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Organics and Nitrogen Removal from Wastewater Across a Plate of Entrapped Mixed Microbial Cells

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ABSTRACT

This work investigated the concurrent removal of organics and nitrogen from wastewater as it passed through a slab of immobilized activated sludge of different thickness. Removals of chemical oxygen demand (COD) by 90 % from feed of 300 mg L^{-1} and of ammonia nitrogen (NH_3-N) by 30 to 50 % from feed of 27 mg L^{-1} in the synthetic wastewater were achieved. Wastewater exited the entrapped mixed microbial cells (EMMC) bed of 0.01 m in depth after a hydraulic retention time of 8 h through the bed. Increasing the bed thickness by up to 5 folds resulted in no enhancement, indicating aerobic processes ceased within the bed depth. The removal of COD was by aerobic respiration and the removal of nitrogen by oxidation via nitrification, both occurring in the aerobic zone of the EMMC bed near the entrance surface. Denitrification occurred deeper into the anaerobic zone of the bed that removed nitrate, leaving behind <0.75 mg L^{-1} of nitrate in the emerging effluent. Apparent first-order rate constants were >0.29 /h and >0.045 /h for COD and NH_3-N removal, respectively.

Key words: *Organic and nitrogen removal, entrapped mixed microbial cells, nitrification, denitrification*

INTRODUCTION

Traditional activated sludge process (ASP) has been applied in wastewater treatment for decades. It consists of an aeration basin to biologically remove dissolved organics and a clarifier to remove the activated sludge, producing an acceptable effluent to the receiving water body. The advance of anaerobic-anoxic-oxic (A2O) process integrates the removal of BOD/COD and nitrogen compounds in anaerobic/anoxic/aerobic chambers. However, the A2O process requires recirculation of wastewater from an aerobic chamber to the anaerobic/anoxic chamber in order to allow denitrification while using available BOD as the carbon source. The A2O process requires highly skilled operation and precise control of dissolved oxygen (DO) in the reactor to ensure nitrification and denitrification processes for total nitrogen removal. The A2O treatment configurations were simplified by the entrapped mixed microbial cell (EMMC) system developed by *Yang et al.* (*1997*). The EMMC method achieved simultaneous removal of carbon and nitrogen from wastewater in a single-pass process as it provided an aerobic/anoxic/anaerobic environment in each EMMC carrier (*Yang et al. 1997; Yang et al. 2002a; Yang et al. 2003a*). The EMMC process offers advantages over conventional ASP and A2O, including a high density of biomass, low suspended solids in the effluent, a short start-up period, and relatively low operation and maintenance costs. In addition, the use of immobilized biomass enables long sludge retention time (SRT) that allows the development and adaptation of slow-growing bacteria to tackle complex organic compounds. In recent years, various types of wastewater have been investigated for the removal of COD and ammonia nitrogen using the EMMC technique. Examples include dilute swine wastewater (*Yang et al. 2003a*), domestic sewage (*Kim and Yang 2004*), and synthetic wastewater (*Yang et al. 2002a, Yang et al. 2003b; Qian et al. 2001*). *Ng et al.* (*2012*) applied the EMMC coupled with membrane to treat food and beverage wastewater (COD of 590 – 1350 mg L^{-1}) and achieved 93-98 % of COD removal. The EMMC process was also experimented for removal of pollutants, including phenol (*Yang et al. 1999*), odorous substances (*Yang et al. 2003b*), dimethyl sulfoxide (*Yang and Myint 2003*), and trimethylamine (*Chang et al. 2004*).

The EMMC medium simulates a local aerobic/anoxic/anaerobic environment akin to A2O, which can be found in natural soil environment. When soil is irrigated with gray-water, biological respiration depletes available DO in the inter space of soil aggregates, creating an anaerobic environment in the intra space of soil aggregates; this effectively creates an environment for the coupled nitrification/denitrification processes resulting from mass transport via convection and diffusion through the macro and micro soil spaces.

