The chemical distributing characteristics and dynamics of iron, cobalt and nickel in 3 different anaerobic digestates

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Abstract:

The enhancement of the availability and process controllability of trace elements (TEs) addition is of significance to improve the anaerobic digestion (AD) performance. In order to understand the bio-availability of TEs, the chemical form distribution patterns of endogenous /exogenous Fe, Ni and Co with different dosage (Fe: 1, 10 and 100 mg/kg; Ni and Co: 0.1, 1 and 10 mg/kg) and different pH condition (6.5; 7.5 and 8.5) were investigated in three different anaerobic digestates. The results showed that the exogenous TEs dosage exerted no obvious effects on the TEs distribution patterns. The chemical fractionation of TEs was more affected by pH and redox potential. Under pH 6.5 and with possibly redox potential rising, the acid-extractable and reducible fractions and of Fe, Ni and Co was increased while the oxidizable fractions were decreased, which enhanced the bio-availability of TEs

Keywords: Trace elements, anaerobic digestion, chemical forms distribution.

Introduction

In the attempts to stabilize and improve the anaerobic digestion (AD) process, the role of TEs has become increasingly recognized since last 3 decades. (Takashima, et al, 1990). It has been revealed that the TEs, including Fe, Co, Ni, Mo, W, Se, et al., are important components of the coenzymes, prosthetic groups or cofactors that are essential for methanogenesis (Zandvoort et al., 2006; Thanh, et al, 2016) and maintaining a healthy AD environment (Demirel, 2011). As a result, the lack of TEs in an AD system is prone to blocking the relevant enzymatic reaction, leading to the uncoupling of the acetogenesis and methanogenesis, and eventually triggering the system to the acidification direction. However, the excessive TEs in an AD system can also cause toxic effects.

In the AD practice, the TEs deficiency in feedstock for AD was reported in numerous cases, such as municipal sewage (Xu et al., 2017), animal manure (Wu et al., 2017), agriculture residues (Schmidt, Nelles et al. 2014) and kitchen waste (Banks, 2012). The supplemental TEs are able to resolve the problems caused by endogenous TEs deficiency (Wandera et al., 2018; Zhang et al., 2015) . To ensure that the microbes in the AD systems obtain sufficient amount of TEs, many studies on the demand for TEs in AD system, the TEs addition dosages, and the effects of the additive TEs on the promotion of AD performance have been carried out (Xu et al., 2017; Wu et al., 2017; Schmidt, Nelles et al. 2014; Banks, 2012). However, the demand and dosage of TEs vary greatly from one report to another. For example, the dosage of 0.5 g Fe/L/d, 0.5 mg Co/L/d and 0.2 mg Ni /L/d was reported to effectively stabilize the mesophilic AD of wheat stillage under organic loading rate(OLR) of 4 g VS/L/d (Gustavsson et al., 2011). In the AD of municipal solid waste and slaughterhouse waste, it was found that 0.4 g Fe/kg, 0.5 mg Co/kg and 0.5 mg Ni/kg benefited the biogas production rate and yield (Moestedt et al., 2015). With the Fe and Ni dosage of 25 mg/L and 5 mg/L, respectively, the highest biogas yield was obtained in the mesophilic batch AD of sludge (Yang et al., 2017). By applying the recommended TEs dosage (Hinken et al., 2008) for corn silage digestion, no significant improving effect for biogas production was found in the batch AD study of rice straw (Mancini et al.,2017). Facchin et al. (2013) reported that the TEs (Co, Mo, Ni, Se, and W) addition showed positive effect in food waste AD, while neutral or slightly negative effects in co-digestion of biowaste and waste activated sludge.

The main reasons for the inconsistency of TEs strategies are as follows: (1) the background values and distributions of TEs are different in different feedstocks; (2) the heterogeneity of feedstocks results in the diversity of the digestate texture, so the specific chemical forms of TEs in a certain AD system vary with the factors including pH, alkalinity, redox potential, sulfur, phosphorus, potassium, extracellular polymeric substance (EPS), soluble microbial products(SMP), et al. (Hullebusch, 2005); (3) the specific requirements of TEs in a certain AD system are related to the its unique microflora and their ability of obtaining certain TEs (Zandvoort et al., 2006); (4) The reference value of the research findings were weakened because much emphasis was put on the significance of total dosage of TEs, other than their actual utilization efficiency (Ortner et al. 2015). Therefore, the optimum dosage obtained in a particular study can not be used as a universal reference when the feedstocks and digestion conditions are changed.

In the AD systems, there are complex interactions between the solid and liquid phases. Generally, before the TEs are contacted and absorbed by microbial cells, they can undergo complex reactions to form precipitates, covalent compounds, and organic complexes. In particular, the interactions between metal and organics triggered by sulfide chemistry are considered to be the critical factor in regulating the TEs bioavailability in the AD systems (Choong, et al., 2016). Williams (1986) found that, although the effluent from chicken manure digestion already contained a significant amount of Ni (253 μM), the addition of another 10 μM Ni could still improve the biogas production. The similar phenomenon was also found in the TEs- sufficient sludge digestion, in which the additional TEs supplement, especially Co addition, led to 9%-50% increase in the acetate methanogenesis rate (Carliell-Marquet et al., 2013). These findings demonstrated that the amount of the additional TEs depends largely on the extent to which elements can be utilized by microorganisms. The total amount of TEs in an anaerobic system is not sufficient to explain the bioavailability and toxicity of these elements (Thanh, et al, 2016). The chemical speciation distribution dynamics and bioavailability of TEs in the AD system need to be studied detailly.

In the past decade, although about 10 times of biogas production has been improved due to the technological innovation in the field of AD, it is remained to be known how to break through the limitation of the low TEs bioavailability on methanogenesis promotion (Fermoso et al., 2015). The TEs distribution characteristics and formation rules in different AD systems are the prerequisites for predicting the bioavailability of TEs. It was deemed that the revelation of the rule of TEs distribution would be helpful for the new development on the basic principle of AD (Choong et al., 2016).

It is generally accepted that the metal elements the natural environment can exist and transform into one another in the following states: dissolved state, adsorbed state, carbonate bound state, phosphate bound state, sulfide precipitation and residual state, with the bioavailability decreases successively (Thanh, et al, 2016). The stepwise extraction using serial chemical reagents is currently the most commonly used method for studying the chemical distribution, bioavailability and mobility of metal elements in solid matrices (Choong, et al., 2016; Hullebusch, 2005).

In the AD system, the Fe content is generally the highest, followed by Ni, Co, Mo, W and Zn (Ortner, et al., 2015). According to some qualitative researches, Fe, Ni and Co are necessary to the growth of almost all of the methanogens (Fermoso et al., 2009). It was also reported that the methanogenic activities are most vulnerable to the Fe, Ni and Co deficiency (Fermoso et al., 2009). Therefore, Fe, Ni and Co might be considered playing the leading role in promoting biogas production among several necessary TEs in normal AD.

The objective of this study was thus to evaluate the dynamical chemical speciation and distribution characteristics of endogenous/exogenous Fe, Ni and Co in 3 different digestate, derived from AD process using maize stalk, pig slurry and waste water from chocolate factory, respectively. The multi-step extraction method established by European Community Bureau of Reference in Brussels (BCR) and inductively coupled plasma-optical emission spectrometry (ICP-OES) are applied to quantitatively describe the chemical form distribution of TEs. The effect of pH value, digestate type, TEs dosage and retention time on the distribution and dynamic changes of TEs chemical form are investigated via static batch AD experiments. Totally, this preliminary study will provide a basic scientific reference for the optimization of the exogenous TEs dosing strategy, the exploitation of the endogenous TEs utilization during AD process and the bioavailability risk assessment of TEs in digestates to their discharging environment.

2. Materials and Methods

2.1 Anaerobic digestate

Three kinds of anaerobic digestats were collected. The anaerobic sludge of a UASB reactor treating waste water (WW) from chocolate factory was collected in Jiaxing, China; The digestate of pig slurry (PS) was collected from a CSTR biogas plant on Chongming island, Shanghai, China; and maize stalk (MS) digestate was from the biochemical methane potential assays carried out in the biomass energy engineering research centre, Shanghai Jiao Tong University. It is worth mentioning that the inoculum of the MS AD was also from the CSTR biogas plant using PS on Chongming island.

