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RESEARCH ARTICLE

INFLUENCE OF INCORPORATED DIGESTATE COMPOSITIONAL PARAMETERS ON CO_2 EMISSION AND CARBON TURNOVER PROCESSES IN SOIL

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ABSTRACT

Anaerobic digestion is considered as the reliable source of renewable energy which provides two valuable products i.e., biogas and digestate slurry. Biogas, an alternative energy, is one of the important forms of clean energy whereas digestate has multipurpose usage, including organic fertilizer in many parts of the world. The compositional parameters such as C:N, cellulose and lignin contents of digestate are important indicators of their qualities that indicate the biodegradability status as well as importance in CO_2 evolution and Carbon (C) turnover process. In a 35-day long incubation study, The six different feedstock sources digestate slurry was applied in silty loam soil to explore the C turnover process that were arranged in a completely randomized block design. Microbial respiration (in terms of CO_2 respired) were measured to understand the biodegradability of different digestates. We found CO_2 evolution was negatively correlated with C:N of digestates. The digestates releasing more carbon dioxide evolution may not be suitable manure but may be utilized by mixing with higher recalcitrance containing organic matter.

KEYWORDS

Anaerobic digestion; digestate; biodegradability; CO2 emission, carbon mineralization

1. INTRODUCTION

Anaerobic digestion yields two end products i.e. biogas and digestate (biogas slurry) (Tani et al., 2006; Zhang et al., 2007). The former one which is also a main product, contributes in supplying clean and renewable energy source of energy at household level and later is used as an important source of manure and a way for soil organic matter (SOM) recycling and plant nutrient supply (KC et al., 2013). The fertilizing efficiency of digestates depends on application technique in the field and the nature of feedstocks (Nkoa, 2014). The feedstock having high organic matter with considerable water content, can be considered for anaerobic digestion (Wellinger et al., 2013). Different types of organic materials, sewage, sludge, and municipal solid wastes could be utilized sole or combined with animal manures or slurries as a feedstock during anaerobic digestion (Makádi et al., 2012).

Energy rich cereal crops, poultry manure and pig slurry are the most promising source of biomass for anaerobic digestion (Callaghan, 2002; Hill, 1983; Möller and Müller, 2012). The nature and quality of the feedstock determine the degradability of digestate (biogas slurry) and its organic matter content (Wolf, 2014). The organic matter fraction of digestate influence physical and chemical characteristics of the soil (Lal, 2001). The feedstock used for anaerobic digestion influence the C dynamics in the soil along with the soluble fraction such as carbohydrates, proteins, cellulose, hemicellulose and lignin content (Sanger et al., 2014; Möller, 2015; Van Soest and Wine, 1967). Most of the easily degradable matters, i.e. soluble organic C are digested first during anaerobic digestion so that digestate contained higher recalcitrance organic matter than feedstock (Gerardi, 2003; Coban et al., 2015; Möller 2015).

Most of the organic matter, like in solid waste, biodegradability can be studied through the biochemical fractionation which helps to describe the microbial transformation patterns of cellulose, hemicellulose and lignin (Masoud, 1995). However, some researcher mentioned that microbial transformation of organic matter is significantly affected not only by the chemical composition on feedstocks but also the environmental factors such as soil temperature, moisture, and air temperature (Rochette and Gregorich, 1998). Carbon and N portion of the organic matter present in feedstock or digestate affect nutrient balance and other agronomical usage such as fertilization, water holding capacity and physical-chemical properties (Havlin et al., 1990). Hence, the suitability of digestate feedstocks significantly depends on composition, digestibility, dry matter contents, nutrients content, methane gas production and other chemical characteristics (Wellinger et al., 2013).

