Development and Scale-up of Bioremediation Technology for Perchlorate Contaminated Water and Soil

by

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Jasmin G. Russel

To my father

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ABBREVIATIONS

ABB	Anaerobic Bio-barrier Unit
AC	Activated Carbon
AFBR	Anaerobic Fixed Film Bioreactor
ALB	Anaerobic Leach Bed Unit
APEP	Ammonium Perchlorate Experimental Plant
APHA	American Public Health Association
ARP	Advanced Reduction Process
BAC	Biologically Activated Carbon
BC	Barrier Compartment
BER	Bioelectrochemical Reactors
BES	Bioelectrochemical System
Bio-MF-RO	Biological-Microfiltration-Reverse Osmosis
BOD	Biological Oxygen Demand
CBED	Carbohydrate Based Electron Donors
CCL	Contaminant Candidate List
cld	Chlorite dismutase
COD	Chemical Oxygen Demand
CSIR	Council for Industrial Research
CSTR	Continuous Stir Tank Reactor
DDE	dichloro-diphenyl-dichloroethylene
DDT	dichlorodiphenyltrichloroethane
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
ED	Electrodialysis
EDR	Electrodialysis Reversal

EISB	Enhanced In-situ Bioremediation
EPA	Environmental Technology Division
ESI-MS	Electron Spray Ionization Mass Spectrometry
ESRS	Ex-situ Soil Remediation System
FBR	Fluidized Bed Reactors
USFDA	United States Food and Drugs Administration
GAC	Granular Activated Carbon
GWRTAC	Ground-Water Remediation Technologies Analysis Center
HRT	Hydraulic Retention Time
IC	Ion Chromatography
IC	Inlet Compartment (in Chapter 6)
IEMB	Ion Exchange Membrane Bioreactor
IIT	Indian Institute of Technology
IMM	Inorganic Mineral Media
ISE	Ion Selective Electrode
ISRO	Indian Space Research Organization
ITRC	Interstate Technology Regulatory Council
IX	Ion Exchange
LC	Liquid Chromatography
MBfRs	Membrane Biofilm Reactors
MBR	Membrane Bioractors
MF	Microfiltration
MGU	Mahathma Gandhi University
MNA	Monitored Natural Attenuation
MS	Mass Spectrometry
MTCC	Microbial Type Culture Collection

NF	Nanofiltration
NIIST	National Institute for Interdisciplinary Science and Research
NIS	Sodium - Iodine Symporter
NOEL	No-Observed Effect Level
OC	Outlet Compartment
OD	Optical Density
ORP	Oxidation Reduction Potential
PBR	Packed Bed Reactor
pcrA	Perchlorate Reductase A
ppb	Parts Per Billion
PRB	Permeable Reactive Bio-barrier
PRM	Perchlorate Reducing Microbes
PVC	Polyvinyl Chloride
R&D	Research and Development
RO	Reverse Osmosis
RPP	Rocket Propulsion Plant
SBR	Sequencing Batch Reactor
SCOD	Soluble Chemical Oxygen Demand
SRM	Sundarraj Manonmani University
STP	Sewage Treatment Plant
T3	Triiodothyronine
T4	Thyroxine
TCOD	Total Chemical Oxygen Demand
TDS	Total Dissolved Solids
TERLS	Thumba Equatorial Rocket Launching Station
TKN	Total Kjeldhal Nitrogen

TMS	Trace Metal Solution
TOC	Total Organic Carbon
TP	Total Phosphate
TPC	Total Plate Count
TS	Total Solids
TSH	Thyroid Stimulating Hormone
TSS	Total Suspended Solids
UF	Ultrafiltration
US	United States
USA	United States of America
USEPA	United States Environmental Protection Agency
VFA	Volatile Fatty Acid
WHO	World Health Organization
ZVI	Zero Valent Iron

Chapter 1

Introduction

1.1. Background

Sustainable Development Goal (SDG) focuses on the universal and equitable access to safe and affordable drinking water. However, water quality is a major challenge that mankind facing today, threatening human health, limiting food production, reducing ecosystem functions, and hindering economic growth. The magnitude of the problem is intensified by large number of emerging contaminants such as pharmaceuticals, personal care products, industrial and household chemicals, changing climate patterns, etc., with still unknown long-term impacts on human health and ecosystems.

Among the large number of emerging contaminants, the presence of a persistent endocrine-disrupting toxic oxyanion, perchlorate (ClO_4^-) is a potential threat to human health (Gullick et al., 2001; Maffini et al., 2016). The environmental presence of ClO_4^- in many countries including India is mainly due to the manufacturing and use of ClO_4^- salts such as ammonium perchlorate (NH_4ClO_4) as oxidizing agent in arms and munitions, for defense R&D and a number of industries (Gullick et al., 2001; Urbansky, 2002; Tikkanen, 2006; Kosaka et al., 2007; Kannan et al., 2009; Iannece et al., 2013; Alomirah et al., 2016; Van Stempvoort et al., 2020). In humans, ClO_4^- can interfere with iodine uptake by thyroid follicle cells, which leads to hypothyroidism and related health and developmental disorders (Wolff, 1998; Lisco et al., 2020). Contaminated drinking water and foodstuffs are the major sources of human exposure to ClO_4^- (Steinmaus, 2016). A number of toxicology studies have reported ClO_4^- induced adverse effects on plants and animals (Mukhi & Patiño, 2007; Chen et al., 2015; Anupama et al., 2017; Niziński et al., 2020). Perchlorate ions are highly stable and resistant to degradation under natural conditions (Stetson et al., 2006). Considering the toxicity associated with ClO_4^- in ppb levels, permissible limits and discharge standards are specified in many countries, as well as the development of treatment technologies for remediating ClO_4^- contaminated matrices is a priority research area in many countries (Tikkanen, 2006; USEPA 2008, WHO 2016; Ma et al., 2016; Niziński et al., 2020)

1.2. Perchlorate ion and its chemical properties

The perchlorate (ClO_4^-) ion is a tetrahedral array of four oxygen atoms with a chlorine atom at the centre. The molecular weight of the ClO_4^- anion is 99.45 g/mol and it can form perchloric acid ($HClO_4$) in combination with an H^+ ion. Common salts of perchloric acid are ammonium perchlorate (NH_4ClO_4), lithium perchlorate ($LiClO_4$), magnesium perchlorate ($MgClO_4$), sodium perchlorate ($NaClO_4$), potassium perchlorate ($KClO_4$) etc. Perchlorate salts are white or clear solid crystals at ambient conditions and among that, NH_4ClO_4 is the commonly used oxidizing agent in rocket fuel and fireworks. Perchlorate salts can easily dissociate in water due to their large molecular volume and single anionic charge (Urbansky, 1998). The properties of selected ClO_4^- salts are presented in Table 1.1.

Table 1.1. Properties of common perchlorate salts

Properties	**NH_4ClO_4**	**$KClO_4$**	**$NaClO_4$**
Molecular weight (g/mol)	117.49	138.5	122.44
Density (g/cm^3)	1.95	2.52	2.53
Solubility in water at 25°C (g/L)	200	2096	15

Due to the +7 oxidation state of chlorine in ClO_4^-, perchlorate is a strong oxidizing agent. The low reactivity of ClO_4^- can be attributed to the strong chlorine–oxygen bonds (Urbansky, 1998). Hence the removal of oxygen is required rather than the direct interaction of chlorine atom with reducing agent to proceed reduction. Under ambient conditions perchlorate compounds are not volatile due to their low vapor pressure (Urbansky, 2002). Perchlorate does not form metal complexes due to its relatively low charge density. Perchlorate is a persistent pollutant due to its high stability, high water solubility, and non-complexing nature (Urbansky, 2000). High solubility and poor adherence to soil and organic matter due to electrostatic repulsion, ClO_4^- present in the soil can easily infiltrate into groundwater. Hence topsoil contamination at places where ClO_4^- is handled in bulk is the major cause of groundwater contamination (Urbansky & Brown, 2003; Gal et al., 2008; Cao et al., 2019; Levakov et al., 2019).

1.3. Sources of perchlorate

There are both natural and synthetic/ anthropogenic sources of ClO_4^- in the environment.

1.3.1. Natural sources of ClO_4^-

The presence of ClO_4^- in nature can be traced back to prehistoric periods (Rao et al., 2007). There are several theories on the natural creation of ClO_4^-. The formation of ClO_4^- by ozone oxidation of aqueous chloride, and electric discharging of chloride aerosol is reported by Dasgupta et.al. (Dasgupta et al., 2005). Atmospheric production of ClO_4^- through reactions during lightning and photochemical conversion of the sea or land-based chloride compounds into ClO_4^- in the presence of ozone is also predicted (Walvoord et al., 2003; Dasgupta et al., 2005; Rajagopalan et al., 2006). However, the actual mechanism of ClO_4^- formation under natural conditions is yet to be discovered. Natural ClO_4^- formation is a very slow process. Naturally occurring ClO_4^- is found mostly in arid environments. The atmospheric origin and deposition of ClO_4^- are supported by the discovery of ClO_4^- in Antarctic dry valleys (Kounaves et al., 2010). A high concentration of ClO_4^- was found in Chilean nitrate deposits of the Atacama Desert, Chile, mineral ore deposits from New Mexico, Canada, Bolivia, and California (Orris et al., 2003). In 2008, Phoenix Mars Lander has detected the presence of ClO_4^- in the form of calcium salts in the Martian soil (Hecht et al., 2009).

1.3.2. Synthetic sources of ClO_4^-

Most of the ClO_4^- found in the environment is of synthetic origin. As already mentioned, its salts have huge applications in various industries. The method for manufacturing ClO_4^- salts begins with the production of sodium perchlorate ($NaClO_4$) by the electrolytic oxidation of sodium chlorite ($NaClO_3$) as the precursor. Potassium perchlorate ($KClO_4$) and ammonium perchlorate (NH_4ClO_4) are produced by reacting the $NaClO_3$ with potassium chloride (KCl) and ammonium chloride (NH_4Cl) respectively. Perchlorate is also found as a contaminant in sodium hypochlorite (Trumpolt et al., 2005).

1.3.3. Distinguishing natural and synthetic ClO_4^- ions

The natural and synthetic ClO_4^- in the environment can be distinguished based on the analysis of the stable isotopic ratio (δ) of chlorine and oxygen in the ClO_4^- ions (Bao & Gu, 2004; Böhlke et al., 2005; Jackson et al., 2010; Zhang et al., 2021). The stable isotopes of Cl are ^{35}Cl and ^{37}Cl with relative abundances of 75.77% and 24.23%, respectively whereas the stable

isotopes of O are ^{16}O, ^{17}O, and ^{18}O with relative abundances of 99.76%, 0.04%, and 0.20% (Sturchio et al., 2012; Zhang et al., 2021).

There are two types of isotopically different natural ClO_4^-. One form has a positive stable chlorine isotope ratio $\delta^{37}Cl$ values of 0 to +6 ‰ and relatively high $^{36}Cl/Cl$ ratios of 3,000 × 10^{-15} to 29,000 × 10^{-15}. The other type has negative $\delta^{37}Cl$ values (-20 to -10 ‰) and relatively low $^{36}Cl/Cl$ ratios (22 × 10^{-15} to 590 × 10^{-15}). The first form of natural ClO_4^- is found at locations across the United States, Namibia, United Arab Emirates, China, and Antarctica, whereas the second form is found only in the Atacama Desert of northern Chile, and which is associated with Chilean nitrate deposits. The synthetic ClO_4^- has a relatively consistent isotopic composition with a positive $\delta^{37}Cl$ value (+0.6 ± 1.0 ‰) and a low $^{36}Cl/Cl$ ratio (1 × 10^{-15} to 40 × 10^{-15}). Similarly, the ClO_4^- from natural sources has stable oxygen isotopic ratio $\delta^{18}O$ values of −24.8 to −4.5 ‰, and the ^{17}O anomaly values of ($\Delta^{17}O$) +4.2 to +9.6 ‰. For synthetic ClO_4^-, the $\delta^{18}O$ value is −24.8 to −12.5 ‰ and the $\Delta^{17}O$ value is only 0.0 ± 0.1 ‰ (Bao & Gu, 2004; Michalski et al., 2004; Dasgupta et al., 2005; Sturchio et al., 2009; Zhang et al., 2021).

1.4. Uses of perchlorate compounds

Perchlorate salts are mainly synthesized for their application in strategic sectors, space R&D, defence R&D, fireworks, and a number of industries. Due to the strong oxidizing property and high stability at moderate temperatures, ammonium perchlorate is used as an oxidizing agent in solid rocket propellent and hence ClO_4^- is often referred to as **'rocket fuel'**(Trumpolt et al., 2005; ITRC 2005). Potassium perchlorate is used in road flares and airbag inflation systems (Mohr, 2007). Perchlorate salts are also used in leather tanning, textile bleaching, electropolishing sectors, etching of brass and copper, photographic flash powder, paints and enamels, matchbox, crackers, etc. (Urbansky, 1998; ITRC 2005). Medically, ClO_4^- was used for the treatment of hyperthyroidism and Graves' disease (Martino et al., 1986; Srinivasan & Viraraghavan, 2009). Chilean nitrate deposits containing ClO_4^- were marketed to the United States of America (USA) as fertilizers (Ericksen, 1983). Different ClO_4^- salts and their industrial uses are given in Table 1.2

Table 1.2. Common uses of perchlorate salts (adapted from PubChem Compound Summary for Chemicals)

Name	Uses
Ammonium perchlorate	Oxidizers in propellants for rockets, fireworks, and highway flares, in explosives, pyrotechnic compositions, analytical chemistry, etching, and engraving agent, radioprotectant
Potassium perchlorate	Treatment of Grave's disease, Automotive Airbags, Explosive materials, road flares, airbag inflation system
Lithium perchlorate	As Oxygen candles in submarines, space crafts for backup oxygen supply, personal and pet care products, sports equipment, etc.
Sodium perchlorate	Explosive Materials, Oxidizing/reducing agents, batteries, Plastic and rubber products, Medication

1.5. Environmental contamination of perchlorate

The use of ClO_4^- salts in various industries, improper handling, storage, and disposal of waste containing ClO_4^- has resulted in the anthropogenic occurrence of ClO_4^- in the environment (Gullick et al., 2001; Urbansky, 2002; Trumpolt et al., 2005). The environmental contamination of naturally occurring ClO_4^- is very rare, and it was only reported from arid environments due to atmospheric deposition. The use of Chilean nitrate fertilizer in the US (United States) has contributed to the contamination of groundwater in many US states. The discharge from ClO_4^- manufacturing and handling sites has resulted in the widespread occurrence of ClO_4^- in surface water, groundwater, soil, and many human consumption products (Mattie, 2005). The presence of ClO_4^- in the public drinking water systems is often attributed to the formation of ClO_4^- as a disinfection by-product formed during the sodium hypochlorite disinfection step (Asami et al., 2009a).

The environmental presence of ClO_4^- attributes to its presence in many human consumption products such as dairy milk, infant formula, fruits and vegetables, beverages, etc (Dyke et al., 2007; Asami et al., 2009b; Her et al., 2010; Calderón et al., 2020). The levels of ClO_4^- in various human consumption products reported from different countries are presented in Table 1.3.

Table 1.3. Levels of perchlorate occurrence in various human consumption products reported from different countries (El Aribi et al., 2006; Dyke et al., 2007; Wang et al., 2009; Calderón et al., 2017)

Country	**Matrix**	**(μg/Kg)**
USA	Lettuce	10.3
	Spinach	115
	Infant formula	3.73 μg/L
	Cow's milk	5.9
Chile	Grapes	38.6
	Apricot	145
Korea	Spinach	190
Japan	Herbs and spinach	419
	Fruits	203
	Soured milk	2.55
	Juices and beverages	2.53
	Egg	6.4
	Cow's milk	9.4
Canada	Cucumbers	48.6
	Tomatoes	44.9
	Melons	536
	Spinach	175
Mexico	Tomato	122.2
Kuwait	Melon	44.2
	Orange	22.4
	Grape	74.9
Italy	Plums	2.79
Guatemala	Cantaloupe	463
France	Beer	21.1 μg/L
Japan	Dairy milk	38.5 μg/L

1.6. Perchlorate: toxicology and human health effects

Perchlorate ions are highly toxic to both plants and animals including humans. Clinical studies have indicated the absorption of ClO_4^- from the gastrointestinal tract and its systemic distribution in plasma (Ting et al., 2006). The harmful effects of ClO_4^- in humans were first observed in the 1960s when fatal aplastic anaemia was reported in cases where $KClO_4$ was used as a medicine to treat hyperthyroidism (Sass, 2004). Thyroxine (T4) and triiodothyronine (T3) are the two hormones produced by the thyroid gland. The functions of thyroid hormones are stimulation of the development and growth of neurons and glial cells, the formation of synapses between neurons, the formation of the myelin sheath, the development of neurotransmitters, and normal growth and development of the skeletal system. Hence, thyroid hormones are necessary for the normal development of the central nervous system in foetuses and infants. Moreover, the T3 and T4 levels are critical in determining the metabolic activity and normal functioning of the organ systems in both adults and infants (Zoeller, 2003).

Molecular Iodide required for the synthesis of thyroid hormones is transported from the bloodstream to the thyroid gland follicular cells with the help of a protein called sodium – iodide symporter (NIS) (Dohan et al., 2003). The NIS molecules have a high affinity for iodide, but the ClO_4^- competes with iodide due to its similar ionic size and charge (Van Sande et al., 2003). The ClO_4^- binds with NIS, which blocks the transport of iodine resulting in intra-thyroid iodide deficiency. Iodide deficiency leads to thyroid hormones (T4 and T3) deficiency leading to hypothyroidism and eventually increases the release of Thyroid Stimulating Hormone (TSH) by the pituitary gland. As a result, thyroid hypertrophy, hyperplasia, and goitre can develop in humans. NIS is also present in the lactating breast epithelium, gastrointestinal tract, placenta, skin, mammary gland, small intestine, and brain; hence the presence of ClO_4^- can affect the normal functioning of these tissues also (Perron et al., 2001). Pregnant women, foetuses, lactating women, infants, and individuals with thyroid problems are more susceptible to the harmful effects of ClO_4^- (Henrichs et al., 2010). Perchlorate can directly impact the neuro-psychological development of foetuses and infants. (Brechner et al., 2000). Direct exposure to a high dosage of ClO_4^- can cause vomiting, diarrhoea, nausea, cough, irritation of eye and skin, etc (ATSDR, 2008). Even though ClO_4^- contaminated drinking water and foodstuffs are the major sources of human exposure, the individuals working in the ClO_4^- manufacturing facilities are more prone to ClO_4^- exposure than others(Mattie, 2005).

There is no known metabolism for ClO_4^- in humans, and the ingested ClO_4^- is normally excreted mainly through urine and breastmilk. However, continuous exposure to high levels of ClO_4^- can lead to high TSH levels and associated health effects. Perchlorate has been detected in human saliva (Kannan et al., 2009), breast milk (Pearce et al., 2007), and urine of population exposed to ClO_4^- contamination (Blount et al., 2006; Alomirah et al., 2016).

In rats, mice, and rabbits exposed to subchronic levels of ClO_4^- have been shown to cause increased thyroid follicular cell hypertrophy and hyperplasia (Keil et al., 1998; Lewandowski et al., 2004). In adult rats, ClO_4^- exposure inhibited iodine uptake and altered the overall functioning of the gland. Haemolytic anaemia and methaemoglobin formation was also observed in rats exposed to ClO_4^- via drinking water. Development of thyroid tumors and (papillary/follicular adenoma and carcinoma) in rats and mice was reported as a consequence of long-term exposure to high doses (928 to 2573 mg/kg body weight per day) of ClO_4^- via drinking water and food (Kessler & Krüskemper, 1966; Rodriguez et al., 1991). Exposure to an environmentally relevant concentration of ClO_4^- resulted in arrested metamorphosis and thyroid dysfunction in amphibians and vertebrates (Tietge et al., 2005). Mukhi et.al. studied the effect of prolonged exposure to ClO_4^- in zebrafish (Mukhi & Patiño, 2007). The presence of ClO_4^- in soil was found to adversely affect the survival and reproduction of earthworms (Landrum et al., 2006).

The accumulation and toxic effect of ClO_4^- in aquatic and terrestrial plants were also reported (Chen et al., 2015). Growth inhibition, decreased chlorophyll content, and morphological difference was noticed in certain plants exposed to ClO_4^- (He et al., 2013; Anupama et al., 2017).

1.7. Perchlorate: regulatory standards

In 1985, the United States Environmental Protection Agency (USEPA) has detected the environmental presence of ClO_4^- in Superfund sites of California in the United States of America (USEPA, 1985). Extensive monitoring by USEPA during 1997-2005 in the southwestern United States (US) has detected the widespread occurrence of ClO_4^- in drinking water supplies. Therefore, ClO_4^- was included in the USEPA Contaminant Candidate List (CCL) of 1998 (USEPA, 1998). The USEPA health advisory limit for ClO_4^- in drinking water is 15 μg/L and the reference dose based on Non-Observed Effect Level (NOEL) is 0.007 mg/kg/day which can be translated to drinking water equivalent of 24.5 μg/L (USEPA, 2008). The World Health Organization (WHO) guideline for ClO_4^- in drinking water is 0.07 mg/L

(WHO 2016). The regulatory limits for ClO_4^- in drinking water by different countries are presented in Table 1.4. In June 2020, US EPA decided not to regulate ClO_4^- levels in the US public drinking water system as only 0.03% of total detection exceeds the limit of 18 μg/L (USEPA Federal Register, 2020). However, many states in the US still follow strict regulations for ClO_4^- in drinking water such as California (6 μg/L), Massachusetts (2 μg/L), etc, (CDPH, 2007, MDEP 2006). Many countries including India, do not have any drinking water standard for ClO_4^-. According to the European Commission Regulations of the European Union, the permissible limit of ClO_4^- and chlorate in fruits and vegetables is 0.05 mg/kg (EU Regulations, 2020)

Table 1.4. Perchlorate regulatory limits for drinking water in different countries

Country	**Permissible Limits (μg/L)**	**Reference**
USA	1 -18	USEPA, 2008
Canada	4	Health Canada (2020)
Korea	15	Ministry of Health, Korea (2010)

1.8. Detection and quantification of perchlorate

Different analytical methods are available for ClO_4^- detection and quantification such as Ion-Selective Electrode (ISE), Ion Chromatography (IC) with suppressed conductivity detector, two-dimensional IC, Electrospray Ionization Mass Spectrometry (ESI-MS) with Liquid Chromatography (LC) or Ion Chromatography Detector, etc. USEPA approved methods for quantification of ClO_4^- are presented in Table 1.5.

Table 1.5. USEPA approved perchlorate detection and quantification methods

Method	Lower Detection Limit
Electrochemical method (ISE)	500 μg/L
IC with background conductivity suppression (EPA 314.0)	<2 μg/L
Two-dimensional IC with suppression of background conductivity (EPA 314.2)	0.012 μg/L
LC – ESI/MS (EPA 331.0) (for food stuffs and water)	0.1 μg/L
IC – ESI/MS (EPA 332.0)	0.02 μg/L
IC – MS/MS, LC – MS/MS (for food stuffs and water)	<5 ng/L

1.9. Perchlorate treatment methods

Perchlorate treatment approaches can be broadly classified into physical (non-destructive), chemical, and biological processes (destructive) (Urbansky, 1998; Srinivasan & Sorial, 2009; Ye et al., 2012; Xie et al., 2018). Physical processes such as adsorption (Parette & Cannon, 2005), ion exchange (Gu et al., 2002), membrane filtration (Huq et al., 2007; Yoon et al., 2009; Heo et al., 2012), and electrodialysis (Roquebert et al., 2000) methods are employed for the removal of ClO_4^- from aqueous streams (Srinivasan & Sorial, 2009). Chemical methods used are metal-based catalytic reduction (Hurley & Shapley, 2007; Wang et al., 2008), electrochemical reduction (Rusanova et al., 2006), photo-catalysis, etc (Theis et al., 2002; Ye et al., 2013; Yang et al., 2016). Compared to Physico-chemical processes, bioremediation methods are eco-friendly, efficient, versatile, and economic (Tekere, 2019). Biological reduction of ClO_4^- uses perchlorate reducing microbes that can completely reduce ClO_4^- into non-toxic chloride (Cl^-) and oxygen (O_2) (Attaway & Smith, 1993; Logan, 1998; Coates et al., 1999; Bardiya & Bae, 2005, 2011). Full-scale ex-situ bioreactors are operational for the microbial reduction of ClO_4^- -containing groundwater in the US (Sutton, 2006). In situ bioremediation methods such as phytoremediation (Nzengung & McCutcheon, 2003), bioaugmentation and bio-stimulation were also reported for the treatment of both water and soil contaminated with ClO_4^- (Hatzinger et al., 2002; Evans & Trute, 2006; Evans et al., 2008).

The prominent methods practiced for ClO_4^- remediation have inherent practical difficulties and the details are presented in Table 1.6. Due to the disadvantages associated with available

technologies, the combinations of different treatment processes are also in practice (Srinivasan & Sorial, 2009). Adsorption followed by biological reduction (Song, et al., 2015; Ren et al., 2015), bio-regeneration of the ion exchange brine and ion exchange membrane (Batista et al., 2002; Gingras & Batista, 2002; Lehman et al., 2008; Sharbatmaleki & Batista, 2012; Sharbatmaleki et al., 2015), biotreatment of the membrane rejects, etc (Giblin et al., 2002). are some of the hybrid treatment methods reported for ClO_4^- remediation. Perchlorate reduction in Bioelectrical Reactors (BER) (Thrash et al., 2007) and Microbial Fuel Cells (MFC) (Butler et al., 2010) are in the budding phase and requires further studies on new bacterial cultures and genetic modification of bacterial strains (Ye et al., 2012).

Table 1.6. Prominent methods reported for perchlorate remediation, and their limitations (adapted from (Russel et al., 2021))

Processes	Limitations	References
Ion Exchange	Generation of concentrated brine, Difficulty in disposal/regeneration of spent brine and saturated resin, Non-specificity	(Hutchison & Zilles, 2018)
Adsorption	Non-selectivity, the requirement for acidic conditions, competitive adsorption by other anions	(Xie et al., 2018)
Membrane filtration	Can treat only low concentrations of ClO_4^-, membrane fouling, non-specificity, high cost of operation	(Xie et al., 2018)
Electrodialysis	Concentrated brine needs further treatment, High cost of operation	(Urbansky & Schock, 1999)
Chemical Reduction	Maintenance of low pH and high pressure, generation of highly reactive species, extreme reaction conditions	(Urbansky, 1998) (Yang et al., 2016)
Biological Reduction		
In-situ bioremediation	Repeated addition of electron donors, growth of Non-perchlorate reducing bacteria, the release of metabolic by-products, etc.	(Hatzinger et al., 2006) (Stroo et al., 2009)
Ex-situ bioremediation	Cannot be applied in drinking water systems as it contains residual microbial load, metabolic by-products, and unused organics, the problem of public acceptance	(Srinivasan & Sorial, 2009) (Ye et al., 2012)

Perchlorate treatment methods are elaborated in detail in Chapter 2.

1.10. Gap areas

Perchlorate (ClO_4^-) being a toxic pollutant, it's continuous monitoring in the environment and human consumption products is highly important., especially at previously reported contaminated places. This will help in minimizing the environmental and public health risks associated with its exposure. Perchlorate was flagged as an emerging contaminant in India recently, and studies in the area are very limited. Regular monitoring of ClO_4^- in the community water resources is required around places like Keezhmad (near to ISRO-APEP) and Thumba (near to VSSC-RPP) that are already reported as highly ClO_4^- contaminated sites.

There is no technological intervention attempted so far to address this environmental and public health issue in India. The reports on research for developing indigenous and techno-economically feasible ClO_4^- remediation methods are also very limited. Implementing technologies that are developed in other countries are high cost involving and need additional research and development to cope up with the local conditions. Therefore, the development of technically competent indigenous technologies with local resources would be a sustainable solution. To bridge these existing gaps the following major objectives are focused on in this study

1.11. Research objectives

Objectives of the present study are:

1. To monitor the status of ClO_4^- contamination of water sources around bulk perchlorate handling sites in Kerala, India.
2. To design and develop treatment systems for ClO_4^- contaminated water and soil.
3. To test and validate the treatment systems in bench-scale or pilot-scale treatment units under field-relevant conditions.

Chapter 2

Review of Literature

2.1. Perchlorate contamination

Perchlorate contamination of water, soil, and foodstuffs has become a major environmental, and public health concern since its toxicity was first reported in 1985 (USEPA, 1985) Advancement in analytical methods and tools helped to detect even trace level (sub ppb level) of ClO_4^- in various matrices. Much of the anthropogenic release of ClO_4^- in water sources is linked to the disposal of ClO_4^- containing wastes by the strategic sector, and several industries such as electroplating, flare manufacturing, cracker manufacturing, and matchbox, etc. that uses ClO_4^- containing chemicals (Urbansky, 2002; Srinivasan & Sorial, 2009).

2.1.1. Global status of perchlorate contamination

Most of the case studies on the origin and occurrence of ClO_4^- in the environment that have been published are based in North America. The first environmental detection of ClO_4^- was reported from the hazardous waste dumping Superfund sites in California, USA in 1985 (Tikkanen, 2006). Later in 1997, the development of a sensitive analytical method by the California Department of Health Services (CDHS) and subsequent environmental monitoring revealed widespread occurrence of ClO_4^- in drinking water sources. It was found that 361 out of ~6800 public drinking-water sources and several private wells in California contain ClO_4^- in detectable limits of > 4 µg/L. In the USA, 34 other states, including Nevada, Arizona, Texas, Utah, New Mexico, Maryland, and Massachusetts, have reported ClO_4^- levels in groundwater or drinking water (Hatzinger, 2005). The lower Colorado River also contains a measurable concentration of ClO_4^- during certain times of the year (~4–9 µg/L) and nearly 15 million people are exposed to this contamination (Hogue, 2003). The nitrate deposits in the Atacama Desert of northern Chile are known to contain naturally occurring ClO_4^- (Urbansky et al., 2001). The use of nitrogen fertilizers from these deposits may provide an explanation for the detection of ClO_4^- in groundwater over a wide area of western Texas where anthropogenic sources are unlikely (Christen, 2003). A study conducted by U.S. Food and Drug Administration (FDA) has detected low levels of ClO_4^- in the milk (3.2–10.4 µg/L) and lettuce (1.0–71.0 µg/L) samples. Groundwater contamination due to ClO_4^- is reported from several military ranges and it can be attributed to the presence of ClO_4^- in munitions (Hatzinger, 2005).

In recent years, several other countries such as China, Kuwait, South Korea, Sri Lanka, India, Japan, Italy, United Kingdom (UK), Germany, France, and Chile, etc. also reported ClO_4^- contamination. The reports from various countries, source of contamination, and type of contaminated matrix with maximum levels detected are presented in Table 2.1.

Table 2.1. Environmental contamination of ClO_4^- reported in different countries

Country	Source of contamination	Matrix	Max concentration (μg/L)	Reference
USA (1997-2005)	Perchlorate manufacturers or users	Well water	420	(USEPA 2005, 2008)
		Drinking water	811	
		Groundwater	3700000	
		Surface water	120000	
		Soil	2000 mg/kg	
Korea (2006)	Unknown	Tap water	35	(Quiñones et al., 2007) (Her et al., 2011)
		Surface water	95.6	
		Wastewater effluent	22	
New Mexico (2006)		Groundwater	200	
Japan (2007-09) *2011	Industrial sources	Natural water	0.57	(Kosaka et al., 2007) (Asami, et.al. 2009b)
		Bottled water	0.53	
		Drinking water	0.92	
		Surface water	2300	
		Industrial effluents	15000	
		*Well water	0.53	

Israel (2009)	Industrial waste ponds	Groundwater	*800000 (avg)	(Levakov et al., 2019)
China (2010) *2014	Firework industry	Surface water Tap water Groundwater *Rainwater	54.4 31.4 22.1 27.3	(Wu et al., 2010) (Qin et al., 2014)
Germany (2011)	Fireworks display	Groundwater Pore water	<1 15000	(Scheytt et al., 2011)
Srilanka (2011)	Unknown	Unknown	0.14	(Guruge et al., 2011)
UK (2011)	Unknown	Drinking water	2.073	(McLaughlin et al., 2011)
India (2009)	Unknown	Groundwater Saliva	6.9 4.7	(Kannan et al., 2009)
India (2012) **NIIST STUDY**	Ammonium perchlorate manufacturing unit & rocket testing facility	Groundwater Surface water Tap water	91.4 19.6 69.2	(Anupama et al., 2012)
Chile (2013)	Chilean deposits	Soil Surface water	2565 1480	(Vega et al., 2018)

France (2015)	Unknown	Water	22	(Vigreux-Besret et al., 2015)
Kuwait (2016)	Unknown	Tap water	18.6	(Alomirah et al., 2016)
		Groundwater	7.02	
		Brackish water	7.99	
		Bottled water	0.70	

2.1.2. Status of perchlorate contamination in India

In India, the presence of ClO_4^- in water resources from different states was first reported in 2009 by Kannan et.al. (State Univ. of New York, USA). They have randomly collected surface, ground, and drinking water samples from six states in India. The highest value reported by them was 6.9 μg/L in a groundwater sample. They have also reported the presence of ClO_4^- in human saliva in the range of 0.2- 4.7 μg/L. This study was not based on any point source of contamination(Kannan et al., 2009).

The first report of high levels of ClO_4^- contamination (91.4 μg/L) in groundwater and surface water sources in India was done through a study conducted by CSIR-NIIST (Anupama et al., 2012; 2015a). Analysis of randomly collected samples from various districts in Kerala, India have revealed considerable levels of ClO_4^- in groundwater samples collected from two districts, Thiruvananthapuram, and Ernakulam. There are two known ClO_4^- inventories in these districts. The major one is the ammonium perchlorate production plant named Ammonium Perchlorate Experimental Plant (ISRO-APEP) situated in Ernakulam district, and the second one is the Rocket Propulsion Plant (VSSC-RPP) a wing of Vikram Sarabhai Space Center in Thiruvananthapuram. A later detailed study conducted by the CSIR-NIIST team has revealed ClO_4^- concentration up to 7270 μg/l in an open well near APEP. Follow up study conducted by the team revealed ClO_4^- concentration up to 43000 μg/l in community wells, and 34000 μg/l in a surface water source (community pond in the region) near APEP.

In 2013, a study conducted by Isobe et.al. (Ehime Univ., Japan) have reported high levels of ClO_4^- in groundwater samples around the cracker industry area in Sivakasi, Viruthunagar district, Tamil Nadu (Isobe et al., 2013). A separate study by Sijimol et al. from Mahatma Gandhi University, Kerala also reported ClO_4^- contamination around APEP and RPP. (Sijimol et al., 2017). Some research organizations from South India, especially from Tamil Nadu are also dealing with ClO_4^- contamination around matchbox and fireworks industries (Balakrishnan et al., 2014; Raj & Muruganandam, 2014; Karthikprabu et al., 2020). The reported studies on ClO_4^- contamination in India are presented in Table 2.2.

Table 2.2. Reports on perchlorate contamination from various states of India

States	Source Type	Sample type	Max concentration (μg/L)	Reference
Tamil Nadu West Bengal Bihar Maharashtra Karnataka Pondicherry	Unknown	Drinking water Groundwater Bottled water Surface water Rainwater Overall Saliva	 6.9 6.9 4.7	(Kannan et al., 2009)
Kerala	Ammonium perchlorate production unit & rocket testing facility	Groundwater Surface water Tap water	91.4 19.6 69.2	(Anupama et al., 2012)
Tamil Nadu Maharashtra West Bengal	Fireworks & Matchbox production	Groundwater	7700	(Sugimoto et al., 2012)
Tamil Nadu	Fireworks industry	Groundwater Surface water Tap water	7690 30.2 0.393	(Isobe et al., 2013)

Tamil Nadu Karnataka Andhra Pradesh		Drinking water Bottled water Groundwater Surface water Industrial Effluents Overall	 93500 93500	(Raj & Muruganandam, 2014)
Kerala	Ammonium perchlorate production unit & Rocket testing facility	Groundwater Surface water Bottled water	7270 355	(Anupama et al., 2015a)
Tamil Nadu Andhra Pradesh	Explosive & fireworks Manufacturing industry	Tap water Groundwater Surface water	319.5 12072 1348.7	(Balakrishnan et al., 2014)
Kerala	Ammonium perchlorate manufacturing unit & rocket testing facility Fireworks manufacturing industry	Tap water Groundwater Bottle water Rainwater	 1172 1067.81 12.8	(Sijimol et al., 2016)
Kerala	Ammonium perchlorate production unit	Groundwater Surface water	43000 34000	(Anupama et al., 2017)

Kerala	Ammonium perchlorate manufacturing unit & rocket testing facility Fireworks manufacturing	Groundwater	32602	(Sijimol et al., 2017)
Gujarat	Firework manufacturing industry and fireworks display	Surface water	65±0.5	(Kumar, 2020)
Tamil Nadu	Fireworks & safety match box production	Groundwater Sludge Soil	95500 98800 97300	(Karthikprabu et al., 2020)

2.2. Treatment methods for perchlorate contaminated matrices

The different approaches for treating and remediating ClO_4^- -contaminated matrices can be categorized mainly into three such as (1) the physical processes, (2) the chemical processes, and (3) the biological processes.

2.2.1. Physical processes for perchlorate remediation

All the physical processes for ClO_4^- removal are non-destructive because the ClO_4^- ion is only physically removed from contaminated matrices. This is applied for contaminated aqueous phases (mostly groundwater). The major physical processes employed for ClO_4^- removal from aqueous streams are ion exchange, adsorption, and different membrane filtration processes.

a) Ion exchange

Ion exchange (IX) is an effective and extensively used method for the removal of ClO_4^- from drinking water (Xie et al., 2018). Cationic resins (styrene-divinylbenzene resins, corn-stalk-based modified-magnetic-biopolymer) are the most commonly used IX material whereas some inorganic materials and their modifications, such as montmorillonite, activated carbon (AC), and permselective membranes were also used (Chen et al., 2012; Song et al., 2017). Resins that are highly specific for ClO_4^- ions are necessary for the removal of trace amounts of ClO_4^- from water along with high concentrations of other anions (Srinivasan & Sorial, 2009). The high price of IX resin, lack of specificity of resin for ClO_4^- ions and the problems associated with regeneration of ClO_4^- -specific resins are the practical difficulties associated with IX processes (Urbansky, 1998; Xie et al., 2018).

b) Adsorption

Adsorption using activated carbon is a widely used method for removing various pollutants including ClO_4^- from drinking water. The virgin Granular Activated Carbon (GAC) is not effective in removing ClO_4^-, hence tailoring of GACs with cationic surfactants is practiced (Srinivasan & Sorial, 2009). Specific chemical interactions between perchlorate and surface functional groups in combination with electrostatic forces are the major mechanism for perchlorate adsorption on activated carbon (Huang & Mahmudov, 2010). But the tailoring and modification of GAC with functional groups for improving its ClO_4^- adsorption and its regeneration is expensive. A variety of other organic, inorganic, and composite materials such as modified chitosan, bentonite, granular iron hydroxide, organoclay, carbon nanotubes, etc.

are also employed for the adsorption of ClO_4^-. Lack of selectivity, disposal of spent media, and regenerative brine are the major drawbacks of adsorption (Xie et al., 2018). Therefore, adsorption is usually practiced along with other ClO_4^- removal technologies.

c) Membrane filtration

Membrane filtration processes such as ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO), and electrodialysis (ED) are used in treating ClO_4^- contaminated water resources (Roquebert et al., 2000; Yoon et al., 2002, 2003, 2009). Lack of selectivity for specific ions, membrane fouling, and high cost of operation are the major drawbacks of membrane processes. The membrane process generates ClO_4^- containing concentrated reject streams/brine which requires further treatment before disposal (Urbansky, 1998; Urbansky & Schock, 1999). The studies that explore the use of membrane filtration technology for the removal of ClO_4^- in recent years are limited to a few bench-scale units and research is progressing to combine this technology with other technologies to deal with ClO_4^- contamination in aqueous matrices (Xie et al., 2018).

2.2.2. Chemical processes for perchlorate remediation

Chemical processes for ClO_4^- include chemical reduction, electrochemical reduction, metal-based catalytic reduction, electrocatalytic reduction, photocatalysis, and photo-electrocatalysis.

a) Chemical Reduction

Chemical reduction is an environment-friendly method for the removal of ClO_4^- by converting it into chloride and oxygen in the presence of a strong reducing agent (Srinivasan & Sorial, 2009; Xie et al., 2018). Thermodynamically chemical reduction of ClO_4^- needs high activation energy of 120 KJ/mol. The reaction equation is as follows:

$$ClO_4^- + 8H^+ + 8e^- \rightarrow Cl^- + 4H_2O \quad E_0 = 1.287\ V$$

Zero Valent Iron (ZVI), Oxidized titanium ions (Ti^{2+}/ Ti^{3+}), etc. are used as reducing agents for chemical reduction of ClO_4^-. Aqueous Ti (II) produced from the oxidative dissolution of zero-valent titanium Ti (0) was used by Park et.al. A low pH was needed to produce Ti(II) from Ti(0) and the amount of acid required was directly proportional to the amount of Ti(0) (Park et al., 2012). Hori et. al. showed that the addition of zero-valent metals (Al, Cu, Zn, Ni, Fe) to pressurized hot water (PHW) system enhanced the decomposition of ClO_4^- to chloride with the highest activity for Fe. This method was successfully used in the decomposition of ClO_4^- in a

water sample contaminated with ClO_4^- because of fireworks display at Albany, New York, USA (Hori et al., 2012). Another chemical reduction method used is Sulfite/Ultraviolet advanced reduction process (ARP) and the rate of ClO_4^- degradation by this method can be enhanced by increasing pH, temperature, and sulfite concentration (Vellanki & Batchelor, 2013). Chemical reduction of ClO_4^- is a slow process. Hence a strong reducing agent with high activity or addition of catalyst for decreasing the activation energy is required. Moreover, the addition of metal-reducing agents is toxic and hence it is not suitable for the treatment of ClO_4^- contaminated drinking water sources (Urbansky, 1998; Xie et al., 2018)

b) Metal-Based catalytic reduction

Perchlorate reduction using mono-metal-based and bi-metallic heterogeneous catalysts was demonstrated under acidic conditions and in the presence of H_2 gas (Hurley & Shapley, 2007). The reaction equation is as follows:

$$ClO_4^- + 4H_2 \xrightarrow[\text{Catalyst}]{} Cl^- + 2H_2O$$

Mono-metal-based catalysts tested for ClO_4^- reduction are 5-10% Pd/AC, Pt/C Raney-Ni, WC, etc. But the activity was very poor at ambient conditions. Reduction on Ti –TiO_2 based catalytic surfaces showed an excellent reduction. (Hurley & Shapley, 2007; Wang et al., 2008). Monometallic catalysts have been generally used for reducing some oxyanions (e.g., BrO_3^-, NO_3^-, and NO_2^-), whereas ClO_4^- reduction always requires a secondary promoter metal. The common promoter metal in ClO_4^- reduction is Re (Rhenium). Bio-inspired catalysts that mimic the enzymatic machinery of bacterial ClO_4^- reduction were also developed (Liu et al., 2015). The most recent bio-inspired catalyst is developed using molybdenum (similar to the Mo cofactor for enzyme activity) as the promoter metal and replaced Rhenium which is a rare metal (Ren et al., 2021). Maintenance of very low pH, high pressure, and requirement of hydrogen gas, etc. are some of the drawbacks associated with catalytic reduction.

c) Electrocatalytic Reduction

Electrocatalytic reduction and catalytic reduction are similar processes. In electrocatalytic processes, the metallic component will act both as an electrode and a catalyst. The electrode materials tested for ClO_4^- reduction are Technetium (Tc), Rhenium (Re), Rhodium (Rh), Ruthenium (Ru), Titanium (Ti), Tin (Sn), Platinum (Pt), etc. (Colom & Gonzalez-Tejera, 1985;

Rhee et al., 1991; Horanyi et.al., 1992; Almeida et al., 1997; Rusanova et al., 2006; Láng et al., 2008) . But the electrocatalytic reduction is a slow process and it is highly dependent on the initial concentration of ClO_4^-, temperature, pH, and the presence of chloride ions formed by the reduction of ClO_4^- (Theis et al., 2002; Yang et al., 2016). Due to the disadvantages such as high cost of operation, safety concerns, consumption of electricity, etc., there are no large scale processes implemented so far (Láng & Horányi, 2003; Ujvári & Láng, 2018).

d) Photocatalytic reduction

Photocatalytic reduction of ClO_4^- using photocatalytic systems such as UV/Cu–TiO_2/SiO_2 system, Ag-doped TiO_2 nanotube arrays, etc. are also reported (Theis et al., 2002; Ye et al., 2013; Jia et al., 2016; Marks et al., 2016).

2.2.3. Biological processes for perchlorate remediation

The biological processes for the remediation of ClO_4^- are mostly bacterial. A specific group of bacteria (Perchlorate Reducing Microbes -PRM), that expresses enzymes that can break down ClO_4^- into Cl^- and oxygen, is applied in most ClO_4^- bioremediation processes. These processes are applied for the treatment of different matrices such as water, soil, resins, membranes, etc. contaminated with ClO_4^-. Among the treatment processes available, microbial processes are the most efficient, cost-effective, and environmentally friendly.

2.2.3.1. Microbial degradation (Reduction) of perchlorate

The degradation of chlorate and perchlorate by soil microorganisms was known decades before the identification of ClO_4^- as an environmental contaminant (Korenkov et al., 1976). Some microorganisms can respire on ClO_4^- as an electron acceptor under anaerobic conditions in the presence of a suitable electron donor (e^-) and carbon source (Attaway & Smith, 1993; Coates & Achenbach, 2004). The enzymes involved in microbial ClO_4^- reduction are perchlorate reductase (pcrA) and chlorite dismutase (cld) (Rikken et al., 1996; Kengen et al., 1999). The biochemical pathway of ClO_4^- degradation is as follows (Figure 2.1).

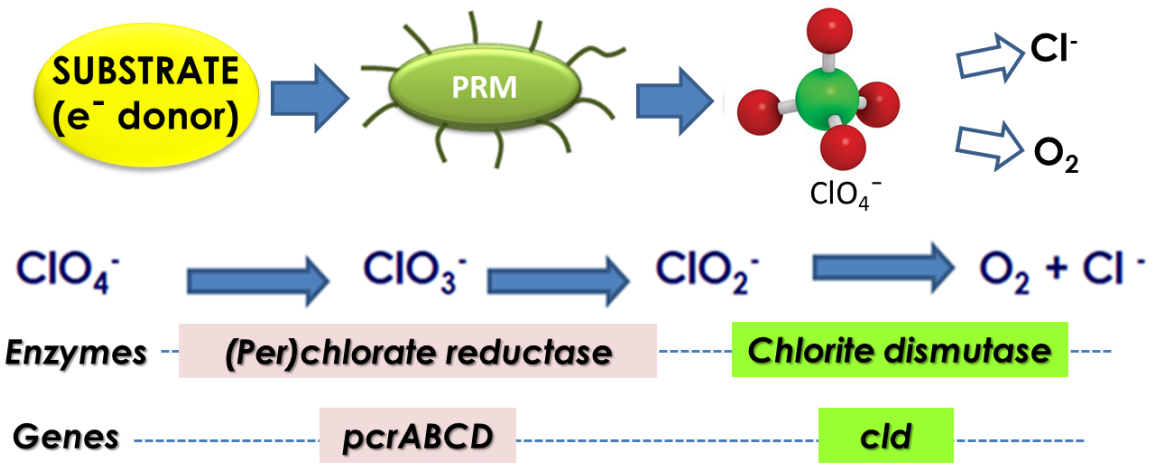


Figure 2.1. Schematic of perchlorate degradation pathway by Perchlorate Reducing Microbes (PRM) and the enzymes involved

2.2.3.2. Perchlorate reducing microorganisms

The first evidence of ClO_4^- respiring microorganisms was obtained when the cell-free extracts from nitrate-adapted *bacillus cereus* were able to reduce ClO_4^- (Hackenthal, 1965). The microorganisms that are capable of utilizing chlorate and perchlorate (denoted as (Per)chlorate) as electron acceptors have been isolated from different environments (Attaway & Smith, 1993; Wallace et al., 1996; Bardiya & Bae, 2011). *Vibrio dechloraticans Cuznesove* B-1168 (γ proteobacteria) which is isolated from industrial wastewater is the first ClO_4^- reducing bacteria that was characterized (Korenkov et al., 1976). A list of ClO_4^- -reducing bacteria that are reported in the literature is presented in Table 2.3.

