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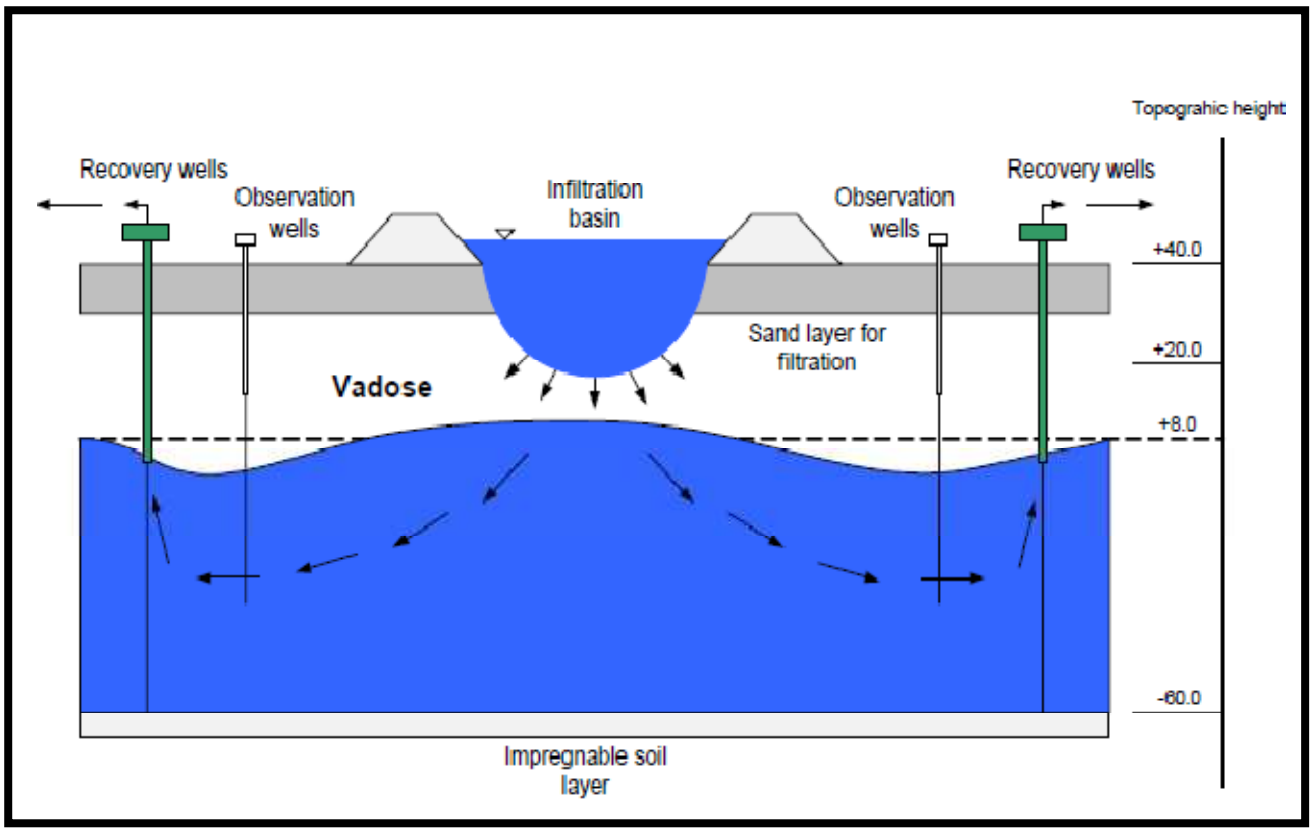


## Effect of Temperature and Redox Conditions on Removal of Contaminants during Soil Aquifer Treatment

**Malolo N Yona**

**MSc Thesis MWI-2011/09**

**April, 2011**

UNESCO-IHE
Institute for Water Education

SWITCH

# Effect of Temperature and Redox Conditions on Removal of Contaminants during Soil Aquifer Treatment

Master of Science Thesis

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The findings, interpretations and conclusions expressed in this study do not neither necessarily reflect the views of the UNESCO-IHE Institute for Water Education, nor of the individual members of the M.Sc. committee, nor of their respective employers.

*This work is dedicated to My Son Ethan.*

## Abstract

Reuse of treated wastewater through groundwater recharge has emerged as an integral part of water and wastewater management in arid and semi arid regions of the world. The potential to augment existing water supplies and reduce reliance on imported surface waters using soil aquifer treatment (SAT) is restricted by lack of information to support rational design and operation of SAT systems. Elimination of uncertainties is necessary to utilize SAT as an alternative sustainable treatment and water resource management option.

Laboratory-scale batch and soil column studies were carried out to study the effect of temperature and redox conditions on removal of bulk organic matter, nutrients (nitrogen and phosphorous) and pathogens with primary effluent (PE) and secondary effluent (SE). PE and SE were collected from Hoek van Holland WWTP, the Netherlands. DOC removal in soil batch tests fed with PE was about 55% under aerobic conditions and about 40% under anoxic conditions. The DOC removal in batch reactors fed with SE was 25 % under aerobic conditions and 15% under anoxic conditions. Likewise in case of soil column studies with PE DOC removal was 46% under aerobic conditions and 31% under anoxic conditions whereas DOC removal was 19% for aerobic conditions and 13% under anoxic conditions in case with SE.

Oxygen availability was an important factor in nitrification (and denitrification) reactions and thus overall ammonium nitrogen removal from infiltrating water. Phosphorus removal efficiency was relatively low by SAT under aerobic and anoxic conditions though it was evident that aerobic conditions are advantageous over anoxic conditions. The removal of phosphorous ranged from 18 to 37% in batch experiments while in soil column experiments the removal ranged from 11 to 31% under both aerobic and anoxic conditions. Removal of pathogens in batch reactors was not substantially affected by redox conditions and ranged from 2.1 to 3.6 log removal. The soil columns were able to remove 2 to 3 log removals of *E-coli* and *total coliform* within 0.3 m column depth under both aerobic and anoxic conditions.

The DOC removal in PE at temperatures 15$^{o}$C, 20$^{o}$C and 25$^{o}$ C was 43%, 50% and 55% respectively. The DOC removal in SE at temperatures 15$^{o}$C, 20$^{o}$C and 25$^{o}$C was 15%, 19% and 23% respectively. The performance of SAT at lower temperatures of 15$^{o}$C in the soil passage did not significantly affect DOC removal but resulted in a diminished nitrification, denitrification rate and a reduced attenuation of nitrogen and phosphorous. It was observed that redox conditions and temperature influence the removal of DOC, nitrogen, phosphorous and pathogens during soil passage. The findings of this research confirm that SAT systems are able to act as a reliable barrier for DOC, nitrogen, and phosphorous regardless of seasonal and flow conditions during SAT providing a sufficient retention time is maintained.



Keywords: Soil Aquifer Treatment, Bulk Organic Matter, Temperature, Redox conditions, Nutrients and Pathogens

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| | |
|---|---|
| BDOC | Biodegradable Dissolved Organic Carbon |
| BOD | Biological Oxygen Demand |
| CAS | Conventional Activated sludge |
| COD | Chemical Oxygen Demand |
| COM | Colloidal Organic Matter |
| DBPs | Disinfection by-products |
| DOC | Dissolved Organic Carbon |
| EBCT | Empty Bed Contact Time |
| EDCs | Endocrine Disrupting Compounds |
| EfOM | Effluent Organic Matter |
| *E-coli* | *Escherichia coli* |
| F-EEM | Fluorescence Excitation Emission Matrix |
| GAC | Granular Activated Carbon |
| HLR | Hydraulic Loading Rate |
| HS | Humic Substances |
| MAR | Managed Aquifer Recharge |
| NBDOC | Non-biodegradable DOC |
| NOM | Natural Organic Matter |
| P | Phosphorous |
| PE | Primary Effluent |
| PhACs | Pharmaceutically Active Compounds |
| POC | Particulate Organic Matter |
| SAT | Soil Aquifer Treatment |
| SE | Secondary Effluent |
| SMPs | Soluble Microbial Products |
| SOC | Synthetic Organic Compounds |
| SP | Sampling Point |
| SS | Suspended solids |

| | |
|---|---|
| SUVA | Specific Ultraviolet absorbance |
| TEA | Terminal Electron Acceptor |
| THMs | Trihalomethanes |
| TKN | Total Kjeldahl Nitrogen |
| TOC | Total Organic Carbon |
| TSS | Total Suspended Solids |
| TOX | Total organic Halides |
| UVA254 | Ultraviolet Absorbance at 254 nm |
| WB | World Bank |
| WWTP | Wasterwater Treatment Plant |

# 1 INTRODUCTION

## 1.1 Background

The rapid population growth and urbanization, the phenomenon which is putting tremendous stress on the world's water resources, especially in the drier climates, requires much more reuse and recycling of water to meet increasing water demand. Creative water management will become essential in many countries in the world in future. Groundwater not only is a major water resource in general, it will also be at risk because rising water demands can lead to over-pumping. This depletes aquifers, increases pumping costs, and may cause land subsidence and water quality problems such as sea water intrusion in coastal areas (Bouwer, 1994).

Water reclamation and reuse provides a unique and viable opportunity to augment traditional water supplies. As a multi-disciplined and important element of water resources development and management, water reuse can help to close the loop between water supply and wastewater disposal (Asano, 2002). The alternatives for water augmentation are the reuse of municipal wastewater to address the ever increasing water demand. Nevertheless, the amount of wastewater that can be reclaimed for this purpose is affected by many factors, ranging from technical possibility to socio-economic and institutional aspects (Yang & Abbaspour, 2007) as cited by Caballero (2010).

Soil Aquifer Treatment (SAT) is defined as a three-component treatment process consisting of the infiltration zone, vadose zone and aquifer storage. This concept can be broadened to a SAT system that adds the additional components of effluent pretreatment, SAT site operation and the recovery of groundwater after infiltration and aquifer storage for water reuse. The SAT technology involves infiltration of secondary effluent through a recharge basin with subsequent extraction through recovery wells, and embodies both treatment, dominant in the vadose (unsaturated) zone, and storage within the saturated zone (aquifer). It is an advanced wastewater treatment process that is both natural and sustainable, and is dominated by biodegradation, initially aerobic and subsequently anoxic (Amy and Drewes, 2007).

Treated wastewater effluents, free from health hazards, must be considered as a valuable water resource for irrigation of certain crops, greening enhancement, landscaping, land reclamation, car washing, industrial process water, and toilet flushing. Treated wastewater may also be used for recharging aquifers in the areas with water shortages or where the aquifers have been depleted by overexploitation to augment water supply for drinking purposes (Akber *et al.*, 2003).

Organic matter is one of the key issues of major concern in potable water as it exerts an oxygen demand and some organic compounds are persistent and carcinogenic when it reacts with other chemicals. Organic matters are derived from plant and animal materials broken

down to small molecules some of which combine to form complex structures of humic substance. The characteristics of organic matter present in the wastewater used for recharge affects water quality after groundwater recharge during SAT. Organic pollutant removal efficiency during SAT depends on the processes conditions, the organic transformations during groundwater recharge, and hydro-geological conditions (Sharma *et al.*, 2007).

Total organic carbon (TOC/DOC) is an analytic technique to measure water quality during the drinking water purification process. Typically, Natural Organic Matter (NOM) is composed of humic substances (humic and fulvic acids) and non-humic materials (e.g., proteins and carbohydrates). Effluent Organic Matter, EfOM represents organic matter found in wastewater effluents, treated to a secondary (or tertiary) level. Typically, EfOM contains a lesser amount of humic substances and more non humic materials than NOM. Within EfOM, there is background (drinking water) NOM, anthropogenic organic compounds derived from the domestic cycle of water use, and SMPs generated during the wastewater treatment process (Fox *et al.*, 2001).

After conventional wastewater treatment, secondary effluent contains wastewater EfOM, consisting of NOM derived from the drinking water source(s) and dominated by humic substances, plus soluble microbial products (SMPs) derived from biological (secondary) wastewater treatment reflecting a microbial origin. In an indirect potable reuse system, the residual humic substances present in EfOM impart color and serve as a precursor to disinfection by-products (DBPs) while the nitrogen-rich SMPs present in EfOM represent a precursor to nitrogenous DBPs (N-DBPs) if extracted water is chlorinated upon recovery (Amy and Drewes, 2007)

Removal of organic matter is a critical parameter in SAT as it governs and influences the removal of other contaminants by biodegradation namely traces organics, nitrogen species and microbes (Sharma *et al.*, 2007).

Reclaimed water through SAT can be used for both direct and indirect potable reuse. Besides, SAT effluent is perceived by public as groundwater rather than sewage water as cycle is essentially not closed. However, SAT efficiency is influenced by type and quality of the wastewater applied, prevailing redox conditions, and hydro geological conditions (Idelovitch *et al.*, 2003). This research was focused on studying the effect of temperature and redox conditions on removal of different contaminants during SAT.

## 1.2 Problem definition

Water scarcity in arid and semi-arid regions along with increasing population growth, increasing per capita water consumption and drought conditions in different parts of the world has exacerbated enormous pressure on the available water resources. Focus on adequate wastewater management is now a crucial requirement that is sought to address the increasing

water demand on limited and scarce water sources through indirect potable and non potable water reuse (Abushbak, 2004).

In the view of rapid population growth with increasing water demands and potential water shortages, Managed Aquifer Recharge (MAR) is becoming increasingly more important all over the world as a sustainable method to save groundwater resources and improve the quality of the infiltrating water. Artificial recharge acts as a purification step due to physical, chemical and biological processes such as filtration of suspended solids, bacteria, viruses or parasites adsorption and biodegradation. The last-named process is often seen as the most important one for the removal of organic pollutants (Massmann *et al.*, 2006).

Application of SAT technology in arid and semi arid regions of the world where groundwater resources have been over exploited augments water supply. SAT is a low cost and appropriate option for wastewater reclamation in developed as well as in developing countries that ensures sustainability of both surface water and groundwater sources within the context of integrated water resources management (Sharma *et al.*, 2007).

According to WB (2003) even with successful urban demand management and increased irrigation efficiency, new and innovative water supplies will be required in the upcoming future. Cost of supplying water from new sources is increasing due to longer conveyance systems, higher pumping costs and higher treatment costs because of poorer water quality as a result of environmental pollution. Groundwater recharge with wastewater effluent during SAT and integration of reclaimed water into national water supply system is rational.

However, there are limitations that include regulations that vary with different countries and require a certain standards to be met by treated wastewater before SAT application and lack of public acceptability of direct potable water use for reclaimed water. In addition, the technology is area specific requiring detailed hydro-geological investigations before SAT application for removal of pollutants like organic matter (Sharma *et al.*, 2007).

Advanced technologies have high capital and operation costs, require educated operators, and generally not well suited for developing countries. There is a need to identify and demonstrate the feasibility of simple low-tech, low cost treatment systems suitable for wastewater reuse in developing communities throughout arid regions of the world (Westerhoff and Pinney, 2000).

Natural groundwater replenishment of aquifers occurs very slowly and for that reason artificial recharge with reclaimed water is an option to increase the rate of replenishment of groundwater in aquifers. This experience has triggered an increasing development to entailing more efficient and well organized use of water resources, both in urban and rural environments. A major mechanism to achieve greater efficiencies is the reuse of municipal wastewater. As a consequence, the application of SAT technology to replenish groundwater resources and augment water supply is justified.

Therefore during this study, the main purpose was to study the influence of temperature and redox conditions on removal of bulk organic matter, nitrogen, phosphorous and pathogens. Many of the concerns associated with organic matter in treated municipal wastewaters are analogous to the health concerns associated with naturally occurring organic matter in surface waters or ground waters that are used for drinking water. The NOM present in drinking water sources is quantified as DOC.

## 1.3 Goal and objectives

The main goal of this study is to examine the influence of temperature and redox conditions on removal various contaminants during SAT with soil column and batch experiments at laboratory-scale.

The following are the specific objectives of this study:

- To explore influence of seasonal variation (temperature) on removal of contaminants (bulk organic matter, nitrogen, phosphorus, pathogens (*E*-coli and *total coliform*) during SAT.
- To analyze the effect of redox conditions on removal of contaminants (bulk organic matter, nitrogen, phosphorus, pathogenic bacteria during SAT.

# 2 LITERATURE REVIEW

## 2.1 Soil aquifer treatment

Water on the earth is constantly on the move, recycling and being used over and over. This is called the water cycle. That means the water that we drink has been used, disposed of, and used again in a never-ending cycle. As part of the cycle, the earth soaks up some of the water and stores it in the ground until it is needed. This underground storage area is called an aquifer or groundwater basin. Some people pump the water from underground aquifers and use it for drinking, household tasks, or landscape or agricultural irrigation. When water soaks into the ground it is purified naturally through physical, chemical and biological processes in the soil, this overall purification process is called soil aquifer treatment ( SAT ) and has occurred since the beginning of time (Fox *et al.*, 2001b).

### 2.1.1 How does SAT work?

SAT is an advanced (generally beyond secondary) wastewater treatment process involving infiltration (percolation) of wastewater effluent through vadose (unsaturated) zone to recharge underlying groundwater aquifer (Amy, 2005).

Prior to its reuse, reclaimed water undergoes two to three stages of cleansing to produce a high quality of water that meets or exceeds standards making it safe to reuse. The cleansing of water by this process is just a speeded-up version of what is done in nature and can be as good as or even better than the natural process (Fox *et al.*, 2001a)

As the applied wastewater moves down through the vadose zone, during the wetting time and drainage time, improvements in its quality can occur as a result of different physical, biological, and chemical mechanisms including filtration, biological degradation, physical adsorption, ion exchange, and precipitation. This combination of removal processes can be very effective in removing organic compounds, nitrogen, phosphorus, pathogens, suspended solids, and trace elements. The efficiency of removal for specific compound or element in the applied water is a function of the type of soil, level of pretreatment of the applied wastewater, and the wetting and drying cycle times. As a result for these mechanisms the physical properties for the soil might be changed: grain size distribution, porosity, and hydraulic conductivity (Abushbak, 2004). Figure 2-1 shows the schematic representation of SAT system

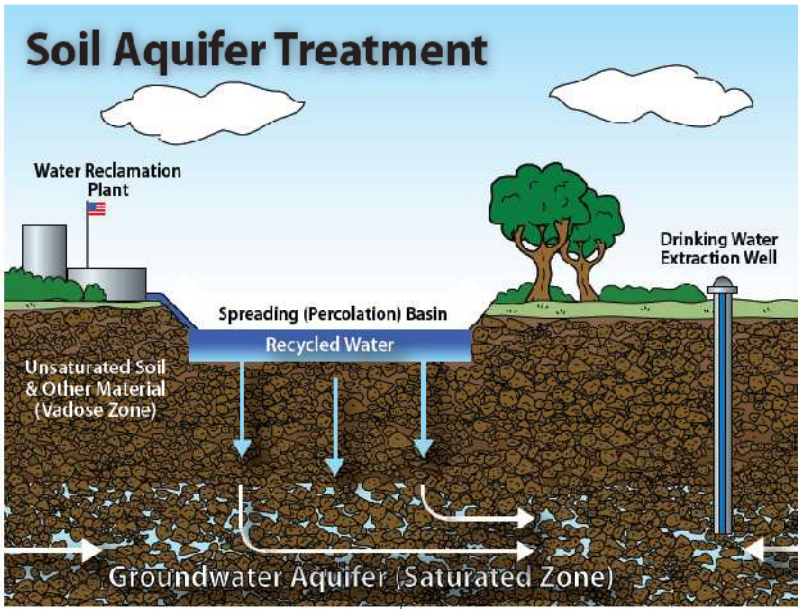



**Figure 2-1**: Schematic representation of SAT system

Source: (Fox *et al.*, 2001)

### 2.1.2 SAT case studies at a global scale

SAT applicability potential is worldwide, depending on geology, soils and hydrology. Presently several SAT projects are in operation in USA, Israel, Australia and Europe (Amy, 2005). Table 2-1 shows the removal of various contaminants by SAT in Dan Region Project.

**Table 2-1:** Removal of various contaminants by SAT in Dan Region Project in Israel

| Parameter | Concentration before SAT | Concentration after SAT | Average relative removal efficiency (%) |
|---|---|---|---|
| SS (mg/L) | 10-80 | 0 | 100 |
| BOD (mg/L) | 5-40 | 0.5 | 98 |
| COD (mg/L) | 40-160 | 10-20 | 85 |
| CODf (mg/L) | 40-80 | 10-20 | 75 |
| DOC (mg/L) | 15-20 | 3-6 | 74 |
| UV absorbance (1/cm x 1000) | 150-400 | 30-80 | 80 |
| Detergents (mg/L) | 0.4-1.0 | 0.05-0.2 | 82 |
| TN (mg/L) | 5-30 | 5-10 | 57 |
| TP (mg/L) | 3-10 | 0.01-0.03 | 99 |

Source: (Idelovitch *et al.*, 2003)

SAT has been applied in several parts of the world typically on a project basis and scaling up is an ongoing process (Viswanathan *et al.*, 1999) as cited by Musabe (2007). Table 2.2 shows some of the SAT sites and their performance in different parts of the world.

**Table 2-2:** Characteristics of selected SAT sites in the world

| **Location** | **Type of wastewater** | **Infiltration rate (m/d)** | **Reported removal efficiency** | **Remarks** |
|---|---|---|---|---|
| Israel; Dan region project | CAS with biological N and P removal | 0.19 - 0.58 | 74 % (DOC) | SAT systems has been in operation since 1977 |
| Mesa (AZ) Northwest Water Reclamation Plant; USA | Nitrification and denitrification CAS plus filtration and disinfection | 0.06 – 0.12 | 83 % (DOC) | Relatively low infiltration rates due to clay soils |
| Sweetwater (Tucson, Arizona);USA | Chlorinated Secondary effluent | 0.13 – 0.17 | 85% (DOC) | Most of the DOC removal is in the top 3 m depth |
| Sulaibiyah, Kuwait | CAS | 0.76 | 70% (COD) | This was done on an experimental in-situ pond scale |
| Belgium (IWVA Torreele) | Secondary effluent from CAS after UF/RO treatment | N/A | 75% (TOC) | A combination of SAT and membrane UF is used |

[Sources: Fox *et al*. (2001a); Idelovitch *et al*. (2003); Viswanathan *et al*., (1999); Yoo *et al*. (2006); WHO (2001)] as cited by (Musabe 2007)

## 2.2 Factors affecting the performance of SAT

### 2.2.1 Influent wastewater quality

The quality of the wastewater applied to SAT ascertains the quality of reclaimed water after soil passage. The quality of applied wastewater plays a vital role in the performance of treatment and in the removal of contaminants. However, the characteristics of raw wastewater in terms of suspended solids determine the settling efficiency and hence primary clarification to make it suitable for SAT. Consequently, the application of SE rather than PE is more practical to minimize clogging layer or schmutzdecke. Near the soil water surface, biological activities in wastewater with high total oxygen demand will utilize all the dissolved oxygen leading to anoxic conditions in the saturated zone (Pescod, 1992). When most of the organic carbon is consumed at the soil water surface the remaining organic carbon is less biodegradable reducing the removal efficiency of SAT system (Chacha, 2007).

### 2.2.2 Effluent pre-treatment

Effluent pretreatment is a key factor that can be controlled as part of SAT system. The greatest impact of effluent pretreatment during SAT is the near soil/water interface where high biological activity is observed. This condition occurs because of both the highest concentration of biodegradable matter and the presence of oxygen. Effluent pretreatment directly impacts the concentration of biodegradable matter that is applied to a percolation basin. Both organic carbon and ammonia may be biologically oxidized and they are the water quality parameters controlling the amount oxygen demand in applied effluents. Near the soil water surface, biological activity with an effluent with high total oxygen demand will utilize all the dissolved oxygen. Aerobic conditions can be maintained with effluents that have low total oxygen demand. It should be noted that the majority of oxygen demand exerted during wetting is the oxidation of organic carbon and ammonia is removed by adsorption (Fox *et al.*, 2001).

### 2.2.3 Hydraulic parameters

The hydraulic parameters which have an effect on SAT systems include infiltration rate, permeability or porosity of the soil and hydraulic retention time.

#### *i. Infiltration rate*

Performance of SAT is significantly depends on the infiltration rate. The slower the infiltration rate the higher will be clogging rate of the system. On the other hand higher infiltration rate declines the quality of the reclaimed water. Reduction in infiltration rates leads to development of anaerobic conditions. This caused by physical clogging due to high-suspended solids (SS) concentration and biological clogging during long flooding or wetting period even under low SS concentration. Infiltration rate has a direct impact on retention time

and hence other DOC removal processes with the time dependant behaviour more pronounced up to 1.5 m depth in the vadose zone (Drewes and Fox, 1999).

Water quality transformations is consistent at sites with infiltration rates greater than 1.8 m/d and do not appear to be affected by variable infiltration rates. At a site with infiltration rates less than 0.3 m/d, wetting/drying cycles do not significantly impact infiltration since the clogging layer did not limit infiltration (Fox *et al.*, 2001a). Typical infiltration rates range from 0.5 to 3 m per day during flooding. Infiltration rates are site specific and are best evaluated on pilot basins or on actual systems. Schedule of flooding for optimum infiltration rates are developed by trial and error (Bouwer, 2002).

#### *ii. Permeability*

SAT systems require permeable soils to get water into the ground and to the aquifer. Permeability depends on type of soil and porosity. The size of pore space and interconnectivity of the spaces help determine permeability. It determines the hydraulic conductivity of the vadose zone and hence the retention time of the applied wastewater. As a result permeability affects the percolation through the unsaturated zone and the treatment processes after rapid infiltration from recharge basins (Fox *et al.*, 2005). Table 2.3 shows typical permeability values for different type of soil

**Table 2-3** Typical permeability values of the various soils

| Soil | Permeability (m/day) |
|---|---|
| Clay soils | <0.1 |
| Loams | 0.2 |
| Sandy loams | 0.3 |
| Loamy sands | 0.5 |
| Fine sands | 1.0 |
| Medium sands | 5.0 |
| Course sands | >10.0 |

Source: (Bouwer, 2002)

#### *iii. Hydraulic loading rate and detention time*

SAT depends on hydraulic conditions in removing contaminants from water and therefore detention times have significant effect on the treatment processes. Microbial activity in degradation of organic matter requires time for growth of microbial population under optimum conditions. Also nitrification-denitrification process and COD conversion will not be complete under short retention time. Removal of refractory organics usually requires long periods. An increase in hydraulic loading rate reduces the EBCT resulting in reduced

microbial formation of adsorbed DOC (Amy, 2005).

Typical hydraulic loading rate for SAT systems ranges from 0.1 to 3 m/day to achieve acceptable effluent quality depending on the hydro geological properties. The distance and travel times between recharge basins and wells should be at least 50-100 m and approximately 6 months for adequate SAT (Asano and Cotruvo, 2004). For the direct injection method a longer detention time is required -12 months but in practice typical detention time for SAT systems is given is this range < 6 to > 12 months.

### 2.2.4 Hydro-geological parameters

Surface infiltration systems require soils with high permeability in which water can infiltrate easily to reduce land requirements. Therefore, key parameters such as infiltration rate, retention time and soil properties are important for determining appropriate sites for artificial recharge of groundwater with this type of systems (Bouwer, 2002).

### 2.2.5 Water depth in the basin

The water depth in the infiltration basin should be kept relatively shallow. Small depths promote fast turnover of the wastewater in the basins during wetting time and minimize growth of suspended algae that can form a filter cake on the surface (Bouwer and Rice, 1984), which causes precipitation of calcium carbonate due to pH increases as the algae remove carbon dioxide from the water during photosynthesis or other biological activities. However, as the water depth is increased, the clogging layer is compressed and becomes less permeable (Bouwer *et al.*, 1984). As a result of this, the increase in infiltration rate may only be moderate or even a decrease. In other words the infiltration rate does not increase in direct proportion to increasing the water depth in the basin. Bouwer and Rice (1989) reported an increase in the hydraulic loading rate from 20 m per year to 100 m per year when the water depth was decreased from 1 m to 0.2 m in basins with fine loamy sand. Unlike this, results from their lab experiments reported an increase in the infiltration rate from 30 cm per day to 40 per cm day in one day as the water depth was increased from 20 cm to 85 cm in columns filled with the same loamy sand. This is a simple illustration how the system under field conditions behaves different from laboratory experiment outcome

### 2.2.6 Soil properties

Soil properties can affect infiltration rates, bacterial attachment, re-aeration rates and adsorption (Fox *et al.*, 2001a). Soils with high hydraulic conductivities provide high infiltration rates during the beginning of wetting cycles during SAT and infiltration rates decrease as clogging layers develop (Pescod, 1992). In addition surface infiltration system requires transmissive aquifers to get lateral flow away from the infiltration system without excessive groundwater mounding (Bouwer, 2002). The nature of soils beneath the vadose zone affects the filtration rate. Previous studies have shown that fine clay result in low

filtration rates (Fox *et al.*, 2001a).

Depending on the soil type, removal of DOC is rapid during percolation through the first 1.5 m (Quanrud *et al.*, 2003). Therefore, SAT technology can be applied in tertiary wastewater treatment without polluting the deeper soil layers. However, soil characteristics vary from place to place with the depth making the rational design and operation of SAT systems difficult since it would require detailed hydro geological investigations for each site.

## 2.3 Removal of different contaminants during SAT

SAT emerged as a natural system not only to solve the problems related to diminishing groundwater level, water intrusion in aquifers and subsidence, but also for playing an important role as a robust multi-barrier for indirect potable reuse due to its great potential on removing diverse classes of contaminants through several physic-chemical processes in the vadose and saturated zone with simplicity in operation and low treatment costs (Amy and Drewes, 2007).

SAT system consists mainly in spreading wastewater effluents in a recharge basin that infiltrate through the upper layers (unsaturated zone) where several mechanisms take place (chemical precipitation, adsorption, biological degradation, nitrification and denitrification, among others) followed by mixing with groundwater and a subsequent recovery by wells (Amy and Drewes, 2007). Through these mechanisms it is possible to achieve considerable removal rates on organic compounds, pathogens and potentially other contaminants such as pharmaceutically active compounds (PhACs) and endocrine disrupting compounds (EDCs) present in the wastewater. Table 2-4 shows the typical removal efficiency of SAT systems for different contaminants.

**Table 2-4:** Typical removal efficiency of SAT systems for different contaminants.

| **Parameter** | **Typical range** |
|---|---|
| DOC (SE) | 10-94% |
| DOC (PE) | 12-62% |
| Total Nitrogen | 25-90% |
| Phosphorous | 70-99% |
| Pathogens | 4-6 log removal |
| $NH_4$-N | >98% |
| $NO_3$-N | 20-70% |

Sources: (Sharma *et al*. 2007; Fox *et al*. 2001; Idelovitch *et al* 2003)

### 2.3.1 Influence of temperature on removal of contaminants during SAT

#### *i. General overview*

The temperature of water is very important parameter because of its effect on chemical reaction and reaction rates, aquatic life and the suitability of the water and for beneficial uses. Increased temperature, for example can cause a change in the species of fish that can exist in the receiving water body. In addition, oxygen is less soluble in warm water than in cold water. The increase in the rate of biochemical reactions that accompanies an increase in temperature, combined with the decrease in quantity of oxygen present in surface waters, can often cause serious depletion in dissolved oxygen concentration in summer months. When significantly large quantities of heated water are discharged to natural receiving waters, these effects are magnified. It should also be realized that a sudden change in temperature can result in a high rate of mortality of aquatic life. Moreover, abnormally high temperatures can foster the growth of undesirable water plants and wastewater fungus (Metcalf and Eddy, 2004).

Physical factors that affect SAT processes include temperature, pH, oxygen concentration and Electrical conductivity. The DOC increases with increasing temperature. Increase in temperature increases the microbial activity resulting in a fast biodegradation process (Sharma *et al.*, 2007). Based on capillary theory, it is assumed that the influence of temperature on the soil water pressure head can be quantitatively predicted from the influence of temperature on surface tension. The temperature dependence of the hydraulic conductivity can be expressed as

$$\boldsymbol{K_{T_S}(h) = \alpha^*_k K_{T_{ref}}(h)} \qquad 2\text{-}1$$

where $\boldsymbol{K_{T_S}}$ $\boldsymbol{and}$ $\boldsymbol{K_{T_{ref}}}$ denote hydraulic conductivities at the reference temperature and soil temperature respectively and $\alpha^*_k$ is the temperature scaling factor for the hydraulic conductivity. This factor depends on the dynamic viscosity $\mu$ [M/TL] and density $\rho$ [M/L$^3$] of soil water at temperatures $\boldsymbol{T_S}$ $\boldsymbol{and}$ $\boldsymbol{T_{ref}}$

$$\boldsymbol{\alpha^*_k = \frac{\mu T_{ref}}{\mu T_s} \times \frac{\rho T_s}{\rho T_{ref}}} \ldots \qquad 2\text{-}2$$

Infiltration rate vary inversely with water viscosity this implies temperature affect infiltration rate (Bouwer, 2002). In recharge basins decrease in infiltration rates is caused by physical and biological clogging. Infiltration rate has direct impact on retention time and hence DOC removal (Drewes and Fox, 1999). On the other hand groundwater table also affects the infiltration rates. When the water table is more than about 1.0 m below the bottom of the recharge basin the infiltration rates are not affected by the changing water levels. If water table rises to less than 1.0 m below the bottom of the recharge basin the infiltration rates decreases linearly with decreasing depth (Bouwer, 2002). Seasonal effects affects infiltration rate, infiltration rate in winter are often less than in summer due to cooler water with higher

viscosity.

Filtration is better at high temperatures because viscosity is low and the van der Waals forces are greater, therefore temperature increases removal efficiency of contaminants during SAT. According to Abushbak, 2004 the optimal temperature for nitrification was investigated in the lab by numerous researchers (Kowalenko & Cameron, 1976; Kassman & Munns, 1980; Malhi & McGill, 1982) and the variation in the predicted optimal temperature, ranged from 20 to 30$^{o}$C, can be attributed to the varying in the climatic conditions of the soil, in which nitrifiers and denitrifiers are adapted to those conditions.

On the other hand at any given temperature, there is a specific concentration of a dissolved mineral's constituents in the groundwater that is in contact with that mineral. The actual concentration is temperature dependent, e.g., at higher temperatures, groundwater can dissolve more of the mineral. Even changes in groundwater temperature of only 5 to 10$^{o}$ C can cause detectable changes in TDS (Nelson, 2002).

### *ii. Temperature influences on nitrate removal.*

Higher temperatures enhance biological and chemical reactions including denitrification. Indeed at all flow rates and in all soil horizons, increasing temperature decreased effluent $NO_3^-$ concentrations. For the surface horizons, temperature had no visible effect on effluent concentration at the lowest flow rates, given an influent concentration of 9.2 mg/L. At the lower flow rates, the denitrification capacity in the surface horizons is sufficient to remove all incoming $NO_3^-$ (9.2 mg/L) even at the lower temperatures. Effluent $NO_3^-$ concentration in both subsurface soils was influenced by temperature at all flow rates, except at the low temperature high flow rate combinations where no $NO_3^-$ removal was predicted and temperature effects could not be observed. The effect of temperature on denitrification rate is highest at the higher flow rates.(Willems *et al.*, 1997)

### *iii. Retention and removal of pathogenic bacteria in wastewater percolating through porous media*

Survival of bacteria decreases with increasing temperature. Incubation of *E. coli* in soil at 5$^{o}$C, 10$^{o}$C, 20$^{o}$C and 37$^{o}$C showed best survivals at 5$^{o}$C. Similar results were observed in an experiment with *E-coli* and *Salmonella typhimurium*. In a survival experiment with Pseudomonas species in soil, Vandenhove found no difference between 5$^{o}$C and 15$^{o}$C, but a significant reduction of the bacterial number at 25$^{o}$C.

Inactivation of microbial cells moving through a porous media is influenced by mechanisms such as physical straining as well as adsorption to porous media. The grain size of porous media and bacterial cell size are important factors affecting the straining of bacteria, as are the hydraulic loading rate or the extent of clogging layer development in the filter. Adsorption of cells to the porous media is influenced by the content of organic matter, degree of biofilm

development, and electrostatic attraction due to ion strength of the solution or electrostatic charges of cell- and particle surfaces.

The rate of inactivation of pathogenic microorganisms, in adsorbed or liquid phases, has been shown to be affected by abiotic and biotic factors such as moisture content, pH, temperature, organic matter, bacterial species, predation, and antagonistic symbiosis between microorganisms in the system. The adsorption of bacteria was substantially greater at higher temperatures. Studies of marine Pseudomonads showed that at a temperature of 3$^{o}$C, the proportion of bacteria attached to polystyrene was decreased compared to that at 20$^{o}$C. The reduction in attachment with decreasing temperature may have several causes: (a) enhancement in the viscosity of the bacterial surface polymer and of the liquid, (b) reduced chemisorption and certain types of physical adsorption and (c) changes in the physiology of the organisms (Kristian Stevik *et al.*, 2004)

### *iv. Optimum temperature for biological activity*

The optimum temperatures for bacterial activity are in the range from 25 to 35$^{o}$C. Aerobic digestion and nitrification stops when temperature rises to 50$^{o}$C. When the temperature drops to about 15$^{o}$C methane-producing bacteria become quite inactive, and at about 5$^{o}$C, the autotrophic-nitrifying practically cease functioning. At 2$^{o}$C even chemoheterotrophic bacteria acting on carbonaceous material become essentially dormant (Metcalf and Eddy, 2004).

