



APPLICATION OF MAGNETIC NANOPARTICLES AND
REACTIVE FILTER MATERIALS FOR WASTEWATER
TREATMENT

RAMNATH LAKSHMANAN

DOCTORAL THESIS

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ALBANOVA UNIVERSITY CENTER
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TO MY FAMILY

DEDICATION

This work is dedicated to my beloved mother, Mrs. Annapoorani Lakshmanan and late grandmother Mrs. Ramayee Ramanathan for their resilience to motivate and educate me, in every part right from childhood.

**“BE THE CHANGE THAT YOU WISH TO SEE IN THE WORLD”
- MAHATMA GANDHI**

ABSTRACT

Lately sewage wastewater treatment processes (WWTP) are facing challenges due to strict regulations in quality of effluent standards and waste production. The reuse of wastewater treatment effluents is rapidly gaining attention as a means of achieving sustainable water supply. Therefore, new methods are required to achieve an efficient WWTP. The foremost emphasis of the present study is to investigate filter materials, synthesis, characterization, and application of magnetic nanoparticles (NPs) for WWTP. Primarily commercially available reactive filter materials such as Polonite and Sorbulite were tested for the effective reduction of contaminants in recirculation batch mode system. Secondly, the magnetic nanoparticles were synthesized using different techniques such as *water-in-oil* (*w/o*) microemulsion and co-precipitation methods and testing for their ability to remove contaminants from wastewater. Thirdly, toxicity test of magnetic NPs were performed using human keratinocytes (HaCaT) and endothelial (HMEC-1) cells (Papers I-VII).

The magnetic iron oxide nanoparticles (MION) synthesized using the co-precipitation method were further functionalized with tri-sodium citrate (TSC), 3-aminopropyl triethoxysilane (APTES), polyethylenimine (PEI) and chitosan. The functionalized MION were further characterized prior to use in removal of contaminants from wastewater. The sewage wastewater samples were collected from Hammarby Sjöstadswerk, Sweden and analyses were performed for the reduction of turbidity, color, total nitrogen, total organic carbon, phosphate and microbial content on the retrieval day.

The experimental results imply that Polonite and Sorbulite require high pH for the efficient reduction of phosphate and the reduction of microbes. Microemulsion prepared magnetic nanoparticles (ME-MION) showed $\approx 100\%$ removal of phosphate in 20 minutes. Results from TEM implied that the size of magnetic Nps were around 8 nm for core (uncoated MION), TSC (11.5 nm), APTES (20 nm), PEI (11.8 nm) and chitosan (15 nm). Optimization studies using central composite face centered (CCF) design showed the potential of magnetic nanoparticles for the removal of turbidity ($\approx 83\%$) and total nitrogen ($\approx 33\%$) in 60 minutes. The sludge water content was reduced significantly by $\approx 87\%$ when magnetic NPs were used whilst compared to the chemical precipitant used in WWTP. PEI coated MION showed $\approx 50\%$ removal of total organic carbon from wastewater in 60 minutes. Effluents from wastewater treated with magnetic NPs were comparable with effluent from the present WWTP. There was no significant change observed in mineral ion concentration before and after treatment with MION. In addition, toxicity results from HMEC-1 and HaCaT cells revealed no formation of reactive oxygen species in the presence of magnetic NPs. Furthermore, laboratory experiments revealed the effectiveness and reusability of magnetic NPs. Thus magnetic NPs are a potential wastewater treatment agent and can be used for effective removal of contaminants, thereby reducing the process time, sludge water content and complex process steps involved in conventional WWTP.

KEYWORDS: Reactive filter materials, magnetic nanoparticles, wastewater treatment process, nutrient reduction, sludge water content, toxicity of magnetic nanoparticles.

LIST OF PUBLICATIONS

This thesis is based on the papers listed below, which will be referred in the text by their Roman numerals (I-VII). The papers are included in the appendix.

- I. Nilsson C, **Lakshmanan R**, Renman G, Rajarao G.K. 2013. Efficacy of reactive materials for phosphate, bacteria, nitrogen and TOC removal – column experiment in recirculation batch mode. *Wat. Res.* 47: 5165-5175.
- II. **Lakshmanan R**, Okoli C, Boutonnet M, Järås S and Rajarao G.K. Microemulsion prepared magnetic nanoparticles for phosphate removal: Time efficient studies (Submitted).
- III. **Lakshmanan R**, Okoli C, Boutonnet M, Järås S, Rajarao G.K. 2013. Effect of magnetic iron oxide nanoparticles in surface water treatment: trace minerals and microbes. *Bioresour. Technol.* 129: 612-615.
- IV. **Lakshmanan R**, Rajaraman P, Okoli C, Boutonnet M, Järås S and Rajarao G.K, 2013. Application of magnetic nanoparticles for the removal of turbidity and total nitrogen from sewage wastewater: Modelling studies (Submitted).
- V. **Lakshmanan R** and Rajarao G.K. Effective water content reduction in sewage wastewater sludge using magnetic nanoparticles. (Submitted).
- VI. **Lakshmanan R**, Dominguez M.S, Matutes Aquino J.A, Wennmalm S and Rajarao G.K. 2013. Removal of total organic carbon from wastewater using polyethylenimine - functionalized magnetic nanoparticles (Submitted).
- VII. **Lakshmanan R***, Bayat N*, Lopes V, Cristobal S and Rajarao G.K. Synthesis, characterization and toxicity assessment of magnetic nanoparticles on skin and endothelial cells *in vitro*: water treatment application (Manuscript).

*These authors share the first authorship

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CONTRIBUTION TO PAPERS

Paper I - Participated in designing and planning, involved in experimental setup, performed part of the experimental analysis, interpretation of results and writing article.

Paper II, III, V & VI - Principal author, involved in planning, performed all experimental work and analysis, interpretation of the results and main part of writing.

Paper IV - Principal author, involved in planning, learnt the modelling tool, performed the experiments and analysis, evaluation of the results and main part of writing.

Paper VII - Shared first authorship, Participated in developing ideas and experimental planning, synthesis of magnetic nanoparticles, involved in nanoparticle characterization and TEM imaging, partly interpreted the results and writing article.

ABBREVIATIONS

APTES	(3-Aminopropyl) triethoxysilane
BA	Bile esculin
BOD	Biological oxygen demand
CCF	Central composite face centered
CCS	Confocal correlation spectroscopy
COD	Chemical oxygen demand
Core	Uncoated MION
DOE	Design of experiments
EMB	Eiosin methylene blue
FT-IR	Fourier transform infrared spectroscopy
HaCaT	Human keratinocytes cells
HCl	Hydrochloric acid
HMEC-1	Human dermal microvascular endothelial cells
H ₂ S	Hydrogen sulphide
ME-MION	Microemulsion prepared magnetic iron oxide nanoparticles
MION	Magnetic iron oxide nanoparticles
NPs	Nanoparticles
NTU	Nephelometric turbidity units
PEI	Polyethylenimine
ROS	Reactive oxygen species
SICS	Scanning interference correlation spectroscopy
SSA	Specific surface area
TEM	Transmission electron microscope
TiO ₂	Titanium dioxide
TOC	Total organic carbon
TOT-N	Total nitrogen
TSC	Tri-sodium citrate
TSS	Total suspended solids
WWTP	Wastewater treatment process
XRD	X-ray diffraction

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1. INTRODUCTION

In recent decades, the availability of clean water is a major concern in both developed and developing countries for use in domestic and industrial applications. It is estimated that 1.1 billion people in the world lack access to improved water supply and 2.6 billion people lack adequate sanitation ¹. The water consumption estimates range from 1,382 km³/year in 1950 to 5,235 km³/ year in 2025. Moreover the water availability in 2025 will be about 872 m³/capita/year, well underneath the limit of 1,000 m³/capita/year, which defines the people who live in water scarce regions ². The increase in water scarcity is one of the most critical environmental problems facing many parts of the world. It has been predicted that in the year 2025, about 3.5 billion people, i.e. 48% of the world population will have an inadequate water supply ³. Considering an increase in the population growth rate, the amount of fresh water availability is expected to reduce with time, which will negatively affect the socio-economic development in many countries. Therefore wastewater reuse can be one of the possible strategies to increase water resources if it can be safely managed ⁴.

Wastewater treatment for industrial applications may involve different treatment steps that are designed to remove contaminants produced by the industry. The existing municipal or sewage wastewater treatment involves three main steps such as primary (physio-chemical processes includes precipitation/sedimentation), secondary (activated sludge or trickling filters) and tertiary (sand filtration/polishing) processes ⁵. Sewage wastewater contains contaminants such as suspended solids, biodegradable organics, microorganisms, nutrients, heavy metals and recently pharmaceutical residuals have been detected ⁶.

Activated sludge process is commonly used in sewage wastewater treatment process (WWTP) and also increases the sludge production, which is difficult to handle both economically and in the environment, for e.g. nearly 9 million tons of sludge was produced by the end of 2005 in Europe. Consequently, the disposal of sludge and treatment can cost up to 50% of the WWTP ⁷. Moreover, the existing treatment processes have challenges in process efficiency, time and operational cost. As an example, the, excess nutrients such as phosphate and ammonium nitrogen in the effluent lead to eutrophication in lakes/rivers, causing a depletion in dissolved oxygen and an increase of aquatic toxicity within receiving waters. Therefore, it is highly essential to control and remove the contaminants for a sustainable environment ⁸.

Considering the importance of sewage wastewater treatment globally, and to meet the rising water demands, there is pressing need to develop new technologies for cost effective, simple, user-friendly, robust and efficient systems. On this basis,

nanotechnology in particular magnetic iron oxide nanoparticles (MION) with their large surface-to-volume ratio and magnetic property could be one of the potential tools in wastewater treatment ^{9,10}. In addition functionalized magnetic NPs are known to display novel and significant physio-chemical properties such as size, surface charge and specific interaction with the complex contaminants in wastewater ¹¹. Other advantages include fast separation, material recovery, recycling and significant reduction of sludge volume. In order to optimize several parameters involved in the WWTP, response surface methodology is one of the methods used for evaluating experimental evidence against computational prediction. Moreover the knowledge in toxicity of NPs is essential prior to their use in treatment of large volumes of wastewater and exposure to environment.

2. BACKGROUND

2.1 Conventional wastewater treatment process

Wastewater treatment process (WWTP) has been constructed to treat both domestic and industrial wastes to meet the effluent standards prior to discharge in the natural environment. The composition and volume of wastewater can also vary within the same area, from hour to hour, from day to day and from year to year. Pollutants in the wastewater can be classified by their particle size as shown in **Table 1**.

Table 1. Particle size range in sewage wastewater.

Particles size	Dissolved	Colloidal	Suspended	Settleable
(μm)	<0.01	0.01-1.0	1-100	>100

The major contaminants found in sewage wastewater are biodegradable organic compounds, volatile organic compounds, xenobiotics, metal ions, suspended solids, nutrients such as phosphorous (P) and nitrogen (N), microbial pathogens and parasites. Moreover the approximate composition of organic carbon found in typical sewage wastewater is: carbohydrates (11-18%), proteins (8-10%), free amino acids (0.5-1.5%), fatty acids (23-25%), dissolved organic acids (7-11%) and other organic compounds (25-28%). However, wastewater is complex and requires detailed characterization of organic substances for an efficient WWTP^{12,13}. The standard methods for quantifying organic substances are determined in the form of biological oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC). BOD is the amount of dissolved oxygen required by microorganisms in wastewater to break down organic matter over a period of 5 days. COD indicates the quantity of pollutants in the water that can be oxidised by a chemical oxidant. TOC is another measure to quantify organic substances by combustion method.

The inorganic components of sewage wastewater mainly consist of nitrogen, phosphorous, chlorides, sulfates, bicarbonates and others. In particular, nitrogen and phosphorous are considered important, as they are the main nutrients that cause eutrophication in the recipient water if not treated properly. The common form of phosphorous in wastewater are orthophosphates or reactive phosphorous, polyphosphates (polymeric form of phosphoric acid) and organic bound phosphates. Polyphosphates or orthophosphates mainly occur in dissolved form, while organic phosphorous are found in solid substances.

The nitrogen present in sewage wastewater is in the form of, ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and nitrous oxide (N_2O). Approximately 40% is in

the form of ammonium and 60% is in organic matter, with insignificant level of nitrate. A schematic representation of the common WWTP is described in **Fig. 1**. The four main steps involved in WWTP are preliminary, primary, secondary and tertiary treatment.

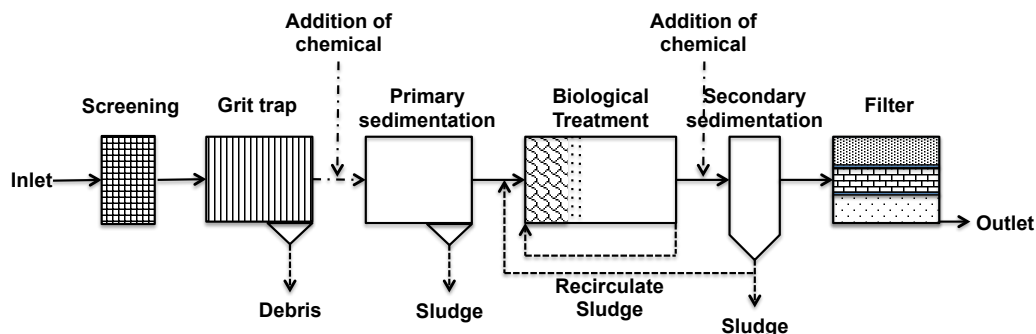


Fig. 1. General sewage wastewater treatment process.

Preliminary treatment in sewage wastewater involves two main steps such as screening and grit trap. Screening is the first treatment station in both surface and wastewater treatment process. The purpose is to easily separate and remove larger matter carried along with raw water while increasing the efficiency of later treatment steps. Grit removal is the next step, which involves removal of gravel, sand, fine mineral particles and sometimes combined with a grease separator.

Sedimentation is one of the primary steps that involve chemical flocculants such as iron salts, aluminium, polyelectrolytes, and lime for precipitation to settle solids from water by gravity. Removal of total suspended particles ($\approx 50 - 60\%$) and biological oxygen demand ($\approx 30 - 40\%$) can be expected in this step. Aluminium or iron salts are an effective coagulating agent for precipitation. Commercially available iron salts used in the WWTP are ferric chloride, ferric sulphate and ferrous sulphate.

The metal (Me^{3+}) salts are generally added in excess to compete with natural alkalinity according to the reaction mentioned in **Eq. 1a** ¹⁴.



Moreover, phosphorous is often removed by chemical precipitation with aluminium or iron salts to form aluminium or ferric phosphate (**Eq. 1b**).



A recent study with aluminium and sea salt as chemical coagulants (160 mg/L) indicated the removal of 87% COD, 94% TSS, 93% BOD₅, 96% total phosphorous and 20% of total nitrogen, whereas without chemical precipitation it was about 40-70% TSS, 25-40% BOD and 5-10% of phosphorous removal from sewage wastewater ^{15,16}. Hence addition of chemicals plays a key role in forming flocs and thereby enhancing the biological treatment process for effective nutrient reduction. Conversely, chemical coagulants (e.g. polyelectrolytes) used after biological processes (i.e. secondary sedimentation) have the ability to further precipitate phosphate and dissolved organic compounds.

Secondary treatment or biological process involves the use of microorganisms to decompose and break down organic pollutants present in sewage wastewater. The Biological treatment process may be classified into aerobic, anaerobic and anoxic conditions. The aerobic process involves oxidising the organic material by microorganisms in the presence of oxygen and the anaerobic process takes place in the absence of oxygen.

Biological phosphorous removal occurs during the activated sludge process where the microorganisms are stressed by switching their environment alternatively between aerobic and anaerobic conditions. In aerobic conditions the microorganisms consume and store phosphorous in the form of energy rich polyphosphates. Whilst in the anaerobic conditions, polyphosphates are released into the solution and again available as an accessible energy source. The phosphorous removal by biological method is lower when compared to nitrogen and carbon removal by microorganisms. Consequently, the conventional treatment process removes a maximum of 20 to 30% of the influent phosphorous.

