

Attenuation of Bulk Organic Matter, Nutrients (N and P), and Pathogen Indicators During Soil Passage: Effect of Temperature and Redox Conditions in Simulated Soil Aquifer Treatment (SAT)

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Abstract Soil aquifer treatment (SAT) is a cost-effective natural wastewater treatment and reuse technology. It is an environmentally friendly technology that does not require chemical usage and is applicable to both developing and developed countries. However, the presence of organic matter, nutrients, and pathogens poses a major health threat to the population exposed to partially treated wastewater or reclaimed water through SAT. Laboratory-based soil column and batch experiments simulating SAT were conducted to

examine the influence of temperature variation and oxidation–reduction (redox) conditions on removal of bulk organic matter, nutrients, and indicator microorganisms using primary effluent. While an average dissolved organic carbon (DOC) removal of 17.7 % was achieved in soil columns at 5 °C, removal at higher temperatures increased by 10 % increments with increase in temperature by 5 °C over the range of 15 to 25 °C. Furthermore, soil column and batch experiments conducted under different redox conditions revealed higher DOC removal in aerobic (oxic) experiments compared to anoxic experiments. Aerobic soil columns exhibited DOC removal 15 % higher than that achieved in the anoxic columns, while aerobic batch showed DOC removal 7.8 % higher than the corresponding anoxic batch experiments. Ammonium-nitrogen removal greater than 99 % was observed at 20 and 25 °C, while 89.7 % was removed at 15 °C, but the removal substantially decreased to 8.8 % at 5 °C. While ammonium-nitrogen was attenuated by 99.9 % in aerobic batch reactors carried out at room temperature, anoxic experiments under similar conditions revealed 12.1 % ammonium-nitrogen reduction, corresponding to increase in nitrate-nitrogen and decrease in sulfate concentration.

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1 Introduction

Contamination of surface water and groundwater, uneven distribution of water resources, and frequent droughts caused by extreme global weather patterns have severely influenced water scarcity (Asano and Cotruvo 2004). Besides, rapid population growth, urbanization, industrial expansion, and sprawling agricultural activity are exacerbating the growing domestic, industrial, and agricultural water demand. Supply of freshwater is limited and cannot meet the growing demand (Wild et al. 2007), and water availability per capita continues to decline (Bakir 2001). Wastewater provides a source of water that could extensively curtail exploitation of valuable natural water resources (Toze 1997). Therefore, wastewater reuse is frequently practiced as a method for water resources management (Vigneswaran and Sundaravadivel 2004). Soil aquifer treatment (SAT) is a managed aquifer recharge (MAR) and wastewater treatment technology which involves physical, chemical, and biological mechanisms through a soil matrix to produce acceptable wastewater quality for indirect reuse. This technology is a low-cost wastewater treatment and polishing alternative which is suitable for both developing and developed countries (Sharma et al. 2007, 2008). Treated municipal wastewater is discharged into recharge basins and infiltrates through the vadose zone (Fox et al. 2001; Montgomery-Brown et al. 2003). The SAT process improves water quality during percolation in the unsaturated zone (Quanrud et al. 2003) before it receives additional purification by dispersion and dilution during its lateral movement in the aquifer (Nema et al. 2001). According to Kanarek and Michail (1996), removal of organic compounds, nitrogen, phosphorus, suspended solids, bacteria, and viruses in a soil infiltration treatment system is achieved through infiltration, percolation, sorption, chemical reaction, biotransformation, die-off, and predation. Phosphorus removal in soil media is influenced by the physical–chemical properties of the media, mainly sorption and precipitation (Vohla et al. 2007). Organic matter is predominantly removed through adsorption and biodegradation in SAT system. These removal mechanisms simultaneously take place in the system, and part of the organic matter adsorbed onto soil grains experiences biological degradation (Xue et al. 2009). Degradation of oxidizable organic substances is facilitated by microbial metabolism (Greskowiak et

al. 2005). Heterotrophic bacteria consume and oxidize organic substances to create new cell material and generate energy for growth and maintenance (van der Aa et al. 2011). Heterotrophic bacteria use dissolved oxygen (DO) to biodegrade natural organic matter (NOM) (van der Kooij et al. 1982). Removal of DO in the unsaturated zone promotes anoxic conditions in the saturated zone (Fox et al. 2001). Furthermore, seasonal variations affect redox processes in groundwater (Massmann et al. 2006). Bacteria are the most commonly found microbial pathogens in recycled water (Toze 1999). *Escherichia coli* (*E. Coli*) and *coliforms* bacteria are used as indicator bacteria to detect fecal contamination (Kretschmer et al. 2000; Pescod 1992). Reduction of pathogens in tertiary treatment plays a pivotal role in minimization of public health risks that arise from exposure to reclaimed water (Costán-Longares et al. 2008).

However, SAT performance is highly influenced by several factors such as wastewater effluent quality, hydrogeological aspects of the site, and process conditions including effluent pre- and post-treatment, hydraulic loading rates, and wetting and drying cycles (Amy and Drewes 2007; Sharma et al. 2008, 2011). Wastewater in many developing countries is either not properly treated or not treated at all before it is discharged back to the environment (Sharma et al. 2011; Wild et al. 2007). Application of primary effluent (PE) in SAT systems in many developing countries has the potential to augment existing water resources to meet the growing water demand and enhance water availability for different uses (Sharma et al. 2011). High ammonium concentration, low nitrate, and relatively high phosphorus are major characteristics of primary effluent (Ho et al. 1992). Nonetheless, there are little data available on the mechanism of different contaminants removal from primary effluent in SAT systems. Therefore, the objective of this research work was to assess the impact of temperature variation and redox conditions on attenuation of bulk organic matter, nitrogen, phosphorus, and pathogen surrogates in laboratory-based soil columns and batch experiments simulating SAT systems.

2 Materials and Methods

2.1 Source and Characteristics of Wastewater Effluent

Nieuwe waterweg wastewater treatment plant (WWTP) located at Hoek van Holland (Netherlands) is an

activated sludge system that contains a nutrients removal unit. Primary effluent was collected from the treatment plant and characterized (Table 1) within 24 h from collection. The effluent was stored in a cold room (4–6 °C) to reduce bioactivity and subsequent change in water quality. The temperature of the water was brought to room temperature by keeping the water at room temperature (20–22 °C) for 2–3 h prior to application to laboratory-based soil columns and batch reactors.

2.2 Experimental Setups

2.2.1 Laboratory-Based Soil Columns

Four double-walled soil columns (SCs) (XK50/30; Amersham Pharmacia Biotech, Sweden) detailed in Fig. 1 were wet-packed by allowing the silica sand grains (0.8–1.25 mm) to settle in deionized water to ensure homogeneous media packing in the column. The porosity and uniformity coefficients ($C_u = D_{60}/D_{10}$) of the porous media were 0.4 and 1.3, respectively. Two columns were used to explore the impact of oxidation–reduction (redox) conditions, while the third column was used to assess the influence of temperature variation