Microbial biomass C is degraded during the anaerobic digestion, whereas hemicellulose could be degraded by more than 80%, cellulose by more than 50% but the most part of the lignin is not degraded (Tambone et al., 2010; Molinuevo-Salces et al., 2013; Möller, 2015). The densely available degradable organic matter is the primary source for the intensive proliferation of the microbial activities in the soil (Alburquerque et al., 2012). Intensive microbial activities bring the priming effect in the soil which defined as the change in inherent soil solution nutrients due to addition of the external nutrient sources (Kuzyakov et al., 2000; Jenkinson et al., 1985). Due to this priming effect, a short fluctuation of C turnover in the soil is witnessed (Kuzyakov et al., 2000).

After applying digestate to soil, a high carbon dioxide (CO2) evolution occur due to earlier digestion of available soluble organic carbon as carbohydrates, amino acids and later CO2 fluxes might be due to digestion

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of available protein contents (Marstorp, 1996). Microorganism take energy from the oxidative decomposition of complex organic compounds. Such organic C is converted to CO2, organic N is mineralized to NH4+. The remaining carbon used by microorganism is temporarily immobilized within microbial tissue (Bernal et al., 1998). For digestate amended soils, carbon turnover is greatly affected by the chemical quality of digestate such as C:N ratio, lignin: nitrogen ratio, carbon concentration, presence of polyphenol compounds (Bending et al., 1998; Trinsoutrot et al., 2000).

The research on constant or varietal effect of temperature on digestate related activities is rather suitable in the incubation experiment to field level (Trinsoutrot et al., 2000; Alburquerque et al., 2012). In an incubation experiment conducted by the different biogas slurries in liquid and solid state gave quite varietal results such as the liquid slurries resulted very fast in a high N availability in the soil (Grigatti et al., 2011). The digestate of raw grass clover induced more microbial activities in the soil, causing more CO2 release and immobilized the mineral nitrogen in soil. Meanwhile, the fact is reveled from the research by that the incorporation of cattle manure composts in soil are very applicable for carbon sequestration due to highly carbon (C) storage in soil (Whalen et al., 2008). Still there is a lack of knowledge about different digestates and their effect on soil C dynamics in the soil. In this experiment, individual digestate from different feedstock were compared for their decomposition characteristic. So, our objective was to evaluate the effects of compositional parameters such as cellulose, hemicellulose, lignin, and C:N of different digestates on C turnover process in the soil.

2. Materials and Methods

2.1 Experimental preparation and incubation

Six digestates viz. Berseem grass slurry (BG), Napier Grass Slurry (NG), poultry manure (PM), Cattle dung slurry (CD) and Food waste slurry (FW) and Buffalo dung slurry (BD) were collected from biogas plants (Table 1) as the treatments. Additionally, no digestates added soil was tested as a control treatment. Soil used for the incubation experiment was top-soil (0-15 cm) ie Alfisols containing a silty loam texture (70% silt, 25% clay and 5% sand) with a pH 6.6 with & 13.25 g kg-1 Total Organic Carbon (TOC). Prior to experiment, the air-dried soil was sieved to 4 mm. For the incubation experiment, TOC was increased to 20% of its original level with aiming to detect sufficient CO2 emissions for C mineralization pattern, corresponds to an addition of 2.65 g C kg-1 soil by individual digestates. The required amount of digestates was thoroughly mixed with 700 g soil and was filled into glass jars. Soil moisture content was set up to 60% of the maximum water holding capacity at the start of incubation. Additionally, glass jars with glued quartz sand were kept as a blank for CO2 evolution.

This experiment was arranged in Randomized complete block design with four repetitions. The jars were incubated in the dark for 35 days at 21±1 °C in a climate chamber. During the incubation period, two beakers were kept inside the glass jar, one with demineralised water to avoid water losses due to evaporation and another one with 1 M sodium hydroxide (NaOH) solution to absorb CO2 evolved from the soil. The NaOH solution was exchanged 10 times (day 1, 2, 3, 5, 7, 10, 14, 17, 21, 28). For soil sampling, two portions of 20 g soil were taken at four days (day 7, 14, 28, 35) to analyse microbial biomass C (Cmic). All jars were aerated every second day for about one minute. Additional soil samples were taken on day 0 (start of incubation) and day 35 (end of incubation) for estimation of moisture content, pH, total C.