Biological nitrogen removal is one of the main processes in WWTP and consists of two main steps, nitrification and denitrification. The first step in the nitrification process involves the sequential oxidation of ammonium to nitrite and nitrite to nitrate (**Eq. 2a,b**). Autotrophic bacteria belonging to the family *Nitrobacteriaceae* perform this process. The growth of microorganisms is strongly dependent upon temperature, i.e., the conversion to nitrate is higher in summer and relative to winter.



The denitrification process takes place in the absence of oxygen and also has nitrite as an intermediate, during the reaction shown in **Eq. 3**.



Nevertheless, microorganisms require oxygen in the aerobic process in order to break down dissolved organic substances and therefore produce excess sludge. The most important operating parameters of the activated sludge process (secondary treatment) are sludge age, oxygen consumption, sludge loading and suspended solids. Moreover the total retention time of the complete biological process is approximately 5 – 10 hours. Later the wastewater is passed on to the secondary sedimentation step.

Secondary sedimentation step involves chemical precipitation, as described earlier for the removal of excess phosphate and suspended solids found in wastewater. This can also be referred as a polishing step in the WWTP. **Filter** materials filled with gravel in two or four sub-layers of intermediate grain size are packed depending on the distribution system (tertiary treatment). The retention time of the filter processes are estimated between 4 – 12 hours.

Wastewater sludge can be obtained from primary and secondary or biological process. The composition of sludge consists of nitrogen, phosphorous, hydrocarbon, fatty acids and microorganisms. Sludge produced by the biological process alone however, accounts for 20% more sludge than primary sedimentation. High water content and the colloidal and compressible nature of the sludge are common characteristics of the different types of sludge and often sludge contains $\approx 90\text{-}95\%$ water ¹⁷. As a consequence it increases the volume and cost for trucking it to the disposal site.

In wastewater engineering, reduction of sludge water content is one of the most challenging tasks. Many techniques have been developed and documented such as vacuum filter, filter press and the addition of chemical flocculants ¹⁸. Among them, mechanical dewatering is often used due to its low energy consumption, reduction of total waste volume while also increasing the caloric value of the product ^{19,20}. However, the presence of organic components (bacterial cells and extracellular polymeric substances (EPS) makes it difficult to dewater even using high-pressure based mechanical techniques such as centrifugation and filtration/compression systems. In the recent years, several techniques have been developed for the minimization of sludge produced during biological wastewater treatment. This has been achieved through different strategies such as metabolism

uncoupling, predation or disintegration by physical or chemical methods such as ultrasound, thermal lysis and ozone ²¹.

Although traditional WWTP are primarily dependent on activated sludge processes, certain micro pollutants such as nitrosamines, anti-inflammatory (e.g. Ibuprofen, diclofenac), antidepressants (e.g. benzodiazepines), β -blockers (e.g. metoprolol, atenolol), antiulcer drugs (e.g. ranitidine), antibiotics (e.g. penicillins, imidazole derivatives) and other substances (e.g. narcotics) ²² are not completely removed. Furthermore, pharmaceuticals such as antibiotics have the potential to create or to proliferate resistant bacterial strains that might affect the activated sludge process or cause problem when discharged in the environment. Therefore additional advanced treatment methods are necessary before the water can be safely released in the environment and/or reused. The key challenges in existing WWTP are the time taken for the complete process (being about 15-20 hours) combined with excess sludge content and sludge handling costs. Therefore new techniques for wastewater treatment need to be developed in order to meet the stringent effluent water quality standards with reduced process time and sludge content ²³.

2.2 Other techniques in WWTP

Apart from the activated sludge process, other conventional methods used in WWTP are aerobic granular sludge, surface aerated basin, filter bed, constructed wetlands, membrane bioreactors and biological aerated filters. Recently developed methods being used in WWTP include filtration, ultrafiltration, reverse osmosis, ion exchange, chlorine, evaporation, ozone, adsorption, photochemical reactions, electrocoagulation and magnetic separation ²⁴. Filtration and magnetic separation are discussed further in section 2.3. This section will be focussed on advanced oxidation processes, electrocoagulation and low cost adsorbents used in both laboratory and large scale WWTP.

2.2.1 Advanced oxidation processes (AOPs)

AOPs involve formation of hydroxy radicals that will accelerate the oxidative degradation of numerous organic compounds dissolved in wastewater. The main techniques of AOPs include ultraviolet (UV) radiation, ozone (O_3) and electrochemical technologies. UV radiation is gaining more attention due to its properties of degrading organic compounds and as a disinfectant. Zhang and Zhou ²⁵ reported that photodegradation aided by TiO_2 allowed more than 90% removal of endocrine disrupting compounds (eg., bisphenol A, 17β estradiol etc.). Furthermore recent studies ²⁶ suggest that supercritical water oxidation is a

promising technology to treat industrial wastewaters. Moreover the reaction occurs within 1 minute and results in reduction of $\approx 99\%$ organic carbon.

According to Petala et al ²⁷, the capacity of ozone to assist in TOC removal was 80% and became saturated at a concentration of 24.2 mg/L. Conversely, maximal removal of 35% ammonium nitrogen was achieved at an ozone concentration of 15.4 mg/L. Alternatively, studies conducted on removal of pharmaceuticals from wastewater by O_3 /hydrogen peroxide (H_2O_2) treatment was found to have 98% mineralization of ibuprofen and diclofenac ²⁸. H_2O_2 is one of the powerful oxidizers for mineralization of pharmaceutical residues. Similar results were reported by Beltran et al ²⁹ for removal of diclofenac using O_3 in the presence of activated carbon with 95% removal in 120 minutes. Other AOPs include combined processes such as UV/ O_3 , UV/ H_2O_2 and O_3 / H_2O_2 ³⁰. The main advantages include reduction of micro pollutants and inactivation of *Cryptosporidium* and *Giardia*.

Application of **electrochemical** technologies in wastewater treatment particularly focuses on electrocoagulation, electroflotation, electrodialysis and electrooxidation ³¹. Electrocoagulation with either aluminium or iron electrodes have been widely used in industrial applications. A recent study ³² demonstrates the efficiency of electrocoagulation process using alumina electrodes for the reduction of phenol, color and chemical oxygen demand (COD) in olive mill wastewater. The results suggest that, there was a fast and effective reduction of pollutants within 25 minutes at 75 mA cm⁻². Another extensive study ³³ performed in purification of wastewater from gelatin production plant exhibited 60% TOC removal with an aluminium anode. However the performance was rigorously affected due to the scaling of electrodes. In order to avoid scale formation, calcium was precipitated using bicarbonate. A coating of TiO_2 on the electrode reduced TOC to 12-15 mg/L from the initial value of 195 mg/L ³³. From this study a combination of electrocoagulation and electro-oxidation were able to achieve 80% TOC removal with an energy consumption of 0.09 Wh/L. The advantages include treatment of large volumes with high organic loads, removal of colloidal and ionic matter and a continuous mode of operation ³⁴.

Consequently, AOPs can be used as pre-treatment or polishing step if they are suitable in large-scale treatment processes ³⁵. Taken together some of their limitations are high-energy consumption (e.g. UV light and ozone generation), dissolution of coatings, need for electrode replacement and H_2O_2 in residues.

2.2.2 Adsorbents

Adsorbents are considered as the utmost wastewater treatment method due to their universal nature, inexpensiveness, waste management and ease of operation. Generally adsorption can be defined as accumulation of a substance at a surface or interface between solid adsorbent and the contaminant. The contaminant being adsorbed is known as adsorbate and the adsorbing phase as adsorbent ³⁶. Gupta et al ²⁴ claim that adsorption and ion exchange involves transfer of one or more solutes between the liquid and solid phase. The physical adsorption occurs when attractive forces are weak (van der Waals forces) resulting in reversible adsorption. Chemisorption occurs when there is a sharing of electrons between the pollutants and the solid surface ³⁷. Other forces controlling adsorption processes are hydrophobicity, hydrogen bonds and $\pi - \pi$ interactions.

Activated carbon has a specific surface area of 500-2000 m²/g and is generally used in wastewater treatment because of its ability to adsorb various types of pollutants such as phenol, metal ions, pesticides, chlorinated hydrocarbons, detergents, humic substances and other organic compounds via formation of carbon-oxygen surface compounds ³⁶. As an example, the organic carbon removal rate was 10% by sand filtration, whereas adsorption onto an activated carbon resulted in more than 80% ²⁷. A recent study revealed that the use of activated carbon for removal of pharmaceuticals did not generate toxic or pharmacologically active products ²².

Several studies have been undertaken for the use of low-cost adsorbents such as peat, fly ash, china clay, maize cob, wood shavings and silica for color removal in wastewater ³⁸. In addition, many carbonaceous materials such as bark, coal, lignite, coconut shells, wood, dead biomass, seaweed, pecan shell, chicken feathers and peat are used in the production of commercial activated carbon, activated charcoal or activated coal ³⁹. However, the preparations of activated carbons are expensive. Nevertheless, their efficiency and applicability in adsorption of various contaminants restrict the economic considerations ²⁴. **Natural zeolites** are microporous materials composed of silicon or aluminium, which has low surface area when compared to synthetic zeolites (700 m²/g) prepared under controlled environments. Some of the examples are clinoptilolite, mordenite, and erionite ⁴⁰.

Another example is the use of **reactive filter materials** obtained from industrial by-products which has the potential for the removal of specific contaminants such as nutrients (e.g. nitrogen, phosphorous), metals (copper, lead, zinc), bacteria (e.g. faecal coliforms) and hydrocarbons. Several filter materials have been investigated and suggested for full-scale applications ^{41,42}. Such materials can be used in

compact filter systems, where the latter design allows for easy replacement and reuse of the material ⁴³⁻⁴⁵.

Concerning the techniques mentioned above, there are still some limitations in regeneration of materials, processing time, material preparation, and operating conditions in real time and these need further investigation. Nevertheless, the future is seeking for a more efficient, eco-friendly and cost effective WWTP ²³. Within the category of treatment and remediation, nanotechnology has the potential to contribute in long-term water quality, availability and viability of water resources ⁴⁶.

2.3 Nanotechnology

The benefits of nanotechnology in water treatment application have been focused in three main areas: treatment and remediation, sensing and detection and pollution control ⁴⁶. It has yielded enormous progress in several areas such as the manufacturing of electronics, telecommunications and medicine. Nanotechnology also plays a major role in addressing fundamental issues of the environment and water sectors ^{46,47}.

2.3.1 Nanotechnology in wastewater treatment

Membrane processes play a significant role in wastewater treatment and the use of nanoreactive membranes and advanced filtration materials helps in water recycling and desalination. Recent studies have demonstrated that membrane technology can be successfully applied to purify wastewater released from textile, leather, food, electronic, dairy industries and municipal wastewater ^{48,49}. Since conventional treatment methods are not able to remove compounds such as organic pollutants and nutrients to a sufficient level required for the effluents to meet the standard quality ⁵⁰. The common types of membrane processes used in water purification systems include microfiltration, ultrafiltration and nanofiltration for water treatment processes (**Fig. 2**). This thesis will highlight the nanofiltration and different types of membrane processes used in the WWTP.

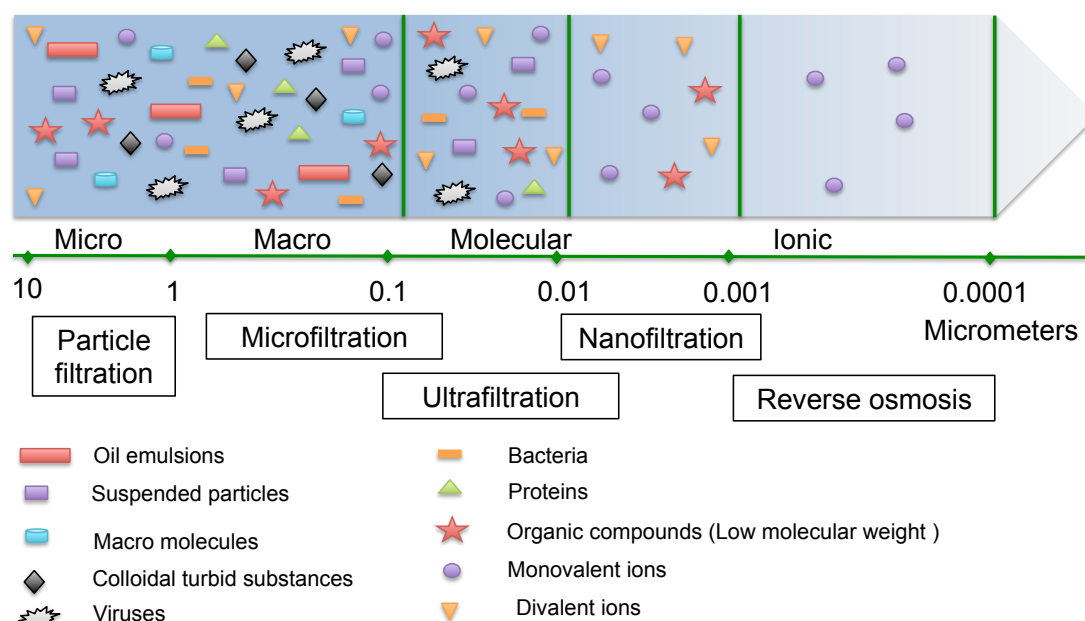


Fig 2. Size dependent filtration methods (Scale: Micro particle range, Macro molecular range, Molecular range, Ionic range). <http://www.watertechnonline.com>

Nanofiltration is a technique based on the use of nanopores present in the membrane used for filtering water samples with contaminants of different size ranges. Two main types of nanomembranes are nanostructured filters and nanoreactive membranes ⁵¹. Pre-treatment of the wastewater is one of the important considerations to prevent fouling and improving membrane performance. Moreover membrane performance can also be affected by oxidizing agents such as chlorine. Some examples of nanostructured and nanoreactive membranes are illustrated in **Table 2**. Commonly used membranes in wastewater treatment are ceramic and polymer supported membranes. Some of the common shapes found in the market are spiral, tubular, hollow fibre and frame membrane modules ⁵².

Table 2. Examples of nanostructures and nanoreactive membranes for removal of pollutants in wastewater treatment at research level ⁵³⁻⁶⁰.

Membrane	Pollutant
Carbon nanotubes	Bacteria and virus
Alumina membrane formed from A-alumoxane	Synthetic dyes
Alumina membrane functionalized with poly(styrene sulphonate)	Divalent cations
Zero-valent Fe laden cellulose acetate membrane	Trichloroethylene
Alumina or polymeric membrane with gold NPs	4-nitrophenol

Polymer-impregnated TiO ₂ filters	Polycyclic aromatic hydrocarbons
Polymer-impregnated ceramic alumina and silicon-carbon filters	Trihalogen methanes and pesticides
Silica and cellulose based membranes functionalized with amino acid homopolymers	Metal ions

Ceramic membranes have high mechanical strength, thermal stability, chemical compatibility, flux, long operational life and are efficient in cross flow filtration. For example, A-alumoxane NPs were used in fabrication of alumina membranes for the removal of synthetic dyes. These were more specific for the removal of dyes such as direct red 81, direct blue 71 and direct yellow 71 as reported earlier ⁵⁴. One of the commercially available ceramic membranes is membralox with a pore size of 20 nm to 0.2 μm and is mostly composed of materials such as alumina, titanium, and zirconium. Some of the disadvantages include capital and operational cost.

Polymeric membranes have specific characteristics such as high flux, resistant to UV, chlorine and ozone. Polymeric membranes consist of cross-linked polyamide, cellulose acetate, polysulfone materials and are used in the treatment of wastewater. Polyamines having primary amines and carboxylic acids in their outer surface have shown improved metal ion binding with a chelating mechanism ⁶⁰. However some of the disadvantages are frequent fouling upon contact with organic contaminants, thus leading to reduced flux rate and poor chemical stability.

