1 **Pilot-scale testing of a leachbed for anaerobic digestion of**
2 **livestock residues on-farm.**

3 **S.D. Yap^a, S. Astals^a, P.D. Jensen^a, D.J. Batstone^a, S. Tait^a**

4 **^a Advanced Water Management Centre, The University of Queensland, St Lucia, QLD**
5 **4072, Australia.**

6 **Corresponding author:** Mr. Shao Dong Yap. Mailing address: Advanced Water
7 Management Centre, The University of Queensland, Level 4, Gehrmann Laboratories
8 Building (60), Brisbane, QLD 4072 Australia. Phone: +61 7 3346 9973. Email:
9 s.yap@awmc.uq.edu.au

ABSTRACT

A leachbed is a relatively simple anaerobic digester suitable for high-solids residues and on-farm applications. However, performance characteristics and optimal configuration of leachbeds are not well-understood. In this study, two 200 L pilot-scale leachbeds fed with spent straw bedding from pigs/swine (methane potential, $B_0 = 195\text{--}218 \text{ L CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{fed}}$) were used to assess the effects of leachate recirculation mode (trickling vs. flood-and-drain) on the digestion performance. Results showed comparable substrate solubilisation extents (30–45% of total chemical oxygen demand fed) and methane conversion (50% of the B_0) for the trickling and flood-and-drain modes, indicating that digestion performance was insensitive to the mode of leachate flow. However, the flood-and-drain leachbed mobilised more particulates into the leachate than the trickling leachbed, an undesirable outcome, because these particulates were mostly non-biodegradable. Inoculation with solid residues from a previous leachbed (inoculum-to-substrate ratio of 0.22 on a VS basis) hastened the leachbed start-up, but methane recovery remained at 50% of the B_0 regardless of the leachate recirculation mode. Post-digestion testing indicated that the leachbeds may have been limited by microbial activity/inhibition. The high residual methane potential of leachate from the trickling (residual $B_0 = 732 \pm 7 \text{ L CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{fed}}$) and flood-and-drain leachbeds ($582 \pm 8 \text{ L CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{fed}}$) indicated an opportunity for further processing of leachate via a separate methanogenic step. Overall, a trickling leachbed appeared to be more favourable than the flood-and-drain leachbed for treating spent bedding at farm-scale due to easier operation.

30 *Keywords: Anaerobic digestion; Leachbed; Solid-phase; Manure; Straw; Inoculation.*

1. Introduction

The use of bedding is common in intensive livestock production. Animal welfare can be maintained with high stocking densities because the bedding absorbs moisture, and reduces odour and ammonia (Aland and Banhazi, 2013). Common bedding materials include straw, barley, woodchips and rice hulls (Kruger et al., 2006). The animals are typically grown in a batch “all in, all out” mode, with batch times varying depending on the type of livestock and farm operations (Aland and Banhazi, 2013). A key by-product from bedded animal housing is a soiled lignocellulosic residue, known as spent bedding, which mainly consists of residual bedding, faeces, urine, spilt water and animal feed (Tait et al., 2009). Spent bedding is typically stockpiled for passive composting prior to re-use as a soil conditioner and fertiliser on-farm. Key concerns from stockpiling are greenhouse gas (GHG) emissions, odour, loss of nutrients, and potential ground and surface water contamination (Sommer and Møller, 2000).

Anaerobic digestion (AD) is a technology option for valorising solid waste (Mata-Alvarez et al., 2000), with significant advantages over conventional stockpiling, such as renewable energy recovery, reduced odour (when operated correctly) and improved nutrient recovery potential. AD is a multistep biological process and for a solid substrate like spent bedding would probably be limited by the rates of both hydrolysis and methanogenesis (Batstone and Jensen, 2011).

Single-stage solid-phase anaerobic leachbeds, also known as percolation or batch solid-phase digesters, is a dry digestion process and thus can operate at relatively high solids content (>20%) (Batstone & Jensen, 2011), making it particularly interesting for solid livestock residues. Further, a leachbed process can be relatively simple to construct and operate and thus cost-effective when compared to technology alternatives such as plug flow or mixed vessel digesters (Deublein & Steinhauser, 2011). For these reasons, leachbeds may be particularly suitable for decentralised agricultural applications. Leachbeds have been mostly

applied to the organic fraction of municipal solid waste (OFMSW) and energy crops, and to a lesser extent of agricultural waste, such as spent bedding (Vandevivere et al., 2003; Deublein & Steinhauser, 2011). Moisture content is a critical factor affecting solid-phase digestion, and which with a leachbed is maintained via exposure of the solid substrate to a leachate (Mussoline, 2013). Good contact between leachate and the solid being digested is thus essential, in order to provide dispersion of inoculum, nutrients, soluble digestion products and pH buffering agents (Jha et al., 2011), and to dilute inhibitors (Chugh et al., 1998).