The researchers previously tested the EMMC process in a reactor packed with spherical bio-carriers, and demonstrated high efficiency of concurrent COD and ammonia nitrogen removals by 97 % (*Yang et al. 2003b; Kim and Yang 2004*) and 95 % (*Yang et al. 2002b, Zhu et al. 2011*), respectively. The physical characteristics such as porosity, pore size, bacteria density/distribution, and bed depth of bio-carriers were presumably important for

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treatment performance. While the granulation model for sequencing batch reactor (SBR) were well described for certain anoxic and anaerobic processes (*Jang et al., 2003*), aerobic conditions is yet to be demonstrated (*Morgenroth et al., 1997; Etterer and Wilderer, 2011*). These studies have shown external and internal mass transfer limitations as being vital in describing the spherical, immobilized biomass in a fixed bed reactor particularly for treatment at high oxygen uptake rate.

Although coupled organics and nitrogen removals have been well reported, the combined process of advection/diffusion/biological transformation through a flat slab of EMMC has not been investigated. We focused on this combined process utilizing a plate EMMC configuration for carbon and nitrogen removal. EMMC plates of various thicknesses (0.01, 0.025, and 0.05 m) were used, and substrate concentrations (BOD/COD, TN) were monitored for kinetic analysis. Though EMMC balls of various sizes were previously found effective for concurrent carbon and nitrogen removals, varying the thickness of the flat plate as investigated in this study allowed us to probe treatment effectiveness of a reduced depth of the entrapped biomass. As hydraulic and kinetic parameters have never been investigated for the plate EMMC system, these parameters as defined in this study would be useful for modeling more complex EMMC configurations.

EXPERIMENTAL METHODS

Plate EMMC and Reactor Operation

The box-shape reactor was built of Plexiglas of 0.33 m, 0.17 m, and 0.05 m in length, width, and depth, respectively (**Figure 1**). At the reactor bottom center was a out opening of 0.15 m length and 0.05 m in width; the opening was fitted with a rectangular plate EMMC of similar dimensions with an extended thickness of 0.01 m, 0.025 m, or 0.05 m and sealed against the reactor bottom opening with silicone. In the previous EMMC studies, we focused bio-balls of 0.05 m in diameter and we wanted to investigate if decreased medium thickness (i.e. less medium) may still support treatment objective. The wastewater dissolved oxygen (DO) of 4.3 – 4.8 mg L^{-1} percolated through the porous EMMC medium under the influence of 0.025 m of constant hydraulic head above the medium. The preparation of the plate EMMC followed procedures described in our previous work (*Wang et al. 2012; Ng et al. 2011; Ng et al. 2010; Yang et al. 2003a*). The activated sludge was obtained from a treatment plant for food processing wastewater. Three working reactors were operated in parallel.

For consistent feed, synthetic wastewater approximating 300 mg L^{-1} of COD was placed in the feed reservoir containing nutrients (in mg L^{-1}): sucrose (270), $(NH_4)_2SO_4$ (130), KH_2PO_4 (140), K_2HPO_4 (290), $MgSO_4{\cdot}7H_2O$ (21), $MnSO_4{\cdot}H_2O$ (2.7), $CaCl_2$ (3.8), and $FeCl_3{\cdot}6H_2O$ (0.14). It was continually recirculated between the reactor and the reservoir by a peristaltic pump to maintain the constant head of 0.025 m above the EMMC plate in the reactor. Air was sparged into the reactor liquid via 3 air stones at 4-5 L min^{-1}. Three reactors with EMMC plates were operated in parallel at the room temperature of 25 ± 3 ◦C. The effluents from the EMMC plates were analyzed to characterize hydraulic parameters and treatment efficiencies.

