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Effects of livestock wastewater variety and disinfectants on the performance of constructed wetlands in organic matters and nitrogen removal

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Abstract

Background, aim and scope Treatment performance of constructed wetlands (CWs) is largely dependent on the characteristics of the wastewater. Although livestock wastewater is readily biodegradable in general, its variety in biodegradability can still be significant in practice. In addition, it is a common practice to periodically use disinfectants in livestock activities for health concerns. Obviously, the residual of the disinfectants in livestock wastewater may have serious inhibitory effect on the microbial activities during wastewater treatment. Thus, the main objective of this study was to examine the variety of livestock wastewater in biodegradability and its effect on the performance of a pilot scale tidal flow CWs (TFCWs) in organic matter and nitrogen removal. Furthermore, investigation of the potential inhibition of the chosen disinfectants on organic matter biodegradation and nitrification was another aim of this study.

Materials and methods The TFCWs system consisted of four-stage down flow reed beds with a hydraulic loading rate of 0.29 m³/m²·d. Long term stored livestock wastewater and fresh livestock wastewater were used, respectively, as feed to the system in different periods. Meanwhile, batch aeration tests were carried out to investigate the difference in biodegradation of the two types of wastewaters. Inhibitions of two types of disinfectants, namely UNIPRED and HYPROCLOREDD, on microbial activities were investigated in laboratory batch tests, with dosage of from 0.05% to 0.5%.

Results With fresh livestock wastewater, removal efficiencies of up to 93% and 94% could be achieved with average of 73% and 64% for COD and TN, respectively. The performance deteriorated when the system was fed with long-term stored wastewater. In the batch tests, the long time stored wastewater was characterized as non-biodegradable or at least very slowly biodegradable, while the fresh wastewater was readily biodegradable. UNIPRED showed very strong inhibition on both heterotrophic organisms and nitrifiers. Tested inhibition started from content of 0.05%, which is 1/10 of the recommended usage rate. Inhibitory effect of HYPROCLOREDD on COD degradation started from 0.1% and complete inhibition occurred from content of 0.3%, while significant inhibition on nitrification started from 0.1%.

Conclusions Livestock wastewater could vary significantly in biodegradability and it may turn to be non-biodegradable after a long-term storage. The variety of the livestock wastewater has a decisive influence on the performance of the CWs system, especially in TN elimination. In addition, the application of disinfectants UNIPRED and HYPROCLOREDD may cause serious inhibition on microbial activities and subsequent system failure.

Keywords Biodegradability, Constructed wetlands, Disinfectant, Livestock wastewater, Nitrogen

1 Background, aim and scope

Constructed wetlands (CWs) as a low-cost technology is becoming a popular alternative for livestock wastewater treatment (Cronk 1996; Hunt et al. 2001; Mankin and Ikenberry 2004; Harringtona and McInnesb 2009; Lee et al. 2010). With typical configurations (surface CWs and subsurface CWs), average removal efficiencies of 65%, 53%, 48%, 42% and 42% were obtained for BOD₅, TSS, NH₄-N, TN and TP, respectively, were reported for livestock wastewater treatment (Knight et al. 2000). More significantly, to overcome wastewater distribution problem and the poor oxygen transfer rate, a so called ‘tidal flow’ CWs (TFCWs) was proposed and developed over the last two decades (Green et al. 1997; Sun et al. 1999; Zhao et al. 2004; Sun et al. 2006; Zhao et al. 2009). The ‘tidal flow’ refers to the rhythmic and fast filling/drainage generated by pumps in the bed matrices. During the draining process, air is drawn into the bed matrices from the atmosphere and thereby oxygen transfer is greatly enhanced. With this operation strategy, treatment capacity can be remarkably enhanced with hydraulic loading of up to 0.43 m³/m²·d and organic loading of 1.055 gCOD/m²·d (Zhao et al. 2004), which almost decouples the general loading rate adopted in most CWs systems (Knight et al. 2000; Sundaravadivel and Vigneswaran 2001).

