The effect of acidogenic and methanogenic conditions on the availability and stability of carbon, nitrogen and phosphorus in a digestate

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Highlights

Acidogenic fermentation increased the availability of phosphorus under acidic condition

Acidogenic fermentation reduced ammonium volatilisation during drying

Acidogenic fermentation increased labile carbon

Methanogenic fermentation increased ammonium volatilisation and reduced labile carbon

Graphical abstract

Abstract

Acidification and drying of digestate are important post-treatment for respectively, improving nutrient availability and hygiene. These approaches are expected to reduce digestate soil application mass and improve the value of a dry product. Whilst this is an important development, there is a need for more studies into an economic and environmentally viable means of improving digestate nutrient availability. This study compared eleven organic substrates under acidogenic and methanogenic fermentation regarding their effects on digestate organic carbon, ammoniacal nitrogen, and inorganic phosphorus concentration. The result showed increases in phosphate concentration under acidogenic conditions and reduction in ammonium nitrogen after drying at 100 °C. The highest phosphate values of 3.2 ± 0.38 g/kg were achieved using whey permeate substrate while the effect of drying on ammonium nitrogen concentration was lowest for acidogenic bird seed fermentation with an ammonium loss of 59.7%. Both results were facilitated by high total volatile fatty acid concentration produced from available carbon-rich agricultural wastes which reached a maximum value of 5.71 ± 0.53 g/L, respectively. Increases in phosphate and ammonium nitrogen stability under acidogenic conditions was a consequence of lower pH, a condition synonymous with acidogenic only fermentation. The accumulated volatile fatty acid contributed to higher carbon to nitrogen ratio under acidogenic fermentation. Higher labile carbon to nitrogen ratio can trigger immobilization of ammonium nitrogen in the soil and this presents a case for subsequent experimentation into acidogenic digestate application in soil.

Keywords

Anaerobic digestion
Acidogenesis
Ammonium nitrogen
Digestate
Methanogenesis
Organic carbon
Phosphorus

1. Introduction

Anaerobic digestion (AD) is currently one of the several technological approaches to realizing a circular economy. The technology is capable of minimizing resource input, greenhouse gas (GHG) emission, energy and nutrient leakage (Zaks et al., 2011). Although since the last

decade the technology has been driven by the economic incentives for renewable energy the nutrient-rich digestate is now receiving more attention (Dahlin et al., 2015). These nutrients have agronomic values and are present in the undigested fraction from the AD process, which is known as digestate (Nkoa, 2014). Despite the agronomic value of the digestate its storage, processing, and application to farmland are often associated with environmental concerns such as ammonia emission and nutrient leaching particular after land application (Perazzolo et al., 2017; Perazzolo et al., 2016). The traditional approach such as dewatering and drying are extensively used to manage AD digestate even though this contributes to ammonia emission and nutrient depletion (Pantelopoulos et al., 2016). In recent times, a more sophisticated technology such as anammox, absorption, membrane separation, struvite, enhanced phosphorus recovery has been investigated with some of them still at the developmental stage while the others are still not financially viable (Romero-Güiza et al., 2016). These sophisticated technologies selectively recover reactive nitrogen and phosphorus excluding valuable constituents such as potassium, organic matter, trace metals, and organic nutrient. These constituents are known to improve soil structural stability creating stable aggregates within the rooting zones and favourable growing conditions for plants (Busari et al., 2008). This promotes the development of soil microbial populations, thereby stimulating both above and below ground floral and faunal biodiversity (Alburquerque et al., 2012a; Gutser et al., 2005). Since the digestate value is not limited to phosphorus and ammonium but macro and micronutrients then a technology that helps stabilize ammonium and increase available phosphate whilst in the digestate should be a better approach. This approach is most suitable for digestate that meet the PAS 110 requirement or other digestate to land regulatory requirements.

A notable approach to achieving this is acidification of the digestate to reduce nitrogen loss and increase phosphorus availability (Pantelopoulos et al., 2016). The drying cost can be offset using the excess heat from the power plant, but the cost of setting up a chemical acidification

process could raise concerns. This batch study focuses on comparing acidogenic and methanogenic fermentation on eleven agricultural wastes and how the two conditions and waste material affects the concentration of labile carbon, ammoniacal nitrogen and inorganic phosphorus in the resulting digestate. The agricultural wastes were selected based on high carbon and nitrogen content using the study carried out by Piveteau et al. (2017). Acidogenesis and methanogenesis have been identified as the key steps for producing, respectively VFAs and methane gas. These two processes occur concurrently within the AD system and can also be separated as in the case of a two-stage AD system. One unique thing about separating these two processes is the distinct variation in their microbial consortia and operating conditions. This approach is expected to lead to several changes in the composition of the resulting digestates. The aim of this studies is to investigate the effect of acidogenic and methanogenic conditions on the nutrient content of a digestates from different agricultural substrates. The following hypotheses were addressed: (i) acidogenesis will increase the organic content of the digestate relative to methanogenesis, (ii) acidogenesis will increase the availability of phosphorus relative to methanogenesis and (iii) acidogenesis will reduce NH_4-N loses during drying relative to methanogenesis

2. Methods and materials

2.1.Sewage sludge digestate

The digestate used as inoculum (IN) was collected from anaerobically treated secondary sludge operated at mesophilic temperature located in the premises of United Utility in Lancaster, UK. The inoculum was divided into two portions to serve the methanogenic and acidogenic setup. The acidogenic reactors were inoculated with a pasteurized digestate autoclaved at 121 °C for 10 mins to inactivate methanogenesis while the methanogenic reactors were inoculated with an unpasteurized digestate (Oh et al., 2003, Park et al., 2005). The physicochemical properties

of the digestates were measured in triplicates according to standard methods described under analytical methods (APHA, 1998). The acidogenic inoculum contained 23.2 ± 0.01 g/kg, total solids (TS), 647 ± 0.1 g/kg volatile solid (VS), 314 ± 0.5 g/kg total carbon (TC), 38.6 ± 0.31 g/kg total nitrogen (TN), 18.6 ± 1.8 g/kg total phosphorus (TP) and 36.06 ± 0.89 g/kg NH_4-N. The pH and alkalinity of the inoculum were 7.76 ± 0.03 and 4.69 mg$CaCO_3$/l respectively. The methanogenic inoculum contained 23.7 ± 2.9 g/kg TS, 591 ± 35 g/kg VS, 314 ± 0.5 g/kg TC, 38.6 ± 0.31 g/kg TN, 18.6 ± 1.8 g/kg TP, and 34.1 ± 0.26 g/kg NH_4-N. The pH and total alkalinity of the inoculum were 7.85 ± 0.02 and 4.89 mg$CaCO_3$/l respectively.