Carbon nanotubes (CNT) are made up of a hexagonal lattice of carbon and fullerene molecules. There are two types namely single (SWCNT) and multi-walled carbon nanotubes (MWCNT) based on the principle of hybridized carbon atom layers on their outer surface. The size range of SWCNT is between 0.3 to 3 nm, whereas MWCNT has diameters up to 100 nm ⁶¹. As an example, CNT's demonstrated the removal of organic pollutants such as benzene, toluene, xylene and ethylbenzene was higher compared to the granular activated carbon ⁶². Other studies have also reported the use of functionalized carbon nanotubes for the removal of pollutants such as p-nitrophenol, heavy metals, trihalomethanes and dimethylbenzene ⁴⁶. Zhang et al ⁶³ revealed that MWCNT have the potential to adsorb 85% bisphenol AP from sewage wastewater and can be reused about 8 times without affecting in efficiency. However synthesis and maintenance in using carbon nanotubes for wastewater treatment is still a challenge.

2.3.2 Nanomaterials as photocatalysts

Titanium dioxide (TiO_2) has been used as a photocatalyst since 1970 due to its stable semiconductor properties for environmental applications. It is one of the frequently reported nanomaterials in consumer products (for e.g. food additives, and clothing). The mechanism of photocatalysis occurs when a light source contacts a semiconductor such as TiO_2 , thereby leading to separation of electrons. These electrons disperse on the surface of the photocatalyst and react with external substances, causing reductions and oxidations (**Fig. 3**). The nano-sized particle of TiO_2 with its high surface area makes it possible to get high reaction rates. Recent studies ⁶⁴ demonstrate the activity of TiO_2 in the degradation of methyl orange, a typical organic micropollutant resulting from the textile industry. The results suggest that a synergetic effect between the electrocatalyst and the intensity of UV radiation promotes the degradation of methyl orange by up to 90%. TiO_2 has also shown the ability to adsorb phosphate ions from aqueous solutions within 60 minutes ⁶⁵. Moreover iron (III) doped TiO_2 NPs are able to degrade phenol under UV light in less than 4 hours ⁶⁶. An added advantage of the process is that disinfection is generally not required since UV light assists elimination of microorganisms. The foremost drawbacks however, would be lower photocatalytic effects in the presence of complex organic mixtures and longer irradiation times are concerns in large-scale applications ⁶⁷.

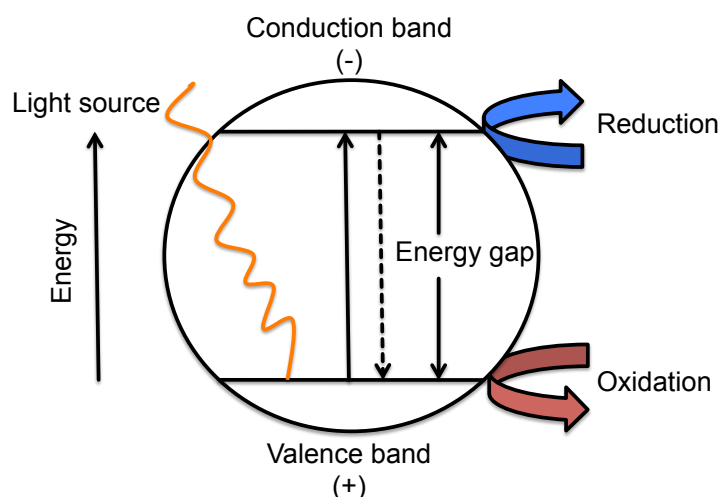


Fig 3. Mechanism of photocatalysis.

2.3.3 Other types of nanoparticles (NPs)

Other types of NPs such as silver, zinc and alumina ⁶⁸ are also widely used in WWTP applications. Farhadi et al ⁶⁹ demonstrated an efficient reduction of various amines, alcohols and phenols in the presence of acetic anhydride using zinc aluminate NPs. Silver NPs have been used extensively in many industrial

applications due to their antibacterial properties. Other nanomaterials such as zinc and carbon nanotubes are considered promising candidates in wastewater treatment for their antimicrobial property and are a possible alternative to chlorine free disinfectants ⁷⁰. Polymer composites such as polyvinyl chloride (PVC) have also been used for sulphate removal in WWTP ⁷¹. Another example includes the amino groups of chitosan, which can strongly adsorb anionic dyes by electrostatic interaction in both basic and acidic conditions ⁷².

Recent pilot study revealed that silver NPs received from household wastewater (i.e., by the usage of cosmetics and lotions) passed through the activated sludge process and ended up in wastewater effluents as silver sulphide ⁷³. These NPs interfere in the biological process, thereby affecting the WWTP efficiency. Another pilot study conducted by Kiser et al ⁷⁴ states that TiO₂ NPs pass through biological process in wastewater treatment and 60% of the NPs were trapped in the biomass. Taken altogether, NPs for example titanium or silver accumulate either in sludge depending on the concentration or released in the environment.

Nevertheless, several challenges still exist for efficient application of such materials in practice, primarily concerning dispersion and retention of nanomaterials. However it is difficult to eliminate nanomaterials from solution since they are too small to be collected by filtration or centrifugation. Further research is essential to apply the nanomaterials in WWTP by improving the immobilization of NPs onto a solid support or recovery of NPs. One such approach will be using magnetic NPs, which can be captured by an external magnet and possibility of recovery and regeneration ⁹⁻¹¹.

2.3.4 Magnetic NPs

Magnetic NPs are very interesting due to their multiple properties such as size effects, surface-to-volume ratio, interaction, magnetic separation, specificity and surface chemistry. Magnetic NPs, in particular nano zero-valent iron, magnetite and maghemite have sparked the application in medicine, molecular biology, and remediation of polluted water ⁹. In most circumstances magnetite or maghemite is used to form the core of magnetic iron oxide nanoparticles (MION).

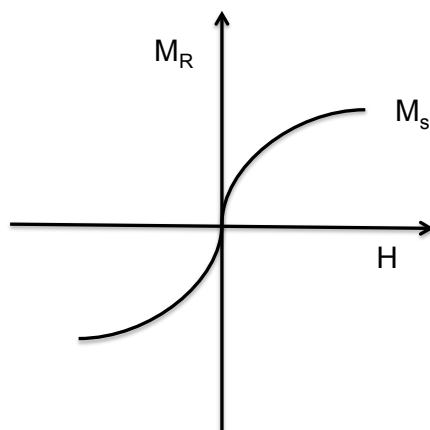


Fig 4. Magnetization hysteresis loops of superparamagnetic NPs. (M_R – zero remanence, M_s – high saturation magnetization, H – coercivity)

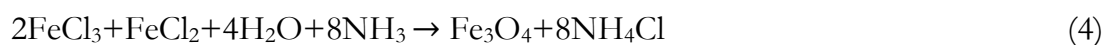
MION's may be broadly divided into three main classes: paramagnetic, ferromagnetic and superparamagnetic behaviour ⁷⁵. Paramagnetic behaviour denotes that the magnetic dipoles are oriented in random directions at normal temperatures due to unpaired electrons, which causes a low positive susceptibility (weak interaction) in a magnetic field. Ferromagnetic materials depend on their domain structure to remain magnetized even in the absence of an applied magnetic field but size decreases to less than the domain size when they undergo a significant change. Superparamagnetism tends to have larger magnetic susceptibility than paramagnets since the magnetic moment of the entire nanoparticle aligns in the direction of the magnetic field (**Fig. 4**).

2.4 Synthesis of magnetic NPs

In recent years a variety of chemical synthesis methods have been developed for preparing monodisperse superparamagnetic NPs with tailored structures, size, surface chemistry and state of aggregation. Particularly during the past few years, many publications have described efficient synthesis methods including chemical co-precipitation, microemulsion synthesis, thermal decomposition and hydrothermal synthesis ⁷⁶.

Co-precipitation is a facile and convenient way to synthesize iron oxide magnetic nanoparticles (MION) from aqueous Fe^{2+} and Fe^{3+} salt solutions by the addition of a base at room temperature or at an elevated temperature. The size, shape and composition of the magnetic NPs depend upon the type of salt (i.e. chloride, sulphate, nitrate), the Fe^{2+} and Fe^{3+} ratio, the reaction temperatures, pH value and ionic strength of the media. Nevertheless, if synthesis conditions are fixed, the quality of the magnetic NPs is fully reproducible ^{77,78}. The synthesis of magnetic NPs can be performed according to **Eq. 4** with complete precipitation of iron oxide (Fe_3O_4) in the pH between 7.5-14 (e.g. pH in the range of 7.5-11 are

obtained when working with microemulsion systems) under inert environment. This method involves preparation of large quantities of NPs in a single batch, but the size distribution is large ⁷⁹.



Microemulsion is a three-component system consisting of water, oil and surfactant forming a thermodynamically stable isotropic solution. Depending on the concentrations of different components, microemulsions can form *water-in-oil* (*w/o*) microemulsion ⁸⁰ or a *oil-in-water* (*o/w*) microemulsion ⁸¹. The surfactants used in this method can be cationic – cetyltrimethylammonium bromide, CTAB, anionic – bis 2-ethylhexyl sulfosuccinate, non-ionic – Berol 050 and polyethylenoxide. The main advantage of this technique is that it allows the formation of NPs in a uniform size distribution. The size of the particles can be tailored with *water-surfactant* molar ratio ⁸². By this method, monodisperesed NPs with various morphologies and surface modification can be prepared for widespread applications ⁷⁶.

Thermal decomposition is a technique that is used mostly in synthesis of high quality semiconductor nanocrystals and oxides in non-aqueous media. Monodisperse magnetic nanocrystals are achieved through thermal decomposition of organometallic compounds in high-boiling organic solvents containing stabilizing surfactants. In this technique, fatty acids such as oleic acid and hexadecylamine are often used as surfactants ^{83,84}. During the synthesis process, reaction time, reaction temperature and aging time are critical parameters for controlled size and morphology of magnetic NPs. One of the main drawbacks in this method is the lack of NPs suspension in aqueous media due to high crystallinity. Moreover, it is a complicated process that requires high temperature and an inert atmosphere during synthesis that lasts for several hours.

Hydrothermal method is considered as one of the most versatile and friendly method ⁸⁵. The strategy is based upon the general phase transfer and separation mechanism occurring at the interface of the liquid, solid and solution phase present during the synthesis. The engineering of nanoparticle surfaces cannot be accomplished and therefore post-processing steps are required. One of the main drawbacks in this method is that at any given temperature, the rate of kinetics is slower when compared to the other synthesis methods ⁷⁶.

The advantages and disadvantages of the four mentioned synthesis techniques are briefly summarized in **Table 3**. Among the methods described, co-precipitation and microemulsion are widely utilized in the synthesis of superparamagnetic NPs for biomedical and environmental application. The working window is quite large

for these two methods and promotes surface chemistry modification of the nanoparticle during synthesis or after synthesis. Another advantage includes reaction temperature and preparation times, which are much lower than hydrothermal and thermal decomposition methods.

Table. 3 Summary of magnetic nanoparticle synthesis methods ⁷⁶.

Method of synthesis	Co-precipitation	Microemulsion	Thermal decomposition	Hydrothermal synthesis
Technique	very simple, ambient conditions	simple	simple, inert atmosphere	simple, high pressure
Component	water	organic compound, water	organic compound	water-ethanol
Reaction period	hours	hours	hours-days	hours
Reaction temp. [°C]	20-90	20-50	100-320	220
Size distribution	relatively narrow	relatively narrow	very narrow	very narrow
Shape control	not good	good	very good	very good
Yield	high/scalable	low	high/scalable	medium

2.4.1 Magnetic separation

Separations using magnetic field are widely used in the areas of drug delivery, molecular biology, diagnostics, immuno assays, catalysis and environmental remediation ^{86,87}. Magnetic NPs are composed of magnetic core, in particular superparamagnetic NPs are easily separated with the help of an external magnet (**Fig. 5**). Indeed magnetic NPs are extensively used in the reprocessing of waste, for example the remediation of heavy metals from wastewater ⁸⁸. This is one of the methods where purification does not generate secondary waste and materials can be recycled. The adsorption mechanisms of magnetic NPs with contaminants are ionic exchange and other weak forces. This approach is commonly referred to as magnetically assisted chemical separation ^{10,89}. Permanent or electro-magnets (for e.g. magnetic separators with a rotating disc and a magnetic drum) are

available commercially for large-scale setups depending on the application and strength of magnetic NPs. Magnetic NPs can be tailored using natural or synthetic polymers for selective adsorptions under laboratory conditions.

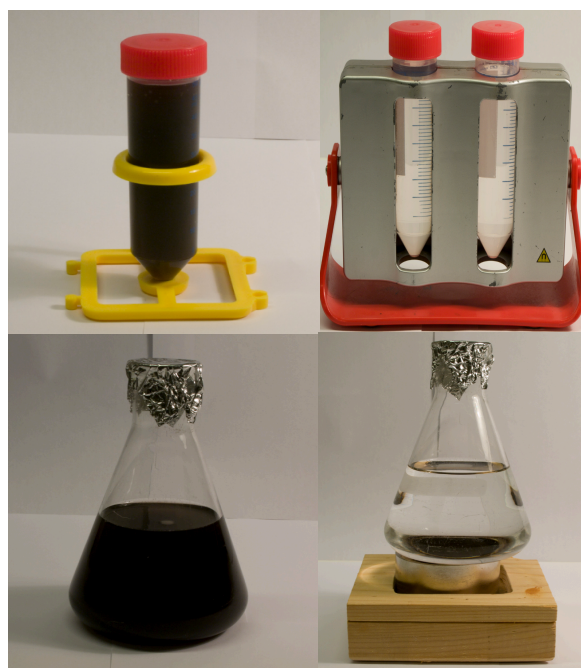


Fig 5. Separation of magnetic nanoparticles at the laboratory scale (50 ml and 500 ml volume). Flask on left shows the NPs in suspension and on right side, NPs collected with an external magnet with ≤ 5 -10 min.

2.4.2 Functionalization and application of magnetic NPs

Although NPs have a high surface-to-volume ratio, stability is a crucial requirement when they are exposed to air in the case of pure metal particles like iron, cobalt and nickel ⁷⁶. Coating of nanomaterials includes surfactant or polymer, precious metal, silica and carbon for various applications. As a result, coating helps not only in protecting the core shell but is also used in addition of functional groups for specific pollutant removal in water treatment ^{9,82}.

Generally, magnetic NPs are surface modified with carboxyl, hydroxyl and amino groups for their specific interactions ⁹⁰. For example, magnetic NPs can be capped with either a positive or negative charge material through surface chemistry in order to increase their stability. Recent studies revealed that microemulsion prepared magnetic iron oxide nanoparticles (ME-MION) with protein binding resulted in reduction of suspended particles and microbes ^{91,92}. Singh et al ⁹³ reported effective removal of >95% heavy metals such as copper, cadmium, nickel, zinc, arsenic and lead from aqueous solution using carboxyl, amino and thiol functionalized magnetic NPs. Therefore, functionalized magnetic NPs have a high degree of interaction and ability to remove specific contaminants in WWTP.

The use of **bimetallic** NPs has been of great interest due to their enhanced stability, inhibition of oxidation and increased reactivity. Normally when two metals are in contact with different electrical potentials, i.e. in the case of iron (Fe) and nickel (Ni), Fe acts as a reducing agent and Ni as a catalyst with hydrogen generated from water. However, Fe/Ni also act a good corrosion stabilizer and lower the cost for on-site wastewater treatment ⁹⁴. The Fe serves as an electron donor to react with the contaminants and the noble metal is protected in the removal of chlorinated aliphatics.

The common techniques used for **characterization of NPs** are transmission electron microscopy (TEM) for determination of size; X-ray diffraction (XRD) to find the crystal structure and to estimate size; Fourier transform infrared spectroscopy (FT-IR) to detect the chemical interaction on the particle surface; zeta potential to find the surface charge and isoelectric point on the surface; dynamic light scattering (DLS) to measure the hydrodynamic diameter of the particles in suspension and magnetization measurements for the magnetic property of the NPs.

2.5 Optimization tool

Response surface methodology (RSM) is a modelling tool for optimization of processes by evaluating experimental evidence against computational prediction. In recent years development & optimization in industrial sectors have become dependent on mathematical statistics and simulation. The main purpose of DOE (Design of experiments) is to gain knowledge, increase understanding and estimate proper operating conditions. In an experimental design, the performance analysis is based on individual factors and the responses are identified. Briefly DOE analysis involves defining factors and responses, creating a design, making the model ($Y = f(x) + e$), interpreting the model and using it for optimization. A common approach in DOE is to define an interesting standard reference experiment and then perform representative experiments around it. Hence the standard reference point is commonly known as *center –point* ⁹⁵. Some of the common design matrices used in response surface methodology are Box Behnken (BB), Central Composite Circumscribed (CCC), Central Composite Face centered (CCF) and full factorial (**Fig. 6**) ⁹⁶. Recently, modelling tools such as BB and CCF have been widely used for environmental remediation studies ^{97,98}.