Different leachate recirculation modes have been trialled previously to provide the necessary solid-liquid contact (Chugh et al., 1988; Kusch et al, 2008; Nizami et al, 2010). In some cases, leachate is sprayed over the solid bed and recovered from the base of leachbed for recirculation (termed trickling) (Deublein & Steinhauser, 2011). In other cases the solid bed is fully flooded with leachate (Nizami et al., 2010), which is left to contact for a period and then drained (termed flood-and-drain) (Clarke and Xie, 2013). Studies to date have suggested that the leachate contact mode can impact on digestion performance (Kusch et al, 2008; Nizami et al, 2010). For instance, Nizami et al. (2010) hypothesized that a flooded system would perform better than a trickling system, because substrate is fully submersed with more intimate contact with leachate. However, there is still a degree of ambiguity as to the preferred leachbed flow arrangement (Kusch et al., 2008; Nizami et al., 2010). Also, to our knowledge, there have not been previous studies of different leachate flow configurations tested in parallel (flooded versus trickling) nor at pilot-scale, which are important to provide insight for future applications of the leachbed technology.

Previous laboratory scale studies (Kusch et al., 2008; Tait et al., 2009) on spent bedding digestion have indicated variable background microbial activity. Inoculation may provide a balanced microbial community and pH buffering necessary for accelerated start-up of a leachbed (Shi et al., 2014; Demirel and Scherer, 2008). Inoculation of leachbeds typically

uses a fraction of digested material or solid residues retained from a previous leachbed batch (Xu et al., 2012). Otherwise, an alternate inoculation technique known as ‘sequencing’ or ‘indirect recirculation’ has been used, which involves cross-circulation of water leachate between a fresh and stabilized waste (Chugh et al., 1998).

The present study evaluates leachbed digestion of spent bedding from pigs/swine at pilot-scale. Two 200L leachbeds are operated in parallel to compare the effects of hydraulic configuration (trickling vs. flood-and-drain) on digestion performance. Also, in two separate tests, a leachbed is operated with and without external inoculation to assess the sufficiency of background microbial activity for leachbed start-up.

2. Materials and methods

2.1 Raw materials

Spent bedding was collected and prepared for analysis according to Test Methods for the Examination of Composting and Compost (U.S. Composting Council, 2002). Samples were collected from stockpiles at two piggeries (site A and B) in Queensland (Australia) and were 0-2 days old at the time of sampling. The two different sites provided some variation in the properties of spent bedding, which was a necessary factor to consider during the testing because spent bedding naturally varies across sites and with local management practices (Tait et al., 2009). At both sites, the spent bedding was from sheds housing smaller pigs (weaners). The pigs were all reared according to a batch “all in, all out” mode and the batch time of site A and B was different at 6 and 4 weeks, respectively. This meant that spent litter from site A was expectedly more soiled. Moreover, bedding at site A consisted only of wheat straw, whilst bedding at site B contained 40 wt% wheat straw, 40 wt% barley straw and 20 wt% sorghum straw (weight basis of fresh bedding added).

2.2 Pilot-scale leachbed trials

Two leachbeds were operated in parallel, which were 200 L stainless steel (SS) vessels (500 mm internal diameter, 1060 mm height) fitted with perforated SS304 base plates to support and retain the spent bedding being digested. The leachate from each leachbed was collected in a separate gastight 120 L polyethylene drum (leachate storage tank), before being pumped back into the leachbed (CP 11, Monopump) at 2 L min^{-1} . When leachate was pumped back into the leachbed, it was first passed through a stainless steel shell-and-tube type heat exchanger (total heat transfer area of 0.6 m^2) to heat the leachate (and thus the leachbed) to a desired temperature of $37 \pm 1^\circ\text{C}$. Pressure transducers were able to detect any blockages of the pipes through which leachate were pumped. The pH of the leachate was measured with a pH probe (Hannah Instrument) mounted through the wall of the leachate storage tank. The temperature of the leachbed was measured with a resistance temperature detector (RTD) (model SEM203P, W&B Instruments) mounted through the wall of the leachbed vessel. Pressure, pH and temperature (4-20mA transmitter) were logged via a PLC system (DirectLogic hardware, Think & Do PC-based control software). Biogas produced by each leachbed/leachate storage tank pair was combined in a single outlet line and was measured with a displacement manometer (Chugh et al., 1999).

The two leachbeds were operated with distinct modes of leachate flow i.e. one leachbed as trickling and a parallel leachbed as flood-and-drain (See Fig. S1 of Supplementary material). In the trickling system, leachate was sprayed over the solid bed via a spray nozzle (3/8YS60130, SprayFlo) for 5 minutes every 20 minutes. With this trickling leachbed, a filter (150 mm internal diameter, 400 mm height, 3 mm mesh size) was placed in the pipeline that carried collected leachate into the leachate storage tank, in order to capture coarse or fibrous material that could have clogged the spray nozzle. However, the amount of material typically retained in this filter was usually minimal, representing a negligible portion of total chemical

oxygen demand fed ($t\text{COD}_{\text{fed}}$). With the flood-and-drain leachbed, the spent bedding content was completely flooded from the base for 20 minutes, followed by a drain and rest for 20 minutes, before the fill/drain cycle was repeated using the same leachate. Three mesh conduits (5 mm mesh size, SS316) were embedded evenly spaced and vertically transverse in the spent bedding of the flood-and-drain leachbed only, to aide free-flow of leachate and improve solid-liquid contact (i.e. mass transfer) (See Fig. S1 of Supplementary material). To minimise the oxidation of organic matter added into the leachbed, the system was flushed with 100% N_2 gas prior to start-up.

