University of Alberta

Removal of model waste-water bacteria by magnetite in water and waste-water treatment processes

By

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DEDICATION

I would like to dedicate this thesis to my grandfather, late Colonel. Jaswant Singh Mann, VSM. He will always be alive in my heart.

I would also like to thank my father, Mr. VIjepal Singh Mann for his never ending support and encouragement which enabled me to have accomplished this task.

ABSTRACT

Conventional disinfection processes used for water and waste-water treatment such as chlorination, and ozonation produce disinfection by products, some of which have been found to be carcinogenic to living organisms. The use of magnetite as an alternative method of removing pathogenic microorganisms from the water streams was proposed as it does not produce any harmful by products. The removal of three model bacteria *Escherichia coli ATCC® 25922™*, *Pseudomonas putida ATCC® 17453™* and *Micrococcus luteus ATCC® 4698™* using magnetite and the mechanism of removal has been studied in this thesis. It was found that the optimal cell : magnetite ratio was 1:50 and could remove bacterial cells as follows: 96.8% for *E. coli,* 94.8% for *P. putida* and 99.7% for *M luteus*. To better understand the removal mechanism the effect of buffers, pH, mixing and contact times were also studied. The addition of buffers reduced the removal efficiency of magnetite by about 10% in most cases but still remains above 90% at a pH of 7.5. Optimum mixing time at 200 RPM was found to be between 10 to 12 minutes. Water and magnetite interaction study indicated that each gram of magnetite releases 1.084 mg/l dissolved oxygen in water. Further the functional groups on the surface of bacteria were also studied to better understand the interaction of magnetite with the bacterial cells. These analyses indicate that magnetite could be efficiently used for disinfection processes in water and waste-water treatment industry.

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LIST OF SYMBOLS AND ABBREVIATION

µm	Micro meter or micron
$Al_2(SO_4)_3$	Aluminum sulfate or Alum
ALNICO	Aluminum Nickel Cobalt
ATCC	American type culture collection
BOD	Biological oxygen demand
$C_{76}H_{52}O_{46}$	Tannic acid
$CaSO_4$	Calcium sulfate
$CaSO_4$	Calcium sulfate
E.	Escherichia
Fe	Iron
Fe_3O_4	Magnetite
H_2S	Hydrogen sulfide
H_2SO_4	Sulfuric acid
HAAs	Haloacetic acids
IEP	Iso-electric potential
M.	Micrococcus
Mg	Magnesium
NaOH	Sodium hydroxide
NH_4	Ammonia
nm	Nano meter
O_2	Oxygen
OH^-	Hydroxyl radical
P.	Pseudomonas
PZC	Point of zero charge
Se	Selenium
T	Tesla
THMs	Trihalomethanes
U_3O_8	Uranium oxide
UV	Ultraviolet
Wt.	Weight

Chapter 1: Introduction

1 INTRODUCTION

1.1 WATER SAFETY

1.1.1 RISK OF WATER BORNE DISEASES

Microbiological contamination of water is related to some of the most terrifying illnesses and death events, which have occurred in history, particularly in relation to risk to human health. It is estimated that in developing countries, almost four-fifths of total illnesses are caused by waterborne microbial contamination (WHO, 2002), including diarrhea, cholera and dysentery. In 1998, an estimated 2.2 million people were killed by diarrhea, most of whom were children under the age of 5 (WHO, 2007). In Southeast Asia and Africa, diarrhea is responsible for as much as 8.5% and 7.7% of all deaths respectively (WHO, 2002). Developed countries have also witnessed some serious water borne disease outbreaks. In the United States in the period from 1971 to 2002, there were 764 documented waterborne outbreaks associated with drinking water, resulting in 575,457 illnesses and 79 deaths (Blackburn et al, 2004).

There are many examples of chronic or fatal water borne infections out of which 88% were caused by microbiological contamination (WHO, 2004). In Canada, twenty four percent of First Nation reserves were found to be at health risk because of poor water quality attributed to inadequate water and sanitation facilities (Health Canada, 2007; INAC, 2003). In Walkerton, Ontario, there was a significant waterborne outbreak that was caused by contamination of a well in a

farm near the city. During the outbreak, more than 2300 people became ill due to *Escherichia coli* O157:H7 and *Campylobacter* species (Clark et al, 2003). Out of the cases reported, 97% were directly due to consumption of water. Internationally, one recent outbreak occurred in 2010/2011 in Haiti, where a cholera outbreak killed almost 5000 people and hospitalized thousands more. The suspected source for the epidemic was the Artibonite River, from which some of the affected people had consumed the water. DNA fingerprinting confirmed that samples of cholera taken from infected patients were *Vibrio cholerae* serogroup O1, serotype - Ogawa, a strain found in South Asia (IPCC, 2007).

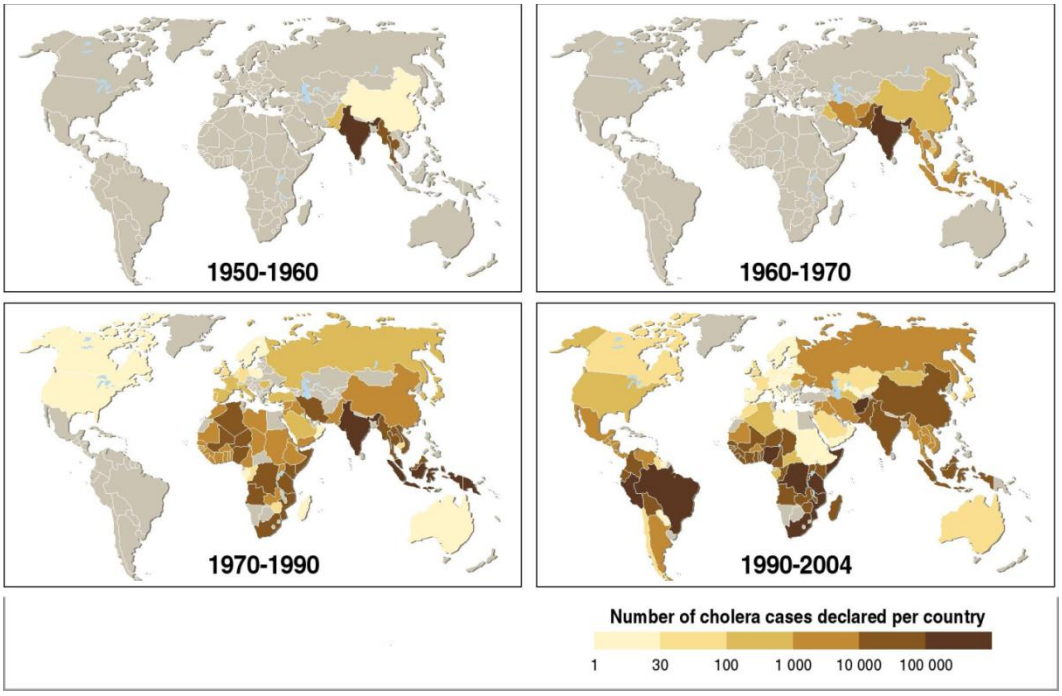

Figure 1.1: Number of cholera cases over the last 50 years. (Adapted from IPCC 2007).

The spread of cholera over the last 55 years is shown in Figure 1.1. According to the trend shown, even developed countries are witnessing an increase in the number of illnesses spread by water systems.

1.2 BASIC AND ALTERNATIVE TECHNOLOGIES OF WATER DISINFECTION

In water and waste-water treatment processes, disinfection is accomplished by two strategies; *removing* microorganisms from water or *inactivating* them. Removal of microorganisms by chemicals is done to kill the bacteria to a certain "safe" level. Inactivation refers to microorganisms being present in water, but are unable to multiply to concentrations required to cause disease.

In water treatment, disinfection refers to two activities: (1) primary disinfection – inactivation of microorganisms in water and (2) secondary disinfection – maintaining a residual concentration of disinfectant in the treated water (distribution system) to prevent further growth. Characteristics of some of the most popular disinfection techniques are summarized below in Table 1.1.

Table 1.1: Characteristics of five most commonly used disinfectants (Adapted from Crittenden et al, 2000).

Argument	Type of disinfectant				
	Basic methods			Alternative Methods	
	Free Chlorine[a]	**Combined chlorine[b]**	**Chlorine Dioxide[c]**	**Ozone**	**Ultraviolet light**
Effectiveness in disinfecting					
Bacteria	Excellent	Good	Excellent	Excellent	Good
Viruses	Excellent	Fair	Excellent	Excellent	Fair
Protozoa	Fair to poor	Poor	Good	Good	Excellent
Endospores	Good to Poor	Poor	Fair	Excellent	Fair
Frequency of use as primary disinfectant	Most common	Common	Occasional	Common	Emerging use

[a]Combined chlorine = NH_2Cl
[b]Chlorine Dioxide = ClO_2
[c]Free Chlorine = OCl^- and/or HOCl

Chlorine still remains one of the most common disinfectants used in water treatment. Nearly 98% of drinking water is chlorinated in the United States, with 0.4% and 0.7% of water disinfected by ozonation and other methods, respectively (AWWA, 1992), mainly because of its economic value and effectiveness. Chlorination provides microbiologically safe drinking water even after water enters the supply system, because of the residual chlorine which has an 'aftereffect'. The 'aftereffect' is the ability of residual chlorine to control biofilms in the distribution system to prevent further growth of microorganisms. UV and ozonation do not have this kind of residual aftereffect and hence need chlorination at some point in the water treatment system. Despite its advantages, chlorine has some disadvantages, including production of disinfection by-products (DBPs). The generation of DBPs is associated with

ozonation and UV as well. Ozonation produces a lot of hydroxyl radicals which react with bromide in water to form bromate, regulated in the USA, and is a potential carcinogen (Richardson et al, 2007). Very few DBPs have been found associated with UV disinfection as they are still emerging.

1.3 CHALLENGE OF DISINFECTION BY-PRODUCTS

The discovery of DBPs has led to reevaluation of chlorine as a disinfectant, as well as reinvestigation of the role of inactivation itself for the control of pathogens. When free chlorine reacts with natural organic matter in water, chlorinated organics are produced; specifically, THMs (trihalomethanes) and HAAs (haloacetic acids) (Richardson et al, 2007). While chlorine remains the dominant disinfectant for water treatment systems, the situation might change in the future because of these concerns. It is very likely that chemical byproducts are formed anytime an oxidant is employed in water treatment, and that many of these byproducts would have to be regulated in future (Trussel, 1993).

The concerns with DBPs are the related health effects. Studies have shown a possible connection between exposure to DBPs in drinking water and cancers and adverse birth effects. Studies have consistently associated the occurrence of bladder cancer by exposure to DBPs (Villanueva et al, 2006). Because of the toxicity of these by-products, the US EPA has given regulatory limits for DBPs, stating maximum allowable concentrations (US EPA, 1991). Reducing or eliminating DBPs, while maintaining high disinfection efficiency, is

one reason to search for new water disinfection technologies. The use of magnetite for disinfection may prove to be efficient, as the process is based on removal of microorganisms by adsorption. Hence, secondary products such as DBPs are not produced.

1.4 MAGNETITE

Magnetite [Iron (II, III) oxide] is a high-grade, naturally-occurring ferromagnetic material with the chemical formula Fe_3O_4. Well-crystalized magnetite is thermodynamically very stable, and has a strong response to an external magnetic field. Magnetite (Fe_3O_4) and Maghemite (γ-Fe_2O_3) have high oxidative stability and are currently the only accepted non-toxic magnetic materials for medical applications (Muller et al, 2004). Pure magnetite has a very unique point of zero charge (PZC) at pH 6.5 (Gregory et al, 1988), meaning that at pH values greater than 6.5, they are negatively charged and can be used to adsorb positively charged species such as cations. Powdered magnetite has been proven to be a very efficient sorbent to remove arsenic (III) and arsenic (V) from water. The efficiency of arsenic removal increases ≈ 200 times when the size of the particle decreases from 300 to 12 nm (Mayo et al, 2007). The use of magnetite to remove charged species from water is known as the "Sirofloc process", which is used for decolourization of water using regenerable magnetite (Prout, 1989; Dixon, 1991). The surface of magnetite has also been manipulated

to adsorb charged biological colloidal matter, such as bacteria, from water (MacRae & Evans, 1983).

1.5 KNOWLEDGE GAP

Magnetite has proven to be a very strong absorbent for heavy metals because of its ability to switch surface charge at an isoelectric potential (IEP) of 6.5. Magnetically-modified microbial cells (using magnetite as a carrier for biosorption) have shown removal of chlorinated hydrocarbons and heavy metals such as nickel from waste-waters (MacRae, 1986). Since magnetite particles have high surface energies, depending on pH, the particle surface can be polarized to target specific contaminants of opposite polarity in water and waste-waters. The ability of magnetite to adsorb microbial cells has never been investigated for commercial disinfection. The adsorption mechanism(s) and kinetics for removal of microbial cells is not understood. The motivation of the research is to understand this adsorption process so that disinfection processes can be better designed.

1.6 RESEARCH OBJECTIVES

The objective of this research is to explore the use of magnetite for water and waste-water treatment (primarily disinfection by adsorption) and explain the possible mechanism(s) for the removal that takes place. This will be investigated using magnetite to remove *Escherichia coli ATCC® 25922™*,

Pseudomonas putida ATCC® 17453™ and *Micrococcus luteus ATCC® 4698™* as model bacteria from solutions. Hence the following research questions arise:

- Can magnetite remove significant amounts of these microorganisms from water?
- If so, what are the mechanisms involved in the removal process?

1.7 THESES OUTLINE

This thesis consists of four chapters. Each chapter will contribute to the overall objectives of this research. Chapter 2 will consist of theoretical background and an extensive literature review on the studies conducted in this field to date. Chapter 3 explains in detail the research methodology and discussion related to the results obtained. Chapter 4 will consist of the main conclusions and recommendations for future research in this field.

1.8 WORKS CITED

1. Aborginal Affairs and Northern Development Canada. "Environment and Natural Resources." 2003. <http://www.aadnc-aandc.gc.ca/eng/1100100034243/1100100034247>.
2. AWWA Water Quality Division Disinfection Committee. "Survey of Water Utility Disinfection Practices." Journal AWWA 84.9 (1992): 121.
3. Blackburn, B. G., et al. "Surveillance for Waterborne-Disease Outbreaks Associated with Drinking Water--United States, 2001-2002." Morbidity

and mortality weekly report.Surveillance summaries (Washington, D.C.: 2002) 53.8 (2004): 23-45.

4. Calderon, R. L., and G. F. Craun. "Estimates of Endemic Waterborne Risks from Community-Intervention Studies." Journal of water and health 4 Suppl 2 (2004): 89-99.
5. Clark, CG, et al. "Characterization of waterborne outbreak–associated *Campylobacter jejuni*, Walkerton, Ontario." 2003. <http://wwwnc.cdc.gov/eid/article/9/10/02-0584.htm>.
6. Crittenden, J., et al. "Disinfection." Waste-water Treatment: Principles and Design. 2nd ed. United States of America: John Wiley and Sons, Inc, 2000.
7. Crittenden, J., et al. "Disinfection." Waste-water Treatment: Principles and Design. 2nd ed. United States of America: John Wiley and Sons, Inc, 2005.
8. Dixon, D. R. "The Sirofloc Process for Water Clarification." Water Supply 9 (1991): S33,S33-S36.
9. Gregory, R., and R. J. Maloney. "Water Treatment using Magnetite: A Study of a Sirofloc Pilot Plant." Water and Environment Journal 2.5 (1988): 532,532-544.
10. Health Canada. "First Nations and Inuit Health." 2007. <http://www.hc-sc.gc.ca/fniah-spnia/promotion/public-publique/water-eau-eng.php>.

11. I.C., Mac Rae, and Susan K. Evans. "Removal of Chlorinated Hydrocarbons from Water and Waste-water by Bacterial Cells Adsorbed to Magnetite." Waste Research 20.9 (1986): 1149,1149-1152.
12. Intergovernmental Panel on Climate Change. “Climate Change: Impacts, Adaption and Vulnerability.” United States of America: Cambridge University Press, 2001.
13. Mac Rae, I. C. "Removal of Chlorinated Hydrocarbons from Water and Waste-water by Bacterial Cells Adsorbed to Magnetite." Water research 20.9 (1986): 1149-52.
14. MacRae, I. C., and Susan K. Evans. "Factors Influencing the Adsorption of Bacteria to Magnetite in Water and Waste-water." Waste Research 17.3 (1983): 271,271-277.
15. Mayo, J. T., et al. "The Effect of Nanocrystalline Magnetite Size on Arsenic Removal." Science and Technology of Advanced Materials 8.1-2 (2007): 71,71-75.
16. Müller, R., et al. "Magnetic Particles for Medical Applications by Glass Crystallisation." Journal of Magnetism and Magnetic Materials 272–276, Part 2.0 (2004): 1539-41.
17. Richardson, L. B., et al. "Residual Oxidant Decay and Bromate Formation in Chlorinated and Ozonated Sea-Water." Water Research 15.9 (1981): 1067,1068,1069,1070,1071,1072,1073,1074.

18. The World Health Organisation. “The World Health Report.” Geneva:, 2000.
19. Trussell, R. "Safety of Water Disinfection: Balancing Chemical & MicrobialRisks." Safety of Water Disinfection: Balancing Chemical & Microbial Risks. Ed. G. F. Graun. Washington D.C.: ILSI Press, 1993. 319.
20. United States Environmental Protection Agency. Status Report on Development of Regulations for Disinfection and Disinfection by-Products. Washington D.C: USEPA, 1991.
21. Villanueva, J., et al. "Differential Exoprotease Activities Confer Tumor-Specific Serum Peptidome Patterns." The Journal of Clinical Investigation 116.1 (2006): 271,271-284.
22. World Health Organisation. “The World Health Report 2002 : Reducing Risks, Promoting Healthy Life.” Geneva: World health Organisation, 2002.
23. The World Health Report 2007 :”A Safer Future : Global Public Health Security in the 21st Century.” France: World Health Organisation, 2007.

Chapter 2: Literature Review and Theoretical Background

2 LITERATURE REVIEW AND THEORETICAL BACKGROUND

2.1 SCALE OF WATER TREATMENT

2.1.1 WATER DEMAND

It is estimated that within the next fifty years, world population will increase by 40-50% (World water council, 2010). Increasing population coupled with industrialization and urbanization would lead to an increasing demand on water. There is more waste-water generated and dispersed today: one out of six people lack safe access to drinking water (WHO/UNICEF JMP, 2004). Water stress causes deterioration of fresh water resources (eutrophication, organic matter pollution, etc.) and consequently reduces water quantity. The water withdrawal to availability ratio, which is the ratio of water withdrawal to natural/renewable water available, indicates high water stress worldwide (Alcamo et al, 1999). A simulation model developed by the Center of Environmental Systems research, University of Kassel, using a water flow modeling program (Figure 2.1) projects fresh water consumption in the next 50 years, which has nearly tripled. (AQUASTAT/FAO, 2004).

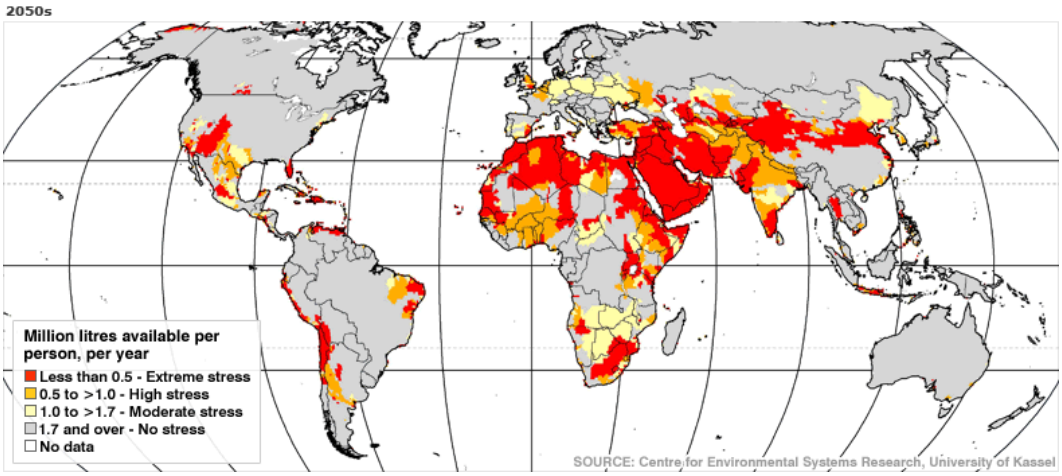

Figure 2.1: Simulated world water stress in 2050s. Source: FAO, 2010

Energy consumption is also accelerating as a function of water demand (The World Water, 2008-09). Changes in lifestyle and eating habits in recent years are requiring more water consumption per capita. This ever increasing demand on water consumption affects the cost of infrastructure and the efficiency of water delivery. Since treated water is a low cost product the cost to benefit ratio in terms of energy consumed will continue to increase every day.

2.1.2 WATER AND ENERGY

The amount of energy required for consistently achieving regulatory criteria for water and waste-water is highly energy intensive and expensive. Municipal water and waste-water treatment systems are among the most energy-intensive facilities owned and operated by local governments, accounting for about 35% of energy consumption (ACEEE, 2012). The cost of water and waste-water treatment involves many factors such as: cost of chemicals used, cost of mixing, cost of sludge treatment, cost of operation of an activated sludge

plant, cost of temperature control for effective biological removal of contaminants such as COD (Chemical oxygen demand), BOD (Biological oxygen demand), and ammonia. The amount of energy required for the treatment of water and waste-water in the United States of America is estimated to be 50,000 GWh, representing 1.4 percent of total national electricity consumed (EPA, 2005). Along with the relatively high energy consumption, there are some important environmental and health problems associated with conventional water and waste-water treatment methods.