2.2 Experimental design

The experimental variables are including: (1) three different pH conditions (6.5; 7.5 and 8.5) adjusted by 6 mol/L HCl or NaOH to simulate the acidified, normal and alkaline digesting conditions; (2) three different Fe, Ni and Co dosage recipes to simulate the different dosage strategies; the Fe, Ni and Co in forms of $FeCl_2.4H_2O$, $CoCl_2.6H_2O$ and $NiCl_2.6H_2O$ are used to give dosages of :(A)1, 0.1 and 0.1 mg/L; (B)10, 1, 1 mg/L; and (C)100, 10, 10 mg/L, respectively; and (3) the 31-days of retention time with 5 time intervals of sampling to investigate the dynamic variations of the chemical distribution.

After pH adjusted and extra TEs recipe given, a 300 g digestates were cultivate at 36°C in a rubber-stopper-sealed 500 mL flask. The biogas was collected by saturated salt solution displacement in a graduated collector, with an airway tube connected to both the flask and the biogas collector. The measured biogas production was recalculated into the STP condition (273.15K, 101.325KPa). The device sketch for digestate cultivation and biogas collection is shown in Figure 1. Each cultivation was duplicated. A sampling of 5 g digestate was carried out at each time intervals, to carry out the BCR sequential extraction and TEs content analysis.

2.3 Analytical methods

For the solid samples, total solid (TS) and volatile solid (VS) content of samples are determined under 105°C and 550°C according to ASTM E1756-08 and ASTM E1755-01. The chemical oxygen demand (COD) (colorimetric dichromate closed reflux method), total nitrogen (TN) and total phosphorus (TP) were analysed according to Standard Methods (APHA, 1995). The pH value was measured by pH meter (Leici, PHS-3C, China). The total (TA), partial (PA) and intermediate alkalinity (IA) analysis are carried out according to previously reported methods (Sun, et al., 2016). The total trace metal and sulfur content of digestates was detected by ICP-OES (Thermo, icap-7600, U.S.A.) at Instrumental Analysis Center of Shanghai Jiao Tong University, after the samples were pretreated by 5 mL of concentrated HNO_3 (MOS grade) and 2 mL of 30% (w/w) H_2O_2 (MOS grade) solution for 10 min in microwave digestion system, and then digested at 200°C for 1h.

Two fractionated extraction methods were applied to separate the chemical fractions of Fe, Co and Ni in digestates. Method-1 is a modified Tessier's method (Tessier et al., 1979; Technical standards for geological survey of the China Geological Survey, DD2005-03), used to fractionate the digestate into 7 fractions of different chemical forms, in order to ascertain the chemical distribution background values of the TEs. In consideration of the economy and operability, Method-2, the BCR sequential extraction scheme (Ure et al., 1993; Rauret et al., 1999), was applied to determine the dynamic changes of 4 TEs chemical fractions during the 31-days AD experiments. The extraction reagents, procedure, and the extracted chemical forms of the two methods was shown in Table 1. After sequential extraction, the acid-microwave digestion process and the ICP-OES was applied to measure the metal contents of each portion.

2.4 Statistical analysis

Duncan's multiple range tests at the 5% level were applied to determine the significance of the differences in chemical form proportions among the different pH and TEs addition groups, respectively.

3. Results and Discussion

3.1 Characteristics of 3 different digestates

The basic characteristics of the three digestates are shown in Table 2. The 7 chemical forms distribution of Fe, Ni and Co in the three digestates are shown in Figure 2. The water content of WW digestate was over 98%, while less than 90% in the MS and PS digestate. This provided different heat and mass transfer environment for the endogenous/exogenous TEs distribution. Despite its higher water content, the percentage of water-soluble fraction of Fe and Ni in the WW digestate was 79.2% and 75.6% lower than in MS digestate, and 86.6% and 65.7% lower than in PS, respectively. However, for Co, the water-soluble fraction percentage in WW was 70.0% and 37.1% higher than that in the MS and PS digestate, respectively.

The pH of MS and PS digestate was more than 8, which related to their high nitrogen content of 2600 and 3083 mg/kg, respectively. Ammonia, the major nitrogen component in AD, contributes alkalinity to the AD system, so the high pH and nitrogen content signified high buffering capability for VFAs surging during the AD process. The complexation and precipitation reaction can happen between the ammonia and ion metals. Not only the Fe^{2+}, Ni^{2+} and Co^{2+} are likely to form precipitation under alkaline condition, the alkaline condition can also activate the acid ligands, which contributes to the form of alkalic coordination complex. All the processes happened under alkaline conditions reduce the bioavailability of the TEs in digestate of PS and MS. In the WW digestate, the pH 7.20 and TN concentration of 141.3 mg/kg were relatively beneficial to the TEs bioavailability when compared with the other two digestates.

The TEs speciation distribution in anaerobic conditions are also controlled by the inorganic ligands of carbonate, phosphate and sulfide. The partial alkalinity (PA) can indicate the available buffering from bicarbonate and ammonia. According to Table 2 and Figure 2, the PA in WW digestate took for 83.9% of the TA, while they were only 76.1% and 74.4% in MS and PS digestate, respectively. This explained the much higher carbonate fraction concentration in the WW digestate. For Fe and Ni in WW digestate, the carbonate fraction accounted for 45.5% and 34.9% of the total Fe and Ni, respectively, while carbonate fraction percentage of Fe and Ni were only 4.5% and 20.2% for MS, 8.3% and 15.5% for PS, respectively. The carbonate fraction is sensitive to the pH changes, as low pH values can release the TEs from carbonate fraction into water-soluble phase.

Both the organic phosphorus and the inorganic phosphate contributed to the total phosphorus content. The phosphate groups, including $H_2PO_4^-$, HPO_4^{2-}, $PO_4^{3-}$and organic phosphate, as well as the sulfur-containing compound, including sulfide, sulfate, sulfite, thiosulfate, thiol, et al., could form coordination complex and precipitate with TEs with relatively stable chemical properties. It was reported that the TEs bioavailability and chemical forms distribution depended largely on the sulfide content (Fermoso et al., 2015). The sulfide takes up a major proportion in the strong organic combination fraction in anaerobic digestate due to the low Eh. The total sulfer content in the WW digestate was 89% lower than that in the other two digeastates, which reflected the very small portion of strong organic combination fraction of Fe and Ni in the WW digestate.

Different from the short of bioavailable TEs in AD feedstock, e.g. residual fraction of 67% Fe, 53% Ni, and 43% Co was found in the slaughterhouse waste (Ortner et al., 2015), residual fraction of 33.8% and 34.2% of Fe, 26.5% and 46.3% of Co was found in MS and PS digestate. Residual Ni was not detected in the three digestate. On the contrary, around 43.9%, 42.5% and 70.4% of bioavailable Fe (water-soluble, ion-exchangeable, carbonate and weak organic combination), 71.0%, 82.1% and 78.9% of bioavailable Ni, and 23.2%, 47.2% and 51.1% of bioavailable Co for MS, PS and WW digestate, respectively, were measured in this study. These values consistent with the conclusion drawn by Ortner et al. (2014), who believed that the TEs fractions of 30%-70% were potentially bioavailable in AD. Gustavsson et al. (2013) also reported the dissolved form of Co was 10-20% of the total Co concentration in the stillage AD, which was consistent with the 12% of water-soluble Co measured in the MS digestate in this study.

3.2 The effect pH on the chemical form distribution of endogenous Fe, Ni, and Co

The dynamics of TEs chemical forms distribution during the 31 days of cultivation was investigated under the BCR sequential extraction scheme, which divided the TEs into 4 chemical forms, namely acid-extractable, reducible, oxidizable, and residual fractions. The pH variation is shown in Figure 3. As shown in Figure 3, the pH value fluctuated around the artificial adjusted pH value of 6.5, 7.5 and 8.5 in all the digestates during the whole cultivation time.

3.2.1 The chemical fractions of Fe under different pH

The proportion variations and dynamics of chemical fractions of Fe under different controlled pH conditions for the three digestate is shown in Figure 4 and 5. For WW digestate, the pH 7.5 exerted significant effects on increasing the reducible and oxidizable form of Fe compared with the groups of pH 6.5 and 8.5($p<0.05$). No significant effect of pH was found on water-soluble and residual fraction distributions of Fe in the WW digestate.