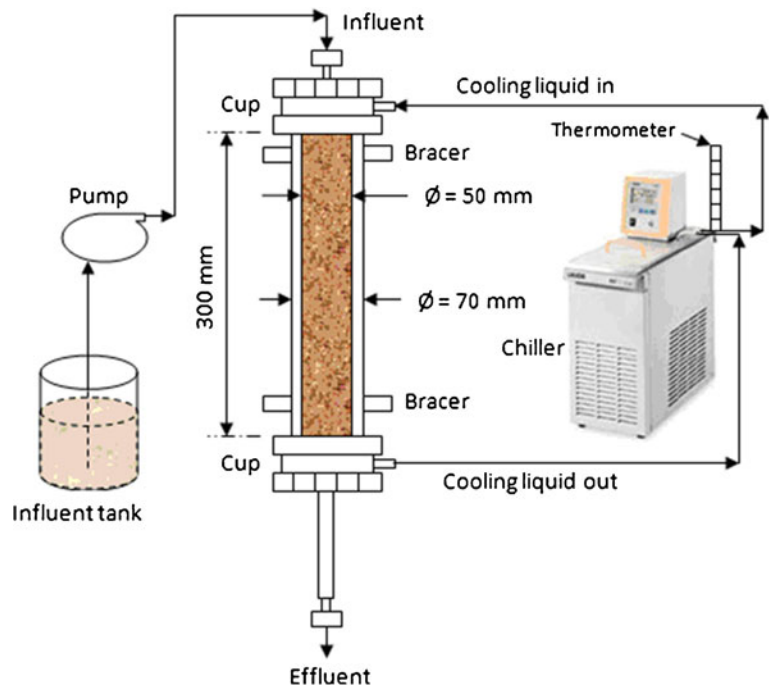
at 15 °C, and the fourth column was operated at 5 °C. Furthermore, the soil column used to examine aerobic conditions was used to conduct continuous loading experiments at 20 and 25 °C. A variable-speed peristaltic pump was connected to each soil column using tygon flexible tubes to continuously feed the primary effluent at constant hydraulic loading rate (HLR) of 0.625 m/day from brown glass bottles to the top part of the column after which it percolated down the column under gravity flow conditions. The column was operated in down flow mode and was therefore assumed to be predominantly unsaturated. The empty bed contact time (EBCT) for the soil columns was 11.5 h. Effluent samples were collected from a sampling port situated at the bottom part of the column after at least 15 h from introduction of feed water to the column to ensure enough retention time and consequently sufficient contact between the water and soil media.

In order to establish a robust sessile microbial population on sand media and ensure steady-state conditions (biostability) in the columns, the columns were acclimated by continuously loading the primary effluent and analyzing the DOC of the influent and effluent samples. The operating conditions during the acclimation process of the system were maintained aerobic through aeration of feed water at room temperature until dissolved oxygen (DO) reached around 8 mg O₂/L after which the aeration process was ceased and application to columns started. The columns were assumed to have reached biostability when a difference of less than ±1 % DOC removal was obtained between each (of three) successive samples measurements. This process was repeated for each new experimental operating condition to ensure that the microorganisms in the biofilms around the media had adapted to the new environmental conditions.

To investigate the effect of temperature variation in the simulated SAT system, an ecoline immersion thermostat chiller (E100 w RE106, Lauda Dr. R. Wobser GmbH & CO. KG, Germany) filled with a cooling liquid (Halfords B.V., Netherlands) was connected to the soil column, and the outer part of the column was used to circulate the liquid to control the test temperature. A thermometer was plugged (perpendicular to liquid flow) into the outlet tube connecting the chiller with the column. Furthermore, the temperature of the water exiting the column was regularly checked using a 330i handheld conductivity meter (Wissenschaftliche-Technische Werkstätten GmbH & CO. KG, Germany)

Table 1 Primary effluent characteristics

Parameter	Units	Measured value
Temperature	°C	13.2±0.4
O ₂	mg O ₂ /L	2.1±0.5
EC	µS/cm	1,497±121.2
pH	–	7.3±0.1
DOC	mg/L	42.5±10.3
UVA254	l/cm	1.3±0.2
SUVA	L/mg m	3.0±0.4
PO ₄ -P	mg/L	5.3±1.7
NH ₄ -N	mg/L	32.0±4.3
NO ₃ -N	mg/L	0.7±0.3
COD	mg/L	296±46
BOD	mg/L	180.7±34.7
<i>E. coli</i>	CFU/100 mL	3.6×10 ⁶ ±1.6×10 ⁶
<i>Total coliform</i>	CFU/100 mL	3.1×10 ⁷ ±1.0×10 ⁷
Alkalinity	mg/L as HCO ₃ ⁻	497.0±46.6
Suspended solids (SS)	mg/L	107.1±30.4
Total dissolved solids (TDS)	mg/L	850.8±76.6

Fig. 1 Soil column setup

equipped with an automatic temperature compensation feature, and the temperature of the cooling liquid was adjusted accordingly to maintain the required temperature in the system. The system was then run at 15, 20, and 25 °C for a period of time (2–3 weeks) to acclimate the microorganisms in biofilms around sand particles to the new environment and establish a stable DOC removal. However, low-temperature experiments were conducted by placing the whole column setup (column, feed water tank, and pump) in a cold room under controlled temperature of 5 °C during which the outer part of the column was filled with the cooling liquid and the opening ports used to circulate the cooling liquid were sealed.

On the other hand, anoxic conditions were created by inserting a fine stream of nitrogen gas into the feed water tank to strip out DO from the influent water. The tank was filled with influent water (no space above water) to ensure that all oxygen escaped the system. The nitrogen stream was ceased when DO concentration of the feed water achieved <0.2 mg O_2/L after which the feed tank was sealed while maintaining the suction pipe (inside the tank) connected to the pump. DOC concentration was monitored at influent and effluent points of the soil column operated under anoxic conditions until a difference of <1 % was attained between successive DOC measurements. Furthermore, oxidation–reduction potential (ORP) was measured

using a redox potential probe connected to calibrated pH meter in mV mode to crosscheck the anoxic conditions in feed and effluent samples. A minimum of four sample repetitions ($n=4$) was collected from the biostable soil columns at various operating conditions.

2.2.2 Laboratory-Based Batch Reactors

To simulate saturated flow conditions in an SAT system at laboratory scale, 15 glass bottles (0.5 L) in triplicate were filled with 100 g of silica sand (similar to the one used in soil columns above) and fed with 400-mL primary effluent every 5 days. The reactors were placed on a horizontal reciprocal table shaker and agitated at 100-rpm shaking speed throughout the entire experimental period. Establishment of a sessile microbial population on the sand and steady-state operating conditions were achieved in the reactors through determination of removable (biodegradable) DOC as the difference between initial DOC concentrations on day 0 (DOC_0) and final day 5 (DOC_5) over 4–6 weeks (data not provided). Aerobic conditions were achieved through aeration of the influent water until DO concentration was 8.0 mg/L. The reactors were considered to have reached steady-state operating conditions when ± 1 % difference between each three consecutive DOC removal values was attained.

To investigate the influence of anoxic operating conditions on removal of bulk organic carbon, nutrients (N and P), and pathogens (surrogates), six reactors were switched to anoxic operating conditions by stripping out DO using nitrogen gas. The nitrogen gas was introduced via a tygon tube that penetrates a cap deep into the influent water while the oxygen was allowed to escape the space above the influent water through another tube that protrudes from the cap. The reactors were tightly capped with screw-type lids when oxygen concentration reached <0.2 mg/L in the water. Continuous measurement of DO was carried out in anoxic batch reactors using an HQ30d meter and LDO101 probe (Hach, Colorado, USA) equipped with data log function at 15-min time interval for 5 days to monitor the change in DO over the retention time when DO concentration on day 5 exceeded 0.2 mg/L. The meters had low and high DO detection limits of 0.01 mg/L and 20 mg O₂/L, respectively. The reactors were tightly capped, and the probe penetrated down into the water from a hole on the cap which was sealed using paraffin. Monitoring of DOC was carried out at 5-day intervals until the reactors were biostable. Samples from the biostable batch reactors were collected (in duplicate) at least three times ($n=6$).