2.2.Substrates

The substrates used in this experiment were sourced from various agricultural farms and bioenergy operating sites in the UK. All samples were collected and stored frozen (-20 °C) until analysis. Prior to the characterization, the required quantities of substrates were thawed overnight at room temperature. The physicochemical characteristics were carried out in triplicates according to standard methods (APHA, 1998). Table 1 shows the representative characteristics of the substrates.

2.3. Experimental batch test design

Batch digestion was carried out using a 0.5 L anaerobic reactor and a freshly collected digestate as inoculum. The substrates used in this study were minced and mixed to form a homogenous mixture prior to inoculation. A similar substrate to inoculum ratio was maintained across the different substrates under methanogenic and acidogenic condition. So that the volume was kept constant within the reactors all volume was made up to 0.23 L with deionized water. Once the reactors were loaded a stream of nitrogen gas was pumped into the reactor through a down tube to remove any oxygen present. All the reactors were sealed with the screwed lid to maintain an

airtight seal and transferred into a water bath set at 37 °C. The reactors were continuously stirred during the experiments through an overhead 12V DC motor connected through a draft tube to a stirrer. The stirring speed was maintained at 30 rpm. The reactors were operated respectively under acidogenic and methanogenic conditions. The acidogenic reactor was operated for 5 days while the methanogenic reactor ended after 15 days using a batch test. See Table 2 for experimental design.

2.4.Analytical methods

The TS and VS content were analysed by heating the samples in an oven (Memmert, Germany) at 105 °C and a furnace (Carbonite, Sheffield UK) at 550 °C for 24 and 5 hrs respectively following the standard method (Apha, 1995). Alkalinity was determined by titration with 0.25 M H_2SO_4 to endpoints of pH 5.7 and 4.3, allowing calculation of total (TA), partial (PA) and intermediate alkalinity (IA). The pH reading was monitored with a Jenway 3010 meter (Bibby Scientific Ltd, UK) with a combination glass electrode, calibrated in buffers at pH 4, 7 and 9.2 after which the samples were centrifuged at 4500 rpm for 15 min and the supernatant was filtered through a cellulose membrane to obtain a soluble fraction. The soluble fractions were used to determine total organic carbon, ammoniacal nitrogen, and total phosphate. Ammonium nitrogen (NH^+_4-N) and available phosphorus were measured in a 1:10 (weight: volume) digestate: water extract after end-over-end shaking for 1 h. NH_4-N and available phosphorus were determined using an auto analyser (Alef & Nannipieri, 1995; Forster, 1995). The elemental determination of total carbon and nitrogen content of the sample was carried out using an elemental analyser (Elementar Vario-EL elemental analyser) (Otero et al., 2011). The samples were dried in an oven at 60 °C for 2 days before elemental analyses. Total organic carbon concentrations were measured with a TOC-V analyser (Shimadzu Corp., Kyoto, Japan). The VFAs were quantified with ion chromatography (IC) (Dionex, ICS-30000, Thermo-

Scientific, USA) using a UV index detector and an Aminex HPX-87H column (Bio-Rad, UK). The separation of VFAs during IC measurement was achieved using a mobile phase of 2.5 mM H_2SO_4 at a flow rate of 0.6 ml min^{-1} and a column temperature of 65 ºC. The detector temperature was 40 ºC. The VFA marker mix containing acetic, propionic, isobutyric, butyric, iso-valeric and valeric acids, each from 0.1- 1.25 mg ml^{-1} (Sigma-Aldrich, UK) were used to calibrate the IC equipment.

3. Result and discussion

3.1.Fermentation of substrates

The acidogenic and methanogenic fermentation was initiated in accordance with the experimental design and the two conditions were evaluated using the VS and total VFA (TVFA) as a function of successful fermentation processes. The VS is a measure of the rate of substrate utilisation or mass reduction and it is often matched with biogas production or VFA accumulation. According to Figure 1, a substantial mass reduction ranging from 10-35% was observed across a broad array of the substrate under an acidogenic and methanogenic condition with exception to fermenter FY, IN, and RH. Mass reduction in a fermentation process in an indication of active microbial population and utilisation of substrate (Brown and Li, 2013). In thes case of FY, IN and RH, VS reduction was less than 10% under both conditions suggesting limited or unavailable organic carbon (Table 1). Equally, a considerable difference was observed between the acidogenic and methanogenic conditions. The methanogenic fermenters recorded higher reduction in VS values which were 4-23% higher than acidogenic fermenters with an exception to fermenter WY, IN and BSG (Fig. 1). This is expected because the methanogenic condition is a four-stage fermentation process and additional carbon is utilised (Madsen et al., 2011). In this case of fermenter WY, IN and BSG, no substantial differences were observed, whilst substrate IN can be ascribed to limited carbon and low solid content in

substrate WY. The comparison between the VS and VFA results showed that VS reduction under acidogenic conditions translated into higher VFA production. These varied with different substrates hence reasons for low TFVA accumulation for FY, IN, and RH.

The VFA is another important indicator for monitoring the fermentation process. According to Brown and Li (2013), the degradation of organic matter during the anaerobic fermentation process dominantly contribute to the production of biogas and intermediate product such as VFAs and other organic nutrients. Biogas production was not measured but VFA accumulation in both systems are well represented in Fig. 2c and 2d. Of the two conditions, the acidogenic fermenters recorded higher concentration of VFAs, particularly acetic and propionic acid (Fig. 2c). Higher VFA accumulation was accompanied by a decrease in the pH of the fermenters and the acidogenic condition recorded the lowest pH values which varied between 5.2 and 6.7 (Fig. 2a and 2b). An exception to this was the IN and FY set up which recorded a pH of 7.54 and 7.2 respectively because no additional carbon source was provided for fermenter IN during the fermentation process and substrate FY was low in available carbon (Table 1 and 2). The WY fermenter recorded the highest TVFA concentration and lowest pH with respective values of 5.71 ± 0.50 g/l and 5.22 ± 0.02 (Fig. 2d). The notable variation in mass reduction and VFA accumulation for the two conditions were driven by the differences in the fermentation processes. The acidogenic condition is a two-step conversion process compared to the four-step methanogenic condition. The latter is necessary to achieve higher mass reduction and biogas production while the former is essential for VFAs and hydrogen gas production (Espinoza-Escalante et al., 2008; Massanet-Nicolau et al., 2013). The measured TVFA concentration is consistent with Espinoza-Escalante et al. (2008) which recorded value of 0.88 -7.2 g/l. Likewise, VS reduction recorded for the methanogenic condition is consistent with one obtained by Massanet-Nicolau et al. (2013), which showed a 13-15% mass reduction.

