

CARBON AND NITROGEN MINERALISATION IN SOIL AMENDED WITH DIGESTATES FROM ANAEROBIC CO-DIGESTION PROCESSES

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1 INTRODUCTION

The modern livestock production systems are characterised by an intensification and concentration of their activities, generating vast amounts of biodegradable wastes which must be managed under sustainable environmental practices (Burton and Turner, 2003). In this context, anaerobic digestion is emerging as a management option for livestock wastes, providing a renewable energy source (biogas). A biogas production potential of 18.5 Mtoe (million tonnes of oil equivalent) has been estimated, taking into account that approximately 1500 million tonnes of animal manure, mainly from cattle and pig farms, is produced every year in the EU-27 countries (Nielsen and Oleskowicz-Popiel, 2008).

On the other hand, the digested materials (digestate) can be used as fertilisers and amendments for agricultural systems. However, the presence of easily-degradable organic matter at a high concentration in digestate can lead to a great increase in soil microbial activity and cause detrimental effects on the plant-soil system such as phytotoxicity (toxic intermediate degradation products), anaerobic soil conditions, etc. Soil amendment with digestate must guarantee both agricultural and environmental benefits, optimising the organic matter balance in soil and increasing soil fertility in the long-term (Ajwa and Tabatabai, 1994; Thuriès et al., 2001). Therefore, the information gained from decomposition studies in digestate-treated soil (potentially mineralised carbon, mineralisation rate, nitrogen immobilisation, etc.) can be very useful to assess the stability of the digestate and decide the most appropriate recovery operation in practice (direct application, stabilisation treatments, optimum application rate, etc.).

The present paper evaluates the carbon and nitrogen mineralisation dynamics in a short-term laboratory study for an agricultural soil amended with some representative digestate samples produced from anaerobic co-digestion processes.

2 MATERIALS AND METHODS

2.1 Soil incubation experiment

Four digestates were selected as representative samples: pig slurry+1.0% sludge from a slaughterhouse wastewater treatment plant and 6.5% biodiesel wastewaters as co-digestion substrates (PS); cattle slurry+4.3% cattle manure and 11.6% maize-oat silage as co-digestion substrates (CS); cattle slurry+4% glycerine (CG4), and cattle slurry+6% glycerine (CG6). Digestate samples (Table 1) were applied to a calcareous sandy-loam agricultural soil from La Alberca (Murcia, Spain) at a rate of 96 m³/ha (4 g of fresh digestate per 100 g of dry soil). The soil used for the experiment was collected from the top-layer (0-20 cm), air-dried and sieved to 2 mm before used in the incubation. Its main characteristics were 23.7% CaCO₃, 7.5 pH and 1.72 dS/m electrical conductivity (saturated paste, with water), 24.3 g/kg organic matter, 14.1 g/kg organic carbon and 1.9 g/kg total nitrogen. The digestate-soil mixtures were incubated under aerobic conditions at 26°C for 56 days, using soil without digestate as the control. Soil moisture was maintained at 60% of the water-holding capacity with deionised water. Each treatment was run in triplicate.

After 2, 7, 14, 28, 42 and 56 days, three soil replicates per treatment were analysed for inorganic-N (NH₄-N and NO₃-N) to follow the nitrogen dynamic of mineralisation, while the dynamic of the organic carbon mineralisation was determined in a separate set of incubations for 56 days. Small vials with 10 ml of 0.1M NaOH were placed inside the incubation vessels to trap the CO₂ evolved during the incubation, and empty vessels were used as blanks. The vessels were closed, but opened during a period of several minutes to restore adequate aerobic conditions when NaOH vials were replaced and measured after 2, 4, 7, 14, 28, 42 and 56 days. The CO₂ was measured by titration of the NaOH solution with 0.1M HCl in an excess of BaCl₂ to precipitate carbonates. The

mineralisation of the organic carbon from the digestate was calculated as the difference between the CO₂-C evolved in the amended soils and that produced in the control (non-amended) soil. Data concerning C-mineralisation evolution in soil were fitted to kinetic models by the non-linear least-square technique (Marquardt-Levenberg algorithm), using the Sigma-Plot computer programme (SPSS Inc.). The statistical significance of curve fitting, the residual mean square (RMS) and F-values were calculated.

TABLE 1 **Main characteristics of the digestate samples (mean value, data expressed on a fresh weight basis).**

Parameter	CG4	CG6	PS	CS
pH	5.64	7.35	8.20	7.50
Electrical conductivity (dS/m)	14.5	11.7	30.3	25.8
Dry matter (g/l)	38.3	72.9	19.5	90.1
Total organic matter (g/l)	26.4	56.4	8.5	66.4
Total nitrogen (g/l)	1.9	2.3	4.0	4.0
NH ₄ -N (g/l)	1.0	0.9	3.3	1.9

2.2 Analytical methods

The following parameters were determined in the digestate samples: electrical conductivity and pH (directly on samples after their homogenisation); moisture content after drying to constant weight at 105°C; the volatile solids which reflect the organic matter content by loss on ignition at 500°C for 24h; total organic carbon (TOC) and total nitrogen (TN) were measured by automatic microanalysis (EuroVector elemental analyser, Milan, Italy). Ammonium was determined by distillation in alkaline medium (MgO). The 5-days biochemical oxygen demand (BOD₅) was measured with OxiTop® equipment.

