# Physical-chemical traits, phytotoxicity and pathogen detection in liquid anaerobic digestates

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**Abstract**

Anaerobic digestates, which are co-products from biogas production, have been recognised as potential biofertilisers for their benefits in nutrient recovery and recycling of different types of organic wastes. Due to the increasing number of different types of organic wastes being used to produce biogas, it is necessary to identify how different types of anaerobic digestates vary in their physical-chemical traits, and how these can impact upon their use as fertilisers. In addition, safe land spreading of anaerobic digestates must be within recommended limits for potentially toxic elements (PTEs) and pathogens. This study analysed physical-chemical traits, phytotoxicity, PTEs and indicator pathogens in a set of eleven different commercial liquid anaerobic digestates from Ireland and the UK, and compared them to the Irish draft standard for digestate. Liquid anaerobic digestates exhibited significant differences (*P*<0.001) for most of the physical and chemical traits evaluated, with higher variability found for dry matter (DM) and K (*CV*= 17.2 and 16.8 respectively), and lower variation for pH and P (*CV*= 1.78 and 3.55 respectively). PTE concentrations were in general within recommended limits; nevertheless, some digestates showed higher concentrations than the recommended limits for Pb, Zn and Cu. Digestate from wastewater treatment feedstock was shown to be high in PTEs. Anaerobic digestates were found to negatively affect early stages of seed germination, but phytotoxicity effects were decreased by dilution in water. Levels of *Salmonella* spp. and *E. coli* were within recommended limits for most of the anaerobic digestates analysed.

**Keywords**: digestate; anaerobic digestion; biofertiliser; phytotoxicity; fertiliser value; potentially toxic elements

## 1. Introduction

The use of renewable energy derived from biogas has risen all over the world stimulated by benefits such as the generation of green energy, low-cost treatment for organic wastes from households, industry and agriculture, reduction of GHG emissions from organic waste degradation, associated methane capture from biological systems, and co-production of potential biofertilisers (Mao et al*.* 2015). These resulting residues, known as anaerobic digestates, are rich in nutrients and have been recognised as potential sustainable alternatives to conventional inorganic and other undigested organic fertilisers (Tambone et al. 2010; Albuquerque et al*.* 2012; Möller and Müller 2012; Walsh et al*.* 2012a).

The utilisation of anaerobic digestates as fertiliser still faces many challenges in terms of uses such as land spreading, due to a broad range of physical-chemical compositions (Albuquerque et al. 2012; Möller and Müller 2012; Nkoa 2014), which make it difficult to establish standard management practices such as fertilisation rates. Physical-chemical and microbiological traits of anaerobic digestate depend on several factors; however, most can be attributed to the type of feedstock utilised (Amani et al. 2010), pre-treatment of the feedstock (Appels et al*.* 2008), the effect of physical-chemical traits of the feedstocks used for digestion on the activity of microbial community within the reactor (Dai et al. 2016), and post-treatment and storage after digestion (Pell Frichmann consultants 2012). Differences between anaerobic digestates directly impact the management practices related to them.

In Europe, many different regulations and guidelines for anaerobic digestate production and use can be found (Holm-Nielsen et al. 2009). In the United Kingdom (UK), the utilisation of anaerobic digestates is subjected to environmental permitting or licenses (BSI, 2010). In Ireland, the Irish Bioenergy Association, in consultation with industry, has developed a draft standard for anaerobic digestate use (IrBEA 2012), based on reviews of standards and quality assurance throughout Europe. These standards deal with environmental impacts, health risks and waste management practices. In order to develop useful standards, it is necessary to better understand how different types of anaerobic digestates vary in their physical-chemical and microbiological composition. Such information can lead to improvements in the regulations about their use and land spreading, and also improve agriculture management practices related to them.

The aim of this study was to analyse physical-chemical traits, total nutrients, PTEs, phytotoxicity and indicator pathogens in a set of eleven different types of commercial liquid anaerobic digestates, and compare them to the concentrations recommended in the draft Irish digestate standards.

## 2. Material and methods

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### 2.1 Digestate sampling

Liquid anaerobic digestate samples from eleven different types of biogas plants were collected from Ireland and United Kingdom in October 2015 (Table 1). DM, ODM, pH, EC and N analyses were carried out within four days of sampling. For microbial analysis, all anaerobic digestate samples were kept refrigerated at 4 ºC and analysed a maximum of of one week from sampling.Samples for elemental composition and PTEs were kept at -20ºC and processed within two months. Samples were analysed according to the methods outlined in the draft IrBEA industry standard for digestate (IrBEA 2013). They were prepared in accordance with European standard EN 16179 (2012). For elemental analysis, samples were air-dried at 40°C until a constant weight.