2.2 Moisture content, TOC measurement

Water content of the mixed soil (day 0 and 35) was determined gravimetrically by weighing. Although the water content was set to 60% of the maximum water holding capacity (WHCmax), it was determined at beginning and end to have a control for potential losses. The pH value of the digested treated soil was measured by pH meter. pH was measured in 1:2.5 soil suspension with 0.01 M CaCl2 buffer solution. Digestates mixed soil pH was measured for the individual sample, taken in initial day '0' and day 35. Similarly, TOC of soil mixture was analysed by using an elemental analyser (Elementar Vario Max (V5.2) auto C:N analyser.

Table 1: Characteristics of applied digestates in the current study (Forage fibre analysis by Goering & Van Soest (1970), Van Soest & Wine (1967).

Digestate	DM	Cellulose	Lignin	TOC	C:N ratio	Digested added
	% FM	% DM	% DM	% DM		g kg-1 FM
Berseem grass Slurry (BG)	8.71	13.8	16.5	38.23	4.9	76.31
Buffalo dung slurry (BD)	5.32	17.4	12.5	41.51	11.36	117.01
Cattle dung slurry (CD)	6.27	20.4	15.6	39.72	6.54	106.23
Food waste slurry (FW)	3.86	<0.90	<0.90	35.73	2.18	192.36
Napier grass Slurry (NG)	8.71	13.8	16.5	38.23	4.9	76.31
Poultry manure Slurry (PM)	5.32	17.4	12.5	41.51	11.36	117.01

DM - dry matter; FM = Fresh Matter, C:N ratio - Carbon and Nitrogen ratio; Hemicellulose = (NDF-ADF); Cellulose = (ADF-ADL); Lignin = ADL; where, NDF - Neutral Detergent fibre; ADF - Acid Detergent Fibre; ADL - Acid Detergent Lignin.

2.3 Carbon dioxide flux

Carbon dioxide evolved from the soil due to microbial respiration, was calculated from the remaining alkalinity in the 1 M NaOH solution titrated with 0.5 M hydrochloric acid (HCl) in surplus with Barium chloride (BaCl2) solution to precipitate carbonates. The amount of CO2-C evolved was measured using equation (1) and expressed as mg CO2-C kg-1 soil. The C-mineralization was measured by the difference between CO2-C evolved from mixed soils and control soils and expressed as mg CO2-C kg-1 dry soil, using equation (2) (Page et al., 1994; Li et al., 2009; Lee et al., 2010).

$$CO_2 - \text{evolution (mg C)} = (B - S) \times C \times E \quad (1)$$

Where, B = ml HCl for reference sample (quartz sand)

S = ml HCl for sample

C = molarity of HCl (0.5 M HCl)

E = equivalent weight of Carbon (C=6)

Adjusted formula for the calculation of CO_2 – evolution (mg C) as following,

$$CO_2 - \text{evolution (mg C)} = (B - S)\ C * E \left(\frac{\text{NaOH ml}}{3}\right)\left(\frac{900}{900 - \text{extracted soil}}\right)\left(\frac{672 \text{ g DS}}{900 \text{ g WS}}\right)\left(\frac{1000 \text{ g DS}}{672 \text{ g DS}}\right) \quad (2)$$

Where, g DS = gram dry soil

g WS = gram wet soil

Conversion factor N_{mic} = 0.54 (Joergensen and Müller, 1996)