3.2.Effect of volatile fatty acid on nutrient availability

The quantity and quality of pH values and VFA concentration as a function of different substrates and fermentation conditions are shown in Figure 2 and 3. Overall there were similarities in the measured NH_4-N concentration between the acidogenic and methanogenic fermenters but differences in the available phosphorus concentration for some of the fermenters. The acidogenic fermenter recorded pH values which varied between 5.2 and 6.76, a TVFA concentration which varied between 1.1 and 4.9 g/l. (Fig. 2). This result showed that the effect of acidification had a negligible effect on the NH_4-N concentration (Fig. 3a). This is expected partly because a higher concentration of organic acid would be required to drive mineralisation of organic nitrogen to NH_4-N (Frandsen et al., 2011; Törnwall et al., 2017). On the other hand, the PW fermenter recorded the highest NH_4-N with a value of 46.72 ± 1.62 g/kg. This could be attributed to the high concentration of TN present in the PW substrate (Table 1). The RH fermenter recorded lowest TN values which also translated in lower values of NH_4-N concentration (Fig. 3a).

Unlike NH_4-N, the effect of VFA accumulation was noticeable for the availability of phosphorus in the acidogenic fermenters as shown in Fig. 3b. Overall the acidogenic conditions increased the availability of phosphorus with exception to fermenter FY and IN (Fig 3b). The differences in the values recorded for available phosphorus under acidogenic and methanogenic conditions for different fermenters could be ascribed to the varying levels of pH. The observed exceptions in the acidogenic fermenters could be attributed to the high buffering capacity of FY fermenter and unavailable carbon to drive VFA formation and pH reduction in IN fermenter (Möller & Müller, 2012). According to Schachtman et al. (1998) depending on the pKs for dissociation, phosphorus is most present as H_3PO_4, $H_2PO_4^-$ and HPO_4^{2-}, the former and latter are prevalent at pH 2.1 and 7.2 respectively. This implies that lower pH increases the

availability of phosphorus species but FY and IN recorded a pH of 7.2 ± 0.01 and 7.54 respectively (Fig 2a). According to Cerozi and Fitzsimmons (2016), when the pH of a medium is above 7 most of the dissolved phosphorus reacts with metals to form metal phosphate and this cause the phosphate to become unavailable. This report can also be used to explain the low phosphate values observed in acidogenic fermenter FY and IN. Acidogenic fermenters WY recorded the highest value for available phosphorus which was 3.2 ± 0.01 g/kg, this corresponded with the low pH value of 5.22 ± 0.02. Again, the availability of phosphorus under acidogenic and methanogenic condition was suggested to have been impacted by pH. Acidogenic fermenters such as PW, BS, and OT which recorded relatively higher value of TVFA of 3.70, 4.14 and 3.52 g/L did not translate into available phosphorus value comparable to WY. In the case of PW, higher buffering is expected to have resisted the acidification as pH remained at 6.68 under acidogenic conditions. However, acidogenic fermenter BS and OT recorded a lower pH of 5.9 ± 0.02 and 5.64 ± 0.05, respectively but it did not translate into comparably available phosphorus as WY was still 4 times higher. The low pH of the WY acidogenic fermenter expected to have contributed to the overall increment in available phosphorus concentration over BS and OT fermenter. Although Piveteau et al. (2017) study showed that the dissolution of phosphorus is achievable between pH of 5.5-6 using lactic acid from fermentation of sucrose. In general, this further shows that acidogenic treatment of organic substrate increases phosphorus availability relative to methanogenic condition. Plants can only absorb phosphorus as free phosphate ion of $H_2PO_4^-$ and HPO_4^{2-} (Becquer et al., 2014). The pH of the acidogenic digestates which varied between 5-6 and increased availability of phosphorus makes it a potential digestate for soil application and plant growth.

3.3.Effect of volatile fatty acid and drying on nutrient stability

Nutrient loss through ammonia volatilisation from digestate takes place in the first week of storage, particularly during the warmer season (Chadwick, 2005; Dinuccio et al., 2008). This is because elevated pH and temperature enhance the dissociation of non-volatile ammonium into ammonia which is eventually removed by the adjacent air (Guštin & Marinšek-Logar, 2011). In this study, the acidogenic fermentation reduced the pH of the digestates more effectively than methanogenesis because of the accumulation of VFAs (Fig. 2). This infers that ammonia volatilisation in an acidogenic digestates is expected to be lesser. Under the temperature-controlled study, 6 of the digestates were randomly selected and dried at 100 °C for 24 hours before measuring the ammonium concentration. The percentage of ammonium reduction for temperature treated under acidogenic and methanogenic conditions are represented in Figure 5. Acidogenic digestates BS, OT, PW, and PT were found to be more efficient in reducing NH_4-N loss. The percentage of NH_4-N reduction after the fermentation process were 59.7%, 79%, 92%, and 74% respectively compared to over 97% NH_4-N reduction for all methanogenic fermenter (Fig 5). Again, this is attributed to VFA accumulation which is higher in the acidogenic fermenter and low pH (Fig 2). For fermenter FY and IN, NH_4-N reduction was similar under acidogenic and methanogenic conditions. Although FY and IN recorded 0.82 and 0.73 TVFA g/L under acidogenic conditions this did not translate into retention of NH_4-N during drying. This was because the concentration TVFA concentration was too low to drive NH_4-N stability to form either ammonium acetate or ammonium phosphate. In addition, acidogenic fermenter FY and IN recorded high pH values which were 6.68 and 7.54, respectively. The reduction in NH_4-N values during drying is similar to Pantelopoulos et al. (2016) study, although they used sulphuric acid to acidify the digestate. There is a cost implication for using chemical agents like sulphuric acid to lower the pH of digestates. Likewise, there are reports that VFAs can inhibit ammonium oxidizing bacteria

responsible for potential ammonium oxidation while another report describes fatty acid as an easily decomposable carbon source for microorganisms in soil (Kirchmann & Lundvall, 1993; Risberg et al., 2017).