Despite the significant development in the CWs process, less attention has been paid in the characteristics of the livestock wastewater itself. However, the treatment performance is largely dependent on the characteristics of the wastewater, especially in biological nutrient removal (Melcer et al. 2003). According to the treatment ability, influent total COD can be divided into two major components: the biodegradable COD and the non-biodegradable (inert) COD. Each of them can be further subdivided into soluble part and particulate part. The inert soluble COD fraction in the
influent bypasses the treatment system without any changes. While the inert particulate fraction can be entrapped in the treatment system, so certain reduction of this part can still be achieved although it is non-biodegradable. Within the biodegradable components, only the soluble biodegradable COD fraction can be readily utilized by microorganisms, whereas the particulate biodegradable fraction has to be converted to soluble fraction before it can be up-taken (Henze et al. 1987; Orhon et al. 1997). Therefore the soluble biodegradable fraction has the prominent influence on the pollutants conversion rates, such as COD degradation and denitrification. When the wastewater passes through the treatment system, most of the biodegradable fractions and the inert particulate fraction will be removed, and the influent inert soluble fraction becomes the major component of the effluent COD. In general, the major COD fraction in livestock wastewater is easily biodegradable. However, its variety in biodegradability can still be significant in practice depending on management and storage conditions before the treatment. This variety may significantly affect the treatment ability of the organic matters themselves and the biological nitrogen removal performance (Boursier et al. 2005). Furthermore, considerable quantities of wastewater are generated on animal farms from washing water and yard runoff. This contains animal faeces and urine as well as parlour washings. It is therefore a common practice to periodically use disinfectants for health concerns during cleaning operation. Obviously, the residual of the disinfectants in wastewater may have serious inhibitory effect on the microbial activities (Bodík et al. 2008).

Thus, the main objective of this study was to examine the variety of livestock wastewater in biodegradability and its effect on the performance of a pilot scale TFCWs in organic matters and nitrogen removal. The potential inhibition of the chosen disinfectants on organic matter biodegradation and nitrification was also investigated.

2 Materials and methods

2.1 Pilot scale alum sludge based tidal flow constructed wetlands

The pilot-scale tidal flow CWs system was located at an animal farm in Newcastle, Co. Dublin, Ireland (Fig. 1). The system consisted of four-stage down flow reed beds. In particular, the system innovatively uses dewatered alum sludge as the main substrate to improve the phosphorus removal. Alum sludge refers to the drinking water treatment residual when aluminium sulphate is adopted as coagulant for purification purpose. Each stage of the CWs was constructed using identical 1100L plastic bins and connected with submersible pumps. In tidal flow operation, there were 3 cycles per day and each cycle consists of 4 hours of wastewater contact and 4 hours of rest (during which wastewater is drained out to the next stage), giving a hydraulic loading rate of 0.29 m³/m²·d. Details of the system set up and operation was described in Zhao et al. (2010). Grab samples were taken once a week from the influent, effluent and each stages and analyzed for COD, BOD₅, TN, NH₄⁺-N, TP, pH and SS.

![Fig. 1 Pilot-scale tidal flow constructed wetlands system](image)

2.2 Livestock wastewater

The farm currently comprises of ca 17,000m² of farm and laboratory buildings with over 2,000 livestock units of sheep, pigs, cattle and horses. Wastewater from the farm activities is collected from the different units and finally stored in a main holding tank with the approximate capacity of 1,000 m³ on the farm before its spreading on the grass of the farmland. Close to the main holding tank, there is a small underground wastewater tank, which is used to temporarily hold the wastewater produced from the piggery before it’s transferred into the main holding tank. The system operation was classified into four periods with different raw wastewater sources. In period 1 (06/02/2009-02/06/2009), the system started up with the long time stored wastewater from the main holding tank (stored wastewater). In period 2 (05/06/2009- 17/08/2009), raw piggery wastewater (fresh wastewater) from the pig unit of the farm was introduced to the CWs system
from the underground tank before it was mixed with the long stored wastewater in the main holding tank. During period 3 (18/08/2009-22/10/2009), no piggery wastewater was produced because the pigs were moved after maturation and the raw wastewater was fed into the system from the main holding tank again. In period 4 (25/10/2009-10/12/2009), raw wastewater was changed back to piggery wastewater produced with the new batch of pigs. Appropriate dilution was carried out using tap water to achieve desired concentration throughout the whole experimental period. The composition of the influent wastewater into the system was summarized in Table 1.