The temperature dependence of the biological reaction- rate constants is very important in assessing the overall efficiency of a biological treatment process. Temperature not only influences the metabolic activities of microbial population but also has a profound effect on such factors as gas- transfer rates and settling characteristics of the biological solids (Metcalf and Eddy, 2004).

The effect of temperature on the reaction rate of a biological process is expressed using the following equation

$$K_T = K_{20}\theta^{T-20} \qquad 2\text{-}3$$

Where $K_T$= reaction-rate coefficient at T, ℃

$K_{20}$=reaction rate coefficient at 20℃

$\theta$ = Temperature activity coefficient

T=Temperature, ℃

### *v. Effect of temperature on removal of DOC, Phosphorous and Nitrogen*

It can be observed that generally for all pollutants, lower removal efficiencies correspond to

lower temperatures and the opposite. For BOD and COD, the temperature dependence is not so significant which implies that the removal of the organic matter is mostly a result of the microbial activity of aerobic and anaerobic bacteria which function even in temperatures as low as 5$^{o}$C. Porous media keeps the wastewater temperature in the winter higher than the air temperature by 2–3$^{o}$C, allowing thus the microbial activity to continue functioning. Phosphorus removal also shows dependence on temperature. There can be seasonal variations and the negative values in phosphorus removals that could be explained by the fact that during winter litter and microbial biomass are decomposed, and phosphorus is released from the precipitates, resulting in phosphorus solubilization in water (Akratos and Tsihrintzis, 2007).

For TKN and ammonia the dependence of removal efficiency on temperature is much more significant, because the microorganisms responsible for nitrogen removal function optimally in temperatures above 15$^{o}$C. The conversion between volatile ammonia and ammonium ions strongly depends on the pH and temperature. At lower pH and temperature levels, the conversion decreases significantly. For a normal condition of 25$^{o}$C and a pH of 7, non ionized ammonia amounts only to 0.6% of the total ammonia present. At a pH of 9.5 and a temperature of 30$^{o}$C, the percentage of total ammonia present in the non-ionized form increases to 72%. Under aerobic conditions, ammonium is oxidized by microorganisms to nitrate, with nitrite as an intermediate product. Temperature has different effects on the growth rate of ammonium and nitrite oxidizers. Only at temperatures above 25$^{o}$C it is possible that the ammonium oxidizers can effectively out-compete the nitrite oxidizers. If this condition is impaired with a low hydraulic retention time and, also a low cellular retention time, nitrite oxidizers can be selectively washed out (Paredes *et al.*, 2007).

**Table 2-5**: Effect of pH, temperature, free ammonia and nitrous acid on nitrification process.

| **Factor** | **Effect** |
|---|---|
| Temperature | |
| T > 15 °C | Ammonium oxidizers grow faster than nitrite oxidizers. |
| T = 25 °C | Ammonium oxidizers can out-compete nitrite oxidizer. |
| pH | |
| 7.0–8.0 | Optimum range for nitrification. |
| 7.9–8.2 | Optimum range for ammonium oxidizers (Nitrosomas). |
| 7.2–7.6 | Optimum range for nitrite oxidizers (Nitrobacter). |
| Free $NH_3$[mg/L] | |
| 150 | Inhibition of ammonium and nitrite oxidizers. |
| 1.0–7.0 | Inhibition of ammonium oxidizers and nitrite accumulation. |
| Long-term . | Nitrite oxidizers (pure cultures of *Nitrobacter* and mixed cultures in biofilms) can be adapted to high free ammonia concentration (40 mg/L) and nitrite accumulation is reduced |
| HNO2 [mg/L] | |
| > 2.8 | Inhibition of ammonium and nitrite oxidizers. |

Source (Paredes *et al.*, 2007).

### 2.3.2 Influence of redox conditions on removal of contaminants during SAT

i. ***General overview***

Redox reactions result in a change of the charge of an ion as it gains or losses an electron. These reactions are almost always facilitated by bacteria that are able to gain energy from the reactions. The solubility of some elements in water depends on whether they are oxidized or reduced. The redox potential can be correlated with the amount of dissolved oxygen. As the oxygen content drops, the environment becomes more reducing (the redox potential drops). The natural environment, therefore, may control in which state the element occurs. For example, iron (Fe) can exist in either as reduced *($Fe^{2+}$)* or oxidized *($Fe^{3+}$)* iron *(Nelson, 2002).*

The most common cause of reducing reactions is organic matter, either in solid form or as DOC. The degradation of oxidisable organic substances is generally mediated by microbial metabolism and is coupled to the reduction of terminal electron acceptors (TEA) such as $O_2$, $NO_3^-$ , Mn- and Fe- *(hydr)* oxides and $SO_4^{2-}$. The TEAs are typically consumed in a sequential order, based on thermodynamic principles. This sequence generally leads to the formation of distinct redox zones along the flow direction. The degradation reactions might trigger further geochemical reactions, which in turn can have a considerable impact on the water quality and on the aquifer matrix, including changes of hydrogeological properties such as porosity and hydraulic conductivity as well as geochemical properties such as mineral reactivity and sorption capacity (Greskowiak *et al.*, 2005).

Dissolved oxygen and redox potential are important parameters for characterizing biologically mediated reactions during SAT. Changes in redox potential directly influence biological reactions. Aerobic, anoxic and anaerobic conditions have been observed during SAT. Aerobic conditions promote the degradation of organic carbon and nitrification. Anoxic conditions promote carbon degradation and denitrification with nitrate as the electron acceptor. Anaerobic conditions promote carbon degradation with other electron acceptors such as sulfate. Changes in redox potential are dependent on the availability of oxygen and alternate electron acceptors (Fox *et al.*, 2001). Mass transfer limitation created by operating wet/dry cycles, soil properties and hydrogeology all affect changes in redox potential.

Typically in groundwater, sulfur exists as sulfate ($SO_4^{-2}$). In this form, it generally has little impact on the potability of groundwater (an exception is at much higher concentrations, i.e.

250 mg/L, sulfate may have a laxative affect on some individuals). If, however, the sulfur is in the form of hydrogen sulfide ($H_2$ S), the distinct and unpleasant rotten egg odor occurs even at concentrations below 10 mg/L. Redox reactions can change sulfate to hydrogen sulfide in oxygen-poor environments in the presence of organic matter or DOC. With decreasing redox potential the following reducing reactions will occur: nitrate to nitrogen gas, $Fe^{3+}$ (insoluble) to $Fe^{2+}$ (soluble), sulfate to hydrogen sulfide and, at very low redox potential,

methane formation (Nelson, 2002).

The following major redox zones can be distinguished:

1. Aerobic respiration zone

$$CH_2O + O_2 \rightarrow CO_2 + H_2O \qquad 2\text{-}4$$

2. Anoxic $Fe^{3+}$, $Mn^{4+}$, $NO^-_3$ reduction zone

$$CH_2O + 8H^+ + 4Fe(OH)_3 \rightarrow 4Fe^{2+} + 11H_2O + C0_2 \qquad 2\text{-}5$$

$$CH_2O + 2MnO_2 + 4H^+ \rightarrow 2Mn^{2+} + 3H_2O + C0_2 \qquad 2\text{-}6$$

$$CH_2O + 4/5H^+ + 4/5NO^-_3 \rightarrow 2/5N_2 + 7/5H_2O + C0_2 \qquad 2\text{-}7$$

3. $SO_4^{2+}$ reduction zone

$$CH_2O + 1/2H^+ + 1/2SO^{2-}_4 \rightarrow 1/2HS^- + H_2O + C0_2 \qquad 2\text{-}8$$

4. $CH_4$ fermentation zone

$$CH_2O + 1/2CO_2 \rightarrow 1/2CH_4 + C0_2 \qquad 2\text{-}9$$

The main part of the microbial community lives within the aerobic zone, and these organisms are primarily responsible for the degradation of organic material. The anoxic zone is characterized by the intense use of alternative electron acceptors and processes like $Fe^{3+}$, $Mn^{4+}$, $NO_3^-$ reduction as well as denitrification. The toxic $SO_4^{2+}$ reduction as well as $CH_4$ fermentation zones with the formation of $H_2S$, $S^{2-}$, and $CH_4$ cannot be tolerated by the benthic community, and thus are unacceptable from the ecological point of view as well as for the drinking water purification process (Gross-Wittke *et al.*, 2010).

Redox conditions govern the particulars for the degradation of constituents present in effluents during SAT. Aerobic conditions only develop in the top 0-1.5 m of the vadose zone at the end of drying cycles. Dissolved oxygen is normally removed during wetting and pore gas oxygen utilized for nitrification during drying. Under aerobic conditions, the nitrification will take place in an unsaturated zone and part of the nitrogen adsorbed on soil particles undergoes nitrification (Fox *et al.*, 2001a).

Changes in reduction/oxidation (redox) potential during SAT are most likely to occur in the vadose zone and can also occur to a lesser extent in the saturated zone. The redox potential decreases as microorganisms utilize electron acceptors during SAT. Typically, oxygen is present in reclaimed water applied to percolation basins. Algae in infiltration basins may

produce additional oxygen. As water percolates through the vadose zone, dissolved oxygen can become rapidly exhausted and the redox potential decreases. Nitrate becomes the next electron acceptor as the redox potential decreases followed by iron or manganese. When no nitrate is present, sulfate-reducing conditions may develop (Fox *et al.*, 2001).

The operation of SAT systems with wet/dry cycles is normally meant to control development of clogging layers at the infiltration interface and maintain optimal infiltration rates, and in some cases disrupt insect life cycles. The drying cycle allows for desiccation of the clogging layer and the recovery of infiltration rates during the next wetting cycle (Pescod, 1992). Wet/dry cycles control redox conditions in the subsurface. Aerobic condition during dry cycle allows oxygen to penetrate to greater depths as drying time is increased. Wet/dry cycle have important effect on nitrogen transformation. Increasing wet cycle increase the depth at which ammonia is adsorbed while increasing dry cycle times increase the depth at which ammonia is nitrified (Pescod, 1992).

With sufficient organic carbon nitrate is converted to nitrogen gas. Denitrification occurs under anoxic conditions and has been reported to occur in limited anaerobic pockets in the aerobic zone making it localized and partial (Idelovitch *et al.*, 2003). In the first application cycle, ammonia is adsorbed to soils during the early part of flooding period after which soil microbes convert ammonia to nitrate under aerobic conditions in the drying period. Nitrifying and denitrifying bacteria are common soil organisms that play a big role in the nitrogen cycle (Fox *et al.*, 2001).

The large amount of adsorbed ammonia prevents pore gas oxygen concentrations from increasing before the next wetting cycle. In cases where the effluent is fully nitrified/denitrified, anoxic conditions develops at a depth of 3 m. Although the majority of oxygen demand is removed in the upper vadose zone during SAT, the total oxygen demand of the applied effluents influences the redox conditions in the saturated zone. If all dissolved oxygen is removed during percolation through the vadose zone, anoxic conditions are likely to develop in the saturated zone since other mechanisms for oxygen transport to the saturated zone are insignificant. The potential for re -aeration of water percolating through deep vadose zones (15-30 m) does exist since the majority of oxygen demanding material is removed in the upper vadose zone (Fox *et al.*, 2001a).

Removal efficiency of Kjeldahl nitrogen and ammonia has been found to reduce after some few years of SAT operation. This has been attributed to the formation of anaerobic conditions in the soil-aquifer system preventing ammonia to nitrate conversion process since growth of nitrifyers requires oxygen. In comparison, the removal efficiency of phosphorous remains stable to even values less than 0.2 mg/L, which indicates high efficiency (Idelovitch *et al.*, 2003).

Anaerobic conditions can develop in the soil-aquifer system as a result of reduction of

infiltration capacity in the upper vadose zone and of lack of proper maintenance and cleaning of the superficial surface of infiltration basis coupled with prolonged wetting or flooding period (Idelovitch *et al.*, 2003). Oxygen concentration in the applied treated wastewater is also important in maintaining aerobic conditions in the soil-aquifer system. The diffusion process dependant on surface area and temperature may also be a major factor during drying and wetting periods.

ii. ***Saturated and unsaturated hydraulic conditions***

The formation of a clogging layer at the bottom of the infiltration basin appears to be the key control on the entire hydraulic system and is responsible for the development of an unsaturated zone below the recharge basin. The development of a clogging layer in recharge basins is a well-known characteristic that can reduce the infiltration rate to as little as 10% of the original infiltration rate. Clogging is caused by a combination of physical (filtration), chemical (precipitation of minerals) and biological (growth of microorganisms, production of polysaccharides) effects which can reduce the sediment's hydraulic conductivity by several orders of magnitude. The hydro chemical characteristics, which develop under either saturated or unsaturated conditions, are predominantly driven by the presence or absence of atmospheric $O_2$. As soon as $O_2$ is completely depleted, successively more reducing conditions developed directly below the recharge basin, including Fe-reducing conditions. During unsaturated conditions, the overall redox environment is controlled by the presence of atmospheric $O_2$ that had intruded from the recharge basin margins. Aerobic conditions became dominant below the recharge basin, except in the vicinity of some suction cups, where $NO^-_3$ -reducing conditions were observed. This means, that the prevailing hydraulic regime controls the overall redox conditions below the basin. Thus, the highly dynamic changes in the redox environments result from the transient hydraulic behavior of this recharge system (Greskowiak *et al.*, 2005)

The development of more reducing conditions appears to have been confined to a narrow zone of approximately 1 m directly below the recharge basin, while less reducing conditions were observed at greater depths and in the groundwater. Distribution of organic matter, the development of distinct and localized redox environments result from both the heterogeneous distribution of sedimentary organic matter and the variability of its degradability (Greskowiak *et al.*, 2005).

### iii. *DOC removal at oxic and anoxic conditions*

Bulk organic matter (DOC) removal is often the main parameter of interest during SAT as it influences the removal of nitrogen species and trace organics by biodegradation. When secondary effluent from wastewater treatment plants were used, in the case of lab-scale soil column studies, DOC removal efficiency ranged from 10% to 73% while for field studies it ranged from 55% to 94%. Regardless of the operating conditions and soil type, when the residence time was more than 30 days, DOC removal was always >80%. However when the residence time was <10 days, DOC removal varied from 30 to 90% depending upon the influent DOC, redox conditions and soil type. It was also found that the average DOC concentrations of SAT product water in field sites were <2 mg/L for both long term SAT of secondary and tertiary effluents. It clearly showed that tertiary treatment before SAT may not be necessary from the organic matter removal point of view as the effluent is comparable to that obtained by SAT of secondary effluent (Sharma *et al.*, 2007).

**Influence of redox conditions on DOC removal during SAT of secondary effluent**

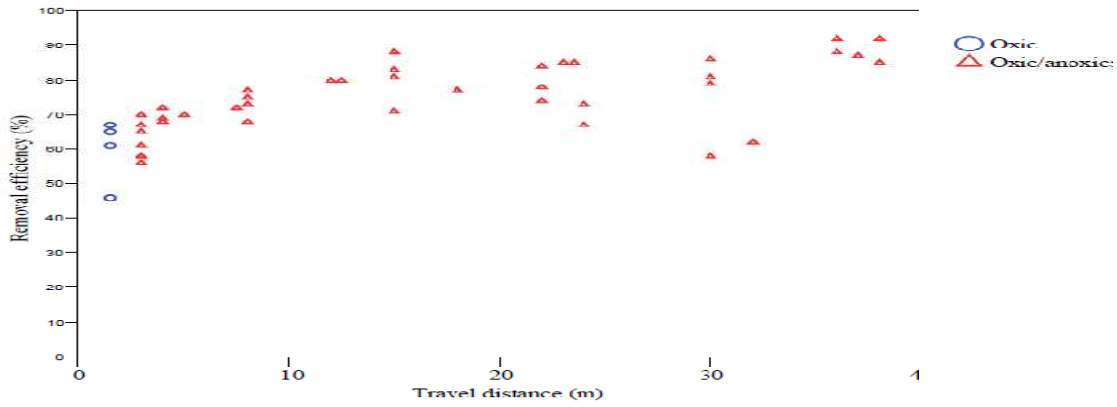



**Figure 2-2**: DOC removal efficiency versus travel distance in the vadose zone during SAT of secondary effluents.

Source: (Sharma *et al.*, 2007).

Figure 2.2 shows that up to about 70% DOC removal is achieved at a travel distance of 1.5 m which is predominantly oxic. Depending on operational schedule (wet and dry cycles) and temperature, oxic condition may extend up to 3 m in depth. After oxic zone, reclaimed water is further purified under anoxic conditions. This suggests that in order to get better DOC removal efficiency, oxic conditions should be maintained up to about 2.0 m below the surface of recharge basin. Table 2.6 presents the analysis of DOC removal from secondary effluents at different depths of aquifer in soil/vadose zone and saturated zone. DOC attenuation under oxic conditions ranged from 10% to 72% while under anoxic/oxic conditions it ranged from 56% to 94%. Most contaminants are significantly removed during percolation of reclaimed water through the vadose zone (Sharma *et al.*, 2007).

**Table 2-6**: Analysis of DOC removal data for SAT systems treating secondary effluents at different redox conditions

Source:(Sharma *et al.*, 2007)

| | **Travel distance (m)** | **Redox conditions** | **Removal efficiency (%)** | | **Standard deviation (%)** | **No. of results** |
|---|---|---|---|---|---|---|
| Vadose | | | Range | Average | | |
| zone | 1-2 | Oxic | 46-68 | 59 | 10 | 6 |
| | 2-10 | Oxic/Anoxic | 56-70 | 63 | 6 | 8 |
| | 10-20 | Oxic/Anoxic | 72-73 | 73 | 1 | 2 |
| | 20-40 | Oxic/Anoxic | 77-88 | 77 | 12 | 9 |
| Saturated zone | >40 | Oxic/Anoxic | 77-94 | 83 | 8 | 7 |

## 2.4 Performance of SAT systems in water quality improvement

When the raw water or wastewater moves through the soil to the groundwater one or more of the following mechanisms take place (Abushbak, 2004).

- Mechanical Straining is the filtering action of the soil.
- Sedimentation results in the retention of the suspended solids smaller than those retained by mechanical straining.
- Adsorption is regarded as the most important purification process during infiltration and percolation.
- Biochemical and bacterial activity involves decomposition of organic matter, build up of an organic filter-skin. This will lead to improve the quality of the percolating water.

For a long-term performance of SAT system, there has to be a balanced interaction between the process conditions. This is attributed to the long term forecast based on the following factors identified by (Idelovitch *et al.*, 2003).

- Proper maintenance of infiltration basins prolongs filtration for removal of suspended solids.
- Biodegradation and nitrification-denitrification for removal of organic matter and nitrogen last forever under optimal conditions for bacterial activity provided a balance between aerobic and anaerobic conditions is maintained.
- This can be achieved by proper design of the SAT systems for wetting and drying cycles.

- Adsorption and chemical precipitation for removal of phosphorous, heavy metals and trace elements depend on the adsorption/precipitation capacity of the soil matrix which is time limited

### 2.4.1 Pathogen removal

*Escherichia coli (E-coli)* are the most common member of fecal coliform bacteria, indigenous to the intestinal tract of humans or other warm-blooded animals. The US Environmental Protection Agency (EPA) recommended that *E-coli* is a better indicator of fecal pollution than fecal coliform for purposes of evaluating ambient freshwater quality. The presence of *E. coli* in drinking water indicates that the water is contaminated by fecal material of humans or other warm-blooded animals, and also indicates the potential for the presence of pathogenic organisms. The source of *E-coli* contamination in surface water includes municipal wastewater discharges, septic leachate, agricultural or storm runoff, wildlife populations, or nonpoint sources of human and animal waste (An *et al.*, 2002).

The use of treated municipal wastewater effluent (reclaimed water) to replenish ground water supplies through artificial recharge is increasing all over the world. The fate of microorganisms and the ability to assess potential contamination and assure the microbiological quality of ground water is critical to protecting public health and obtaining public and regulatory acceptance of managed ground water recharge programs. The length of time that viruses and viral genetic material may persist in groundwater is important to planning and designing recharge projects and understanding the additional health protection provided by soil filtration (Fox *et al.*, 2001).

One of the principal issues associated with indirect potable reuse are potentially harmful microorganism that pass through conventional and advanced water treatment systems (Drewes *et al.*, 2003). Microbial activity in the subsurface is mainly driven by the net decomposition of organic matter, which provides microorganisms with the energy, nutrients and electrons they need to synthesize biomass and sustain life functions (Hunter *et al.*, 1998) as cited by Chacha (2007).

The removal of pathogens in a SAT system depends on a variety of factors including the physical and chemical characteristics of the soil, the size and nature of organisms, and the environmental conditions of the soil whether aerobic or anaerobic. The removal of virus is controlled by number of factors, the key factors are type of soil, type of virus, infiltration rate and the degree of soil saturation (Quanrud *et al.*, 2003). Bacteria are also filtered but in addition it may be adsorbed to the soil solid material during the wetting time and decay during the drying time. It was observed that SAT system can remove fecal coliform effectively up to 99% by filtration in the upper few centimeters of the soil profile. Viruses are too small to be filtered by most soil pores and it is removed only by sorption and decay, but still they are effectively removed. It was observed that high virus removal occurred after low infiltration

rates were achieved in the system. This may take place after the development of the clogging layer, which leads to increase the retention time. The lowest removal was observed when infiltration was high and retention time low (Abushbak, 2004).

Although theoretical considerations strongly suggest that *coliphage* may represent the best choice for a microbial indicator of fecal contamination in groundwater, field studies have not yet provided substantial evidence to support the selection of any single indicator. USEPA is currently examining *E-coli, Enterococci* and *coliphage* as potential indicators of fecal contamination in groundwater for the developing groundwater rule. Current microbial standards for drinking water in a distribution system are based on the *total coliform* rule. Current thought, however, is that *total coliforms* lack adequate specificity to be an appropriate indicator of fecal contamination in groundwater. For similar reasons, *clostridium perfringens*, another potential indicator of fecal contamination that has been used more often in some European countries, was also ruled out as a potential indicator of groundwater contamination. Nevertheless, there is not adequate data available to support the acceptance or rejection of any potential microbial indicator to detect fecal contamination in groundwater (Fox *et al.*, 2001).

### 2.4.2 Nitrogen removal

#### *i. Nitrification*

This is an important biological process in wastewater treatment which occurs by two step oxidation of ammonia. There are a number of autotrophic nitrifying bacteria that perform nitrification but the most important genera are *nitrosomonas* and *nitrobacter* (Metcalf and Eddy, 2004) Ammonia oxidation is an aerobic process that requires oxygen. The two step reaction starts with oxidation of ammonia to nitrite by *nitrosomonas* species followed by conversion of the nitrites to nitrates by *nitrobacter* species.

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + \quad 4H^+ + H_2O \qquad 2\text{-}10$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad 2\text{-}11$$

The nitrates formed can be assimilated by other organisms or can be denitrified to dinitrogen gas. Besides denitrification, nitrates can also be transformed to other nitrogen forms known as assimilatory nitrate reduction and dissimilatory nitrate reduction. Assimilatory nitrate reduction entails the reduction of nitrate to ammonia by bacteria. For nitrification to occur, sufficient oxygen must be present. For complete oxidation of 1g of $NH_4^+$-N, 4.57g of oxygen and 7.14g alkalinity (Calcium Carbonate) is required. Other factors that affect nitrification process include temperature, pH, BOD, toxic compounds and high concentration of other forms of nitrogen (Metcalf and Eddy, 2004).

### *ii. Denitrification*

This is the biological reduction of nitrate to nitric oxide, nitrous oxide and nitrogen gas by microorganisms. It is a vital process in wastewater treatment plant where prevention of eutrophication and $NO_3$-N pollution of ground water is required. In nature, denitrification is an important process because it closes the loop of the nitrogen cycle. Without this process the atmospheric nitrogen would be depleted. Denitrification is favored in the absence of oxygen (anoxic or anaerobic) although most denitrifiers are facultative (Metcalf and Eddy, 2004).

### *iii. Removal of Nitrogen during SAT*

The total nitrogen levels in conventionally treated domestic wastewater are on the order of 20 mg-N/L unless effluent is at least partially nitrified. The dominant nitrogen species are ammonium ion and organic nitrogen. During SAT nitrogen conversions tend to produce oxidized nitrogen forms (predominantly nitrate) that may have groundwater quality implications (Fox *et al.*, 2001). Nitrogen removal from the percolated water in the SAT system is important because nitrogen may contaminate the groundwater and cause serious health problems if consumed by humans. Biological denitrification has received much attention as a method of removing nitrogen because it returns nitrogen to the atmosphere as inert $N_2$ gas. The main difficulty in using denitrification is that N in the applied wastewater is mostly in the $NH^+_4$ and organic N forms, which must be first oxidized to $NO_3$ before denitrification can proceed (Abushbak, 2004).

Nitrogen is very important in wastewater management because nitrogen has many effects in environment like eutrophication, oxygen depletion and toxicity. Ammonia is extremely toxic to fish and other aquatic organism, it is also oxygen consuming compound which can deplete dissolved oxygen in aquatic environment (Chacha, 2007).

Suspended solids are removed by filtration through the upper soil layer and the largest part is of organic nature in form of volatile suspended solids. Particulate kjeldahl nitrogen is also removed by filtration in the upper soil layer and the dissolved part by adsorption onto soil particles. Ammonia is removed by adsorption and nitrification-denitrification biological process while phosphorous is removed through chemical precipitation and adsorption (Idelovitch *et al.*, 2003).

Nitrogen removal has been observed during SAT at many sites recharging effluent containing ammonia-nitrogen. A common hypothesis for this nitrogen removal is the two-step process of autotrophic nitrification and heterotrophic denitrification. Recharge basins are typically operated to consist of a wetting cycle when water is applied followed by a drying cycle. Due to the net positive charge of the ammonium ion, it is adsorbed onto the soil in the upper region of the vadose zone during the wetting cycle. During wetting, oxygen is not available for nitrification. As the soil dries and air/oxygen enters the soil, the oxidation of ammonia to

nitrate by autotrophic nitrifiers may occur. This process results in a high nitrate concentration at the beginning of the following wetting cycle. This nitrate, which tends to be more mobile, is transported with the water deeper into the vadose zone. Once the nitrate reaches an anoxic zone, heterotrophic denitrification may convert the nitrate to nitrogen gas in the absence of oxygen and in the presence of an organic carbon electron donor (Fox *et al.*, 2001).

In order to achieve denitrification, wetting period must be long enough to obtain anaerobic conditions that allow adsorption of the ammonium to the clay particles. The adsorbed ammonium will be nitrified under aerobic conditions during drying part of cycle while at the same time the nitrate will be denitrified in micro anaerobic zones present in the aerobic vadose zone. At the beginning of the wetting time of the basin sufficient oxygen is available. This leads the organic matter (usually stated as $C_5H_7O_2N$ to be degraded aerobically to form ammonium as described by the reaction:

$$C_5H_7O_2N + 5O_2 \rightarrow NH_4^+ + \quad 4CO_2 + H_2O + HCO_3 \qquad 2\text{-}12$$

Amount of produced and original $NH_4^+$ is adsorbed to the clay particles in the soil. The amount to be adsorbed depends on the cation exchange capacity of the soil. As the drying process starts, oxygen from the atmosphere starts to enter the soil to create the aerobic conditions in the system. The adsorbed ammonium is oxidized by the *nitrosomonas* and *nitrosococcus* bacteria (nitrifying bacteria) as described by the chemical reaction:

$$NH_4^+ + 3/2O_2 + H_2O \rightarrow NO_2^- + \quad HCO^+{}_3 \qquad 2\text{-}13$$

The total nitrification of $NH_4^+$ during the aerobic or drying time is desirable to promote the denitrification process during subsequent anaerobic or wetting time and for avoiding $NH_4^+$ accumulation in the soil. This accumulation can happen if the amount of $NH_4^+$ applied during the wetting time exceeds that which can be nitrified during the following dry time. This causes a subsequent increase in the $NH_4^+$ content of the water during the next wetting time.

The formed nitrite $NO_2^-$ in soil environment is so quickly oxidized to nitrate $NO_3^-$ that it rarely accumulates: In most soils $NO^-{}_3$ is not adsorbed to the clay particles. It moves readily with the soil solution. If large quantities of wastewater are applied to the land $NO_3^-$ will move downward and may eventually reach the groundwater. However, whether $NO_3^-$ is formed from oxidizing $NH_4^+$ or is initially present in the wastewater, it is subject to denitrification under the denitrification conditions of the soil which may prevent at least some of it from moving downward:

◄

$$5CH_2O + 4H^+ + 4NO_3^- \rightarrow 5CO_2 + 7H_2O + N_2 \uparrow \quad 2\text{-}14$$

This mechanism of denitrification is known as heterotrophic denitrification. When sulfide, iron, or manganese replaces organic carbon as the electron donor the mechanism for denitrification is known as autotrophic. Both mechanisms were found to take place when oxygen levels are limited, but heterotrophic mechanism was found to be the dominant in the SAT systems. Therefore, denitrifiers prefer very wet soil conditions, where there is available organic carbon. The un-denitrified $NO^-_3$ from this process will leach out to the groundwater by the next wetting process. The remaining nitrate that may leach out during the wetting period of the following cycle will be removed with distance of travel and dilution in aquifer(Abushbak, 2004). Under the same studies, it was also found out that most nitrogen transformations take place in the upper 1m of the vadose zone.

**Table 2-7:** The effect of the wetting time on the nitrogen percent removed by soil column with 5 days drying.

| **Wetting days** | **N in sewage (mg)** | **N in column effluent (mg)** | **N removed ( %)** |
|---|---|---|---|
| 2 | 1641.5 | 1714.8 | -4 |
| 9 | 4298.1 | 3108.9 | 28 |
| 16 | 6811.2 | 4547.3 | 33.2 |
| 23 | 9893.4 | 6685.7 | 33.9 |

Source: (Abushbak, 2004).

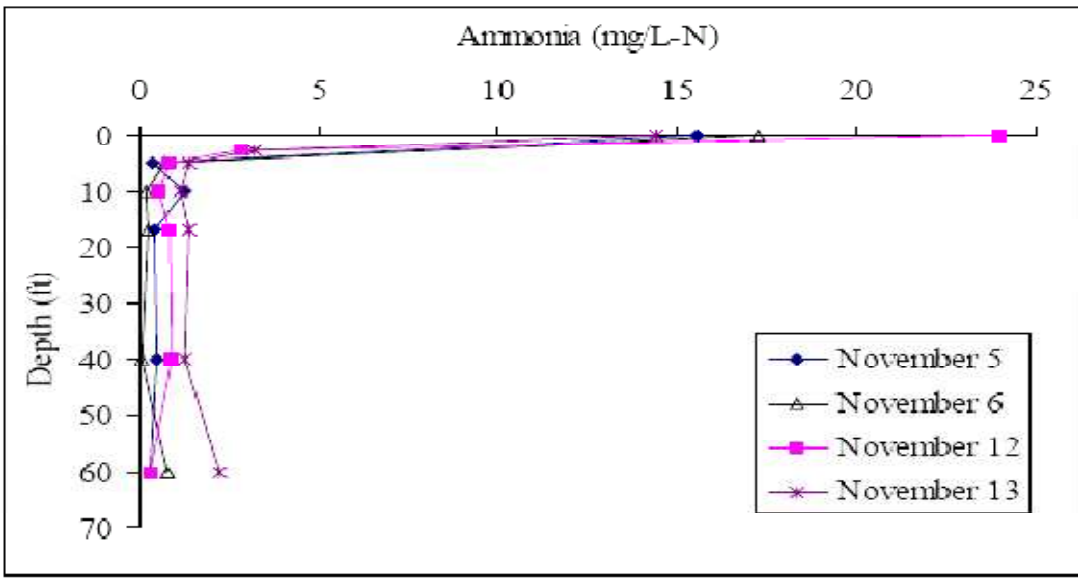



**Figure 2-3:** Removal of ammonia from secondary effluent during soil aquifer treatment (SAT) field site recharge basin in Tucson, Arizona in USA

Source:(Fox *et al.*, 2001)

Once nitrate reaches deeper (Figure 2.3) into the anoxic zone, heterotrophic denitrification may convert nitrate into nitrogen gas in the presence of organic carbon as electron donor (Fox *et al.*, 2001b). C: N ratio influence nitrogen removal, C: N ratio greater than 3 is necessary to sustain high nitrogen removal efficiency (Fox *et al.*, 2001b). In field sites the removal efficiency of nitrogen is site specific, for example the Dan region recharge project in Israel reported nitrogen removal efficiency of 45% with C:N ratio 2:1 while the Tucson Sweetwater site in USA reported a removal efficiency 25-90% with C:N ratio less than 1:1 (Fox *et al.*, 2001b). According to Fox *et al.* (2001b) the Dan region recharge project has sufficient organic carbon to sustain heterotrophic denitrification. The high removal efficiency of nitrogen species may be attributed to anaerobic ammonium oxidation (ANAMMOX) at Tucson Sweetwater site.

Previous laboratory-scale studies conducted at UNESCO-IHE are shown in Figures 2.4 and 2.5 presenting nitrate and oxygen profiles along the depth of the column, respectively. It is clear from Figure 2.4 that in the top one meter of the column there is nitrification of ammonium present, leading to an increase in nitrate concentration. As there is depletion of oxygen in the subsequent depth of the column, the denitrification of the nitrate starts. As expected, at higher HLR, nitrification and denitrification activities shifted deeper down in the soil column. It can be concluded that when proper redox conditions and adequate depth of the soil column is provided, both nitrification and denitrification can be achieved during SAT.

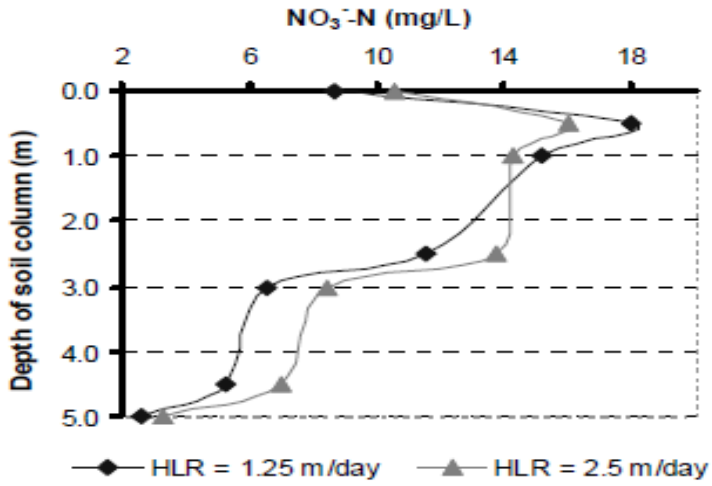



**Figure 2-4**: $NO_3^-$-N profile along the soil column at different HLR when operated under aerobic conditions with settled primary effluent.

Source: (Katukiza, 2006)

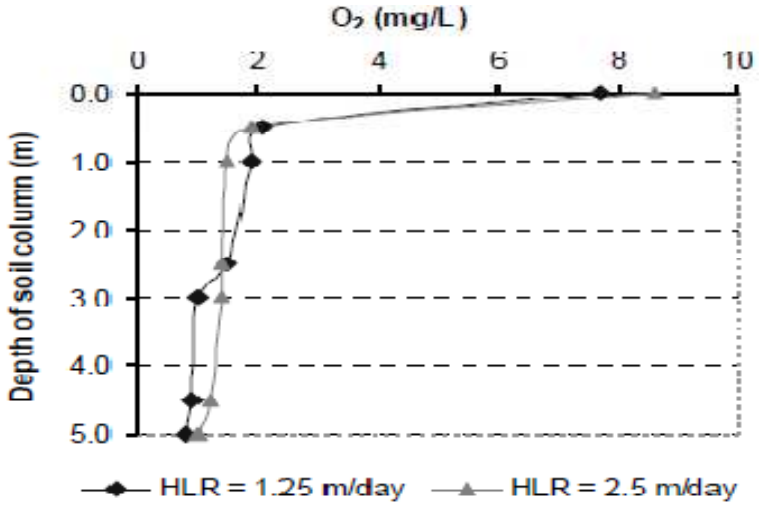



**Figure 2-5**: Oxygen profile along the soil column at different HLR when operated under aerobic conditions with settled primary effluent**.**

Source: (Katukiza, 2006)

### 2.4.3 Phosphorous removal

Release of phosphorus (P) from anthropogenic sources such as municipal wastewater, runoff from agricultural areas and landfill leachate to lakes, rivers, and coastal areas constitutes the main risk for reduced water quality and eutrophication. Eutrophication may increase significantly the cost of water treatment in surface water treatment plants. At the same time, P is an essential nutrient for all forms of life and cannot be replaced by any other element. In

order to reduce the negative effects of overloading the ecosystems with P as well as reducing the high costs that accompany the mining and processing of P, it is necessary to investigate various techniques and materials that could contribute to the removal as well as recycling of P.

Earlier studies have shown that the short-term P-sorption may be overestimated in experiments using only a pure P solution, as the competitions with other negative ions are not considered. The studies also showed that higher pH values led to higher P removal and that the P removal decreased in the presence of competitive ions such as $Cl^-$, $CO_3^{2-}$, $SO_4^{2-}$, and humic acid (Ádám *et al.*, 2007).