There are heterotrophic as well as autotrophic PRMs reported based on the nutritional and electron donor requirement. The majority are heterotrophic and can utilize organic substances like acetate as electron donor for ClO_4^- reduction (Rikken et al., 1996; Chaudhuri et al., 2002). Even though PRMs can utilize a variety of organic electron donors such as lactate, pyruvate, casaminoacids, fumarate, succinate, methanol, ethanol, fructose, cellobiose, 1-hexane, propane, ethane, methane, mannose, xylose, pectin, n-alkanes, etc., many are not able to use carbohydrates, benzoate, catechol, glycerol, benzene, citrate, etc. (Wallace et al., 1996; Bruce et al., 1999; Coates & Achenbach, 2004; Shrout & Parkin, 2006; Cai et al., 2010; Luo et al., 2015; Lai et al., 2021). Autotrophic PRMs can use various inorganic electron donors such as hydrogen gas, ferrous ion, zero-valent iron, sulfur, hydrogen sulfide, thiosulphate, etc (Nerenberg et al., 2002; Coates & Achenbach, 2004; Sahu et al., 2009).

Table 2.3. Perchlorate reducing bacteria reported in the literature so far

Organism	**Division**	**Source**	**Reference**
Azospirillum sp. AJ2, ABL1, PMS1, PMS2, SN1A, SN1B, SN2	Alpha	Aquifer and groundwater	(Waller et al., 2004)
Azospirillum sp. cl-19-Sarno river	Alpha	Surface water	(Vigliotta et al., 2010)
Dechlorospirillum sp. cl-31 Sarno river	Alpha	Surface water	(Vigliotta et al., 2010)
Dechlorospirillum anomalous WD	Alpha	Swine water lagoons	(Michaelidou et al., 2000)
Dechlorospirillum anomalous JB116	Alpha	Sewage treatment plant	(Bardiya and Bae, 2008)
Magnetospirillum bellicus VDY	Alpha	Cathodic chamber of the bioelectrical system	(Thrash et al., 2010a)
Magnetospirillum sp. VITRJS5	Alpha	Freshwater sediment	(Jacob et al., 2018)
Azospira oryzae (Dechlorosoma suillum) GR-1	Beta	Activated sludge	(Rikken et al., 1996)
Azospira oryzae (Dechlorosoma suillum) type KJ, PDX	Beta	Primary digestor sludge	(Logan et al., 2001)
Azospira oryzae (Dechlorosoma suillum) PS	Beta	Soil and groundwater	(Achenbach et al., 2001; Bruce et al., 1999)
Azospira oryzae (Dechlorosoma suillum) JPLRND	Beta	Groundwater	(Farhan and Hatzinger, 2009)
Azospira sp. OGA 24.	Beta	Polluted site	(Guarino et al., 2020)

Azospira. PMJ	Beta	Wastewater treatment plant	(Nam et al., 2016)
Azospira suillum JB524	Beta	Tidal flats of yellow sea	(Bardiya and Bae, 2016)
Dechloromonas aromatica type: CCO, SIUL, MissR	Beta		(Coates and Achenbach, 2004)
Dechloromonas hortensis MA-1	Beta	Garden soil	(Wolterink et al., 2005)
Dechloromonas sp. HZ	Beta	Bioreactor	(Zhang et al., 2002)
Dechloromonas EAB1, EAB2, EAB3, ABL2, PMC	Beta	Groundwater aquifer	(Waller et al., 2004)
Dechloromonas JDS5 and JDS6	Beta	Soil, groundwater	(Shrout et al., 2005)
Ideonella dechloratans	Beta	Activated sludge of municipal wastewater treatment plant	(Malmqvist et al., 1994)
Propionivibrio militaris MP, CR	Beta	Cathodic chamber of the bioelectrical system	(Thrash et al., 2010b)
Wolinella succinogenes HAP - 1	Epsilon	Anaerobic sewage enrichment culture	(Wallace et al., 1996)
Acinetobacter bereziniae strain GWF	Gamma	Anaerobic activated sludge	(Zhang et al., 2016)
Aeromonas	Gamma	Soil, groundwater	(Kesterson et al., 2005)
Citrobacter farmeri JB109	Gamma	Sewage treatment plant	(Bardiya and Bae, 2004)
Citrobacter sp. Iso Cock 1	Gamma	Hydrocarbon oxidizing enrichment culture	(Okeke et al., 2002)
Citrobacter amalonacticus JB101	Gamma	Sewage treatment plant	(Bardiya and Bae, 2004)

Dechlorobacter hydrogenophilus LT-1	Gamma	Soil, groundwater	(Thrash et al., 2010b)
Pseudomonas stutzeri A1	Gamma	Soil	(Shete et al., 2008)
Paracoccus halodenitrificans	Gamma	Groundwater aquifer	(Okeke et al., 2002)
Serratia marcescens strain NIIST 5	Gamma	Perchlorate sequencing batch reactor	(Anupama et al., 2013)

2.2.3.3. Factors affecting bacterial perchlorate reduction

i) Electron donor

The availability of an electron donor is necessary for the reduction of ClO_4^- by heterotrophic and autotrophic PRM. Acetate, ethanol, methane, glycerol, glucose, etc. are the commonly used organic electron donor and carbon sources for heterotrophic ClO_4^- reduction (Attaway & Smith, 1993; Achenbach et al., 2001; Upadhyaya et al., 2015; Lv et al., 2019; Nair et al., 2020). Among the electron donors, acetate is the most preferred due to its high ClO_4^- removal rates and decreased biomass production (Brown et al., 2005; Dugan et al., 2009; He et al., 2019). But the problem with organic electron donors is that they can be consumed by Non-PRMs and can lead to secondary contamination. Inorganic electron donors such as hydrogen, elemental sulfur, thiosulphate, zero-valent iron, etc. are also used in autotrophic processes (Son et al., 2006; Yu et al., 2006; Huang & Sorial, 2007; Sahu et al., 2009; Wang et al., 2014; Zhang et al., 2017). Low-cost organic electron donors such as molasses, whey, yeast extract are also employed in case of ClO_4^- degradation of high strength wastewaters (ITRC, 2008, NASA JPL, 2006)

ii) pH

Perchlorate reduction normally takes place under the neutral pH range (6.6 – 7.5) and the optimum pH is 7 – 7.1 (Attaway & Smith, 1993; Wang et al., 2008; Shang et al., 2018). Perchlorate reducing isolates (CKB, perc1ace, HAP 1, *Acinetobacter)* and mixed cultures are known to survive at pH ranging from 6 – 8.5 (Attaway & Smith, 1993; Wallace et al., 1996; Bruce et al., 1999; Herman & Frankenberger, 1999). There are reports on ClO_4^- reduction at pH ranging from 5 – 9. But the rate of ClO_4^- reduction was much slower for more acidic and alkaline conditions (Wang et al., 2008; Wu et al., 2008; Zhu et al., 2016). Hatzinger et.al. observed that there was no ClO_4^- reduction in soil microcosmos amended with electron donor when the pH was 4.3 (Hatzinger et al., 2006). Recently, Xu et.al. have reported that a pH in the range of 7.2 – 8 stimulated bacterial growth and accelerated ClO_4^- reduction (Xu et al., 2015). The effect of pH on ClO_4^- reduction can be due to its effect on enzymes involved in the biochemical pathway. A change in pH can alter the three-dimensional structure of the enzyme and ionic group of the substrate thereby changing the affinity for enzyme or by changing the acid or base group on the active site of the enzyme (Shuler & Kargi, 1992; Wang et al., 2008).

iii) Oxidation-Reduction Potential

An Oxidation Reduction Potential (ORP) of -110 mV or less is required for the bacterial reduction of ClO_4^- (Attaway & Smith, 1993; Giblin et al., 2000). A positive ORP indicates the presence of oxygen and hence aerobic respiration is favored under such conditions (ITRC 2002). Denitrification can occur at slightly aerobic and anaerobic conditions (0 – 100 mV) and hence depletion of dissolved oxygen and nitrate must be accomplished before ClO_4^- degradation takes place. Perchlorate degradation requires strictly anaerobic conditions but the ORP levels necessary for sulfate reduction and methanogenesis (- 250 mV) are not necessary (ITRC 2008). Recently Shrout et.al. observed a ClO_4^- reduction of 35% and 32% at ORP - 50 mV and +180 mV respectively. But the reduction was complete when the ORP was - 220 mV. They suggested that the addition of excess electron donor than the required amount may be required if the ORP is >0 mV (Shrout & Parkin, 2006).

iv) Competitive electron acceptors

Dissolved oxygen (DO) and nitrate (NO_3^-) are the common competitive electron acceptors that can affect ClO_4^- reduction (Bardiya & Bae, 2011). Most of the ClO_4^- reducing bacteria are either facultative anaerobes or microaerophilic, hence they utilize O_2 in preference to ClO_4^- (Shrout & Parkin, 2006). Complete inhibition of ClO_4^- reduction was reported for bacterial isolates such as *A. sillium* and *Azospira sp.* KJ when DO was present at a concentration of 2 mg/L and 6-7 mg/L respectively (Chaudhuri et al., 2002; Song & Logan, 2004; Xu et al., 2015). In the absence of oxygen, ClO_4^- was shown to degrade without any lag phase (Xu et al., 2015). Studies have reported inhibition of ClO_4^- reduction after aeration by an adapted microbial system (Attaway & Smith, 1993; Song & Logan, 2004). Meanwhile, ClO_4^- degradation in the presence of DO was also reported in a few bioreactors studies (Brown et al., 2002, 2003; Choi, 2007). A mixed consortium enriched using lactate as an electron donor could reduce ClO_4^- in the presence of 4.8 mg/L of DO. Hence oxygen inhibition of bacterial ClO_4^- reduction is not absolute (Shrout & Parkin, 2006). The reduction potential (E^0) of NO_3^-/N_2 pair and ClO_4^-/Cl^- is 1.25 V and 1.28 V respectively. Hence nitrate is a strong competitor of ClO_4^- (Bardiya & Bae, 2011). Simultaneous as well as sequential reduction of both ClO_4^- and NO_3^- are reported for pure and enriched cultures (Giblin & Frankenberger, 2001; Chaudhuri et al., 2002; Matos et al., 2006; Lehman et al., 2008; Hutchison & Zilles, 2018). *D. agitata sp.* CKB, *W. succinogens* HAP I, and Perc1ace are the pure cultures that are capable of reducing nitrate and ClO_4^- simultaneously(Wallace et al., 1996; Giblin & Frankenberger, 2001; Chaudhuri et al.,

2002). Like DO, the presence of nitrate causes a longer lag phase and the complete removal of nitrate is necessary for ClO_4^- reduction to start (Tan, et al., 2004). Nitrate is ubiquitous and found along with ClO_4^- as co-contaminant at a concentration several magnitudes higher than that of ClO_4^- in ground and surface water (Van Ginkel et al., 2008). Even though nitrate is a competitive electron acceptor, the presence of nitrate enhances the growth rate of ClO_4^- reducing bacteria and thereby increasing the overall ClO_4^- degradation rate (Xu et al., 2004). It is also observed that the density of ClO_4^- reducing bacteria is higher in high nitrate-containing soils (Nozawa-Inoue et al., 2005).

2.2.4. Bioremediation approaches for perchlorate contaminated water and soil

There are both ex-situ and in-situ bioremediation approaches reported for the treatment of ClO_4^- contaminated water and soil.

2.2.4.1. Ex-situ bioremediation approaches for perchlorate contaminated water

Ex-situ biological treatment processes are mostly, pump and treat systems designed for the remediation of ClO_4^- contaminated water in suitable engineered bioreactors. The bioreactor configuration and the operating parameters are determined based on the nature of contaminated water, amount of ClO_4^-, presence of competitive electron acceptors, the type of microbial communities present, etc.

a) Continuous Stir Tank Reactors (CSTR)

Full-scale CSTRs are reported for the treatment of ClO_4^- in concentrated wastewater generated during the hog out procedure for removing solid fuels from rockets and missiles using high-pressure water (Coppola & McDonald, 2000). CSTRs are also being tested for the treatment of nitrate and ClO_4^- -laden IX brine generated during groundwater treatment using IX technology (Hatzinger, 2005; NASA JPL, 2006). The concentration of ClO_4^- that can be treated in CSTRs are in the range of 1 – 15000 mg/L (Coppola & McDonald, 2000, ITRC 2008).

b) Packed Bed Reactors

There are autotrophic and heterotrophic packed bed reactors reported for ClO_4^- degradation. High ClO_4^- removal rates and small reactor size are the advantages of packed bed reactors. The main disadvantage of packed bed-type reactors is the plugging at the reactor entry and clogging due to biomass build-up. Backwashing and air scouring are recommended to prevent reactor clogging and channeling (Sutton, 2006). The different packed bed reactors reported, the scale of operation, the packing material, and the electron donors used are presented in Table 2.4.

Table 2.4. Packed Bed Reactors reported in the literature, the scale of operation, packing material, and electron donor and carbon source used

Type	Scale	Packing material	Electron Donor and Carbon source	Reference
Autotrophic	Bench	Elemental Sulphur with crushed oyster shell	S^0 and organic carbon from oyster shells	(Sahu et al., 2009)
Autotrophic	Bench	Glass beads	H_2 and CO_2 gas	(Logan & LaPoint, 2002)
Autotrophic	Bench	Glass beads	H_2 and CO_2 gas	(Miller & Logan, 2000)
Autotrophic	Pilot	Elemental Sulphur with crushed oyster shell	S^0 and organic carbon from oyster shells	(Boles et al., 2012)
Heterotrophic	Bench	Sand	Acetate	(Kim & Logan, 2001)
Heterotrophic	Bench	Celite	Acetate	(Losi et al., 2002)
Heterotrophic	Bench	Plastic	Acetate	(Chung et al., 2010)
Heterotrophic	Pilot	Sand, Plastic	Acetic acid	(Min et al., 2004)
Heterotrophic	Pilot	GAC	Acetic acid	(Brown et al., 2005)
Heterotrophic	Bench	Celite	Acetate	(Giblin et al., 2002)
Heterotrophic	Pilot	Plastic	Acetate 50 mg/L	(Zhang et al., 2005)
Heterotrophic	Bench	Celite	BYF -100 (Brewer's yeast)	(Wallace et al., 1998)

c) Fluidized Bed Reactors

Fluidized Bed Reactors (FBR) are fixed film bioreactors in which the ClO_4^- degrading microbes are immobilized on hydraulically fluidized media particles and degradation is promoted by maintaining conditions such as, low reduction potential and good mass transfer (Sutton & Mishra, 1994; Hatzinger et al., 2000). They are presently being used at full scale to treat ClO_4^- in groundwater at several locations (Hatzinger, 2005; McCarty & Meyer, 2005)The slow growth rate of cells in the reactor, excess biomass production are the main disadvantages of FBRs.

d) Membrane Biofilm Reactors (MBfR)

Membrane Biofilm Reactors (MBfRs) are used for the delivery of gaseous electron donors for the degradation of electron acceptors such as nitrate, ClO_4^-, etc. (Lee & Rittmann, 2000; Nerenberg et al., 2002; Luo et al., 2015; Lai et al., 2021). In MBfRs, the interior of the fiber is connected to a pressurized gaseous electron donor supply at one end and sealed at the other end. The contaminated water circulates outside of the fiber, and electron donor diffuses from the lumen of the fiber, through the wall, and towards the bulk liquid. A biofilm grows on the outside of the membrane and attached biofilm will effectively degrade the contaminant (Nerenberg et al., 2002). Hydrogen-based, hollow-fiber membrane MBfR is ideal for ClO_4^- reduction in drinking water treatment systems, as hydrogen gas is inexpensive, non-toxic, and leaves little or no residual in the treated water. MBfRs are tested in bench-scale and pilot-scale units for treating low levels of ClO_4^- (55 μg/L) in the presence of nitrate (5 mg/L) and DO (8 mg/L) (Nerenberg et al., 2003). Methane-based membrane biofilm reactors (MBfRs) are also tested for ClO_4^- remediation (Luo et al., 2015; Xie et al., 2018; Lv et al., 2019; Wu et al., 2019). Recently, ClO_4^- degradation was successfully demonstrated using ethane and propane as gaseous electron donors in bench-scale MBfRs (Lai et al., 2021). Among the gaseous electron donors used H_2/CO_2 mixture is the most studied and efficient electron donor used for perchlorate remediation.

2.2.4.2. In-situ bioremediation approaches for perchlorate contaminated water and soil

In situ ClO_4^- remediation methods are employed for the treatment ClO_4^- contaminated soil and water. Enhanced In-situ Bioremediation (EISB), Phytoremediation, and constructed wetlands are the methods practiced so far.

a) Enhanced In-situ Bioremediation

Enhanced In-situ Bioremediation is the process by which, the ClO_4^- biodegradation is achieved either by bio-stimulation or bioaugmentation (Hatzinger et al., 2006). Bioaugmentation is the introduction of specialized ClO_4^- reducing bacterial isolates along with carbon sources and nutrients into the contaminated groundwater or soil for the degradation of ClO_4^-. But in most cases, augmentation with ClO_4^- reducing microbes are not necessary (Deitsch et al., 2005; Stroo et al., 2009). Perchlorate-reducing microbes are ubiquitous and hence instead of bio-augmentation, indigenous PRMs can be bio-stimulated to degrade ClO_4^- to below detection by adding suitable substrate (carbon source, electron donor and nutrients) to these environments (Coates et al., 1999). Permeable Reactive Bio-barriers (PBRs) and Horizontal Flow Injection Well are the two strategies used in the bio-stimulation of PRM for in-situ groundwater remediation (Hatzinger, 2005; Stroo & Ward, 2008). Permeable reactive bio-barriers are in-situ physical barriers constructed across a contaminated groundwater plume for the microbial remediation of the contaminant when a suitable substrate is provided as a carbon source and electron donor (Borden, 2007). In-situ anaerobic soil composting and soil flushing and subsequent treatment of the groundwater etc, are practiced in case of soil contaminated with ClO_4^- (ITRC 2002, 2005, 2008; Battey et al., 2007)).

b) Phytoremediation

Phytoremediation of ClO_4^- using terrestrial woody and vascular plants is an alternative bioremediation approach for ClO_4^- removal. Phyto-accumulation, phyto-degradation, or rhizo-degradation are the mechanisms by which plants remove the toxic chemical from the environment where they grow (Nzengung et al., 1999; Nzengung & McCutcheon, 2003). Woody and rooted wetland plants are shown to have ClO_4^- removal properties (Yu et al., 2004; Fang & Chen, 2011; Liang et al., 2021). Phytoremediation using free-floating macrophytes such as *Eichornia, Pistia*, *Salvinia*, and *Lemna* was also reported in the literature (Bhaskaran et al., 2013).

c) Constructed wetlands

In a constructed wetland, the plants and their associated rhizospheric microflora removes ClO_4^- from groundwater (Krauter, 2001). Several wetland plant species (Bulrush, Canna Indica, Cattails, Sedges, etc.) are tested for their ClO_4^- removal efficiency in lab-scale units and field-scale units (Tan, et al., 2004; Krauter et al., 2005; He et al., 2013; Li et al., 2021).

2.2.5. Ex-situ remediation approaches for perchlorate contaminated soil

a) Thermal desorption

In a thermal desorption system, the evaporation and volatilization property of the ClO_4^- was utilized for its removal (ITRC 2008). Perchlorate impacted soil was excavated and heated in a drum to 500 – 1100˚F to remove the contaminants. The exhaust is caught and further treated in filters and afterburners for the destruction of ClO_4^- and other contaminants. High energy and temperature requirements are the major drawbacks of the thermal desorption process (Gangopadhyay et al., 2005, 2010).

b) Anaerobic Soil composting

In anaerobic soil composting, excavated soil is amended with carbon sources such as soluble organic carbon sources (acetate, lactate, molasses), slow carbon release compounds (vegetable oil, emulsified vegetable oil, hydrogen release compound), and solid carbon sources (mulch, compost, poultry waste, cow manure), etc. and anaerobically composted inside lined treatment cells (ITRC 2005, 2008). The ClO_4^- reducing microbes present in the soil will biodegrade the ClO_4^- by utilizing the substrates. The efficiency of this approach depends on the presence of appropriate microflora and the ability of the substrate to stimulate sufficient growth and activity (Amin et al., 2015).

2.2.6. Hybrid processes for perchlorate remediation

Due to the disadvantages associated with the available processes, certain hybrid processes are tested for the removal and destruction of ClO_4^- from contaminated matrices. Table 2.5. summarizes the hybrid ClO_4^- remediation processes reported in the literature.

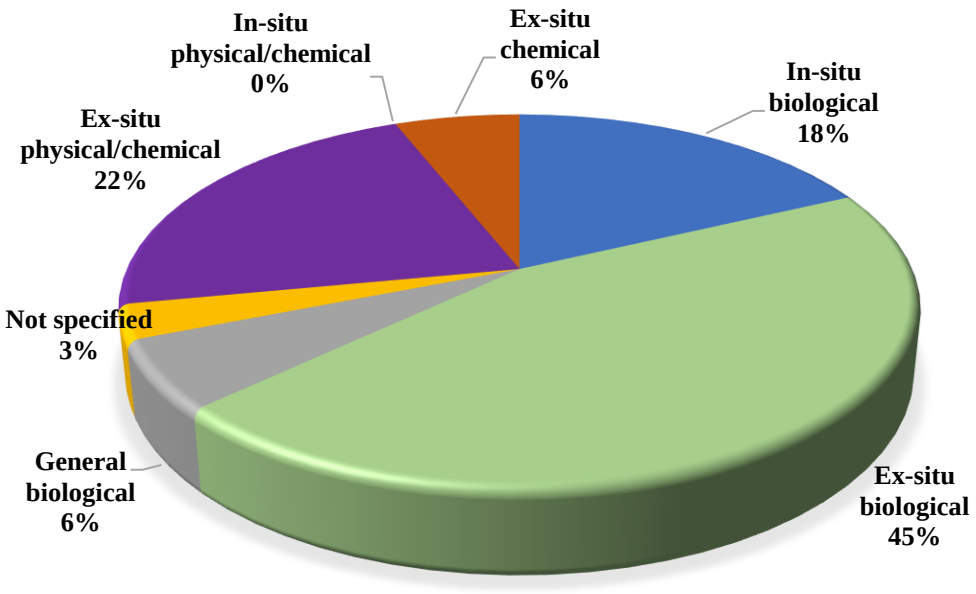
Table 2.5. List of hybrid processes reported for perchlorate remediation

Hybrid process	**Process Type**	**Remarks**	**Reference**
Biologically Activated Carbon (BAC)	Bio-physical	Adsorption on Granular Activated Carbon followed by biological reduction of perchlorate in fixed bed biological reactors	(Brown et al., 2002, 2005) (Choi et al., 2008)
Bio-regeneration of ion exchange brine	Bio-physical	Ion exchange followed by biological regeneration of the concentrated brine, in sequencing batch reactors, fluidized bed reactors, and membrane biofilm reactors	(Lin et al., 2007) (Lehman et al., 2008) (Van Ginkel et al., 2008) (Chung et al., 2010)
Ion Exchange Membrane Bioreactor (IEMB)	Bio-physical	Ion exchange membrane is regenerated using microbial reduction of perchlorate laden resin	(Gao et al., 2012) (Matos et al., 2005, 2006) (Ricardo et al., 2012) (Fox et al., 2014)
Electrodialysis and Reverse Osmosis	Physical	Electrodialysis followed by reverse osmosis for perchlorate removal	(Yang et al., 2020)

Reverse osmosis (RO) and biological reduction	Bio-physical	Reverse osmosis followed by biological reduction of perchlorate in the RO rejectate in a packed bed reactor inoculated with perchlorate reducing Dechloromonas sp. Perc1ace bacteria.	(Giblin et al., 2002)
Adsorption/Ultrafiltration	Physical	Adsorption of perchlorate onto Chitosan followed by ultrafiltration to recover pure water	(Xie et al., 2011)
Microbial Fuel Cells (MFC) Microbial Electrolysis Cells (MEC)	Bio-electrochemical systems (BES)	Electrochemically active microorganisms serve as catalysts for the reduction of perchlorate (Biocathode)	(Thrash et al., 2007) (Shea et al., 2008) ((Butler et al., 2010) . (Wang et al., 2014) (Li et al., 2015) (Lian et al., 2016, 2017)

2.2.7. Status of perchlorate remediation technologies

According to the status report compiled by the Ground-Water Remediation Technologies Analysis Center (GWRTAC), out of the 65 case studies reported for ClO_4^- remediation in the United States, 69 % of the pilot and full-scale projects for ClO_4^- remediation are based on biological methods (Figure 2.2). Among those 65 projects, only 11% are full scale, 40 % are field/pilot scale and 47 % are laboratory/ bench scale operations (the rest 2% is unknown).



Total number of case studies = 65

Figure 2.2. Different treatment technologies employed for perchlorate remediation (GWRTAC, 2001)

Among the biological treatment methods employed 19% are fluidized bed reactors and 11% are packed bed reactors (Figure 2.3). The selection of the ClO_4^- remediation method depends on the type and the initial ClO_4^- concentration in the contaminated matrix. Physical remediation processes are not employed when the ClO_4^- levels are >100000 ppb (GWRTAC, 2001) (Figure 2.4). Chemical treatment methods are the least explored. The concentration of ClO_4^- in the drinking water was less than 1000 ppb in all the case studies and high levels of ClO_4^- were usually observed in the case of soil, groundwater, and waste streams.

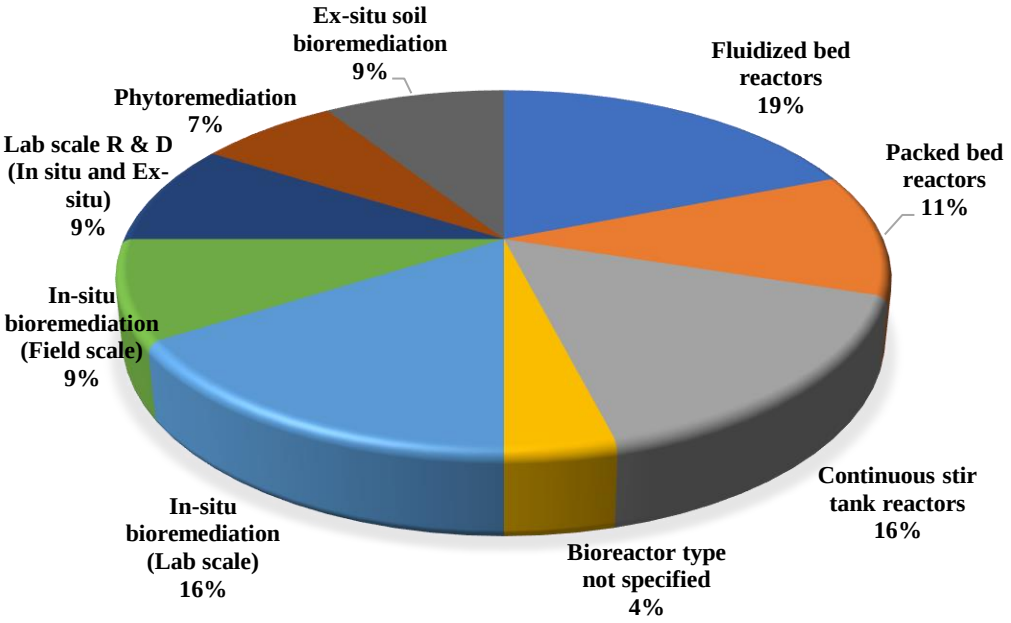


Figure 2.3. Different bioremediation methods employed for perchlorate contaminated water and soil (GWRTAC, 2001)

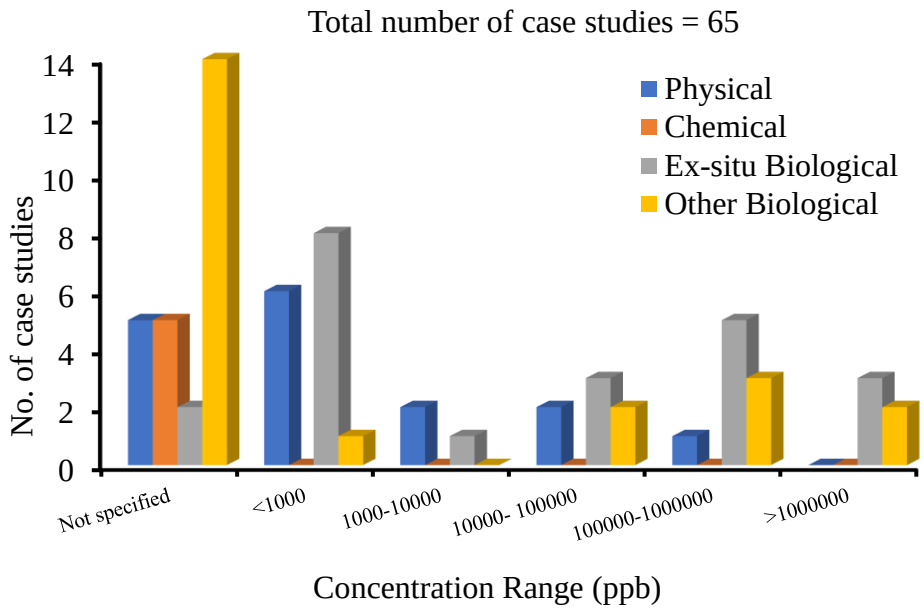


Figure 2.4. The number of case studies reported based on the concentration range of perchlorate and the type of treatment technology implemented (GWRTAC, 2001).

2.2.8. Patents on perchlorate treatment methods and technology

Several patents disclose the processes and technologies available for the remediation of perchlorate-containing matrices. A thorough patent search was done using common patent databases and search engines and the results are presented in Table 2.6.

Table 2.6. List of patents disclosed for perchlorate treatment methods and technology

Technology	Inventors	Assignee	Patent No	Published Year
A bacterial consortium for reducing perchlorate and/or nitrate and the process thereof	Krishnakumar & Anupama	CSIR-NIIST	US20210147269A1	2021
Methane oxidation coupled to perchlorate reduction in Membrane Biofilm Batch Reactor	Chen et. al.	Univ Zhejiang	CN106830354B	2020
Electrodialysis Ion exchange Membrane Bioreactor	Liu et.al.	Univ Henan Technology	CN105253992A	2019
Hybrid anaerobic ammonia oxidation coupled to electrocatalytic – catalytic oxidation followed by ultrafiltration and reverse osmosis	Ming et.al.	Shanghai Mideapure Environmental Engineering Co. Ltd.	CN104276720A	2017
Electrochemical reduction and microbial hydrogen autotrophy	Liu et.al.	Univ Henan Technology	CN104843953A	2017
Bioremediation using commercial bacterial culture	Saul et.al.		US9802230	2017
Electrochemical reduction	Gao et.al.	Univ Nankai	CN107162117A	2017
In-situ perchlorate bioremediation of water and soil	Borden		US9365441B2	2016

Methane substrate biomembrane reactor	Hongxue et. al.	Univ Hunan	CN105271513A	2016
Adsorption on magnetic resin followed by biological reduction	Gao et.al.	Univ Tongji	CN105906072A	2016
Multi-chamber fixed-bed biomembrane reactor	Guo et.al.	Univ Tianjin Chengjian	CN105984943A	2016
Biological reduction using biodegradable composite materials as solid carbon sources	Fan et.al.	Univ Jinan	CN103709694B	2015
Adsorption of tailored earth materials and bio regeneration	Nzengung	MuniRem Environmental LCC. US	US9067808	2015
Biological treatment of ion exchange brine	Roberts et.al.	The University of Houston System, US	US8772014	2014
Biological reduction	Christine et.al.	Safran Seramics. SA	ES2398062T3	2013
Electrode biofilm reactor	Jie Gao		CN102616942A	2012
Fluidized Bed Reactor	Canzano et.al	Envirogen Technologies, US	US2010/0089825 A1	2010
Metabolic primers for detection of perchlorate reducing bacteria	Bender et.al.	Southern Illinois University	US7700756	2010
Adsorption	Gurol et.al.	PURE O TECH Inc., US	US7850854	2010

Ion exchange	Jensen et.al.	Envirogen Technologies, US	US7754071	2010
Ion exchange	Qian et.al.	Univ Shangai, China	CN101456616B	2009
Autotrophic Packed Bed Reactor	Sengupta et.al.	University of Massachusetts	US7575686B2	2009
Ion exchange	Jensen et.al.	Basin Water Inc, US	US0116124A1	2008
Ion exchange	Clarke et.al.	Applied Intellectual Capital, US	US7399725	2008
Bio-degradation of perchlorate in ion exchange resins	Batista	Basin Water Inc, US	US7407581	2008
Membrane Biofilm Reactor using Hydrogen gas as substrate	Bowman	Perkins Coie LLP, US	US2008302720A1	2008
Ion exchange	Jensen et.al.	Basin Water Inc, US	US7309436	2007
Ion exchange	Coppola et.al.	Applied Research Associates Inc. New Mexico	US0114178A1	2007
Adsorption on GAC	Cannon et.al.	The Penn State Research Foundation, US	US7157006B2	2007
Membrane Biofilm Reactor	Rittmann et.al.	Northwestern University, US	US7186340 B1	2007

Biodegradation of perchlorate in ion exchange resins	Guter et.al	Basin Water Inc, US	EP1567455B1	2006
Biological reduction using elemental sulfur	Bentley et.al.	Hydro Geochem Inc	US0292684A1	2006
Catalytic reduction	Gu et.al.	U.T. Battelle, US	US6800203	2004
Chemical reduction	Gurol et.al.	San Diego State University Foundation, US	US6531065	2003
Isolation of perchlorate and nitrate-reducing bacteria (DM – 17)	Gearheart et.al.		US6423533	2002
Packed Bed Reactor	B. E. Logan	The Penn State Research Foundation, US	US6214607B1	2001
Biological purification of perchlorate	Gaudre-Longerinas et.al.	SNPE, France	US6328891	2001
Continuous Stirred Tank Reactors	Coppola et.al.	Applied Research Associates Inc. New Mexico	US6077432A	2000
Ion Exchange and biological treatment of regenerant brine followed by reverse osmosis or nanofiltration	Venkatesh et.al.	Calgon Carbon Corporation, US	US6066257A	2000

Biological reduction of perchlorate and nitrate using Perclace culture	Frankenberger et.al.	The Regents of the University of California, US	US6077429	2000
Biological reduction in hydrogen gas lift reactor	Attaway III et.al.		US5948260	1999
Biological reduction of chlorite	Van Ginkel et.al.	Akzo Nobel N.V.	US5891339	1999
Biological reduction using HAP 1 culture	Attaway et.al.		US5302285	1994
Biological treatment	Yakovlev et.al.		US3755156	1973
Biological reduction of perchlorate and chlorate using Vibrio dechloraticans Cuznesove B-1168	Korenkov et.al.		US3943055	1976

2.2.9. Perchlorate remediation research in India

The studies on ClO_4^- remediation from India are very limited. In an early study, Ghosh et.al. (IIT, Guwahati) have reported ClO_4^- degradation by an enrichment culture under anaerobic conditions in batch studies (Ghosh et al., 2011).

The CSIR-NIIST is the pioneer in the extensive research on ClO_4^- contamination in India, including periodical surveillance of ClO_4^-, and development of ClO_4^- remediation methods (Anupama et al., 2012, 2013, 2015a 2015b, 2017). NIIST has screened randomly collected water samples from different states/Union Territories (UTs) such as Kerala, Tamil Nadu, Maharashtra, Assam, Goa, Uttar Pradesh, Jammu & Kashmir, and New Delhi over the past few years. On the remediation aspect, NIIST has developed a microbial system, and a patent on the remediation of perchlorate/nitrate contaminated matrices (US20210147269A1). The inputs from preliminary studies were the basis for the development and scale up ClO_4^- remediation processes detailed in this thesis. Phytoremediation of ClO_4^- using floating macrophytes, as well as the development of organic functionalized clay-based adsorbent for ClO_4^- removal was also reported from NIIST (Bhaskaran et al., 2013; Sankar et al., 2014).

Recently few more research groups in India have also started reporting studies in this area. Adsorption of ClO_4^- using quaternary ammonium functionalized chitosan beads was reported from SRM University (Sowmya et al., 2020). Sijimol et.al. (MG University, Kerala) demonstrated ClO_4^- degradation (~30%) through a Fenton-type chemical process (Sijimol et al., 2020).

Chapter 3

Surveillance of perchlorate contamination around major ammonium perchlorate inventories in Kerala, India

3.1. Introduction

In India, the first report on the presence of ClO_4^- in water and human saliva samples from five different states/Union Territories (UTs) (Tamil Nadu, West Bengal, Bihar, Maharashtra, Karnataka, and Pondicherry) was in 2009. However, the observed mean value (6.9 μg/L) was below the international guidelines (Kannan et al., 2009). Subsequently, in 2012, studies by Anupama et al., have reported for the first time, high levels (up to 91.4 μg/L) of ClO_4^- in groundwater samples in India (Anupama et al., 2012). Later few more studies have also reported significant levels of ClO_4^- in water samples from different states in India (Isobe et al., 2013; Anupama, et al., 2015a; 2017; Sijimol et al., 2016). Among those studies, high levels of perchlorate were detected in groundwater samples (up to 7690 μg/L) from perchlorate handling sites such as firework manufacturing industries at Sivakasi, Tamil Nadu district in India (Isobe et al., 2013).

Unlike the other states in India, Kerala has two known bulk ClO_4^- handling sites, one is the Ammonium Perchlorate Experimental Plant (ISRO-APEP) located in Keezhmad panchayath, Aluva, in Ernakulam district, and the other is Rocket Propulsion Plant (VSSC-RPP), in Thumba, Thiruvananthapuram district. The former produces ammonium perchlorate in bulk, whereas the latter routinely uses it for space R&D activities. A study conducted by Anupama et.al. has reported ClO_4^- levels up to 7270 μg/L in public well water samples near to APEP, as well as ~300 μg/L ClO_4^- in well water samples near to RPP (Anupama, et al., 2015a). A later study by the same group in 2017, has revealed ClO_4^- levels up to 43,000 μg/L (maximum level reported in India so far) in community well water near APEP. The study also reports the presence of ClO_4^- up to 1.6 km away APEP (Anupama et al., 2017). Meanwhile, an independent study conducted by the Kerala State health department observed a higher incidence of hypothyroidism among people exposed to ClO_4^- contaminated well water in Keezhmad Panchayath near APEP (Keezhmad Project Report, 2015).

Being a recently identified environmental and public health problem in India, the continuous monitoring of ClO_4^- at severely contaminated regions is highly important. This will leverage the initiatives for developing technologies for addressing the pollutant. Therefore, the objectives covered in this study includes (i) assessing the present status of ClO_4^- levels in selected community water resources around the highly contaminated regions around APEP and (ii) to study the possibility of natural attenuation of ClO_4^- by indigenous microflora.

3.2. Materials and Methods

3.2.1. Study area, sampling points, and sample collection

The study area, and sampling points for this study were determined based on the previous studies conducted by Anupama et.al. during 2009 – 2015. The earlier studies have shown higher levels of ClO_4^- in the Ernakulam and Thiruvananthapuram districts of Kerala. More specifically around the ISRO - APEP unit in Keezhmad Panchayath, Aluva, Ernakulam, and near VSSC - RPP, Thumba, Thiruvananthapuram (Anupama, et al., 2015a, 2017). Hence in this study focus was given in analyzing water samples from these locations

Site 1: Keezhmad Panchayath, Aluva, Ernakulam district.

The Ammonium Perchlorate Experimental Plant (ISRO-APEP) is located near a heavily populated rural area in Keezhmad Panchayath, Aluva, Ernakulam district. Kerala. During the previous studies by CSIR-NIIST, remarkably high levels of ClO_4^- were observed in three community open wells (PW1, PW2, and PW3), several household open wells, and a community pond (Kulakkad Pond) in this area. The contaminated community pond Kulakkad pond is located nearly 550 meters away from APEP. An infiltration stream of unknown origin that carries a high concentration of ClO_4^- to the pond was spotted in this area. There is also an outflow canal that is directed towards Periyar River which is hardly 3.1 km away from APEP.

To determine levels of ClO_4^-, water samples were collected from the household open wells, community wells (PW1, PW2, and PW3), and the Kulakkad pond during January 2018 and March 2021. The samples from community wells (PW1, PW2, and PW3) and the Kulakkad Pond (P1) (collected during 2021) were analyzed in detail for pH, Total Dissolved Solids (TDS), Dissolved Oxygen (DO) Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), nitrate (NO_3^-), Total Plate Count (TPC) The location of APEP and the sampling points are shown in Figure 3.1. An ariel view of APEP, PW1, PW2, PW3, and Kulakkad pond is shown in Figure 3.2. The sampling points from the Kulakkad pond are shown in Figure 3.3. The infiltration stream is shown as P4, and the outflowing canal is marked as P5 (Figure 3.3).

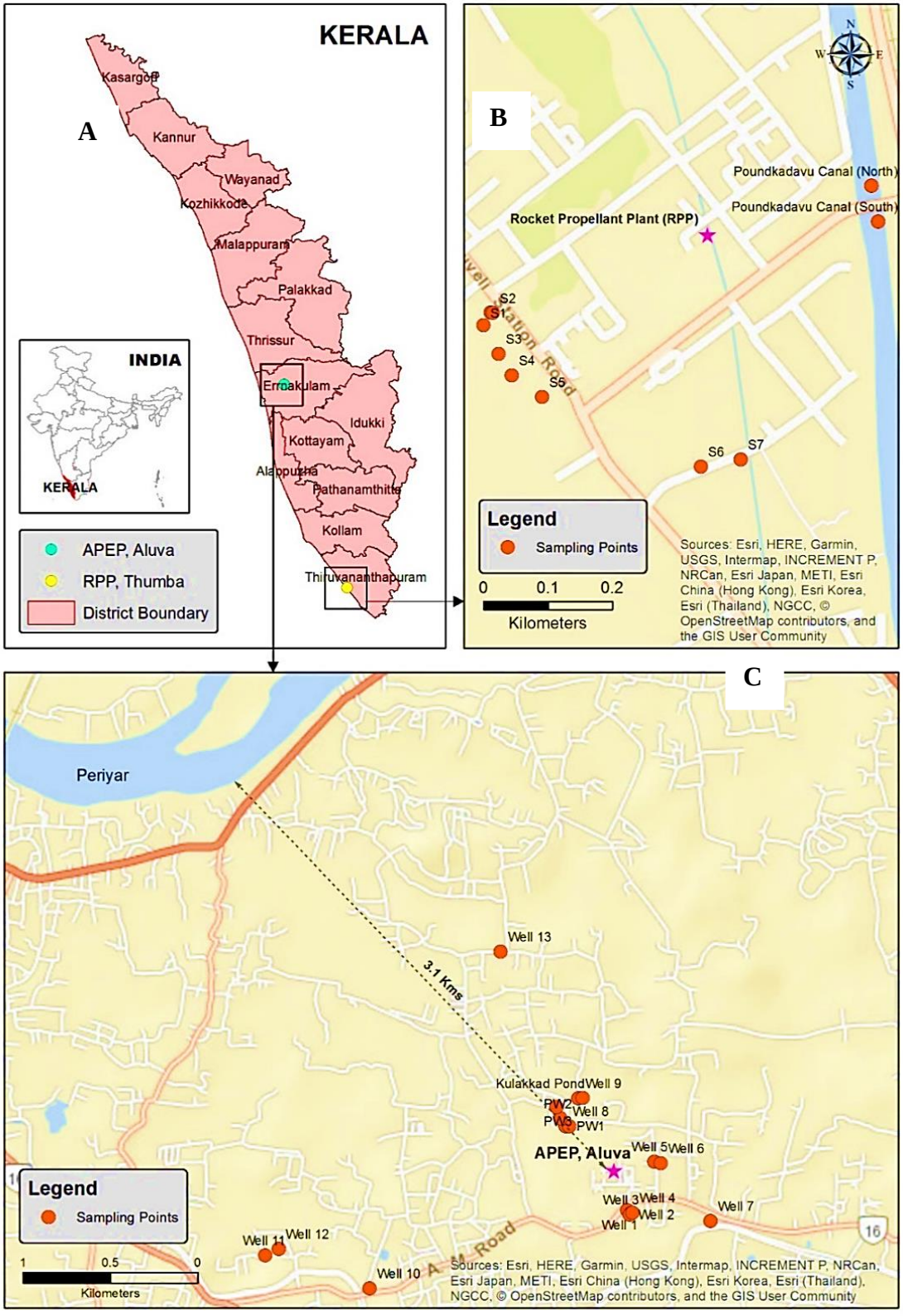


Figure 3.1. (A) Map of Kerala showing the location of Ammonium Perchlorate Experimental Plant in Aluva, Ernakulam (cyan circle) and Rocket Propulsion Plant in Thumba, Thiruvananthapuram (yellow circle), in Kerala and the area map showing sampling points around RPP **(B)** and APEP **(C)** (in orange circles)

Figure 3.2. Ariel view of APEP in Aluva, and the three community wells (PW 1, 2, and 3), and the heavily contaminated community pond (Kulakkad Pond) in the area. (Courtesy: Google Earth ©)

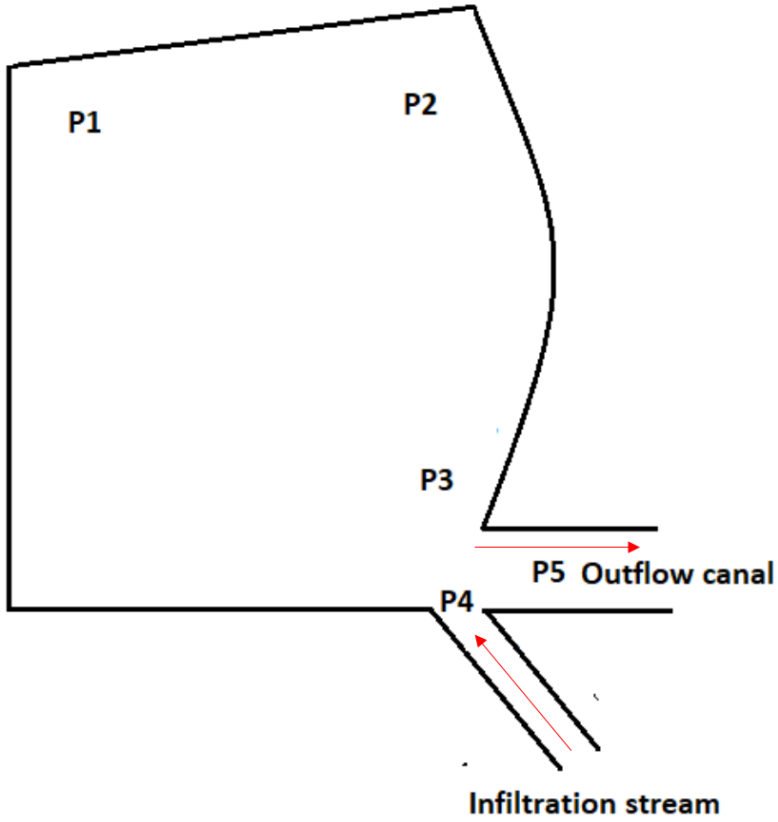


Figure 3.3. A schematic of the perchlorate contaminated community pond (Kulakkad pond) with sampling points (P1-P5).

Site 2: Thumba region, (near to VSSC-RPP), Thiruvananthapuram District

The Rocket Propulsion Plant (VSSC-RPP) is located near a populated coastal area in the Thumba region, Thiruvananthapuram. The sampling points around RPP were also determined based on previous data. Water samples were collected from a nearby canal (Poundkadavu Canal, 8°31'29"N, 76°52'54"E) and well water (household open wells and a bore well) sources near RPP in Thumba, Thiruvananthapuram during March 2021 (Figure 3.1 **B**).

The total number of well water and surface water samples collected from each site and sampling period is presented in Table 3.1. Approximately one liter of water was collected from all the sampling points and brought into the laboratory for detailed analysis. Sterile bottles were used for the samples for microbial analysis.

