**Recycling separated liquid-effluent to dilute feedstock in anaerobic digestion
of dairy manure**

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Abstract

The major concern of recycling anaerobic digestion (AD) effluent in the digester centers on accumulation of total ammonia nitrogen (TAN) and salinity, both of which can potentially inhibit methane production. In the current study, 30%, 50%, and 80% of separated-liquid AD effluent, were recycled in a series of batch AD experiments. The inhibitions to specific methane potential (SMP) caused by TAN and salinity were evaluated. Recycling up to 80% of un-treated effluent resulted in the best SMP averaging $0.265 \pm 0.005 \text{ m}^3 [\text{CH}_4] \text{ kg}^{-1} [\text{volatile solids}]$, which averaged 10% more compared to recycling 80% treated effluent and 5% more compared to no recycling (the control). After acclimation, up to 6.39 g N L^{-1} increase in TAN resulted in SMP averaging $0.112 \pm 0.002 \text{ m}^3 [\text{CH}_4] \text{ kg}^{-1} [\text{volatile solids}]$ and up to 12 parts per thousand increase in salinity resulted in SMP averaging $0.163 \pm 0.005 \text{ m}^3 [\text{CH}_4] \text{ kg}^{-1} [\text{volatile solids}]$. A mass balance for a hypothetical 5,000 cows dairy farm showed effluent recycle of up to 66% for maintaining 8% solids in anaerobic digester. Moreover, in the proposed system, the effluent going off-farm was on w/w basis 64% less water, 66% less solids, and 52% less nitrogen compared to the effluent produced with no recycle facility.

Keywords: Specific methane production, Anaerobic digestion, Dairy manure, Effluent recycling, Inhibition.

Nomenclature

AD	Anaerobic digestion
TAN	Total ammonia nitrogen (g N L^{-1})
SAL	Salinity (ppt)
SMP	Specific methane potential ($\text{m}^3 \text{CH}_4 \text{ kg}^{-1}$ volatile solids)
ppt	Parts per thousand
LF	Loading factor
AS	Acclimated seed
UAS	Un-acclimated seed
TE	Treated effluent
UTE	Un-treated effluent

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44 **1. Introduction**

45 Anaerobic digestion (AD) is a proven manure management technology that
46 mitigates emissions of odor, pathogens, and greenhouse gases (Ma et al., 2013).
47 Moreover, the production of bio-methane as renewable energy and nutrient rich
48 digestate are additional benefits of AD technology (Pöschl et al., 2010). In U.S, AD
49 technology mostly utilizes a slurry approach (i.e. complete mix, plug-flow),
50 receiving influents with total solids (TS) in the range of 4–12% resulting from typical
51 “flushing” or “scraping” manure collection systems (Frear et al., 2011). Either way,
52 the AD influent requires some degree of dilution, using either fresh, parlor, or
53 lagoon water. The dilution of manure and operation of the slurry digester yields

benefits to microbial action and engineering design (Powers et al., 1997). However, it also results in greater wastewater volume (2–6 times that of as-produced manure) requiring eventual disposal to fields or treatment in potentially expensive post-AD nutrient separation/recovery systems to meet environmental regulations (Spellman and Whiting, 2007; Ma et al., 2013). In 1999, USDA and USEPA developed a unified strategy to address the environmental and public health concerns related to animal feeding operations. Accordingly, all animal feeding operations were expected to develop and implement a farm specific Comprehensive Nutrient Management Plan (CNMP) to reduce the environmental and public health risks posed by animal feeding operations (USDA and USEPA, 1999). A CNMP is a site-specific conservation plan that documents on farm conservation practices (for example, manure handling, storage, and land application) dealing with the concerns related to soil erosion, livestock manure, and disposal of organic by-products. To further reduce the public health and environmental impacts of animal feeding operations, particularly, the concentrated animal feeding operations the National Pollutant Discharge Elimination System (NPDES) permits are required to show the compliance with clean water act. These permits ensure the proper handling and utilization of the animal manure generated on-farm and its proper land application. These regulatory interventions are devised not only for environmental and public health safety related to animal manure but the compliant farms also get tax incentives (USDA and USEPA, 1999). Thus, timely interventions to mitigate the environmental and economic concerns associated with voluminous amounts of wastewater generated by livestock industry are required to enhance

the sustainability of on-farm organic waste management (Tiwary et al., 2015). One potential method to alleviate this wastewater volume concern is to utilize the liquid stream of digested effluent as return or dilution water to dilute the next batch of feedstock entering the digester. This can reduce fresh water inputs to the digester, leaving a portion of the effluent continually within the system and, therefore, not in need of disposal to fields. This practice can be particularly beneficial to high fiber content substrate like dairy manure. For high fiber substrates, about 25–30% of the methane potential is estimated to remain intact even after AD (Jagadabhi et al., 2008). Thus, efficient utilization of this residual methane potential can be realized through the proposed recycling of AD effluent.

The concerns surrounding recycling of the digestate during AD is the potential for accumulation of key chemical inhibitors to the AD microbiology like ammonia and salinity (McCarty and McKinney, 1961; Chen et al., 2008; Yenigün and Demirel, 2013). In the AD process, ammonia is a product of breakdown of organic compounds and exists either as ammonia (NH_3), ammonium (NH_4^+), or combination of both, commonly referred to as total ammonia nitrogen, TAN ($\text{NH}_3 + \text{NH}_4^+$), depending on pH-conditions. Bacteria need some of this ammonia for their growth; however, excessive accumulation can be inhibitory for methanogens (Chen et al., 2008). High concentrations of ammonia can disturb the optimum C/N in the digester resulting in loss of methanogens and lowering of biogas production (Sakar et al., 2009). Salinity is another important factor which can cause inhibition of the AD process (McCarty and McKinney, 1961; Yenigün and Demirel, 2013). While moderate concentration of salts is necessary for methanogenic growth, high salt

content may cause dehydration of methanogenic cells (Yerkes et al., 1997). Thus, a viable solution to the accumulation of ammonia and salinity is necessary in order to recycle AD digestate with minimal effect on either biogas or methane yield.

