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"KEY ELEMENTS FOR A SUSTAINABLE WORLD: ENERGY, WATER AND CLIMATE CHANGE"

Evaluation of the Pollutant Removal Mechanisms of a Reed Bed System: Biochemical Parameters

A. M. Islam ^a, T. Tudor ^b, M. Bates ^c

a. School of Applied Sciences, University of Northampton,
ashiq.islam@northampton.ac.uk

b. School of Applied Sciences, University of Northampton,
terry.tudor@northampton.ac.uk

c. School of Applied Sciences, University of Northampton,
margaret.bates@northampton.ac.uk

Abstract

This study was aimed to evaluate the key biochemical mechanisms that occur within a reed bed system or constructed wetland during the treatment of landfill leachate. Soil respirations, dehydrogenase activities and urease activities within a horizontal subsurface flow reed bed were extensively examined to determine these mechanisms. Variations in biochemical parameters because of change in space and leachate applications were investigated. Correlations among the biochemical parameters and between biochemical parameters and pollutant removal efficiency were undertaken.

No biochemical activities showed any horizontal variations across the reed bed. For both preloading and during-loading conditions, soil respirations and dehydrogenase activities did not have any vertical variations whereas urease activities at 5cm depth were significantly higher ($P < 0.001$) than those at 50cm and 90cm depth. When during-loading conditions were compared with preloading conditions, soil respirations showed no variation at any depth, whereas significant reductions were observed at 50cm ($P = 0.034$) for dehydrogenase activities and at 50cm ($P = 0.018$) and 90cm ($P = 0.004$) depths for urease activities. A modest correlations ($r = 0.474$, $P = 0.023$) between soil respirations and dehydrogenase activities was observed. A strong correlation ($r = 0.777$, $P < 0.001$) was found between dehydrogenase activities and urease activities. No correlation existed between the biochemical parameters in the reed bed soil and the pollutant removal efficiencies for chemical oxygen demand (COD) or total kjeldahl nitrogen (TKN). Aerobic microbial activity showed equal potential for the degradation of pollutants in the wastewater which suggests the importance of creating suitable conditions for aerobic microbes within the root zone in the reed bed. Significant reduction of total microbial activities at the middle depth suggests that it was influenced more by heavy metals due to more exposure to leachate. The top layer reed bed soil needs to be properly utilised to maximise the reduction of nitrogenous pollutants from leachate. A biochemical activity can be utilised to predict another biochemical activity but not the removal of COD and TKN.

Keywords: Reed beds, leachate, respiration, dehydrogenase, urease

1 Introduction

Reed bed systems started to be utilised for wide varieties of wastewater treatment more than three decades ago. It has been used for the treatment of landfill leachate, storm water runoff, municipal, agricultural, industrial wastewater etc.

Reed bed systems are used for secondary or tertiary treatments of wastewaters (Vymazal, 2009). Reed beds are low-cost, easy to construct, operate and maintain and have ecological values (Chen et al., 2009; Chen et al., 2009a; Nivala et al., 2007; Tsihrintzis et al., 2008). They have been proved to be efficient in reducing different undesired constituents, such as BOD, COD, NH₄-N, PO₄-P, heavy metals from wastewaters. Biochemical transformations, adsorptions, precipitations, volatilisation and plant uptake of pollutants are the main pollutant removal mechanism in a reed bed system (Gui et al., 2007; Imfeld et al., 2009; Stottmeister, 2003; Ugurlu et al., 2009; Vymazal, 2009a). Pollutant transformations, removals and transports in reed bed systems have been a matter of great interest to the scientific community (Vymazal, 2009).