<table>
<thead>
<tr>
<th>Influent</th>
<th>Period 1 (06/02-02/06)</th>
<th>Period 2 (05/06-17/08)</th>
<th>Period 3 (18/08-22/10)</th>
<th>Period 4 (25/10-10/12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mgL⁻¹)</td>
<td>stored wastewater</td>
<td>fresh wastewater</td>
<td>stored wastewater</td>
<td>fresh wastewater</td>
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<tr>
<td>BOD₅ (mgL⁻¹)</td>
<td>463</td>
<td>527</td>
<td>723</td>
<td>1306</td>
</tr>
<tr>
<td>TN (mgL⁻¹)</td>
<td>45</td>
<td>302</td>
<td>261</td>
<td>716</td>
</tr>
<tr>
<td>NH₄-N (mgL⁻¹)</td>
<td>61</td>
<td>117</td>
<td>211</td>
<td>149</td>
</tr>
<tr>
<td>TP (mgL⁻¹)</td>
<td>41</td>
<td>75</td>
<td>165</td>
<td>131</td>
</tr>
<tr>
<td>SS (mgL⁻¹)</td>
<td>175</td>
<td>115</td>
<td>177</td>
<td>365</td>
</tr>
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</table>

To explore the difference between the long time stored wastewater and the fresh piggery wastewater in biodegradability, series of batch tests were conducted to investigate the reduction of soluble COD with alum sludge (Al-S) samples (collected from the field CWs system after three months operation) and activated sludge (AS) samples (collected from a local municipal wastewater treatment plant) in a series of beakers at room temperature. Unlike the particulate COD fractions, which can be entrapped in a porous medium, soluble COD can only be reduced through biological conversion. Therefore, the reduction of soluble COD in the aerobic batch tests indicates the amount of soluble biodegradable COD fraction. Furthermore, the residual soluble COD in the aerobic batch tests represents the amount of inert soluble fraction of the influent organic matters, which determine directly the extent of the treatment ability of the wastewater.

Two types of disinfectants, UNIPRED and HYPROCLOR ED (HYPRED, France) were used for cleaning and disinfection purpose in the dairy unit (the equipment, pipelines and bulk tanks) in the farm. UNIPRED contains over 50% concentrated phosphoric acid and high levels of surfactants. The recommended usage rate is 0.4-0.6%. HYPROCLOR ED consists of 444 gL⁻¹ sodium hypochlorite together with sodium hydroxide. Usage rate is recommended as 0.5%. In practice, UNIPRED was applied once a week with the dosage of 1% (2 liters UNIPRED in 200 liters water), while HYPROCLOR ED was applied every day with the usage rate of 0.5% (1 liter per day in 200 liters water). The residual waters from the cleaning & disinfection process were then mixed with the dairy wastewater (approx. 2m³·day⁻¹) and finally transferred into the main holding tank. To investigate the potential inhibition of these two disinfectants on microbial activities, lab batch tests were performed with the fresh piggery wastewater and activated sludge in series of 1000 mL beakers at room temperature. Aeration was supplied with diffusers placed at the bottom of each beaker to keep DO above 3 mgL⁻¹. Experimental conditions were summarized in Table 2 and Table 3 with UNIPRED and HYPROCLOR ED, respectively.

<table>
<thead>
<tr>
<th>Beaker No.</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS (gL⁻¹)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>UNIPRED (% V/V)</td>
<td>0</td>
<td>0.05</td>
<td>0.1</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>pH_initial</td>
<td>7.3</td>
<td>4.6</td>
<td>3.2</td>
<td>2.6</td>
<td>2.4</td>
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</table>
COD, TN, NH₄⁺-N, TP and SS were analyzed using a Hach DR/2400 spectrophotometer according to its standard operating procedures. BOD₅ was measured with a Hach BODTrak instrument. pH was measured with a pH meter (Orion 920 A+, Thermo). DO was monitored with a microprocessor oximeter (Oxi 325, WTW).