Past studies evaluating the effects of recycling AD digestate on methane production present contradicting results. For instance, Jagadabhi et al. (2008) observed a decrease in methane production during recycling of alkali treated solid portion of the digestate, whereas Estevez et al. (2014) observed a 16% increase in methane yield with liquid digestate recycling as compared to no recycling. In the latter case, ammonia or volatile fatty acids did not inhibit the process although the solids accumulation in the digester over time resulted in a reduction in methane production. In a recent study, accumulation of ammonia caused by digestate recycling during AD was associated with 43% reduction in biogas production (Wu et al., 2016). However, subsequent treatment of pH adjusted digestate (ammonia air stripping), to remove ammonia, resulted in biogas recovery. In general, inhibition to biogas production due to solids accumulation, ammonia accumulation, or chemical input to adjust pH, for that matter, can limit the amount of digestate that can be recycled.

This study investigated the recycling of only the separated-liquid portion of the digestate after solids removal through centrifugation. The hypothesis was that a combination of AD and post-AD solids separation would effectively control potential inhibitory components, particularly salinity and TAN, which would allow substantial re-use of separated liquid-effluent as dilution water for dairy manure entering AD.

The specific objectives of this study were to: (1) determine the effects of salinity

and TAN accumulation due to recycling of separated liquid-effluent on methane production; and (2) present mass balances of water, solids, and nutrients for a typical dairy farm with an effluent-liquid recycle system.

2. Materials and methods

2.1. Feedstock and inoculum

Fresh “as excreted” dairy manure collected from the Knotts Dairy Center, a Research facility at Washington State University, Pullman, WA., USA was used as substrate in this study. Total solids (TS), volatile solids (VS), pH, and total ammonia nitrogen (TAN) of the manure were $13.72 \pm 0.08\%$, $12 \pm 0.07\%$, 7.80 ± 0.02 , and $1.30 \pm 0.02 \text{ g N L}^{-1}$, respectively. Mesophilic anaerobic digested sludge (inoculum) with $1.54 \pm 0.14\%$ TS and $1.11 \pm 0.01\%$ VS was collected from a local Municipal Wastewater Treatment Plant located in Pullman, WA.

2.2. Test setup and experimental design

A series of batch AD tests were performed following the principles described by Owen et al. (1979) and modified by others (Labatut et al., 2011; Ma et al., 2013). During these tests, specific methane potential (SMP) was used as the performance indicator of the substrate’s methane production. The SMP for this study was defined as the total volume of methane produced over the course of digestion period per unit weight of the substrate’s volatile solids (i.e. $\text{m}^3 \text{CH}_4 \text{kg}^{-1}$ substrate VS). The use of SMP as a performance indicator remedied the skew of methane production caused by some input changes or volatilization of VS for the treated recycle-liquid during TAN treatments.

The substrate and anaerobic digester sludge were inoculated at 1:1 (substrate VS: inoculum VS) in 500-mL serum bottles. The digesters were then flushed with nitrogen for 3 min and sealed immediately with screw caps equipped with rubber septa. Afterwards the digesters were placed in temperature-controlled room for 20 d at mesophilic temperature ($37\pm1^{\circ}\text{C}$) and 150 RPM mixing. Previous research indicates that the 20 d digestion time of dairy manure yields 80–90% of total potential biogas production (Kafle and Chen, 2016). To measure background methane production from the inoculum, two additional bottles with “inoculum only” were also placed in the temperature-controlled room.

After 20 d digestion time, 100 mL of mixed liquor effluent (20% v/v) was saved as seed for the subsequent digestion, while the remaining 400 mL mixed liquor effluent (total discharge volume) was centrifuged at 4500 rpm for five minutes to remove fine suspended solids. Following centrifugation, 30%, 50%, and 80% of the separated liquid-effluent (v/v) were recycled in the next digestion cycle as three separate treatments. All treatments were studied in duplicates and the resulting TAN, salinity, and SMP values expressed as means and standard deviations. To determine the inhibitory effects caused exclusively by salinity and TAN, two different sets of AD digesters were studied with the same recycle ratios. To study the effects of salinity only, the digestate was treated (aerated) before centrifugation and recycling to remove up to 40% TAN according to the methods described by Zhao et al. (2015). Briefly, air was pumped into the bottom of the respective digester through an air stone (Aqua-Mist, Hauppauge, NY) using a peristaltic pump (Masterflex L/S 7524-40, Fisher Scientific, Pittsburg, PA) at a constant flow rate of

400 mL min⁻¹. Twelve hours of aeration at 55°C under such conditions was found to remove up to 40% of TAN from digestate. On the other hand, untreated digestate was centrifuged and recycled for TAN inhibition studies. The two loading factors used during these experiments were 10 and 20 g VS L⁻¹. The protocol used for the digestion experiment is summarized in Table 1.