Because of toxicity, landfill leachate is classified as a problematic wastewater and dangerous source of pollution for the environment (Kjeldsen et al., 2002). The EU Water Framework Directive (2000/60/EC) set a number of deadlines for the European Union states to meet. The European Integrated Pollution Prevention and Control (96/61/EC) made it an obligation for all the member states to implement the best available techniques (BAT) by the 30th October, 2007. Different environmental bodies in the UK, such as Environment Agency, Scottish Environmental Protection Agency have adopted a technical guidance (Sector Guidance Note IPPC S5.03) where reed beds were included as one of the best available techniques for treating landfill leachate. There have not been extensive studies on the biochemical mechanisms in the horizontal subsurface used for removal of landfill leachate. In this study, biochemical characteristics of a horizontal subsurface flow reed bed were examined for the treatment of landfill leachate as the reed bed systems have become an important tools for the treatment of landfill leachate in the UK. Soil respiration, dehydrogenase activity and urease activity were utilised as biochemical responses of the of the reed bed soil substrate to prior to and during application, as they have been recognised as important biochemical indicators in soil (Gagnon et al., 2007; Hallberg et al., 2005; Liang et al., 2003; Zhou et al., 2005). This study included determination the changes in the biochemical parameters, correlations within the biochemical parameters and between biochemical parameters and removal of COD and TKN. The purpose of the study was to have a good understanding of the efficient evaluation and utilisation of the biochemical activities in a horizontal subsurface flow reed bed system for treating landfill leachate.

2 Materials and methods

2.1 Site description and sampling

The experimental horizontal subsurface flow reed beds were installed in The University of Northampton (Park Campus), UK. There were nine parallelly connected reed beds, each of 5.4m length, 2.0m width and 1.0m depth. They were planted with *Phragmites australis* (common reed). For this study one of the nine reed beds was selected for the experimental works. The experimental reed bed was divided into six equal blocks, from each blocks one point was randomly selected (Fig. 1) and from each point soil samples were collected from three different depths (5, 50, 90cm) for preloading and during-loading conditions.

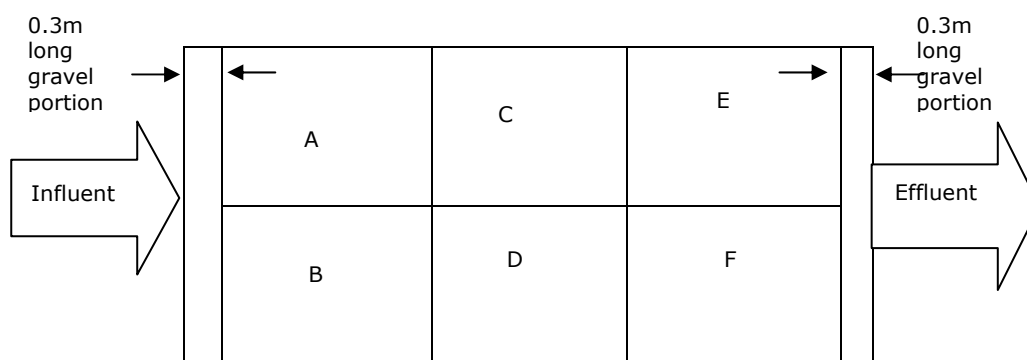


Fig.1. Plan of the experimental reed bed (Top view, 5.4m x 2.0m)

Sampling was done within 4.8m x 2.0m area of the non-gravel portion of the reed bed. The depths were selected based primarily on the total depth of the reed bed (1m), expected differences of availability of oxygen (due to changes in diffusion and root zone) and soil structure (due to compaction and preferential flow of wastewater).

Soil samples were collected with a soil auger when the reed bed was not loaded with leachate (preloading conditions) and on the 14th day of leachate application (during-loading conditions) to determine soil respiration, dehydrogenase activity and urease activity. The reed bed used to be loaded with leachate in every few days during the during-loading conditions. Effluent samples were collected to determine pH, COD and TKN (total kjeldahl nitrogen) on the 7th, 14th and 21st day of the leachate applications.

2.2 Preparing the synthetic leachate

Parameters of a landfill leachate change significantly from landfill to landfill. Even within a landfill there are significant changes due to spatial, temporal variations and changes in a waste type and amount (Christensen et al., 2004; Robinson et al., 2005). In this study, synthetic leachate was used to have better control over the availability and concentrations of the target parameters. To develop the synthetic leachate, sugar (200mg/L), peptone (200mg/L), urea (50mg/L) and potassium hydrogen phosphate (50mg/L) were used as sources of saccharose, protein, nitrogen, phosphorous respectively (Tsihrintzis et al., 2007). Maximum UK concentrations in landfill leachate were chosen for heavy metals (0.03, 0.56, 0.16, 0.28, 0.67mg/L for Cd, Cr, Cu, Pb and Zn respectively) (Christensen et al., 2004). All materials were mixed with tap water and the synthetic leachate was applied on the reed bed immediately after preparation. The pH, COD and TKN of the synthetic leachate were 6.9, 288mg O₂/L and 51.59mg NH₃-N/L respectively.

