually occurred with Fe. It is a component of over 100 minerals but is not found naturally in its pure or elemental form (HHS, 2012). Mn usually occurs naturally in many groundwater sources and in soils that may erode into these waters. Human activities are also responsible for Mn contamination in groundwater which will affect the water quality (WHO, 2011). Figure 2.4 shows the Mn elements that naturally occurred in Earth's crust.

Figure 2.4: Manganese (Tomihahndorf, 2006)

2.4 Groundwater Treatment

In general, Fe and Mn exist in the soluble form as Fe (II) and Mn (II) and converted to insoluble form as Fe (III) and Mn (IV) before the removal is achieved (Cho, 2005). There are several methods for Fe and Mn removal from drinking water like ion exchange and water softening (Vaarmaa and Lehto, 2003), activated carbon and other filtration materials (Munter et al., 2005), supercritical fluid extraction (Andersen and Bruno, 2003), bioremediation (Berbenni et al., 2000) and limestone treatment (Aziz et al., 2004) , oxidation by aeration, chlorination, ozonation followed by filtration (Ellis et al., 2000), by ash (Das et al., 2007), by aerated granular filter (Cho, 2005) and by adsorption (Tahir and Rauf, 2004).

2.4.1 Limestone

Limestone is one of the easily available and cheap materials that help in the removal of Fe and Mn. Limestone also acts as a pH controller (Sim et al., 2001). Limestone has been proven that it is effective in removing metals from water and wastewater.

Limestone is an alkaline agent with the ability to neutralize, or partially neutralize strong acids. In this research project, limestone is used to conduct a limestone filter model with small scale by using the size range of 0.6-2.36 mm. Based on the previous research paper, the experiment by using limestone size of 0.6-2.36 mm has the highest removal efficiency of Fe (Adlan et al., 2016). Figure 2.5 shows the percentage of Fe removal against limestone size.

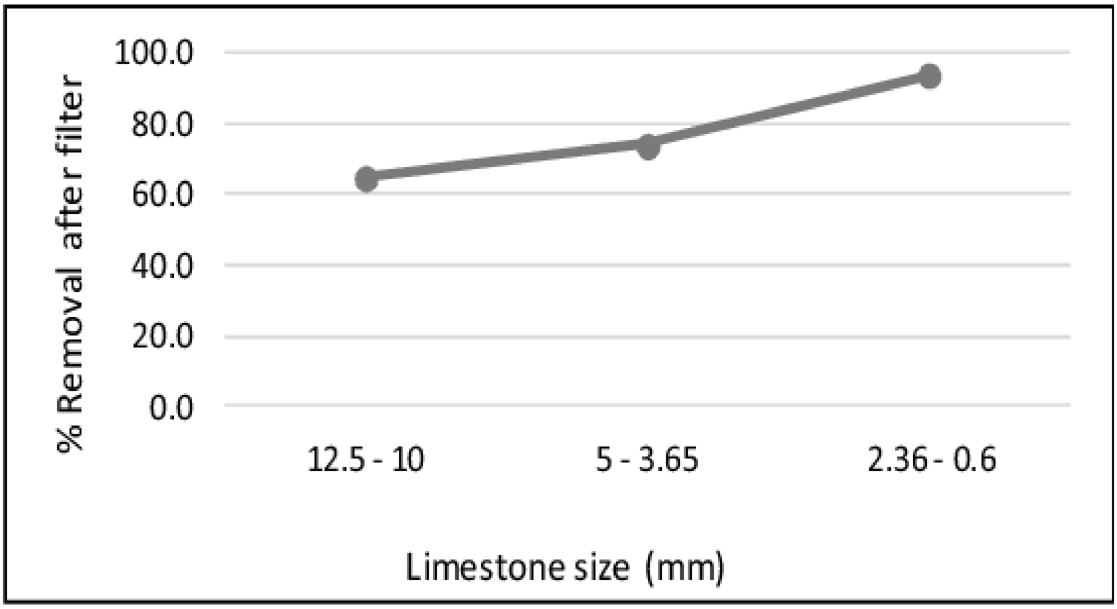

Figure 2.5: Percentage Removal of Iron against Limestone Size (Adlan et al., 2016)

Limestone is capable in removing heavy metals such as Copper (Cu), Zinc (Zn), Cadmium (Cd), Lead (Pb), Nickel (Ni), Chromium (Cr), Iron (Fe) and Manganese (Mn), and the removal capability is reported at up to 90% (Aziz et al., 2004).