2.3. Manual loading of TAN and salinity in digesters

An additional set of biological methane potential tests was conducted to better determine the inhibitory effects of total ammonia nitrogen (TAN) and salinity on AD of dairy manure. Desired amounts of TAN and salinity (mentioned in table 1) were maintained in the digesters by externally adding ammonium chloride (NH₄Cl) and potassium chloride (KCl) salts, respectively, in the TAN and salinity digesters. Two treatments of this experiment included manual dosing of: (1) an acclimated seed obtained from the 'digestion and recycling' experiment in this study, and; (2) fresh un-acclimated seed obtained from Municipal Wastewater Treatment Plant located in Pullman, WA. Inoculum obtained from the 'digestion and recycling' experiment (used only for the first acclimated seed digestion) had already gone through more than four months of digestion and was expected to have achieved some level of acclimation (Rajagopal et al., 2013). For the rest of acclimated seed digestions 20% v/v of the digestate from each digester was used as inoculum for the successive digestion cycle. On the other hand, all un-acclimated digestions were simultaneously carried out with a fresh inoculum obtained from a local Municipal Wastewater Treatment Plant. All the digestions in this experiment were carried out

at a loading factor of 20 g VS L⁻¹. Before start of each digestion the digesters in this experiment were set to a neutral pH using 1M NaOH.

2.4. Analytical methods

Total solids and volatile solids of the dairy manure and inoculum were measured according to Standard Methods (APHA, 1998). Daily biogas produced in each bottle was measured using a 250-mL industrial luer lock glass syringe equipped with 20-guage needle (Tomopal Inc., Sacramento, CA, USA). Gas samples were collected every 6 d during digestion for a total of three times per digestion and stored in 12-mL evacuated borosilicate vials (Extainer, Labco Limited, Wycombe, England). The CH₄ and CO₂ contents of the biogas were determined via a Varian gas chromatograph (Palo Alto, CA, USA) equipped with a thermal conductivity detector according to a method described by Wen et al. (2007). A Restek (Bellefonte, PA, USA) Shincarbon column (2 × 1/16 inch) with silicosteel packing material (100/120 mesh) was used, and nitrogen served as the carrier gas. Each type of gas was quantified based on calibration curve. At the end of each digestion run, liquid samples from the digestate were analyzed for total ammonia nitrogen as grams of nitrogen per liter (g N L⁻¹) according to APHA (1998) Standard Methods using a Tecator 2300 Kjeltac Analyzer (Eden Prairie, MN, USA; 4500-NorgB; 4500NH3BC), and salinity was measured as parts per thousand (ppt) according to APHA (1998) Standard Methods using a conductivity meter (Metler Toledo SevenGo Pro, AG 8603 Schwerzenbach, Switzerland).

2.5. Statistical analysis

One-way Analysis of variance (ANOVA) procedures were performed using Minitab[®] Statistical Software (version 16) at the significance level (α) of 0.05 to determine statistically significant differences ($P < 0.05$) between treatments. The mean values for TAN, salinity, and SMP obtained for different treatments were compared to find statistically significant differences using Tukey method. A summary of the statistical results for control and 80% effluent recycle treatment is presented in Table 2.

3. Results and discussion

3.1. Effects of digestate recycling on TAN, salinity, and SMP

Fig. 1 represents the average TAN, salinity, and SMP values for anaerobic digestions of dairy manure with recycle of separated liquid-effluent at 10 and 20 g VS L⁻¹ loading factors. Fig. 1a and 1b show that the TAN and salinity values for the 80% un-treated were significantly different ($P < 0.05$) from 80% treated and other effluent-recycle treatments yet the SMPs for all treatments were not significantly different ($P > 0.05$) from each other (Fig. 1c). The average SMPs for treated 30%, 50%, and 80% separated liquid-effluent treatments were 0.223 ± 0.018 , 0.251 ± 0.022 , and 0.241 ± 0.007 m³ CH₄ kg⁻¹ VS, respectively, while the average SMPs for corresponding un-treated effluent treatments were 0.220 ± 0.012 , 0.262 ± 0.012 , 0.265 ± 0.005 m³ CH₄ kg⁻¹ VS. The average SMP for the control across all digestions was 0.252 ± 0.005 m³ CH₄ kg⁻¹ VS. These SMP values are similar to values of methane production reported in the previous studies for uninhibited batch mode anaerobic digestion of dairy manure. Ma et al. (2013)

reported yields of $0.200 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$, while Kafle and Chen (2016) observed productions of $0.240 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$. Thus, based on the scope of our study, only the results related to 80% liquid-effluent recycle (treated and untreated) were discussed further in next sections.

Fig. 2a presents the TAN values for the 80% liquid-effluent recycle (treated and untreated) and the control. On average, for seven digestion-cycles, the TAN values were $0.470 \pm 0.012 \text{ g N L}^{-1}$ for the control, $0.568 \pm 0.010 \text{ g N L}^{-1}$ for the 80% treated effluent, and $1.112 \pm 0.016 \text{ g N L}^{-1}$ for the 80% un-treated effluent treatment. The TAN values for the 80% un-treated effluent recycle were significantly higher ($P < 0.05$) than for the 80% treated effluent recycle and the control (Table 2). In general, a decreasing trend for TAN was observed in successive digestion-cycles. The decrease in TAN was attributed to the removal of fine solids from the digestate through centrifugation before recycling in the next digestion. The AD of dairy manure increases TAN concentration in the effluent by as much as 35% (Frear et al., 2012) and most of this TAN is adsorbed on the solid surface (Zhang and Westerman, 1997; Neerackal et al., 2015). Neerackal et al. (2015) observed a 56% reduction in the TAN concentration of AD effluent after liquid-solid separation. Thus, an important conclusion that can be drawn for AD of dairy manure practicing 20% sludge recycle followed by solids removal is that under the simulated commercial conditions of AD treatment, TAN concentrations may not accumulate.