2.3 Analytical Methods

DOC concentration of all pre-filtered samples collected from the WWTP and laboratory-based set-ups was determined within 3 days by the combustion technique using total organic carbon analyzer (TOC-VCPN (TN), Shimadzu, Japan) with a precision range of 0.5 to 20 mg/L. Since aromatic unsaturated bonding structures in organic compounds are responsible for ultraviolet (UV) light absorption over the range of 200–300 nm (Michail and Idelovitch 1981), UV absorbance at 254-nm wavelength (UVA₂₅₄) for all pre-filtered samples was measured in a quartz cell (cuvette) with a 1-cm path length using a computer-controlled UV-VIS spectrophotometer (UV-2501 PC, Shimadzu, Japan). The instrument was auto-zeroed prior to samples measurements to obtain zero absorbance (reference) using ultra-pure water (Milli-Q). Specific ultraviolet light absorbance (SUVA) was calculated to explore the contribution of aromatic structures of DOC of the samples using their respective UVA₂₅₄ measurements and DOC values. Since DOC contains chromophoric (light absorbing) and fluorophoric (light emitting)

molecules, fluorescence excitation–emission matrices (F-EEM) were used in water samples analysis (Baker 2001). DOC concentrations of all samples were diluted with ultra-pure (Milli-Q) water to obtain 1-mg/L DOC concentration. No pH adjustment was carried out. F-EEM spectra were then obtained through collection of a series of emission spectra at different excitation wavelengths using a FluoroMax-3 spectrofluorometer (HORIBA Jobin Yvon, Edison, NJ, USA). Procedures for F-EEM measurement are elaborated in Maeng et al. (2011). The area under the Raman scatter peak (at excitation wavelength of 350 nm) of Milli-Q water sample was used to calibrate fluorescence spectra, followed by removal of the Raman signal through subtraction of normalized Milli-Q EEM (Stedmon et al. 2003). In order to account for earlier DOC dilution, an order of magnitude at which the DOC was diluted (dilution factor) was used to carry out correction of intensities in MATLAB (version 7.9, R2009b) used to illustrate organic matter fractions of humic-, fulvic-, and protein-like as identified by Amy and Drewes (2007). These fractions were identified in MATLAB contour maps as Peaks of an excitation–emission (Y-X) matrix. These peaks were referred to by a combination of letters and numbers as in Maeng et al. (2011). Peak 1 (P1) was assigned to (primary) humic-like, peak 2 (P2) was given to (secondary humic) fulvic-like, while peak 3 (P3) corresponded to protein-like.

Chemical reagents used to determine ammonium as nitrogen (NH₄-N), nitrate as nitrogen (NO₃-N), and phosphorus (PO₄-P) were of analytical grade and were purchased from Merck KGaA, Germany and J.T. BAKER, Netherlands. NH₄-N, NO₃-N, and PO₄-P were determined using colorimetric automated techniques with a spectrophotometer according to Eaton et al. (2005). Standard calibration line (in 5 concentration range) was prepared for NH₄-N, NO₃-N, and PO₄-P to calculate their concentrations in various water samples. Sulfate (SO₄²⁻) was measured using an ion chromatography system (ICS-1000, Dionex Corporation, USA).

The plate count method was used to enumerate indicator bacteria (*E. coli* and *total coliforms*) in unfiltered water samples from soil columns and batch reactors. Chromocult agar (26.5 g, Merck KGaA, Germany) was dissolved in 1 L of deionized water in a round-bottom flask and placed in a water bath at 99 °C for 30 min. The flask was then removed from the water bath and kept at an ambient temperature of 50 °C in an oven

for 30 min; after which, it was decanted into two smaller (0.5 L each) round-bottom flasks. Finally, the warm liquid agar was poured into test plates and left to solidify. Well-centrifuged primary effluent (0.1 mL) was transferred (using pipette) to test plates (in triplicate) and cultured in test plates containing chromocult agar for 24 h at 37 °C ambient temperature.

Statistical package for the social sciences IBM SPSS® (v19.0 statistics) was used to determine if there was difference in a given contaminants removal/reduction under certain operating conditions using one-way analysis of variance (ANOVA). The removal/reduction was considered significant when $P < 0.05$.

3 Results and Discussion

This section addresses the influence of temperature and redox operating conditions under constant hydraulic loading rate in laboratory-based soil columns simulating unsaturated conditions in SAT system. Furthermore, it presents results from laboratory-scale batch experiments simulating saturated conditions in SAT.

3.1 Influence of Temperature on Contaminants Removal in Soil Columns

3.1.1 DOC, SUVA, and F-EEM

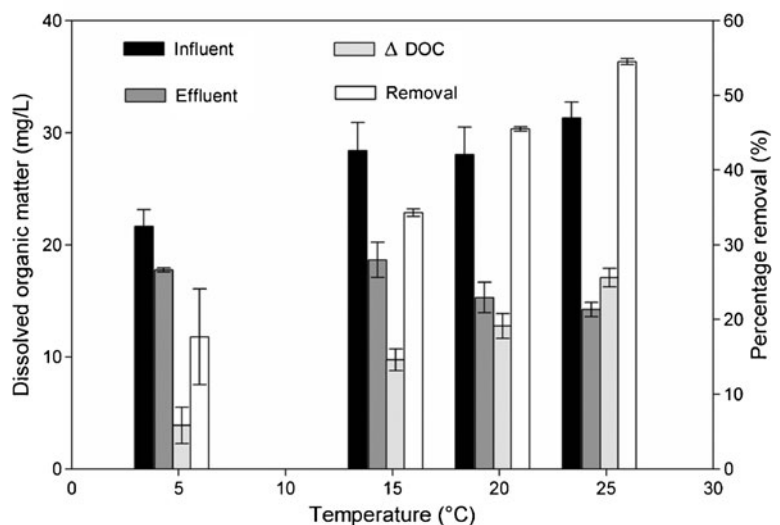
Figure 2 shows removal of DOC ($n=4$) in soil column experiments conducted at 5, 15, 20, and 25 °C under

aerobic conditions. The percentage and magnitude of DOC removed (expressed as ΔDOC) increased with an increase in temperature. The soil column removed DOC by $17.7 \pm 6.4\%$ ($\Delta\text{DOC} = 3.9 \pm 1.6$ mg/L) at 5 °C, $34.3 \pm 0.5\%$ ($\Delta\text{DOC} = 9.7 \pm 1.0$ mg/L) at 15 °C, $45.5 \pm 0.3\%$ ($\Delta\text{DOC} = 12.8 \pm 1.1$ mg/L) at 20 °C, and $54.5 \pm 0.4\%$ ($\Delta\text{DOC} = 17.1 \pm 0.8$ mg/L) at 25 °C.

Furthermore, SUVA values proportionally increased with increase in temperature by 9.5 ± 5.7 , 39.4 ± 2.3 , 46.8 ± 4.9 , and $69.6 \pm 9.1\%$ at 5, 15, 20, and 25 °C, respectively. These significant increase ($P < 0.0001$) in DOC removal and increase in SUVA values with an increase in temperature imply that biodegradable substances were preferentially removed due to increased microbial activity with an increase in temperature. According to Grünheid et al. (2005), non-degradable DOC increases during cold winter months at recharge sites. Low DOC removal at 5 °C suggests that the activity of heterotrophic bacteria that rely on DOC for growth and metabolism was severely affected by low temperature, leading to lower DOC removal and subsequently higher DOC concentration exiting the soil column compared to 15, 20, and 25 °C columns.

Fluorescence intensities were identified in three regions in 3D F-EEM spectra. These intensities were differentiated based on the range of excitation and emission wavelengths at which they occurred. Humic-like peak (P1) was observed in wavelength range of ($\lambda_{\text{Ex/Em}} = 240\text{--}250/430\text{--}450$), fulvic-like peak (P2) covered the wavelength range of ($\lambda_{\text{Ex/Em}} = 320\text{--}330/420\text{--}430$), and protein-like peak (P3) appeared at

Fig. 2 Summary of change in DOC concentration with temperature in aerobic soil column experiments fed with primary effluent (EBCT=11.5 h)



the wavelength range of ($\lambda_{Ex/Em}=270\text{--}280/308\text{--}330$). As shown in Table 2, fluorescence intensities have revealed 2 % reduction in P1, while P2 remained unchanged at 5 °C. Additionally, reduction in P1 was in the range of 8–12 % at 20 and 25 °C, while P2 exhibited reduction of 5.3–8.6 % at 20 and 25 °C, while P3 was reduced by 26.3, 26.9, and 50.1 % at 5, 20, and 25 °C, respectively. However, higher reduction in P1 (35.4 %), P2 (27.4 %), and P3 (33.0 %) was observed at 15 °C.