Table 3.1. The number of well water and surface water samples analyzed from Site 1 and Site 2 during 2018 and 2021 as part of this study

Site 1 (APEP)				**Site 2 (RPP)**	
2021 (March)		2018 (January)		2021 (March)	
No of well water	No of Surface water	No of well water	No of Surface water	No of well water	No of Surface water
12	5	10	4	8	2

3.2.2. Enrichment of perchlorate reducing consortia from contaminated well water samples

During an enrichment procedure we expose the microbes to higher concentration of contaminants (selection pressure) so that the microorganism that can tolerate and metabolize the contaminant will only get selectively enriched. The water sample from PW1 (close to APEP) was used for developing ClO_4^- reducing enrichment culture, and for substantiating natural degradation of ClO_4^-. The PW1 water was chosen because, it was heavily contaminated with perchlorate up to 43000 mg/L previously and microflora present in the PW1 water was

already exposed to higher levels of perchlorate. Hence, we assumed that under suitable conditions the enrichment will be faster. Under laboratory conditions, microcosm studies were performed using the water from PW1 as inoculum. Around 250 mL water sample from PW1 (~2.2 mg/L of ClO_4^- was already present) was amended with ~ 15 mg/L of ClO_4^- and 60 mg/L of acetate (perchlorate: electron donor ratio, 1:4) and 0.025% yeast extract in triplicate. A laboratory control using tap water (non-sterile) was also kept by spiking with 10 mg/L of ClO_4^- and 40 mg/L of acetate. The experiments were conducted under ambient conditions.

After 3 days, when the ClO_4^- concentration was below the detection limit in the bottles with PW1 water, the culture was repeatedly spiked with ClO_4^- (~50 &100 mg/L), acetate (200 & 400 mg/L), and nutrients (inorganic minerals and trace metals) for confirming the ClO_4^- reduction potential of enrichment culture. The enrichment culture was subsequently maintained in the laboratory by spiking with 50 mg/L of ClO_4^- and acetate in the ratio of 1:2 on every 2 days. The enrichment culture was also sub-cultured onto Inorganic Mineral Media supplemented with Trace Metals (Russel et al., 2021) for further studies such as community analysis by conducting V3-V4 metagenomics analysis. The results of the metagenomic analysis are anticipated to provide further insights into the marginal decline in ClO_4^- levels in the community wells (natural attenuation) that are being observed in the field.

3.2.3. Sample preparation and analysis

The pH, TDS and DO of the samples were measured using pH probe (Eutech Instruments, UK), TDS probe (Hanna Instruments, USA) and DO probe, (Thermo scientific, USA), respectively APHA standard methods (APHA 1998) was followed for the analysis of following parameters: Chemical Oxygen Demand (COD, 5220 B - Open Reflux Method), Biological Oxygen Demand (BOD, 5-Day BOD Test – 5210B), nitrate (4500 NO_3^- E, Cadmium Reduction Method), Total Plate Count (TPC, 9215C - Spread plate method). The samples were analyzed for ClO_4^- ions using Ion-Selective Electrode (ISE) and Ion Chromatography system (IC). The samples were filtered using 0.2 μm filter paper (Millipore). The field samples were initially screened with ISE and if necessary, it was subsequently diluted to 500 μg/L level for accurate analysis using the IC system.

a) Ion-Selective Electrode (ISE) Method

A combination ISE specific for ClO_4^- (Cat No. WW-27504-24; Cole Palmer, USA) was used in this method. A three-point standard curve was established using potassium perchlorate (Sigma Aldrich). The detection range of ISE was 500 μg/L – 20,000 mg/L

b) Ion Chromatography Method

Lower concentration (< 500 μg/L) of ClO_4^- in the samples were analyzed using Ion Chromatography (IC) as per the method for ClO_4^- detection in drinking water recommended by USEPA methods 314.0 and 314.1. An IC system (IC – 5000, Dionex) with a separation column-Ion Pac AS 16 (2x250 mm and 4x250 mm), guard column-Ion Pac AG 16 (2x50mm and 4x50 mm), and an anion self-regenerating suppressor ASRS 300 (4mm) was used in this method. 50 mM NaOH (Sigma Aldrich) was used as eluent at a flow rate of 1.5 mL/min. The injected sample volume was 1000 ml. Perchlorate standards were prepared using high purity $KClO_4$ (Sigma Aldrich) by diluting 1000 mg/L primary standard. Samples were also diluted before analysis. All solutions were prepared using ultrapure Milli-Q water (Millipore). The lower detection limit of ClO_4^- through this method (Method Detection Limit – MDL) was 0.5 μg/L.

Quality Assurance and quality control (QA/QC) for IC

A calibration curve (1 – 500 μg/L) was generated and laboratory reagent blank and fortified samples were analyzed for QC. The mean recovery of ClO_4^- with the AS 16 column and analytical condition was 100±10.

3.3. Results and Discussion

3.3.1. Assessment of perchlorate levels samples from Site 1, (Keezhmad, Ernakulam)

In order to assess the present status of ClO_4^- level at site 1, water from both community open wells as well as private open wells were collected and analyzed and the results are presented in subsequent sections. Previous studies recorded a very high concentration of ClO_4^- in the community wells (up to 43 mg/L) and the community pond (up to 34 mg/L) near APEP (Anupama, Puthiyaveettil, et al., 2015; Anupama et al., 2017).

(i) Perchlorate levels in community open wells

The three community wells PW1, PW2, and PW3 are located in the Kulakkad region of Keezhmad Panchayath. These community wells were the drinking water source for 180 families and due to severe ClO_4^- contamination, the people are prohibited from using water from those wells. Even though alternative drinking water was provided, water availability is a major problem in this area. In 2018, the wells were in closed condition and the people were not using the well water (Figure 3.4). Presently, we have observed that the people started using the well water for purposes other than drinking, like washing clothes and utensils and for construction works. But PW1 was almost in an abandoned condition with plants growing on the inside wall of the well. The position of the wells with respect to APEP and the level of ClO_4^- in the wells during 2018 and 2021 are presented in Table 3.2.

During the previous study conducted by Anupama et. al. in 2017, the maximum ClO_4^- levels in PW1, PW2, and PW3 were 43, 23, and 9 mg/L respectively (Anupama et al., 2017). A marginal decline in ClO_4^- concentration in PW1 was observed during follow-up studies. This may be due to the dilution effect or microbial degradation of ClO_4^- by indigenous microflora. Results of the study to verify microbial degradation of ClO_4^- is discussed in the subsequent section.

Table 3.2. A comparison of perchlorate concentration in community wells near Site 1, during 2018 and 2021.

Sl. No.	Sample ID	Distance from APEP (m)	ClO_4^- (µg/L)	
			2018	2021
1	PW1	350	13200	2270
2	PW2	450	6800	7230
3	PW3	550	1340	1130

Figure 3.4. Closed community wells PW1, PW2 and PW3 contaminated with ClO_4^- in Kulakkad region, Keezhmad, Ernakulam District

(ii) Perchlorate levels in household open wells

The concentration of ClO_4^- in household open wells during January 2018 and March 2021 is given in Table 3.3.

Table 3.3. Perchlorate concentration in household open wells at Site 1, Keezhmad in Aluva during January 2018 and March 2021.

Sl No.	Sample ID	Distance from APEP (m)	ClO_4^- (mg/L)	
			2018	2021
1	Well 1	100	12593	NA
2	Well 2	100	NA	605
3	Well 3	150	140	29
4	Well 4	175	NA	4481
5	Well 5	100	37	24
6	Well 6	100	22	NA
7	Well 7	200	NA	9141
8	Well 8	350	13093	NA
9	Well 9	600	4516	NA
10	Well 10	1000	220	NA
11	Well 11	1200	240	BDL
12	Well 12	1200	NA	BDL
13	Well 13	1300	142	NA

NA – Samples not available, BDL – Below Detection Limit

The well water samples from almost the same wells were tested during 2018 and 2021. However, some of the well water was not accessible in 2021 due to the absence of owners or the well was in dried condition, etc. Hence at this point, temporal comparison between the household well water samples are not possible. The present analysis results clearly indicate that most of the wells are severely contaminated with ClO_4^- and need follow-up. Most of the people are not using water from these wells for drinking. Even then at this time of water scarcity, it is of utmost importance to reclaim the available water resources.

The results of the water quality assessment done for the community wells are presented in Table 3.4. Previous data on water quality parameters are not available for the community wells.

Table 3.4. Water quality parameters of the community wells

Sample	pH	TDS (mg/L)	DO (mg/L)	COD (mg/L)	BOD_5 (mg/L)	Nitrate (mg/L)	Total plate count (CFU/ml)
PW1	5.21	78.43	5.65	11.97	1.37	3.6	8×10^2
PW2	4.53	76.88	4.94	8.7	0.6	5.2	1.19×10^4
PW3	5.73	119.1	6.31	15.23	1.45	5.5	2.06×10^4

(iii) Perchlorate levels in surface water samples at Site 1:

Kulakkad pond is a community pond in the Keezhmad panchayath and it was a major surface water source for the local people (Figure 3.5). The approximate area of the pond is 640 m^2 and the average depth is 5 m. A previous study conducted by Anupama et.al., have reported ClO_4^--induced toxicity to a submerged water plant, *H. veticillata*, a major water plant in the pond. Massive decay and altered growth parameters were evident in the ClO_4^- exposed plants (Anupama et al., 2017). Many nearby household wells were also found contaminated with ClO_4^-. The results of the ClO_4^- levels in pond water samples are presented in Table 3.5. The ClO_4^- level was around 10 mg/L during 2018. The source of ClO_4^- contamination to the pond was a small infiltration stream (Figure 3.6) of unknown origin (P4 in Figure 3.3) that carries a high concentration of ClO_4^- (16.2 mg/L in 2018 and 26.78 mg/L in 2021). The outflow from the pond as a canal (P5 in Figure 3.3), that contained ClO_4^- at 4.97 mg/L level and flows towards the Periyar river which is hardly 3.1 km away.

Figure 3.5. Photograph of the perchlorate contaminated Kulakkad pond near ISRO-APEP (Site 1)

Figure 3.6. Photographs of the small stream flowing towards the pond and the canal flow out of the pond

Table 3.5. Perchlorate concentration in pond water samples during 2018 and 2021.

Sl. No.	Sample Name	ClO_4^- in January 2018 (μg/L)	ClO_4^- in March 2021 (μg/L)
1	Pond water P1	10200	365
2	Pond water P2	10600	NA
3	Pond water P3	10800	1840
4	Infiltration (P4)	16200	26780
5	Outflow canal (P5)	11400	4970

NA - Sample not analyzed

During the previous study, the highest level of ClO_4^- observed in the pond and the infiltration stream was 5 mg/L and 34 mg/L respectively (Anupama et al., 2017). Sijimol et. al. reported groundwater ClO_4^- levels up to 1.172 mg/L in 2016 and 32.06 mg/L in 2017 near APEP in Aluva (Sijimol et al., 2016, 2017). A difference in the level of ClO_4^- at two ends of the pond was observed in previous studies. As shown in Figure 3.3. sampling points P3 and P4 are near to APEP and always had higher ClO_4^- at that end of the pond. The perchlorate concentration in the infiltration stream (P4) was not constant and depends on the climatic factors like rain that can leach more perchlorate or can dilute the infiltration stream. March is the beginning of summer and that might be the reason for increased ClO_4^- concentration. Whereas points P1 and P2 are opposite ends and the $ClO_4^-$was much lower. But during this study, in 2018 the pond water had more or less the same ClO_4^- levels, and this may be due to the thorough mixing of the pond water. In 2021 it was much lower at the P1 end (365 μg/L) and this may be due to the construction of a bund separating the pond water and the contaminated infiltration stream. Moreover, the pond was cleaned by draining the water completely.

In 2021, the pH of the pond water was 5.31 and TDS was 39.48 mg/L. The COD and BOD values were 14.14 and 1.5 mg/L, respectively. The nitrate concentration in the pond was 1.7 mg/L and the total bacterial count was 2.1 x 10^3 CFU/mL. Previously, (in 2015) the pH and nitrate concentration was 7.4±3 and 8.52 mg/L respectively. Previous data on other parameters are not available (Anupama et al., 2017).

Keezhmad panchayath is listed among the places in Ernakulam dist. where there is severe water scarcity and acute water shortage during the summer months (NAQUIM, 2018). Even though separate drinking water is supplied for the affected people, they find it difficult to meet their daily water need for household purposes. This may be the reason why the people have started using the contaminated well water and pond water despite the warnings from the concerned authorities.

Previous studies recorded ClO_4^- levels up to 355 μg/L in the Periyar river which is the longest river in Kerala (Anupama, et. al., 2015a). A major water treatment plant is located upstream in Aluva, from where potable water is distributed to various parts of the Ernakulam District (TOI, 2020). Therefore, close monitoring of ClO_4^- contamination in this region is very important.

3.3.2. Assessment of perchlorate levels in samples from Site 2, Thumba Region, Thiruvananthapuram

Perchlorate contamination around RPP is not widespread when compared to that around APEP. Among the samples collected from Site 2, only 3 groundwater samples contained ClO_4^- above the detection limit. The results of the ClO_4^- assessment of water samples collected from water resources near RPP are presented in Table 3.6. The concentration of ClO_4^- of 1290 μg/L was observed in a household bore well sample which is close to ISRO's Thumba Equatorial Rocket Launching Station (TERLS), Thumba, Thiruvananthapuram. The same household had an open well that had a ClO_4^- level of 745 μg/L. Previous studies conducted by NIIST around this site reported maximum ClO_4^- levels of 91 μg/L and 300 μg/L. The ClO_4^- level in a small running surface water body (Poundkadavu Canal) was BDL whereas a previous study recorded a value of 13 μg/L (Anupama et al., 2015a). A study conducted by Sijimol et.al. found 3133 μg/L of ClO_4^- near TERLS in 2017 (Sijimol et al., 2017). Hence, it is highly important to continuously monitor the levels of ClO_4^- near water resources near RPP and TERLS.

Table 3.6. Level of perchlorate in the water samples collected from Site 2

Sl No.	Sampling points	Distance from RPP (M)	ClO_4^- (μg/L)
1	S1	495	10
2	S2 (open well)	493	745
3	S2 (bore well)	478	1290
4	S3	450	BDL
5	S4	486	BDL
6	S5	463	BDL
7	S6	412	BDL
8	S7	317	BDL
9	Pondukadavu Canal North	150	BDL
10	Pondukadavu Canal South	150	BDL

Perchlorate contamination in groundwater and surface water resources is being regularly reported by many countries. The details are presented in Table 2.1. (Chapter 2). There are few reports on ClO_4^- contamination in India also (Refer to Table 2.2). This is indicative of the emerging concern regarding ClO_4^- contamination globally. Since the 1980's it was evident that the ClO_4^- is leaching from military dumpsites as USEPA detected groundwater ClO_4^- levels ranging from 380 – 811 μg/L in the Pacific and Southwest region of the USA. USEPA has identified 4 closed ClO_4^- manufacturing facilities, one operating manufacturer, and at least 100 ClO_4^- users in 40 states of the US as a potential anthropogenic source of contamination in the USA. Severe contamination of groundwater up to 3700 mg/L and surface water up to 120 mg/L was reported near ClO_4^- inventories in the USA (USEPA 2005). Similarly, in Israel, an average ClO_4^- level of 800 mg/L was reported in groundwater due to leaching from industrial waste ponds (Levakov et al., 2019). Even though there are a greater number of industries and ClO_4^- production facilities in India, there is no recommended standard for ClO_4^- levels in the water, foodstuffs, or discharges.

3.3.3. Perchlorate reduction by enrichment consortia from contaminated well

The water from PW1 amended with acetate as electron donor and carbon source could reduce ClO_4^- (externally spiked) from 17.34 ± 1.13 mg/L to below detection limit in 3 days with the help of enriched indigenous microbes that were present in the well water. The perchlorate concentration in the non-sterile laboratory control did not get reduced even after 10 days. The enrichment microcosm could reduce ClO_4^- on subsequent spiking with electron donor, and nutrients. The results of ClO_4^- reduction during the repeated spiking of the enrichment culture are presented in Figure 3.7. The initial lag in ClO_4^- reduction and requirement of excess electron donor is due to the time required for the multiplication of sufficient ClO_4^- reducing microbes (PRM) as well as due to the presence of dissolved oxygen (5.65 ± 0.1 mg/L) and nitrate (3.6 ± 0.2 mg/L) in the well water (Table 3.5). The enrichment consortia sub-cultured and maintained in 50 mg/L of ClO_4^- and 100 mg/L of acetate is also showed ClO_4^- reduction within 24 hours of spiking. This culture will be further used for community analysis using V3-V4 metagenomics.

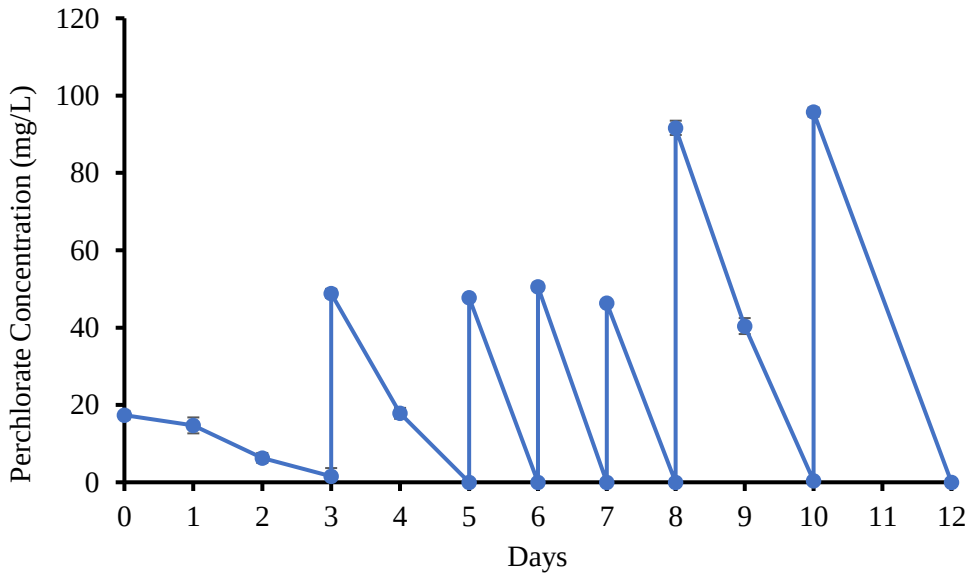


Figure 3.7. Perchlorate reduction using enrichment consortium from public well 1 water amended with an electron donor (acetate and nutrients). Each spike represents the addition of perchlorate into the enriched media.

The formation of an enrichment culture capable of reducing ClO_4^- is indicative of the exposure of the natural (indigenous) organisms to higher levels of ClO_4^- for a long period in the past. Observations of the enrichment culture experiment reconfirm the fact that though PRMs are present in the environment, natural degradation of ClO_4^- is highly limited due to the reasons such as PRMs are not present in sufficient number, lack of substrates (carbon and electron donor), optimum redox conditions and presence of competitive inhibitors like nitrate, O_2, etc. Even though PRMs are ubiquitous, the enrichment from anaerobic sludge, pristine soils, and sediments takes several weeks and months and exposure to a higher initial concentration of ClO_4^- (500 – 1000 mg/L) is usually required (Coates et al., 1999; Wu et al., 2001). Our initial studies on the enrichment of ClO_4^- reducing consortia from anaerobic sludge from existing wastewater treatment facility took one month for complete degradation of ClO_4^- from 500 mg/L to below detection limit using 1000 mg/L of acetate in the absence of oxygen. In this study, the growth of PRMs was stimulated by the presence of a low initial concentration of ClO_4^- even in the presence of competitive electron acceptors.

Monitored natural attenuation (MNA) is a strategy that is being evaluated for its effectiveness in remediating ClO_4^- in groundwater. Natural attenuation of ClO_4^- in the environment depends on the biogeochemical conditions of the contaminated groundwater aquifer. For MNA of ClO_4^-, factors such as low dissolved oxygen (DO) (anaerobic or microaerophilic conditions), a negative oxidation-reduction potential (ORP), pH between 5 and 8, nitrate concentrations <5 mg/L, and total organic carbon (TOC) concentrations > 2 mg/L are required. Microcosms and bench-scale column studies can be used to demonstrate that natural attenuation is occurring and to estimate the rate and extent of ClO_4^- biodegradation similar to that we did in this study (ESTCP 2008).

The indigenous PRMs can be bio-stimulated to degrade ClO_4^- to below detection by providing carbon and nutrient source for enhancing bacterial growth as reported earlier (Coates et al., 1999). A study conducted by Vigliotta et.al. across the heavily polluted Sarno River Basin in Italy, observed the presence of ClO_4^- in the headwater but found no evidence of ClO_4^- ions along the river course. They have found that the indigenous microbes that were present in the polluted river water reduced ClO_4^- and that could be the possible explanation of the absence of ClO_4^- downstream (Vigliotta et al., 2010). Similarly, Borden et.al. observed the natural degradation of ClO_4^- in a shallow alluvial aquifer when the plume migrates through the organic-rich littoral zone. They have also observed an increase in the relative gene copy number of the perchlorate reductase A (pcrA) gene which is responsible for the enzymatic reduction of

ClO_4^-. They also speculated that natural attenuation can clean up the contaminated area within 11 – 27 years without any active remedial measures (Borden et al., 2014). But considering the toxic effects of long-term ClO_4^- e exposure and scarcity of freshwater the time required for natural attenuation is relatively high and hence cannot be considered as a remedial strategy for the highly populated region.

Conclusions

It is almost a decade (first reported in 2012) since severe ClO_4^- contamination was reported around two major known ClO_4^- inventories in Kerala and observations of the present study indicate that it is still a live public health and environmental issue that need follow up action. At site 1 (Keezhmad, Ernakulam Dist.) the open wells (both community and household) which were the primary drinking water source for the village community around APEP are still contaminated with high levels of ClO_4^- (>7230 μg/L) which is a serious threat to public health. Similarly, the ClO_4^- level in the community pond in the area is still very high (365 μg/L), and continuous infiltration of ClO_4^- contaminated water into the pond was observed and that needs special attention and control. At site 2 (Thumpa, Thiruvananthapuram Dist.) also, ClO_4^- was detected in wells near to RPP and the highest value recorded was 1290 μg/L in a household bore well. A marginal decline in ClO_4^- level was observed in some of the contaminated wells (PW 1 at site 1), and a ClO_4^- reducing consortium was enriched from the well water and under optimum conditions the consortium repeatedly reduced ClO_4^- up to 100 mg/L in a short interval of time of 24 h. This indicates the possibility of natural attenuation of ClO_4^- only under optimum conditions such organic, nutrients, electron donor, redox conditions, etc.

Chapter 4

Development of a bio-physical treatment system for perchlorate contaminated water, and its testing in a pilot-scale unit

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4.1. Introduction

As presented in previous chapters, the presence of high levels of ClO_4^- in community water resources is emerging as public health as well as an environmental problem in India. In states like Kerala with high population density, people in villages and semi-urban areas depends mostly on open wells for daily water requirement. Therefore, well water contamination with toxic contaminants like ClO_4^- needs special attention.

Among different states in India, the highest level of groundwater ClO_4^- contamination (~45 mg/L) was reported from the surroundings of ISRO-APEP, in Keezhmad Panchayat, Ernakulam, Kerala, India (Anupama et al., 2017). The APEP unit produces ammonium perchlorate (NH_4ClO_4) in bulk quantity for space R&D activities. The community open wells (drinking water source) in this region were found contaminated with ClO_4^- several magnitudes higher than international guidelines for ClO_4^- in drinking water (Keezhmad Project Report, 2015). Moreover, a higher incidence of hypothyroidism among the people (in Kulakkad region, Keezhmad) exposed to contaminated well water was also reported in that study. Subsequently, as suggested by CSIR-NIIST, the Kerala state health department temporarily closed the contaminated wells, and the people were supplied with alternative drinking water, which is continued till date.

As detailed in chapter 2, physical, chemical, and biological processes were reported for the treatment of ClO_4^- contaminated matrices. Among these methods, Ion exchange (IX), Reverse Osmosis (RO), and microbial processes have gained much attention for the treatment of ClO_4^- contaminated water. There are several reports on the treatment of drinking water contaminated with ClO_4^- using IX membranes (Lehman et al., 2008; Sanyal, 2015). Recently, IX membranes having higher affinity for ClO_4^- ions were also reported (Li et al., 2020). However, the higher cost of the IX membrane, lack of specificity of the membrane for ClO_4^- ions, and regeneration and disposal of the resin are the major challenges with this approach. Membrane filtration processes such as reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), etc. were also practiced for removing ClO_4^- from drinking water resources (Giblin et al., 2002; Yoon et al., 2003, 2009). Membrane fouling and disposal of reject and wash water are the disadvantages associated with all membrane processes. Furthermore, in all the physical processes mentioned above, the detoxification (breakdown) of ClO_4^- into non-toxic components (Cl^- and O_2) never happens. The membrane processes were generally adopted when the ClO_4^- contamination level was less than 1 mg/L. In the present study, since the level of ClO_4^- in contaminated well water

was ~15 mg/L (based on our 2018 analysis), it was decided to choose a combination of a microbial and physical process for the treatment. In the past, microbial processes are combined with physical processes mainly for the regeneration of the resin/membrane, or the treatment of rejects. (Brown et.al, 2002; Giblin et al., 2002; Lin et al., 2007; Srinivasan and Sorial, 2009; Ye et al., 2012; Fox et al., 2014; Kim and Choi, 2014; Song et al., 2015; Yang et al., 2020). To the best of our knowledge, ClO_4^- remediation processes/technologies were never reported from India.

The major objectives of this study were (1) the development of an ex-situ remediation system for treating ClO_4^- contaminated well water, and (2) to test the proof of concept in a pilot-scale unit under laboratory conditions. The fouling associated with membranes and practical solutions to address the same were covered in this study. Field-relevant concentration of ClO_4^- (~15 mg/L) observed in community wells near APEP (Aluva) was adopted in this study.

4.2. Materials and methods

In this study, initially, a Reverse Osmosis (RO) unit was tested for removing ClO_4^- (primary treatment) and an Anaerobic Fixed Film Bioreactor (AFBR) was used for treating the reject and the RO membrane backwash water. But based on preliminary results the bioreactor was used for the primary treatment, and the residual ClO_4^- was removed with the RO module. Subsequent experiments with this combination indicated frequent membrane fouling and to manage this problem a microfiltration unit (MF) was introduced in between the AFBR and RO units.

The combined bio-physical treatment system consists mainly of three units: (1) an Anaerobic Fixed Film Bioreactor (AFBR) for the biological reduction of ClO_4^- into non-toxic chloride and oxygen, (2) a ceramic Microfiltration (MF) unit for removing suspended solids including bacterial biofilm, and (3) a Reverse Osmosis (RO) unit for removing the residual trace ClO_4^- and dissolved solids and organics. The schematic representation of the entire experimental setup and a photograph of the pilot-scale treatment unit are presented in Figure 4.1. and 4.2 respectively.

4.2.1. Anaerobic Fixed Film Bioreactor (AFBR)

The primary treatment of ClO_4^- contaminated water was done in the Anaerobic Fixed Film Bioreactor (AFBR). The AFBR unit was made up of a 60 L capacity PVC barrel, having a working volume of 55 L, and it is filled with charcoal pieces as a biofilm support matrix (Figure 4.3a).

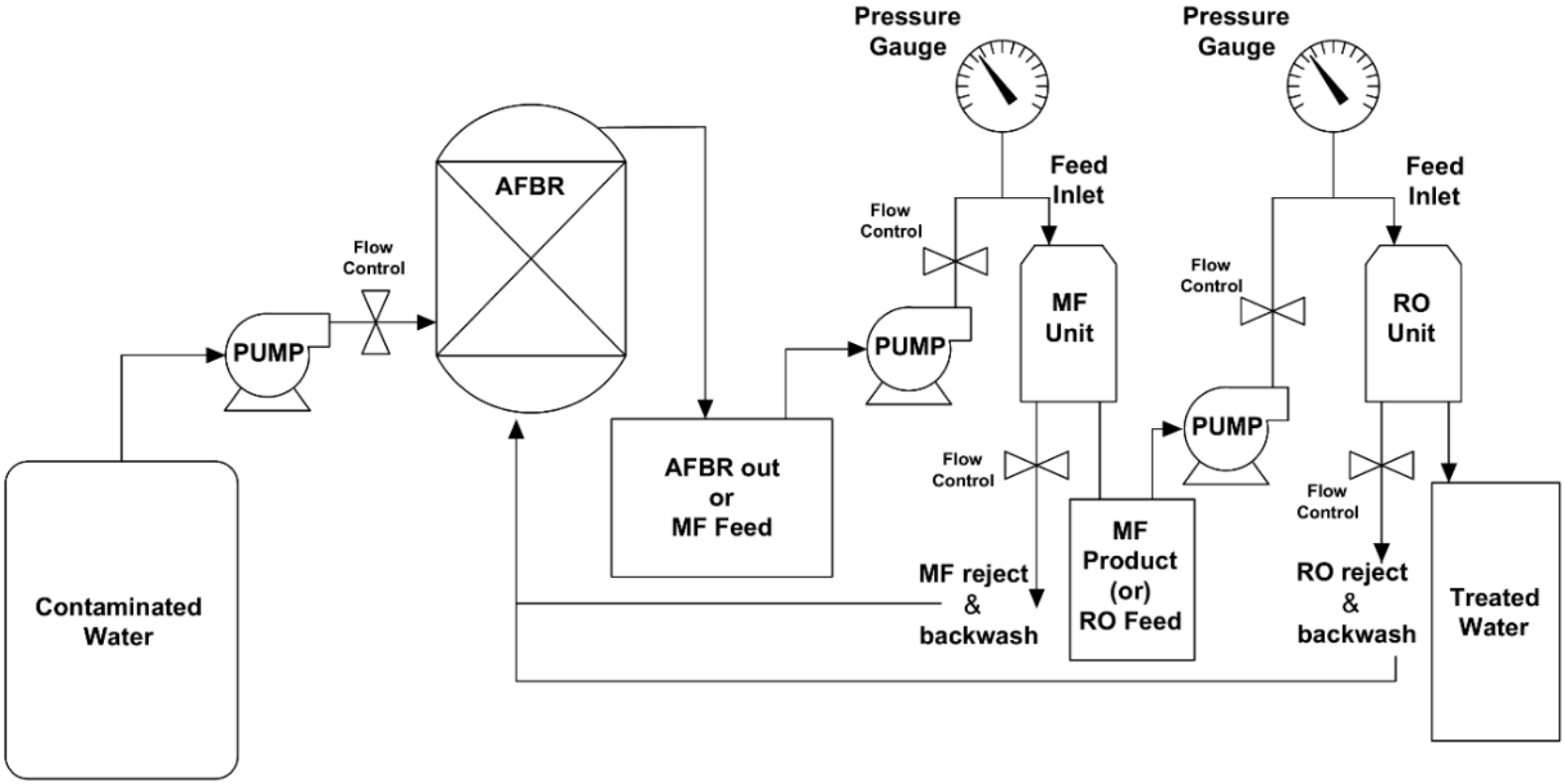

Figure 4.1. Schematic representation of the combined Bio-MF-RO unit for ClO_4^- treatment.

Figure 4.2. Photograph of the pilot-scale combined Bio-MF-RO unit for ClO_4^- treatment (1 - Perchlorate feed tank, 2- Pump (for bioreactor feed) 1, 3 – Anaerobic Fixed film Bioreactor (AFBR), 4 – Reactor effluent collection tank, 5 – Pump 2 (for MF and RO feed), 6 – Microfiltration unit (MF), 7 – Reverse Osmosis unit (RO), 8 – MF permeate collection tank, 9 – RO permeate collection tank)

Figure 4.3a. Anaerobic Fixed Film Bioreactor inside showing charcoal filter bed

Figure 4.3b. *Serratia marcescens* strain NIIST5 colonies on nutrient agar medium

In the beginning, the bioreactor was inoculated with an enrichment culture of the ClO_4^- reducing bacterium, *Serratia marcescens* strain NIIST5 (a proprietary culture of CSIR-NIIST, MTCC 5821, Genbank JQ807993) maintained at CSIR-NIIST Environmental technology division (Anupama et al., 2013). A photograph of *S. marcescens* colonies on nutrient agar medium is shown in Figure 4.3b. Isolated colonies of *Serratia marcescens* maintained in nutrient agar plates containing 10 mg/L of ClO_4^- ($KClO_4$) were sub-cultured onto 25 mL of nutrient broth with 10 mg/L of ClO_4^-. This mother culture was then inoculated in 50 mL of inorganic mineral medium (IMM), supplemented with trace elements (Trace Metal Solution – TMS) (Table 4.1), 10 mg/L levels ClO_4^-, and 40 mg/L of acetate (CH_3COONa). After 72 hours of incubation, this enrichment culture was transferred to 250 mL of IMM spiked with ClO_4^- and acetate (concentration as specified above) in 500 mL conical flasks (4 nos). When the ClO_4^- concentration in the medium declined to <2 μg/L, the contents of all the flasks were pooled, and the 1 L culture obtained was used as inoculum for scaleup of the culture (9 L of IMM with ClO_4^- and acetate). At the end of incubation for another 72 hours, the bulk enrichment culture obtained was used for inoculating the AFBR.

At the start-up, 110 L of well water supplemented with $KClO_4$ (equal to 25 mg/L level ClO_4^-), and CH_3COONa (equal to 100 mg/L level acetate) and 10 L of bacterial culture at log phase (OD 0.317 at 600 nm; Eppendorf Biophotometer plus, Germany) in IMM and Trace Minerals Solution was slowly pumped (~5 L/h) into the AFBR using a peristaltic pump (Watson Marlow, USA. In the beginning, the reactor was operated in recirculation mode (5 L/h), to facilitate the build-up of bacterial biofilm on the charcoal. The perchlorate was degraded to <2 μg/L after four days of operation. Then the AFBR was switched over to continuous mode.

The water from an open well in the CSIR-NIIST campus was used for preparing the synthetic contaminated wastewater. $KClO_4$ stock solution (10 X) was prepared and the required volume of this was added to the well water for making the contaminated water for the treatment study. The characteristics of the well water used are presented in Table 4.2. The optimum pH for perchlorate reduction is in the neutral range. The pH of the contaminated public well water at Aluva, was in the acidic range (~5). While field implementation, neutralization of well water using an alkali is required prior to bioreactor treatment.

Table 4.1. Composition of modified Inorganic Mineral Media (IMM) and Trace Metal Solution (TMS) used in this study

Compounds in 1X IMM	**Weight (mg/L)**
$MgSO_4.7H_2O$	12
K_2HPO_4	10.0
KH_2PO_4	6.0
NH_4Cl	67
TMS	0.1 ml/L
Compounds in 1X TMS	**Weight (mg/L)**
EDTA	63.68
$ZnSO_4.7H_2O$	3.916
$CaCl_2$	5.5
$MnSO_4.H_2O$	4.83
$FeSO_4.7H_2O$	5.0
$Na_2MoO_4.2H_2O$	2.12
$CuSO_4.5H_2O$	1.57
$CoCl_2.6H_2O$	6.1
Boric Acid	0.50
$NiCl_2$	0.1363

Table 4.2. Characteristics of open well water used as synthetic feed in this study.

Parameter	**Concentration**
pH	7.1±0.2
Dissolved O (mg/L)	5.6-6.8
TDS (mg/L)	55-60
ORP (mV)	+230-350
TKN (mg/L)	24±3
TP (mg/L)	0.2±0.1
Nitrate (NO_3^- - N mg/L)	5

4.2.2. Optimization of electron donor concentration and Hydraulic Retention Time

To achieve an effluent ClO_4^- concentration < 1 mg/L, optimization studies were conducted with different ClO_4^- to acetate ratios, and at different hydraulic retention time (HRT). To optimize the ratio of ClO_4^- to acetate, the feed water ClO_4^- (influent) was maintained at 15 mg/L, and four different ClO_4^- to acetate ratios such as 1:1, 1:2, 1:3, 1:4, and 1:5 was tested in continuous feed mode in the AFBR. This corresponds to acetate (sodium salt) concentrations of 15 mg/L, 30 mg/L, 45 mg/L, 60 mg/L, and 75 mg/L respectively in the feed water. To optimize the HRT, the feed water was pumped into the AFBR under three different flow rates (2.5 L/h, 5.5 L/h, and 8.5 L/h) to achieve different HRT such as 22 hours, 10 hours, and 6.5 hours. Samples were taken daily to assess the performance of the bioreactor in terms of ClO_4^- removal, pH, total dissolved solids (TDS), total suspended solids (TSS), total chemical oxygen demand (TCOD), and microbial load in the AFBR out water. The ORP inside the reactor was also monitored regularly to assess the anoxic status of the bioreactor. Based on the optimization results, the AFBR was operated with 15 mg/L of ClO_4^- and 60 mg/L of acetate constituting a ratio of 1:4 of ClO_4^- to acetate and an HRT of 6.5 hours (flow rate of 8.5 L/h). The removal of ClO_4^- in the AFBR at different initial concentrations (20-50 mg/L) was also tested. The optimized ClO_4^-/acetate ratio and HRT were maintained in these studies.

4.2.3. Microfiltration (MF), and Reverse Osmosis (RO) Unit

The MF unit used in this study was a ceramic tubular membrane (25 cm long and 34 mm outer diameter) made of alumina. This was obtained from the Ceramic Research Laboratory, Material Science and Technology Division, CSIR-NIIST, Thiruvananthapuram, India. The average pore size of the membrane was 1.5 μm and the total surface area was 0.12 m^2. The MF membrane had a pure water flux of 12.5 x 10^{-4} m/s at ~15 psi. The AFBR treated water was pumped into an MF unit at a flow rate of 50 L/hr at 50 psi using a diaphragm booster pump (Zuanli, China). The purpose of the MF unit was to remove the suspended solids and bacterial cells present in AFBR treated water.

A commercially available RO membrane (polyamide thin film composite) module (Dupont, Film Tec, BW-60-1812-75) with a total surface area of 0.38 m^2 was used as the RO unit in this study. The filtered water from the MF unit was pumped into the RO unit using a second diaphragm booster pump (Zuanli, China) at a flow rate of 40 L/h, and pressure at this unit was 50 psi. According to the product data sheet of the RO unit, permeate flow rate is 12 L/h at 50 psi for inlet water containing ~250 mg/L of TDS at 25 $^{\circ}$C. The ratio of reject to permeate was 7:3. Hence, this condition was chosen in ClO_4^- rejection studies and for treating the AFBR effluent. Perchlorate rejection efficiency of the RO unit was evaluated by varying the inlet ClO_4^- concentration from 1 to 100 mg/L at a feed flow rate of 40 l/h at 50 psi. The pressure and water flow rates were continuously monitored in both the MF and RO units. Samples of product water were taken daily from both the units for the analysis of ClO_4^-, TDS, and viable bacterial cell count.

The membrane flux in both MF and RO units was calculated using the general formula:

$$Flux = \frac{volumetric\ flowrate}{surface\ area}\ m/s \qquad [1]$$

4.2.4. MF and RO Membrane fouling, and control measures

Manual backwashing and forward flushing methods using pure product water from the RO unit were adopted for controlling the fouling associated with MF and RO units. Backwashing was done by passing pure water through the permeate channel of the MF and RO units and collecting the backwashed water from the retentate side while keeping the feed closed. Forward flushing was done by fully opening the reject valve so that all the feed gets collected as reject by flushing the deposited residues. Backwash/forward flushing was performed at different time

intervals (1, 1.5, 2, and 2.5 h) using different volumes of pure water (1, 2, and 2.5 L), at different wash water flow rates (20, 25, and 30 L/h). The conditions that produced the best result in terms of recovery of membrane flux after backwashing/forward flushing were selected. The flowrates were optimized based on flux recovery.

4.2.5. Treatment of membrane wash water and rejects

The RO and MF reject along with backwashed and forward flushed water contained ClO_4^-. To degrade ClO_4^- present, it was pooled and mixed with the fresh feed and pumped into the AFBR. Samples of wash water and rejects were taken daily from both units for ClO_4^- and TDS analysis.

4.2.6. Analysis

The outlet water from AFBR, MF, and RO units was analyzed for ClO_4^- concentration and water quality parameters such as pH, TDS, TSS, TPC, and TCOD.

Analysis of perchlorate

Perchlorate concentration in the samples was measured using Ion-Selective Electrode (ISE) and Ion Chromatography (USEPA methods 314.0 and 314.1). (Detailed in Chapter 3, Section 3.1)

Analysis of water quality parameters

Oxidation-Reduction Potential (ORP) of samples was measured using an ORP meter (Eutech Instruments, ORP tester10). The TDS content of samples was measured using a TDS conductivity meter (Eutech Instruments, model no CON700). TSS, TCOD, and Total Plate Count (TPC) in the samples were estimated by APHA approved standard methods 2540 D, 5220 B (Open Reflux Method), and 9215 C (Spread Plate Method for heterotrophic plate count) respectively (APHA 1998).

Statistical Analysis

The statistical analysis of the data generated was done using MS Excel. The primary data from the bioreactor performance, as well as operation of the MF and RO units presented, are an average of a minimum of three readings, expressed with standard deviation at a significance level of $P< 0.05$.

4.3. Results and discussions

4.3.1. Performance of the Anaerobic Fixed-film Bioreactor (AFBR)

The perchlorate reducing bacteria applied in the AFBR in this study was *Serratia marcescens* that was studied well, and reported (Anupama et al., 2013). In the present study, a translation of the knowledge base for treating ClO_4^- contaminated well water was done for practical application. During the start-up stage, the *Serratia marcescens* inoculated AFBR was operated in recirculation mode at a slow flow rate. This helped in the build-up of perchlorate reducing bacterial biofilm in the filter bed (charcoal). Charcoal is selected for the biofilm support matrix due to the reasons such as low cost, availability, adsorptive property, and high pore space (surface area). It can provide higher surface area for the biofilm growth and it is also an inert material without any leaching problems. Reported studies in this area have used supporting media like sand, celite, pall rings, etc (Logan & LaPoint, 2002; Losi et al., 2002; Min et al., 2004).

The treatable limit (inlet concentration) of ClO_4^- concentration for the RO membrane used in this study was <2 mg/L. However, the average ClO_4^- level observed in the field (near APEP) was ~15 mg/L. Therefore, the primary role of the bioreactor operation was to reduce the ClO_4^- concentration from 15 mg/L to < 1 mg/L, so that the RO membrane can remove this residual ClO_4^- concentration.

The results of ClO_4^- removal under different acetate levels and HRT are presented in Figure 4.4. It was found that ClO_4^-/acetate ratio 1:4 and HRT 6.5 hours (flow rate of 8.5 L/h) were suitable for ClO_4^- removal in the present AFBR.

Under this condition, the AFBR treated 200 L of contaminated (externally added) well water per day and reduced ClO_4^- from the initial 15 mg/L by 0.4±0.35 mg/L (97.33% removal). The average redox potential (ORP) inside the AFBR was –101 ± 26 mV, and pH was about the neutral range (7.3±0.5) without any external correction. The performance of the AFBR reactor after optimizing the ClO_4^-/acetate ratio (1:4) and HRT (6.5 h) from day 1 to 54 is shown in Figure 4.5.

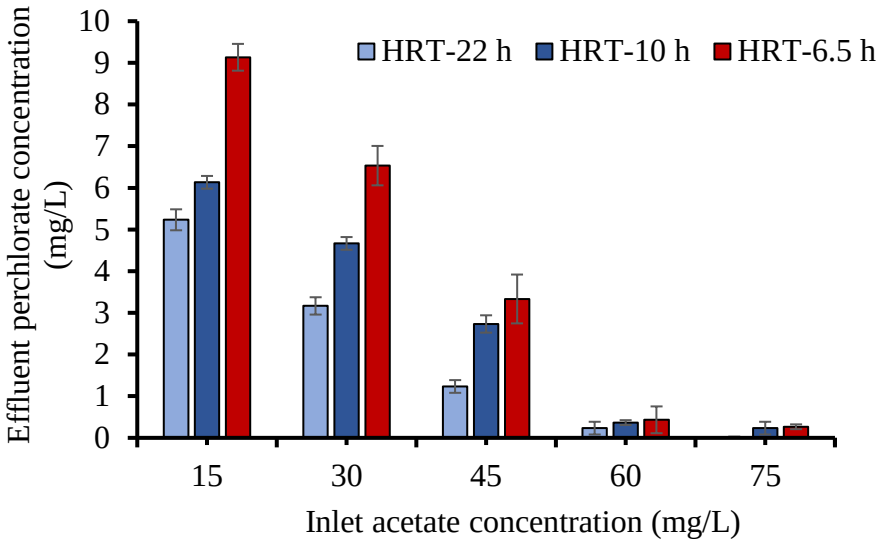

Figure 4.4. Effluent perchlorate concentrations at different acetate concentrations, and HRT for an influent perchlorate concentration of 15 mg/L.

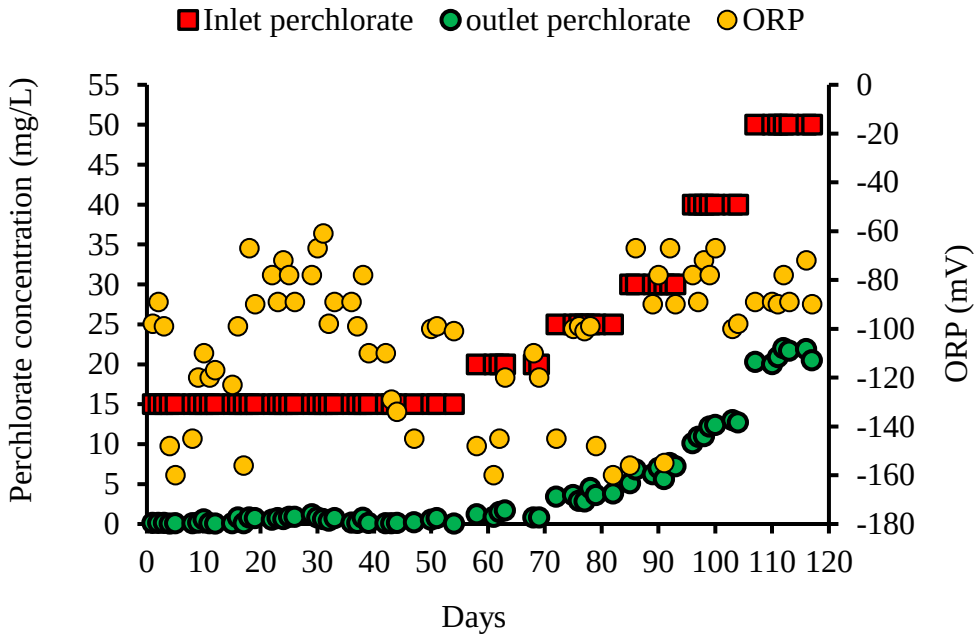

Figure 4.5. Inlet and outlet concentrations of ClO_4^-, and ORP level of the AFBR under optimum conditions of ClO_4^-/acetate ratio (1:4) and HRT (6.5 h).

Previous batch experiments with *Serratia marcescens*, pure culture of the perchlorate reducing bacteria used in this study, revealed the equimolar consumption of acetate for ClO_4^- reduction

(Anupama et al., 2013). But, in AFBR, the requirement of a higher concentration of acetate was observed. The optimum ClO_4^-/acetate ratio for maximum ClO_4^- removal was found to be 1:4. The higher acetate requirement for maximum ClO_4^- removal in AFBR could be due to the presence of non-perchlorate reducing heterotrophs proliferating along with the inoculated *S. marcescens*. The presence of viable heterotrophic bacteria (other than S. *marcescens*) was evident from the spread plating of AFBR outlet samples. The whole experimental setup was operated under conditions similar to the field (not maintained under sterile conditions), including the feed well water used was not sterilized. This can lead to the natural proliferation of heterotrophs in the AFBR. Higher acetate requirements up to six times of stoichiometric requirement for ClO_4^- removal in bioreactors with different ClO_4^- reducing microbes have been reported earlier (Kengen et al., 1999; Kim & Logan, 2001; Farhan & Hatzinger, 2009).