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#### 2.2 Physical-chemical, elemental composition and PTEs

For DM analysis, samples were oven dried at 105°C according to European standard EN 13040 (2007). The organic dry matter (ODM) was determined by loss on ignition according to European standard EN 15935 (2012). Total organic carbon (TOC) was calculated based on the OM analysis, estimating that TOC was approximately 58% of the OM (Bernal et al. 1998). Total Kjeldahl nitrogen (TKN) was measured using a Buchi Kjeldahl apparatus according to European standard EN 16169 (2012). The C/N ratio was calculated using the ratio of the TOC and the TKN. For pH and electrical conductivity (EC), samples were extracted with deionised water at a ratio of 1:5 (v/v) according to European standard EN 15933 (2012). pH was measured with by probe (Mettler Toledo, Switzerland). After pH measurement, samples were centrifuged at 4500 rpm for 10 min, then the supernatant was filtered and measured for EC using a probe (CON-700, EUTECH), according to CEN/TS 15937 (2013). Total concentrations of the following chemical elements (P, Ca, K, Mg, Na, Mn, B, Co, Se, Al, Fe) and PTEs (Cd, Cr, Cu, Pb, Ni, Zn) were analysed using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) VARIAN model 710-ES, according to guidelines of CEN/TS 16170 (2012). The extracts analysed were produced after total digestion of dried anaerobic digestates (Section 2.1) in aqua regia (6 ml HCl + 2 ml HNO3) using a microwave digester (Mars 240/50, CEM) in accordance with the guidelines described in European standard EN 16174 (2012). For Hg, samples were sent to an external laboratory and analysed using ICP-MS.

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#### 2.3 Phytotoxicity

Seed germination assays were carried out by adapting the methodology described by Albuquerque et al. (2012). Seed germination tests were performed in square Petri dishes, where two filter papers moistened with 1 ml of solution served as an environment for seed germination. Ten cress (*Lepidium sativum*) seeds were sown in between filter papers. The dishes were sealed with parafilm and incubated in darkness at 23°C for 72 hours. Anaerobic digestates were diluted with deionised water to solution concentrations of 10%, 25%, 50%, and 100%. After incubation, the number of germinated seeds was noted, and germination was calculated as a percentage of the control (deionised water).

#### 2.4 Detection of pathogens

*Salmonella* spp. were enumerated in digestate samples by enrichment in selenite-cystine broth, followed by most-probable-number (MPN) analysis using Rappaport-Vassiliadis broth, and confirmed by streaking on XLD and Rambach agar, in accordance with CEN/TR 15215-2 (2006). *Escherichia coli* were enumerated by most-probable-number (MPN) analysis in Fluorocult lauryl sulphate broth confirmed by Kovac’s reagent, according to CEN/TR 16193 (2013).

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## 2.5 Statistical analysis

Physical-chemical trait data were tested for normality and equal variance (Levene's test), and analysed using one-way ANOVA. Seed germination data were analysed by descriptive statistics. Phytotoxicity was correlated with physical-chemical traits of anaerobic digestates using Pearson's correlation test (P>0.05 and P>0.01) in SPSS. Magnitudes of correlation follow: if | r | < 0.20, non-existent correlation; 0.20 < | r | < 0.40, weak correlation; 0.40 < | r | < 0.60, moderate correlation; 0.60 < | r | < 0.80 strong correlation; if | r | > 0.80 very strong correlation. Relationships among physical-chemical characteristics were analysed by principal component analysis (PCA) using XLSTAT (Addinsoft Software).

## 3. Results and discussion

### 3.1 Physical-chemical traits

All traits related to organic matter (DM, ODM, N, C, TOC, C/N) exhibited significant differences between anaerobic digestates (*P*<0.001). Liquid anaerobic digestates were found to have DM contents varying from 1.50 to 7.75% (Table 2), with an average of 3.61%. The ODM average was 57.8%, corresponding to an average of approximately 40% of mineral content in the DM in liquid anaerobic digestates. Total nitrogen in liquid anaerobic digestates varied between 6.58 to 24.11%, with an average of 11.69% and total organic carbon (TOC) from 18.3 to 41.9 %, with an average of 32.22%. The C/N ratio average was 3.49. Among these organic matter traits, the lowest coefficient of variation was found for ODM (*CV*= 6.28%), while the highest detected was for DM (*CV*=17.2%). The low *CV* found for ODM indicated that despite differences in DM content among liquid anaerobic digestates, there was a tendency for lower variability in the ratio between organic compounds and mineral content, although the range varied from 31.46 to 72.09%.

In a literature review, Nkoa (2014) reported that ODM in liquid anaerobic digestates varied between 38.6 to 75.4%, similar results to the ones found in the present study. In agriculture, DM is sometimes used a means of standardising application rates between different types of digestates (i.e., the volume of each digestate will be varied so that the same amount of DM of each is spread). However, since there is high variability in ODM content, applying the same amount of total DM of different digestates can result in varying amounts of ODM being provided. These differences in ODM can have a substantial impact on nutrient supply and also environmental issues, especially PTE accumulation. It is recommended that anaerobic digestates should have their ODM contents analysed before fertilisation, especially when fertilisation rates were established based on DM contents. Variations in dry/organic matter in liquid anaerobic digestates are generally due to a combination of factors, such as: feedstock used for biogas production, initial C/N ratios, pre-and post-treatment, and/or efficiency of the anaerobic digestion process (microbial activity, HRT and temperature) (Yadvika et al. 2004). Another factor that causes variability in anaerobic digestates is the fact that biogas plants use different types of feedstocks available in the moment, generally using mixtures of different feedstocks. Common combinations that can be found in biogas plants are mixtures of animal slurries and food wastes (Nkoa 2014). In the present study, most of the anaerobic digestates evaluated were from mixtures of animal slurry and food wastes (Table 1), which might have contributed to the variability found in many of the traits evaluated.