Fig. 2b shows the salinity values for the 80% effluent recycle (treated and untreated) and the control in parts per thousand (ppt). On average for seven digestion-cycles, the salinity values were $2.750 \pm 0.047 \text{ ppt}$ for the control,

4.090±0.095 ppt for the 80% treated effluent, and 6.630±0.162 ppt for the 80% un-treated effluent treatments. The salinity values for the 80% un-treated effluents were significantly higher ($P<0.05$) than for the 80% treated effluent and the control (Table 2). Similar to TAN, salinity also decreased with increasing number of recycles (Fig. 2b). This lack of salinity accumulation can also be attributed to removal of fine solids from the digestate through centrifugation before the next digestion-cycle. On average the centrifuged liquid-effluent was 36% lower in salinity for the 80% treated effluent recycle and 30% lower in salinity for the 80% un-treated effluent recycle as compared to unseparated digestate (data not shown).

Fig. 2c presents the SMP values for the 80% effluent recycle (with and without treatment) and the control. On average, for seven digestion-cycles, the SMP values were $0.252\pm0.005 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$ for the control, $0.241\pm0.007 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$ for the 80% treated effluent, and $0.265\pm0.005 \text{ m}^3 \text{ CH}_4 \text{ kg}^{-1} \text{ VS}$ for the 80% un-treated effluent recycle. Thus, on average the 80% un-treated recycle produced 10% more SMP compared to the 80% treated effluent recycle and 5% more SMP than the control. Table 2 summarizes the mean TAN, salinity, and SMP values for the control and the 80% recycle treatments. In general, the SMP increased in each successive digestion-cycle for the control and the 80% effluent recycle treatments at both loading factors (Fig. 2c). However, after increasing the loading factor to 20 g VS L^{-1} at 5th-cycle, there was a sudden drop in SMP for all the treatments. At this point TAN and salinity values also increased for all the treatments compared to previous digestions at 10 g VS L^{-1} loading factor (Fig. 2a). As apparent in the Figs. 2a and 2b, however, these increased TAN and salinity values, were lower than

2.00 g N L⁻¹ for TAN and below 8 ppt for salinity described previously as inhibitory levels of TAN and salinity for AD (Liu and Sung, 2002; Chen et al., 2008). Thus, the sudden drop in SMP at elevated loading factor may be attributed to significant increase in solids content of the reactor according to previous research (Estevez et al., 2014). However, in the current study, the SMP started to rise in successive digestion-cycles at 20 g VS L⁻¹ loading factor (Fig. 2c). The trend of rising SMPs in successive digestion-cycles can be associated with undigested volatile solids carried over to next digestion in the recycled effluent, which possibly increased the overall SMP performance of the digester.

The observed data for the 80% un-treated effluent recycle suggests that after a series of seven digestion-cycles, even with no TAN treatment, there was no inhibition in methane production. Moreover, the observation that SMP values for the 80% un-treated effluent recycle treatment closely resembled the methane production values reported in literature (Ma et al., 2013; Kafle and Chen, 2016) indicates that recycling of untreated separated liquid-effluent is viable for AD of dairy manure.

3.2. Effects of TAN and salinity on SMP

Figs. 3a and 3c summarize the SMP for acclimated seed digestions with manual addition of TAN and salinity. It is evident in Fig. 3a that the first digestion of acclimated seed with initial TAN concentration of 0.88 g N L⁻¹ resulted in a SMP value of 0.237±0.006 m³ CH₄ kg⁻¹ VS. Subsequently, 13% and 54% drop in SMP was observed with increase in TAN concentrations to 1.86 and 2.79 g N L⁻¹, respectively. Starting at 2.79 g N L⁻¹ the next four TAN increases of 3.30, 3.98,

5.13, and 6.39 g N L⁻¹ showed an average SMP of 0.112±0.002 m³ CH₄ kg⁻¹ VS which is about 64% SMP drop compared to digestion with 0.88 g N L⁻¹. However, these five digestions showed a consistent SMP performance even with successive increase in TAN concentration from 2.79 g N L⁻¹ to 6.39 g N L⁻¹. This resistance to inhibition (between TAN concentrations of 2.79 g N L⁻¹ and 6.39 g N L⁻¹) can be attributed to acclimation of the microbial community in the digesters. Acclimation in anaerobic digesters is achieved within a few months with gradual increase in dosing of inhibitory agents like TAN or salinity (Rajagopal et al., 2013). Further drop in SMP was observed when TAN concentration was increased to 8.50 g N L⁻¹ with a corresponding SMP of 0.081±0.000 m³ CH₄ Kg⁻¹ VS and finally for 11.52 g N L⁻¹ the SMP was 0.054±0.000 m³ CH₄ Kg⁻¹ VS (Fig. 3a).

The saline dosed digestions with acclimated seed showed the highest SMP value of 0.281±0.006 m³ CH₄ Kg⁻¹ VS at a salinity concentration of 3 parts per thousand (ppt) (Fig. 3c). The SMP dropped by 36% in next digestion with 4 ppt of salinity. However, compared to TAN digesters the salinity digesters showed lower drop in SMP for similar increases in salinity. For instance, the SMP drop for about 2 ppt increase in salinity concentration going from 3 ppt to 5 ppt salinity was 43% (Fig. 3c) against 54% SMP drop for 2 g L⁻¹ increase in TAN concentration going from 0.88 g N L⁻¹ to 2.79 g N L⁻¹ (Fig. 3a). Saline dosed digestions showed a resistance to depression in SMP between 4 ppt and 12 ppt of salinity (Fig. 3a). The average SMP for 4, 5, 6, and 12 ppt of salinity was 0.163±0.005 m³ CH₄ Kg⁻¹ VS. Again the acclimation of microflora in the salinity digesters against slowly accumulating salinity can be presented as a reason for depressed but consistent

SMP performance. Further drop in SMP for salinity digesters was observed at very high salinity dosages of 24, 36, and 48 ppt of salinity with respective SMPs at 0.088 ± 0.001 , 0.048 ± 0.001 , and 0.016 ± 0.00 $\text{m}^3 \text{CH}_4 \text{Kg}^{-1} \text{VS}$.