The non- perchlorate reducing heterotrophic microflora in AFBR will help to maintain a lower redox potential (by scavenging dissolved oxygen) that favors conditions for ClO_4^- reduction (-110 mV). After two months, when the inlet ClO_4^- concentration was increased from 15 mg/L to 20 mg/L, the percentage of ClO_4^- reduction declined to 94%. Further, at 50 mg/L ClO_4^- concentration and from day 58 to 117 the removal was only 58% (Figure 4.5). The ClO_4^-: CH_3COO^- was maintained at 1:4 in all these cases to avoid substrate limitation. Under stable performance conditions, the TCOD, TSS, and TDS levels of the AFBR treated water were 45±21 mg/L, 1± 0.25 mg/L, and 202±10 mg/L respectively. The TCOD of the AFBR effluent was higher and that could be due to the presence of soluble microbial products and suspended organic particles. The bacterial load in the treated water from AFBR was 1.2×10^7 CFU/mL. The decline in ClO_4^- removal at higher concentrations could be due to the shock loading effect, or insufficient ClO_4^- reducing biomass level in the AFBR. However, by providing more acclimatization period and HRT, it may be possible to arrive at better removal efficiency even at a higher concentration of ClO_4^-.

4.3.2. Performance of RO membrane module

The ClO_4^- removal by the RO membrane module at a flow rate of 40 L/h and 50 psi is shown in Figure 4.6, where Jv represents the permeability of the RO membrane. The RO membrane could remove 99.1% of ClO_4^- when the inlet ClO_4^- concentration was 1 mg/L. As the inlet ClO_4^- concentration increased, the permeate ClO_4^- level also increased. The ClO_4^- rejection was 98.9% when the inlet ClO_4^- was 10 mg/L. Therefore, it was evident that to achieve a ClO_4^-

concentration <15µg/L in the product water, the inlet ClO_4^- concentration should be less than 1 mg/L

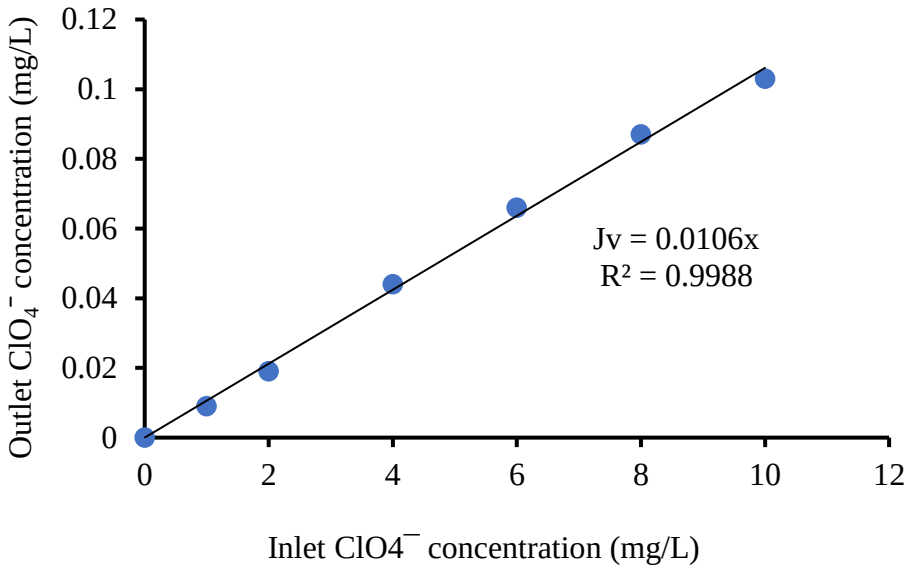

Figure 4.6. Perchlorate removal by RO membrane for different inlet ClO_4^- concentrations at a flow rate of 40 l/h and 50 psi.

These observations were similar to previously reported studies on ClO_4^- removal through RO membranes. Several lab-scale studies have reported ClO_4^- rejection using the RO process. Yoon et. al. showed 80-95% rejection of ClO_4^- when the initial concentration was 100 µg/L (Yoon et al., 2004, 2005). In a recent study, Yang et.al reported a decline in ClO_4^- rejection from 99.9% to 95.6% when the inlet ClO_4^- concentration was increased from 0.2 mg/L to 2 mg/L at ~30 psi (B. M. Yang et al., 2020). The commercial RO membranes that have a higher ClO_4^- rejection efficiency were also reported. Sanyal et. al. have reported a ClO_4^- rejection of 93% with BW30 membrane at ~70 psi for an inlet ClO_4^- concentration of 10 mg/L, and a 95% removal when SW30 type RO membrane was used at the same pressure (Sanyal et al., 2015). From the previously reported studies, it can be concluded that the percentage rejection of ClO_4^- depends on the initial ClO_4^- concentration, the type of membrane used, and the transmembrane pressure. Perchlorate removal by various membrane processes reported so far is summarized in Table 4.3.

Table 4.3. Comparison of perchlorate removal by various membrane processes

Membrane process	**Inlet ClO_4^- (μg/L)**	**% Removal**	**Reference**
Reverse osmosis	1000	99.1	Present study
Reverse osmosis	200	99.9	(Yang et al., 2020)
Reverse osmosis	10000	BWRO - 93 SWRO - 95	(Sanyal et al., 2015)
Reverse osmosis	1000	96	(Heo et al., 2012)
Nanofiltration	1000	82	(Heo et al., 2012)
Reverse osmosis	100	>90	(Yoon et al., 2009)
Ultrafiltration	99450	95	(Huq et al., 2007)
Reverse osmosis	100	80 - 95	(Yoon et al., 2005)
Nanofiltration	100	75 - 90	(Yoon et al., 2004)
Ultrafiltration	100	75 - 90	(Yoon et al., 2004)
Reverse osmosis	800	95-98	(Giblin et al., 2002)

4.3.3. Combined AFBR-MF-RO unit, and its performance:

During initial studies, when RO membrane was used for primary treatment, the maximum concentration of ClO_4^- that can be removed by the membrane was 2 mg/L. Meanwhile, the ClO_4^- concentration in the well water was 15 mg/L. To overcome this difficulty, the AFBR unit was introduced before the RO unit. The AFBR removed ~97% of the initial ClO_4^- concentration, the residual ClO_4^- (0.4±0.35 mg/L) was removed by the RO unit. However, a decreased flux and low ClO_4^- rejection were observed at this stage due to membrane fouling. The high bacterial cell count (~10^7 CFU/mL) in the AFBR treated water can easily clog the membrane. To control fouling, the ceramic MF unit as a pretreatment to RO membrane was introduced. The MF passed water had only 200±60 CFU/mL. Bacterial cells were not completely removed in the MF unit used, probably due to the large pore size (1.5 μm) of the ceramic membrane used. The TDS and ClO_4^- concentration remained as 202±10 mg/L and 0.4±0.35mg/L respectively without any quantifiable TSS in the MF treated water. The MF unit

produced 20 liters of permeate and 30 liters of reject in 1 hour. Integrating the terminal RO unit reduced the ClO_4^- concentration to <10 μg/L, TDS value to <25 mg/L, and TCOD below the detection limit. The RO unit produced 12 liters of permeate and 28 liters of reject in one hour. The overall performance of the combined treatment system at optimized working conditions is summarized in Table 4.4.

Table 4.4. The concentration of perchlorate and other water quality parameters in feed water and at different stages of the combined treatment system at optimized working conditions.

Parameter	**Feedwater**	**AFBR treated water**	**MF treated water**	**RO treated water**
ClO_4^- (mg/L)	15	0.4±0.35	0.4±0.35	<10 μg/L
TCOD (mg/L)	*NA	45±21	<20	**BDL
pH	7±0.4	7.3±0.5	7±0.5	6.3±0.5
TSS (mg/L)	NA	1±0.25	BDL	BDL
TDS (mg/L)	210±15	202±10	202±10	<25
Total plate count (CFU/mL)	NA	1.2×10^7	200±60	0

*Not Applicable; **Below Detection Limit

In previously reported studies, ClO_4^- in water was initially removed through membranes (NF, UF, RO or Electrodialysis (ED)) or IX unit, and a microbial process was adopted separately either for regenerating the resin or for treating the reject (Yoon et al., 2009; Sharbatmaleki & Batista, 2012; Sharbatmaleki et al., 2015; Qi et al., 2017). Adsorption with a quaternary amine-functionalized bio-resin and biological/chemical regeneration of the resin was reported recently for treating ClO_4^- contaminated groundwater (Pan et al., 2019). The lower bio-regeneration (26-89%) of the resin was one of the drawbacks observed in this study. Similarly, poor bio-regeneration capacity (84.9% in 5 days) of a surface-modified bio-sorbent for removing ClO_4^-,

and further the requirement of sterilization of resin before the next adsorption step was also reported (Ren et al., 2017). Increased fouling after bio-regeneration of the membrane due to the accumulation of soluble microbial products and extra polymeric substances during the bio-regeneration step was also reported in this study. The removal of ClO_4^- in groundwater through a combined electrodialysis reversal (EDR) and the RO method was reported recently (Yang et al., 2020). Perchlorate at an initial 10.5 mg/L was removed to a non-detectable limit through this approach. However, at a higher initial ClO_4^- concentration, a lower removal through EDR was observed in this study. Compared with the different approaches reported, particularly for water contaminated with higher levels of ClO_4^-, the method adopted in this study would be a better option. Since most of the ClO_4^- is removed in the AFBR, a small capacity RO membrane would be sufficient for the final treatment. This will bring also down the operational cost of the entire treatment system.

4.3.4. Membrane fouling, and treatment of wash water, and rejects

The major issue observed during the operation of the integrated Bio-MF-RO system was a significant decrease in membrane flux of both MF and RO units due to fouling. A considerable decline in permeate flux after one hour of MF and RO operation was observed. The variation in membrane flux and permeate flow rate during the operation of the MF and RO membrane is given in Table 4.5.

Table 4.5. The variation in membrane flux and permeate flow rate in one hour of MF and RO membrane operation

Time (min)	MF flow rate (L/h)	MF membrane flux (10^{-5} m/s)	Normalized MF Flux	RO flow rate (L/h)	RO membrane flux (10^{-6} m/s)	Normalized RO flux
0	20.0	4.63	1.0	12.0	8.77	1.0
10	19.2	4.44	0.98	11.9	8.70	0.99
20	18.9	4.38	0.94	11.58	8.46	0.96
30	17.6	4.07	0.88	11.388	8.32	0.94
40	16.2	3.75	0.81	10.98	8.03	0.91
50	13.8	3.19	0.69	10.74	7.85	0.89
60	12.4	2.87	0.62	10.67	7.80	0.88

The results of feed water flux through the MF unit over one cycle (50 L feed/h) are presented in Figure 4.7. The membrane flux after 1 h was only 60% of the initial flux i.e., 2.87 x 10^{-5} m/s

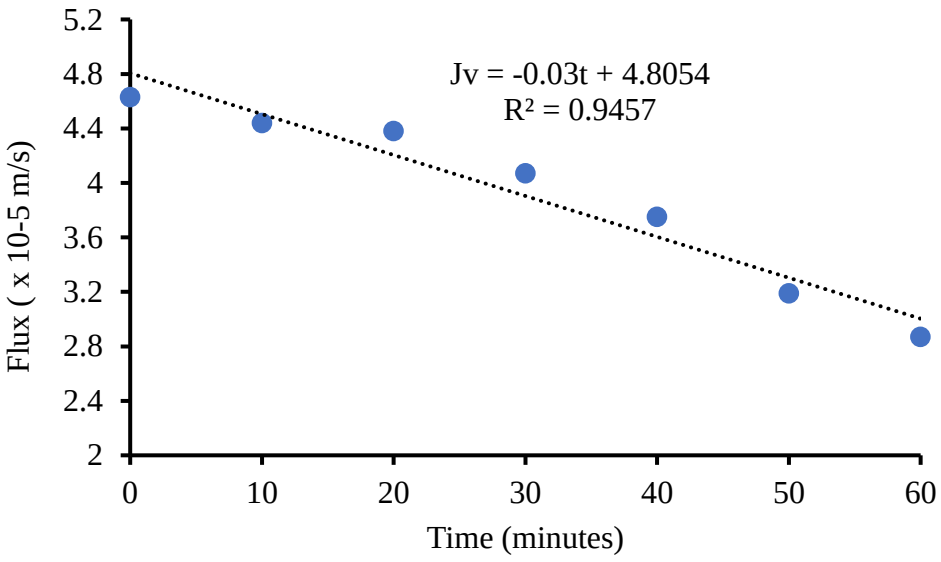

Figure 4.7. Feedwater flux through the MF unit over one cycle (50 L of feed per hour; Jv is the permeability of the membrane)

The fouling associated with MF and RO systems is very common, and few studies have specifically reported fouling associated with NF, UF, and RO membranes in ClO_4^- removal studies (Yoon et al., 2009; Heo et al., 2012; Qi et al., 2017; Yang et al., 2020). Suspended cells, dissolved organic matter, soluble microbial products, and extra polymeric substances are mainly responsible for membrane biofouling (Nguyen et.al, 2012). Physical, chemical, and biological approaches are practiced for controlling the fouling of different membranes (Bagheri & Mirbagheri, 2018). Specifically, for ClO_4^- removal in a hybrid Electrodialysis-RO system, acid (HCl) treatment was adopted for controlling fouling associated with the RO membrane (Yang et al., 2020). Among the various methods to control biofouling, backwashing and forward flushing are simple, cost-effective, and environment friendly. Biofouling control through backwash as well as forward flush with pure water under optimum backwash time interval and wash water volume used was reported earlier (Chang et al., 2017; Shao et al., 2018). However, this approach was never reported in membrane-based ClO_4^- removal studies.

In this study, it was found that forward flushing was not effective to control fouling in the MF unit. This could be due to lower pressure built-up as observed (3-5 psi). Since ceramic membranes are made of mineral oxides with high surface tension, low pressure will not remove most of the adhered particles and hence high pressure needs to be applied to remove all the adhered particles (Yue et al., 2018). For every 50 L of feed passed, the most effective condition to control fouling in the MF membrane was backwashing at every hour using 2 L pure water at a flow rate of 25 L/h at 60 psi. After backwashing, the MF membrane was regenerated, and the initial flux was regained (Figure 4.8.). Hence only a backwashing technique was adopted for MF membrane regeneration. The MF backwash water contained <10 μg/l of ClO_4^-.

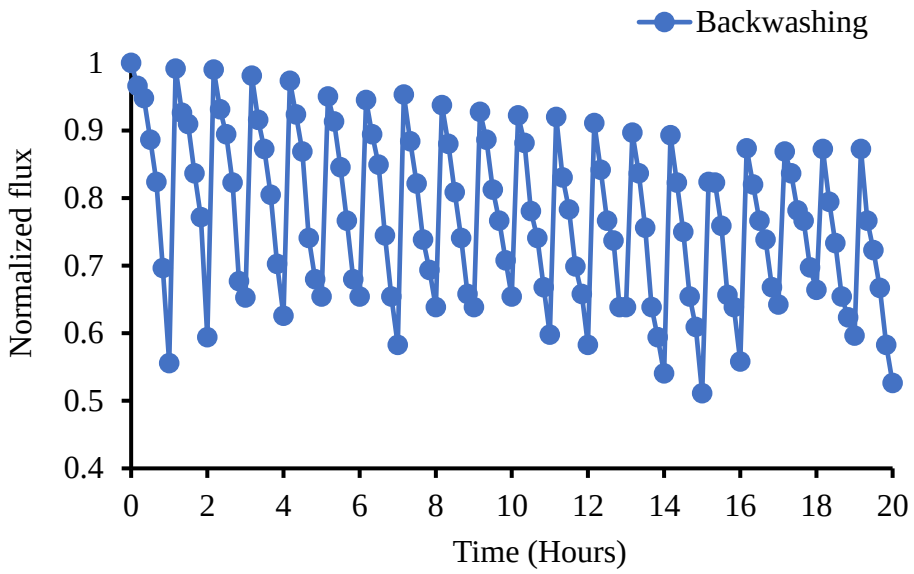

Figure 4.8. Effect of backwashing in the recovery of MF Membrane flux (20 cycles, 1000 L of feed)

However, on prolonged use, (i.e., 120 hours of operation), the flux after backwashing could regain only 85% of the initial flux. Hence, chemical washing is recommended to regain the initial flux after prolonged use. Various membrane regeneration strategies such as washing with chemicals, backwashing with hot water, and dipping the membranes in an acidic solution can be performed to regain the membrane flux in the case of ceramic membranes with high surface tension (Akhtar et al., 2020).

The initial product flux declined from 8.77×10^{-6} m/s to 7.8×10^{-6} m/s in one hour. The results of feed water flux through RO unit over one cycle at 40 L/h feed are presented in Figure 4.9.

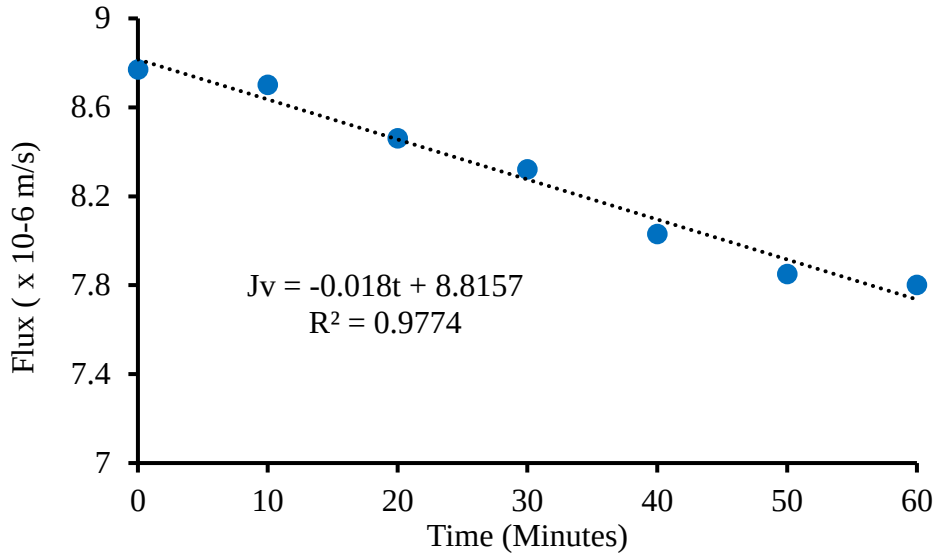

Figure 4.9. Feedwater flux through the RO unit over one cycle at 40 L/h feed (Jv is the permeability of the membrane).

Unlike in the MF unit, both backwashing and forward flushing with pure water were found equally efficient in regaining the flux through the RO membrane (Figure 4.10).

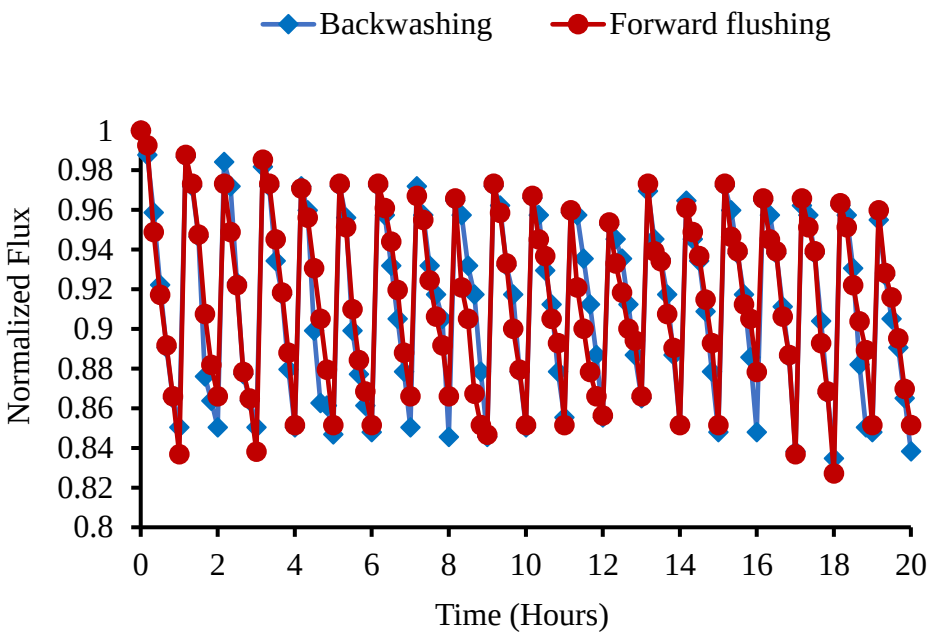

Figure 4.10. Effect of backwashing and forward flushing in the recovery of RO Membrane flux (20 cycles, 800 L of feed)

It was found that for every 40 L of feed passed, hourly backwashing with one-liter pure water at 30 L/h, and 70 psi or hourly forward flushing at 30 L/h and 3 to 5 psi regained the RO membrane flux. However, since product recovery was almost the same in both methods, and forward flushing consumes less pressure (3 to 5 psi at 30 L/h) it was chosen for RO membrane recovery.

The optimized conditions for the regeneration of MF and RO membranes are summarized in Table 4.6.

Table 4.6. The optimized conditions for the regeneration of MF and RO membranes

	Washing type	**Washing interval (h)**	**Wash water Volume (L)**	**Flow rate (L/h)**	**Pressure (psi)**
MF membrane	Backwashing	1	2	25	60
RO membrane	Forward flushing	1	1	30	3-5

Out of 12 liters of product water produced from the RO module per hour, three liters were used for MF and RO membrane regeneration. Hence, at this permeate flow rate from RO, the combined system produced ~200 L of treated potable quality water per day. The integrity of both the MF and RO membranes was constant for ~5000 liters of water treated. The MF and RO reject as well as backwash and forward flush water that contained ClO_4^- (<1 mg/L), dissolved organics, and bacterial cells were pooled daily and mixed with fresh feed and pumped into the AFBR for complete degradation of ClO_4^- to achieve a zero-discharge status for the combined system. The TDS build-up due to recycling was negligible as the backwash/forward flush water and reject water was mixed with fresh feed and hence there was a dilution in overall TDS. Compared with the previously reported methods, the novel approach tested in this study was found to be more effective for treating ClO_4^- contaminated groundwater. Since ~99% of inlet ClO_4^- was degraded into innocuous byproducts through a less energy-intensive anoxic bio-treatment as pre-treatment, the stress on subsequent membranes was low and they can be operated at lower pressure (less energy input), There was no need for secondary treatment of brine, resin or membranes in this approach. Furthermore, the fouling associated with membranes in his approach was controlled through simple and cost-effective mechanisms. All these aspects make the process unique with minimum environmental interventions. This

method can be adopted for treating and recovery of reuse quality water from ClO_4^- contaminated discharge and wash water from ClO_4^- handling sites like APEP or RPP.

Conclusions

In this study, a novel bio-physical process for treating ClO_4^- contaminated well water was developed, and it was tested in a pilot-scale unit under field-relevant conditions. The integrated Biological-Microfiltration-Reverse Osmosis (Bio-MF-RO) system developed effectively treated ClO_4^- contaminated well water and generated potable quality water meeting the standards. In this translational research, the primary treatment was microbial in which the potential of a bioprocess unit to degrade higher concentrations of toxic ClO_4^-, into non-toxic Cl^- and O_2 was mainly applied. After the biotreatment, the quality of water was improved to a potable level by passing through MF and RO membranes in series. Membrane fouling was one of the practical problems observed with both MF and RO units. However, the fouling associated with both the membranes was studied separately and it was found that backwashing and forward flushing techniques were effective to control it in both units respectively. Eventually, the reject and backwash water from the filtration process was also bio-treated, making the process a zero discharge one. The small-scale units will be ideal for individual houses in the affected areas, whereas large-scale units can generate enough drinking water for a large community. Installation and continuous operation of this system will gradually remove the ClO_4^- in groundwater in the affected area that will control further spreading of the persistent contaminant. This scalable process will find direct application at highly ClO_4^- contaminated places ensuring public health and environmental safety. The present process may also find application for treating similar toxic oxyanions such as chlorate, chlorite, and nitrate.

Chapter 5

Development of an ex-situ remediation system for perchlorate contaminated soil, and its validation in a pilot-scale unit.

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5.1. Introduction

Contamination of soil (top soil and vadose zone soil) is a major environmental concern, since it can significantly contribute to the ClO_4^- contamination of groundwater and vegetation. (Smith et al., 2004; Tan et al., 2004; ITRC 2008). Due to the high solubility in water, and poor sorption to soil particles, ClO_4^- is highly mobile in the environment (Urbansky, 1998). The penetration of ClO_4^- through soil is highly dependent on the type and texture of the soil, and it can easily transported to groundwater via infiltration (Urbansky & Brown, 2003; Gal et al., 2008). Due to capillary force and surface tension, the dissolved ClO_4^- can be trapped within soil pores (ITRC, 2008). Severe contamination of the vadose zone and subsequent groundwater contamination due to infiltration is a major problem around ClO_4^- inventories (Gal et al., 2008; Cao et al., 2019; Levakov et al., 2019). Improper disposal of ClO_4^- containing scraps and debris of solid propellants and explosives and rejected rocket motors has resulted in the contamination of soil (Trumpolt et al., 2005). The Bermite site, north of Los Angeles, California, a former explosive manufacturing site, is an example of perchlorate contaminated soil containing site. The perchlorate concentration in the soil at the site was highly variable and was as high as 316 mg/kg (Evans et.al., 2008). Natural ClO_4^- has been detected in soil samples from arid and semi-arid regions (Jackson et al., 2015; Vega et al., 2018).

As detailed in Chapter 2, In-situ Bioremediation (anaerobic composting, soil flushing and bio-stimulation) (Battey et al., 2007; Gal et al., 2008; Höhener & Ponsin, 2014) phytoremediation ex-situ bioremediation (anaerobic composting of the excavated soil piles) (Nzengung et al., 1999; P. Krauter et al., 2005; Evans et al., 2008) and thermal desorption (Gangopadhyay et al., 2010) are the methods adopted for treating ClO_4^- contaminated soil. The in-situ bioremediation approaches reported were either through bio-stimulation (addition of a substrate as electron donor and nutrient source) or bio-augmentation (addition of ClO_4^- reducing bacteria along with a bio-stimulant). Mechanisms like phytoextraction, phyto-degradation, and rhizo-degradation (with rhizospheric microflora) were also reported in the case of soil remediation (Fang & Chen, 2011). The ex-situ remediation of ClO_4^- contaminated soil reported so far include excavation of the polluted soil followed by its treatment in which a combination of the substrate (glycerine as the electron donor) and nutrient (Diammonium hydrogen phosphate) was applied to the soil. An average ClO_4^- removal rate achieved through this approach was 200 μg/Kg/day (Evans et al., 2008).

The efficiency of soil remediation method depends on factors like the level of ClO_4^- in the soil, the presence of viable ClO_4^- reducing bacteria, redox potential, type and level of substrate available, and the presence of competitive electron acceptors (Tipton et al., 2003; Krauter et al., 2005; Gal et al., 2008). The rate of ClO_4^- degradation reported in soil remediation studies is from many days to years (Deitsch et al., 2005).

Our preliminary studies on bio-stimulation of the soil using STP secondary sludge as substrate for enhancing microbial activity, and the application of ClO_4^- reducing bacterial culture for bioaugmentation were not satisfactory. Even though there was a reduction in the soil ClO_4^- the results of the batch studies were not satisfactory in terms of the amount of soil that can be treated, and the time required for completing the treatment. Hence the major objective of this study was to develop a novel process for treating bulk quantity of ClO_4^- contaminated soil within a short period. Through this study, a novel ex-situ remediation approach was developed and is successfully validated on a pilot-scale unit. The high-water solubility of ClO_4^-, as well as its poor adsorption to soil/ organic matter, was the basis for the approach. In this approach, the ClO_4^- contaminated soil is washed with water (soil washing) and the wash water with ClO_4^- (leachate) was bio-treated in a bioreactor using ClO_4^- reducing microbial system.

5.2. Materials and methods

5.2.1. Soil bio-stimulation and bioaugmentation

The degradation of ClO_4^- in contaminated soil was tested through both bio-stimulation and bioaugmentation methods. In bio-stimulation, external substrate (organic and nutrient) was provided to stimulate indigenous ClO_4^- reducing microbes present in the contaminated soil. In bioaugmentation, along with substrate, an enrichment culture of ClO_4^- reducing microbes will also be applied to the soil. Biosolids (secondary sludge) from a Sewage Treatment Plant (STP) was used as the substrate for bio-stimulation. An enrichment culture of ClO_4^- reducing bacteria was used for the bioaugmentation studies. Both the experiments were conducted in PVC pipes of height 50 cm and diameter 10 cm. The ends of the pipes were closed using end caps to maintain anaerobic conditions inside the treatment unit. The experimental details are given in the following sections.

a) Characterization of the soil

The soil for conducting the soil remediation experiments was collected from the CSIR-NIIST campus. To remove gravel and other particles, the soil samples were sieved using a 6 mm sieve. The soil composition (sand, silt, clay), pH, moisture content, levels of chloride, sulfate, and nitrate were determined by the standard protocol of soil sampling and methods of analysis (Canadian Society of soil science Carter and Gregorich., 2nd edition).

b) Collection and characterization of sludge

Secondary sludge (biosolid) without the addition of chemical polymers (for thickening) was collected from the Municipal Sewage Treatment Plant, located at Muttathara, Thiruvananthapuram City, Kerala, India. Characteristics of the sludge, such as pH (Eutech Instruments, United Kingdom), Total Suspended Solids (TSS, 2540 D), Chemical Oxygen Demand (COD, 5200 B), and Biological Oxygen Demand (BOD, 5210 B), were determined using Standard Methods (APHA, 1998). Volatile Fatty Acid (VFA) and alkalinity analysis was done by titration method (Anderson & Yang, 1992). Redox potential (ORP) was analyzed using a waterproof ORP tester (Eutech Instruments, Singapore). The sludge was stored at 4°C for further use.

c) Bio-stimulation experiments using different ratios of soil and sludge

In this experiment, different ratios of soil and sludge were mixed to test the effect of sludge addition on ClO_4^- removal. The ratio was determined based on the available BOD and COD in the secondary sludge. When supplementing complex carbon sources there is a requirement for excess substrate. Hence higher to lower concentration of substrate was provided and for further studies optimized soil to sludge ratio was followed (Cox et al., 2000). Initially the soil samples were spiked with ClO_4^- stock solution such that the ClO_4^- level in the soil was 500 mg/kg of soil (dry weight). Into this soil different volumes of STP sludge like 1 L, 0.5 L, and 0.250 L (sludge: soil ratio of 1:1, 1:2, and 1:4) was added. This corresponds to 14, 7, and 3.5 g/L of COD and 8, 4, and 2 g/L of BOD in terms of the sludge added. A negative control sample was kept with 1 kg of soil and no added sludge. The soil sludge mixture spiked with ClO_4^- was then filled inside the PVC pipe and maintained under anaerobic conditions. All the treatments were done in triplicates and soil samples were taken during, 0, 1, 3, 5, 7, and 9 days for the analysis of residual ClO_4^- in the soil.

d) Bioaugmentation experiments using perchlorate reducing bacterial isolates

In this experiment, 1 kg of soil was mixed with 500 mL of sludge (sludge: soil ratio of 1:2). The sludge–soil mixture was then augmented with ClO_4^- reducing bacterial isolates. The list of ClO_4^- reducing bacterial isolates used for the bioaugmentation is presented in Table 5.1. The isolated cultures that were maintained in nutrient agar plates containing 10 mg/L of ClO_4^- were sub-cultured onto 25 mL of nutrient broth with 10 mg/L of ClO_4^-. This mother culture was then inoculated in 50 mL of IMM (contains K_2HPO_4, NH_4SO_4, $MgSO_4$, $CaCO_3$, and $FeSO_4.7H_2O$) supplemented with trace elements, 10 mg/L of ClO_4^-, and 40 mg/L of acetate as the electron donor. The culture was then centrifuged at ~6869 g force for 10 minutes (Hermle Z 383 K, Germany) and the cell pellet was resuspended in 50 mL of IMM without ClO_4^- and acetate before adding it to the treatment soil samples. The cultures are either added singly or in consortium. The OD of the bacterial culture was adjusted to 0.4 – 0.5 to ensure equal cell density in all the treatment units. The final moisture content of the mixture was 35 – 45 %. All the treatments were done in triplicates and soil samples were taken during, 0, 1, 2, 3, 4, and 5 days for the analysis of residual perchlorate in the soil.

Table 5.1. List of perchlorate reducing bacterial isolates used for soil bioaugmentation studies

Perchlorate reducing NIIST Isolates	**GenBank Accession No:**
Micrococcus sp (MC)	KJ410671
Bacillus pumilus (BP)	JQ820452
Serratia marcescens (SA)	JQ807993
Halomonas sp. (HA)	JN935775
Bacillus Safensis (BS)	JN935774

5.2.2. Soil washing and Bio-regeneration of the wash water

a) Preliminary soil washing experiment

To assess the ClO_4^- recovery from the soil, preliminary soil washing experiments were conducted under different conditions such as number of washes, water holding time, and ideal soil column height. Two types of experimental washing units were used for this purpose, one was a box-type unit and another one was a cylindrical type of unit.

The box-type unit was made up of a transparent polycarbonate sheet of base length 35 cm, width 35 cm and height 50 cm. A photograph of the experimental unit is given in Figure 5.1. The volume of the unit was 0.061 m^3. It had a drain valve at the bottom for collecting the wash water. The loading and unloading of the soil were done by keeping the top of the box open. To ensure uniform distribution of water through the soil column, the box was internally divided into four compartments using a polycarbonate sheet. The first set of experiments were conducted using the box type unit and 20 kg of the garden soil was taken for this purpose. The height of the soil column was 23 cm. The soil was spiked with ClO_4^- by spraying ClO_4^- stock solution (334.3 mg $KClO_4$ in 100 ml water, containing 240 mg ClO_4^-). A garden sprayer was used for spraying the solution uniformly on the surface of the soil column. Then the soil column was completely covered by adding 30 L of tap water and collected the leachate by opening the bottom valve. The ClO_4^- in the leachate was estimated using a ClO_4^- ion-selective electrode (Cole Parmer, USA). From the concentration of ClO_4^- in the leachate, the residual ClO_4^- in the soil was estimated. To recover all the ClO_4^- from the soil, the washing was repeated with fresh water. The experiment was repeated for different water holding times (0-90 minutes).

Figure 5.1. Photographs of the box type unit (A) and filling up of the unit with perchlorate spiked soil for washing experiments (B).

The cylindrical, column type unit used was made up of bottom PVC pipe and its top part was an acrylic column. The diameter of the unit was 20 cm, and the height was 125 cm with a volume of 0.039 m^3. A photograph of the experimental unit is given in Figure 5.2. Similar to the box type unit, there was a drain valve at the bottom to collect wash water and the top of the

column was kept open for loading and unloading the soil. In the cylindrical column, 35 kg soil was taken with a soil column height of one meter. Perchlorate stock solution equivalent to 240 mg of ClO_4^- was spiked on the surface of the soil using the garden sprayer. The soil was logged with 15 L of water and a holding time of 1 hr was given based on the results of the experiment conducted with box-type unit. During the holding time, ~10 cm of the water column was visible on the top of the soil. The leachate sample was collected after each wash and was analyzed for ClO_4^-. To recover all the ClO_4^- from the soil, a total of three washes were required. To test the removal efficiency of higher ClO_4^- levels such as 480 mg/L and 960 mg/L were also tested similarly.

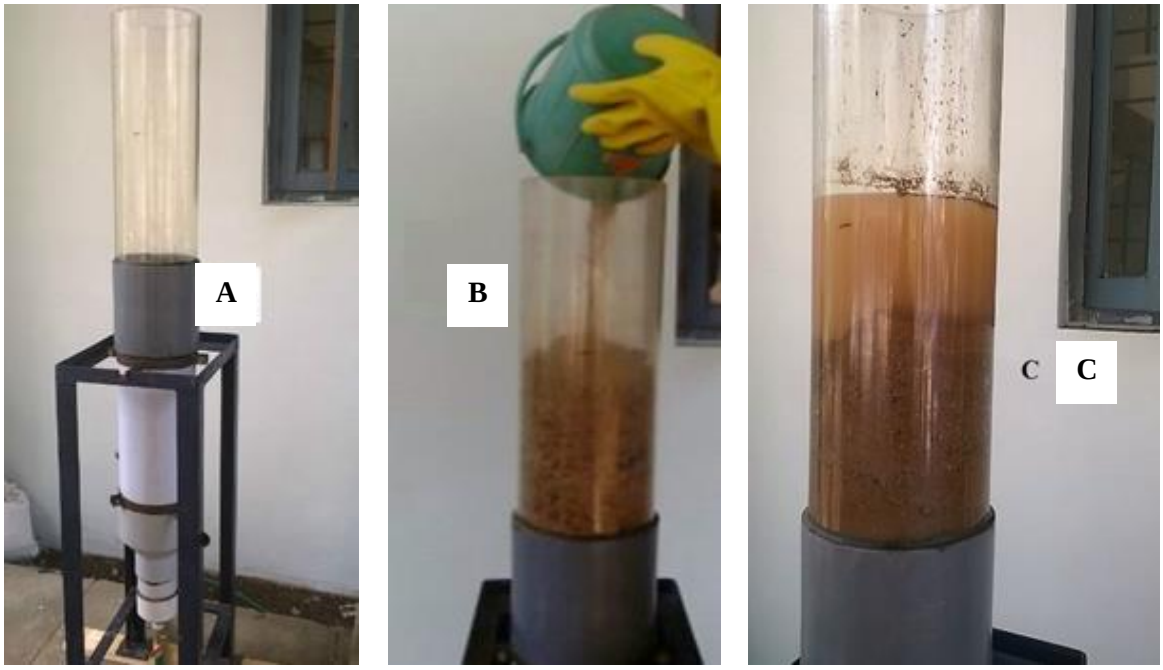

Figure 5.2. Photographs of the cylindrical soil washing unit (A), soil filling (B), and the soil-filled unit with water for washing (C)

b) Pilot-scale setup for soil washing and wash water bio-treatment

The pilot-scale treatment system for soil washing and wash water bio-regeneration (Ex-situ Soil bio-Remediation System -ESRS) consisted of two units, (i) a soil washing unit for eluting the ClO_4^- from contaminated soil and (ii) an anaerobic packed-bed bioreactor system for the biological reduction of ClO_4^- in the wash water. The schematic of the complete treatment system and the photograph of ESRS are presented in Figures 5.3 and 5.4.

Pilot-scale soil washing setup

The pilot-scale soil washing unit was made up of mild steel of breadth 0.8m, length 0.7 cm, and height 1.1 m. The volume of the reactor was 0.62 m^3. Six shower taps were mounted uniformly on top of the tank for spraying the water and at the bottom, there was a valve for draining the wash water from the soil. The unit was filled with 670 kg of garden soil from the institute campus. The soil column had a height of ~1 meter. The wash water was collected in a 300 L PVC tank. Initially, water containing 2.5 g of ClO_4^- was sprayed uniformly over the soil surface. The perchlorate solution for spiking was prepared by dissolving 3.485 grams of $KClO_4$ in 1 liter of distilled water. After spiking the soil with ClO_4^-, the soil was flushed with tap water using the overhead shower taps (Figure 5.5.). The amount of water for flushing was given such that it can fill the soil with a water column remaining just above the soil column. A holding time of one hour was given before eluting the wash water. After one hour, the wash water was collected in a PVC reservoir tank (leachate reservoir) by opening the drain valve from the bottom of the washing unit. The leachate reservoir also functioned for the setting of solids present in the wash water. Since the wash water contained different levels of ClO_4^-, there was a second equalization tank into which treated water from the bioreactor was also mixed. This helped to maintain a constant known concentration of ClO_4^- in the inlet water for biotreatment. The perchlorate concentration in the leachate was analyzed using ISE and the washing was repeated until 99.5% of ClO_4^- was recovered. A total of 360 liters of water was used in 3 washing steps for complete recovery of ClO_4^- from the contaminated soil. The entire washing (three cycles, each with one hour holding time) was completed in 6.3 hours.

c) Extraction and analysis of perchlorate from soil samples

One gram of soil sample (dry weight) was taken in a 50 mL sterile centrifuge tube and 50 mL of deionized water was added. The tube was vigorously shaken for 15 min. and subjected to centrifugation at ~ 6037 g force for 10 minutes (Hermle Z 383 K, Germany). The resulting supernatant was collected, filtered through a 0.45 μm nylon filter, and stored at 4°C until analysis. Repeated extractions were done to ensure that all the ClO_4^- ions leach out from the sample. Samples were analyzed for ClO_4^- using Perchlorate Ion-Selective Electrode (Cole Palmer, USA) and Ion Chromatography (Dionex ICS 1100, Thermo scientific) .as detailed in Chapter 3, Section 3.2.

The final concentration was calculated using the formula as given below:

Final ClO_4^- in soil samples (in mg/kg) = (Concentration in extract from calibration curve (mg/ L ClO_4^-) x Final volume of sample extraction solution (L) x Dilution factor)/Mass of initial sample extracted

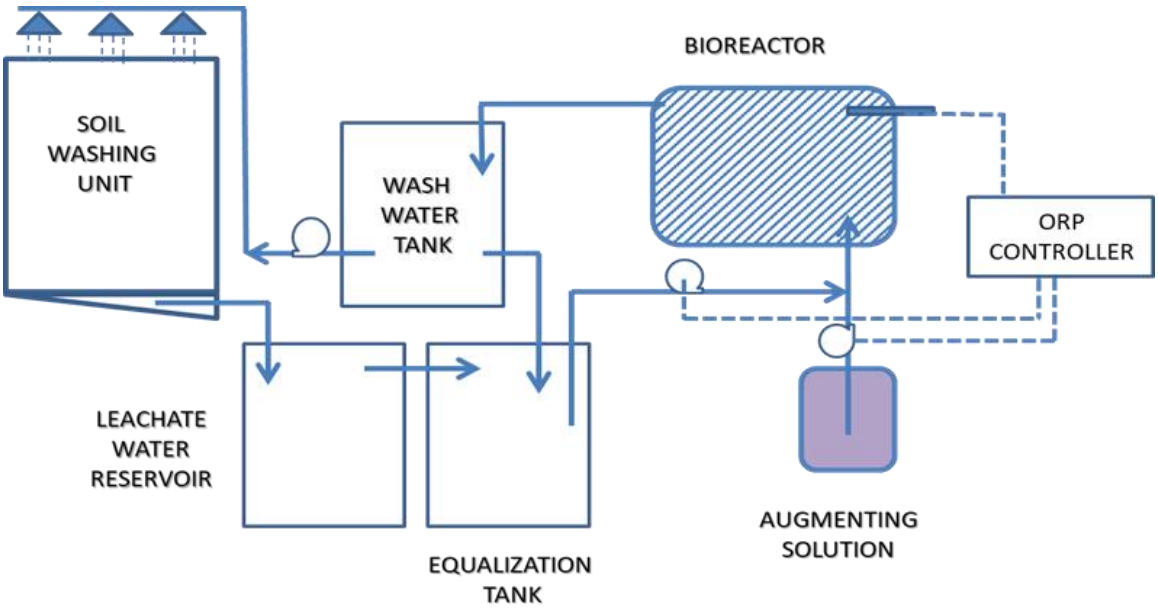

Figure 5.3. Schematic diagram of the pilot-scale soil washing unit coupled with wash water treating bioreactor.

Figure 5.4. Pilot-scale soil washing unit coupled with wash water treating bioreactor.

Figure 5.5. Photograph of the overhead shower taps for spraying wash water for soil washing (Left) experiment. The figure also shows the water column logged with water (Right)

d) Start-up of the bioreactor setup and continuous operation

The bioreactor used for the degradation of ClO_4^- contained in the soil wash water was a fixed film type packed bed bioreactor. The reactor was made up of four PVC tanks of 50 L capacity each and they were connected in parallel (Figure 5.4). The biofilm support media used were needle felt coir fiber and activated carbon. The working volume of the whole reactor set up was 140 L. The filter bed was then augmented with the proprietary ClO_4^- reducing bacterial consortium from NIIST.

The consortium was comprised of *Serratia marcescens (Gen bank no. HM751096), Bacillus pumilus (Gen bank no. JQ820452), and Micrococcus sp. (Gen bank no. KJ410671).* The isolated bacterial colonies were maintained on ClO_4^- (10 mg/L) containing Nutrient Agar plates. Initially, they were sub-cultured onto ClO_4^- (10 mg/L) containing nutrient broth (25 mL of each culture) and subsequently enriched in ClO_4^- (10 mg/L) containing Inorganic Mineral Media (contains K_2HPO_4, NH_4SO_4, $MgSO_4$, $CaCO_3$, and $FeSO_4.7H_2O$) supplemented with trace metals and glucose as the electron donor in the ratio 1:4 (250 mL of each) (Figure 5.6). Further, the cultures were mixed (750 mL) and inoculated into 3.25 L of IMM to make a 4L enriched ClO_4^- reducing bacterial consortia for reactor augmentation The Figure 5.7. shows the bioreactor filling and bioaugmentation with ClO_4^- reducing bacterial isolates from NIIST.

At the start-up, 140 L of tap water spiked with 50 mg/L of ClO_4^- and 500 mg/L of glucose (9.75 g $KClO_4$ and 70 g glucose), 4 L of the ClO_4^- reducing bacterial enrichment culture, and

1L of inorganic mineral solution were mixed and was recirculated in the bioreactor using a peristaltic pump (Watson Marlow, USA) at a flow rate of 90 mL/min. After three days of operation, when the ClO_4^- level was below the detection limit, the reactor was switched over to continuous flow mode. A synthetic feed solution containing ClO_4^-, glucose, and minerals were used for maintaining the bioreactor. The bioreactor was operated for three months, and its performance in terms of ClO_4^- degradation for different initial concentrations of ClO_4^- (10-50 mg/L) was monitored.

Initially, glucose was supplied as the substrate (electron donor) for the bacterial activity. at a glucose/ ClO_4^- ratio of 2, but the ClO_4^- degradation was not complete. To optimize the ratio of glucose to ClO_4^-, 10 mg/L of influent ClO_4^- solution and three different concentrations of glucose such as 20 mg/L, 40 mg/L, and 50 mg/L were tested in continuous feed mode in the reactor. The feed flow rate was 30 L/h and HRT was 4.5 h.

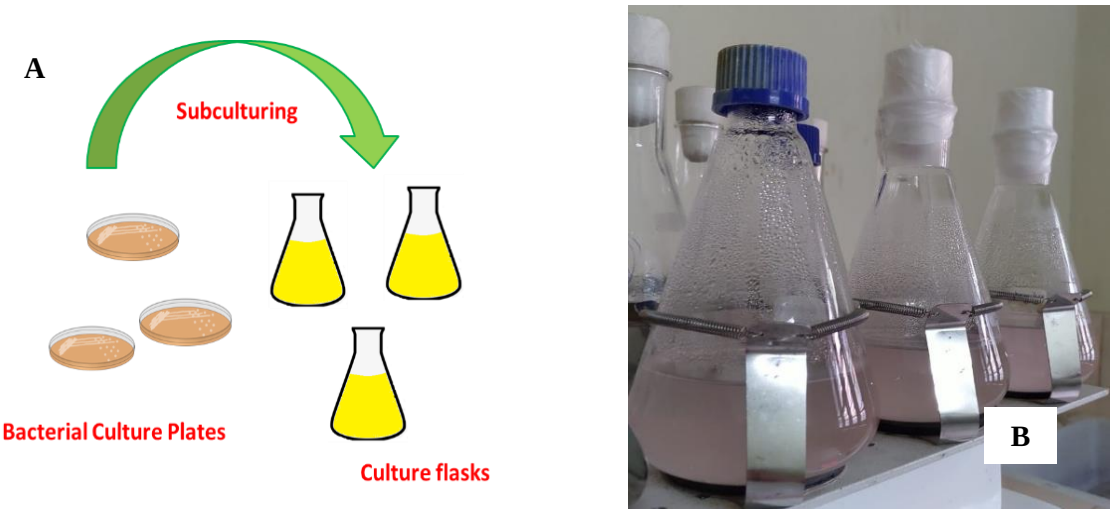

Figure 5.6. Schematic showing the subculturing of perchlorate reducing NIIST isolates (A) and photograph of enrichment in IMM with perchlorate (10 mg/L) and electron donor glucose in the ratio 1:4 (B)

Figure 5.7. Photographs showing bioreactor start-up steps.

e) Biotreatment of soil wash water (leachate)

After two months of bioreactor start up, when it showed stable performance, the pooled soil wash leachate containing ClO_4^- (8.5 mg/L) was treated in the bioreactor. The wash water was fed to the reactor at a flow rate of 30 L/h using a peristaltic pump (Watson Marlow, USA). The HRT was 4.5 hours. An augmenting solution containing glucose and minerals was mixed with the feedwater using a separate peristaltic pump before the feed was pumped into the bioreactor. The glucose/ ClO_4^- ratio was maintained at 5. The perchlorate concentration in the bio-treated water was analyzed using Ion-Selective Electrode (Cole Plamer, USA) and Ion Chromatography (Dionex IC 1100) as described earlier. The pH and Oxidation Reduction Potential (ORP) of the bioreactor influent was continuously monitored using in- line probes (Thermo scientific, Alpha PH 560) connected to the bioreactor. The treated wash water was collected separately, and it was used for the subsequent soil washing cycle.