Humic substances are broadly defined as large negatively charged molecules that constitute humic and fulvic acid (Laangmark et al. 2004). While these substances resist biodegradation due to their hydrophobicity, they could be removed through adsorption in the subsurface environment (Quanrud et al. 1996). Silica sand used as media in column experiments conducted at 5, 15, 20, and 25 °C may have undergone humification during the ripening period as a result of deposition of high molecular (>20,000 Da) humic substances leading to exhaustion of adsorption sites within the media. According to Xue et al. (2009), reduction in protein-like substances in soil column experiments simulating SAT is attributed to breakdown of these substances into non-fluorescent structures. High reduction of protein-like fluorescence intensities at 25 °C could be ascribed to degradation of fluorescent materials due to increase in microbial activity pertaining to increase in temperature. Schreiber et al. (2007) stipulated that the adsorption process generally decreases at higher temperature. Additionally, high reduction in peak 3 is ascribed to possible increase in microbial activity at 25 °C compared to 5 °C.

3.1.2 Ammonium-Nitrogen and Nitrate-Nitrogen

$\text{NH}_4\text{-N}$ concentration was attenuated fairly significantly ($P<0.0001$) at high (15, 20, and 25 °C) experimental temperatures. However, $\text{NH}_4\text{-N}$ reduction rates decreased considerably at 5 °C. An average reduction of

8.8 % (28.3 ± 3.7 to 25.8 mg N/L) was achieved at 5 °C, 89.7 % (32.5 ± 5.9 to 3.36 ± 1.1 mg N/L) at 15 °C, while >99 % $\text{NH}_4\text{-N}$ was reduced at 20 and 25 °C. A decrease in $\text{NH}_4\text{-N}$ concentration resulted in an increase in $\text{NO}_3\text{-N}$ concentration which marginally increased from 0.3 ± 0.1 to 0.6 ± 0.1 mg N/L at 5 °C (Fig. 3). Nonetheless, $\text{NO}_3\text{-N}$ concentration increased from less than 2 mg N/L in influent samples to 25.0 ± 2.9 , 20.4 ± 1.4 , and 23.3 ± 1.1 mg/L at 15, 20, and 25 °C, respectively.

Low $\text{NH}_4\text{-N}$ removal and corresponding slight increase in $\text{NO}_3\text{-N}$ at 5 °C implied that nitrifying bacteria were affected by low temperature. However, these bacteria remained active and provided limited removal of $\text{NH}_4\text{-N}$. This is contrary to the findings of Yamaguchi et al. (1996) who stipulated that growth of nitrifying bacteria ceases at 10 °C. Furthermore, $\text{NH}_4\text{-N}$ removal was 10 % less at 15 °C than that at 20 and 25 °C. Conversely, $\text{NO}_3\text{-N}$ concentration in effluent samples from 15 °C soil column was somewhat higher than that of 20 and 25 °C. These results suggest that another removal mechanism (presumably adsorption) contributed to relatively high removal of $\text{NH}_4\text{-N}$ at high temperatures due to higher molecular activity that increased collision between positively charged $\text{NH}_4\text{-N}$ ions and negatively charged silica sand. These results diverge from the findings of Yamaguchi et al. (1996) who observed a decrease in $\text{NH}_4\text{-N}$ adsorption at 30 °C compared to 10 °C in a soil column filled with granite mixed with clayey silt.

3.1.3 Phosphorus Removal

Mass loading rate (MLR) for $\text{PO}_4\text{-P}$ at various soil columns operated at different temperatures ranged from 4.9 ± 0.8 to 8.0 ± 0.1 mg/day. Figure 4 shows change in $\text{PO}_4\text{-P}$ concentration at feed and effluent points of the soil columns at different water temperatures. Phosphorus is poorly removed in sandy soils (Reemtsma et al. 2000). Reduction of 4.9 ± 0.7 % (0.4 ± 0.0 mg/day) was observed in $\text{PO}_4\text{-P}$ concentration at 5 °C, 11.4 ± 2.3 % ($0.8\pm$

Table 2 Change in intensity characteristics of fluorescence peaks with temperature in soil column experiments (EBCT=11.5 h)

Peak	$\lambda_{Ex/Em}$ (nm)	Reduction in F-EEM (%)			
		5 °C	15 °C	20 °C	25 °C
P1 (humic-like)	240–250/430–450	2.0	35.4	7.8	12.0
P2 (fulvic-like)	320–330/420–430	0.0	27.4	8.6	5.3
P3 (protein-like)	270–280/308–330	26.3	33.0	26.9	50.1

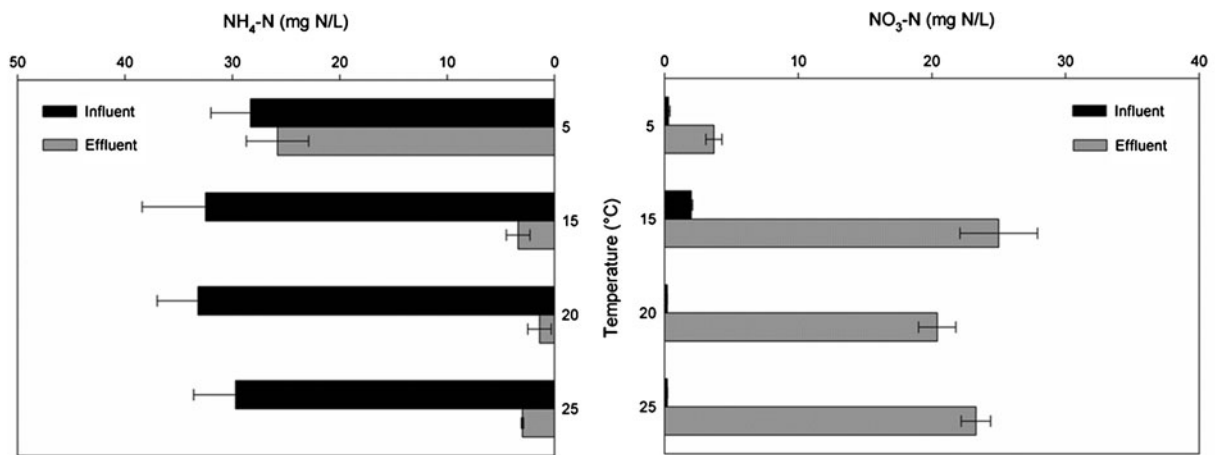


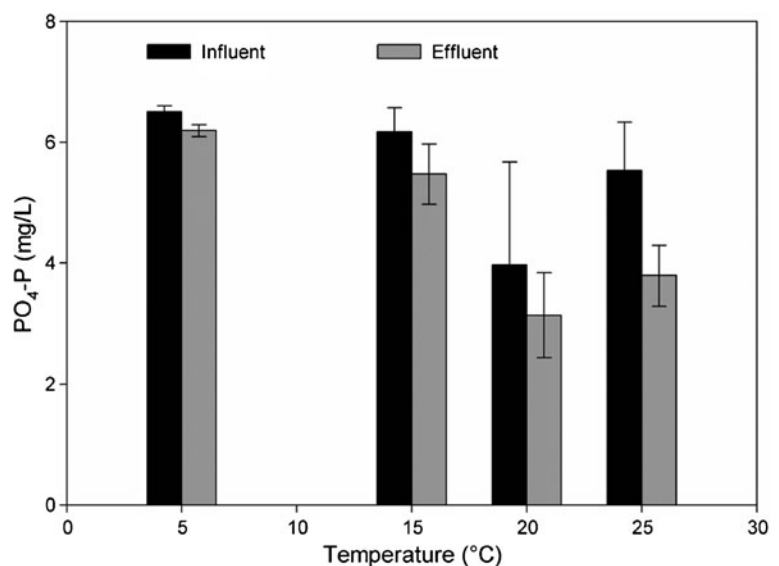
Fig. 3 Impact of temperature variation on nitrogen removal from primary effluent in soil column experiments under aerobic conditions (EBCT=11.5 h)

0.1 mg/day) at 15 °C, while $23.2 \pm 4.7\%$ (1.0 ± 0.2 mg/day) PO₄-P was removed at 20 °C, and $31.5 \pm 1.2\%$ (2.1 ± 0.3 mg/day) removal was achieved at 25 °C. These results indicate that higher PO₄-P removal took place at higher ambient temperature.