The acid-extractable fraction is corresponding to the sum of water-soluble, ion-exchangable fraction, carbonate and maybe some weak organic combination forms fractionated by the modified Tessier's method. Ortner et al. (2015) concluded that the fractions of soluble and exchangeable fractions directly impacted the anaerobic digestion performance. Nges et al. (2012) suggested the usage of dissolved fraction, instead of total TEs concentration, to give a rough indication of bioavailability for AD process. Bougrier et al. (2018) also adopted the remaining TEs concentrations in the soluble phase of digestate, to achieve a better indication of the exogenous TEs dosage. Figure 4 and 5 shows that the acid-extractable fraction of Fe in the WW digestate accounted for 65.0%-68.8% at the start of the cultivation, while accounted for 51.5%-63.5% at the end, despite the pH differences. The acid-extractable fraction of Fe in the WW digestate was about 5-10 times of that in MS and PS. From Figure 1, it was shown that in the WW digestate, the major proportion of Fe in the acid-extractable fraction was the carbonate form, which could be brought down by lower pH. However, the pH didn't bring about significant difference on the acid-extractable fraction distribution of Fe in PS and WW digestate ($p>0.05$). It was likely that the probably diminished carbonate combination form of Fe in WW digestate transferred into water-soluble and/or ion-exchangeable state. The pH of 6.5 significantly enhanced the acid-extractable fraction proportion of Fe in the MS digestate ($p<0.05$)

In the soil colloid chemistry, the Fe-Mn oxides possess typical colloid physical and chemical characteristics, such as porosity and large specific surface area with the positive and negative charges on surface. By co-precipitation or adsorption, they can selectively absorb and enrich some TEs in the surroundings. The reducible form fractionated during BCR method is corresponding to the Fe-Mn oxides combination form in the modified Tessier's method. This fraction can only be dissolved under extremely low pH conditions (pH lower than 1.5). The anaerobic conditions exert influence on the exist of Fe-Mn oxides. Under the anaerobic reduction condition, the ferric iron in the Fe-Mn oxides can be reduced to ferrous iron. The latter is of better solubility and mobility, thereby the TEs absorbed by Fe-Mn oxides released into the more mobile phases. It was shown by Figure 2, 4 and 5 that the reducible fraction of Fe in MS digestate increased sharply from 8.5% of the total Fe to 48.4%, 56.0% and 46.6% under pH 6.5, 7.5 and 8.5, respectively, during the first 3 days of cultivation. For PS digestate, they were from 19.2% to around 60% under pH 6.5 and 7.5, while 36.6% under pH 8.5. However, the reducible fractions of Fe in the WW digestate were declined from 26% of the total Fe to 14.9-19.3%. Then the sharp decline of the reducible Fe fraction to less than 5% was found in the MS and PS digestate under pH 7.5 and 8.5. The pH 6.5 significantly retarded the shrinkage of reducible Fe fraction in MS and PS digestate ($p<0.05$). This might be due to the lower pH condition of 6.5 made part of the oxidizable fraction of Fe converted into the reducible form, which was indicated in the Figure 5 (Oxidizable fraction).

The oxidizable fraction of Fe in MS and PS digestate went through the tendency of increasing during first 10 days of cultivation and then decreasing gradually. The pH of 6.5 significantly reduced the oxidizable fraction of Fe ($p<0.05$). Sulfides and some organic matters are the main component of the oxidizable fraction. The partial dissolution of metal sulfide under relatively lower pH might account for the lower concentration of oxidizable Fe in both MS and PS digestate, when compared with pH 7.5 and 8.5.

The residual fraction contains the most difficult form of TEs to be taken up and utilized by organisms. This stable form of TEs can be beneficial when consider the potential heavy metal pollution brought by anaerobic digestate discharge. It was reported that the pH reducing shock was able to result in translocation of Fe, Ni, Co and S from the residual to the organic sulfide fraction (Zandvoort et al., 2006). Correspondingly, the pH 6.5 lowered the residual Fe fraction in MS and PS significantly ($p<0.05$) in this study. There was no significant difference of residual fraction variation in WW digestate under the different pH ($p>0.05$). From Figure 5, the residual fraction of Fe in MS and PS digestate kept increasing from 52.2-104 mg/kg to 320-486 mg/kg since the 3rd day of cultivation until the end of the experiment. It was shown in Figure 2 that the initial residual fraction of Fe was 428 mg/kg and 224 mg/kg for MS and PS digestate, respectively. The residual fraction in MS and PS digestate underwent drastic decline during the first three days of cultivation.

3.2.2 The chemical fractions of Ni and Co under different pH

The chemical fraction proportion variations of Ni and Co in the MS and PS digestate under different pH are shown in Figure 6. In the WW digestate, as the tatol Ni and Co content was only 0.946 and 0.119 mg/kg, respectively, most of its chemical fractions of Ni and Co were below the detectable limit in this study. In MS digestate, nearly no residual form Ni and Co was detected, except in the pH 8.5 group at the end of the experiment. Similar to Fe, the pH 6.5 significantly enhanced the acid-extractable and reducible fraction of Ni and Co in MS and PS digestate, and decreased the oxidizable fraction of Ni and Co in the two degstate ($p<0.05$). Also, the reducible fraction of Ni and Co underwent the variation tendency of sharp increasing at day 3 followed by falling down gradually. Despite the effection of pH, the cultivation increased the acid-extractable fraction of Ni in both MS and PS digestate. Similar phenomenon was also discovered by Dong et al. (2013), who found the high-solid AD process much or less increased the bioavailability of Ni.

It was also signified by Figure 6 that, the oxidizable fraction took a major percentage in the total Ni and Co. The higher the pH condition, the more oxidizable and residual fractions could form. Under pH 8.5, most of the Fe, Ni and Co in oxidizable form in MS and PS digestate reached their highest point of around 60%-80% at day 9. The lower pH of 6.5 could help increasing the formation of acid-extractable Ni and Co. These results were consistent with many studies reported that reducing the pH in AD lead to increased solubilization of metals (Zandvoort et al. ,2005; Lopes et al.,2008; Gonzalez-Gil et al.,2012). Compared with Fe, the increase of residual fraction during the cultivation was not obvious in the Ni and Co. However, between pH 6 and 8, Osuna, et al.(2004) found no significant differences in the Co sorption in the carbonates, organic matter+sulfides, and residual fractions.

With the semi-continuously fed biogas tank reactors dealing with sulfur-rich stillage, Gustavsson et al. (2013) found that the Ni was entirely associated with organic matter/sulfides while Co were more easily accessible by the microorganisms, which was opposite to the findings with total sulfer content of 1.2-1.3 g/kg in this study, although concentrations of sulfur compounds as low as 13 mg/L was acclaimed to significantly influenced the TEs retention/accumulation (Zandvoort et al., 2006). Except day 3, a bigger proportion of oxidizable form of Co than Ni along the whole cultivation time could be illustrated by Figure 6. van Hullebusch et al. (2005) reported that Co and Ni were found in both the organic/sulfide and the residual fraction of granular anaerobic sludge at neutral pH, which was consistent with the results in PS digestate.

3.3 The chemical form distribution dynamics of exogenous Fe, Ni and Co

As TEs addition strategy was often applied in the biogas production plants, in order to improve the bioactivity and overall performance of AD. The additional Fe, Co and Ni was given to the three digestate, to describe the chemical distribution dynamics and bioavailability variations of the exogenous TEs. The variation of the average pH of the MS, PS and WW digestate with different TEs dosage is shown in Figure 7. The pH of MS and PS digestate fell to below 8 at day 4, and then reached to and fluctuated around 8.1-8.3 during the rest time of cultivation. The pH of WW digestate climbed from 7.1 to 7.8 at day 19, and then declined to 7.3 at the end of cultivation. In this study, the first sampling time was changed from day 3 to day 1, and the second sampling time was the 4th day, so as to observe the dramatic increase pattern of the reducible and oxidizable fraction during the first few days of cultivation, of which phenomena was found in the previous study of the pH effect. The influence of antagonisis among TEs including but limited to Fe, Co and Ni on the exogenous metal fractionation in anaerobic granular sludge was reported by Osuna, et al. (2004). For instance, the FeS can potentially adsorb and/or co-precipitate Ni and Co, to form the complexes of Fe-Ni/Co-S (Choong, et al., 2016). However, the interactions among TEs was not quantified in this study.