2.2 PROBLEMS WITH CONVENTIONAL WATER AND WASTE-WATER TREATMENT METHODS

Water and waste-water treatment both include physical processes such as settling and filtration and chemical processes such as coagulation and disinfection. The environmental and health impacts of chemicals added for water and waste-water treatment for the purpose of coagulation and disinfection are briefly discussed below.

2.2.1 ENVIRONMENTAL IMPLICATION OF COAGULANTS

The objective of chemical coagulation is to destabilize particles so that they form larger particles in the size range of 0.01 to 1 μm. This aggregation of particles then facilitates the removal of the contaminant, which cannot be achieved through physical processes. The contaminant might be suspended, colloidal or dissolved organic matter. Typical coagulants used are alum, ferric

chloride and ferric sulphate (Crittenden et al, 2000). Alum is one of the most widely used coagulants, and produces a waste commonly known as sludge. It is estimated that approximately 1 million tonnes of alum sludge is produced in the US annually (Water Pollution Control Federation, Gruninger, 1975). In addition, alum sludge discharge into aquatic environments has adverse effects through the development of anaerobic conditions near the point of discharge if the water velocity is low (Cornwell et al. 1987).

The Alberta Environmental Center (1987) conducted a comprehensive study on drinking water treatment plants in Calgary and Edmonton, Alberta, concluding that sludges tested were non-toxic using MicroTox and subacutely non-toxic to rainbow trout. Conversely, a study in the USA reported a significant reduction in reproduction rates in freshwater fleas (*Cladoceran* and *Ceriodaphnia dubia*) exposed for 7 days to 100 % aluminum sludge effluent (Hall, 1989). It was suggested that the effects were likely due to the combined effects of decreases in pH and dissolved oxygen concentrations, physical stress due to high levels of suspended solids, and possibly the presence of aqueous aluminum. The study also observed high mortality rates in fathead minnow, exposed to 100 % effluent and a test concentration of 6.3 % dissolved aluminum (Environment Canada, 2011; Hall, 1989). Studies conducted to test sludge toxicity on plants concluded that alum sludge may have direct effects of aluminum on plants (limiting plant growth), but also with indirect effects (fixation of phosphorus by precipitation) related to phosphorus deficiencies (Jonasson, 1996; Cox et al,

1997; Quartin et al, 2001). The European water committee has stressed the importance of recycling the sludge from treatment plants as much as possible because redox conditions are still active around the aluminum flocs which allow it to effectively be used as a coagulant and to phase out the disposal of sludge to surface water (European commission, council directive of May 1991, section 271).

2.2.2 Environmental Implication of Disinfectants and Disinfection by Products

Disinfection of drinking water is very important to protect the public from water borne infections and diseases. Table 2.1 summarizes the advantages and disadvantages of the most common disinfectants used in water and waste-water.

Table 2.1: Advantages and disadvantages of the most common disinfectants used for water and waste-water treatment (*Crittenden et al*, 2000; *Standard of Russian federation*, 2008)

Disinfectant	Activity spectrum			Disinfection by-products	Comments
	Biological	Organic	Inorganic		
Chlorine	Bacteria, Cysts (Giardia, cryptosporidium)	Phenolics, cyanides, other nitrogen compounds	Fe, Mg, H_2S, NH_4	THMs[a], HAAs[b], bromates and brom-organic disinfection	Most widely used disinfectant
UV	Cysts (Giardia, Cryptosporidium)			None so far.	Turbidity reduces efficiency.
Ozone	Bacteria, Viruses	Taste and odor compounds,	Improves turbidity removal	Bromoforms, peroxides, quinone and brominated by products	Strong oxidant,
Chlorine Dioxide	Bacteria, Cysts (Giardia, cryptosporidium)	Destroys some THM precursors	Fe, Mg	Chlorates and chlorites, Taste and odour compounds	Works in small doses and doesn't react with Ammonia.
Chloramine	Prevents biofilm formation, bacteria.	Lesser formation of THMs and HAAs.		Chloral Hydrate and Nitrogen based compounds.	Persistent residual disinfectant.

[a]THMs = Trihalomethanes
[b]HAAs = Haloacetic acids

Theoretically every chemical used in water treatment which reacts with natural organic matter will produce a byproduct. Disinfection byproducts have received attention recently as some have been found to be carcinogenic (EPA, 1990). Chlorination byproducts were among the first ones to be discovered in the 1970s, when volatile halogenated organic compounds, such as chloroform, were identified in chlorinated surface waters containing high levels of natural

organic material (Rook, 1971). Several studies have focused on the health effects of exposure to disinfection by products, and have shown that bromate, chlorite, trihalomethanes and haloacetic acids are carcinogenic (EPA, 2011). Later studies in the Scandinavian countries reported defects in neural tube and urinary tract of newborn children, pregnant women and older populations, caused by exposure to chlorination by products, specifically chloroform found in local water supply subject to chlorination for disinfection (Hwang, 2003; Magnus, 1999; Klotz, 1999; Bove, 1995; Aschengru, 1993). Strong evidence of an elevated risk of cardiac and ventricular septal defects was associated with long-term exposure to trihalomethanes on lab mice (Cedergen, 2002; Nieuwenhuijsen, 2008).

At this point there is no concrete basis to suggest that disinfectant alternatives to chlorine are any safer from a toxicological point of view. The dimensions of related health effects are unclear as of now, because ninety percent of these experiments were conducted on lab mice (Hwang, 2008). The hazard seems to revolve around the susceptible populations such as women at reproductive age or those with endocrine problems. It is important to understand that as long as there is some quantity of organic material present in water, all disinfectants will produce by products. Some advanced treatment methods are available which can control the formation of disinfection by products because these are capital intensive physical processes and involve no chemical reaction, with the exception of ozonation.

2.2.3 USE OF ADVANCED TREATMENT TO REMOVE CONTAMINANTS

Pollution from disinfection byproducts and other micro pollutants, such as pharmaceutical contaminants, are regarded as a major environmental issue at a regional and a global scale (Oliver, 2007). Although advanced treatment methods such as advanced oxidation (oxidation of organic compounds using free OH radicals), GAC (Granular activated carbon - deodorization and separation of organic components by adsorption) and membrane filtration significantly reduce the quantity of micro-pollutants but they also carry a large energy and financial cost. The cost of some of these advanced methods was compared for a similar sized population and has been summarized in Table 2.2.

Table 2.2: Total operational cost of large sized water works calculated by application of TR61. (Adapted from Oliver et al, 2007).

Treatment Method	Total Capital cost (£ million)	Total operation cost per year (£ million)	Total cost per m^3 treated (£)
GAC[a] and ozone (O^3)	40.7	1.16	1.17
MF[b] and RO membranes	54.9	4.23	1.65

[a]GAC = Granular Activated Carbon
[b]MF and RO = Micro Filtration membrane and Reverse Osmosis

The obstacle for their practical application is the relatively high cost of the process, including the cost of the membranes themselves, the energy cost for operation, and the cost of chemicals required for membrane cleaning. An energy intensive GAC or ozone plant, running 24 h a day, 365 days a year, would therefore indirectly contribute a large amount of CO_2 to the atmosphere, with associated ramifications for global warming and climate change (Oliver et al, 2007). Hence, we need to work on comparatively inexpensive water treatment

techniques, which are also effective. Magnetite could be an efficient and affordable alternative to reduce or eliminate some of the problems related to conventional water and waste-water treatment methods mentioned above.

2.3 MAGNETITE

Magnetite is a naturally-occurring iron ore, and is represented by either Fe_3O_4 or $FeO{\cdot}Fe_2O_3$ [one part wüstite (FeO), one part hematite (Fe_2O_3)]. This refers to the different oxidation states of iron in one structure. Magnetite is a black solid with a metallic luster, and has a specific gravity of 5.2 and Mohs hardness of 6.0 (Handbook of Mineralogy, 1985). Magnetite occurs in igneous, metamorphic, and sedimentary rocks, typically as crystals or grains comprising less than 1 % of their host rock (Gem & Mineral Miners, Inc., 2009). Magnetite may be commonly found in plutonic igneous rocks as grains or crystals, and because of its magnetic nature they may aggregate making it easy to extract/mine (Gem & Mineral Miners, Inc., 2009). Crystals of magnetite have also been found in some bacteria (e.g. *Magnetospirillum magnetotacticum*) and have been reported to be found in the brains of bees, fish, birds and humans to give them a sense of orientation to the earth's magnetic field (Baker et al, 1983).

Magnetite also can be prepared by chemical precipitation (also called the Massart Method) of a aqueous mixture of Fe(II) chloride, Fe(III) chloride, and sodium hydroxide with mechanical agitation. The dark precipitate formed is sometimes called ferrite and consists of micro- and nano-particles of magnetite

(Harrison et al, 2002; Massart, 1981). The ratio of Fe (II) to Fe (III) controls the magnetic susceptibility of synthetic magnetite. Higher magnetic strength of magnetite is desired for quick separation using an external magnetic field.

2.3.1 MAGNETIC STRENGTH

The magnetism exhibited by magnetite is controlled by the molar ratio of ferrous to ferric ions in each particle of magnetite. Ferrous and ferric ions have magnetic properties because they are part of the spinel group. The spinel group consists of oxides with the general formula AE_2O_4, (for magnetite AE = Fe), where they crystallize in a cubic lattice with cations A and E occupying all the octahedral sites. A and E could be divalent, trivalent or even the same metal with different charge; for magnetite Fe(II) and Fe(III) (Hill, 1979). The change in the ratio of ferric and ferrous ions can reduce or augment the force of attraction to an applied magnetic field. Work carried out at Imperial College, London (White et al, 2000), was to make a coagulant that is magnetically separable. The '*Sirofloc*' process (CSIRO, Australia) was developed in Australia and was of a similar nature. The experiment used consisted of batch sedimentation of flocs or magnetite complexes in a water sample using synthetic magnetite to determine its magnetic strength.

The approximation of the total forces exerted on the flocs in the sample holder was also determined. The force on the floc is given by Equation (1) as follows:

$$F = m*g \quad (1)$$

Here *m* is the mass of the floc and *g* is the gravitational constant (9.81 m/s^2). When a magnetic force is applied to the sedimentation column; the force on flocs increases because of magnetic attraction. An inverse square law is applicable for the effect of magnetism on the floc, the force on the sample in a magnetic field (F_m) is given by Equation (2):

$$F_m = m*g + m*k/h^2 \quad (2)$$

In this expression, *h* is the distance of the sample from the magnet and *k* is a constant summing up all other magnetic field variables in the system. This would assume that the magnet was a point source. The magnetic force was plotted as a function of ferrous to ferric ion molar ratio, and is presented in Figure 2.2.

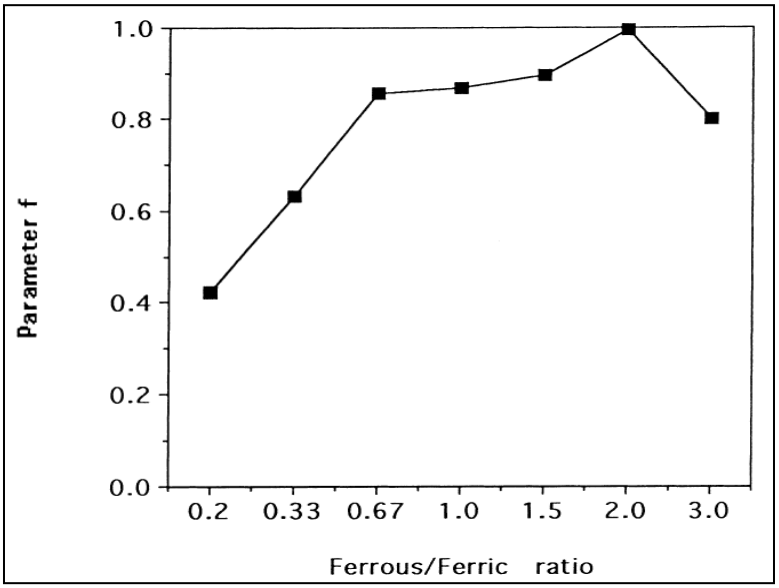

Figure 2.2: The effect of ferrous to ferric ion molar ratio to the magnetic susceptibility of synthetic magnetite (*f*). (Adapted from White et al, 2000).

Figure 2.2 shows the relationship between the magnetic force parameter *f* as a function of ferrous to ferric ion molar ratio. The value of *f* is highest at a ferrous/ferric molar ratio of 2.0; this means that the effect of magnetic field will be highest at that ratio. This data would help in predicting the magnetic strength of synthetic magnetite and also some of its related electrochemical properties.

2.3.2 ELECTROCHEMICAL PROPERTIES OF MAGNETITE

Magnetite has unique electrochemical properties, which can be applied for water and waste-water treatment. Magnetite is an iron oxide with a band gap of 0.1eV (band gap is a measure of energy of the oxidation state of the metal), which is quite low compared to other iron oxides such as FeO (2.0eV), making magnetite very stable (Jung et al, 2010). This results in a unique electrochemical property, giving it a point of zero charge (PZC) of 6.5 (Cornell et al, 2006). PZC can be defined as the point at which the net potential of adsorbed ions is zero. Figure 2.3 shows the changing zeta potential of magnetite as a function of pH. Although some studies found the PZC of magnetite to be around 8, there is a range depending on the characteristics of the solution. The point of zero charge is influenced by the presence of ions such as calcium and magnesium in water (MacRae, 1981).

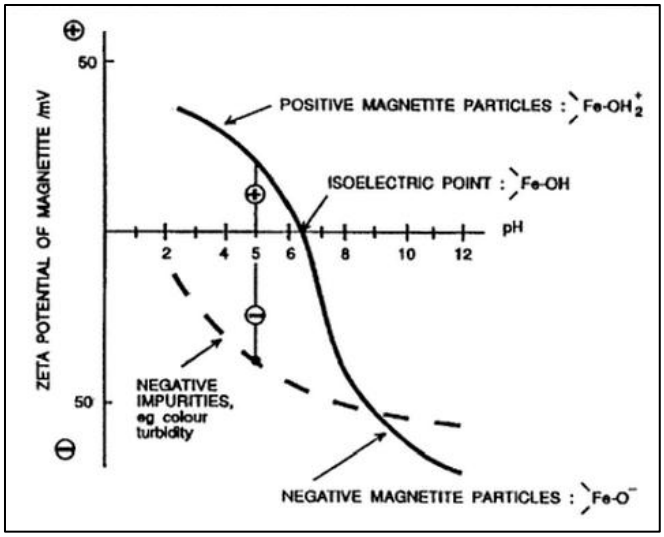

Figure 2.3: Zeta potential of 99 % pure clean magnetite plotted as a function of pH (Adapted from Kolarik, 1983)

Figure 2.3 is a good representation of magnetite reversing its potential as a function of pH (the ionic environment). Results reveal that magnetite has great affinity for OH^-, and OH^- strongly adsorbs on magnetite (Jung, 2010; Pierrefeu, 2010). Reduction of H_2O molecules was observed on the magnetite surface by interaction of H_2O with magnetite and generation of H^+ atoms at the magnetite surface when the solution was made acidic (Sung and Jung, 2010). This explains how the zeta potential of magnetite reverses depending on pH in water.

Inconsistencies were rising on the chemical effect of magnetic field on the physical and spectral properties of water. A satisfactory answer and evidence was presented in soviet journals as reviewed in the work of Maggard (Maggard, 1989). The review summarized changes in physical and spectroscopic properties of solutions when they are exposed to a magnetic field. These properties included propagation of ultrasonic waves, dielectric properties, Brownian

motion, magnetic susceptibility, and volume of solution. The only physical property that is consistently reported to not change is the surface tension of water. This possibly suggests a combined effect of magnetite and magnetic field to yield such unique properties, which are attributed to its use as a sorbent in water and waste-water treatment applications.

2.4 APPLICATION OF MAGNETITE AS A SORBENT

Magnetite (Fe_3O_4) has been used for groundwater remediation due to its high reductive capacity for groundwater and soil contaminants, including metals such as arsenic, chromium and some organic colloidal compounds as well. Due to their persistence in natural environments and toxicity to humans and animals, efforts have been made to remove these contaminants using magnetite.

2.4.1 METALS

Heavy metals are regulated as water contaminants. Magnetite has been tested for adsorption and recovery of heavy metals. One of the first studies were carried out by Anand et al (1985) to examine the performance of magnetite for metal removal under a magnetic field. Their experimental data on the effective removal of metals such as calcium, copper, nickel and zinc under the effect of magnetic field showed that increasing the magnetic field and decreasing the flow velocity led to increase in separation. The most important conclusion to be drawn from the result is that the effective removal of most of the metals, up to

95 percent, from water using magnetite and magnetic separation was accomplished.

Ferrite is a generic term used for a class of crystalline magnetic iron oxide compounds that possess the property of magnetization similar to magnetite (Reynolds, 1980). Ferrites and natural magnetite were used in batch modes for actinide and heavy metals removal from waste-water consistently achieving more than 90 % removal efficiency (Boyd et al, 1986; Kochen & Navratil, 1987; Navratil, 1989).

Investigation into the adsorption process revealed that iron atoms in magnetite can be replaced by many other metal ions without seriously altering its spinel structure (Boyd et al, 1986). Studies also show that the removal capacity of plutonium and americium from waste-water was enhanced by using an external magnetic field (Kochen & Navratil, 1997). Removal of heavy metals such as uranium and selenium using magnetite was over 99% (Navratil et al, 2009). Table 2.3 summarizes and compares the removal efficiency of in situ ferrite (synthetic magnetite) and natural magnetite pretreated with deionized water and acid (CuCl).

Table 2.3: Efficiency of magnetite to adsorb uranium (U_3O_8) and selenium (Se). (Adapted from Navratil et al, 2009).

Conditions	Metal removed	Concentration after treatment (mg/l)	% Removed
Deionized water washed magnetite	Se	0.023	99.8
CuCl acid washed magnetite	Se	0.012	99.9
Deionized water washed in situ ferrite (1mMol)	U_3O_8	<0.2	>98
Deionized water washed magnetite	U_3O_8	2.5	75

Recent studies have demonstrated removal of arsenic, chromium, cobalt, iron and uranium from simulated waste-water to be over 90% (Cotton et al, 1999; Navratil, 2008). These experiments were performed at a pH above the PZC of magnetite (i.e. pH > 6.5, but pH > 8 was found more efficient). These observations were explained by the high-gradient magnetic separation effect, as americium, plutonium, and other hydrolytic metals are known to form colloidal particles with magnetite at elevated pH levels (ph = 8) (Ebner et al, 1999). Research conducted on chromate removal from waste-water generated in cooling nuclear reactors, found that the removal efficiency of magnetite to adsorb chromium ions (IV & III) was 99.8% at pH 8 (Anderson et al, 1984).

Studies investigating nano-magnetite for effluent processing concluded that magnetic separation and flocculation by electrostatic interaction can be used for removal of heavy metals and release dissolved oxygen in water simultaneously (Vatta et al, 2006). Higher surface area of powdered micron size magnetite increases its adsorption capacity. The adsorption capacity of

magnetite to remove arsenic (III) and arsenic (V) increases ≈ 200 times when the size of magnetite particle decreases from 300 to 12 nm (Mayo et al, 2007). Heavy metal removal would take place at a pH above 6.5, as magnetite gets negatively charged, generally around 8 for efficient removal. The reverse is true for adsorption of colloidal particles and organic material from water and waste-waters.

2.4.2 COLLOIDAL PARTICLES

Colloidal particles are dispersed phase particles ranging from 1 to 1000 nm in size (Levine, 2001). Colloidal particles are so small that their surface area in relation to mass is very large (Crittenden, 2005). All colloidal particles are electrically charged. If the electrodes are immersed in the fluid then the particles migrate towards the pole of opposite charge (Crittenden, 2005). Colloids have high surface area and hence have a lot of active surface for adsorption to occur. The stability of colloids is mainly due to preferential adsorption of ions (Crittenden, 2005). Magnetite has been used to remove suspended solids in municipal waste-water (Johan et al, 2004).

Adsorption of colloids such as very fine suspended kaolinite clay particles has also been successfully achieved using synthetic magnetite (Oliveira et al, 2003). The adsorption features of clays (clays are non-magnetic in nature) with the magnetic properties of iron oxides were combined in a composite to produce a magnetic adsorbent. One of these magnetic adsorbents developed by Hayashi

et al (2004) exhibits good adsorption capacity for metal ions contaminants in water and show excellent chemical stability in a wide pH range (Figure 2.4). This indicates that magnetite could be efficiently used for adsorption of colloidal clay in water and waste-water.

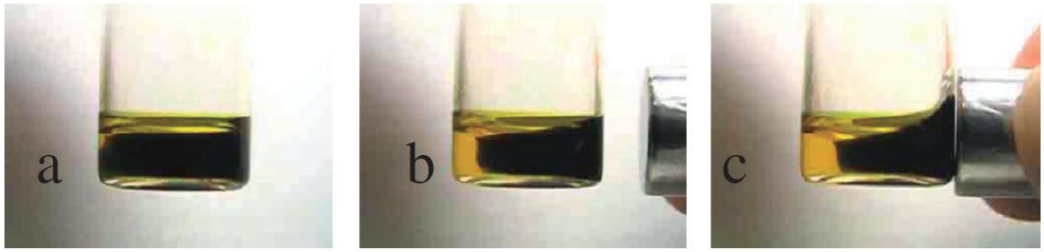

Figure 2.4: The effect of a permanent magnet on the magnetic adsorbent made using $FeCl_4^-$. (a) Settling of the magnetic adsorbent under gravity, (b) and (c) are demonstrations of a simple magnet being able to attract the adsorbent in a test tube. (Hayashi et al, 2004)

The use of magnetite for mine water treatment also revealed that magnetite is capable of removing a large percentage of dissolved and suspended organic matter, such as clay and color from untreated water (Kolarik et al, 1977). It has been found that separation of suspended particles can be enhanced using different magnetic strengths and flow rates. The study concluded that higher magnetic strength (4.0 T) and magnetic field contact time (4 mins) enhance settling of suspended particles. Applied magnetic field and low flow rates are also responsible for affecting the formation of scales in distribution systems.