Un-acclimated seed digestions were simultaneously carried out at five different TAN and salinity doses—1.20, 2.47, 4.83, 7.99, and 10.51 g N L^{-1} for TAN and 3, 4, 12, 24, and 48 ppt for salinity study. Figures 3b and 3d depict the SMP of un-acclimated seed digestions at respective TAN and salinity concentrations.

According to Fig. 3b it is apparent that the un-acclimated TAN digester with 1.20 g N L^{-1} TAN concentration showed an SMP of $0.153 \pm 0.000 \text{ m}^3 \text{CH}_4 \text{Kg}^{-1} \text{VS}$. This represents a 29% reduction in SMP compared to acclimated TAN digester at 1.86 g NL^{-1} ($0.215 \pm 0.001 \text{ m}^3 \text{CH}_4 \text{Kg}^{-1} \text{VS SMP}$) and 41% reduction in SMP compared to the corresponding control SMP ($0.258 \pm 0.008 \text{ m}^3 \text{CH}_4 \text{Kg}^{-1} \text{VS}$). Similarly, other un-acclimated TAN digesters with 2.47, 4.83, 7.99, and 10.51 g N L^{-1} showed lower SMP values compared to respective acclimated TAN digesters and the control (Figs. 3a and 3b). Similar findings of SMP inhibition in un-acclimated digesters due to increased TAN concentrations are reported in literature (Yenigün and Demirel, 2013; Chen et al., 2008).

In Fig. 3d it can be seen that the SMP for un-acclimated digester at 3 and 4 ppt salinity concentration is 0.153 ± 0.001 and $0.173 \pm 0.001 \text{ m}^3 \text{CH}_4 \text{Kg}^{-1} \text{VS}$, respectively, which is comparable to $0.173 \pm 0.003 \text{ m}^3 \text{CH}_4 \text{Kg}^{-1} \text{VS SMP}$ for acclimated salinity digester at 4 ppt salinity concentrations. However, each successive increase in salinity concentration in un-acclimated digesters resulted in further inhibition in SMP. Overall, the un-acclimated salinity digesters showed

lower SMP values than the corresponding controls and acclimated digesters with comparable salinity concentrations. However, the un-acclimated salinity digesters showed comparable SMP inhibitions at much higher concentrations than un-acclimated TAN digesters. For instance, at 1.20 g N L^{-1} the un-acclimated TAN digester showed $0.153 \pm 0.002 \text{ m}^3 \text{ CH}_4 \text{ Kg}^{-1} \text{ VS SMP}$, whereas, the un-acclimated salinity digester showed $0.173 \pm 0.001 \text{ m}^3 \text{ CH}_4 \text{ Kg}^{-1} \text{ VS SMP}$ even at 4 ppt of salinity concentration (Fig. 3b and 3d).

In the current study, irrespective of TAN or salinity treatment, all acclimated seed digestions showed higher SMP values compared to respective un-acclimated seed digestions. Acclimation of microbial population is achieved by gradually increasing the concentration of ammonia or other salts in the digester (McCarty and McKinney, 1961). This acclimation can be a consequence of changes in intracellular mechanisms in dominant species or growth of specific methanogens now adapted to higher concentrations of the inhibitory agents (Zeeman et al., 1985). Zeeman et al. (1985) reported inhibition in methane production for an un-acclimated cow manure digestion at a TAN concentration of 1.70 g N L^{-1} . However, after acclimation they noticed a consistent methane production at TAN concentration of 3.30 g N L^{-1} . Hashimoto (1986) also reported stable methane production for an acclimated seed at 4 g N L^{-1} TAN concentration whereas an inhibited performance for an un-acclimated seed was reported at only 2.50 g N L^{-1} TAN concentration. In a similar study, Angelidaki and Ahring (1994) reported consistent methane production for an acclimated thermophilic anaerobic digestion of cattle manure at a TAN concentration of 6.00 g N L^{-1} . As for salinity,

methanogens have shown different requirements for growth and inhibition to salts. For instance, Ca^{2+} and Na^{+} ions are required for an optimal growth of methanogens at a concentration of 0.20 ppt while a severe inhibition was caused by both ions at 8.00 ppt (Kugelman and McCarty, 1964). With respect to K^{+} , less than 0.40 ppt is reported as beneficial for microbial growth (Chen et al., 2008), while higher concentrations result in neutralization of the cell membranes (Jarrell et al., 1984). Here again an acclimated microflora was found to give better methane production performance compared to an un-acclimated one. Chen et al., (2008) reported that the lethal concentration of Na^{+} affecting the growth of methanogens for acclimated methanogens increased from 12.70 ppt to 22.80 ppt and also the lag phase to methane production was shortened. Nonetheless, once the acclimation is achieved, the microbial population can tolerate higher concentrations of TAN or salinity and show consistency in methane production.

