Benefits and risks of agricultural reuse of digestates from plastic tubular digesters in Colombia

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Abstract

The aim of this study is to characterize the digestates from three plastic tubular digesters implemented in Colombia fed with: i) cattle manure; ii) cattle manure mixed with cheese whey; iii) pig manure. All the digesters worked under psychrophilic conditions. Physico-chemical characteristics, heavy metals, pathogens, and agronomic quality were investigated.

All the digestates were characterized by physico-chemical characteristics and nutrients concentration suitable for their reuse as biofertilizer. However, these digestates may only partially replace a mineral fertilizer due to the high nutrients dilution. Heavy metals were under the detection limit of the analytical method (Pb, Hg, Ni, Mo, Cd, Chromium VI) or present at low concentration (Cu, Zn, As, Se) in all the digestates. Biodegradable organic matter and pathogens (coliform, helminths and *Salmonella* spp.) analysis proved that all the digestates should be post-treated before soil application in order to prevent environmental and health risks, and also to reduce residual phytotoxicity effects. The digestate from pig manure had a higher nutrient percentage (0.2, 0.6 and 0.05 % w/w of total N, P_2O_5 and K_2O, respectively), but also higher residual phytotoxicity than the other digestates. Co-digestion seemed not to significantly improve the digestate fertilizing potential. Finally, further studies should address how to improve fertilizing potential of digestates from plastic tubular digesters, avoiding environmental and health risks.

Keywords: Biofertilizer; biogas; low-tech digester; anaerobic digestion; nutrients recovery; manure

1. Introduction

Anaerobic digestion is a natural process in which microbes degrade organic waste in sealed containers (digesters) producing a clean renewable fuel (biogas), which can be used for cooking, heating, and electricity production, and an effluent (digestate) that can be used in agriculture. Anaerobic digestion can be implemented on a macro-scale (for instance in cities) and by means of high-tech solutions, as well as on a small to medium-scale (for example in rural communities, households) using low-tech technologies. In this sense, low-cost biogas digesters are considered a sustainable technology, which can help to achieve nine of the Sustainable Development Goals, including generating clean energy, mitigating the effects of climate change, and reducing poverty (WBA, 2020). Low-cost digesters have been spreading around the world since the 1970s (Bond and Templeton 2011; Feng et al., 2012). In particular, in Latin America the implementation of low-cost digesters was spurred after the oil crisis in the 1970s and several recent successful experiences have been reported, especially in rural communities (Garfí et al., 2016; Garfí et al., 2019; Jaimes-Estévez et al., 2020; Martí-Herrero et al., 2014; Martí-Herrero et al., 2019; Mendieta et al., 2021; Silva-Martínez et al., 2020). Indeed, in Latin America around 83 million people still lack access to modern and healthy cooking, especially in rural areas where the economy is mainly based on self-sufficient agriculture and family farming (ECLAC, 2019).

In Latin America, the plastic tubular digester is the most common digester model due to its low-cost and ease of implementation and handling, since it does not require specialised skills for construction and maintenance (Botero and Preston, 1987; Garfí et al., 2011a). It consists of a tubular polyethylene or PVC bag (the digester), buried in a trench, and fed with diluted feedstock, which flows from the inlet to the outlet. There is neither mixing (to avoid material sedimentation inside the reactor) nor heating (to increase the temperature). Even though these digesters often operate under psychrophilic conditions (15-20 °C), they are able to produce enough biogas to cover users' needs due to the presence of a microorganism consortia that is well-adapted to these conditions (Garfí et al., 2011a; Jaimes-Estévez et al., 2021).

The biogas produced is a clean fuel, mainly composed of methane and carbon dioxide, which can replace traditional biomass (i.e. firewood, dried cattle dung) for cooking. The use of a biogas cook stove significantly improves people's health by preventing air pollution in confined and unventilated kitchen spaces and avoiding harmful emissions (for example particulate matter, sulphur oxides) caused by the combustion of traditional biomass (ECLAC, 2019; Garfí et al., 2012). In addition, anaerobic digestion of animal manure decreases the harmful potential of inadequate handling of animal waste that can seriously affect the soil and water quality (for instance direct spreading on land of slurries) (Zhang et al., 2021).

Digestate is the other product of anaerobic digestion and it is rich in nutrients (including nitrogen, phosphorus, potassium, calcium and magnesium) and can be used in agriculture as biofertilizer to improve crop productivity. Digestate reuse in agriculture appears to be as important as biogas for rural households and small-scale farms of Latin America (Martí-Herrero et al. 2014).

Despite the fact that the technical feasibility, environmental and socio-economic benefits of biogas production from plastic tubular digesters have already been investigated, information about the potential effect of the digestate on crops fertilization is still scarce. Preliminary studies have proved that digestates had a fertilization efficiency higher than manure (Garfí et al., 2016). However, the digestate may not be completely safe especially in terms of pathogens and heavy metals (Chang et al., 2021;

Nakamya et al., 2020; Surendra et al., 2014). In the context of small-scale farms of Latin America, the risk from heavy metals could be considered negligible with respect to pathogens, since feedstocks are mainly manure from low-intensity farming.