2.3 Soil respiration, dehydrogenase activity and urease activity in reed bed soil

The non-buffered method described in Alef (1998) was followed to determine soil respiration. In a closed jar, 25mL NaOH solution was pipetted. A beaker with 30g sieved soil (<2.0mm) was placed in an 1-L jar and the top was sealed. After 3 days of incubation at 20°C, the NaOH solution was titrated with standard HCl in the jar. A control was carried out without soil sample.

Dehydrogenase activity was determined according to the TTC method in Thalmann (1968). In a test tube, 5g sieved soil (<2.0mm) was mixed with

triphenyltetrazolium chloride (TTC), stoppered and incubated for 24h at 30°C. Then 40mL acetone was to the tube, shaken at room temperature for 2h. The soil suspension was filtered and optical density of the supernatant was measured against the blank at 546nm in a spectrophotometer to determine Triphenyl formazan (TPF). The blank was carried out without soil sample.

Urease activity was determined according to Kandeler et al. (1988). In an Erlenmeyer flask 5g sieved soil (<2.0mm) and 2.5mL urea solution were placed. The flask was stoppered and incubated for 2h at 37°C. Then 50mL of KCl solution was added and the flask was shaken for 30min. The soil suspension was filtered and 1mL of filtrate was mixed with 9mL distilled water, 5mL Na-salicylate/NaOH solution and 2mL sodium dichloroisocyanurate solution in a different Erlenmeyer flask. The solution was allowed to stand for 30min before the optical density against the blank at 690nm was measured to determine ammonia. A control without urea was carried out.

2.4 COD and TKN in leachate and effluent

COD was determined according to Closed Reflux, Titrimetric Method (APHA, 1998). The sample (2.5mL) was mixed with 1.5mL standard potassium dichromate and 3.5mL sulfuric acid in a tube and was digested in a block digester at 150°C for 2h. A blank was prepared with distilled water instead of sample. The digested sample was allowed to cool and was titrated with standard ferrous ammonium sulphate (0.1M) solution using ferroin indicator. A blank was carried out using distilled water instead of sample.

Macro-Kjeldahl, Titrimetric Method (APHA, 1998) was followed to determine TKN. The sample (50mL) was mixed with 50mL digestion solution (mixture of sulphuric acid, potassium sulphate and copper sulphate). The sample was allowed to cool, diluted to 300mL. Sodium hydroxide-sodium thiosulphate reagent (50mL) was added to the sample and distilled. In indicating boric acid mixed with methyl red and methyl blue indicator, 250mL distillate was absorbed. The ammonia in the distillate was titrated with standard sulphuric acid (0.02N). A blank was carried out with distilled water instead of the sample.

2.5 Statistical analysis

Statistical software SPSS 11.5 was used for the statistical analyses of the experimental data. The normality of distribution was tested by means of the Kolmogorov-Smirnov test of normality ($\alpha=0.05$). Significant differences were then assessed using ANOVA for normally distributed data, while the non-parametric Wilcoxon rank test was used to assess significant difference ($P<0.05$) for any data that were not normally distributed. Pearson correlation coefficients will be determined between the parameters (Tack et al., 2007).

3 Results

3.1 Spatial variations

Statistical analyses show no significant horizontal difference for any of the parameters (see section 3.2, 3.3, 3.4). But for a few cases, there were differences between the depths for an individual loading condition (see section 3.4) and/or due to changes in loading conditions (see section 3.3, 3.4).

3.2 Soil respiration

No significant spatial variation for soil respiration was found in any individual loading condition. Moreover, at any depth, there was no significant difference between preloading and during-loading conditions. Fig. 2 shows the trend of soil respirations at the different depths within the experimental reed bed.

