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Acidogenic digestion of organic municipal solid waste in a pilot scale reactor: Effect of waste ratio and leachate recirculation and dilution on hydrolysis and medium chain fatty acid production

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ABSTRACT

The purpose of this investigation is to study the effect of 1) leachate recirculation (LR) and dilution (LD), 2) the increment of the waste ratio, and 3) co-digestion using poultry waste as co-substrate for enhancing the acidogenesis process of the organic municipal solid waste (OMSW), thereby increasing the medium chain fatty acids (MCFAs) production. The results of the experiment carried in a batch pilot reactor show that LR increases hydrolysis as well as the OMSW conversion efficiency. Meanwhile, the highest total MCFA production of around 62,000 mg/L is shown for LR with a high OMSW ratio. Thus, a high concentration of hexanoic acid is shown in LR lysimeters (5475 mg/L, 6627 mg/L, and 10,889 mg/L respectively). However, a metabolic shift toward the production of heptanoic and octanoic acids is reported for LD samples. Nonetheless, the use of poultry waste as co-substrate in the co-digestion process multiplied the concentration of the produced MCFAs.

1. Introduction

The percentage of food and green waste in municipal solid waste (MSW) for low- and middle-income countries is very high, constituting 53 to 57%. In these countries, landfilling is the most used option to dispose of waste (Kaza et al., 2018). However, the high organic amount in the MSW leads to leachate generation during the landfilling process (Maguiri et al., 2017). A majority of landfills in low- and middle-income countries are not state-of-the-art, so there is a risk that leachate treatment measures are completely lacking (Kaza et al., 2018; Maguiri et al., 2017). If these polluted leachates are not treated properly, they can enter the soil and contaminate both soil and groundwater (Maguiri et al., 2017). One of the solutions to this problem is the implementation of treatment measures to reduce the organic load in the leachate. The use of leachate as feedstock in biorefining processes is a chance to produce valuable bio-based materials, such as carboxylic acids (including volatile fatty acids (VFA) or even medium chain fatty acids (MCFA)), from waste (Agler et al., 2011; Hussain et al., 2017; Kannengiesser et al., 2015). This possibility to gain valuable products from organic waste converges with the main goal of circular bioeconomy (CBE). CBE is defined as the enhanced recirculation of materials or, in this case, waste materials (especially organic materials) into the economic cycle (Stegmann et al., 2020). Therefore, CBE aims to generate products of equal or, in terms of upcycling, even higher value from organic waste. The main goal of CBE is to achieve a sustainable and resource-efficient valorization of biomass or, in this case, organic waste materials (Stegmann et al., 2020). As mentioned before, the recovery of MCFAs from leachate is one of many ways to realize a CBE.

MCFAs are essential intermediate products in the hydrolysis and acidogenesis steps when organic materials are degraded under anaerobic conditions (Hussain et al., 2017). In the last decades, those acids gained a lot of interest due to the fact that they are extremely useful in the chemical industry; carboxylic acids are precursors of concentrated chemicals and derivatives (esters, ketones, aldehydes, alcohols, and alkanes) in conventional organic chemistry (Dahiya et al., 2015). However, the anaerobic digestion process has been studied for the purpose of

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acid production using different feedstocks: food waste (Hussain et al., 2017; Luo et al., 2019), agricultural waste and lignocellulosic biomass (Esteban-Gutiérrez et al., 2018; Scarborough et al., 2018) and OMSW (Garcia-Aguirre et al., 2017; Jankowska et al., 2018), using different types of reactors. Moreover, many parameters have been studied for the purpose of enhancing their production. The MCFA production process is strongly dependent on the microorganisms' activities (Agler et al., 2011), which can be improved through several parameters, including organic loading rate (Moretto et al., 2019), using different electron donors (e.g. ethanol and methanol) (Saadoun et al., 2020), co-digestion with other wastes (Jankowska et al., 2018), and feeding patterns. Nonetheless, the most relevant factor that influences MCFAs production is the pH. Different studies identified an increment of the production yields near the neutral phase (Jiang et al., 2013). Huang et al. (2016) showed high acid yields from swine manure at slightly alkaline pH values (8–10) for a short hydrolysis retention time. Thus, pH is an influencing factor that induces acidification and inhibits methanogens for the selective production of acids (Garcia-Aguirre et al., 2017). Along with pH, the temperature is a key parameter for acidogenic digestion, due to its direct involvement in both microbial growth and metabolism (Zhou et al., 2017). Jiang et al. (2013) reported maximum acid yield from food waste at lower temperatures (35 °C–45 °C), demonstrating low solubilization and high acidogenesis, with a sharp decrease at high-temperature conditions (>55 °C). Therefore, temperature impacts the microbial population, leading to selective acid formation depending on the type of waste (Bhatt et al., 2020). Also, the hydrolysis retention time and the organic loading rate have an impact on MCFA production. However, depending on the type of waste, the hydrolysis retention time should be adjusted to promote acidification, because hydrolysis and acidogenesis are known to be the rate-limiting step in the digestion of particulate organics like food waste and restrict methanogen activity for distributed acid production (Hussain et al., 2017; Tałałaj et al., 2019). Although, for enhancing acids production, hydrolysis and acidification must be accelerated (Hussain et al., 2017).

Leachate recirculation (Calabrò et al., 2018; Luo & Wong, 2019) and dilution (Kullavanijaya & Chavalparit, 2019) are in-situ cost-effective approaches for enhancing hydrolysis and acidogenesis (Hussain et al., 2017). The recirculation of leachate is a widely used technique applied in landfills for accelerating the decomposition rate of OMSW (Patil et al., 2016). Further, co-digestion is another parameter that has proved its positive impact on improving acid production. Meanwhile, abundant literature about the utilization of co-digestion exists, such as co-digestion of OMSW and agricultural residues (Converti et al., 1997; Kübler et al., 2000), organic solid wastes and sewage sludge (Edelmann et al., 2000), and more specific wastes (Rabii et al., 2019). For enhancing MCFAs production, inhibition of methanogenesis is required, which for example can be achieved with residues with a high ammonia content, such as poultry waste (Plácido & Zhang, 2018). However, besides poultry waste, substrates with high lipid content can also inhibit methanogenesis (Fernández et al., 2005; Plácido & Zhang, 2018). Nonetheless, this type of waste has not been evaluated in the context of MCFA production. Only one study (Plácido & Zhang, 2018) studied and proved VFA production from slaughterhouse waste but by adding an enzymatic pre-treatment and additives.