pH values in liquid anaerobic digestate had an average of 8.23 and had low variability among different types of liquid anaerobic digestates (*CV*= 1.78), although significant differences were detected (*P*<0.0001). The pH of anaerobic digestates in this study varied from slightly (7.73) to moderate alkaline (8.49). Due to this alkaline nature, the land spreading of anaerobic digestates has been associated with increases in soil pH (Makádi et al. 2012; Voelkener et al. 2015). Nevertheless, the presence of acid compounds in liquid anaerobic digestates might also cause pH decreases due to organic acid condensation, physical-chemical transformations, and connections to other organic and inorganic colloids (Makádi et al. 2012). The process of nitrification of NH4-Nfrom anaerobic digestate after land spreading can also result in the release of considerable amounts of H+ in the soil, as anaerobic digestates are generally composed of 40 to 70% NH4-N (Albuquerque et al. 2012).

The EC of liquid anaerobic digestates ranged from 152.7 to 595.7 μS/cm, averaging 384.5 μS/cm, with significant differences among values (*P*<0.001). The EC variability can be mostly explained by differences in the number of free ions in the solution, salinity level and physical properties of the liquid digestates. EC of anaerobic digestates should be considered when using them as fertiliser because their land spreading might affect directly soil electrical properties. Voelkener et al. (2015) reported that fertilisation with anaerobic digestates was associated with increases in electrical conductivity in loamy and sandy soils, while Albuquerque et al. (2012) cautioned that special attention should be given when using excessive doses or continuous applications of anaerobic digestates, especially when salt concentrations are high. In the literature, considerable variability of results can be found for EC in liquid anaerobic digestates. For example Voelkener et al. (2015) reported EC of liquid anaerobic digestates ranging from 77 to 91 μS/cm, while in the work of Albuquerque et al. (2012), the results ranged from 5,200 to 30,800 μS/cm. Considering the variability of EC found for anaerobic digestates in different studies, the results found in the present trial were closest to the ones reported in four recent European studies (Bougnom et al. 2012; Walsh et al. 2012a; Walsh et al. 2012b; Pokój et al. 2015).

Elemental analysis of the liquid anaerobic digestates showed that nutrients with highest concentrations were K, Ca, Na, P and Fe (61.53, 32.84, 27.27, 17.39 and 10.60 mg kg-1 DM respectively) (Table 3). K had the highest variability among the elements (*CV*= 16.82), due to the large range of differences in K concentrations (7.49 to 173.48 g kg-1 DM). In contrast, P had lower variability (*CV* = 3.55), with concentrations ranging from 8.10 to 32.80 g kg-1 DM. Significant differences (*P*<0.001) were found between the digestate samples for all elements analysed, with the exception of the elements that were below the detection limit such as B and Co.

Anaerobic digestion has been recognised as an excellent option for recycling and recovering essential nutrients from a variety of organic wastes, and these recycled elements, especially plant macronutrients (N, P and K), can contribute to reducing agriculture costs by decreasing artificial fertiliser use (Albuquerque 2012; Möller and Müller 2012; Nkoa 2014). Nevertheless, the use of anaerobic digestates as fertiliser faces many issues related to high variability in their chemical composition. The results found in the present study indicated that high variability in essential macro and micronutrients in liquid anaerobic digestates can lead to different supplementation requirements to meet the specific needs of different types of agriculture crops (Sheets et al. 2015). For example, the ratios of N, P and K varied widely, with some having almost the same concentrations of P and K, while others had more P than K or vice-versa. Farmers must keep this variability in mind and perform an analysis of the macro nutrients such as N, P and K present prior to using digestate as fertiliser. Several different fertilisation trials have reported the need for supplementation of nutrients when using anaerobic digestates as fertilisers. Liedl et al. (2006) reported anaerobic digestate was an incomplete fertiliser for the set of crops evaluated, and supplementation of nutrients was necessary to meet specific crop growth requirements, while Svensson et al. (2004) reported that P was the main supplementation requirement when anaerobic digestates were used as fertilisers. One of the main challenges for the use of anaerobic digestate as fertiliser is to produce standard fertilisation rates for different crops, which depends on research trials aiming to address crop growth responses to different types of anaerobic digestates.