Two separate trials (termed Test 1 and 2) were carried out, each with the two leachbeds operating in parallel. Each trial ran for about 50 days (dictated by levelling off of biogas production over time), which could roughly be the expected operating time in practice, because leachbeds are batch systems. Table 1 summarises the initial set-up condition of Tests 1 and 2. Test 1 was started without external inoculum, relying on native microbial activity of manure present in the spent bedding. Test 2 was inoculated with solid residues and leachate from the trickling leachbed of Test 1. In Test 1, the mesh sizes of the supporting base plate used in the trickling and the flood-and-drain leachbeds were 3 mm and 5 mm, respectively. The larger mesh size in the flood-and-drain leachbed was to facilitate free-flow of leachate during the flood and drain cycles. However, midway through Test 1 (day 25), the leachate recirculation line of the flood-and-drain leachbed was continuously clogged by fibrous material that had passed through the mesh base plate when the leachate drained. This issue was resolved in Test 2 by instead using a 3 mm baseplate for both leachbeds to better retain the fibrous material. The trickling leachbed in Test 1 did not experience such operational problems. During the course of Tests 1 and 2, 10 mL leachate samples were intermittently collected from the leachate storage tank for analysis (see Section 2.5). At the same time, samples of headspace gas were collected from an inline gas sample point for composition

analysis by GC (see Section 2.5). At the end of each trial, leachate was drained from the remaining solid residue in each leachbed over a 24hr period before the remaining solid residues and collected leachates were separately further analysed (see Section 2.4).

2.3 Biochemical methane potential tests

Biochemical methane potential (BMP) tests followed the methods of Angelidaki et al. (2009) to determine spent bedding biodegradability extent and kinetics at $37\pm1^\circ\text{C}$. The BMP testing was performed in 250 mL non-stirred media bottles. The BMP results provided baseline specific methane yield (B_0), with which the performance of the leachbeds could be compared. Inoculum for the BMPs was from a mixed mesophilic digester in South East Queensland (Australia) treating primary and secondary municipal sludge, and was added at an inoculum-to-substrate ratio (ISR) of two on a volatile solids (VS) basis. Bottles were flushed with 100% N_2 gas for about 1 min (4 L min^{-1}), after which they were immediately sealed with a rubber septum which was retained with an open top screw cap. Tests were performed in triplicate and background methane production from blanks (substrate-free assay) were subtracted. Biogas samples were periodically collected from the headspace of each vial for measurement of methane produced. Biogas volume was measured using a manometer and methane content was determined by gas chromatography (GC) (see Section 2.5). Tests were mixed by swirling before and after every sampling event, but not in-between sampling events. The BMP test data were analysed using the package Aquasim 2.1d to perform a non-linear least-squares fit of a simple first-order kinetic model (Eq. 1) to the measured cumulative methane (B_t) produced at incubation time (t) (Jensen et al., 2010):

$$B_t = B_0(1 - e^{-k_{\text{hyd}} \cdot t}) \quad (1)$$

where B_0 has units of $\text{L CH}_4 \text{ kgVS}_{\text{fed}}^{-1}$ and k_{hyd} is a fitted first-order kinetic rate coefficient with units of d^{-1} .

2.4 Residual methane potential tests

Residual methane potential was measured for the remaining solid residue and leachate collected at the end of each leachbed trial. These measurements used the BMP methods described in Section 2.3, but with and without added inoculum. B_0 was quantified as measured cumulative methane normalised to the amount of VS present in the residue. In the case without added inoculum, the solid residues were diluted with milliQ water at a dilution factor of 0, 2 or 4 or with leachate at a dilution factor of 2 or 4. These additional tests were performed to assess suspected effects of chemical inhibitors and mass transfer limitations.

2.5 Analytical methods

For total fractions, the analysis was performed directly on samples as collected. For soluble fractions, samples were immediately filtered through 0.45 μm syringe filters (PES membrane) and stored at 4°C until analysis.

Volatile fatty acids (VFAs) and alcohols were measured using a Perkin–Elmer GC with a flame ionisation detector (GD-FID) and a DB-FFAP column. Total ammonium nitrogen (TAN) was measured using a Lachat Quik-Chem 8000 Flow Injection Analyser (Lachat Instrument, Milwaukee). Free ammonia nitrogen concentration (Z_{NH_3} g $\text{NH}_3\text{-N L}^{-1}$) was calculated for inhibition analysis, using Eq. 2 (Batstone et al., 2002):

$$Z_{\text{NH}_3} = \frac{K_{\text{a,NH}_4} \cdot S_{\text{TAN}}}{K_{\text{a,NH}_4} + 10^{-\text{pH}}} \quad (2)$$

Where S_{TAN} is the TAN concentration (g $\text{NH}_4\text{-N L}^{-1}$). The acid-base equilibrium coefficient for ammonium ($K_{\text{a,NH}_4}=10^{-9.25}$) was corrected for leachbed temperature T (degrees K) using the constant enthalpy form of van't Hoff with a heat of reaction of 51.965 kJ Mole^{-1} (Batstone et al., 2002).

Total solids (TS) and volatile solids (VS) were measured according to Standard Methods procedures 2540G (Franson et al., 2005). VS values were corrected for evaporation of volatile fatty acids (VFAs) and alcohols by the method of Peces et al. (2014). Total and soluble chemical oxygen demand (tCOD and sCOD, respectively) were determined for the total and soluble fractions, respectively, using a Merck Spectroquant® cell and a Spectroquant® Move 100 mobile colorimeter (Merck, Germany). Biogas composition (CH₄, CO₂, H₂) was determined using a Shimadzu GC-2014 with a HAYESEP Q80/100 as described by Astals et al. (2015b).