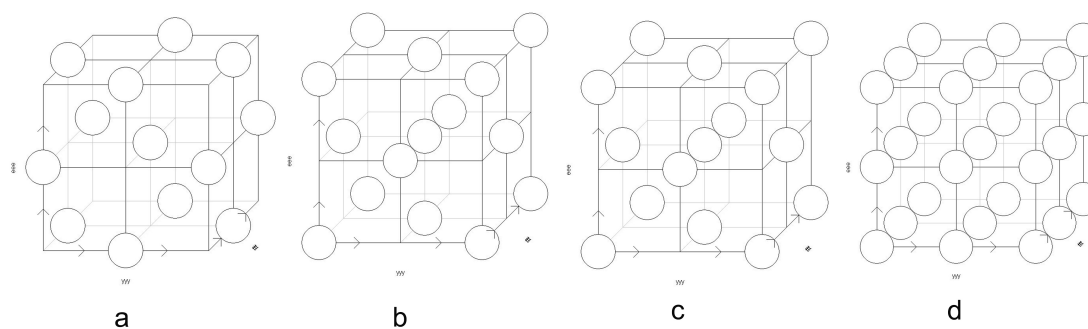


Fig 6. Different types of design matrices in response surface methodology. (a – Box Behnken, b – Central Composite Circumscribed (CCC), c – Central Composite Face centered (CCF), d – Full Factorial)

2.6 Toxicity of engineered nanomaterials

The characteristic properties of magnetic NPs include surface coating, charge, size and morphology. These influence the interaction between the NPs and biological organisms typically at their surface. In order to enhance the potential application of magnetic NPs in development of sustainable wastewater treatment technology, toxicity studies on the fate of magnetic NPs are essential for risk assessment ⁴⁶. However understanding the mechanism of toxicity and biocompatibility depends upon numerous parameters such as NPs size, shape, surface property, concentration, type of cell and nanomaterial ^{9,99}. Magnetic NPs can be distributed to various organs, tissues and cells, whilst MION's (<10 nm) are usually removed through renal clearance and > 200 nm are sequestered by the spleen via mechanical filtration. A typical final bio-distribution of magnetic NPs is 80-90% in liver, 5-8% spleen and 1-2% bone marrow ¹⁰⁰. Some examples of NPs on toxicity studies are illustrated in **Table 4**.

The main mechanistic injury pathways ¹⁰¹ caused by NPs are:

- Redox activity and reactive oxygen species (e.g. TiO₂, CuO)
- Dissolution and shedding of toxic ions (e.g. ZnO, CuO)
- Cationic toxicity (e.g. Polystyrene, PEI)
- Lung fibrosis (e.g. CNT)
- Inflammasome activation (e.g. CNT, CeO₂ rods)
- Photoactivation (e.g. TiO₂)
- Embryo hatching interference (e.g. CuO)
- Membrane lysis (e.g. SiO₂ and Ag-plates)

Table 4. Summary of recent toxicity studies reported¹⁰⁰.

Coating material	Cell types/lines	Size (nm)	Concentration (mg/mL)	Results
Dextran	Macrophages (human)	100-150	0.1	Only 20% cell viability observed after 7 days
Silica	Human lung adenocarcinoma epithelial cells	50	4	Dose and size dependent damage
Amine-surface	Human liver carcinoma cells	61-127	3	Cause severe cytotoxicity due to positive charge
Chitosan	Human hepatocellular carcinoma cells	13.8	0.12	Only 10% cell viability observed after 12 hours
Zinc oxide	Bronchial epithelial cells	25	0.2	Ion shedding, Oxidative stress due to local ion concentration
Silver	Gill fish cell line	40	0.05	Membrane damage

Endothelial cells are versatile and multifunctional cells that play a key role in many physiological processes such as wound healing, modulation of vascular bone, blood flow, and regulation of immune and inflammatory responses¹⁰². NPs that succeed in crossing the epithelial barrier, whether through inhalation or dermal absorption, may get into the bloodstream, raising the possibility of direct contact with the vascular endothelium. Alternatively, they can be translocated to other organs such as liver, potentially causing disease¹⁰³. Skin is one of the largest organs exposed to a variety of environmental stress, including NPs. The question of NPs ability to penetrate healthy skin is debated among researchers. It is therefore potentially, the main entry routes of NPs into the body. Presently, there is a limited body of literature available regarding the effects of MION on skin cells. Recent studies have also reported that MION induce cytotoxicity, genotoxicity and oxidative stress in human skin cells with concentration of cells in a dose dependent manner¹⁰⁴.

Although magnetic NPs have potential application in wastewater treatment, some technical challenges still exist such as recovery, efficiency in removal of contaminants, regeneration of magnetic NPs and the potential risk of these magnetic NPs to environment are yet to be explored.

2.7 Objectives of the present work

The overall scope of this thesis is to investigate the use of reactive filter materials and magnetic NPs for wastewater treatment processes (WWTP). In order to explore the potential application of materials in WWTP, there are primarily two commercial adsorbents, Polonite and Sorbulite were selected and recirculation batch mode column experiments were conducted for the removal of phosphate, inorganic nitrogen, total organic carbon and microbial pathogens. Secondly the proposed methodology is based on a bottom up approach i.e. the synthesis, characterization and application of magnetic NPs for wastewater treatment.

The first goal was to synthesize and obtain suitable magnetic NPs using the microemulsion and co-precipitation methods. Later, microemulsion prepared magnetic iron oxide nanoparticles (ME-MION) were investigated for the removal of phosphate from sewage wastewater. Furthermore, possible recovery and reuse of ME-MION was also investigated. Mineral ion analysis was carried out in lake water samples to study and understand the influence of NPs after treatment. Magnetic iron oxide nanoparticles (MION) prepared with co-precipitation methods were functionalized and characterized to find their physio-chemical properties. An optimization study for the removal of contaminants by MION was performed using response surface methodology. The investigation was carried out for the efficiency of MION on the reduction of nutrients, TOC, microbes and sludge water content.

Toxicity of MION was tested with human endothelial (HMEC-1) and keratinocyte (HaCaT) cells. The toxicity analyses of MION were performed for cell viability, DNA strand damage and generation of reactive oxygen species (ROS).

Lastly, sequential process steps were tested using functionalized magnetic NPs for the removal of contaminants from sewage wastewater under laboratory conditions. The effluents obtained from magnetic NPs treatment were compared with effluents from existing WWTP. In addition, the magnetic NPs concentration was investigated before and after treatment with surface water to ascertain the possible recovery.

3. MATERIALS AND METHODS

3.1 Synthesis and preparation of materials

3.1.1 Reactive filter materials

Two filter materials such as Polonite and Sorbulite were used for the investigation of the removal of contaminants, Polonite is a commercial product derived from the siliceous sedimentary rock Opoka after calcination ⁴³. Sorbulite is a brand owned by Biopotech AB, Sweden, and is produced from scrap material from the manufacture of autoclaved aerated concrete (AAC).

3.1.2 Magnetic iron oxide nanoparticles (MION) prepared microemulsion (ME-MION)

Magnetic NPs were prepared in *water-in-oil* (*w/o*) microemulsion system (ME-MION) using a single-step mode of preparation ¹⁰⁵. Briefly, a precursor solution containing 2:1 molar ratio of iron salts was dissolved in distilled water. The addition of precursor solution to the mixture of CTAB/1-butanol/n-octane resulted in the formation of a microemulsion. The pH of the mixture was adjusted to 11 with ammonia and the formation of ME-MION was subsequently observed. Protein binding was achieved by equilibrating the ME-MION (6-10 nm size) with 10 mM ammonium acetate buffer, pH 6.7 containing coagulant protein as described earlier ⁹¹. The developed protein-functionalized magnetic NPs were further suspended in ammonium acetate buffer and kept at 4 °C prior to use.

3.1.3 MION prepared from co-precipitation method

Magnetic iron oxide nanoparticles (MION) were prepared using co-precipitation method with OH⁻ functional surfaces as mentioned earlier ¹⁰⁶. Briefly a precursor solution containing 1 M FeCl₃ and 0.5 M FeCl₂ was dissolved in 35 ml distilled water. Later the iron source containing 35 ml distilled water was added to the 20 ml of 0.7 M ammonia solution at 70°C. The resultant MION (**Fig. 7**) were washed and suspended in distilled water for further use.

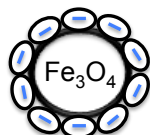


Fig 7. Schematic illustration of uncoated MION.

3.1.6 APTES – coated MION

3-Aminopropyltriethoxysilane (APTES) was used as an amino-silane coupling agent with -NH_2 surface functional groups on the MION¹⁰⁸. The MION suspension (150 mg) was dispersed in 100 ml of distilled water. APTES was added drop-wise into the reaction mixture in the presence of atmospheric N_2 at 70°C for 3 hours (**Fig. 10**). The prepared APTES coated MION were collected with an external magnetic field and washed with ethanol followed by distilled water. Finally APTES coated MION were re-suspended and stored at 4°C prior to use.

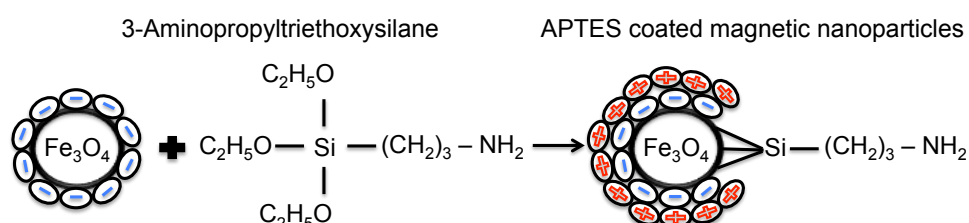


Fig 10. Schematic illustration of APTES coated MION.

3.1.7 Chitosan – coated MION

Chitosan is a cationic biopolymer with -NH_2 groups obtained from alkaline N-deacetylation of chitin. Chitosan has specific properties like selectivity and hydrophilicity together with biocompatibility and biodegradability. As mentioned earlier⁷⁹, MION suspension was dispersed in distilled water. Consequently, 0.68 g of chitosan was dissolved in 25 ml of acetic acid (CH_3COOH) and added to the MION suspension (**Fig. 11**). The reaction mixture was kept at room temperature for 24 hours without shaking. Later, the chitosan coated MION were separated using an external magnet and washed with ethanol followed by distilled water. Finally chitosan coated MION were resuspended and stored at 4°C prior to use. Total concentrations of magnetic NPs were expressed in terms of dry weight of particles per unit volume in suspension.

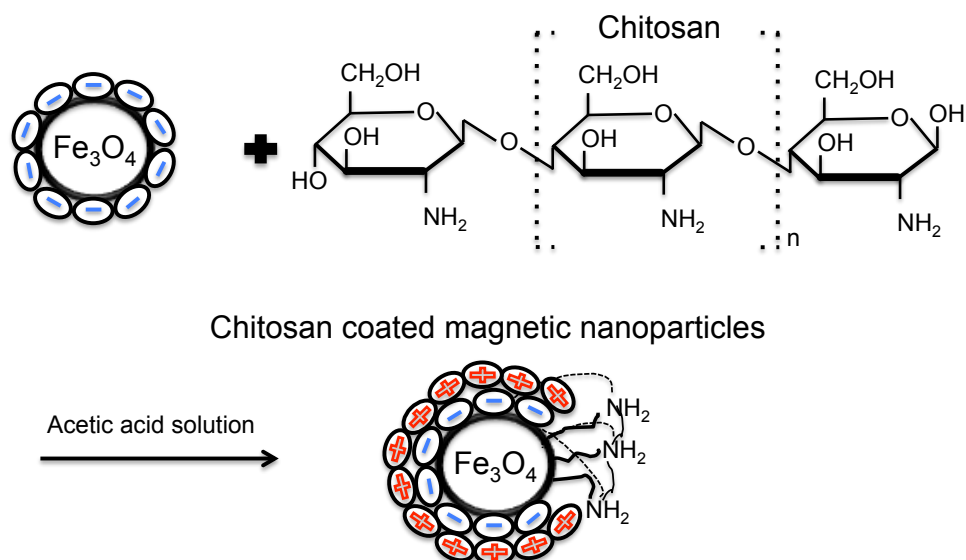


Fig 11. Schematic illustration of chitosan coated magnetic NPs.

3.2 Characterization of magnetic NPs

3.2.1 TEM

High Resolution Transmission Electron Microscopy (HRTEM) was performed for analyses of the particle size, morphology, and crystallinity. The sample was prepared as follows: 0.5 mg of magnetic NPs were dispersed in water (4 ml) and sonicated; the large agglomerates were removed with a magnet.

3.2.2 FT-IR analysis

Fourier Transformed Infrared Spectroscopy (FT-IR) was performed in order to identify organic functional groups present on the NPs, as well as the molecular interactions between the adsorbed molecules and the NPs (FT-IR; Nicolet 5700 spectrometer). *In situ* FT-IR analysis was performed under the different conditions such as room temperature, 50°C, 100°C and 150°C.

3.2.3 Magnetization studies

Magnetization curves at room temperature were determined using a physical properties measurement system from quantum design with a maximum applied magnetic field of 20,000 Oe.

3.2.4 TGA analysis

Thermogravimetric analysis (TGA) was carried out by heating few mg of each sample from 25 °C to 800 °C in a TGA-50 instrument from Shimadzu, using a heating rate of 10 °C/min.

3.2.5 CCS and SICS analysis

Confocal Correlation Spectroscopy (CCS), ¹⁰⁹ as well as Scattering Interference Correlation Spectroscopy (SICS), ¹¹⁰ are novel techniques that resemble Fluorescence Correlation Spectroscopy (FCS). As in FCS, the diffusion coefficient of particles is estimated from the transit time of the diffusing particles through the confocal detection volume. However, in contrast to FCS which is based on fluorescence, SICS is based on light scattering plus interference and CCS is based on light scattering only. CCS-measurements were performed on a homebuilt microscope as described earlier ¹¹⁰.

3.2.6 Zeta potential and dynamic light scattering (DLS)

Zeta potential is an electrical potential obtained on the particle at the hydrodynamic plane of shear. Any change in the ion concentration or pH would affect the zeta potential. On the other hand, the dynamic light scattering is used to determine the size distribution of particles in an aqueous solution. In addition, polydispersity index (PDI) was assessed with all five different NPs in solution using a Malvern Zetasizer Nano series V5.03 Worcestershire, UK.

3.3 Response surface methodology

The Central Composite Face-centered design (CCF) was used to create a set of designed experiments by Modde software (Version 9.0). The CCF design is composed of full or fractional factorial design and center points placed on the face sides of the design matrix. The statistical prediction was established according to **Eq. 5** and fitted with the MLR (multiple linear regression method).

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \varepsilon \quad (5)$$

Where, y is the response (yield), $\beta_0, \beta_1, \beta_2, \beta_3$ are the regression coefficient constants, X_1, X_2, X_3 are variables and ε is the experimental error.

3.4 Experimental setup

3.4.1 Column experiment with recirculation batch mode system

The recirculation batch mode system was investigated to find the efficiency of reactive filter materials such as Polonite and Sorbulite in the removal of contaminants from sewage wastewater. A schematic representation is shown in **Fig. 12** and detailed information can be found in Paper I.