3.3. Mass balance calculation

A hypothetical mass balance considering water, solids, and nutrients for a dairy farm with 5,000 cows is presented in Figs. 4 and 5. The manure production for a dry lot dairy manure collection system was assumed to be $22.7 \text{ Kg. cow}^{-1} \text{ d}^{-1}$ with total solids (TS) content of 17.3%. In addition, a parlor water stream of $65.6 \text{ Kg. cow}^{-1} \text{ d}^{-1}$ with TS of 0.7% was also considered for mass balance. Two scenarios were evaluated in this mass balance study. First, majority part of the digester effluent-liquid was recycled to the digester to dilute the vacuumed feed lane manure to achieve a TS content of 8%. Moreover, the parlor water was discharged directly supposedly with its own storage and treatment (Fig. 4). Second, part of

parlor water was used to dilute vacuum collected dry manure to achieve 8%TS content in the digester. For the latter scenario, the digester effluent was discharged without recycle (Fig. 5). The former is a mass balance depicting the current study, while the latter is a control more indicative of typical dairy digester projects. In both scenarios, a dissolved air floatation unit in addition to coarse solid separation (primary and secondary screening) are included to represent fine solids removal process.

An itemized comparison of the water and nutrient flow through and after the two scenarios is summarized in Table 3. The scenario with effluent recycle produced a liquid-effluent stream with less nutrients and solids with more nutrients from the post digestion processing. On the contrary, the scenario without effluent-liquid recycle produced an effluent stream with more nutrients and solids with less nutrients from post digestion processing.

The total reduction in effluent nitrogen as well as increased nutrient concentration of the recovered solids depicted in scenario 1 is a result of the proposed recycle concept. Repeated recycle with control of TAN and salinity through solid separation increased nutrient loadings to both the digester and solid separation, leading to more efficient digestion and nutrient removal upon solid separation, ultimately achieving a steady state that produces more concentrated nutrient solids as well as reduced nutrient discharge. The combination of recycle, AD, and solid separation can lead to a wastewater discharge that on w/w basis is has 64% less water, 66% less solids, 52% less nitrogen, and 66% less phosphorus compared to the effluent produced with no recycle facility.

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420 **4. Conclusions**

421 In the current study the effects of recycling separated liquid-effluent, salinity, and
422 total ammonia nitrogen on anaerobic digestion (AD) of dairy manure were
423 investigated. The results revealed that AD of dairy manure followed by solid
424 separation produced an effluent-liquid suitable for up to 80% recycle. For
425 acclimated seed digestions, both total ammonia nitrogen and salinity dosed
426 digesters showed a resistance to drop in SMP against shock loading of ammonia
427 and salinity. On the other hand, un-acclimated seed digesters showed a constant
428 drop of SMP at similar concentrations of total ammonia nitrogen and salinity as that
429 of acclimated seed digesters. The mass balances performed for a farm scale
430 anaerobic digester facilitated with liquid-effluent recycle revealed a reduction of
431 64%, 66%, 52%, and 66% in water, solids, nitrogen, and phosphorus, respectively,
432 in the effluent going off-farm compared to the no recycle scenario. The reduction in
433 volume and nutrients load with the proposed liquid-effluent recycle system thus
434 presents dairy farmers with an alternative for meeting current and potential
435 wastewater regulatory requirements for their operations.

436

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References

- Angelidaki, I. and Ahring, B.K., 1994. Anaerobic thermophilic digestion of manure at different ammonia loads: effect of temperature. *Water Research*, 28(3), pp.727-731.
- APHA, A., WEF (American Public Health Association, American Water Works Association, and Water Environment Federation). 1998. *Standard methods for the examination of water and wastewater*, 19.
- Chen, Y., Cheng, J.J. and Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. *Bioresource technology*, 99(10), pp.4044-4064.
- Estevez, M.M., Sapci, Z., Linjordet, R., Schnürer, A. and Morken, J., 2014. Semi-continuous anaerobic co-digestion of cow manure and steam-exploded *Salix* with recirculation of liquid digestate. *Journal of environmental management*, 136, pp.9-15.
- Frear, C., Ewing, T., Yu, L., Ma, J., Chen, S., 2012. Two novel floor-scale anaerobic digester systems for processing food waste. Part 1: Multi-reactor, liquid recycle system for high solids. Part 2: Moderate solids system. Olympia, WA, Washington State Department of Ecology.
- Frear, C., Wang, Z.W., Li, C. and Chen, S., 2011. Biogas potential and microbial population distributions in flushed dairy manure and implications on anaerobic digestion technology. *Journal of Chemical Technology and Biotechnology*, 86(1), pp.145-152.
- Hashimoto, A.G., 1986. Ammonia inhibition of methanogenesis from cattle wastes. *Agricultural Wastes*, 17(4), pp.241-261.

466 Jagadabhi, P.S., Lehtomäki, A. and Rintala, J., 2008. CO-DIGESTION OF GRASS
 467 SILAGE AND COW MANURE IN A CSTR BY RE-CIRCULATION OF
 468 ALKALI TREATED SOLIDS OF THE DIGESTATE. *Environmental*
 469 *technology*, 29(10), pp.1085-1093.

470 Jarrell, K.F., Sprott, G.D. and Matheson, A.T., 1984. Intracellular potassium
 471 concentration and relative acidity of the ribosomal proteins of methanogenic
 472 bacteria. *Canadian journal of microbiology*, 30(5), pp.663-668.

473 Kafle, G.K. and Chen, L., 2016. Comparison on batch anaerobic digestion of five
 474 different livestock manures and prediction of biochemical methane potential
 475 (BMP) using different statistical models. *Waste Management*, 48, pp.492-
 476 502.