PO₄-P is mainly sorbed or precipitated in filter media (Vohla et al. 2007). Dissolved organics are removed through adsorption and biodegradation (Idelovitch et al. 2003), and removal of PO₄-P might be affected by organic carbon content in PE through competition for adsorption sites. Experiments conducted at 20 °C preceded those at 25 °C, but the latter exhibited higher phosphorus removal. Part of the

organic matter adsorbed onto soil particles is biologically degraded (Idelovitch et al. 2003). Increasing PO₄-P removal at high temperature may be due to an increase in degradation of the sorbed materials or increase in degradation of organic matter at 25 °C compared to 20 °C. Furthermore, increase in temperature might have increased PO₄-P adsorption following easy diffusion of PO₄-P into biofilm environment due to decrease in water viscosity at high temperatures. Nonetheless, the role of direct adsorption of PO₄-P to binding sites within silica sand is assumed to be minimal due to negative charges on both the sand and PO₄-P.

Fig. 4 Impact of temperature variation on the removal of phosphorus from primary effluent in soil column (EBCT=11.5 h)

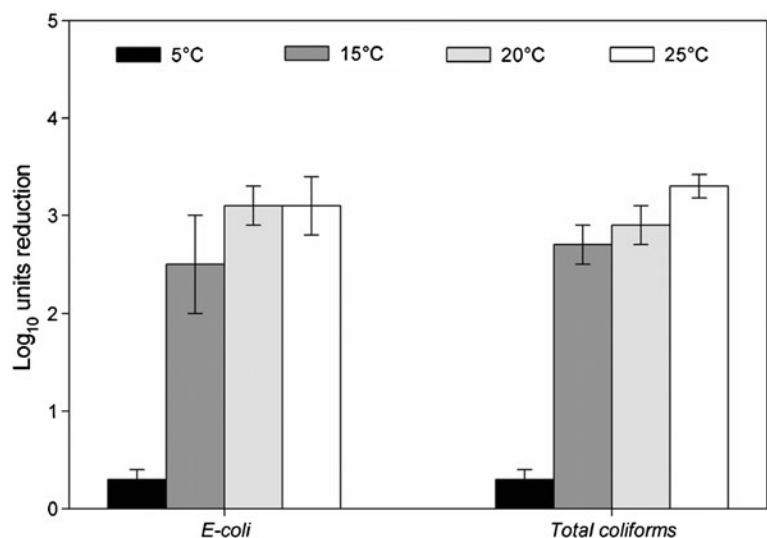


3.1.4 *E. coli* and Total Coliforms Removal

Mean concentrations of indicator microorganisms in influent wastewater fed to the setups ranged from 5.9×10^6 to 6.7×10^6 CFU/100 mL for *E. coli* and 19.9×10^6 to 37.3×10^6 CFU/100 mL for *total coliforms*. These concentrations were attenuated in the soil column under different operating temperatures (Fig. 5). Both microorganisms were equally removed by $0.36 \log_{10}$ units ($n=6$) at 5 °C. However, removal of both microorganisms increased at 15 °C, and *E. coli* was removed by $2.5 \log_{10}$ units ($n=6$), while *total coliforms* revealed a total removal of $2.7 \log_{10}$. Furthermore, removal of *E. coli* was identical and exhibited no change at 20 and 25 °C and showed $3.1 \log_{10}$ units ($n=6$), while *total coliforms* removal revealed increase from $2.9 \log_{10}$ units ($n=6$) at 20 °C to $3.3 \log_{10}$ units at 25 °C.

Biomass in a low-temperature environment excretes extracellular polymers substances (EPS) which enhance clogging of a biofilter (Characklis 1973; Le Bihan and Lessard 2000). Presence of EPS in high amounts could adversely affect adsorption of bacteria through blockage of adsorption sites. Inactivation plays a major role in removal of microorganisms in managed underground system (NRC 2007). Low removal of *E. coli* and *total coliforms* at 5 °C suggests that this low temperature caused inactivation of *E. coli* and *total coliforms*. Furthermore, some microorganisms become viable but non-culturable at low temperature, leading to less bacteria counts.

Fig. 5 Indicator microorganisms inactivation (\log_{10} units reduction) at different temperatures in soil column using primary effluent (EBCT=11.5 h)



3.2 Influence of Redox Reactions on Contaminants Removal in Soil Columns

3.2.1 DOC, SUVA, and F-EEM

DOC removals of 46.4 ± 2.0 % (34.3 ± 6.0 to 19.6 ± 4.5 mg/L) and 31.3 ± 0.3 % (29.4 ± 3.8 to 19.7 ± 2.4 mg/L) were attained in acclimated soil column experiments under aerobic and anoxic conditions, respectively. SUVA values increased from 1.8 to 2.2 L/mg m under aerobic and from 2.1 to 2.6 L/mg m under anoxic operating conditions. These results show that removal under aerobic conditions was 15 % higher than that observed for anoxic conditions. This significant ($P < 0.0001$) difference is attributed to the presence of oxygen which is used by the microorganisms to degrade the organic matter in aerobic soil column experiments. On the other hand, DOC removal under anoxic conditions could be ascribed to combination of anoxic degradation, adsorption, and presumably aerobic biodegradation in aerobic pockets along the media depth. These results are consistent with the findings of Grünheid et al. (2005) who observed slower degradation of DOC in an anoxic zone compared to aerobic zone during soil passage at a bank filtration site. Increase in SUVA values under both redox conditions indicated that aliphatic substances were removed in both aerobic and anoxic soil columns.

Results of F-EEM (Fig. 6 and Table 3) show reduction in P1, P2, and P3 under aerobic conditions, while the same peaks increased under anoxic conditions.