### 3.2 Potentially toxic elements

Although PTE averages were generally below or close to the recommended limits suggested by Irish agencies (Table 4), some anaerobic digestates did exceed the limits for Zn and Pb. For Zn, four anaerobic digestates showed higher concentrations than recommended: AD1, AD4, AD5 and AD11 (434.03, 515.63, 1155.23 and 445.73 mg kg-1, respectively). For Pb, average concentrations were in general lower than the recommended limits in most of the anaerobic digestates analysed. However, one sample (AD5) stood out from the other anaerobic digestates due to its high Pb concentration (1959.83 mg kg-1). This digestate was from a wastewater treatment plant. Wastewater treatment sludge is known for being a source of concentrated heavy metals (Fu and Wang 2011; Barakat 2011). Total concentrations of heavy metals in this study were similar to other recent published findings that evaluated different types of anaerobic digestates (Albuquerque et al. 2012; Kupper et al. 2014), where most of the anaerobic digestates evaluated had PTE concentrations below or close to the recommended limits cited in Table 4.

Safe environmental application of anaerobic digestates, in terms of PTEs, depends upon the chemical composition and availability of these elements in the anaerobic digestate. Although most of the anaerobic digestates investigated were within the recommended limits for PTE, the total concentration is only an indication of the potential for toxicity. Many other factors and interactions between anaerobic digestates, soil, and plants can influence the level of heavy metal bioavailability, and therefore toxicity (Tchounwou et al. 2012). According to Zhu et al. (2014), only when heavy metals are in their ionic form or in the exchangeable fraction of the soil do they migrate and accumulate in plants and other living organisms; therefore, heavy metals in the water-soluble fraction of the anaerobic digestates (Cu, Zn, Mn, Ni and Cd) deserve more attention, due to direct toxicity to the environment. Another factor to consider about PTEs in anaerobic digestates is leaching and accumulation in agricultural soils due to constant application of anaerobic digestates (Bonten et al. 2008; Möller and Müller 2012; Nkoa 2014).

3.3 Relationships among physical-chemical traits

Principal component analysis was conducted in order to determine relationships between digestates and their physical-chemical characteristics (Figure 1). DM content was correlated with the C/N ratio, indicating that the anaerobic digestates with lower dry matter content tended to have higher values of total N. Both DM and C:N are related to the type of feedstock used in the anaerobic digestion process. Digestates AD1 and AD9 were most highly correlated with total N. AD1 originated from a biogas plant that processes dairy industry wastes, mostly composed of whey, and AD9 was produced from a mixture of animal slurries, including chicken manure. Whey and chicken manure are known for their considerable concentrations of N and low C/N ratios compared to other organic wastes commonly used in anaerobic digestates (Wang et al. 2012; Carlini et al. 2015). The total N concentration in liquid digestates was also correlated with K and pH. Albuquerque et al. (2012) reported high correlation coefficients (r=0.90) between total N and K in liquid anaerobic digestates. The positive correlations between N and pH can be explained by the fact that during anaerobic digestion of organic feedstocks, the pH is increased by the production of ammonia (Melamane et al. 2007; Tambone et al. 2009). Ammonia concentration in liquid anaerobic digestates is associated with the total N content or low C/N ratios (Albuquerque et al. 2012; Wang et al. 2012). In relation to the PTEs, it was observed that they were mostly correlated among themselves. The anaerobic digestate most strongly correlated with PTE concentrations was (AD5), produced from wastewater treatment. As discussed above, wastewater treatment sludge is known to be high in heavy metals (Fu and Wang 2011; Barakat 2011).

### 3.4 Phytotoxicity

Phytotoxicity results showed that in general, concentrations of liquid anaerobic digestates greater than 50% completely suppressed cress seed germination. The anaerobic digestate AD7 showed the lowest phytotoxicity effects, exhibiting high germination at concentrations of 50 and 100% (Figure 2). Many factors, such as electrical conductivity, can influence seed germination when in contact with anaerobic digestates. Correlation analysis showed that EC was the only variable that had a significant (P<0.05) strong positive correlation with phytotoxicity (r= 0.76), although moderate positive correlations were also detected for DM (r=0.46), pH (r=0.50), and Na (r=0.44). The relationship between EC and phytotoxicity was seen in AD7, which had one of the lowest values for EC (20.53 mS/cm), and the highest germination rate. Three digestates (AD4, AD9 and AD11) suppressed germination completely at all dilutions tested; these digestates also had high EC μS/cm (595.7, 425.3 and 412, respectively). Similar results have been reported in the literature, with Albuquerque et al. (2012) finding that germination of cress and lettuce seeds was inversely correlated with electrical conductivity, and McLachlan et al. (2004) reporting a negative correlation between the germination index of cress and radish seeds and electrical conductivity of anaerobic digestates. Abdullahi et al. (2008) found that seed germination can be increased by diluting anaerobic digestates, which was also observed for the anaerobic digestates in this study; according to Möller and Müller (2012), once anaerobic digestates are spread on a field site, the possible risks and negative effects of phytotoxicity can quickly decrease.