Based on the lack of studies on the use of poultry waste as co-substrate for MCFA production as well as the effect of leachate recirculation and dilution on MCFAs production, several questions arise:

1) What are the effects of the OMSW ratio and co-digestion using poultry waste as co-substrate on MCFA production?
2) How does leachate recirculation or dilution influence MCFA production on a pilot scale reactor?

Subsequently, the scope of this study is 1) to investigate the impact of the ratio of OMSW and the use of poultry waste as co-substrate on MCFAs production and 2) to evaluate the effect of leachate recirculation and dilution on the yield and spectrum of MCFAs productivity.

2. Material and methods

2.1. Pilot reactor description

Six similar pilot reactors were assembled for the assay. The pilot reactors were made up of high-density polyethylene (HDPE). Due to the high density, the reactors were very resistant to acids, alkalis, and other chemicals. The dimensions of the reactors were 180 cm in height and 44 cm in diameter with an approximate total volume of 120 L (Fig. 1). The pilot reactors were made following the lysimeter concept based on the experiment duration and the sizes of the samples. At the bottom of the lysimeters, a layer of gravel was fitted to allow a better flow of the leachate into the valve for collection. The lysimeters had a device for a gas collecting system. Furthermore, for the system of recirculation (Fig. 1a) or dilution through water addition (Fig. 1b), a device to recirculate the leachate or add water consisted of a stainless steel tube at the upper end, outside the lysimeters. The six lysimeters were filled with a different ratio of OMSW (Table A.1).

2.2. Operational condition of the lysimeters

Two irrigation systems were conducted for the six lysimeters (lysimeters 1 to 6). For lysimeters 1, 3, and 5, leachate dilution (LD) and for lysimeters 2, 4, and 6, leachate recirculation (LR) was applied as an irrigation system. The six lysimeters were filled with a layer of gravel (13 cm), and waste was put on this layer. The lysimeters were in a temperature-controlled room during the experiment, and the incubation temperatures were above 30 °C. The lysimeters performed for one year under uncontrolled pH. For lysimeters 2, 4, and 6, the leachate was recirculated at a rate of 1 l per day and for lysimeters 1, 3, and 5, 3.6 l of water was injected per month. Table A.1 presents the detailed experimental conditions and the used waste compositions for the six lysimeters. At regular intervals, the leachate samples were collected and analyzed regarding pH, electrical conductivity (EC), chemical oxygen demand (COD), MCFAs, alcohols, and ammonium nitrogen.

2.3. Pilot feeding

The six lysimeters were filled with different MSW compositions from a controlled landfill in Marrakech, Morocco (see Table A.1) without any pretreatment. The lysimeter composition included total volatile solids (TVS) of 13 ± 2%; 20 ± 3% and 25 ± 2% for lysimeters 1 and 2; lysimeters 3 and 4; and lysimeters 5 and 6 respectively.

2.4. Analytical methods

The TVS, COD, and ammonium analyses were performed according to Standard Methods (APHA, 2005; Rodier, 2009). The pH and electrical conductivity (EC) were measured using multi-parameter HANNA, while the MCFAs and alcohols (the sum of methanol and ethanol production) were measured on an Agilent Gas chromatograph equipped with a flame ionization detector (FID) as described already in Saadoun et al. (2020).

2.5. Performance indicators

The lysimeters' performance was assessed using some indicators, such as dissolved organic matter in the leachate, based on the soluble chemical oxygen demand (sCOD) and the conversion efficiency. According to Hussain et al. (2017), the latter was determined by calculating two essential parameters: hydrolysis (Eq. (1)) and acidification yield (Eq. (2)).

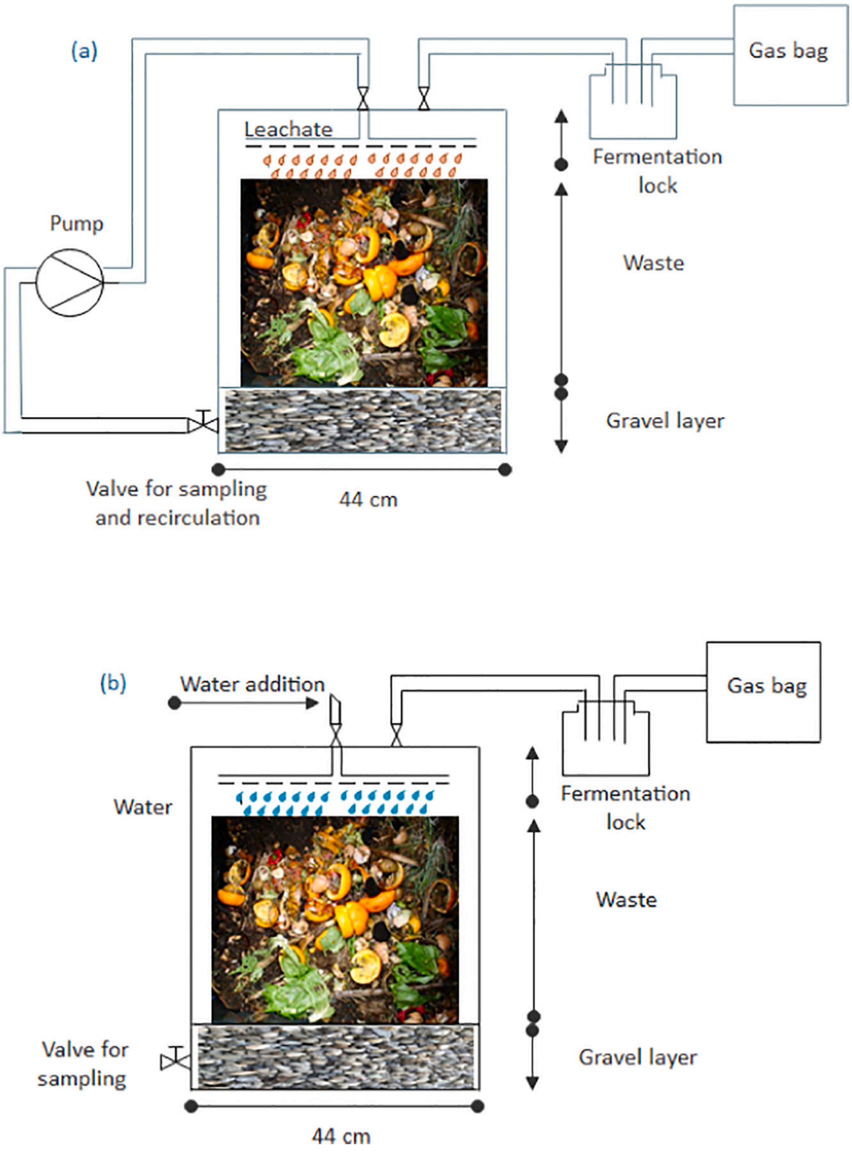

Fig. 1. Pilot reactors with leachate recirculation (LR) (a) and leachate dilution (LD) (b).