Stabilization of organic matter is another major concern for the digestate agricultural use. Tambone et al. (2019) have recently reported that organic carbon (C) from anaerobic digestate is biologically stable because of the preservation of recalcitrant material during full-scale anaerobic co-digestion of manure and energy crops. Nevertheless, when the anaerobic digestion is performed under psychrophilic or mesophilic (25-35 °C) conditions, large concentrations of easily degradable organic compounds (like sugars and volatile fatty acids) can remain in the digestate due to slow process kinetics. In a recent study on the psychrophilic anaerobic digestion of food wastes, Muñoz et al. (2019) have shown that despite a high biomethane production, digestate from psychrophilic processes was still rich of dissolved organic C such as volatile fatty acids. Besides the known phytotoxic effect of volatile fatty acids (Di Maria et al., 2014), these compounds can be readily mineralized by soil microorganisms after digestate application, leading to soil quality depletion (Cucina et al., 2018a; Solé-Bundó et al., 2017).

Usually, the digestate from low-tech digesters is spread on agricultural land by farmers without analysing its quality or treating it further, thus increasing risks for human health, soil quality and plant growth. Garfí et al. (2011b) reported the preliminary results of a field study where potatoes and forage were fertilized with digestate from a plastic tubular digester fed with guinea pig manure. They indicated that both potatoes and forage yields increased significantly due to the digestate fertilization but also claimed the need for further investigation on digestate quality.

The aim of this study is to characterize the digestates from three plastic tubular digesters implemented in Colombia fed with: i) cattle manure; ii) cattle manure mixed with cheese whey; iii) pig manure. Physico-chemical parameters, agronomic quality, heavy metals and pathogens were analysed. The influence of the different feedstock and co-digestion on the digestate quality was also evaluated.

2. Material and Methods

2.1 Experimental sites and anaerobic digestion processes

Three different digestates obtained from full-scale plastic tubular digesters implemented in Colombia fed with different substrates were studied: cattle manure (digester 1), cattle manure and cheese whey (digester 2) and pig manure (digester 3). The main design and operational parameters of the anaerobic digestion processes and feedstock properties are reported in Table 1.

Specifically, the digestate obtained from cattle manure (D1) was collected from a plastic (polyethylene) tubular digester implemented in a small-scale farm located in the Andean region (7°01’0.07”N 73°08’13.3”W, 959 m.a.s.l, 23 ± 5 °C average ambient temperature). The digester had a useful volume of 7.1 m^3 and a hydraulic retention time (HRT) of 35 days. This digester produced 0.13 m^3 biogas per m^3 of digester per day (Table 1).

The digestate obtained from the co-digestion of cattle manure and cheese whey (D2) was collected from a polyethylene digester also implemented in the Andean region (7°44’10”N 73°03’03”W, 1882 m.a.s.l, 17 ± 3 °C average ambient temperature). The useful volume was 5.2 m^3 and it operated with a HRT of 75 days. The average production of biogas from this digester was 0.54 m^3 biogas per m^3 of digester per day (Table 1).

The digestate obtained from pig manure (D3) was collected from a tubular digester also located in the Colombian Andes (6°27’45.0”N 72°24’43.0”W, 2963 m.a.s.l., 17 °C average ambient temperature). The digester was a low-density polyethylene tubular reactor with a useful volume of 70.9 m^3 and a HRT of 25 days, and the biogas production rate was 0.06 m^3 biogas per m^3 of digester per day (Table 1).

Digestate samples were collected from the storage tank of each digester. To obtain a representative sample, five sub-samples of the same digestate were collected during two weeks from each storage tank. Sub-samples were then carefully mixed to obtain the final samples, which were stored at 4°C before analytical measurements.

2.2 Digestate characterization

2.2.1 Physico-chemical characterization

Total solids (TS), volatile solids (VS), chemical oxygen demand (COD) and total organic C (TOC) were determined following standard procedures (APHA, 2015). Total volatile fatty acids (TVFA) and total alkalinity (ALK) were quantified by using potentiometric H_2SO_4 titration and expressed as acetic acid and calcium carbonate equivalents, respectively (Di Maria et al., 2014). pH and electrical conductivity (EC) were measured by using a glass electrode and a conductivity probe, respectively.

Biochemical Methane Potential (BMP) tests were carried out at 37 ± 2 °C following the guidelines described by Holliger et al. (2016).

Total nitrogen (N) and ammonium N were determined following standard methods (APHA, 2015) and then organic N was calculated as the difference between total and ammonium N. The other plant nutrients (phosphorus, P, potassium, K, calcium, Ca, magnesium, Mg, sodium, Na, sulphur, S, and iron, Fe) were determined following standard procedures (APHA, 2015).

2.2.2 Pathogens and heavy metals

Pathogen analyses (coliforms, helminth eggs and *Salmonella* spp.) were carried out following the methods reported by Rivera et al. (2012).

In order to determine the heavy metals concentration (copper, Cu, zinc, Zn, lead, Pb, mercury, Hg, arsenic, As, nickel, Ni, selenium, Se, molybdenum, Mo, and cadmium, Cd), samples were digested in HCl-HNO_3 (3:1, v/v) (200°C, 15 min). Elements were then determined on mineralized samples by atomic absorption spectrometry. Chromium (Cr) VI was analysed by colorimetric method in aqueous extracts prepared from dry samples (Loubna et al., 2015).

2.2.3 Residual phytotoxicity

The Germination Index (GI) was determined by modifying the phytotoxicity test described by Solé-Bundó et al. (2017). Briefly, pure digestates (100%) and four dilutions (10, 20, 50 and 70% v/v in deionised water) were used as germination media, whereas deionised water was used as a control. 10 seeds of cress (*Lepidium sativum* L.) were placed on a paper filter wetted with 1 mL of each germination solution and then placed in a Petri dish. Each treatment was replicated five times. Petri dishes were closed with plastic film to avoid water loss and kept in the dark for 48 hours (20 ± 2 °C). At the end of the incubation, the GI was determined by measuring the number of germinated seeds and the length of the primary root, and expressed as percentage of the control.