2.4 Microbial biomass of carbon (C_{mic})

Soil microbial biomass C and N were determined by the chloroform fumigation extraction (Vance et al., 1987; Müller et al., 2006). The two portions of 20 g soil samples were taken from the incubated soil mixture. One portion was used for fumigation with ethanol free chloroform (CHCl3) in the dark for 24 hours at 25 °C. The other 20 g soil portion was immediately extracted for the non-fumigated sample by adding 80 ml 0.5 M K2SO4 and shaking for 30 min at 225 revolutions per min in an electric shaker (SM25, Edmund Buhler). Afterwards, the mixture was filtrated with 1617 ½ filter papers. Likewise, the fumigated portion was extracted after chloroform removal. All extracts were stored at -20 °C in a freezer until further analysis. Cmic was calculated using equation 3 and 4.

$$C_{mic} = \frac{(EC_{fum} - EC{:}N\ n_{non\ fum})}{0.45} \quad (3)$$

Where, C_{mic} = microbial biomass C

EC_{fum} = extractible organic C in fumigated sample

EC:N $_{non\ fum}$ = extractible organic C in non-fumigated sample

Conversion factor C_{mic}= 0.45 (Joergensen, 1996)

2.5 Statistical analysis

The effect of treatments was tested by analysis of variance (ANOVA) for repeated measures by using GLIMMIX procedure at significance level of α = 0.05. Daily CO2 release were log10 transformed to achieve normal distribution and repeated measures one-way ANOVA was performed using GLIMMIX procedure. LSMEANS method was used for mean comparison (where least significant difference (LSD) was shown at α = 0.05). All statistical analysis was performed by using SAS (Statistical Analysis System university version 9.4).

3. Results

3.1 Carbon turnover in terms of CO2 flux

A rapid development of microbial activities after the incorporation of different digestates was reflected by the immediate high-rate CO2 fluxes from all digestate treated soil (Figure 1). Peak CO2 flux in all the digestates occurred at the first day of incubation. Food waste digestate (FW) produced the CO2 peak of 184.1 mg kg-1 soil. The magnitude of peak fluxes for all other treatments varied slightly. The CO2 fluxes were in the decreasing order: BG (112.8 mg kg-1 soil) > PM (105.3 mg kg-1 soil) > NG (100.5 mg kg-1 soil) > BD (94.5 mg kg-1 soil) > CD (92.7 mg kg-1 soil) (Table 2). The lowest CO2 peak of 17.9 mg kg-1 soil was observed in the control treatment. Initial CO2 peak of all treatments dropped after second day, except food waste (FW), which declined sharply in the third day. For FW, two small intermediate peaks could be seen and only one intermediate rise in CO2 for other treatment. Finally, after 28 days of incubation CO2 emissions subside and there were only negligible CO2 fluxes afterward.

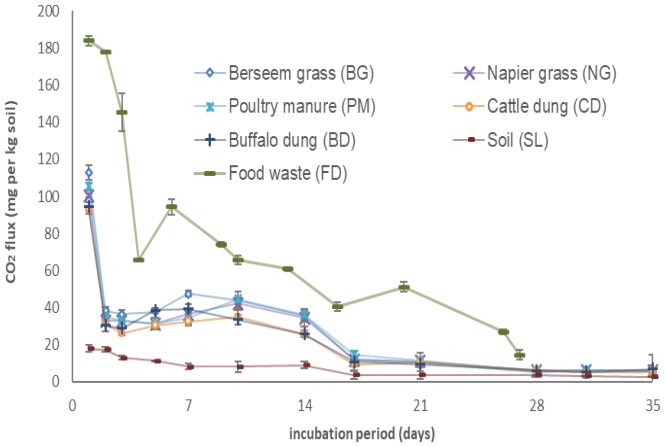

Figure 1: CO2 fluxes production rate (mean ± SE) during 35 days of incubation under different sources of digestates. BG; Berseem grass slurry, NG; Napier grass slurry, PM; poultry manure slurry, CD; Cattle dung slurry, FW; food waste slurry, BD; Buffalo dung slurry, and SL; soil.

Table 2: Carbon dioxide emissions (log transformed value) at different days of incubation.