### 3 Results and discussion

#### 3.1 CWs system performance in organic and nitrogen removal

The overall performances in organic matters and nitrogen removal are illustrated in Fig. 2 and Fig. 3, respectively. The results demonstrate that the COD removal efficiency is largely dependent on the source of the wastewater. In the start-up period with the long time stored wastewater (Period 1), only 26% COD removal in average (average effluent 355 mg L⁻¹) was achieved over the first 3 months. In most cases, insufficient DO is the main reason for the poor biological COD removal. In such case, nitrification is restrained more seriously than COD reduction because oxygen is utilized for carbon oxidation prior to nitrification due to the much faster growth rate of heterotrophic organisms than nitrifiers (Henze et al. 1987). However, very good nitrification had been established during this period since NH₄⁺-N removal had reached to 93% with the average of 85% (average effluent 7.7 mg L⁻¹) in the third month (Fig. 3). This suggests that DO is not the reason for the poor COD removal performance in this case. Instead, other factors such as biodegradability of the wastewater and toxicity should be examined. After fresh piggery wastewater was fed to the CWs system (Period 2), COD removal increased steadily and reached to 94% (with effluent of 44 mg L⁻¹) one and half month later. At the same time, nitrification still maintained above 80%. This indicates that the tidal flow operation strategy can provide sufficient oxygen for both COD oxidation and nitrification. Thereafter, COD removal dropped again when the influent was changed back to the long time stored wastewater during Period 3. Finally, good COD removal of up to 84% (with effluent of 197 mg L⁻¹) was restored with fresh piggery wastewater produced by the new batch of pigs in Period 4. In spite of the changes of the wastewater/source, the CWs system performed more stable in BOD₅ removal (data not shown). Average BOD₅ removal was recorded as 65%, 79%, 63% and 73% with average effluent of 28, 60, 113 and 191 mg L⁻¹ from Period 1 to Period 4, respectively. This could be explained with the fact that BOD represents the biodegradable part of organic matters, wherefore there is no difficulty for microorganisms to degrade this part if DO is adequate.

![Fig. 2 System performance in organic matter removal](image-url)
The total nitrogen elimination showed the same trend as COD removal. During period 1, poor TN removal efficiency with an average of only 27% (average effluent 52 mgL.\(^{-1}\)) was achieved in despite of good nitrification performance. In period 2, the total nitrogen removal increased markedly from 50% to over 90% with the average of 64% (average effluent 32 mgL.\(^{-1}\)) after the fresh piggery wastewater was introduced into the CWs system. After that, it dropped dramatically to 13.5-47.8% (average effluent 150 mgL.\(^{-1}\)) due to the lack of carbon source for denitrification with the long stored wastewater in period 3. In period 4, it rapidly jumped to around 80% (average effluent 40 mgL.\(^{-1}\)) after the influent source was changed back to the fresh piggery wastewater. However, the drop after 23 Nov. 2009 should not be explained with the variety of the wastewater since the system was still fed with the same piggery wastewater. Instead, it was in accordance with the decrease in nitrification performance due to low temperature (below 5 °C) as shown in Fig. 3 (Wiesmann et al. 2007). TN elimination showed more sensitive to the wastewater variety comparing with COD removal. Average TN removal decreased from 66% in Period 2 to 32% in period 3, while COD removal only dropped from 72% to 65%. This is mainly because carbon source is always oxidized firstly with oxygen rather than utilized for denitrification. Unless sufficient carbon is available, denitrification is limited due to lack of carbon source (Henze et al. 1997). Furthermore, less DO was consumed with the stored wastewater, and therefore it was more difficult to create anoxic condition for denitrification.