2.6 Data analysis

The leachbed performance was assessed based on extents of substrate solubilisation and methane production. Substrate solubilisation was quantified as the sum of the COD contributions by methane produced and by soluble COD measured in leachate samples (sCOD_{leachate}) normalized to tCOD originally fed into the leachbed:

$$\text{Extent of substrate solubilisation (\%)} = \left(\frac{\text{COD}_{\text{CH}_4} + \text{sCOD}_{\text{leachate}}}{\text{tCOD}_{\text{fed}}} \right) \times 100 \quad (3)$$

Methane productivity was quantified as a ratio to tCOD originally fed into the leachbed:

$$\text{Extent of methane conversion (\%)} = \left(\frac{\text{COD}_{\text{CH}_4}}{\text{tCOD}_{\text{fed}}} \right) \times 100 \quad (4)$$

These methods were adapted from Jensen et al. (2011). tCOD originally fed to the leachbed also included COD contributed by any added inoculum. The extents of substrate solubilisation and methane production were calculated over time for each sample event.

Extent of COD mobilisation to leachate was similarly quantified using Eq. (5), but only at the end of each leachbed trial because periodic leachate samples had erratic solids content.

$$\text{Extent of COD mobilisation to leachate (\%)} = \left(\frac{\text{COD}_{\text{CH}_4} + \text{tCOD in leachate}}{\text{tCOD}_{\text{fed}}} \right) \times 100 \quad (5)$$

As a check of data quality, a COD mass balance was also performed at the end of each leachbed trial (Eq. 6):

$$\text{COD mass balance (\%)} = \left(\frac{\text{COD}_{\text{CH}_4} + \text{tCOD left over in residual solid and leachate}}{\text{tCOD}_{\text{fed}}} \right) \times 100 \quad (6)$$

to confirm that tCOD initially fed into the leachbed was accounted for by COD contributions of methane recovered during the leachbed trial plus COD remaining in the residual solid and leachate recovered at the end of the trial.

To check for biogas/methane leaks that may have occurred during the leachbed trial (difficult to detect directly because of low prevailing gas pressures), a methane contribution balance was also performed at the end of each leachbed trial using Eq. (7). This methane contribution balance summed up the respective B_0 s of residual solids and leachate recovered at the end of the trial together with total methane recovered from the leachbed during the trial (COD_{CH_4}), and compared it to the B_0 of substrate originally fed to the leachbed:

$$\text{Methane balance (\%)} = \left(\frac{\text{BMP of recovered leachate and residual solids} + \text{COD}_{\text{CH}_4}}{\text{BMP of original substrate fed to the leachbed}} \right) \times 100 \quad (7)$$

When data are presented with error bars, these data are average values for triplicate analysis, with error bars representing errors estimated at the 95% confidence level using a two-tailed t -test. Where values are the result of calculations in eq.3-7, errors were propagated analytically as described by Batstone (2013).

3. Results and Discussion

3.1 Raw materials characterisation

Table 2 summarises key characteristics of raw spent bedding from sites A and B. VS/TS ratios and k_{hyd} were similar for bedding from sites A and B. B_0 was only slightly lower for

bedding from site A than for bedding from site B. Measured B_0 values were comparable with those reported elsewhere (Kusch et al., 2008; Tait et al., 2009; Tong et al., 1990).

3.2 Pilot-scale leachbed performance

Figs. 1 and 2 present time series results for the leachbed trials with and without inoculation, respectively. Overall, the COD mass balance closed to within 10% of the expected value (Eq. 6), indicating high data quality and negligible methane losses. Further, $94 \pm 8\%$ of B_0 (Eq. 7) could be recovered in the leachbed and residual methane tests, again indicating that the data was sound.

In Test 1, up to day 25 (Figs. 1a, b), the trickling system and flood-and-drain systems had comparable performance, but at day 25, the leachate recirculation pipelines in the flood-and-drain leachbed became clogged. Once leachate could not be recirculated through the heat exchanger due to the clogging, the measured operating temperature of the leachbed rapidly decreased from 37 °C to room temperature (25 °C). As expected, this decrease in operating temperature disrupted methanogenic activity, and biogas production ceased (Fig. 1b) (Batstone and Jensen, 2011). Propionate rapidly accumulated, but stopped soon after, possibly due to product inhibition (Veeken and Hamelers, 1999). Due to these issues, the discussion below will consider the full dataset for the trickling bed in Test 1, but only data collected up to day 25 for the flood-and-drain leachbed in Test 1. All the data for both leachbeds of Test 2 were deemed valid and were used in the discussion and interpretation.

Initially in Test 1, soluble organics (measured as sCOD) rapidly accumulated in both leachbeds (Figs. 1a, b), possibly due to rapid mobilisation of water-soluble organic matter present in the raw spent bedding. Soluble organic matter gradually declined and stabilised after 40 days. VFA accumulation was detected shortly after start-up, with acetic acid being initially prominent before declining as propionic acid accumulated (Figs. 1c, d). In the

267 trickling system, all VFAs were consumed to exhaustion within 40 days with no net
268 production observed thereafter (Figs. 1c, d). VFA accumulation decreased the leachate pH to
269 below 6.0, but pH recovered as VFAs were converted into methane (Figs. 1e, f). Methane
270 production appeared to be initially delayed by low pH (Figs. 1a, b). Methane production in
271 the trickling system levelled off at around 40 days, with an overall extent of substrate
272 solubilisation of 44% ($\text{CH}_4 + \text{sCOD}$) and a methane conversion of 51% of the B_0 (195 LCH_4
273 $\text{kg}^{-1} \text{ VS}_{\text{fed}}$) (Figs. 1a, b). Methane concentration measured in the headspace gas was also
274 reasonable at up to 58 ± 3 % by volume (See Fig. S2 of Supplementary material).