3.3.1 The distribution patterns of the exogenous Fe, Ni and Co in three digestates

The dynamics of different chemical fractions of exogenous Fe, Ni and Co in the MS, PS and WW digestate are shown in Figure 8-10. It was indicated from the Figure 7-9 that the amount of exogenous TEs dosage had no effect on the distribution pattern of different chemical fractions, as the variation tendency of different chemical forms of the same TE among different dosage groups was basically consistent with each other. As result of the similar microbial flora originated from the same biogas plant and thus probably very similar AD conditions, the MS and PS shared very similar TEs distribution patterns of each chemical form. However, the variation tendencies and distributions of some chemical fractions of the WW digestate are different from the other two. Corresponding to the high water content of 98% and low TS content of 2% in WW digestate, the acid-extractable fraction content of TEs was much higher and the residual fraction was much lower when compared with the other two digestates. However, the variation tendency of the residual fraction of exogenous Fe, the oxidizable and residual fraction of exogenous Ni, and the acid-extractable and reducible fraction of exogenous Co still paralleled to the MS and PS digestate. These phenomena indicated that the exogenous TEs complied to certain distribution rules in the AD conditions.

Besides pH, the chemical forms distribution of TEs is mainly regulated by the inorganic ligands of carbonate, phosphate and sulfides in anaerobic conditions, which are generated by the microbial mineralization of organic matter (Yekta et al., 2017). The shaping factor to the TEs distributing patterns are included but not limited to these physicochemical properties. In this study, the acid-extractable Fe in the WW digestate gradually decreased from around 400 mg/kg to 307 and 332 mg/kg with the TEs addition recipe (A) and (B), respectively, and from 500 mg/kg to 387 mg/kg with the recipe (C). In MS and PS digestate, there was no obvious changes of acid-extractable Fe. After a short-time increase at the beginning of the cultivation, the acid-extractable Ni and Co was relatively stable and consistent to the initial concentration in the three digestates. For the reducible fraction, the Fe, Ni and Co in MS and PS digestate maintained at similar level to their initial value except a peak formed during the first 4 days. However, in the WW digestate, the reducible fraction was on the rise after a slight decline at the beginning. The recipe (C) resulted in the largest increase in reducible TEs of WW digestate, with the increase of 72.4 mg/kg and 1.7 mg/kg for reducible Fe and Ni, respectively. The oxidizable Fe fraction in MS and PS digestate reached their highest point after a drop in the first 4 days, and then decreased gradually to less than 200 mg/kg. The oxidizable Ni and Co kept to their initial level after a drop at the beginning. In WW digestate, no obvious changes of oxidizable TEs was observed. Very similar variation patterns were found in the residual Fe of three digestates. The decrease of residual Fe in the first 4 days was followed by a rapid increase. The residual Ni and Co was very limited in the three digestates. With recipe (C), the variation pattern similar to residual Fe was found in the digestates.

3.3.2 The effect of redox potential shock on the chemical distribution

The most noticeable feature of the chemical distribution pattern in this study was the sharp increase of the acid-extractable (Ni and Co) and reducible fraction (Fe, Ni and Co), as well as the corresponding sharp decrease of the oxidizable and residual fraction of Fe, Ni and Co found within the first 4 days in the MS and PS digestate. Because the inoculation in this study was not done in an anaerobic environment, it is assumed that oxygen was mixed into the pervious anaerobic digestive system along with the process of digestate transfer, the addition of the concentrated recipe of exogenous TEs, and the following vigorous stirring in purpose of homogenization. During the first 4 days, the pH falling from 8.16 and 8.46 to 7.81 and 7.92 in MS and PS digestate, respectively, could also give evidence to redox potential increasing shock. The empirical value in the soil chemistry was that, the redox potential could increase 60 mV along with the pH value decrease of 1 (30°C). It is generally believed that the high Eh and low pH value promote the release of trace metals. The low Eh and high pH value are beneficial to the redeposition of metals and their re-complexation with organic matters. under anaerobic conditions, trace metals are more difficult to mobilize and mainly precipitate as sulphides and carbonates (Thanh et al., 2016). Owing to the possible drastic increase of the redox potential brought by air inpouring, it was assumed that plenty of Fe, Co and Ni transferred from the oxidizable and residual fraction into the acid-extractable and reducible fraction in MS and PS digestate.

During the redox potential shock, the augment for the percentage of reducible fraction in total Fe were 35.9%-44.3% in MS digestate, and 32.7%-38.4% in PS digestate, despite the dosage difference; for Ni, they were 25.9%- 28.5% and 25.6%-50.7%; and for Co, they were 21.9%-30.6% and 30.2%-34.3%, respectively. The loss for the percentage of oxidizable fraction in total Fe were 16.1%-26.3% in MS digestate, and 4.0%-19.4% in PS digestate; for Ni, they were 30.5%-35.3% and 37.5-52.9%; and for Co, they were 40.3%-54.1% and 58.3%-60.1%, respectively. The redox potential shock exerted stronger influence on the increase of reducible Fe and the decrease of oxidizable Ni and Co. The Fe in MS digestate was more affected by the redox potential shock while the Ni and Co in PS digestate were more sensitive to the shock.

The WW digestate with high water content and good flowability avoided the stirring frequency and thus severe oxygen contamination. This was probably an important reason explained the absence of the reducible peak and oxidizable valley in the WW digestate. The redox potential condition was one of the key factors in regulating the TEs chemical distribution in AD, which was also proved by the conclusion addressed in the research on heavy metals distribution during AD of high solid sewage sludge (Dong, et al. 2013). Consequently, in view of the obvious influence of redox potential on the TEs chemical distribution pattern, it is highly suggested that the anaerobic environment with low redox potential and short sample preparation time should be adopted when study the TEs chemical speciation of the samples from AD process.

4. Conclusions

The chemical distribution patterns of Fe, Ni and Co was not influenced by the amount of exogenous TEs dosage, but by the pH, redox potential variation and water content. The pH of 6.5 showed negative effect on the recovery of high proportion of reducible TEs fraction lead by the possible redox potential shock. It was speculated that the redox potential exerted great influence on the chemical fractionating of Fe, Ni and Co, which resulted in the sharp increase of reducible TEs and decrease of oxidizable TEs in the first few days of cultivation. The research on the TEs chemical speciation was suggested to be carried out under anaerobic environment within short preparation time to ensure its native low redox potential.

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Table 1 The sequential extraction schemes of the BCR and modified Tessier's method

BCR method		Modified Tessier's method	
Acid extractable	HOAc (0.11 mol/L)	Water-soluble	distilled water
		Ion-exchangable	$MgCl_2$(1 mol/L, pH 7)
		Carbonate	NaAc (1 mol/L, pH 5)
		Weak organic combination (humic acid)	$Na_4PO_7 \cdot 10H_2O$ (0.1 mol/L, pH 10)
Reducible	$NH_2OH \cdot HCl\text{-}HNO_3$ (0.5 mol/, pH 1.5)	Fe-Mn Oxidates	$NH_2OH \cdot HCl$ -HCl (0.25 mol/L, pH 1.5)
Oxidizable	H_2O_2 (15%)	Strong organic combination	NH_4OAC (3.2 mol/L) +HNO_3+H_2O_2
Residual	HNO_3+HF+$HClO_4$	Residual	HNO_3+HF+$HClO_4$

Table 2 The basic characteristics of the three digestates (wet weight (ww) basis)

	digestate source		
items	maize stalk	pig slurry	waste water of chocolate factory
pH	8.16	8.46	7.20
TS, g/kg	147.8	113.1	21.23
VS, g/kg	56.79	69.20	10.68
COD, g/kg	52.95	72.49	9.38
VFAs, mg/kg	1282	1766	unfound
TP, mg/kg	132.4	117.6	2.12
TN, mg/kg	2600	3083	141.3
S, mg/kg	1274	1231	139.5
TA, g $CaCO_3$/kg	24.70	26.40	12.31
PA, g $CaCO_3$/kg	18.81	19.63	10.32
IA, g $CaCO_3$/kg	5.89	6.76	1.99
IA/PA	0.31	0.34	0.19
Total Fe, mg/kg	673.7	652.8	652.7
Total Ni, mg/kg	2.242	1.947	0.946
Total Co, mg/kg	0.672	0.513	0.119