3.4.Effect of acidogenesis on organic carbon and l soil nutrient

An additional benefit of acidogenic fermentation is the high carbon to nitrogen ratio. As shown in Figure 4a, is the concentration of total organic carbon as a function of different fermentation process and substrate. Overall there was a large difference between the measured total organic carbon (TOC) values for acidogenic and methanogenic fermentation for different substrates. The acidogenic fermenters, PT and WY recorded the highest TOC values of 644.43 ± 1.87g/kg and 686.12 ± 5.11 g/kg which were 3.3 and 6 times higher than the methanogenic counterpart. This could be attributed to the VFA concentration and other undigested organic carbon as this is common with acidogenic fermentation. An extensive portion of the organic carbon in the acidogenic fermenters are from the VFA production. This instantly increases the C/N of the digestates and acidity can influence the soil properties. On application of the digestate to land, the stability of ammonium can easily be influenced depending on carbon to nitrogen ratio or acidity. Sánchez-Rodríguez et al. (2018) showed that cumulative ammonia volatilization losses were significantly reduced by the acidification of the digestate. With regards to carbon to nitrogen ratio, the acidogenic digestates varied between 1.8 and 61 while the methanogenic digestates were less than 0.5 (Fig. 4b). This indicates that the acidogenic digestates with C:N ratio above 25 will encourage immobilization of ammonium, particularly OT, BS, GS, MC, and BSG while all the methanogenic digestates will result in mineralisation of ammonium which will increase nutrient loss (Alburquerque et al., 2012b; Jat et al., 2012). There is a need for further studies on the best form to utilise the acidogenic digestate in order to avoid soil acidification, inhibition of soil microbial activity and immobilisation of ammonium nitrogen

4. Conclusion

Acidogenic anaerobic digestion of was successfully achieved after the operational time of 5 days and acidification was observed as a response to VFAs build-up. The reduction of pH in the acidogenic fermenter resulted in an increase in phosphate, soluble organic carbon and reduction in ammonium volatilisation during drying. The evaluation of the batch process under acidogenic condition suggests the need for a substrate with high soluble carbon to facilitate

build- up of VFAs aimed at overcoming the buffering resistances. However, the added benefit of organic carbon infers a potential increase in ammonium immobilisation and soil acidification. There is a need for further studies on the best form to utilise the acidogenic digestate application to land even though nutrient stability and availability are shown to be more efficient than traditional methanogenic fermentation.

5. References

1. Alburquerque, J., De la Fuente, C., Campoy, M., Carrasco, L., Nájera, I., Baixauli, C., Caravaca, F., Roldán, A., Cegarra, J., Bernal, M. 2012a. Agricultural use of digestate for horticultural crop production and improvement of soil properties. European Journal of Agronomy, 43, 119-128.

2. Alburquerque, J.A., de la Fuente, C., Bernal, M.P. 2012b. Chemical properties of anaerobic digestates affecting C and N dynamics in amended soils. Agriculture, Ecosystems & Environment, 160, 15-22.
3. Alef, K., Nannipieri, P. 1995. Methods in applied soil microbiology and biochemistry. Academic Press.
4. Apha, A. 1995. WPCF, Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.
5. APHA. 1998. Standard methods for the examination of water and wastewater. In: Clesceri, L.S., Greenberg, A.E.,Eaton, A.D. (Eds.), 20th ed. American Public Health Association, Washington, D.C.
6. Becquer, A., Trap, J., Irshad, U., Ali, M.A., Claude, P. 2014. From soil to plant, the journey of P through trophic relationships and ectomycorrhizal association. Frontiers in Plant Science, 5(548).
7. Busari, M., Salako, F., Adetunji, M. 2008. Soil chemical properties and maize yield after application of organic and inorganic amendments to an acidic soil in southwestern Nigeria. Spanish Journal of Agricultural Research, 6(4), 691-699.
8. Brown, D., Li, Y. 2013. Solid state anaerobic co-digestion of yard waste and food waste for biogas production. Bioresource Technology, 127, 275-280.
9. Cerozi, B.d.S., Fitzsimmons, K. 2016. The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. Bioresource Technology, 219, 778-781.
10. Chadwick, D.R. 2005. Emissions of ammonia, nitrous oxide and methane from cattle manure heaps: effect of compaction and covering. Atmospheric Environment, 39(4), 787-799.
11. Dahlin, J., Herbes, C., Nelles, M. 2015. Biogas digestate marketing: Qualitative insights into the supply side. Resources, Conservation and Recycling, 104, 152-161.

12. Dinuccio, E., Berg, W., Balsari, P. 2008. Gaseous emissions from the storage of untreated slurries and the fractions obtained after mechanical separation. Atmospheric Environment, 42(10), 2448-2459.
13. Espinoza-Escalante, F.M., Pelayo-Ortiz, C., Gutiérrez-Pulido, H., González-Álvarez, V., Alcaraz-González, V., Bories, A. 2008. Multiple response optimization analysis for pretreatments of Tequila's stillages for VFAs and hydrogen production. Bioresource Technology, 99(13), 5822-5829.
14. Forster, J. 1995. Soil nitrogen. Methods in applied soil microbiology and biochemistry, 79-87.
15. Frandsen, T.Q., Rodhe, L., Baky, A., Edström, M., Sipilä, I., Petersen, S.L., Tybirk, K. 2011. Best available technologies for pig manure biogas plants in the Baltic Sea Region.
16. Guštin, S., Marinšek-Logar, R. 2011. Effect of pH, temperature and air flow rate on the continuous ammonia stripping of the anaerobic digestion effluent. Process Safety and Environmental Protection, 89(1), 61-66.
17. Gutser, R., Ebertseder, T., Weber, A., Schraml, M., Schmidhalter, U. 2005. Short-term and residual availability of nitrogen after long-term application of organic fertilizers on arable land. Journal of Plant Nutrition and Soil Science, 168(4), 439-446.
18. Jat, R.A., Wani, S.P., Sahrawat, K.L. 2012. Chapter Four - Conservation Agriculture in the Semi-Arid Tropics: Prospects and Problems. in: Advances in Agronomy, (Ed.) D.L. Sparks, Vol. 117, Academic Press, pp. 191-273.
19. Kirchmann, H., Lundvall, A. 1993. Relationship between N immobilization and volatile fatty acids in soil after application of pig and cattle slurry. Biology and fertility of soils, 15(3), 161-164.