Chemical Analysis

Influent and effluent samples of the plate EMMC system were analyzed daily for total chemical oxygen demand

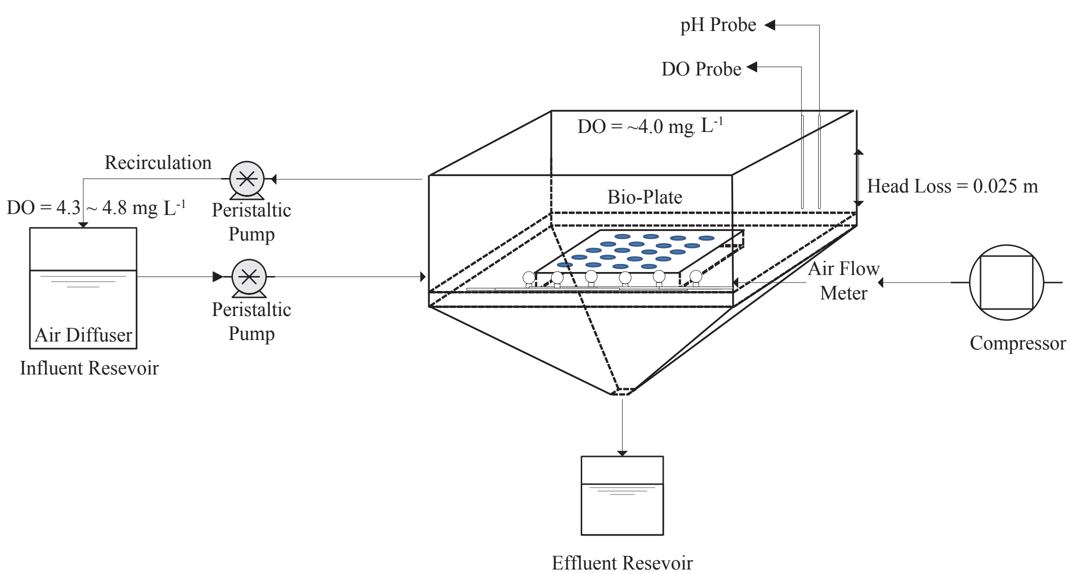

Figure 1. Experimental setup consisting of a reactor with the EMMC plate installed at the bottom, along with the air diffuser and reservoir.

(TCOD), soluble chemical oxygen demand (SCOD), ammonia nitrogen (NH_3-N), and nitrate nitrogen (NO_3^--N). DO and pH in the reactor were recorded daily with a portable dissolved oxygen/pH meter (HACH HQ20). COD was analyzed by HACH closed reflux colorimetric method with a spectrophotometer (HACH DR 2800) in the detection range of 20 - 1500 mg L^{-1}. NH_3-N was analyzed according to the colorimetric Nessler method (*Hach 2005*) at 0.02 – 2.50 mg L^{-1} detection range. NO_3^--N was analyzed according to the cadmium reduction method at 0.1 – 10.0 mg L^{-1} detection range.

RESULTS AND DISCUSSION

COD and NH3-N removal of bio-plate

The synthetic feed water containing 300 mg L^{-1} of COD and 26-28 mg L^{-1} of NH_3-N was fed into three reactors installed with EMMC plates of individual thickness of 0.01, 0.025, and 0.05 m; it percolated through the EMMC medium at 0.025 m of hydraulic head. There are removals of COD, NH_3-N, and residual NO_3^--N, respectively, in the effluent at HRT of 8 h (**Figures 2** and **3**). Based on effluent COD as shown in **Figure 2**, the removal of COD across the EMMC plate began with 45-55 % removal during initial days of operation, and it rose steadily over the next few days reaching at the end of three weeks of operation 80 % COD removal for the 0.025 m and 0.05 m plates and 90 % COD removal for the 0.01 m plate. The initial rise in removal was attributed to a period of acclimation of the EMMC to the feed. The steady-state removal appeared to have been established within a week.