2.4.3 HARDNESS CONTROL

Gehr et al (1995) carried out work on the saturated solution of $CaSO_4.2H_2O$ under a magnetic field to assess the effect of magnetic field on

hardness in water under well controlled laboratory conditions. The two independent variables tested were magnetic field strength and rotation speed. These variables were set at two levels, (4.75 T (Tesla) and 1200 rpm respectively). Their results show that the magnetic field had a significant effect on the separation of $CaSO_4$ crystals. Calcium ions showed an increase in precipitation in the form of stable crystals with magnetic field applied. These crystals were not the scale forming type but could be separated using a filter.

Efficiency of magnetic fields to control or decrease water hardness is debated. Although there are many different arguments on the mechanism of hardness removal, the proposed mechanism of hardness removal is that an increase in ionicity, caused by restructuring the water molecule in the liquid that passes through the magnetic field, modifies the crystallization mode of the minerals in water and prevents nucleation (Hibben, 1973).

This type of change in crystallization mode could be of great importance since it involves scaling problems associated with common industrial units such as boilers, heat exchangers and pipelines. It was demonstrated in the work of Wang et al (1997) that a phenomenon of crystallization is taking place in such units and resulted in a greater number of crystals with smaller sizes having irregular shapes. The crystallization and consequently the precipitation were found to occur faster under magnetic field than without the use of these fields. In these units, the magnetic field was observed to create turbulence effect in

fluid flow. The key to scaling control by magnetic water treatment lies in destabilization of the ion by passing it through a magnetic field of high intensity and not allowing it to form a crystal (Lipus et al, 2001).

The reported effects of magnetic water treatment for hardness control are varied and often contradictory. When calcium and magnesium ions pass through the magnetic field, a force is exerted on each ion. The forces on ions of opposite charges are in opposite directions. The redirection of the particles tends to increase the frequency with which ions of opposite charge collide and combine to form a mineral precipitate or insoluble compound (American Petroleum Institute, 1985). No magnesium or calcium is removed from the water by magnetic treatment. Instead, the claims are that the magnetic field decreases the tendency of the dissolved minerals to form scale. While the dissolved mineral concentration indicates the water is still hard, magnetically treated water supposedly behaves like soft water because crystallization of calcium and magnesium ions takes place.

2.5 ADSORPTION OF MICROORGANISMS

Adsorption of microorganisms on the surface of magnetite was first reported in the seventies. Use of magnetite for adsorption of bacteria was first reported by CSIRO (Commonwealth Scientific and Industrial Research Organization) in Australia. The fact that magnetite is effective at adsorbing bacteria from turbid natural waters is summarized in Table 2.4 (MacRae, 1981).

Pretreated magnetite caused a 5-log reduction in *E. coli* UQM 70 when magnetite was acid-washed to remove calcium and magnesium ions (MacRae and Evans, 1983). Acid washing removes adsorbed ions such as Ca^{2+} and Mg^{2+}, as these ions can interfere with the adsorption process and decrease the efficiency of magnetite to adsorb microorganisms (Anderson et al, 1981).

Table 2.4: Effect of magnetite pretreatment to remove bacteria from suspension (Adapted from MacRae & Evans, 1981)

Bacterium	Removal of Bacteria (%)	
	Untreated Magnetite	*Treated Magnetite
Escherichia coli	98.07	99.77
Serratia marcescens	98.98	99.75
Enterobacter aerogenes	99.74	99.92
Bacillus subtilis	99.97	96.40

*Treated magnetite refers to eight cycles of pre-treatment with alternating magnetic fields. The magnetite was exposed to 0.1 M NaOH and then washed with water followed by 8 applications of alternating magnetic fields to demagnetize magnetite, so that the surface area of magnetite remains optimum.

Rhodopseudomonas sphaeroides was adsorbed on magnetite surface using the method described by MacRae (1981) in Table 2.4, to be used for biological degradation of chlorinated hydrocarbons using the magnetite adsorbed cells. Algae removal from lake water using magnetite has been reported; 94% of algal cells were removed at a pH of 6.5 (Bitton et al, 1975).

Removal of other biological matter has also been reported in the early 80s. Adsorption of MS 2 bacteriophage of *E. coli* has also been reported using alkali-activated magnetite by Atherton and Bell (1983). Addition of 1% magnetite to suspensions (pH = 6) of radioactive bacteriophage resulted in loss of 99.9% infectivity and radioactivity from the supernatant (Bell & Atherton, 1983). Ninety

percent removal of bacteriophage infecting *Escherichia coli* from water by adsorption onto magnetite in the presence of $CaCl_2$ and subsequent separation of magnetite with adsorbed cells using a magnetic field was successfully achieved (Bitton and Mitchell, 1974). Confirmatory evidence of the disruption of virus particles was observed when the cells were desorbed at pH 10, suggesting that most of the virus protein was retained on the surface of magnetite. The ability of magnetite to retain and disrupt the virus protein may turn out to be a useful property for possible disinfection.

2.5.1 Model Bacterial Strains

To demonstrate the ability of magnetite to adsorb bacteria some model bacterial strains were chosen. The model bacteria chosen for this study are non-pathogenic ATCC (American Type Culture Collection) strains - *Escherichia coli ATCC® 25922™*, *Pseudomonas putida ATCC® 17453™* and *Micrococcus luteus ATCC® 4698™*. Specific strains were chosen based on their category such as Gram negative/positive, size and surface characteristics to help widen the scope of this study.

The classification of bacteria in terms of their staining (Gram staining) largely divides bacteria into two big groups: Gram negative and Gram positive. Gram staining divides bacterial species on the basis of the chemical and physical properties of their cell walls.

Gram positive bacteria have a high amount of peptidoglycan in the form of a thick layer on its surface (Madigan et al, 2000). The outer surface (which is the layer of peptidoglycan) of a gram positive cell is not protected by any outer membrane due to which the peptidoglycan layer is well exposed to the environment. Peptidoglycan layer in Gram positive bacteria are cross linked by enzymes, whereas in Gram negative bacteria there is a direct covalent bond between the enzymes and peptidoglycan layer (Madigan et al, 2000). The peptidoglycan layer also constitutes of a compound called lipoteichoic acid, which serves as a chelating agent and could also assist in adherence of cells (Madigan et al, 2000). Lipoteichoic acid is widely available on the surface of Gram positives.

On the other hand Gram negative cells have a different outer cell structure. Generally, Gram negative bacteria have an outer cell wall layer comprising of lipopolysaccharides and proteins which protect the peptidoglycan layer between the periplasmic spaces. So unlike Gram positive cells, the peptidoglycan layer of Gram negative cells is protected and is not directly interacting with the surrounding environment.

E. coli and *P. putida* species were chosen because they are good representatives' of Gram negative bacteria found in water and waste-water as well. They are rod shaped organisms with a size of about 2/1.7 µm in length and 1/0.7 µm in diameter respectively (ATCC). *E. coli* are widely studied as a model

organism (Fux et al, 2005), hence the species served a healthy model organism for this study. Both the bacterial strains have aerobic metabolism, and are able to grow on a wide variety of organic substances (ATCC MSDS). On the other hand, *M. luteus* is a Gram positive type of bacteria, has facultative metabolism, is spherical in shape with a typical diameter of 2 μm, and can grow in a wide variety of organic substances.

2.6 KNOWLEDGE GAP AND HYPOTHESIS

Magnetite has the property to form complexes with charged bio-colloidal matter in water because of the difference in polarity, which lends itself to be used as a disinfection aid or as a primary disinfectant. There is very little work that has looked in detail at optimizing adsorption of microorganisms on the surface of magnetite. The disadvantages suffered by using chemicals for conventional disinfections techniques (as described in Table 2.1) can be lessened or eliminated if magnetite could be successfully used as an adsorbent for microorganisms. It will reduce the overall cost of water treatment, because magnetite is re-generable and widely available. Since a process involving magnetite uses adsorption instead of chemical oxidation, there is very low susceptibility of production of disinfection by products. The magnetic nature of magnetite, which is desirable, will decrease the hydraulic retention time as no sedimentation would be required. This would result in increased production of clean water for the same amount of energy used.

This gives rise to two research questions and hypotheses:

- *Can magnetite remove significant amounts of microorganisms from water?*

Since a microorganism ranges in size from 0.2 to 2 microns in width or diameter, and up to 1- 10 microns in length for the non-spherical species (Robert et al, 2004), they will behave like small biological colloids in water. The surface of the microorganism will carry an electrostatic charge depending on pH and other organic matter in water. Most of the organic colloidal matter in natural water is negatively charged (Crittenden, 2005). Because of the protein nature of their surface, bacteria might also have complex forming functional groups on their surface along with the electrostatic charge (MacRae, 1981).

Finely divided magnetite (1 – 5 micron, 99%) has very high surface area, and has the property of being positively charged below the PZC of magnetite. Magnetite has a very similar colloidal behavior to bacteria in terms of having surface charge as a function of pH (Amirhor, 1975). Magnetite being positively charged at a pH below its PZC of 6.5 should electrostatically adsorb microorganisms in water. The cell and magnetite complex formed can then be settled with the help of a permanent magnet.

To prove this concept, at first will be to check the adsorption of colloidal species such as fine clay particles. Fine clay particles are present in natural waters during spring runoff making the water very turbid. Turbidity removal will

serve as a preliminary experiment for this study. If magnetite is able to remove turbidity from natural waters then it has potential to remove microbial colloids from water as well.

To prove the adsorption of bacteria, three model bacteria will be chosen based on their size, shape and type. The model bacteria chosen are non-pathogenic ATCC (American Type Culture Collection) strains - *Escherichia coli ATCC® 25922™*, *Pseudomonas putida ATCC® 17453™* and *Micrococcus luteus ATCC® 4698™*.

- *If so, what are the mechanisms involved in the removal process?*

We first must understand the mechanisms involved in this process prior to taking any steps for optimization of this type of disinfection process. Electrostatic attraction of bacteria on the surface of magnetite could be one of the dominant mechanisms, but due to the functional groups on the surface of bacterial cells, there is a possibility of some other adsorption processes taking place. To find out if electrostatic charge is one of the dominant mechanisms, experiments will be run at different pH conditions which are above and below the PZC of magnetite. The functional groups on the surface of bacterial cells will be scanned to determine if there is any other form of complexation happening on the cell and magnetite interface.

As the literature suggests that the PZC of magnetite can shift with the interference of ions such as Ca^{2+} and Mg^{2+} (Anderson et al, 1981), experiments

will be conducted in solutions free of such ions. This will help in determining the most probable reason for the adsorption of bacterial cells on the surface of magnetite. Solutions used will be – (1) Sterile standard saline (0.85% by weight NaCl) and (2) Phosphate buffered saline (Buffered using 0.1 M monosodium phosphate and disodium phosphate solutions).

2.7 WORKS CITED

1. Alcamo, J., T. Henrichs, and T. Roesch. World Water in 2025: Global Modeling and Scenario Analysis for the World Commission on Water for the 21st Century. University of Kassel,Germany: Center for Environmental Systems Research (CESR), 1999.
2. American Council for an Energy Efficient Economy. "Water and Waste-water." <https://www.aceee.org/topics/water-and-waste-water>.
3. American Petroleum Institute. "Non-Chemical Technologies for Scale and Hardness Control." <http://www.api.org/>.
4. Anand, P. "Heavy Metals Removal by High Gradient Magnetic Separation." IEEE Transactions on Magnetics 21.5 (1985): 2062.
5. Anderson, N. J., B. A. Bolto, and L. Pawlowski. "A Method for Chrome Removal from Cooling Tower Blowdown Water." Nuclear and Chemical Waste Management 5 (1984): 125,125-129.
6. Anthony, JW, et al. "Magnetite." Handbook of Mineralogy. 1985th ed. Chantilly, Virginia: Mineralogical Society of America, 1985.

7. Aschengrau, A., S. Zierler, and A. Cohen. "Quality of Community Drinking Water and the Occurrence of Late Adverse Pregnancy Outcomes." Archives of Environmental Health 48 (1993): 105. Abstract.
8. Atherton, JG, and SS Bell. "Adsorption of Viruses on Magnetic particles—II. Degradation of MS2 Bacteriophage by Adsorption to Magnetite." Water Research 17.8 (1983): 949.
9. Baker, RR, JG Mather, and JH Kennaugh. "Magnetic Bones in Human Sinuses." Nature 301 (1983): 79,79-80. Abstract.
10. Bitton, G., and R. Mitchell. "the Removal of *Escherichia Coli*-Bacteriophage T_7 by Magnetic Filtration." Water Research 8.8 (1974): 549.
11. Bitton, G. "Adsorption of Viruses Onto Surfaces in Soil and Water." Water Research. 9.5 and 6 (1975): 473.
12. Bove, F., MC Fulcomer, and JE Sarrin. "Public Drinking Water Contamination and Birth Outcome." American Journal of Epidemiology 141.9 (1995): 850,850-861.
13. Boyd,TE, Cusick,MJ, and JD Navratil. "Ferrite Separation Science and Technology." Recent Developments in Separation Science 8 (1986).
14. Cedergren, MI, et al. "Chlorination Byproducts and Nitrate in Drinking Water and Risk for Congenital Cardiac Defects." Environmental Research 89 (2001): 124,124-130. Abstract.

15. Cornell, R. M., and U. Schwertmann. the Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses, 2nd, Completely Revised and Extended Edition. John Wiley and Sons, 2006.
16. Cornwell, D. A., et al. Handbook of Practice, Water Treatment Plant Waste Management. United States of America: American Water Works Assosciation, 1987.
17. Cotten, GB, and JD Navratil. "Magnetic Adsorption Method for the Treatment of Metal Contaminated Aqueous Waste." Proceedings of Waste Management.
18. Cox, A. E., J. J. Camerata, and B. R. Smith. "Phosphate Availability and Inorganic Transfonnation in an Alum Sludge-Affected Soil." Journal of Environmental Quality 26.5 (1997): 1393,1393-1398.
19. Crittenden, J., et al. "Disinfection." Waste-water Treatment: Principles and Design. 1st ed. United States of America: John Wiley and Sons, Inc, 2000.
20. Crittenden, J., et al. "Disinfection." Waste-water Treatment: Principles and Design. 2nd ed. United States of America: John Wiley and Sons, Inc, 2005.
21. CSIRO. "Sirofloc." <http://www.csiropedia.csiro.au/display/CSIROpedia/SIROFLOC>.

22. Ebner, A.D., et al. "New Magnetic Field-Enhanced Process for the Treatment of Aqueous Wastes." Separation Science and Technology 34.6 (1999): 1277.
23. Environment Canada. Aluminum Salts (Final Content)., 2011.
24. Fux, C. A., et al. "Survival Strategies of Infectious Biofilms." Trends in microbiology 13.1 (2005): 34-40.
25. Gehr, R., et al. "Reduction of Soluble Mineral Concentrations in $CaSO_4$ Saturated Water using a Magnetic Field." Water Research 29.3 (1995): 933.
26. Gem and Mineral Miners Inc. "Magnetite: Mineral Information Page." 2009. <http://www.mineralminers.com/html/magminfo.htm>.
27. Gibson, V. C., et al. "New Unsymmetrical Thioether- and Thiolate-Substituted Ferrocene Ligands and an Unusual Bridged-Pd Dimer Complex." Chemical Communications 23 (2000): 2359.
28. Gruninger, Robert M., "Disposal of Waste Alum Sludge from Waste-water Treatment Plants." Jouranl of Water Pollution Control Federation 74.3 (1975). "Disposal of Waste Alum Sludge from Water Treatment Plants." Journal of the Water Pollution Control Federation 74.3 (1975): 543,543-552.
29. Hall, W. S., and L. W. Hall. "Toxicity of Alum Sludege to Ceriodaphnia Dubia and Pimephales Promelas." Bulletin of Environmental Contamination and Toxicology 42.5 (1989): 791,791-798.

30. Hayashi, S., "A New Class of Magnetic Fluids: Bmim[$FeCl_4$] and Nbmim[$FeCl_4$] Ionic Liquids." IEEE Transactions on Magnetics 42.1 (2004): 12.
31. Hibben, S.G., “Magnetic Treatment of Water. Advanced Research Projects Agency of the Department of Defense” National Technical Information Service, 1622–4, (1973).
32. Hill, R. J., James R. Craig, and G. V. Gibbs. "Systematics of Spinel Structure Type." Physics and Chemistry of Minerals 4.4 (1979): 317.
33. Hwang, B.F., Jaakkola, J.K., "Water Chlorination and Birth Defects: A Systematic Review and Meta-Analysis." Archives of Environmental Health 58 (2003): 83,83-91.
34. Hwang, B.F., J.K. Jaakkola, and H.R. Guo. "Water Disinfection Byproducts and the Risk of Specific Birth Defects: A Population-Based Cross-Sectional Study in Taiwan." Environmental Health 7.23 (2008).
35. N., Levine, and N. Ira. *Physical Chemistry*. 5th ed. Boston: Mc-Graw Hill, 2001.
36. I.C., Mac Rae. "Removal of Pesticides in Water by Microbial Cells Adsorbed to Magnetite." Water Research. 19.7 (1985): 825,825-830.
37. Ip, S.Y., et al. "Physicochemical Treatment of an Australian Municipal Waste-water." Water Research 11.2 (1977): 173.
38. Jonasson, B., "Phosphorus Transformation in Alum Sludge Amended Soils." Swedish Journal of Agricultural Research 26.2 (1996): 69,69-79.

39. Jones, O. A. H., et al. "Questioning the Excessive use of Advanced Treatment to Remove Organic Micropollutants from Waste-water." Environmental Science and Technology 41.14 (2007): 5085,5085-5089.
40. Jung, K.S., and L.D. Pierrefeu. "Electrochemical Characterization of Sintered Magnetite Electrode in LiOH Solution." Corrosion Science 52.3 (2010): 817.
41. Jung, K.S., and K.W. Sung. "Magnetite: Electrochemical Properties and its role on Flow Accelerated Corrosion." Magnetite: Structure, Properties and Applications. Ed. DM Angrove. Haupauge,New York: Nova Publishers, 2010.
42. Klotz, J.B., and LA Pyrch. "Neural Tube Defects and Drinking Water Disinfection by-Products." Epidemiology 10.4 (1999): 383,383-390.
43. Kolarik, L. O., A. J. Priestley and D. E. Wiess. "The Sirofloc Process for Turbidity and Colour Removal." 7th Federal Convention AWWA. Canberra.
44. Lipus, L.C., J. Kropeb, and L. Crepinsek. "Dispersion Destabilization in Magnetic Water Treatment." Journal of Colloid and Interface SciencE 236.1 (2001): 60.
45. Lowenberger, A., et al. the Efficiency Boom: Cashing in on the Savings from Appliance Standards. Vol. A123. American Council for Energy Efficient Economy, 2012.

46. MacRae, I. C., and Susan K. Evans. "Factors Influencing the Adsorption of Bacteria to Magnetite in Water and Waste-water." Water Research. 17.3 (1983): 271.
47. Madigan, M. T., J. M. Martinko, and J. Parker. Brock Biology of Microorganisms. 9th ed. Prentice Hall College Div, 2000
48. Maggard, S. M., A Chemometric Analysis of a Magnetic Water Treatment Device. PhD University of Michigan,Ann Harbor, 1989.
49. Magnus, P., et al. "Water Chlorination and Birth Defects.." Epidemiology 10 (1999): 513. Abstract.
50. Massart, R., "Preparation of Aqueous Magnetic Liquids in Alkaline and Acidic Media." IEEE Transactions on Magnetics 17.2 (1981): 1247-8.
51. Moniwa, S., H. Shiire, S. Ebihara, et al. Water Treatment System. KK Toshiba, assignee.
52. Navratil, J.D., "Mine Water Treatment using Iron Ferrites and Magnetite." Mine Water and the Environment (2008): 305,305-308.
53. Nieuwenhuijsen, M.J., et al. "Chlorination Disinfection by-Products and Risk of Congenital Anomalies in England and Wales." Environmental Health Perspectives 116.2 (2008): 216,216-222.
54. Olegsander, P., Phosphorous Removal and Recovery from Waste-water using Magnetite. Masters of Science Royal Institute of Technology,Sweden, 2010.

55. Oliveira, L., et al. "Clay–iron Oxide Magnetic Composites for the Adsorption of Contaminants in Water." Applied Clay Science 22.4 (2003): 169,169-177.
56. Oliver, A.H., et al. "Questioning the Excessive use of Advanced Treatment to Remove Organic Micropollutants from Wastewater." Environmental Science and Technology 41.14 (2007): 5085,5085-5089.
57. Quartin, V. M. L., et al. "Mineral Imbalance due to Manganese Excess in Triticales." Journal of Plant Nutrition 24.1 (2001): 175,175-189.
58. Reynolds, T. G. "Ferrites." Encyclopedia of Chemical Technology., 1980.
59. Rook, J. J. "Formation of Haloforms during Chlorination of Natural Waters.." Water Treatment Exam 23.2 (1971): 234,234-243.
60. United States Environmental Protection Agency. Status Report on Development of Regulations for Disinfection and Disinfection by-Products. Washington D.C: USEPA, 1991. " A Summary of Publications on the Development of Mode-of-Action Information and Statistical Tools for Evaluating Health Outcomes from Drinking Water Disinfection by-Product (Dbp) Exposures." USEPA, 2011.
61. Vatta, LL, Ron D. Sanderson, and KR Koch. "Magnetic Nanoparticles: Properties and Potential Applications." Pure Applied Chemistry 78.9 (2006): 1793.
62. Wang, Y., et al. "Rapid Onset of Calcium Carbonate Crystallization under the Influence of a Magnetic Field." Water research 31.2 (1997): 346-50.

63. WHO/Unicef Joint Monitoring Programme. MDG Assessment Report (2006). Switzerland: World Health Organisation Press, 2006.