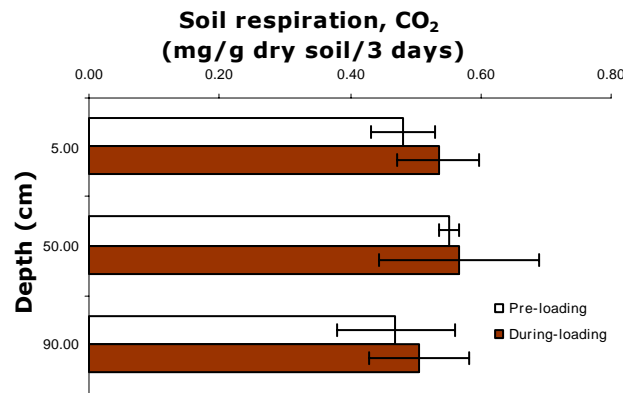


Fig. 2. Soil respirations across the depths

3.3 Dehydrogenase activity

Dehydrogenase activities showed no significant horizontal or vertical differences in any individual loading condition. At 50cm depth, dehydrogenase activities were significantly lower ($P=0.034$) in during-loading condition than preloading condition. Fig. 3 shows dehydrogenase activities at different depths within the experimental reed bed.

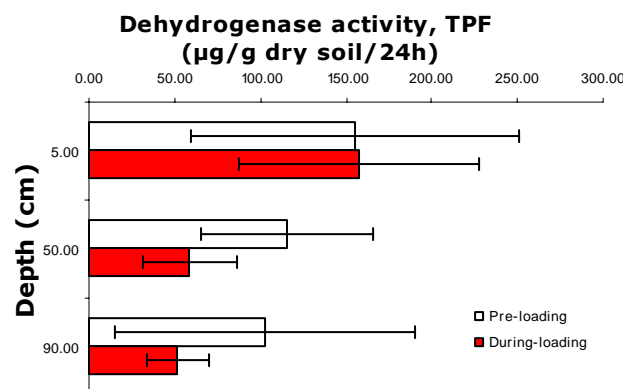


Fig. 3. Dehydrogenase activities across the depths

3.4 Urease activity

In both loading conditions, urease activities at 5cm depth were significantly higher ($P<0.001$) than those at 50cm and 90cm depths. Significantly lower urease activities were found at 50cm ($P=0.018$) and 90cm ($P=0.004$) depths in during-loading condition than preloading condition.

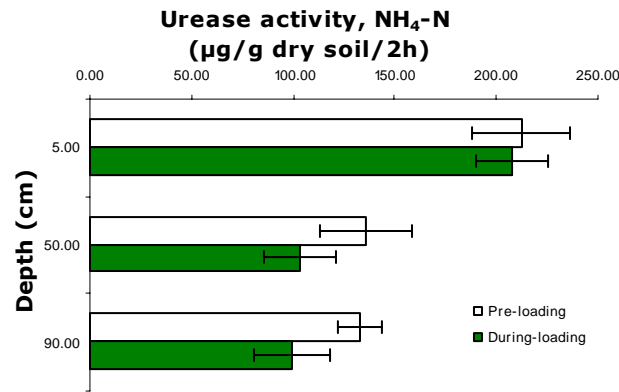


Fig. 4. Urease activities across the depths

3.5 Correlations

A modest correlation ($r= 0.474$, $P=0.023$) between soil respirations and dehydrogenase activities was observed. A strong correlation ($r=0.777$, $P<0.001$) was found between dehydrogenase activities and urease activities. No correlation existed between soil respirations, dehydrogenase activities and urease activities in the reed bed soil and the pollutant removal efficiencies for COD or TKN.

4 Discussion

4.1 Composite sampling is a viable option

Trends of spatial variations in the reed bed soil suggests that in a horizontal subsurface reed bed system, (1) vertical variations rather than horizontal variations should be the main focus, and (2) soil samples in each depth can be composited to get the representative values for the parameters. In a horizontal reed bed, the two far end sides are constructed with larger gravels (Fig. 1). The few centimeters at the bottom consist of sands which are placed on the impervious polythene. The rest (where the samplings were conducted) makes up the most of the reed bed and is constructed with normal soil which is mixture of sand, clay, silt, smaller gravels and soil organic matter. This soil is normally brought from same location or source. Gradually, the changes in soil layers in reed bed take place due to variations in compactions, root zone, biochemical activities and preferential flow of water or wastewater through the reed bed. These phenomena cause more vertical variations than horizontal variations. So, using composite samples for each depth can facilitate time, energy and cost-efficient evaluation or research for reed beds characteristics and performance.