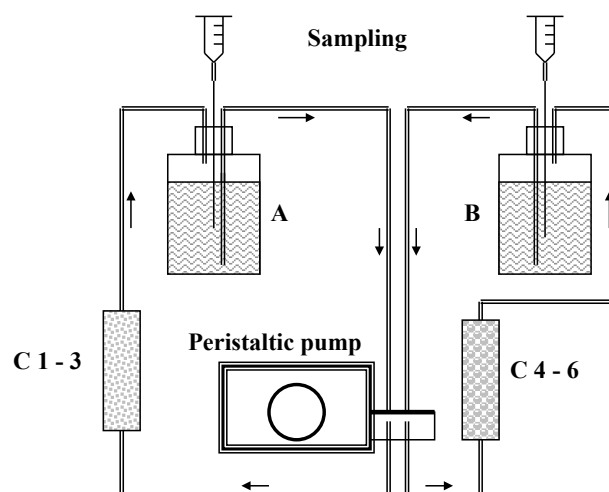


Fig 12. Schematic representation of the experimental set-up under laboratory conditions with columns filled with Polonite (C1-3) and Sorbulite (C4-6). The wastewater from individual bottles was recirculated through the columns.

3.4.2 Wastewater treatment with magnetic NPs

The purpose of this study was to demonstrate the potential use of magnetic NPs in the removal of contaminants from sewage wastewater. An overall schematic setup, employed in the present study is illustrated in **Fig. 13**. For detailed procedures and conditions refer Papers II, IV-VI.

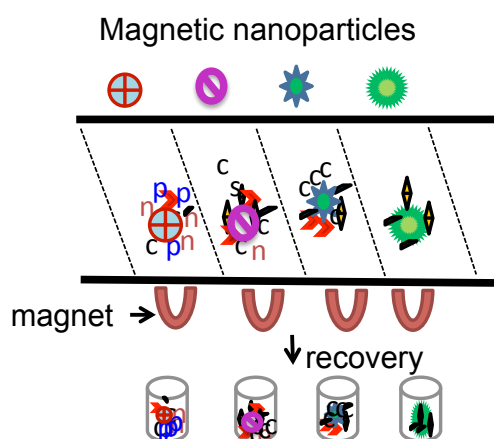


Fig 13. Removal of contaminants using different magnetic NPs.

Sewage wastewater samples were collected from Hammarby Sjöstadverk, Sweden and experiments were performed on the same day of sample retrieval (refer Papers II, IV-VI). Surface water samples were obtained from Lake Brunnsviken/Örlången, Sweden. Detailed experimental conditions are reported in Paper III.

3.4.3 Regeneration of ME-MION

Regeneration and reuse of ME-MION after adsorption of contaminants from sewage wastewater under laboratory conditions is illustrated in **Fig. 14**. A detailed procedure can be found in paper II.

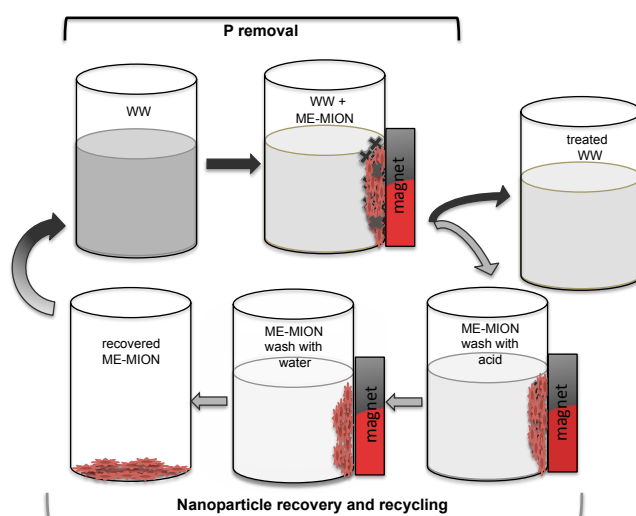


Fig 14. Schematic representation of the experimental setup as an example for phosphate removal, recovery and reuse of ME-MION (WW – wastewater; P – phosphate; ME-MION – microemulsion prepared magnetic NPs; acid washed – NPs washed with 0.01 M HCl; washed with water – NPs washed with de-ionized water).

3.5 Optimization studies

Optimization studies were carried out for magnetic NPs in order to find the suitable concentration and time required for the efficient removal of contaminants from sewage wastewater.

3.5.1 ME-MION and phosphate removal

ME-MION concentrations ranging from 0 to 0.88 g L⁻¹ were tested in 50 ml volume of sewage wastewater. Later 0.44 g L⁻¹ of ME-MION was used to determine the time needed for the removal of phosphate (0, 5, 10, 15, 20, 30, 40, 50 and 60 minutes). ME-MION was separated from the treated samples with an external magnet within 2-3 minutes. In order to reuse the ME-MION after phosphate adsorption from sewage wastewater (0.44 g L⁻¹, 20 minutes mixing), the

collected ME-MION+Phosphate NPs were dispersed in 1 ml of 0.01M hydrochloric acid (HCl) and kept under shaking conditions for 10 minutes (paper II).

3.5.2 MION and nitrogen removal

Three independent variables such as concentration of MION from 2.5 to 25 mg (X_1), time 10 to 60 minutes (X_2) and pH range of 4 to 7 (X_3) were optimized for the removal of total nitrogen with a constant volume (50 ml) of sewage wastewater. A work sheet was generated with a total of 17 experiments with different parameters and 3 center points. Consequently extra data points were conducted under optimum conditions to validate the statistical model obtained. Finally, 320 mg of MION were treated with 1L of sewage wastewater to determine the maximum reduction of total nitrogen and turbidity (Paper IV).

3.5.3 Comparison of ferrous sulphate, protein and MION for reduction of sludge water content

An experiment was performed with different concentrations of flocculants such as ferrous sulphate, MION (5, 10 and 25 mg/L) and coagulant protein (0.25, 0.5 and 1.25 mg/L) were tested to determine the efficiency of the reduction of sludge water content in 100 ml wastewater with a mixing speed of 100 rpm for 5-20 minutes. The experiments were performed in duplicates. After mixing with respective coagulants, the ferrous sulphate and protein treated wastewater were allowed to settle for 60 min; whereas the sludge from MION treated wastewater was separated in less than 5 min using an external magnet (Paper V).

3.5.4 Removal of total organic carbon using PEI coated MION

In this study, experiments were performed in two stages. Firstly, PEI coated MION in different concentrations (10, 50, 100, 200, 300, 400 and 500 $\mu\text{g/ml}$) were mixed with a final volume of 10 ml wastewater. Samples were collected at different time intervals (20, 40, 60, 90 and 120 minutes) and the removal efficiency of TOC was calculated as shown in **Eq. 6**. Secondly, 20 $\mu\text{g/ml}$ of PEI coated MION were incubated with 0.5 L of wastewater for 60 minutes in triplicates to assess the removal efficiency in a large-scale (Paper VI).

$$\text{Reduction (\%)} = \left(\frac{\text{Initial} - \text{Final}}{\text{Initial}} \right) * 100 \quad (6)$$

3.6 Experimental analysis

The analyses of different parameters involved in the present study are described in the following sub-sections (Papers I-VI).

3.6.1 Chemical ion analysis

Chemical analysis of 17 compounds (nitrate, nitrogen, phosphorous, potassium, magnesium, calcium, sulphur, sodium, chloride, manganese, boron, copper, iron, zinc, molybdenum, silicon and aluminum), pH and conductivity of the treated and untreated water sample were carried out at LMI (Lennart Månsson International AB), Helsingborg, Sweden using ICP-OES (Inductively coupled plasma optical emission spectroscopy).

3.6.2 Turbidity, pH and color analysis

Turbidity of water samples was measured using a Eutech (TN100IR) and Hach (2100Q) portable turbidimeter according to ISO standard. Measurements of pH were made using a pH meter of model PHM 95, Radiometer, Copenhagen. Color was measured using a UV-Vis spectrophotometer (Aquamate Thermospectronic, England) at 420 nm.

3.6.3 Phosphate, nitrogen and total organic carbon analysis

Two different methods were used for analysis of phosphate and total nitrogen in water samples. Firstly samples were filtered through a 0.45 μm Sartorius filter prior to analysis of phosphate and inorganic species of nitrogen, i.e. nitrite, nitrate and ammonium ($\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{+N}$). This analysis was performed using Flow Injection Analysis (FIA, Aquatec-Tecator, Sweden). Secondly phosphate content was analysed in the samples before and after treatment using PhosVer 3 phosphate reagent pillow from the HACH Company. Total nitrogen high range set was obtained from VWR International; Sweden (with a persulfate digestion method 10 to 150 mg/L N) for the detection of total nitrogen content in water samples. Total organic carbon was analysed using TOC-5000 equipment from the Shimadzu Corporation, Japan.

3.6.4 Langmuir Isotherm

Generally Langmuir and Freundlich adsorption models represent adsorption isotherm. These methods are performed to investigate the coverage or adsorption of molecules on a solid surface at a fixed temperature. Among them Langmuir isotherm is represented as shown in **Eq. 7**.

$$\frac{Y}{M} = \frac{abC}{1 + aC} \quad (7)$$

Where,

Y = concentration of pollutant adsorbed, mg/L

M = concentration of adsorbent used, mg/L

C = remaining concentration in treated water samples, mg/L

a,b= constant (graphically determined)

3.6.5 Sludge water content

Flocculants such as ferrous sulphate and protein treated samples were allowed to settle for 1 hour followed by centrifugation. Sludge with MION was separated by magnet and the water present in the sludge was removed by centrifugation. See paragraph 3.5.3.

3.6.6 Microbial analysis

The microbial content (Colony Forming Units; CFU/ml) of the water samples was analysed by plating untreated and treated water samples on nutrient agar plates that were subsequently incubated at different conditions (Room Temperature (RT), 30°C & 37°C) in triplicates. Average colony count was then recorded. In surface water samples, the number of colonies representing the Gram negative and Gram-positive organisms were observed after 48 hours on nutrient agar; MacConkey and phenyl ethyl agar plates respectively. A selective medium eosin methylene blue (EMB) agar was used for the detection of *Escherichia coli* (forming metallic green) sheen and bile esculin (BA) agar for the detection of *Enterococci* (change of the medium color to black).

3.7 Toxicity assessment on magnetic NPs

The toxicity of functionalized MION was evaluated using human dermal microvascular endothelial (HMEC-1) and keratinocytes (HaCaT) cells. Two different cell densities i.e. 1×10^5 and 5×10^4 cells/mL were used for both the cell lines. All five magnetic NPs (core, TSC, PEI, APTES and chitosan coated) were tested with a concentration range of 0 – 200 mg/L. The toxicity test assayed multiparameter apoptosis, cell viability, intracellular reactive oxygen species, DNA strand damage and nuclear morphology. The behaviour of magnetic NPs in cell culture media was studied and subsequently compared with NPs in solution (Paper VII).

4. RESULTS AND DISCUSSION

The overall aim of the thesis is to investigate the application of reactive filter materials and magnetic NPs in wastewater treatment. Although some of the data presented in this section is not yet published, they reflect outcomes appended in papers I-VII. This section is focussed on treatment of lake/wastewater, highlighting on five aspects; (i) reactive filter materials, (ii) microemulsion prepared magnetic iron oxide nanoparticles (ME-MION) (iii) magnetic iron oxide nanoparticles (MION) prepared from co-precipitation method, (iv) toxicity of MION in human cells under *in vitro* conditions and (v) sequential treatment process/recovery of magnetic NPs under laboratory conditions.

4.1 Application of reactive filter materials (Paper I)

The results from the present study shows that Polonite has an average removal of 80% phosphate compared with 75% in Sorbulite over a 90 day period (**Fig. 15**). The initial pH of Polonite and Sorbulite was 13.4 and 9.1 respectively.

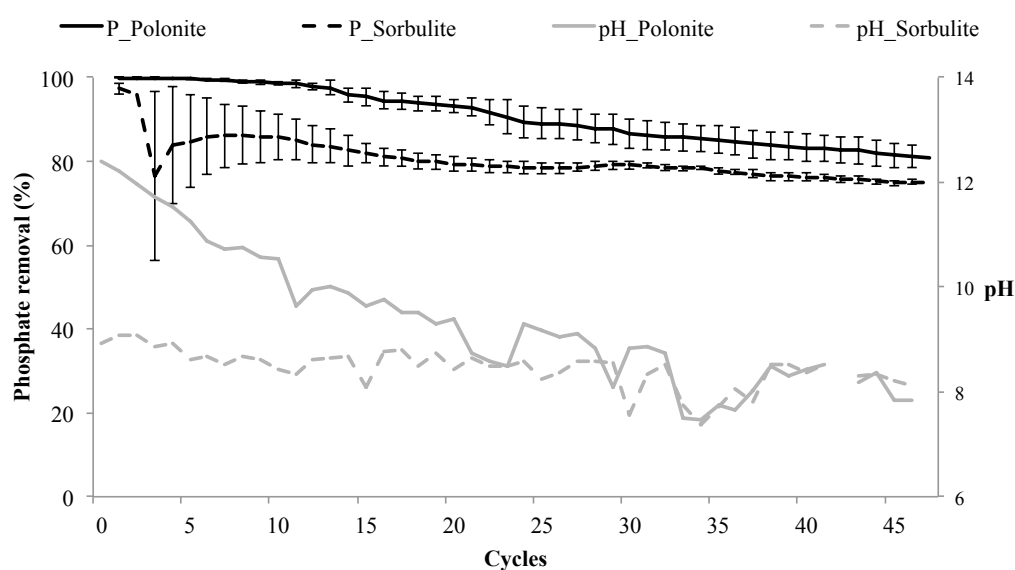


Fig 15. Average reduction of pH and phosphate (P) removal in Polonite and Sorbulite.

The differences in phosphate removal with these two reactive materials were mainly due to chemical composition, size and pH of the materials. Although the removal of phosphate was higher with Polonite as compared to Sorbulite, the amount of phosphate adsorbed to the materials using mass-balance calculations were higher in Sorbulite. This was due to the difference in physical properties of the Sorbulite such as its high specific area and higher porosity¹¹¹.

Other filter materials used for phosphate removal include Filtrate P, Shellsand and Turkish Zeolite with a wide range of particle diameters. Several studies have been reported for the removal of phosphate using filter materials in synthetic aqueous solutions, compared to real wastewater samples ¹¹¹⁻¹¹³. The results from these studies suggest that an increase in phosphate adsorption occurs due to the high concentrations of calcium content present in the materials. Moreover in the present study, the average reduction of total inorganic nitrogen and organic carbon was found to be 11% -51% and 23% -1% in Polonite and Sorbulite respectively over a period of 90 days. The negative effect was due to the higher load of organic carbon in wastewater from each batch owing to column recirculation for a minimum of 24 hours. Detailed information about the removal of organic carbon and total nitrogen can be found in paper I. As the age of the column material increases, the pores on the material surface were filled with particles from wastewater, thereby leading to change in flow rate and a clogging of the columns. It was also observed that as the number of batches increased, there was a direct impact on removal efficiency of the contaminants.

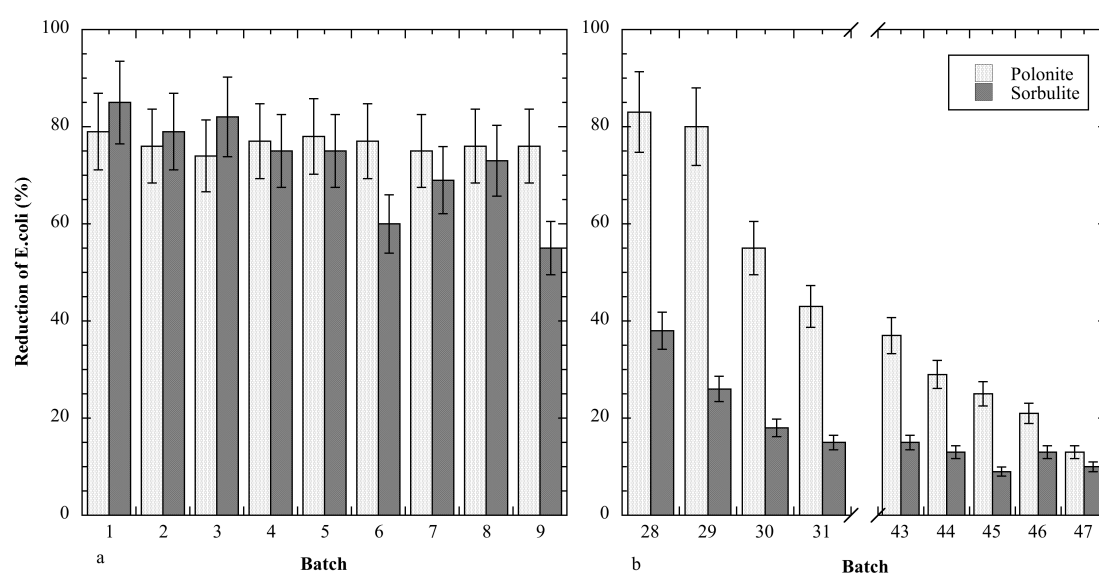


Fig 16-A. Percentage reduction in *E. coli* using Polonite and Sorbulite, (a) batches 1-9; (b) batches 28-31 and 43-47.