5.3. Results and discussions

5.3.1. Characteristics of soil and sludge

The characteristics of soil used for bio-stimulation and bioaugmentation studies as well as soil washing experiments are presented in Table 5.2. The garden soil used in this study was composed of 33% sand, 43% silt, and 3.3% clay (w/w). The moisture content was 10% (w/w), and nitrate concentration was 0.15 mg/kg.

Table 5.2. Characteristics of the soil used for soil remediation experiments

Parameter	Observed value
pH	7
Moisture (%)	10
Clay (%)	3.3
Silt (%)	43
Sand (%)	33
Chloride (mg/L)	5.7
Sulphate (mg/L)	0.2
Phosphorous (mg/L)	0.006

Table 5.3 presents the characteristics of secondary sludge used as the substrate for bio-stimulation studies. The BOD and VFA indicate the availability of organic carbon in the sludge that can be utilized by the bacterial community.

Table 5.3. Characteristics of sludge used for bio-stimulation experiments

Parameter	Observed Value
pH	7.07
VFA (m.eq/L of CH_3COOH)	12.227
Alkalinity (m.eq/L HCO_3^-)	6.45
TSS (g/L)	27.72
BOD (g/L)	8
COD (g/L)	14
ORP (mV)	-178

Physicochemical and biological properties of the soil matrix determine the success of its remediation approach, especially for ClO_4^-. Perchlorate penetration through soil is highly dependent on soil texture (Urbansky & Brown, 2003; Gal et al., 2008). The percolation of water

through the soil column depends on the soil composition. Since the soil used in this study had less clay content (3.3%), the water could percolate through the soil column and the ClO_4^- was easily mobilized from the soil particles to the aqueous phase The nitrate present in the soil (0.15 mg/kg) can affect ClO_4^- degradation during the bio-treatment of the wash water. However, this could be compensated by providing sufficient substrate.

5.3.2. Perchlorate degradation through bio-stimulation and bioaugmentation

Perchlorate-reducing bacteria are ubiquitous, and the addition of biostimulants (substrates) to enhance the growth and activity of indigenous microbes is a method used in the in-situ bioremediation approach. (Coates et al., 1999; Wang et al., 2013; Höhener & Ponsin, 2014). Low-cost substrates such as cow manure, chicken manure, compost, mulch, hay, etc. are used in case of ex-situ as well as in-situ soil composting process for ClO_4^- remediation (ITRC 2005). In this study complete degradation of ClO_4^- was achieved within 10 days when STP secondary sludge was added to the soil as substrate in the ratio 1:1 and 1:2. This corresponds to 8000 and 4000 mg of BOD respectively available for the degradation of 500 mg of ClO_4^- (perchlorate: electron donor ratio of 1:16 and 1:8 respectively). Perchlorate degradation was not complete when the sludge: soil ratio was 1:4. This may be due to the low availability of organic carbon and electron donors (corresponds to only 2000 mg/L of BOD available for 500 mg of ClO_4^-). In the case of complex electron donors, an excess of COD and BOD may be needed for providing sufficient electron donors. Cox et.al provided simple electron donor (ethanol) in the ratio of 1:2, whereas complex electron donor (molasses) in the ratio 1:4.5 in their microcosm studies (Cox et al., 2000). Secondary sludge is a low-cost abundant substrate with high content of biologically available organic carbon (Seiple et al., 2017). Our studies suggest that secondary sludge, which is waste from sewage treatment plants can be a potential candidate to be considered as a carbon and nutrient source for in-situ ClO_4^- bioremediation. Figure 5.8. presents the results of the time course of ClO_4^- degradation using different ratios of sludge and soil mixtures in our studies.

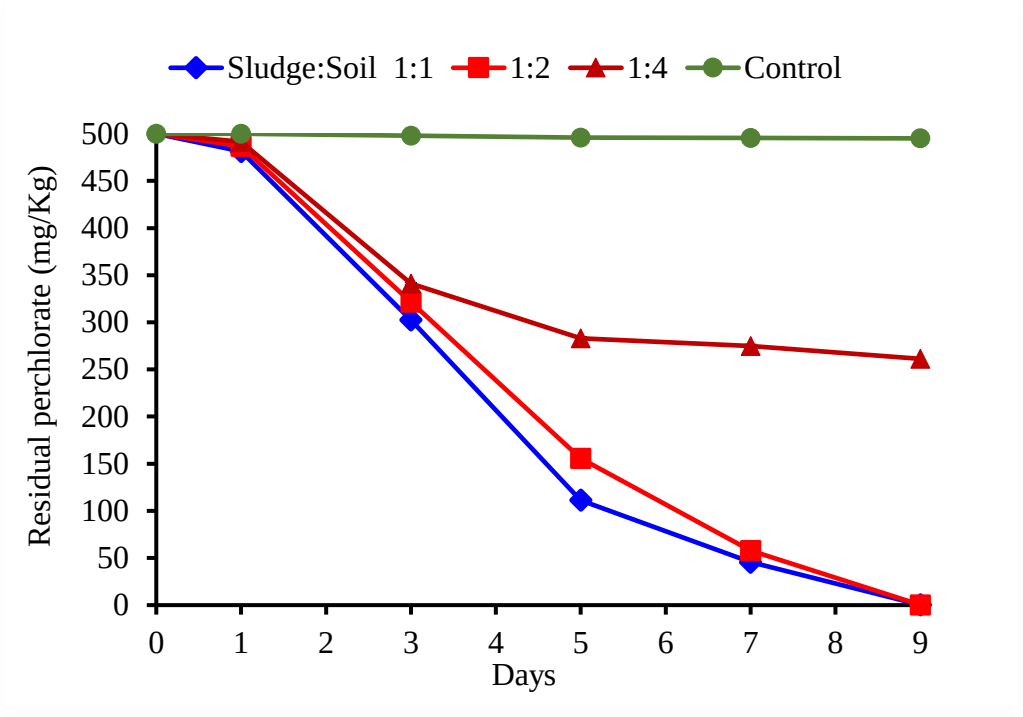

Figure 5.8. Time course of perchlorate degradation is soil samples amended with different ratios of secondary sludge as bio-stimulant

In bioaugmentation studies, compared with uninoculated soil, a considerable decline in the time required for complete removal of ClO_4^- in the augmented soil was observed. To remediate 1 kg of soil spiked with 500 mg of ClO_4^- and 0.5 L of sludge as substrate, it took 5 days with bioaugmentation and 10 days without bioaugmentation. Figure 5.9. presents the time course of ClO_4^- degradation using sludge and soil in the ratio 1:2 and bioaugmentation with ClO_4^- reducing isolates either singly or as a consortium

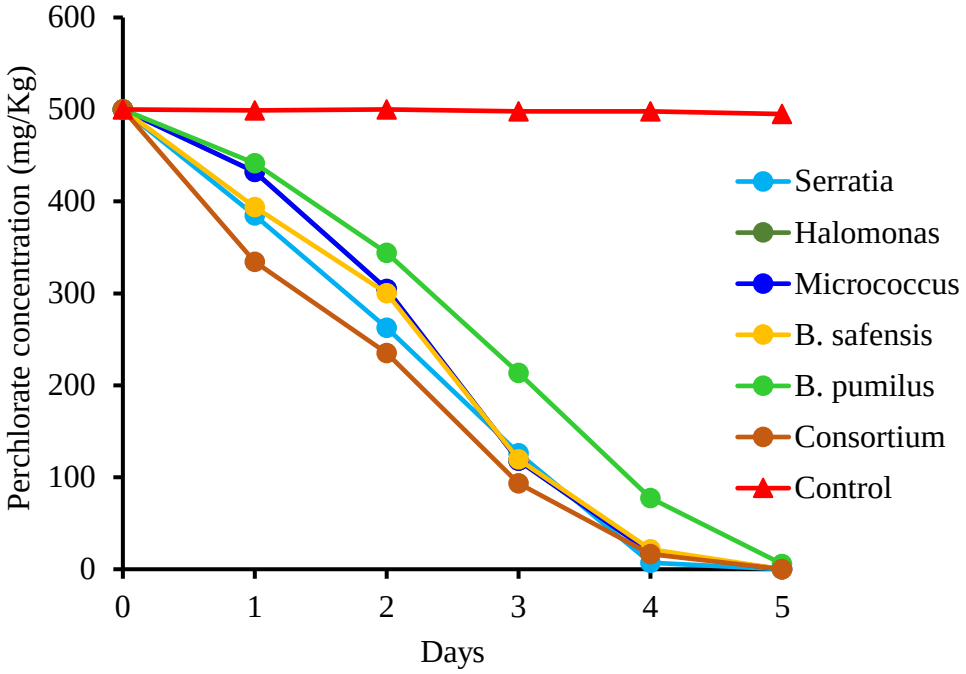

Figure 5.9. Time course of perchlorate degradation in soil samples amended with sludge in the ratio 1:2 and bio-augmented with perchlorate reducing bacterial isolates either singly or as a consortium.

Cox et.al. have used soil samples contaminated with 30 to 40 mg/kg of ClO_4^- and substrates such as ethanol, manure, molasses etc. as carbon source for soil microcosm studies. In the soil microcosms amended only with the carbon source, a lag period of 20 to 40 days was observed before the start of ClO_4^- degradation. Whereas complete reduction in ClO_4^- was observed within four days when the soil samples were augmented with a ClO_4^- reducing bacterial isolate KB-1(Cox et al., 2000). Hence it can be concluded that bioaugmentation can reduce the lag period and can enhance ClO_4^- degradation. In this study both bio-stimulation and bioaugmentation approaches are shown to be effective for in-situ remediation of ClO_4^- contaminated soil. However, it took 5-9 days for a marginal decline in ClO_4^- level.

5.3.3. Soil washing and Bio-regeneration of wash water

i) Preliminary soil washing experiments

The preliminary soil washing studies were conducted to test the effect of water holding time, ClO_4^- concentration in the soil, and the height of soil column on ClO_4^- removal from soil.

Figure 5.10 presents the results of ClO_4^- removal under different water holding times in the soil column

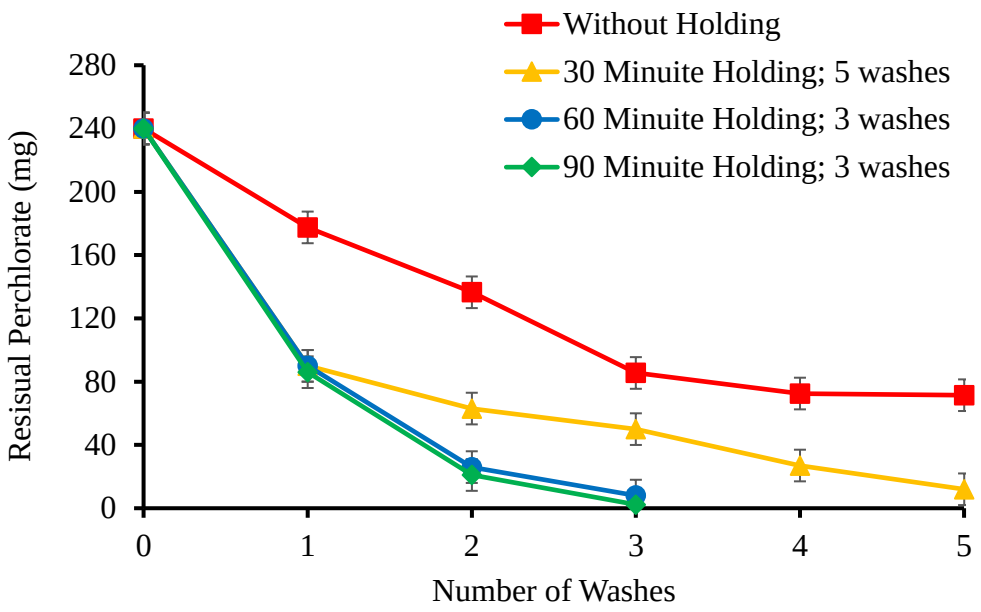

Figure 5.10. Residual ClO_4^- in soil under different water holding times and washing cycles in the box-type soil washing unit.

The ClO_4^- recovery was poor when the water holding time was minimum (no holding) and around 65 L water in five washes removed only 70% ClO_4^- that was added to the soil. With a water-holding time of 30 minutes and 5 washes using 35 L of water, 80% of ClO_4^- got eluted from the soil. The recovery was further improved (96.7 %) when the holding time was increased to 60 minutes. The water consumption was 35 L and only three washes were needed. The elution efficiency increased to 99 %, when the holding time was 90 minutes, with the same amount of water (35L) and the same number of washes. Based on these results, 60 min holding time was taken as the optimized condition for the subsequent experiments.

As the ClO_4^- concentration in the soil varied, the number of washes required, as well as the amount of water required for the washing also varied. The results of the experiment on ClO_4^- removal from the soil at different initial concentrations are presented in Figure 5.11. Three washes with 25 L of water were sufficient to achieve a removal percentage of 98.5 % and 97.9 % for 240 mg and 480 mg of ClO_4^-, respectively. The number of washes, as well as the quantity

of water, required increased to five and 35 L respectively when the ClO_4^- concentration was 960 mg and the recovery achieved was ~ 98.2%.

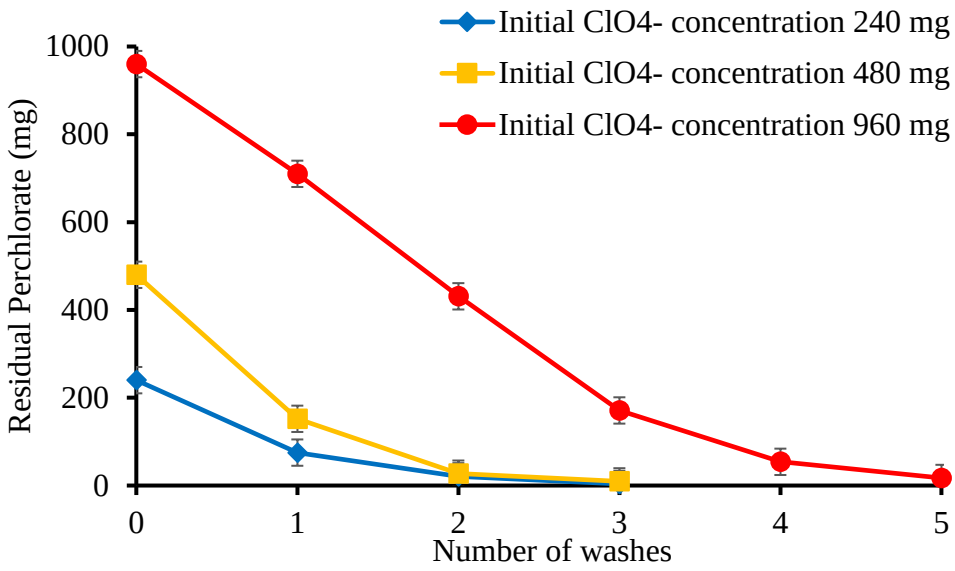

Figure 5.11. Washing efficiency with respect to initial perchlorate concentration in the soil

Two different soil column heights of 20 cm and 100 cm were also tested in this study. The washing efficiency was almost the same for the soil column height up to 100 cm at the optimized water holding time of 60 minutes. In soil sample spiked with 500 mg of ClO_4^-, 98% removal efficiency was achieved by three washing cycles with 25 L water at 1 m soil column height.

ii) Pilot-scale soil washing study

Based on the results from the preliminary soil washing test, water holding time of 60 minutes, soil column height of 1 m, and three washing cycles were considered for the pilot-scale soil washing experiments. At this condition, 2.5 g of ClO_4^- spiked in soil was recovered to the aqueous phase using a total of 360 litres of tap water. The amount of water used for each washing cycle, the volume of leachate produced, the concentration of ClO_4^- in the wash water, and the time taken for washing are presented in Table 5.4. The percentage recovery of ClO_4^- to the aqueous phase was ~ 99.84 %. The pooled wash water (360 L) was fed to the bioreactor for treatment.

Table 5.4. Pilot-scale Soil washing data

	Water used for washing (L)	**Leachate water (L)**	**Total washing time (min)**	**ClO_4^- Concentration in wash water (mg/L)**
1st wash	150±5	118±5	90	13.85
2nd wash	110±5	90±4	130	8.64
3rd wash	100±5	83±4	160	1.02

Soil washing followed by treatment of the wash water has been reported as an effective method for cleaning soil contaminated with few organic pollutants such as pesticides, hydrocarbons, etc. But in none of these approaches, biological treatment was reported for treating the wash water. Soil washing with surfactants like Triton X-100, Tween 80, etc. coupled with Photo-Fenton oxidation of the wastewater obtained was previously reported for remediating soil contaminated with DDT, DDE, hydrocarbons, etc. (Villa et al., 2010; Huguenot et al., 2015; Befkadu & Quanyuan, 2018). The ionic properties of ClO_4^-, and high water solubility, eliminate the requirement of surfactant and only simple washing with water is required for completely eluting ClO_4^- from the soil.

iii) Bioreactor start-up, continuous operation, and wash water treatment

In the beginning, the bioreactor was operated in recirculation mode which helped in the gradual build-up of an active ClO_4^- reducing biofilm on the biofilm support media. Glucose was used as the substrate (carbon source and electron donor). The initially spiked 50 mg/L ClO_4^- was reduced to <2 μg/L in 3 days (99.9 % removal). The result of ClO_4^- removal in the bioreactor at different glucose / ClO_4^- ratios is presented in Figure 5.12. The highest removal of ClO_4^- (99%) was observed when the glucose / ClO_4^- ratio was at 5. The reduction got declined to 89% and 50% respectively when glucose/ ClO_4^- ratios reduced to 4 and 2.

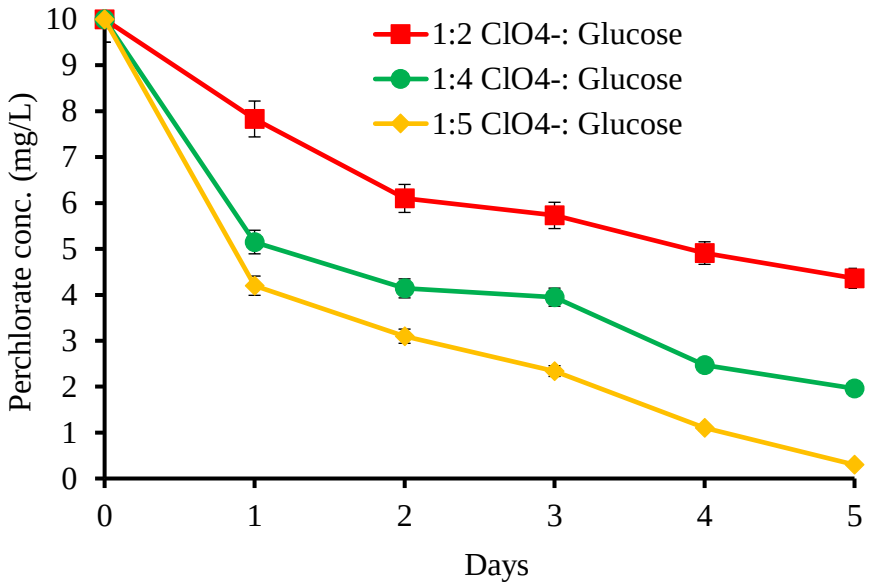

Figure 5.12. Perchlorate degradation in the bioreactor at different ratios of ClO_4^- and glucose.

The higher glucose requirement (than a stoichiometric requirement) for ClO_4^- degradation could be due to the presence of heterotrophic microflora are normally present in the soil wash water. The excess glucose will scavenge the oxygen and nitrate in the wash water and create a more favourable (anoxic) environment in the bioreactor for ClO_4^- reduction. The level of ClO_4^- in the wash water was the basis for glucose level in the augmenting solution along with other nutrients. This will help to avoid excess organic loading into the soil, which was the case with in-situ and ex-situ remediation approaches reported earlier.

The performance of the bioreactor during the first three-month of operations is presented in Figure 5.9a. During this period, the influent ClO_4^- concentration (tap water spiked with ClO_4^-) was in the range of 10-50 mg/L. To ensure complete ClO_4^- reduction, the glucose level was maintained proportionally. In all the concentrations tested, around 99% removal of ClO_4^- was observed during the period. Throughout the study, the reactor pH was around 7±0.5 without any external pH correction (Figure 5.13). The optimum pH range of ClO_4^- removal in bioreactors has been reported as 6.5 - 7.5 (Waller et al., 2004; Balk et al., 2010). The performance of the bioreactor directly depends on the oxidation-reduction potential (ORP). Decreasing ORP, which leads to an increase in anoxic/ anaerobic microbial activity. At the start-up stage, the ORP was around -50 mV, and gradually it decreased to -150 to -300 mV range (Figure 5.14). Previously reported studies have shown retarded ClO_4^- reduction under the presence of molecular oxygen (Waller et al., 2004). The perchlorate removal is inversely

proportional to ORP and a complete reduction of ClO_4^- is observed when the ORP was -220 mV (Shrout & Parkin, 2006).

The microbial ClO_4^- reduction is a sequential process with chloride and oxygen as the end products. The intermediates like chlorite (in particular) and chlorate as unstable and will be reduced further into chloride and oxygen. The chlorite and chlorate were not detected in the ion chromatography (IC) analysis of the treated water.

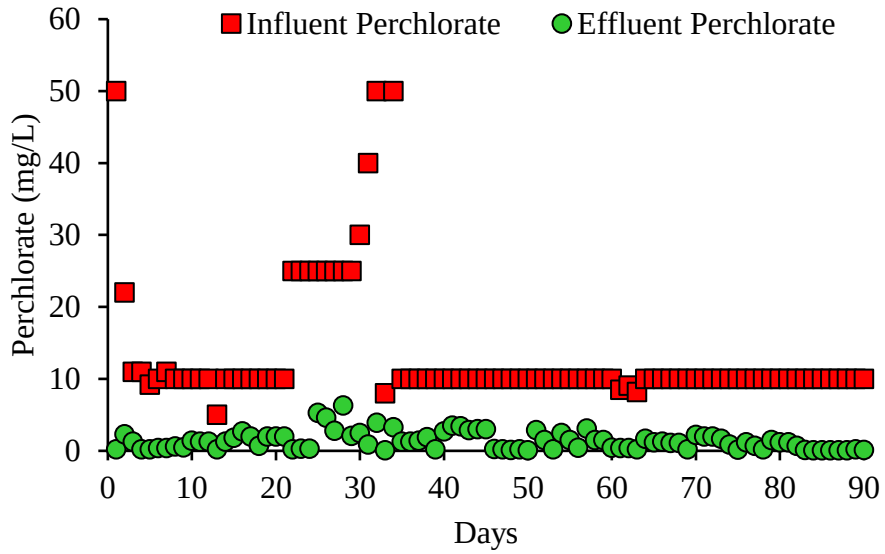

Figure 5.13. Concentration of influent and effluent ClO_4^- in the bioreactor for first 90 days of operation, each data point represents daily analysis result.

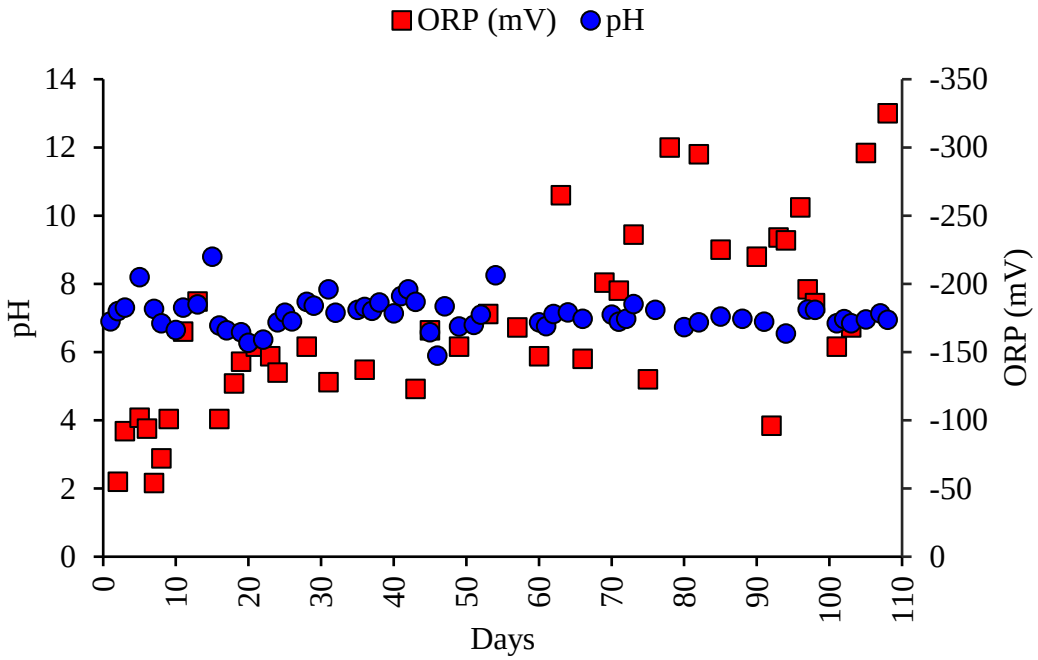

Figure 5.14. pH and ORP profile of the Bioreactor during the first 90 days of operation, each data point represents the daily analysis result.

The soil wash water containing ClO_4^- was treated completely in the bioreactor used in this study. The total volume of water used in three different washing cycles, the corresponding level of ClO_4^- in the pooled wash water, and ClO_4^- in the treated water are summarized in Table 5.5. The level of ClO_4^- in the treated water was very low (2-4 µg/L), and that was achieved within a short Hydraulic Retention Time (HRT) of 4.5 hours. The combined washing (total 6.3 hours, including 60 min holding time) and subsequent wash water (9.7 hours) treatment could be finished within a maximum of 16 hours. This is comparatively very short compared with other (in-situ and ex-situ) approaches reported in the past, where it took a few days to many months to complete the remediation of soil contaminated with ClO_4^- (Deitsch et al., 2005; ITRC 2005, 2008; Sarria et al., 2019). The biotreated wash water can be used for the next washing next lot of contaminated soil and thus the wastage of freshwater can be avoided.

Table 5.5. Performance data of ClO_4^- containing soil wash water treatment in the bioreactor

Experiment	wash water volume (L)	ClO_4^- concentration in leachate (mg/L)	ClO_4^- concentration in treated leachate (µg/L)
1	291	8.59	4
2	306	8.17	2
3	276	9.06	4

The present strategy has many advantages when compared with the in-situ (including in-situ soil flushing and treatment) and ex-situ remediation approaches reported so far. A comparison of the soil remediation method reported so far is presented in Table 5.6. This process may find application at places where ClO_4^- is handled in bulk, and that leads to topsoil contamination. The major advantages of the present approach are (i) Perchlorate from the contaminated soil can be completely removed by recovering it to an easy to treat aqueous phase, (ii) the entire treatment process of soil washing and bio-treatment of the wash water can be completed within a few hours, (iii) the practical difficulties and adverse impacts of adding organic substrates (secondary contamination) to soil can be avoided, (iv) adverse change in soil properties can be avoided, (v) there is no requirement of a pre or post-treatment of the contaminated soil, (vi) this approach can be adapted to any soil types, provided a proper mechanism to enhance water percolation in the case of soil with high clay content (poor water penetration), (vii) the biotreated wash water can be reused and hence freshwater wastage is minimized. Instead of glucose, cheap and economical, locally available substrates like leachate from agro-residues, organic wastes, etc. can also be considered as substrates for the microbial activity in this treatment. A point of concern in this approach is the fate of natural soil microflora and nutrient levels that may be affected through the washing step. But this can be compensated through mixing the treated soil with organic manure or compost.

Table 5.6. Comparison of different soil remediation processes reported so far (ITRC 2005; Evans & Trute, 2006; Cai et al., 2010)

Process	**Scale of study**	**Perchlorate concentration in the soil**	**Electron donor**	**Remarks**
Ex-situ anaerobic composting	Pilot-scale	57 mg/kg	Horse stable compost	The soil was excavated, and soil piles were treated to <7.8 mg/kg
Anaerobic landfarming inlined, flooded cell.	Full scale	5000 mg/kg	Citric acid	The soil was excavated and logged with water to maintain moisture content. Perchlorate got reduced to <0.1 mg/kg
Ex-situ anaerobic composting	Full scale	100 mg/kg	Mulch and hay	12 months to treat 1500 cubic yards of soil with a residual perchlorate of 0.01 mg/kg
Ex-situ anaerobic composting	Full scale	23 mg/kg	NA	14 days for treating 20 cubic yards of soil with a residual perchlorate of 0.1 mg/kg
Ex-situ anaerobic composting	Full scale	175 mg/kg	Horse stable compost	90 days for treating 200 cubic yards of soil with a residual perchlorate of <1 mg/kg
In situ anaerobic composting	Full scale	450 mg/kg	Cow manure and calcium magnesium acetate	Residual perchlorate of 1.4 mg/kg
Enhanced in-situ bioremediation	Pilot-scale	300 mg/kg	Chicken and horse manure, ethanol	9 days for pilot scale
	Full scale	6.7 mg/kg		10 months for full-scale treatment

Enhanced in-situ bioremediation using gaseous electron donors	Microcosm study	9.6 mg/kg	Hydrogen, ethyl acetate, Liquified Petroleum Gas, ethanol, 1-Hexane	7 – 184 days for different electron donors with ethyl acetate not showing any substantial reduction
Enhanced in-situ bioremediation using gaseous electron donors	Pilot-scale	2.6 – 750 mg/kg	Hydrogen, Liquified Petroleum Gas	Reduced to < 0.013 mg/kg to 8.8 mg/kg in 35 to 42 days
In situ soil flushing and treatment of groundwater	Pilot-scale	72.4 mg/L	NA	Water is flushed through the Vadose zone contaminated with perchlorate and it is captured in the below-ground water aquifer. The water is treated above ground and injected back to the aquifer
Ex-situ soil washing and bio-treatment of the wash water **(This study)**	Pilot-scale	3.7 mg/kg (2.5 g perchlorate in 670 kg of soil)	Glucose	Perchlorate was completely recovered from the soil to the aqueous phase and the perchlorate was reduced from 8.5 mg/L to <0.002 mg/L. The whole process was completed within 16 h

Conclusions

In this study, a novel, ex-situ (bio)-remediation approach for ClO_4^- contaminated soil was developed and successfully validated in a pilot-scale unit. In this approach, ClO_4^- in the contaminated soil was directly eluted with water, and the wash water was treated in a bioreactor. The bio-regenerated wash water was used for the next lot of soil washing and the cycle was continued. This approach will find application, especially for treating contaminated topsoil, which is very common at places where ClO_4^- is handled in bulk. Compared with the existing approaches in this field, the soil washing approach developed in this study has many advantages and therefore, can be a better substitute. The major highlight of this approach is, the entire soil washing procedure and bio-regeneration can be completed in a few hours. This will prevent the infiltration of highly persistent ClO_4^- into the underlying groundwater.

Chapter 6

Development of a low-cost permeable reactive bio-barrier system for in-situ perchlorate remediation – A bench-scale study

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***Best Oral Presentation**, (Engineering Science and Technology), Kerala Science Congress 2021*

6.1. Introduction

Groundwater aquifer contamination of ClO_4^- particularly around its bulk handling sites is a known environmental problem (Trumpolt et al., 2005; Tiemann, 2006; Steinmaus, 2016). Perchlorate being highly stable in aqueous environment, and its natural attenuation is very difficult, it can migrate substantial distances from the site of contamination (Gullick et al., 2001). A spatio-temporal study conducted by CSIR-NIIST has shown ClO_4^- contamination around the Ammonium Perchlorate Experimental Plant (APEP) Aluva, Ernakulam Dist. and the presence of ClO_4^- up to 2 km around the plant (Figure 6.1) (Anupama et al., 2017).

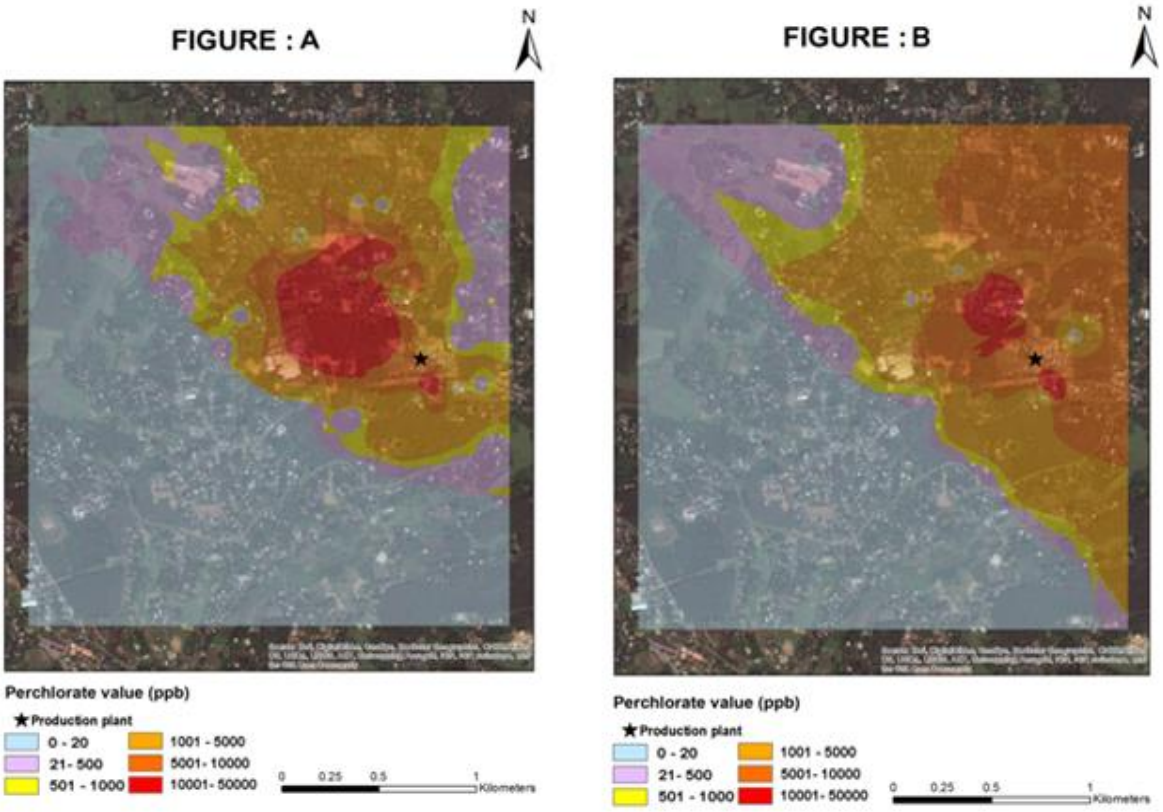

Figure 6.1. Contour map showing the spatial variation in ClO_4^- concentration in groundwater samples during (A) 2014 July and (B) 2015 June (adapted from (Anupama et al., 2017)).

Another observation during this study was an infiltration stream (drain) containing high concentration of ClO_4^- (34 mg/L) as a prime source contaminating a community pond, (Kulakkad Pond) in the area (detailed in Chapter 3). Considering these scenarios, practical solutions to control the mobility of ClO_4^- laden aqueous phase (underground or surface) is highly important. In-situ remediation systems are recommended to address these kinds of

problems. Permeable Reactive Bio-Barriers (PRB) is a concept practiced in this field where a treatment unit will be installed upgradient or around the boundary of the contaminant inventory (ClO_4^- in this case) to prevent plume migration to wider regions (Borden, 2007; Henry et al., 2009).

Permeable Reactive Bio-barriers can be of three configurations like, active, semi-passive or passive (injected and trench biowall) (USEPA 2005; ITRC 2005; Borden, 2007; Stroo & Norris, 2009). In an active or a semi-passive bio-barrier, a mobile soluble amendment (substrate for the bio-barrier) is delivered into the contaminated aquifer through injection wells and the groundwater mixed with the water-soluble amendment is recirculated through extraction – injection wells. In semi-passive system the groundwater or substrate is recirculated only intermittently. The common substrates used are acetate, lactate, citrate, ethanol etc. (Parr, 2002; Hatzinger, 2005; Stroo & Ward, 2008; Krug et al., 2009; Taraszki, 2009). Alternatively gaseous electron such as hydrogen, ethyl acetate etc. were also reported (Evans & Trute, 2006; Evans et al., 2009, 2011; Cai et al., 2010). Passive bio-barrier systems that uses low-cost substrates are the cheapest in-situ method, and it requires low operational and maintenance cost (Stroo & Norris, 2009).

The application of a biofilm support matrix (biofilter medium) to improve the efficiency of bio-barrier was also reported. The availability, cost, longevity, environmental compatibility, and low operational and maintenance cost are factors considered while choosing these filter media. The common media reported are granular materials such as gravel, sand, quartz, pumice, perlite, granular activated carbon etc (Di Lorenzo et al., 2005; Liu et al., 2006; Careghini et al., 2013). Substrate such as mulch, compost etc. are often mixed with the filter media to avoid compaction and for better hydraulic conductivity in passive trench biowalls for ClO_4^- removal (Ahmad et al., 2007; Lu et al., 2007; Shen et al., 2010). Polypropylene fleece and natural coconut fibre are also proposed as trench filling material (Careghini et al., 2013).

The cost of electron donors is another contributor to the economics of both in-situ as well as ex-situ bioremediation process (Okeke & Frankenberger, 2005). A major practical challenge is excess or limited organic release leading to organic contamination or a contaminant breakthrough respectively. (Zhao et al., 2021). Improper mixing of the substrate is another difficulty reported (Stroo & Norris, 2009). In-situ remediation studies of ClO_4^- was never reported from India.

With this background, the main objective of this study was to develop a low-cost in-situ remediation system (Permeable Reactive Barrier) using cheap and locally available agro-residues as substrate (organic, nutrient and electron donor) as well as biofilm support matrix. As part of this study, screening of different ligno-cellulosic waste biomass such as rice straw, rice husk, sugarcane bagasse and peanut shell as substrate for ClO_4^- removal was done. Organic waste derived leachate was tested as sole substrate for ClO_4^- removal in a bio-barrier set up. Furthermore, we have tested high lignin containing natural fibre as support matrix for the ClO_4^- reducing bacterial biofilm in this study. The proof of concept was tested in a bench scale unit under field relevant conditions.

6.2. Materials and Methods

6.2.1. Perchlorate reduction using organic waste derived leachate as sole substrate in a bench scale bio-barrier unit.

The bench-scale bio-barrier treatment system used in this study consisted of two components,
(1) An anaerobic leach bed unit for generating leachate from vegetable waste, and

(2) Anaerobic bio-barrier unit for ClO_4^- degradation.

a) The Anaerobic Leach Bed Unit (ALB)

The ALB unit was made of poly vinyl chloride pipe of length 30 cm, and inner diameter 15 cm. A metal mesh (5 mm) was placed 12 cm above the bottom outlet. The working volume of the ALB unit was 5.3 liters. Initially 1 kg of organic waste (heterogenous kitchen vegetable waste without any pretreatment) was loaded into the ALB unit, and 5 L of well water was dribbled from its top at a constant flow rate of 2.5 L/h using a peristaltic pump (Watson Marlow 530 Du, UK). The mixed vegetable waste was composed of beetroot peels, potato peels, cucumber peels, discarded portions of lady's finger, carrot, pumpkin etc. The leachate collected from the ALB bottom outlet was recirculated continuously. After 48 hours of recirculation, leachate sample was taken for the analysis of pH, Volatile Fatty Acid (VFA), alkalinity, Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Total Chemical Oxygen Demand (TCOD), Soluble Chemical Oxygen Demand (SCOD), Total Kjeldahl Nitrogen (TKN), Total Phosphate (TP), ammonia (NH_3 -N), nitrate (NO_3^- - N), nitrite (NO_2^- - N), phosphate (PO_4^{3-} - P), sulfide (S_2^-) and total plate count (TPC). The recirculation of leachate was continued until its SCOD concentration reached ~50 mg/L. This experiment was repeated with different

quantity of waste (1 kg and 2 kg) and volume of water used (2.5 L and 5 L) for the leachate generation. The photograph of heterogenous vegetable waste loaded on to the ALB, the crude leachate produced and the residue after 18 days of leaching is present in Figure 6.2, 6.3 and 6.4 respectively. The results of this preliminary experiment were used for further treatment studies in the bio-barrier unit.

Figure 6.2. Photograph of heterogenous vegetable waste loaded into the Anaerobic Digestion Unit (AD)

Figure 6.3. Photograph of the organic rich leachate obtained from the anaerobic digestion of the vegetable waste

Figure 6.4. Photograph of the vegetable waste residue after 18 days of leaching

b) The Anaerobic Bio-barrier Unit (ABB)

In this study a chamber type bio-barrier unit was designed to simulate the field conditions. The ABB used was a rectangular tank (50 x 10 x 20 cm), made up of 3 mm mild steel sheet. The total volume of the tank was 10L and it was internally divided into 3 compartments. There was an inlet and outlet compartment (IC and OC), both 3 L on either side of the bio-barrier compartment (BC) which was of 4 L capacity. The schematic of the complete experimental set up is given in Figure 6.5a, and the photograph of the bio-treatment unit is presented in Figure 6.5b.

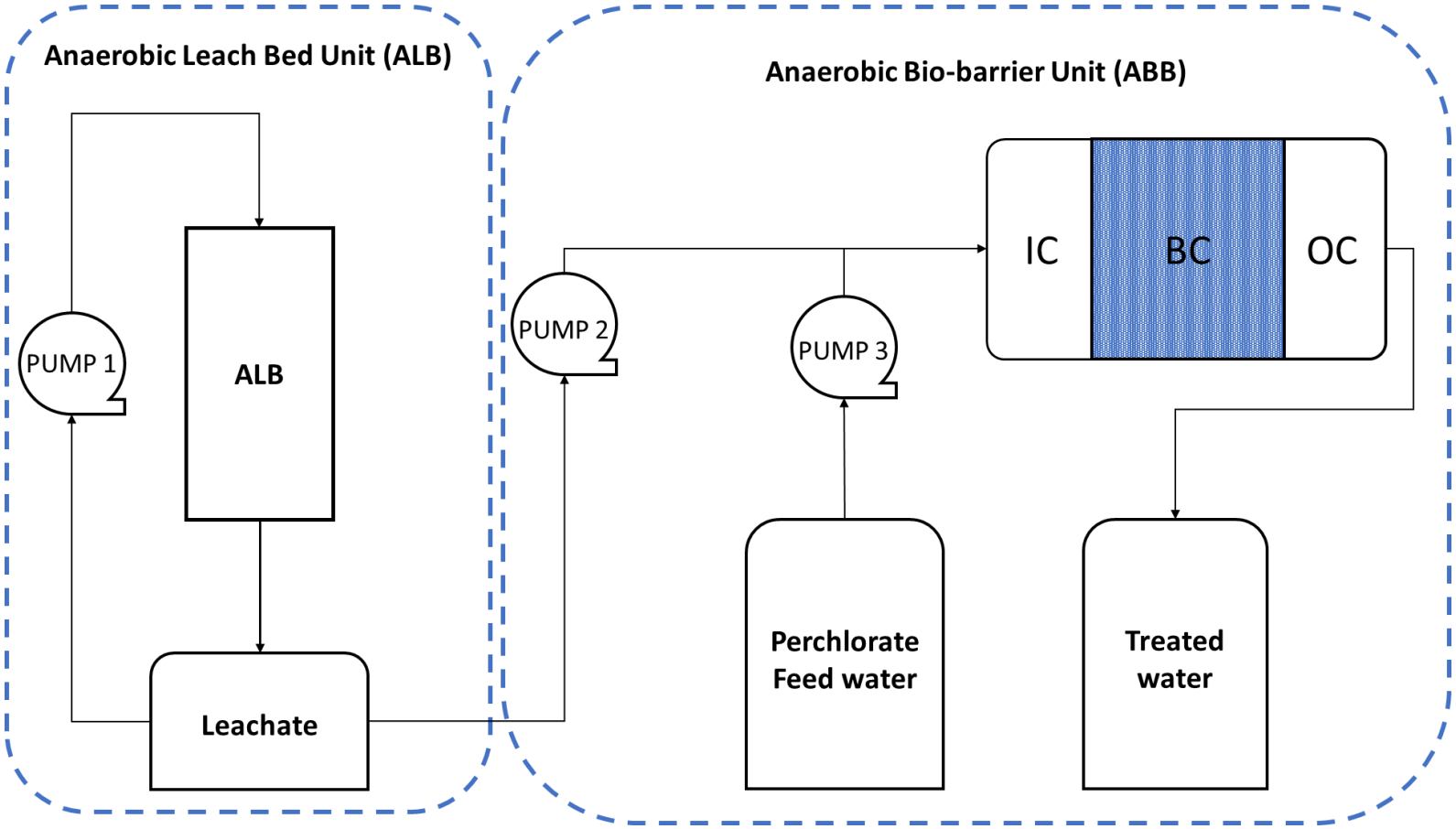

Figure 6.5a. Schematic representation of the combined leach-bed-bio-barrier treatment system. (IC – Inlet chamber, BC – Barrier Chamber, OC – Outlet chamber)

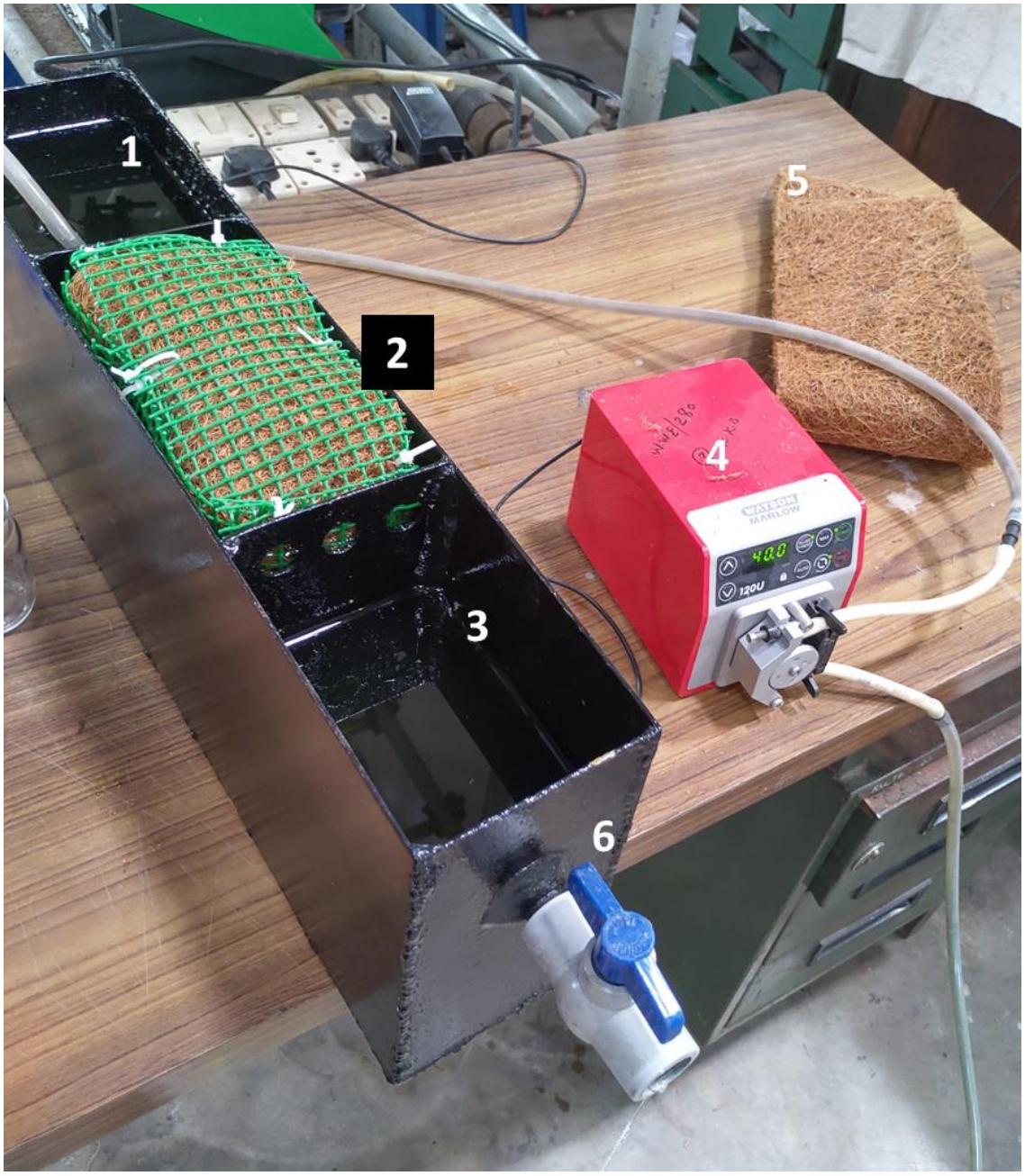

Figure 6.5b. Photograph of the Anaerobic Bio-barrier unit (ABB) (1 - Inlet Compartment (IC), 2 - Barrier Compartment (BC), 3 - Outlet Compartment (OC), 4 - Peristaltic pump, 5 – Needle felt coir fiber, and 6 - Outlet.