$$(\text{g cum.sCOD/kg TVSadded})\text{Hydrolysis yield} = \frac{\text{cumalative soluble COD}}{\text{TVS}} \tag{1}$$

$$\left(\text{g}\frac{\text{COD}}{\text{kg}}\text{TVSadded}\right)\text{Acidification yield} = \frac{\text{concentration of all acid products}}{\text{TVS}} \tag{2}$$

2.6. Statistical analysis

For the statistical analysis, R software package from FactoMineR was used to check the differences between LR and LD in MCFAs production. Also, to visualize the effect of the OMSW ratio on the distribution of MCFAs, the data were subjected to principal component analysis (PCA). Then, Pearson linear correlation was applied to show the correlations between several parameters for both systems (LR and LD).

3. Results and discussion

The results of the experiment are described and discussed in this chapter. First, the evaluation of the process performance was assessed through hydrolysis and conversion efficiency results (see Section 3.1). Then, the total production of MCFAs and alcohols during the experimental period was presented (see Section 3.2). Next, the spectrum of MCFAs was explained according to the system used (LR and LD) and the effect of co-digestion (see Section 3.3). The last part of the results was devoted to the statistical analysis (see Section 3.4).

3.1. Hydrolysis and conversion efficiency during process performance

3.1.1. Effect of time and pH evolution

Hydrolysis and acidification of OMSW in a lysimeter were expressed by the changes in the conversion indicators. The results of conversion indicators of the OMSW are shown in Fig. 2. The hydrolysis and acidification yields took into account the sCOD produced and all acid products respectively in the TVS added to the reactor initially. The indicators' values vary between the lysimeters based on the pH evolution during the process. Although the most promising process conditions for acids formed during the OMSW digestion occurred in the second semester of the process, when the pH was around 5 and 5.5, the hydraulic retention time was the longest (Fig. 2), and the acidification yield (Ya) was the highest (above 200 gCOD/kgTVS$_{\text{added}}$).

For the six lysimeters, the hydrolysis yield (Yh) increased at the start

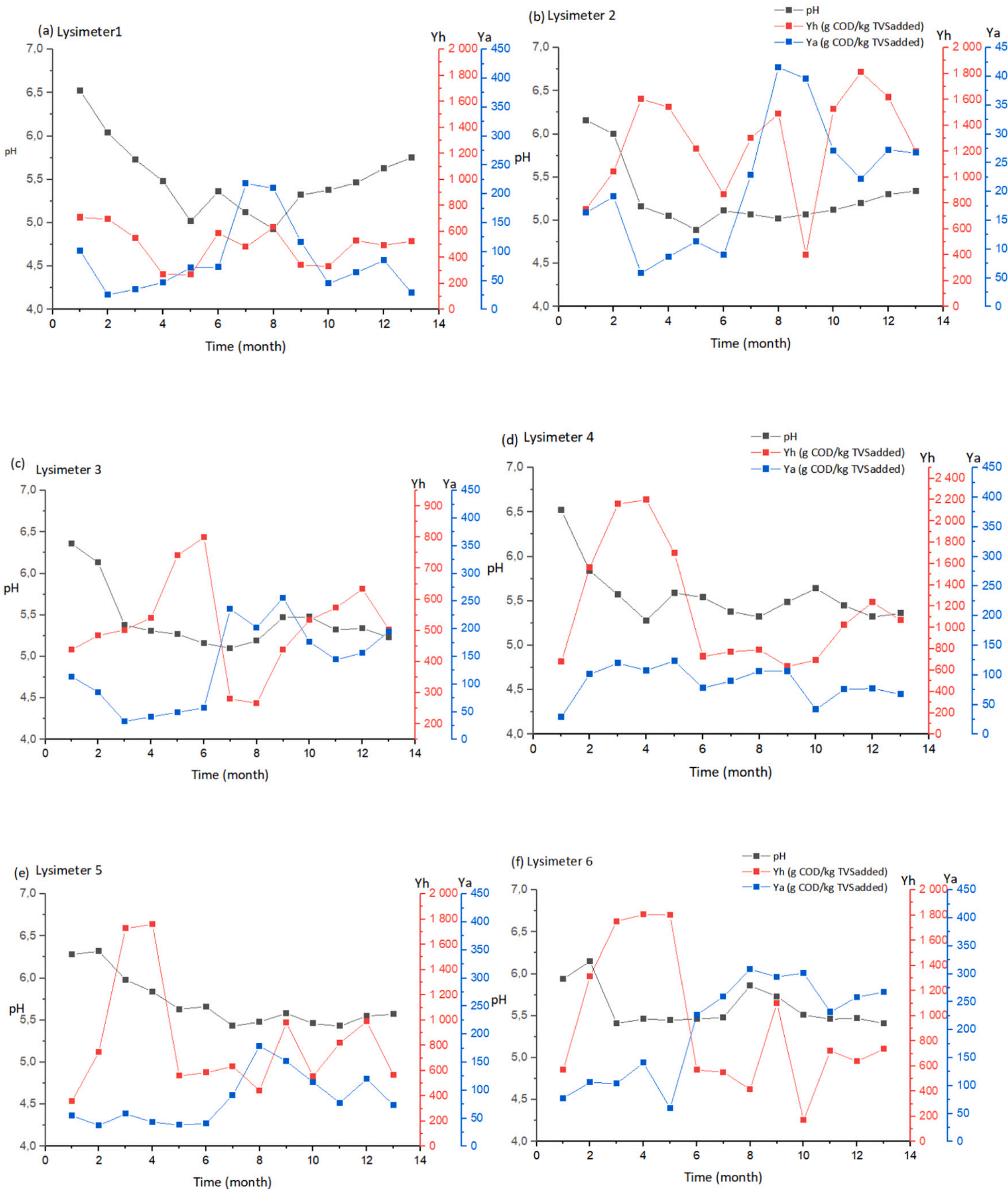

Fig. 2. Hydrolysis (Yh) and acidogenesis (Ya) yields variation with pH evolution during one year acidogenesis process for the six lysimeters under analysis.