Treatment	Mean CO_2 release (mg C kg^{-1} soil) with incubation day							
	1	2	3	7	14	21	28	35
Berseem grass slurry (**BG**)	111^{b}	39^{b}	34^{c}	94^{b}	136^{b}	41^{bc}	37^{ab}	34^{a}
Napier grass slurry (NG)	100^{cd}	33^{b}	33_{c}	73^{de}	138^{b}	44^{bc}	43_{a}	34^{a}
Poultry manure slurry (PM)	105^{bc}	35^{b}	33^{c}	69^{de}	145^{b}	47^{b}	40^{a}	37^{a}
Cattle dung slurry (CD)	93^{de}	31^{b}	26^{c}	66^{e}	103^{c}	42^{bc}	42^{a}	36^{a}
Food waste slurry (FW)	184^{a}	178^{a}	145^{a}	189^{a}	182^{a}	204^{a}	29^{b}	35^{a}
Buffalo dung slurry (**BD**)	94^{de}	30^{b}	29^{c}	78^{de}	103^{c}	37^{bc}	42^{a}	36^{a}

Note: Means followed by letters are significantly different within each column among treatments ($p < 0.05$).

3.2 Microbial biomass growth

Microbial biomass C (Cmic) increased in most of the treatments after 28 day of incubation except in food waste. Food waste gained Cmic peak development in 14 days with mean value 358.43 mg C kg-1 dry soil (Figure 2). Other digestates peak value of Cmic was recorded in order of NG (367.83 mg kg-1 soil) > PM (359.64 mg kg-1 soil) > BD (315.57 mg kg-1 soil) > BG (298.77 mg kg-1 soil) > CD (284.98 mg kg-1 soil) respectively. The peak microbial biomass growth in 28 days of incubation has positive correlation (Cmic= 0.48) with cellulose content of the digestate. However, the correlation was not significant with cellulose content of digestate.

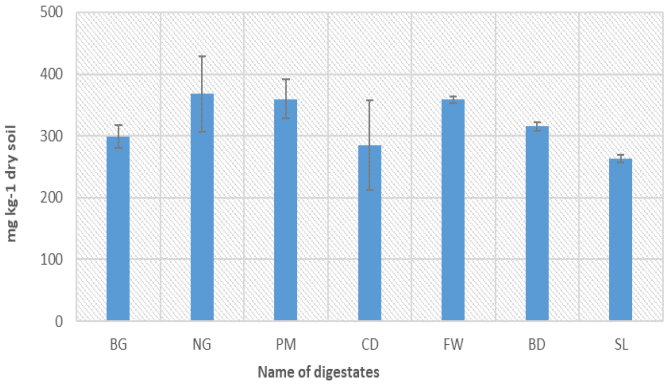

Figure 2: Peak microbial biomass C (MBC) growth under different sources of digestates. BG: Berseem grass, NG; Napier grass, PM; poultry manure, CD: cattle dung, FW: food waste, BD: Buffalo dung slurry, and SL: un-amended soil.

3.3 Correlation of digestate composition on C turnover

Cumulative CO2 evolution was highly significant with C:N ratio of digestate with negative correlation (Table 4).

Table 4: Pearson correlation coefficient among the digestate composition and C mineralization.

Parameters	Cellulose	Lignin	C:N ratio	Cum. CO_2
Cellulose	1			
Lignin	0.73*	1		
C:N ratio	(0.78)*	(0.90)**	1	
Cum. CO_2	-0.92***	-0.89***	(-0.88†)**	1

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

n= 6; except where † is mentioned (n= 5); C:N ratio; carbon and nitrogen ratio of digestate, Cum. CO2; cumulative CO2 evolution from the digestate treated soil after 35 days of incubation (mg kg-1dry soil), # excluding the value of Buffalo dung slurry (BD) as outlier, * significant at probability level p<0.05, ** significant at probability level p<0.01, *** significant at probability level p<0.001.