3. Results and discussion

3.1 Physico-chemical characterization and organic matter stabilization

The fertilizing potential of digestates mainly depends on their physico-chemical characteristics, which in turn depends on feedstock characteristics and operational parameters of the anaerobic digestion process. Table 2 reports the results of the physico-chemical characterization of the three digestates.

All the digestates had a TS content of 2.1 to 4.1 %, classifying them as liquid products. The absence of a mixing system in the plastic tubular digester may prompt solids deposition at the bottom of the reactors, leading to a TS decrease from the influent to the effluent. Although the management of liquid fertilizers increases transport costs with respect to solid fertilizers, it should not represent an issue if the utilization of the digestate takes place within the same farm (Garfí et al., 2016).

The VS percentage was also low (1.4 - 3.2 %), while the VS/TS ratio ranged from 66.7 % in D1 (digestate from cattle manure) to 78.0 % in D3 (digestate from pig manure). This indicates that a large amount of organic matter was still present in the digestate. In fact, the VS/TS ratio usually ranges between 40-50% in digestates from anaerobic processes operating under mesophilic conditions (Castro et al., 2017; Garfí et al., 2011a; Solè-Bundó et al., 2017). The high VS/TS ratio in these digestates could be related to several factors, including slow digestion kinetics under psychrophilic conditions that can result in poor VS removal in digesters with an HRT of 25-75 days.

In agreement with the high VS/TS ratio, high COD and TOC concentrations were found in the digestates (average values were 23.0 g L^{-1} and 0.9 % on a fresh weight basis, respectively). Organic matter is one the most important parameters to assess the fertilizing properties of the digestate, since it plays a key role in soil fertility. Nevertheless, the application of poorly stabilized organic matter to the soil can cause adverse effects such as a reduction in soil oxygen, an increase of greenhouse gases emissions, or the development anoxic conditions in soil with subsequent fermentation processes and phytotoxic effects (Cucina et al., 2018a). Anaerobic digestion processes running in unfavourable conditions (for example with excessive OLR) (Di Maria et al, 2014) were often characterized by lack of organic matter stabilization in digestates, whereas anaerobic digesters performing properly (in terms of HRT and OLR) produce more stabilized digestates (Tambone et al., 2019).

The concentration of TVFA measured in the digestates confirmed the low stabilization. Indeed, TVFA are readily biodegradable organic compounds produced during the anaerobic digestion that can be rapidly converted into biomethane under optimal conditions. On the other hand, the abundance of TVFA represents a further obstacle for the agricultural reuse of the digestates, since these compounds have been correlated to phytotoxic effects (Alburquerque et al., 2012; Cucina et al., 2017; Di Maria et al., 2014; Risberg et al., 2017; Solé-Bundó et al., 2017).

The residual biochemical methane potential of the three digestates also confirmed the low organic matter stabilization. The BMP ranged from 0.066 m^3 CH_4 $kgVS^{-1}$ in D2 (digestate from the co-digestion) to 0.077 m^3 CH_4 $kgVS^{-1}$ in D1 (digestate from cattle manure), which are higher than previously reported (Menardo et al., 2011). The high BMP measured highlighted that these digesters working under psychrophilic conditions with HRT values of 25-75 days produced digestates still rich in biodegradable organic matter that could be converted into biomethane. Large amount of biodegradable organic matter in digestate represents an environmental issue for their reuse, since biomethane production can occur during its management, both in the storage and upon soil application, leading to greenhouse gases and odour emissions. Moreover, there was no evidence of a higher organic matter stabilization in the digestate obtained from the process that produced more biogas (co-digestion, D2, Table 1), with respect to the other digestates. Although the lack of stabilization can be due to the higher OLR in this digester, this result is in contrast with common findings. For instance, Solé-Bundó et al.

(2017) reported that the digestate from the co-digestion of pretreated microalgae and primary sludge was characterized by a higher stabilization than the one from microalgae mono-digestion, according to the higher production of biogas in the former. These findings suggest that even when the biogas production is noticeable, psychrophilic anaerobic digestion in plastic tubular reactors cannot produce stabilized digestates with HRT up to 75 days.

All the digestates were characterized by alkaline pH values that were compatible with agricultural euse (Solé-Bundó et al., 2017). The digestate pH ranged from 7.1 to 8.7, which is in accordance with Alburquerque et al. (2012) who reported that the pH of digestates obtained from animal manure falls in alkaline values (about 8). The alkaline values are mainly caused by the anaerobic digestion process that lead to the release of ammonia from the hydrolyzation of protein, and subsequent pH increase. In addition, TVFA produced in the first phases of AD are converted to biogas during methanogenesis, increasing the pH of digestate. The digestate from the co-digestion of cattle manure and cheese whey (D2) had the highest pH and it was probably related to the increased buffer capacity that is typical of co-digestion processes and depends on the co-substrate characteristics (Rabii et al., 2019). In fact, the digestate from the co-digestion of cattle manure and cheese whey (D2) also had a higher alkalinity (2.2 g L^{-1}) than the other digestates. The digestate from the mono-digestion of cattle manure (D1) had the lowest alkalinity and the highest concentration of TVFA, resulting in the lowest pH value measured (7.1).

Excessive soluble salts application can have a negative effect on soil properties, leading to salinization, colloid dispersion, loss of soil structure and inhibition of plant growth (Daliakopoulos et al., 2016). All the digestates were characterized by moderate EC values (2.9 – 6.8 dS m^{-1}), in accordance with those reported by Alburquerque et al.