Calcium carbonate ($CaCO_3$), which is the main component of the limestone, provides an alternative means of neutralizing acid water and the production of smaller sludge (Vu et al., 2003). Limestone contains $CaCO_3$ will increase pH to the level at which Fe and Mn became insoluble and precipitate in the form of metal carbonate for easier removal (Aziz and Smith, 1992b). Limestone filter acts as good adsorbent media and can be a good alternative of groundwater treatment because of the low cost of the media. In addition, the use of limestone could help to overcome the excessive Fe and Mn problem in water treatment plants (Akbar et al., 2015a).

Table 2.3 shows that various adsorbent performance in Fe and Mn removal. From the table, activated carbon has a higher removal efficiency of 94.43% to 98.89%, which carbon is one of the main elements in the limestone ($CaCO_3$).

Table 2.3: Various Adsorbent Materials Removal Efficiency

Sr. No.	**Name of Adsorbent**	**Concentration (mg/L)**	**pH**	**Removal efficiency (%)**
1.	Curry tree carbon	25 to 200	5.5	> 90%
2.	Curry tree carbon	25 to 200	6.0	> 90%
3.	Mahogany leaves	0.002	6.4	81% for Cr (III), 80% for Fe (II)
4.	Processed wooden charcoal (PWC)	5	5.5	50 – 54%
	Processed sand (PS)			
5.	Pine fruit	22.22 of Fe and 57.6 of Cu	Fe^{2+} 5.0, Cu^{2+} 7.0	Fe^{2+} 96.3 – 97.3%, Cu^{2+} 94.1 – 96%
6.	Recinius Communis Linn	20	5.0 to 7.0	45 to 90%
7.	Modified mangrove bark	10 – 100	2 to 10	Ni – 7.25, Cu – 6.95
8.	Eucalyptus bark	25 – 300	2 to 5	14.53 to 16.47 at temp. 20 to 50ºC
9.	Activated carbon	1.3, 5 & 10	2.0 to 7.0	94.43 to 98.89%
10.	Tea leaves	5 – 100	5	96, 91, 72 and 58% for Pb > Fe > Zn > Ni
11.	Biofilm of Escherichia coli supported on NaY zeolite	10, 25, 50, 70, 80 and 100	7.2	100% for iron
12.	Typha domingensis	7, 10, 4.1	2.5	-
13.	Husk of tur dal (Cajamus cajan)	20 – 100	2, 2.5	-
14.	Crude Olive Stones	5 – 100 mg dm-3		30 to 70%
15.	E. coli biofilm supported on kaolin	10, 25, 50, 70, 80, and 100	4.6, 5.1 (cr), 2.7, 3.5 (fe), 5.6, 6.0 (cd), 5.7, 6.2	100% (fe), cadmium (70%), nickel 74 – 40% and chromium 100% - 20% Fe > Cd > Ni > Cr

2.4.2 Iron-Oxidizing Bacteria (IOB)

IOB is non-harmful bacteria that produce their energy by oxidizing the Fe from Fe^{2+} to Fe^{3+}, which is naturally present in some soils. The oxidized Fe gives the bacteria their rusty colour. The bacteria grow in mats, or large clumps, and will remain in a certain location until a large pulse of water washes them away or until other environmental parameters change.

An Fe bacterium is among the first prokaryotes to be observed and recorded by pioneer microbiologists, such as Ehrenberg and Winogradsky, in the 19th century. They were originally considered to be bacteria that catalyzed the oxidation of Fe^{2+}, ferrous iron to Fe^{3+}, ferric Fe, often causing the latter to precipitate and accumulate as extensive, ochre-like deposits (Hedrich et al., 2011). Figure 2.6 shows the phylogenetic tree which shows the relationship of *acidophilic* (**red**), *neutrophilic* aerobic (**green**), nitrate-dependent (**black**) and phototrophic iron-oxidizing (**blue**) proteobacteria.