275 At ISR of 0.22 (VS basis), the two leachbeds in Test 2 achieved comparable methane
276 recovery, although the extent of substrate solubilisation was slightly higher in the flood-and-
277 drain system. VFAs rapidly accumulated at start-up but stabilised after day 16 with no net
278 production observed after that. Acetic and propionic acid were the most notable VFAs (Figs.
279 2c, d). Unlike Test 1, VFA accumulation in Test 2 caused a comparatively minor decrease in
280 pH (Figs. 2e, f), likely due to effects of the added inoculum (alkalinity and microbial
281 activity). For the same reason, methane production in Test 2 commenced near instantaneously
282 (Figs. 2a, b). Methane production in Test 2 was initially steady but ceased after day 16 (Figs.
283 2a, b). Nevertheless, the leachbed with added inoculum (Test 2) achieved the same methane
284 conversion within half the period taken by the trickling leachbed without added inoculum
285 (Test 1). The shorter processing time of the trickling leachbed could be desirable as this could
286 reduce leachbed size and cost. Overall, both the leachbeds in Test 2 achieved an extent of
287 substrate solubilisation of around 30% ($\text{CH}_4 + \text{sCOD}$) and methane recovery of about 50% of
288 the expected B_0 ($194 \text{ LCH}_4 \text{ kg}^{-1} \text{ VS}_{\text{fed}}$).

3.3 Post-digestion analysis

Table 3 and 4 summarized the key characteristics of the solid residue and residue leachate from leachbed trials, respectively. Characteristic of residues from flood-and-drain leachbed in Test 1 were not presented as the test failed to reach completion due to continual clogging.

Fig. 3 shows results of the fractionation of $t\text{COD}_{\text{fed}}$ as methane, leachate and solid residues for each leachbed at the end of Test 2. This data indicated that the flood-and-drain system mobilised more COD into the leachate than the trickling system (Fig. 3A). However, this COD that was mobilised by the flood-and-drain leachbed contained a large fraction of non-biodegradable material (Fig. 3B), which is unwanted because of little added benefit via further leachate processing into methane, and could cause materials handling issues at full-scale. It was suspected that the stainless steel conduits used in the flood-and-drain leachbed (Section 2.3) could have increased the entrainment of solids. Previous studies have shown that lignocellulosic waste can act as a viable filter media to promote separation of particulate solid from the liquid phase (Zhang & Lorimor, 2000). Thus, the absense of lignocellulosic materials in the conduits was likely the cause of migration of solid particle with leachate in the flood-and-drain system. Accordingly, a flood-and-drain bed without conduits may sufficiently deter the migration of suspended solids.

Moisture recovery from leachbed at the end of each experiment was 96 to 98% of the initial process water input, with the remaining process moisture likely retained in the saturated solid residues (Aikaterini, K., 2015). The residual B_0 was high for leachate recovered from the leachbed ($732 \pm 10 \text{ L CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{fed}}$ for trickling and $582 \pm 8 \text{ L CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{fed}}$ for flood-and-drain), indicating good potential for further processing into methane. These values corresponded to about 25 and 30% of the B_0 of the original raw spent bedding, respectively. With on-farm applications, this might involve further treating of leachate in existing methanogenic systems such as covered lagoons or digesters.

Fig. 4 presents results from residual analysis without added inoculum (water or leachate dilution only), performed on solid residue collected from the leachbeds. Based on the results, the full residual B_0 could only be recovered when an external inoculum was provided; indicating that background microbial activity in the spent bedding was insufficient. However, in the absence of external inoculum, the solid residues did continue to degrade in the post-methane tests (Fig. 4), even though methane production had ceased in the leachbeds of origin (Figs. 1, 2). Higher methane productivity was observed when the solid residues were diluted with more water or leachate, indicating mass transfer limitations in the leachbed, likely due to effects of leachate channelling through the solid bed. Inhibition testing (Astals et al., 2015a) was performed on samples of solid residue from the leachbeds and the results suggested that background ammonia in the leachbed was not at inhibitory levels (Figure S3 of Supplementary material). The composition of the leachate and solid residues matrices are expected to be complex and a range of other inhibitors could be present. While most compounds may be at low concentrations, some compounds can be inhibitory at very low levels (Chen et al., 2008). The effects of chemical inhibition (by compounds other than ammonia) and background microbial activity merit further investigation.

3. 4 Application

A leachbed can be particularly suitable for anaerobic treatment of solid agricultural residues on-farm, due to its relative simplicity compared to technology alternatives. Specifically, leachbeds have:

- no need for complex in-reactor mixing, because the leachbed relies on convective liquid movement for mass transfer (Vandevivere et al., 2003);
- the substrate largely handled in the original solid state without requiring any pre-treatment or conditioning (slurrying up), reducing system capital operating costs;

- flexible operation, because the leachbed could probably tolerate variations in substrate composition;
- efficient water-reuse, because of the recovery and reuse of leachate; and
- could allow use of equipment already available on-farm, such as front loaders and covered lagoons.