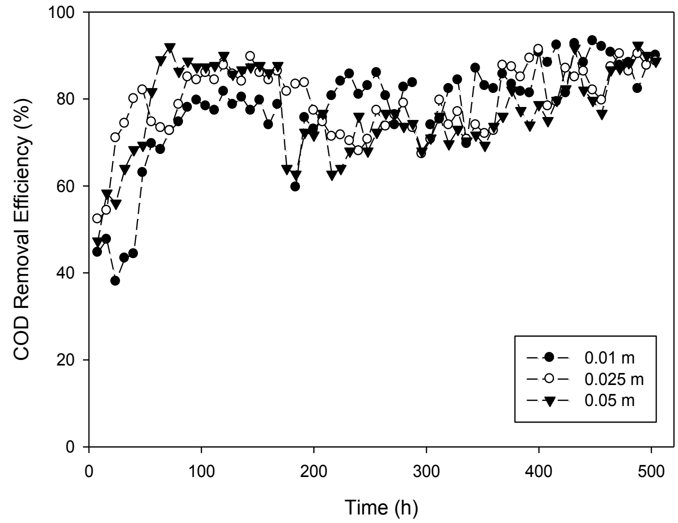

Figure 2. Removal of COD from stream flowing across EMMC plate over three weeks of operation.

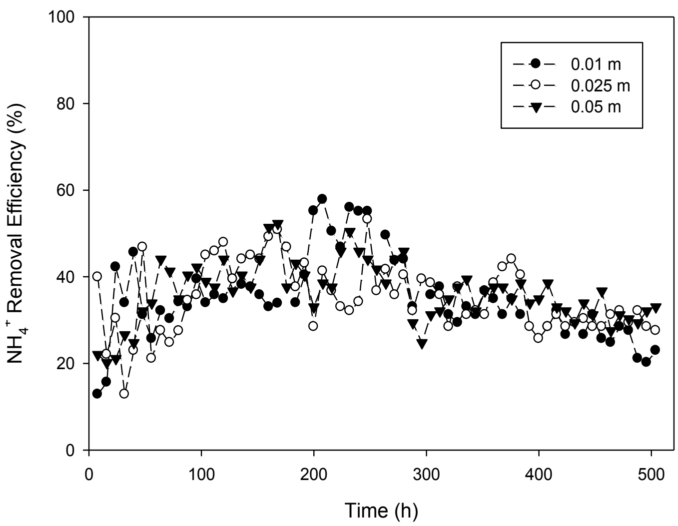

Figure 3. Removal of ammonia nitrogen from stream flowing across EMMC plate.

Based on effluent NH_3-N , the removal of NH_3-N across the EMMC plate began at about 20-30 % initially to about 30 % near the end of three weeks, although the removal had been varying much more widely between 30 and 55 % throughout the operation period with no apparent dependence on plate thickness (**Figure 3**). Likewise, residual NO_3^--N concentrations in the effluents varied widely between 0.2 and 0.75 mg L^{-1} throughout operation, again with no apparent dependence on plate thickness. The significant removal of NH_3-N (>30 % from 34 mg L^{-1} of influent NH_3-N) with low levels of effluent NO_3^--N (<0.74 mg L^{-1}) indicated a strong likelihood of denitrification being a significant mechanism of nitrogen removal.

The occurrence of denitrification requires an anaerobic environment. While COD was removed by 90 % from the stream as it exited the EMMC plate, the DO in the collected effluent was about 1.3-2.0 mg L^{-1}, indicating a substantial decrease from the influent level of >4.3 mg L^{-1} but not complete depletion. This was inconsistent with an anaerobic environment in the EMMC bed for denitrification; it was further inconsistent with the 90 % reduction of COD from an influent sucrose concentration of 300 mg L^{-1}, which would have demanded an approximately equal mass of DO for complete mineralization. The inconsistency was explained by sufficient replenishment of DO for biomass near the plate's entrance surface to support biodegradation of organics as well as nitrification of ammonium nitrogen. However, upon further transport into the bed, the environment became anoxic/anaerobic that enabled denitrification to occur. The substantial presence of DO in the collected effluent was attributed to leakage of atmospheric oxygen into the effluent sample during collection and handling of time span typically >8 h. It was also conceivable that short-circuiting channeling might have occurred in the EMMC plate that resulted in the residual DO and COD.