477 Kugelman, I.J. and McCarty, P.L., 1964. Cation toxicity and stimulation in
 478 anaerobic waste treatment. *Journal (Water Pollution Control Federation)*,
 479 37(1), pp.97-116.

480 Labatut, R.A., Angenent, L.T. and Scott, N.R., 2011. Biochemical methane
 481 potential and biodegradability of complex organic substrates. *Bioresource*
 482 *technology*, 102(3), pp.2255-2264.

483 Liu, T. and Sung, S., 2002. Ammonia inhibition on thermophilic aceticlastic
 484 methanogens. *Water Science and Technology*, 45(10), pp.113-120.

485 Ma, J., Yu, L., Frear, C., Zhao, Q., Li, X. and Chen, S., 2013. Kinetics of
 486 psychrophilic anaerobic sequencing batch reactor treating flushed dairy
 487 manure. *Bioresource technology*, 131, pp.6-12.

488 McCarty, P.L. and McKinney, R.E., 1961. Salt toxicity in anaerobic digestion.
489 Journal (Water Pollution Control Federation), pp.399-415.

490 Neerackal, G.M., Ndegwa, P.M., Joo, H.S., Wang, X., Harrison, J.H., Heber, A.J.,
491 Ni, J.Q. and Frear, C., 2015. Effects of Anaerobic Digestion and Solids
492 Separation on Ammonia Emissions from Stored and Land Applied Dairy
493 Manure. *Water, Air, & Soil Pollution*, 226(9), pp.1-12.

494 Owen, W.F., Stuckey, D.C., Healy, J.B., Young, L.Y. and McCarty, P.L., 1979.
495 Bioassay for monitoring biochemical methane potential and anaerobic
496 toxicity. *Water research*, 13(6), pp.485-492.

497 Pöschl, M., Ward, S. and Owende, P., 2010. Evaluation of energy efficiency of
498 various biogas production and utilization pathways. *Applied Energy*, 87(11),
499 pp.3305-3321.

500 Powers, W.J., Wilkie, A.C., Van Horn, H.H. and Nordstedt, R.A., 1997. Effects of
501 hydraulic retention time on performance and effluent odor of conventional
502 and fixed-film anaerobic digesters fed dairy manure wastewaters.
503 *Transactions of the ASAE*, 40(5), pp.1449-1455.

504 Rajagopal, R., Massé, D.I. and Singh, G., 2013. A critical review on inhibition of
505 anaerobic digestion process by excess ammonia. *Bioresource technology*,
506 143, pp.632-641.

507 Sakar, S., Yetilmezsoy, K. and Kocak, E., 2009. Anaerobic digestion technology in
508 poultry and livestock waste treatment—a literature review. *Waste
509 management & research*, 27(1), pp.3-18.

510 Spellman, F.R. and Whiting, N.E., 2007. Environmental management of
 511 concentrated animal feeding operations (CAFOs). CRC Press.
 512 Tiwary, A., Williams, I.D., Pant, D.C. and Kishore, V.V.N., 2015. Assessment and
 513 mitigation of the environmental burdens to air from land applied food-based
 514 digestate. *Environmental Pollution*, 203, pp.262-270.
 515 USDA, and USEPA. 1999. Unified national strategy for animal feeding operations.
 516 United States Department of Agriculture and United States Environmental
 517 Protection Agency, Washington, DC.
 518 Comprehensive nutrient management plan (CNMP). Retrieved October 30, 2016,
 519 from
 520 [http://www.nrcs.usda.gov/wps/portal/nrcs/detail/wi/water/?cid=nrcs142p2_02](http://www.nrcs.usda.gov/wps/portal/nrcs/detail/wi/water/?cid=nrcs142p2_020843)
 521 0843.
 522 Wen, Z., Frear, C. and Chen, S., 2007. Anaerobic digestion of liquid dairy manure
 523 using a sequential continuous-stirred tank reactor system. *Journal of*
 524 *Chemical Technology and Biotechnology*, 82(8), pp.758-766.
 525 Wu, S., Ni, P., Li, J., Sun, H., Wang, Y., Luo, H., Dach, J. and Dong, R., 2016.
 526 Integrated approach to sustain biogas production in anaerobic digestion of
 527 chicken manure under recycled utilization of liquid digestate: Dynamics of
 528 ammonium accumulation and mitigation control. *Bioresource technology*,
 529 205, pp.75-81.
 530 Yenigün, O. and Demirel, B., 2013. Ammonia inhibition in anaerobic digestion: a
 531 review. *Process Biochemistry*, 48(5), pp.901-911.

532 Yerkes, D.W., Boonyakitsombut, S. and Speece, R.E., 1997. Antagonism of
 533 sodium toxicity by the compatible solute betaine in anaerobic methanogenic
 534 systems. *Water Science and Technology*, 36(6-7), pp.15-24.
 535 Zeeman, G., Wiegant, W.M., Koster-Treffers, M.E. and Lettinga, G., 1985. The
 536 influence of the total-ammonia concentration on the thermophilic digestion of
 537 cow manure. *Agricultural Wastes*, 14(1), pp.19-35.
 538 Zhang, R.H. and Westerman, P.W., 1997. Solid-liquid separation of animal manure
 539 for odor control and nutrient management. *Applied Engineering in*
 540 *Agriculture*, 13(3), pp.385-393.
 541 Zhao, Q.B., Ma, J., Zeb, I., Yu, L., Chen, S., Zheng, Y.M. and Frear, C., 2015.
 542 Ammonia recovery from anaerobic digester effluent through direct aeration.
 543 *Chemical Engineering Journal*, 279, pp.31-37.