Phosphorous present in municipal wastewater is in form of orthophosphate, polyphosphate and organic phosphate. Nema et al. (2004) found out that orthophosphate is removed biologically but stressed that phosphorous removal depends on chemical reactions that must be renewed. Treatment plant effluent discharge limits have ranged from 0.10 to 2.0 mg/L of phosphorous depending on plant location and potential impact on receiving waters (Metcalf and Eddy, 2004).

The main sources of phosphorus in municipal sewage are excreta and detergents. Raw sewage can contain 5 mg/l to 50 mg/L of phosphorus depending on diet and water use on local population. The activated sludge treatment plants can reduce significantly the phosphorus concentration in wastewater. The basic configuration of activated sludge treatment plant consist of anaerobic and aerobic zone, phosphorus removal is through biological process in aerobic zone (Pescod, 1992).

The most important media characteristics for P adsorption are good hydraulic conductivity and chemical composition of the adsorption media as well as their Ca content. Because phosphorous is removed via sorption and precipitation processes, Ca, Fe and Al content is important in efficient P removal. However, P removal efficiency is often high initially and then decreases after sometime as the P-sorption capacity of the sand is exhausted. The amount of P adsorbed increase with increase the pH and removal efficiency is predominantly affected by porous media size and type (Vohla *et al.*, 2011).

The phosphorus removal during SAT is through adsorption to soil as reclaimed wastewater percolates the soil and sediments. Other mechanisms include filtration and microbial uptake. However, phosphorus adsorption is controlled by the interaction of redox potential, pH, native iron, calcium and aluminum minerals and the iron to P ratio. The main removal process for phosphorus is chemical precipitation reaction with the calcium and magnesium ions present in the soil and adsorption (Idelovitch *et al.*, 2003). Furthermore, phosphorus is removed by adsorption onto iron and aluminum containing minerals and precipitation with these minerals (Reemtsma *et al.*, 2000).

### 2.4.4 Bulk organic matter

#### i. *General*

TOC is a measure of the total mass of organic carbon (particulate and dissolved). Most TOC analyzers involve a chemical (wet oxidation) or thermal (combustion method) conversion of organic-C to an equivalent amount of carbon dioxide ($CO_2$) which is measured by a $CO_2$-specific detector.

DOC is defined as dissolved organically bound carbon, classified by filtration through a 0.45µm filter. TOC is comprised of DOC and POC (particulate organic carbon). During SAT, there is virtual complete removal of POC through the top-most layer of the vadose zone and hence, in the subsurface, TOC and DOC are essentially equivalent.

Biodegradable Organic Carbon (BDOC) is the biodegradable fraction of DOC. The remainder of the DOC is deemed the non-biodegradable DOC (NBDOC). Non-biodegradable portion of DOC remaining after SAT simulated in a soil-column set-up is operationally defined as residual DOC. The persistent, recalcitrant part of organic matter, resistant to biodegradation and not sustainable adsorbed during SAT, is defined as refractory DOC.

UVA is a spectrophotometric measurement based on the absorbance of UV light by organic (and inorganic) molecules. Over the UV wavelength range of 200 to 400 nm, the most commonly used wavelength is 254 nm (UVA-254), where there is strong absorbance by molecules containing unsaturated and double-bonded carbon (e.g., aromatic carbon). At low wavelengths near 200 nm, there are interferences caused by inorganic compounds (e.g., nitrate).

SUVA representing the ratio of UVA-254/DOC is an index of aromaticity. Since SUVA is normalized to DOC concentration, increasing SUVA indicates a larger percentage of unsaturated carbon-carbon double bonds (i.e., increasing aromaticity). SUVA correlates well with the aromaticity and the hydrophobicity of the organic carbon, SUVA values 4 and higher indicates that the organic matter is dominated by higher molecular weight and by hydrophobic humic acid fraction, SUVA between 2 and 4 indicates that the organic matter is a mixture of hydrophobic and hydrophilic fractions of different molecular weight, humic and fluvic acids and SUVA less than 2 indicates that mostly non-humic, low molecular weight and substances with low hydrophobicity.

Fluorescence is a spectrophotometric measurement involving two steps, excitation of molecules that fluoresce (fluorophores) at a UV wavelength (excitation wavelength) and subsequent measurement of their fluorescence at a (higher) emission wavelength. Fluorescence spectroscopy offers an additional tool for characterizing bulk, or isolated, DOC. Advantages of fluorescence over UV absorbance include reduced interference from inorganic compounds and more opportunities to optimize the signal through the combination of

excitation/emission wavelengths. Generally, an excitation wavelength, providing a maximum emission signal, is first determined from an excitation wavelength scan. Next, the maximum emission wavelength (λmax) is determined with the response reported as (relative) fluorescence intensity. Molecules that fluorescent with reasonable efficiency are aromatic molecules and those with highly unsaturated aliphatics (i.e., molecules with extensive π systems). The general rule is that electron-withdrawing functional groups (e.g., carboxyl) reduce fluorescence, and electron donating functional groups (e.g., phenolic or amine) enhance fluorescence. Fluorescence spectroscopy may provide a tool for assessing structural bonding features, functional groups, and polarity of DOC (Fox *et al.*, 2001).

***Fluorescence quenching*** refers to any process which decreases the fluorescence intensity of a molecule. A variety of processes can cause fluorescence quenching, such as excited state reactions, energy transfer, complex formation and molecular collision. This phenomenon may pose challenges for the implementation of fluorescence spectroscopy for water quality monitoring method due to the complex and variable water matrices that exists within water recycling systems and distribution networks. The potential influence on fluorescence measurements from temperature variations, pH, metal ions and oxidation processes need to be considered.

***Temperature*:** Fluorescence intensity is highly dependent on temperature. A rise in temperature increases the likelihood that an excited electron will return to its ground state by radiationless decay, leading to reduced fluorescence intensity. For example, fluorescence intensity can increase by 1% with a 1$^{o}$C decrease in temperature, within the range 10–45$^{o}$C, for tryptophan-like, humic-like and fulvic-like substances depending on colloid size and fluorophores. Within the context of recycled water schemes, water temperature may vary across a range of 20$^{o}$C or more between summer and winter, leading to a corresponding minimum in fluorescence intensity during the summer by 20%. However, no research has yet been undertaken on the thermal quenching properties of recycled waters (Henderson *et al.*, 2009).

### *ii. Removal of organics during SAT*

Wastewater is classified as strong, medium, or weak depending on the concentration of contaminants. In medium strength wastewater, about 75% of suspended solids and 40% of the filterable solids are organic in nature. Organic compounds are normally composed of a combination of carbon, hydrogen and oxygen. The principal groups of organic substances found in wastewater are proteins (40 to 60%), carbohydrates (25 to 50%), and fats and oils 10%. In addition to that, Organic matter in wastewater is highly heterogeneous, containing molecules of various molecular weights ranging from the simple compounds like acetic acid to very complex polymers.

Bulk organic matter in wastewater contains a heterotrophic mixture of hydrophobic and hydrophilic organic compounds with a wide range of molecular weight, including non homogeneous organic compounds such as humic substances, amino acids, sugars, aliphatic and aromatic acids. DOC can be broadly divided into two fractions: humic substances (HS) and non-humic substances (non-HS), which include carbohydrates, lipids and amino acids. HS are considered resistant to bacterial degradation, where as non humic substances are biodegradable and often referred to as BDOC. Organics in wastewater can be best characterized by TOC, the concentration of DOC, soluble organics measured by gross parameters such as biological oxygen demand (BOD) and chemical oxygen demand (COD), total organic halides (TOX), and absorbance at wavelength254 nm (UV254) (Abushbak, 2004).

Residual organic compounds in reclaimed water originate from three major sources: (1) anthropogenic organic compounds added by consumers, (2) NOM already present in drinking water, and (3) SMPs generated during the wastewater treatment process due to the decomposition of organic material (Drewes *et al.*, 2003).

One of the major aims of drinking water treatment is to remove NOM from raw water. NOM may react with disinfection chemicals and cause undesirable microbial growth in distribution systems and formation of disinfection by products. Rook first noted the production of organo-halides via the reaction of free chlorine with dissolved organics during chlorine-based disinfection of water. The first DBPs to be widely recognized and regulated were the THMs. These compounds are suspected human carcinogens (Quanrud *et al.*, 2003).

The fractionation of DOC based on previously developed methods separated DOC into hydrophobic acids consisting of fulvic and humic acids, hydrophilic acids and ultra hydrophilic acids. Under same studies, it was also found out that humic substances constitute a significant part of soluble organic substances produced during SAT treatment process, which together with Fulvic acids form EfOM in reclaimed water (Drewes and Fox, 1999).

EfOM comprises of NOM dominated by humic substances originating from drinking water and SMPs that include proteins and polysaccharides (Amy, 2005). Concentrations of NOM and hence EfOM are in the order of higher magnitude in comparison to synthetic organic compounds with concentrations in the order of few micrograms per litre. Therefore, the TOC variations in domestic effluents are attributed to the characteristics of drinking water sources (groundwater or surface water), the type of wastewater (domestic or industrial effluents) and the wastewater treatment technology (Drewes and Fox, 1999).

The bulk organic carbon fractions are used in investigating the fate of EfOM. They include: Hydrophilic organic matter (HPI), hydrophobic acids (HPO-A), colloidal organic matter (COM), and SMPs and were used by Rauch and Drewes (2004) in assessing bulk organic matter removal. During artificial groundwater recharge, NOM is removed by physical,

chemical and microbial processes. In conventional basin infiltration, part of the removal occurs in sediment bio films of the basin. In sprinkling infiltration, forest soil also releases NOM to the subsurface. NOM is then removed in the vadose and saturated zones.

Transport of large quantities of NOM into aquifers is a crucial issue of artificial groundwater recharge due to the potential risks of clogging and break-through. Either of these would adversely affect aquifers. Microorganisms may play a key role in preventing these adverse effects and providing a method of environmentally sustainable drinking water production. Several studies have focused on the different NOM removal mechanisms in both artificial groundwater recharge and SAT illustrating the importance of biodegradation. Bacterial abundance in the subsurface varies in different zones depending on the hydrological, physical, and geochemical conditions. In the vadose zone, the decline of nutrients results in a decline in bacterial numbers. However, possibly due to mixing of oxygen and recently recharged nutrients, the groundwater interface has a higher number of bacteria. In the subsurface, most of the bacteria are attached to soil particles as bio films (Kolehmainen *et al.*, 2007). Several studies have shown that TOC in wastewater effluent is significantly reduced during SAT. Table 2-8 shows reductions in total organic carbon during SAT of wastewater effluent from different studies.

**Table 2-8:** Reductions in total organic carbon during SAT of wastewater effluent

| **Authors (Year)** | **Reduction (%)** | **Co (mg/L)** | **Sediment column thickness (m)** |
|---|---|---|---|
| Bouwer et al. (1974) | 73 | 10–30 | 3.3 |
| Bouwer and Rice (1984) | 70-71 | 10.2–11.7 | 18 |
| Idelovitch and Michail (1984) | 82 | 18 | 25 |
| Nellor et al. (1984) | 66 | 10 | 2.4 |
| Amy et al. (1993) | 50[1] | 10.7–12.0[1] | 6.1 |
| Wilson et al.(1995) | 90 | 15, 12 [1,2] | 37 |
| Drewes and Fox (1999) | 72 | 5.7 [1] | 20 |

Source: (Quanrud *et al.*, 2003)

[1] dissolved organic carbon.

[2] annual averages for two recharge seasons

## 2.5 SAT challenges and limitations

### 2.5.1 Challenges

Using reclaimed municipal wastewater for artificial recharge of groundwater presents a wide spectrum of technical and health challenges. One of the major issues associated with SAT leading to potable and non potable reuse of reclaimed wastewater is the presence of potentially harmful organic compounds, trace elements and pathogens that eventually may be consumed by public. Incidental or unplanned indirect potable reuse of polluted water may expose people to health risks. Non potable uses such as irrigation have less potential for human exposure to hazardous agents and the risks are therefore significantly lower (Asano and Cotruvo, 2004).

Pathogenic organisms and trace organic compounds are by far a predominant concern to public health, it is important to design multiple barriers systems to assure continuous production of safe water. Therefore, SAT process should be designed and managed to avoid encroachment into the native groundwater (Asano and Cotruvo, 2004). However, the infiltration of reclaimed wastewater via soil percolation generally removes microorganisms such bacteria, viruses and therefore serves as a protection against direct health effects(Asano and Cotruvo, 2004).

### 2.5.2 Limitations

The following are some of the limitations of SAT systems:

- SAT systems are site-specific and require detailed hydro-geological studies to confirm the suitability of the soil matrix at their proposed locations. These studies are expensive and time consuming.
- No reliable transfer of experience to others. SAT is restricted by lack of information on rational design and operation of the system (Nema *et al.*, 2004).
- Only limited barrier for certain contaminants because trace unregulated and uncharacterized organics still present in small concentrations in the treated water may be toxic and this hinders the acceptability and sustainability of the product water.
- Space limitation important for future research.
- Possible release of iron and manganese.

# 3 MATERIALS AND METHODS

## 3.1 Introduction

This chapter gives details of the experimental setup, experimental procedures, data collection and analysis. Soil column and batch experiments were carried out at UNESCO-IHE laboratory to simulate SAT with respect to organic matter, nutrients (nitrogen and phosphorous) and pathogen (*E-coli* and *total coliform*) removal. During the research period, data were collected from the tests carried on batch and soil column setups for analysis and removal trends were established. The materials, experimental setups and experimental procedures that were used during this research are as delineated below

## 3.2 Experimental setup during laboratory studies

To determine the biodegradability of effluents from Hoek Van Holland WWTP, two experimental setups were used.

### 3.2.1 Batch experimental setup

Batch experimental setups were used to simulate the removal efficiency of SAT in removing bulk organic matter, nutrients (N & P) and pathogens. The source water used for the experiments is both Secondary effluent (SE) and Primary effluent (PE) collected from Hoek van Holland Wastewater Treatment Plant (WWTP), the Netherlands.

Batch experimental setup consisted of transparent glass bottles of 500 mL containing 100 g acid washed silica sand. The sand was initially inoculated using Hoek Van Holland WWTP PE and SE. This established a robust microbial population on the sand particles. Acclimation of microbial populations was achieved by dosing the reactors with 400 mL of Hoek Van Holland WWTP PE and SE over multiple 5-day cycles. Glass bottles were continuously agitated using a shaker table.

Twenty soil batch reactors with bio-acclimated silica sand with grain size ranging from 0.8 mm to 1.25 mm were arranged to simulate SAT. The experiments were carried out with source water at aerobic and anoxic conditions. Figure 3-1 shows batch reactors placed on table shaker.


**Figure 3-1:** Batch reactors on a shaker table

### 3.2.2 Short soil column experimental setup

Prior to experiments, laboratory-scale short soil columns containing silica sand were biologically acclimated. Silica sand with grain size ranging between 0.8 mm and 1.25 mm was packed (wet) into XK 50/30 column. This column consists of borosilicate glass tube with 300 mm length and 50 mm inner diameter, in addition to acrylic plastic tube thermostat with threaded ends. The end pieces of the column are made of reinforced acetal plastic and contain O-ring, sealing ring, washer and locking ring. Besides, a tubing connector through which water enters or leaves the column is interconnected with the end pieces. Peristaltic pump was used to introduce the influent water in down flow mode after which the water infiltrate into the media under the effect of gravity. Four short soil columns (as shown in Figure 3-2) were deployed to simulate the subsurface conditions and ascertain the removal of bulk organic matter, nutrients (N & P) and pathogens and establishing the dominant removal mechanism during soil passage. Two columns were connected to a chiller, whereby water temperatures were adjusted from 25°C, 20°C and 15°C to analyze the effect of seasonal variation of temperature using PE and SE. All four columns were filled with biologically active silica sand (0.8 – 1.25 mm), ripened with SE and PE for 12 weeks.

**Figure 3-2:** Laboratory scale (short) soil column setup

## 3.3 Experimental procedures

PE and SE from Hoek van Holland WWTP were used as influent water. The main water quality parameters of these waters were characterised and measured during SAT simulation studies. Short soil column experiments were carried out at different redox conditions and temperatures to assess SAT performance while other parameters remaining constant. Batch experiments were carried out only at different redox conditions.

### 3.3.1 Batch experimental procedures

Procedures of batch reactor experiments were as designated below:

1. Influent water was collected, characterised and stored in a cold room under ambient temperature of 4$^{o}$C before it is used in the experiments.

2. Influent water was brought out of the cold room and kept under room temperature for at least 1 to 2 hours before it is used in the experiments.

3. Bio-active sand was prepared to allow the growth of bacteria on sand with SE and PE. Approximate 100 g of silica sand 0.8-1.25 mm was added to 0.5 litre glass bottle, PE and SE were filled up to 0.45 litre levels. Batch reactors were run in triplicate. The water inside the reactor was constantly mixed and renewed by emptying it away and refilling the reactor with a new batch of water after every 5 days. DOC concentration was checked on day 0 and day 5 until steady state (approximately constant DOC removal) conditions were attained.

4. Batch experiments were then carried out at different process conditions by using the already ripened 100 g bio-sand with PE and SE.
5. Analysis of bulk organic matter, (DOC, UVA-254, F-EEM, $NH_4$-N, $NO_3$-N, $PO_4$-P, *E-coli* and *total coliform*) was carried out by taking samples from each batch reactor over the period of operation of the batch experiments to establish removal efficiency of SAT and the degradation of the BDOC component.
6. Of all twenty batch reactors, eight batch reactors were control batch reactors four with PE and other four with SE influent. The controls were spiked with 2.0 mmol/L sodium azide ($NaN_3$) to inhibit aerobic microbial activity
7. The difference between initial DOC and DOC at the last day of the experiment is biodegradable DOC (BDOC) that has been removed and the DOC at the last day is NBDOC.

### 3.3.2 Soil columns (short) experimental procedure.

Seasonal variation in ground water temperature was simulated by carrying out laboratory-scale soil columns experiments. These experiments were carried out at different operating temperatures controlled by chiller to evaluate the effect of seasonal variations on the removal of bulk organic matter, nutrients mainly nitrogen and phosphorous and pathogenic bacteria. SE and PE were used as influent water for soil columns experiments.

Experimental procedures of soil columns experiment were as designated below:

1- Source water was stored at an ambient temperature of $4^oC$ prior to application to the soil columns experiment to minimizing the microbial activities.

2- Soil columns' filter material were kept under continuous influent water flow to stabilize biological activity in the fixed bed media during ripening period and measurements of DOC in influent and effluent were frequently done based on the EBCT. The EBCT for the soil columns experiments was approximately 12 hours.

3- Having attained maturation of soil columns after ripening phase, the characterized influent water was applied to soil columns at different operating temperatures.

4- Samples were collected from influent and effluent water applied to the columns and filtered with 0.45 μm cellulose membrane filter and stored at $4^oC$ prior to analysis.

5- Analysis of various parameters (DOC, UVA 254, FEEM, $NH_4$-N, $NO_3$-N, $PO_4$-P *E-coli* and *total coliforms* ) was performed from the collected samples

6- Temperature of soil columns was lowered or increased using a chiller. These experiments were used to assess bulk organic matter, nutrients (N & P) and pathogens removals by simulated SAT.

### 3.3.3 Experimental process conditions

The experiments with both soil columns and batch reactors simulating SAT were carried out at a range of process conditions to determine their effect on DOC, nutrients (N &P) and pathogen removal from wastewater effluents.

#### *i. Aerobic and anoxic conditions*

Aerobic conditions were maintained by aeration of influent water for the aerobic biodegradation of bulk organic matter. During aeration dissolved oxygen concentration was maintained to above 8.0 mg/L. Under oxic condition the electron acceptor is the oxygen ($O_2$).

Anoxic conditions were established during the experiment by constantly stripping off $O_2$ using nitrogen gas until the dissolved oxygen level was less than 0.2 mg/L. Fine diffusers were connected to $N_2$ line so that the $N_2$ gas distributed evenly in the reactor. In comparison to aerobic biodegradation process, in the anoxic condition $NO_3^-$ is the electron acceptor. Anoxic batch reactors were previously ripening under aerobic conditions and after ripening the same bio sand were used. Anoxic conditions were maintained using a constant flow of nitrogen gas in both reactors to remove the available dissolved oxygen usually present in SE and PE. Table 3-1 summarize the experimental process conditions of the batch and soil short columns experiments respectively

**Table 3-1:** Experimental process conditions

| Type of experiment | Water type | Temperature | Redox conditions | HLR (m/day) |
|---|---|---|---|---|
| Batch | PE | Room temperature (20-22$^o$C) | Oxic, Anoxic | N/A |
| | SE | Room temperature (20-22$^o$C) | Oxic, Anoxic | N/A |
| Short column | PE | 15 ºC, 20ºC & 25ºC | Aerobic | 0.625 |
| | SE | 15ºC, 20ºC & 25ºC | Aerobic | 0.625 |

#### *ii. Hydraulic loading rate*

To study the effect of temperature and redox conditions on the removal of bulk organic matter, nutrients and selected pathogenic bacteria during soil column studies a hydraulic loading rate of 0.625 m/day was adopted. The flow rate was controlled and maintained using a measuring cylinder and a stop watch. The hydraulic loading rate was then established using the flow rate and the cross sectional area of the soil column. The EBCT corresponding to a hydraulic

loading rate of 0.625 m/d is 12 hours for a column depth of 0.3 m and internal diameter of 50mm.

***iii. Filter media***

The filter media that will be used in this study consisted of silica sand of size 0.8 mm-1.25 mm. The effective filter bed will be 300 mm deep for each column used to simulating SAT system. The porosity of silica sand media of size 0.8-1.25 mm diameter used for this was assumed 0.4.

## 3.4 Analytical methods

The techniques, reagents and apparatus used to measure different parameters during the study are delineated below.

### 3.4.1 Measurement of Temperature, EC, pH and $O_2$

The electrical conductivity and temperature of all influent and effluent water was measured with WTW cond 330i conductivity meter. During measurement the probe of the meter was inserted in the sample, the sample was stirred to ensure uniform mixing and when stable reading obtained, the reading was recorded. Dissolved oxygen was measured with the specific HACK HQ10 oxygen meter. For short soil columns the dissolved oxygen of samples was measured from inlet and outlet of the soil columns. The probe of the oxygen meter was inserted in the collected sample at the outlet of the column, A stable reading when obtained and recorded.. Measurement of pH was carried out by using Metrohm-691 pH meter which was calibrated prior to the measurement. Samples were collected in plastic cups from the influent and sampling points of the soil columns and the batch reactors and the samples were placed on a magnetic stirrer to ensure uniformity. Then the meter probe or the electrode was immersed in the sample after rinsing it thoroughly by spouting demineralised water from plastic wash bottle. The stable final reading was read.

### 3.4.2 Measurements of DOC /TOC

TOC/DOC was measured using Shimadzu TOC-VCPN total organic carbon analyzer. The samples of DOC were prepared by filtering through 0.45µm of cellulose membrane. TOC was not measured for this case DOC was the main parameter of the research. The DOC analyzer was calibrated to measure within the range 0.01µg/L to 20 mg/L. The 40 ml glasses (vials) were used to place samples in auto-sampler of DOC analyzer. The collected samples during the batch and soil experiments were diluted for PE influent and filtered through 0.45 µm to vials. The glass vials were acid washed and dried in 70˚C oven to remove any residual carbon on it. The cellulose acetate membrane was cleaned by soaking with milli-Q water at least for 24 hours to eradicate the leaching DOC of the filter. Before measuring of samples, DOC

analyzer was run with milli-Q water (with two determination) to clean the system and to make sure the given results are correct. Figure 3-3 shows the TOC/DOC analyzer used in this study.

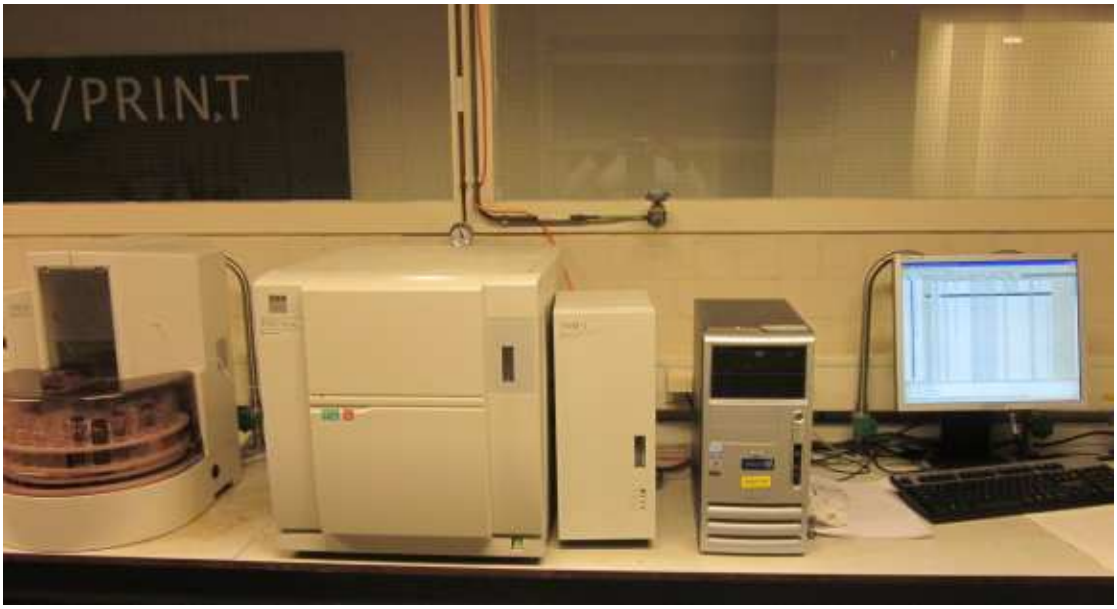



**Figure 3-3:** Shimadzu TOC-VCPN total organic carbon analyzer

### 3.4.3 Measurement of Ammonia, $NH_4$-N

Ammonium nitrogen was measured using Dichloroisocyanurate method, which consisted of the preparation of reagents and standards. Ammonium ($NH_4^+$-N) was determined using the standard curve after measuring the absorbance at 655 nm of standards and samples using Shimadzu UV-2501 PC UV-VIS spectrophotometer. The calibration curve ($NH_4^+$-N Vs absorbance) was prepared using the standard stock solution of $NH_4Cl$. The standards were made by diluting the stock solution to 50 ml. Preparation of reagents and the procedure of samples preparation are describes below.

**Reagents**

*Salicylate reagent*: 130 g of sodium salicylate ($NaC_7H_5O_3$) and 130 g of Sodiumcitratedihydrate ($Na_3C_6H_5O_7.2H_2O$) were dissolved in about 650 mL of deminaralized water in 1000 mL volumetric flask. The pH was checked and adjusted to a maximum 8 using HCl. Finally, 0.970 g of disodiumpentacyano nitrosyl ferrate (III) ($Na_2Fe$ (CN) $5NO.2H_2O$) was mixed and filled up with demi water to the mark to make the solution 1000 mL. The solution was stored in glass bottle and it had to be used within two months.

*Dichloroisocyanurate reagen*t: 32.0 g of NaOH was dissolved in 500 mL of demineralised water, cooled to room temperature and 2.00 g of Sodiumdichloroisocyanurate ($NaC_3N_3O_3Cl_2$) was added. The volume was made up to 1000 mL with demi water and mixed. Lastly, it was

stored in the refrigerator and it had to be used within 15 days.

*Stock* $NH_4Cl$: 3.819 g of anhydrous $NH_4Cl$ was dissolved in deminaralized water and diluted to 1000 mL.

*Standard* $NH_4Cl$: 10 ml of stock solution was diluted to 1000 ml (1.0 Ml = 0.01 mg N=10 µg N)

**Procedure**

a) 40 ml diluted and filtered samples were transferred to a 50 ml volumetric flask.
b) 4.0 ml of Salicylate reagent was added and mixed.
c) 4.0 ml of Dichloroisocyanurate reagent was added and mixed.
d) The mixture was then filled up to the mark with deminaralized water and mixed. Absorbance at 655 nm of both the standard solutions and the samples were determined using a spectrophotometer between 1 and 3 hours.
e) Calibration curve was then prepared for the readings of the standard solutions ($NH_4$-N versus Absorbance at 655 nm).
f) From the mathematical expression of the calibration line, the $NH_4$-N concentration of the samples was determined for a particular absorbance.

### 3.4.4 Measurement of Anions ($NO_3^-$-N, $PO_4$-P)

Anions were measured using DIONEX, ICS-1000 Ion Chromatography system coupled with ISA-100 automated sample injector. A filtered sample of about 1 ml was used for the analysis in the Ion Chromatography system. Mili-Q and control sample of known anions concentration were analyzed prior to the samples during each analysis. Figure 3-4 shows Ion Chromatography assembly.

**Figure 3-4:** Ion Chromatography system

### 3.4.5 Measurement of UVA 254 nm/SUVA

Shimadzu UV-2501 PC UV-VIS recording spectrophotometer was used to measure the UV absorbance at wavelength of 254 nm. Quartz cuvettes were used to contain the samples and the blank, which was a Milli-Q water sample. Two Milli-Q water samples were first used to auto-zero the spectrophotometer. A tap water sample was measured as a control to ensure that the system was working properly. Then the undiluted samples, first filtered through a 0.45 µm filter were analysed. Before measuring, the cuvette was properly cleaned and dried with tissue paper and rinsed once more with the sample. Between measurements a tap water was checked intermittently as a control. The orientation of the cuvette was kept the same for all samples to the auto-zeroed sample. Figure 3-5 shows the UV absorbance equipment.


**Figure 3-5:** Shimadzu UV-2501 PC UV-VIS spectrophotometers

### 3.4.6 Measurement of FEEM

The removal of different fractions of organic matter was analysed by measuring the fluorescence excitation-emission matrix (F-EEM) spectra of different samples of batch and soil column experiments. F-EEM was measured using Horbia Jobin Yvon Flouormax-3 spectrofluorometer. The samples were prepared as in case DOC and UV-254 measurement; in addition to that it was diluted to 1 mg/L of DOC. because the instrument was calibrated to measure the maximum of 1 mg/L DOC samples.

During measurement of FEEM first the xenon lamp and Raman peak performance was verified. Next as a blank Milli – Q water sample whose pH was adjusted to 2.8 ± 0.1 was analysed. The range of the wavelength used in the FEEM matrixes are; Excitation: 240 –450 nm (10 nm intervals) and Emission: 290 – 530 nm (2 nm intervals). EEM of the blank was subtracted from each sample to remove raman scatter peaks. Correction steps were applied to each blank-subtracted EEM using emission and excitation correction factors provided by the

manufacturer. Then the EEM contours were plotted in MATLAB software using the code written for this purpose. This enabled the DOM fractions to be categorized in terms of protein – like material, humic – like material and fulvic – like material. Figure 3-6 shows the measuring equipment of F-EEM.


**Figure 3-6:** FluoroMax-3 for fluorescence measurements

### 3.4.7 Measurement of pathogens (*E-Coli* and *total coliform* bacteria)

Unfiltered samples from influent and effluent at inlet and outlet points of the soil columns and batch experiments were used to measure *E-coli* and *total coliforms*. A liquid chromocult agar (growth media) from (Merck KGaA - Germany) was added to three triplicate test plates. 26.5 g of chromocult agar was dissolved in 1000 mL of demineralised water in round-bottom flask and put in a water bath at 99°C for 30 minutes. The hot liquid agar was then transferred to two smaller 500 mL round-bottom flasks and its temperature was reduced to 50°C by keeping the flasks in an oven at 50$^{o}$C. The liquid agar will then be transferred to test plates.

A volume of 0.1 mL from well shacked sample was added to plate and brought to contact with the agar by using a glass distributor which was sterilized after each sample using a flame. Test plates were kept under an ambient temperature of 37°C in an incubator for 24 hours. Enumeration of both *E-coli* and *total coliform* bacteria was carried out on the following day and obtained number of bacteria will be multiply with a dilution factor to express it in number of bacteria as CFU/mL. Dilution of samples was decided upon based on the initial results of diluted and undiluted tests. The chromocult agar was inoculated by spreading the sample on the surface of the plates and the plates were then taken into the incubator for 24 hours at a temperature of 37 degrees celicius. After 24 hours CFU/mL appeared as dark blue to violet colonies on the plates for E-coli and pink red for total coliform.

# 4 RESULTS AND DISCUSSION

## 4.1 Introduction

Attenuation of bulk organic matter, nutrients (nitrogen and phosphorus) and pathogens during different SAT process conditions was assessed using laboratory-scale batch and short soil column experiments. Experiments were carried out with PE and SE under different reduction-oxidation conditions. Additionally, the effect of temperature in removing contaminants under aerobic conditions was assessed in soil columns studies. Results obtained from these experiments are presented in the following sections.

## 4.2 Characteristics of effluent wastewater

Both PE and SE collected from Hoek van Holland WWTP, were characterized prior to application to the laboratory-scale batch reactors and soil columns. Characterization of the effluent wastewater is imperative to assess change in wastewater quality with regard to bulk organic matter, nitrogen, phosphorous and pathogens. The PE and SE were regularly characterized shortly after collection from the WWTP. The average quality characteristics of wastewater effluent applied to column and batch set-ups are presented in Table 4-1.

**Table 4-1:** Wastewater quality of primary and secondary effluents

| **Parameters** | **Units** | **Secondary Effluent** | **Primary Effluent** |
|---|---|---|---|
| Temperature | $^{o}$C | 11±2.2 | 13.2±0.4 |
| *E-coli* | CFU/mL | 170±70 | 35600±15500 |
| $NO_3^-$-N | mg/L | 4.32 ± 1.27 | 0.72 ± 0.32 |
| *Total coliform* | CFU/mL | 1600±990 | 310600±104000 |
| BOD | mg $O_2$/L | 6.2±1.3 | 180.67±34.68 |
| Dissolved Oxygen | mg $O_2$/L | 5.9±1.2 | 2.1±0.46 |
| TDS | mg/L | 720.89±84.36 | 850.84±76.61 |
| EC | µS/cm | 1079±176.2 | 1497±121.2 |
| DOC | mg/L | 12.75±2.3 | 42.49±10.26 |
| $UVA_{254}$ | 1/cm | 0.419±0.1 | 0.523±0.15 |
| PO4-P | mg/L | 3.86±1.63 | 5.28±1.66 |
| pH | - | 7.2±0.1 | 7.29±0.1 |
| $NH_3^+$-N | mg/L | 0.2 ± 0.1 | 32.02 ± 4.28 |
| COD | mg $O_2$/L | 51.3±14 | 296±46 |
| SUVA | L/mg-m | 3.15±0.3 | 1.83±0.1 |
| Redox | mV | I84±27.22 | 79±14.75 |
| SS | mg/L | 4.54±1.39 | 107.06±30.35 |
| Alkalinity | mg/L as HCO3- | 301.34±28.75 | 497.03±46.64 |
| | mg/L as CaCO3 | 247±23.57 | 407.40±38.23 |

## 4.3 Ripening of laboratory-scale batch and soil column set-ups

DOC was constantly measured and monitored for influent and effluent of batch and soil columns until steady state conditions with respect to DOC removal was reached. DOC concentrations of wastewater fed to batch reactors was measured at 5-days intervals while hydraulic retention time was used to collect samples from soil columns. In case of soil column studies, four short soil columns were used. Two columns were used with PE (labeled PE-C1 and PE-C2) and the other two columns were used with SE (labeled SE-C1 and SE-C2). After ripening, the columns were used to study effect of different process conditions. PE-C2 and SE-C2 were used to explore the effect of temperature on removal of contaminants and the other two columns (PE-C1 and SE-C1) were used to study the effect of redox conditions on contaminants removal.

### 4.3.1 Ripening of laboratory-scale soil columns

Ripening of soil column was carried out continuously for 12 weeks using PE and SE under aerobic conditions until DOC concentrations exiting the soil columns were relatively constant (±2%). Influent and effluent samples were collected once every 2 or 3 days due to the short EBCT for the soil columns. Figure 4-1 shows the DOC removals during ripening period in both PE and SE.