64. World Water Council. "Water Crisis." <http://www.worldwatercouncil.org/index.php?id=25>.

Chapter 3: Experimental work

3 EXPERIMENTAL WORK

3.1 INTRODUCTION

Few studies have been done to investigate the possible engineering application of magnetite in water and waste-water treatment to remove different contaminants. As a result, research conducted on the use of magnetite for water and waste-water treatment has been limited because it is still looked at as an emerging process. The one exception is the use of magnetite for removing heavy metals in waste-water. Navratil (2009) describes the application of magnetite and ferrites for mine water treatment and remediation. The results show that magnetite can achieve a removal efficiency greater than 95% for heavy metals such as selenium, chromium (VI), and uranium (Navratil, 2009). Metal ion removal by adsorption to magnetite is gaining popularity in the waste-water treatment industry. The removal of zinc and nickel from effluents of metal polishing industries has also been investigated and found to be promising (Oskay, 2003). Studies on the utilization of magnetite for chromium (VI) (Yuan et al., 2010) and mercury (Girginova et al., 2010) removal on a bench scale plant showed potential for a practical engineering solution to water and waste-water treatment. The effect of size of magnetite has also been investigated (Mayo et al, 2007). The adsorption efficiency of magnetite to adsorb arsenic ions from waste-water increased by 200 times when the size of magnetite was decreased from 300 nm to 12 nm (Mayo et al, 2007).

The use of magnetite for disinfection in water and waste-water treatment is an emerging technology. There are some convincing reports of magnetite adsorbing bacterial and algal cells from surface waters, up to 94% of algal cells were adsorbed (Bitton et al, 1975) but not enough to support the statement that magnetite could potentially be used as a disinfectant. MacRae and Evans (1981) reported adsorption of *E. coli* by 5 log cycles, which is very high, and is comparable to modern disinfection techniques such as UV and ozonation. Adsorption of 99.4% of polio virus type 1 (sabin) (Bitton et al, 1976) using magnetite has also been reported by the same author.

Magnetite for waste-water treatment presents several advantages. Its main advantage is that magnetite can be settled using a simple magnetic field. This particular property can reduce the footprint of a water or waste-water treatment plant, thus resulting in shorter sedimentation times and higher flow rates. Another advantage of using magnetite is that it can be manufactured on site and spent magnetite can be regenerated on site as well.

This research will focus on investigating the capability of magnetite to be used as a disinfectant aid or a primary disinfectant. The research will also entail the different factors which affect the performance of magnetite to adsorb microorganisms. The purpose of this research is to look for alternative water and waste-water treatment methods which minimize the use of chemicals and release of chemical by products in the municipal water stream.

3.2 MATERIALS AND METHODS

This section of chapter 3 will explain in detail the materials and methods used for all experiments conducted.

3.2.1 SAMPLING OF RIVER WATER

The water used for testing the ability of magnetite to control turbidity was obtained from the North Saskatchewan River, at the base of Emily Murphy Park, Edmonton, Alberta in June, 2011. The samples were saved in cold storage at $4^{o}C$ and appropriate amount of sample was brought to room temperature before testing. The pH of the river water was recorded when acquired. pH was adjusted with stock solutions of NaOH (0.1M) and H_2SO_4 (0.1M) to required levels when experiments were conducted. Water samples were treated at a pH of 5.6. The value 5.6 was adapted from CSIRO division as it is the pH at which electrostatic attraction of magnetite is highest for organic matter (Anderson et al, 1983).

3.2.2 FACTORIAL DESIGN OF EXPERIMENTS

The concentration of magnetite used for treating river water varied and the range was determined by some preliminary experiments. Different range concentrations of magnetite, alum and poly acrylamide were used. The factorial design used for turbidity control was a partial factorial design consisting of 3^2 values. The chart of the values used is given in Table 3.1. A = Commercial grade Alum: Aluminum sulfate $Al_2(SO_4)_3$, 26.8% Wt. in water (10 mg/l and 60 mg/l), P = Polyacrylamide: Polyacrylamide (acrylamide-co-diallyldimethylammonium

chloride) 10% Wt. in water, density of 1.02 gm/ml at 25°C (1 mg/l to 3 mg/l), M = Magnetite (100 mg/l to 300 mg/l), the values in brackets indicate the upper and lower limit of the concentrations used for the appropriate combination used. The letter N indicates that no chemical was added and acts as a control. The letters L and H indicates its lower concentration limit and upper concentration limit used. For example: Combination 15 (NA, NP, and HM) means that no alum, no polyacrylamide but only magnetite, upper limit concentration of 300 mg/l was used for the experiment.

A total of 16 combinations between alum, polyacrylamide and magnetite were used. Controls used were: positive control = No chemicals added and negative control = Clean deionized water (turbidity = 0.01NTU).

Table 3.1: Combinations used to determine the removal efficiency of magnetite, alum and polyacrylamide. L = Lower limit of concentration, H = Higher limit of concentration and N = No chemical added.

Combination	A (Commercial grade Alum)	P (polyacrylamide)	M (Magnetite)
1	*LA*	*LP*	*LM*
2	*HA*	*LP*	*LM*
3	*LA*	*HP*	*LM*
4	*HA*	*HP*	*LM*
5	*LA*	*LP*	*HM*
6	*HA*	*LP*	*HM*
7	*LA*	*HP*	*HM*
8	*HA*	*HP*	*HM*
Control	*NA*	*NP*	*NM*
9	*LA*	*LP*	*NM*
10	*LA*	*NP*	*HM*
11	*NA*	*HP*	*NM*
12	*NA*	*HP*	*HM*
13	*HA*	*NP*	*HM*
14	*HA*	*HP*	*NM*
15	*NA*	*NP*	*HM*

3.2.3 RIVER WATER TREATMENT SETUP

A schematic of the setup used for the treatment of river water is shown in Figure 3.1. Untreated raw river water volume of 1L was mixed with magnetite at 200 RPM for 10 mins using a paddle mixer on a jar tester (Phipps and Bird, PB - 700^{TM} Jar tester, Virginia, USA). After treatment the sample was pumped through a magnetic field (see section 3.2.5) to trap the flocs formed (Approximate time of contact with the magnetic field was 23s, flow rate was adjusted to achieve maximum time of contact) and then the clear water was collected in a collection jar.

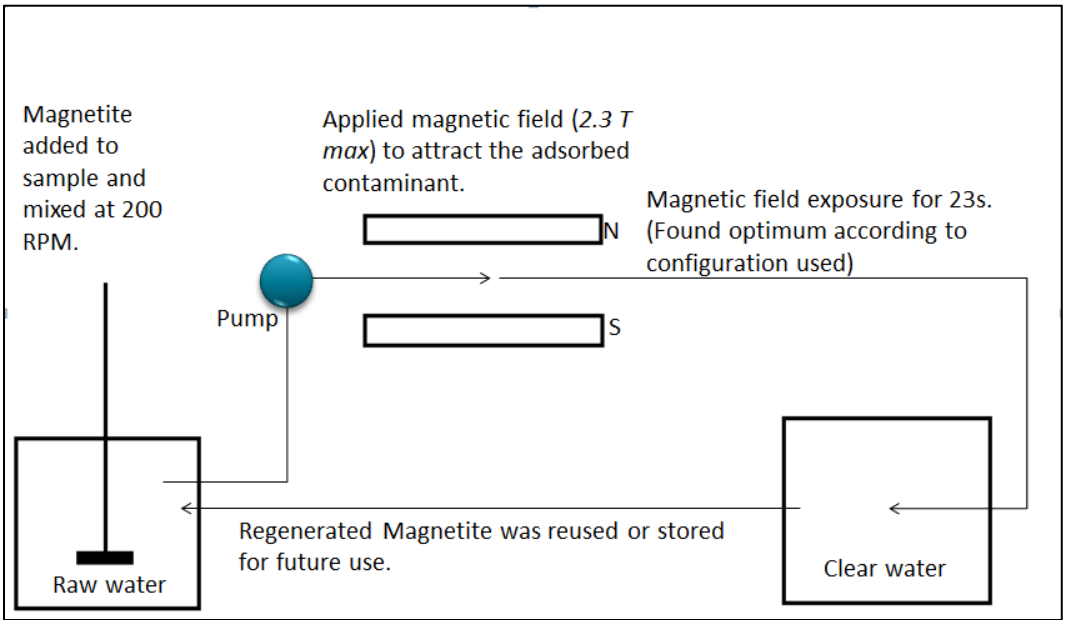

Figure 3.1: Schematic of the setup used for treatment of river water using magnetite. The arrows indicate the flow of water unless otherwise mentioned in the text. Volume of water used as sample was kept 1 L at all times to maintain consistency.

The tubes were rinsed with deionized water after each experiment and the magnetite was flushed at high flow rate (after removing the magnetic field). The turbidity of the sample was measured after the clear water was collected in

the clean jar. The pHs of the samples were checked before and after as alum consumes some alkalinity during the treatment process (Crittenden et al, 2005).

3.2.4 MEASUREMENTS OF TURBIDITY OF RIVER WATER

Turbidity of river water was measured using a Digital Direct reading field turbidity meter (Orbeco – Hellige, New York). The turbidity of sample was measured in duplicates before and after treatment with magnetite. In cases where no magnetite was added to the samples, the flocs were allowed to settle for 20 mins before being pumped through the magnetic field (Figure 3.1).

3.2.4.1 Statistical Analysis of data

To ensure accuracy, reliability and reproducibility of results from the factorial design statistical analysis of the factorial design were run. Statistics were analyzed using Regression analysis to solve data for significance of factors using Microsoft Excel 2010. All data were normally distributed before running regression analysis. Regression was run at 95% confidence level ($P<0.05$).

3.2.5 SPECIFICATIONS OF MAGNETS USED

The magnets used for settling magnetite were high strength ALNICO (Aluminum Nickel Cobalt) disk magnets from UNITED NUCLEAR SCIENTIFIC, MI, USA. The dimensions of which were ¼" x 3.5" (thickness x diameter) and a strength of 2.3 T (1Tesla = 10000 Gauss) at the poles. Average strength of about 1.8T was measured using a gauss meter.

3.2.6 BACTERIAL STRAINS AND GROWTH CONDITIONS USED

All cultures used in this study were obtained from ATCC® (American Type Culture Collection). Cultures used were *Pseudomonas putida* ATCC® 17453™, *Escherichia coli* ATCC® 25922™, and *Micrococcus luteus* ATCC® 4698™. These specific strains were chosen as they are non-pathogenic model organisms to simulate bacteria typically found in waste-water. All bacterial cultures were inoculated from a frozen stock at -80^{o}C in appropriate growth media. *P. putida* and *E. coli* were streaked on a petri dish with nutrient agar (BD DIFCO^{TM}) overnight at 37°C, and a single isolated colony from the same petri dish was inoculated in nutrient broth (BD DIFCO^{TM}) for 18 hs at 37°C. *M. luteus* was streaked on tryptic soy agar (BD BACTO™) for 48 hs at 37°C and a single isolated colony was inoculated in tryptic soy broth (BD BACTO™) for 48 hs at 37°C.

All cultures were incubated at 37°C without shaking (concentration achieved was approximately 10^6 cfu/ml, see Appendix C). Cells were harvested by centrifugation at 10,000*g* for 15 min (GYROZEN 1730MR) at room temperature. The supernatant was discarded and the cell pellet was washed with Phosphate buffered Saline (PBS) or Saline (0.85% NaCl) (depending on the experiment being conducted) solution at pH 7.5 and suspended in the same solution. The wet weight of the pellet was recorded prior to suspension in PBS. The percent dry weight of *E. coli*, *P. putida* and *M. luteus* has been reported to be 31.4%, 48.4% and 34.9% respectively (Bratback et al, 1984; Carstensen et al, 1965). These approximate dry weight values were used to calculate the

appropriate amount of magnetite required for experiments (see section 3.2.7). The optical densities of cultures were used to determine the concentration using the standard curves. Optical density of all cultures was measured at 600nm UV spectrum using NANODROP 2000c spectrophotometer (Thermo Scientific). The accuracy of this method was cross checked for every experimental run by correlating the optical density to viable plate count to ensure accuracy.

3.2.7 CALCULATIONS OF MAGNETITE DOSAGES USED IN EXPERIMENTS

Magnetite (Iron (II, III) Oxide) used was <5 µm 95%, MW = 231.53 g/mol and obtained from SIGMA ALDRICH, MO, USA. The cells and appropriate amount (by % Wt.) of magnetite were added to 50 mL of solution either buffered with phosphate buffers or sterile saline at pH 7.5, depending on the experiment being conducted. The amount of magnetite added to solutions was calculated depending on the dry weight of the cells (see Appendix A). This was done to achieve a direct correlation between the concentration of magnetite and bacteria.

To achieve a direct correlation between the concentration of cells and magnetite added, dry weight of the cell pellet was kept directly proportional to the weight of magnetite added. The term cell : magnetite ratio is used throughout this chapter, and represents the ratio of dry cell weight to magnetite weight. For example if the cell : magnetite ratio is 1 : 5, this means that for every 1 g of cells (dry weight percent), 5 g of dry magnetite was added. Different ratios

used throughout this study were 1 : 0, 1 : 1, 1 : 5, 1 : 10, 1 : 50, 1 : 100 and 1 : 200. Since the dry weight of each culture is different, the magnetite added to the solutions was proportionate by weight.

3.2.8 MIXING OF BACTERIAL CELLS AND MAGNETITE

Magnetite added was washed twice with deionized water only. The harvested cells were added to 50 ml volume of standard saline or phosphate buffered saline, whichever is applicable, for adsorption to take place. Sample volume was kept same for all experiments to maintain consistency. All the solutions containing magnetite were mixed at 200 oscillations per min for 10 min at room temperature on a bench top shaker (Sartorius Certomat SII, Gottingen, Germany). After mixing, the magnetite was allowed to settle with the help of a permanent magnet (ALNICO ¼" x 3", average strength 1.8 T and Max at 2.3 T) and the supernatant analyzed for viable cells.

Negative controls were also examined to check the effect of permanent magnet on bacterial cells. The settling by magnetic field was only about 5% max and <1% min, which is most probably aseptic technique error. Positive control for this experiment was to check if magnetite added any microorganisms to the solutions, and none were found.

3.2.9 MAGNETITE AND WATER INTERFACE STUDY

A stock solution of Tannic acid ($C_{76}H_{52}O_{46}$, J.T Baker Chemical Co, Phillipsburg, NJ, USA) was used at a concentration of 1000 mg/l (Appendix E).

The temperatures of the solutions were allowed to come in equilibrium with room temperature for a day. Dissolved oxygen was measured using YSI Model 52 DO meter, YSI Inc. Ohio, USA. The dissolved oxygen metering probe was stabilized overnight and calibrated for elevation and pressure according to the manual with the instrument.

3.2.10 PHOSPHATE BUFFERS

Stock solutions for phosphate buffers were prepared. Stock solution is a solution that is used for preparing all working solutions in the experiments done. Monosodium (monobasic) phosphate (Na_2HPO_4) and disodium (dibasic) phosphate (NaH_2PO_4) were used to make two phosphate buffering stock solutions. A volume of 1 L at 1M concentration were prepared using deionized water and stored at 4°C.

3.2.11 BUFFERED AND NON BUFFERED SALINE

The working solutions used in all the experiments related to bacterial cells were brought to working osmotic pressure by using salt (NaCl). The salt concentration in all the working solutions was kept at 0.85% by weight (8.5 gm/l) and is referred to as 'standard saline' in this document. The pH of non-buffered saline was 7.5. The solutions were buffered by adding appropriate volume of the buffer solutions in adapted from SIGMA Aldrich, MO, USA (Appendix B). The pH was checked (AR50, Accumet Research, Fisher Scientific, USA) and recorded after the required solutions were buffered.

3.2.12 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR)

Fourier Transform Infrared Spectroscopy was carried out to have a deeper understanding of the functional groups on the surface of the bacteria used. Bacterial cells (5 ml, concentration of 10^6 cells/ml) were harvested by centrifugation at 10,000*g* for 15 min and pellet washed with PBS (pH 7.5) and suspended in PBS (pH 7.5). The solution was freeze-dried at -50^{o}C and vacuum pressure of 1400 mbar for 24 hs. Infrared spectra were obtained with BIO-RAD, FTS 6000 and Varian Resolutions Pro software with a N_2 purging system equipped with a Mercuric Cadmium Telluride (MCT) detector.

The dried biomass and a KBr (Potassium Bromide) powder were mixed, keeping a culture weight percentage of 0.7%. The instrument was allowed to purge for 20 min before the scan was obtained. This ensured minimum background noises in the scan. The mixture of cells and KBr was homogenized using a mortar and pestle and then scanned under the following conditions: 20 kHz speed, 5 filter, 4 cm^{-1} resolution, 8 sensitivity and 128 scans. Background was obtained with a scan of pure KBr powder and was automatically subtracted from the sample spectra by the software. The wavenumber was used to determine the functional group shown on the scan, absorption correlates to the abundance of the measured chemical bond. Pure magnetite was run as a control.

3.3 RESULTS AND DISCUSSION

To better understand the capacity of magnetite as a disinfectant, river water samples from the North Saskatchewan were treated with magnetite. To further optimize the removal process three model bacteria were chosen to understand the effect of magnetite ratios, buffers, pH, mixing and contact time on the process. Finally, tests were used to determine the surface functional groups that may play a role in the adsorption mechanism.

3.3.1 TREATMENT OF RIVER WATER WITH MAGNETITE

a. Turbidity Control with Magnetite

Control of turbidity from North Saskatchewan River water was the first step taken to compare the efficiency of magnetite to current day turbidity control measures such as flocculation by coagulant and polymer. Colloidal organic matter is generally negatively charged in natural water (Crittenden et al, 2005). On the basis of which it was hypothesized that if magnetite is positively charged at a pH below its PZC (6.5 to 7) it will effectively be able to electrostatically adsorb colloidal clay in natural water. The coagulant and polymer used for these comparisons were alum (Commercial grade, see section 3.2.11) and polyacrylamide respectively. Alum and polymer flocculation is one of the most widely used methods for turbidity control (Crittenden et al, 2005).

The turbidity of the river water samples was measured for before and after treatment with magnetite. The positive control used for this experiment

was river water alone, which had an initial turbidity of 115.2 NTU (Nephelometric Turbidity Units) at room temperature. The negative control used was deionized water alone, which had a turbidity of 0.01 NTU (99.99%).

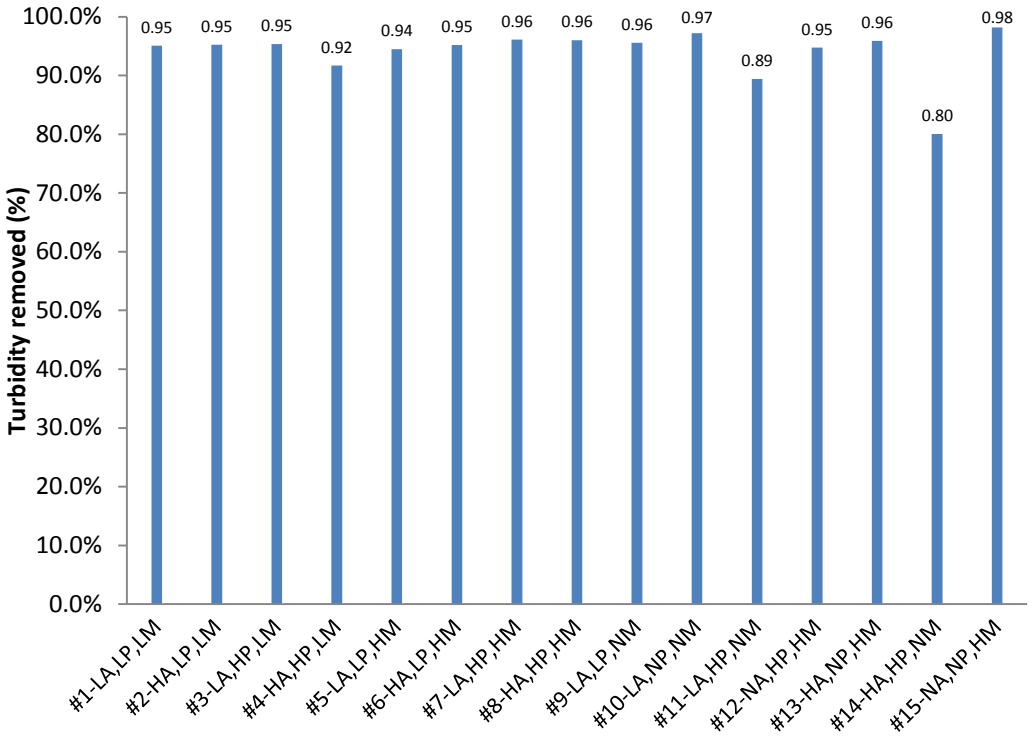

Figure 3.2: Percent turbidity removed based on different combinations of Alum, Polyacrylamide and Magnetite (refer to Table 3.1). L = Lower limit concentration of chemical used, H = Higher limit concentration of chemical used, N = No chemical used, A = Alum, P = Polyacrylamide, M = Magnetite and #X = combination number. E.g. #15 NA, NP, HM means that no alum, no polyacrylamide, Higher limit (300mg/l) magnetite was used for the run. ph of the sample was adjusted from 8 to 5.6. Mixing times for all combinations were 10 mins, with the exception of samples where magnetite is not used the flocs were allowed to settle for 20 mins after mixing (Total time of 30 mins).

The removal efficiency of magnetite alone (#15 in Figure 3.2) was found to be highest at 98% among all the other combinations tested. Alum alone at a concentration of 10mg/l (#10) is very close at 97% removal. Combinations 1 through 4 use lower level limit of magnetite (100mg/l) and different concentration combinations of polyacrylamide and alum. The turbidity removed

from 1 to 3 is 95% and about 92% for combination #4. The difference of removal efficiency between combination #4 at 92% and #14 at 80% is most probably because of the addition of magnetite. The test runs #5 through #8 consistently removed up to 96% of turbidity. Test runs 9, 10, 11 and 14 have only the different combinations of alum and polyacrylamide and have 96%, 97%, 89% and 80% removal, with 80% being the lowest out of all the combinations used.