The thickness of EMMC plate has not significantly influenced treatment outcomes (**Figures 2** to **4**). These suggest that the aerobic environment at or near the surface of the EMMC plate was sufficient for the aerobic microorganisms to remove the carbonaceous contents, enabling significant removal of the feed COD. A sufficient thickness of the aerobic zone needed for effective COD removal was apparently met by the thinnest plate of 0.01 m used in the study; thus the additional thickness in the 0.025 m and 0.05 m plates resulted in no improved outcomes. This was consistent with our previous study that found EMMC plate being capable of providing an aerobic environment to sustain microbial activities leading to biodegradation of organic matters (*Ng et al. 2010*). In their study of microbial activities in soil, *Chen et al.* (*2001*) reported the distribution of nitrifying microorganisms across the soil aggregates and active nitrification process occurring at the surface of soil aggregates. The present study confirms that the removal of ammonia nitrogen (nitrification) has not been enhanced by plate thickness (0.01, 0.025, and 0.05 m) because nitrification process requires aerobic condition that is available only near the plate surface. As pointed out by *Arie et al.* (*2005*), microbial consumption and diffusion limitation of DO acted to hinder nitrification in the inner bed of immobilized microbial cells.

Bakti and Dick (*1992*)*; Yang et al.* (*1997*)*; Pochana and Keller* (*1999*)*; Holman and Wareham* (*2005*) postulated that denitrification took place in the inner part of sludge flocs or biofilm even under highly aerated environment because of a very steep DO gradient within the flocs. The explanation was supported by *Jang et al.* (*2003*) who found ammonia-oxidizing bacteria being primarily in the upper and middle layers of the granule and the nitrification process being significantly influenced by the granular depth that the DO had to diffuse through.

Effluent NO_3^--N concentrations were relatively steady without being influenced by the EMMC plate thickness (**Figure 4**). The nitrate ion was produced via nitrification within the EMMC plate and the process weakened as

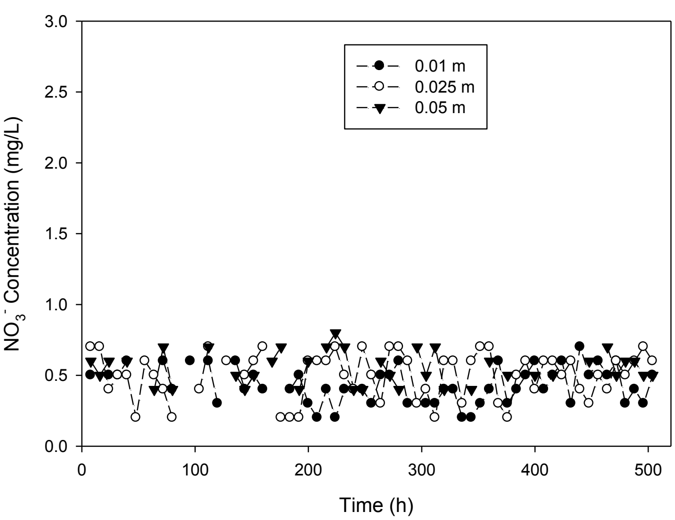

Figure 4. Residual nitrate nitrogen in effluent of EMMC plate during three weeks of operation.

DO decreased with increasing depth into the bed. The nitrification results were consistent with those of COD removal via aerobic heterotrophic bacteria that resided in the aerobic zone near the entrance surface of the EMMC plate, beyond which zone the EMMC bed became anoxic and was not conducive to nitrification. As a result, NO_3^--N was removed via denitrification in the anoxic/anaerobic zone (*Jang et al. 2003*). Thus, microbial activities of the EMMC plate enabled the coupled removal of COD via the aerobic process and of ammonia nitrogen via the concerted nitrification/denitrification processes.