### 3.5 Pathogen detection

*Salmonella* spp. was not detected in most of the anaerobic digestates (Table 5); only one sample (AD1) contained a low level (7 CFU 10 g-1 fresh mass). Low detection of *Salmonella* spp, meets current legislation for animal by-product (ABP) handling and processing (IrBEA 2013). *Salmonella* is one of the most common pathogens that can be spread in the environment through animal slurries and sewage sludge (Sahlström 2003). *Salmonella* strains that can be harmful to humans mostly originate from animals used in food production such as pigs, cattle and poultry (Kagambèga et al. 2013). As most of the anaerobic digestates evaluated in this study were from animal slurry feedstocks, the low detection of *Salmonella* spp. indicated that despite differences in operational parameters, temperature and HRT, the inactivation of this pathogen has been achieved by the biogas plants. Thermophilic conditions, combined with longer HRT and pre/post pasteurisation, are the main components in *Salmonella* spp. inactivation in biogas tanks. Additionally, volatile fatty acids play an important role in the inactivation of *Salmonella* spp, with high concentrations of organic acids such as acetic, propionic and butyric acid produced during the AD process directly reducing this pathogen (Salsali et al. 2006).

The concentration of *E. coli* varied from <0.3 (not detected), to 2400 CFU g-1 fresh mass (Table 5). Except for one digestate (AD3), all digestates met Irish recommended limits (IrBEA, 2013) for *E. coli* detection in anaerobic digestates. AD3 was not pasteurised pre- or post-digestion, which may have contributed to its relatively high levels of *E. coli*. Anaerobic digestion in general is known to reduce or inactivate *E. coli* (Aitken et al. 2007; Massé et al. 2011; Pandey and Soupir 2011); however, this effect seems to vary according to digestion temperature. Massé et al. (2011) reported that *E. coli* concentrations in pig slurry were decreased to undetectable levels by psychrophilic anaerobic digestion in sequential batch reactors operated at 7 and 14 days. Pandey and Soupir (2011) demonstrated that batch anaerobic digestion of dairy cattle manure affected *E. coli* in different ways according to the temperature level, with higher temperatures requiring shorter times for inactivation. All anaerobic digestates tested in the present study were produced under mesophilic conditions with varied HRTs ranging from 14-70 days; the majority were carried out for over 40 days, which should encourage inactivation of *E. coli*.

**4. Conclusion**

* The anaerobic digestates analysed in this study were shown to be potentially useful biofertilisers due to their concentrations of plant essential nutrients such as N (6.6 to 24.1%, average 11.7%), P (8.1 to 32.8 g kg-1 DW, average 17.4), and K (8.1 to 173.5 g kg-1 DW, average 61.5). However, the proportions of N-P-K in each digestate were widely variable.
* All anaerobic digestates analysed were below recommended limits for the concentrations of the following potentially toxic elements: Cr, Cd, Ni, and Hg (limits 92, 1.3, 56, 0.4 mg kg-1 DW, respectively). However, three PTEs were over limit in some of the digestates analysed: Pb (limit 149 mg kg-1 DW; AD5=1959); Zn (limit 397 mg kg-1 DW; AD1=434, AD4=516, AD5=1155, AD6=755, AD11=456); and Cu (limit 149 mg kg-1 DW; AD4=307, AD5=224, AD6=210, AD11=281). AD5 was derived from wastewater treatment feedstock, which may be responsible for its higher concentrations of PTEs.
* Phytotoxicity was associated with EC and decreased with anaerobic digestate dilution.
* Levels of *Salmonella spp.* and *E. coli* in the anaerobic digestates analysed were within the suggested limits recommended.
* In conclusion, the liquid anaerobic digestates evaluated showed substantial differences in terms of nutrients and physical-chemical characteristics. Due to the complexity of anaerobic digestates and especially their widely variable composition, it may be difficult to produce standard fertilisation rates for different digestates. Therefore, it is strongly recommended that their land spreading should be preceded by a physical-chemical and nutrient analysis.

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Table 1. Feedstock composition and operational aspects of biogas plants supplying the set of anaerobic digestates evaluated. HRT= Hydraulic retention time

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Digestate** | **Feedstock** | **Operation**  | **Temperature (ºC)** | **HRT (days)** | **Volume (m3)** | **Pasteurisation** |
| AD1 | Food waste (dairy industry) | Continuous | Mesophilic | 70 | 1200 | Pre-digestion |
| AD2 | Food waste, pig slurry | Continuous | 40 | 90 | 2000 | Post-digestion |
| AD3 | Food waste (farm and food)  | Continuous | 38 | 54 | 600 | No |
| AD4 | Food waste, municipal sludge | Continuous | 37-42 | 60 | 1850 | Post-digestion |
| AD5 | Waste water treatment  | Batch | Mesophilic | 14 | 1700 | Pre-digestion |
| AD6 | Food waste, garden waste  | Continuous | Mesophilic | 26 | 5200 | Pre-digestion |
| AD7 | Whole cattle slurry | Continuous | 27 | 22 | 220 | No |
| AD8 | Whole grass  | Continuous | 40 | 70 | 0.2 | No |
| AD9 | Cattle slurry, chicken manure, food waste | Continuous | Mesophilic | 40 | 265 | No |
| AD10 | Whole cattle slurry | Continuous | Mesophilic | 40-50 | 870 | No |
| AD11 | Food waste (kitchen), garden waste | Continuous | Mesophilic | 70 | 0.2 | No |