4. Discussion

4.1 Carbon turnover

In the current experiment, the release of CO2 fluxes was immediate and varied between the treatments (Figure 1). This might be attributed to the degradation of liable carbon (C) fraction of organic matters. Correspondingly, a group researcher found more CO2 emission due to fast degradation of easily available C fraction during initial stage of incubation

experiment with co-digestion mixture (Alburquerque et al., 2012). Owing to the increase in microbial activities, digestate treated soil shows very fast decomposition within few days in soil even in a matter of a day after incorporation (Kirchmann and Lundwall, 1993; Marcato et al., 2009). Soil microorganisms use the available organic C as energy source during biodegradation process, which afterwards is used for building cell wall or released as CO2 (Teglia et al., 2011).

The addition of C into the system is responsible for increasing CO2 (Rochette and Gregorich, 1998). Other Small intermediate CO2 peaks could be the result of successive decomposition of compounds that are previously resistant to decomposition. Residue fraction such as fats, waxes, resins and oils are utilized once readily soluble fractions including sugars, starch, proteins and organic acids are used up (Reber and Schara, 1971). Low CO2 fluxes after day 28 of incubation could be due to less available of easily degradable organic fraction which was vastly decomposed in the beginning of incubation experiment. A group researchers also studied that carbon mineralization rate in the digested treated (pig slurry) soil was decreased after 30 days of aerobic incubation due to exhaustion of readily available organic carbon (Plaza et al., 2007). After 28 days of incubation, there was no significant difference between the treatments cessation microbial respiration.

The difference in magnitude of CO2 fluxes among the treatments could be due to the difference in the chemical composition of the digestate (Rochette and Gregorich, 1998). However, the incubation experiment Conducted also found that compositional differences of digestates play a minor influence on digestate performance after soil application but its C/N ratio, NH4+-N concentration and fibre composition greatly influence in whole C mineralization process (Häfner et al., 2022). Higher CO2 flux from food waste in first week of incubation, might be explained by lower C:N ratio (i.e., 2.2, Table 1). As total organic carbon does not much, narrow C:N ratio is due to high N content (Tambone et al., 2010). Subsequently, high amount of N increases the rate of decomposition where microbes utilize O2 and respire CO2 (Williams and Gray, 1974; Zibliske, 1994). This result is well corroborated by where material with lower C:N ratio containing materials evolved more CO2, thus showed more C mineralization (Riffaldi et al., 1996).

Furthermore, high CO2 release from food waste might be priming effects. More CO2 release due to containing of highly degradable substrates addition indicate the priming effects as like observed after amendment of energy rich substrates in incubation experiments (Kuzyakov et al., 2002; Falchini et al., 2003). However, priming effect was not considered in this experiment. Undigested cattle slurry evolved comparatively less CO2, could be due to higher C:N ratio. Other digestates from different feedstocks sources such as clover grass, maize, poultry manure, grass showed higher carbon mineralization than undigested cattle slurry, which could also be explained by relatively lower C:N ratio. This result is correlated with the study of where high CO2 evolution rate from the different fertilizers amended soil was influenced by the presence of easily decomposable fraction as well as less C:N ratio of the fertilizers (Müller et al., 1998). This could be justified that the negative correlation between cumulative CO2 evolution and C:N ratio of the digestate was strong as well as highly significant ($r = -0.88$, $p < 0.01$). Negative correlation indicates that lower lignin containing digestates lead to higher cumulative CO2 release during incubation.

Food waste digestate showed the highest CO2 flux among the treatments which might be due to lowest lignin content (i. e. <0.9, below the detection limit, Table 1). Food waste digestate was significantly different among other digestate which indicate that food waste has lowest recalcitrance of organic matter. Komilis and Ham described the role of lignin as the decomposition retarding material (Komilis and Ham, 2003). The higher lignin contents of the materials retards the rate of decomposition (Vikman et al., 2002). So low lignin content implies a lower recalcitrant nature. A group researchers reported that the digestates with low recalcitrance of organic matter, released more CO2 during aerobic incubation (Alburquerque et al., 2012). Contrastingly, less cumulative CO2 releasing treatments, Cattle dung slurry, Buffalo dung slurry showed more recalcitrance of organic matter could be explained by more lignin containing digestates.