(2012). The highest value was measured in the digestate from the co-digestion of cattle manure and cheese whey (D2), as cheese whey is rich in soluble salts (Prazeres et al., 2012).

Finally, the physico-chemical characteristics of the digestates may be improved by increasing the digester temperature or using longer HRT. Indeed, increasing HRT may reduce the biodegradable organic matter and obtain a more stabilized digestate.

3.2 Nutrients concentration

The digestate fertilizing potential is related to its N, P, K and other meso- and micro-nutrients concentration. Indeed, the higher the nutrients concentration, the higher the digestate fertilizing potential. The nutrients concentration in the digestates analysed is shown in Table 3. The digestate obtained from pig slurry digestion (D3) were characterized by a higher concentration of total N with respect to the other digestates due to the high concentration of this nutrient in the feedstock (pig slurry). Nevertheless, all the digestates from plastic tubular digesters were characterized by high N concentration (mean value of 0.9 g L^{-1}), similar to those reported by Tambone et al. (2017) for digestates obtained from animal manure and energy crops digestion. Partition of total N into organic and ammonium N in organic fertilizers is important because the latter acts as a readily available N source for crops, whereas the former contributes to medium and long-term N turnover in soil (Cucina et al., 2018a). On the other hand, high concentrations of ammonium N may raise environmental issues (for instance ammonia volatilization or nitrate leaching after digestate application in the field). The digestate from co-digestion of cattle manure and cheese whey (D2) was characterized by an organic N/total N ratio of 30.9%, which was significantly lower than the values of digestates from cattle manure (D1) (88.9%) and pig manure digestion

(D2) (73.5%). During anaerobic digestion, hydrolytic processes lead to ammonium release from organic matter and a decrease of the organic N/total N ratio (Tambone et al., 2010). The differences among the digestates in terms of N partition may be related to different performance of the anaerobic process in terms of biogas production. Indeed, the highest ammonium N concentration was measured for the digestate from the co-digestion (D2), which was obtained from the anaerobic digester characterized by the highest biogas production rate (Table 1).

As measured for total N, the digestate from pig manure (D3) had the highest concentration of total P (2.80 g L^{-1}) among the digestates. Again, this was probably related to the large concentration of P in pig slurry. All the digestates were characterized by high amounts of phosphates (mean value of 0.20 g L^{-1}), indicating that digestate use in agriculture could also provide available P to the crops (Tambone et al., 2010). Total N and total P concentrations in digestates decreased in the following order D3 > D2 > D1. These findings are in accordance with Alburquerque et al. (2012) who indicated that N and P in digestates from pig slurry were at least double than those measured in cow slurry digestates.

The digestates from plastic tubular digesters were all characterized by a large concentration of total K, ranging from 0.5 gK_2O L^{-1} (D3) to 1.2 gK_2O L^{-1} (D2). These values are comparable or even higher than those reported for digestates obtained from the anaerobic treatment of animal manure (Alburquerque et al., 2012; Tambone et al., 2010). The highest concentration of K was measured in D2 digestate (co-digestion of cattle manure with cheese whey) and it was attributed to the large concentration of cheese whey in the feedstock, which is a K-rich substrate (Prazeres et al., 2012).

The meso- and micronutrients (Ca, Mg, Na, S and Fe) concentration of the three digestates from plastic tubular digesters are also reported in Table 3. All digestates were characterized by a high concentration of meso- and micronutrients, if compared to literature (Alburquerque et al., 2012). Among the three digestates, the one obtained from pig manure (D3) had the highest amount of Ca (4.3 g L^{-1}), Mg (0.4 g L^{-1}) and Fe (300 mg L^{-1}). The results of this study were in accordance with Qi et al. (2018) who found that the concentrations of Ca and Mg in digested cattle slurry were about 1.6 and 0.6 g L^{-1}, respectively. In addition, Ca and Mg concentrations in the digestates obtained from plastic tubular digesters were higher than those reported for digestates from other feedstock (for instance microalgae biomass, co-digestion of microalgae biomass with primary sludge) (Solé-Bundó et al., 2017). The Na concentration in digestates and, more in general, in all fertilizers should be carefully evaluated since this element acts as a plant micronutrient but excessive concentrations can produce phytotoxic effects (Bożym et al., 2020). The Na concentration in the digestates ranged from 0.1 g L^{-1} in D3 (digestate from pig manure) to 0.3 g L^{-1} in D1 and D2 (digestates from cattle manure and cattle manure co-digestion with cheese whey, respectively). These values are lower than those reported to have phytotoxic effects (Bożym et al., 2020). Moreover, the other nutrients (S and Fe) concentrations were comparable to those reported by Alburquerque et al. (2012) for pig slurry and cattle manure digestates.

Since national and international regulations do not take into account meso- and micronutrients to evaluate the fertilizing potential of the digestate, it could be useful to compare the aforementioned results with the nutrient requirements of a widespread crop in Latin America, for instance cocoa. *Theobroma cacao* L. (cocoa) cultivation usually implies the utilization of large amounts of mineral fertilizers to supply K, N, P, Ca, Mg, S, Fe and other micronutrients (Snoeck et al., 2016). Although the concentrations of plant nutrients in the digestates appear inadequate to completely replace the use of mineral fertilizers in cocoa cultivation, it is evident that the use of digestate may allow for a partial recycling of plant nutrients required by cocoa. In any case, the use of digestates from plastic tubular digesters as biofertilizer should be preceded by field trials to assess the effectiveness of digestate fertilization on crop yields. To the best of the authors' knowledge, only Garfí et al. (2011b) have investigated the effect of digestate obtained from plastic tubular digester fed with guinea pig manure on potatoes and forage yields. Although the results were positive, the authors stated the need for more field trials considering different soils, crops and digestate management.