20. Madsen, Michael, Jens Bo Holm-Nielsen, and Kim H. Esbensen. "Monitoring of anaerobic digestion processes: A review perspective." Renewable and sustainable energy reviews 15, no. 6 (2011): 3141-3155.
21. Massanet-Nicolau, J., Dinsdale, R., Guwy, A., Shipley, G. 2013. Use of real time gas production data for more accurate comparison of continuous single-stage and two-stage fermentation. Bioresource Technology, 129, 561-567.
22. Möller, K., Müller, T. 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. Engineering in Life Sciences, 12(3), 242-257.
23. Nkoa, R. 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. Agronomy for Sustainable Development, 34(2), 473-492.
24. Otero, M., Lobato, A., Cuetos, M.J., Sánchez, M.E., Gómez, X. 2011. Digestion of cattle manure: Thermogravimetric kinetic analysis for the evaluation of organic matter conversion. Bioresource Technology, 102(3), 3404-3410.
25. Pantelopoulos, A., Magid, J., Jensen, L.S. 2016. Thermal drying of the solid fraction from biogas digestate: Effects of acidification, temperature and ventilation on nitrogen content. Waste management, 48, 218-226.
26. Perazzolo, F., Mattachini, G., Riva, E., Provolo, G. 2017. Nutrient Losses during Winter and Summer Storage of Separated and Unseparated Digested Cattle Slurry. Journal of environmental quality, 46(4), 879-888.
27. Perazzolo, F., Mattachini, G., Tambone, F., Calcante, A., Provolo, G. 2016. Nutrient losses from cattle co-digestate slurry during storage. Journal of Agricultural Engineering, 47(2), 94-99.
28. Piveteau, S., Picard, S., Dabert, P. and Daumer, M.L., 2017. Dissolution of particulate phosphorus in pig slurry through biological acidification: A critical step for maximum phosphorus recovery as struvite. Water research, 124, pp.693-701.

29. Risberg, K., Cederlund, H., Pell, M., Arthurson, V., Schnürer, A. 2017. Comparative characterization of digestate versus pig slurry and cow manure – Chemical composition and effects on soil microbial activity. Waste Management, 61, 529-538.
30. Romero-Güiza, M.S., Mata-Alvarez, J., Chimenos Rivera, J.M., Garcia, S.A. 2016. Nutrient recovery technologies for anaerobic digestion systems: An overview. Revista Ion, 29(1), 7-26.
31. Sánchez-Rodríguez, A.R., Carswell, A.M., Shaw, R., Hunt, J., Saunders, K., Cotton, J., Chadwick, D.R., Jones, D.L., Misselbrook, T.H. 2018. Advanced Processing of Food Waste Based Digestate for Mitigating Nitrogen Losses in a Winter Wheat Crop. Frontiers in Sustainable Food Systems, 2(35).
32. Schachtman, D.P., Reid, R.J., Ayling, S.M. 1998. Phosphorus Uptake by Plants: From Soil to Cell. Plant Physiology, 116(2), 447-453.
33. Törnwall, E., Pettersson, H., Thorin, E., Schwede, S. 2017. Post-treatment of biogas digestate – An evaluation of ammonium recovery, energy use and sanitation. Energy Procedia, 142, 957-963.
34. Zaks, D.P.M., Winchester, N., Kucharik, C.J., Barford, C.C., Paltsev, S., Reilly, J.M. 2011. Contribution of Anaerobic Digesters to Emissions Mitigation and Electricity Generation Under U.S. Climate Policy. Environmental Science & Technology, 45(16), 6735-6742.
35. Oh, S.-E., Van Ginkel, S., Logan, B.E. 2003. The relative effectiveness of pH control and heat treatment for enhancing biohydrogen gas production. *Environmental science & technology*, **37**(22), 5186-5190.
36. Park, W., Hyun, S.H., Oh, S.-E., Logan, B.E., Kim, I.S. 2005. Removal of headspace CO2 increases biological hydrogen production. *Environmental science & technology*, **39**(12), 4416-4420.

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Figure captions

Fig. 1. The initial and final volatile solid profile of different substrates and digestates under acetogenic and methanogenic fermentation.

Fig.2. The pH and VFA evolution of digestates from different substrates under acidogenic and methanogenic fermentation. PA: Propionic acid and AA: Acetic acid

Fig. 3. Water extractable ammonium and phosphorus concentration of digestates from different substrates under acidogenic and methanogenic fermentation.

Fig. 4. Water extractable total carbon concentration and soluble N:C of digestates from different substrates under acidogenic and methanogenic fermentation.

Fig. 5. Changes in ammonium content from acidogenic and methanogenic digestates of different substrates during drying at 100 °C.

Graphical abstract

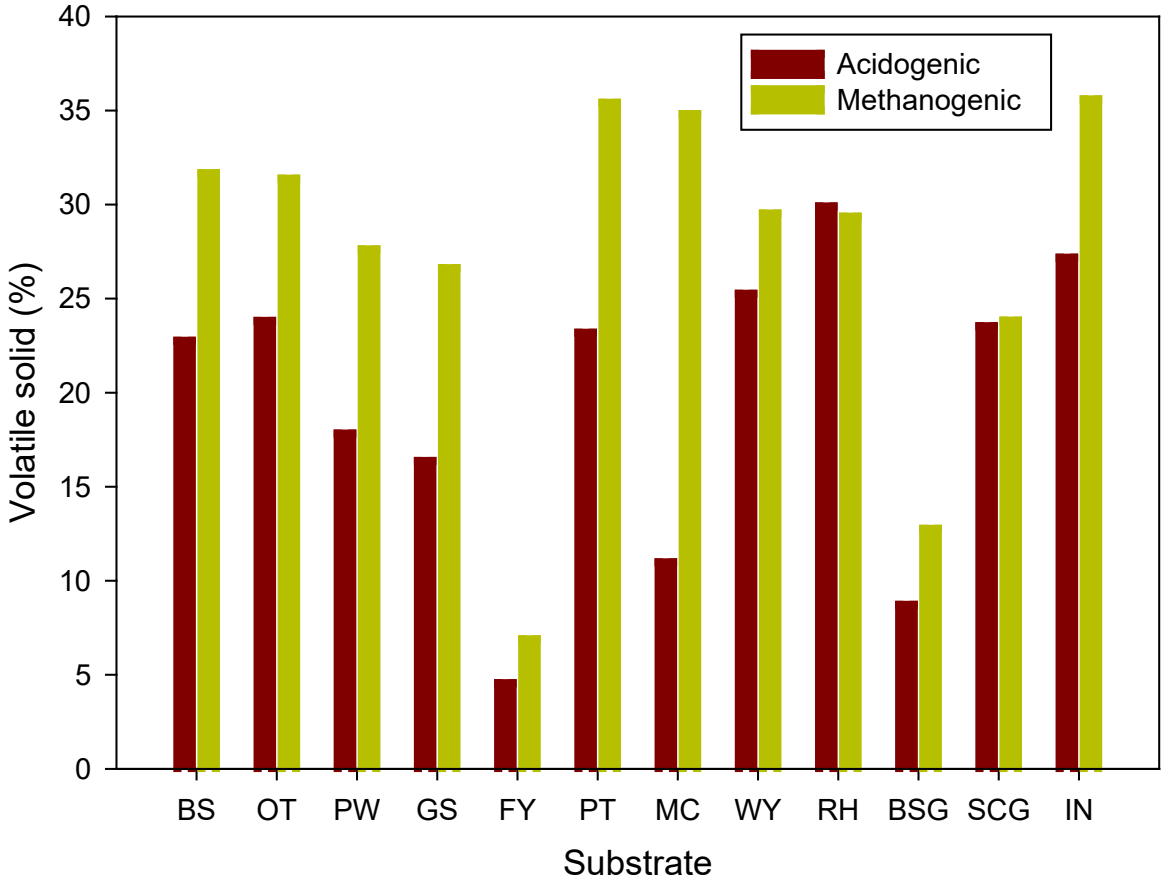
Acidogenic
Methanogenic
Volatile solid (%)
0
5
10
15
20
25
30
35
40
BS
OT
PW
GS
FY
PT
MC
WY
RH
BSG
SCG
IN
Substrate

Fig. 1.