of the experiment, and it was higher than the Ya, while the pH was more than 6 (Fig. 2). Then, for lysimeter 1 (Fig. 2a), the Yh started decreasing, while the pH dropped; however, the acidification increased slightly. After six months, the Yh showed an increment that corresponded with the fall of the pH to 5. Additionally, the acidification showed the highest yield value of more than 200 gCOD/kgTVS$_{added}$. Meanwhile, for lysimeter 3 (Fig. 2c), an inversely proportional correlation between hydrolysis and acidification was detected. When a high Yh appeared (around 800 gCOD/kgTVS$_{added}$) after four months of running the experiment, the lowest Ya resulted in a decrement of the pH from 6.25 to 5.5. Nonetheless, after six months, the highest Ya appeared when the lowest Yh was shown. For lysimeter 5 (Fig. 2e), the uppermost Yh showed up in a small hydraulic retention time while the pH was decreasing from 6.4 to 5.8. In the meantime, the Ya was at its lowest level. After six months, Ya started increasing, while the pH was slightly stable at 5.5. For lysimeter 2 (Fig. 2b), a high hydrolysis performance was approximately shown during the whole experiment, while the pH dropped from 6.25 to 5. However, the acidification yield reached the highest value after six months, while the pH was around 5. A high hydrolysis yield appeared in lysimeter 4 (Fig. 2d) from the start of the experiment, while the pH dropped from 6.5 to 5.25. In the meantime, the acidification performance started increasing from the second month and maintained the same level during the whole experiment. For lysimeter 6 (Fig. 2f), the hydrolysis yield showed a high increment during the five first months, while pH dropped from 6.10 to 5.5. However, considerable increment of acidification was shown after the fifth month of running the experiment.

In general, the results of Fig. 2 demonstrated a clear dependency of OMSW solubilization on time and pH evolution. A high hydrolysis performance appeared at the first semester of the experiment and when the pH was between 6.5 and 5.5, which is in agreement with several authors' findings. Hussain et al. (2017) found an improved hydrolysis performance with an increase in leachate pH from acidic to neutral. Also, Browne et al. (2013) and Cysneiros et al. (2012) found an increment of hydrolysis performance of 20 and 35% respectively by raising the pH to 6.5 and 7.5. These improvements in hydrolysis performance can be attributed to the activity of hydrolytic microorganisms toward a high pH value in terms of enhancing the conversion of complex insoluble organic particulates to simpler monomers (Xu et al., 2014).

However, regarding acidification, a later occurrence was noticed during the process, especially when hydrolysis yield decreased. Therefore, our results agree with the findings of Jankowska et al. (2018) that an increment in hydrolysis yield was accompanied by a decrement in acidification yield, which can be explained by the large production of acids, ranging from acetic to octanoic acids. However, any disturbance in the acidification process resulted in an accumulation of the hydrolysis products. Consequently, the results indicate the influence of pH evolution and increase in the hydraulic retention time with regards to enhancing hydrolysis and the conversion of OMSW to acids, thus offering an improvement of MCFAs production.

3.1.2. *Effect of leachate recirculation and dilution*

Hydrolysis and acidogenesis are recognized as the rate-limiting step in the degradation of particulate organic waste (Dogan et al., 2009). Several strategies to improve the solubilization of solid waste have been reported in the literature. LR is one of the critical operational parameters affecting the degradation of organic waste (Hussain et al., 2017). In our results (Fig. 2), lysimeters 2, 4, and 6, working with the LR system, showed a higher hydrolysis and acidification yield than the LD operating lysimeters 1, 3, and 5. These enhancements in the hydrolysis and acidogenesis performance can be attributed to better distribution of hydrolytic enzymes and microorganisms population and better access to organic waste, which improves the hydrolytic performance and acid production (Browne et al., 2013; Xu et al., 2014). Our results related to the positive effect of LR on hydrolysis and acidogenesis confirmed the findings of other authors (Hussain et al., 2017; Luo & Wong, 2019). Hussain et al. (2017) found out that recirculation of leachate improved the hydrolytic performance of biowaste in a leach bed reactor. Also, Luo and Wong (Luo & Wong, 2019) achieved a higher hydrolysis-acidogenesis efficiency and lower energy loss in a leach bed reactor with higher leachate recirculation ratio.

3.2. *Effect on total production of MCFA and alcohols*

3.2.1. *Total MCFAs production*

The tMCFAs concentrations measured in mg/L for all lysimeters during the experimental period are shown in Fig. 3a. The tMCFAs concentrations reflected a clear dependency on hydraulic retention time and pH, like hydrolysis. However, our results (Fig. 2) showed a slight ability of pH self-regulation at acidic conditions between 5 and 5.5 and an increasing tMCFA concentration from the fifth month. Therefore, this result revealed an interesting outcome about the optimum pH value (around 5.5) for OMSW digestion, which is in line with findings from previous studies (Cheah et al., 2019; Jiang et al., 2013; Moretto et al., 2019; Valentino et al., 2018).

The six lysimeters showed the lowest tMCFAs concentrations at the start of the experiment. The tMCFAs concentration reflected some changes in terms of increasing and decreasing concentrations during the experimental period. The highest tMCFAs concentration was noticed in the second semester of the experiment for all lysimeters, whereas lysimeter 6 showed the highest tMCFAs concentration (62,000 mg/L). In general, we noticed that the hydraulic retention time had a positive impact on accumulating MCFAs. However, for some lysimeters, 1, 4, and 6, the tMCFAs concentration decreased by the end of the experiment period. For lysimeters 4 and 6, this evolution may be attributed to the use of the LR system, which inhibits the start of hydrolysis and acidogenesis due to the high tMCFAs concentration in the leachate (Jankowska et al., 2018); this can also be noticed in Fig. 2. But also, the accumulation of volatile fatty acids can affect the activity of some specific enzymes and bacteria, leading to the inhibition of hydrolysis and acidogenesis (Wang et al., 2009). For lysimeter 1, the washing out of the microorganisms responsible for hydrolysis and acidogenesis by the water dilution, according to Nordberg et al. (2007), could be a reason for the decrease. Moreover, the tMCFA concentrations of lysimeters 2, 4 and 6 (47,900 mg/L, 55,000 mg/L and 62,000 mg/L respectively) (Fig. 3a) had the highest values in comparison to lysimeters 1, 3 and 5 (19,000 mg/L, 21,000 mg/L and 40,000 mg/L respectively), which confirmed the result of the acidogenesis yield in Fig. 2. This finding could be attributed to the positive effect of LR, which also has been proven by Luo and Wong (2019) and Hussain et al. (2017). Therefore, the positive impact of LR can also be explained by the better distribution of acidogenic microorganisms, which allowed more accessibility to the soluble hydrolytic fragments, resulting in improved MCFA production (Zhang et al., 2007).