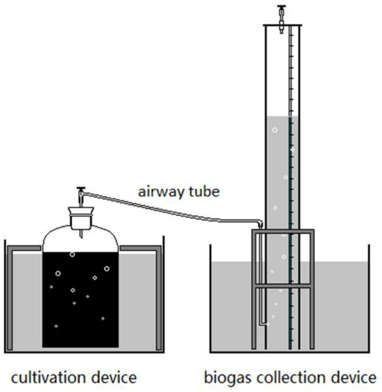
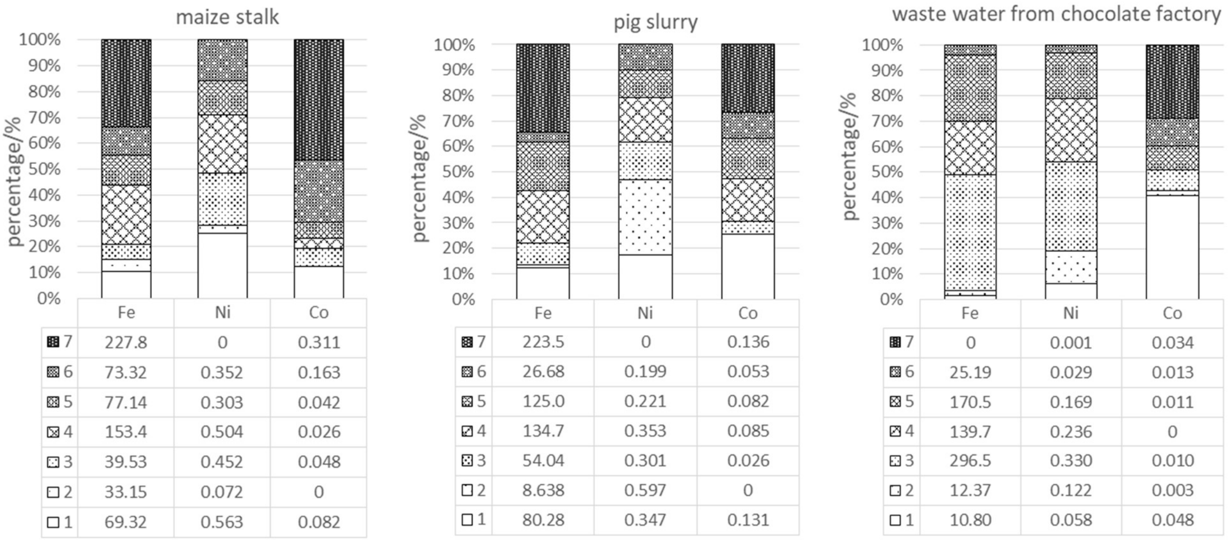


Figure 1 The device for digestate cultivation and biogas collection



maize stalk

	Fe	Ni	Co
▩ 7	227.8	0	0.311
▩ 6	73.32	0.352	0.163
▩ 5	77.14	0.303	0.042
▩ 4	153.4	0.504	0.026
▨ 3	39.53	0.452	0.048
☐ 2	33.15	0.072	0
☐ 1	69.32	0.563	0.082

pig slurry

	Fe	Ni	Co
▩ 7	223.5	0	0.136
▩ 6	26.68	0.199	0.053
▩ 5	125.0	0.221	0.082
▩ 4	134.7	0.353	0.085
▨ 3	54.04	0.301	0.026
☐ 2	8.638	0.597	0
☐ 1	80.28	0.347	0.131

waste water from chocolate factory

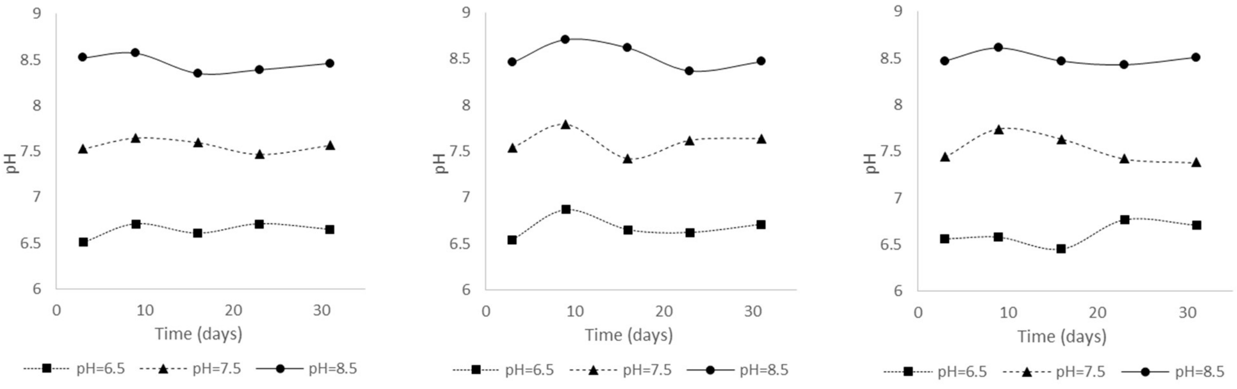
	Fe	Ni	Co
▩ 7	0	0.001	0.034
▩ 6	25.19	0.029	0.013
▩ 5	170.5	0.169	0.011
▩ 4	139.7	0.236	0
▨ 3	296.5	0.330	0.010
☐ 2	12.37	0.122	0.003
☐ 1	10.80	0.058	0.048

Figure 2 The chemical forms distribution of Fe, Ni and Co in the three digestates (mg/kg ww)

Notes: 1: water-soluble fraction; 2: ion-exchangable fraction;

3: carbonate fraction; 4: weak organic combination fraction;

5: Fe-Mn oxides fraction; 6: strong organic combination fraction; 7: residue fraction.



Maize stalk Pig slurry Waste water of chocolate factory

Figure 3 The pH variations of the three digestates with artificial pH adjustments at beginning

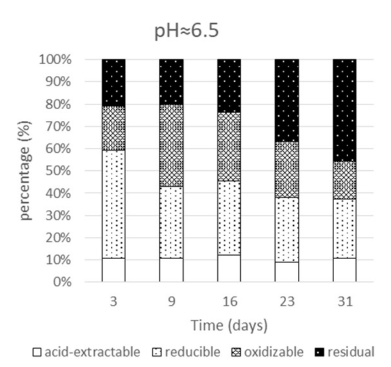
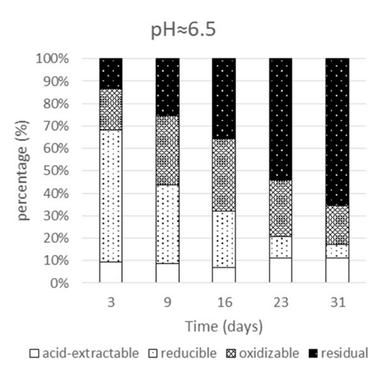
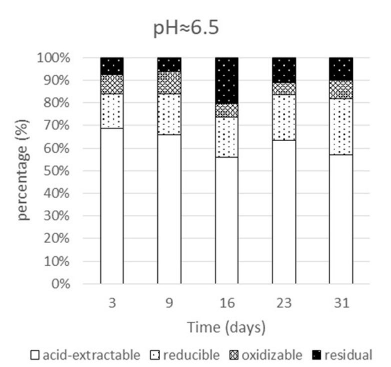
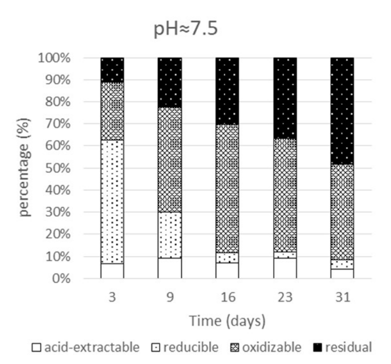
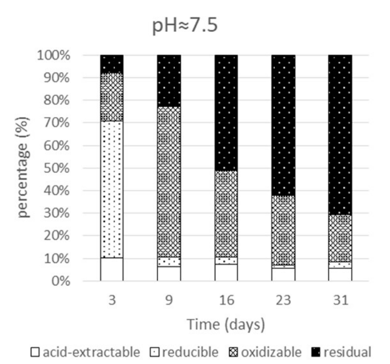
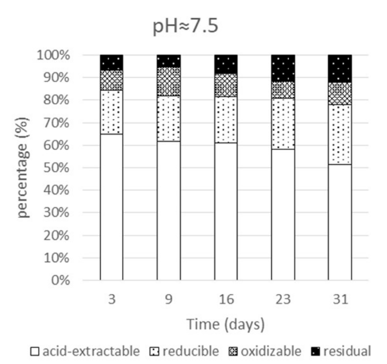
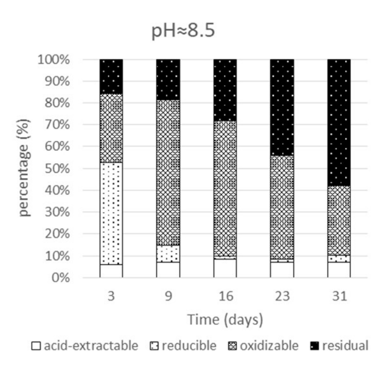
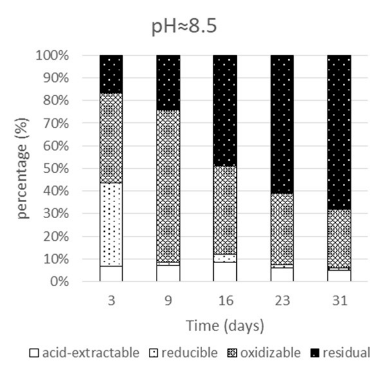
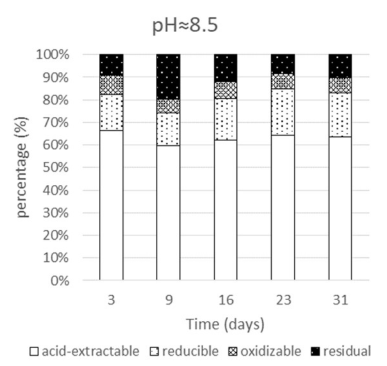
	Maize stalk	Pig slurry	Waste water of chocolate factory
pH≈6.5			
pH≈7.5			
pH≈8.5			