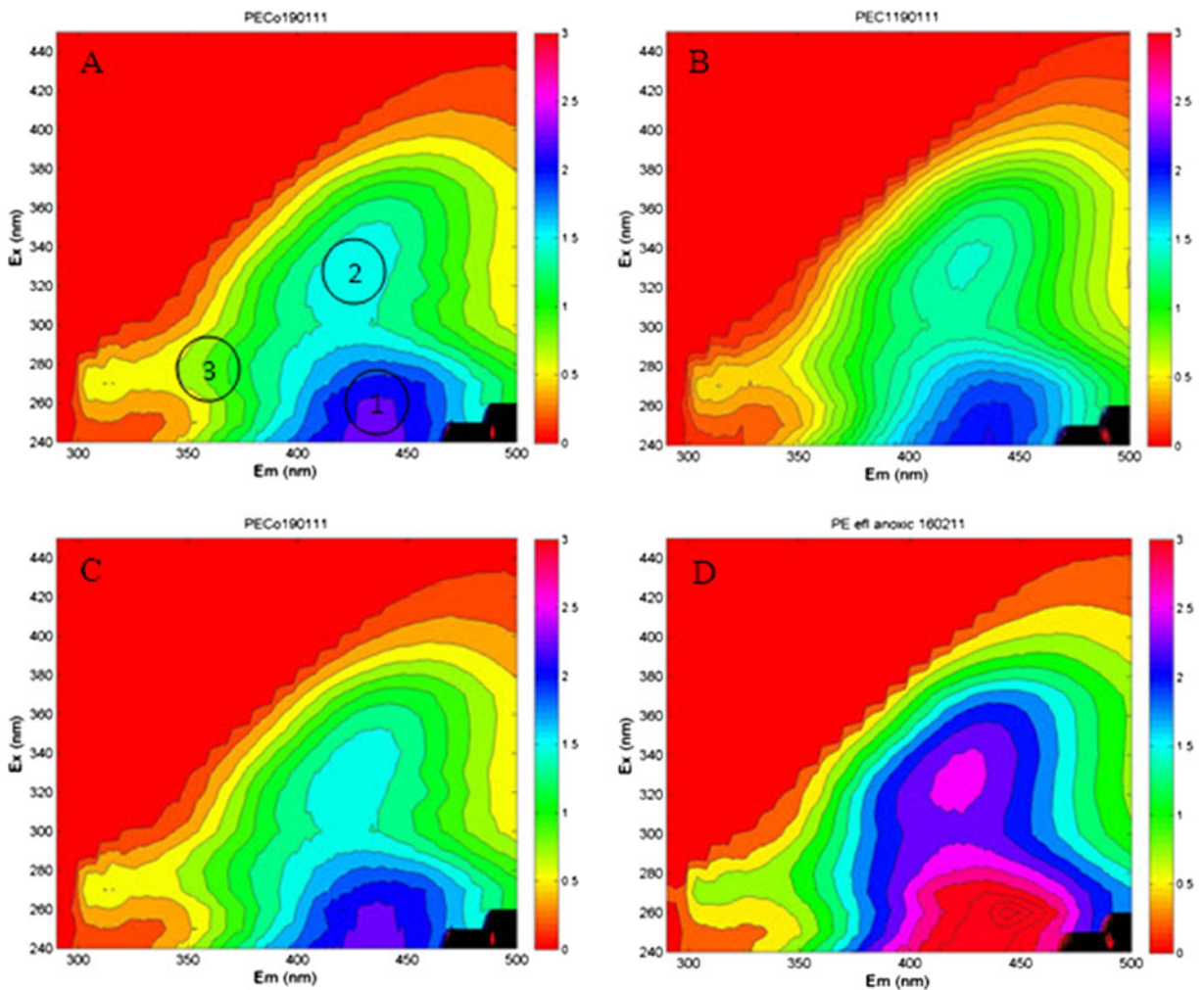


Fig. 6 F-EEM spectra of soil column experiments conducted at different redox conditions: **a, c** influent; **b** effluent aerobic, **d** effluent anoxic (feed water: primary effluent, EBCT 11.5 hrs)

These results are in agreement with the findings of a study carried out by Saadi et al. (2006) to monitor effluent dissolved organic matter in the Haifa wastewater treatment plant effluent using fluorescence, UV, and DOC. The authors ascribed decrease in fluorescence intensities to degradation of fluorescing material or dampening of dissolved organic matter (DOM) fluorescence by newly formed organic molecules. However, they postulated that increase in fluorescence intensities was due to formation of new fluorescence material affiliated with DOM biodegradation and/or degradation of organic components able to quench fluorescence. Results of a study carried out by Maeng et al. (2008) using soil columns (HRT=5 days retention time) under anoxic conditions did not reveal

increase in fluorescence intensities for P1 and P2. Anoxic conditions in this research work appear to have induced increase in fluorescence intensities for the same peaks in soil column experiments with shorter HRT (11.5 h). This increase in P1 and P2 could be ascribed to humification caused by continuous loading of humic-like fractions at rates higher than the removal in column.

3.2.2 Ammonium-Nitrogen and Nitrate-Nitrogen

$\text{NH}_4\text{-N}$ decreased by 99.5 ± 0.2 % (33.2 ± 3.8 to 0.2 ± 0.0 mg N/L) in soil column operated under aerobic conditions, while anoxic conditions reduced $\text{NH}_4\text{-N}$ by 71.8 ± 2.0 % (33.2 ± 3.8 to 9.3 ± 0.7 mg N/L).

Table 3 Changes in intensities of (organic matter) fluorescence peaks in primary effluent through aerobic and anoxic primary in soil columns fed with primary effluent (silica sand, EBCT=11.5 h)

Redox condition	Fluorescence Peaks		
	P1 ($\lambda_{Ex}/\lambda_{Em}$ = 240–250/430–440 nm)	P2 ($\lambda_{Ex}/\lambda_{Em}$ = 320–330/420–430 nm)	P3 ($\lambda_{Ex}/\lambda_{Em}$ = 270–280/310–320 nm)
Aerobic			
Influent	2.28	1.59	0.75
Effluent	2.10	1.49	0.55
Change (%)	7.8 (-)	8.6 (-)	26.9 (-)
Anoxic			
Influent	2.28	1.59	0.75
Effluent	3.45	2.55	0.90
Change (%)	92.4 (+)	111.3 (+)	57.3 (+)

- decrease, + increase

Decrease in $\text{NH}_4\text{-N}$ concentrations yielded substantial increase in $\text{NO}_3\text{-N}$ concentrations from 0.2 ± 0.0 mg N/L in the influent of the aerobic column to 20.4 ± 1.4 mg N/L in the effluent, whereas $\text{NO}_3\text{-N}$ concentration increased from 1.9 ± 0.1 to 16.3 ± 2.7 mg N/L in effluent anoxic soil column samples. Furthermore, an average DO concentration of 0.46 mg/L and ORP of 38.2 ± 1.3 mV were measured in water samples exiting the anoxic column. Figure 7 shows change in $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations under aerobic and anoxic operating conditions.

High reduction in $\text{NH}_4\text{-N}$ concentration and corresponding increase in $\text{NO}_3\text{-N}$ concentration in

influent and effluent samples taken from aerobic soil column indicate that the nitrification process took place in the column. However, decrease in $\text{NH}_4\text{-N}$ concentration in anoxic samples might be due to adsorption of $\text{NH}_4\text{-N}$ by soil media or nitrification in aerobic pockets along the column depth resulting from oxygen entrapment in the media. Reduction of NH_4^+ under anoxic conditions in the presence of organic matter was observed in a previous study conducted by Sabumon (2007) in a 700-mm-high and 50-mm-diameter column (continuous reactor) seeded with digested cow dung.