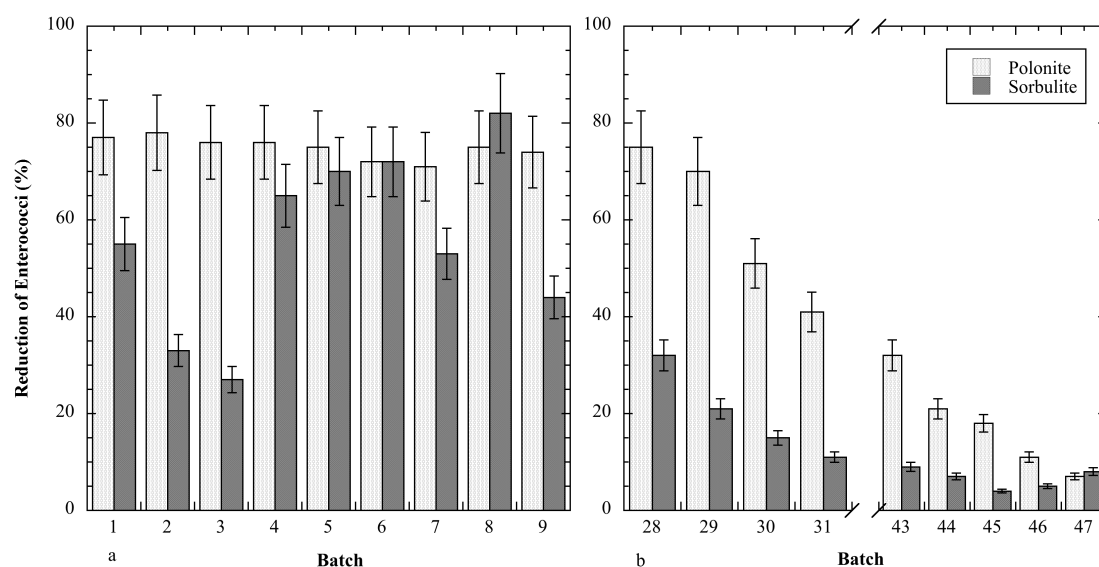


Fig 16-B. Percentage reduction in *Enterococci* using Polonite and Sorbulite, (a) batches 1-9; (b) batches 28-31 and 43-47.

Approximately 80% reduction of *E. coli* was observed in the beginning of the recirculation batch systems using Polonite and Sorbulite, whilst the efficiency was decreasing as the number of batches increases. Polonite exhibited around 80% reduction of *Enterococci* until batch 28, whereas in the case of Sorbulite, the reduction efficiency was around 60% in the batches 1, 4 to 6 and 8 (**Fig. 16A,B**). The removal efficiency of *E. coli* and *Enterococci* decreased with time as compared to initial batches. The reduction of organic carbon and bacteria was shown to possess a close relationship with surface loading on the materials, thus affecting the adsorption sites. In addition, materials packed in a column can have less interaction with the contaminant, thereby resulting in a decrease of process efficiency per unit of time. Other limitations include high circulation time, higher effluent pH, surface porosity and regeneration of the reactive filter materials.

4.2 Application of ME-MION (Paper II&III)

Microemulsion prepared magnetic iron oxide nanoparticles (ME-MION) were synthesized and characterized according to Okoli et al ¹⁰⁵. In order to evaluate ME-MION for wastewater treatment, initial screening study was performed using sewage wastewater collected from Hammarby Sjöstadswerk, Sweden and the initial concentration of phosphate was found to be approximately 8-10 mg/L in sewage wastewater. At optimum dosage of ME-MION (0.44 g/L), $\approx 100\%$ phosphate removal was achieved in 20 minutes. FT-IR analysis confirmed the interaction of ME-MION+Phosphate in simulated water (i.e. potassium phosphate dissolved in distilled water). Earlier studies have reported that 99% phosphate removal was achieved in 1 minute when using a mesoporous silicon support (Fe-EDA-SAMMS) tested in an aqueous solution ^{114,115}. However, it is a fact that phosphate

precipitates at higher pH ¹¹⁶ in solution but in the present study, initial wastewater was in the range of pH 7 to 8.5. For this reason it could be speculated that the hydrophilic interaction with ME-MION enhances the phosphate removal efficiency in a shorter period of time, thereby forming a $\approx\text{Fe}_2\text{PO}_4$ complex. After determining the effective removal of phosphate from sewage wastewater, regeneration studies and reusability of ME-MION was studied (**Fig. 17**). From this experiment, it was noted that ME-MION exhibits a minimum reusability potential for 5 times and subsequently in removing the adsorbed impurities with mild cleaning solutions.

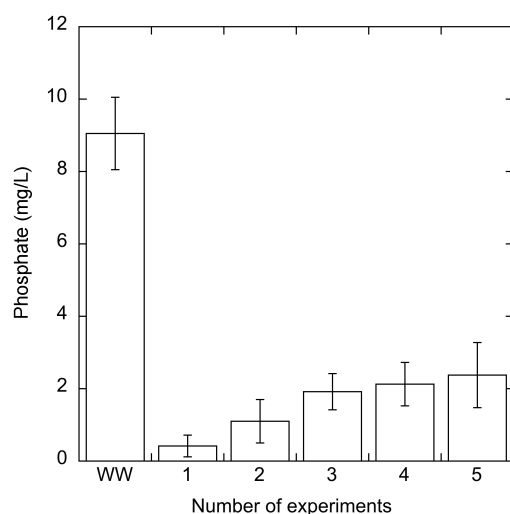


Fig 17. Re-use of ME-MION for 5 times after phosphate adsorption.

As a next step, ME-MION was tested on two different lake water samples to further investigate any change in mineral ion concentration before and after treatment. From earlier studies ⁹² protein functionalized ME-MION (MOCP+ME-MION) were shown to remove suspended particles and therefore MOCP+ME-MION were also examined in this study to find out the differences in mineral ion composition. Results suggest that there was no significant change in mineral ion composition. The pH and conductivity was similar in all the samples. It was also observed that low concentrations of phosphate ions were found in lake water samples. Conversely, ME-MION was not as efficient in removing phosphate in the case of lake water samples. This might be due to the very low concentration of phosphate in the lake water with varied chemical composition and different complexity ⁹¹.

From the microbial analysis, it was predicted that Gram-negative microorganisms were predominant in both lake water samples. The possible mechanism of interaction between ME-MION and microorganisms would be van der Waals forces and irreversible adsorptions ¹¹⁷. The ME-MION tested in this study was owing to the simple synthesis method and possible reusability. ME-MIONs are

therefore potential candidates for phosphate removal in WWTP. Nevertheless some drawbacks include the use of organic solvents in synthesis of NPs and low yield compared to the co-precipitation method.

4.3 Application of MION (Papers IV-VI)

4.3.1 Optimization studies using response surface methodology (Paper IV)

As a first step, optimization studies were performed using real sewage wastewater with a central composite face centered (CCF) model for the evaluation of turbidity and total nitrogen reduction. For clarity, core NPs in this investigation were prepared by the co-precipitation method. The multiple regression fit obtained from theoretical and experimental evidence for turbidity and total nitrogen content was 0.93 and 0.95 respectively. The co-efficient plot describes the significant model term regarding the investigation in efficiency of magnetic NPs for the maximum reduction of turbidity and total nitrogen (**Fig. 18**). The optimal pH was around 5.5 and at this pH, the core NPs are protonated and become stable due to adsorption on the surface. Since NPs have a large surface-to-volume ratio and they tend to form complexes with other contaminants that also promote the binding efficiency.

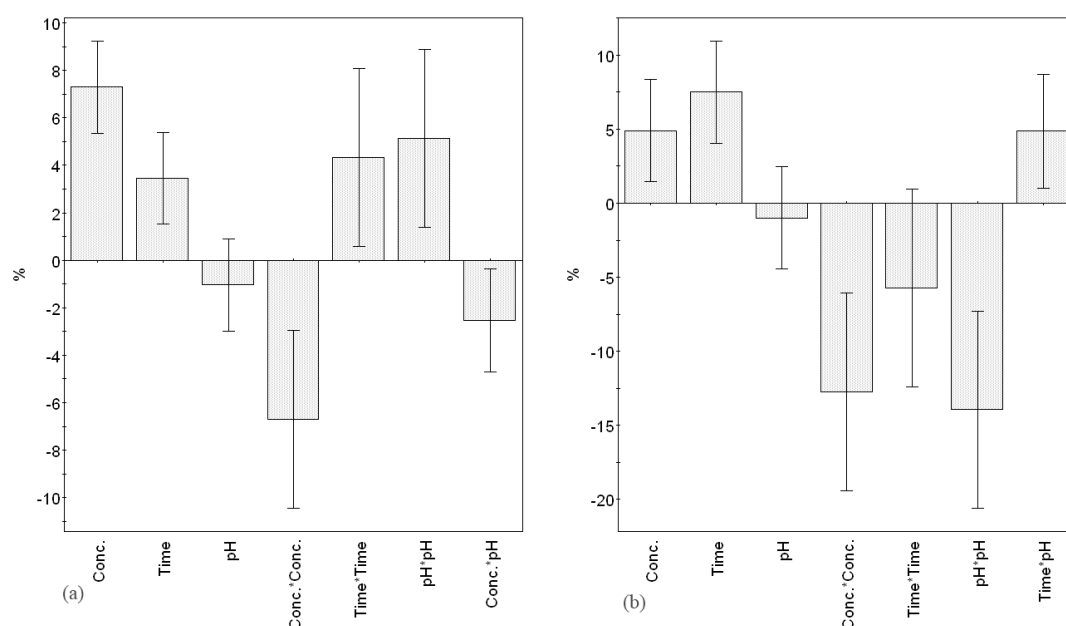


Fig 18. Coefficients plot for significant model terms (a) turbidity and (b) total nitrogen (X-axis – Interaction; Y-axis – Percent change in confidence interval).

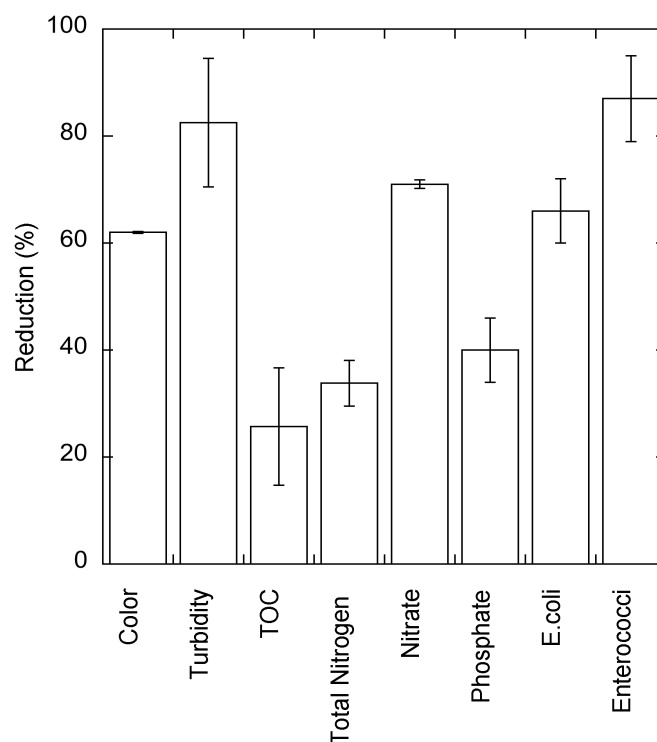


Fig 19. Removal of contaminants at optimum conditions using core NPs.

The maximum removal of turbidity and total nitrogen was $\approx 83\%$ and $\approx 34\%$ respectively when using 320 mg/L of core NPs within 60 minutes. In addition, other contaminants such as color ($\approx 62\%$), phosphate ($\approx 25.5\%$), nitrate ($\approx 71\%$), organic carbon ($\approx 40\%$) and microbial content ($\approx 75\%$) were also removed (**Fig. 19**). Hwang et al ¹¹⁸ stated that nitrogen removal generally occurs due to adsorption, size exclusion and volatilization along with complex organic substances in the presence of NPs. In contrast, the activated sludge process involves anaerobic and aerobic digestion to accomplish the removal of nitrogen compounds with excess sludge production. From this perspective, the advantages of magnetic NPs include the removal of several contaminants within one hour and the possibility of recovery of the NPs after treatment.

In reality, the usage of 320 mg/L core NPs in wastewater treatment corresponds to high NPs concentrations that might limit their practical feasibility. Additionally the excessive amount of iron oxide present in the sludge is concerning given that it may lead to corrosion or influence the sludge handling process. Conversely, the usage of core NPs has an advantage, that they permit the removal of $\approx 30\%$ total nitrogen within 60 minutes. Earlier studies suggest that when iron (III) phosphate was added, reduction of H_2S was observed due to binding of sulphides in the sludge digester and making them insoluble ¹¹⁹. Moreover in conventional WWTP, sludge contains $\approx 90\text{-}95\%$ water content ¹⁷ and as a consequence it increases the volume and cost of trucking to the disposal site. Therefore in light of the present

study, it is of interest to investigate the effect of MION on reduction of sludge water content.

4.3.2 Reduction of sludge water content using MION (Paper V)

Sludge from sewage wastewater was taken into consideration because of the expense involved in total treatment cost ¹²⁰. According to Appels et al ¹²¹ sludge treatment and disposal constitutes up to 50% of the total treatment cost. In the present study flocculants like coagulant protein ¹²², core NPs ⁹⁰ and ferrous sulphate were employed to find the optimum conditions in the reduction of water content in sludge. Several parameters were investigated for the optimization of flocculants including water content in sludge and the wet and dry weight of sludge and turbidity. Concentrations of flocculants used were approximately similar with the addition of chemical used in WWTP. Protein concentrations were 20 times lower than chemical (ferrous sulphate) and core NPs concentrations. It was observed that the water content after treatment with core NPs was significantly lower and quick separation of sludge by an external magnet in less than 10 minutes was achieved (**Fig. 20**). As discussed earlier, tense formations surrounding NPs avoided in trapping water molecules and thereby increased the separation of sludge from effluents.

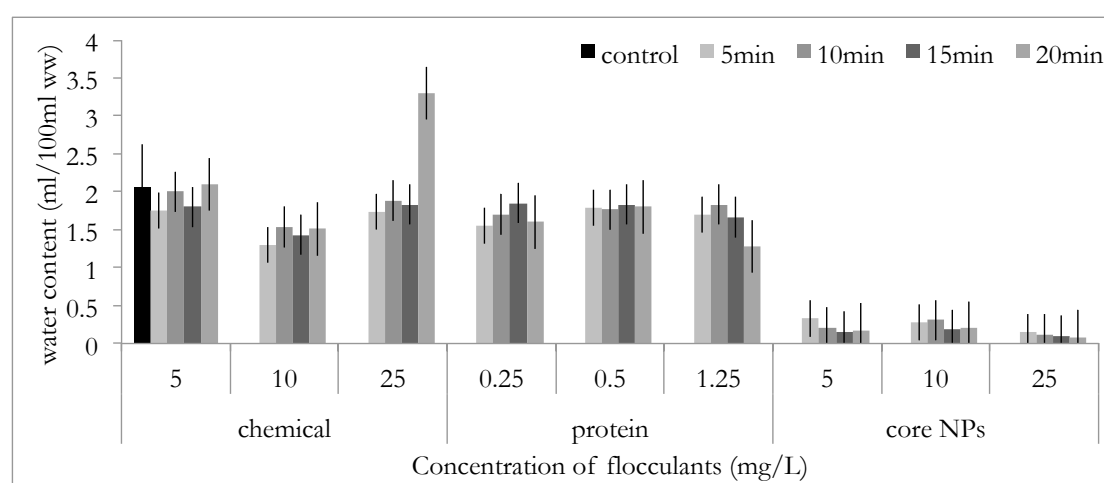


Fig 20. Concentration and time kinetics of flocculants (chemical, protein and Core NPs) on sludge water content.