Fig. 1. (a) Total ammonia nitrogen (TAN), (b) salinity, and (c) specific methane potential (SMP) during recycle of 30%, 50%, and 80% of separated liquid-effluent, respectively. Error bars represent the standard deviations, ppt represents parts per thousand, TE represents treated effluent (TAN removed), UTE represents untreated effluent (no TAN removed). Different letters on top of the vertical bars indicate statistically significant differences at $p < 0.05$.

Fig. 2. Variations in (a) total ammonia nitrogen (TAN), (b) salinity, and (c) specific methane potential (SMP) during recycle of 80% of separated liquid-effluent. Each cycle represents a 20 d digestion and error bars represent the standard deviations, ppt represents parts per thousand, TE represents treated effluent (TAN removed), UTE represents untreated effluent (no TAN removed), and LF represents loading factor. The loading factor was switched from 10 to 20 g VS L⁻¹ at cycle number 5.

Fig. 3. Effects of total ammonia nitrogen (TAN) and salinity (SAL) on specific methane potential (SMP) of dairy manure with acclimated seed (AS) and un-acclimated seed (UAS): (a) SMP vs. TAN for AS digestion; (b) SMP vs. TAN for UAS digestion; (c) SMP vs. SAL for AS digestion; (d) SMP vs. SAL for UAS digestion. Error bars represent standard deviations, ppt represents parts per thousand. Each point on a graph represents one complete 20 d digestion at a loading factor of 20 g VS L^{-1} .

Fig. 4. Mass balances of water, solids, and nutrients for a typical 5,000 herd of dairy cows farm whose anaerobic digestion system is operated with effluent-liquid recycle.

Fig. 5. Mass balances of water, solids, and nutrients for a typical 5,000 herd of dairy cows farm whose anaerobic digestion system is operated without effluent-liquid recycle.

Table 1. Summary of the experimental design for the repeated recycle and manually dosed anaerobic digestion experiment.

Parameter	Units	Recycle experiment	AS experiment	UAS experiment
Purpose		^[a]	^[b]	^[b]
Inoculum	(v/v)	^[c] 20% digestate recycled as seed	^[c] 20% digestate recycled as seed	^[d] Fresh seed
Loading factor	(g VS L ⁻¹)	10, 20	20	20
Volume of effluent recycled	(v/v)	30%, 50%, 80%	0	0
Digestion time	(days)	20	20	20
Manual TAN loading	(g N L ⁻¹)	0	0.88, 1.86, 2.79, 3.30, 3.98, 5.13, 6.39, 8.50, 11.52	1.20, 2.47, 4.83, 7.99, 10.51
Manual salinity loading	(ppt)	0	2, 3, 4, 5, 6, 12, 24, 36, 48	3, 4, 12, 24, 48

AS: Acclimated seed; UAS: Un-acclimated seed; TAN: Total ammonia nitrogen; SAL: Salinity; ppt: Parts per thousand.

^[a] Determine the effects of TAN and SAL on SMP of dairy manure with repeated recycle of various amounts of solid separated digestate.

^[b] Determine the difference in SMP response of acclimated and un-acclimated seed against shock loadings of various concentrations of TAN and SAL.

^[c] Following the preparatory digestion 20% volume/volume of the digestate was recycled for the successive digestions in repeated recycle and AS digestion experiments.

^[d] All UAS digestions were simultaneously carried out with different shock loadings of TAN and SAL and inoculum used for these digestions was a fresh seed obtained from Municipal Wastewater Treatment Plant located in Pullman, Washington.

Table 2. Multiple comparisons of means using Tukey method.

Treatment	Mean TAN (g N L ⁻¹)	Mean salinity (ppt)	Mean SMP (m ³ CH ₄ Kg ⁻¹ VS)
UTE (80%)	1.112 ± 0.016 ^{A*}	6.630 ± 0.162 ^(A)	0.265 ± 0.005 ^A
TE (80%)	0.568 ± 0.010 ^B	4.090 ± 0.095 ^(B)	0.241 ± 0.007 ^A
Control	0.470 ± 0.012 ^B	2.750 ± 0.047 ^(C)	0.252 ± 0.005 ^A

Means with different upper case letter in the same column were significantly different (P < 0.005). TAN: Total ammonia nitrogen; ppt: Parts per thousand; SMP: Specific methane production; UTE: Un-treated effluent; TE: Treated effluent.

Table 3. Water and nutrients discharge comparison of 5,000 cows dairy farm operating anaerobic digester facilitated with and without effluent recycle.

	Parameter	Unit	With effluent recycle	Without effluent recycle
Liquid discharge	Flow rate	Kg d ⁻¹	3.55×10^5	3.56×10^5
	TN	Kg d ⁻¹	3.44×10^2	4.71×10^2
	TP	Kg d ⁻¹	3.60×10	4.5×10
Post primary screen solids	Flow rate	Kg d ⁻¹	1.81×10^4	1.72×10^4
	TN	Kg d ⁻¹	1.81×10^2	1.36×10^2
	TP	Kg d ⁻¹	9.07	9.07
Post second screen solids	Flow rate	Kg d ⁻¹	3.63×10^3	3.63×10^3
	TN	Kg d ⁻¹	4.5×10	3.6×10
	TP	Kg d ⁻¹	9.07	9.07
Post dissolved air flotation solids	Flow rate	Kg d ⁻¹	9.07×10^3	8.16×10^3
	TN	Kg d ⁻¹	2.35×10^2	1.72×10^2
	TP	Kg d ⁻¹	8.10×10	8.10×10

TN: Total nitrogen; TP: Total phosphorus.