The BC was packed ~ 200 g of needle felt coir fibre procured from a local market. The lignin rich (45%) coir fibre functioned as the biofilm support medium. The working volume of the BC was 3.1 L. At the start-up, the bio-barrier filter bed was inoculated with an enrichment culture of ClO_4^- reducing *Serratia marcescens* strain NIIST5 (MTCC 5821, Genbank JQ807993)(Anupama et al., 2013).

One litre of bacterial culture at log phase (OD 0.306) in 15 L of deaerated well water containing 15 mg/L of ClO_4^- (as $KClO_4$) and 60 mg/L of CH_3COO^- (as sodium acetate) supplemented with inorganic minerals, trace metals and 0.5% yeast extract (based on our previous studies on ClO_4^- reduction in a fixed bed reactor) (Russel et al., 2021) was pumped into the IC of bio-barrier in recirculation mode at a flow rate of 0.5 L/h using a peristaltic pump (Watson Marlow 120 U, UK). This helped in the gradual build-up of ClO_4^- reducing biofilm in the filter bed. After 48 hours when complete reduction of ClO_4^- <2µg/L was observed, the reactor was switched over to continuous feed mode. The feed solution at this stage contained 10 mg/L of ClO_4^- and 40 mg/L of CH_3COO^- and the flow rate was 1.5 L/h. After 10 days of operation and assessing the ClO_4^- removal efficiency of the system, instead of acetate, organic waste derived leachate as was supplied to the system.

For testing the ClO_4^- reduction by the Anaerobic Bio-barrier Unit (ABB) using organic waste leachate as soluble substrate, well water spiked with ClO_4^- was used as feed solution. The influent ClO_4^- was maintained at 10 mg/L (field relevant concentration observed around site 1) The perchlorate feed solution and the crude leachate (leachate with SCOD concentration >1500 mg/L) were pumped separately using two different peristaltic pumps and supplied to the inlet compartment (IC) using a 'Y' joint as shown in Figure 6.5a. This helped in the proper mixing of ClO_4^- feed solution with the crude leachate (termed as mixed influent in subsequent sections). Based on the SCOD concentration of the crude leachate, its flowrate was adjusted to get the desired SCOD concentration in the mixed influent.

Performance of the system at different influent SCOD (20, 40, 60 and 80 mg/L) for an inlet ClO_4^- concentration of 10 mg/L was tested during this study. Crude leachate with a SCOD concentration of ~1750 mg/L was chosen for this purpose. The HRT within the BC at this point was calculated based on the combined flow rate of ClO_4^- feed (510 – 492 mL/h) and the crude leachate (6 – 24 mL/h) into the BC. At this stage the combined flowrate was 0.516 L/h, and the HRT was 6 hours. To check the effect of HRT on ClO_4^- reduction at constant SCOD concentration of 40 mg/L and influent ClO_4^- concentration of 10 mg/L, different feed flow rates, 0.486, 0.504 and 0.540 L/h corresponds to HRT 6.3, 6.15, 5.7 hours was also tested.

The performance of the combined unit was tested for a period of 30 days to assess sustained ClO_4^- reduction using organic waste leachate. During this period feed solution contained 10 mg/L ClO_4^- and SCOD at 40 mg/L and the feed flowrate and HRT were 12 L/h and 6.15 hours respectively. The mixed influent and effluent were analyzed for ClO_4^- concentration, pH, Oxidation Reduction Potential (ORP), TDS, DO, TKN, TP, NH_3 -N, NO_3^- - N, NO_2^- - N, PO_4^{3-}

- P, S_2^-, and TPC. The photograph of the entire bench scale experimental unit is presented in Figure 6.6.

Figure 6.6. Photograph of the combined Anaerobic Leach Bed and Bio-barrier unit used in this study (1 - Anaerobic Leach Bed unit, 2 – Crude leachate collection tank, 3 - Perchlorate feed tank, 4 - Anaerobic Bio-barrier Unit, 5 - Treated water collection tank, 6,7,8 - Peristaltic pumps).

6.2.2. Screening of ligno-cellulosic biomass for perchlorate reduction in the bio-barrier unit

Ligno-cellulosic biomass such as rice straw, rice husk, sugarcane bagasse and peanut shell were selected as solid slow carbon releasing substrates for ClO_4^- reduction in bio-barrier unit. The characterization of the biomass was done in terms of its total solid (TS) content, total organic carbon (TOC) and soluble organic carbon releasing efficiency.

a) Static leaching test

To analyze the performance of rice straw, rice husk, sugarcane bagasse and peanut shell in terms of slowly released organic carbon, a static leaching test was conducted for determining soluble chemical oxygen demand (SCOD) of the leachate produced from each substrate. SCOD is an indirect measurement of dissolved organic carbon (DOC) in the leachate. The experiments were conducted with both sterile and non-sterile substrate. Sterile static leaching test was conducted by suspending 5 g of sterilized material (121°C for 15 minutes) to 100 mL of sterile distilled H_2O under aseptic conditions. For the non-sterile static leaching test, 1 g of substrate (sun-dried and non-sterile) was added to 100 mL of well water and allowed to leach for 48 hours. The supernatant was analysed for pH, ORP and SCOD (Okeke & Frankenberger Jr, 2005; Y. Xie et al., 2017) .

b) Perchlorate Reduction with rice straw – Batch scale studies

Based on the preliminary static leaching experiments, rice straw was chosen as solid carbon source for ClO_4^- reduction. To evaluate the ClO_4^- reduction efficiency of rice straw, 5 g of the substrate was added to 100 mL Inorganic Mineral Media (IMM) and Trace Metal Solution spiked with 10 mg/L ClO_4^-. The conditions tested for ClO_4^- reduction in batch experiments using rice straw are given in Table 6.1. In order to test the presence of ClO_4^- reducing microbes on the substrate surface, an indigenous control (C) was kept using non-sterile substrate. Indigenous control was not augmented with ClO_4^- reducing consortium. The ClO_4^- consortium for augmentation was obtained from a ClO_4^- acclimatized consortium maintained in our laboratory.

Table 6.1. Treatment conditions tested for batch perchlorate degradation studies using rice straw

Sample	Testing condition
A	Negative control – IMM without substrate and inoculum
B	Sterile control – IMM with the sterile substrate
C	Indigenous control – IMM with non-sterile substrate
D	IMM with sterile substrate and PRM consortia
E	IMM with non-sterile substrate and PRM consortia
G	Adsorption control – Sterile substrate in sterile d.H_2O

The experiments were conducted in 100 mL screw cap bottles and for testing each condition 10 bottles were kept. Every 48 hours two bottles were opened and residual ClO_4^-, ORP, SCOD and pH were measured. An adsorption control (G) was also kept for testing if the ClO_4^- is adsorbed by the rice straw. For that purpose, 5 g (dry weight) of sterile rice straw was added to 100 ml of sterile distilled water spiked with 10 mg/L of ClO_4^-. After 48 hours the ClO_4^- concentration in the supernatant was measured. The experiments were carried out under ambient conditions.

c) Perchlorate reduction using rice straw as substrate in bench scale bio-barrier unit

The experimental ABB unit used for ClO_4^- degradation with vegetable waste derived leachate was used for the rice straw-based experiments with some modifications. In this experiment, the inlet compartment was filled with 100 g (wet weight) of fresh rice straw, and 25 g (wet weight) of rice straw from batch experiment bottles as inoculum for initiating degradation of rice straw and easy release of organic carbon. The middle BC was packed with high lignin containing natural fibre (200g) as a biofilm adhering matrix (Figure 6.5b, Section 6.1.1.). Around 120 L of well water spiked with 40 mg/L level of ClO_4^- was pumped into the inlet compartment using a peristaltic pump at a flow rate of 2.8 ml/min (HRT of 31 h). The treated water was collected in the OC and the overflow from OC was collected and pooled for further analysis (termed as pooled effluent in subsequent sections). The samples from the OC were analysed for pH, TCOD, SCOD, TDS and ORP, every 2 – 3 days. The pooled effluent (~110 L) was also analysed for water quality parameters such as TSS, TCOD, SCOD, TKN, TP, orthophosphate

(PO_4^{3-} -P), ammonia (NH_3 – N), nitrate (NO_3^- - N), nitrite (NO_2^- - N), sulfide (S_2^- F), Phenol and Total Plate Count (TPC) after 33 days of experiment.

Analysis of perchlorate

Perchlorate concentration > 0.5 mg/L was analysed using perchlorate Ion Selective Electrode (Cole Palmer, USA). Perchlorate concentration < 0.5 mg/L measured using Ion Chromatography (USEPA methods 314.0 and 314.1). (Detailed in Chapter 3)

Analysis of water quality parameters

The pH, ORP, TDS and DO of organic waste leachate, bio-barrier influent and effluent were estimated using Horiba Multi-Parameter water quality probe U-50 series (Horiba, Japan). The pH and ORP of samples from static leaching test and ClO_4^- batch degradation studies were analyzed using Eutech pH probe (Thermo Scientific, USA) and Pinpoint ORP tester (American Marine Inc. USA). VFA and alkalinity analysis was done by titration method (Anderson & Yang, 1992). APHA standard methods were used for the estimation of TSS (2540 D), TCOD, SCOD (5200 B), TKN (4500 – N_{org} B), TP, orthophosphate (PO_4^{3-} -P) (4500-P E), ammonia (4500 NH_3 – N C), nitrate (4500 NO_3^- - N E), nitrite (4500 NO_2^- - N B), sulfide (4500 S_2^- F) and TPC (9215 C) (APHA 1998). The TOC of solid carbon sources were analysed using TOC analyzer (Analytik Jena, Germany). The phenol concentration in the rice straw bio-barrier effluent was estimated using Continuous Flow Analyser (Skalar, Germany).

6.3. Results and Discussions

6.3.1. Perchlorate reduction using organic waste derived leachate as soluble amendment in the bench scale bio-barrier.

i) Performance of Anaerobic Leach Bed Unit (ALB)

The ALB unit was capable of digesting 1 to 2 kg of heterogeneous vegetable waste. The TCOD and SCOD of the leachate were maximum during 4 to 6 days of digestion. When 2 kg of organic waste was leached with 2.5 L of water in recirculation for 48 hours, the SCOD was maximum (3500 mg/L) on the 4^{th} day. For 1 kg of organic waste the maximum SCOD of the crude leachate was 2300 mg/L on the 4^{th} day. The TCOD and SCOD profile of the leachate during one complete batch operation of the ALB is presented in Figure 6.7.

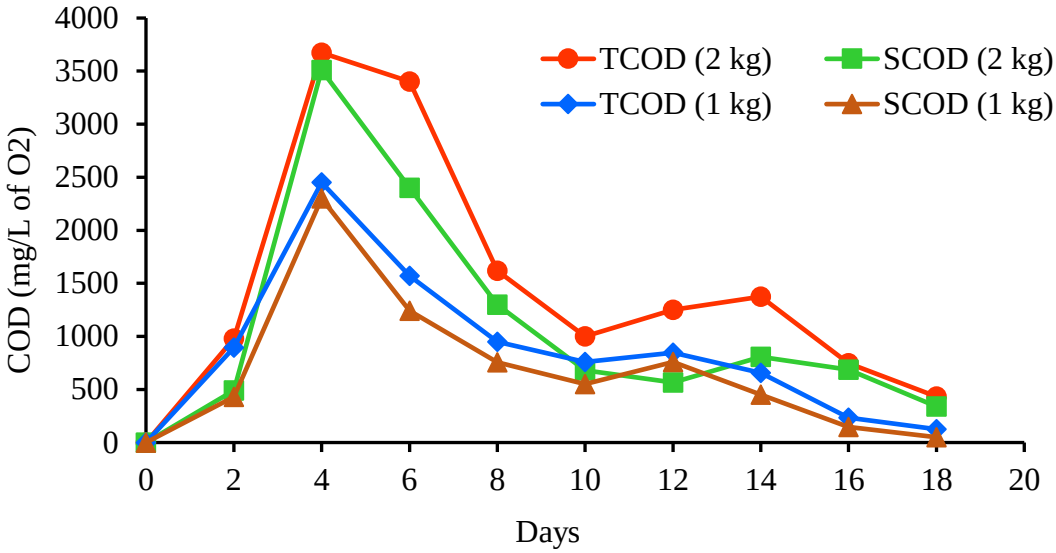

Figure 6.7. TCOD and SCOD profile of the leachate during first 18 days of digestion. Samples were collected every 48 hours and the leaching process was continued using 2.5 L of fresh well water.

With 5 liters of fresh water every 48 hours produced leachate with SCOD concentration upto 900 and 1500 mg/L for 1 and 2 kg of organic waste, respectively. Detailed characterization of this leachate was not performed due to its low SCOD content. Hence leaching with 2.5 L of water was chosen for further studies. The physico-chemical properties of the leachate obtained during 18 days of leaching with 1 kg and 2 kg waste using 2.5L of water are presented in Table 6.2.

Total Organic Carbon (TOC) is directly proportional to total COD (Dubber & Gray, 2010) and SCOD is taken as a measure of the Dissolved Organic Carbon available in the leachate that can be a surrogate for the electron donor (Sarria et al., 2019). The leachate contained Volatile Fatty Acids (6-17 m.eq/L of CH_3COOH) which was produced from the anaerobic digestion (acidogenesis stage) of the organic waste (Krishania et al., 2013). VFAs such as acetic acid, citric acid, lactic acid etc. are known electron donors for ClO_4^- degradation (Wu et al., 2001). Carbohydrate Based Electron Donors (CBED) like molasses, corn syrup, cellobiose are used as a substitute for acetic acid in ClO_4^- degrading fixed bed reactors and fluidized bed reactors (Upadhyaya et al., 2015; He et al., 2019). These substrates are fermented by microorganisms such as *Lacto bacilli* to lactic acid and which can be utilized as an electron donor by Perchlorate Reducing Microbes (PRM) (He et al., 2019). Perchlorate Reducing bacterial isolates such as

Dechloromonas sullium and *Dechloromonas agitata* are known to use lactate as electron donor (Achenbach et al., 2001).

Table 6.2. Characteristics of the leachate obtained during 18 days of leaching.

Amount of vegetable waste loaded (Kg)	**1**	**2**
Volume of water used for leaching (L)	2.5	2.5
Leaching duration (h)	48	48
Volume of leachate collected every 48 h (L)	2.3 - 2.5	2.4 - 2.5
pH	4.6 – 6.9	4.5 – 6.5
VFA (m.eq/L of CH_3COOH)	6 – 14	9-17
Alkalinity (m.eq/L HCO_3^-)	2 – 12	3-16
TDS (mg/L)	120 - 900	250 – 1300
TSS (mg/L)	3 – 8	3 – 15
TCOD (mg/L)	125 - 2500	430– 3600
SCOD (mg/L)	50 - 2300	340 - 3500
TKN (mg/L)	40 - 240	70 - 320
TP (mg/L)	5 – 28	4- 39
Ammonia (NH_3-N mg/L)	4- 10	6 - 14
Nitrate (NO_3^- - N mg/L)	4 – 6	5 -9
Nitrite (NO_2^- - N mg/L)	0.08 – 0.2	0.09 – 0.4
Orthophosphate (PO_4^{3-} -P mg/L)	1.2 – 12.7	2.5 – 15.9
Sulfide (S^{2-}mg/L)	2.2 – 5	3.2 – 8
Total Plate Count (CFU/mL)	10^7 - 10^8	10^7 - 10^9

Perlmutter et.al. have studied the ClO_4^- degradation using fruit juice wastes in a CSTR. A decreased ClO_4^- reduction rate was observed in this study, due to the fermentation of the fruit juice waste(Perlmutter et al., 2001). The VFA accumulation in an anaerobic system can

eventually lead to acidification and can negatively affect the growth of PRM (He et al., 2019). In the present study, at the initial stage, we used 5 liters of well water for leaching 2 kg of waste continuously for 4 days. Even though it produced leachate with a high concentration of COD (4400 mg/L), the pH was <4.5. The ORP of the leachate was also in the range of -230 to -300 mV which promoted sulfate reduction which was evident by the smell of H_2S. When the pH was adjusted using 1M NaOH, the precipitation of ferrous sulfide (black precipitate) occurred as reported in a previous study by Nielson et. al. (Nielsen et al., 2005). But the use of fresh well water for leaching every two days minimized the drop in pH as well as production of H2S (no sulphide smell) (Figure 6.8) as well as production of H_2S. The two-stage process helped in minimizing the impact of pH variation and there was no need for adjusting the pH with alkali. The controlled delivery of the leachate into the ABB also prevented pH fluctuations. Wu et.al studied the use of different substrates such as acetate, lactate, citric acid and molasses as electron donors for ClO_4^- reduction, and have observed that using molasses it took seven days to reduce 200 mg/L of ClO_4^- to below detection limit whereas for citric acid, acetate and lactate it took only 5 days (Wu et al., 2001).

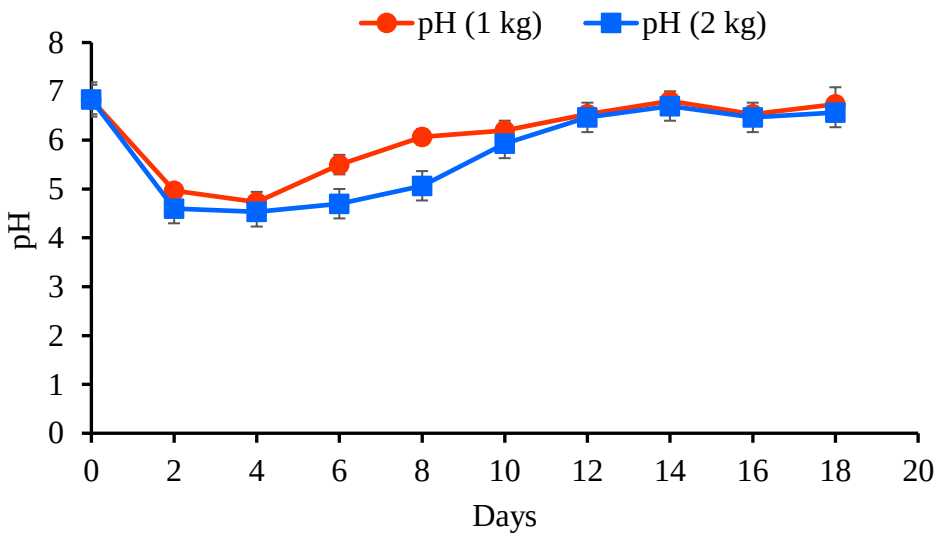

Figure 6.8. pH profile of the leachate during 18 days of leaching. Every 48 hours fresh well water was used for leaching process

The leachate from 4 to 8 days of digestion was found suitable as a substrate for ClO_4^- reduction because it contained a sufficient concentration of VFA, nitrogen, phosphate, and other nutrients (Table 6.3) for sustaining bacterial activity. Even though the leaching experiment was

conducted for 18 days the leachate after the 8th day of leaching was not used for further experiments due to low concentration of SCOD and other nutrients present in it.

Table 6.3. Characteristics of the leachate used as substrate.

Parameter	**Concentration in Leachate**
TCOD (mg/L)	1500 – 3600
SCOD (mg/L)	1200 – 3500
VFA (m.eq/L of CH_3COOH)	14 – 17
TKN (mg/L)	180 – 320
TP (mg/L)	17 – 39

ii) Performance of the Anaerobic Bio-barrier system

Effect of influent SCOD and HRT in ClO_4^- reduction

The results of the effect of influent SCOD concentration on ClO_4^- reduction are presented in Figure 6.9. At 40 mg/L SCOD, 98.2 % of ClO_4^- reduction was achieved from an initial concentration of 10 mg/L at an HRT of 6 hours. However, when the SCOD concentration was increased from 40 to 60 and then to 80 mg/L > 99% reduction in ClO_4^- was observed (Figure 6.9). When the inlet SCOD was >40 mg/L the treated effluent from the bio-barrier had high residual COD (>20 mg/L). Therefore, an influent SCOD of 40 mg/L of O_2 was maintained for further experiments. At an influent ClO_4^- concentration of 10 mg/L and SCOD concentration of 40 mg/L, the percentage of ClO_4^- reduction was inversely proportional to the HRT. When the HRT was 6.3 h the ClO_4^- reduction was 99.3%. However, when the HRT was decreased to 6.15 and 5.7 h the ClO_4^- reduction declined to 98.2% and 94.2%, respectively. The effect of HRT on ClO_4^- removal is presented in Figure 6.10.

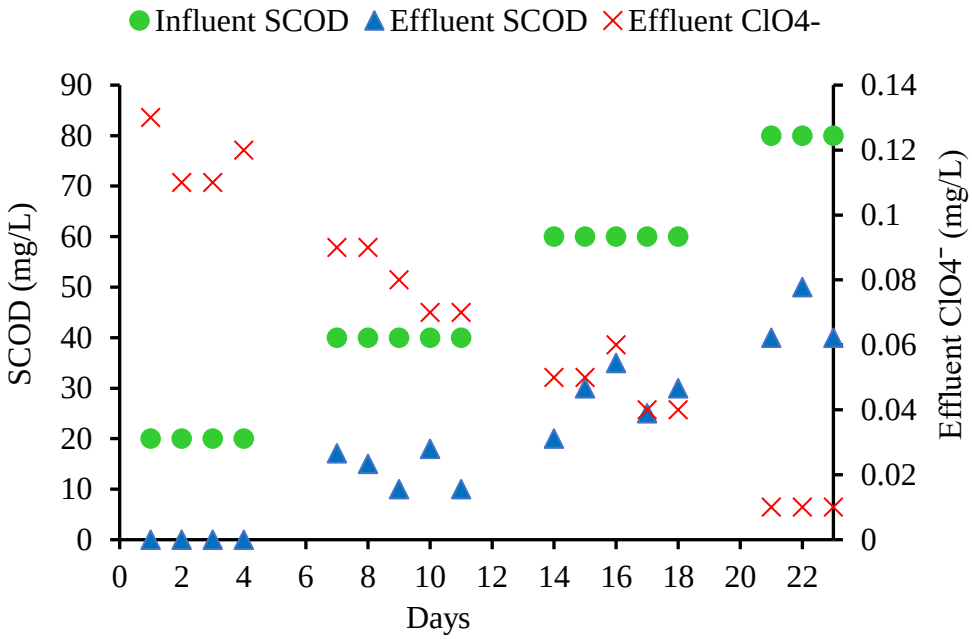

Figure 6.9. The residual perchlorate (ClO_4^-) concentration and soluble chemical oxygen demand (SCOD) in the bio-barrier effluent at different influent SCOD concentration.

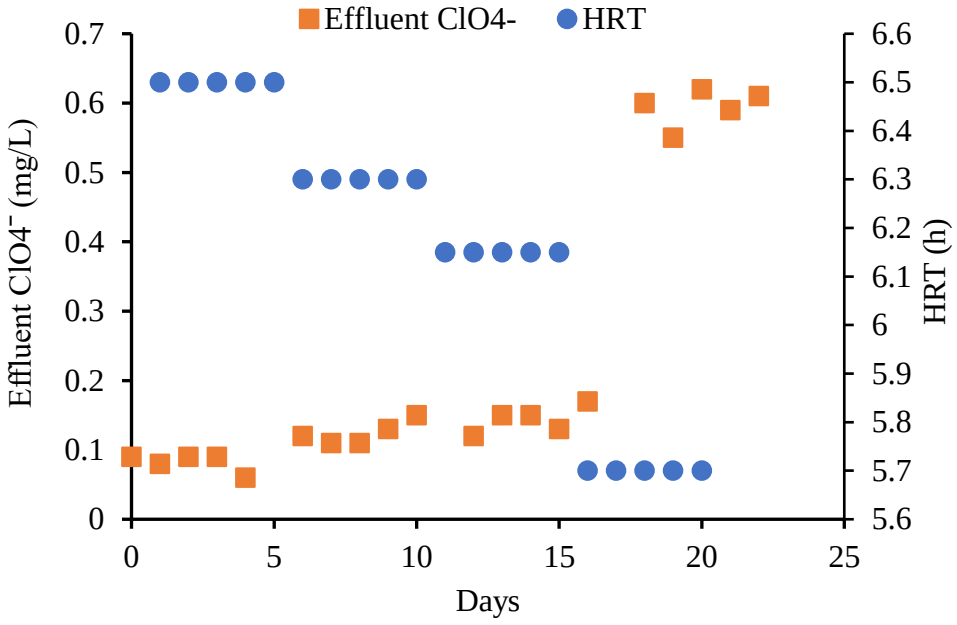

Figure 6.10. The residual perchlorate (ClO_4^-) concentration in the bio-barrier effluent at different hydraulic retention time (HRT)

The effect of HRT on ClO_4^- reduction was reported previously for Packed Bed Reactors. Min et. al. studied ClO_4^- reduction (at 75 μg/L) in a plastic bed reactor at two different HRT of 56 and 28 min and found that, for lower HRT more residual ClO_4^- in the treated water (Min et al., 2004). Similarly, Giblin et.al. observed at influent ClO_4^- concentration of 8 mg/L, for an HRT of 2 h the effluent ClO_4^- concentration was <0.4 mg/L where as a decreased HRT of 0.8 h resulted in effluent concentration of 0.8 mg/L in a bench scale packed bed reactor (Giblin et al., 2002). In a study conducted by Sahu et.al. higher concentration of ClO_4^- (5-8 mg/L) was reduced to <0.5 mg/L at an HRT of 13.5 h whereas low concentration of ClO_4^- (60 -120 μg/L) was reduced to <4 μg/L at an HRT of 7.5 h in an autotrophic Sulphur packed bed reactor (Sahu et al., 2009).

In the present study, during continuous operation of the bio-barrier at 6.15 h of HRT and 40 mg/L SCOD, ~98.5% (0.15±0.05 mg/L) of ClO_4^- reduction was achieved from an initial concentration of 10 mg/L. The volumetric ClO_4^- loading at this stage was 39 mg/L/day and the effluent SCOD concentration was below 20 mg/L. The bio-barrier was operated for 30 days and the physico-chemical characteristics of mixed influent and effluent during this operational period are presented in Table 6.4.

Upadhyaya et.al. have studied the use of Carbohydrate Based Electron Donors (CBED) for ClO_4^- and NO_3^- removal in fixed and fluidized bed reactors. They have used 150-250 mg/L of COD equivalent of CBED for the reduction of 25 mg/L of nitrate and 200 μg/L of ClO_4^-. They have reported complete removal of nitrate and the presence of 3 and 6 μg/L of residual ClO_4^- in fixed and fluidized bed reactors, respectively. The empty bed contact time was 80.5 minutes. Even though HRT was low, they have observed sulphate reduction and presence of acetic acid and residual COD (35-60 mg/L) in the effluent (fermentation product of excess CBED) as a consequence of high influent COD concentration >125 mg/L (Upadhyaya et al., 2015).

In a pilot scale semi-passive bio-barrier for field demonstration used lactate as electron donor. For an average ClO_4^- concentration of 171 mg/L (8 – 430 mg/L), 380 mg/L of lactate (1:2.2) was supplied. The groundwater pH was 4.3, and hence they had to supply bicarbonate buffer for elevating the pH to 7. A residual lactate concentration <0.5 mg/L was observed after 111 days of operation with complete reduction of ClO_4^- to <5 μg/L. They have also noticed excess requirement of electron donors due to the presence of nitrate and dissolved oxygen and sulphide was detected by odour from certain injection wells with a negligible reduction of sulphate (Hatzinger et al., 2006).

Table 6.4. Physico-chemical characteristics of influent and effluent at optimized conditions. The data presented is the average of observed values during 30 days of operation.

Parameters	**Mixed influent**	**Effluent**
Perchlorate (ClO_4^-, mg/L)	10 ± 0.5	0.15 ± 0.05
pH	6.74 ± 0.64	6.70 ± 0.64
TDS (mg/L)	145±63	165±59
DO (mg/L)	1.8±0.8	1.4±0.5
ORP (mg/L)	90±59	-120±14
TCOD (mg/L)	75±21	50±18
SCOD (mg/L)	30±10	20±10
TKN (mg/L)	27±2	25±5
Nitrate (mg/L)	0.6±0.2	BDL
Nitrite (mg/L)	0.07 ± 0.01	0.09±0.01
Ammonia (mg/L)	0.6±0.4	5.5 ± 0.5
TP (mg/L)	2.5±0.5	1.5±0.5
Phosphate (mg/L)	1.5±0.5	0.5 ± 0.2
Sulphide (mg/L)	BDL	BDL
Total Plate Count (CFU/mL)	10^3 - 10^5	10^2 - 10^4

The use of organic waste leachate did not cause any drastic decline in pH in the filter bed. The mixed influent pH was 6.74 ± 0.64 and that of effluent was 6.79 ± 0.64 (Figure 6.11). The optimum pH range reported for ClO_4^- removal is in the range of 6.2 – 7.5 (Wallace et al., 1998). Hence there was no need for external pH correction at any stage. ORP is indicative of the anaerobic status of the system and the presence of molecular O_2 can cause a retarded ClO_4^-

removal. An ORP less than -110 mV or less is required for complete ClO_4^- reduction(Attaway & Smith, 1993). The average ORP of the effluent was -120±14 mV (Figure 6.11). In this study the feed water contained an influent DO > 5 mg/L and the mixed influent DO was 1.8±0.8 mg/L. The effluent DO was 1.4 mg/L, and it is because the outlet chamber was open and was in contact with the atmospheric oxygen. Sustained ClO_4^- reduction was observed in the ABB using organic waste leachate as the sole carbon and nutrient source. By increasing, HRT and SCOD concentration >99 % removal of ClO_4^- can be achieved.

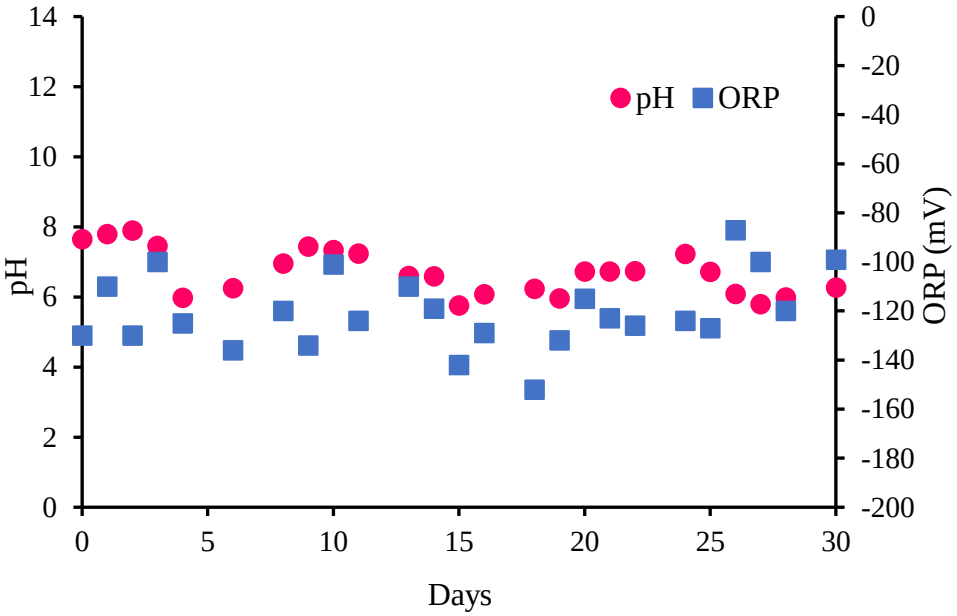

Figure 6.11. pH and ORP profile of the ABB effluent during 30 days of operation at an HRT of 6.15h. Each data point represents daily analysis results.

Okeke et.al. have studied the use of starch and potato peels for ClO_4^- degradation in batch studies. They have suggested the use of a two-stage reactor with cell-free amylolytic enzymes in one reactor for starch hydrolysis and sequential ClO_4^- degradation using the carbon-rich effluent from a hydrolytic reactor to minimize the competition between PRMs and hydrolytic bacteria. (Okeke & Frankenberger Jr, 2005).

Unlike the traditional passive bio-barriers using mulch, sawdust, pecan shells, compost etc. the biofilm support matrix used in this study is unique, structured natural fiber with high lignin (>45%) (Yao et al., 2011). This will minimize the chance of excess carbon and nutrient release from the trench filling material. Coir fibre has a high specific surface area and wetting ability,

which is suitable for microorganism's adhesion and biofilm formation. In south Asian countries, biofilm attached coconut fibre treatment system is a popular and reliable method for wastewater treatment process because of its high durability (low biodegradability due to high lignin content) and local availability (Dharmarathne et al., 2013). The coir-fiber based filter bed is highly durable as evident by the appearance even after several months of operation. Due to the high permeability of the filter media, the bio-barrier was free form internal clogging and channeling. In India, Kerala is the main producer and supplier of coir fiber to the world market (Mathai, 2005). Hence the use of locally available low-cost coir fiber will considerably minimize the cost of bio-barrier.

The two-stage bio-barrier process for ClO_4^- degradation in this study is a novel concept. The semi-passive approach without recirculation will minimize the operational and maintenance cost by avoiding the need for active recirculation through injection extraction wells. Like in passive injected type bio-barriers, the natural groundwater flow will help in the mixing of the substrate in the bio-barrier proposed in this study. By providing an inert filter media as permeable barrier and biofilm matrix there is no organic contamination due to the degradation of trench filling material. The contaminated plume mixes with adequate level of substrate and is allowed to flow through the permeable filter media via natural hydraulic gradient where ClO_4^- degradation takes place. The electron donor supply can be controlled by monitoring the groundwater flowrate, ClO_4^- concentration and the concentration of co-contaminants. This helps to minimize too much residual organics in the treated water. The use of heterogenous vegetable waste derived leachate as low-cost soluble amendment also will bring down the operational cost of bioremediation considerably.

6.3.2. Screening of ligno-cellulosic biomass for perchlorate degradation.

i) Characteristics of selected ligno-cellulosic biomass

The characteristics of selected ligno cellulosic biomass in terms of total solids, moisture content and total organic carbon is presented in Table 6.5. Among these the organic carbon was in the range of 32 – 39%. However, the TS level varied from 34 (peanut shell) to 88% (rice husk)

Table 6.5. Characteristics of lignocellulosic biomasses selected for the study

Sample	Moisture (%)	Total Organic Carbon (%)	Total Solids (%)
Sugarcane Bagasse	61.90	39.79	38.10
Rice husk	11.99	32.57	88.01
Rice straw	15.34	32.21	84.66
Peanut shell	65.72	38.81	34.28

Static leaching test

The results of static leaching test conducted for rice straw, rice husk, sugarcane bagasse and peanut shell is presented in Table 6.6.

Table 6.6. The pH, Oxidation Reduction Potential (ORP) and Soluble Chemical Oxygen Demand (SCOD) of the leachate produced from the lignocellulosic biomasses during static leaching test.

Substrate	pH	ORP (mV)	SCOD (mg/L)
Rice Straw	6.62	-230	46
Bagasse	4.32	-340	310
Rice husk	6.7	-180	9
Peanut shell	6.70	-210	14

The SCOD concentration of the rice straw leachate was optimum (46 mg/L per gram) compared to other substrates. Sugarcane bagasse produced a higher SCOD than required (310 mg/L per gram), while peanut shell (9 mg/L per gram) and rice husk (14 mg/L per gram) produced leachate with low concentration of SCOD in 48 hours. Based on the results of static leaching test rice straw was chosen as potential solid slow carbon releasing substrate for ClO_4^- reduction.

ii) Perchlorate reduction with rice straw as carbon source- Batch scale studies

Based on the results of static leaching test, rice straw was evaluated as substrate for ClO_4^- removal in batch cultures. Figure 6.12 presents the time course of ClO_4^- reduction in bottles with added rice straw at different test conditions. There was no ClO_4^- reduction observed in bottles without the straw as well as sterilized straw. The adsorption of ClO_4^- by the rice straw was also not observed. Perchlorate removal was 59%, 90% and 95% in bottles with non-sterile straw, sterile straw inoculated with PRM consortium, and non-sterile straw inoculated with PRM consortium. The uninoculated bottles showed slight decline in ClO_4^- level after an initial lag of 4 days. This could be due to the low numbers of natural ClO_4^- reducing bacteria present in the rice straw. Even then ClO_4^- degradation was observed, and which is suggestive of the ubiquitous presence of ClO_4^- degraders in various environments. As mentioned earlier, ClO_4^- reducing microbes are ubiquitous and they have been isolated from pristine environments (Coates et al., 1999; Okeke & Frankenberger, 2005; Bardiya & Bae, 2011). Perchlorate degradation was rapid in bottles with non- sterile straw inoculated with PRM consortia. This may be due to the presence of cellulolytic bacteria in the non-sterile straw which helps in the breakdown of cellulose to simple organic carbon residues which can be easily utilized by the PRMs.

These observations were in concurrence with the ClO_4^- degradation studies conducted by Okeke and Frankenberger, using potato peels as carbon source. They have also observed a rapid degradation in samples with non-sterile potato peels inoculated with ClO_4^- degrading Dechloromonas sp. perc1ace and a degradation with lag phase in samples with only non-sterile potato peels and no ClO_4^- reducing bacteria. They have also added enriched amylolytic cultures for the easy degradation of starch from potato as well as shown the presence of natural amylolytic microbial population on potato peels (Okeke & Frankenberger, 2005). In this study non-sterile rice straw was added to enrich the natural cellulolytic microbial population that can degrade cellulose.

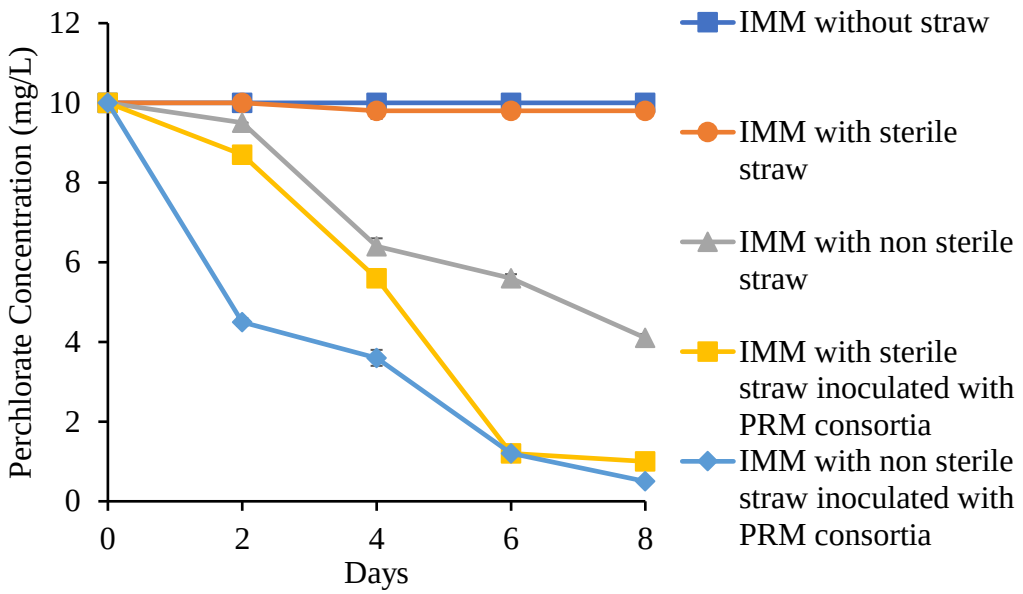

Figure 6.12. Time course of perchlorate reduction in batch cultures with rice straw as substrate at different test conditions

iii) Perchlorate degradation using rice straw as substrate in the bench scale bio-barrier unit:

The bench scale anerobic bio-barrier system was packed with rice straw as sole substrate for ClO_4^- reduction and was operated for a period of 33 days. During the period of operation, the system could treat 120 L of well water with a ClO_4^- concentration of 40 mg/L (4.8 g of ClO_4^-) to less than 0.58 mg/L (98.75% reduction) using rice straw as sole carbon and nutrient source for microbial activity. The system was operated at an HRT of 31 days. The effluent started flowing into the OC of the system on the 3rd day. The TDS, TCOD and SCOD of the effluent were high during initial days and started declining from day 5 onwards. The performance of rice straw bio-barrier during 33 days of operation is presented in Figure 6. 13. The change in TDS, TCOD, and SCOD of the effluent are presented in Figure 6.14. The overall characteristics of the rice straw bio-barrier effluent is given in Table 6.7.

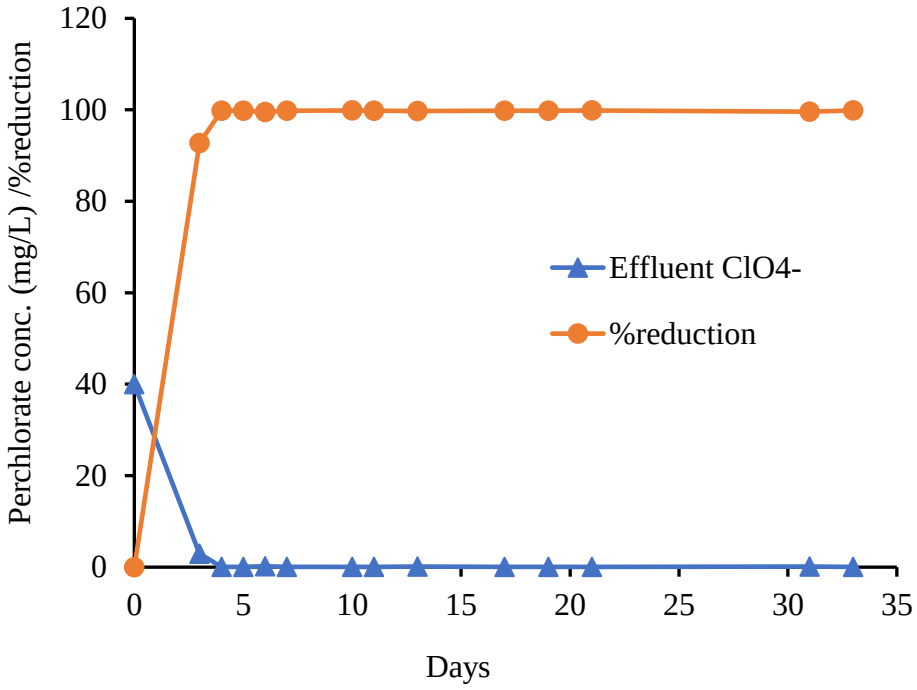

Figure 6.13. The performance of the rice straw-based bio-barrier system in terms of effluent perchlorate concentration and percentage reduction of ClO_4^-. The influent perchlorate concentration was 40 mg/L.

The ORP of the bio-barrier unit attained a negative potential from day 3 and remained negative until day 29 and became positive from day 30 onwards (Figure 6.14). A decline in effluent pH was observed from day 20 and the DO concentration in the effluent was less than 1 mg/L until day 29. The increase in DO concentration is in concurrence with an increase in ORP observed from day 29. This may be due to the decline in sufficient dissolved organic carbon that is released form the rice straw. Subsequent refilling of the barrier unit with enough rice straw will be necessary for the continuous supply of organic carbon. The change in DO and pH is presented in Figure 6.15.

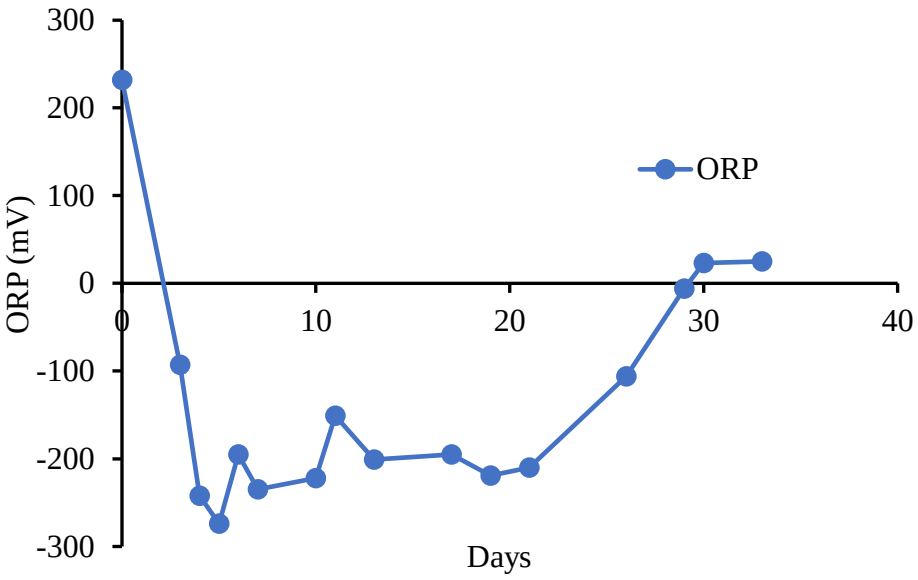

Figure 6.14. ORP profile of the rice straw bio-barrier during 33 days of operation

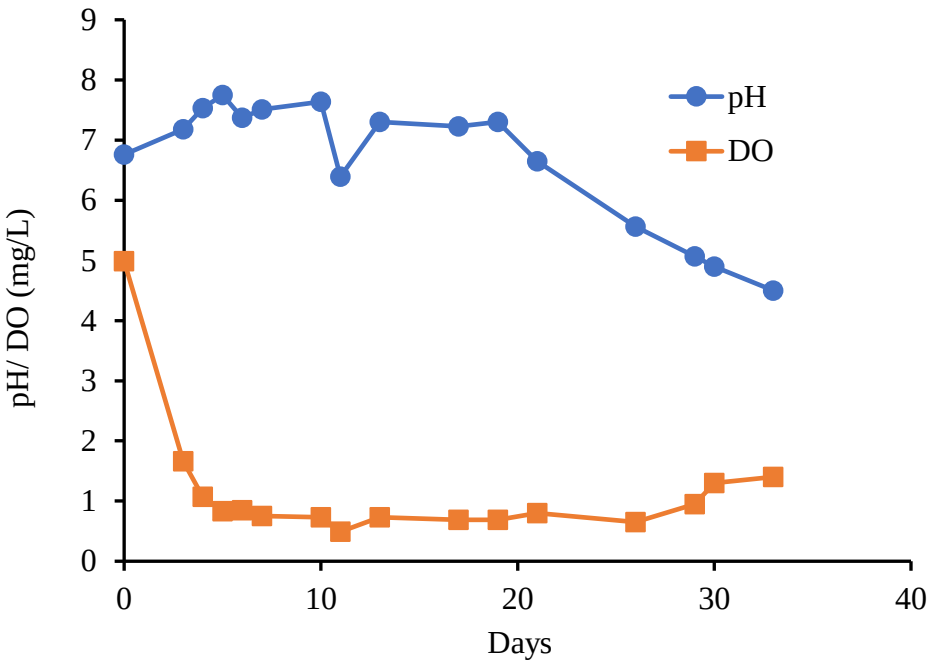

Figure 6.15. Change in pH and DO of the rice straw bio-barrier during 33 days of operation

The treated water from the bio-barrier was dark in colour possibly due to the presence of phenolic compounds released from the rice straw during fermentation. The total phenol concentration of the pooled effluent was 638 mg/L. A similar kind of observation was reported in case of denitrification using wheat straw as solid carbon source in bench scale bio-barrier

studies. The problem of excess COD generation during initial days and the dark coloration of the effluent can be tackled by installing trickling sand filters or activated carbon as post treatment units (Soares & Abeliovich, 1998; Della Rocca et al., 2005). The use of cotton as an alternative carbon source can also avoid the generation of dark colored effluent as in the case of nitrate reducing bio-barrier study by Della Rocca et.al. (Della Rocca et al., 2005)

Table 6.7. Overall characteristics of the rice straw bio-barrier influent and the pooled effluent.