Figure 4 The chemical fraction proportions of endogenous Fe in the three digestate under different pH

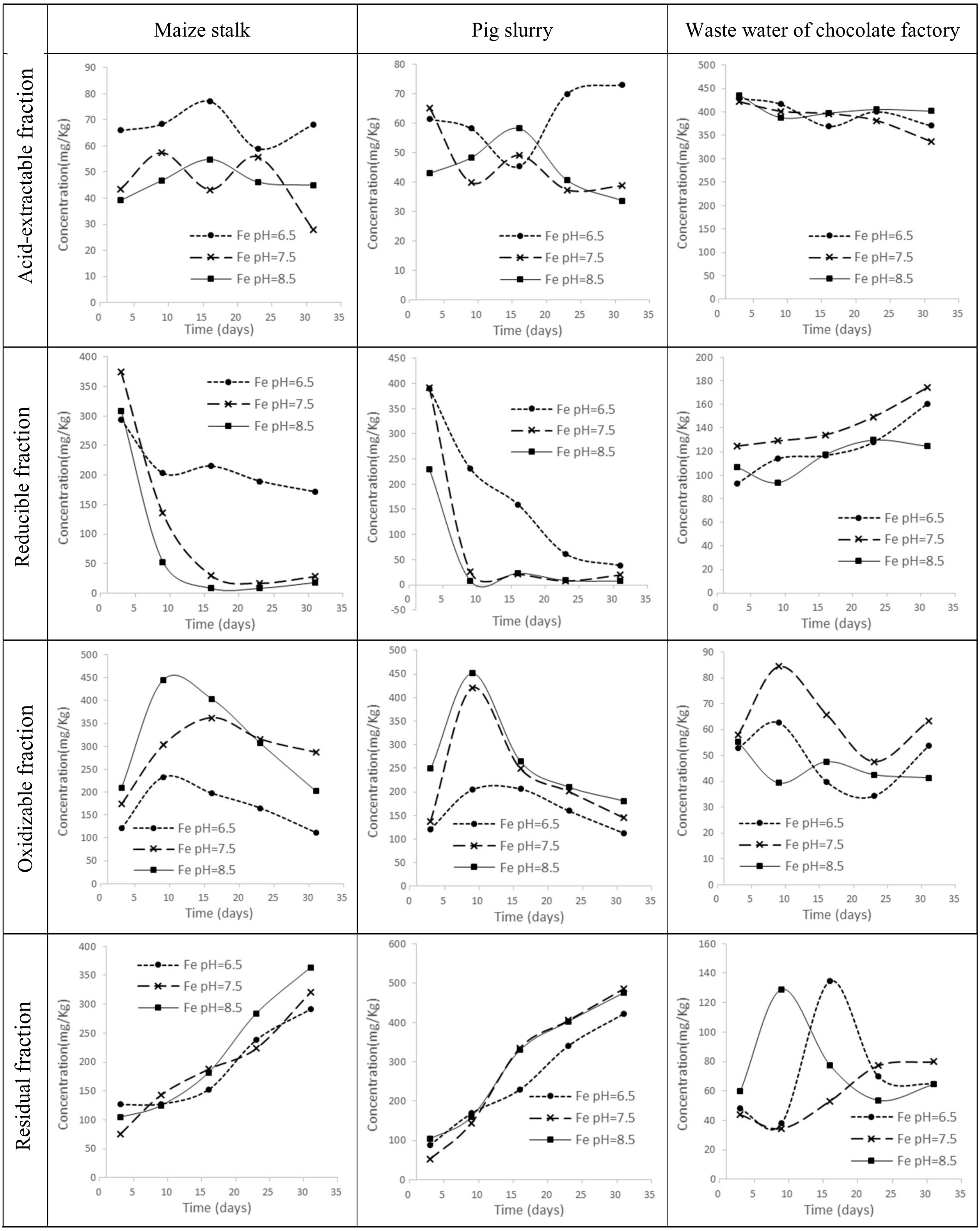
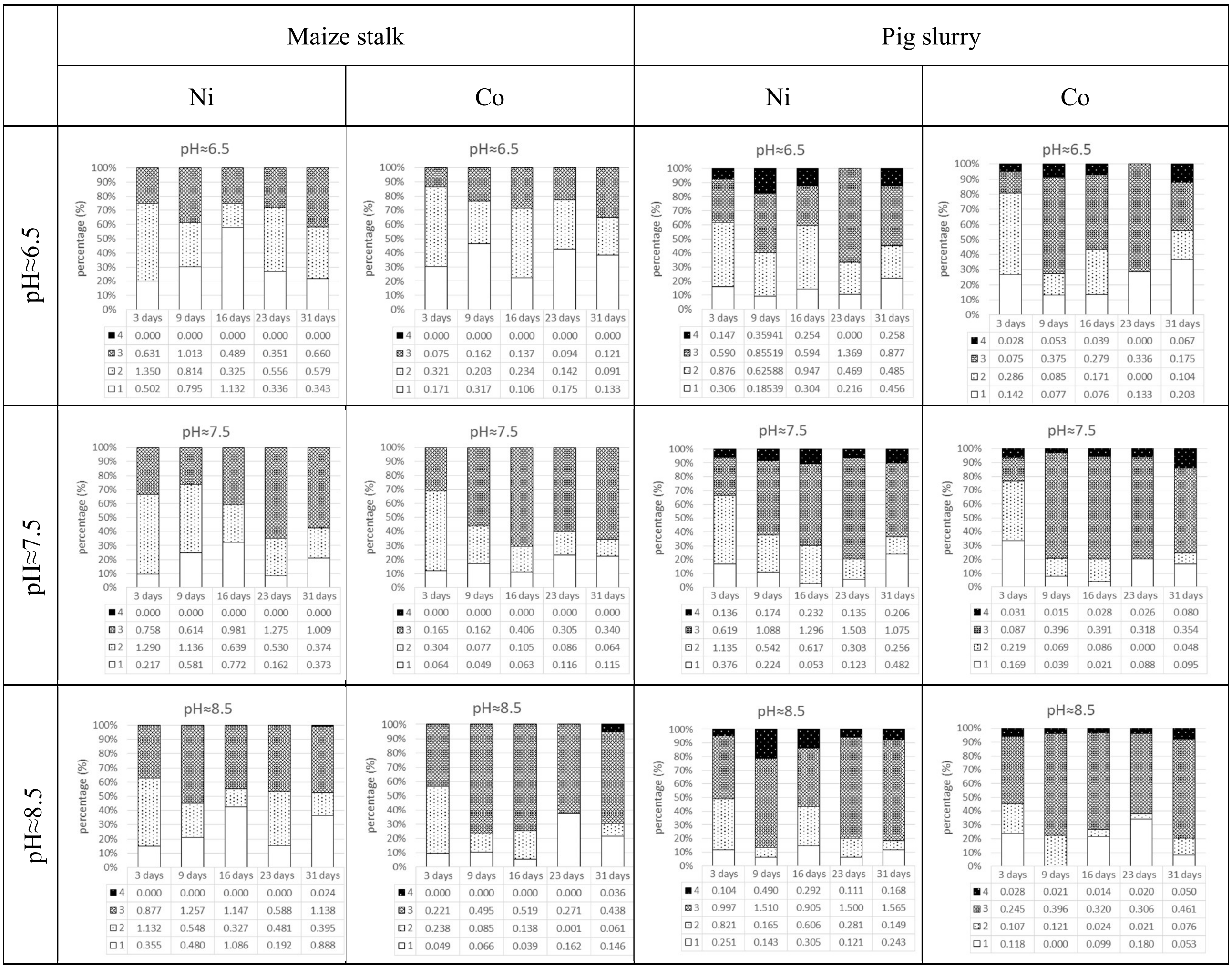