4.2 No variations in the potential for aerobic microbial activity

No significant variations in the soil respiration means at any depth aerobic microbial degradation can become equally active provided creation of oxic condition. In the experimental reed bed system, *Phragmite australis* root zone could extend as far as 90cm deep which could increase the hydraulic conductivity and oxygen release in the root zone. Oxic-anoxic interfaces are changed dynamically in the constructed wetlands by fluctuations of water level, oxygen diffusion/advection through the water column and soil, and active oxygen transports throughout the rhizosphere via plant tissues (D'Angelo, 2002). Plants have highly significant affect on respiration (Singh et al., 2009). So, the utilisation of plants like *Phragmite australis* with extensive root zones can be a good practice in reed bed constructions.

4.3 Significant changes in overall microbial activities

As dehydrogenase activity is an intracellular process that occurs in every proper microbial cell, it can be indicative of the overall biochemical activity (Nannipieri et al., 1990; Tam, 1998). Dehydrogenase activity is also linked to C cycle of the pollutants (Hernández et al., 2001) and heavy metals have inhibitory effects on it (Sauvé et al., 2008). During the application of the leachate in the experimental reed bed, from time to time the leachate used to evaporate from the topmost layer and made the layer unsaturated. The lowest depth might have been subjected to less contact with leachate due to compaction. The middle depth was always subjected to leachate and hence, was exposed to heavy metals more than the topmost and lowest portion of reed bed. This might have caused the significant reduction of total microbial activities in the reed bed system.

4.4 Utilisation of total reed bed soil substrate

The enzymes urease exist both in intra and extracellular location (Nannipieri et al., 2003; Tabatabai et al., 1999). The extracellular enzymes have different characteristics than the intracellular enzymes and are not affected by inhibition or stimulation of microbial activities. Moreover, some extracellular enzymes may be immobilised on soil organic and mineral substances due to adsorption and chemical binding. These immobilised or stable enzymes are less affected by changes of factors (such as loading condition) than the free enzymes (Bremner et al., 1978; Shaw et al., 2009). Urease activity contributes to N cycle of the pollutants. Presence of sufficient mineral nitrogen significantly reduces urease activity due to the reduction nitrogen demand in the soil. Whease organic substances can immobilise the extracellular enzyme (Hernández et al., 2001). Because of the hydrolyses of nitrogenous organic pollutants in the leachate, especially urea for 14 days, resulted in increased mineral nitrogen in the reed bed. Inhibitory effects of heavy metals reduced the intracellular urease activity. Organic substances in the leachate immobilised extracellular urease enzymes. But the significant reduction of urease activities in the during-loading condition suggests that the inhibitory effects of increased mineral nitrogen and heavy metals were much more than the stimulatory effects due to stabilisation of enzymes. The extracellular enzyme activity decreases significantly with depth (Baldrian et al., 2008) and same was demonstrated by the experimental results. So, for treating wastewaters which contain nitrogenous pollutants, such as leachate, its will be a good practise to make the best use of the top layer of reed bed soil substrate. Inundation of reed bed with leachate and recirculation of the effluent can increase the efficiency of nitrogenous pollutant removal.

4.5 Correlations as indicators

Liang et al. (2003) examined the correlations of urease activity with pollutant removal efficiencies for different parameters in an vertical subsurface flow reed bed system and observed strong correlation for only TKN. Correlations among the three biochemical parameters can be utilised to predict one of the parameters which has not been determined. Such predictions can be made qualitatively, if not quantitatively. Again, the lack of correlations between biochemical parameters in soil and pollutant removal efficiencies for COD and TKN means that using these biochemical parameters are not a reliable option for predicting removal efficiencies for COD and TKN.

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