Table 2. Physical-chemical characterisation of liquid anaerobic digestates. Means are followed by standard errors in parentheses. ANOVA: \*\*\*: significant at probability level *P* < 0.001. DM= Dry matter; ODM= Organic dry matter; TKN= Total Kjeldahl N%; TOC= Total organic carbon; C: N= Carbon/nitrogen ratio; EC= Electrical conductivity; *CV*= Coefficient of variation

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **DM (%)** | **ODM %** | **TKN%** | **TOC** | **C/N** | **pH** | **EC (μS/cm)** |
|  |  |  |  |  |  |  |  |
| **AD1** | 2.82 | 64.17 | 16.54 | 37.4 | 2.37 | 8.49 | 442.3 |
| *SE* | (0.06) | (1.66) | (1.37) | (0.50) | (0.09) | (0.27) | (8.5) |
| **AD2** | 5.08 | 61.18 | 10.62 | 35.6 | 3.35 | 8.25 | 559.3 |
| *SE* | (0.07) | (5.77) | (0.44) | (3.21) | (0.19) | (0.06) | (95.8) |
| **AD3** | 3.27 | 53.78 | 7.47 | 31.27 | 4.18 | 8.13 | 227.2 |
| *SE* | (0.35) | (3.72) | (0.25) | (7.21) | (1.02) | (0.10) | (31.7) |
| **AD4** | 7.75 | 68.05 | 10.41 | 41.1 | 3.96 | 8.28 | 595.7 |
| *SE* | (0.08) | (3.58) | (0.20) | (0.10) | (0.85) | (0.10) | (8.3) |
| **AD5** | 1.92 | 55.45 | 10.37 | 32.2 | 3.11 | 7.73 | 152.7 |
| *SE* | (0.30) | (1.51) | (0.21) | (0.85) | (0.13) | (0.03) | (6.7) |
| **AD6** | 3.62 | 47.48 | 10.36 | 23.2 | 2.30 | 8.17 | 529.3 |
| *SE* | (0.08) | (0.45) | (2.31) | (0.01) | (0.51) | (0.01) | (21.7) |
| **AD7** | 2.36 | 62.82 | 10.10 | 35.8 | 3.54 | 7.88 | 205.3 |
| *SE* | (0.85) | (3.06) | (0.44) | (1.69) | (0.06) | (0.05) | (8.5) |
| **AD8** | 1.50 | 31.46 | 13.60 | 18.3 | 1.24 | 8.33 | 296.7 |
| *SE* | (0.08) | (4.67) | (0.49) | (2.62) | (0.10) | (0.04) | (21.9) |
| **AD9** | 1.73 | 48.99 | 24.11 | 28.5 | 1.16 | 8.85 | 425.3 |
| *SE* | (0.14) | (4.35) | (0.33) | (2.49) | (0.14) | (0.27) | (47.5) |
| **AD10** | 4.78 | 72.09 | 6.58 | 41.9 | 6.40 | 8.33 | 384.0 |
| *SE* | (0.20) | (3.34) | (0.42) | (1.96) | (0.72) | (0.23) | (14.9) |
| **AD11** | 4.89 | 70.68 | 8.39 | 41.1 | 4.93 | 8.07 | 412.0 |
| *SE* | (1.83) | (4.31) | (0.61) | (2.51) | (0.61) | (0.01) | (59.5) |
|  |  |  |  |  |  |  |  |
| **Average** | 3.61 | 57.83 | 11.69 | 32.22 | 3.49 | 8.23 | 384.5 |
| **ANOVA** | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* |
| ***CV*** | 17.2 | 6.28 | 7.00 | 9.06 | 14.89 | 1.78 | 10.35 |

Table 3. Elemental composition (g kg-1 dry weight) of liquid anaerobic digestates. ANOVA: \*\*\*: significant at probability level *P* < 0.001. *CV*= Coefficient of variation; (<)= under limit of detection; NA= Not analysed