Leachbed process typically requires operating multiple leachbeds, parallel in single-stage, or feeding to a single methanogenic reactor (such as an existing anaerobic lagoon or mixed digester) in dual stage configuration (Nizami et al., 2010; Browne et al, 2013) to regulate biogas flow and quality. Based on the current study (Trial 2), a proposed operation of the leachbed could involve insertion of feedstock to the leachbed on a weekly basis. For example, a system composed of 4 batch leachbeds; the operating cycle might be 28 days with the solid retention time for about 21 days and an additional 7 days for substrate loading/unloading via front loaders, and then cleaning and maintenance for start-up of a new batch. The major drawback of this operation is that it may be labour-intensive. However, the skill level requirements could be tolerable for on-farm applications.

A sufficient amount of solid residues for inoculation provides the necessary alkalinity and microbial activity (Section 3.2) for leachbed start-up. The ISR used in the present study was comparable to the optimum ISR of 0.25 recommended by Cui et al. (2011) for leachbed start-up. The same study by Cui et al. (2011) also found that $ISR > 0.25$ did not yield any process improvement. However, the use of solid residues for inoculation decreases treatment capacity for fresh waste and might exacerbate clogging in a leachbed (Xu et al., 2012). Alternatively inoculation of a leachbed may be via ‘sequencing’ (Chugh et al., 1998). For example, a study by Kusch et al. (2008) demonstrated comparable AD performance between a leachbed system inoculated with solid residues at 20% inoculum (v/v) and via ‘sequencing’ when treating

spent bedding from horse stalls. Results from the present study suggested the use of a ‘sequencing’ technique for inoculation could be considered for a trickling type leachbed, but may not be an option for a flood-and-drain type leachbed because excessive entrainment of solids could be an issue (Section 3.3). Otherwise, solid particulates from leachate maybe separated via filtration or a solid trap prior to sequencing or recirculation. Shahriari et al. (2012) noted that inhibitors, such as ammonia, accumulated in leachate after multiple sequencing steps and recommended dilution of leachate with fresh make-up water before re-use. As the dilution approach would inevitably increase water requirements in the system, ammonia accumulation in the leachate may need to be addressed in other ways such as via air stripping (Abrood et al., 2014).

The performance of leachbeds did not greatly differ in the present study even with the use of markedly different leachate flow configurations (trickling versus flood-and-drain), both with the aim to promote mass transfer in solid bed (Section 3.2). Based on current leachbed design and operation, energy requirement (for instance, temperature control and leachate recirculation) between trickling and flood-and-drain should be comparable. However, as suggested by the residues analysis (Section 3.3), process performance of the leachbeds was still affected by mass transfer limitation, possibly due to leachate channelling. These are of particular concern for larger scale systems where short-circuiting or pooling/trapping of leachate are more likely to occur (Mussoline, 2013). It has been suggested that an increase in leachate recirculation rate could increase mass transfer (Vavilin et al. (2002), improve solubilisation (Chugh et al., 1998; Vavilin et al., 2002) and promote transport between acidogenic and methanogenic regions (Veeken and Hamelers, 2000). However, increased recirculation rates may not resolve leachate channelling (Morris et al., 2003). Other approaches to improve mass transfer in leachbed have included solid bed agitation by rotation (Vandevivere et al., 2003), aggressive pretreatments prior to digestion (Yang et al., 2015), or

the addition of bulking agents or reduction of particle size to improve microbial access to the substrate (Mata-Alvarez et al., 2000; Yang et al., 2015). However, such approaches are unlikely to be cost competitive or sufficiently simple for decentralized agricultural applications (Mata-Alvarez et al., 2000; Yang et al., 2015).

Based on the current study results, the trickling arrangement appeared to be preferred over the flood-and-drain arrangement. While the methane recovery via trickling and flood-and-drain were similar (Section 3.2), water requirement for flood-and-drain system for treatment of spent bedding (due to the need for substrate to be remained submersed) is more likely to become significantly higher than trickling system when operating at full-scale with multiple leachbeds configuration. This may complicate leachate handling at full scale due to increased leachate throughput, which can expect to increase capital and operating costs. Further, solids entrainment in leachate of a flood-and-drain system can be an operational nuisance, which implies that flood-and-drain may require more maintenance than trickling leachbed at full-scale.

At present there is no known farm-scale leachbed operating on spent bedding. While leachbeds have the potential to be economically viable on a small-scale farm even with a methane recovery at 50% of the B_0 , site-specific assessments should still be conducted because the feasibility of the process would depend on specific considerations such as on-site power usage and electricity costs, solid waste characteristics, government policy as well as opportunities for new revenues stream, such as nutrient recovery from treated waste streams (Wilkinson, 2011).