3.3 *Pathogens and heavy metals*

Faecal indicators and pathogens concentration in the digestates are shown in Table 4. All the pathogens and indicators studied were detected in all the digestates, with the exception of *Salmonella* spp. that was absent in D3 (digestate from pig slurry) and this was probably related to the longer activity of these digester (eight years) with respect to the others. Coliforms were present in the range of 10^3-10^5 CFU g^{-1} and 100-300 CFU g^{-1}, respectively. Helminths eggs were identified in all the digestates, with viable eggs ranging from 6.7 (D2, co-digestion) and 21.7 (mono-digestion of cattle manure) eggs in 4 g of dry matter. Temperature and HRT are the main operational parameters of anaerobic digestion that affect pathogens' presence in digestate. It is known that psychrophilic and mesophilic temperature regimes are not as efficient as thermophilic digestion at inactivating pathogens, making post-treatment of digestates mandatory to eliminate them (Alburquerque et al., 2012; Costa et al., 2017). Pathogens could cause severe morbidity or even mortality for human beings by inflicting respiratory diseases, gastroenteritis, conjunctivitis, cystitis, genital disease, skin and soft tissue infections (Zhao and Liu, 2019). Consequently, a high risk of pathogen transfer into the food chain can rise when digestates are applied to the land.

Heavy metals concentration of the digestates is reported in Table 5 Most of the heavy metals analysed were under the detection limit of the method used for the analysis (Pb, Hg, Ni, Mo, Cd and Chromium VI). As was detected at low concentration only in the digestate from cattle manure (D1) (0.4 mg kg^{-1}), Se was detected in the digestate from co-digestion of cattle manure with cheese whey (D2) (1.2 g L^{-1}) and pig manure (D3) (2.1 g L^{-1}), whereas Cu was found only in the digestate from pig manure (D3) (5.3 g L^{-1}). The only heavy metal found in all the digestates was Zn, which ranged from 12.1 g L^{-1} in D2 (digestate from co-digestion of cattle manure with cheese whey D2) to 85.3 g L^{-1} in D3 (digestate from pig manure). These results were expected since the digesters were fed with animal manure and cheese whey produced in rural communities, where heavy metals contamination is not likely to occur. The results were also in accordance with Tambone et al. (2017) who reported that digestates from animal manure and energy crops co-digestion were characterized by low concentration of heavy metals. Interestingly, heavy metals concentrations in the digestates were in line with those reported for poultry manure, lower that those reported for compost and much lower than those reported for sewage sludge, which are feedstocks commonly used in agriculture as organic fertilizers and amendments (Alvarenga et al., 2015; Tambone et al., 2017).

3.4 *Residual phytotoxicity*

The evaluation of GI was carried out to assess residual phytotoxicity of the digestates from plastic tubular digesters. Different dilutions of the digestates (100%, 70%, 50%, 20% and 10%) were used as media for cress seeds (*Lepidium sativum* L.) germination and results were reported as % of the control (deionized water) (Fig. 1). Generally, GI values below 50% indicate high phytotoxicity, values between 50% and 80% indicate moderate phytotoxicity, and values above 80% indicate the absence of phytotoxicity

(Barral and Paradelo, 2011).

Phytotoxicity decreased with increasing dilution. At 100% dilution, only the digestate from cattle manure (D1) was characterized by reduced phytotoxicity (GI was 59 %), whereas no germination was observed for the other digestates. High GI values were measured for the digestate from cattle manure (D1) and co-digestion of cattle manure and cheese whey (D2) at 50% dilution (87.6 and 97.4%, respectively), whereas the digestate from pig slurry (D3) had a strong phytotoxicity (GI was 6.6%). At higher dilutions (20% and 10%), all the digestates were characterized by GI values higher than 100%.

Residual phytotoxicity is often found in anaerobic digestates and is related to several factors (for example high concentrations of soluble salts, ammonium N, TVFA, phenols) (Alburquerque et al., 2012; Cucina et al., 2018a; Cucina et al., 2017). Solé-Bundó et al. (2017) reported that residual phytotoxicity of co-digested microalgae biomass and primary sludge was significantly correlated to ammonium N, TVFA and EC. These results showed that GI was found to be negatively correlated with several parameters (TVFA, COD, ammonium N, EC and Na concentration). The negative effects of the COD and TVFA on the GI confirmed the influence of anaerobic digestion operational parameters on the digestate quality. Indeed, in plastic tubular digesters working under psychrophilic conditions and without mixing systems, the residual biodegradable organic matter can compromise the feasibility of agricultural reuse of the digestate.

None of the digestates should be used directly on soil due to their residual phytotoxicity. Digestates may be used in agriculture by means of fertirrigation (dilution of digestate in the irrigation water). Dilution of the digestates in irrigation water in a 1:2 or 1:5 ratio could be a feasible solution to remove the phytotoxic effects from D2 and D3 and, at the same time, provide nutrients to the crops. In addition, dilution may promote phytostimulant and phytonutrient effects of the digestates increasing their GI above 100% (Barral and Paradelo, 2011). Within the digestates, D1 (digestate obtained from cattle manure) and D2 (digestate obtained from co-digestion of cattle manure and cheese whey) would require less water for dilution with respect to D3 (digestate obtained from pig manure). However, digestate dilution can make handling more difficult in rural communities and water availability is not always ensured in this context. Moreover, dilution would decrease the fertilizing potential of digestates due to nutrients dilution. Increasing the efficiency of anaerobic digestion processes or implementing a digestate post-treatment (for example liquid/solid separation, composting) could be suitable strategies for a more efficient digestate reuse in agriculture.