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **P** | **K** | **Ca** | **Mg** | **Na** | **Fe** | **Al** | **Mn** | **B** | **Co** | **Se** |
|  | (g kg-1 DW) |
| **AD1** | 12.06 | 45.15 | 36.55 | 3.10 | 61.08 | 6.32 | 1.51 | 0.12 | <0.0006 | <0.0006 | <0.0008 |
| *SE* | (0.72) | (23.65) | (39.97) | (0.35) | (32.49) | (0.41) | (0.09) | (0.006) | (0) | (0) | (0) |
| **AD2** | 29.71 | 58.37 | 33.90 | 12.09 | 39.33 | 2.28 | 8.26 | 0.24 | <0.0006 | <0.0006 | 0.0083 |
| *SE* | (10.06) | (13.06) | (0.57) | (0.53) | (11.73) | (0.12) | (0.21) | (0.004) | (0) | (0) | (0.0014) |
| **AD3** | 32.80 | 32.69 | 22.34 | 12.69 | 7.28 | 16.01 | 20.87 | 0.26 | <0.0006 | <0.0006 | <0.0008 |
| *SE* | (0.67) | (31.32) | (63.98) | (0.93) | (11.37) | (0.46) | (0.78) | (0.003) | (0) | (0) | (0) |
| **AD4** | 23.80 | 7.49 | 24.04 | 3.57 | 8.06 | 15.94 | 21.80 | 0.28 | <0.0006 | <0.0006 | <0.0008 |
| *SE* | (0.42) | (16.94) | (0.41) | (0.39) | (54.47) | (0.90) | (10.17) | (0.016) | (0) | (0) | (0) |
| **AD5** | 21.65 | 8.09 | 32.42 | 9.37 | 5.62 | 13.14 | 12.40 | 0.28 | 0.0028 | <0.0006 | 0.0039 |
| *SE* | (0.35) | (0.63) | (18.81) | (0.26) | (0.31) | (0.44) | (0.48) | (0.006) | (0.003) | (0) | (0.006) |
| **AD6** | 20.55 | 75.84 | 48.68 | 7.92 | 72.70 | 5.17 | 2.29 | 0.41 | <0.0006 | 0.00160 | 0.0039 |
| *SE* | (15.23) | (24.24) | (52.48) | (11.78) | (35.84) | (0.38) | (0.16) | (0.007) | (0) | (0.0004) | (0.006) |
| **AD7** | 9.78 | 38.18 | 24.62 | 11.98 | 7.55 | 2.57 | 1.76 | 0.29 | <0.0006 | <0.0006 | 0.0014 |
| *SE* | (0.32) | (44.38) | (0.65) | (0.31) | (12.47) | (0.02) | (0.07) | (0.006) | (0) | (0) | (0.002) |
| **AD8** | 9.21 | 173.48 | 30.81 | 3.22 | 25.07 | 23.39 | 6.87 | 0.25 | <0.0006 | <0.0006 | 0.0216 |
| *SE* | (0.04) | (43.04) | (0.21) | (0.18) | (0.62) | (0.14) | (0.08) | (0.002) | (0) | (0) | (0.03) |
| **AD9** | 11.51 | 119.90 | 25.67 | 2.34 | 47.42 | 22.30 | 19.47 | 0.25 | <0.0006 | <0.0006 | 0.0115 |
| *SE* | (0.007) | (13.08) | (0.17) | (0.08) | (0.55) | (0.12) | (0.10) | (0.001) | (0) | (0) | (0.001) |
| **AD10** | 8.10 | 63.93 | 28.50 | 5.48 | 7.86 | 3.05 | 1.50 | 0.19 | <0.0006 | <0.0006 | 0.0100 |
| *SE* | (0.41) | (63.85) | (11.27) | (0.41) | (0.80) | (0.17) | (0.27) | (0.004) | (0) | (0) | (0.001) |
| **AD11** | 12.11 | 53.75 | 53.77 | 3.14 | 18.01 | 6.48 | 1.45 | 0.34 | <0.0006 | <0.0006 | 0.0130 |
| *SE* | (0.15) | (267.2) | (23.52) | (0.86) | (94.29) | (0.43) | (0.12) | (0.029) | (0) | (0) | (0.001) |
|  |  |  |  |  |  |  |  |  |  |  |  |
| **Average** | 17.39 | 61.53 | 32.84 | 6.81 | 27.27 | 10.60 | 8.92 | 0.26 | 0.0003 | 0.00015 | 0.0067 |
| **ANOVA** | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* | \*\*\* | N/A | N/A | \*\*\* |
| ***CV*** | 3.55 | 16.82 | 9.16 | 8.65 | 15.31 | 3.84 | 4.87 | 4.24 | N/A | N/A | 94.99 |
|   |