Table 3.2: Results from Regression analysis on results obtained using MS Excel, 2010. Grey cell highlights the P value of magnetite as a significant factor (P < 0.05).

Regression Statistics		Factor	P-value
Multiple R	0.923	Alum	0.365
R Square	0.851	Polyacrylamide	0.133
Significance F	0.093	Magnetite	0.075

Regression analysis reveal that magnetite has no significant difference (P=0.075, P>0.05, see table 3.1) from the other factors tested but the time taken for removal of turbidity using magnetite was 10 minutes compared to 30 minutes for alum and polyacrylamide combinations. In table 3.1, the intercept explains the statistically that the effect of magnetite is not significant (P = 0.075, P>0.05). The other values such as Multiple R and R square explain the accuracy of the regression analysis. R square 0.85, means that the regression analysis has an accuracy of 85%. Significance F parameter means that the probability of predicting the wrong P-value is 9.3%, at 95% confidence. This means that the overall accuracy of the regression analysis is 93%.

The above results closely confirm previous studies where 99.63% of turbidity control from Yarra River, Victoria, Australia (raw water turbidity in excess of 190 NTU) was achieved by using 10000mg/l of pretreated magnetite (Anderson et al, 1981). Previous studies used a very high concentration of pretreated magnetite (0.5 to 2% v/v = 5000mg/l to 20000mg/l) to achieve more than 99% of turbidity control (Kolarik et al, 1983). Magnetite concentration used in this study ranges from 100 mg/l to 300 mg/l, achieving a maximum efficiency of 98% at 300 mg/l magnetite.

This suggests that the efficiency of magnetite is highest compared to the other treatment methods. The difference to look at is the time used for treatment of river water – magnetite (#15) = 10 mins, whereas alum (#10) = 30 mins. The time taken for alum to treat the same sample was three times higher relative to time taken by magnetite to achieve the same efficiency of 98%. The removal efficiency of polyacrylamide alone (#10) is around 90% and is lower than the rest of the combinations. Interesting point to note is the difference of removal efficiency between the combined effect of alum + magnetite (#12 = 95%) and polyacrylamide + magnetite (#13 = 96%). There is almost no difference, which suggests that magnetite reacts faster than the chemicals itself.

3.3.2 ADSORPTION OF BACTERIAL CELLS

The removal of bacterial cells by adsorption was first observed by plating river water samples treated with magnetite. The samples were plated on

nutrient agar (100 µl on a petridish) under aerobic conditions at 37°C and visual observation was made. To confirm this observation a preliminary experiment was carried out with a pure culture, *P. putida* ATCC® 17453, inoculated into the river water sample at approximately 10^8cells/ml. The same conditions as above were used: pH = 5.6, mixing time = 10 mins, and magnetite concentration of 300 mg/l. A removal efficiency of 1.12 logs (approx. 93%) was observed for *P. putida* ATCC® 17453 (Figure 3.3). There was no published data available which confirmed the adsorption of this particular strain (ATCC 17453) on the surface of magnetite. There were no studies conducted to check the toxicity of magnetite on bacterial cells, hence there is a possibility of a producing a false negative or a false positive in the results. So then adsorption of bacterial cells on the surface of magnetite was studied in detail.

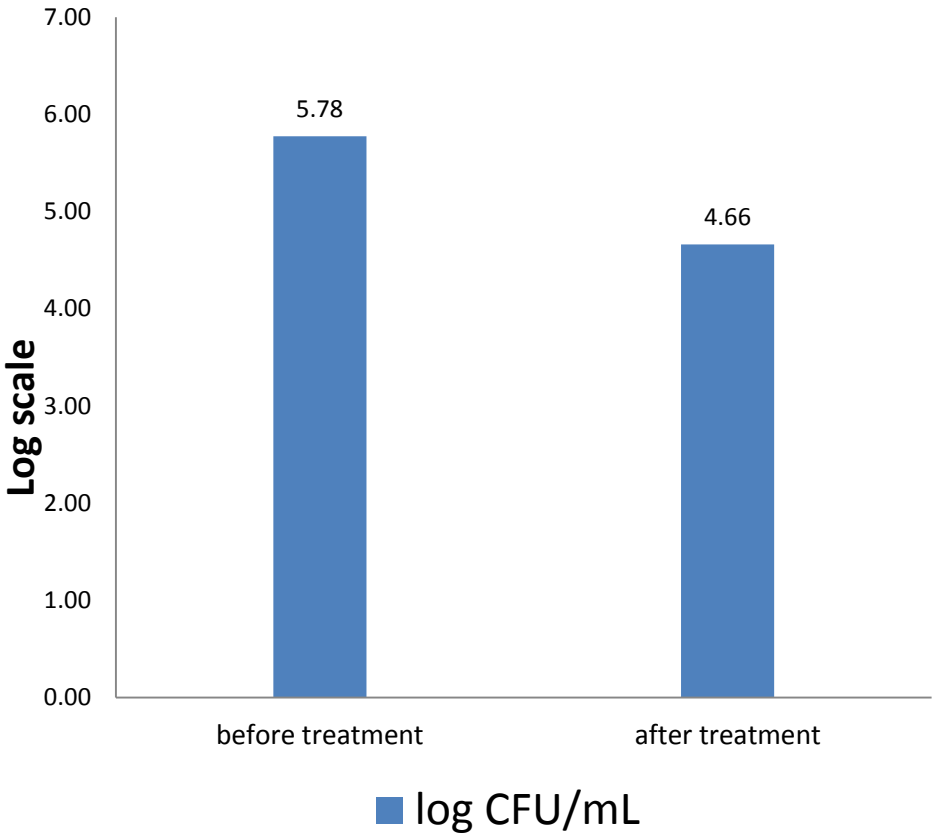

Figure 3.3: Histogram showing average values with one standard deviation for log removal of *P. putida* at pH = 5.6, mixing time = 10 mins, and magnetite concentration of 300 mg/l.

To better elucidate and optimize the capability of magnetite to adsorb bacteria the effect of magnetite concentration (cell : magnetite ratios), buffers, pH, mixing/contact time and different bacteria types was tested.

a. Optimization of Magnetite Performance

Results from the preliminary experiment were very interesting and led to the research objectives listed in chapter 1. To further look into the ability of magnetite to adsorb microorganisms, three model bacteria were chosen. The model bacteria chosen were based on their size, surface characteristics, and type of group (Gram negative or Gram positive). The three model bacteria chosen

were: *Pseudomonas putida* ATCC® 17453™, *Escherichia coli* ATCC® 25922™, and *Micrococcus luteus* ATCC® 4698™.

E. coli and *P. putida* are Gram negative type of bacteria. They are rod shaped with a size of about 2/1.7 µm in length and 1/0.7 µm in diameter respectively (ATCC). Both the bacterial strains have aerobic metabolism, are motile and are able to grow on a wide variety of organic substances (ATCC). On the other hand, *M. luteus* is a Gram positive type of bacteria, has facultative metabolism, is spherical in shape with a typical diameter of 2 µm, very low motility, and can grow in a wide variety of organic substances (ATCC).

One of the first steps to optimize the use of magnetite was to check the effect of changing concentration of magnetite with respect to bacteria.

b. Effect of Varying the Cell : Magnetite Ratio

This particular experiment was carried out to find out the amount of magnetite required for the adsorption of bacterial cells in a non-buffered 0.85% by Wt. saline solution (Figure 3.4). The positive control used for this experiment was settling by magnetic field alone was 0.3%, 1.1% and 2.8% (mean value reported) for *E. coli, P. putida* and *M. luteus* respectively. Negative control was used to check if magnetite is not contaminated in any way, the values of which were 0% for all the three cultures. The controls show a 2.8% removal under the influence of a magnetic field, which suggests that the species (*M. luteus*) could have been affected by the magnetic field applied. Studies show that applied

static magnetic field creates a dissociation of ion protein complexes on the surface of bacteria leading to an anti-bacterial effect (Binhi et al, 2001).

Conditions for the experiment were kept very similar to the preliminary experiment with the exception of pH which was neutral (7.5) in this case. The suspending solution used was Standard 0.85% by Wt. saline with a mixing time of 10 mins at 200 RPM at room temperature.

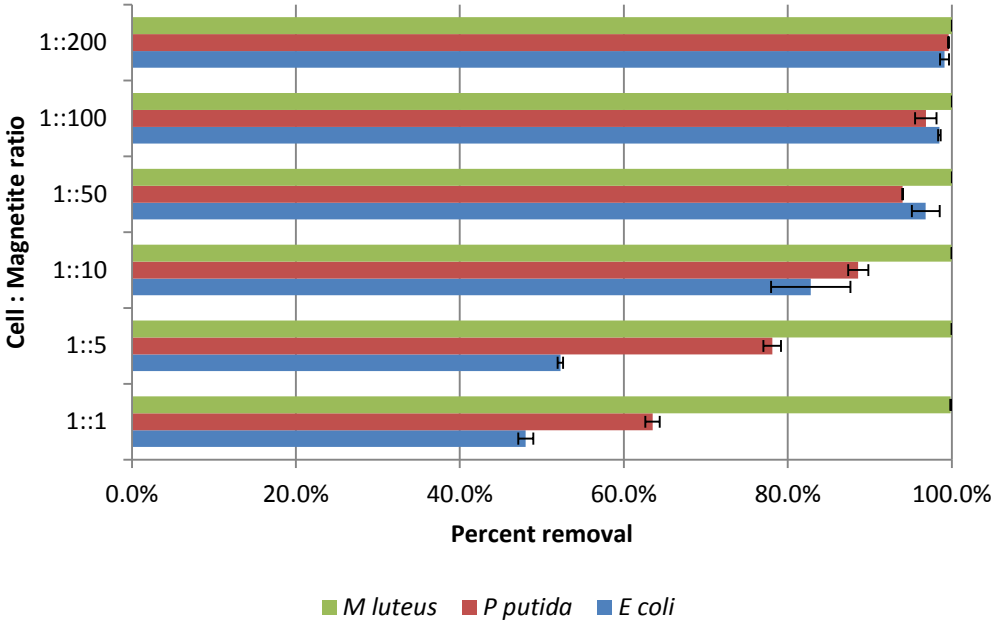

Figure 3.4: Effect of magnetite concentration as a function of percentage removal of *M. luteus, P. putida* and *E. coli* by adsorption to magnetite in 0.85% saline at pH 7.5. Values are average of triplicates with +/- one standard deviation.

It is worth noticing that the concentration of magnetite is somewhat proportional to the adsorption of *E. coli* and *P. putida*. The increase in percent removal is from 50% to 100% for the cell : magnetite ratio increasing from 1:1 to 1:200 follows a trend (approximation). Approximately the increase in percent removal came out to be about .25 percent increase per 1 unit increase in

concentration of magnetite. The removal efficiency of *M. luteus* is very high compared to the two other strains reaching above 99% removal for all conditions tested. The adsorption capacity plateaus all the way from cell : magnetite ratio 1:1 to 1:200, and no reasonable proportionality relationship is observed. From cell : magnetite ratio 1:1 to 1:5 there is no huge difference observed in the removal efficiency of *E. coli*, but for *P. putida* there is a 15% increase. On the other hand percent removal for *M. luteus* is 99% and is very high comparatively. From cell : magnetite ratio 1:10 to 1:200 the adsorption percentage continues to increase from 99% and reaches a maximum of 100%. One important thing to note is that the adsorption percentage of *M. luteus* does not change too much, it remains in excess of 99% at 1:1 ratio and is 99.9% at the highest cell : magnetite ratio of 1:200. The removal of *P. putida* is more than *E. coli* at lower doses of magnetite (upto 1:10 ratio) but then removal of *E. coli* increases slightly at higher magnetite doses (1:50 and above ratio). Such little changes in removal efficiency could have been due to an error in weighing magnetite or an error in timing the contact time of magnetite with bacterial cells, and therefore was ignored.

There are no previous studies which have checked the adsorption capability of magnetite for the three particular strains used in this study. Studies conducted on some other species (Table 2.4, chapter 2) indicate 98.07% adsorption of *Escherichia coli* using 4% v/v magnetite and a pH of 4.0 (MacRae, 1981). This concentration of magnetite is roughly equal to (assume density of water = 1000 kg/m^3) 40 gm/l, which is very high compared to the concentrations

tested in this study. The conversion for concentrations from cell : magnetite ratio of 1:50 is roughly 44 mg/l (SG = 5.1) and at the highest cell : magnetite ratio of 1:200 being roughly about 180 mg/l. There is no pH adjustment in this experiment and the pH was measured to be 7.5, which is a contrary point as it is very close to the PZC (6.5-7) of magnetite.

Bitton et al (1973) conducted experiments to study the adsorption of *E. coli* bacteriophage T_7 and reported 99% removal at a magnetite concentration of 500 mg/l and a mixing time of 30 mins at 100 RPM. Even at 500 mg/l, the amount of magnetite used for adsorption of *E. coli* bacteriophage T_7 is much greater than the highest cell : magnetite ratio of 1:200 (approx. 180 mg/l for *E. coli*).

Previous studies which reported the use of magnetite to adsorb microorganisms were conducted at a pH between 4 to 5 (MacRae et al, 1981; Bitton et al, 1973). The experiments in the present study were conducted at a pH of 7.5, which is very close to the point of zero charge (PZC) of magnetite (Ranges between 6.5 to 7.0, average value considered 6.7). Based on this information electrostatic interaction between magnetite and bacteria will be of a very low magnitude, and therefore will likely not be a dominant mechanism for adsorption. For this reason some additional work was completed to determine the mechanisms for adsorption.

The cell : magnetite ratio of 1:50 was chosen for further experiments as the removal percentage for all three strains at this particular ratio was above 90% (Figure 3.3). Any higher than this ratio the amount of magnetite required for treatment will be doubled for a very little increase in efficiency, which makes it an economically unviable solution.

c. Effect of Buffers on Adsorption to Magnetite

The effect of buffers was tested to check if the buffers (phosphate buffers) interfered with the adsorption process. The same test conditions were used as were used for the non-buffered solution of standard saline, with the exception of buffering the pH at 7.5 using phosphate buffers. There was very little difference found in the removal efficiency of magnetite between phosphate buffered saline (PBS) and standard saline (Figure 3.5, 3.6, 3.7). The addition of buffers decreased the capability of magnetite for all three bacterial cultures.

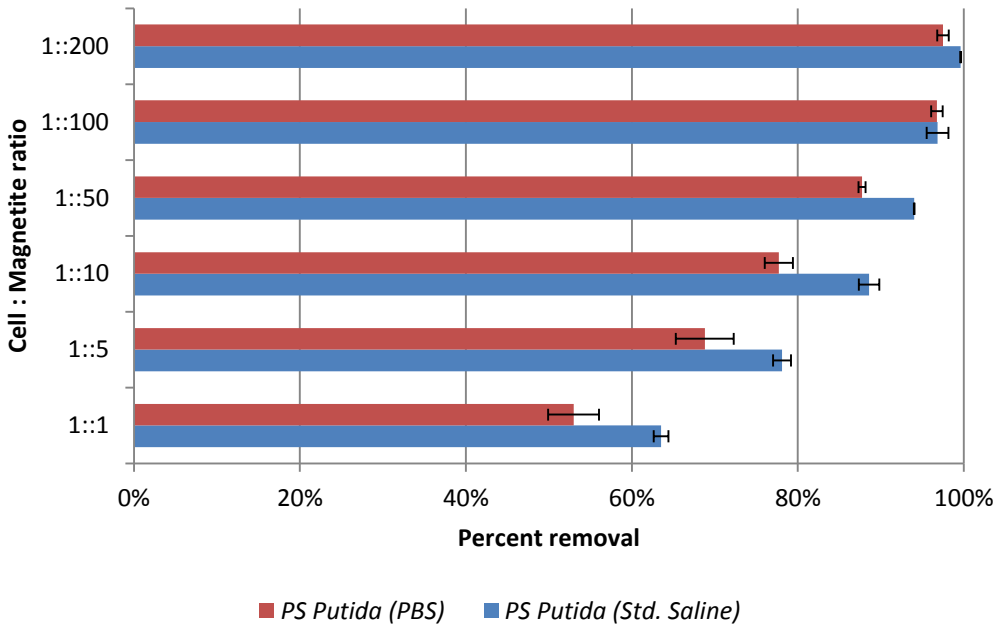

Figure 3.5: Comparison of percent removal of *P. putida* in phosphate buffered saline (PBS) and standard saline at a pH of 7.5. Values are average of triplicates with +/- one standard deviation.

The percentage of adsorbed *P. putida* cells (Figure 3.5) in the presence of buffers is lesser (by 10%) at lower magnetite doses (upto a cell : magnetite ratio of 1:50), equalizes at 1:100 and above with a little difference (of 2%) at 1:200. *E. coli* cells also show a very similar trend of decreasing percentage of adsorbed cells. From magnetite concentrations 1:1, 1:5, 1:10, 1:50, 1:100 and 1:200 the effect of phosphate buffers decreases the amount of adsorbed cells by 8, 5, 2, 6, 3 and 2 percent (average values in triplicates with one standard deviation) respectively. *M. luteus* cells also have decreased number of adsorbed cells (less by 8%) at a magnetite concentration of 1:1. The difference (between percent adsorbed cells in PBS and 0.85% saline) decreases as the concentration of magnetite increases.

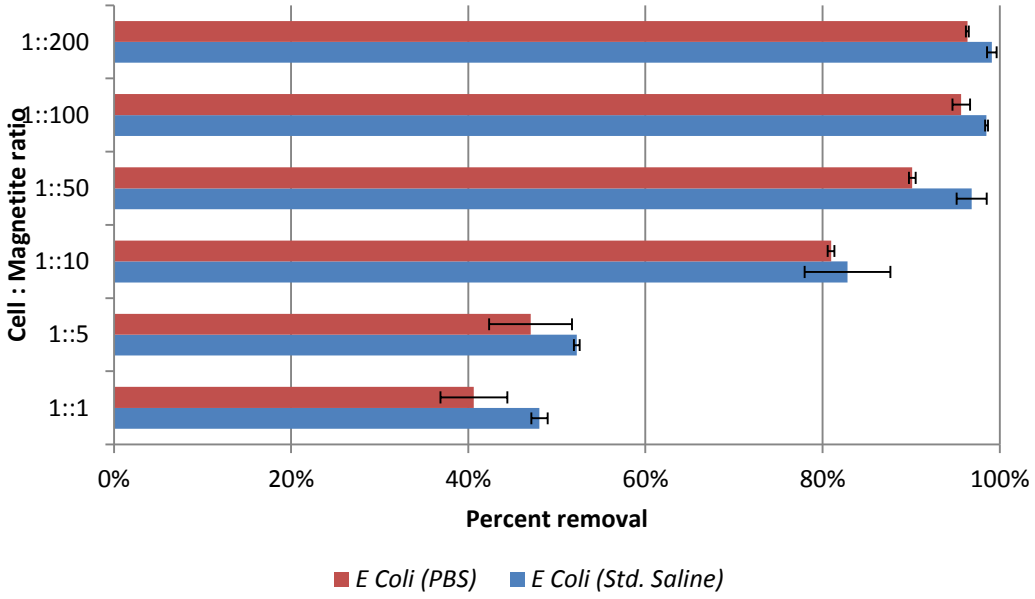

Figure 3.6: Comparison of percent removal of *E. coli* in phosphate buffered saline (PBS) and standard saline at a pH of 7.5. Values are average of triplicates with +/- one standard deviation.

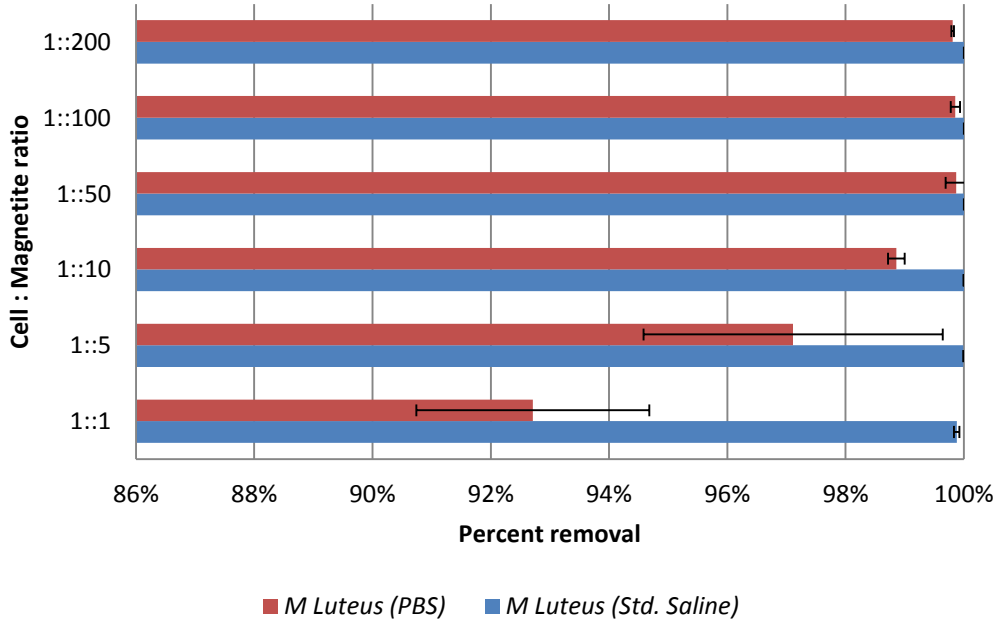

Figure 3.7: Comparison of percent removal of *M. luteus* in phosphate buffered saline (PBS) and standard saline at a pH of 7.5. Values are average of triplicates with +/- one standard deviation.

These observations led to a confirmation that PBS might have an interfering effect on the adsorption of Gram negative and Gram positive cells. The overall removal efficiency decreased due to the addition of an external buffer. Figure 3.8 compares the differences between the three bacterial cultures in PBS. *P. putida* is removed more than *E. coli* in general with the exception of cell : magnetite ratios 1:10 and 1:50, which is very similar to the adsorption of the same cells in 0.85% saline.