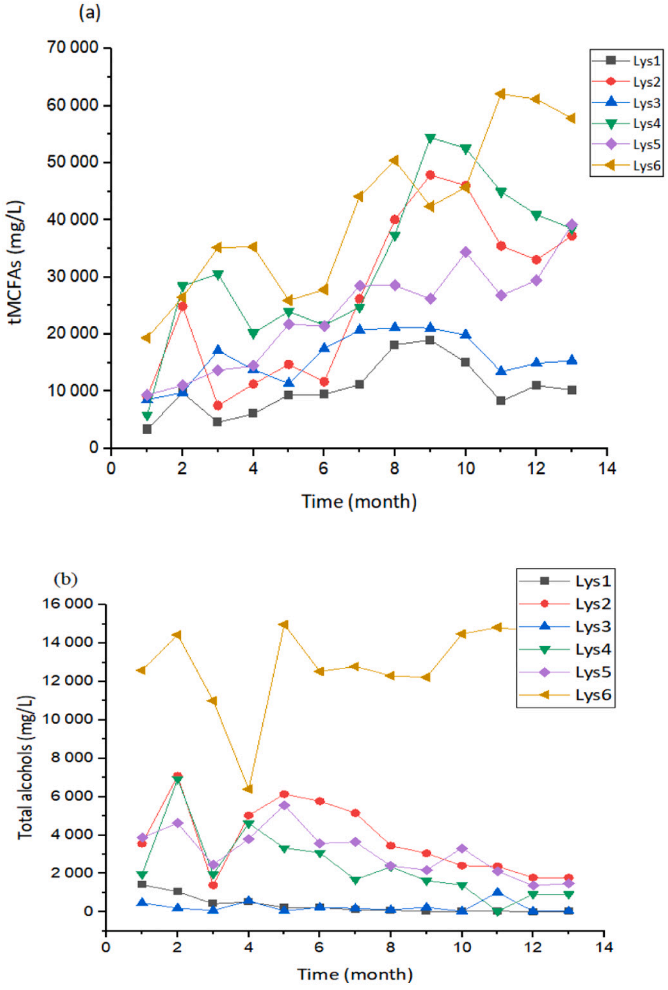

Fig. 3. The evolution of the total medium chain fatty acids (tMCFAs) (a) and total alcohols (b) production during the experimental period for the six lysimeters (1 to 6).

3.2.2. *Alcohol production*

In addition to the above metabolic products (tMCFAs) (Fig. 3a), the total alcohol production (the sum of the methanol and ethanol concentrations produced) of the leachate was examined. After acetic, butanoic, and propionic acid, the total alcohol was the fourth most dominant product in the lysimeter experiments. Fig. 3b showed the evolution of alcohol production in the six lysimeters during the whole experimental period. While the alcohol profile showed an increment in production for lysimeters 2, 4, 5, and 6 at the beginning of the experiment, it decreased for lysimeters 1 and 3 (1420 to 32 mg/L; 1020 to 32 mg/L respectively). The highest concentrations of alcohols (6140 mg/L, 3070 mg/L, 5600 mg/L, 15,000 mg/L for lysimeters 2, 4, 5 and 6 respectively) appeared in the second semester of the experimental period, similar to acidification (Fig. 2) and tMCFAs production (Fig. 3a). This shift in alcohol concentration could be explained by the positive effect of LR or the increment of the OMSW ratio. The main reason for this result could be attributed to the accumulation of short chain fatty acids, which could be used as a carbon source in the alcohol production pathways. Also, improved alcohol production with the increment of acidification activity was previously reported by Gheshlaghi et al. (2009). Dogan et al. (2009) and Hussain et al. (2017) found that an increase in alcoholic products coincided with increased butyric and acetic acid production. Furthermore, the improved concentration of alcohols could explain the launching of the chain elongation process and the apparition of longer carbon chain acids, such as hexanoic, heptanoic, and octanoic acids (Fig. 4), with interesting concentrations (Agler et al., 2012; Spirito et al., 2014).