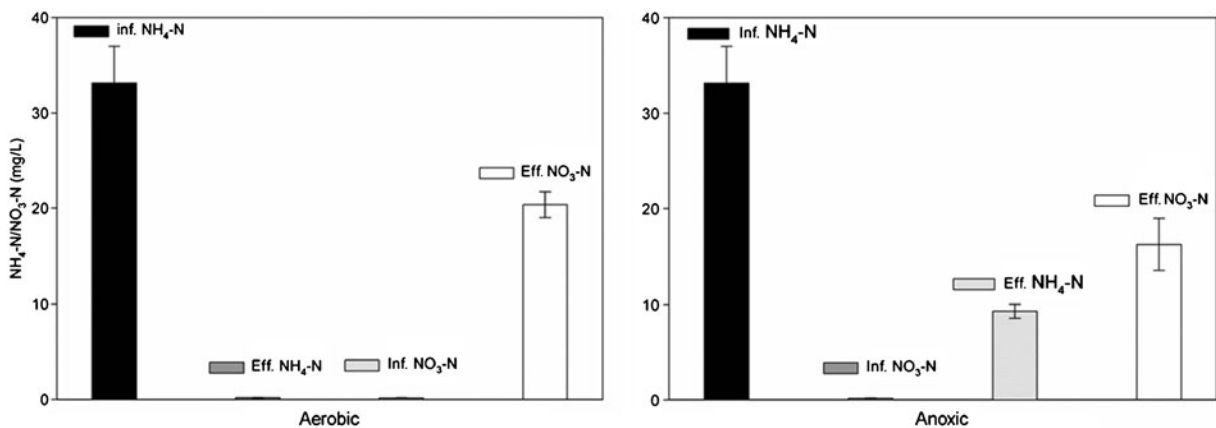
3.2.3 Phosphorus

MLR of 4.9 ± 0.8 mg/day was applied to aerobic and anoxic soil columns. $\text{PO}_4\text{-P}$ removal in aerobic soil column under experiments was 23.2 ± 4.7 % (1.0 ± 0.2 mg/day), while anoxic conditions resulted in 22.5 ± 2.9 % (1.1 ± 0.3 mg/day) $\text{PO}_4\text{-P}$ removal. This comparable ($P=0.74$) $\text{PO}_4\text{-P}$ removal under both aerobic and anoxic conditions in soil columns implies that redox conditions did not affect $\text{PO}_4\text{-P}$ removal.

3.3 Influence of Redox Operating Conditions on Removal of Contaminants in Batch Experiments

3.3.1 DOC, SUVA, and F-EEM

Aerobic biostable batch reactors exhibited DOC removals of 54.5 ± 0.3 % ($\text{DOC}_0=35.4 \pm 2.5$ mg/L and $\text{DOC}_5=16.1 \pm 1.2$ mg/L). However, anoxic batch reactors showed DOC removal of 46.7 ± 0.7 %

**Fig. 7** Change in nitrogen concentration of primary effluent in soil column operated at room temperature under different redox conditions (EBCT=11.5 h)

($\text{DOC}_0=35.4\pm 2.5$ mg/L and $\text{DOC}_5=19.1\pm 3.0$ mg/L). SUVA values increased from 1.65 L/mg m in aerobic water samples to 2.87 L/mg m, whereas SUVA of anoxic water samples increased from 1.65 to 2.68 L/mg m. Increasing SUVA values during aerobic soil passage were observed by Grünheid et al. (2005) in a full-scale bank filtration study to monitor DOC. The authors attributed this increase to preferential removal of aliphatic carbon substances. However, increase in SUVA values under anoxic conditions suggests anoxic degradation of aliphatic substances. This is contrary to the findings of the same authors who revealed decrease in SUVA in the anoxic zone. On the other hand, results of F-EEM (Fig. 8) for aerobic and anoxic

experiments showed higher reduction of P1 by 27 % in aerobic tests compared to 23 % in anoxic batch experiments, while P2 exhibited less reduction of 15 % in aerobic batch samples compared to 17.3 % in anoxic samples. However, P3 was substantially reduced by 61.5 % under aerobic conditions, and anoxic batch experiments revealed 38.9 % reduction. Correspondence of high P3 reduction to high DOC removal under aerobic conditions implies that biodegradation has an influential role in reduction of protein substances. However, slight differences between reductions of P1 and P2 suggest that adsorption could be the mechanism that dominated the reduction of these peaks.

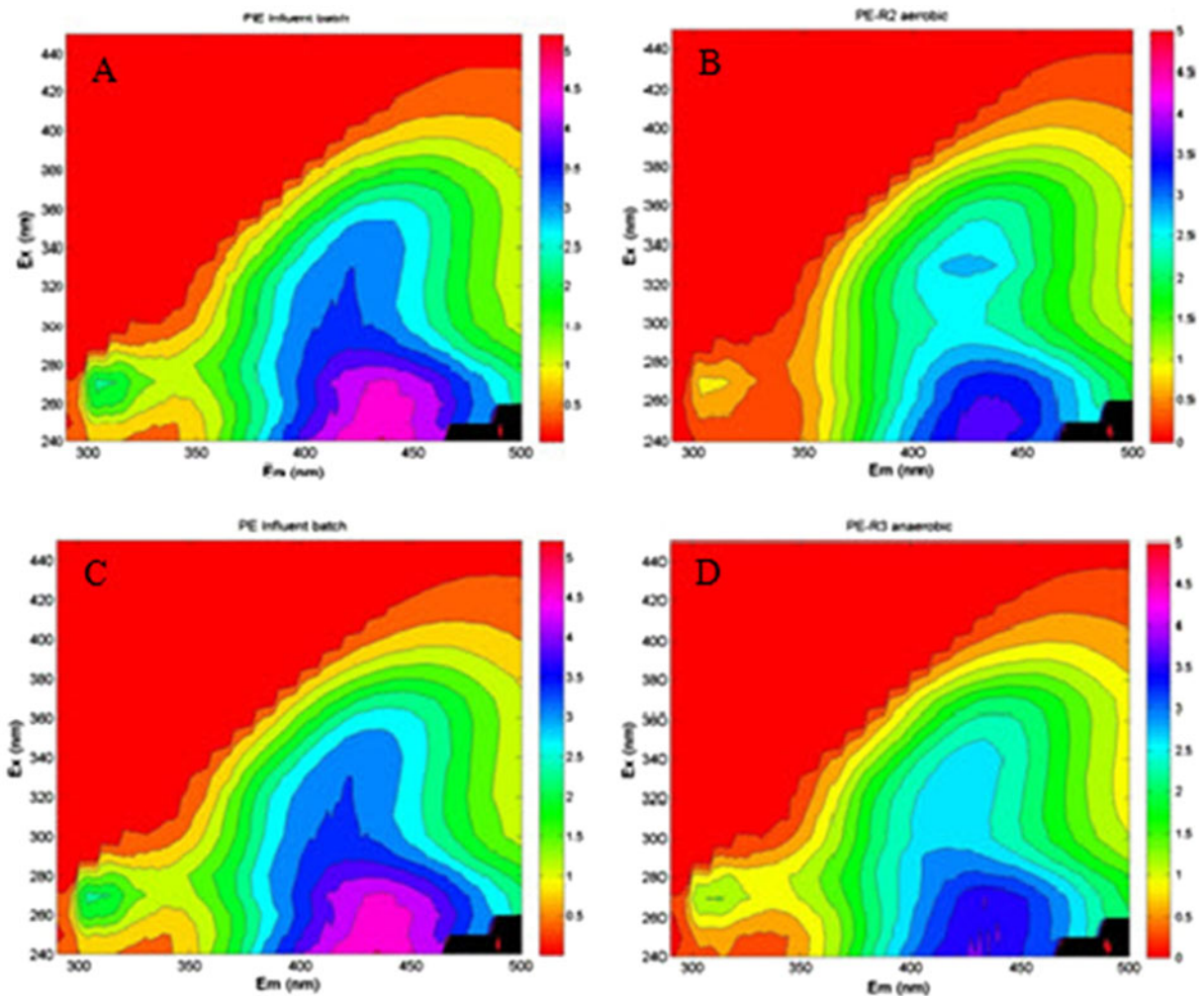


Fig. 8 F-EEM spectra of batch experiments conducted at different redox conditions: **a, c** influent; **b** effluent aerobic, **d** effluent anoxic (feed water: primary effluent, influent: day 0, effluent: day 5)