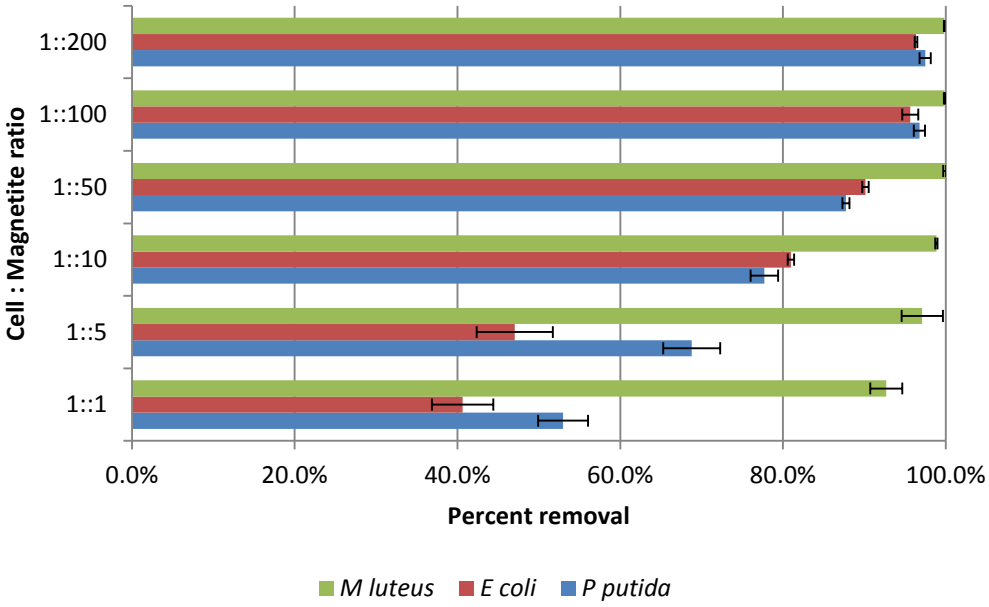

Figure 3.8: Comparison of percentage removal of *M. luteus, P. putida* and *E. coli* by adsorption to magnetite. Y – Axis = Cell : magnetite ratio and X – Axis = Percent removal. Test conditions: pH = 7.5, Solution used = phosphate buffered saline, mixing time = 10 mins at room temperature. Values are average of triplicates with +/- one standard deviation.

Some of the key differences to note would be the adsorption percentage of *M. luteus*, which has come down from 99% to 92%. Although this dip in the removal efficiency is not large, but it suggests that chemical interaction might play an important role in the adsorption mechanism of bacterial cells. Another

thing to note between Figure 3.3 and Figure 3.4 is that the Gram positive cells (*M. luteus*) are more efficiently removed when compared to the Gram negative cells tested.

There is no data to compare the effect of phosphate buffers on the adsorption of microorganisms, but the effect of cations such as Ca^{2+} and Mg^{2+} has been tested before (MacRae, 1985; Bitton et al, 1973). The reason to compare buffers with cations is that buffers also contain ions in water (HPO_4^{2-}, H^+, $H_2PO_4^-$) and provide alkalinity which gives the solution its buffering capacity. The presence of Ca^{2+} and Mg^{2+} decreases the removal efficiency of magnetite for microorganisms such as *E. coli* bacteriophage T_7 and *E. coli* probably due to bridging of the (divalent or trivalent) cations to magnetite particles (MacRae, 1985; Bitton et al, 1973). The effect of buffers was similar to cations as the overall removal efficiency of magnetite decreased with the addition of buffers.

This particular observation led to reason that chemical interaction at the surface could be one of the forms of adsorption. Phosphate buffers could be a form of interference to the process of adsorption taking place. To reason the previous sentence it was necessary to check the effect of pH to check if there is any electrostatic interaction between magnetite and bacteria

d. Effect of pH

To confirm the degree of electrostatic interaction between magnetite and bacterial cells, the effect of pH was studied as well. The hypothesis here is

that, 'if electrostatic potential is a dominant mechanism in the adsorption process, then the adsorption capacity will drastically drop when magnetite goes above its PZC (Figure 3.9). An experiment was carried out at a cell : magnetite concentration of 1 : 50, and the range of pH chosen was from 5 to 8. This specific range was chosen as it is the most common range to work in during the treatment of natural waters. PH of the solutions was buffered using phosphate buffers. The actual values of the four pH point were measured as 5.62, 5.92, 6.94 and 8.24 at 97% slope on the pH meter (Accumet Research, AR50). Other test parameters: mixing time = 10 mins at 200 RPM at room temperature.

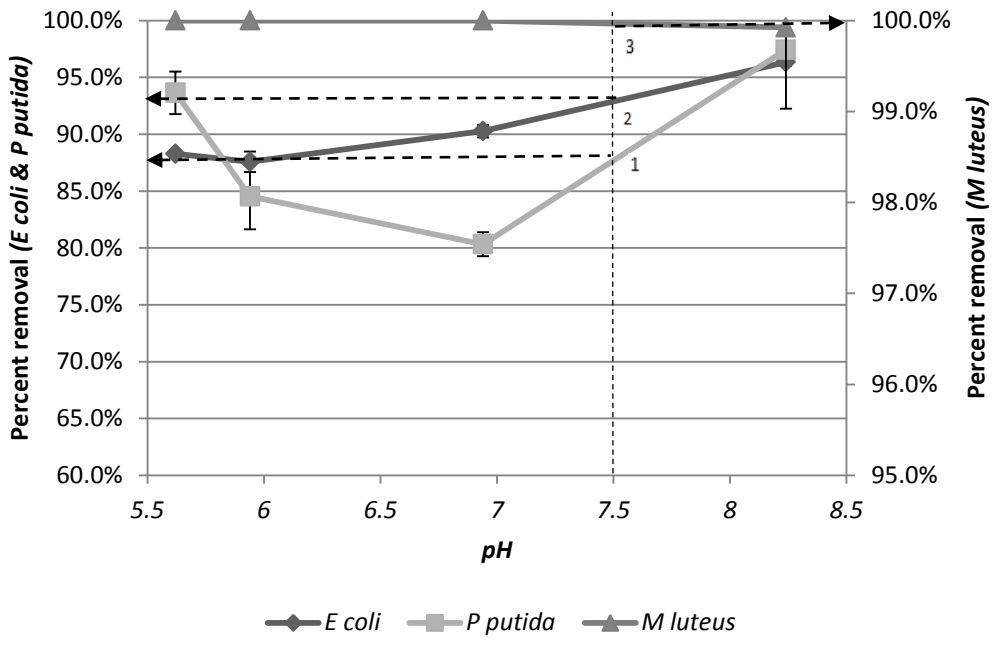

Figure 3.9: Effect of pH on adsorption capacity of magnetite at cell : magnetite ratio of 1 : 50. Left hand Y – axis = Percentage removal of *E. coli* and *P. putida*, X – axis = ph (buffered at 5.5, 6.0, 7.0 and 8.0) and right hand Y – axis = percentage removal of *M. luteus*. Values are average of triplicates with +/- one standard deviation.

The results from this experiment concluded that electrostatic interaction between magnetite and bacterial cells is not the dominant mechanism. The

removal efficiency for *M. luteus* (Δ) dropped by very little, from almost a 100% to >99.5% (Right hand Y axis, Figure 3.9) over the whole range of pH tested. The Gram negative cells (□ and ◊) had a different trend when compared to the Gram positive (Δ) cells. The percent removal of *P. putida* decreases up to pH 7 and then starts to increase and reaches a maximum of 97% at pH 8.0. *E. coli* followed a similar trend but the increase in percent removal starts from a pH 6.0 reaching a maximum of 96% at pH 8.0.

The most interesting observation to note here was the removal efficiency at pH 7.5 (points 1, 2 and 3 marked on dotted line). In fact, this led us to believe that chemical interaction between the two particles (cells and magnetite) is a likely mechanism for removal. The most probable answer is that interactions of surface functional groups on bacterial cells are reacting with magnetite to form some type of complex. This might be a case of a reversible chemical adsorption. To reason the formation of complexes it was thought that if the mixing times for magnetite and cells are changed the complexation would be of greater extent, in turn increasing the efficiency of magnetite. Therefore a test was run to check if changing the mixing time made any difference in the adsorption of cells.

e. Effect of Mixing/Contact Time on Adsorption to Magnetite

To check for the effect of mixing time, a time range of 1 to 20 mins was chosen. The sampling interval was set lower around the 10 minute mark. The reason for this being like that was, that 10 mins of mixing time was being used

for all experiments where magnetite was used. The close sampling interval also served as a reference mark and could be used to compare to the previous results.

Sampling intervals chosen were at 0, 2, 5, 8, 10, 12, 14, 17 and 20 mins (Figure 3.10, 3.11, and 3.12). The pH of further experiments was chosen to be at 7.5 as there was no significant change found in the efficiency of magnetite. This minimized the use of chemicals from an application point of view, as most natural waters are at a neutral pH of 7.5. The sampling interval was kept same for all the three strains to maintain consistency.

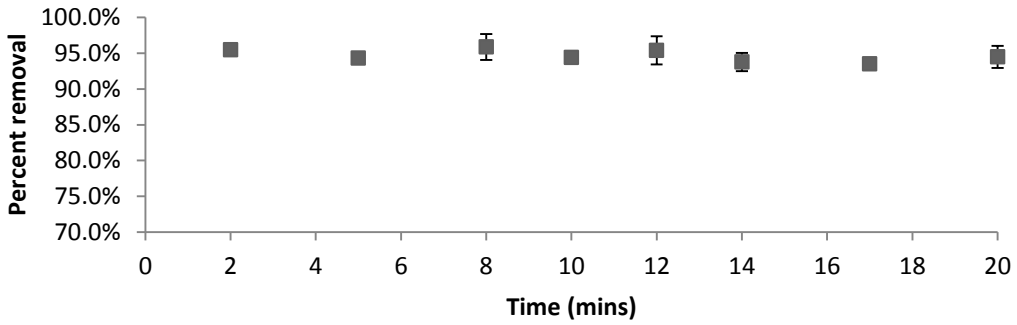

Figure 3.10: The effect of mixing/contact time between magnetite and bacterial cells (*P. putida*) over a range of 0 to 20 mins. Test conditions: pH = 7.5 buffered using phosphate buffers, mixed at 200 RPM over 0, 2, 5, 8, 10, 12, 14, 17 and 20 minute time intervals. Values are average of triplicates with +/- one standard deviation.

The difference in the removal percentage is very small over the whole range of mixing time tested. This concluded that adsorption took place in the first 2 mins of contact between magnetite and bacterial cells. The trend is very similar to Gram positive bacteria (Figure 3.6), where there is almost no significant difference between the removal efficiency of Gram negative and Gram positive cells (*P. putida* and *M. luteus*, Figure 3.10 and 3.11).

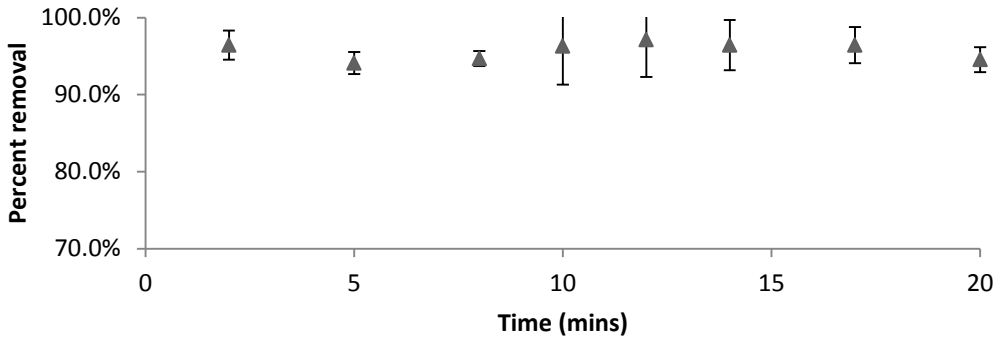

Figure 3.11: The effect of mixing/contact time between magnetite and bacterial cells (*M. luteus*) over a range of 0 to 20 mins. Test conditions: pH = 7.5 buffered using phosphate buffers, mixed at 200 RPM over 0, 2, 5, 8, 10, 12, 14, 17 and 20 minute time intervals. Values are average of triplicates with +/- one standard deviation.

The same was the case with *E. coli* (Figure 3.12). Very little difference in removal efficiency for *E. coli* was found when compared to the other two model bacteria (*P. putida* and *M. luteus*). The reason for small fluctuation in data (marked in an oval, Figure 3.12) is unknown, and is attributed to an error in weighing magnetite at ratio 1 : 50 for each sample. But the overall trend (Figure 3.12) is very similar to the other model bacteria tested.

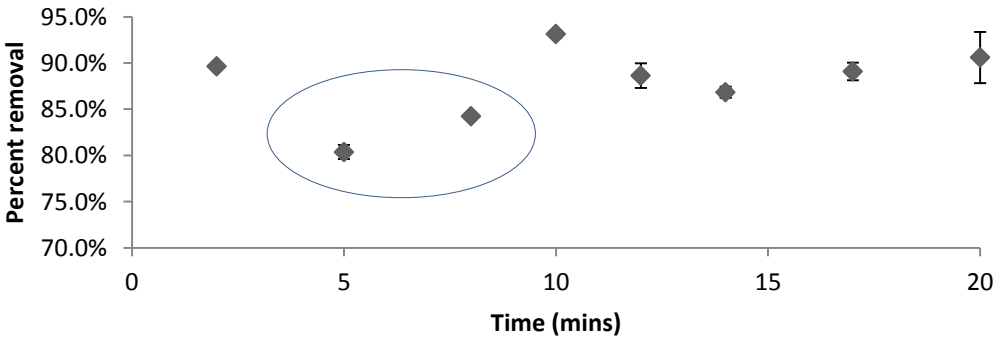

Figure 3.12: The effect of mixing/contact time between magnetite and bacterial cells (*E. coli*) over a range of 0 to 20 mins. Test conditions: pH = 7.5 buffered using phosphate buffers, mixed at 200 RPM over 0, 2, 5, 8, 10, 12, 14, 17 and 20 minute time intervals. Values are average of triplicates with +/- one standard deviation.

The effect of mixing/contact time was successfully studied and proved that the time of contact has very little or no significant impact on the adsorption

capability of magnetite. These observations made it necessary to check which functional group could possibly interact with magnetite. To find confirmatory evidence, a small experiment was thus conducted to check for the chemical behavior of magnetite crystal in water.

3.3.3 WATER AND MAGNETITE INTERFACE STUDY

Literature suggests that magnetite has an affinity for hydroxyl groups in solution (Jung et al, 2010). There are two competing theories which explain the ability of magnetite to bind with hydroxyl groups. According to one of them magnetite replaces one oxygen atom in its octahedral lattice to an external hydroxyl group (Roonasi, 2007). According to this theory, out of the 32 oxygen occupied sites in the structure of magnetite crystal there is partial oxidation of some iron oxygen double bonds, when adsorption of hydroxyl groups takes place, by the release of oxygen rich molecules. This means that magnetite is partially reduced from wustite (FeO, section 2.3) to iron (Roonasi, 2007). Thermogravimetric analysis done in this study reveals loss of weight in the magnetite crystal, showing evidence that magnetite crystal replaces an oxygen atom for an external hydroxyl group.

The second theory however states that dissociative adsorption is more likely taking place on the surface of magnetite (Parkinson et al, 2011). According to this theory each adsorbing hydroxyl species occurs on the vacant oxygen sites on the surface of magnetite (Parkinson et al, 2011). In both cases however there

is release of oxygen in the solution. According to Parkinson et al (2011) there is splitting of water at the magnetite surface by interaction with hydroxyl species possibly via the creation of O_2 (Parkinson et al, 2011). Both theories have confirmatory evidence of release of oxygen when magnetite is added to water. This suggested that interaction of bacteria with the surface of magnetite could be of a similar nature. Bacteria have numerous types of functional groups on their surface (section 3.3.3a). To confirm the affinity of magnetite for hydroxyl species, a preliminary experiment was run. To test the release of O_2 and the adsorption of hydroxyl groups on the surface of magnetite, color adsorption from green tea as a preliminary experiment was tested (Figure 3.13).

Green tea is a rich constituent of polyphenols (chosen because of the abundance of hydroxyl group), the color in green tea is derived from Tannin (type of polyphenol) which is rich in hydroxyl groups. The results from this preliminary experiment were as expected (Figure 3.13). The experiment proved that there is interaction between hydroxyl groups (in this case polyphenols) and magnetite and is of a very strong nature. The adsorbed hydroxyl groups (in the form of color) were released when 0.1M NaOH was added to the settled magnetite (after the supernatant had been decanted). A very high proportion of the color was suspended back in the solution.

This preliminary experiment led to designing a new experiment to quantify and explain the degree of affinity for hydroxyl groups. A solution of

Tannic acid ($C_{76}H_{52}O_{46}$) was used this time. The reason to choose tannic acid was that it is a type of polyphenol, and has a very good solubility, 1 gm in 0.35 ml of water (Halkens et al, 2001). The removal of tannic acid color would correspond to the adsorption of hydroxyl groups.

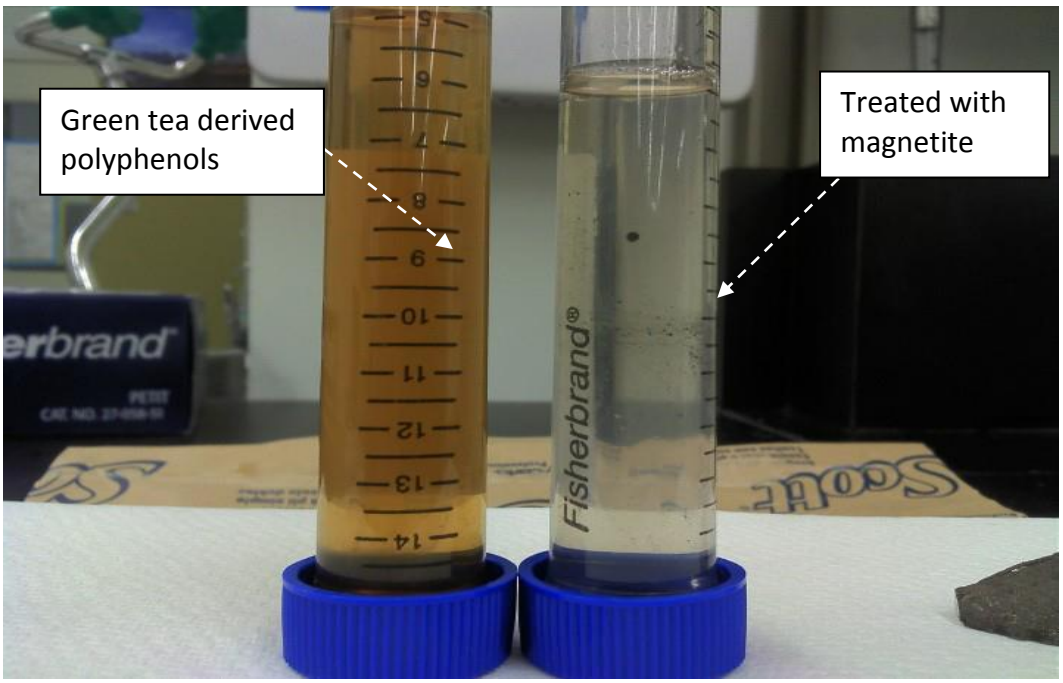

Figure 3.13: A picture showing the difference between original tea sample (on the left) and the treated tea sample (on the right). Test conditions: Magnetite = 300 mg/l, pH = 7.5, mixing time = 10 mins at 200 RPM at room temperature.

Test conditions for this particular experiment were fairly simple. The pH of solutions was 7.5 in a non-buffered, non-saline (no salt added), deionized water solution. A stock solution of tannic acid at a concentration of 1000 mg/l was made. Solution was allowed to equilibrate with room temperature for 30 mins. Controls for this experiment were (1) Deionized water alone (negative control) and (2) Tannic acid solution (Positive control). To quantify the removal

of color a standard curve using multiple dilutions of the stock tannic acid solution was made (see Appendix E).

On conducting this experiment it was found that magnetite on contact with deionized water increases the dissolved oxygen content in water. Each gram of magnetite increased the O_2 content by 1.0834 mg. This phenomenon was not observed with any of the tannic acid solutions and neither was any color removal (0%) observed. This experiment supports the claims made in the second theory, which states that hydroxyl species get adsorbed on the surface of magnetite. If the first theory were true then magnetite would have gotten reduced from wustite to iron, and tannic acid in the solution phase would have been adsorbed. There was no observed effect of magnetite on tannic acid.

The release of oxygen in water could be possibly because of two reasons: (1) the water molecule is being dissociated on the surface of magnetite or (2) the magnetite crystal replaces an oxygen atom to form a hydroxylated layer on its surface by some type of complexation reaction, as discussed above in the competing theories. Claims made by some researchers are that magnetite is able to reduce the BOD (Biological oxygen demand) and phosphorous in waste-waters (Panasuik, 2010). These claims were based on the argument that magnetite adsorbs organic compounds in waste-water because of its surface charge, whereas the oxygen production would have largely remediated BOD in waste-

waters as well. The control of BOD from waste-waters by introducing oxygen is a more concrete explanation than the theory of electrostatic adsorption

The observations from all previous experiments suggest that magnetite has good capability of adsorbing bacteria (Gram negative - *E. coli* and *P. putida* and Gram positive - *M. luteus*). Earlier it was presumed that bacterial surface is negatively charged and magnetite surface is positively charged and hence electrostatic adsorption takes place. But due to the fact that the point of zero charge for magnetite is within a range of 6.5 to 7.0 (Cornell et al, 2006), the degree of electrostatic interaction is very small. Although there will be change in the surface density charge with change in pH of the sample. As observed in the experiments which test the effect of pH on the adsorption of microorganisms, the surface charge density of bacteria would have also changed as a function of pH.