Parameters	**Influent**	**Pooled effluent**
Volume of water (L)	120	110
Perchlorate (mg/L)	40	0.58
Amount of perchlorate (g)	4.8	0.063
pH	7.19	7.37
DO (mg/L)	4.24	2.41
TDS (mg/L)	96	150
TSS (mg/L)	NIL	30
ORP (mV)	232	202
TCOD (mg/L of O_2)	BDL	80
SCOD (mg/L of O_2)	BDL	20
TP (mg/L)	0.2	1.38
Phosphate (mg/L)	BDL	0.53
TKN (mg/L)	24	28
Nitrate+ Nitrite (mg/L)	BDL	0.54
Ammonia (mg/L)	BDL	4
Sulphide (mg/L)	BDL	BDL
Total phenol mg/L	BDL	658

Total plate count (CFU/mL)	Nil	10^4

Even though there are reports on the use of sawdust, mulch etc. as solid carbon source in ClO_4^- treating bio-barriers in full scale units there are no reports addressing the issues such as excess carbon release and the presence of phenols in the effluent (Hine & Smith, 2009; Morris, 2009). There are several reports on the use of solid carbon sources for denitrification, but there are no such reports on the use of solid carbon sources for ClO_4^- remediation (Volokita et al., 1996; Della Rocca et al., 2005). Nitrate and perchlorate are co-occurring toxic oxy anions with some similarity and hence any treatment for any one of the contaminants may find application for the other contaminant also.

Conclusions

This study focused on the development and testing at bench scale the proof of concept of a low-cost permeable reactive bio-barrier system for in-situ remediation of ClO_4^- contaminated plumes and running water bodies (drains). The Anaerobic Bio-barrier System developed could reduce 98.5% of ClO_4^- (from an initial concentration of 10 mg/L) using a leachate derived from the anaerobic digestion of vegetable waste. The leachate functioned as the as sole carbon and electron donor as well as nutrient source for the bioprocess. The experimental unit could treat a volumetric ClO_4^- loading rate of 39 mg/L/day at a lower HRT of 6.15 hours. The novelty components of the study were (i) the use of vegetable waste derived leachate as sole substrate for the sustained microbial activity in the treatment unit, and (ii) the use of natural fibre-based biofilm support matrix. These factors will minimize the installation and maintenance cost of remediation system to a greater extend. Unlike the traditional PRBs for in-situ groundwater treatment, the unique and compact design of the bio-barrier unit makes it suitable for installation across ClO_4^- contaminated streams (point sources) around bulk handling locations. Moreover, the controlled release of electron donor can avoid excess organic contamination or substrate limited conditions. The installation of the present system can prevent the spreading of similar contaminant such as nitrate, chlorate etc. Even though the preliminary studies with agro-residues such as rice straw as sole substrate for ClO_4^- removal in bio-barrier are encouraging more detailed study is required for further implementation of such substrates as carbon source for ClO_4^- degradation.

Chapter 7

General Discussion and Future Perspectives

The present study involves the environmental surveillance of ClO_4^-, an endocrine-disrupting persistent, micro-pollutant at two previously contaminated sites in Kerala, India. Furthermore, the development of sustainable technologies for the remediation of ClO_4^- contaminated water as well as soil were also covered. Perchlorate is considered a toxic oxyanion because it can interfere with iodine uptake by the thyroid gland causing hypothyroidism and related health disorders in humans (Lisco et. al., 2020). Perchlorate contaminated drinking water and foodstuffs are the major ways of human exposure to ClO_4^- (Gullick et al., 2001; Smith et al., 2004; Steinmaus, 2016; Calderón et al., 2020). For reducing the risks associated with ClO_4^- toxicity regulatory standards are set for by many countries (USEPA 2008, Ministry of Health, Korea, 2010; WQA 2016; Health Canada 2020). Moreover, continuous monitoring of ClO_4^- in drinking water, foodstuffs, etc. are being practiced in several countries (Alomirah et al., 2016). But in India, there are no environmental regulations for ClO_4^- in drinking water as well as no discharge standard specified for this toxicant.

Since 2009, the research group from CSIR-NIIST started monitoring ClO_4^- contamination in India (Anupama et al., 2012). The findings that relatively higher levels of ClO_4^- at places like Keezhmad in Ernakulam and Thumpa in Thiruvananthapuram were the basis of the study presented in this thesis (Anupama et al., 2015, 2017). The present study revealed the persistence of this toxic contaminant in community water resources at the contaminated region even after 9 years of its first detection, which is a matter of great concern. Even though there is an overall decline observed in ClO_4^- levels near APEP, the present levels of ClO_4^- in many well water samples are still higher than the international guidelines. The USEPA recommended level of ClO_4^- in drinking water is 15 μg/L (2019). As mentioned earlier the ClO_4^- is chemically inert and therefore the natural attenuation of this contaminant is quite difficult. The limitation of natural degradation of ClO_4^- was evident from the laboratory experiment results with enrichment culture during this study. It may take several years for the chemical to reach the permissible levels. But during the course of time, it will pollute more and more water resources and the surface infiltration as observed in this study will spread to more areas, increasing the public health threat.

Even though severe groundwater contamination due to ClO_4^- was reported from India, studies on its remediation approaches from India are very limited. Microbial reduction of ClO_4^- into innocuous chloride and oxygen is reported to be the most economic and environmentally friendly approach for decontaminating ClO_4^- laden water, soil, and industrial effluents (Srinivasan & Sorial, 2009; Ma et al., 2016). As part of this research study, we have developed

three indigenous and low-cost remediation technologies for the treatment of ClO_4^- contaminated water and soil. The ClO_4^- remediation technologies reported in this study are the first of their kind in India. The input from the previous biodegradation studies conducted in batch and bench-scale bioreactor units were adapted for the development of these technologies.

The hybrid Bio-MF RO system tested on a pilot-scale unit for the remediation of ClO_4^- contaminated well water was unique in several aspects. A primary microbial process, followed by microfiltration and reverse osmosis units to produce potable quality water from ClO_4^- contaminated well water was never reported in the literature. Already reported studies on hybrid ClO_4^- treatment processes either use biodegradation as a secondary treatment step for decontaminating ClO_4^- containing concentrated rejects from membrane filtration units or for the regeneration of ion exchange resins or regenerant brine solution. Physical processes are usually preferred for the remediation of ClO_4^- contaminated drinking water sources. But, for higher ClO_4^- levels (as observed in this study) physical processes are not effective and hence microbial reduction was adopted initially for bringing down the high ClO_4^- concentration so that the physical processes can remove the residual to potable level. The biofilm support medium used in the bioreactor (AFBR) was charcoal and it was different from the support media already reported such as sand, celite, GAC, pall rings, etc (Logan & LaPoint, 2002; Losi et al., 2002; Min et al., 2004). Channeling and clogging are the major drawbacks of packed bed reactors reported (Sutton, 2006) and in the present study such problems were never observed in the charcoal-packed AFBR. The AFBR was inoculated with ClO_4^- reducing bacterium *Serratia* sp. which was isolated in our laboratory during previous studies and the MF unit was also fabricated within CSIR-NIIST. The system is now being scaled up to produce 2000 L of potable water per day and laboratory validation of the system is going on prior to its installation and field demonstration for treating one of the contaminated community wells in Kulakkad colony, Aluva. This is anticipated to provide a solution for the water scarcity issues of the people of Keezhmad panchayath, where APEP is situated.

Perchlorate contamination of soil followed by infiltration and surface runoff is the major contributor to groundwater and surface water contamination at places where the chemical is manufactured or consumed in large quantities (Smith et al., 2004; Tan et al., 2004). Ex-situ treatment methods such as bio-stimulation and bioaugmentation (anaerobic soil composting by providing substrates and conditions for ClO_4^- reduction) of the contaminated soil by excavating large quantities of soil are the most common methods reported. Similarly, in-situ remediation methods are also practiced for remediating the soil (ITRC 2005, 2008). Compared with the

existing approaches reported, the soil remediation approach developed in this study was novel and has many advantages in terms of less time and operational costs involved. The ex-situ soil washing and regeneration of wash water through biotreatment was the new concept established. The treated wash water can be for subsequent soil washing. The whole process can be completed within a day, while the already reported methods are known to take days or months for the same quantity of ClO_4^- contaminated soil (ITRC 2005, 2008). In India, we have several ClO_4^- handling industries, and this soil treatment approach will find a direct application at these sites. This will prevent the infiltration of ClO_4^- into groundwater resources and thereby minimize the chance of widespread contamination.

The low-cost permeable reactive bio-barrier system developed in this study can be installed around the handling sites or across the contaminated groundwater plume or drains and that will prevent the spreading of contamination from point sources. The use of low-cost substrates such as organic waste leachate will bring down the operational and maintenance cost of the bio-barrier system. The controlled release of the organic substrate as practiced in this study will minimize the chances of secondary pollution and biofouling as observed in the case of active bio-barrier systems. The operational costs can also be highly reduced because there is no need for active recirculation of groundwater and substrate for its mixing. The natural fiber-based biofilter media used in the treatment bed is a low-cost material and more environment-friendly. The lignin-rich biofilter media will minimize the chance of secondary organic contamination (organic leach out) which is often observed in the case of traditional solid substrate-filled trench bio-walls (Zhao et al., 2021). The design of the bio-barrier system was such that it can be installed across ClO_4^- contaminated running water bodies (drains) as well as underground trench bio-walls also. The release of excess dissolved organics and a higher concentration of phenols was observed when lignocellulosic biomasses were tested as solid carbon sources and trench filling biofilter media in the bio-barrier. Even though there reports on the use of lignocellulosic biomasses as the substrate for denitrification, important aspects such as the release of phenolic compounds and excess residual organics were not covered in such studies. Further, research in this area can consider the development of poly hydroxy alkanoate (PHA) based lignocellulosic composites as slow carbon releasing substrates in bio-barriers removing ClO_4^- and similar contaminants.

In brief, the presence of toxic ClO_4^- around major its inventories in Kerala continues to be an environmental as well as public health hazard. Considering the persistent nature of the contaminant as clearly established through this study, there is a requirement for regular

monitoring of ClO_4^- in water sources as well as human consumption products around the areas where it is handled in bulk. Moreover, the surveillance of ClO_4^- needs to be extended to other regions major industries and R&D units, where ClO_4^- salts are handled in bulk. The assessment of ClO_4^- in foods and vegetation and periodic checking of the thyroid gland functioning (TSH, T3, T4, etc.) of people in severely contaminated regions can minimize the risks associated with ClO_4^- toxicity. The intervention of regulatory agencies can help in setting up appropriate directions for the disposal of wastes containing ClO_4^-, discharge standards, and drinking water regulations for ClO_4^- so that its impact on society can be minimized.

The installation of the scalable technologies developed during this study can be taken forward as a sustainable solution for the remediation as well as prevention of contamination. The bio-physical system can be implemented at places like Keezhmad where the contaminated well water can be treated to a potable level and distributed to the local people. This can alleviate the existing burden on the people in the affected region. The soil remediation as well as the in-situ bio-barrier type remediation approach developed in this study will find application for controlling the environmental release (including unintentional, accidental, or fugitive release) through soil contamination from bulk handling sites. This can include industries including clusters (like cracker units) and/or space R&D, defense research units, etc. handling ClO_4^- salts. Even though the microbial remediation approaches developed in this study targets ClO_4^-, these technologies will also find application for similar toxic oxyanions such as chlorate, chlorite, and nitrate.

Chapter 8

Summary and Conclusions

The entire research study covered in this thesis is about Perchlorate (ClO_4^-), a persistent, endocrine-disrupting, micropollutant, and the contamination of which has been reported in India in the recent past. Two major aspects about ClO_4^- are covered in this study, such as monitoring of ClO_4^- levels in community water resources at two previously reported, highly contaminated sites in Kerala. One of the sites was Keezhmad Panchayat, Aluva in Ernakulam district where ISRO-APEP is located. The second site was Thumba region in Thiruvananthapuram district, where VSSC-RPP is located. The aspect of the study was the development and validation (pilot scale or bench scale) of innovative bio-remediation technologies for ClO_4^- contaminated water and soil. Studies in this area are very limited in India, especially on the remediation aspects of ClO_4^-.

As first part of the present study, an assessment of the present status of ClO_4^- concentration in community water sources at previously contaminated sites was done. The possibility of natural attenuation of the contaminant was also studied through detailed analysis of field samples, followed by laboratory experiments under simulated conditions. It was found that even after nine years since the contamination was reported in 2012, the level of ClO_4^- in most of the community wells at both the studied region (also a community pond at site 1) are well above the international guidelines. An enrichment microbial system was developed from a ClO_4^- contaminated well water, and subsequent studies with the enrichment culture indicated ClO_4^- degradation can happen only under favorable conditions of substrate, nutrients and other conditions that are highly limited under natural conditions.

The second part of the study focused mainly on the development of sustainable remediation approaches for ClO_4^- contaminated water and soil. Three innovative bio-remediation approaches were developed and tested in pilot or bench scale in this study. A bio-physical ex-situ remediation process for ClO_4^- contaminated well water treatment, an ex-situ remediation process for soil contaminated with ClO_4^-, and an in-situ remediation process for ClO_4^- contaminated plumes and/or drains.

The bio-physical, ex-situ remediation process developed in this study comprised of an anaerobic fixed-film bioreactor (for primary treatment) inoculated with a ClO_4^- reducing bacterium, *Serratia marcescens* (proprietary culture of CSIR-NIIST, MTCC 5821, Genbank JQ807993) that degrade the toxic ClO_4^- into non-toxic chloride and oxygen. This was followed by a series of Micro-Filtration and Reverse Osmosis units (secondary treatment) for polishing the biotreated water to potable standards. The pilot-scale unit was capable of producing 200 L

of water per day. The fouling associated with the membranes, as well as treatment of rejects were studied in detail, and practical solutions to address these problems, such as backwashing and forward flushing were also demonstrated in this study.

Soil contamination of ClO_4^- at the handling sites has been reported as a potential source of groundwater and surface water contamination. Due to high water solubility of ClO_4^-, surface runoff and infiltration can significantly contribute to ClO_4^- in the water resources around. The innovative ex-situ soil remediation approach developed in this study includes washing of the contaminated soil in a confined system, followed by bio-treatment of the ClO_4^- containing wash water for repeated soil washing. This novel concept was tested in a pilot-scale unit. The processing unit consisted of a soil washing unit capable of handling 670 Kg of soil per batch, and a fixed-film bioreactor (200 L) for the treatment and regeneration of the wash water. The whole soil remediation process could be completed within 16 hours while already reported processes for ClO_4^- contaminated soil treatment takes several days to years.

The in-situ remediation approach developed was mainly targeted for restricting the mobility of ClO_4^- contaminated groundwater (plumes) or contaminated streams, drains, canals, etc. A low-cost Permeable Reactive Bio-barrier was developed and tested in a bench-scale unit. The major highlights here were the application of vegetable waste-derived, organic leachate as substrate (carbon, nutrient, and electron donor) for the ClO_4^- reducing microbial activity, and the application of more environmentally benign natural fiber-based biofilm support media. The experimental unit could treat a ClO_4^- loading rate of 39 mg/L/day at a lower HRT of 6.15 hours. Moreover, different agro-residue based biomass were tested as solid substrates for ClO_4^- remediation in the bio-barrier unit. Though the results are promising more studies are required in this field.

In conclusion, the ClO_4^- contamination around the major perchlorate inventories in Kerala, India, is a live problem and a potential threat to public health, and the environment in these places. This underlines the need for continuous monitoring of the level of ClO_4^- especially in drinking water sources in these areas. Moreover, environmental surveillance for ClO_4^- should be extended to other places where ClO_4^- salts are handled in bulk. The scalable remediation approaches developed in this study will find applications in the field, that will provide a sustainable solution for the ClO_4^- contamination and associated environmental and public health problems. The direct beneficiaries of these developments will be local communities in the affected regions, and industries or R&D agencies involved in bulk handling of ClO_4^- salts

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ABSTRACT

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Title of the thesis: Development and Scale-Up of Bioremediation Technology for Perchlorate Contaminated Water and Soil

Perchlorate (ClO_4^-) is a toxic endocrine-disrupting contaminant of water, soil, vegetation, and many human consumption products. In the recent past, severe groundwater contamination of ClO_4^- has been reported from bulk ClO_4^- manufacturing and handling sites in India. This study focuses on the environmental surveillance of ClO_4^- in ground and surface water resources around two such sites such as the Ammonium Perchlorate Experimental Plant (ISRO) in Aluva, Ernakulam, and Rocket Propulsion Plant (VSSC) in Thumba, Thiruvananthapuram, Kerala, India. Moreover, innovative and sustainable remediation approaches for ClO_4^- contaminated water and soil were developed and tested at a pilot scale in this study.

Detailed analysis of water samples from the contaminated sites revealed severe ClO_4^- contamination, which indicates even after nine years (first reported in 2012), it is a live environmental issue and potential threat to public health at these places. To address the ClO_4^- -associated contamination problems, three remediation approaches were developed in this study. A novel ex-situ bio-physical system comprising of a packed bed bioreactor and membrane filtration units for the treatment of ClO_4^- contaminated well water was developed and validated in a pilot-scale unit. The pilot-scale system could generate ~200 L of potable water daily from ClO_4^- contaminated well water. Infiltration and surface runoff from ClO_4^- contaminated topsoil is a major source of ground and surface water contamination. To address this, a novel ex-situ soil remediation system was developed and validated its efficiency in a pilot-scale unit. The treatment unit consists of a soil washing unit (~670 kg soil), followed by the treatment and regeneration of wash water in a bioreactor. The present soil remediation approach could be completed within 16 hours, unlike the existing approaches that will take several months to years. Perchlorate is highly mobile in the environment and spatio-temporal studies have revealed the spreading of the contamination from a concentrated plume to nearby areas. To address the problem, we have developed a low-cost in-situ remediation approach, “permeable reactive bio-barrier”, and it was tested in a bench-scale unit. Low-cost substrates such as organic waste leachate and solid lignocellulosic biomass were used, and the results of this study are highly encouraging for further scale-up and development.

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Russel, J.G., Thulasiraman, V., Nair, R.R., Ravindran, S.C., Hareesh, U.S.., Bhaskaran, K., 2021. A novel bio-physical approach for perchlorate contaminated well water treatment. Environ. Adv. 100058. https://doi.org/https://doi.org/10.1016/j.envadv.2021.100058

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Oral presentation (virtual platform) at 33rd Kerala Science Congress 2021 from January 25 – 30, **2021** organized by KSCSTE. *Perchlorate Remediation in a Bench Scale Bio-Barrier System*. **Jasmin Godwin Russel** and Krishnakumar B.

Poster presentation at international conference on New Horizons in Biotechnology, NHBT 2019, November 20 – 24, **2019**, organized by CSIR-NIIST and Biotech Research Society, India. *An integrated bio-physical process for generating potable water from rocket fuel contaminated groundwater for community distribution*, **Jasmin Godwin Russel**, Venkatesh T., Rothish R. Nair, Sayana C. R., Hareesh U. S., and Krishnakumar B.

Oral presentation at 30th Kerala Science Congress 2018 from January 28 – 30, **2018**, at Government Brennen College, Kannur, organized by KSCSTE. *An integrated bio-physical process for generating potable water from rocket fuel contaminated groundwater for community distribution*, **Jasmin Godwin Russel**, Venkatesh T., Rothish R. Nair, Sayana C. R., Hareesh U. S. and Krishnakumar B. **(Best Paper Award)**

Oral presentation at Kerala Environment Congress 2017 on December 6 – 8, **2017**, at Center for Environment and Development, Thiruvananthapuram, Kerala, organized by Centre for

Environment and Development. *An integrated bio-physical process for generating potable water from rocket fuel contaminated groundwater*, **Jasmin Godwin Russel**, Venkatesh T., Rothish R. Nair, Sayana C. R., Hareesh U. S. and Krishnakumar B.

MANUSCRIPTS UNDER PREPARATION

Russel, J. G., Bhaskaran K. - Perchlorate reduction in a bench-scale bio-barrier system using organic waste-derived leachate as soluble amendment for microbial activity.

OTHER PUBLICATIONS

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A novel bio-physical approach for perchlorate contaminated well water treatment

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ABSTRACT

A novel bio-physical approach for treating well water contaminated with perchlorate (ClO_4^-) at 15 mg/L is reported in this study. In this process, the ClO_4^- was initially treated in an anaerobic fixed-film bioreactor (55 L), followed by a ceramic Micro-Filtration (MF) unit (1.5 μm pore size, 0.12 m^2 surface area) and a Reverse Osmosis (RO) unit (0.38 m^2 surface area) connected in series. The bioreactor inoculated with a ClO_4^- reducing bacterium *Serratia marcescens* (Gen bank no. JQ807993) removed ~97% of the ClO_4^- using acetate as substrate (acetate/ClO_4^- ratio = 4). Subsequently, the MF and RO units removed ClO_4^- to <10 μg/L, Total Dissolved Solids (TDS) to <25 mg/L and Total Chemical Oxygen Demand (TCOD) to below detection Limit. The fouling associated with membranes was controlled (88–100%) through hourly manual backwashing with 2 L pure water at 25 L/h, and 60 psi, and forward flushing with 1 L pure water at 30 L/h and 3–5 psi for MF and RO units, respectively. The rejects and membrane wash water were also treated in the bioreactor, resulted in complete removal of ClO_4^- through this approach. This is the first report where biotreatment is adopted as a pre- and post-treatment to membrane process for removing ClO_4^-, and this will find field application for treating ClO_4^- contaminated ground as well as surface water sources.

Introduction

Perchlorate is an emerging endocrine disrupting contaminant released into the environment mainly due to its extensive use in aerospace programs, defense research and development, and few industries like fireworks, bleaching, etc. (Isobe et al., 2013; Urbansky, 2002; Wolff, 1998). The presence of ClO_4^- has been reported in soil, water, and many human consumption products (Calderón et al., 2020; Liao et al., 2020). Perchlorate contamination of groundwater was reported from many countries including India (Anupama et al., 2012; Kannan et al., 2009). Among different states in India, the highest level of groundwater ClO_4^- contamination (~50 mg/L) was reported from bulk ClO_4^- handling sites in Kerala, India (Nadaraja et al., 2017). Among people exposed to ClO_4^- contaminated (~50 mg/L) drinking water, an elevated thyroid-stimulating hormone (TSH) level was observed in a study in Kerala, India (Keezhmad Project Report, 2015).

Perchlorate is resistant to degradation under natural conditions, and the available technologies in practice for treating ClO_4^- contaminated water can be broadly categorized into physical (ion exchange, membrane process, adsorption, etc.), chemical (catalytic reduction), biological (mostly microbial), and bio-electrochemical (Cecconet et al., 2018; Srinivasan and Sorial, 2009; Stetson et al., 2006; Xie et al., 2018; Ye et al., 2012). Among these, ClO_4^- removal through Ion exchange (IX) and microbial methods have gained much attention due to their techno-economic feasibility (Batista et al., 2002; Kim and Logan, 2001). There are several reports on IX membranes treating ClO_4^- contaminated drinking water (Lehman et al., 2008; Sanyal et al., 2015). Ion exchange membranes with higher affinity for ClO_4^- was reported recently (Li et al., 2020). However, the higher cost of the IX membrane, the non-specificity of the membrane to selectively remove ClO_4^- along with other oxyanions, and regeneration and disposal of the resin are the major challenges. In the microbiological approach, the ClO_4^- is degraded biochemically into non-toxic chloride (Cl^-) and oxygen (O_2) by perchlorate reducing bacteria (PRB), which expresses perchlorate reducing enzymes, perchlorate reductase and chlorite dismutase (Kengen et al., 1999; Rikken et al., 1996). In the bio-catalysis approach, these enzymes

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Table 1.
A comparison of available technologies for treating ClO_4^- contaminated water.

Processes	Limitations	References
Ion Exchange	Generation of concentrated brine, difficulty in disposal/regeneration of spent brine and saturated resin, non-specificity	(Hutchison and Zilles, 2018)
Adsorption using Granular Activated Carbon	Non-selectivity, the requirement for acidic conditions, competitive adsorption by other anions	(Xie et al., 2018)
Membrane filtration Reverse Osmosis Ultrafiltration Nanofiltration	Can treat only low concentrations of perchlorate, membrane fouling, non-specificity, high cost of operation	(Xie et al., 2018; Huq et al., 2007)
Electrodialysis	Concentrated brine needs further treatment	(Urbansky and Schock, 1999)
Metal-based Catalytic Reduction, Electrochemical Reduction	Maintenance of low pH and high pressure, generation of highly reactive species, extreme reaction conditions	(Urbansky, 1998; Yang et al., 2016)
In-situ bioremediation	Repeated addition of electron donors, growth of Non-PRB, the release of metabolic by-products, etc.	(Hatzinger et al., 2006; Stroo et al., 2009)
Ex-situ bioremediation	Cannot be applied in drinking water systems as it contains residual microbial load, metabolic by-products, and unused organics, the problem of public acceptance	(Srinivasan and Sorial, 2009; Ye et al., 2012)

immobilized on matrices were employed for removing ClO_4^- in drinking water (Hutchison and Zilles, 2015). A comparison of prominent methods for treating ClO_4^- is presented in Table 1.

Perchlorate remediation infield practices may require a combination of one or more approaches (hybrid processes) to achieve the desired product water quality, or to regenerate the resin/membrane, or to treat the reject (Srinivasan and Sorial, 2009; Ye et al., 2012). Different combinations of adsorption, ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) have been reported in the past for removing ClO_4^- and similar oxyanions in aqueous systems (Han et al., 2012; Xie et al., 2011; Yoon et al., 2009, 2005). Most of the studies reported cover the treatment of groundwater contaminated with ClO_4^- at sub ppm level. Moreover, microbial processes were adopted in some of these studies mainly for regenerating the resin, or for treating the reject (Giblin et al., 2002; Lin et al., 2007). Ion exchange combined with resin regeneration through chemical reduction or bio-regeneration is reported in few cases (Kim and Choi, 2014; Li et al., 2020; Ebrahimi et al., 2017; Yang et al., 2020). Similarly, combined adsorption and microbial reduction, and integrated ion exchange membrane bioreactor were also reported for removing ClO_4^- in groundwater (Brown et al., 2002; Fox et al., 2016; Song et al., 2015). Unlike the studies reported previously, a novel approach is practiced in this study where ClO_4^- was initially treated in a bioreactor, followed by a series of MF and RO systems for attaining safe limits of ClO_4^- in the treated water. Furthermore, the treatment of open well water highly contaminated with ClO_4^- is targeted in this study. Therefore, this study investigated in pilot-scale, a novel approach for removing field relevant concentration of ClO_4^- in well water. Moreover, practical solutions for the fouling associated with the MF and RO membranes used in the process were also studied. The level of ClO_4^- selected in this study was similar to the average ClO_4^- concentration observed in well water at a contaminated site (Keezhmad, Kerala, India).

Materials and methods

The pilot-scale combined system for treating the contaminated well water consists of three units: (1) an Anaerobic Fixed Film Bioreactor (AFBR) for the bacterial reduction of ClO_4^-, (2) a ceramic Micro Filtration (MF) unit for removing suspended solids and (3) a final Reverse Osmosis (RO) unit for removing residual ClO_4^-, and dissolved solids. The schematic of the entire experimental setup and the photograph of the pilot-scale treatment unit is presented in Fig. 1a. and b, respectively.

The anaerobic fixed film bioreactor (AFBR)

The AFBR was made up of a PVC barrel of 60 L capacity (working volume 55 L). It was packed with charcoal as a biofilm support matrix. In the beginning, the reactor was inoculated with an enrichment culture of the ClO_4^- reducing bacteria *Serratia marcescens* strain (MTCC 5821, Genbank JQ807993). A photograph of *S. marcescens* colonies on agar medium is shown in Supplementary Fig. S1. Even though ClO_4^- contaminated well water at Keezhmad in Ernakulam (India) was targeted in this study, due to practical reasons, the well water for experimental purposes was collected from an open well in the CSIR-NIIST campus, Thiruvananthapuram, India. The characteristics of the well water used are presented in Supplementary Table S1. At the start-up, 110 L of well water supplemented with $KClO_4$ (equal to 25 mg/L level ClO_4^-), and CH_3COONa (equal to 100 mg/L level acetate) and 10 L of bacterial culture at log phase (OD 0.317 at 600 nm, Eppendorf Biophotometer plus, Germany) in Inorganic Mineral Media and Trace Minerals Solution (composition of the mineral media modified to minimize total dissolved solids in the influent is given in Supplementary Table S2) was slowly pumped (~5 L/h) into the AFBR using a peristaltic pump (Watson Marlow, USA). The entire mixture was run in recirculation mode. After four days, when complete degradation of ClO_4^- was observed, the AFBR was switched over to continuous mode.

To achieve an effluent ClO_4^- concentration of < 2 mg/L (treatable limit of RO membrane used in this study) from an initial ClO_4^- concentration of 15 mg/L (average ClO_4^- concentration found in the field) optimization studies were conducted with different ClO_4^- to acetate ratio and hydraulic retention time (HRT). To optimize the ratio of ClO_4^- to acetate, the feed water ClO_4^- (influent) was maintained at 15 mg/L, and four different acetate concentrations such as 30 mg/L, 45 mg/L, 60 mg/L and 75 mg/L were tested in continuous feed mode in the reactor. This corresponds to ClO_4^- to acetate ratio of 1:2, 1:3, 1:4 and 1:5, respectively. To optimize the HRT, the feed water was pumped into the AFBR under three different flow rates (2.5 L/h, 5.5 L/h and 8.5 L/h) to achieve different HRT such as 22 h, 10 h and 6.5 h. Samples were taken daily to assess the performance of the bioreactor in terms of ClO_4^- removal, pH, total dissolved solids (TDS), total suspended solids (TSS), total chemical oxygen demand (TCOD) and microbial load in the AFBR out water. The ORP inside the reactor was also monitored regularly to assess the anaerobic status of the bioreactor. Based on the optimization results, the reactor was operated with 15 mg/L of ClO_4^- and 60 mg/L of acetate constituting a ratio of 1:4 of ClO_4^- to acetate at an HRT of 6.5 h (flow rate of 8.5 L/h). The removal of ClO_4^- at different initial concentrations (20–50 mg/L) was also tested. The optimized ClO_4^-/acetate ratio and HRT were maintained in these studies.

The microfiltration (MF) and reverse osmosis (RO) units

The MF unit used in this study was a ceramic tubular membrane (25 cm long and 34 mm outer diameter) made of alumina. This was obtained from the Ceramic Research Laboratory, Material Science and Technology Division, CSIR-NIIST, Thiruvananthapuram, India. The average pore size of the membrane was 1.5 μm and the total surface area was 0.12 m^2. The MF membrane had a pure water flux of

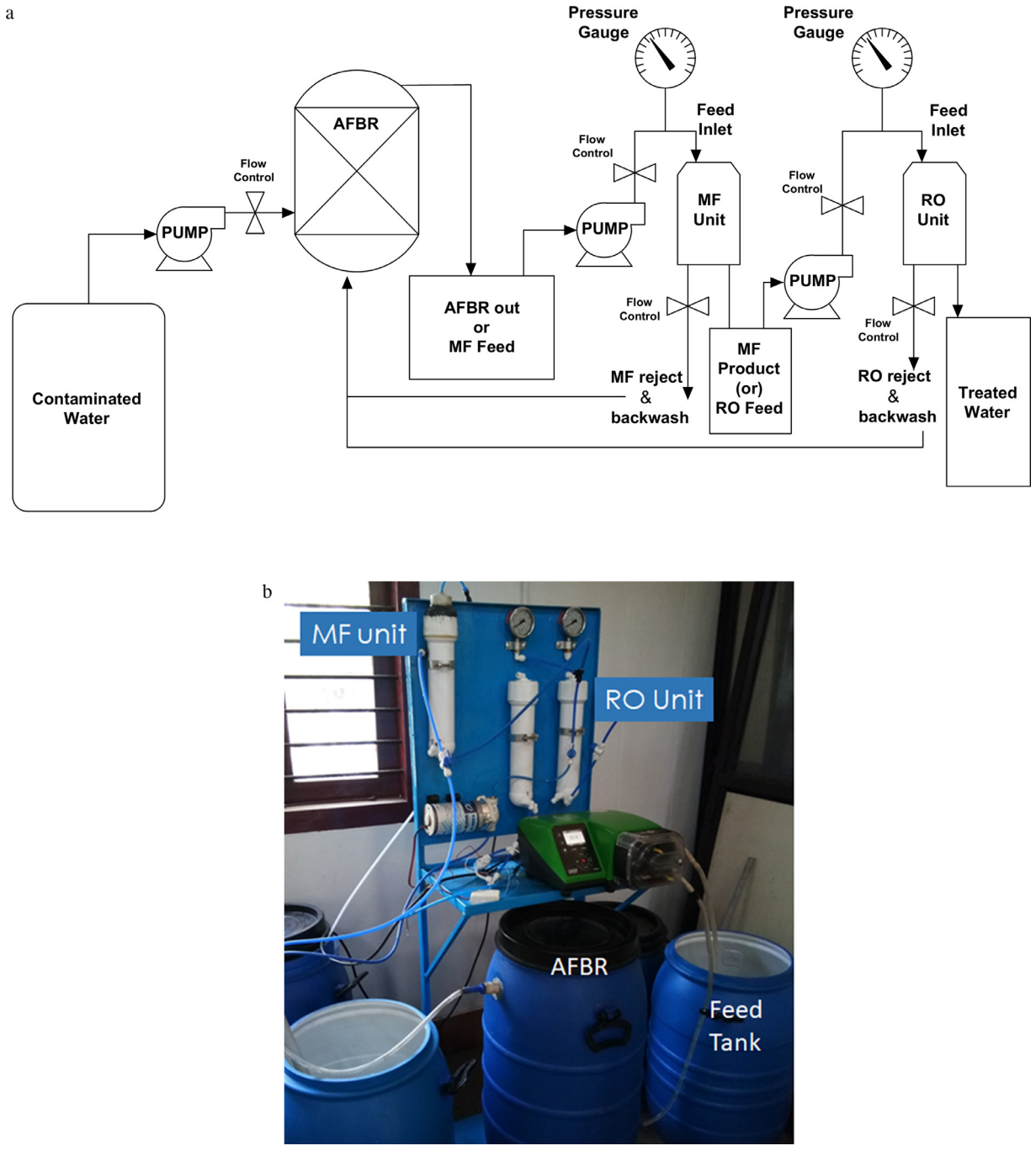

Fig. 1. a. Schematic representation of the combined Bio-MF-RO unit for ClO_4^- treatment. b. Photograph of the pilot-scale combined Bio-MF-RO unit for ClO_4^- treatment.

12.5×10^{-4} m/s at ~15 psi. The AFBR treated water was pumped into an MF unit at a flow rate of 50 L/hr at 50 psi using a diaphragm booster pump (Zuanli, China) for the removal of suspended solids and bacterial cells present in AFBR treated water.

Commercially available RO membrane (polyamide thin film composite) module (Dupont, Film Tec, BW-60-1812-75) was used as the RO unit and the total surface area was 0.38 m^2. The filtered water from the MF unit was pumped into the RO unit using a diaphragm booster pump (Zuanli, China). The flow rate and pressure at this unit were 40 L/h, and 50 psi, respectively. According to the product data sheet of the RO unit, permeate flow rate is 12 L/h at 50 psi for inlet water containing ~250 mg/L of TDS at 25 °C. The ratio of reject to permeate was 7:3. Hence, this condition was chosen in ClO_4^- rejection studies and for treating the AFBR effluent. Perchlorate rejection efficiency of the RO unit was evaluated by varying the inlet ClO_4^- concentration from 1 to 100 mg/L at a feed flow rate of 40 l/h at 50 psi. The pressure and water flow rates were continuously monitored in both the MF and RO units. Samples of product water were taken daily from both the units for the analysis of ClO_4^-, TDS and viable bacterial cell count.

The membrane flux in both MF and RO units was calculated using the general formula:

$$Flux = \frac{volumetric\ flowrate}{surface\ area}\ m/s \quad (1)$$

Membrane fouling, and treatment of wash water and reject

In this study, the fouling associated with MF and RO units was controlled by manual backwashing and forward flushing methods using pure product water from the RO unit. For backwashing, pure water was passed through the permeate channel of MF and RO units and backwashed water was collected through the retentate side while keeping the feed closed. For forward flushing, the reject valve was opened fully so that all the feed gets collected as reject by flushing the deposited residues along with the outlet. Backwash at different time intervals (1, 1.5, 2, and 2.5 h) different volumes of pure water (1, 2 and 2.5 L) for flushing, and different wash water flow rates (20, 25 and 30 L/h) were experimented. The conditions that produced the best result in terms of recovery of membrane flux after backwashing/forward flushing were selected. For backwashing/forward flushing, pure water from RO was used. The RO and MF rejects along with backwashed and forward flushed water were pooled with the fresh feed and pumped into the AFBR for the degradation of ClO_4^- present in it. Samples of wash water and rejects were taken daily from both units for ClO_4^- and TDS analysis.

Analysis

The outlet water from AFBR, MF and RO units was analyzed for ClO_4^- concentration and water quality parameters such as pH, TDS, TSS, TPC and TCOD.

Estimation of perchlorate

Perchlorate concentration in the samples was measured using Ion Chromatography (USEPA methods 314.0 and 314.1). The Ion Chromatographic (IC) unit (DIONEX) was equipped with a self-regenerating anion suppressor (ASRS 300) and a conductivity detector. IC column and guard column (AS 16 and AG 16, DIONEX) specific for ClO_4^- analysis at sub ppb level were used in this study. The eluent used was 50 mM NaOH at a flow rate of 1.5 ml/min. The injection volume was 1000 μl. All reagents were purchased from Sigma Aldrich and standards were prepared in ultra-pure Milli Q water (Millipore).

Estimation of water quality parameters

Oxidation-Reduction Potential (ORP) of samples was measured using an ORP meter (Eutech Instruments, ORP tester10). The TDS content of samples was measured using a TDS conductivity meter (Eutech Instruments, model no CON700). TSS, TCOD and Total Plate Count (TPC) in the samples were estimated by APHA approved standard methods 2540 D, 5220 B (Open Reflux Method) and 9215 C (Spread Plate Method for heterotrophic plate count), respectively.

Statistical analysis

The statistical analysis of the data generated was done using MS Excel. The primary data from the bioreactor performance, as well as operation of the MF and RO units presented, are an average of minimum of three readings, expressed with standard deviation at a significance level of $P < 0.05$.

Results and discussions

Perchlorate reduction in the anaerobic fixed-film bio-reactor

The results of ClO_4^- removal under different acetate levels and HRT are presented in Fig. 2. It was found that ClO_4^-/acetate ratio 1:4 and HRT 6.5 h (flow rate of 8.5 L/h) were suitable for ClO_4^- removal in the present AFBR.

Under this condition, the AFBR treated 200 L of contaminated well water per day and reduced ClO_4^- from the initial 15 mg/L by 0.4 ± 0.35 mg/L (97.33% removal). The average redox potential (ORP) inside the AFBR was −101 ± 26 mV, and pH was about the neutral range (7.3 ± 0.5) without any external correction. The performance of the AFBR

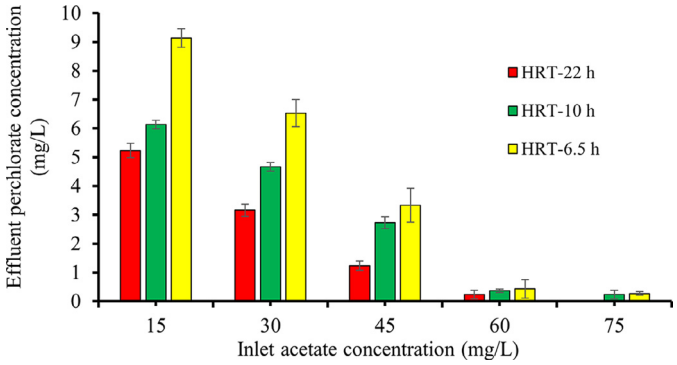

Fig. 2. Effluent perchlorate concentrations at different acetate concentrations, and HRT for an influent perchlorate concentration of 15 mg/L.

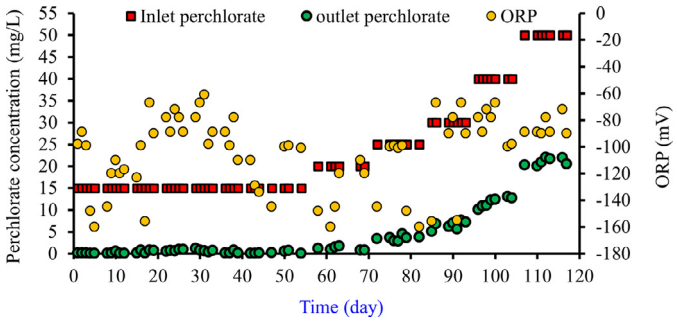

Fig. 3. Inlet and outlet concentrations of ClO_4^-, and ORP level of the AFBR under optimum conditions of ClO_4^-/acetate ratio (1:4) and HRT (6.5 h) from day 1 to 54.

reactor after optimizing the ClO_4^-/acetate ratio (1:4) and HRT (6.5 h) from day 1 to 54 is shown in Fig. 3.

Our previous batch experiment with *Serratia marcescens* in pure culture revealed the equimolar consumption of acetate for ClO_4^- reduction (Vijaya Nadaraja et al., 2013). But, in AFBR, the requirement of a higher concentration of acetate was observed. The optimum ClO_4^-/acetate ratio for maximum ClO_4^- removal was found to be 1:4. The higher acetate requirement for maximum ClO_4^- removal in AFBR could be due to the presence of non-perchlorate reducing heterotrophs proliferating along with the inoculated *S. marcescens*. The presence of viable heterotrophic bacteria (other than S. *marcescens*) was evident from the spread plating of AFBR outlet samples. The whole experimental setup was operated under conditions similar to the field (not maintained under sterile conditions), including the feed well water used was not sterilized. This can lead to the natural proliferation of heterotrophs in the AFBR. Higher acetate requirements up to six times of stoichiometric requirement for ClO_4^- removal in bioreactors with different ClO_4^- reducing microbes have been reported (Farhan and Hatzinger, 2009; Kengen et al., 1999; Kim and Logan, 2001).

The non- perchlorate reducing heterotrophic microflora in AFBR will help to maintain a lower redox potential (by scavenging dissolved oxygen) that favors conditions for ClO_4^- reduction. After two months, when the inlet ClO_4^- concentration was increased from 15 mg/L to 20 mg/L, the percentage of ClO_4^- reduction declined to 94%. Further, at 50 mg/L ClO_4^-concentration and from day 58 to 117 the removal was only 58% (Fig. 3). The ClO_4^-: CH_3COO^- was maintained at 1:4 in all these cases to avoid substrate limitation. Under stable performance conditions, the TCOD, TSS and TDS levels of the AFBR treated water were 45 ± 21 mg/L, 1 ± 0.25 mg/L and 202 ± 10 mg/L, respectively. The TCOD of the AFBR effluent was higher and that could be due to the presence of soluble microbial products and suspended organic particles. The bacterial load in the treated water from AFBR was 1.2×10^7 CFU/mL. The

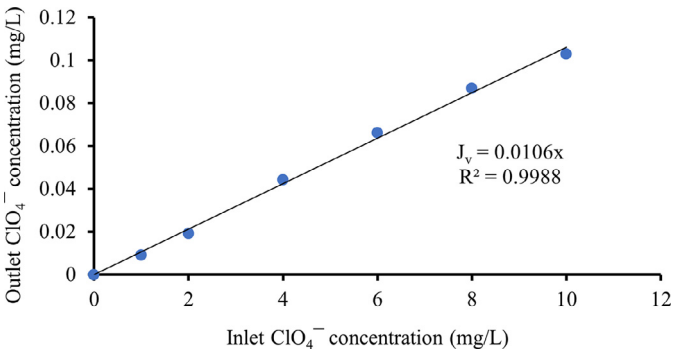

Fig. 4. Perchlorate removal by RO membrane for different inlet ClO_4^- concentration at a flow rate of 40 l/h and 50 psi.

decline in ClO_4^- removal at higher concentrations could be due to the shock loading effect, or insufficient ClO_4^- reducing biomass level in the AFBR. However, by providing more acclimatization period and HRT, it may be possible to arrive at better removal efficiency even at a higher concentration of ClO_4^-.

Performance of RO membrane

The ClO_4^-removal by the RO membrane at a flow rate of 40 L/h and 50 psi is shown in Fig. 4, where Jv represents the permeability of the RO membrane. The RO membrane could remove 99.1% of ClO_4^- when the inlet ClO_4^- concentration was 1 mg/L. As the inlet ClO_4^- concentration increased, the permeate ClO_4^- level also increased. The ClO_4^- rejection was 98.9% when the inlet ClO_4^- was 10 mg/L. Therefore, it was evident that to achieve a ClO_4^- concentration <15 μg/L in the product water, the inlet ClO_4^- concentration should be less than 1 mg/L.

These observations were similar to previously reported studies on ClO_4^- removal through RO process. Several lab-scale studies report ClO_4^- rejection using the RO process. Yoon et al. (2005, 2004) showed 80–95% rejection of ClO_4^- when the initial concentration was 100 μg/L. Yang et al. (2020) reported a decline in ClO_4^-rejection from 99.9% to 95.6% when the inlet ClO_4^- concentration was increased from 0.2 mg/L to 2 mg/L at ~30 psi. The commercial RO membranes have a higher ClO_4^- rejection efficiency. Sanyal et al. (2015) have reported a ClO_4^- rejection of 93% with BW30 membrane at ~70 psi for an inlet ClO_4^-concentration of 10 mg/L, and a 95% removal when SW30 type RO membrane was used at the same pressure. From these reports, it can be concluded that the percentage rejection of ClO_4^- depends on the initial ClO_4^- concentration, the type of membrane used and the transmembrane pressure. Perchlorate removal by various membrane processes reported so far is summarized in Table S3 (supplementary material).

Combined AFBR-MF-RO unit, and its performance

In this study, initially, a RO unit alone was tested for removing ClO_4^- in the contaminated well water, and the AFBR was used for treating the RO reject and backwash water. However, preliminary studies revealed that the maximum concentration of ClO_4^- that can be removed by the RO membrane used was 2 mg/L, whereas ClO_4^- concentration in the well water was 15 mg/L. To overcome this difficulty, the AFBR unit was introduced prior to the RO unit. The AFBR removed ~97% of the initial ClO_4^- concentration, the residual ClO_4^- (0.4 ± 0.35 mg/L) was removed by the RO unit. However, a decreased flux and low ClO_4^- rejection were observed at this stage due to membrane fouling. The high bacterial cell count (~10^7 CFU/mL) in the AFBR treated water can easily clog the membrane. To decrease fouling, the ceramic MF unit as a pretreatment to RO membrane was introduced. The MF passed water had only 200 ± 60 CFU/mL. Bacterial cells were not completely removed in the MF unit used, probably due to the large pore size (1.5 μm) of the ceramic membrane used. The TDS and ClO_4^- concentration remained as 202 ± 10 mg/L and 0.4 ± 0.35 mg/L, respectively without any quantifiable TSS in the MF treated water. The MF unit produced 20 L of permeate and 30 L of reject in 1 h. Integrating the terminal RO unit reduced the ClO_4^- concentration to <10 μg/L, TDS value to <25 mg/L and TCOD below the detection limit. The RO unit produced 12 L of permeate and 28 L of reject in one hour. The overall performance of the combined treatment system at optimized working conditions is summarized in Table 2.