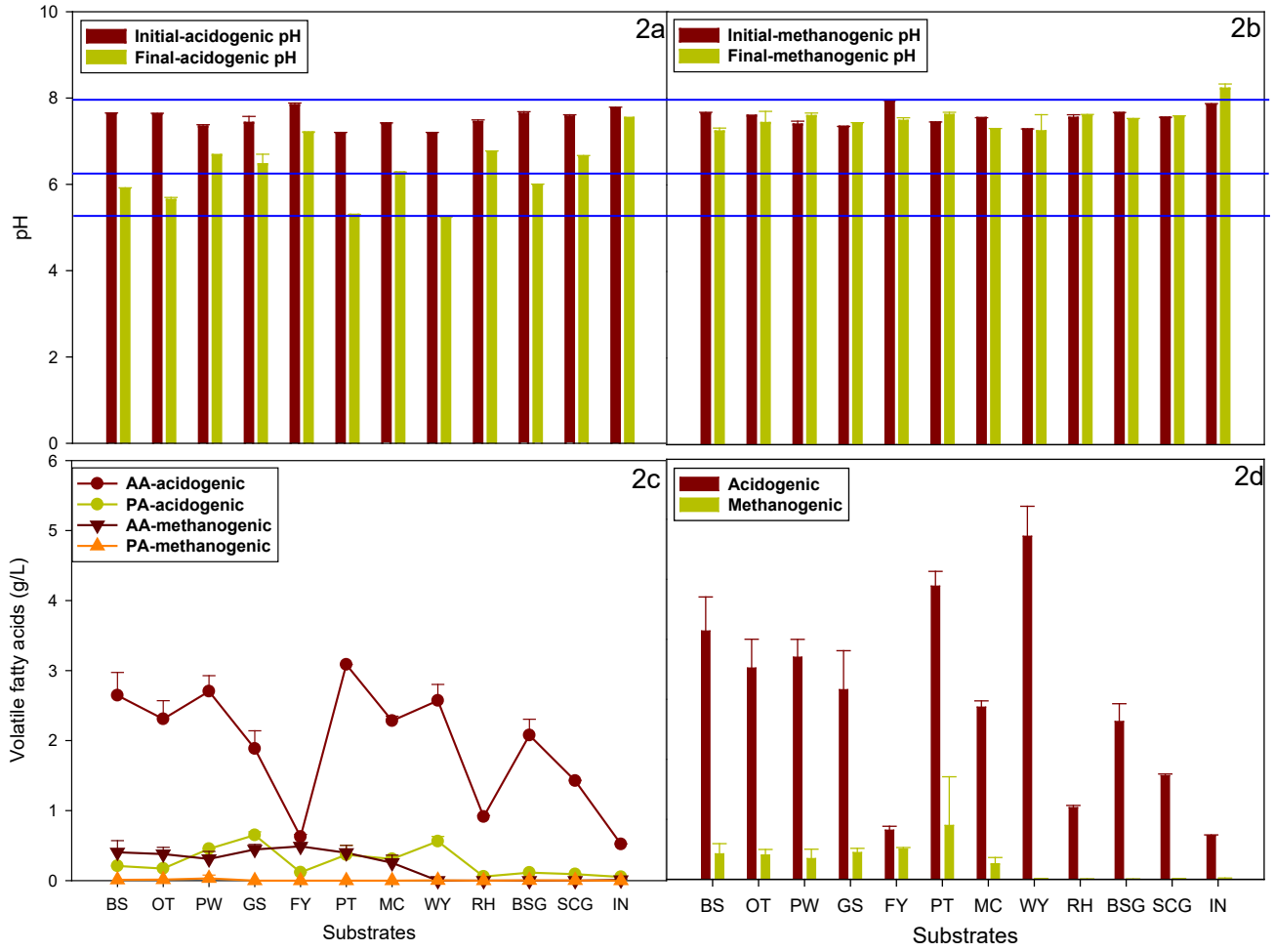
2a
Initial-acidogenic pH
Final-acidogenic pH
pH
2b
Initial-methanogenic pH
Final-methanogenic pH
2c
AA-acidogenic
PA-acidogenic
AA-methanogenic
PA-methanogenic
Volatile fatty acids (g/L)
2d
Acidogenic
Methanogenic
BS OT PW GS FY PT MC WY RH BSG SCG IN
Substrates

Fig.2.