Due to the huge complexity of the possible removal mechanism, there is still no concrete evidence or theory that explains the type of interaction between magnetite and bacterial species. Bacterial surface is very complex in nature which makes it hard to pin point the most probable adsorption mechanism.

These developments led to look for hydroxyl functional groups on the surface of bacterial cells.

a. Study of Surface Functional Groups (Fourier Transform Infrared Spectroscopy)

Fourier Transform Infrared Spectroscopy (FTIR) was conducted for all the three model bacteria used previously. An FTIR spectrum is a popular method used for detecting vibrational frequency changes to differentiate between different functional groups on surface of bacterial cells. The wavenumber (X - axis) is a very good indication of the type of functional group that might be present in the sample. The positive/negative values of absorbance (Y-axis) show a phase shift. The negative values can occur most likely due to photons escaping the sample port by reflection and affect the measurement of the reference (KBr) (Swann et al, 2010). The negative values can be attributed to instrument error because of misalignment of the sample port and detector.

Qualitative analysis of FTIR data reveals the different functional groups on the surface of the specific bacterial strains: *Pseudomonas putida* ATCC® 17453™, *Escherichia coli* ATCC® 25922™, and *Micrococcus luteus* ATCC® 4698™.

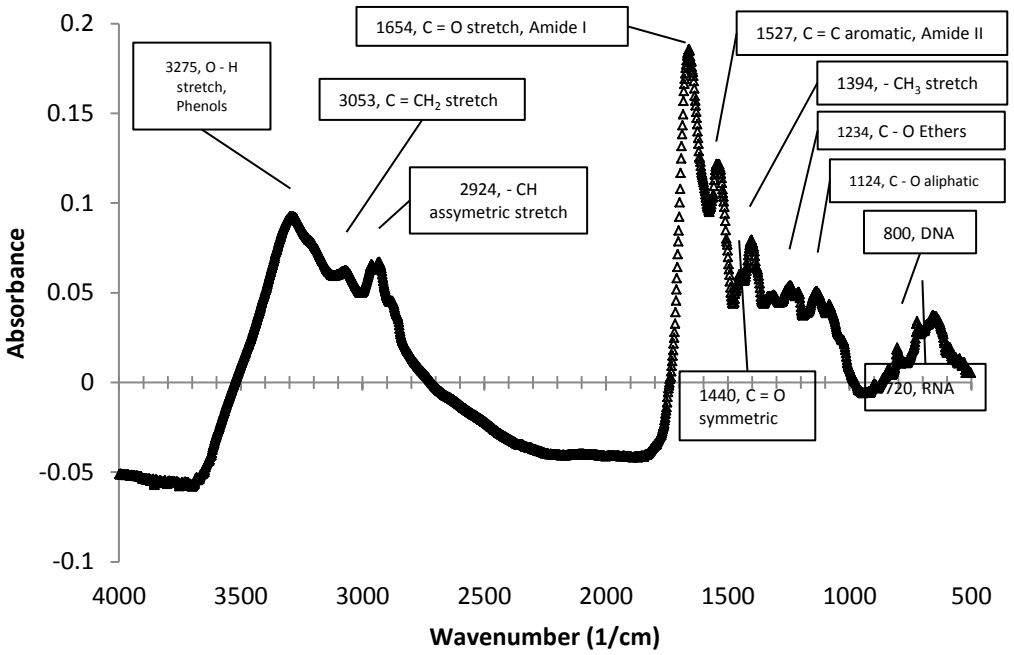

Figure 3.14: FTIR scan of freeze dried *M. luteus* (128 scans, 8 sensitivity and 4cm^{-1} resolution).

Eleven different types of functional groups were detected in the FTIR scan of *M. luteus*. The most important to discuss are the stretches of O–H groups. The reason to discuss the intensity of the O–H stretch bands is that they are most probable suspects which interact with magnetite surface. The overall O–H peak lies between wavenumber 3600 – 3000 cm^{-1}, this includes the alcohols as well as the carboxylic acids (Filip et al, 2008). The range between 3200 and 3400 cm^{-1} represents a high concentration of O–H groups in alcohols and phenols (Filip et al, 2008).

The OH group peak in the scan of Gram positive bacterial cell (Figure 3.14), *M. luteus*, is at 3275 cm^{-1}. The range value means that there is a high concentration of phenolic or alcoholic groups on the surface of *M. luteus*. On the other hand, FTIR scans for Gram negative cells, *P. putida* and *E. coli,* are not

hugely different from the scan of Gram positive bacteria. Hydroxyl species in alcohol and phenols form a wide and stable peak, 3271 cm^{-1} for *P. putida* (Figure 3.15) and 3267 cm^{-1} for *E. coli* (Figure 3.16). These scans confirm the availability of hydroxyl groups on the surface of bacterial cells. Hydroxyl groups are an integral part of the polysaccharides which form the outer layer of the cell wall, and are most probably responsible for the adsorption on the surface of magnetite.

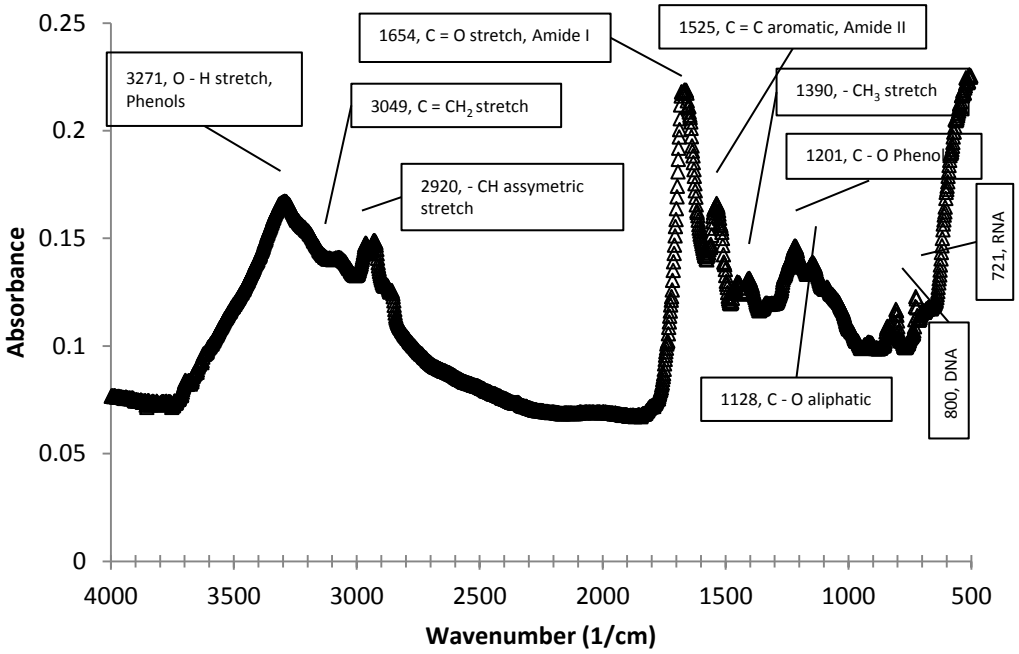

Figure 3.15: FTIR scan of freeze dried *P. putida* (128 scans, 8 sensitivity and 4cm-1 resolution).

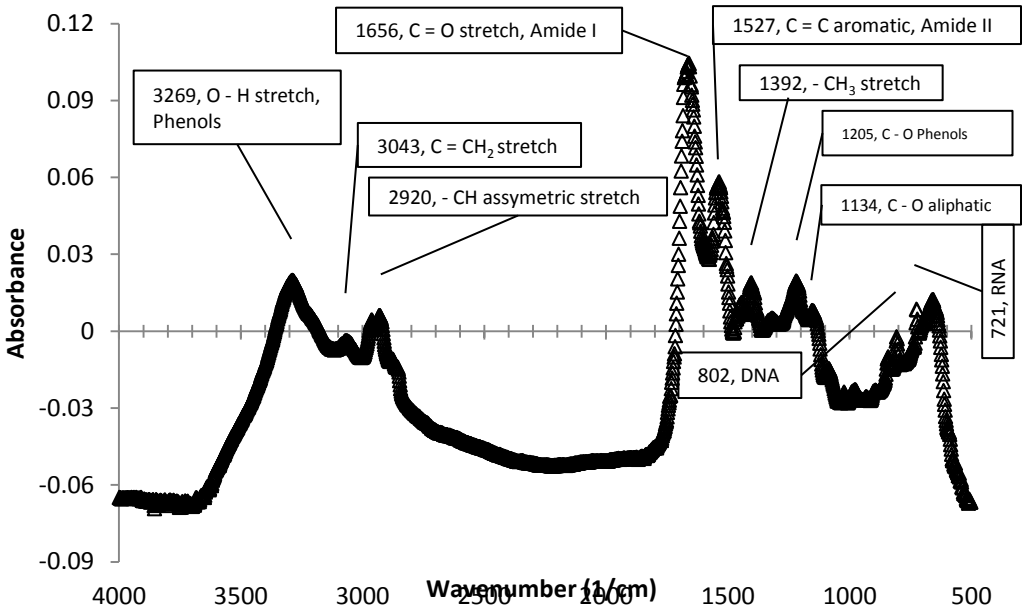

Figure 3.16: FTIR scan of freeze dried *E. coli* (128 scans, 8 sensitivity and 4cm-1 resolution).

The overall combined analysis of all the results concludes that electrostatic interaction is most likely not the dominant mechanism for the adsorption of bacterial cells. The most probable reason for the adsorption is some sort of chemical and colloidal interaction of O–H groups on cell walls (Figure 3.14, 3.15, & 3.16), and have also seen that some polyphenols are readily adsorbed when contacted with magnetite (Figure 3.13). The same type of polyphenol being desorbed when strong base is added to settles magnetite, shows that affinity to $O–H^-$ is higher that O– H group. Hence in slightly alkaline natural water, the removal efficiency of magnetite might be somewhat higher, independent of its PZC.

3.4 RESULTS ACCURACY

There are several sources or error which were observed during the experiments

- Dilution error (concentration and volume mistake)
- Equipment error (weighing balance)
- Human error
- Method error

Dilution error occurred every time when volume of solutions was being transferred. The dilution error has been identified from the standard deviation, which has been taken into account for the results displayed, and comes out to be about 0.5%. The error in weighing magnetite for experiments, in spite of being very careful, occurred sometimes. Percentage error value of which should not be more than 0.5%. The probability of having a miscalculation error is very small and can be neglected. All measures to be precise were taken.

To establish accuracy and reliability of the collected data, all samples were studied in triplicates. Regression analysis (MS Excel 2010) was used to evaluate the significance of factors (for the factorial design, section 3.2.2). Confidence level was kept 95% in all cases and data was normalized before analysis. All error bars in figures are plus and minus one standard deviation.

3.4 CONCLUSIONS

After preforming this study it can be concluded that magnetite has a very good ability to adsorb microorganisms. Magnetite has very good efficiency to adsorb the three model bacteria chosen: *Pseudomonas putida* ATCC® 17453™, *Escherichia coli* ATCC® 25922™, and *Micrococcus luteus* ATCC® 4698™. The method of adsorption of microorganisms for disinfection of water and waste-water might have a potential engineering application, as this method involves much less time for treatment.

To be able to use magnetite as a primary disinfectant, the ability of magnetite has to be tested for viruses, protozoa, and some other types of commonly found bacteria. The property of magnetite to be attracted to a permanent magnet is of great use as it reduces settling time and also the foot print of the water treatment plant, at the same time providing higher flow rates through the plant. Also the effect of temperature might be helpful in optimizing this process. Regeneration of magnetite to be efficiently used in an engineering application is also very important. The main point of using magnetite for water treatment applications is that it can be magnetically settled and then can be recycled into the same system.

The results conclude that magnetite has great potential for it to be used as a disinfectant/disinfectant aid and serves as a good alternative to full disinfection of water treatment plants.

3.5 WORKS CITED

1. Anderson, N. H., et al. "Colour and Turbidity Removal with Reusable Magnetite particles—VI: Pilot Plant Operation." Water research 17.10 (1983): 1235-43.
2. Anderson, N. J., and A. J. Priestley. "Colour and Turbidity Removal with Reusable Magnetite particles—V: Process Development." Water research 17.10 (1983): 1227-33.
3. Bitton, G., O. Pancorbo, and G. E. Gifford. "Factors Affecting the Adsorption of Polio Virus to Magnetite in Water and Waste-water." Water Research. 10.11 (1976): 973.
4. Binhi, V. N., Ye D. Alipov, and I. Ya Belyaev. "Effect of Static Magnetic Field on E. Coli Cells and Individual Rotations of Ion-Protein Complexes." Bioelectromagnetics 22.2 (2001): 79-86.
5. Bratbak, G., and I. Dundas. "Bacterial Dry Matter Content and Biomass Estimations." Applied And Environmental Microbiology 48.4 (1984): 755,755-77.
6. Carstensen, E. L., et al. "Passive Electrical Properties of Microorganisms: I. Conductivity of Escherichia Coli and Micrococcus Lysodeikticus." Biophysical journal 5.3 (1965): 289-300.
7. Cornell, R. M., and U. Schwertmann. "Magnetite." the Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses, 2nd, Completely Revised and Extended Edition. 2nd ed. United States: Wiley, 2006.

8. Crittenden, J., et al. "Turbidity." Waste-water Treatment: Principles and Design. “1st ed. United States of America: John Wiley and Sons, Inc, 2000.
9. Crittenden, J., et al. "Disinfection." Waste-water Treatment: Principles and Design.” 2nd ed. United States of America: John Wiley and Sons, Inc, 2005.
10. Filip, Zdenek, Susanne Herrmann, and Jaromir Kubat. "FT-IR Spectroscopic Characteristics of Differently Cultivated Bacillus Subtilis." Microbiological research 159.3 (2004): 257-62.
11. Girginova, Penka I., et al. "Silica Coated Magnetite Particles for Magnetic Removal of Hg2+ from Water." Journal of colloid and interface science 345.2 (2010): 234-40.
12. Halkes, S. B. A., et al. "The use of Tannic Acid in the Local Treatment of Burn Wounds: Intriguing Old and New Perspectives." Wounds 13.4 (2001): 144.
13. JT Mayo, JT, et al. "The Effect of Nanocrystalline Magnetite Size on Arsenic Removal." Science and Technology of Advanced Materials 8.1-2 (2007): 71.
14. Navratil, JD. "Mine Water Treatment using Iron Ferrites and Magnetite." Mine Water and the Environment (2008): 305,305-308.
15. Oskay, E. “Treatment of Waste-water using Magnetite.” Masters of Science, Doyuz Eylul University,Turkey, 2003 Izmir.

16. Parkinson, GS, et al. "Room Temperature Water Splitting at the Surface of Magnetite." Journal of American Chemical Society 133 (2011): 12650-5.
17. Roonasi, P. Adsorption and Surface Reaction Properties of Synthesized Magnetite Nano-Particles. PhD Luleå University of Technology, 2007 <http://pure.ltu.se/portal/files/1223719/LTU-LIC-0767-SE.pdf>.
18. Swann, G. E. A., and S. V. Patwardhan. "Application of Fourier Transform Infrared Spectroscopy (FTIR) for Assessing Biogenic Silica Sample Purity in Geochemical Analyses and Palaeoenvironmental Research." Climate of the Past discussion (CP) 6 (2010): 1629.
19. Yuan, Peng, et al. "Removal of Hexavalent Chromium [Cr(VI)] from Aqueous Solutions by the Diatomite-supported/unsupported Magnetite Nanoparticles." Journal of hazardous materials 173.1–3 (2010): 614-21.

Chapter 4: Conclusions, Recommendations and Future Work

4 CONCLUSIONS, RECOMMENDATIONS AND FUTURE WORK

4.1 CONCLUSIONS

The use of magnetite in water and waste-water treatment processes such as flocculation and disinfection could prove to be of great potential. This is important as the use of magnetite will eliminate/reduce the use of chemicals added to water and waste-water treatment systems. To better understand the capacity and mechanisms involved in removing bacteria with magnetite this research used three different types of microorganisms, namely *Escherichia coli* ATCC® 25922™, *Pseudomonas putida* ATCC® 17453™ and *Micrococcus luteus* ATCC® 4698™.

This study has confirmed with adequate evidence that magnetite can be efficiently used as a primary disinfectant/disinfectant aid for treatment of surface waters and waste-waters as well. Some of the main conclusions of this research have been summarized below:

4.1.1 TURBIDITY CONTROL

Magnetite effectively controls turbidity by adsorbing more than 98% of suspended clay particles from North Saskatchewan River water. Studies to compare magnetite performance to popular methods such as chemical coagulation and flocculation were also conducted. The removal efficiency of magnetite alone is very close to coagulation with alum alone (97%); however treatment with magnetite is 3 times faster than conventional coagulation by

alum. The settling time for alum flocs is generally 20 mins, whereas magnetite is mixed for 10 mins and was simply pulled out with the help of a permanent magnet, eliminating the requirements for a settling tank or a clarifier. The use of magnetite also reduces/eliminates any sludge production, which means that sludge handling and treatment costs will be low. At the same time higher flow rates can be achieved as treatment with magnetite does not need larger settling times.

4.1.2 ADSORPTION OF BACTERIAL CELLS

The ability of magnetite to adsorb microorganisms was successfully tested in this study. Three model microorganisms: *Escherichia coli* ATCC® 25922™, *Pseudomonas putida* ATCC® 17453™ and *Micrococcus luteus* ATCC® 4698™ were chosen for this study. The adsorption of microorganisms was studied and optimized for factors such as Magnetite concentration (Cell : Magnetite ratio), effect of buffers, pH and mixing times. The study also focused on elucidating the adsorption mechanism by studying functional groups on the surface of bacterial cells and their interaction with magnetite.

a. Effect of Varying the Cell : Magnetite Ratio

The effect of cell : magnetite ratios was studied to find out the most optimal amount of magnetite required for adsorption of microorganisms. Six different cell : magnetite ratios were chosen and they were 1 : 0, 1 : 1, 1 : 5, 1 : 10, 1 : 50, 1 : 100 and 1 : 200. Suspension solution used for this test was 0.85% sterile saline.

The cell : magnetite ratio of 1:50 was found to have more than 90% of removal efficiency for all three strains in the neutral pH range (more specifically 7.5). At 1:50 ratio, the percentage removal of Gram positive bacteria (*M. luteus*) is 99.7%, which is higher than the Gram negative bacteria: 96.8% for *E. coli* and 94.8% for *P. putida*. The ratio 1:50 was chosen as for higher ratios the amount of magnetite doubles for a very small increase in percentage removal for the three strains making it relatively expensive. The reason for high removal of Gram positive bacteria is yet unknown; therefore more experimental work is needed. This use of magnetite can significantly reduce disinfection costs when compared to present methods, such as chlorination, UV and ozonation. For example if magnetite is able to remove >90% of the bacteria, the chlorination or ozonation will be needed to remove the remaining 10% of microorganisms. This translates to saving costs of chemicals used, while also reducing issues such as disinfection byproducts and the environmental implications of the same chemicals added to the water treatment system.

f. Effect of Buffers on Adsorption to Magnetite

The effect of addition of Phosphate buffers was studied to look for any changes in the removal efficiency of magnetite. All six cell : magnetite ratios were tested again to learn any effect of buffers on the removal efficiency. It was concluded that addition of buffers decreases the removal efficiency of magnetite to adsorb microorganisms. At 1:50 ratio the removal efficiencies of *P. putida*, *E.*

coli and *M. luteus* were 87.8%, 90.1% and 99.7% respectively, which are lesser than the results found using 0.85% saline.

The overall removal efficiency at cell : magnetite ratio of 1:50 was very close to 90% for all the three cases with the addition of buffers, hence was not changed for further experiments.

b. Effect of pH

The effect of pH on the capacity of magnetite to remove the three model bacteria was also tested. The pH was tested at 5, 6, 7 and 8 using phosphate buffers. The removal efficiency for *M. luteus* at the four pH points 5, 6, 7 and 8 was 99.9%, 99.9%, 99.8% and 99.5% respectively. The removal efficiency for *E. coli* at the four pH points 5, 6, 7 and 8 was 88.3%, 87.6%, 90.3% and 96.3% respectively, but the removal percentage increases as a result of increase in pH. For *P. putida* the removal efficiency at the four pH points 5, 6, 7 and 8 was 93.6%, 84.5%, 80.3% and 97.4% respectively. The removal efficiency at neutral pH of 7.5 remains above 90% in the three strains tested and confirmed with above mentioned results.

c. Effect of mixing/contact time

The effects of mixing times were studied over a range of 20 mins at neutral pH of 7.5 using phosphate buffers. Samples were analyzed for cell count at intervals of 0, 2, 5, 8, 10, 12, 14, 17 and up to the 20^{th} minute. The data does not show any significant differences between removal efficiencies over the 20

minute time range. Experiments were run at a cell : magnetite ratio of 1:50 and was found that the percentage removal for all three model bacteria was well above 90%. For *P. putida*, *E. coli* and *M. luteus,* at the 10^{th} minute were 94%, 93.1% and 96% respectively. Reaching the highest percentage removal at the 10^{th} minute for *E. coli* (93.1%), at the 8^{th} minute for *P. putida* (96%) and at the 12^{th} minute for *M. luteus* (97%).