If digestates have more lignin content, other decomposable fractions such as hemicellulose, cellulose is kept by bounding around and lead to slow decomposition (Tambone et al., 2009). Similarly, other digestates, Napier grass (NG) and poultry manure (PM) are not significantly different with digestates CD and BD which indicate that both NG, PM has low recalcitrance of organic matter. However, similar lignin containing digestates BG (i.e., 16.5) was slightly less recalcitrant of OM which could be due to lower lignin:N ratio. This was supported by the study of showed that initially high CO2 evolution rate from the soil is influenced by the presence of easily decomposable fractions as well as low C:N ratio of the substrates, afterward, less CO2 release is influenced by high lignin contents and lignin:N ratio (Müller et al., 1998). This is further justified by the correlation between cumulative CO2 and lignin content of the digestate. There was negative correlation with highly significant ($r = -0.89$, $p < 0.001$). Negative correlation indicates that higher lignin containing digestates lead to lower cumulative CO2 release during incubation and high recalcitrance of organic matters.

4.2 Microbial biomass

Increasing microbial biomass growth could be due to increasing microbial population and microbial activities. Microbial activities enhance the potential growth of microbial biomass in the soil (Griffiths et al., 1998; Saviozzi et al., 1997). A group researcher recorded in an incubation experiment with pig slurry amended soil, that microbial biomass carbon could increase during first 30 days, however microbial biomass growth may not always be correlated with increasing microbial activities (Plaza et al., 2007). Accumulation of highly degradable organic matters in the soil can favour condition for increasing microbial activities, which can promote the growth of autochthonous microorganism in soils (Sakamoto and Oba, 1994).

Additionally, outer sources of microorganism might be available in amended materials, help to increase microbial activities in soil (Perucci, 1992). Microbial carbon of food waste was more in day 14 of incubation which could be due to lowest amount of cellulose and lignin (i.e., < 0.9). Similar increase of microbial carbon due to biodegradation of easily available organic compounds also appeared within the first 14 days in an experiment of analysing organic waste amended soils (Moreno et al., 1999). Other digestates reached a peak Cmic development only after 28 days of incubation. The later Cmic peak could be due to higher amount of cellulose and lignin than food waste. Organic fertilizers which have comparatively higher cellulose and lignin content lead to a later increase of microbial activities due to hindrance for a certain time by cellulose and lignin (Müller et al.,1998). However, we observed a high cellulose and lignin contents delayed microbial growth.

5. Conclusion

The current study provides some insights into C turnover process in the anaerobic digestate treated soil. The higher microbial respiration was appeared in the lower C:N ratio containing digestate. Lignin content of the digestate showed distinguishable results in the recalcitrance of the organic matter in digestates. Higher recalcitrance of organic matter in digestate, was found in higher lignin containing digestate. Higher cellulose content of the digestate delayed the microbial biomass growth. Therefore, Food waste that is higher carbon dioxide emission digestate may not be suitable as organic fertilizers because it can't supply the nutrients to plant but may harmful in environment or carbon sequestration. Buffalo dung slurry has relatively stable organic matter than food waste so it is potential for humus development.

Food waste can be good N fertilizer sources but only if managing soil by adding some carbon or with right crops which provide crop residue in field. Beside this, there may be needed further research for some more digestates such as food wastes, domestic organic household wastes because composition may differ in different seasons. In conclusion, the compositional parameters of the digestate such as C:N ratio, cellulose and lignin contents play a significance role for defining the C turnover processes after addition of digestate in the soil. The digestate having less C:N released more CO2 evolution enhanced C mineralization. Therefore, the compositional parameters of the digestate such as C:N, cellulose and lignin contents play the vital role for determining the C turnover processes after the addition of digestate in the soil.

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