In previously reported studies, ClO_4^- in water was initially removed through membranes (NF, UF, RO or ED) or IX unit, and a microbial process was adopted separately either for regenerating the resin or for treating the reject (Qi et al., 2017; Sharbatmaleki et al., 2015; Sharbatmaleki and Batista, 2012; Yoon et al., 2009). Adsorption with a quaternary amine-functionalized bio-resin and biological/chemical regeneration of the resin was reported recently for treating ClO_4^- contaminated groundwater (Pan et al., 2019). The lower bio-regeneration (26–89%) of the resin was one of the drawbacks observed in this study. Similarly, poor bio-regeneration capacity (84.9% in 5 days) of a surface-modified bio-sorbent for removing ClO_4^-, and further the requirement of sterilization of resin before the next adsorption step was also reported (Ren et al., 2017). Increased fouling after bio-regeneration of the membrane due to the accumulation of soluble microbial products and extra polymeric substances during the bio-regeneration step was also reported in this study. The removal of ClO_4^- in groundwater through a combined electrodialysis (EDR) and the RO method was reported recently (Yang et al., 2020). Perchlorate at an initial 10.5 mg/L was removed to a non-detectable limit through this approach. However, at higher initial ClO_4^- concentration, a lower removal through EDR was observed in this study. Compared with the different approaches reported, particularly for water contaminated with higher levels of ClO_4^-, the method reported in this study would be a better option. Since most of the ClO_4^- is removed in the AFBR, a small capacity RO membrane would be sufficient for the final treatment. This will bring also down the operational cost of the entire treatment system.

Membrane fouling, and treatment of wash water and rejects

The major issue observed during the operation of the combined system was a significant decrease in membrane flux of both MF and RO units due to fouling. The fouling associated with MF and RO systems is very common, and few studies have specifically reported fouling associated with NF, UF and RO membranes in ClO_4^- removal studies (Han et al., 2012; Qi et al., 2017; Yang et al., 2020; Yoon et al., 2009). Suspended cells dissolved organic matter, soluble microbial products, and extra polymeric substances are mainly responsible for membrane biofouling (Nguyen et al., 2012). Physical, chemical and biological approaches are practiced for controling the fouling of different membranes (Bagheri and Mirbagheri, 2018). Specifically, in ClO_4^- removal studies, acid (HCl) treatment was adopted for controling fouling associated with the RO membrane in a hybrid Electrodialysis-RO system (Yang et al., 2020). Among the various methods to control biofouling, backwashing and forward flushing are simple, cost-effective, and environment friendly. Biofouling control through backwash/forward flush with pure water under optimum backwash time interval and wash water volume used was reported earlier (Chang et al., 2017; Shao et al., 2018). However, this approach was never reported in membrane-based ClO_4^- removal studies.

In this study, a considerable decline in permeate flux after one hour of MF and RO operation was observed. The variation in membrane flux and permeate flow rate during the operation of the MF and RO membrane is given in Table 3.

The MF membrane flux after one hour was only 60% of the initial flux i.e., from 4.63×10^{-5} m/s declined to 2.87×10^{-5} m/s (Fig. 5).

In this study, it was found that forward flushing was not effective to control fouling in the MF unit (Fig. S2). This could be due to lower pressure built-up as observed (3–5 psi). Since ceramic membranes are made

Table 2.
Concentration of perchlorate and other water quality parameters in feed water and at different stages of the combined treatment system at optimized working conditions.

Contaminant	Feed water	AFBR treated water	MF treated water	RO treated water
ClO_4^- (mg/L)	15	0.4 ± 0.35	0.4 ± 0.35	<0.01
TCOD (mg/L)	*NA	45 ± 21	<20	**BDL
pH	7 ± 0.4	7.3 ± 0.5	7 ± 0.5	6.3 ± 0.5
TSS (mg/L)	NA	1 ± 0.25	BDL	BDL
TDS (mg/L)	210 ± 15	202 ± 10	202 ± 10	<25
Total plate count (CFU/mL)	NA	1.2×10^7	200 ± 60	0

*Not Applicable
**Below Detection Limit

Table 3
The variation in membrane flux and permeate flow rate in one hour of MF and RO membrane operation.

Time (min)	MF flow rate (L/h)	MF membrane flux (10^{-5} m/s)	Normalized MF Flux	RO flow rate (L/h)	RO membrane flux (10^{-6} m/s)	Normalized RO flux
0	20.0	4.63	1.0	12.0	8.77	1.0
10	19.2	4.44	0.98	11.9	8.70	0.99
20	18.9	4.38	0.94	11.58	8.46	0.96
30	17.6	4.07	0.88	11.388	8.32	0.94
40	16.2	3.75	0.81	10.98	8.03	0.91
50	13.8	3.19	0.69	10.74	7.85	0.89
60	12.4	2.87	0.62	10.67	7.80	0.88

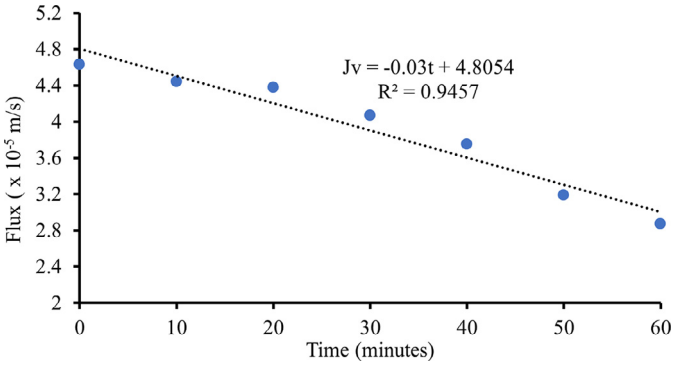

Fig. 5. Feed water flux through the MF unit over one cycle (50 L of feed per hour; Jv is the permeability of the membrane).

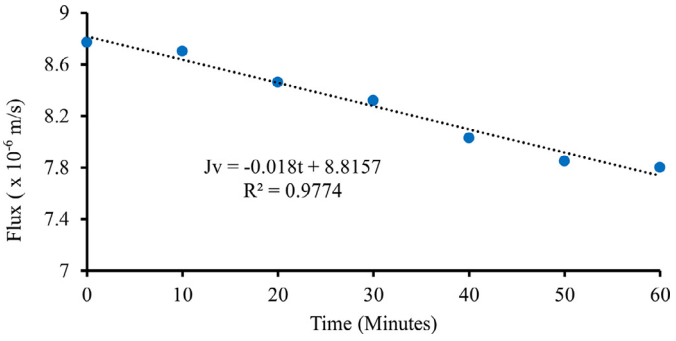

Fig. 7. Feed water flux through the RO unit over one cycle at 40 L/h feed (Jv is the permeability of the membrane).

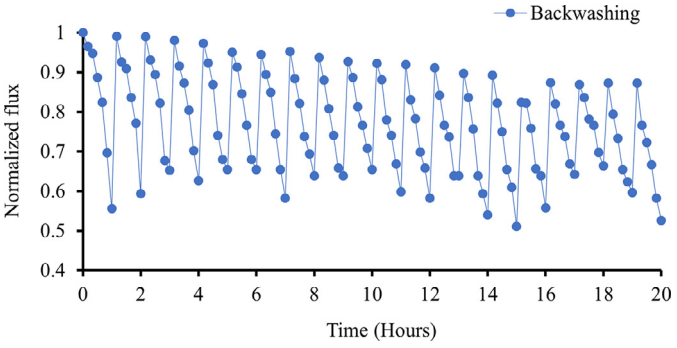

Fig. 6. Effect of backwashing in the recovery of MF Membrane flux (20 cycles, 1000 L of feed).

of mineral oxides with high surface tension, low pressure will not remove most of the adhered particles and hence high pressure needs to be applied to remove all the adhered particles (Yue et al., 2018). For every 50 L of feed passed, the most effective conditions to control fouling in the MF membrane was backwashing at every hour using 2 L pure water at a flow rate of 25 L/h at 60 psi. After backwashing, the MF membrane was regenerated, and the initial flux was regained (Fig. 6.). Hence only backwashing technique was adopted for MF membrane regeneration.

However, on prolonged use, (i.e., 120 h of operation), the flux after backwashing could regain to only 85% of the initial flux. Hence, chemical washing is recommended to regain the initial flux after prolonged use. Various membrane regeneration strategies such as washing with chemicals, backwash with hot water and dipping the membranes in an acidic solution can be performed to regain the membrane flux in the case of ceramic membranes with high surface tension (Akhtar et al., 2020). The MF backwash water contained <10 μg/l of ClO_4^-.

Similarly, in the RO membrane, the initial product flux declined from 8.77×10^{-6} m/s to 7.8×10^{-6} m/s in one hour (Fig. 7).

Unlike in MF unit, both backwashing and forward flushing with pure water was found equally efficient in regaining the flux through the RO membrane (Fig. 8). It was found that for every 40 L of feed passed, hourly backwashing with one-liter pure water at 30 L/h, and 70 psi or hourly forward flushing at 30 L/h and 3 to 5 psi regained the RO membrane flux. However, since product recovery was almost the same in both methods, and forward flushing consumes less pressure (3 to 5 psi at 30 L/h) it was chosen for RO membrane recovery. One liter of RO forward flush water contained 16 ± 2 mg ClO_4^-.

The optimized conditions for the regeneration of MF and RO membranes are summarized in Table 4.

Table 4.
The optimized conditions for the regeneration of MF and RO membranes.

	Washing type	Washing interval (h)	Wash water Volume (L)	Flow rate (l/h)	Pressure (psi)
MF membrane	Backwashing	1	2	25	60
RO membrane	Forward flushing	1	1	30	3–5

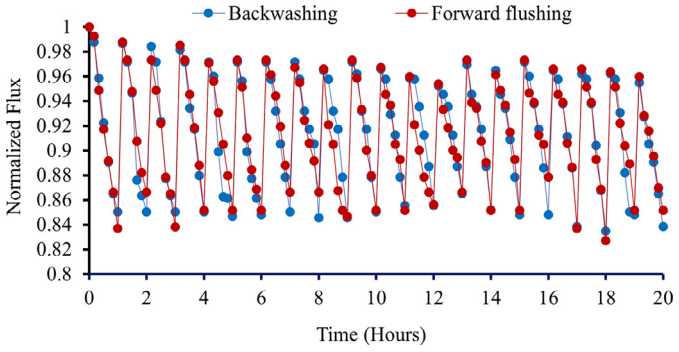

Fig. 8. Effect of backwashing and forward flushing in the recovery of RO Membrane flux (20 cycles, 800 L of feed).

Out of 12 L of product water from RO produced per hour, three liters were used for MF and RO membrane regeneration. Hence, at this permeate flow rate from RO, the combined system produced ~200 L of treated water per day. The integrity of both the MF and RO membranes was constant for ~5000 L of water treated. The MF/RO reject as well as backwash and forward flush water that contained ClO_4^-, dissolved organics and bacterial cells were pooled daily and mixed with fresh feed and pumped into the AFBR for complete degradation of ClO_4^- to achieve a zero-discharge status for the combined system. The TDS build-up due to recycling was negligible as the backwash/forward flush water and reject water was mixed with fresh feed and hence there was a dilution in overall TDS. Compared with the previously reported methods with their disadvantages as presented in Table 1, the novel approach tested in this study was found to be more effective for treating ClO_4^- contaminated ground water. The application of ClO_4^- reducing *Serratia sp.* as potential bacteria for the degradation of perchlorate in an engineered treatment system is established in this study. Since, ~99% of inlet ClO_4^- was degraded into innocuous biproducts through a less energy intensive anoxic bio-treatment as pre-treatment, the stress on subsequent membranes was low and they can be operated at lower pressure (less energy input). There was no need of a secondary treatment of brine, resin or membranes in this approach. This can be applied for treating even highly ClO_4^- contaminated groundwater. Furthermore, the fouling associated with membranes in his approach was controlled through simple and cost-effective mechanisms. All these aspects make the process unique with minimum environmental interventions.

Conclusions

In the present study, initial microbiological followed by a combination of MF and RO membranes in series was effective for treating well water contaminated with higher levels of ClO_4^- (15 mg/L). The anaerobic biotreatment reduced ~97% of the ClO_4^- in well water into non-toxic chloride and oxygen. The subsequent MF and RO membrane removed the residual ClO_4^- level to <10 µg/L. This study also demonstrates the effectiveness of cost-effective, and environment-friendly approaches like backwashing and forward flushing with pure water to manage fouling associated with the MF and RO membranes. The ClO_4^- present in the rejects and wash water from the membranes were also treated so that there is no need for secondary treatment. This kind of treatment system will be useful for addressing localized, higher ClO_4^- contamination of water sources. Results from the present two-stage approach would help design a scale-up system for field application.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2021.100058.

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A novel ex-situ bio-remediation process for perchlorate contaminated soil

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HIGHLIGHTS

- A novel ex-situ approach for treating perchlorate contaminated soil.
- The process includes a combined soil washing and wash-water bio-treatment.
- The wash water can be reused several times.
- The entire process can be completed in ~6 h.

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ABSTRACT

A novel, ex-situ remediation process for perchlorate contaminated soil is reported in this study. This approach comprises washing the contaminated soil with water, followed by treatment of the wash water in a bioreactor. The treated water reused for the next batch of soil, and the cycle continued. The pilot-scale treatment unit comprising of a soil washing unit (0.75 m3) and a fixed-film bioreactor (140 L), both connected in series for continuous operation for a period of three months. The bioreactor was inoculated with a novel perchlorate reducing microbial consortium comprising *Serratia marcescens* (Gen bank no. HM751096), *Bacillus pumilus* (Gen bank no. JQ820452) and *Micrococcus sp.* (Gen bank no. KJ410671). The microbial activity was supported by glucose (glucose/perchlorate ratio = 5), and trace mineral solution. In a typical washing cycle, 2.5 g perchlorate (KClO4) spiked in 670 kg soil was completely removed in three washing cycles, that completed in 6.3 h consuming ~360 L water. The pooled wash water containing perchlorate at 8.5 mg/L was treated completely in the bioreactor operated at 4.5 h HRT and −200 mV ORP. Compared with both in-situ and ex-situ remediation methods reported, the present approach has many advantages for treating perchlorate contaminated soil.

1. Introduction

Perchlorate is a toxic oxyanion (ClO_4), identified as an emerging endocrine disrupting contaminant. Its presence has been reported in soil, water and many human consumption products (Kumarathilaka et al., 2016; Pearce et al., 2007; Trumpolt et al., 2005). The anthropogenic contamination of perchlorate is caused by its widespread applications in strategic sector, and industries like fireworks, highway flares, match sticks, etc. Perchlorate have high solubility (15–200 g/L) and mobility in water, but poor sorption to soil particles due to electrostatic repulsion (Urbansky, 1998). Vadose zone around perchlorate handling sites encounter severe ClO_4 contamination (conc. up to 30,000 mg/L), and that leads to contamination ground water (up to 800 mg/L) (Cao et al., 2019; Gal et al., 2008; Levakov et al., 2019). Perchlorate penetration through soil is highly dependent on soil type and texture (Urbansky and Brown, 2003). It is easily transported to ground water via infiltration (Gal et al., 2008), meanwhile, dissolved ClO_4 can be trapped within soil pores due to capillary force and surface tension (ITRC, 2008).

Different approaches including in-situ bioremediation (Battey et al., 2007; Gal et al., 2008; Hohener and Ponsin, 2014; ITRC., 2008), ex-situ bioremediation (Evans et al., 2008), ex-situ thermal desorption (Gangopadhyay et al., 2010) and phytoremediation (Krauter et al., 2005; Nzengung et al., 1999) have been reported for

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treating perchlorate contaminated soil. Some of the approaches have been successfully tested under field conditions. The in-situ bioremediation approaches reported were either through bio-stimulation or bio-augmentation. In bio-stimulation, the native microflora will be activated for degrading ClO_4. Perchlorate reducing bacteria (PRB) are ubiquitous, and by providing necessary conditions like substrate level (organic carbon source and electron donor), optimum redox conditions, availability of macro/micro nutrients and absence of competitive electron acceptors (like nitrate, sulphate, etc.) that favour the microbial activity for attenuating the ClO_4 level in soil (Levakov et al., 2019; Tyagi et al., 2010). Bio-augmentation on the other hand depends on externally introduced specific microflora for the clean-up activity. In practical situations, bio-augmentation is reported to have limitation in delivering the culture to the site of action, mainly due to impervious clay/sand layers underneath (Scheutz et al., 2010). Phytoremediation approach for treating perchlorate contaminated soil and ground water includes mechanisms like phyto-extraction, phyto-degradation and rhizo-degradation (with rhizospheric microflora) (Fang and Chen, 2011). The ex-situ remediation of perchlorate contaminated soil reported so far include excavation of the polluted soil followed by its treatment in which a combination of substrate (glycerine as the electron donor) and nutrient (Diammonium hydrogen phosphate) were applied to the soil. An average perchlorate removal rate achieved through this approach was 200 μg/Kg/day (Evans et al., 2008).

Both in-situ and ex-situ soil remediation approaches reported have inherent practical difficulties during field implementations. The efficiency of both in-situ and ex-situ soil remediation methods are depends on factors like concentration of perchlorate in soil, and availability of viable microflora capable of degrading perchlorate in soil. Also there are some necessary conditions for the microbial activity in soil remediation like redox potential, preferential substrate (organic carbon and electron donor) type and concentration, supporting nutrients, presence of competitive electron acceptors like nitrate (very common ground water contaminant), etc. (Gal et al., 2008; Krauter., 2005; Tipton et al., 2003). Due to the negative impact of these factors, the rate of perchlorate degradation reported in soil remediation studies were very low (days to few years) (Deitsch et al., 2005). In this scenario, a novel ex-situ remediation approach is successfully validated in a pilot scale unit in this study. In this approach, the perchlorate contaminated soil is initially flushed with water (soil washing) and the wash water (leachate) is subsequently treated completely in a continuously fed bioreactor with a perchlorate reducing microbial system. The high-water solubility of ClO_4, as well as its poor adsorption to soil/organic matter favours soil washing approach to clean the contaminated soil. Soil washing coupled with Photo-Fenton oxidation was reported for remediating soil contaminated with pollutants like DDT (dichlorodiphenyltrichloroethane), DDE (dichlorodiphenyldichloroethylene), hydrocarbons, etc. (Befkadu and Chen, 2018; Huguenot et al., 2015; Villa et al., 2010). But, this study reports for first time soil washing, and wash water biotreatment as a better substitute for approach for cleaning perchlorate contaminated soil.

2. Materials and methods

2.1. Soil characterization

The soil washing experiments were conducted with garden soil collected from the institute campus. The soil samples were initially sieved using 6 mm sieve to remove gravel and other large particles. The characterisation of the soil such as pH, moisture, composition (sand, silt, clay), chloride, sulphate and nitrate levels were done by the standard protocol of soil sampling and methods of analysis (Canadian society of soil science, Carter and Gregorich, 2006., 2nd edition).

2.2. Preliminary soil washing experiment

Preliminary soil washing experiments were done to assess perchlorate recovery from soil under different conditions such as number of washes, water holding time and ideal soil column height. The experiments were conducted in two types of washing unit, one box type and another cylindrical type. The first set of experiments were done in the box type wash unit made up of a transparent polycarbonate sheet. The unit has 35 cm base length, 35 cm width and 50 cm height (volume of 0.061 m3). The top of the box was kept open for loading and unloading the soil, and a drain valve at the bottom for draining the leachate (wash water). The unit was divided internally into four compartments using a polycarbonate sheet to ensure uniform water penetration through the soil column. Around 20 kg of garden soil was taken in this unit, and the soil column height was 23 cm. Perchlorate stock solution (334.3 mg KClO4 in 100 ml water, contains 240 mg ClO_4 was prepared, and using a garden sprayer the solution was sprayed uniformly on the surface of the soil in the box. Subsequently, 30 L of tap water was sprayed over the soil, so that the water level completely covered the soil. The bottom valve was opened to collect the wash water (leachate). The perchlorate in the wash water was determined using an ion-selective electrode method (Cole Parmer, USA). The estimation of residual perchlorate in soil was made by measuring the perchlorate in wash water, and subtracting it from the originally spiked perchlorate in soil. The washing was repeated with fresh water, and total five washes were required to recover all the perchlorate from the soil. In a separate experiment, to improve the ClO_4 recovery from the soil, different water holding time (0–90 min) was given in the box type unit. The washing steps were done as described earlier.

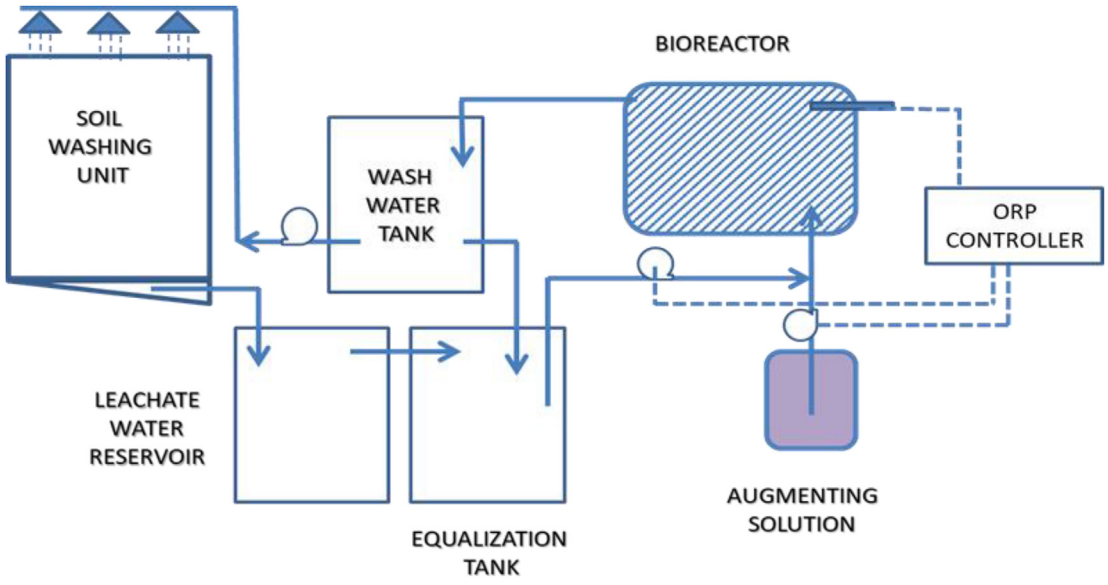
Soil washing experiment was subsequently conducted in a cylindrical, column type unit. The unit was made up of bottom PVC pipe and top acrylic column with a diameter 20 cm and height 125 cm, (volume of 0.039 m3) (Supplementary Fig. 1). Top of the column was kept open for loading and unloading the soil, and a valve at the bottom to collect the soil leachate. 35 kg soil was taken in this unit (soil column height 1 m), and KClO4 stock solution equivalent to 240 mg of ClO_4 was spiked on the surface of the soil as described earlier. Based on previous experiment results, 1-h water holding time was provided here. 15 L of tap water was sprayed on top of the soil, and it formed ~10 cm water column above soil level during the holding time. Leachate sample was collected after each wash and was analysed for perchlorate. Total three washes required to recover all the perchlorate from the soil. In a separate set of experiment, the removal efficiency of higher ClO_4 such as 480 mg/L and 960 mg/L were also tested, respectively.

2.3. Pilot-scale soil washing and leachate treating bioreactor setup

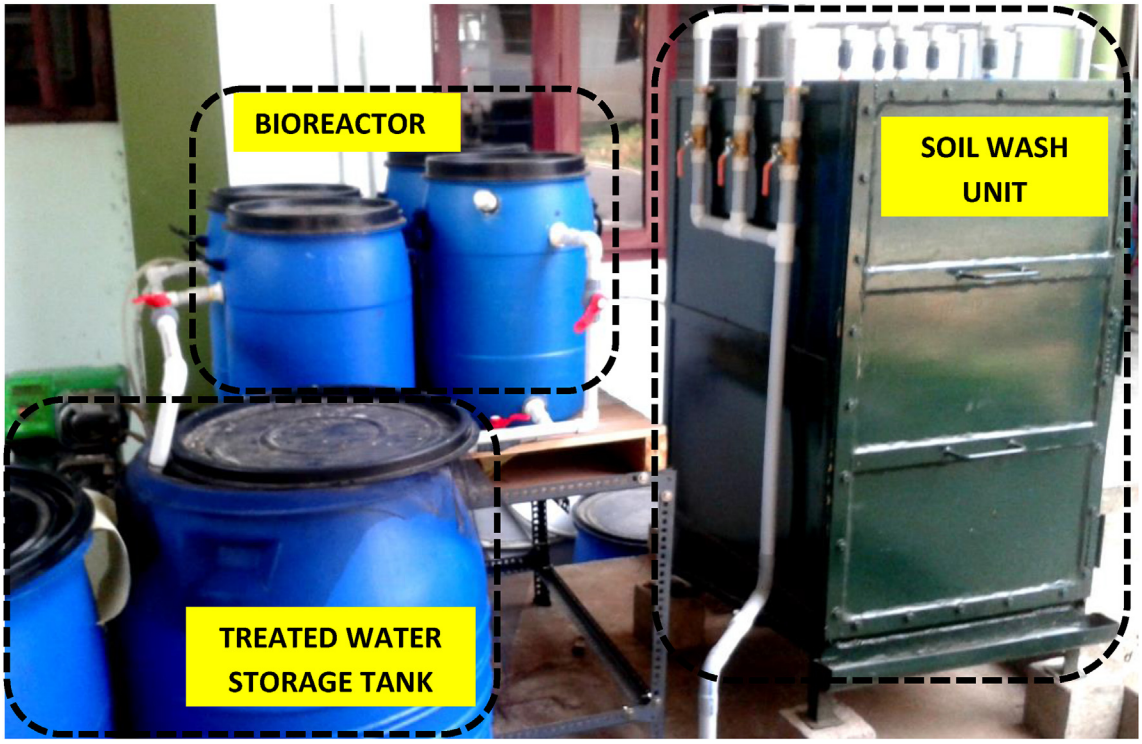
The pilot-scale ex-situ soil remediation system (ESRS) consisting of an (i) soil washing unit to elute perchlorate from the soil and (ii) a continuously fed fixed-film type bioreactor system for treating the wash water. A schematic of the complete treatment system and the actual pilot-scale treatment system are presented in Fig. 1a and b.

2.3.1. Pilot-scale soil washing setup

The pilot-scale soil washing unit was a metal tank made up of mild steel of (0.8 m breadth, 0.7 m length and 1.1 m height; 0.62 M3 volume). For spraying water, six shower taps were mounted uniformly on top of the tank. At the bottom, there was a valve to drain



(a)



(b)

Fig. 1. (a): Schematic diagram of the pilot-scale soil washing unit coupled with wash water treating bioreactor. (b): Pilot-scale soil washing unit coupled with wash water treating bioreactor.

wash water completely from the soil column. Soil washing unit was filled with 670 kg of garden soil from institute campus. The height of the soil column was ~1 m. A 300 L Polyvinyl chloride (PVC) tank was used to collect the wash water from the soil column for subsequent bio-treatment. The soil surface was uniformly sprayed with 2.5 g perchlorate containing water. The solution was prepared by dissolving 3.485 g of KClO4 in 1 L of distilled water. Tap water was then flushed through the showerheads. Water was given in such an amount that it will have an above soil column head. One-hour water holding time was given. After 1 h, the bottom drain valve was opened to collect the leachate in the PVC reservoir tank. Perchlorate was measured as described earlier. Based on perchlorate concentration in wash water, the washing step was repeated until 99.5% recovery was obtained.

A total of 360 L of water was used in 3 washing steps for complete recovery of perchlorate from the contaminated soil. The entire washing (three cycles, each with 1 h holding time) was completed in 6.3 h.

2.3.2. Bioreactor setup and continuous operation

A fixed film type, continuous flow bioreactor was used in this study. The reactor consists of four PVC tanks of 50 L capacity each, connected in parallel (Fig. 1 b). Needle felt coir fibre was used as the biofilm support matrix within the bioreactor. The working volume of the entire reactor setup was 140 L. The bioreactor was inoculated with perchlorate reducing bacterial (PRB) consortia developed at NIIST. The consortium comprising of *Serratia marcescens (Gen bank no. HM751096), Bacillus Pumilus (Gen bank no. JQ820452) and Micrococcus* sp. *(Gen bank no. KJ410671).* Continuous monitoring of pH and Oxidation Reduction Potential (ORP) was done (Thermo scientific, Alpha PH 560) through probes connected to bioreactor.

In the beginning, 9.75 g KClO4 and 70 g glucose were mixed in 140 L tap water. Four litres of the PRB enrichment culture and 1 L of an inorganic mineral solution (contains K_2HPO_4, NH_4SO_4, $MgSO_4$, $CaCO_3$, $FeSO_4 \cdot 7H_2O$ and Trace metal solution) were also mixed with the above solution, and the combined synthetic feed solution was recirculated in the bioreactor using a peristaltic pump (Watson Marlow, USA) at 90 ml/min flow rate. After three days of operation, when the ClO_4 level was below the detection limit, the fresh feed solution was prepared, and the reactor was switched over to continuous flow mode. The synthetic feed solution containing ClO_4, glucose and minerals was used for maintaining the bioreactor. The bioreactor was operated for a period of three months, and its performance in terms of ClO_4 degradation at different initial ClO_4 concentration (10–50 mg/L) was continuously monitored.

For the whole bioreactor operation, glucose was supplied as the substrate (electron donor) for the bacterial activity. Initially, the glucose/ClO_4 ratio was maintained at 2, but the perchlorate degradation was not complete. To optimise the ratio of glucose to perchlorate, 10 mg/L of influent perchlorate solution and three different concentrations of glucose such as 20 mg/L, 40 mg/L and 50 mg/L was tested in continuous feed mode in the reactor. The different feed flow rate was also tested to optimise the minimum HRT required for complete degradation of tested ClO_4 concentration.

2.4. Biotreatment of soil wash leachate

The pooled soil wash leachate (wash water) containing perchlorate (8.5 mg/L) was treated in the bioreactor after two months of its operation. Till that period the bioreactor was maintained with the synthetic feed solution as mentioned earlier. The wash water was continuously fed to the reactor using a peristaltic pump (Watson Marlow, USA) at a flow rate of 30 L/h (HRT 4.5 h). Using a separate peristaltic pump, an augmenting solution consists of glucose and minerals (as explained earlier) was mixed with the wash water before fed to the bioreactor. As stated, the glucose/ClO_4 ratio was maintained at 5. The perchlorate concentration in the treated wash water from the bioreactor was analysed initially with Ion-selective electrode (ISE) and then with Ion Chromatography (IC). The bioreactor treated wash water was collected separately, and the same was used for the subsequent soil washing cycle.

2.5. Analytical methods

2.5.1. Ion-selective electrode (ISE)

Perchlorate concentration in the soil wash leachate was measured using an Ion-selective electrode (Cole Pamer, USA, Model No. K-27502-35) according to the manufacturer's protocol. The lower detection limit of perchlorate electrode was 0.7 mg/L.

2.5.2. Ion Chromatography (IC)

Perchlorate concentration in the bioreactor treated water was analysed using Ion Chromatography (USEPA methods 314.1). The IC was equipped with a self-regenerating anion suppressor (ASRS 300) and a conductivity detector. The Ion Pac AS 16 columns with AG 16 guard column specific for ClO_4 with a lower detection limit of 2 μg/L was used in this study. The eluent used was 50 mM NaOH at a flow rate 1.5 ml/min. The injection volume was 1000 μL. All reagents were purchased from Sigma Aldrich and standards were prepared in ultra-pure Milli-Q water (Millipore).

3. Results and discussions

3.1. Characterisation of soil

The garden soil used in this study contained 33% sand, 43% silt and 3.3% clay (w/w). The moisture level was 10% (w/w), and nitrate at 0.15 mg/kg. The detailed soil properties are presented as supplementary material (Table S1). The physical, chemical and biological properties of the contaminated soil determine the success of a soil remediation approach, especially for ClO_4.The soil used in this study has less clay content (3.3%). Therefore, water percolation through the soil will be fast that mobilise ClO_4 from soil particles to the aqueous phase. Perchlorate penetration through soil is highly dependent on soil texture (Gal et al., 2008; Urbansky and Brown, 2003). The nitrate present in soil in this study (0.15 mg/kg) could have a negative impact on ClO_4 degradation during the biotreatment of the wash water. However, this could be compensated by providing sufficient substrate (glucose as electron donor) level, as we have practised in this study.

3.2. Preliminary soil washing experiments

The preliminary soil washing studies focused mainly to test the effect of soil-water contact time, initial perchlorate concentration in soil, and soil column height on perchlorate removal from soil. The results of ClO_4 removal under different water holding time in soil column is presented in Fig. 2.

It shows a comparison of washing efficiency with respect to the soil-water contact time. Without holding (water pass through), the ClO_4 recovery was poor. Around 65 L water in five washes removed only 70% ClO_4 initially added to the soil. But with 30 min holding time, using 35 L water in 5 washes, the removal increased to 80%. When the holding time was increased to 60 min, the recovery

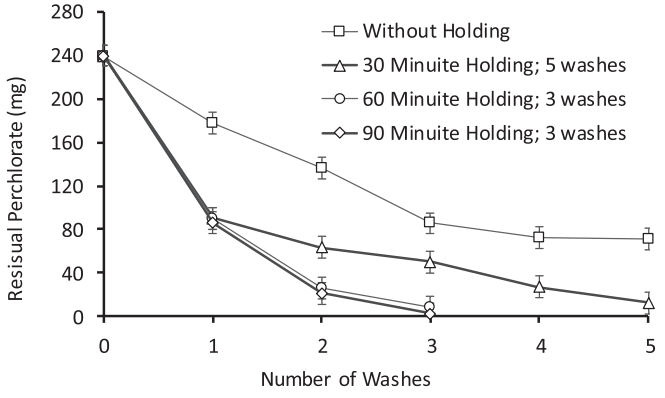


Fig. 2. Residual ClO_4 in soil under different water holding time and washing cycle in box type soil washing unit.

improved further to 96.7%, with the same amount of water consumption (35 L), but in three washes. At 90-min holding time, efficiency increased to 99% with the same amount of water (35 L) and the same number of washes. Based on these results, 60 min holding time was taken as the optimised one in the subsequent experiments.

Depending on the ClO_4 concentration in soil, the number of washes required, as well as the amount of water required for the washing changed. The result of the experiment on ClO_4 removal from the soil at different initial concentration is presented in Fig. 3. At 240 mg and 480 mg ClO_4, three washes with 25 L of water was sufficient to achieve removal percentage of 98.5% and 97.9% respectively. But, at 960 mg ClO_4, the number of washes, as well as the quantity of water, required increased to five and 35 L respectively. Nevertheless, the recovery achieved was only 98.2%.

In this study, we have tested two different soil column heights, 20 cm and 100 cm. Soil column height up to 100 cm has similar washing efficiency with the optimised 60 min water holding time. At 1-m soil column height, when 500 mg ClO_4 was spiked on soil, ~98% removal was achieved with 25 L water in three washes. Soil column height above 1 m was not tested due to practical difficulty in handling the soil under laboratory conditions.

3.2.1. Pilot-scale soil washing study

Based on the observations of the preliminary soil washing studies, we have considered 60 min holding time, 1-m soil column height, and three washing cycles for the pilot-scale soil treatment experiment. The complete recovery of 2.5 g ClO_4 spiked on soil was achieved in three cycles of washing using a total of 360 L of tap water.

The first washing with 150 L of water produced 118 L of leachate that contained 13.89 mg/L (1.639 g) of ClO_4 (65.5% recovery). During the second wash using 110-litre tap water, 90 L of leachate came out, containing 8.64 mg/L (0.77 g) ClO_4. During the third and final wash with 100-litre water, 83 L of leachate containing 1.02 mg/l (0.084 g) of ClO_4.After three washing steps, the total ClO_4 recovery was 2.497 g of ClO_4 out of 2.5 g (99.8%). The total wash water was used 360 L. The entire leachate was pooled (291 L), and the ClO_4 concentration in the pooled water was 8.5 mg/L. The complete soil washing, including holding time of 1 h in each washing, took around 6.3 h. The results are summarised in Table 1. The pooled water was fed to the bioreactor for treatment.

Soil washing followed by treatment of the wash water has been reported as an effective method for cleaning soil contaminated with many organic pollutants. Soil washing (with surfactants like Triton X-100, Tween 80 etc.) coupled with Photo-Fenton oxidation of the wastewater obtained was previously reported for remediating soil contaminated with DDT, DDE, hydrocarbons, etc. (Befcadu and Chen, 2018; Huguenot et al., 2015; Villa et al., 2010). On the other hand, the ionic properties of ClO_4 eliminate the requirement of surfactants, and its high water solubility is an added advantage to recover completely from the soil through simple washing with water as we have practised in this study.

3.3. Bioreactor start-up, continuous operation and leachate treatment

At the start-up stage, the bioreactor was operated in recirculation mode. This helps in a gradual build-up of an active ClO_4 reducing biofilm in the support media. The initially added 50 mg/L ClO_4 concentration was reduced to <2 μg/L in 3 days (99.9% removal). In this study, glucose was used as the substrate (carbon and electron donor). The result of ClO_4 removal in the bioreactor at different glucose/ClO_4 ratio is presented in Fig. 4. The highest ClO_4 removal (99%) was observed when glucose/ClO_4 ratio at 5. However, the removal declined to 89% and 50% when glucose/ClO_4 ratios reduced to 4 and 2, respectively.

Both organic (acetate, lactate, glycine, glucose, ethanol, ethyl acetate, etc.) and inorganic (hydrogen, sulphur, Fe°) substrates (electron donor) have been reported for bacterial ClO_4 degradation in water as well as soil remediation studies (Cai et al., 2010; Jackson et al., 2004; Luo et al., 2015). Among the organic substrates, many PRBs reported having a preference for acetate (Cox et al., 2000;

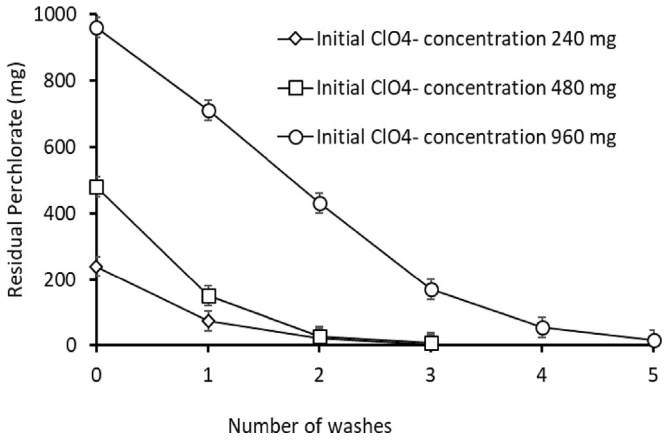


Fig. 3. Washing efficiency with respect to perchlorate concentration.

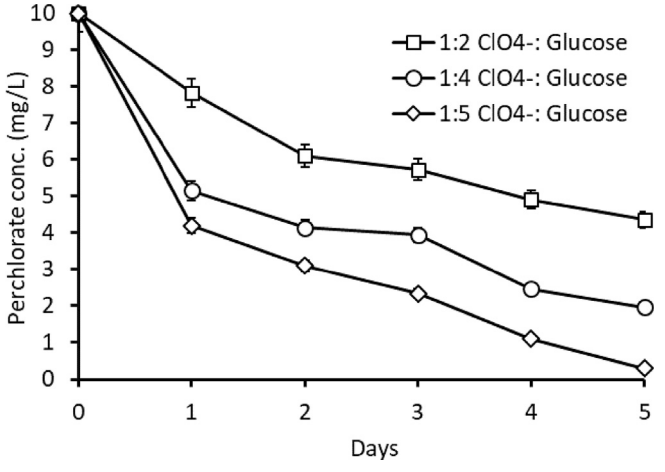


Fig. 4. Perchlorate degradation in the bioreactor at different ratios of ClO_4 and glucose.

Table 1
Pilot scale Soil washing data.

	Water used for washing (L)	Leachate water (L)	Total washing time (min)	ClO_4 Concentration (mg/L)
1st wash	150 ± 5	118 ± 5	90	13.85
2nd wash	110 ± 5	90 ± 4	130	8.64
3rd wash	100 ± 5	83 ± 4	160	1.02

Perlmutter et al., 2000; Rikken et al., 1996). Interestingly, the stoichiometric requirement of acetate for ClO_4 degradation varied from 1:2 to 1:7 (ClO_4: acetate) among the different PRBs reported (Farhan and Hatzinger, 2009; Rikken et al., 1996). In soil remediation studies, application of external electron donor found to have a positive effect on ClO_4 removal (Avishai et al., 2017). Simple substrates like acetate to more complex and cheap substrates have been reported in ClO_4 contaminated soil remediation studies (Gal et al., 2008). However, for field applications, slow-releasing substrates that are low cost, and available in bulk (like emulsification oil, mulch extract, etc.) are attractive (Mayra et al., 2018). Alternatively, gaseous electron donors like hydrogen, 1-hexene, ethyl acetate, and liquefied petroleum gas (LPG), were also tested in microcosm studies for treating ClO_4 contaminated soil (Cai et al., 2010; Evans and Trute, 2006). The higher glucose requirement (than a stoichiometric requirement) for ClO_4 degradation could be due to the presence of heterotrophic microflora that are normally present in soil wash water. The excess glucose will scavenge the oxygen and nitrate in the soil wash water and creates a more favourable environment in the bioreactor for ClO_4 reduction. The analysis of ClO_4 level in the wash water will help to decide required glucose level to be added in the augmenting solution along with other nutrients. This will help to avoid excess organic loading into the soil, which was the case with in-situ and ex-situ remediation approaches reported earlier.

The performance of the bioreactor during its three-month period of operation is presented in Fig. 5a. During this period, the influent perchlorate concentrations were in the range of 10–50 mg/L. To ensure complete ClO_4 reduction, the glucose level was maintained proportionally. In all the concentration tested, around 99% removal of ClO_4 was observed. IC analysis of the treated water showed only chloride (Cl) as the end product, and intermediate products like Chlorate (ClO_3) or Chlorite (ClO_2) were not detected. Bacterial perchlorate reduction is a sequential enzymatic process with chloride and oxygen as the end products. The intermediates Chlorite (in particular) and Chlorate are unstable and will be reduced further into terminal products like chloride and oxygen. Throughout the period of study, the reactor pH was around 7 ± 0.5 without any external pH correction (Fig. 5b). The optimum pH range of ClO_4 removal in bioreactors has been reported as 6.5–7.5 (Balk et al., 2010; Waller et al., 2004). Perchlorate reduction was highly depended on the oxidation-reduction potential (ORP) status of the bioreactor. Lower ORP levels favoured higher ClO_4 reduction. At start up stage, the ORP was around −50 mV, but gradually it declined to −150 to −300 mV range in two weeks period, and provided a better condition for bacterial ClO_4 reduction in the bioreactor (Fig. 5b).

Shrout and Parkin (2006) have also reported similar observations in a bioreactor study where 100% removal of ClO_4 observed when the ORP was −220 mV.

The soil wash water (containing ClO_4) was treated completely in the bioreactor. The total volume of water used in three different

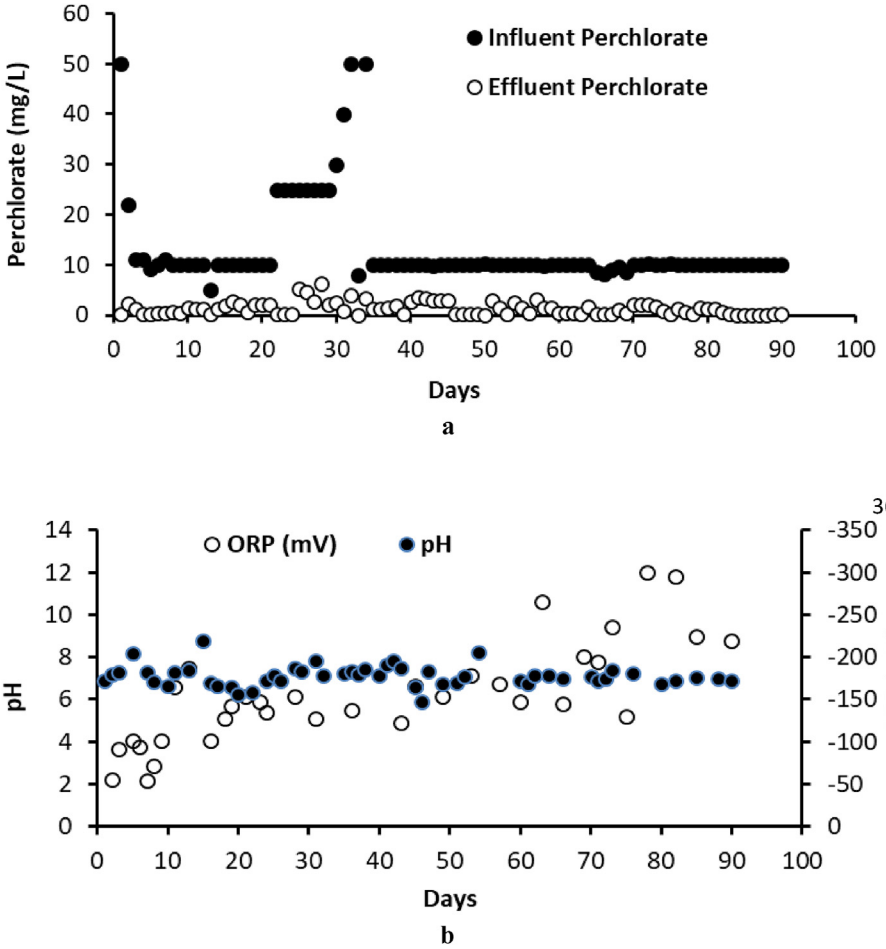


Fig. 5. a: Concentration of influent and effluent ClO_4 in the bioreactor for first 90 days of operation, each data point represents daily analysis result. **b:** pH and ORP profile of the Bioreactor during first 90 days of operation, each data point represents the daily analysis result.

Table 2
Performance data of ClO_4 containing soil wash water treatment in the bioreactor.

Experiment	Total wash water volume (L)	ClO_4 concentration in leachate (mg/L)	ClO_4 concentration in treated leachate (μg/L)
1	291	8.59	4
2	306	8.17	2
3	276	9.06	4

washing cycle, the corresponding level of ClO4 in the pooled wash water, and ClO_4 in the treated water are summarised in Table 2. The level of ClO_4 in the treated water was very low (2–4 μg/L), and that was achieved within a short Hydraulic Retention Time (HRT) of 4.5 h. The combined washing (total 6.3 h, including 60 min holding time) and subsequent wash water (9.7 h) treatment could be finished within a maximum of 16 h. This is comparatively very short compared with other (in-situ and ex-situ) approaches reported in the past, where it took few days to many months to complete the remediation of soil contaminated with ClO4 (Deitsch et al., 2005).

Compared with the in-situ (including in-situ soil flushing and treatment) or ex-situ remediation approaches reported, the present strategy has many advantages. This may find the major application at places where ClO_4 is handled in bulk, and that leads to topsoil contamination. The major advantages of the present approach are (i) complete removal of ClO_4 from the contaminated soil, (ii) the entire treatment (including soil washing and wash water treatment) can be completed within few hours, (iii) the practical difficulties and adverse impacts due to directly adding organic substrates to soil can be avoided, (iv) adverse change in soil properties can be avoided, (v) there is no requirement of a pre or post-treatment of the contaminated soil, (vi) this approach can be adopted to any soil types, provided proper mechanism to enhance water percolation in the case of soil with high clay content (poor water penetration). Instead of glucose that we have successfully validated in the pilot-scale treatment studies, more economical and locally available substrates like leachate from agro residues can also be considered as substrates for the microbial activity in this treatment.

4. Conclusions

This study demonstrates a novel, ex-situ remediation approach for ClO_4 contaminated soil in a pilot-scale unit. In this approach, ClO_4 in the soil is directly eluted with water, and the wash water is regenerated in a bioreactor, and the cycle is continued. This approach will find application, especially for treating contaminated topsoil, which is very common at places where ClO_4 is handled in bulk. Considering the limitations associated with the existing approaches reported, the soil washing approach is a better alternative. The entire remediation can be completed in a few hours that will prevent the infiltration of highly persistent ClO_4 into underlying groundwater.

CRediT authorship contribution statement

Rothish R. Nair: Investigation, Formal analysis, Writing - review & editing. **Jasmin G. Russel:** Investigation, Formal analysis, Writing - original draft. **S. Pradeep:** Investigation. **S.V. Ajay:** Investigation. **B. Krishnakumar:** Conceptualization, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.125947.

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