3.3.2 Ammonium-Nitrogen and Nitrate-Nitrogen

An average removal of 99.9 % $\text{NH}_4\text{-N}$ (influent = 31.7 ± 4.7 mg N/L) resulted in substantial increase in $\text{NO}_3\text{-N}$ concentration from 1.9 ± 0.1 mg N/L in influent samples to 36.0 ± 1.8 mg N/L in the effluent under aerobic conditions causing DO reduction from 8 to 2.1 ± 0.6 mg/L. However, $\text{NH}_4\text{-N}$ decreased by 12.1 % (31.7 ± 4.7 to 27.9 ± 1.8 mg N/L) under anoxic conditions causing $\text{NO}_3\text{-N}$ to increase from 1.9 ± 0.1 to 4.9 ± 1.1 mg N/L in influent and effluent, respectively. Furthermore, ORP values of 199 ± 9.8 and 27.4 ± 8.1 mV were measured under aerobic and anoxic conditions. According to Tebbutt (1982), aerobic reactions show ORP values of $> +200$ mV, while anaerobic reactions occur below $+50$ mV. A higher ($P < 0.0001$) decrease in $\text{NH}_4\text{-N}$ concentration in aerobic batch reactors in the presence of organic matter is ascribed to its nitrification to $\text{NO}_3\text{-N}$ by heterotrophic nitrifying bacteria due to the prevalence of high oxidation conditions manifested by the presence of high DO concentration and ORP in influent water samples. However, lower DO and ORP in effluent samples suggested the prevalence of anoxic conditions. Furthermore, the slight decrease observed in $\text{NH}_4\text{-N}$ concentration and corresponding increase in $\text{NO}_3\text{-N}$ under anoxic conditions imply that some nitrification took place. Such anoxic nitrification of ammonium has been reported by Sabumon (2007) who conducted batch studies to assess anaerobic ammonium removal. The author stipulated that anoxic oxidation of ammonium in the presence of organic matter by mixed bacteria cultures was a result of facultative nitrifiers, methanogenesis, and SO_4^{2-} reduction. Removal of $\text{NH}_4\text{-N}$ under anoxic conditions in this study could be due to ANAMMOX process since $\text{NO}_3\text{-N}$ concentration in the feed water was relatively low (1.9 ± 0.1 mg N/L) to support denitrification process. Measurements of SO_4^{2-} concentrations in influent (primary effluent) water and effluents from aerobic and anoxic batch reactors revealed significant increase in SO_4^{2-} concentration by 13.3 mg/L (86.5 ± 2.3 to 99.8 ± 3.1 mg/L) in aerobic reactors and decrease in anoxic reactors by 10.5 mg/L (86.5 ± 2.3 to 76.5 ± 3.1 mg/L). Since oxidation in the absence of oxygen is brought about by reduction of inorganic salts such as sulfate (Samorn 1996), reduction in SO_4^{2-} concentration in anoxic batch reactors indicated utilization of SO_4^{2-} reduction to promote anoxic $\text{NH}_4\text{-N}$ nitrification.

Furthermore, continuous measurement of DO in the batch reactor exhibited steady increase in DO level on day 4 and day 5 from 0.2 to 0.8 mg/L. The used silica sand was relatively free of iron and manganese oxides. An increase in sulfate concentration in aerobic batch reactors may be ascribed to the release of sulfate bound to organic matter due to degradation of the organic matter.

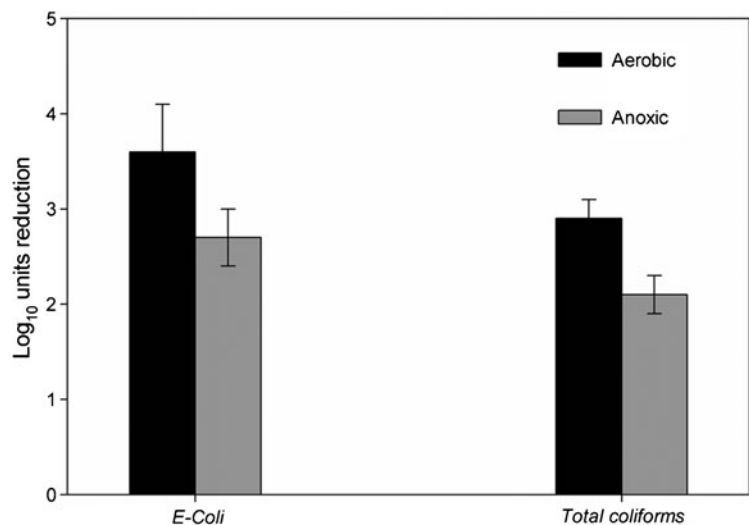
3.3.3 Phosphorus

To probe the impact of redox conditions on the removal of $\text{PO}_4\text{-P}$ in batch experiments with hydraulic retention time (HRT) of 5 days, a maximum MLR of 2.67 ± 0.0 mg was applied to batch reactors on day 0. Measurements of $\text{PO}_4\text{-P}$ at the effluent of batch reactors on day 5 showed no significant difference ($P = 0.237$) between removal in aerobic and anoxic batch experiments. A total removal of 32.2 ± 3.4 % (0.86 ± 0.09 mg) was achieved under aerobic conditions, while $\text{PO}_4\text{-P}$ was reduced by 26.5 ± 6.2 % (0.71 ± 0.16 mg) under anoxic conditions. Relatively high $\text{PO}_4\text{-P}$ removal under aerobic conditions could be attributed to increase in the degradation of previously sorbed materials and subsequently mineralization of the $\text{PO}_4\text{-P}$ attached on these materials.

3.3.4 *E. coli* and Total Coliforms Removal

E. coli and total coliforms were removed in laboratory-based batch experiments with 5-days hydraulic retention time under aerobic and anoxic conditions. Since bacteria survival in soil matrix ranges from a few weeks to a few months (Yavuz Corapcioglu and Haridas 1984), the removal of *E. coli* and total coliforms in batch reactors might be ascribed to adsorption of bacteria strains. *E. coli* were reduced by 3.6 \log_{10} units in aerobic batch reactors, while 2.7 \log_{10} units removal was attained in anoxic reactors. On the other hand, total coliforms were attenuated by 2.9 \log_{10} units and 2.1 \log_{10} units under aerobic and anoxic conditions, respectively. Organic matter competes with bacteria for adsorption sites resulting in less bacterial adsorption (Stevik 2004). High bacterial removal (Fig. 9) under aerobic conditions as compared to anoxic is attributed to high degradation of organic matter under aerobic conditions, predation, or die-off due to competition with heterotrophic microorganisms. However, low natural growth rates of *E. coli*

Fig. 9 Indicator microorganisms inactivation (\log_{10} units reduction) under different redox conditions in batch experiments using primary effluent (retention time = 5 days)



and *total coliforms* as compared to their extinction rates in the reactor might influence the removal rates in the system.

4 Conclusions

Assessment of the influence of temperature variation and redox conditions on the removal of bulk organic matter, nutrients, and pathogens led to the following conclusions:

- Removal of bulk organic matter in aerobic soil columns was significantly higher at high temperature. An increment of 10 % was noted with each 5 °C increases in temperature in the range 15 to 25 °C, while the lowest removal was obtained at 5 °C. This was due to increasing microbial activity manifested by an increase in SUVA values that exhibited a substantial increase in aliphatic substances removal with increase in temperature. Furthermore, reduction in F-EEM peaks shows that the protein-like peak was better reduced at high temperature.
- Ammonium-nitrogen removal at 15 °C was at least 10 % less than that at 20 and 25 °C. Nonetheless, experiments carried out at 5 °C revealed 8.8 % removal, suggesting that nitrifying bacteria were sensitive to low temperature levels.
- Low removal of *E. coli* and *total coliforms* at 5 °C compared to that at 15, 20, and 25 °C was influenced by competition between these pathogen

indicators with poorly removed organic matter and blockage of adsorption sites by EPS secreted by heterotrophic bacteria at low temperature. Inactivation of the pathogen indicators is assumed to have contributed to the removal.

- Aerobic operating conditions in laboratory-based soil column exhibited DOC removal of 15 % higher than that under anoxic conditions. However, this difference was only 8 % in aerobic and anoxic batch experiments.
- Ammonium-nitrogen concentration was attenuated above 99.9 % in aerobic batch reactors, while only 12.1 % was reduced in anoxic reactors.

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