3.5 Quality assessment matrix of the digestates

Table 6 shows a matrix to be used for digestate quality assessment from the perspective of agricultural use. The main results of digestates' characterization were reported as intervals and compared to recommended ranges, for instance values found in literature or regulations.

Concerning the physico-chemical characteristics and organic matter stabilization, it can be concluded that the digestates from plastic tubular digesters working under psychrophilic conditions are not suitable for agricultural reuse due to their high concentration of biodegradable organic matter. The VS/TS ratio, COD and TVFA values largely exceed recommended values (40-60%, < 0.5 g L^{-1} and < 0.5 g L^{-1}, respectively) and this result may represent an environmental and agronomic issue that cannot be neglected (Garfi et al., 2011a; Res. 00150, 2003; Risberg et al., 2017). Improving the efficiency of converting soluble organic matter into biogas may solve the issue of poor digestate stabilization. As mentioned above, a feasible strategy to improve digestate stabilization could be to increase the HRT of the digester in order to enhance the biogas production and mineralize the highest amount of organic matter (Meegoda et al., 2018). Castro et al. (2017) have proposed the implementation of a degasification tank to recover retained biogas from digestate obtained from a plastic tubular digester fed with cattle manure. This strategy may also increase the digestate stabilization, making its agricultural reuse suitable. Finally, digestate recirculation may be another suitable way to enhance organic matter conversion into biogas, as reported by Sambusiti et al. (2015).

Pathogens exceeded the recommended range established by European and American normatives (EU Reg. 2019/1009; US EPA, 2016), representing one of the major concerns for agricultural reuse of the digestates from plastic tubular digesters. Since pathogens spread from anaerobic digestate represents an emerging issue (Nag et al., 2020), a post-treatment of digestates for pathogens elimination (for example composting, solid/liquid separation) appears mandatory.

Heavy metals do not represent a concern for the agricultural reuse of the digestates. Among all the legislations, it was decided to compare the results obtained in this study with the last European Regulation concerning fertilizers (EU Reg. 2019/1009) because it is the most complete and restrictive regulation available worldwide. All the heavy metals analysed were within the limits established with the exception of Zn, but this was mainly due to the fact that limit values are reported as mg kg^{-1} of dry matter. In fact, when liquid products as the digestates are analysed on fresh samples, the conversion of the results from fresh weight basis to dry weight basis can result in high values due to the low percentage of TS.

EU Reg. 2019/1009 establishes a minimum concentration of nutrients (organic C, N, P and K) to define marketable organic fertilizers. Although the digestates were characterized by high concentrations of all the plant nutrients, these values were far from those required. The low percentage of TS in the digestates means an excessive dilution of the nutrients and, consequently, they cannot be classified as organic fertilizers (EU Reg. 2019/1009). Nevertheless, digestate agricultural reuse may represent a valuable strategy for nutrient recycling, which can help to partially replace the mineral fertilizer consumption. As reported by other authors (Garfí et al., 2016), avoiding the sedimentation of TS inside the plastic tubular digesters (for instance by means of a simple liquid mixing device) could increase the TS concentration in the digestates, leading to a more concentrated product with even greater fertilizing potential.

On the other hand, according to the phytotoxicity results, the digestate should be used in agriculture after a proper dilution (up to 20-50%) to avoid phytotoxic effects on crops. Nevertheless, digestate dilution would increase water consumption, digestate handling difficulty, and reduce the fertilizing potential due to nutrients dilution. Consequently, other management strategies should be explored for digestate reuse. Composting may represent a suitable post-treatment since this treatment is known to inactivate pathogens and weed seeds, increase organic matter stabilization, and decrease phytotoxicity and moisture percentage (Cucina et al., 2018b). Despite this, the low TS concentration of digestates represents a serious barrier for composting due to the high need for bulking materials (for example wood chips, tree-pruning residues).

Besides the potential benefits (plant nutrient recovery, replacement of mineral fertilizers), the digestates were characterized by several agronomic and environmental risks (pathogens, lack of stabilization, phytotoxicity) that need to be solved to ensure their safe reuse. A post-treatment of the digestate seems to be mandatory in order to avoid negative effects of digestate application. For instance, solid/liquid separation represents a widespread system for digestate management, for instance using a sand filter, which is simple and requires low investment and maintenance costs. Indeed, Patil and Husain (2019) pointed out that sand filtration is a simple and effective technology to improve digestate quality and make its management easier. Tambone et al. (2017) reported that solid/liquid fractionation of digestates led to obtain a potential substitute for mineral N fertilizers (the liquid fraction) and a NP-organic fertilizers (the solid fraction). After solid/liquid separation, pathogens are expected to remain in the solid fraction (Barampouti et al., 2020), which can be treated aerobically through composting in order to obtain a complete hygenization. Aerobic treatments of the solid fraction may also improve organic matter stabilization and lead to a complete removal of phytotoxic effects (Cucina et al., 2018a, b).