Table 4. Potentially toxic elements (PTEs) (mg kg-1 DM) in liquid anaerobic digestates. ANOVA: \*\*\*: significant at probability level *P* < 0.001. *CV*= Coefficient of variation; (<)= under limit of detection. Hg and Cd were not analysed statistically since most values were below detection limits.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** |  | **Pb** | **Zn** | **Cu** | **Cr** | **Cd** | **Ni** | **Hg** |
|  |  | (mg kg-1 DW) |
|  |  |
| **AD1** |  | 5.87 | 434.03 | 54.70 | 16.00 | <0.00002 | 8.6 | 0.0002 |
| *SE* |  | (0.46) | (38.45) | (4.78) | (0) | (0) | (0) | (0) |
| **AD2** |  | 1.37 | 359.90 | 60.93 | 11.80 | <0.00002 | 5.93 | <0.000001 |
| *SE* |  | (2.36) | (12.75) | (1.55) | (0) | (0) | (3.25) | (0) |
| **AD3** |  | 9.47 | 300.97 | 112.97 | 68.77 | <0.00002 | 27.63 | 0.0002 |
| *SE* |  | (2.13) | (5.82) | (1.13) | (0) | (0) | (0) | (0) |
| **AD4** |  | 17.23 | 515.63 | 306.77 | 20.77 | <0.00002 | 13.7 | 0.0004 |
| *SE* |  | (1.69) | (37.11) | (28.40) | (0) | (0) | (0) | (0) |
| **AD5** |  | 1959.83 | 1155.23 | 223.97 | 46.83 | <0.00002 | 27.93 | 0.0006 |
| *SE* |  | (93.15) | (13.90) | (1.34) | (0) | (0) | (0) | (0) |
| **AD6** |  | 15.67 | 755.00 | 209.57 | 15.20 | <0.00002 | 20.23 | <0.000001 |
| *SE* |  | (3.63) | (7.10) | (2.47) | (0) | (0) | (0) | (0) |
| **AD7** |  | 0.60 | 319.07 | 47.70 | 4.70 | <0.00002 | 2.37 | <0.000001 |
| *SE* |  | (1.03) | (20.11) | (1.90) | (0) | (0) | (0) | (0) |
| **AD8** |  | 19.03 | 237.93 | 91.10 | 4.47 | <0.00002 | 6.87 | <0.000001 |
| *SE* |  | (1.22) | (2.10) | (1.77) | (0) | (0) | (0) | (0) |
| **AD9** |  | 7.17 | 251.40 | 70.37 | 9.83 | <0.00002 | 10.60 | <0.000001 |
| *SE* |  | (0.83) | (2.00) | (0.90) | (0) | (0) | (0) | (0) |
| **AD10** |  | 1.80 | 153.47 | 42.27 | 8.43 | <0.00002 | 4.27 | <0.000001 |
| *SE* |  | (0.45) | (15.01) | (3.68) | (0) | (0) | (0) | (0) |
| **AD11** |  | 48.43 | 445.73 | 281.10 | 6.37 | <0.00002 | 33.20 | 0.0002 |
| *SE* |  | (1.85) | (87.60) | (54.75) | (0) | (0) | (0) | (0) |
|  |  |  |  |  |  |   |  |  |
| **Average** |  | 189.7 | 448.0 | 136.5 | 19.4 | 0 | 14.7 | 0.0001 |
| **ANOVA** |  | \*\*\* | \*\*\* | \*\*\* | \*\*\* | N/A | \*\*\* | N/A |
| ***CV***  |  | 14.83 | 7.25 | 13.72 | 3.35 | N/A | 40.4 | N/A |
| **Limits for PTEs** | **149** | **397\*** | **149\*** | **92** | **1.3** | **56** | **0.4** |
| **Irish Bioenergy Association (IrBEA, 2013).** | **\*Note**: Copper and Zinc are plant micro nutrients and limit values are not absolute. Should these values be exceeded, specific labelling/provision of information to the end user is required. Absolute levels must not exceed 30% above limit values. |
|  |
| Table 5. Detection of *Salmonella* spp. (MPN CFU 10 g-1 FW) and *Escherichia coli* (MPN CFU g-1 FW)in liquid anaerobic digestates. FW= fresh weight

|  |  |  |
| --- | --- | --- |
| **Sample** | ***Salmonella* spp.****(MPN CFU 10 g-1 FW)** | ***Escherichia coli*** **(MPN CFU g-1 FW)** |
| AD1 | 7 | <0.3 (not detected) |
| AD2 | <10 (not detected) | 8 |
| AD3 | <10 (not detected) | 2400 |
| AD4 | <10 (not detected) | <0.3 (not detected) |
| AD5 | <10 (not detected) | 460 |
| AD6 | <10 (not detected) | <0.3 (not detected) |
| AD7 | <10 (not detected) | 15 |
| AD8 | <10 (not detected) | 9 |
| AD9 | <10 (not detected) | <0.3 (not detected) |
| AD10 | <10 (not detected) | <0.3 (not detected) |
| AD11 | <10 (not detected) | 23 |
| Draft digestate standard limits**Irish Bioenergy Association (IrBEA, 2013).** | Not detected in 25 g | < 1000 CFU/g fresh mass |

C:\Users\20068905.WIT\Dropbox\Janerson_Nabla\Publications\Articles\Digestate physical-chemical\PCA graph updated symbols.jpg**Figure 1**. Principal component analysis of physical-chemical traits in liquid anaerobic digestates. Physical-chemical traits as arrows and digestate samples (*n*=3) as symbols. AD= Anaerobic digestate; DM= Dry matter; OM= Organic matter; ODM= Organic dry matter; TKN= Total Kjeldahl N%; TOC= Total Organic Carbon; C:N= Carbon/nitrogen ratio; EC= Electrical conductivity. |

**Figure 2**. Anaerobic digestate phytotoxicity under different concentrations (100, 50, 25 and 10%) of anaerobic digestate, calculated based on the germination (%) of cress seeds (*Lepidium sativum*) in relation to the control (distilled water).