Hydraulic conductivity of the EMMC bed

The flow of water through the EMMC plate can be described by Darcy's law for one-dimensional flow through porous media:

$$Q = AK\frac{(\Delta h)}{L} \qquad (1)$$

Where Q = volumetric flowrate ($m^3\ h^{-1}$), A = EMMC plate area (m^2), K = hydraulic conductivity ($m\ h^{-1}$), Δh = hydraulic head (m), and L = EMMC bed thickness (m).

Volumetric flow data collected using a hydraulic head of 0.025 m above the EMMC bed (water depth) was used to determine the bed's hydraulic conductivity at $6.9 \times 10^{-8}\ m\ s^{-1}$, as shown in **Figure 5**. The measured hydraulic conductivity did not vary significantly with plate thickness (0.01, 0.025, and 0.05 m), corresponding to flow through the porous bed at the superficial velocity of $1.7 \times 10^{-7}\ m\ s^{-1}$ (or $3.4 \times 10^{-7}\ m\ s^{-1}$ in the pore space assuming porosity of 0.5). Thus, the HRT was 8 h for bed depths of 0.01 m, 0.025 m, and 0.05 m, respectively.

Roles of advection, diffusion, and biodegradation in the EMMC plate

The treatment of COD and NH_3-N in wastewater as it passes through the EMMC plate over an extended period can be approximated by a one-dimensional diffusion/advection/reaction equation at steady state:

$$D\frac{d^2C}{dx^2} - v_x\frac{dC}{dx} - K_rC = 0 \qquad (2)$$

Where D = diffusivity ($m^2\ h^{-1}$), vx = advection velocity ($m\ h^{-1}$), kr = 1st-order rate constant of degradation (1/h), C = concentration ($mg\ L^{-1}$), and x = bed depth (m). The general solution for Eq. 2 is:

$$c(x) = A_1 e^{\lambda_1 x} + A_2 e^{\lambda_2 x} \qquad (3)$$

Where λα (1/m) are the eigenvalues of Eq. 1, with given as (*Schwarzenback et al. 2003*):

$$\lambda_\alpha = \frac{1}{2D}\left[v_x \pm \{v_x + 4Dk_r\}^{\frac{1}{2}}\right], \alpha = 1, 2 \quad (4)$$

A relevant non-dimensional number in the eigenvalues, the Peclet Number (Pe):

$$Pe = \frac{|v_x|L}{D} \quad (5)$$

measures the relative pace of transport by advection and diffusion. When Pe is >>1, advection exceeds diffusion; such is particularly the case when the distance L is significantly large. With flow velocity vx = 3.4 x 10^{-7} m s^{-1}, thickness L = 0.01 m, and molecular diffusivity D estimated in the order of 10^{-9} m^2 s^{-1} (for chemical compounds in water), the Pe amounts to be 3.4 (i.e. Pe is >1). This shows advection is more important in our EMMC plate even at 0.01 m thickness. Alternatively, the values of D and vx are compared in a ratio that defines L_{crit} (*Schwarzenback et al. 2003*):

$$L_{crit} = \frac{D}{|v_x|} \quad (6)$$

The L_{crit} represents a critical distance into a slab at which advection and diffusion are equally important but beyond which the role of diffusion can be neglected. Our experimental conditions suggest L_{crit} ~ 0.003 m. This distance is smaller than the EMMC plate thickness (smallest in this study at 0.01 m). Thus the role of diffusion can be neglected in the reactive transport equation, rewritten as:

$$v_x \frac{dC}{dx} + k_r C = 0 \quad (7)$$

with the solution:

$$C = C_0 e^{-\frac{k_r}{v_x}x} = C_0 e^{-k_r t} \quad ln\frac{c}{c_0} = -k_r t \quad (8)$$

Where C_0 is the influent concentration and x/v_x = t the HRT for flow across the EMMC plate.