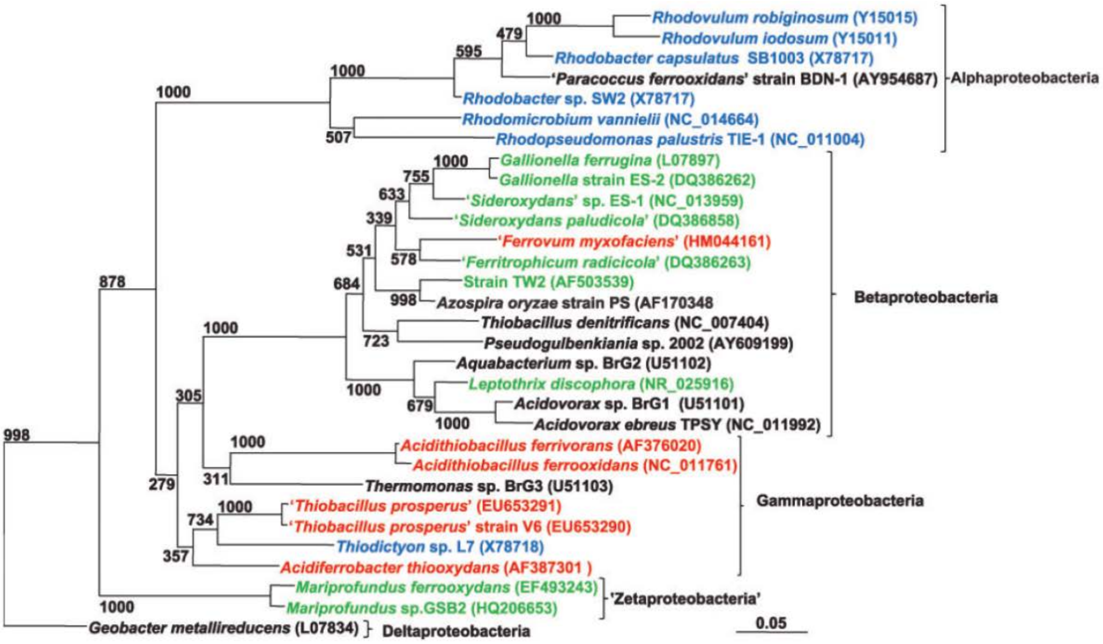

Figure 2.6: Phylogenetic Tree of Iron-Oxidizing Bacteria (Hedrich et al., 2011)

IOB has a major influence on the geochemical evolution of our planet and continues to have a significant impact on terrestrial and aquatic environments (Hedrich et al., 2011). Fe oxidation typically occurs at the anoxic-oxic interface, in waters with dissolved oxygen (DO) content of less than 10% (Liang et al., 1993). At pH levels greater than 5, Fe^{2+} will rapidly and spontaneously oxidize to Fe^{3+} (Ehrlich, 1999). In fully aerated freshwater at pH 7, the half-life of Fe^{2+} oxidation is less than 15 minutes (Stumm and Morgan, 1981). In order to thrive in these transition zones, bacteria must out-compete the rapid autoxidation of ferrous Fe. The extent to which Fe oxide mats result from biological or abiotic oxidation must be determined on a case-by-case basis (Rohrssen et al., 2007).

2.5 Drinking Water Parameter

2.5.1 Drinking Water Standard

A drinking water quality guideline value represents the concentration of a constituent such as suspended solids, heavy metals and others that do not result in any significant health risk to the consumer over a lifetime of consumption. Drinking water must be suitable for human consumption and for all usual domestic purpose. The cause should be investigated and corrective action should be taken when a guideline value is exceeded (WHO, 1997).

In the determination of the national standard for drinking water quality, various local, geographical, socioeconomic and cultural factors are taken into account. In conclusion, national standards may differ appreciably from the guideline values (WHO, 1997).

2.5.2 pH

pH is a measure where it can detect and differentiate the acidity or alkalinity of a solution with giving the ranges of 0 to 14. The aqueous solutions at 25ºC with a pH less than 7 are acidic and those with a pH more than 7 are alkaline or basic. pH level with 7 at 25ºC is neutral because the concentration of H_3O^+ equals the concentration of OH^- in pure water (Helmenstine, 2018). Figure 2.7 shows the pH of the acidic, neutral and alkaline states.

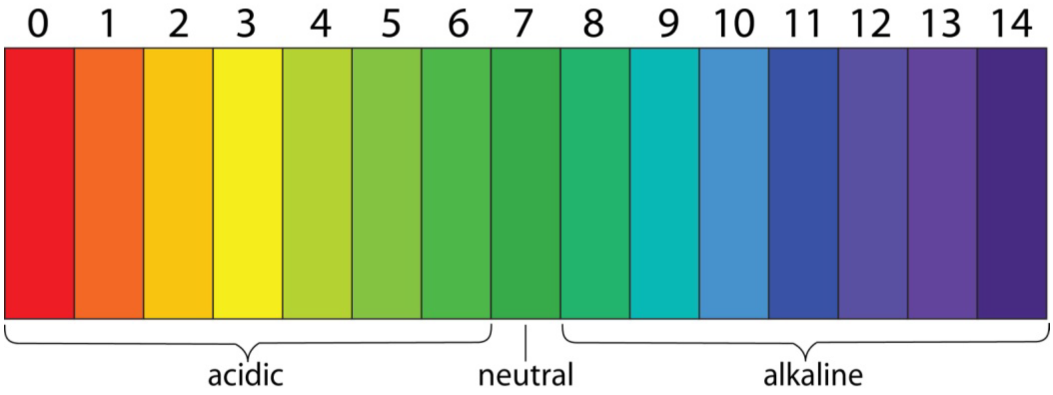

Figure 2.7: pH Scale (Robert, 2018)