4. Conclusion

In the present study, three digestates from plastic tubular digesters implemented in Colombia and operating under psychrophilic conditions were characterized to assess their potential reuse as biofertilizers. All the digestates were characterized by physico-chemical characteristics, nutrients and heavy metals concentrations suitable for their reuse as biofertilizer. However, digestates might only partially replace the mineral fertilizer due to their low nutrients and total solids concentration. Biodegradable organic matter concentration and pathogens were not suitable for digestate reuse in agriculture, showing that all the digestates should be post-treated before soil application in order to prevent environmental and health risks and reduce residual phytotoxicity effects. Further studies should be addressed in order to identify sustainable strategies to improve the fertilizing potential of the digestate from plastic tubular digesters. For instance, organic matter stabilization could be improved by implementing a degasification tank or by recirculating the digestate into the digester. Composting represents a suitable post-treatment for the solid fraction of the digestate, to enhance organic matter stabilization and hygenization. Besides, sand filtration has been proved to allow for a simple and safe management of anaerobic digestates.

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Table 1: Main design and operational parameters of the anaerobic digestion and feedstock properties in three plastic tubular digesters implemented in Colombia.

Parameter	*Unit*	Digester 1 (CM)	Digester 2 (CM+CW)	Digester 3 (PM)
Process parameter				
Useful volume	m^3	7.1	5.2	70.9
Average ambient temperature	°C	23	17	17
OLR	kgVS m^{-3} day^{-1}	0.7	1.0	0.5
HRT	d	35	75	25
Biogas production rate	m^3 m$^{-3}_{dig}$ day^{-1}	0.13	0.54	0.06
Feedstock characteristics (after dilution)				
Feedstock composition (w/w)		100% CM	30% CM + 70% CW	100% PM
Dilution (manure:water, w/w)		1:3	-	1:6
TS	%	4.5 ± 0.6	6.7	4.8 ± 1.4
VS	%	3.6 ± 0.6	5.1	3.6 ± 1.3
VS/TS	%	80	76	75
COD	g L^{-1}	5.4 ± 0.7	92.2	27.3 ± 0.1

CM: cattle manure, CW: cheese whey, PM: pig manure.
OLR: organic loading rate, HRT: hydraulic retention time, TS: total solids, VS: volatile solids, COD: chemical oxygen demand.
Mean value ± SD, n = 3.
Data are expressed on a fresh weight basis.

Table 2: Physico-chemical characterization of the digestates obtained from three plastic tubular digesters implemented in Colombia.

Parameter	Unit	D1 (CM)	D2 (CM+CW)	D3 (PM)
TS	%	2.1 ± 0.1	2.2 ± 0.1	4.1 ± 0.5
VS	%	1.4 ± 0.1	1.6 ± 0.1	3.2 ± 0.4
VS/TS	%	66.7	72.3	78.0
COD	g L^{-1}	17.0 ± 0.1	25.8 ± 2.4	26.1 ± 1.1
TOC	%	0.6	1.0	1.0
TVFA	g L^{-1}	0.60 ± 0.06	0.22 ± 0.03	0.22 ± 0.03
BMP	m^{3}CH$_4$ kgVS^{-1}	0.077 ± 0.001	0.066 ± 0.002	0.070 ± 0.009
pH	-	7.1 ± 0.3	8.7	7.6 ± 0.3
ALK	g L^{-1}	1.3 ± 0.1	2.2 ± 0.2	1.8 ± 0.0
EC	dS m^{-1}	2.9	6.8	4.5

CM: cattle manure, CW: cheese whey, PM: pig manure.
TS: total solids, VS: volatile solids, COD: chemical oxygen demand, TOC: total organic C, TVFA: total volatile fatty acids (gAcetic Acid L^{-1}), BMP: biochemical methane potential, ALK: total alkalinity (gCaCO$_3$ L^{-1}), EC: electrical conductivity.
Mean value ± SD, n = 3.
Data are expressed on a fresh weight basis.

Table 3: Plant macro-, meso- and micronutrients concentration in the digestates obtained from three plastic tubular digesters implemented in Colombia.

Parameter	Unit	D1 (CM)	D2 (CM+CW)	D3 (PM)
Total N	g L^{-1}	0.36	0.68	1.70
Total N	%	0.04	0.07	0.17
Ammonium N	g L^{-1}	0.04 ± 0.00	0.47 ± 0.00	0.45 ± 0.01
Organic N	g L^{-1}	0.32	0.21	1.25
Organic N/Total N	%	88.9	30.9	73.5
Total P	g L^{-1}	0.13	0.32	2.80
Total P (P_2O_5)	g L^{-1}	0.27	0.67	5.88
Total P (P_2O_5)	%	0.03	0.07	0.59
Phosphate-P	g L^{-1}	0.20 ± 0.00	0.14 ± 0.00	0.26 ± 0.10
Total K	g L^{-1}	0.6	1.0	0.4
Total K (K_2O)	g L^{-1}	0.7	1.2	0.5
Total K (K_2O)	%	0.07	0.12	0.05
Total Ca	g L^{-1}	0.6	0.5	4.3
Total Ca (CaO)	g L^{-1}	0.8	0.7	6.0
Total Mg	g L^{-1}	0.2	0.2	0.4
Total Mg (MgO)	g L^{-1}	0.3	0.3	0.7
Soluble Mg	g L^{-1}	0.10	0.10	0.04
Total Na	g L^{-1}	0.3	0.3	0.1
Total S	g L^{-1}	0.25	0.10	0.1
Sulphate	g L^{-1}	0.75	0.30	0.3
Total Fe	mg L^{-1}	100	43	300

CM: cattle manure, CW: cheese whey, PM: pig manure.
Mean value ± SD, n = 3.
Data are expressed on a fresh weight basis.