Assuming a uniform downward flow of water carrying COD and NH_3-N to be removed via first-order kinetics over the HRT of 8 h through the EMMC bed, the apparent first order rate constants were obtained at 0.29 h for COD removal (based on 90 % removal in 8 h) and 0.045 h for nitrification (based on 30 % removal in 8 h). However, increasing thickness (as with 0.025 and 0.05 m in bed depth) resulted in no increased removals. It was likely that, due to other limiting factors such as DO, substrate removal occurred but ceased long before reaching the plate bottom exit (i.e. actual reaction time being shorter than HRT). Thus, these values represent the minimum first-order removal rate constants of the processes, which were subjected to limiting factors such as availability of DO as observed by *Kremen et al.* (*2005*).

CONCLUSIONS

As the wastewater migrated across a porous EMMC plate, concurrent removals of COD by 90 % and ammonium nitrogen by 30 % from a synthetic wastewater containing 300 mg L^{-1} and 27 mg L^{-1} of the two substrates, respectively, were achieved, leaving 0.2-0.75 mg L^{-1} of residual nitrate nitrogen in the effluent. The concurrent removal of COD by aerobic respiration and oxidation of nitrogen to nitrate via nitrification occurred in the aerobic zone of the EMMC

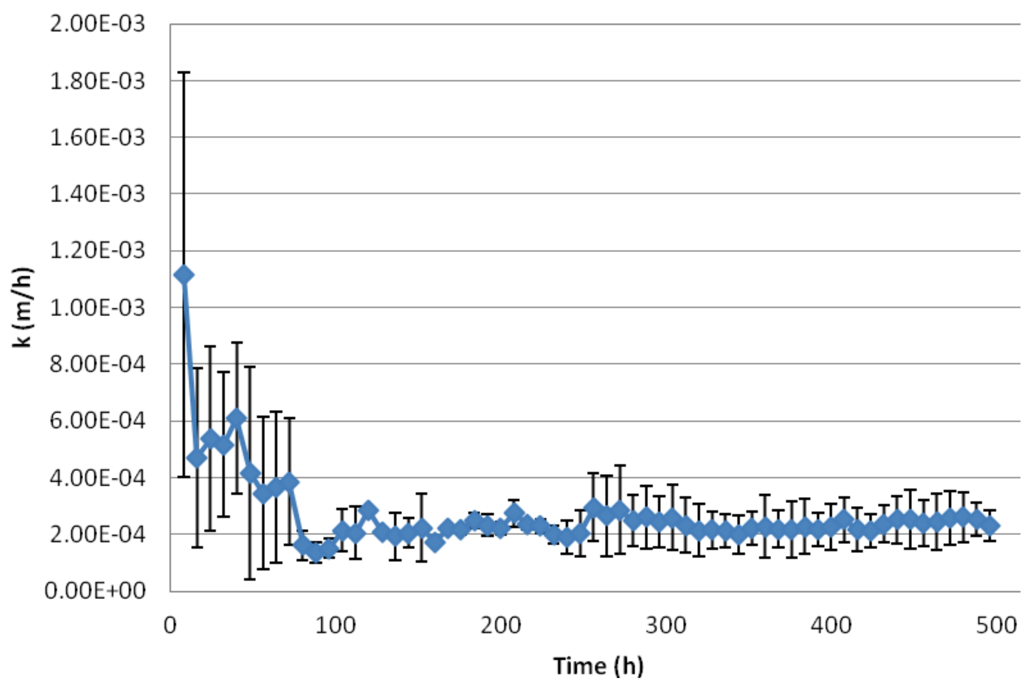

Figure 5. Mean hydraulic conductivity measured for the EMMC plates of different thicknesses (0.01, 0.025, and 0.05 m).

bed near the entrance surface. Upon further migration into the anoxic/anaerobic zone, denitrification occurred that removed nitrate, resulting in residual nitrate in the effluent. The removal efficiency was not influenced by increase of plate thickness from 0.01 to 0.05 m, which suggested that while denitrification was substantial in nitrate removal nitrification was likely limited by availability of DO within the EMMC bed. Based on kinetics obtained from the 0.01-m plate, the minimum apparent first-order rate constants were estimated at 0.29 h and 0.045 h for COD and NH_3-N removal, respectively.

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