pH is an important parameter since it can determine whether the groundwater sample is under suitable conditions and guidelines for the drinking purpose or not. In addition, the pH value given that is used to predict the concentration of the metals ions that soluble in water and giving suggestion for the removal of heavy metals.

2.6 Experimental Parameters

2.6.1 Zeta Potential

Zeta potential is a method for the measurement of the electrostatic potential at the electrical double layer surrounding a nanoparticle in solution. Nanoparticles with a zeta potential between -10 and +10 mV are considered approximately neutral, while nanoparticles with zeta potentials of greater than +30 mV or less than -30 mV are considered strongly cationic and strongly anionic, respectively. Since most cellular membranes are negatively charged, zeta potential can affect a nanoparticle’s tendency to permeate membranes, with cationic particles generally displaying more toxicity associated with cell wall disruption (Clogston and Patri, 2011).

2.6.2 Scanning Electron Microscopy (SEM)

SEM can image and analyze bulk specimens. The electrons will form a thermionic and field-emission cathode are accelerated through a voltage difference between cathode and anode that may be as low as 0.1 keV or as high as 50 keV (Hawkes and Reimer, 2013). Advantages of SEM include its wide-array of applications, the detailed three-dimensional and topographical imaging and the versatile information garnered from different detectors.

2.6.3 X-Ray Powder Diffraction (XRD)

XRD uses a collimated monochromatic X-ray beam to analyze a crystalline solid and reveal highly accurate, critical information about the size, shape, and symmetry of unit cells and the position of atoms within the lattice framework. This XRD method allows for efficient, rapid analysis of powder crystalline samples. The output is provided in the form of diffraction peaks, which show the unique “fingerprints” of different crystal phases that may be present. But XRD analysis isn’t just restricted to the identification and quantification of crystallites. XRD is also useful for the positive identification of a contaminant or corrosion product, as well as the identification of foreign phases for purity analyses of crystalline powders (Gorges, 2019).

2.7 Summary of Literature Review

Groundwater becomes an alternative source to complement the human needs for drinking purposes. It has been extracted to complement the increasing demand, but it is not readily suitable for human consumption due to the presence of high levels of heavy metals especially Fe and Mn which the groundwater required further treatment. Limestone is found that it is capable in removing Fe and Mn from groundwater, which it acts as an adsorption medium for Fe (II) and Mn (II) ions which the ions will be adsorbed onto the catalytic surface of the limestone. In the presence of oxygen, the adsorbed soluble form of Fe (II) and Mn (II) is converted to the insoluble form of Fe (III) and Mn (IV) respectively, which will further react to form metal oxide for easier removal. Throughout the findings, ammonia-oxidizing bacteria (AOB) have been used to immobilize on the limestone surface in Kampung Chap Water Treatment Plant, Kelantan for groundwater treatment and the performance is better than the conventional treatment by using limestone alone. Thus, IOB has been suggested in this research due to the easier cultivation. IOB can contribute to the Fe and Mn removal from groundwater by the oxidation process of the soluble form of Fe (II) and Mn (II) to the insoluble form of Fe (III) and Mn (IV).

CHAPTER 3

METHODOLOGY

3.1 Overview

This chapter describes the methodology and testing used in this research. The methodology can be divided into 4 sections: characterization of raw groundwater; cultivation of iron-oxidizing bacteria (IOB); treatment of groundwater under different treatment methods (limestone filter alone and limestone assist with IOB); and last is the column study at different operation time. Figure 3.1 shows the flowchart of the methodology throughout the research.