4.1.3 WATER AND MAGNETITE INTERFACE STUDY

Behavior of magnetite in deionized water was also tested. Each Gram of magnetite increased the dissolved oxygen content in water by 1.0834 mg. The reason for this unreported phenomenon has not been confirmed yet, but it appears that a temporary splitting of water occurs at the magnetite surface (Parkinson et al, 2011). The study conducted by Parkinson et al supports this observation where the adsorption behavior could be splitting water at room temperature at one of the reactive surfaces of the magnetite crystal. This finding has importance as it supports claims made by other researchers that magnetite is able to reduce the BOD and phosphorous in waste-waters (Panasuik, 2010). These claims were based on the argument that magnetite adsorbs organic compounds in waste-water because of its surface charge, whereas the oxygen production could have controlled BOD in waste-waters as well. The control of BOD from waste-waters by introducing oxygen is a more concrete explanation than the theory of electrostatic adsorption.

b. Study of Surface Functional Groups (Fourier Transform Infrared Spectroscopy)

To help elucidate the adsorption mechanism, experiments were conducted to check for O – H functional groups on the surface of the model bacteria cells. Fourier Transform Infrared Spectroscopy (FTIR) was performed on all three model bacteria and the scans show strong peaks for O – H groups. Interestingly, there is very little difference between the concentrations of O – H groups between the model bacteria. More evidence is required to come up with a clearer explanation for this type of adsorption. All the studies conducted to find the ability of magnetite to adsorb microorganisms show that the process of adsorption is not entirely electrostatic, but is a mix of electrostatic and chemical interaction. Largely the adsorption is attributed to chemical interaction between the surface functional groups (O–H) and magnetite surface

The work carried out in this study has confirmed that magnetite has the capacity to adsorb microorganisms and remove them from solution to the order of 95% or more. The mechanism(s) involved in the removal process have not been fully elucidated. The next section will provide recommendations that will help move this research forward.

4.2 RECOMMENDATIONS

This study had several obstacles that were not anticipated at the beginning of the work. Some improvements that could help in the experimental

design and approach to improve the work for future purposes have been summarized below:

1. A better method is needed to quantify the magnetite. A particle basis could be used where a relationship could be established to find out exactly how many particles of magnetite (by weight, mg) are required to adsorb 1 mg (dry weight) microorganisms in water. This can be established by counting the number of bacterial cells prior to addition to the solution using a flow cytometer and estimating the number of magnetite particles based on their density (assuming magnetite and bacteria particles are spherical in nature). This relationship will be able to calculate the exact amount of magnetite based on particle basis required for the adsorption of bacterial cells.

   Although, the dry weight basis is a good estimate, but does not draw attention to the formation of any lumps (in bacteria and magnetite) by aggregation. The aggregation results in lower removal efficiency as magnetite does not have enough surface area for adsorption. Surrounding magnetic fields from laboratory equipment such as shakers and motors for long periods of time can have an impact on aggregation of magnetite particles. A particle based analysis (cell : magnetite particle) would give a better idea of how much magnetite is required to precisely measure the required amounts.

2. Better quantification of magnetite's surface properties. Adequate information was available on magnetite's physical properties, but there was lack of adequate confirmatory information on the water and magnetite surface. For the specific type of magnetite used for this study, there was very little data provided by the supplier about its manufacturing process, ferric to ferrous ion ratio, and chemical properties in water. Knowing the chemical properties of magnetite, would add in understanding the adsorption behavior. For future experiments it is recommended that several chemical properties of magnetite such as water-magnetite interface, affinity for functional groups and magnetic susceptibility be determined prior to testing its use in water and waste-water treatment. Properties such as adsorption of charged species should be analyzed.
3. This research has demonstrated the capacity of magnetite to act as a disinfectant. More model bacteria such as *Salmonella Enterica*, and some *E. coli* species that harbors toxin genes and represent indication of typical pathogenic organisms found in surface water or sewage should be used for tests. This can also be extended from bacteria to other organisms such as algae, protozoa, and viruses.
4. If these additional experiments show successful removal via magnetite, then it can be suggested to develop a small bench top system to test the capability of magnetite in a flow through system. Parameters such as the

magnetic field required for settling flocs, and the point of introduction of magnetite in the water stream can then be determined.

These recommendations will facilitate future work involving the use of magnetite for adsorption of microorganisms. Overall, this method of treatment using magnetite has unique applications to the water and waste-water treatment industry. Magnetite adsorbs target species (specifically microorganisms) quickly, which saves energy and cost related to the treatment of surface waters. Magnetite does not require any settling chambers/tanks as it can be separated with the help of a simple magnet, which does not require a big sedimentation tank, therefore reducing the foot print of water and waste-water treatment plants. Magnetite does not produce any harmful byproducts in water and has been medically tested as safe and non-toxic (Ficai, 2011). The ability of magnetite to be regenerated (MacRae, 1981) is of great potential as all magnetite used can be recycled in the treatment stream. Additionally, the ability of magnetite to release dissolved oxygen in water can help remediate waste-waters of excess BOD, in turn reducing the load on activated sludge plants.

These are some of the advantages of magnetite over conventional chemical treatment systems. The use of magnetite, for remediation or treatment of surface waters and waste-waters has the potential for further studies. In addition, there are other possible applications for the use of magnetite. Some

ideas for future studies involving magnetite and its applications have been discussed in the next section.

4.3 FUTURE WORK

Future work involving the use of magnetite should focus on understanding the overall ability of magnetite for varied applications. Some of the research ideas/questions for future work have been listed below:

1. *Can magnetite's surface properties be altered to adsorb multiple contaminants in complex waste-waters*?

Experiments investigating a study for the use of magnetite to adsorb more than one type of contaminant in water or waste-water would be a great contribution. This could possibly be achieved by choosing appropriate chemical compositions that coat the magnetite surface. This will not only help in stabilizing the magnetite surface over an extreme pH, but could adsorb contaminants, which are in the ppb (parts per billion) range. This study will then show how magnetite can be tailored for treating specific contaminants of a particular waste-water stream. The study could also confirm the affinity of magnetite to different functional groups, thus making the process of water and waste-water treatment very specific for target contaminants and efficient as well.

2. *What is the most effective method for the regeneration of spent magnetite*?

The engineering application of magnetite is not feasible if it cannot be regenerated efficiently. The approach to apply and test different regeneration techniques will come after a deeper understanding of the chemical properties of magnetite is achieved. Regeneration by heat could be a possibility but magnetite might lose some of its adsorption capability, as heat can alter its chemical structure. Another method of regeneration could be to contact spent magnetite with concentrated sodium hydroxide and replace the adsorbed functional groups with OH as magnetite has high affinity for OH group.

This would be one of the most important studies that should be conducted to check the feasibility of magnetite as an adsorbent for contaminants in water and waste-water. If magnetite cannot be regenerated efficiently then studying any further would be entirely futile, as there is no practical application for this method.

3. *Check the difference in adsorption capability of magnetite as a function of its size.*

The magnetite used in this study was 1 to 5 micron in size. A study to find out the difference in adsorption efficiency when the size of magnetite is increased (up to 1mm) and decreased (up to 100nm), would be of great importance as this will further help in tailoring magnetite for specific

contaminants in treatment systems. It might be possible to adsorb dissolved organic species such as naphthenic acids, if the size of magnetite is in the lower colloidal range (i.e. 1 - 200nm).

4. *Does the magnetic field affect the adsorption capability of magnetite?*

The effects of magnetic fields were not studied in this paper, but a change in the field strength might reveal something of interest. This particular objective can be fulfilled by designing a simple experiment involving a liquid nitrogen cooled solenoid high strength electromagnetic field. A super cooled solenoid produces a superconducting magnetic field (in the order of 4 to 8T) and might have the capability of partially magnetizing the organic contaminant itself. The magnetic fields achieved by cooling the electromagnetic solenoid can be very high. Temperature of the solenoid can control the strength of the magnetic field applied which can answer the above research question with adequate evidence.

The above mentioned future work will contribute to our understanding of the behavior of magnetite in water and waste-water treatment. The purpose of doing this future work is to check the feasibility of this technology as an alternative to disinfection in water and waste-water treatment systems. If the method proves to be efficient and practical to existing water and waste-water treatment technologies, there will be no environmental implication of chemicals added to the treatment system.

4.4 WORKS CITED

1. Ficai, D., et al. "Synthesis of Rod-Like Magnetite by using Low Magnetic Field." Digest Journal of Nanomaterials and Biostructures 6.3 (2011): 943-51.
2. Olegsander, P. “Phosphorous Removal and Recovery from Waste-water using Magnetite.” Masters of Science Royal Institute of Technology,Sweden, 2010.
3. Parkinson, GS, et al. "Room Temperature Water Splitting at the Surface of Magnetite." Journal of American Chemical Society 133 (2011): 12650-5.

CHAPTER 5: APPENDICES

APPENDIX A: Sample calculation for cell : magnetite
APPENDIX B: Volume required for adjustment of pH with phosphate buffers.
APPENDIX C: Summary of standard curves for bacterial concentrations.
APPENDIX D: Zeta potential of magnetite as a function of pH.
APPENDIX E: Calibration curve for serial multiple dilutions of Tannic acid.

APPENDIX A

SAMPLE CALCULATION FOR CELL : MAGNETITE RATIO

The procedure for calculating the amount of magnetite required for each experiment was carried out in the following way.

1. Weight of empty micro centrifuge tube recorded (W_I).
2. Weight of micro centrifuge tube + bacterial pellet (W_F).
3. Weight of wet bacterial pellet noted ($W_B = W_F - W_I$).
4. Calculated the percent dry weight of the bacterial pellet using values from table A-1 (W_D = %dry weight x W_B)
5. The calculated value (W_D) was multiplied by the appropriate ratio factor RF (W_M = RF x W_D).
6. Ratio factors used were 1, 5, 10, 50, 100, and 200.

Table A-1: Table of percent dry weight of bacterial species adopted from Bratback et al (1984) and Carstensen et al, (1965)

Bacterial Species	% Dry weight of bacteria	% Intracellular water	% Carbon in dry cells
Escherichia coli	31.4 +/- 0.36	35.1 +/- 1.1	47.9 +/- 0.2
Pseudomonas putida	48.4 +/- 6.6	71.95 +/-5.9	45.7 +/- 0.8
Micrococcus luteus	34.1 +/- 0.4	NA	NA

APPENDIX B

VOLUME REQUIRED FOR ADJUSTMENT OF pH WITH PHOSPHATE BUFFERS

The table of different volumes required was adapted from SIGMA ALDRICH, MO, USA. The volumes shown in table B-1 are based on using 1M concentration of the buffering agent.

Table B-1: Table of different volumes used to buffer pH for solutions. Adapted from SIGMA ALDRICH, MO, USA.

pH	Na_2HPO_4 (ml)	NaH_2PO_4 (ml)
5.4	3	97.0
5.6	5	95.0
5.8	7.8	92.2
6.0	12.0	88.0
6.2	18.5	81.5
6.4	26.6	73.5
6.6	37.5	62.5
6.8	50.0	50.0
7.0	61.1	38.9
7.2	71.5	28.5
7.4	80.4	19.6
7.6	86.8	13.2
7.8	91.4	8.6
8.0	94.5	5.5

APPENDIX C

SUMMARY OF STANDARD CURVES FOR BACTERIAL CONCENTRATIONS

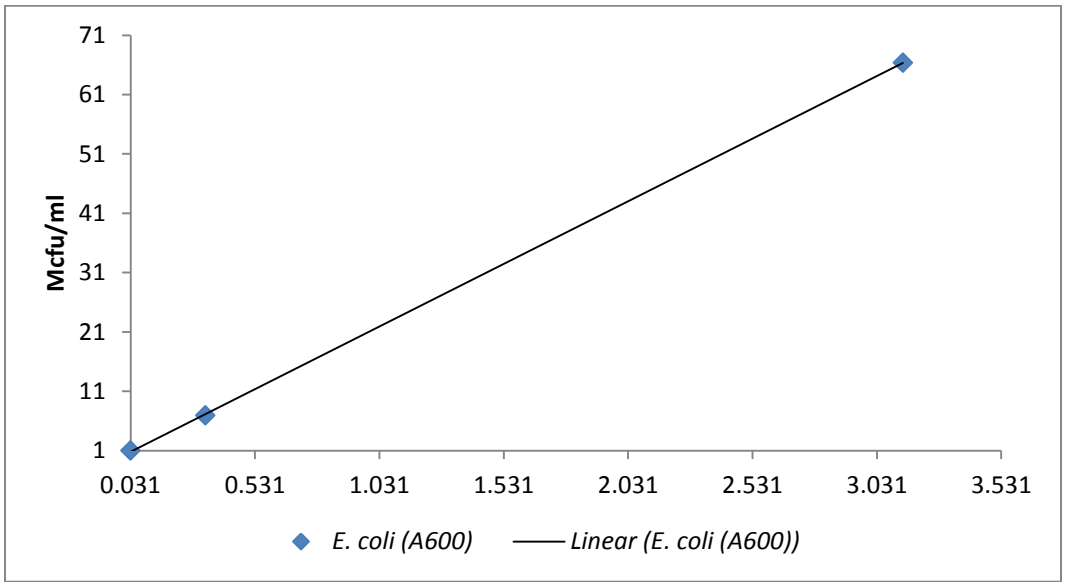

Figure C-1 : Standard curve of optical density v/s Mcfu/ml for *Escherichia coli* ATCC 25922.

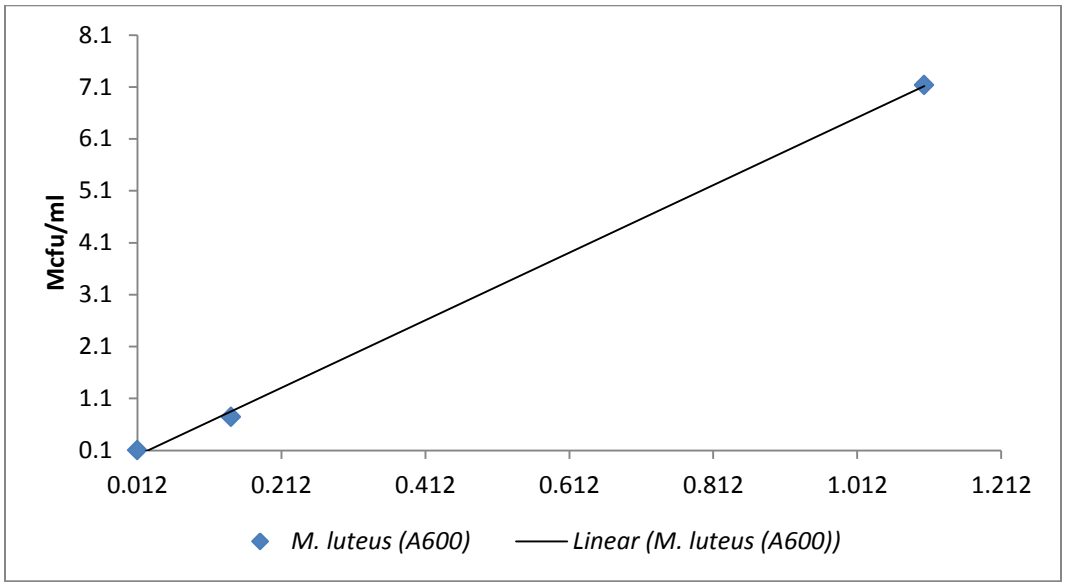

Figure C-2 : Standard curve of optical density v/s Mcfu/ml for *Micrococcus Luteus* ATCC 4698.

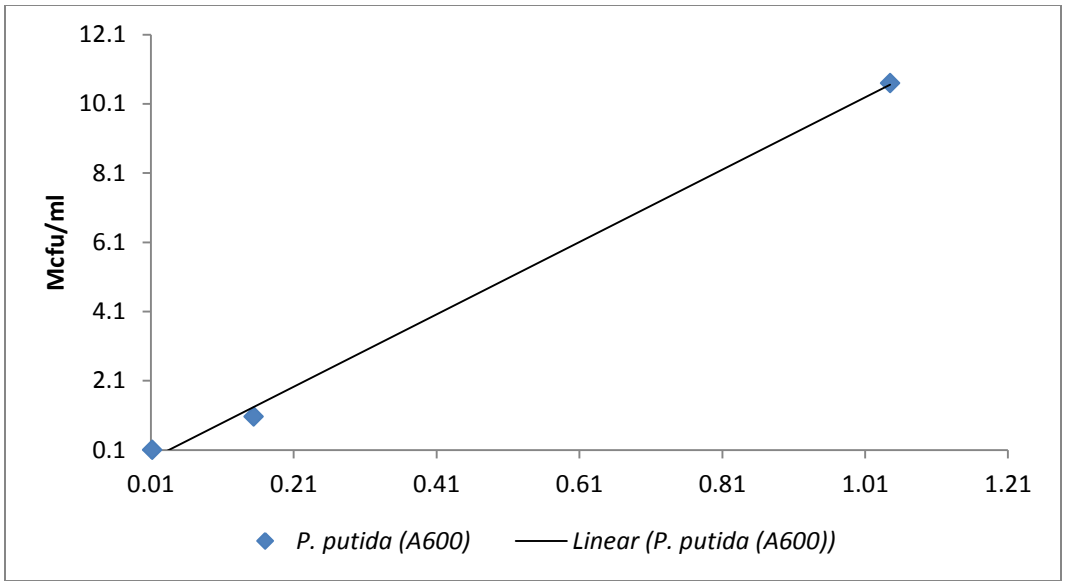

Figure C-3 : Standard curve of optical density v/s Mcfu/ml for *Pseudomonas putida* ATCC 17453.

APPENDIX D

ZETA POTENTIAL OF MAGNETITE AS A FUNCTION OF PH.

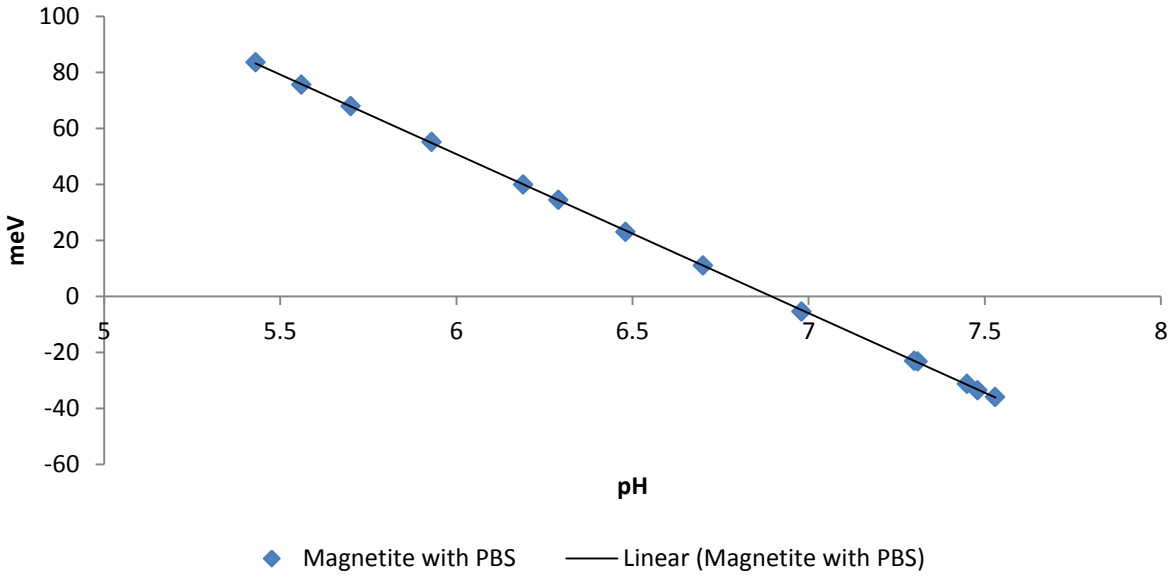

Figure D-1: Zeta potential of magnetite as a function of pH.

APPENDIX E

CALIBRATION CURVE FOR SERIAL MULTIPLE DILUTIONS OF TANNIC ACID

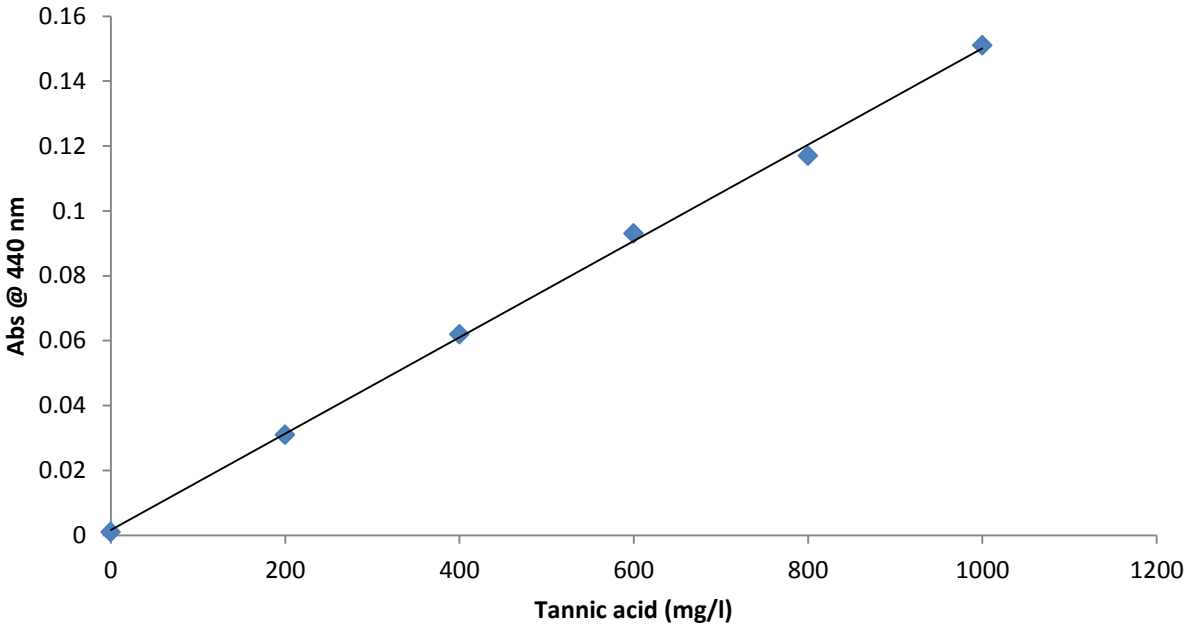

Figure E-1: Calibration curve used to quantify color removal using serial dilutions of 1000 mg/l stock tannic acid solution.