Table 4: Pathogens concentration in the digestates obtained from three plastic tubular digesters implemented in Colombia.

Parameter	Unit	D1 (CM)	D2 (CM+CW)	D3 (PM)
Total coli	CFU g^{-1}	435000 ± 50000	3450 ± 350	2970 ± 379
Faecal coli	CFU g^{-1}	263 ± 61	210 ± 14	175 ± 21
E. coli	presence/absence	presence	presence	presence
Total helminths eggs	eggs/4g d.m.	38.7 ± 5	21.3 ± 4	19.3 ± 2
Viable helminths eggs	eggs/4g d.m.	21.7 ± 1	6.7 ± 1	13 ± 4
Salmonella spp	presence/absence in 25 g	presence	presence	absence

CM: cattle manure, CW: cheese whey, PM: pig manure.
CFU: colony forming unit, d.m.: dry matter.
Mean value ± SD, n = 3.
Data are expressed on a fresh weight basis.

Table 5: Heavy metals concentration in the digestates obtained from three plastic tubular digesters implemented in Colombia.

Parameter	Unit	D1 (CM)	D2 (CM+CW)	D3 (PM)
Total Cu	mg kg^{-1}	< 5*	< 5*	5.3 ± 1.0
Total Zn	mg kg^{-1}	15.4 ± 1.0	12.1 ± 1.0	85.3 ± 1.0
Total Pb	mg kg^{-1}	< 10*	< 10*	< 10*
Total Hg	mg kg^{-1}	< 0.15*	< 0.15*	< 0.15*
Total As	mg kg^{-1}	0.4 ± 0.1	< 0.3*	< 0.3*
Total Ni	mg kg^{-1}	< 10*	< 10*	< 10*
Total Se	mg kg^{-1}	< 0.3*	1.2 ± 0.1	2.1 ± 10.1
Total Mo	mg kg^{-1}	< 10*	< 10*	< 10*
Total Cd	mg kg^{-1}	< 5*	< 5*	< 5*
Chromium VI	mg L^{-1} O$_2$	< 0.05*	< 0.05*	< 0.05*

CM: cattle manure, CW: cheese whey, PM: pig manure.
* = detection limit of the method.
Mean value ± SD, n = 3.
Data are expressed on a fresh weight basis.

Table 6: Quality assessment of the digestates obtained from three plastic tubular digesters implemented in Colombia.

	Parameter	*Unit*	*Range (this study)*	*Recommended range*	*Reference*	*Evaluation*
Stability	VS/TS	%	65 - 80	40-60	Garfì et al., 2011	Not suitable
	COD	g L^{-1}	15 - 30	< 0.5	Res. 00150, 2003	
	TVFA	g L^{-1}	0.2 – 0.6	< 0.5	Riesberg et al., 2017	
	BMP	m^{3}CH$_4$ kgVS^{-1}	0.07 – 0.08	n.d.		
Hygenization	Total coli	CFU g^{-1}	10^3 – 10^5	< 10^3	EU Reg. 2019/1009; US EPA, 2016	Not suitable
	Salmonella spp	-	Presence	Absence in 25 g	EU Reg. 2019/1009	
	Helminths eggs	eggs in 4 g	5 - 50	-		
Heavy metals	Total Cu	mg kg^{-1} d.m.	< 5* - 200	< 300	EU Reg. 2019/1009	Suitable
	Total Cd	mg kg^{-1} d.m.	< 5*	< 1.5	EU Reg. 2019/1009	
	Total Zn	mg kg^{-1} d.m.	400 - 2000	< 800	EU Reg. 2019/1009	
	Total Pb	mg kg^{-1} d.m.	< 10*	< 120	EU Reg. 2019/1009	
	Total Hg	mg kg^{-1} d.m.	< 0.15*	< 1	EU Reg. 2019/1009	
	Total As	mg kg^{-1} d.m.	< 0.3* - 15	< 40	EU Reg. 2019/1009	
	Total Ni	mg kg^{-1} d.m.	< 10*	< 50	EU Reg. 2019/1009	
	Total Se	mg kg^{-1} d.m.	< 0.3* – 2.5	n.d.		
	Total Mo	mg kg^{-1} d.m.	< 10*	n.d.		
Plant macronutrients	Total organic C	%	1.0 – 1.5	> 5.0	EU Reg. 2019/1009	Suitable, but not classifiable as organic fertilizer
	Total N	%	0.04 – 0.2	> 2.0	EU Reg. 2019/1009	
	Total K (K$_2$O)	%	0.05 – 0.15	> 2.0	EU Reg. 2019/1009	
	Total P (P$_2$O$_5$)	%	0.03 – 0.65	> 1.0	EU Reg. 2019/1009	
	Ammonium N	g kg^{-1}	0.10 – 0.50	< 1 - 2	Di Maria et al., 2014	
	Phosphate P	g kg^{-1}	0.05 – 0.30	n.d.		
Agronomic quality	GI 10%	%	> 100	> 80	Barral and Paradelo, 2011	Suitable, if properly treated
	GI 20%	%	> 100			
	GI 50%	%	5 - 100			
	GI 70%	%	0 - 70			
	GI 100%	%	0 – 60			

n.d.: not defined.
VS: volatile solids, TS: total solids, COD: chemical oxygen demand, TVFA: total volatile fatty acids, SMA: specific methanogenic activity, BMP: biomethanisation potential, GI: germination index, CFU: colony forming unit, d.m.: dry matter.
* = detection limit of the method.