3.3. *MCFAs distribution*

3.3.1. *Effect of recirculation and dilution on MCFAs distribution over time*

The MCFAs distribution profile for every lysimeter is shown in Fig. 4a. Acetic and butanoic acids were the most predominantly produced acids for the lysimeters. Acetic acid was the primary product formed; it had the highest concentration for most lysimeters (except for lysimeters 5 and 6) during the second semester of the experimental period. This was also confirmed by the result of the acidogenesis yield (Fig. 2) and tMCFAs concentration (Fig. 3). For lysimeters 5 and 6, butanoic acid was the foremost product. This qualitative profile can be explained by the characterization of the feedstock rather than the digestion conditions (Moretto et al., 2019). Meanwhile, propionic acid was the third most dominantly produced acid, but with lower concentrations (3145 mg/L; 4496 mg/L; 4575 mg/L; 7356 mg/L; 3738 mg/L and 5863 mg/L respectively for lysimeters 1, 2, 3, 4, 5 and 6). Our results are in line with previous works (Hussain et al., 2017; Jiang et al., 2013; Moretto et al., 2019) on acidogenesis of food waste, which indicated that a pH range of 4–5 supported acetic acid production, while a higher pH range of 6–7 resulted in a shift toward the generation of butanoic acid. Also, Valentino et al. (2018) found that butanoic acid was usually predominant (40–60%) in food waste fermentation, followed by acetic acid (25–40%). Pentanoic acid was produced from the start of the experiment, but high concentrations appeared at the fifth month of running the experiment. The highest concentration of pentanoic acid was noticed in lysimeter 4, which can be explained by the effect of feedstock composition and the recirculation of leachate. Hexanoic acid was detected in the six lysimeters but with big variability in terms of concentration. The hexanoic acid concentrations in all lysimeters were less than 1500 mg/L at the start of the experiment. From the sixth month, a high increment of the concentration was noticed in lysimeters 4, 5, and 6 (1434–5475 mg/L; 1825–6627 mg/L; and 4493–10,889 mg/L respectively), which can be attributed to the high ratio of organic fraction inside the lysimeters. This finding agrees with the results of Hussain et al. (2017) and Moretto et al. (2019), which showed a higher level of butanoic acid at acidic pH for food waste digestion. Nonetheless, low concentrations of longer MCFAs (heptanoic and octanoic acids) were detected in all lysimeters during the last semester of the experiment. However, for lysimeters 1, 3, and 5, running with LR, higher concentrations of heptanoic (367 mg/L, 460 mg/L, and 878 mg/L, respectively) and octanoic acids (151 mg/L, 100 mg/L, and 485 mg/L, respectively) were noticed in comparison to the LD lysimeters. These results are in line with Luo and Wong's (Luo & Wong, 2019) findings; they reported that in the absence of leachate recirculation or low recirculation ratios, no longer carbon chain acids were detected. This implies that high LR ratios promote the degradation of long chain fatty acids (Luo & Wong, 2019), but in contrast, the results of Hussain et al. (2017) indicated that the rate of LR did not impact the profile of production or cause considerable changes in the percentage composition of individual MCFAs. However, the results of the LD effect agree with the results of He et al. (2005) and Jagadabhi et al. (2010) on enhancing the production of MCFA with a long carbon chain by reducing the inhibitory effect of MCFAs accumulation and improving the solubility and hydrolysis of OMSW. Meanwhile, Spirito et al. (2014) reviewed chain elongation pathways in an anaerobic process and reported that pentanoic and hexanoic acids can be generated through the reverse β oxidation pathway from short chain fatty acids (e.g. acetate and propionate). Additionally, when a leach bed reactor operates at higher leachate recirculation ratios, more metabolic intermediates accumulate in the leachate, which can simplify the chain elongation process of the metabolic products. Therefore, longer carbon chain acid concentrations provide evidence for the existence of fatty acid elongation reactions, under high leachate recirculation conditions. Overall, the possible reason for the observed changes in metabolic product formation under LR and LD could be understood by investigating the changes in microbial communities' structure caused by changes in the organic fraction (Jankowska et al., 2018; Luo & Wong, 2019).

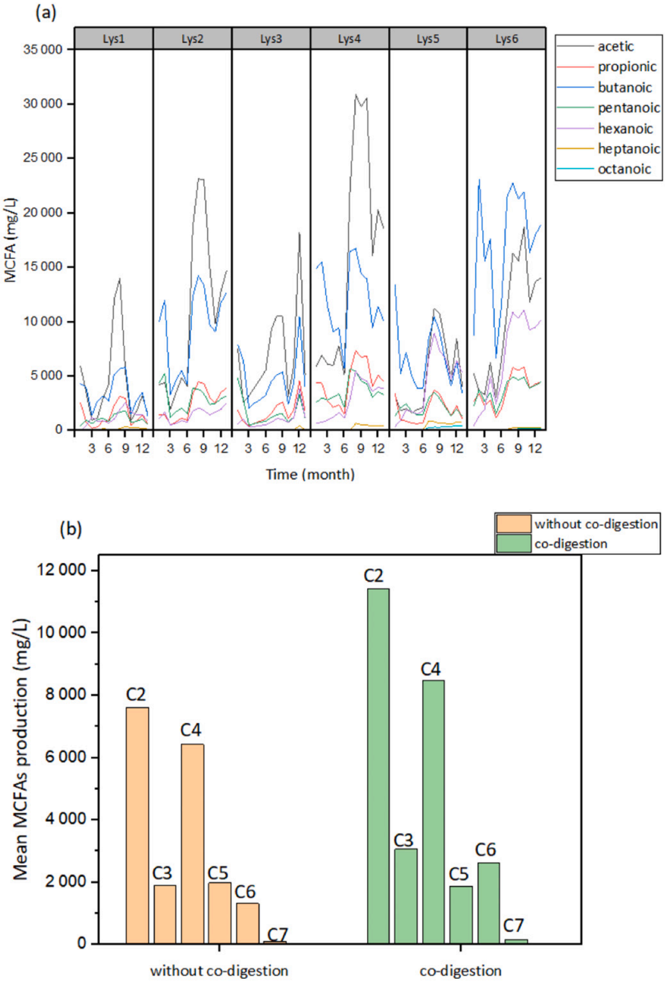

Fig. 4. MCFA distribution for the six lysimeters: (a) the effect of time and systems (LR and LD) and (b) the effect of co-digestion on MCFAs spectrum.

3.3.2. *Effect of co-digestion on MCFAs production*

Fig. 4b showed the effect of co-digestion on the spectrum of MCFAs production. Co-digestion was applied for lysimeters 3 and 4 by adding poultry waste, as mentioned in Table A.1; lysimeters 1 and 2 operated without co-digestion. As shown in Fig. 4b, co-digestion had a significant effect on the distribution of MCFAs. As mentioned previously (see Section 3.3.1), acetic and butanoic acids were predominantly produced by the process. However, with co-digestion, their concentrations were multiplied by around one and a half times in comparison to lysimeters without co-digestion. While the concentration of propionic acid showed a slight increment due to the co-digestion process, pentanoic acid showed the highest concentration for lysimeters without co-digestion. However, MCFAs with longer carbon chains (hexanoic and heptanoic acids) were mostly produced with co-digestion. Therefore, co-digestion displayed a high positive impact on MCFAs production. These results confirmed that the use of poultry waste as co-substrate in the co-digestion process had a good buffering capacity with rising MCFAs concentration. This can be attributed to the lipids found in industrial poultry waste; these were mainly triacylglycerides (Fernández et al., 2005), consisting of glycerol attached to three fatty acid chains, which were broken down by extracellular lipases excreted by acidogenic bacteria (Fernández et al., 2005). The glycerol fraction was then fermented to propionate, while the long chain fatty acids were sequentially oxidized to acetic acid and others carbon acids components (Fernández et al., 2005). Additionally, our findings converge with those of Jain et al. (2015) and Luo et al. (2019), who explained that the degradation of organic macromolecules like proteins, carbohydrates, and fats in poultry waste may increase MCFA concentration to levels that inhibit the production of biogas, especially in combination with low pH values.