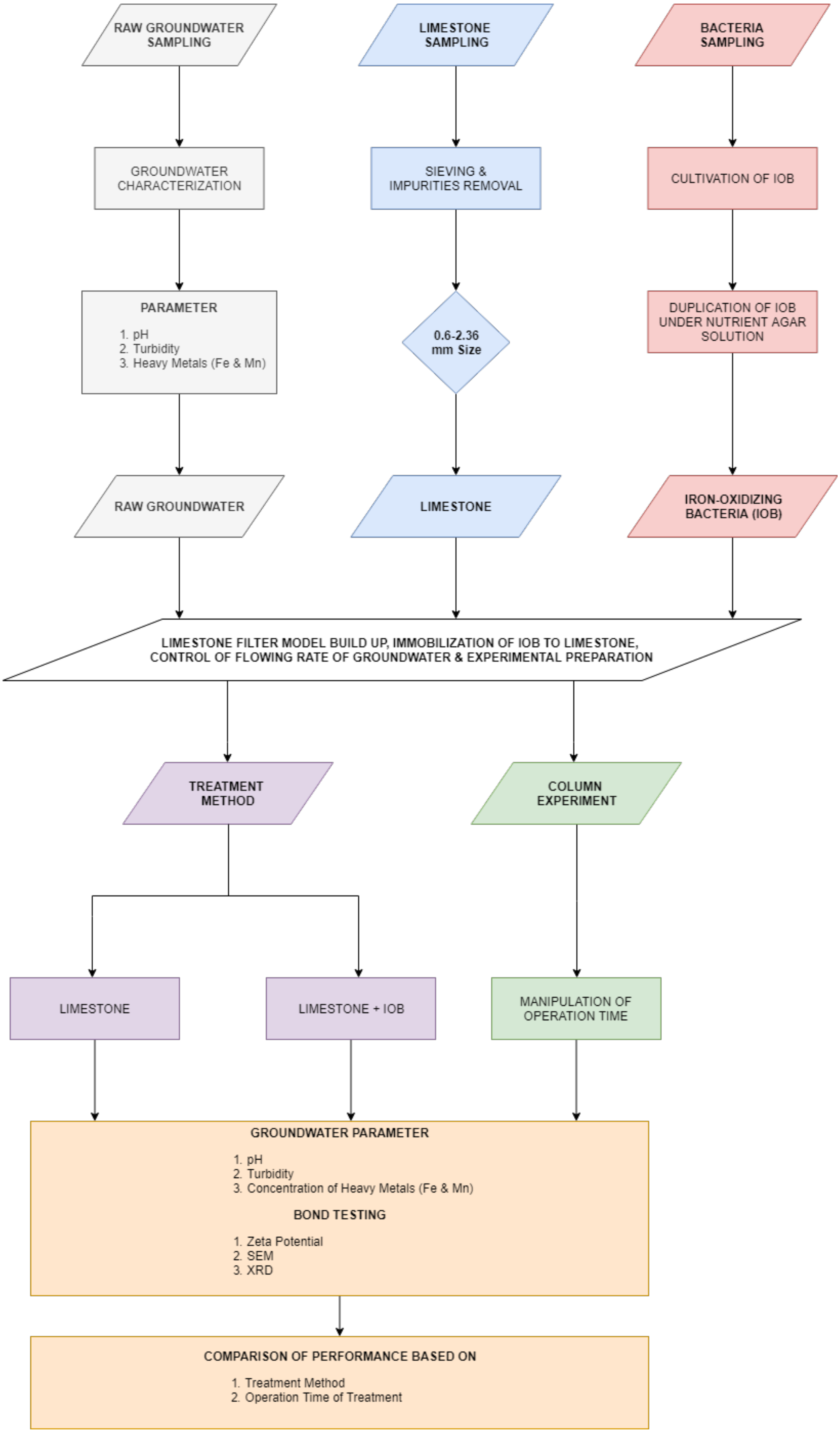

Figure 3.1: Flowchart of Research Methodology

In this research, the main parameters of the groundwater were pH, turbidity, Fe and Mn concentration. The summary of the equipments used throughout this research was tabulated in the Table 3.1.

Table 3.1: Summary of the Equipment Used

Equipment	**Module Number/Reference**	**Parameter**
pH Meter	APHA 4500 H^+ B	pH
Turbidity Meter	APHA 2130 B.	Turbidity
Atomic Adsorption Spectrometer (AAS)	APHA 3500-Fe APHA 3500-Mn	Fe and Mn
Zetasizer	Malvern Zetasizer Nano ZS	Zeta Potential
Field Emission Scanning Electron Microscopy (FESEM)	Zeiss Supra 35 VP FESEM	SEM
X-Ray Diffractometer	X-Ray Diffractometer Bruker D8 Advance	XRD

3.2 Characterization of Raw Groundwater

The raw groundwater was taken from the USM borehole (5º08′50.5″N, 100º29′34.7″E) (Figure 3.2 and Figure 3.3). The characterization of the raw groundwater was carried out for each batch taken of the sample. Characterization work was done for groundwater parameter as follows: pH, turbidity, iron (Fe) and manganese (Mn) concentration.