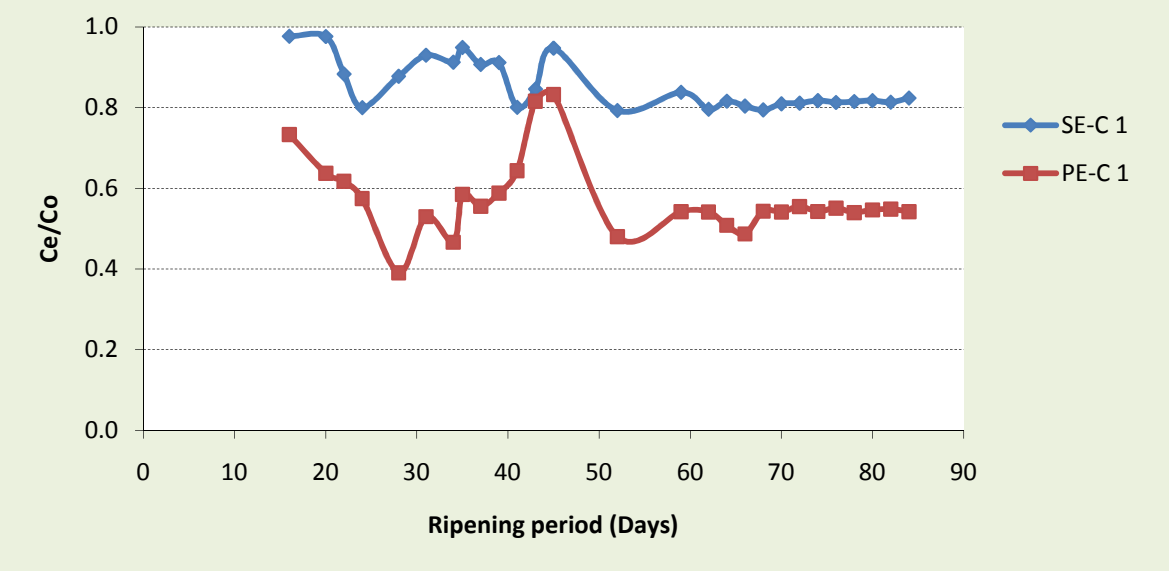



**Figure 4-1:** DOC removal during ripening period for SE- C1 and PE- C2 (influent: PE and SE, media size: 0.8-1.25 mm, HLR=0.625 m/d, column depth 0.3 m, aerobic conditions)

Throughout the study, PE and SE DOC concentrations entering the columns ranged from 25.86 to 52.92 mg/L (average=34.29±6.01 mg/L; n=30) and 10.73 to 16.54 mg/L (average=13.75±1.44 mg/L; n=30) respectively. The average effluent DOC concentrations during the ripening period were 19.62±4.51 mg/L in PE-C1 and 18.42±3.96 mg/L in PE-C2, corresponding to average removals of 42.49±10.79 and 45.96±9.55 % in PE-C1 and PE-C2 respectively. Similarly, the average effluent DOC concentrations during the ripening period

were 11.75±1.51 mg/L in SE-C1 and 11.84±1.50 mg/L in SE-C2, corresponding to average removals of 14.55±6.11 and 13.93±6.21 % in SE-C1 and SE-C2 respectively.

The operation of the soil column system was continuous at a hydraulic loading rate of 0.625 m/day with the infiltration rate controlled by peristaltic pump. Clogging effects were minimized by applying pre-settled wastewater, the filtration rate reduced considerably to about 0.45 m/day after 1 week for PE. However, flow rates were controlled at influent and effluent points of the column using the pump and adjustment of the opening at column effluent. Tubes (tygon hose tubes used for conveyance of PE to the soil column) were frequently replaced.

### 4.3.2 Ripening of laboratory- scale batch reactors

Ripening of batch reactors was carried out continuously for 10 weeks using PE and SE under aerobic conditions until the residual DOC concentrations in the batch reactors were relatively constant with time. During ripening process, biodegradable DOC was determined as the difference between the initial (day 0) and final (day 5) DOC values Figure 4-2 shows the removal of DOC in batch reactors during the ripening period in both PE and SE.

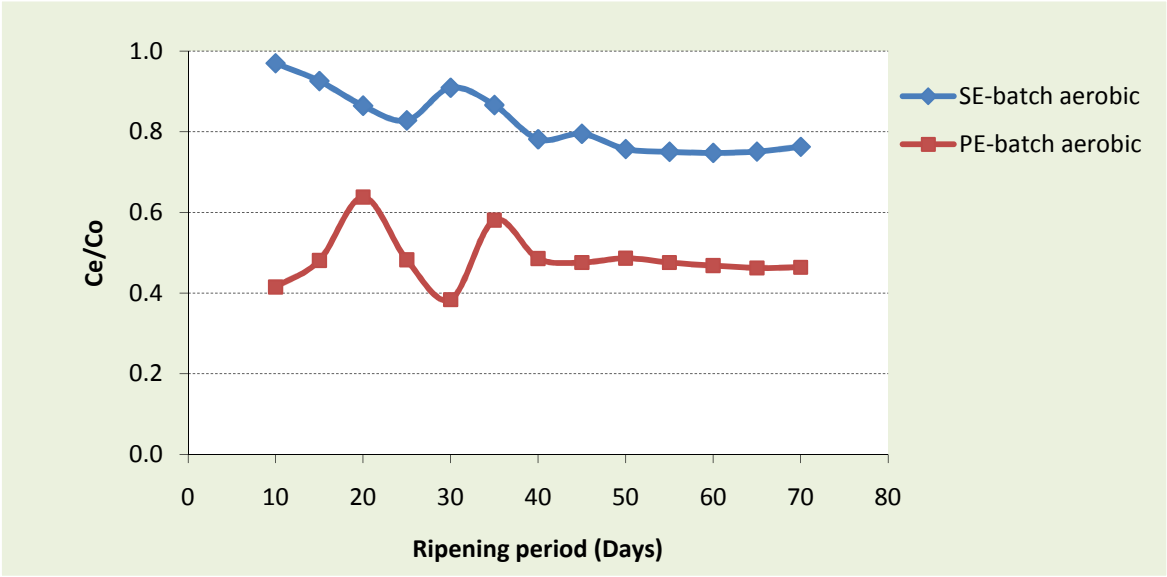



**Figure 4-2:** DOC removal during ripening of batch reactors (Influent: PE and SE, mass of sand media =100 g and media size: 0.8-1.25 mm, HRT=5 days, aerobic conditions)

During the study PE and SE DOC concentrations in wastewater effluent used as influent water ranged from 12.14 to 16.65 mg/L (average=14.07±1.40 mg/L; n=19) and 26.01 to 42.38 mg/L (average 32.80±4.78 mg/L; n=19) respectively. The average effluent residual DOC concentrations during the ripening period were 15.63±2.35 and 11.38±1.30 mg/L in PE and SE batch reactors respectively, corresponding to average removals of 52.09±5.86% and 18.93±7.38%.

To maintain aerobic conditions influent water were aerated to obtain the dissolved oxygen concentration at least 8.0 mg $O_2$/L throughout the experiments.

## 4.4 Effect of redox conditions on removal of organic matter, nitrogen, phosphorus and pathogens

To determine the bulk organic matter, nitrogen, phosphorus and pathogens removal after the ripening period, DOC, nitrogen, phosphorous and pathogens concentrations were measured at inlets and outlets points of the laboratory-scale soil columns system simulating SAT and influent and effluent concentrations in the batch reactors.

### 4.4.1 Effect of redox conditions on bulk organic matter removal

DOC attenuation of wastewater effluent was studied for both aerobic and anoxic conditions in batch and soil columns experiments. In order to determine bulk organic matter removal after ripening period, measurements of DOC, F-EEM and UVA-254 for samples from laboratory-scale soil columns and batch reactors were conducted. Additionally, the redox potential was monitored.

**a- Batch experiments**

The changes in DOC concentration with time and trends in DOC biodegradation are presented in the following subsections.

**i- Removal of organic matter under aerobic and anoxic conditions**

PE and SE were aerated prior to application in to batch reactors was aerated to obtain the dissolved oxygen concentration of at least 8.0 mg $O_2$/L while the residual dissolved oxygen concentration was 2.1 and 5.3 mg/L respectively. The DOC, F-EEM and UVA-254 were then monitored over the duration of the batch experiments. Figure 4-3 shows the DOC removal with time under aerobic and anoxic conditions after ripening period with PE as influent water

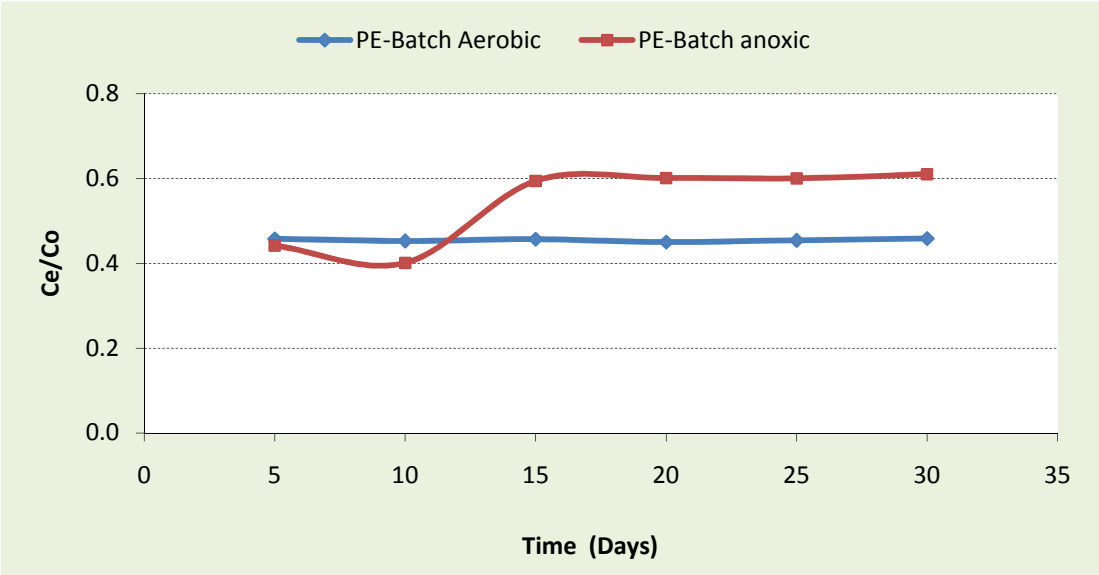



**Figure 4-3:** DOC degradation with time during batch experiment under aerobic and anoxic conditions after ripening period (Influent: PE and SE after 1-2 hour settling, volume of batch: 500 mL, mass of sand media =100 g and media size: 0.8-1.25 mm, HRT=5 days)

During the course of batch experiments, PE and SE DOC concentrations in the wastewater effluent used as influent water in the batch reactors had an average concentration of 35.40±2.54 mg/L and 15.38±1.11 mg/L respectively. The average effluent residual DOC concentrations after ripening period in PE and SE were 16.11±1.20 and 11.58±0.85 mg/L respectively, corresponding to average removals of 54.49±0.32% and 24.72±0.16% for aerobic conditions.

Figure 4-4 shows the DOC removal with time under aerobic and anoxic conditions after ripening period with SE as influent water. Under anoxic conditions with PE and SE, the average DOC concentration in the influent water was 35.40±2.54 mg/L and 15.38±1.11 mg/L respectively. The average effluent residual DOC concentrations under anoxic conditions after ripening period with PE and SE were 19.10±3.01 and 12.87±1.23 mg/L respectively, corresponding to average removals of 39.85±0.65% and 14.52±0.24%.

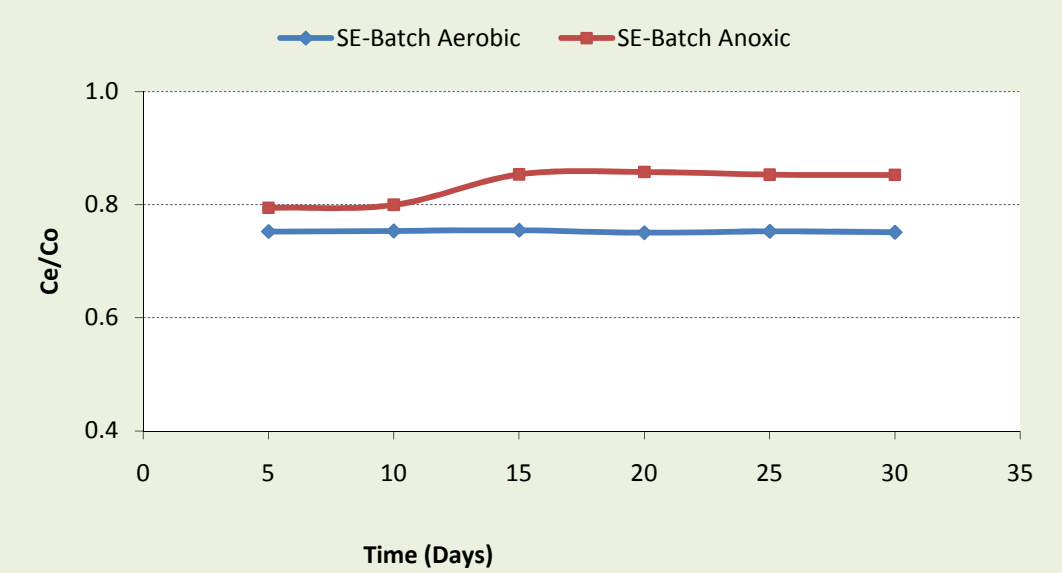



**Figure 4-4:** DOC degradation with time during batch experiment under aerobic and anoxic conditions after ripening period (Influent: PE and SE after 1-2 hour settling, volume of batch: 500 mL, mass of sand media =100 g and media size = 0.8-1.25 mm, HRT=5 days)

The high DOC removal from PE for both aerobic and anoxic conditions was attributed to the high BDOC concentration composed mainly of non-humic substances. The biodegradation of bulk organic matter under aerobic conditions in both PE and SE is more rapidly than that of anoxic conditions and more favourable for heterotrophic microorganisms (Grünheid *et al.*, 2005) and this explains the reason for higher removals of DOC in PE and SE under aerobic conditions.

**ii- SUVA changes under aerobic and anoxic conditions**

The UVA at 254 nm is a gross measure of humic substances with an aromatic character and increases as BDOC is biodegraded since UVA absorbing substances are refractory to biodegradation. SUVA that characterizes relative aromaticity increased due to removal of BDOC since it's the ratio of UVA at 254 nm to DOC. UVA decreased from 0.584 $cm^{-1}$ to 0.463 $cm^{-1}$ in PE under aerobic conditions and from 0.419 $cm^{-1}$ to 0.349 $cm^{-1}$ in SE under aerobic conditions whereas the UVA decreased from 0.584 $cm^{-1}$ to 0.550 $cm^{-1}$ in PE under anoxic conditions and from 0.419 $cm^{-1}$ to 0.349 $cm^{-1}$ in SE under anoxic conditions

The SUVA values increased from 1.78 to 3.08 L/mg.m under aerobic conditions in batch reactors fed with PE, while it increased in SE reactors from 2.88 to 3.19 L/mg.m. For anoxic conditions the trend was similar but the increment was not as significant as for aerobic conditions; SUVA values increased from 1.78 to 2.75 L/mg.m in PE and from 2.88 to 3.00 L/mg.m in SE for anoxic conditions. The corresponding changes in SUVA values are as shown in Figure 4-5 for aerobic and anoxic conditions in batch experiments.

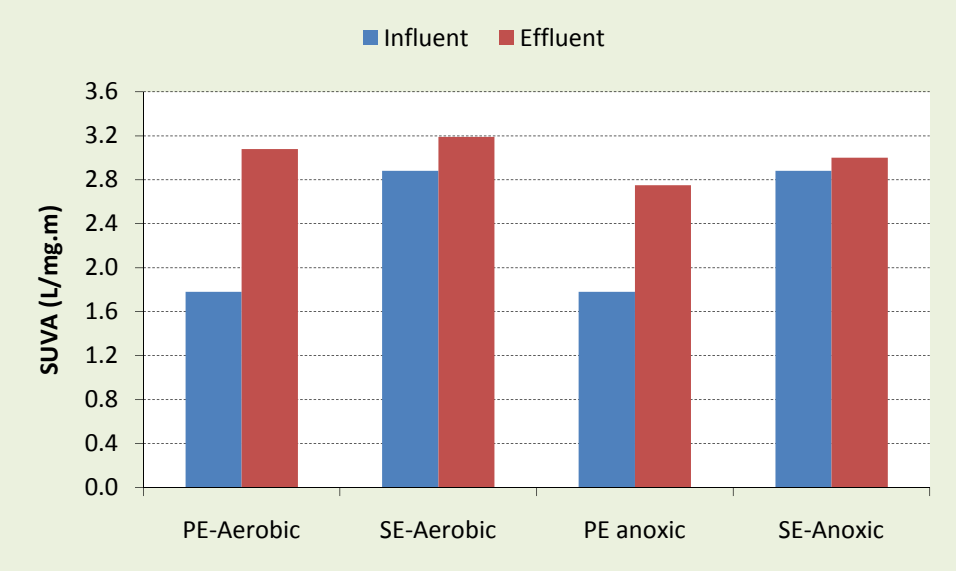
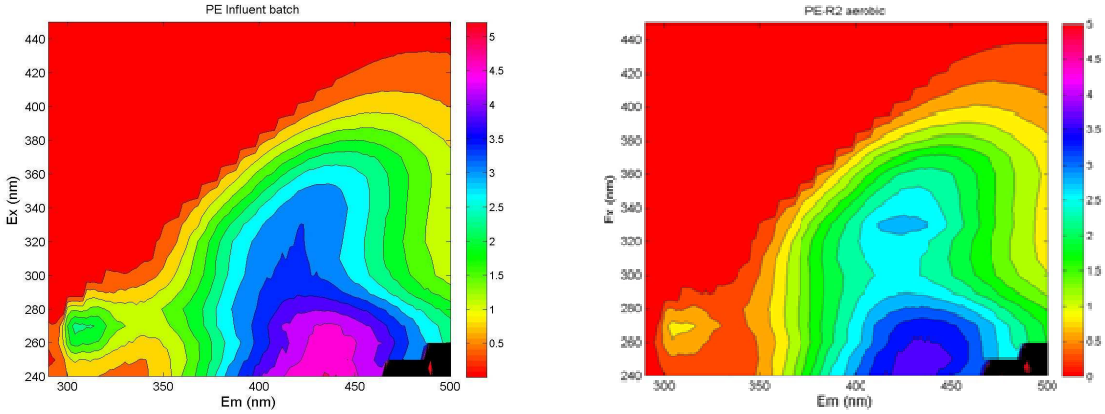



**Figure 4-5:** Influent and effluent SUVA changes in laboratory-scale batch reactors (Influent: PE and SE after 1-2 hours settling, volume of batch: 500 mL, mass of sand media =100 g and media size = 0.8-1.25 mm, HRT = 5 days, aerobic and anoxic conditions)

Biodegradation of lower molecular weight compounds leads to increase of SUVA values, while sorption of larger molecular weight, and more hydrophobic constituents has an opposite effect on SUVA; the results above indicates that easily biodegradable (aliphatic) compounds are better removed under aerobic conditions than anoxic conditions. The results above are consistent with observations made by Xue *et al.*, (2009) in a study of behavior and characteristics of dissolved organic matter during column studies of soil aquifer treatment where he found that aerobic degradation seem to preferentially remove non aromatic components and responsible for SUVA increase during SAT.

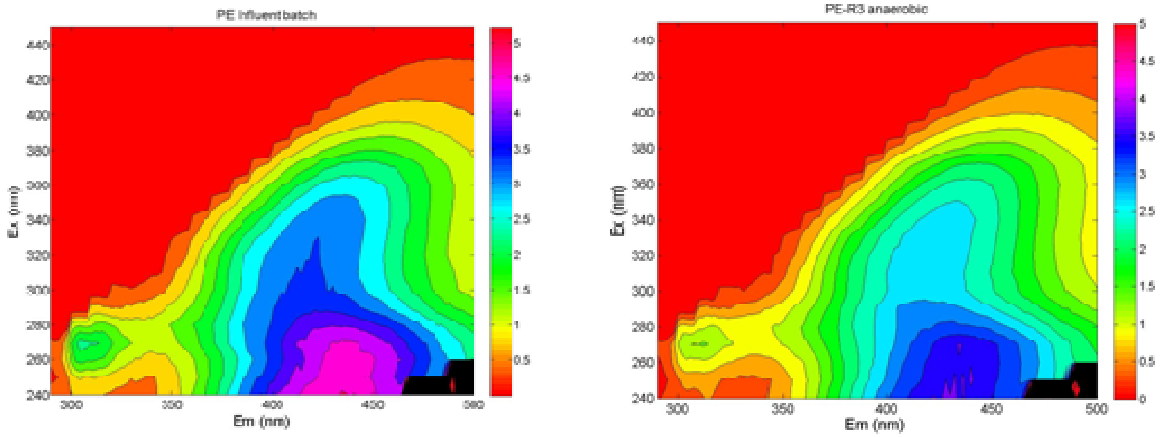
### iii- Change in Fluorescence EEM during batch studies

Results of F-EEM for aerobic and anoxic batch reactors with PE are presented in Figure 4-6, Figure 4-7, and Table 4-2. The fluorescence EEM spectra for SE are presented in Appendix A6. Fluorescence EEM spectra of influent and effluent samples were measured to investigate NOM characteristics. Three peaks were identified namely, humic/fulvic-like, humic-like and protein-like peaks related to maximum excitation and maximum emission. The presence of humic/fulvic-like, humic-like and protein-like compounds were determined using three peaks on the graphs. Peak 1 is found between Ex 330-350 nm and Em 420-480 nm, Peak 2 is found Ex 250-260 nm and Em 380-480 nm and peak 3 is found Ex 250-280 nm and Em 280-350 Em as a humic /fulvic, humic and protein-like compounds respectively (Leenheer and Crowe, 2003). The expected removal of the compounds was measured by comparing the intensities of the peaks in EEM spectra. The differences between fluorescence intensities for day zero and day 5 were identified.




Influent Effluent (b)

**Figure 4-6** F-EEM spectra for influent (day 0, (a)), and effluent (day 5, (b)); (Influent: PE after 1-2 hours settling, volume of batch = 500 mL, HRT = 5 days, mass of sand media =100 g and media size = 0.8-1.25 mm, aerobic conditions)




Influent (a) Effluent (b)

**Figure 4-7** F-EEM spectra for influent (day 0, (a)), and effluent (day 5, (b)); (Influent: PE after 1-2 hours settling, volume of batch = 500 mL, HRT = 5 days, mass of sand media =100 g and media size = 0.8-1.25 mm, anoxic conditions)

Figure 4-6 and Figure 4-7 show reduction in intensities for each peak during batch experiments with PE under aerobic and anoxic conditions. The reduction of peaks for humic /fulvic fractions was 15% under aerobic conditions and 17% under anoxic conditions. Humic-like fractions were more reduced under aerobic conditions (27%) than under anoxic conditions (24 %). Protein-like fractions were more reduced by 62 % under aerobic conditions and 39 % under anoxic conditions implying that protein like materials are more biodegradable compared to humic like substances and humic/ fulvic like substances. Table 4-2 gives more details about the reductions of peaks of different fractions of the DOC.

**Table 4-2** Fluorescence EEM analysis of influent and effluent in batch experiments fed with PE under aerobic and anoxic conditions

| **Peak 1 Humic/Fulvic-like organic matter fractions** | | **Excitation (nm)** | **Emission (nm)** | **Intensity** | **Reduction in Intensity ( % )** |
|---|---|---|---|---|---|
| PE-aerobic batch | Influent | 330 | 422 | 3.41 | 15.0 |
| | Effluent | 330 | 422 | 2.90 | |
| PE-anoxic batch | Influent | 330 | 422 | 3.41 | 17.3 |
| | Effluent | 330 | 422 | 2.82 | |
| **Peak 2 Humic-like organic matter fractions** | | | | | |
| PE-aerobic batch | Influent | 240 | 436 | 4.96 | 27.0 |
| | Effluent | 240 | 436 | 3.62 | |
| PE-anoxic batch | Influent | 240 | 436 | 4.96 | 23.6 |
| | Effluent | 240 | 436 | 3.79 | |
| **Peak 3 Protein-like organic matter fractions** | | | | | |
| PE-aerobic batch | Influent | 270 | 308 | 2.39 | 61.5 |
| | Effluent | 270 | 308 | 0.92 | |
| PE-anoxic batch | Influent | 270 | 308 | 2.39 | 38.9 |
| | Effluent | 270 | 308 | 1.46 | |

Additionally, the details of reduction in intensities for each peak during batch experiments with SE under aerobic and anoxic conditions are shown in appendices A6 and A7. The humic /fulvic-fractions were reduced by 12% under aerobic and 11% under anoxic conditions. Humic peaks were reduced by 11% under aerobic conditions and 7% under anoxic conditions. Protein like fractions were more removed under aerobic conditions ( 33%) than under anoxic conditions ( 25% ) implying that protein like materials are more biodegradable compared to humic like substances. As observed in case with PE where humic/ fulvic-like organic matter fractions were better removed under anoxic conditions, in SE the removal of humic/fulvic-like fractions were more or less the same under aerobic and anoxic conditions.

**b- Soil columns experiments**

Four short soil columns were biologically acclimated using PE and SE under aerobic conditions. Aerobic conditions were maintained by continuous aeration of the influent. After aerobic ripening stage of the columns, two columns were operated at different temperatures to study the effect of temperature change on the removal of different contaminants. The temperature was controlled by the chiller connected to the columns. The other two columns

were operated under anoxic conditions to study the effect of redox conditions on removal of contaminants.

The anoxic conditions were maintained by sparging fine stream of nitrogen gas into the container with influent water until the dissolved oxygen was reduced to less than 0.2 mg $O_2$/L. Redox potential was monitored through inlet and outlet of the columns to ensure that water flowing through the media is in anoxic conditions.

**i- Removal of organic matter under aerobic and anoxic conditions**

Table 4-3 shows the SUVA changes in soil columns under aerobic conditions whereas Figure 4-9 shows DOC degradation with time during column experiment under aerobic and anoxic conditions after ripening period in PE and SE.

**Table 4-3:** DOC and SUVA values of different effluents before and after SAT under aerobic conditions.

| Effluent applied | **Before SAT** | | **After SAT** | |
|---|---|---|---|---|
| | DOC (mg/L) | SUVA (L/mg.m) | DOC (mg/L) | SUVA (L/mg.m) |
| PE-C1 | 29.13 | 1.68 | 15.91 | 2.24 |
| PE-C2 | 26.17 | 1.97 | 14.34 | 2.52 |
| SE-C1 | 12.03 | 2.78 | 9.79 | 3.31 |
| SE-C2 | 13.15 | 2.81 | 9.82 | 3.26 |

Average DOC attenuation in PE-C1 and PE-C2 under aerobic conditions after ripening period was 46.35±2.04% and 50.40±1.65% respectively. Likewise the average DOC attenuation in SE-C1 and SE-C2 after steady states conditions was 18.80±0.79% and 18.74±1.11% respectively. UVA decreased from 0.515 $cm^{-1}$ to 0.356 $cm^{-1}$ in PE under aerobic conditions and from 0.370 $cm^{-1}$ to 0.320 $cm^{-1}$ in SE under aerobic conditions and related SUVA changes are shown in Table 4-3.

Table 4-4 shows the SUVA changes in soil columns under anoxic conditions in PE and SE

**Table 4-4:** DOC and SUVA values of different effluents before and after SAT for anoxic conditions.

| Effluent applied | **Before SAT** | | **After SAT** | |
|---|---|---|---|---|
| | DOC (mg/L) | SUVA (L/mg.m) | DOC (mg/L) | SUVA (L/mg.m) |
| PE-C1 | 28.53 | 2.14 | 18.86 | 2.60 |
| SE-C1 | 14.57 | 2.92 | 12.59 | 3.23 |

Average DOC attenuation in PE-C1 and SE-C1 under anoxic conditions after ripening period was 31.28±0.33 and 13.09±0.26 % respectively. DOC concentrations entering the columns ranged from 12.05 to 15.43 mg/L (average=13.71±1.29 mg/L; n=10) in SE and from 25.13 to 32.49 mg/L (average 28.94±2.74 mg/L; n=10) in PE. The UVA decreased from 0.611 $cm^{-1}$ to 0.490 $cm^{-1}$ in PE under anoxic conditions and from 0.419 $cm^{-1}$ to 0.398 $cm^{-1}$ in SE under anoxic conditions and subsequent SUVA changes are shown in Table 4-4

The observed increase in SUVA as indicated in Figure 4-8 was attributed to preferential biodegradation of lower molecular weight compounds in PE and SE under both aerobic and anoxic conditions indicating the DOC present in wastewater effluents can be removed during SAT under aerobic and anoxic conditions.

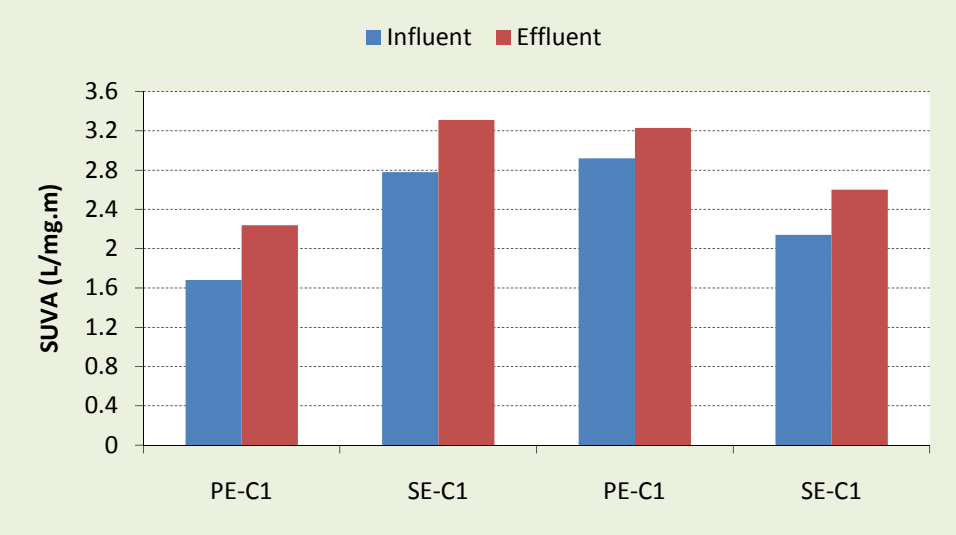



**Figure 4-8:** Influent and effluent SUVA changes in laboratory-scale soil columns in aerobic and anoxic conditions (Influent PE and SE, media size= 0.8-1.25 mm, HLR = 0.625 m/d, columns depth =0.3 m)

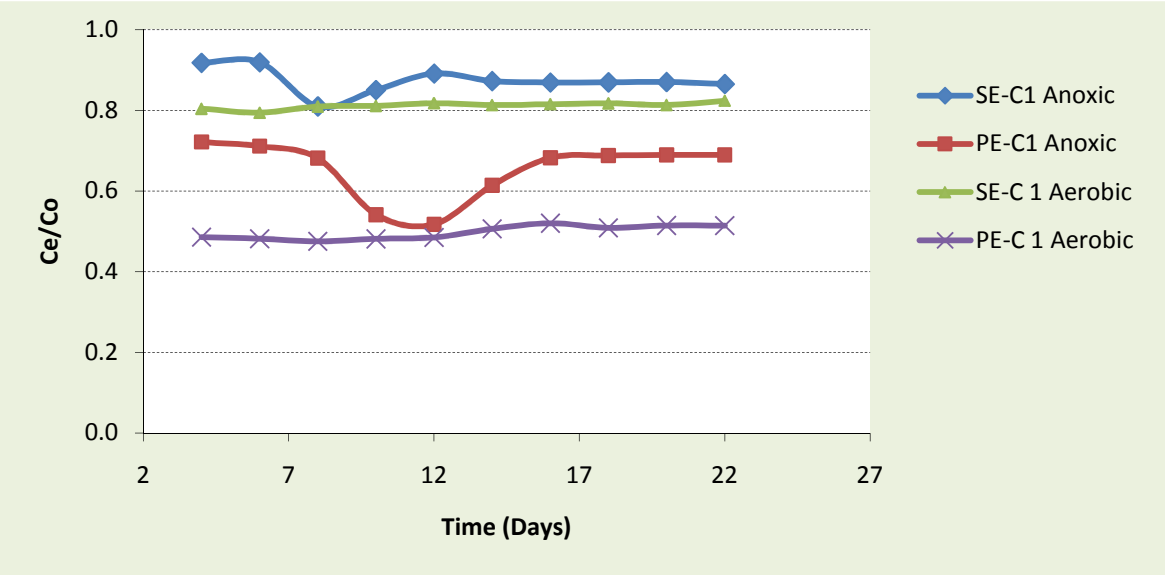



**Figure 4-9:** DOC degradation with time during column experiment under aerobic and anoxic conditions after ripening period (Influent: PE and SE, media size = 0.8-1.25 mm, HLR =0.625 m/d, column depth = 0.3 m)

### ii- FEEM of soil columns under aerobic and anoxic conditions

The results of FEEM analysis and reduction of intensities of different peaks for soil column studies with PE and SE are tabulated in Tables 4-5 and 4-6. Figures in Appendix B 5 show the Fluorescence EEM spectra for different samples taken from soil columns which were operated under aerobic and anoxic conditions. In all samples three peaks representing humic/fulvic-like (peak 1), humic-like (peak 2) and protein-like substance (peak 3) were identified and analyzed. The difference between influent and effluent fluorescence intensity of the peaks were compared and considered to represent a percentage reduction of intensity during soil passage in the columns with PE and SE.

**Table 4-5:** Fluorescence EEM analysis of influent and effluent in soil columns with both PE and SE under aerobic conditions

| **Peak 1 Humic/Fulvic-like organic matter fractions** | | **Excitation (nm)** | **Emission (nm)** | **Intensity** | **Reduction in Intensity ( % )** |
|---|---|---|---|---|---|
| PE-C1 | Influent | 330 | 430 | 1.59 | 8.6 |
| Aerobic | Effluent | 330 | 430 | 1.46 | |
| SE-C1 | Influent | 350 | 480 | 0.51 | 28.6 |
| Aerobic | Effluent | 350 | 480 | 0.37 | |
| **Peak 2 Humic-like organic matter fractions** | | | | | |
| PE-C1 | Influent | 250 | 434 | 2.28 | 7.8 |
| Aerobic | Effluent | 250 | 434 | 2.10 | |
| SE-C1 | Influent | 250 | 480 | 0.72 | 21.9 |
| Aerobic | Effluent | 250 | 480 | 0.56 | |
| **Peak 3 Protein-like organic matter fractions** | | | | | |
| PE-C1 | Influent | 270 | 312 | 0.75 | 26.9 |
| Aerobic | Effluent | 270 | 312 | 0.55 | |
| SE-C1 | Influent | 270 | 342 | 0.04 | 38.2 |
| Aerobic | Effluent | 270 | 342 | 0.02 | |

The reduction of humic/fulvic-like organic matter fractions in PE was 8.6% and humic-like organic matter fractions were reduced by 7.8% with 26.9% reduction of protein-like organic matter fractions under aerobic conditions whereas the reduction of humic/fulvic like substances in SE was 28.6% and humic like substances were reduced by 21.9% with 38.2% reduction of protein like substances. The reductions of intensities in humic like substances peaks are more or less comparable under aerobic and anoxic conditions with PE as indicated in Table 4-5 and Table 4-6. Furthermore there were substantial reductions of intensities of both peaks under aerobic conditions than under anoxic conditions with SE indicating that reduction of peaks of different DOC fractions are favorable under aerobic conditions than anoxic conditions.

**Table 4-6:** Fluorescence EEM analysis of influent and effluent in soil columns with both PE and SE under anoxic conditions

| **Peak 1 Humic/Fulvic-like organic matter farctions** | | **Excitation (nm)** | **Emission (nm)** | **Intensity** | **Reduction in Intensity ( % )** |
|---|---|---|---|---|---|
| PE-C1 Anoxic | Influent | 330 | 424 | 2.10 | -62.4 |
| | Effluent | 330 | 424 | 3.42 | |
| SE-C1 Anoxic | Influent | 340 | 430 | 3.00 | 7.1 |
| | Effluent | 340 | 430 | 2.79 | |
| **Peak 2 Humic-like organic matter fractions** | | | | | |
| PE-C1 Anoxic | Influent | 260 | 472 | 3.89 | 7.2 |
| | Effluent | 260 | 472 | 3.61 | |
| SE-C1 Anoxic | Influent | 250 | 430 | 4.07 | 4.4 |
| | Effluent | 250 | 430 | 3.89 | |
| **Peak 3 Protein-like organic matter fractions** | | | | | |
| PE-C1 Anoxic | Influent | 270 | 310 | 0.91 | -33.5 |
| | Effluent | 270 | 310 | 1.21 | |
| SE-C1 Anoxic | Influent | 270 | 308 | 0.94 | 24.1 |
| | Effluent | 270 | 308 | 0.72 | |

The reduction/increase of humic/fulvic-like organic matter fractions in PE was -62.4% and humic-like fractions were reduced by 7.2% with 33.5% increase of protein-like fractions under anoxic conditions whereas the reduction of humic/fulvic-like fractions in SE was 7.1 % and humic-like fractions were reduced by 4.4% with 24.1% reduction of protein-like fractions. The reductions of intensities in humic like substances peaks are more or less comparable under aerobic and anoxic conditions with PE. However humic/fulvic-like fractions and protein-like fractions in PE increased by 62.4% and 33.5% respectively suggesting selective degradation of non-fluorescing constituents or formation of new fluorescing material associated with DOC biodegradation and/or degradation of certain organic components capable of quenching DOC fluorescence according to Saadi et al. (2006).

### 4.4.2 Effect of redox conditions on nitrogen removal

A set of batch reactors were used to simulate saturated aerobic and anoxic conditions in order to assess the extent of ammonium nitrogen oxidation. A common hypothesis for nitrogen removal is the two-step process of autotrophic nitrification and heterotrophic denitrification.

Redox potential was mostly used to indicate aeration conditions, and it is an important factor influencing the nitrification–denitrification process.

**a- Batch experiments**

**i- Removal of nitrogen under aerobic conditions**

Figure 4-10 shows the changes in concentrations of $NH_4$-N and $NO_3$-N on day 0 and day 5 after ripening of batch reactors in PE and SE. The high influent ammonium nitrogen concentration in PE (31.73±4.69 mg N/L) was completely oxidized to nitrate. The concentration of nitrate in influent increased substantially from 1.87±0.05 mg/L to of 36.04±1.75 mg/L in effluent. Also, the low ammonium nitrogen concentration in SE (0.35±0.3 mg N/L was completely oxidized to nitrate. Nitrate concentration of effluent increased from 5.96±1.5 to 6.91±3 mg/L.