3.4. *Effect of leachate recirculation and dilution on MCFAs production: statistical analysis*

Principal component analysis (PCA) was done to identify the effects of LR and LD on MCFAs production by grouping individuals based on the GC-FID dataset (Fig. 5a). The two dimensions explained 84.8% of the total MCFAs production variability [Dim 1 (58.8%) and Dim 2 (26%)]. Both systems, LR and LD, have positive contributions to Dim 1. The LR system showed a strong correlation to the different MCFAs individuals. However, an insignificant negative correlation was noticed with Dim 2. These results can be explained by the high enhancing impact of recirculation on the production of MCFAs with carbon chains below six carbons, in contrast to acids with longer carbon chains, such as heptanoic and octanoic acids. For the LD system, a strong correlation is shown with Dim 2, whereas Dim 1 was less positively correlated compared with the LR system. This finding can be explained by the high production of longer carbon chain acids for lysimeters with LD. However, low production of MCFAs below hexanoic acid appeared. Additionally, the mean difference between the two systems (LR and LD) regarding the variability of distribution of MCFAs production is shown in the PCA cosine squared plot (Fig. 5b). It was observed that octanoic and heptanoic acids have high cos2 values, with more than 0.90, indicating that they were the most important variables affected by the two systems. Nevertheless, acetic acid was the least affected variable by the changes between LR and LD systems (cos 2 value < 0.75). Therefore, Fig. 5a and b confirm the positive effect of LR and LD on MCFAs production. While LR showed a high enhancing effect on MCFAs production (especially for hexanoic and short carbon chain acids), LD showed a positive correlation with longer carbon chain fatty acids production, such as heptanoic and octanoic acids. These results can be explained by the positive effect of LD on hydrolysis and the reduction of the inhibitory effect of MCFAs accumulation as a consequence, allowing the launching of chain elongation pathways from shorter carbon chain acids (He et al., 2005; Jagadabhi et al., 2010; Spirito et al., 2014).

3.5. *Effect of OMSW ratio on MCFAs production*

Individual factor map, as presented in Fig. 5c, showed the effect of the various OMSW ratios on MCFAs production. The individual factor map (Fig. 5c) identified three end-members represented by the different ratios of OMSW [70% OMSW (in pink), 70% OMSW + Co-digestion (in green) and 100% OMSW (in blue)]. The blue plot (100% OMSW) was highly, positively correlated with both dimensions. From this plot, it was inferred that there was a strong correlation between the variables of MCFAs and the blue plots, which means that high production of MCFAs was found in 100% OMSW samples. The green plot was ranked in second place in terms of MCFA production; it was positively and negatively correlated with both dimensions. This result can be explained as follows: there is a positive effect on MCFAs production in general, but some factors interfere with the use of poultry waste as a co-substrate. The pink plot (70% OMSW) yielded positive values for MCFA production, but it was negatively correlated with Dim 1 and positively correlated with Dim 2. This result was defined as a partial correlation between MCFAs and the associated samples. As a consequence, the OMSW ratio highly influences MCFAs production, and by increasing the OMSW ratio from

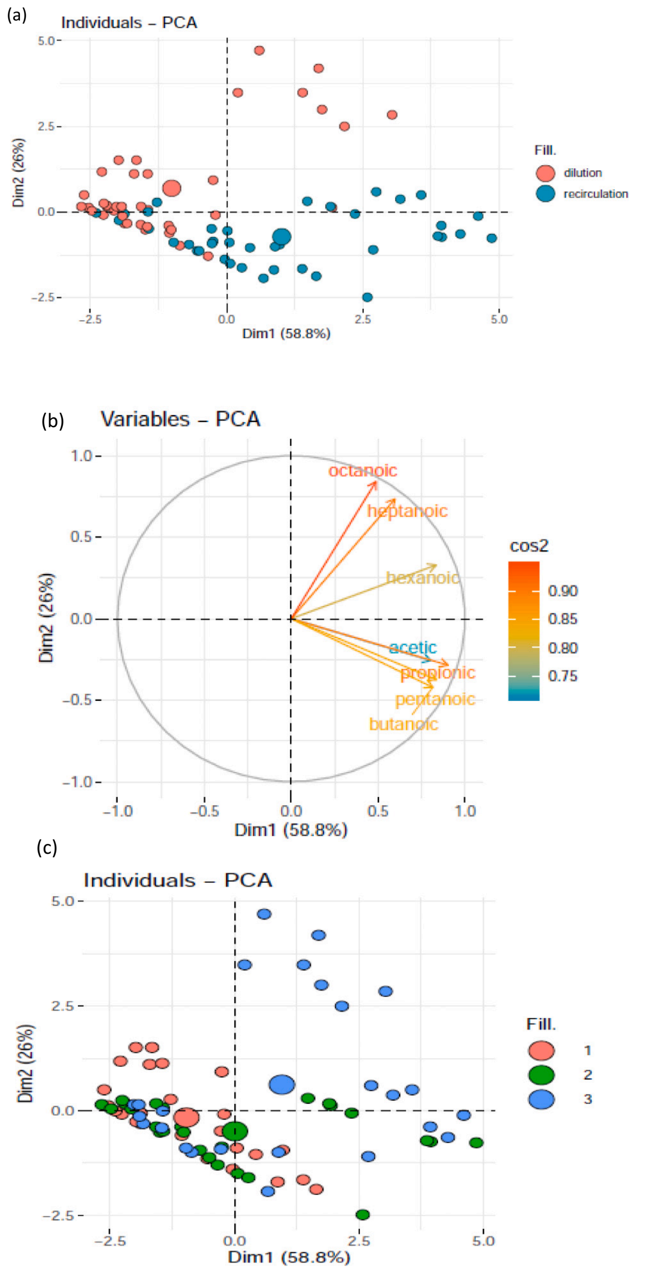

Fig. 5. PCA results on the effect of leachate recirculation and dilution (a, b) and OMSW ratio (c) (where 1: 70% OMSW; 2: 70% OMSW + Co-digestion; 3: 100% OMSW) in MCFAs production.

70% to 100%, the potential for production rises. Also, co-digestion influenced partially the production of MCFAs, especially in terms of the distribution of the acids as mentioned previously (see Section 3.3.2).