4. Conclusions

Single-stage pilot-scale leachbeds recovered 50% of the B_0 ($100 \text{ L CH}_4 \text{ kg}^{-1} \text{ VS}_{\text{fed}}$) for spent bedding from pigs/swine. Results showed methane production rate and yield were insensitive

to leachate flow configurations (trickling vs. flood-and-drain). Inoculation from a previous leachbed at ISR of 0.22 accelerated start-up of a subsequent leachbed, but the B_o of the spent bedding could not be fully recovered. Flood-and-drain mode did mobilise more of unwanted non-biodegradable particulates into leachate compared trickling mode, which may hinder performance at full-scale. Overall trickling is a preferred mode for leachate recirculation compared with flood-and-drain due to easier handling. The study highlighted a future need/opportunity to reach the ultimate methane potential by targeted research on microbial inhibition and inoculation techniques.

Acknowledgments:

This research was funded by the Australian Government Department of Agriculture, Quantum Power Limited, Australian Egg Corporation Limited and the CRC for High Integrity Australian Pork (Pork CRC). Australian Pork Limited was an in-kind contributor.

REFERENCE

- Abrood, A.R., Bao, J., Du, J., Zheng, D., Luo, Y., 2014. Non-biodegradable landfill leachate treatment by combined process of agitation, coagulation, SBR and filtration. *Waste Manag.*, 34, 439-447.
- Aikaterini, K., 2015. Characterizing water holding capacity and total solids of manure-bedding mixtures. PhD thesis, University of Illinois.
- Aland, A., Banhazi, T., 2013. *Livestock Housing: Modern Management to Ensure Optimal Health and Welfare of Farm Animals*, Wageningen Academic Publishers, Wageningen, Netherlands.
- Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Sci. Technol.*, 59, 927-934.
- Astals, S., Batstone, D.J., Tait, S., Jensen, P.D., 2015a. Development and validation of a rapid test for anaerobic inhibition and toxicity. *Water Res.*, 81, 208-215.
- Astals, S., Musenze, R.S., Bai, X., Tannock, S., Tait, S., Pratt, S., Jensen, P.D., 2015b. Anaerobic co-digestion of pig manure and algae: Impact of intracellular algal products recovery on co-digestion performance. *Bioresour. Technol.*, 181, 97-104.
- Batstone, D.J., 2013. Teaching uncertainty propagation as a core component in process engineering statistics. *Educ. Chem. Eng.*, 8, e132-e139.
- Batstone, D.J., Jensen, P.D., 2011. Anaerobic Processes, in: Wilderer, P. (Eds.), *Treatise on Water Science*. Elsevier. Oxford, pp. 615-639.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T., Siegrist, H., Vavilin, V.A., 2002. The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Sci. Technol.*, 45, 65-73.

Browne, J.D., Allen E., Murphy, J.D., 2013. Improving hydrolysis of food waste in a leached reactor. *Waste Manag.*, 33, 2470-2477.

Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: A review. *Bioresour. Technol.*, 99, 4044-4064.

Chugh, S., Chynoweth, D.P., Clarke, W., Pullammanappallil, P., Rudolph, V., 1999. Degradation of unsorted municipal solid waste by a leach-bed process. *Bioresour. Technol.*, 69, 103-115.

Chugh, S., Clarke, W., Pullammanappallil, P., Rudolph, V., 1998. Effect of recirculated leachate volume on MSW degradation. *Waste Manag. Res.*, 16, 564-573.

Clarke, W., Xie, S., 2013. Rapid digestion of shredded MSW by sequentially flooding and draining small landfill cells. *Sardinia_2013: 14th International Waste Management and Landfill Symposium*. Cisa Publisher.

Cui, Z., Shi, J., Li, Y., 2011. Solid-state anaerobic digestion of spent wheat straw from horse stall. *Bioresour. Technol.*, 102, 9432-9437.

Demirel, B., Scherer, P., 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. *Environl. Sci. Bio/Technol.*, 7, 173-190.

Deublein, D., Steinhauser, A., 2011. *Biogas from Waste and Renewable Resources : An Introduction*, fourth ed. Wiley, Hoboken.

Franson, M.A.H., Eaton, A.D., Association., A.P.H., Association., A.W.W., Federation., W.E., 2005. Standard methods for the examination of water & wastewater, 21st ed. American Public Health Association, Washington, DC.

Jensen, P.D., Ge, H., Batstone, D.J., 2011. Assessing the role of biochemical methane potential tests in determining anaerobic degradability rate and extent. *Water Sci. Technol.*, 64, 880-886.

Jha, A.K., Li, J.Z., Nies, L., Zhang, L.G., 2011. Research advances in dry anaerobic digestion process of solid organic wastes. *African J. Biotechnol.*, 10, 14242-14253.

Kruger, I., Taylor, G., Rosese, G., Payne, H., 2006. Primefact 68, Deep- litter housing for pigs. NSW DPI, Sydney, Australia.

Kusch, S., Oechsner, H., Jungbluth, T., 2008. Biogas production with horse dung in solid-phase digestion systems. *Bioresour. Technol.*, 99, 1280-1292.

Mata- Alvarez, J., Macé, S., Llabrés, P., 2000. Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresour. Technol.*, 74,3-16.

Morris, J.W.F., Vasuki, N.C., Baker, J.A., Pendleton, C.H., 2003. Findings from long-term monitoring studies at MSW landfill facilities with leachate recirculation. *Waste Manag.*, 23, 653-666.

Mussoline, W., 2013. Enhancing the methane production from untreated rice straw using an anaerobic co-digestion approach with piggery wastewater and pulp and paper mill sludge to optimize energy conversion in farm-scale biogas plants. PhD thesis, Université Paris-Est.