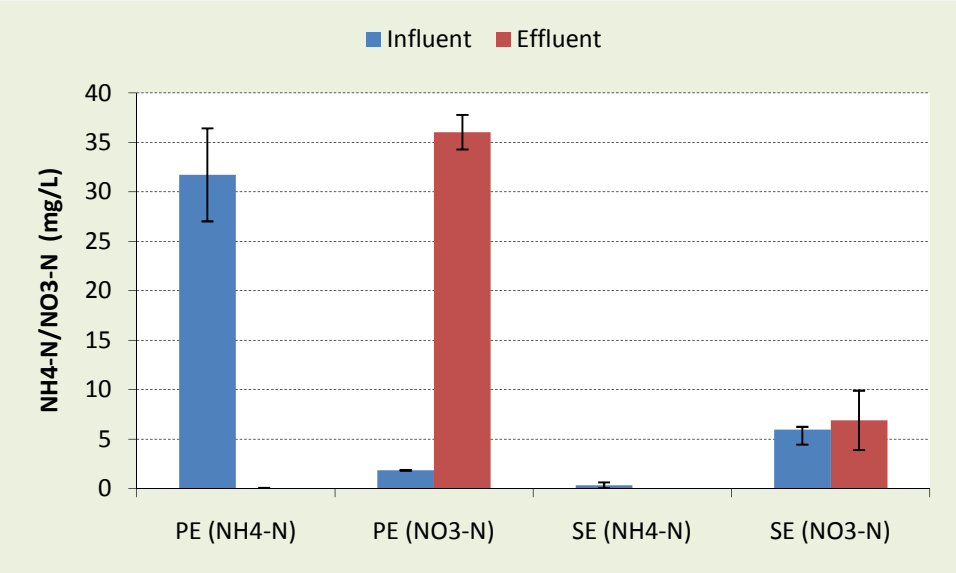



**Figure 4-10:** Changes in ammonium nitrogen and nitrate concentrations in batch reactors (Influent: PE and SE after 1-2 hour settling, volume of batch: 500 mL, mass of sand media =100 g and media size = 0.8-1.25 mm, aerobic conditions)

The removal of ammonium nitrogen in the aerobic batch reactors was through nitrification process which is considered to be a dominant removal mechanism of ammonium nitrogen in aerobic batch reactors. The observed removal of ammonium nitrogen through nitrification in batch reactors with PE and SE respectively was 99.91 and 100%. Dissolved oxygen concentration of the effluent water after five days in PE was 2.1±0.6 mg/L and in SE was 4.4±0.8 mg/L. It was well established that ammonium nitrogen was ultimately converted to nitrate through nitrification process and was possibly assimilated into the cells through biomass synthesis. Thus a significant decrease in ammonium nitrogen could be caused by both its conversion and assimilation, especially with the help of air being able to penetrate into batch reactors during the agitation. A concurrent decrease in the dissolved oxygen also

supports the active nitrification of ammonium nitrogen.

The concentration of dissolved oxygen coupled with nitrate concentration of the effluent proves that there was no denitrification process taking place in aerobic batch reactors. The observed high ammonium nitrogen oxidation in reactors fed with PE is in agreement with the study made by Reemtsma *et al.* (2000) during the research on infiltration of combined sewer overflow and tertiary municipal wastewater: an integrated laboratory and field study on nutrients and dissolved organics where ammonium concentration of 23±9 mg N/L was completely oxidized to nitrate.

**ii- Removal of nitrogen under anoxic conditions**

Figure 4-11 shows the concentrations of $NH_4$-N and $NO_3$-N in batch reactors with PE and SE respectively under anoxic conditions. The ammonium nitrogen and nitrate concentrations in Figure 4-11 provide no indication for a significant denitrification in the batch reactors, which may partly be due to the fact that there was insufficient oxygen demand to create anoxic conditions and nitrogen removal was not apparent.

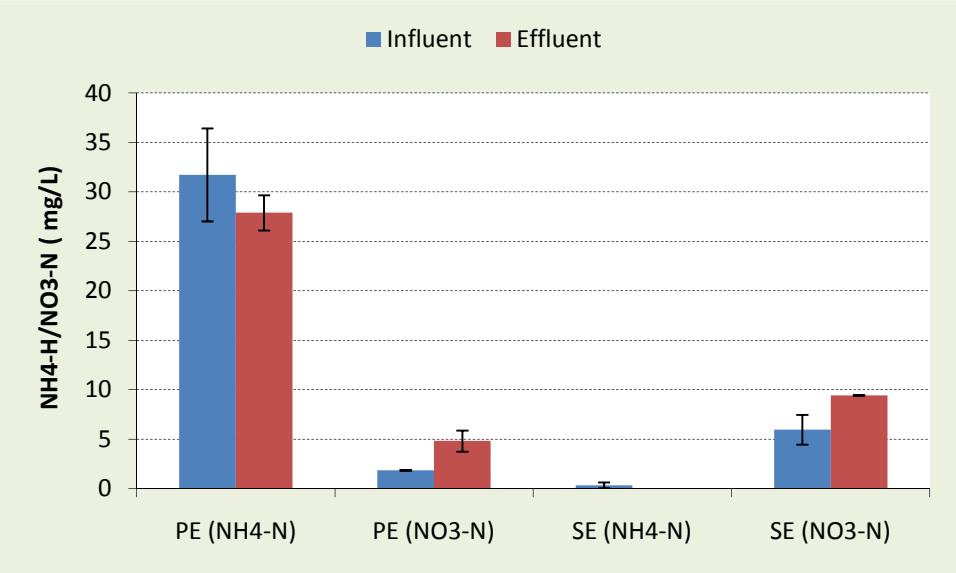



**Figure 4-11:** Change in ammonia and nitrate concentrations in batch reactors (Influent = PE after 1-2 hour settling, volume of batch = 500 mL, mass of sand media =100 g and media size = 0.8-1.25 mm, anoxic conditions)

The removal of ammonium nitrogen in batch reactors was very less due to less oxygen environment that limits nitrification process in anoxic batch reactors. About 12.1% of ammonium nitrogen was removed in batch reactors fed with PE under anoxic conditions whereas in SE the ammonium nitrogen was completely oxidized to nitrate due to less concentration of influent ammonium nitrogen. The concentration of ammonium nitrogen in PE was 31.73±4.69 mg N/L and final effluent concentration was 27.89±1.78 mg N/L. Concentration of ammonium nitrogen in SE was 0.35±0.3 mg N/L whereas final effluent

concentration was zero. Subsequent nitrate concentration of influent water in PE was 1.87±0.05 mg/L while final effluent concentration increased to 4.87±1.07 mg/L while initial concentration of influent water in SE was 5.96±1.50 mg/L which increased to 9.44±0.05 mg/L.

Redox potential measurements verified that the water was shaking in anoxic zone with the average reading of 27.4±8.1 mV conversely to aerobic conditions where average redox potential was 199±9.8 mV. It seems that nitrification was still taking place under anoxic zone because ammonium nitrogen decreased somehow and corresponding nitrate in effluent water increased which justifies there was some nitrification in SE and PE. It is expected that denitrification is taking place under anoxic conditions, which means nitrate concentration would have decreased. These results are in agreement with Sabumon (2007) in a study for anaerobic ammonia removal in presence of organic matter where he reported sequential batch studies that confirmed the possibility of anaerobic ammonia removal in presence of organic matter, but ammonia was oxidized anoxically to nitrate (at oxidation reduction potential; =−248±25 mV) by an unknown mechanism. The oxygen required for oxidation of ammonia might have been generated through catalase enzymatic activity of facultative anaerobes in mixed culture.

### b- Soil column experiments

#### i- Removal of nitrogen under aerobic conditions

Figure 4-12 and Figure 4-13 show the changes in ammonium nitrogen concentration in soil columns (PE-C1, PE-C2, SE-C1 and SE-C2). The concentration of ammonium nitrogen in influent water with PE was 33.15±3.83 mg N/L and final effluent concentration exiting the two columns namely PE-C1 and PE-C2 was 1.37±2.10 and 0.16±2.10 mg N/L respectively. Concentration of ammonium nitrogen in SE was 0.13±0.03 mg/L N and final effluent concentration of SE-C1 and SE-C2 was 0.06 and 0.04 mg N/L respectively.

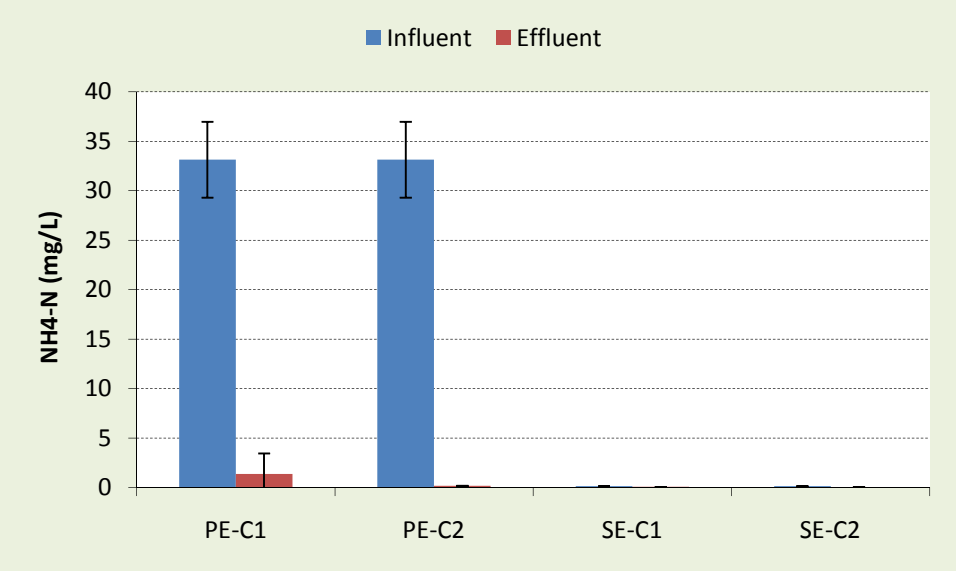



**Figure 4-12:** Change in ammonium concentrations in soil column (Influent = PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3m, media size = 0.8-1.25 mm, aerobic conditions)

The observed ammonium nitrogen removal in PE-C1 and PE-C2 was 95.9 and 99.5% respectively, likewise in SE-C1 and SE-C2 the ammonium nitrogen removal was 53.9 and 69.2% respectively. This might be attributed by optimum conditions for ammonium nitrogen oxidizers. Over the test period, the average values of the influent pH and temperature were 7.9±0.08 and 21.1±1.3˚C respectively. It is evident from these studies that oxygen availability is an important factor in nitrification (and denitrification) reactions and thus overall nitrogen removal from infiltrating water.

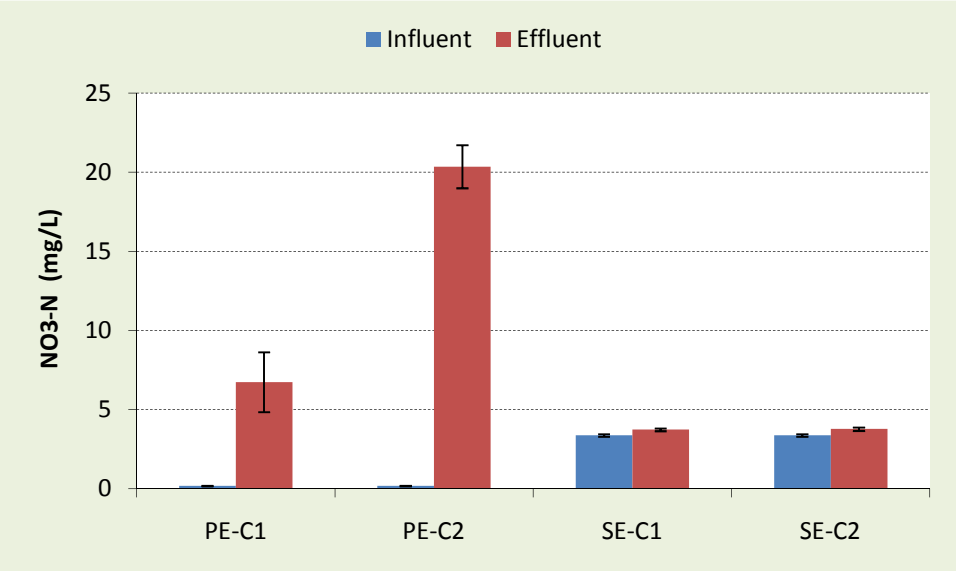



**Figure 4-13:** Change in nitrate concentrations in soil columns (Influent = PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

However, nitrate nitrogen was not completely removed from the system due to the fact that effluent water exiting the columns had nitrate concentration of 6.74±1.89 mg/L in PE-C1 and 20.36±1.36 mg/L and in PE-C2. The nitrate concentration in the effluent indicates that fully denitrification could not take place due to limited depth and retention time of columns used for this study; though in PE-C1 denitrification was achieved to some extent due to less nitrate concentration in the effluent.

**ii- Removal of nitrogen under anoxic conditions**

Figure 4-14 and Figure 4-15 show change in ammonium nitrogen and nitrate concentration under anoxic conditions.

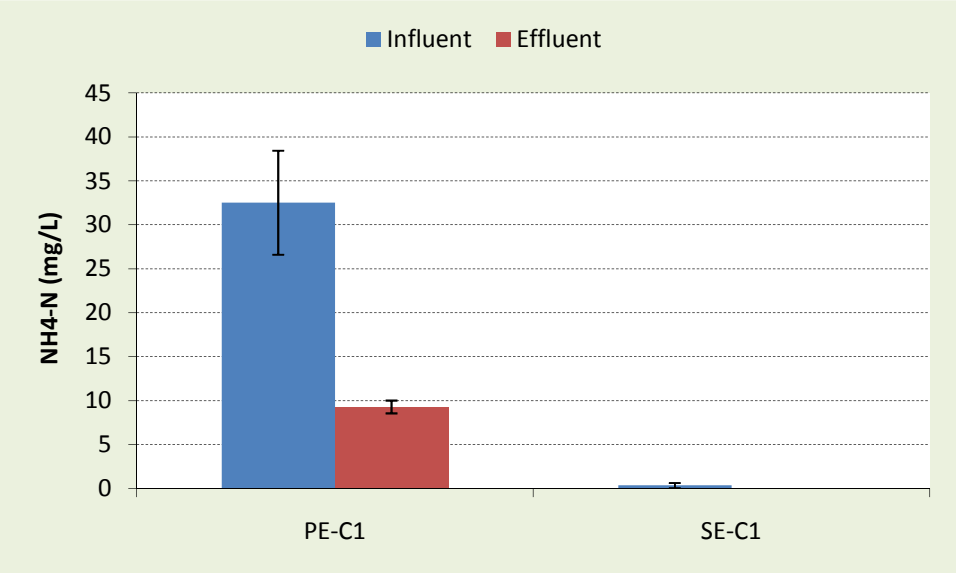



**Figure 4-14:** Change in ammonium nitrogen concentrations in soil columns (Influent = PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3, media size = 0.8-1.25 mm, anoxic conditions)

The ammonium removal in PE was 71.4% under anoxic conditions. Denitrification requires the presence of nitrate and organic carbon (an energy source for denitrifying bacteria) under anaerobic conditions. The concentration of ammonium nitrogen of influent water in PE was 32.53±5.92 mg N/L whereas final effluent concentration exiting the column was 9.30±0.73 mg N/L. The concentration of ammonium nitrogen of influent water in SE was 0.36±0.3 mg N/L while final effluent concentration exiting the column was zero.

Redox potential was regularly used to indicate aeration conditions, and it is a significant aspect to determine the nitrification–denitrification process. Redox potential measurements and dissolved oxygen in PE was monitored and the average readings were 38.22±1.3 mV and 0.46 mg $O_2$/L in effluent water exiting the soil columns. Nitrate concentrations in influent and effluent water for SE decreased from 5.16±0.94 mg/L to 3.89±0.73 mg/L $NO_3$-N indicating that denitrification was taking place across the column. Nevertheless, in anoxic operating

conditions where nitrification is not favored, the decrease of ammonium nitrogen concentrations from 32.53±5.92 mg N/L to 9.30±0.73 mg N/L suggests that there was another significant mechanism for ammonium removal. This could possibly be ammonium nitrogen was oxidized anoxically to nitrate in the presence of organic matter as reported by Sabumon (2007). The corresponding nitrate concentration increased substantially from 1.87±0.05 mg/L in the influent to 16.27±2.70 mg/L in effluent water as shown in Figure 4-15. Nitrogen removal mechanisms in columns media include nitrification–denitrification and adsorption.

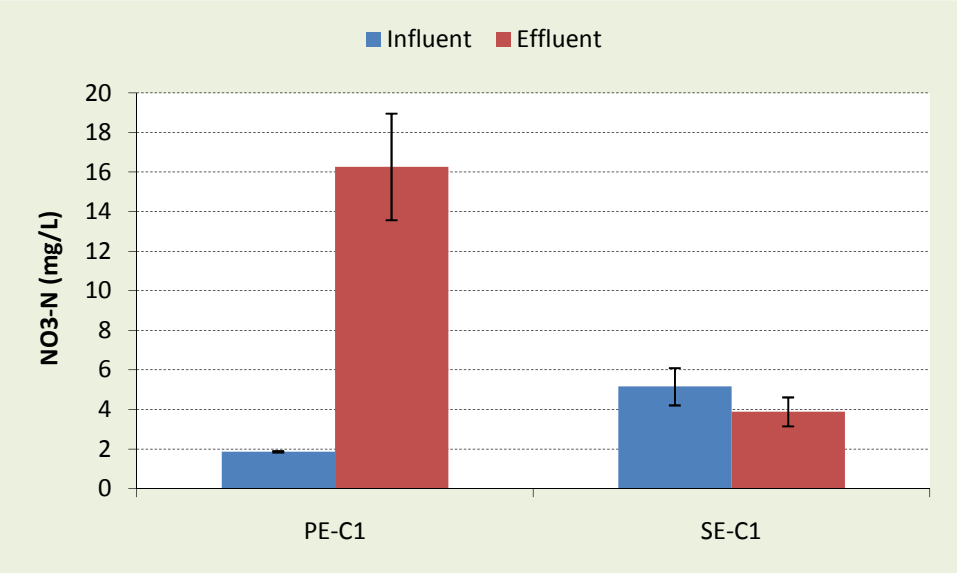



**Figure 4-15:** Change in nitrate concentrations in soil columns (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, anoxic conditions)

Table 4-7 and 4-8 provide the summary on removals of ammonium nitrogen, nitrate and total nitrogen in soil columns and batch studies under different redox conditions with both PE and SE.

**Table 4-7:** Summary table on removal of ammonium nitrogen and nitrate in soil columns and batch studies under different redox conditions in PE

| System | Redox conditions | | $NH_4$-N removal (%) | $NO_3$-N removal (%) | Total nitrogen removal (%) |
|---|---|---|---|---|---|
| Batch tests | Aerobic | | 99.9 | -1827 | 1.26 |
| | Anoxic | | 12.1 | -160 | 6.5 |
| Soil column tests | Aerobic | PE-C1 | 95.9 | -3644 | 76 |
| | | PE-C2 | 99.5 | -11211 | 38 |
| | Anoxic | | 71 | -770 | 26 |

- Means increase

**Table 4-8:** Summary table on removal of ammonium nitrogen and nitrate in soil columns and batch studies under different redox conditions in SE

| System | Redox condition | | $NH_4$-N removal (%) | $NO_3$-N removal ( % ) | Total nitrogen removal (%) |
|---|---|---|---|---|---|
| Batch tests | Aerobic | | 100 | -16 | 46.8 |
| | Anoxic | | 100 | -58 | -49.4 |
| Soil column tests | Aerobic | SE-C1 | 53.9 | -11 | 8.0 |
| | | SE-C2 | 69.2 | -12 | 8.8 |
| | Anoxic | | 100 | 25 | 29.5 |

- Means increase

### 4.4.3 Effect of redox conditions on phosphorus removal

The P removal was measured with batch and short soil columns experiments. The maximum removal is observed in batch experiments under aerobic conditions for both PE and SE than in columns studies partly due to longer hydraulic retention in batch reactors. Phosphorus concentration in the wastewater effluent varied between 3.07 and 6.37 mg/L in PE and varied between 1.75 and 6.25 mg/L in SE during the research period for both batch reactors and soil columns. Phosphorus concentration after SAT was 4.53±0.19 mg/L in PE and 2.50±0.42 in SE (Figure 4-15 and 4-16). The main processes responsible for phosphorus removal are chemical precipitation and adsorption.

#### a- Batch experiments

The phosphorous removal in the batch experiments was studied for aerobic and anoxic conditions as depicted below

#### i- Removal of phosphorus under aerobic conditions

Figure 4-16 shows the phosphorous removal in batch experiment for both PE and SE. The phosphorous concentration of the influent water in PE was 6.29±0.79 mg/L whereas concentration of effluent after the hydraulic retention time of five days in batch was 4.53±0.19 mg/L accounting to 27.98±5.4% removal. On the other hand, the phosphorous concentration of the influent water in SE was 3.94±1.04 mg/L and concentration of effluent after the hydraulic retention time of five days was 2.50±0.42 mg/L accounting to 36.54±4.8% removal.

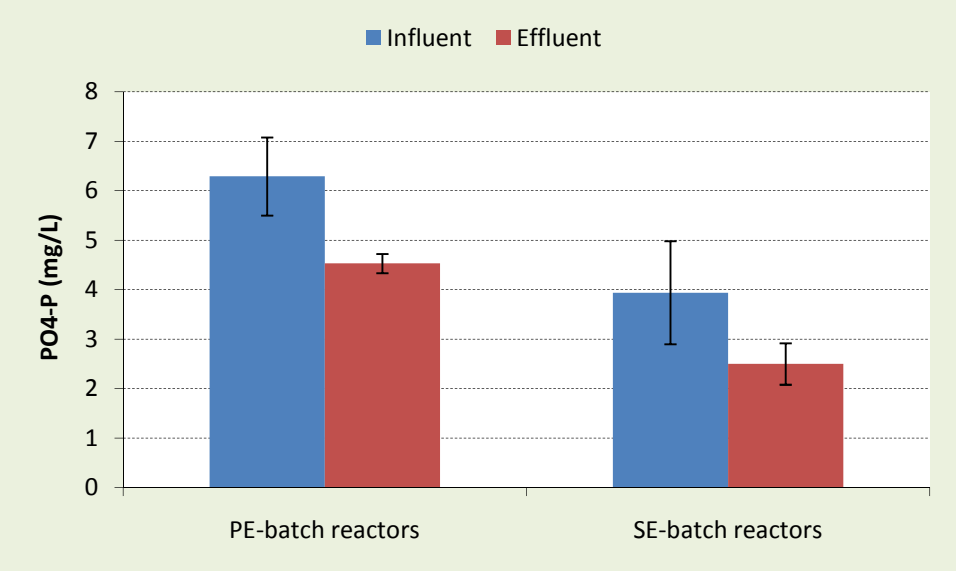



**Figure 4-16:** Change in phosphorous concentrations in batch reactors (Influent: PE and SE after 1-2 hours settling, volume of batch: 500 mL, mass of sand media =100 g and media size = 0.8-1.25 mm, HRT = 5 days, aerobic conditions)

The above results indicate very low efficiency of phosphorus removal by SAT possibly due to the small soil volume, soil type and size participating in the adsorption process. Furthermore the amount of carbon in PE may have an effect on P removal in two ways: by (1) blocking the adsorption sites and (2) competing with phosphorous ions for the adsorption sites. This can explain why the removal for this case is higher in SE than in PE under aerobic conditions.

Prior to application of influent water to the batch reactors, the influent was aerated to at least 8.0 mg $O_2$/L to maintain aerobic conditions and the average effluent $O_2$ concentration in PE after 5 days was 2.1±0.6 mg $O_2$/L and in case with SE was 4.4±0.8 mg $O_2$ /L

**ii- Removal of phosphorus under anoxic conditions**

Figure 4-17 shows the phosphorous removal in both PE and SE in batch reactors under anoxic conditions. The phosphorous concentration of the influent water in PE was 6.37±0.85 mg/L and concentration of effluent after the hydraulic retention time of five days was 4.97±0.42 mg/L accounting to 22±3.03 % removal. On the other hand, the phosphorous concentration of the influent water in SE was 5.43±0.57 mg/L and concentration of effluent after the hydraulic retention time of five days was 4.47±0.42 mg/L accounting to 17.68±4.92 % removal.

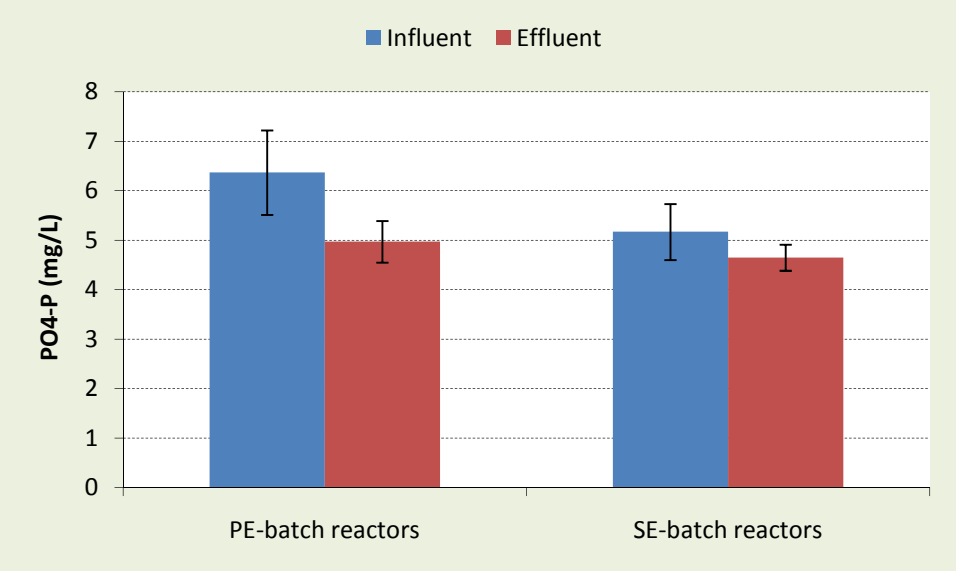



**Figure 4-17:** Change in phosphorous concentrations in batch reactors (Influent: PE and SE after 1-2 hours settling, volume of batch: 500 mL, HRT = 5 days, mass of sand media =100 g and media size = 0.8-1.25 mm, anoxic conditions)

The above results indicate the very low efficiency of phosphorus removal by SAT under anoxic conditions than in aerobic conditions showing that aerobic conditions are advantageous with respect to the retention of phosphorous. Despite that the retention time in batch experiments was 5 days; the results for this study are contrary to findings by Vohla et al (2007) in the study of dynamics of phosphorus, nitrogen and carbon removal in a horizontal subsurface flow constructed wetland. The reported findings were that during the first 4 months of the ash filter experiment, the efficiency of P removal was about 71%, resulting in average concentration 1.9 mg P /L in the outlet with hydraulic retention time (1.5–2 days).

### b- Soil column experiments

The phosphorous removal as in case of batch experiments was also studied with soil columns experiments under aerobic and anoxic conditions.

#### i- Removal of phosphorus under aerobic conditions

Figure 4-18 shows change in phosphorous concentrations in soil columns with PE and SE under anoxic conditions.

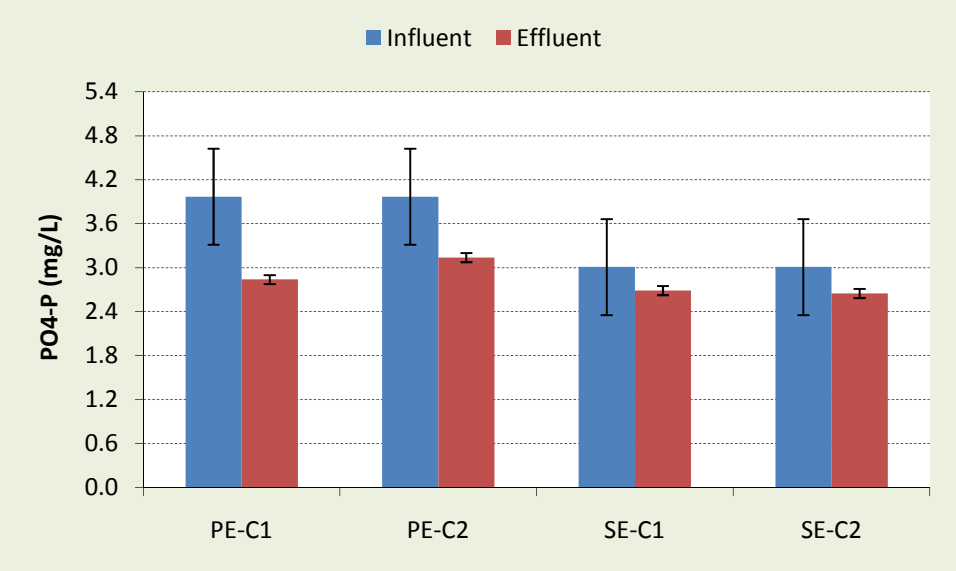



**Figure 4-18:** Change in phosphorous concentrations in soil columns (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3m, media size = 0.8-1.25 mm, anoxic conditions)

Influent phosphorous concentration in PE was 3.97±1.65 mg/L whereas concentration exiting the soil columns in the two soil columns PE-C1 and PE-C2 operating under aerobic conditions was 2.84±0.63 and 3.14±0.66 mg/L corresponding to 31.09±2.54 and 23.16±4.71 % removal. Similarly, the influent phosphorous concentration in SE was 3.01±0.2 mg/L whereas the concentration exiting the soil columns in the two soil columns SE-C1 and SE-C2 operating under aerobic conditions was 2.69±0.1 mg/L and 2.65±0.1 mg/L corresponding to 10.46±3.87 and 11.00±4.33 % respectively. The above results indicate even very low efficiency of phosphorus removal in soil columns than in batch reactors most likely due to short hydraulic retention time in columns, media type and size.

**ii- Removal of phosphorus under anoxic conditions**

Figure 4-19 shows change in phosphorous concentration in both PE and SE under anoxic conditions. Influent phosphorous concentration in PE was 6.15±0.75 mg/L and concentration exiting the soil column PE-C1 operating under anoxic conditions was 4.84±0.49 corresponding to 21.10±3.69% removal. Similarly, the influent phosphorous concentration in SE was 3.65±0.91 mg/L and concentration exiting the soil column SE-C1 operating under anoxic conditions was 3.32±0.78 corresponding to 8.79±1.72% removal.

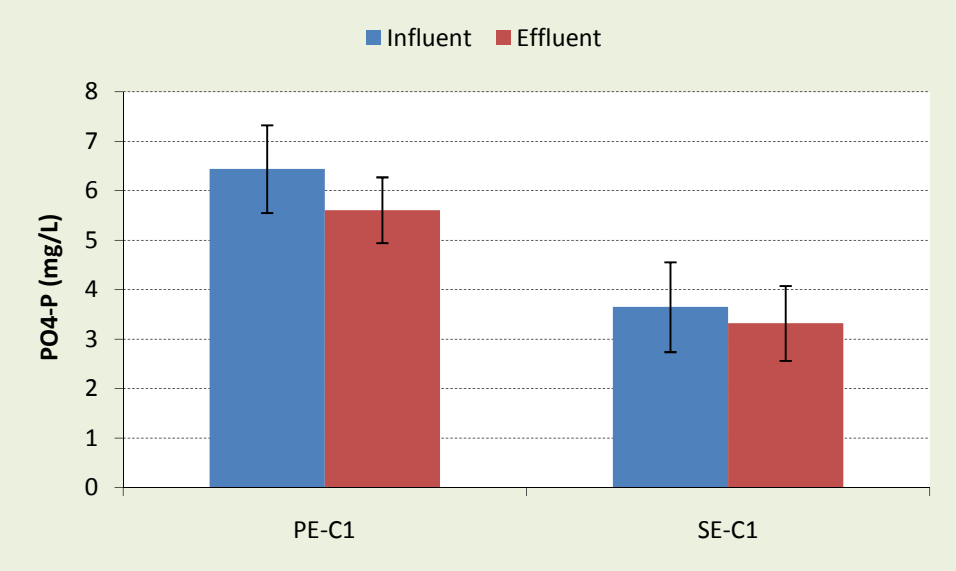



**Figure 4-19:** Change in phosphorous concentrations in soil columns (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0 .8-1.25 mm, anoxic conditions)

The phosphorus removal by SAT in both batch reactors and soil columns under both aerobic and anoxic conditions are quite low most likely due to short hydraulic retention time in columns, media type and size. These results are consistent with Vohla et al (2011) in a paper reviewing filter materials for phosphorus removal from wastewater in treatment wetland where removals reported were very low to about 22.44%. The removal efficiency was predominantly affected by porous media size and type.

Table 4-9 below gives the summary of phosphorous removal under different redox conditions during batch and soil column experiments.

**Table 4-9:** Summary table on phosphorous removal under different redox conditions in both PE and SE during batch and soil column experiments.

| **System** | **Redox conditions** | **PE ($PO_4$-P removal %)** | | **SE ($PO_4$-P removal %)** | |
|---|---|---|---|---|---|
| | | PE-C1 | PE-C2 | SE-C1 | SE-C2 |
| Soil column | Aerobic | 31 | 23 | 10.5 | 11 |
| | | **PE ($PO_4$-P removal %)** | | **SE ($PO_4$-P removal %)** | |
| | Anoxic | 21 | | 9 | |
| Batch tests | Aerobic | 28 | | 37 | |
| | Anoxic | 22 | | 18 | |

### 4.4.4 Effect of redox conditions on pathogen removal

The fate of micro-organisms and the ability to assure the microbial quality of groundwater is vital to protecting public health and to obtain public acceptance of managed groundwater recharge programs. The capacity of the soil columns and batch reactors was assessed in removing of *E-coli* and *total coliform* bacteria from wastewater during soil passage with respect to redox conditions. Plate count tests were conducted for influent and effluent samples from soil columns operating at hydraulic rate of 0.625 m/day and retention time of 5 days in batch reactors.

#### a- Batch experiments

Low levels of pathogens were detected in the effluent water especially in SE where pathogens were removed in 2 to above 2.5 log removal units. Contrary to PE, there were still some pathogens in the effluent water despite the fact that high removals were observed accounting from 3 to 3.5 log removals. This was attributed by the fact that the influent water in SE had initially less pathogens as compared to PE. So from health point of view removal of pathogens in SE is more valuable as the produced effluent water was of drinking water quality microbiologically as *E-coli* and *total coliform* were absent in 100 mL water.

#### i- Removal of pathogens under aerobic and anoxic conditions

The results indicate that the removal of pathogens in batch reactors is not significantly affected by redox conditions. The results suggest a 2-3-log reduction of *E-coli* and *total coliform* for both aerobic and anoxic conditions though there were some fluctuations in PE for aerobic conditions to removals of 4-log reduction that was characterized by the instability in the influent concentration. Figure 4-18 shows the log removal of *E-coli* and *total coliform* in the batch reactors for both aerobic and anoxic conditions.

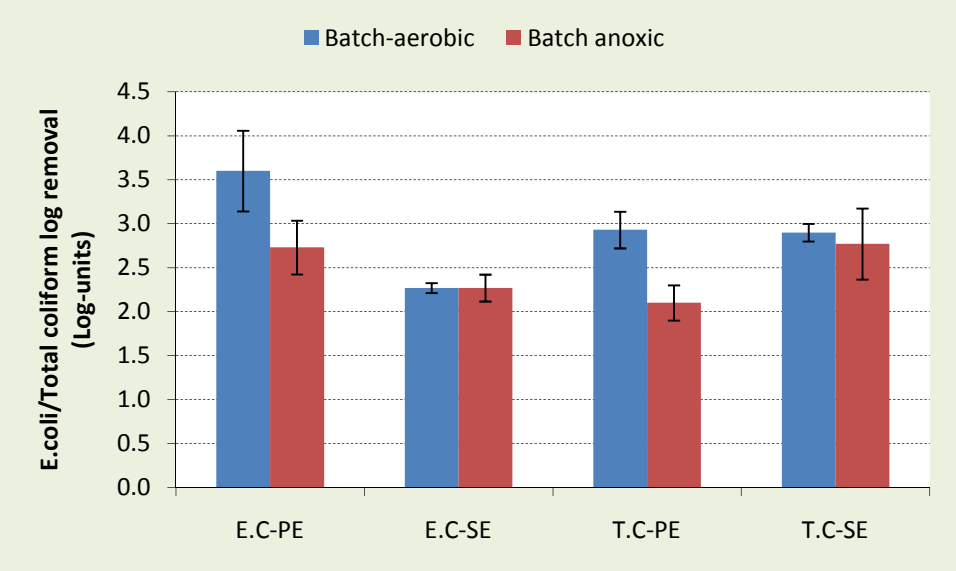



**Figure 4-20**: *E-coli and total coliform* log removal in batch reactors (Influent: PE and SE after 1-2 hours settling, volume of batch =500 mL, HRT = 5 days, mass of sand media =100 g and media size = 0.8-1.25 mm, aerobic and anoxic conditions)

The dominating factors that influence retention and removal of pathogens include physical straining as well as adsorption to porous media. The grain size of porous media, pH, temperature, organic matter and bacterial cell size are important factors affecting the removal of pathogens as report by Stevik *et al.*( 2004).