![Graph showing TN and NH-N Removal](image)

**Fig. 3 System performance in total nitrogen removal**

### 3.2 Biodegradability of long time stored livestock wastewater and fresh piggery wastewater

To facilitate the understanding of the performance of the CWs system, laboratory batch tests on the biodegradation of the long time stored livestock wastewater and the fresh piggery wastewater were conducted and the results are illustrated in Fig. 4. Soluble COD of the long stored wastewater kept more or less constant over 9 hrs aeration with activated sludge while it even increased slightly with the alum sludge sample obtained from the CWs system. However, the fresh piggery wastewater showed similar trend with NaAc in biodegradation. Significant reduction of soluble COD from 310 mgL.\(^{-1}\) to 45 mgL.\(^{-1}\) after 9 hrs aeration was observed with activated sludge. The results clearly revealed the significant difference between the two types of wastewaters in biodegradability. The stored livestock wastewater can be characterized as non-biodegradable or at least very slowly biodegradable, while the fresh piggery wastewater is readily biodegradable as NaAc. The dramatic variety of the livestock wastewater during the long term storage might be attributed to the fact that the main holding tank was served as a facultative lagoon, which has been widely applied for the treatment of high strength agricultural wastewaters (Hart and Turner 1965; Schulz and Barnes 1990). Aerobic zone formed in the top layer of the tank with DO diffused from the air and produced through photosynthesis with algae on the water surface, while the middle and bottom layer turned to be facultative or anaerobic. Readily biodegradable fraction of the livestock wastewater degraded in both of the aerobic zone and anaerobic zone via oxidation and anaerobic conversions. As a result, the livestock wastewater changed to be slowly biodegradable or non-biodegradable (Hamilton et al. 2006).
Fig. 4 Degradation of the long stored livestock wastewater (Stored) and fresh piggery wastewater (Fresh) with field alum sludge (Al-S) and activated sludge (AS)

3.3 Inhibition of UNIPRED on COD degradation and nitrification

The effects of UNIPRED on COD biodegradation and nitrification are shown in Fig. 5(a, b). UNIPRED showed very strong inhibition on both heterotrophic organisms and nitrifiers. Only 0.05% of UNIPRED, which is 1/10 of the recommend usage rate, was strong enough to completely inhibit COD biodegradation and nitrification. The main inhibition mechanism is probably the acidic condition created by the concentrated phosphoric acid, as the initial pH showed in Table 2. For carbonaceous removal with aerobic biological oxidation, the tolerable pH range is 6-9. For nitrification, rates decline significantly at pH below 6.8 (Tchobanoglous et al. 2003). While the pH values were 4.2, 3.6, 2.6 and 2.4 with 0.05%, 0.1%, 0.3% and 0.5% of UNIPRED, respectively (Table 2) in the batch tests, which exceeded the tolerable range for both heterotrophic organisms and nitrifiers. The increasing of the initial soluble COD with the addition of UNIPRED may be caused by the surfactants contained in the disinfectant. The significant increase of soluble COD with high content of UNIPRED along with time might result from the cell death and the consequent lysis, similar to COD release caused by microbial cell disruption with high salt concentrations (Kincannon and Gaudy 1966), toxic compounds (Aquino and Stuckey 2004) and ultrasonic treatment of biological sludge (Tiehm et al. 1997).
3.4 Inhibition of HYPROCLOR ED on COD Degradation and nitrification

The effect of HYPROCLOR ED on carbonaceous oxidation is demonstrated in Fig. 6. The results showed that the inhibitory effect existed from 0.1% of HYPROCLOR ED. Although soluble COD reduced to the same level at HYPROCLOR ED content of 0.1% as the contents of 0 and 0.05%, the degradation rate reduced. When the content was above 0.1%, COD degradation was completely inhibited. Dramatic increase of soluble COD with high content of the disinfectant was observed, probably due to cell death and lysis, as discussed above. This was justified with test No. 5 with 0.5% HYPROCLOR ED but without activated sludge, in which the soluble COD maintained more or less constant through the whole test period.
Fig. 6 Inhibition of HYPROCLORED (Hyp) on COD degradation