The total organic carbon and total nitrogen concentrations of soil were determined with an automatic microanalyser. The CaCO₃ content was measured with a Bernard calcimeter. As incubation progressed, inorganic-N was determined in 1:5 (w/v) soil:water extracts for NO₃-N and soil:2M KCl extracts for NH₄-N. Nitrate-N was measured using a nitrate-ion selective electrode (EPA, 2007), while NH₄-N was determined by a colorimetric method based on Berthelot's reaction (Sommer et al., 1992). All values refer to soil dried at 105°C for 24h.

3 RESULTS AND DISCUSSION

3.1 Carbon mineralisation in digestate treated-soil

All digestates showed the largest release of CO₂ during the first day of incubation due to the mineralisation of the most easily-degradable organic fraction of digestate samples. Mineralisation rates rapidly decreased until the end of the incubation where they became nearly constant, ranging from 5 to 10 µg C/g soil and day, and close to control values. Digestates from CG showed the highest production of CO₂-C in the soil, favoured by the glycerine addition which increased the labile pool of organic-C in these treatments. The total amount of CO₂-C evolved after 56 days of incubation increased significantly in the order (µg C/g soil): 730 < 948 < 1027 < 1679 for PS, CS, CG4 and CG6, respectively. The results obtained were conditioned by the amount of organic carbon added to soil in the digestate samples (237, 1350, 712 and 1713 mg TOC per kg of dry soil for PS, CS, CG4 and CG6, respectively) and its stability against microbial degradation. Then, BOD₅ values were clearly higher in CG samples (930.7 and 1420.5 g O₂/kg OM for CG6 and CG4, respectively) than in CS and PS digestates (90.4 and 252.3 g O₂/kg OM, respectively).

The pattern of organic-C mineralisation varied considerably amongst digestates. At the end of the incubation period (56 days), the mineralised-C (% of TOC) in CS was 30%, much lower than for the CG digestates (60 and 63% of TOC for CG4 and CG6, respectively) and indicating the presence of a large easily degradable C fraction in the latter (lower stability degree), while more than 100% of the organic-C added with PS was mineralised, indicating the degradation of some native soil organic-C during incubation (priming effect).

The mineralisation of the total organic-C (TOC) coming from the digestates was calculated as the difference between the CO₂-C evolved in the amended soils and that produced in the control, these results were fitted to kinetic functions (Table 2). CG samples followed a combined first- and zero-order kinetic model which suggested the presence of two different fractions of organic matter: an easily-degradable pool which was

mineralised quickly, representing about 50% of the added organic-C, and another more resistant to microbial degradation and, hence, degraded at a much lower rate during incubation. The CS digestate mineralisation pattern fitted a first order model, which meant that organic matter mineralisation followed an exponential rise to a maximum growth, while the PS dynamic needed an independent parameter (B) to show the initial flux of CO₂-C evolved.

TABLE 2 Parameters of the kinetic models used to describe C-mineralisation of the digestates (\pm standard error).

Combined first- and zero-order function: $C_m = C_R(1 - e^{-k_R t}) + At$						
Digestate	C_m (56 days)	C_R	k_R	A	RMS	F
CG4	59.8 \pm 2.4	52.0 \pm 0.6	0.33 \pm 0.01	0.14 \pm 0.02	0.4985	3015***
CG6	63.0 \pm 0.8	46.5 \pm 0.5	0.36 \pm 0.01	0.31 \pm 0.02	0.4357	3577***
First order: $C_m = C_0(1 - e^{-kt})$						
Digestate	C_m (56 days)	C_0	k	B	RMS	F
PS	108.2 \pm 4.4	106.4 \pm 10.0	0.02 \pm 0.01	30.2 \pm 1.9	4.6248	568***
CS	30.1 \pm 1.2	28.9 \pm 0.6	0.08 \pm 0.01	-	0.7567	1447***

***: Significant at probability level $P < 0.001$.

C_m : mineralised-C (% of TOC) at the end of incubation (56 days) and t : incubation time (days). In the combined first- and zero-order function: C_R , rapid potentially mineralisable-C (% of TOC); k_R , rapid rate constant (day^{-1}); A ($\% \text{ day}^{-1}$), slowly mineralisable-C rate (equivalent to " $C_S \times k_S$ "; C_S : slowly mineralisable-C and k_S : the slow rate constant). In the first order: C_0 , potentially mineralised-C (% of TOC) and k , rate constant (day^{-1}). For PS, a constant term (B) was included to consider the initial mineralisation flux detected in this treatment.