**b- Soil column experiments**

Similarly, for soil columns operating under aerobic and anoxic conditions with hydraulic flow rate of 0.625 m/day, no apparent effect of redox conditions on pathogens removal was observed, strengthening the results obtained from the batch reactors.

**i- Removal of pathogens under aerobic conditions**

Figure 4-19 shows the log removal of *E-coli* and *total coliform* in the batch reactors for both aerobic and anoxic conditions. The results from column studies indicate a 2-3-log reduction of *E-coli* and *total coliform* for both aerobic and anoxic conditions though there were some fluctuations in both aerobic and anoxic conditions where high removals were observed under aerobic conditions and vice versa.

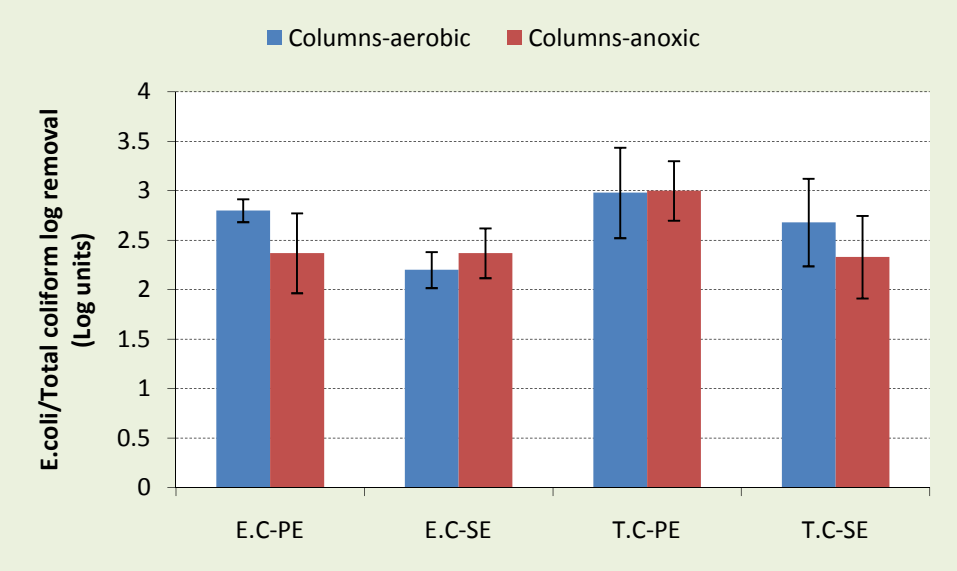



**Figure 4-21**: *E-coli and total coliform* log removal in soil columns (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, columns depth = 0.3 m, media size = 0.8-1.25 mm, aerobic and anoxic conditions)

The results of *E-coli* and *total coliform* removal in soil columns under aerobic and anoxic conditions could not reveal the dependence of redox conditions on the removal of pathogenic bacteria as shown in Figure 4-21. The trend is that *total coliform* in PE were removed to 3-log in both aerobic conditions and anoxic conditions whereas *total coliform* in SE was removed to 2.8- log under aerobic conditions while it was removed to 2.4-log under anoxic conditions. Additionally, *E-coli* in PE were better removed under aerobic conditions while *E-coli* were better removed under anoxic conditions in case with SE.

Table 4-10, Table 4-11 and Table4-12 below show the summarized removals of different parameters at different redox conditions during batch and soil column experiments with both PE and SE.

**Table 4-10**: Summary table on E-coli and total coliform removal under different redox conditions in PE and SE

| **System** | **Redox conditions** | **PE ( Log removal )** | | **SE ( Log removal )** | |
|---|---|---|---|---|---|
| | | *E-coli* | *Total coliform* | *E-coli* | *Total coliform* |
| Batch tests | Aerobic | 3.6 | 2.9 | 2.3 | 2.9 |
| | Anoxic | 2.7 | 2.1 | 2.3 | 2.8 |
| Soil columns | Aerobic | 2.8 | 3.0 | 2.2 | 2.7 |
| | Anoxic | 2.4 | 3.0 | 2.4 | 2.3 |

**Table 4-11:** Summary table indicating removal of different parameters at different redox conditions in batch reactors fed with PE and SE.

| Influent | Parameter | Redox conditions | |
|---|---|---|---|
| | | **Aerobic** | **Anoxic** |
| **PE** | Organic matter | 55 % | 40 % |
| | Ammonium nitrogen | 99.9 % | 12.1% |
| | Phosphorous | 28 % | 22 % |
| | Pathogens *E-coli* | 3.6 Log | 2.7 Log |
| | *Total coliform* | 2.9 Log | 2.1 Log |
| **SE** | Organic matter | 25 % | 15 % |
| | Ammonium nitrogen | 100 % | 100 % |
| | Phosphorous | 37 % | 18 % |
| | Pathogens *E-coli* | 2.3 Log | 2.3 Log |
| | *Total coliform* | 2.9 Log | 2.8 Log |

**Table 4-12:** Summary table indicating removal of different parameters at different redox conditions in soil columns with PE and SE.

| Influent | Parameter | Redox conditions | |
|---|---|---|---|
| | | **Aerobic** | **Anoxic** |
| **PE** | Organic matter | 46 % | 31 % |
| | Ammonium nitrogen | 95.9 % | 71.4 % |
| | Phosphorous | 31 % | 21 % |
| | Pathogens *E-coli* | 2.8 Log | 2.4 Log |
| | *Total coliform* | 3.0 Log | 3.0 Log |
| **SE** | Organic matter | 19 % | 13 % |
| | Ammonium nitrogen | 69 % | 100 % |
| | Phosphorous | 11 % | 9 % |
| | Pathogens *E-coli* | 2.2 Log | 2.4 Log |
| | *Total coliform* | 2.7 Log | 2.3 Log |

## 4.5 Effect of temperature on removal of organic matter, nitrogen, phosphorus and pathogens

The effect of temperature was studied in laboratory-scale soil columns on removal of bulk organic matter, nutrients and pathogens. Two borosilicate glass columns with internal diameter of 50 mm and 300 mm depth were packed with 0.8 mm to 1.25 mm silica sand short columns labeled as PE-C2 and SE-C2. The columns were initially acclimated biologically at room temperature (20-22 $^{o}$C), and after ripening the temperature effect was studied at different temperatures controlled by chiller with a cooling liquid. The hydraulic flow rate for both columns was 0.625 m/d with EBCT of 12 hours.

### 4.5.1 Effect of temperature on removal of organic matter in soil columns

After columns had ripened under room temperature 20-22$^{o}$C, columns were set under different temperatures using a cooling liquid in a chiller to get the required temperature. Once the temperature was changed, DOC measurements were monitored for about three weeks to allow microbes to get used to a new environment with respect to the operating temperature.

#### i- Removal of organic matter at 25°C

Figure 4-22 shows the DOC degradation with time across the column operating at 25$^{o}$ C for both PE and SE under aerobic conditions.

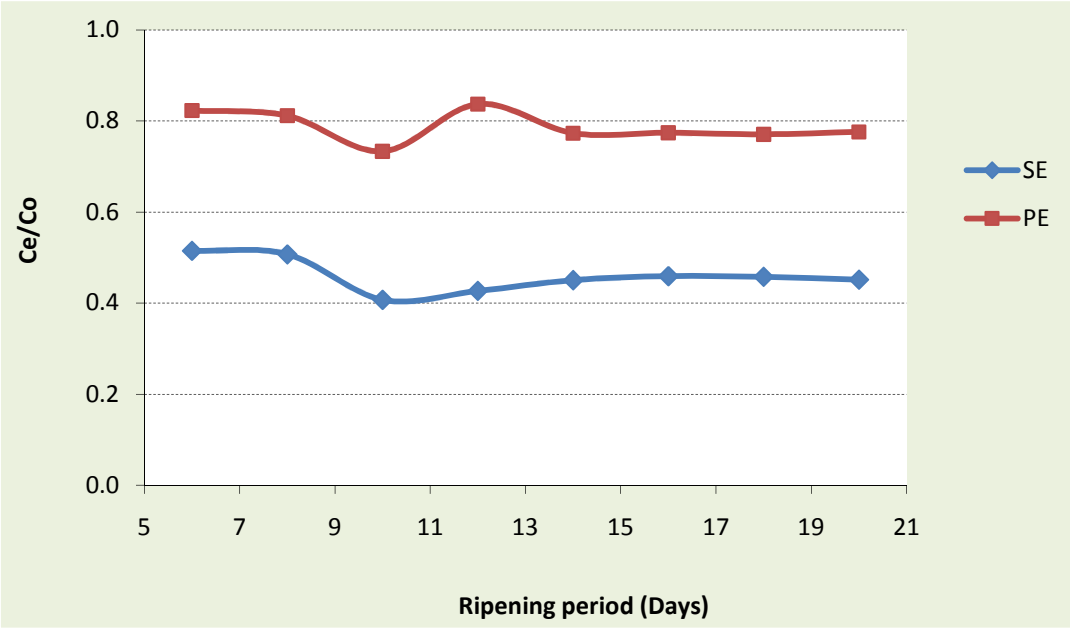



**Figure 4-22:** DOC degradation with time in column at 25$^{o}$C (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

The average influent concentration of DOC in SE under 25$^{o}$ C was 13.73±0.81 mg/L and the residual DOC concentration of the effluent exiting the column was 10.80±0.68 mg/L accounting to a removal of 21.27±3.39 % during ripening. After ripening the DOC removal was 22.66±0.22 %. Also the average influent concentration of DOC in PE for 25$^{o}$C was 29.89±3.27 mg/L and the residual DOC concentration of the effluent exiting the column was 13.64±0.91 mg/L accounting to a removal of 54.06±3.64 % during ripening. After ripening the DOC removal was 54.52±0.44 %. The dependence of temperature on DOC removal for this case is not very visible as removals achieved after ripening at 20$^{o}$ C are quite comparable. The DOC removal ranged from 17.65 to 20.61% in SE at a temperature of 20-22$^{o}$C and from 44.94 to 52.02% in PE at same temperature. UV254 absorption decreased by 25% in PE and 9.2% in SE showing substances with aromatic humic and fulvic structures are less reduced in SE than in PE.

### ii- Removal of organic matter at 20°C

This operating temperature was termed 20$^{o}$ C but it was actually the room temperature where the columns were ripening under. The range of room temperature was 20-22$^{o}$C. DOC attenuation after ripening in PE under aerobic conditions after ripening period was 46.35±2.04 and 50.40±1.65 % for PE-C1 and PE-C2 respectively. Likewise for C2 DOC attenuation after ripening in SE was 18.80±0.79 and 18.74±1.11 % for SE-C1 and SE-C2 respectively. The UVA decreased from 0.515 $cm^{-1}$ to 0.356 $cm^{-1}$ for PE under aerobic conditions and from 0.370 $cm^{-1}$ to 0.320 $cm^{-1}$ in SE under aerobic conditions whereas related SUVA changes are shown in Table 4-3

### iii- Removal of organic matter at 15°C

Figure 4-23 shows the DOC degradation with time in soil column at a temperature of 15$^{o}$C. The average influent concentration of DOC in SE for temperature 15$^{o}$C was 13.88±1.34 mg/L and the residual DOC concentration of the effluent exiting the column was 11.75±0.68 mg/L accounting to a removal of 15.07±4.10 %. Also the average influent concentration of DOC in PE for the temperature 15$^{o}$C was 31.29±3.57 mg/L and the residual DOC concentration of the effluent exiting the column was 17.65±1.65 mg/L accounting to a removal of 42.93±8.27 %.

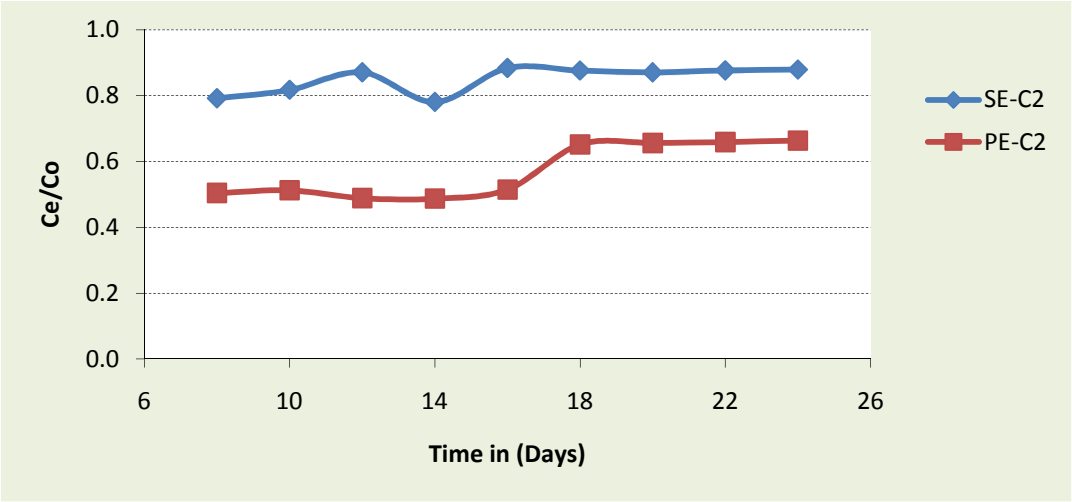



**Figure 4-23:** DOC degradation with time in column at 15$^{o}$C (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

The performance of SAT for seasonal variations did not notably affect DOC removal, which entails that the removal of bulk organic matter is normally a result of aerobic and anaerobic microbial activity which function even in temperatures as low as 5$^{o}$C. The results are consistent with observation reported by Hoppe-Jones *et al (* 2010) in the study for attenuation of total organic carbon and unregulated trace organic chemicals in U.S. riverbank filtration systems and Akratos and Tsihrintzis (2007) in a study for effect of temperature, HRT, vegetation and porous media on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands.

### iv- SUVA changes in laboratory-scale soil columns at different temperatures

Figure 4-24 shows the SUVA changes across the soil columns at temperatures 15$^{o}$C, 20$^{o}$C and 25$^{o}$C. Increase of SUVA values justifies the removal of easy biodegradable aliphatic compounds available in influent water and it shows a direct correlation where lower SUVA values correlated with higher DOC values at both temperatures. SAT in laboratory-scale soil columns reduced the concentration of DOC present in influent water and increased its aromaticity. SUVA changes increased significantly across the SAT columns over the course of the study. The influent SUVA values in PE ranged from 1.76 to 1.92 L/ (mg-m) at both temperatures, and SUVA values exiting the soil column in PE were 2.67 to 2.96 L/(mg-m) higher than influent levels in both temperatures. The percentage increase in SUVA values in PE for temperatures 15$^{o}$C, 20$^{o}$C and 25$^{o}$C is 39.1%, 48.1% and 68.2% respectively.

Similarly in SE, the influent SUVA values ranged from 2.87 to 3.45 L/ (mg-m) at both temperatures, and SUVA values exiting the soil column in SE were 3.22 to 4.05 L/(mg-m) higher than influent levels in both temperatures. The percentage increase in SUVA values in SE for temperatures 15$^{o}$ C, 20$^{o}$ C and 25$^{o}$ C is 9.9%, 12.2% and 17.4% respectively.

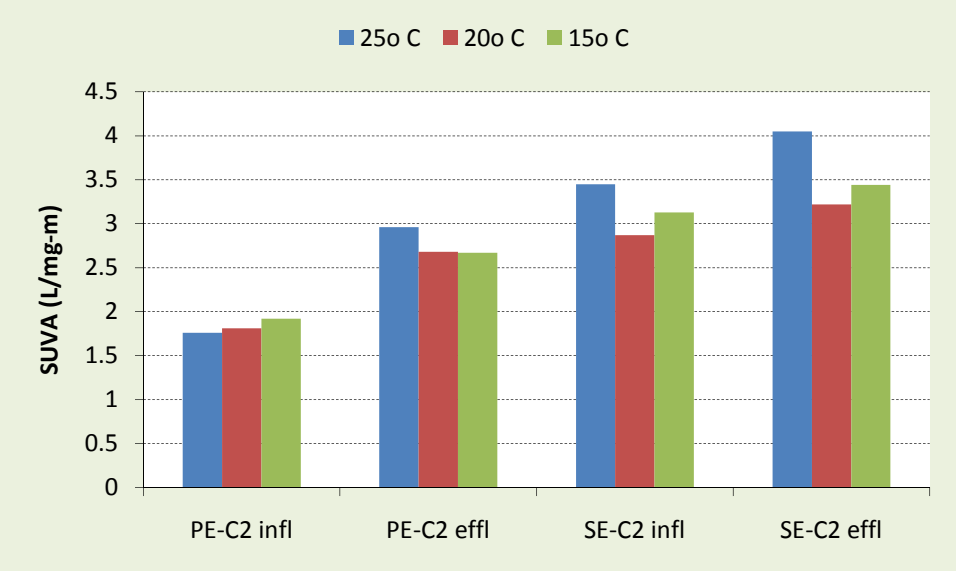



**Figure 4-24:** Influent and effluent SUVA changes in laboratory scale soil columns at temperatures 25$^{o}$C, 20$^{o}$C and 15$^{o}$C. (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

The above results indicate that easily biodegradable aliphatic compounds are better removed at higher temperatures than lower temperatures and the influent water for PE has higher easily biodegradable compounds than in SE. Increased SUVA values across the soil columns may be attributed to preferential removal of non-aromatic compounds during soil infiltration.

### v- Fluorescence EEM of soil columns at 15$^{o}$C, 20$^{o}$C and 25$^{o}$C

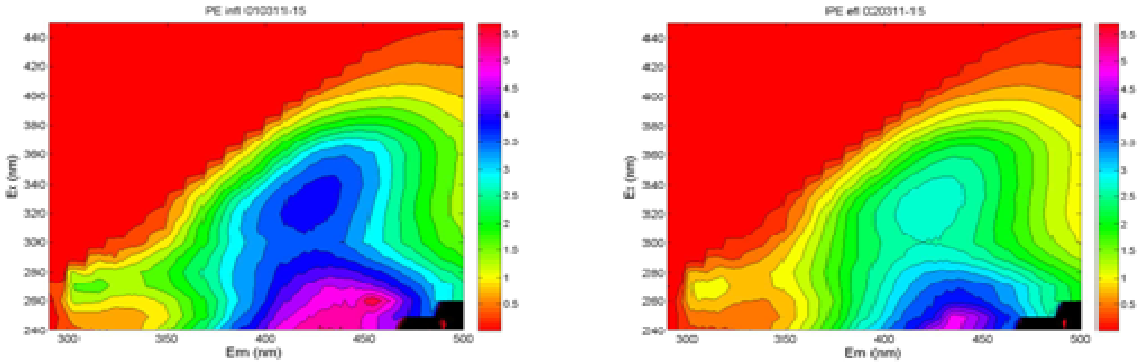
The results of FEEM analysis and reduction of intensities of different peaks from PE and SE are tabulated in Table 4-13 and Table 4-14. F-EEM spectra of PE are shown in Figures 4-25 and 4-26. Details of F- EEM spectra plot is provided in Appendices C4 and C5 for both PE and SE. Three peaks were identified namely, humic/fulvic-like, humic-like and protein-like peaks related to maximum excitation and maximum emission.

**Table 4-13** Fluorescence EEM analysis of influent and effluent of soil columns with PE at different temperatures under aerobic conditions

| **Peak 1 Humic/Fulvic-like organic matter fractions** | | **Excitation (nm)** | **Emission (nm)** | **Intensity** | **Reduction in Intensity ( % )** |
|---|---|---|---|---|---|
| PE-soil column 15$^{o}$C | Influent | 330 | 420 | 4.00 | 27.4 |
| | Effluent | 330 | 420 | 2.91 | |
| PE-soil column 20$^{o}$C | Influent | 330 | 430 | 1.59 | 8.6 |
| | Effluent | 330 | 430 | 1.46 | |
| PE-soil column 25$^{o}$C | Influent | 330 | 424 | 3.79 | 5.3 |
| | Effluent | 330 | 424 | 3.59 | |
| **Peak 2 Humic-like organic matter factions** | | | | | |
| PE-soil column 15$^{o}$C | Influent | 260 | 454 | 5.72 | 35.4 |
| | Effluent | 260 | 454 | 3.69 | |
| PE-soil column 20$^{o}$C | Influent | 250 | 434 | 2.28 | 7.8 |
| | Effluent | 250 | 434 | 2.10 | |
| PE-soil column 25$^{o}$C | Influent | 240 | 436 | 5.27 | 12.0 |
| | Effluent | 240 | 436 | 4.64 | |
| **Peak 3 Protein-like organic matter fractions** | | | | | |
| PE-soil column 15$^{o}$C | Influent | 270 | 308 | 1.77 | 33.0 |
| | Effluent | 270 | 308 | 1.19 | |
| PE-soil column 20$^{o}$C | Influent | 270 | 312 | 0.75 | 26.9 |
| | Effluent | 270 | 312 | 0.55 | |
| PE-soil column 25$^{o}$C | Influent | 270 | 308 | 3.96 | 50.1 |
| | Effluent | 270 | 308 | 1.98 | |

The peaks reductions were higher at temperature 15$^{o}$C compared to other temperatures in PE which means rise in temperature led to reduced fluorescence peak intensity. These results support the hypothesis that fluorescence intensity is highly dependent on temperature. A rise in temperature increases the likelihood that an excited electron will return to its ground state by radiationless decay, leading to reduced fluorescence intensity. For example, fluorescence intensity can increase by 1% with a 1$^{o}$ C decrease in temperature, within the range 10–45 $^{o}$C, for tryptophan-like, humic-like and fulvic-like substances depending on colloid size and fluorophores (Henderson *et al.*, 2009).

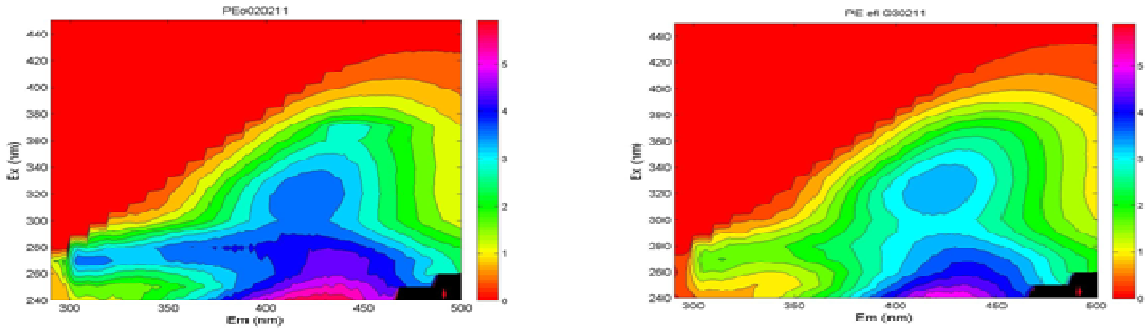
PE-spectrum at a temperature of 15$^{o}$C



Influent (a) Effluent (b)

**Figure 4-25:** F-EEM spectra for influent (a), and effluent (b) in soil columns at 15$^{o}$C (b); (Influent: PE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

PE-spectrum at a temperature of 25$^{o}$C



Influent (a) Effluent (b)

**Figure 4-26:** F-EEM spectra for influent (a), and effluent (b) in soil columns at 25$^{o}$C, (b); (Influent: PE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

Peak 1 was reduced by 27.4%, 8.6% and 5.3% at 15$^{o}$C, 20$^{o}$C and 25$^{o}$C respectively. Peak 2 was reduced by 35.4%, 7.8% and 12.0% at 15$^{o}$C, 20$^{o}$C and 25$^{o}$C respectively. Peak 3 was reduced by 33%, 27% and 50% at 15$^{o}$C, 20$^{o}$C and 25$^{o}$C respectively. The results shows protein like substances were better removed in at higher temperature. Additionally, peak 1 and peak 2 were better reduced at low temperature, indicating that fluorescence intensity is dependent on temperature.

**Table 4-14** Fluorescence EEM analysis of influent and effluent of soil columns with SE at different temperatures

| **Peak 1 Humic/Fulvic-like organic matter fractions** | | **Excitation (nm)** | **Emission (nm)** | **Intensity** | **Reduction in Intensity ( % )** |
|---|---|---|---|---|---|
| SE-soil column | Influent | 340 | 430 | 3.57 | 13.1 |
| 15$^{o}$C | Effluent | 340 | 430 | 3.10 | |
| SE-soil column | Influent | 350 | 480 | 0.51 | 28.6 |
| 20$^{o}$C | Effluent | 350 | 480 | 0.37 | |
| SE-soil column | Influent | 330 | 420 | 4.19 | 12.4 |
| 25$^{o}$C | Effluent | 330 | 420 | 3.67 | |
| **Peak 2 Humic-like organic matter fractions** | | | | | |
| SE-soil column | Influent | 250 | 434 | 4.75 | 9.4 |
| 15$^{o}$C | Effluent | 250 | 434 | 4.31 | |
| SE-soil column | Influent | 250 | 480 | 0.72 | 21.9 |
| 20$^{o}$C | Effluent | 250 | 480 | 0.56 | |
| SE-soil column | Influent | 240 | 436 | 8.18 | 41.9 |
| 25$^{o}$C | Effluent | 240 | 436 | 4.75 | |
| **Peak 3 Protein-like organic matter fractions** | | | | | |
| SE-soil column | Influent | 270 | 308 | 1.32 | -25.3 |
| 15$^{o}$C | Effluent | 270 | 308 | 1.66 | |
| SE-soil column | Influent | 270 | 342 | 0.04 | 38.2 |
| 20$^{o}$C | Effluent | 270 | 342 | 0.02 | |
| SE-soil column | Influent | 270 | 308 | 2.60 | -11.3 |
| 25$^{o}$C | Effluent | 270 | 308 | 2.90 | |

On the other hand, soil columns studies were concurrently conducted with SE where in this case the peaks response to temperature was quite contrary as a case with PE. In this case protein-like peaks and humic/fulvic peaks were more reduced at a temperature 20$^{o}$C. Humic-like peaks were reduced better at a temperature 25$^{o}$C. Some samples showed increase in protein-like peaks at temperature 15$^{o}$C and 25$^{o}$C possibly due to formation of new fluorescing material associated with DOC biodegradation. Details of these results are shown in appendix C5 and Table 4-14

### 4.5.2 Effect of temperature on nitrogen removal in soil columns

The effect of temperature on removal on nitrogen during soil passage was studied at laboratory-scale soil columns for various temperatures. The soil column tests were carried out to ascertain dependence of nitrification process on temperature in removing nitrogen and other contaminants during SAT and establish overall removal efficiency of SAT at low and high temperatures.

#### i- Removal of nitrogen at 25°C

Figure 4-27 shows the reduction in concentration of ammonium nitrogen and nitrate at operating temperature of 25$^{o}$ C.

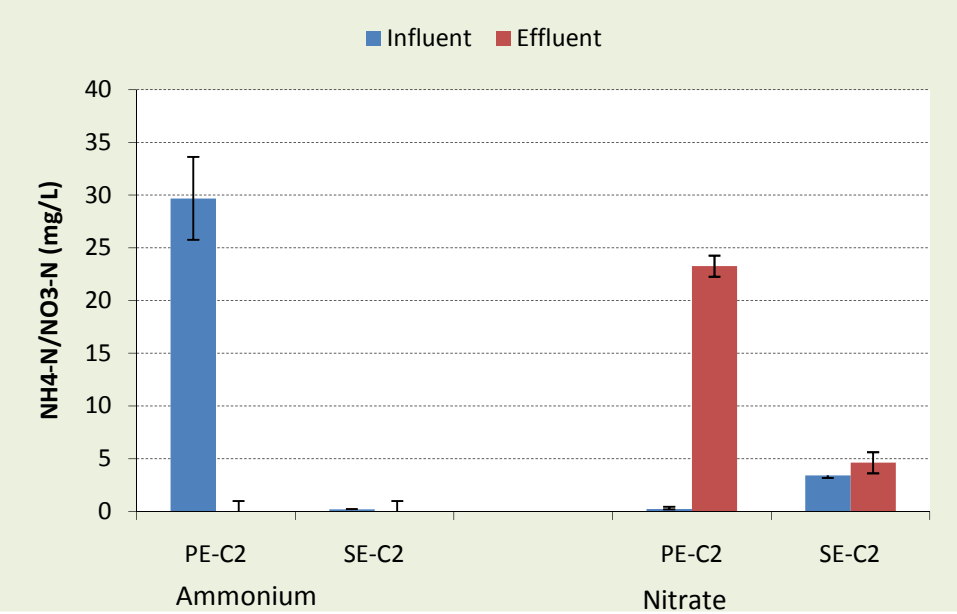



**Figure 4-27:** Change in ammonium and nitrate concentrations in soil columns at 25$^{o}$C (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

The average ammonium nitrogen influent concentration in PE and SE was 29.69±3.93 mg/L and 0.21±0.03 mg/L respectively. The effluent ammonium nitrogen concentration exiting the columns for both PE and SE was zero with effluent nitrate concentration going up to 23.26±1.12 mg/L in PE. This implies that the removal of ammonium nitrogen was 100% and the effect of temperature change is evident at a temperature of 25$^{o}$ C compared to temperature 20$^{o}$ C and 15$^{o}$ C.

#### ii- Removal of nitrogen at 20°C

Figure 4-12 and Figure 4-13 shows the changes in concentration of ammonium nitrogen in soil columns (PE-C1, PE-C2, SE-C1 and SE-C2) at 20$^{o}$C temperature under aerobic conditions. It is obvious from this work that temperature is a significant aspect in nitrification

process and thus nitrogen removal from SAT systems. The ammonium nitrogen removal for PE-C1 and PE-C2 was 95.9 and 99.5% respectively, likewise for SE-C1 and SE-C2 the ammonium removal was 53.9 and 69.2% respectively.

### iii- Removal of nitrogen at 15°C

Figure 4-28 shows change in ammonium nitrogen and nitrate concentrations in soil columns at 25° C.

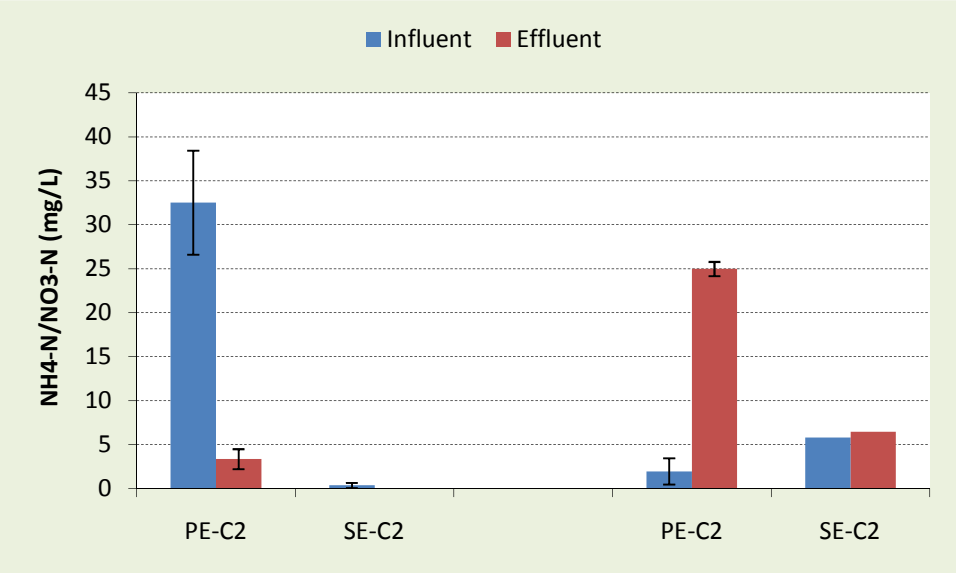



**Figure 4-28**: Change in ammonium and nitrate concentrations in soil columns at 25° C (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

Ammonium nitrogen oxidation was visibly affected by temperature in case with PE and SE. At a temperature of 25°C the ammonium nitrogen was fully oxidized to nitrate and at 20°C the oxidation of ammonia in PE was 95.5% for PE- C1 and 99.5% for PE- C2. Likewise in SE at 20°C the removal of ammonium nitrogen was 53.9 and 69.2% for SE-C1 and SE-C2 respectively. At 15°C the ammonium nitrogen oxidation in PE was 89.67% and the removal of ammonium nitrogen in SE was 100% possibly due to very low concentration in the influent. These results reinforce the findings by Stefanakis et al (2011) in the research for effect of wastewater step-feeding on removal efficiency of pilot-scale horizontal subsurface flow constructed wetlands. Details showed that the microorganisms responsible for nitrogen retention function optimally at temperatures above 15°C and consistent with results by Akratos and Tsihrintzis (2007).

### 4.5.3 Effect of temperature on phosphorus removal in soil columns

The effect of temperature in removal of phosphorous during soil passage was conducted in laboratory-scale soil columns with PE and SE as delineated below.

#### i- Removal of phosphorus at 25°C

Figure 4-29 shows the change in concentration of phosphorous for influent and effluent water in PE and SE at a working temperature of 25$^{o}$C.

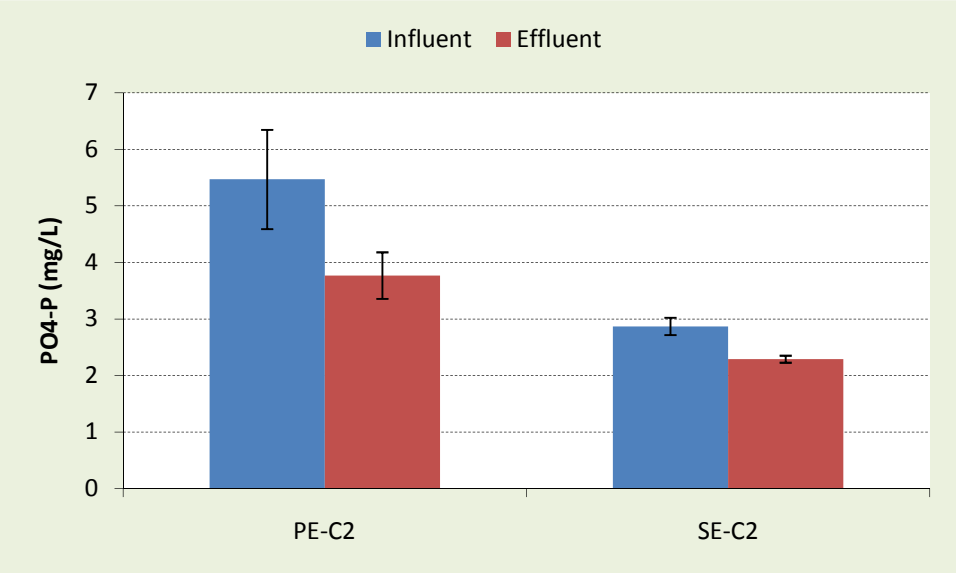



**Figure 4-29:** Change in phosphorous concentrations in soil columns at 25$^{o}$C (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

The phosphorous concentration of influent water in PE was 5.53±0.77 mg/L and the final effluent concentration was 3.79±0.52 mg/L accounting to a removal of 31.49±1.22%. On the other hand, the phosphorous concentration of influent water in SE was 2.87±0.15 mg/L and the final effluent concentration was 2.28±0.14 mg/L accounting to a removal of 20.61±0.76%.

#### ii- Removal of phosphorus at 20°C

Figure 4-18 shows the change in phosphorous concentration in soil columns experiments for both PE and SE at 20$^{o}$C. Influent phosphorous concentration in PE was 3.97±1.65 mg/L and concentration exiting the soil columns in the two soil columns PE-C1 and PE-C2 operating under aerobic conditions was 2.84±0.63 and 3.14±0.66 mg/L corresponding to 31.09±2.54 and 23.16±4.71% removal. Similarly, the influent phosphorous concentration in SE was 3.01±0.2 mg/L and the concentration exiting the soil columns in the two soil columns SE-C1 and SE-C2 operating under aerobic conditions was 2.69±0.1 mg/L and 2.65±0.1 mg/L corresponding to 10.46±3.87 and 11.00±4.33% respectively. The above results indicate even very low efficiency of phosphorus removal in soil columns than in batch reactors most likely

due to short hydraulic retention time in columns, media type and size.

### iii- Removal of phosphorus at 15°C.

Figure 4-30 shows the change in phosphorous concentrations in soil columns at 15$^{o}$C.