Conversely, other parameters such as turbidity and color did not show much difference when compared to sludge water content. In the large volume experiments, i.e. 500 ml, a similar trend was observed for all the flocculants tested on the reduction of sludge water content. The flocs formed by different flocculants after mixing with wastewater were observed under the microscope. The formation of flocs was very different in all three cases; ferrous sulphate, coagulant protein and core NPs treated wastewater samples. According to Leong et al ¹²³ particle size within 10 to 100 μm are classified as normal whereas, in the

present case, flocs formed by the chemical and protein flocculants was between 50 μm to 200 μm . In addition samples treated with core NPs indicated particles sizes above 200 μm and this confirms the strong electrostatic interaction between the core NPs and contaminants present in wastewater. When compared with optimization studies performed using response surface methodology and sludge water content, experimental data revealed that the concentration of core NPs did not influence the microbial reduction. However there was no significant reduction of turbidity and color at 25 mg/L of core NPs used, whereas the reduction of turbidity was $\approx 81\%$ within 60 minutes when 12.5 times higher concentration (i.e. 320 mg/L) of core NPs was used. Other studies reported that aluminium and silica based polyelectrolyte materials used in wastewater treatment showed $\approx 84\%$ turbidity removal at a dosage of 50 mg/L ¹²⁴. Earlier studies have also reported that magnetic NPs act as a catalyst in the presence of hydrogen peroxide as a Fenton process ¹²⁵. Therefore the dosage of MION plays a major role in effective removal of contaminants from wastewater. Keeping in mind the dosage of magnetic NPs used for the removal of contaminants in wastewater, it would be interesting to functionalize magnetic NPs and study their efficiency in the removal of specific contaminants.

4.3.3 Functionalized MION for removal of contaminants

In order to stabilize the core NPs and increase the removal efficiency of contaminants in wastewater, functionalization of MION plays a vital role ¹²⁶. As mentioned earlier ¹²⁷, proper surface coating also stabilizes iron oxide NPs, thereby preventing dissolution and release of toxic ions.

MION were coated with TSC, APTES, PEI and chitosan to investigate their potential in the removal of contaminants. Results from the preliminary studies suggest that APTES and chitosan coated MION showed a lower significance in removal of specific contaminants in wastewater samples (**Fig. 21**). Hence, detailed study of MION is required in order to understand and enhance the reduction efficiency. However, there was an increase in the reduction of turbidity and organic carbon when treated with uncoated MION and PEI coated MION was observed. A detailed investigation of uncoated MION was performed earlier (see section 4.2 and 4.3). Hence, PEI coated MION have been selected for further study in order to find out the efficiency in the removal of contaminants for potential application in wastewater treatment.

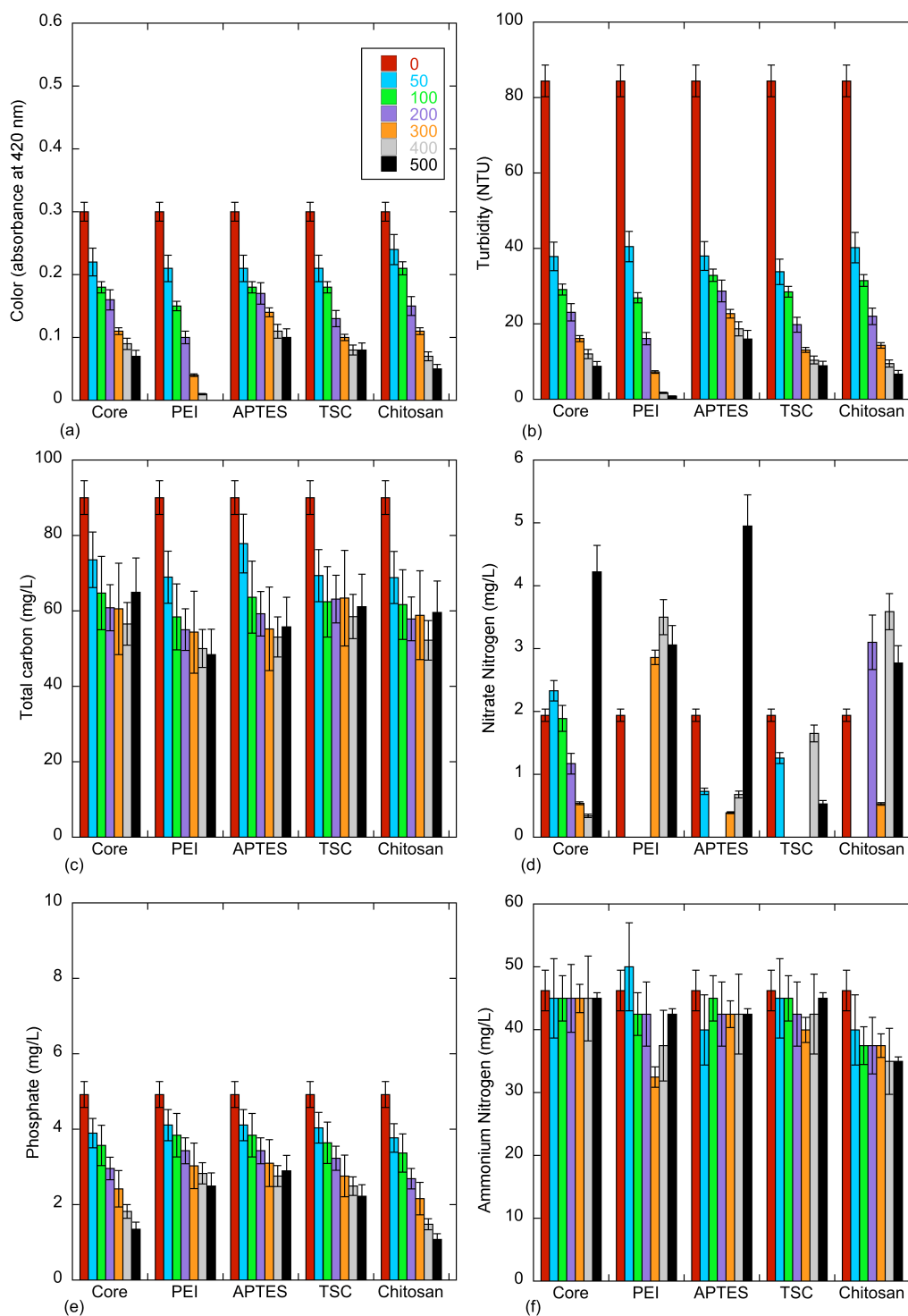


Fig 21. Preliminary treatment using all five different functionalized/coated MION (Core, PEI, APTES, TSC and Chitosan coated). Reduction of contaminants observed were (a) color, (b) turbidity, (c) total carbon, (d) nitrate nitrogen, (e) phosphate, (f) ammonium nitrogen. The concentration of NPs used for this screening study was 0-500 µg/ml. (Core represents the uncoated MION)

4.3.4 PEI coated MION in WWT (Paper VI)

The PEI coated MION were synthesized and extensively characterized by TEM, XRD, SICS, CCS, TGA, FT-IR, Zeta potential and BET analysis. TEM showed an average diameter of 12 nm with dense aggregates and quasi-spherical morphologies (**Fig. 22a**). Moreover aggregation of NPs was mainly due to their surface energy, and dependent upon experimental conditions such as temperature, pH, reaction environment and surface chemistry ^{106, 128}.

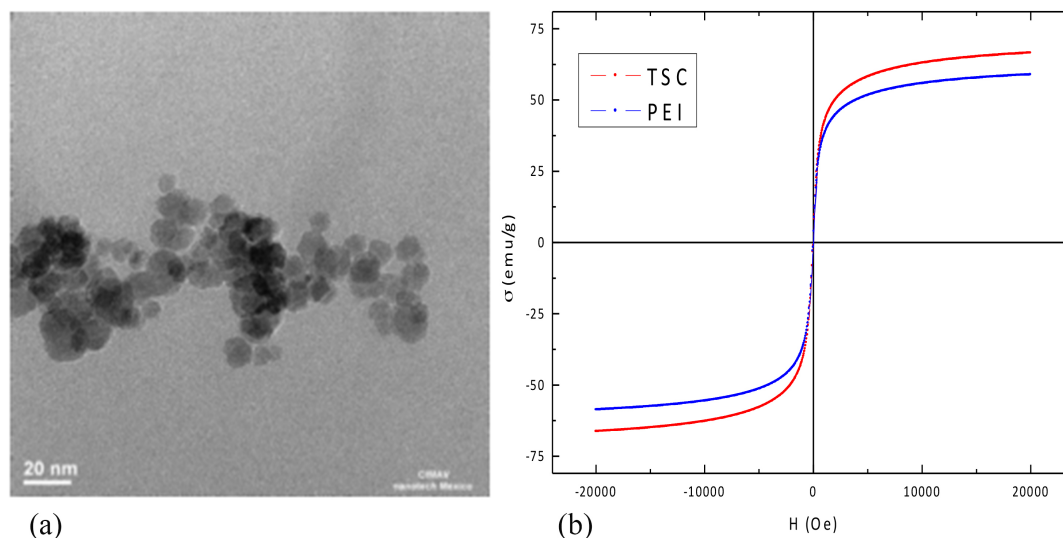


Fig 22. TEM image (a) and superparamagnetic property (b) of PEI coated MION.

Results from magnetization properties of TSC and PEI coated MION are presented in **Fig 22b**. It can be seen that both TSC and PEI coated MION revealed a superparamagnetic behaviour. The TSC and PEI coated MION depicts maximum specific magnetization $\sigma_{\max}=66.4$ emu/g and $\sigma_{\max}=58.8$ emu/g respectively. Conversely, PEI coated MION exhibited lower magnetization than TSC coated MION due to the thickness on the magnetite surface. The zeta potential of PEI coated magnetic MION was found to be 33.14 mV at pH 7, which is in correlation with an earlier report ¹²⁹. FT-IR analysis confirmed the carboxylate group $\nu_{\text{asym}}(\text{COO}^-)$ at 1470 cm^{-1} from TSC and C-N stretching for PEI at 1556 cm^{-1} vibration peak as reported earlier ¹³⁰⁻¹³². Specific surface area (SSA) of PEI coated MION was found to be $85.1\text{ m}^2/\text{g}$.

The laboratory experiments were performed using PEI coated MION for the concentration (0-500 $\mu\text{g}/\text{ml}$) and time (0-120 minutes) kinetics studies in the removal of TOC from sewage wastewater. At higher concentrations of PEI coated MION (i.e $\geq 100\text{ }\mu\text{g}/\text{ml}$), lead to an increase in the total organic carbon and nitrogen content in the treated samples (**Fig. 23a**). It was observed that 20-40 $\mu\text{g}/\text{ml}$ of PEI coated MION was optimum for removal of TOC (**Fig. 23b**).

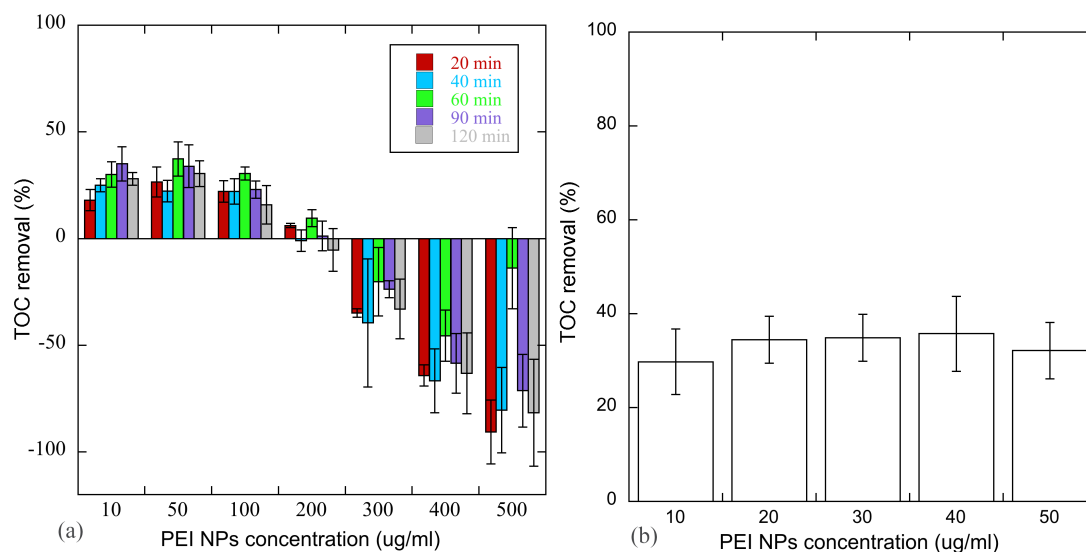


Fig 23. Study of the removal of total organic carbon (TOC) from sewage wastewater, (a) Concentration and time kinetics (b) Low concentration kinetics at 60 minutes.

Earlier studies have also reported that the presence of amine groups on magnetite NPs enhances their potential to remove heavy metals such as copper, cadmium, nickel, zinc, lead and arsenic from synthetic solutions ⁹³. As described earlier ¹³², adsorption and complex formation on NPs depend upon the pH of the solutions. Moreover, it was evident that apart from TOC, other contaminants were also removed (**Fig. 24a,b**). This was correlating from SICS and CCS analyses indicating an average increase of size (aggregation), 20 times more than in PEI coated MION after treatment.

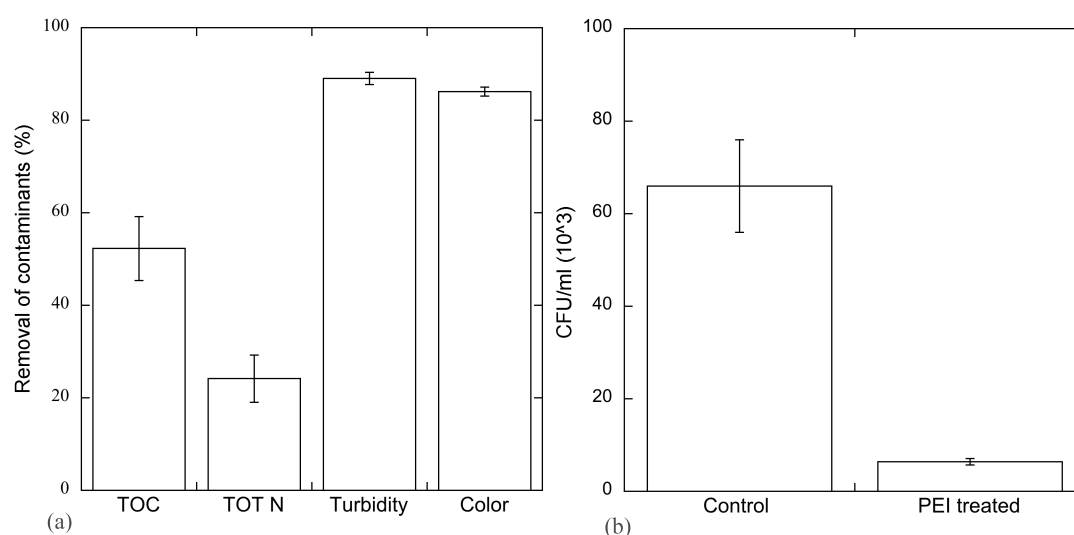


Fig 24. Removal of contaminants (a) TOT N, TOC, color, turbidity and (b) microbial content at optimum conditions using PEI coated MION. (TOC – Total organic carbon; TOT-N – Total nitrogen)

As compared to uncoated MION (section 4.3.1), microbial reduction in PEI treated samples was significant due to their positive charge (NH_2^+) surface. Moreover, the concentration used was considerably lower than in any other NPs tested in this study. For instance, the concentration of ME-MION and core NPs used in the removal of contaminants are approximately 10 to 12 times higher than PEI coated MION. Although 50% of organic carbon was reduced when using PEI coated MION at optimum conditions, further sequential step processes could enhance the removal efficiency. Conversely, it is also essential to assess the toxic effects of NPs before application in wastewater treatment ^{6,46,101}. Though magnetic NPs will be recovered along with contaminants using an external magnet, it would be of interest to test the toxicity of MION for sustainable WWTP.