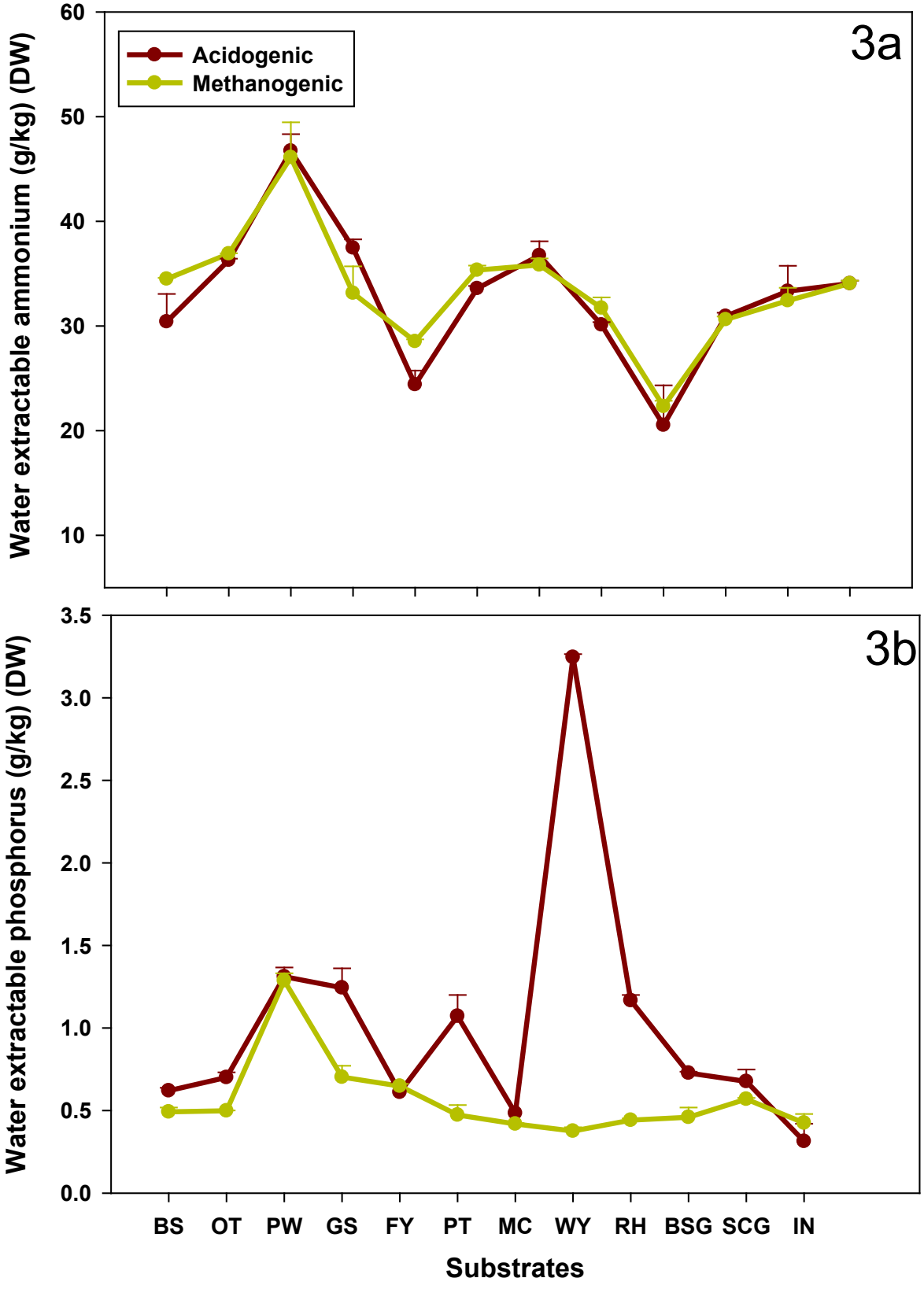
3a
Acidogenic
Methanogenic
Water extractable ammonium (g/kg) (DW)
60
50
40
30
20
10
3b
Water extractable phosphorus (g/kg) (DW)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
BS
OT
PW
GS
FY
PT
MC
WY
RH
BSG
SCG
IN
Substrates

Fig. 3.

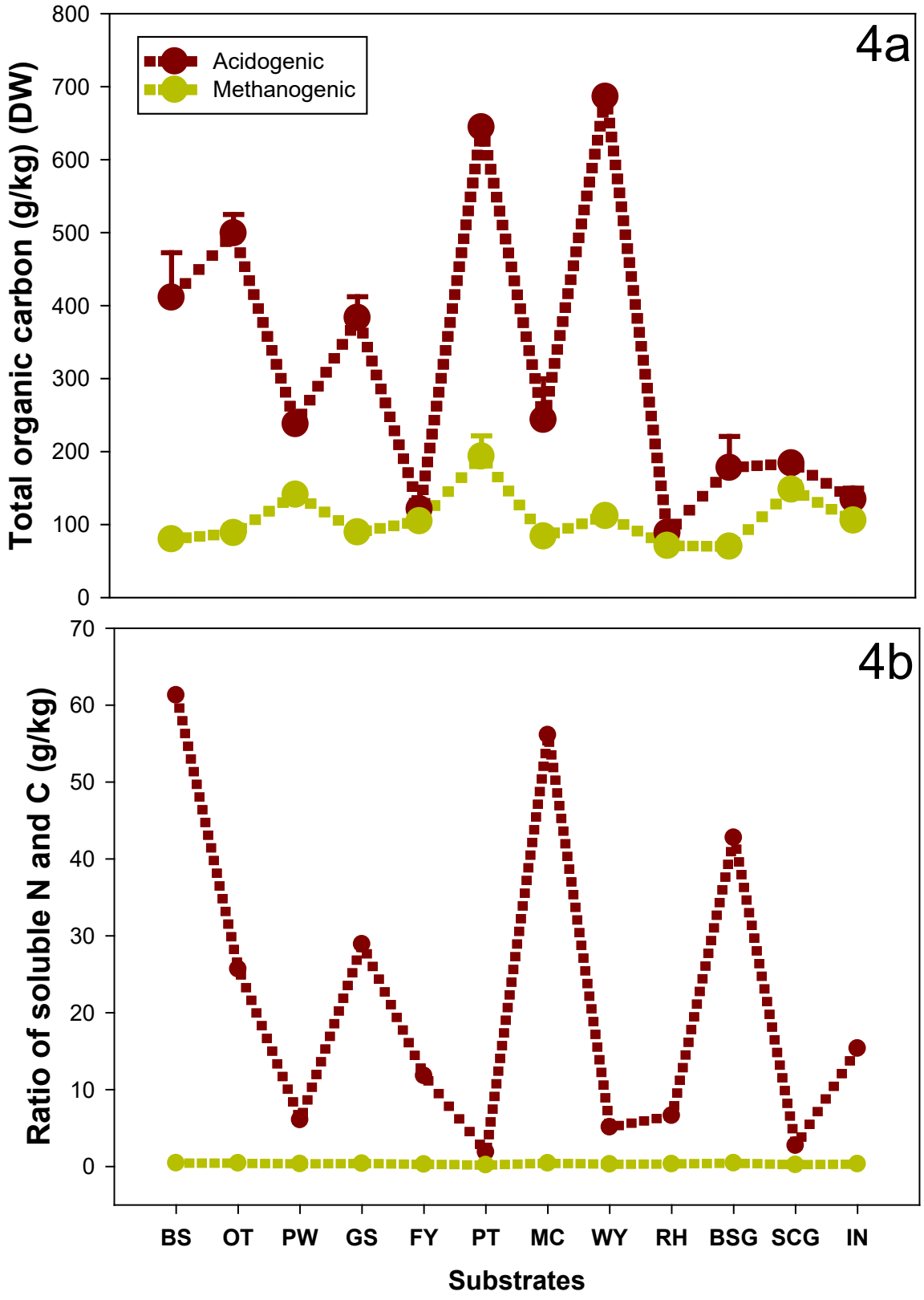
4a
Acidogenic
Methanogenic
Total organic carbon (g/kg) (DW)
800
700
600
500
400
300
200
100
0
4b
Ratio of soluble N and C (g/kg)
70
60
50
40
30
20
10
0
BS
OT
PW
GS
FY
PT
MC
WY
RH
BSG
SCG
IN
Substrates