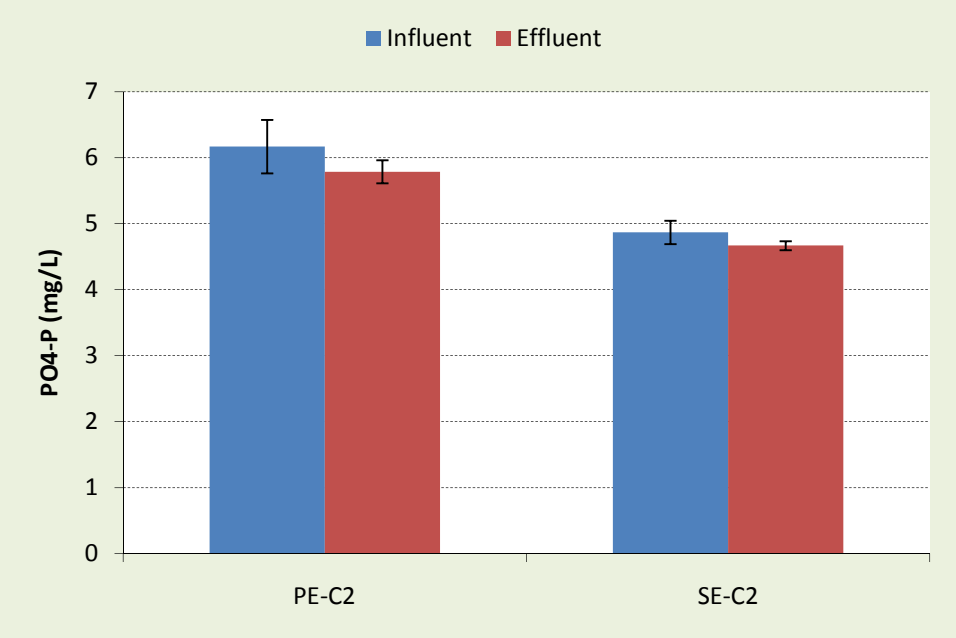



**Figure 4-30:** Change in phosphorous concentrations in soil columns at 15$^{o}$C (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, columns depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

Phosphorous concentration of influent water was 6.17±0.41 mg/L whereas the final effluent concentration was 5.47±0.47 mg/L in PE accounting to a removal of 11.37±2.27%. Likewise for SE, the concentration of phosphorous in the influent water was 4.87±0.18 mg/L while the final effluent concentration was 4.47±0.19 mg/L accounting to a removal of 8.10±0.84%.

The considerable less removal of phosphorous at 15$^{o}$C indicates that phosphorous removal efficiencies proved to have dependence on temperature though the overall removal efficiency at both temperatures was very low.

### 4.5.4 Effect of temperature on removal of pathogens in soil columns

Figure 4-29 shows the log removals of *E-coli* and *total coliform* at various temperatures as indicated below. *E-coli* and *total coliforms* were monitored at different temperatures from the laboratory-scale soil columns. *E-coli* removal in PE at temperatures 15$^{o}$C, 20$^{o}$C and 25$^{o}$C was 2.5, 3.1 and 3.07 log removals respectively. *E-coli* removal in SE at temperatures 15$^{o}$C, 20$^{o}$C and 25$^{o}$C was 2.0, 2.4 and 2.2 log removals respectively. *Total coliform* removal in PE at temperatures 15$^{o}$C, 20$^{o}$C and 25$^{o}$C was 2.7, 2.9 and 3.3 log removals respectively *Total coliform* removal in SE at temperatures 15$^{o}$C, 20$^{o}$C and 25$^{o}$C was 2.0, 2.1 and 2.2 log removals respectively

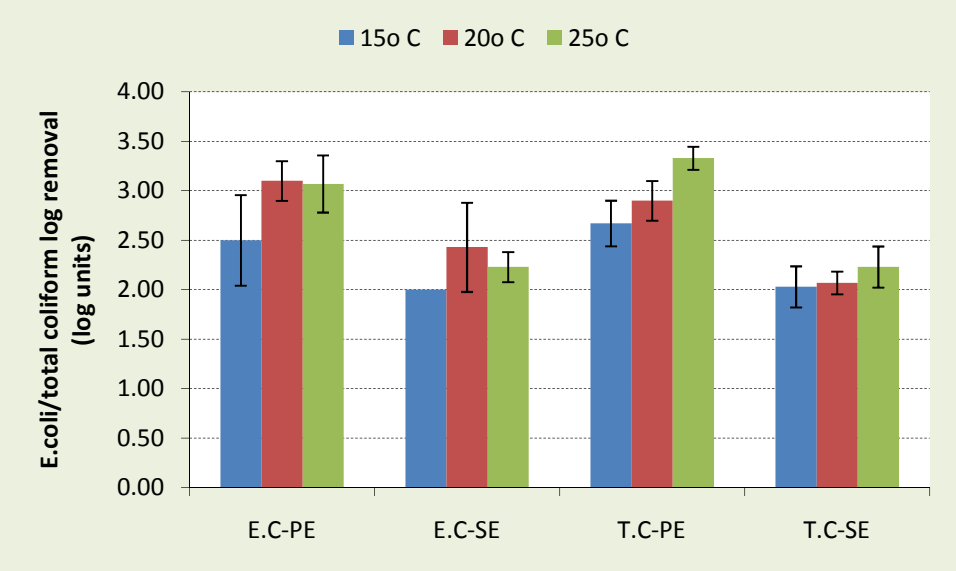



**Figure 4-31**: *E-coli and total coliform* log removal in soil columns at different temperatures. (Influent: PE and SE after 1-2 hours settling, HLR = 0.625 m/d, column depth = 0.3 m, media size = 0.8-1.25 mm, aerobic conditions)

The general trend is that log removals of *E. coli* and *total coliform* at 25$^{o}$ C is slightly higher than log removals at 15$^{o}$C and 20$^{o}$C in both PE and SE. In SE the removal of both *E-coli* and *total coliform* at both temperatures seem to be more or less the same and this is attributed by less pathogen in the effluent water as compared to PE. However there is no notable difference in terms of log removal between 20$^{o}$C and 25$^{o}$C in PE and SE for both *E- coli* and *total coliform*.

The above results suggest that adsorption of bacteria was slightly greater at higher temperatures. Hendricks et al (1979) suggested that adsorption of bacteria was substantially greater at higher temperatures in study on adsorption of bacteria on soils. Also studies of marine *Pseudomonads* showed that at a temperature of 3$^{o}$C, the proportion of bacteria attached to polystyrene was decreased compared to that at 20$^{o}$C

Table 4-14 shows the summarized results on removal of different parameters at different temperature.

**Table 4-15**: Summary table showing effect of temperature on removal of different parameters in soil column studies.

| Influent | Parameter | | Temperature | | |
|---|---|---|---|---|---|
| | | | **15°C** | **20°C** | **25°C** |
| PE | Organic matter | | 43% | 46% | 55% |
| | Ammonium nitrogen | | 90% | 96% | 100% |
| | Total nitrogen | | 18% | 38-75% | 22% |
| | Phosphorous | | 11% | 23% | 32% |
| | Pathogens | *E-coli* | 2.5 Log | 3.1 Log | 3.1 Log |
| | | *Total coliform* | 2.7 Log | 2.9 Log | 3.3 Log |
| SE | Organic matter | | 15% | 19% | 23% |
| | Ammonium nitrogen | | 100% | 69% | 100% |
| | Total nitrogen | | 8.9% | 8.8% | 27.6% |
| | Phosphorous | | 8.1% | 11% | 21% |
| | Pathogens | *E-coli* | 2.0 Log | 2.4 Log | 2.2 Log |
| | | *Total coliform* | 2.0 Log | 2.1 Log | 2.2 Log |

## 4.6 Practical implication of the study

- It is intended that SAT will become an important technology in urban water management that becomes standard practice wherever it is viable and cost-effective, as it has potential to contribute to longer term-benefits. In practice SAT process has an enormous capacity for removing a wide range of contaminants including organics, nitrogen compounds, phosphorus and pathogens and can play an important role in a multi-barrier, indirect potable reuse system.
- Experimental results showed that SAT is suitable for treating PE with respect to organic matter removal and other contaminants; therefore pretreatment of the raw wastewater is necessary in preventing clogging of the upper part of the vadose zone and producing pathogen free effluent during SAT. Sand filtration and settling of applied effluent can be used as a pretreatment before infiltration through onsite soil layers to avoid clogging and UV disinfection as post treatment to producing pathogen-free effluent.
- Soil column studies and batch experiments with PE and SE verified that SAT can remove up to 99-100 % of *total coliforms and E-coli* from PE and SE influent. In practice, 4–6 log removal of microorganisms, such as *enteric viruses*, *E-coli* and *total*

*coliforms*, have been reported to be removed in natural filtration through alluvial aquifer sediments.

- High removal efficiencies of DOC, nitrogen and phosphorous can be achieved under aerobic and anoxic conditions. However adequate retention time is a very important factor for contaminants removal during SAT. Additionally for pathogen removal, UV disinfection if applied as post treatment will help to produce pathogen-free effluent during SAT.
- Based on the results from this study redox conditions were very sensitive with respect to carbon, nitrogen and phosphorous removal than temperature. This is because change in redox conditions from aerobic to anoxic had a substantial effect on removal of these contaminants unlike for the case in temperature.
- Currently SAT renovated water is being used in USA, Israel, Australia and Europe, however SAT is potential worldwide depending on geology, soils and hydrology. A limitation of MAR systems, like in cases of GAC, membranes and advanced oxidation is that it's only limited barrier for certain contaminants. Additionally, the results from previous studies have evidence that MAR systems are proven barrier for microbes. The other limitations of MAR systems are that there is no reliable transfer of experiences to others and the possibility of release of Fe and/or Mn at some sites.

# 5 CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusions

After the analysis and discussion of results acquired through short soil column and batch experiments, DOC, nutrients and pathogens attenuation in PE and SE, the following conclusions can be made:

**Batch experiments**

1. DOC removal in soil batch tests with PE was about 55% under aerobic conditions and 40% under anoxic conditions while in SE, DOC removal was 25% under aerobic conditions and 15% under anoxic conditions.

2. The SUVA values in PE increased from 1.78 L/mg m to 3.08 L/mg m while that in SE increased from 2.88 to 3.19 L/mg m across soil column for aerobic conditions. SUVA values for anoxic conditions showed similar trend (1.78 to 2.75 L/mg.m in PE and 2.88 to 3.00 L/mg.m in SE) indicating that DOC present in wastewater was removed in both aerobic and anoxic conditions.

3. Ammonium nitrogen removal in batch reactors in PE and SE under aerobic conditions was above 99 %. About 12.1% of ammonium nitrogen was removed in batch reactors fed with PE under anoxic conditions. There was no indication for a significant denitrification in the batch reactors. Total nitrogen removal in batch reactors fed with PE was 1.26% and 6.5% under aerobic and anoxic conditions respectively. Total nitrogen in batch reactors with SE was removed by 47% under aerobic conditions and was not removed under anoxic conditions.

4. The phosphorous removal was relatively low for both PE and SE. The phosphorous removal in batch reactors for PE and SE were approximately 28 and 37% under aerobic conditions 22% and 18% under anoxic conditions respectively.

5. The removal of pathogens in batch reactors was not substantially affected by redox conditions. There was a 2-3-log reduction of *E-coli* and *total coliform* for both aerobic and anoxic conditions.

**Short soil column experiments**

**a. Redox conditions**

6. DOC removal under aerobic conditions in two soil columns PE-C1 and PE-C2 was 46 and 50% respectively. Under anoxic conditions the DOC removal was 31% with PE. Consequently in SE, DOC removal under aerobic conditions with two soil columns SE-C1 and SE-C2 gave the same percent removal of about 19%. Under anoxic conditions in SE the DOC removal was 13%.

7. The SUVA values in PE for the two columns increased 1.68 to 2.24 L/mg m and from 1.97 to 2.52 L/mg m in PE-C1 and PE-C2 respectively. In SE, SUVA increased from 2.78 to 3.31 L/mg m and from 2.81 to 3.26 L/mg.m in soil columns indicating that during aerobic and anoxic soil passage aliphatic carbon sources are preferentially used.

8. Soil column experiments showed that nitrification can remove up to 96 % of ammonium nitrogen within a column of 0.3 m depth and also some denitrification can be achieved during soil passage. Total nitrogen removal in soil column with PE ranged from 38 to 76% under aerobic conditions and 26% under anoxic conditions. Total nitrogen removal in soil column with SE was 8.8% under aerobic conditions and 30% under anoxic conditions.

9. Phosphorous removal was generally very low (23 to 31% for PE and 10 to 11% for SE under aerobic conditions) possibly due to short hydraulic retention time, less media, size and type of media used. Also phosphorus removal during SAT is not sustainable since its removal mechanism is mainly adsorption and there breakthrough is most likely when the soil adsorption capacity is exceeded.

10. The removal of pathogens in soil columns studies under aerobic and anoxic conditions showed a range of 2.2 to 3 log removals. The results indicate that there is no redox dependence on pathogens removal.

**b. Effects of temperature.**

11. DOC removal in PE at temperatures $15^{o}$ C, $20^{o}$ C and $25^{o}$ C was 43%, 50% and 55% respectively. The DOC removal in SE at temperatures $15^{o}$C, $20^{o}$C and $25^{o}$C was 15%, 19% and 23% respectively. The performance of SAT for seasonal variations did not remarkably affect DOC removal, which entails that the removal of bulk organic matter is normally a result of aerobic and anaerobic microbial activity which function even in temperatures as low as $5^{o}$C

12. The percent increase in SUVA values in PE at temperatures $15^{o}$C, $20^{o}$C and $25^{o}$C is 39%, 48% and 68% respectively. SUVA changes in SE also increased by 10%, 12% and 17% at temperatures $15^{o}$C, $20^{o}$C and $25^{o}$C respectively. The results indicate that easily biodegradable aliphatic compounds are better removed at higher temperatures than lower temperatures.

13. Ammonium nitrogen oxidation showed to have great dependence in temperature despite the low hydraulic retention time in the soil columns. Conversion of ammonium nitrogen was above 95% at a temperature of $25^{o}$C. The maximum total nitrogen removal in soil columns observed was 76% with PE at a temperature of $20^{o}$C.

14. Phosphorous removal in soil columns with PE at temperatures $15^{o}$C, $20^{o}$C and $25^{o}$C was 11%, 23% and 32% respectively. Phosphorous removal in soil columns with SE at temperatures $15^{o}$C, $20^{o}$C and $25^{o}$C was 8%, 11% and 21% respectively.

15. Soil columns were effective in removing *E-coli and total coliforms* removal and can produce product water to the level of public acceptance in PE and SE. The log removal of *E-coli* and *total coliform* PE and SE at temperatures $15^{o}$C, $20^{o}$C and $25^{o}$C ranged from 2 to 3.5 log removals. However the temperature effect on removal of pathogens was very little.

## 5.2 Recommendations

The following recommendation are made, based on the findings of this study

1. Further study is required to analyze the effect of temperature on removal of DOC, nitrogen, phosphorous and pathogens using long soil columns, longer retention and different media size and type to get a better understanding on the effect of seasonal variations in removing different contaminants.

2. Longer-term studies should be done on removal of multiple contaminants during SAT in PE. The findings could encourage developing countries to apply SAT technology as it is cheaper to treat wastewater to primary level prior to SAT.

3. Further study is also required to analyze the effect of low temperatures like $5^{o}C$ on removal of DOC, nitrogen, phosphorous and pathogens. The effect of temperature on removal of contaminants under anoxic conditions should be carried to understand its influence on redox conditions during SAT.

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# 7 APPENDICES

## Appendix A: Measurements in batch experiments.

**A1:** The average influent and effluent DOC concentrations of batch reactors during ripening period

**A2:** Changes in DOC, UVA-254 and SUVA values of batch reactors with PE after ripening period

**A3**: DOC, UVA and SUVA values for soil batch experiments in PE after ripening under aerobic and anoxic conditions

**A4**: DOC, UVA and SUVA values for soil batch experiments in SE after ripening under aerobic and anoxic conditions.

**A5:** (a) *E-coli* and *total coliform* removals in batch reactors under aerobic conditions

**A5:** (b) Summary of *E-coli* and *total coliform* removal under different redox conditions in batch experiments

**A6:** Fluorescence EEM spectra plots in batch reactors under aerobic and anoxic conditions in SE

**A7**: Fluorescence EEM analysis of influent and effluent in batch experiments fed with

SE under aerobic and anoxic conditions

**A8:** Measurements of Ammonium nitrogen and nitrate in batch experiments under aerobic conditions

**A9:** Measurements of Ammonium nitrogen and nitrate in batch experiments under anoxic conditions

## Appendix B: Measurements in soil columns-Redox conditions

**B 1:** DOC concentration of influent and Effluent during ripening and after ripening for SE-C1 under aerobic conditions

**B 2:** DOC concentration of influent and Effluent during ripening and after ripening for PE-C1 under aerobic conditions

**B 3:** DOC concentration of influent and Effluent during ripening and after ripening for PE-C1 and SE-C1 under anoxic conditions

**B 4**: Fluorescence EEM spectra plots in soil column under aerobic and anoxic conditions in both PE and SE.

**B5**: (a) *E-coli* and *total coliform* removals in soil columns under anoxic conditions

**B5**: (b) Summary of *E-coli* and *total coliform* removal under different redox conditions in soil column experiments for both PE and SE

**B6**: Ammonium nitrogen measurements in soil column experiments under aerobic conditions

**B7**: Ammonium nitrogen measurements in soil column experiments under anoxic conditions

**B8**: Phosphorous measurements in soil column experiments under aerobic conditions for both PE and SE

**Appendix C: Measurements in soil column-Temperature effect**

**C1:** DOC removals in PE and SE at a temperature $25^{o}$ C in soil column studies under aerobic conditions

**C2:** DOC removals in PE and SE at a temperature $15^{o}$ C in soil column studies under aerobic conditions

**C3**: DOC, UVA and SUVA values for soil columns experiments at different temperature in PE and SE after ripening and under aerobic condition

**C4**: Fluorescence EEM spectra plots in soil column at different temperatures in both PE & SE.

**C5**: Fluorescence EEM analysis of influent and effluent in batch experiments fed with

SE under aerobic and anoxic conditions indicating reduction in different peaks

**C6**: E-coli and total coliform removal at different temperatures in soil column experiments

**C7**: Ammonium nitrogen and nitrate measurements in soil column experiments at temperature $25^{o}$C in both PE and SE

**C8**: Ammonium nitrogen and nitrate measurements in soil column experiments at temperature $15^{o}$C in both PE and SE

**C9**: Measurements of phosphorous in soil column experiments at $25^{o}$C with both PE and SE

**C10**: Measurements of phosphorous in soil column experiments at $20^{o}$C with both PE and SE

**C11**: Measurements of phosphorous in soil column experiments at $15^{o}$C with both PE and SE

**A1**: The influent and effluent DOC concentrations of batch reactors during ripening period (influent: SE after 1-2 hours settling, changing of influent at every 5 days, volume of batch =500 ml, mass of sand media =100 g and sand size =0.8-1.25 mm

| **Days** | **SE-Aerobic batch reactors** | | | |
|---|---|---|---|---|
| | Influent (mg/L) | Effluent (mg/L) | Removal (%) | Ce/Co |
| 10 | 12.30 | 11.93 | 3.01 | 0.97 |
| 15 | 14.73 | 13.64 | 7.40 | 0.93 |
| 20 | 12.71 | 10.99 | 13.53 | 0.86 |
| 25 | 12.69 | 10.51 | 17.18 | 0.83 |
| 30 | 14.55 | 13.23 | 9.07 | 0.91 |
| 35 | 14.89 | 12.90 | 13.36 | 0.87 |
| 40 | 15.57 | 12.17 | 21.84 | 0.78 |
| 45 | 12.14 | 9.65 | 20.51 | 0.79 |
| 50 | 12.57 | 9.52 | 24.26 | 0.76 |
| 55 | 12.86 | 9.65 | 24.96 | 0.75 |
| 60 | 14.87 | 11.12 | 25.22 | 0.75 |
| 65 | 15.50 | 11.64 | 24.90 | 0.75 |
| 70 | 13.69 | 10.44 | 23.74 | 0.76 |
| 75 | 14.97 | 11.27 | 24.72 | 0.75 |
| 80 | 14.21 | 10.71 | 24.63 | 0.75 |
| 85 | 16.86 | 12.73 | 24.50 | 0.76 |
| **AVG** | **14.07** | **11.38** | **18.93** | |
| **STDEV** | **1.40** | **1.30** | **7.38** | |

**A2**: The influent and effluent DOC concentrations of batch reactors during ripening period (Influent: PE after 1-2 hours settling, changing of influent at every 5 days, volume of batch =500 ml, mass of sand media =100 g and sand size =0.8-1.25 mm

| **Days** | **PE-Aerobic batch reactors** | | | |
|---|---|---|---|---|
| | Influent (mg/L) | Effluent (mg/L) | Removal (%) | Ce/Co |
| 10 | 42.38 | 17.60 | 58.47 | 0.42 |
| 15 | 39.02 | 18.77 | 51.90 | 0.48 |
| 20 | 31.69 | 20.23 | 36.16 | 0.64 |
| 25 | 31.53 | 15.22 | 51.73 | 0.48 |
| 30 | 41.52 | 15.94 | 61.61 | 0.38 |
| 35 | 33.06 | 19.22 | 41.86 | 0.58 |
| 40 | 34.68 | 16.86 | 51.38 | 0.49 |
| 45 | 30.48 | 14.51 | 52.40 | 0.48 |
| 50 | 28.41 | 13.83 | 51.32 | 0.49 |
| 55 | 26.01 | 12.38 | 52.40 | 0.48 |
| 60 | 30.03 | 14.07 | 53.15 | 0.47 |
| 65 | 26.46 | 12.24 | 53.74 | 0.46 |
| 70 | 29.82 | 13.84 | 53.59 | 0.46 |
| 75 | 32.53 | 14.89 | 54.23 | 0.46 |
| 80 | 33.99 | 15.39 | 54.72 | 0.45 |
| 85 | 33.22 | 15.03 | 54.76 | 0.45 |
| **AVG** | **32.80** | **15.63** | **52.09** | |
| **STDEV** | **4.78** | **2.35** | **5.86** | |

**A3**: DOC, UVA and SUVA values for soil batch experiments in PE after ripening under aerobic and anoxic conditions

| | **PE batch Reactors** | **DOC (mg/L)** | **Avg.DOC (mg/L)** | **UVA-254 (cm-1)** | **Avg.UVA (cm-1)** | **SUVA (L/mg-cm)** |
|---|---|---|---|---|---|---|
| Influent | | 32.74 | 32.74 | 0.584 | 0.584 | 1.78 |
| Effluent | R1- Aerobic | 15.07 | | 0.479 | | |
| | R2 -Aerobic | 15.77 | | 0.485 | | |
| | R3-Aerobic | 14.19 | 15.01 | 0.424 | 0.463 | 3.08 |
| | R1-Anoxic | 18.47 | | 0.559 | | |
| | R2-Anoxic | 20.66 | | 0.541 | | |
| | R3-Anoxic | 20.81 | 19.98 | 0.549 | 0.550 | 2.75 |

**A4**: DOC, UVA and SUVA values for soil batch experiments in SE after ripening under aerobic and anoxic conditions

| | **SE batch Reactors** | **DOC (mg/L)** | **Avg.DOC (mg/L)** | **UVA-254 (cm-1)** | **Avg.UVA (cm-1)** | **SUVA (L/mg-cm)** |
|---|---|---|---|---|---|---|
| Influent | | 14.57 | 14.57 | 0.419 | 0.419 | 2.88 |
| Effluent | R1- Aerobic | 11.37 | | 0.338 | | |
| | R2 -Aerobic | 11.23 | | 0.353 | | |
| | R3-Aerobic | 10.25 | 10.95 | 0.357 | 0.349 | 3.19 |
| | R1-Anoxic | 11.88 | | 0.378 | | |
| | R2-Anoxic | 11.74 | | 0.361 | | |
| | R3-Anoxic | 13.67 | 12.43 | 0.381 | 0.373 | 3.00 |

**A5**: (a) *E-coli* and *total coliform* removals in batch reactors under aerobic conditions (Influent: PE and SE after 1-2 hours settling, changing of influent at every 5 days, volume of batch =500 ml, mass of sand media: 100 g and sand size: 0.8-1.25 mm)

i. *E-coli* removal from aerobic batch reactors with PE

| **Date** | **Influent (CFU/mL)** | **Effluent (CFU/mL)** | | | | | **Log Removal** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Aerobic reactors | | | Mean | STDEV | Aerobic reactors | | | Mean | STDEV |
| 01/02/11 | 45,000 | 0 | 40 | 0 | 14 | 23 | 4.7 | 3.1 | 4.7 | 4.1 | 0.9 |
| 03/02/11 | 40,000 | 40 | 30 | 10 | 27 | 15 | 3.0 | 3.1 | 3.6 | 3.2 | 0.3 |
| 28/02/11 | 28,000 | 10 | 0 | 60 | 24 | 32 | 3.4 | 4.4 | 2.7 | 3.5 | 0.9 |

ii. *E-coli* removal from aerobic batch reactors with SE

| **Date** | **Influent (CFU/mL)** | **Effluent (CFU/mL)** | | | | | **Log Removal** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Aerobic reactors | | | Mean | STDEV | Aerobic reactors | | | Mean | STDEV |
| 01/02/11 | 160 | 0 | 0 | 0 | 0 | 0 | 2.2 | 2.2 | 2.2 | 2.2 | 0.0 |
| 03/02/11 | 220 | 0 | 0 | 0 | 0 | 0 | 2.3 | 2.3 | 2.3 | 2.3 | 0.0 |
| 28/02/11 | 200 | 0 | 0 | 0 | 0 | 0 | 2.3 | 2.3 | 2.3 | 2.3 | 0.0 |

iii. *Total coliform* removal from aerobic batch reactors with PE

| **Date** | **Influent (CFU/mL)** | **Effluent (CFU/mL)** | | | | | **Log Removal** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Aerobic reactors | | | Mean | STDEV | Aerobic reactors | | | Mean | STDEV |
| 01/02/11 | 238,000 | 240 | 190 | 130 | 187 | 55 | 3.0 | 3.1 | 3.3 | 3.1 | 0.1 |
| 03/02/11 | 218,000 | 230 | 230 | 220 | 227 | 6 | 3.0 | 3.0 | 3.0 | 3.0 | 0.0 |
| 28/02/11 | 202,000 | 340 | 440 | 330 | 370 | 61 | 2.8 | 2.7 | 2.8 | 2.7 | 0.1 |

*iv.* *Total coliform* removal from aerobic batch reactors with SE

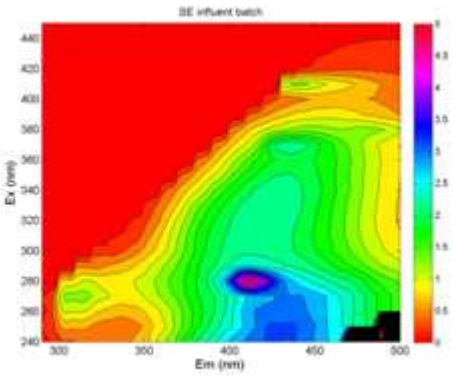
| **Date** | **Influent (CFU/mL)** | Effluent (CFU/mL) | | | | | Log Removal | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Aerobic reactors | | | Mean | STDEV | Aerobic reactors | | | Mean | STDEV |
| 01/02/11 | 830 | 0 | 0 | 0 | 0 | 0 | 2.9 | 2.9 | 2.9 | 2.9 | 0.0 |
| 03/02/11 | 1110 | 0 | 0 | 0 | 0 | 0 | 3.0 | 3.0 | 3.0 | 3.0 | 0.0 |
| 28/03/11 | 1680 | 0 | 20 | 0 | 7 | 11 | 3.2 | 1.9 | 3.2 | 2.8 | 0.8 |

**A5:** (b) Summary of *E-coli* and *total coliform* removal under different redox conditions in batch experiments

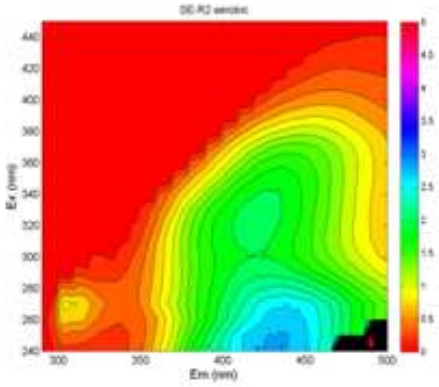
| **Log removal in batch experiments under aerobic conditions** | | | | | |
|---|---|---|---|---|---|
| **Category** | Plate 1 | Plate 2 | Plate 3 | Average | STDEV |
| *E-coli* in PE | 4.1 | 3.2 | 3.5 | 3.60 | 0.46 |
| *E-coli* in SE | 2.2 | 2.3 | 2.3 | 2.27 | 0.06 |
| *Total coliform* in PE | 3.1 | 3.0 | 2.7 | 2.93 | 0.21 |
| *Total coliform* in SE | 2.9 | 2.8 | 3.0 | 2.90 | 0.10 |
| **Log removal in batch experiments under anoxic conditions** | | | | | |
| | Plate 1 | Plate 2 | Plate 3 | Average | STDEV |
| *E-coli* in PE | 2.8 | 2.4 | 3.0 | 2.73 | 0.31 |
| *E-coli* in SE | 2.4 | 2.1 | 2.3 | 2.27 | 0.15 |
| *Total coliform* in PE | 2.1 | 1.9 | 2.3 | 2.10 | 0.20 |
| *Total coliform* in SE | 3 | 2.3 | 3.0 | 2.77 | 0.40 |

**A6:** Fluorescence EEM spectra plots in batch reactors under aerobic and anoxic conditions in SE

i. SE-aerobic batch reactors

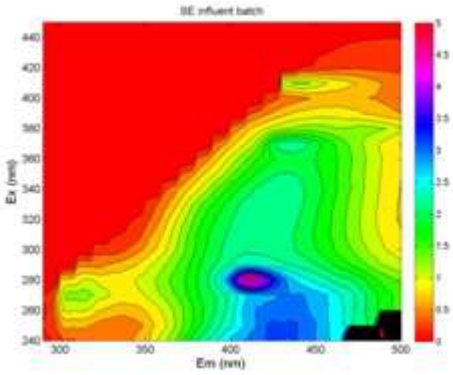



Influent

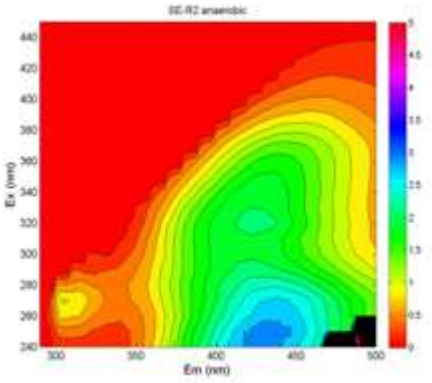



Effluent

ii. SE-anoxic batch reactors




Influent




Effluent

**A7**: Fluorescence EEM analysis of influent and effluent in batch experiments fed with SE under aerobic and anoxic conditions indicating reduction in different peaks

| **Peak 1 Humic/Fulvic-like organic matter fractions** | | **Excitation (nm)** | **Emission (nm)** | **Intensity** | **Reduction in Intensity ( % )** |
|---|---|---|---|---|---|
| SE-aerobic batch | Influent | 340 | 430 | 2.26 | 12.1 |
| | Effluent | 340 | 430 | 1.99 | |
| SE-anoxic batch | Influent | 340 | 430 | 2.26 | 11.0 |
| | Effluent | 340 | 430 | 2.01 | |
| **Peak 2 Humic-like organic matter fractions** | | | | | |
| SE-aerobic batch | Influent | 250 | 430 | 3.22 | 10.9 |
| | Effluent | 250 | 430 | 2.87 | |
| SE-anoxic batch | Influent | 250 | 430 | 3.22 | 7.2 |
| | Effluent | 250 | 430 | 2.99 | |
| **Peak 3 Protein-like organic matter fractions** | | | | | |
| SE-aerobic batch | Influent | 270 | 310 | 1.36 | 32.6 |
| | Effluent | 270 | 310 | 0.92 | |
| SE-anoxic batch | Influent | 270 | 310 | 1.36 | 24.7 |
| | Effluent | 270 | 310 | 1.03 | |

**A8:** Measurements of Ammonium nitrogen and nitrate in batch experiments under aerobic conditions

| | **1st-reading** | **2rd-reading** | **3rd-reading** | **AVG** | **STDEV** |
|---|---|---|---|---|---|
| PE influents ($NH_4$-N) | 31.97 | 26.92 | 36.3 | 31.73 | 4.69 |
| PE effluents ($NH_4$-N) | 0.10 | 0.00 | 0.00 | 0.03 | 0.06 |
| PE influents ($NO_3$-N) | 1.91 | 1.88 | 1.82 | 1.87 | 0.05 |
| PE effluents ($NO_3$-N) | 36.49 | 37.53 | 34.11 | 36.04 | 1.75 |
| | | | | | |
| SE influents ($NH_4$-N) | 0.59 | 0.02 | 0.45 | 0.35 | 0.30 |
| SE effluents ($NH_4$-N) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SE influents ($NO_3$-N) | 4.54 | 7.53 | 5.82 | 5.96 | 1.50 |
| SE effluents ($NO_3$-N) | 10.01 | 4.02 | 6.71 | 6.91 | 3.00 |

**A9:** Measurements of Ammonium nitrogen and nitrate in batch experiments under anoxic conditions

| | **1st-reading** | **2rd-reading** | **3rd-reading** | **AVG** | **STDEV** |
|---|---|---|---|---|---|
| PE influents ($NH_4$-N) | 31.97 | 26.92 | 36.3 | 31.73 | 4.69 |
| PE effluents ($NH_4$-N) | 29.28 | 25.89 | 28.51 | 27.89 | 1.78 |
| PE influents ($NO_3$-N) | 1.91 | 1.88 | 1.82 | 1.87 | 0.05 |
| PE effluents ($NO_3$-N) | 3.63 | 5.10 | 5.72 | 4.82 | 1.07 |
| | | | | | |
| SE influents ($NH_4$-N) | 0.59 | 0.02 | 0.45 | 0.35 | 0.30 |
| SE effluents ($NH_4$-N) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SE influents ($NO_3$-N) | 4.54 | 7.53 | 5.82 | 5.96 | 1.50 |
| SE effluents ($NO_3$-N) | 9.48 | 9.39 | 9.44 | 9.44 | 0.05 |

**B1:** DOC concentration of influent and Effluent during ripening and after ripening for SE-C1 under aerobic conditions

| SE-C1 | | | | |
|---|---|---|---|---|
| **Days** | **Influent (mg/L)** | **Effluent (mg/L)** | **Removal (%)** | **Ce/Co** |
| 16 | 12.30 | 12.01 | 2.36 | 0.98 |
| 20 | 11.62 | 11.34 | 2.41 | 0.98 |
| 22 | 13.57 | 11.98 | 11.72 | 0.88 |
| 24 | 12.69 | 10.15 | 20.02 | 0.80 |
| 28 | 14.52 | 12.74 | 12.26 | 0.88 |
| 31 | 14.55 | 13.52 | 7.08 | 0.93 |
| 34 | 16.54 | 15.09 | 8.77 | 0.91 |
| 35 | 15.34 | 14.55 | 5.15 | 0.95 |
| 37 | 14.89 | 13.50 | 9.34 | 0.91 |
| 39 | 14.28 | 13.01 | 8.89 | 0.91 |
| 41 | 15.40 | 12.33 | 19.94 | 0.80 |
| 43 | 15.57 | 13.17 | 15.41 | 0.85 |
| 45 | 13.12 | 12.42 | 5.34 | 0.95 |
| 52 | 12.38 | 9.81 | 20.76 | 0.79 |
| 59 | 10.73 | 8.99 | 16.22 | 0.84 |
| 62 | 14.19 | 11.29 | 20.44 | 0.80 |
| 64 | 14.55 | 11.87 | 18.42 | 0.82 |
| 66 | 15.18 | 12.20 | 19.63 | 0.80 |
| 68 | 14.36 | 11.40 | 20.61 | 0.79 |
| 70 | 15.83 | 12.81 | 19.08 | 0.81 |
| 72 | 13.37 | 10.84 | 18.92 | 0.81 |
| 74 | 13.14 | 10.74 | 18.26 | 0.82 |
| 76 | 13.40 | 10.89 | 18.73 | 0.81 |
| 78 | 12.76 | 10.40 | 18.50 | 0.82 |
| 80 | 12.03 | 9.83 | 18.29 | 0.82 |
| 82 | 13.15 | 10.69 | 18.71 | 0.81 |
| 84 | 11.90 | 9.80 | 17.65 | 0.82 |
| **AVG** | **13.75** | **11.75** | **14.55** | |
| **STDEV** | **1.44** | **1.51** | **6.11** | |
| **AVG after ripening** | **13.61** | **11.04** | **18.80** | |
| **STDEV after ripening** | **1.25** | **0.95** | **0.79** | |