4.4 Toxicity studies using MION (Paper VII)

Paper VII focussed on toxicity study on the core (uncoated MION), TSC and PEI coated MION. This section also includes toxicity studies on APTES and chitosan coated MION. The influence of surface charge on the NPs and media composition was observed using zeta potential, dynamic light scattering and poly dispersibility (**Table. 5**). In the present study MION dispersed in water were stable however, a corona layer formed on the surface of MION when exposed to cell culture media. In particular, the entire positive surface charge was transformed to negative due to the strong electrostatic interaction ¹⁰¹. Therefore this effect would have also caused interference in the toxicity assessment. Although the concentration used for toxicity studies are higher, it is still necessary to see the effect and behaviour of both selected cell lines.

Table 5. Magnetic NPs effect with surface charge and media composition.

NPs	Size (Z-average d. nm)			Zeta potential			PDI		
	H ₂ O	HMEC-1 medium	HaCaT medium	H ₂ O	HMEC-1 medium	HaCaT medium	H ₂ O	HMEC-1 medium	HaCaT medium
uncoated	5845	8858	9913	-27.1	-8.25	-7.68	0.98	0.68	0.66
MION (core)	±873.9	±934.7	±698.9	±1.1	±1.3	±1	±0.1	±0.3	±0.2
TSC	810.4 ±42.2	1370 ±43.1	1217 ±66.7	-32.1 ±1.7	-8.46 ±0.7	-7.4 ±0.9	0.51 ±0.1	0.55 ±0.2	0.81 ±0.1
PEI	3459 ±157.6	5255 ±212.5	4081 ±464.1	21.8 ±0.2	-3.9 ±1.2	-4.45 ±0.5	0.64 ±0.1	0.25 ±0.1	0.86 ±0.2
APTES	5338 ±189.3	5675 ±1074	4882 ±455.9	4.37 ±0.4	-8.3 ±0.8	-9.7 ±0.6	1 ±0.0	0.63 ±0.2	0.72 ±0.5
Chitosan	1223 ±71	2553 ±148.0	1597 ±897.7	40.5 ±0.5	-5.37 ±0.9	-9.5 ±1.5	0.6 ±0.1	0.47 ±0.1	0.59 ±0.3

The experimental results suggest that cell proliferation was slightly lowered in both cells depending on the concentration of MION tested (Table. 6). Cytotoxicity of magnetic NPs decreased with higher levels of confluence obtained with endothelial cells, whereas keratinocytes cells showed an opposite behaviour. This could also be due to the different cell type and cell culture medium ^{133,134}.

Table 6. Summary of magnetic NPs (200 mg/L) in toxicity assessment.

HMEC - 1 (1*10⁵)					
Test	Core	TSC	APTES	PEI	Chitosan
Cell viability	X	X	X	XX	X
Mitochondria membrane potential	XXX	★	★	★	★
Commet assay	XXX	XXX	XXX	★	★
Intra cellular ROS	★	★	★	★	★
Cell free ROS	★	★	★	★	★
HaCaT (1*10⁵)					
Cell viability	#	#	#	#	XXX
Mitochondria membrane potential	XXX	XXX	XXX	XXX	★
Commet assay	XXX	★	#	★	XXX
Intra cellular ROS	★	★	★	★	★
Cell free ROS	★	★	★	★	★

(X - Slight decrease; XX - Medium decrease; XXX - Highest decrease; ★ - No significant effect; # - Improved cell viability).

TEM images were taken from the cells treated with 200 mg/L of MION (Fig. 25), it was noted that the nuclear membrane was not damaged and MION resulted in forming either small or large vesicles close to the nucleus. The uptake

of MION with negative surface charge is favourable in endothelial cells (HMEC-1) compared to keratinocyte (HaCaT) cells. Though a corona layer formed for positively charged surfaces of the MION, it was observed that HaCaT cells have more internalization of MION than HMEC-1 cells. The experimental results suggest that cell proliferation was slightly lowered in endothelial cells depending on the functional surface of the MION. However, the cell viability was not affected in keratinocyte cells except chitosan coated MION. Moreover, when compared to the positive control (H_2O_2), magnetic NPs did not produce ROS though high concentration (200 mg/L) was used. More detailed information can be found in paper VII.

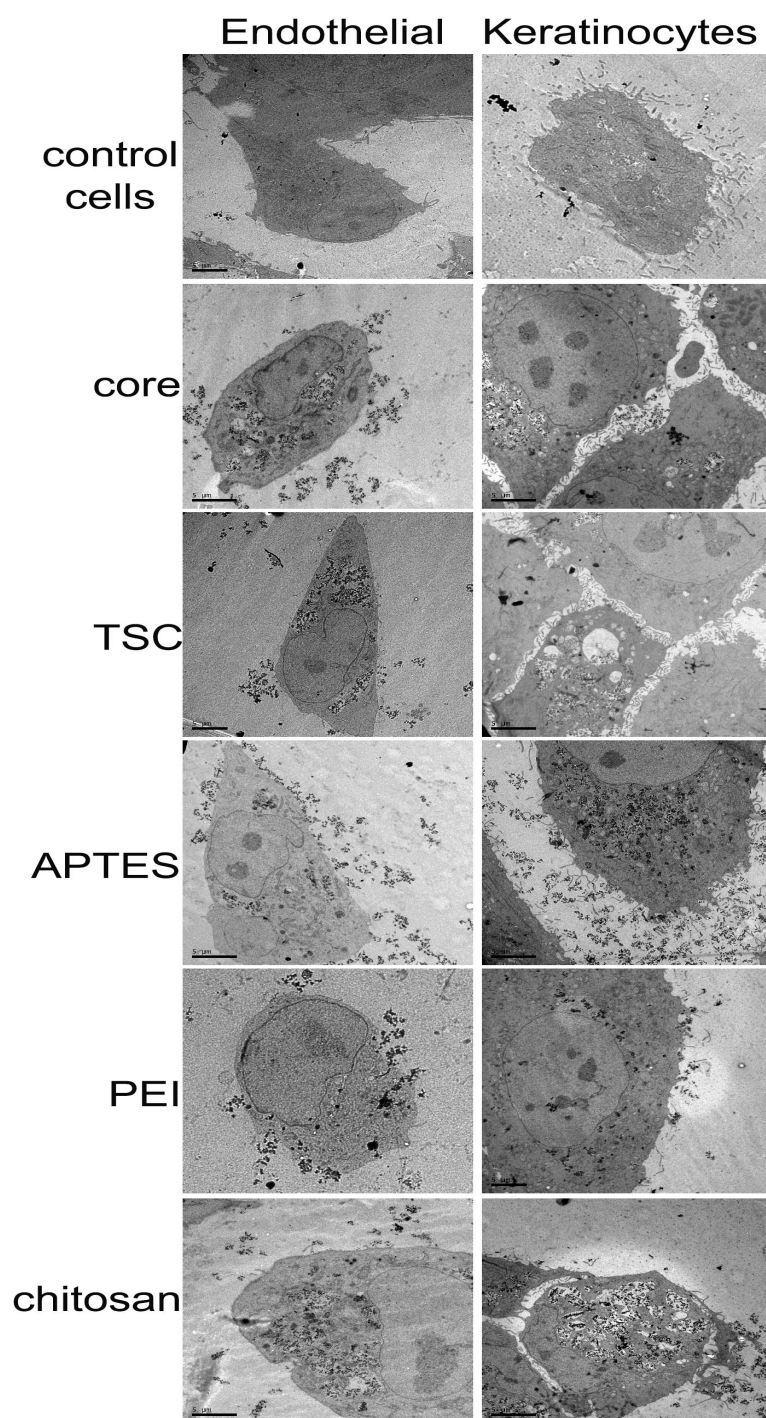


Fig 25. TEM images showing the uptake of magnetic NPs (200 mg/L) in endothelial and keratinocytes cells (Core represents uncoated MION).

4.5 Sequential treatment steps using magnetic NPs

In order to investigate the use of MION in WWTP, randomized processes were conducted as shown in **Fig 26**. Several concentrations of different surface modified MION were tested under laboratory conditions for the removal of contaminants. As discussed in section 4.3.2, the present investigation helps to obtain low water content in the sludge when compared to the activated sludge

Table 7. Comparison of WWTP with activated sludge process and magnetic NPs.

Process parameters	Inlet wastewater	Effluent - activated sludge process	Effluent - magnetic nanoparticles process
Phosphate (mg/L)	7-11	1	0
Nitrate nitrogen (mg/L)	18-25	50	5±1
Turbidity (NTU)	90-120	4	≤5
Color @ 420nm	greyish white	white	white
Total carbon (mg/L)	120-150	25±4	57±5
Ammonium nitrogen (mg/L)	42-50	8±3	40±8
Total suspended solids (mg/L)	800	10	5±2

The question of NPs recovery is always critical and therefore an investigation was conducted on the recovery of all five types of magnetic NPs before and after treatment. The surface water was preferred due to the limited ions and not enriched with nutrients like wastewater samples. It was evident that due to the magnetic property of NPs $\geq 99\%$ were recovered (**Fig. 27**).

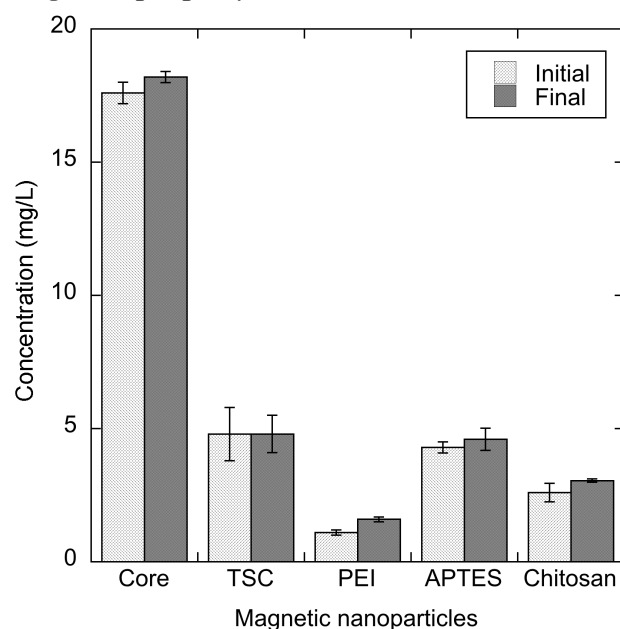


Fig 27. Recovery of magnetic NPs after treatment with lake water. The lake water was taken and different functionalized MION were added, incubated and separated i.e. Initial and Final concentration of NPs (Core represents uncoated MION).

In summary MION have potential benefits in the reduction of contaminants from sewage wastewater due to their large surface-to-volume ratio. For instance in the

case of process steps mentioned in section 4.5, it was evident that except ammonium nitrogen, other contaminants were removed within 90 minutes when compared to existing WWTP where the approximate time taken was 15-20 hours with excess sludge production. Conversely, when compared to chemical flocculent (ferrous sulphate), there was a substantial decrease in sludge water content using MION. Therefore, benefits include the lowering of sludge handling costs and excess sludge production. In addition, magnetic separation helps in recovery, regeneration and recycling of MION. From the toxicity studies, it was clear that the effect of magnetic NPs on cell lines were dependent on dosage and culture media. Even though surface modified MION have a wide pH tolerance, it is crucial to maintain the pH above 7 for efficient removal of contaminants in wastewater treatment. By using magnetic NPs, reduction in volume of secondary waste would be significant, and hence, desirable for potential application in WWTP.

5. CONCLUSIONS

The work presented in this thesis has been focussed on the efficient use of magnetic iron oxide nanoparticles (MION) in sewage wastewater treatment processes. Magnetic NPs with different surface chemistry were synthesized by microemulsion and conventional co-precipitation methods. Three main surface charges such as hydroxyl, carboxyl and amino groups were used to functionalize the NPs and characterized for their physical and chemical properties. All the synthesized magnetic NPs were in the size range of 10 – 40 nm. The efficiency of reactive filter materials in recirculation batch mode was also studied in the light of contaminant reduction.

Two filter materials such as Polonite and Sorbulite resulted in high removal of phosphorous as well as nitrogen, therefore having a potential use in WWTP in recirculation batch mode. However high effluent pH and organic loads needs further investigation. In the case of ME-MION, $\approx 100\%$ phosphate removal was achieved in 20 minutes from sewage wastewater under laboratory conditions. The interaction mechanism using FT-IR and *Langmuir* isotherm validates the experimental (*in vitro*) data in terms of adsorption. To the best of our knowledge and according to published data, ME-MION is the first report on removal of phosphate in real wastewater samples. No significant difference was observed in mineral ion composition before and after ME-MION treatment.

From the modelling studies, at optimum concentration of core NPs, the maximum removal of turbidity and total nitrogen achieved was $\approx 83\%$ and $\approx 34\%$ respectively. Conversely, experimental data obtained in the present study are in agreement with the predicted values. On the other hand, at an optimum dosage of 10 mg/L core NPs, $\approx 87\%$ removal of sludge water content was observed in large volume experiments when compared to ferrous sulphate used in today's chemical flocculent process. When using PEI coated MION at a concentration of 20 mg/L, it was clear that 50% total organic carbon could be reduced within 60 minutes. Furthermore, using PEI coated MION, it was possible to reduce turbidity and microbial contaminants by $\approx 80\%$. Equally, no increase of the iron concentration was observed in the treated samples that also confirm the stability and superparamagnetic behavior of PEI coated MION. The recovery of magnetic NPs with the help of an external magnet is an additional benefit.

Toxicity of magnetic NPs was concentration dependent when exposed to keratinocytes and endothelial cells. A change in surface charge of the magnetic NPs was observed due to the interference from cell culture medium. However, MION did not produce ROS and did not influence in DNA strand breakage.

Though there is a concern regarding the effect of NPs in the environment, the present study indicates that: magnetic NPs can be recovered, MION has the potential to reduce sludge water content even at low concentrations, PEI coated MION exhibited reduction of $\approx 50\%$ TOC, $\approx 80\%$ turbidity, $\approx 80\%$ color and $\approx 80\%$ microbial content in 60 minutes and ME-MION revealed $\approx 100\%$ phosphate removal with regeneration and reusability for at least 5 consecutive times. In summary, magnetic NPs have the potential to reduce process time, complexity, sludge production and use of additional chemicals in the wastewater treatment processes. The approaches and treatment methods described in this thesis are simple to use, robust and environment friendly.

PERSPECTIVES

This work validates the application of magnetic NPs and their potential application in the reduction of contaminants from sewage wastewater. In order to strengthen the present findings practical systems, pilot and large-scale studies are required. However, an in-depth study is deemed necessary for the effective reduction of total nitrogen using magnetic NPs. In addition the stability of the nanoparticles upon storage and the initial wastewater composition needs detailed study. Among five synthesized magnetic NPs, only uncoated MION and PEI-coated MION were optimized under laboratory conditions in the present study. Therefore TSC, APTES and chitosan coated MION requires further optimization in terms of effective contaminant removal. Adsorption isotherms can heighten the present findings and also find optimum conditions for maximal contaminant removal. In this manner, economic feasibility and material consumption can be optimized when treating higher volumes of wastewater. Upon optimization, improved sequential process steps could then be built for efficient and robust WWTP. A study on varying pH would be interesting as magnetic NPs are pH dependent at critical point and keeping in mind the natural conditions, it is necessary to check a wide range of pH values. Under optimal conditions, higher volume of wastewater should be tested for superparamagnetic behaviour since it is also important to understand the required strength of electro magnet in real time applications. Furthermore point of accumulation and uptake of NPs from time scale experiments with respect to toxicity studies would be beneficial in understanding the mechanisms inside the cell. Prospective systematic studies on the removal and recovery of MION can provide a practical solution for wastewater industry. In addition this knowledge can also benefit other industrial effluents such as those originating from the textile, dairy and leather industries, which are also faced with stringent effluent standards.

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