3.2 Inorganic nitrogen dynamics in digestate-treated soil

At the beginning of the incubation, NH₄-N was the predominant nitrogen form provided by the digestates to the soil. As incubation progressed, nitrification of this NH₄-N occurred in all treatments. However, changes in the NH₄-N and NO₃-N concentrations in digestate-treated soil were clearly influenced by the stability of the added material (Fig. 1).

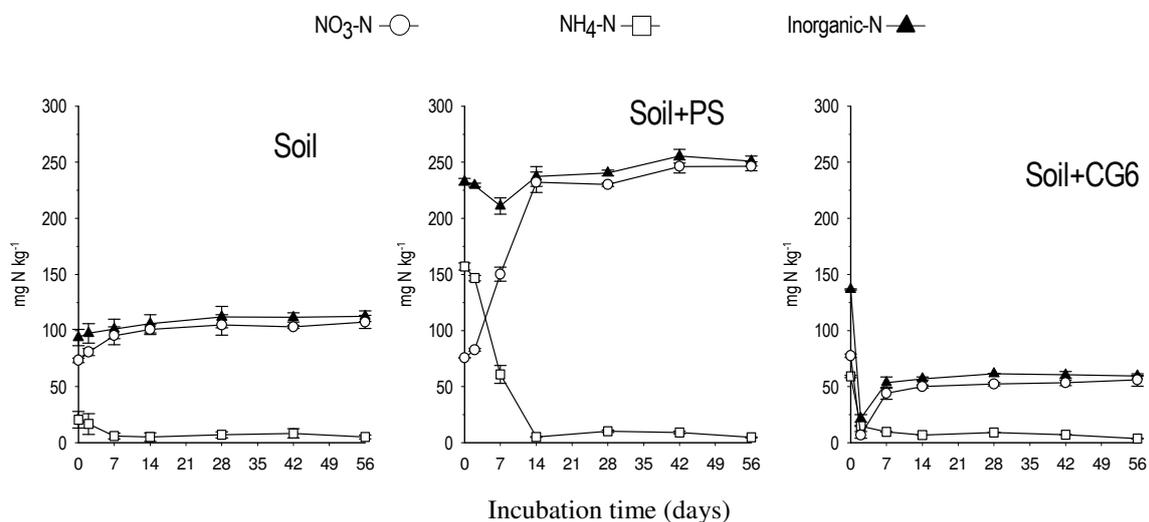


FIGURE 1 Dynamics of inorganic-N in soil during incubation with two representative digestate samples - PS and CG6 (average \pm standard deviation; where absent, bars fall within symbols).

Therefore, two different trends in the mineral-N dynamic were observed during incubation. Soil treated with CG digestates, having a high proportion of labile organic matter, showed a large decrease in both mineral form (NH₄-N and NO₃-N) concentrations during the first week of incubation. These findings suggest that CG addition favoured the microbial immobilisation of inorganic-N in soil, due to the development of microorganisms in soil, as observed by the subsequent intense microbial respiration (high respiration rates were detected initially in these

treatments), which could also induce a lack of oxygen in the soil leading to denitrification (Dendooven et al., 1998). After this intense microbial activity, nitrification occurred but inorganic-N concentrations remained clearly lower than at the beginning of incubation.

In contrast, nitrogen immobilisation/denitrification phenomena were not observed when PS (Fig. 1) and CS digestates were added to soil. Nitrate production was higher in the soil treated with PS than in that treated with CS due to the higher concentration of $\text{NH}_4\text{-N}$ added by PS (Table 1) and also to the lower C/N ratio of PS digestate (1.5 and 8.5 for PS and CS, respectively). At the end of the incubation period, the percentage of added N that had been converted into nitrate “ $100 \times [(\text{NO}_3\text{-N}_{\text{soil+digestate}} - \text{NO}_3\text{-N}_{\text{soil}}) / \text{added N}]$ ” was 55% and 85% for CS and PS, respectively.

4 CONCLUSIONS

The digested materials tested in this experiment showed a great variability in their organic matter content and a low stability degree. The digestates from cattle slurry-glycerine mixtures showed a high organic load and the lowest degree of stability. This fact conditioned the nitrogen and carbon mineralisation processes, leading to higher $\text{CO}_2\text{-C}$ production and N-immobilisation/denitrification. In these cases a further stabilisation process, such as the exhaustion of the easily degradable organic matter in anaerobic digestion, is recommended to assess the maximum agricultural and environmental benefits.

In stabilised digestates the nitrogen immobilisation process hardly occurred in soil, and a considerable fraction of the added nitrogen was converted into nitrate during incubation. This constitutes an important source of available nitrogen for plants which must be taken into account in fertilisation programmes.

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