Figure 5 The chemical form distrition dynamics of endogenous Fe in the three digestate under different pH



	3 days	9 days	16 days	23 days	31 days
■ 4	0.000	0.000	0.000	0.000	0.000
▨ 3	0.631	1.013	0.489	0.351	0.660
⊡ 2	1.350	0.814	0.325	0.556	0.579
□ 1	0.502	0.795	1.132	0.336	0.343

	3 days	9 days	16 days	23 days	31 days
■ 4	0.000	0.000	0.000	0.000	0.000
▨ 3	0.075	0.162	0.137	0.094	0.121
⊡ 2	0.321	0.203	0.234	0.142	0.091
□ 1	0.171	0.317	0.106	0.175	0.133

	3 days	9 days	16 days	23 days	31 days
■ 4	0.147	0.35941	0.254	0.000	0.258
▨ 3	0.590	0.85519	0.594	1.369	0.877
⊡ 2	0.876	0.62588	0.947	0.469	0.485
□ 1	0.306	0.18539	0.304	0.216	0.456

	3 days	9 days	16 days	23 days	31 days
■ 4	0.028	0.053	0.039	0.000	0.067
▨ 3	0.075	0.375	0.279	0.336	0.175
⊡ 2	0.286	0.085	0.171	0.000	0.104
□ 1	0.142	0.077	0.076	0.133	0.203

	3 days	9 days	16 days	23 days	31 days
■ 4	0.000	0.000	0.000	0.000	0.000
▨ 3	0.758	0.614	0.981	1.275	1.009
⊡ 2	1.290	1.136	0.639	0.530	0.374
□ 1	0.217	0.581	0.772	0.162	0.373

	3 days	9 days	16 days	23 days	31 days
■ 4	0.000	0.000	0.000	0.000	0.000
▨ 3	0.165	0.162	0.406	0.305	0.340
⊡ 2	0.304	0.077	0.105	0.086	0.064
□ 1	0.064	0.049	0.063	0.116	0.115

	3 days	9 days	16 days	23 days	31 days
■ 4	0.136	0.174	0.232	0.135	0.206
▨ 3	0.619	1.088	1.296	1.503	1.075
⊡ 2	1.135	0.542	0.617	0.303	0.256
□ 1	0.376	0.224	0.053	0.123	0.482

	3 days	9 days	16 days	23 days	31 days
■ 4	0.031	0.015	0.028	0.026	0.080
▨ 3	0.087	0.396	0.391	0.318	0.354
⊡ 2	0.219	0.069	0.086	0.000	0.048
□ 1	0.169	0.039	0.021	0.088	0.095

	3 days	9 days	16 days	23 days	31 days
■ 4	0.000	0.000	0.000	0.000	0.024
▨ 3	0.877	1.257	1.147	0.588	1.138
⊡ 2	1.132	0.548	0.327	0.481	0.395
□ 1	0.355	0.480	1.086	0.192	0.888

	3 days	9 days	16 days	23 days	31 days
■ 4	0.000	0.000	0.000	0.000	0.036
▨ 3	0.221	0.495	0.519	0.271	0.438
⊡ 2	0.238	0.085	0.138	0.001	0.061
□ 1	0.049	0.066	0.039	0.162	0.146

	3 days	9 days	16 days	23 days	31 days
■ 4	0.104	0.490	0.292	0.111	0.168
▨ 3	0.997	1.510	0.905	1.500	1.565
⊡ 2	0.821	0.165	0.606	0.281	0.149
□ 1	0.251	0.143	0.305	0.121	0.243

	3 days	9 days	16 days	23 days	31 days
■ 4	0.028	0.021	0.014	0.020	0.050
▨ 3	0.245	0.396	0.320	0.306	0.461
⊡ 2	0.107	0.121	0.024	0.021	0.076
□ 1	0.118	0.000	0.099	0.180	0.053

Figure 6 The chemical fraction proportions of endogenous Ni and Co in the maize stalk and pig slurry digestate under different pH

Notes: 1: acid-extractable; 2: reducible; 3: oxidizable; 4: residual fractions

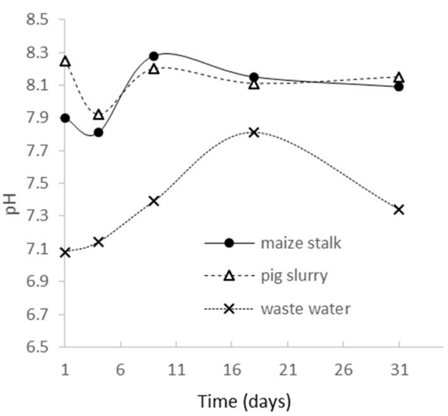


Figure 7 The variation of the average pH of the maize stalk, pig slurry and waste water digestate

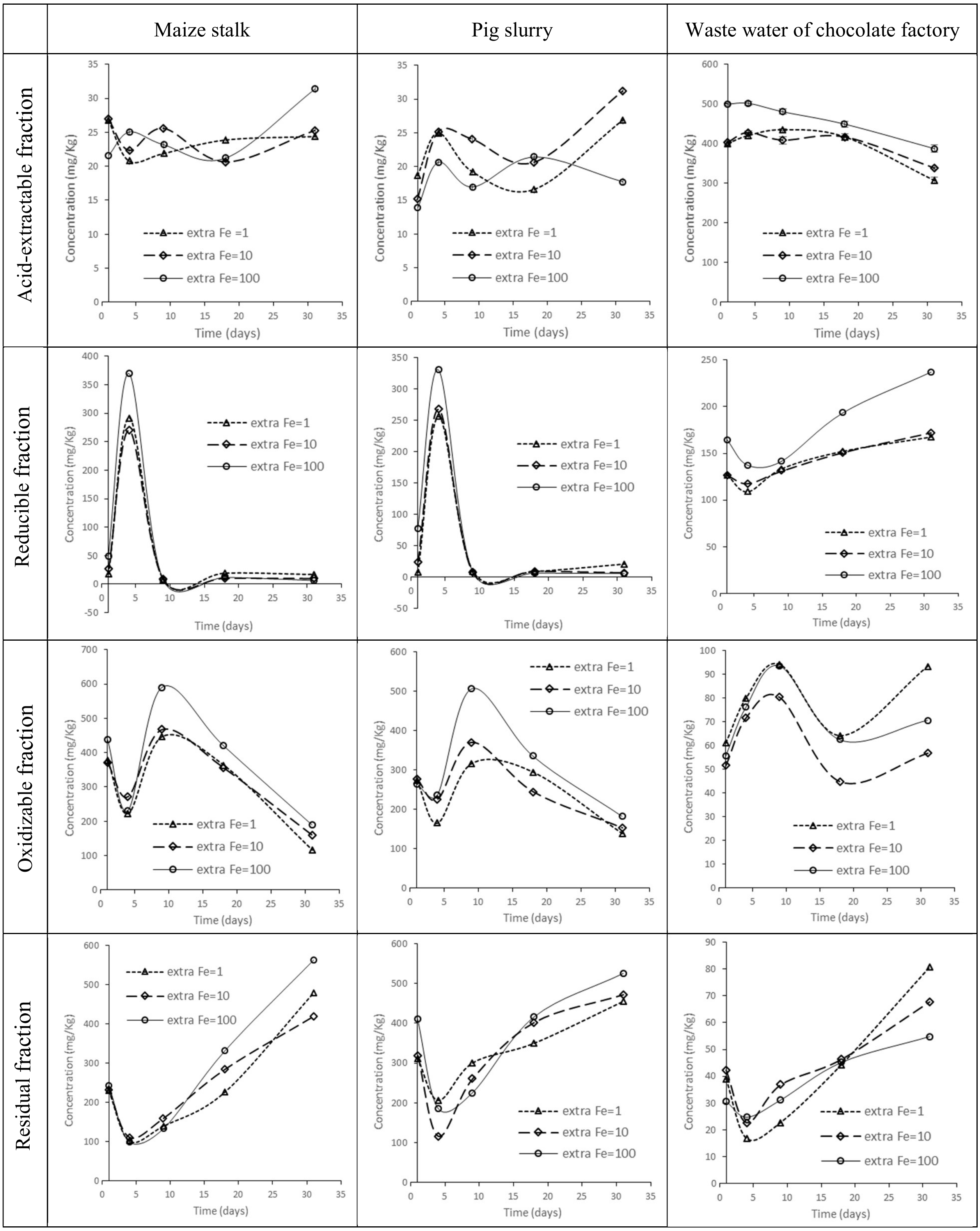


Figure 8 The chemical form distrition dynamics of exogenous Fe in the three digestate