Fig. 4

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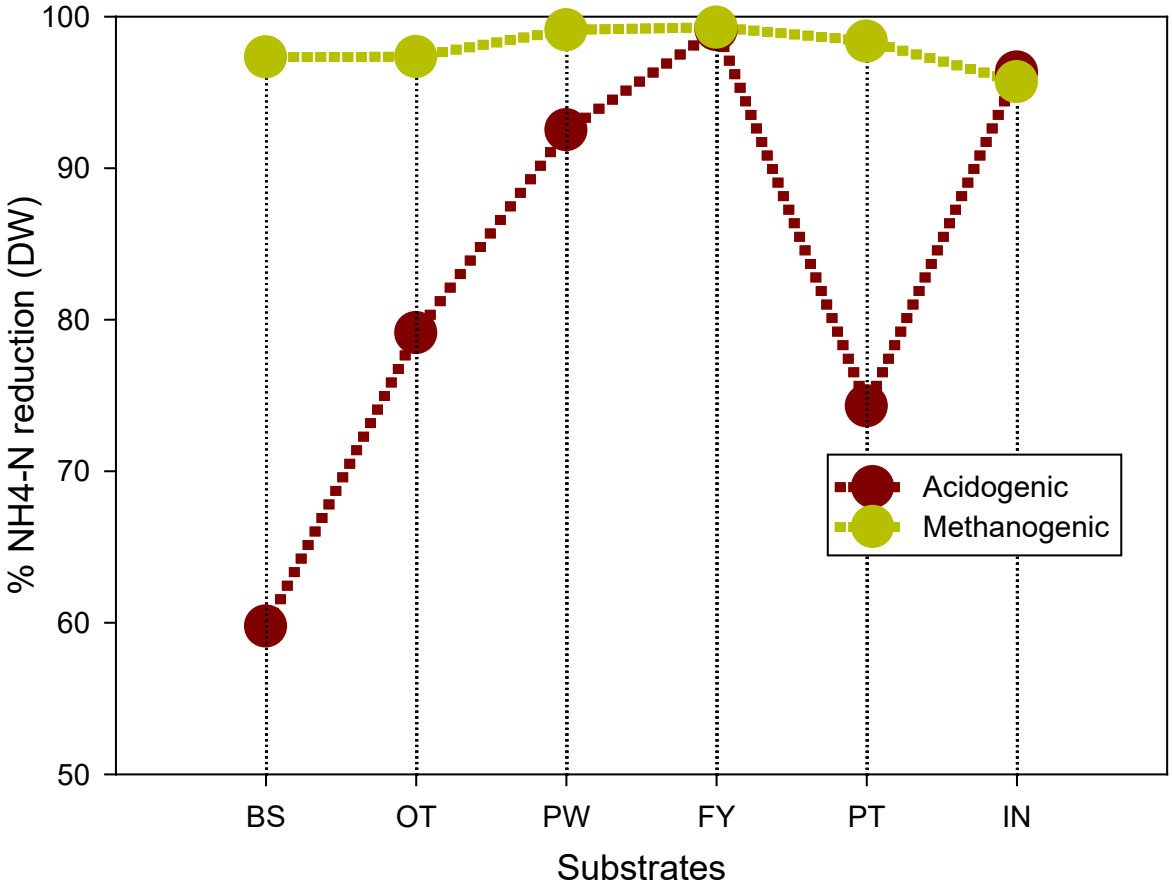
100
90
80
70
60
50
% NH4-N reduction (DW)
BS
OT
PW
FY
PT
IN
Substrates
Acidogenic
Methanogenic

63

64 Fig. 5

Table caption

Table 1: Characteristics of substrates in the batch trial

Table 2: Experimental design

Table 1

Sample ID	Sample	TS (g/kg)	VS (g/kg)	TC (g/kg)	TN (g/kg)	TP (g/kg)
BS	Bird seeds	793.4 ± 0.02	759.3 ± 0.02	451.9 ± 1.12	20.3 ± 1.30	0.91 ± 0.01
PW	Poultry manure	525.3 ± 0.19	465.3 ± 0.17	441.5 ± 0.84	47.5 ± 0.45	6.54 ± 0.66
OT	Oat grain	830.3 ± 0.01	810.4 ± 0.02	407.8 ± 2.60	16.1 ± 0.29	0.94 ± 0.01
GS	Grass silage	153.4 ± 0.02	135.7 ± 0.03	409.1 ± 2.03	19.0 ± 1.07	2.76 ± 0.05
FY	Farm yard manure	274.3 ± 0.06	196.1 ± 0.14	354.6 ± 0.39	17.1 ± 0.43	2.66 ± 0.14
PT	Potatoes	188.1 ± 0.01	177.5 ± 0.01	391.3 ± 0.84	12.3 ± 0.38	0.35 ± 0.01
MC	Maize cob	256.4 ± 0.01	250.7 ± 0.01	438.1 ± 1.40	10.9 ± 0.10	1.05 ± 0.03
WY	Whey Yorkshire	132.9 ± 0.12	118.2 ± 0.01	369.6 ± 0.55	9.90 ± 0.36	1.98 ± 0.03
RH	Rice husk	913.2 ± 0.06	737.2 ± 0.11	397.9 ± 0.62	9.60 ± 0.64	2.54 ± 0.09
BSG	Brewery spent grain	229.7 ± 0.05	221.6 ± 0.03	474.8 ± 0.84	34.0 ± 0.25	0.61 ± 0.02
SCG	Spent coffee grounds	443.6 ± 0.03	435.5 ± 0.01	512.1 ± 1.57	22.4 ± 0.52	0.69 ± 0.03

Values are expressed in mean and standard error (n = 3)

Table 2

Sample ID	Sample	Sample weight (g)	Sample weight (gVS)	Sample weight (g)	Sample weight (gVS)
IN	Inoculum	200	2.79	200.00	3.01
BS	Bird seed	3.67	2.79	3.96	3.01
OT	Oat grain	3.42	2.79	3.70	3.01
PW	Poultry waste	5.99	2.79	6.47	3.01
GS	Grass silage	20.54	2.79	22.18	3.01
FY	Farm yard manure	14.21	2.79	15.34	3.01
PT	Potatoes	15.70	2.79	16.95	3.01
MC	Maize cob	23.45	2.79	25.32	3.01
WY	Whey Yorkshire	23.58	2.79	25.47	3.01
RH	Rice husk	3.78	2.79	4.08	3.01
BSG	Brewery spent grain	12.58	2.79	13.59	3.01
SCG	Spent coffee grounds	6.40	2.79	6.91	3.01