**B2:** DOC concentration of influent and Effluent during ripening and after ripening for PE-C1 under aerobic conditions

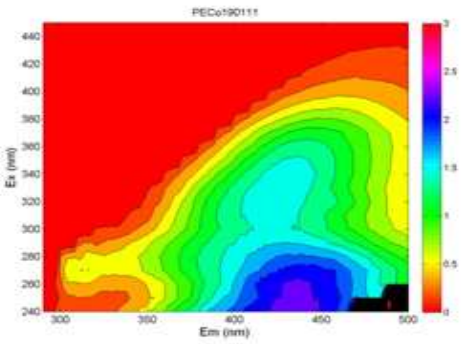
| PE-C 1 | | | | |
|---|---|---|---|---|
| **Days** | **Influent (mg/L)** | **Effluent (mg/L)** | **Removal (%)** | **Ce/Co** |
| 16 | 44.38 | 32.53 | 26.70 | 0.73 |
| 20 | 38.04 | 24.22 | 36.33 | 0.64 |
| 22 | 35.43 | 21.86 | 38.30 | 0.62 |
| 24 | 31.53 | 18.11 | 42.56 | 0.57 |
| 28 | 42.27 | 16.49 | 60.99 | 0.39 |
| 31 | 41.52 | 21.98 | 47.06 | 0.53 |
| 34 | 52.92 | 24.66 | 53.40 | 0.47 |
| 35 | 39.84 | 23.28 | 41.57 | 0.58 |
| 37 | 33.06 | 18.36 | 44.46 | 0.56 |
| 39 | 36.69 | 21.56 | 41.24 | 0.59 |
| 41 | 32.70 | 21.04 | 35.66 | 0.64 |
| 43 | 34.04 | 27.76 | 18.45 | 0.82 |
| 45 | 28.17 | 26.26 | 6.78 | 0.93 |
| 52 | 35.13 | 16.85 | 52.04 | 0.48 |
| 59 | 31.41 | 17.01 | 45.85 | 0.54 |
| 62 | 36.39 | 19.69 | 45.89 | 0.54 |
| 64 | 29.70 | 15.09 | 49.19 | 0.51 |
| 66 | 35.64 | 17.34 | 51.35 | 0.49 |
| 68 | 29.65 | 16.10 | 45.70 | 0.54 |
| 70 | 29.40 | 15.91 | 45.88 | 0.54 |
| 72 | 32.53 | 18.03 | 44.57 | 0.55 |
| 74 | 30.34 | 16.45 | 45.78 | 0.54 |
| 76 | 31.55 | 17.37 | 44.94 | 0.55 |
| 78 | 32.58 | 17.58 | 46.04 | 0.54 |
| 80 | 29.13 | 15.91 | 45.38 | 0.55 |
| 82 | 26.17 | 14.34 | 45.20 | 0.55 |
| 84 | 25.86 | 14.01 | 45.82 | 0.54 |
| **AVG** | **34.30** | **19.62** | **42.49** | |
| **STDEV** | **6.01** | **4.51** | **10.76** | |
| **AVG after ripening** | **30.23** | **16.19** | **46.35** | |
| **STDEV after ripening** | **2.83** | **1.33** | **2.04** | |

**B3:** DOC concentration of influent and Effluent during ripening and after ripening for PE-C1 and SE-C1 under anoxic conditions

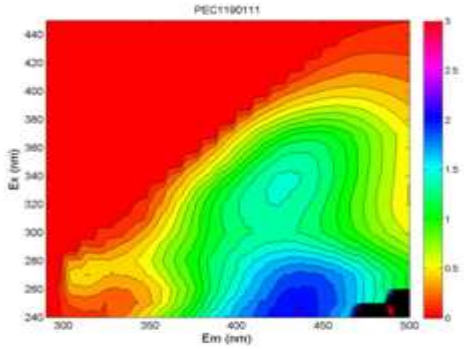
| **Days** | **SE C 1 Anoxic** | | | **PE C 1 Anoxic** | | |
|---|---|---|---|---|---|---|
| | **Influent (mg/L)** | **Effluent (mg/L)** | **Removal (%)** | **Influent (mg/L)** | **effluent(mg/L)** | **Removal (%)** |
| 4 | 12.65 | 11.61 | 8.22 | 30.26 | 21.83 | 27.86 |
| 6 | 12.05 | 11.07 | 8.13 | 29.11 | 20.70 | 28.89 |
| 8 | 12.55 | 10.16 | 19.04 | 28.92 | 19.70 | 31.88 |
| 10 | 12.42 | 10.56 | 14.98 | 27.02 | 14.62 | 45.89 |
| 12 | 13.21 | 11.77 | 10.90 | 27.02 | 13.97 | 48.30 |
| 14 | 15.43 | 13.46 | 12.77 | 31.52 | 19.35 | 38.61 |
| 16 | 15.32 | 13.31 | 13.12 | 32.45 | 22.14 | 31.77 |
| 18 | 14.54 | 12.64 | 13.07 | 32.49 | 22.35 | 31.21 |
| 20 | 14.91 | 12.97 | 13.01 | 25.13 | 17.32 | 31.08 |
| 22 | 14.01 | 12.12 | 13.49 | 25.46 | 17.55 | 31.07 |
| **AV** | **13.71** | **11.97** | **12.67** | **28.94** | **18.95** | **34.66** |
| **STDEV** | **1.29** | **1.14** | **3.17** | **2.74** | **3.02** | **7.16** |
| **AV after ripening** | **14.84** | **12.90** | **13.09** | **29.41** | **19.74** | **31.28** |
| **STDEV after ripening** | **0.58** | **0.54** | **0.26** | **3.78** | **2.42** | **0.33** |

**B4**: Fluorescence EEM spectra plots in soil column under aerobic and anoxic conditions in both PE and SE.

i. PE- soil columns aerobic conditions

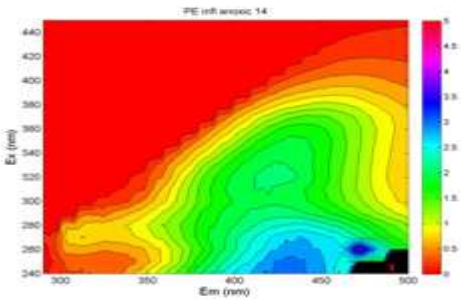



Influent

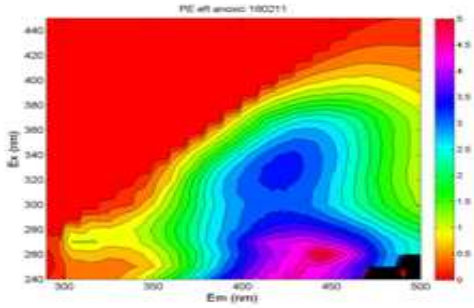



Effluent

ii. PE- soil columns anoxic conditions

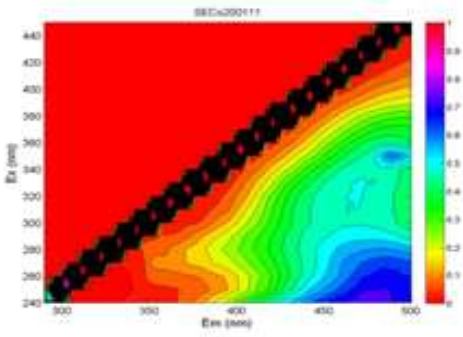



Influent

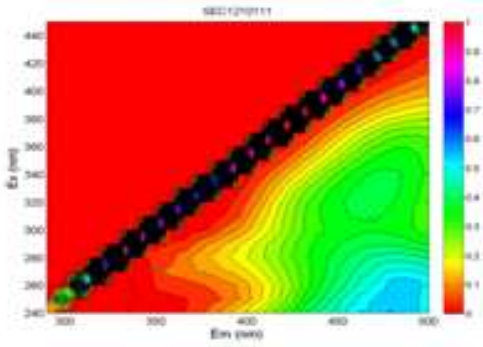



Effluent

iii. SE-soil column aerobic conditions

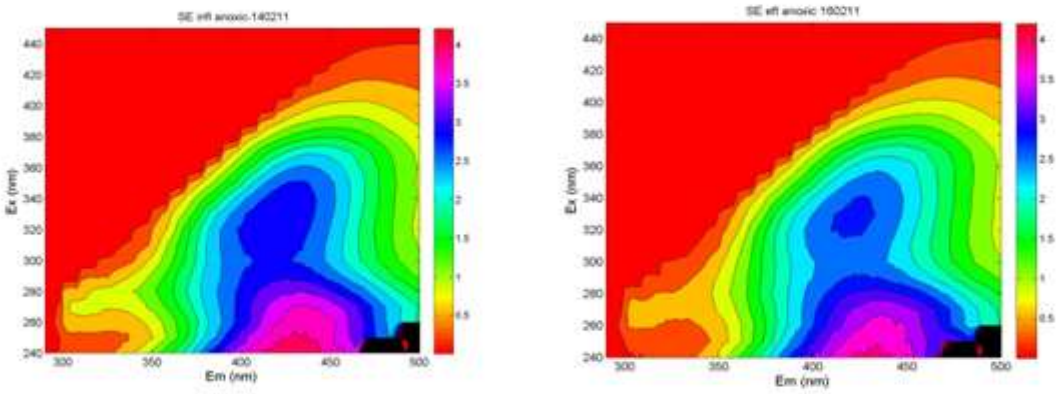



Influent




Effluent

iv. SE-soil column anoxic conditions




Influent Effluent

**B 5:** (a) *E-coli* and *total coliform* removals in soil columns under anoxic conditions (Influent: PE and SE after 1-2 hours settling, HLR=0.625 m/d, column depth = 0.3 m, media size = 0 .8-1.25 mm, anoxic conditions)

i. *E-coli* removal from soil short columns with PE-Anoxic

| Date | Influent | Effluent (CFU/mL) | | | | | Log Removal | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | (CFU/mL) | Column 1 | | | Mean | STDEV | Column 1 | | | Mean | STDEV |
| 14/02/11 | 32,000 | 280 | 310 | 300 | 297 | 15 | 2.1 | 2.0 | 2.0 | 2.0 | 0.0 |
| 16/02/11 | 29,000 | 30 | 50 | 90 | 57 | 31 | 3.0 | 2.8 | 2.5 | 2.8 | 0.2 |
| 18/02/11 | 30,000 | 120 | 140 | 160 | 140 | 20 | 2.4 | 2.3 | 2.3 | 2.3 | 0.1 |

*ii.* *E-coli* removal from soil short columns with SE-Anoxic

| **Date** | **Influent** | **Effluent (CFU/mL)** | | | | | **Log Removal** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **(CFU/mL)** | Column 1 | | | Mean | STDEV | Column 1 | | | Mean | STDEV |
| 14/02/11 | 120 | 0 | 0 | 0 | 0 | 0 | 2.1 | 2.1 | 2.1 | 2.1 | 0.0 |
| 16/02/11 | 370 | 0 | 0 | 0 | 0 | 0 | 2.6 | 2.6 | 2.6 | 2.6 | 0.0 |
| 18/02/11 | 240 | 0 | 0 | 0 | 0 | 0 | 2.4 | 2.4 | 2.4 | 2.4 | 0.0 |

*iii.* *Total coliform* removal from soil short columns with PE-Anoxic

| **Date** | **Influent** | **Effluent (CFU/mL)** | | | | | **Log Removal** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **(CFU/mL)** | Column 2 | | | Mean | STDEV | Column 2 | | | Mean | STDEV |
| 14/02/11 | 320,000 | 580 | 560 | 590 | 577 | 15 | 2.7 | 2.8 | 2.7 | 2.7 | 0.0 |
| 16/02/11 | 361,000 | 400 | 370 | 330 | 367 | 35 | 3.0 | 3.0 | 3.0 | 3.0 | 0.0 |
| 18/02/11 | 350,000 | 170 | 190 | 190 | 183 | 12 | 3.3 | 3.3 | 3.3 | 3.3 | 0.0 |

iv. *Total coliform* removal from soil short columns with SE-Anoxic

| Date | Influent (CFU/mL) | Effluent (CFU/mL) | | | | | Log Removal | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Column 2 | | | Mean | STDEV | Column 2 | | | Mean | STDEV |
| 14/02/11 | 2,070 | 30 | 20 | 20 | 23 | 6 | 1.8 | 2.0 | 2.0 | 2.0 | 0.1 |
| 16/02/11 | 1,470 | 0 | 10 | 0 | 4 | 5 | 3.2 | 2.2 | 3.2 | 2.8 | 0.6 |
| 18/02/11 | 1,890 | 10 | 10 | 20 | 13 | 6 | 2.3 | 2.3 | 2.0 | 2.2 | 0.2 |

**B5**: (b) Summary of *E-coli* and *total coliform* removal under different redox conditions in soil column experiments for both PE and SE

| **Log removal in soil column experiments under aerobic conditions** | | | | | |
|---|---|---|---|---|---|
| **Category** | **Plate 1** | **Plate 2** | **Plate 3** | **Average** | **STDEV** |
| *E-coli* in PE | 2.9 | 2.9 | 2.7 | 2.83 | 0.12 |
| *E-coli* in SE | 2.1 | 2.4 | 2.3 | 2.27 | 0.15 |
| *Total coliform* in PE | 2.4 | 2.9 | 3.1 | 2.80 | 0.36 |
| *Total coliform* in SE | 2.4 | 3.0 | 3.1 | 2.83 | 0.38 |
| **Log removal in soil column experiments under anoxic conditions** | | | | | |
| **Category** | Plate 1 | Plate 2 | Plate 3 | Average | STDEV |
| *E-coli* in PE | 2.0 | 2.8 | 2.3 | 2.40 | 0.57 |
| *E-coli* in SE | 2.1 | 2.6 | 2.4 | 2.35 | 0.35 |
| *Total coliform* in PE | 2.7 | 3.0 | 3.3 | 2.85 | 0.21 |
| *Total coliform* in SE | 2.0 | 2.8 | 2.2 | 2.40 | 0.57 |

**B6**: Ammonium nitrogen and nitrate measurements in soil column experiments under aerobic conditions for PE and SE

| | | **1st-reading** | **2rd-reading** | **3rd-reading** | **AVG** | **STDEV** |
|---|---|---|---|---|---|---|
| PE ($NH_4$-N) | Influent | 36.3 | 34.27 | 28.88 | 33.15 | 3.83 |
| | PE-C1 effl | 3.79 | 0.14 | 0.17 | 1.37 | 2.10 |
| | PE-C2 effl | 0.14 | 0.17 | 0.16 | 0.16 | 0.02 |
| PE ($NO_3$-N) | Influent | 0.17 | 0.16 | 0.20 | 0.18 | 0.02 |
| | PE-C1 effl | 4.56 | 7.9 | 7.77 | 6.74 | 1.89 |
| | PE-C2 effl | 19.54 | 21.93 | 19.60 | 20.36 | 1.36 |
| | | | | | | |
| SE ($NH_4$-N ) | Influent | 0.15 | 0.14 | 0.10 | 0.13 | 0.03 |
| | SE-C1 effl | 0.06 | 0.06 | 0.05 | 0.06 | 0.01 |
| | SE-C2 effl | 0.04 | 0.04 | 0.05 | 0.04 | 0.01 |
| SE ($NO_3$-N ) | Influent | 3.39 | 3.30 | 3.45 | 3.38 | 0.08 |
| | SE-C1 effl | 3.63 | 3.8 | 3.78 | 3.74 | 0.09 |
| | SE-C2 effl | 3.65 | 3.83 | 3.85 | 3.78 | 0.11 |

**B7**: Ammonium nitrogen and nitrate measurements in soil column experiments under anoxic conditions for both PE and SE

| | **1st-reading** | **2rd-reading** | **3rd-reading** | **AVG** | **STDEV** |
|---|---|---|---|---|---|
| PE-C1 influents ($NH_4$-N) | 31.97 | 26.92 | 38.71 | 32.53 | 5.92 |
| PE-C1 effluents ($NH_4$-N) | 10.12 | 9.03 | 8.74 | 9.30 | 0.73 |
| PE-C1 influents ($NO_3$-N) | 1.91 | 1.88 | 1.82 | 1.87 | 0.05 |
| PE-C1effluents ($NO_3$-N) | 13.23 | 17.19 | 18.38 | 16.27 | 2.70 |
| | | | | | |
| SE-C1 influents ($NH_4$-N) | 0.59 | 0.02 | 0.47 | 0.36 | 0.30 |
| SE-C1 effluents ($NH_4$-N) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SE-C1 influents ($NO_3$-N) | 6.25 | 4.70 | 4.54 | 5.16 | 0.94 |
| SE-C1 effluents ($NO_3$-N) | 4.67 | 3.22 | 3.77 | 3.89 | 0.73 |

**B8**: Phosphorous measurements in soil column experiments under aerobic conditions for both PE and SE

| | **1st-reading** | **2rd-reading** | **3rd-reading** | **4th-reading** | **AVG** | **STDEV** |
|---|---|---|---|---|---|---|
| Influents ($PO_4$-P) | 4.83 | 3.96 | 3.28 | 3.79 | 3.97 | 0.65 |
| PE C1 effluents | 3.74 | 2.81 | 2.31 | 2.50 | 2.84 | 0.63 |
| PE C2 effluents | 3.97 | 3.05 | 2.37 | 3.15 | 3.14 | 0.66 |
| Influents ($PO_4$-P) | 2.95 | 3.30 | 2.93 | 2.85 | 3.01 | 0.20 |
| SE C1 effluents | 2.72 | 2.78 | 2.61 | 2.64 | 2.69 | 0.08 |
| SE C2 effluents | 2.66 | 2.73 | 2.64 | 2.58 | 2.65 | 0.06 |

**C1:** DOC removals in PE and SE at a temperature $25^o$ C in soil column studies under aerobic conditions

| **Days** | **SE C 2-25℃** | | | **PE C2-25℃** | | |
|---|---|---|---|---|---|---|
| | **Influent (mg/L)** | **Effluent (mg/L)** | **Removal (%)** | **Influent (mg/L)** | **Effluent(mg/L)** | **Removal (%)** |
| 6 | 12.98 | 10.68 | 17.72 | 24.24 | 12.48 | 48.51 |
| 8 | 12.47 | 10.12 | 18.85 | 25.71 | 13.04 | 49.28 |
| 10 | 13.75 | 10.08 | 26.69 | 30.82 | 12.55 | 59.28 |
| 12 | 14.17 | 11.86 | 16.30 | 33.14 | 14.14 | 57.33 |
| 14 | 13.00 | 10.05 | 22.69 | 31.02 | 13.97 | 54.96 |
| 16 | 14.52 | 11.24 | 22.59 | 32.07 | 14.73 | 54.07 |
| 18 | 14.43 | 11.12 | 22.94 | 29.41 | 13.46 | 54.23 |
| 20 | 14.54 | 11.28 | 22.42 | 32.70 | 14.77 | 54.83 |
| **AVG** | **13.73** | **10.80** | **21.27** | **29.89** | **13.64** | **54.06** |
| **STDEV** | **0.81** | **0.68** | **3.39** | **3.27** | **0.91** | **3.64** |
| **AVG after ripening** | **14.12** | **10.92** | **22.66** | **31.30** | **14.23** | **54.52** |
| **STDEV after ripening** | **0.75** | **0.59** | **0.22** | **1.44** | **0.63** | **0.44** |

**C2:** DOC removals in PE and SE at a temperature $15^{o}$ C in soil column studies under aerobic conditions

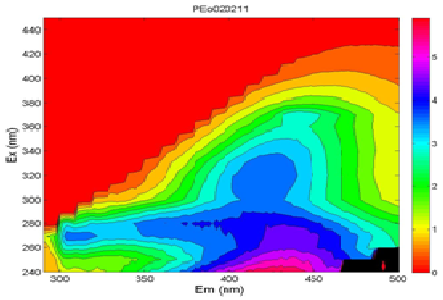
| Days | SE C 2-15℃ | | | PE C2-15℃ | | |
|---|---|---|---|---|---|---|
| | **Influent (mg/L)** | **Effluent (mg/L)** | **Removal (%)** | **Influent (mg/L)** | **Effluent (mg/L)** | **Removal (%)** |
| 8 | 16.65 | 13.18 | 20.84 | 36.37 | 18.34 | 49.57 |
| 10 | 15.37 | 12.56 | 18.28 | 35.71 | 18.30 | 48.75 |
| 12 | 12.96 | 11.28 | 12.96 | 31.82 | 15.55 | 51.13 |
| 14 | 14.17 | 11.06 | 21.95 | 33.14 | 16.14 | 51.30 |
| 16 | 13.64 | 12.05 | 11.66 | 31.02 | 15.97 | 48.52 |
| 18 | 13.52 | 11.84 | 12.43 | 32.07 | 20.89 | 34.86 |
| 20 | 13.41 | 11.67 | 12.98 | 26.37 | 17.30 | 34.40 |
| 22 | 12.69 | 11.12 | 12.37 | 27.37 | 18.03 | 34.12 |
| 24 | 12.54 | 11.02 | 12.12 | 27.70 | 18.37 | 33.68 |
| **AVG** | **13.88** | **11.75** | **15.07** | **31.29** | **17.65** | **42.93** |
| **STDEV** | **1.34** | **0.74** | **4.10** | **3.57** | **1.65** | **8.27** |

**C3**: DOC, UVA and SUVA values for soil columns experiments at different temperature in PE and SE after ripening and under aerobic condition

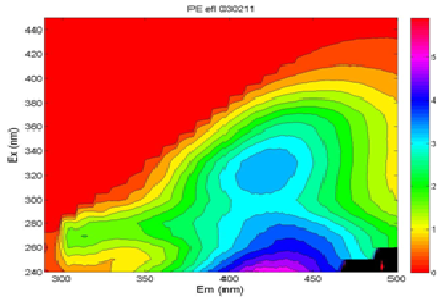
| Influent water | Temp | DOC (mg/L) | | Avg.DOC (mg/L) | | UVA-254 (cm-1) | | Avg.UVA (cm-1) | | SUVA (L/mg-cm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Influent | Effluent | Influent | Effluent | Influent | Effluent | Influent | Effluent | Influent | Effluent |
| PE-C2 | 25° C | 32.07 | 14.73 | 31.82 | 14.32 | 0.581 | 0.425 | 0.562 | 0.424 | 1.76 | 2.96 |
| | | 33.99 | 14.77 | | | 0.644 | 0.452 | | | | |
| | | 29.41 | 13.46 | | | 0.460 | 0.396 | | | | |
| | | | | | | | | | | | |
| SE-C2 | 25° C | 14.52 | 11.24 | 14.50 | 11.21 | 0.508 | 0.419 | 0.500 | 0.454 | 3.45 | 4.05 |
| | | 14.43 | 11.12 | | | 0.504 | 0.479 | | | | |
| | | 14.54 | 11.28 | | | 0.488 | 0.464 | | | | |
| | | | | | | | | | | | |
| PE-C2 | 20° C | 29.13 | 14.81 | 27.05 | 13.85 | 0.490 | 0.358 | 0.488 | 0.371 | 1.81 | 2.68 |
| | | 26.17 | 13.46 | | | 0.515 | 0.396 | | | | |
| | | 25.86 | 13.29 | | | 0.460 | 0.360 | | | | |
| | | | | | | | | | | | |
| SE-C2 | 20° C | 12.03 | 9.79 | 12.36 | 10.08 | 0.335 | 0.324 | 0.354 | 0.325 | 2.87 | 3.22 |
| | | 13.15 | 10.68 | | | 0.370 | 0.325 | | | | |
| | | 11.90 | 9.78 | | | 0.358 | 0.325 | | | | |
| | | | | | | | | | | | |
| PE-C2 | 15° C | 26.37 | 17.30 | 27.15 | 17.90 | 0.457 | 0.509 | 0.520 | 0.478 | 1.92 | 2.67 |
| | | 27.37 | 18.03 | | | 0.548 | 0.514 | | | | |
| | | 27.70 | 18.37 | | | 0.556 | 0.412 | | | | |
| | | | | | | | | | | | |
| SE-C2 | 15° C | 13.41 | 11.67 | 12.88 | 11.27 | 0.406 | 0.382 | 0.403 | 0.387 | 3.13 | 3.44 |
| | | 12.69 | 11.12 | | | 0.402 | 0.389 | | | | |
| | | 12.54 | 11.02 | | | 0.400 | 0.391 | | | | |

**C4**: Fluorescence EEM spectra plots in soil column at different temperatures in both PE and SE under aerobic conditions

i. PE-at a temperature of 25$^{o}$C

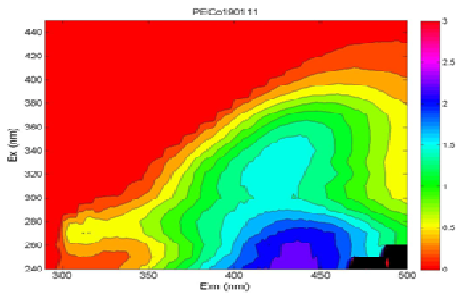



Influent

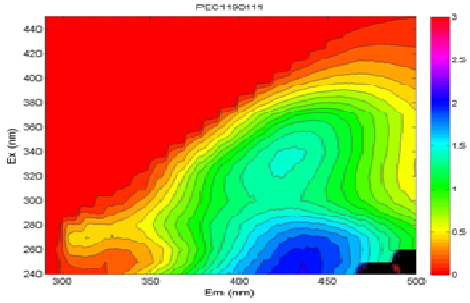



Effluent

ii. PE-at a temperature of 20$^{o}$C

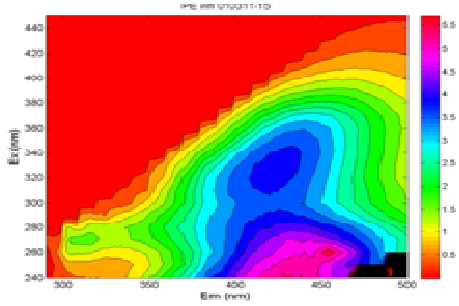



Influent

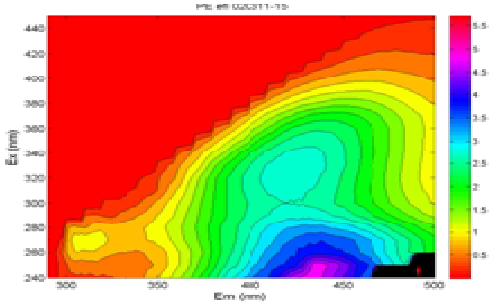



Effluent

iii. PE- at a temperature of 15$^{o}$C

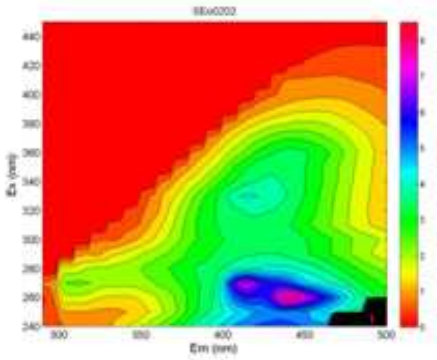



Influent

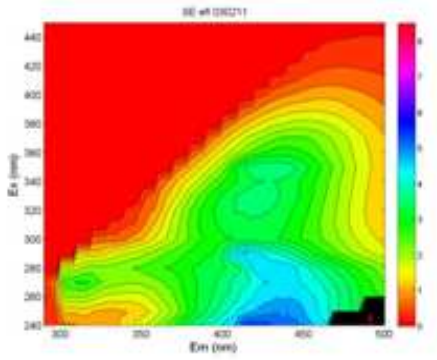



Effluent

iv. SE-at a temperature of 25$^{o}$C

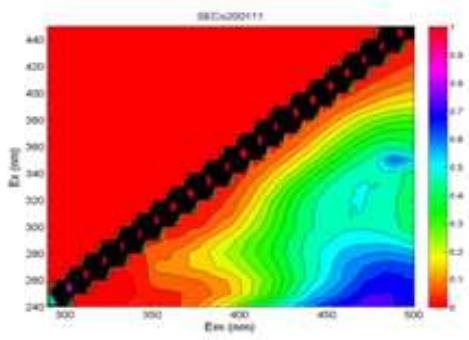


Influent

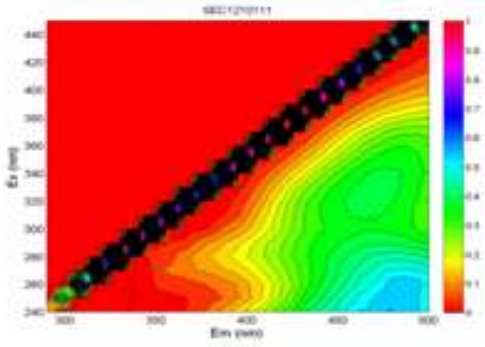


Effluent

v. SE-at a temperature of 20$^{o}$C

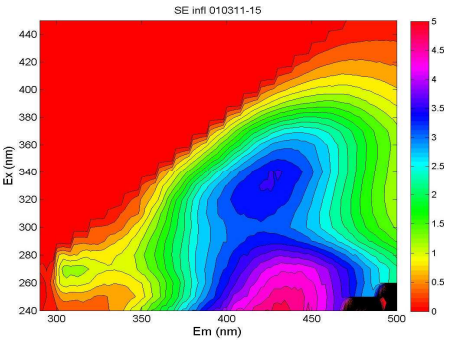


Influent

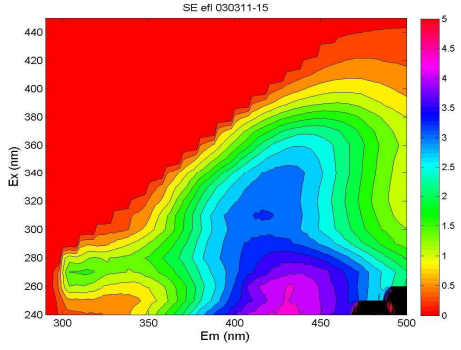


Effluent

vi. SE- at a temperature of 15$^{o}$C




Influent




Effluent

**C5**: Fluorescence EEM analysis of influent and effluent in batch experiments fed with SE under aerobic and anoxic conditions indicating reduction in different peaks

| **Peak 1 Humic/Fulvic-like organic matter fractions** | | **Excitation (nm)** | **Emission (nm)** | **Intensity** | **Reduction in Intensity ( % )** |
|---|---|---|---|---|---|
| SE-soil column | Influent | 340 | 430 | 3.57 | 13.1 |
| 15$^{o}$C | Effluent | 340 | 430 | 3.10 | |
| SE-soil column | Influent | 350 | 480 | 0.51 | 28.6 |
| 20$^{o}$C | Effluent | 350 | 480 | 0.37 | |
| SE-soil column | Influent | 330 | 420 | 4.19 | 12.4 |
| 25$^{o}$C | Effluent | 330 | 420 | 3.67 | |
| **Peak 2 Humic-like organic matter fractions** | | | | | |
| SE-soil column | Influent | 250 | 434 | 4.75 | 9.4 |
| 15$^{o}$C | Effluent | 250 | 434 | 4.31 | |
| SE-soil column | Influent | 250 | 480 | 0.72 | 21.9 |
| 20$^{o}$C | Effluent | 250 | 480 | 0.56 | |
| SE-soil column | Influent | 240 | 436 | 8.18 | 41.9 |
| 25$^{o}$C | Effluent | 240 | 436 | 4.75 | |
| **Peak 3 Protein-like organic matter fractions** | | | | | |
| SE-soil column | Influent | 270 | 308 | 1.32 | -25.3 |
| 15$^{o}$C | Effluent | 270 | 308 | 1.66 | |
| SE-soil column | Influent | 270 | 342 | 0.04 | 38.2 |
| 20$^{o}$C | Effluent | 270 | 342 | 0.02 | |
| SE-soil column | Influent | 270 | 308 | 2.60 | -11.3 |
| 25$^{o}$C | Effluent | 270 | 308 | 2.90 | |

**C6**: *E-coli* and *total coliform* removal at different temperatures in soil column experiments

| **Log removal in soil columns at temperature 15$^{o}$C** | | | | | |
|---|---|---|---|---|---|
| **Category** | **Plate 1** | **Plate 2** | **Plate 3** | **Average** | **STDEV** |
| *E-coli* in PE | 2.1 | 2.4 | 3.0 | 2.50 | 0.46 |
| *E-coli* in SE | 2.0 | 2.0 | 2.0 | 2.00 | 0.00 |
| *Total coliform* in PE | 2.8 | 2.8 | 2.4 | 2.67 | 0.23 |
| *Total coliform* in SE | 1.8 | 2.2 | 2.1 | 2.03 | 0.21 |
| **Log removal in soil columns at temperature 20$^{o}$C** | | | | | |
| Category | Plate 1 | Plate 2 | Plate 3 | Average | STDEV |
| *E-coli* in PE | 3.3 | 3.1 | 2.9 | 3.10 | 0.20 |
| *E-coli* in SE | 2.9 | 2.4 | 2.0 | 2.43 | 0.45 |
| *Total coliform* in PE | 2.7 | 2.9 | 3.1 | 2.90 | 0.20 |
| *Total coliform* in SE | 2.2 | 2.0 | 2.0 | 2.07 | 0.12 |
| **Log removal in soil columns at temperature 25$^{o}$C** | | | | | |
| Category | Plate 1 | Plate 2 | Plate 3 | Average | STDEV |
| *E-coli* in PE | 3.4 | 2.9 | 2.9 | 3.07 | 0.29 |
| *E-coli* in SE | 2.4 | 2.1 | 2.2 | 2.23 | 0.15 |
| *Total coliform* in PE | 3.4 | 3.4 | 3.2 | 3.33 | 0.12 |
| *Total coliform* in SE | 2.4 | 2.3 | 2.0 | 2.23 | 0.21 |

**C7**: Ammonium nitrogen and nitrate measurements in soil column experiments at temperature 25$^{o}$C in both PE and SE

| | **1$^{st}$-reading** | **2$^{rd}$-reading** | **3$^{rd}$-reading** | **AVG** | **STDEV** |
|---|---|---|---|---|---|
| PE influents ($NH_4$-N) | 31.05 | 32.76 | 25.26 | 29.69 | 3.93 |
| PE effluents ($NH_4$-N) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PE influents ($NO_3$-N) | 0.19 | 0.24 | 0.22 | 0.22 | 0.03 |
| PE effluents ($NO_3$-N) | 22.08 | 24.31 | 23.4 | 23.26 | 1.12 |
| | | | | | |
| SE influents ($NH_4$-N) | 0.25 | 0.20 | 0.19 | 0.21 | 0.03 |
| SE effluents ($NH_4$-N) | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| SE influents ($NO_3$-N) | 3.53 | 3.55 | 3.14 | 3.41 | 0.23 |
| SE effluents ($NO_3$-N) | 4.69 | 4.67 | 4.50 | 4.62 | 0.10 |

**C8**: Ammonium nitrogen and nitrate measurements in soil column experiments at temperature 15$^{o}$C in both PE and SE

| | 1st-reading | 2rd-reading | 3rd-reading | AVG | STDEV |
|---|---|---|---|---|---|
| PE influents ($NH_4$-N) | 31.97 | 26.92 | 38.71 | 32.53 | 5.92 |
| PE effluents ($NH_4$-N) | 3.80 | 2.07 | 4.21 | 3.36 | 1.14 |
| PE influents ($NO_3$-N) | 1.91 | 1.88 | 2.05 | 1.95 | 0.09 |
| PE effluents ($NO_3$-N) | 28.10 | 24.54 | 22.30 | 24.98 | 2.92 |
| | | | | | |
| SE influents ($NH_4$-N) | 0.59 | 0.02 | 0.47 | 0.36 | 0.30 |
| SE effluents ($NH_4$-N) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SE influents ($NO_3$-N) | 7.53 | 5.06 | 4.85 | 5.81 | 1.49 |
| SE effluents ($NO_3$-N) | 7.40 | 6.10 | 5.92 | 6.47 | 0.81 |

**C9**: Measurements of phosphorous in soil column experiments at 25$^{o}$C with both PE and SE

| | 1st-reading | 2rd-reading | 3rd-reading | AVG | STDEV |
|---|---|---|---|---|---|
| PE influents ($PO_4$-P) | 5.76 | 4.68 | 6.16 | 5.53 | 0.77 |
| PE effluents ($PO_4$-P) | 4.02 | 3.20 | 4.15 | 3.79 | 0.52 |
| Removal (%) | 30.21 | 31.62 | 32.63 | 31.49 | 1.22 |
| SE influents ($PO_4$-P) | 3.04 | 2.81 | 2.75 | 2.87 | 0.15 |
| SE effluents ($PO_4$-P) | 2.44 | 2.22 | 2.17 | 2.28 | 0.14 |
| Removal (%) | 19.74 | 21.00 | 21.09 | 20.61 | 0.76 |

**C10**: Measurements of phosphorous in soil column experiments at 20$^{o}$C with both PE and SE

| | 1st-reading | 2rd-reading | 3rd-reading | 4th-reading | AVG | STDEV |
|---|---|---|---|---|---|---|
| Influents ($PO_4$-P) | 4.83 | 3.96 | 3.28 | 3.79 | 3.97 | 0.65 |
| PE C1 effluents | 3.74 | 2.81 | 2.31 | 2.50 | 2.84 | 0.63 |
| PE C2 effluents | 3.97 | 3.05 | 2.37 | 3.15 | 3.14 | 0.66 |
| Influents ($PO_4$-P) | 2.95 | 3.30 | 2.93 | 2.85 | 3.01 | 0.20 |
| SE C1 effluents | 2.72 | 2.78 | 2.61 | 2.64 | 2.69 | 0.08 |
| SE C2 effluents | 2.66 | 2.73 | 2.64 | 2.58 | 2.65 | 0.06 |

**C11**: Measurements of phosphorous in soil column experiments at 15$^{o}$C with both PE and SE

| | **1$^{st}$-reading** | **2$^{rd}$-reading** | **3$^{rd}$-reading** | **AVG** | **STDEV** |
|---|---|---|---|---|---|
| PE influents ($PO_4$-P) | 6.24 | 5.73 | 6.53 | 6.17 | 0.41 |
| PE effluents ($PO_4$-P) | 5.66 | 4.94 | 5.81 | 5.47 | 0.47 |
| Removal (%) | 9.29 | 13.79 | 11.03 | 11.37 | 2.27 |
| SE influents ($PO_4$-P) | 4.75 | 4.78 | 5.07 | 4.87 | 0.18 |
| SE effluents ($PO_4$-P) | 4.32 | 4.41 | 4.69 | 4.47 | 0.19 |
| Removal (%) | 9.05 | 7.74 | 7.50 | 8.10 | 0.84 |