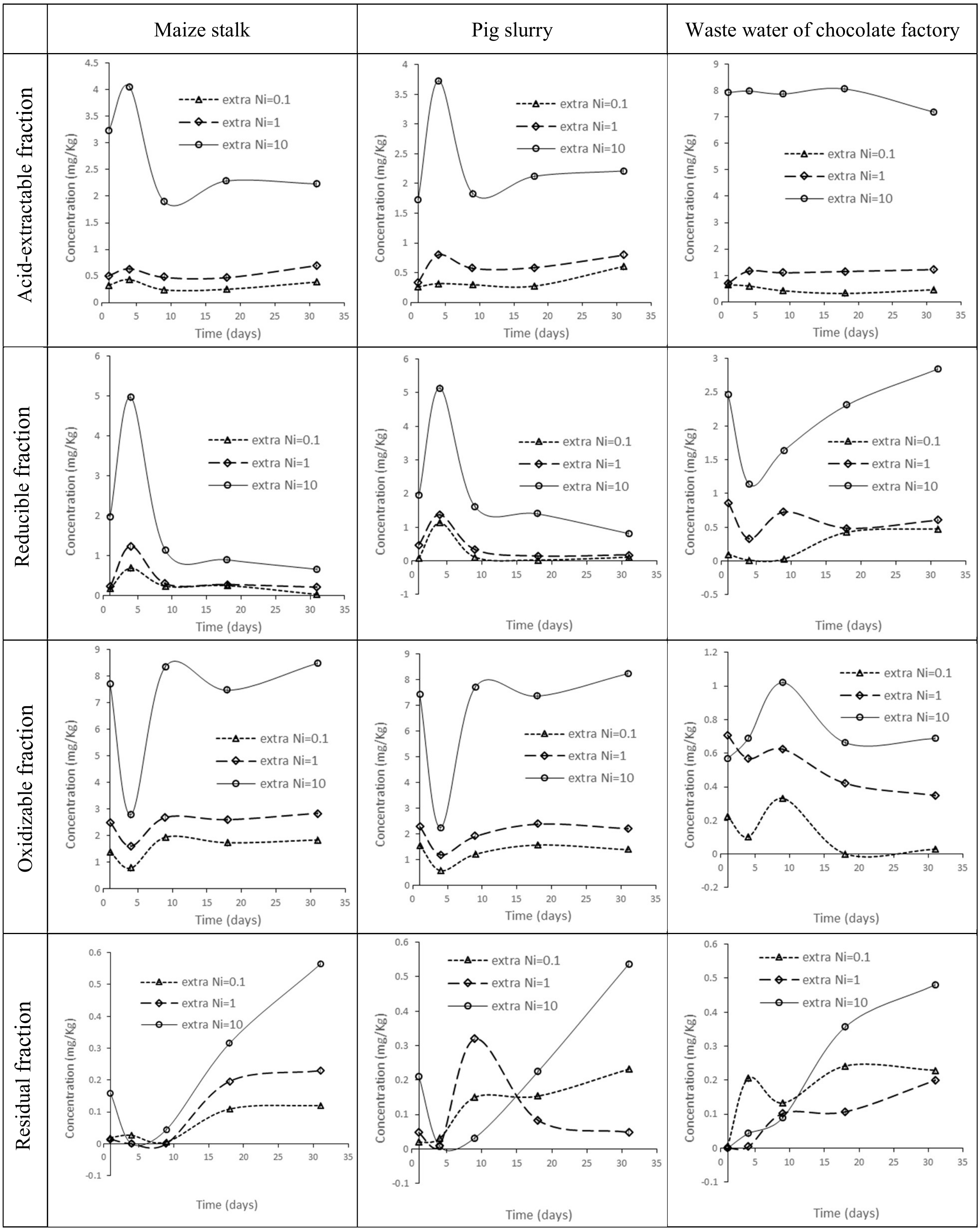


Figure 9 The chemical form distrition dynamics of exogenous Ni in the three digestate

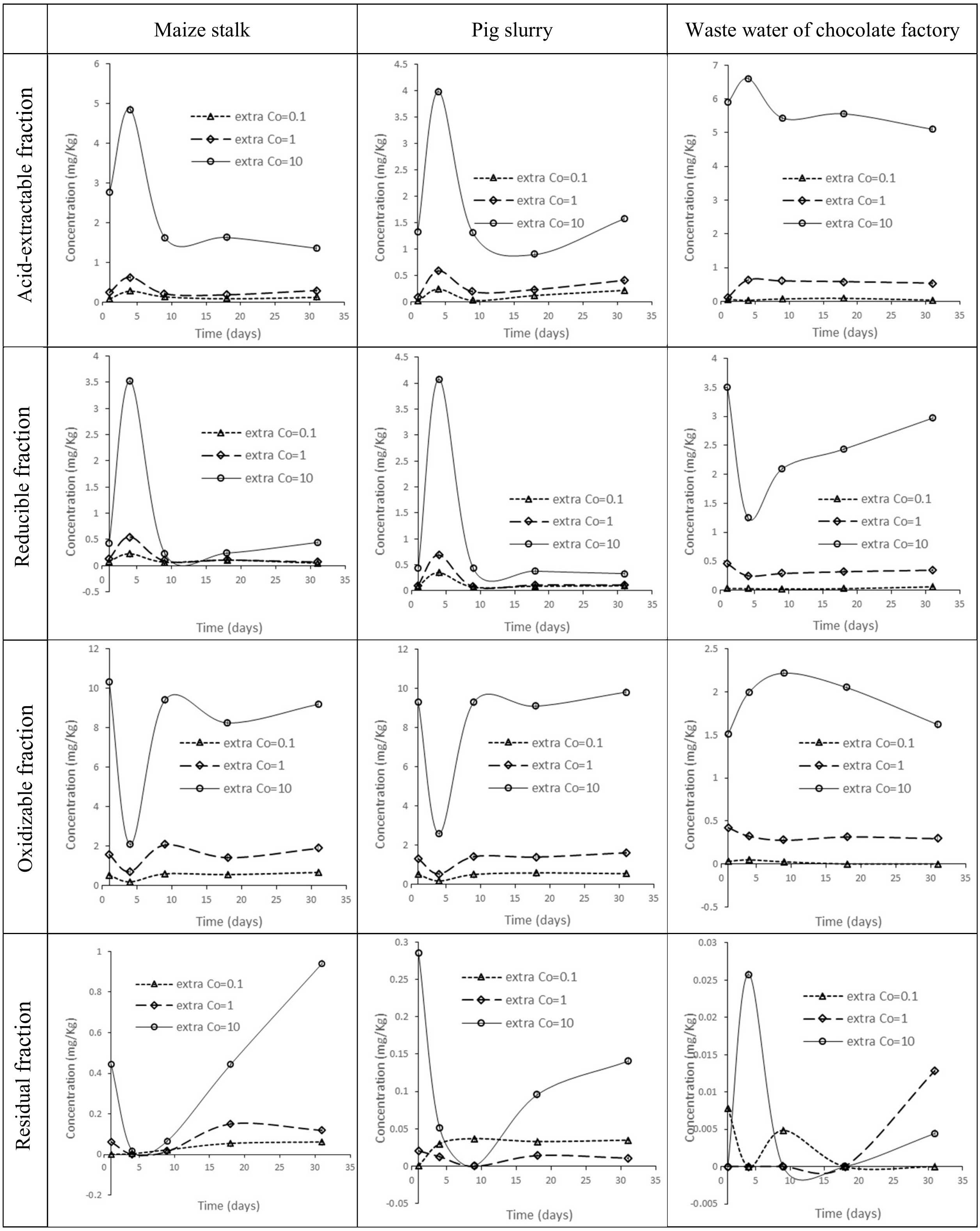


Figure 10 The chemical form distrition dynamics of exogenous Co in the three digestate