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Microbial removal from the separated liquid fraction of anaerobically digested pig manure in meso-scale integrated constructed wetlands

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ABSTRACT

The aim was to investigate microbial removal from the liquid fraction of anaerobically digested pig manure in meso-scale integrated constructed wetlands (ICW's) over a 13 month period. Four treatments were investigated: T1 (standard), T2 (effluent recycling), T3 (high nutrient loading), and T4 (high flow rate). Mean counts of yeasts and moulds and spore-forming bacteria were higher in T3 and T4 than in T1 and T2 ($P < 0.05$). Flow through the cells reduced mean counts of coliform, yeasts and moulds and spore-forming bacteria across all treatments ($P < 0.01$). Counts varied with season; coliform were highest in the Summer ($P < 0.001$), with yeasts and moulds highest in the Summer and Autumn ($P < 0.01$) and spore-formers lowest in the Autumn ($P < 0.001$). As *Salmonella* was undetectable in the influent and *Escherichia coli* and *Enterococcus* were rarely detected it is difficult to make conclusions regarding pathogen removal. Further investigations using marked strains would allow pathogen tracking within the ICW's.

1. Introduction

Each year, the pig industry produces substantial amounts of manure worldwide, with land spreading the most common disposal method. However, intensification of pig production has resulted in large numbers of pigs concentrated in specific geographic locations. This means that in these 'pig dense' regions, surrounding land areas are often insufficient to deal with the quantities of manure nutrients generated. Additional restraints have been placed on the pig industry by the Nitrates Directive 91/676/EEC (EC, 1991) first implemented in Ireland in 2006 and currently interpreted by S.I. No. 610 (S.I., 2010). This has compelled the industry to examine alternatives to land spreading for pig manure.

Anaerobic digestion (AD) involves bacteriological breakdown of organic material into biogas (mostly methane and carbon dioxide) and digestate. Mesophilic AD, which employs temperatures of 30–40 °C, is commonly used to treat sewage sludge (Taricska et al., 2007) and there is now increasing interest in the use of on-farm anaerobic digesters for manure treatment. The benefits of this technology are that the digestate, compared with manure, has fewer viable weed seeds, less odor and lower pathogen counts. The methane gas produced is an excellent source of renewable energy that may be used on farms or exported as gas, electricity and/or heat for use elsewhere. In Ireland there are five functioning small-scale on-farm anaerobic digesters treating farm waste and utilizing the energy generated on-farm (EPA, 2006).

Another manure management option for pig farmers is the mechanical separation of manure into solid and liquid fractions, either before or after AD (Burton, 2007). The separated solid fraction can be land spread, composted and used as an organic fertilizer (Burton, 2007; Nolan et al., 2011) or further processed into added-value products such as a solid biofuel. The nitrogen-rich liquid fraction also requires disposal and is usually land spread (Hjorth et al., 2010). However, integrated constructed wetlands (ICW's) may offer a low-cost sustainable alternative to land spreading.

ICW's are horizontal, surface flow constructed wetlands (CW's) specifically designed to incorporate the surrounding landscape (Babatunde et al., 2008; Dunne et al., 2005). They are biological wastewater treatment systems in which contaminants are removed by way of sedimentation, filtration, microbial degradation and plant uptake (Babatunde et al., 2008; Hunt and Poach, 2001). Integrated constructed wetlands consist of a series of linked ponds or 'cells'. The influent material is pumped directly into the first pond and from there flows sequentially through the ponds. The effluent is usually discharged to waterways. In Ireland, CW's were designed primarily for the treatment of municipal or domestic wastewater with around 140 in operation in 2005 (Babatunde et al., 2008). However, their popularity as a treatment option for

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agricultural wastewater is growing and in Ireland, a number of on-farm ICW's are in operation in the Anne Valley in Co. Waterford (Dunne et al., 2005; Harrington and McInnes, 2009). The primary concern is the removal of nutrients. Previous studies on these and other ICW's, demonstrate that they are successful in reducing nitrogen, phosphorus, suspended solids and chemical and biological oxygen demand in pig and dairy wastewaters (Dunne et al., 2005; Hunt et al., 2003). Nutrient removal is influenced by factors such as nutrient loading and flow rate, the latter being inversely proportional to the retention time. These parameters also influence the efficacy of pathogen removal (Cronk, 1996; Díaz et al., 2010; Hill and Sobsey, 2001).

From an experimental point of view, meso-scale ICW systems are useful as they allow investigation of the effects of varying operational parameters. They enable a high degree of treatment replication while using a relatively small land area. Experimental meso-scale ICW's were installed at Teagasc, Pig Development Department, Animal & Grassland Research & Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland to treat the separated liquid fraction of AD pig manure. Nutrient removal has been investigated in these systems (Harrington and Scholz, 2010). The aim of the present study was to investigate removal of enteric indicator bacteria and other micro-organisms from the separated liquid fraction of AD pig manure in these meso-scale systems, using varying operational parameters.