Fig. 7 reveals the effect of HYPROCLORED on nitrification. Significant inhibition on nitrification was recorded from HYPROCLORED of 0.1%, indicating nitrifiers are more sensitive to HYPROCLORED comparing with heterotrophic organisms. Although ammonia-N reduction was also observed at high contents of HYPROCLORED (0.3% and 0.5%), this should not be explained as a result of microbial activities. If these reductions at high disinfectant contents were also caused by nitrification, it should be less than the value at HYPROCLORED of 0.1% since the inhibition increased with the increasing of the disinfectant content. However, the results showed an opposite situation. Instead, these reductions were probably as a result of the ammonia gas stripping. The initial pH values were recorded as 10.4 and 11.5 with HYPROCLORED contents of 0.3% and 0.5 respectively (Table 3). When pH is above 10, more than 80% ammonia-N exists as ammonia gas (Tchobanoglous et al. 2003), which will be stripped when aeration is supplied. This was validated with test No. 5 with 0.5% HYPROCLORED but without activated sludge, in which almost the same reduction rate with HYPROCLORED content of 0 was observed. The difference between the tests with the same 0.5% HYPROCLORED but with/without activated sludge (Hyp 0.5% and Hyp 0.5%, AS 0) might also be attributed to cell death and lysis of the activated sludge (as significant soluble COD release showed in Fig. 6). In the both tests, ammonia-N reduced due to ammonia gas stripping. But ammonia-N was also continuously produced during the experimental period due to cell death and lysis of the activated sludge in the former, while no ammonia-N was produced in the latter since it didn’t contain activated sludge. Consequently, more significant ammonia reduction took place in the test without activated sludge (Hyp 0.5%, AS 0).
3.5 Potential impacts of disinfectants on CWs performance

Although the laboratory batch tests showed strong inhibitions of the chosen disinfectants on COD degradation and nitrification, no direct impacts were recorded in the field study. This is because the disinfectants were highly diluted in the main holding tank. Without regard to other wastewaters (from other units), the residual disinfectants concentrations in the main holding tank were less than 0.02% for UNIPRED and near 0.05% for HYPROCLOR ED, based on the consideration of the usage rate of 2 L a week and 1 Ld⁻¹ for UNIPRED and HYPROCLOR ED, respectively, and the flow rate of around 2.200 Ld⁻¹ from the dairy unit. In fact, the amount of other units wastewaters entering the main holding tank were significant at times of the dairy wastewater. This means that the actual concentrations in the feed to the CWs system were less than 0.01% for UNIPRED and 0.025% for HYPROCLOR ED. This explains why no direct inhibitions were observed in the field CWs study. However, there is still a great risk of system failure due to the inhibitions of the disinfectants if the dairy wastewater was introduced into the CWs system directly. Two potential impacts can be defined depending on the flow rate of the dairy wastewater, which are loss in activity and cell death. When the flow rate is between 1 m³d⁻¹ and 2 m³d⁻¹, UNIPRED will be 0.02-0.03% and HYPROCLOR ED will be 0.05-0.1%. Within this range, microbe may still keep alive, but theirs activities will be significantly reduced as showed in Fig. 6 and Fig. 7. As a result, pollutants removal rate will be decreased significantly. When the flow rate reduces to below 1 m³d⁻¹, cell death could be significant and irreversible system failure happens consequently. Therefore, direct introduction of the dairy wastewater into the CWs system should be avoided in this case.

4 Conclusions

Significant variety of livestock wastewater in biodegradability during long time storage was observed in this study. Results of the laboratory batch tests showed that fresh livestock wastewater was readily biodegradable, while it turned to be non-biodegradable after long time storage. The variety of the livestock wastewater has a decisive influence on the performance of the tidal flow CWs system regarding the removals of organic matters and nitrogen. With fresh livestock wastewater, removal efficiencies up to 93% and 94% could be obtained with average of 73% and 64% for COD and TN, respectively. However, the performance deteriorated when the system was fed with long time stored livestock wastewater. The application of disinfectants UNIPRED and HYPROCLOR ED may cause serious inhibition on microbial activities. Both heterotrophic organisms and nitrifiers could be inhibited with UNIPRED content from 0.05%, which is 1/10 of the recommended usage rate. Inhibitory effect of HYPROCLOR ED on COD biodegradation exists from 0.1% and complete inhibition occurs from content of 0.3%, while significant inhibition on nitrification starts from content of 0.1%.

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