3.6. *Correlation between physicochemical parameters and leachate recirculation and dilution*

Fig. 6 presents graphically all linear Pearson correlation coefficients among five parameters of the leachate running in the two different irrigation systems (LR and LD). The correlations were based on 24 measurements for each system; the measurements were performed during a one-year experiment. For both irrigation systems, the pH showed a slight variability between the samples measurement's and a negative correlation to total MCFAs, ammonium, and total correlation. However, between pH and EC, a negative correlation only appeared for the LD samples, which can be explained by the pH-self regulation in the experiment (Jankowska et al., 2017). Therefore, any high effect was reflected in total MCFAs production. Meanwhile, a big variability in terms of EC measurements was shown (around 20 ms/cm and 40 ms/cm respectively for LD and LR). Nonetheless, EC showed a strong correlation with all evaluated parameters for both irrigation systems. Moreover, a positive correlation between the pH and total alcohol production appeared in both systems, but the strongest correlation was identified in LR. Thus, recirculation enhanced hydrolysis and acid production, which can be utilized as a carbon source for alcohol production (Hussain et al., 2017). Hence, the total alcohol and MCFA production showed a strong total correlation (cor: 0.428). The relation between MCFA production and alcohols can be attributed to the high positive effect of recirculation on hydrolysis and acidification and increased accessibility of microorganisms to degrade organic matter. Nonetheless, ammonium and total MCFAs revealed a strong correlation, especially in the LR system. This was because of the accumulation of ammonium due to recirculation and addition of poultry waste, which inhibits methanogenesis and boots acidogenic microorganism's activities (Bae et al., 2019; Plácido & Zhang, 2018).

4. Conclusions

Leachate recirculation and dilution show a positive effect on the acidogenesis process by improving the hydrolysis and acidification yields. The tMCFAs production shows a higher effect for LR (62,000 mg/L) than LD (40,000 mg/L). Acetic and butanoic acids are the dominant products of both systems, while heptanoic and octanoic acids are mostly found in LD. Moreover, co-digestion with poultry waste enhances MCFAs productivity. Additionally, the statistical analyses confirm the positive effect of increasing the ratio of OMSW. Therefore, the positive effect of LR and LD can be combined in future investigations to optimize MCFA production.

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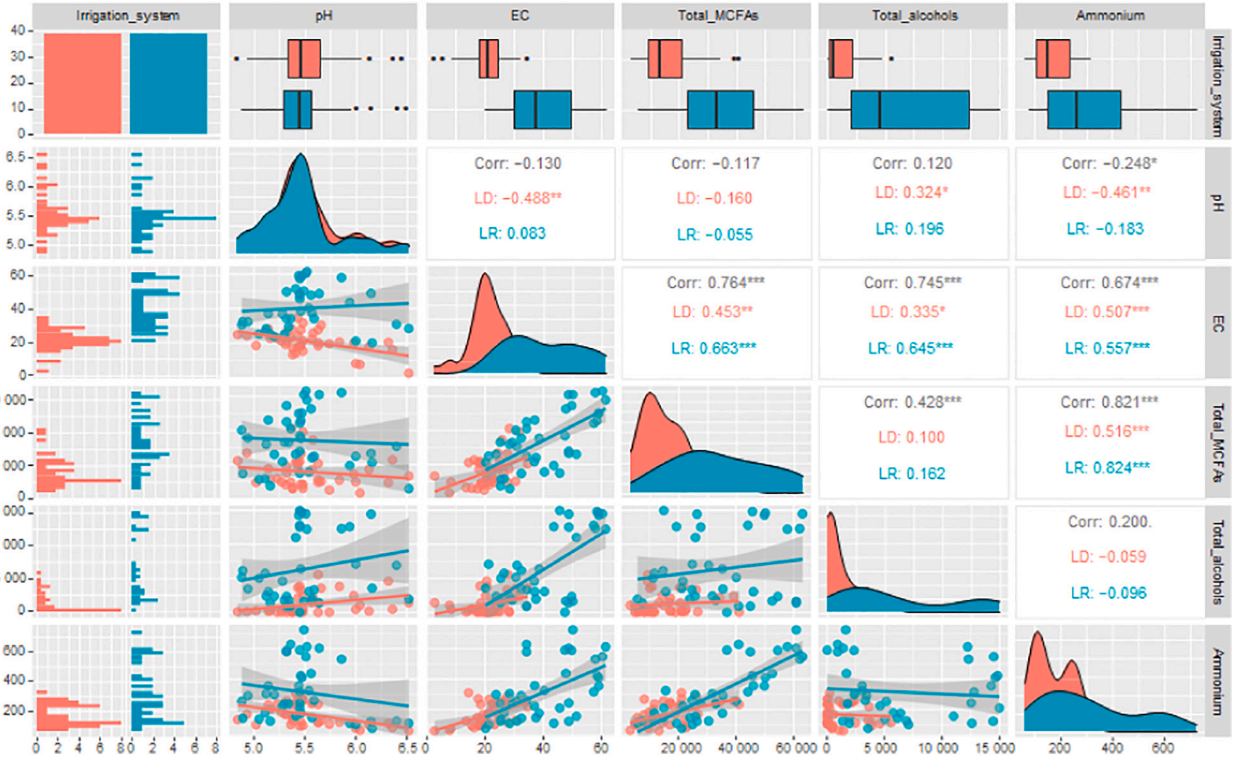

Fig. 6. Scatter-plot matrix among parameter concentrations and values of LR and LD based on 48 samples collected during the experimental period (pH in pH units, EC in ms/cm and all other units in mg/L).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A

Table A.1
Experiment conditions and waste composition for the six lysimeters under study.

Lysimeter	1	2	3	4	5	6
Organic fraction (%)	69.67	68.08	59.78	61.89	100	100
Poultry waste (%)	0	0	11	11	0	0
Paper (%)	16.3	15.98	14.02	12.8	0	0
Plastic (%)	7.17	6.97	7.04	7.42	0	0
Glass (%)	1.6	1.49	1.49	1.86	0	0
Leather and skin (%)	0.15	1.16	0.15	0.78	0	0
Textile (%)	5	5.4	4.8	4.14	0	0
Residues (%)	0.11	0.92	1.72	0.11	0	0
Irrigation system	LD	LR	LD	LR	LD	LR
Water volume for LD (l/month)	3.6	–	3.6	–	3.6	–
Leachate volume for LR (l/day)	–	1	–	1	–	1

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