2. Methods

2.1. Meso-scale ICW systems

The experimental meso-scale ICW systems were sited at Teagasc, Pig Development Department, Animal & Grassland Research & Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland. The mean seasonal temperatures and rainfall recorded at the Moorepark weather station over the sampling period (April 2009 to May 2010) were as follows: Spring, 7.3 °C, 84.4 mm; Summer, 12.8 °C, 68.2 mm; Autumn, 14.3 °C, 94.8 mm; and Winter, 5.3 °C, 138.8 mm. The design and operation of the ICW systems has been described in detail by Harrington and Scholz (2010). Briefly, there were 16 ICW systems in total, each consisting of four wetland cells, with an overall surface area of 0.788 m^2. These 16 ICW systems were assigned to four different treatments as follows: (T1) standard (100 mg/L NH_3 at a hydraulic loading rate of 37 m^3/ha); (T2) effluent recycling (100 mg/L NH_3 at a hydraulic loading rate of 37 m^3/ha with 100% recycling from cell 3 to cell 1 weekly); (T3) high nutrient loading (200 mg/L NH_3 at a hydraulic loading rate of 37 m^3/ha) and (T4) high flow rate (100 mg/L NH_3 at a hydraulic loading rate of 74 m^3/ha). Each treatment was replicated four times. All treatments except T3 received the separated liquid fraction of AD pig manure diluted 1:32 with untreated tap water of potable quality. The high nutrient loading treatment (T3) received the same material diluted 1:16 with water. All wetland systems were planted with the same quantities and species of plants, as outlined by Harrington and Scholz (2010).

2.2. Sampling of the meso-scale ICW's

The effluents from the first, second and third cells and the final effluent from the fourth cell were sampled monthly from each of the four treatment systems. For the first 3 months one replicate of each treatment was sampled and thereafter two replicates were sampled. Samples were also taken from the storage tanks containing the influent material (both the 1:16 and 1:32 dilutions of the liquid fraction of AD pig manure). Samples (both influent and ICW) were taken monthly from April 2009 to May 2010 (excluding January 2010 when all of the ICW systems were frozen). All samples (~500 ml) were collected in sterile containers and stored at 4 °C until microbiological analysis was performed (within 24 h).

2.3. Microbiological analysis

Meso-scale ICW samples (25 ml) were homogenized in 225 ml of buffered peptone water and a 10-fold dilution series was performed in maximum recovery diluent (MRD; peptone 1 g/l; sodium chloride 8.5 g/l). Relevant dilutions were pour-plated in duplicate on the following media; kanamycin azide aesculin (KAA) agar incubated at 45 °C for 24 h for enterococci; chromoCult® tryptone bile X-glucuronide (CTBX) agar incubated at 37 °C for 24 h for *Escherichia coli*; McConkey agar incubated at 37 °C for 24 h for total coliforms and yeast glucose chloramphenicol agar incubated at 28 °C for 4 days for yeasts and moulds. Colonies were counted and the counts averaged and presented as CFU/ml of the original sample. At certain time points, *Enterococcus* and *E. coli* were also enumerated by membrane filtration according to standard methods (APHA, 1992), using 0.45 μm pore size 47 mm diameter sterile cellulose nitrate filters (Whatman, Kent, UK). Filters were incubated on CTBX agar at 37 °C for 24 h and on modified KAA agar (Audicana et al., 1995) at 45 °C for 24 h for enumeration of *E. coli* and *Enterococcus*, respectively. Counts were presented as CFU/100 ml of sample. To enumerate spore-forming bacteria, 5 ml of the initial 1 in 10 dilution of each of the samples was heated to 80 °C for 10 min, cooled on ice and serially diluted 10-fold in MRD. Relevant dilutions were pour-plated in duplicate on nutrient agar and the plates were incubated at 37 °C for 48 h. All microbiological media and the MRD were obtained from Merck (Darmstadt, Germany). The presence/absence of *Salmonella* in 25 ml ICW samples was also determined according to standard procedures (ISO, 2007), with modifications, as outlined by McCarthy et al. (2011).

2.4. Statistical analysis

Microbial counts were log-transformed and analyzed for repeated measures using the PROC mixed procedure of SAS (Cary, NC, USA). Fixed effects were season of sampling, treatment and sampling point (i.e. cell). The microbial count in the influent was used as a covariate where appropriate and sampling point (i.e. cell, nested within sampling season) was considered the repeated measure. The experimental unit was the individual ICW system (four in-line cells). Multiple pair-wise comparisons were performed using the Tukey–Kramer test. Statistical significance was assumed at $P < 0.05$.

3. Results and discussion

The use of meso-scale ICW's to examine treatment of the separated liquid fraction of AD pig manure is novel, both in terms of the scale of the systems and the influent material used. These systems have been validated with respect to nutrient removal (Harrington and Scholz, 2010), and the present study investigates microbial removal from this material.

3.1. Effect of operational parameters on micro-organisms within the ICW's

As *Salmonella* was not detected in the influent or in the effluent from any of the ICW cells at any time point during the 13-month sampling period (Table 1), no conclusions can be made regarding its removal. However, in a large-scale on-farm ICW treating the same influent material, *Salmonella* Typhimurium DT104b was detected in the influent as well as in the first and mid-cell effluent,