Nizami, A.S., Thamsiroj, T., Singh, A., Murphy, J.D., 2010. Role of leaching and hydrolysis in a two-phase grass digestion system. *Energy Fuels*. 24, 4549-4559.

Peces, M., Astals, S., Mata-Alvarez, J., 2014. Assessing total and volatile solids in municipal solid waste samples. *Environ. Technol.*, 35, 3041-3046.

Shahriari, H., Warith, M., Hamoda, M., Kennedy, K.J., 2012. Effect of leachate recirculation on mesophilic anaerobic digestion of food waste. *Waste Manag.*, 32, 400-403.

Shi, J., Xu, F., Wang, Z., Stiverson, J.A., Yu, Z., Li, Y., 2014. Effects of microbial and non-microbial factors of liquid anaerobic digestion effluent as inoculum on solid-state anaerobic digestion of corn stover. *Bioresour. Technol.*, 157, 188-196.

Sommer, S.G., Møller, H.B., 2000. Emission of greenhouse gases during composting of deep litter from pig production – effect of straw content. *J. Agri. Sci.*, 134, 327-335

Tait, S., Tamis, J., Edgerton, B., Batstone, D.J., 2009. Anaerobic digestion of spent bedding from deep litter piggery housing. *Bioresour. Technol.*, 100, 2210-2218.

Tong, X., Smith, L.H., McCarty, P.L., 1990. Methane fermentation of selected lignocellulosic materials. *Biomass*. 21, 239-255.

U.S. Composting Council, 2002. Test methods for the examination of composting and compost. Composting Council Research and Education Foundation, Bethesda.

Vandevivere, P., Baere, L.D., Verstraete, W., 2003. Types of anaerobic digesters for solid wastes, in: Mata-Alvarez, J. (Eds), *Biomethanization of the organic fraction of municipal solid wastes*. IWA London, UK, pp 111-137.

510 Vavilin, V.A., Shchelkanov, M.Y., Rytov, S.V., 2002. Effect of mass transfer on
 511 concentration wave propagation during anaerobic digestion of solid waste. *Water Res.*,
 512 36, 2405-2409.

513 Veeken, A.H., Hamelers, B.V., 2000. Effect of substrate-seed mixing and leachate
 514 recirculation on solid state digestion of biowaste. *Water Sci. Technol.*, 41, 255-62.

515 Wilkinson, K.G., 2011. A comparison of the drivers influencing adoption of on-farm
 516 anaerobic digestion in Germany and Australia. *Biomass and Bioeng.*, 35: 1613-1622 e.

517 Xu, S.Y., Karthikeyan, O.P., Selvam, A., Wong, J.W.C., 2012. Effect of inoculum to
 518 substrate ratio on the hydrolysis and acidification of food waste in leach bed reactor.
 519 *Bioresour. Technol.*, 126, 425-430.

520 Yang, L., Xu, F., Ge, X., Li, Y., 2015. Challenges and strategies for solid-state anaerobic
 521 digestion of lignocellulosic biomass. *Renewable Sustainable Energy Rev.*, 44, 824-834.

522 Zhang, M., Lorimor, J., 2000. Manure solids separation by filtration with four crop
 523 residues. *Trans. Am. Soc. Agric. Eng.*, 43, 981-986.

524 **Tables and figures**

525 **Table 1** Initial start-up conditions of leachbeds in Test 1 and 2.

526 **Table 2** Measured spent bedding characteristics from deep litter housing at site A and B in
527 Queensland, Australia.

528 **Table 3** Measured solid residue characteristics from pilot-scale leachbed trials.

529 **Table 4** Measured residue leachate characteristics from pilot-scale leachbed trials.

530 **Fig. 1** Leachbeds performance from Test 1: soluble substrate (measured as $sCOD_{leachate}$) and
531 cumulative methane (COD_{CH_4}) (a-b), VFAs (c-d), and pH (e-f) profiles of leachbed reactors
532 from Test 1.

533 **Fig. 2** Leachbed performance from Test 2: soluble substrate ($sCOD_{leachate}$) and cumulative
534 methane (COD_{CH_4}) (a-b), VFAs (c-d), and pH (e-f) profiles

535 **Fig. 3** Residue analysis: (a) Methane, leachate and solid residues in g COD as percentage of g
536 COD_{fed} of pilot-scale experiments after 50 days of operation; (b) Post-digestion residual
537 methane analysis of leachate from Test 2 to assess ultimate biodegradability.

538 **Fig. 4** Post-digestion residual methane analysis of solid residue diluted with leachate and
539 water at different dilution factors to assess the mass transfer limitation and biological
540 inhibition of leachbeds in Test 2: (a) trickling; (b) flood-and-drain.

541 **Supplementary material**

542 **Fig. S1.** Detailed schematic of pilot-scale leachbed system setup: (a) trickling; (b) flood-and-
543 drain.

544 **Fig. S2.** Methane and CO_2 concentration profile of trickling and flood-and-drain type pilot-
545 scale leachbed in (a) Test 1; (b) Test 2

546 **Fig. S3** Inhibition testing for ammonia using solid residues from Test 2 leachbeds as
547 inoculum: (a) trickling; (b) flood-and-drain. Inhibition coefficient (KI_{50}) indicated biostatic
548 inhibition potential of ammonia, representing the concentration at which the uptake rate is
549 half the maximum.