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Potential of medium chain fatty acids production from municipal solid waste leachate: Effect of age and external electron donors

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ABSTRACT

A large quantity of leachate is generated during municipal solid waste collection operation and in landfills due to the large amount of organic waste and high humidity. The content of medium chain fatty acids (MCFAs) in the leachate is a low cost feedstock for bio-based chemical and fuel production processes. The aim of this study is to investigate the MCFA production potential of three leachate ages through chain elongation process under uncontrolled pH batch test. Moreover, the effect of using different external electron donors (ethanol, methanol and a mixture of both) is studied. The experiment consists of characterizing the samples then adding external electron donors with a specific ratio to leachate samples under mesophilic temperature. For this investigation, also a statistical analysis is done, which shows the production of MCFAs is highly influenced by leachate age. The results indicate that the production of even-numbered acids increase from 600 to 1,000 mg/L by the end of the ethanol chain elongation experiment for young leachate. However, a higher MCFA production of more than 1,000 mg/L is achieved by using the mixture of methanol and ethanol as electron donor. Furthermore, all methanol chain elongation experiments lead to an odd-numbered production of MCFAs, such as pentanoic and heptanoic acids. These results confirm the potential improvement of MCFA production from leachate through choosing the optimal leachate age and electron donor. Overall, producing MCFAs from leachate is a good example of circular bio-economy because waste is used to produce biochemicals, which closes the material cycle.

1. Introduction

The global solid waste production is rising annually due to increasing population and economic growth. According to the World Bank, the world's cities generate annually 2.01 billion tons of municipal solid waste (MSW). Also, 33 percent of these are not environmentally managed (Shawn Burke et al., 2018). The waste generated worldwide per person and day is about 0.74 kg, but ranges widely from 0.11 to 4.54 kg. By 2050, global waste is expected to reach 3.40 billion tons per year (Kaza et al., 2018).

As consequences of high urbanization rates, economic development and population growth, waste generation is generally found to increase at a faster rate for incremental income changes at low income levels than at high income levels (Shawn Burke et al., 2018). The open dumping of waste and leachate leaking on the streets during waste collection and in the soil and groundwater during disposal are common occurrences in low income countries (Maguiri et al., 2017; Shawn Burke et al., 2018). They have negative impacts on human health, local and global environment and create a damaging effect on the economy (Shawn Burke et al., 2018). Nevertheless, waste collection is achieved at high costs in these countries, which have poor waste management systems (Kaza et al., 2018). According to the World Bank Data (2019), Morocco is categorized as a lower-middle income country and its total disposed MSW at the national level in 2015 was estimated at 7.4 million

Abbreviations: AD, Anaerobic digestion; ANOVA, Analysis of Variance; BL, Blank; BL-FL, Blank of fresh leachate; BL-OL, Blank of old leachate; BL-YL, Blank of young leachate; COD, Chemical oxygen demand; E, Ethanol; E/M, Ethanol and methanol mixture; FFA, Free fatty acid; FL, Fresh leachate; GC-FID, Gas chromatograph with flame ionization detector; M, Methanol; MCFA, Medium chain fatty acid; MSW, Municipal solid waste; MT, Million Tons; OL, Old leachate; PCA, Principal Component Analysis; YL, Young leachate.

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tons. However, only Marrakech, one of the largest cities in Morocco, generates around 1 million tons of MSW per year. This amount will increase due to the mismanagement associated with the very low rate of collection (less than 40% especially in peri-urban communes), which causes open dumps and the dispersion of garbage on the streets (State Secretariat to the Minister of Energy et al., 2019).

Hence, during the waste collection, a large quantity of contaminated water can leak from the collection trucks due to the huge amount of organics and the high moisture content (Maguiri et al., 2017; Ouchen, abd elghani, 2018). The contaminated water damages properties in the city and pollutes the air. Another leachate source is the disposal of waste in landfills (Maguiri et al., 2016). This is considered a highly-contaminated wastewater, which favors greenhouse gases production during the anaerobic digestion (AD) process and pollutes soil and groundwater with mineral and organic compounds (Ezyske and Deng, 2018; Hafidi, 2016; Merzouki et al., 2018). In order to meet the discharge limits, the treatment of leachate is necessary, which involves evaporating it (Maguiri et al., 2016). This process leads to offensive odor near the treatment site and the deterioration of surface and ground water quality (Hafidi, 2016; Maguiri et al., 2016). The absence or complexity of leachate treatment systems has led researchers to focus on ways of valorizing this liquid substrate. Moreover, the valorization of leachate into valuable products through a cascade of steps is a good example of putting circular bio-economy into praxis. According to Stegmann et al. (2020) "[...] circular bio-economy focuses on the sustainable, resource-efficient valorization of biomass in integrated, multi-output production chains (e.g. biorefineries) while also making use of residues and wastes and optimizing the value of biomass over time via cascading." In the last few decades, several studies to valorize landfill leachate were conducted. Zupančič and Grilc (2018) investigated the valorization of leachate for biogas production as a final product of the AD process, whereas Agler et al. (2011 and 2012) analyzed leachate regarding intermediate products of the AD process, such as carboxylic acids platform (Agler et al., 2012, 2011). The most important intent of leachate valorization is to gain MCFAs with chain length of six to eight carbons due to their large range of applications.

The MCFAs can be produced through chain elongation process (Levy et al., 1981) using short chain fatty acids with an externally supplied electron donor (Agler et al., 2012). This chain elongation pathway has been studied by several researchers at different levels. Several experiments were conducted using different external or internal electron donors (Magdalena et al., 2020). A large number of reduced organics have been studied as external electron donors including, ethanol (Agler et al., 2012; Grootscholten et al., 2013; Grootscholten et al., 2013; Kannengiesser et al., 2016; Roghair et al., 2018), lactic acid (Weimer et al., 2016; Jiajie Xu et al., 2018), methanol (Chen et al., 2017, 2016), propanol and succinate (Kenealy and Waselefsky, 1985), D-galactitol and galactose (Jeon et al., 2013, 2010). In addition, these electron donors have been utilized to generate MCFAs at large production disparities 0.74–12.8 g/L (Wu et al., 2018). The chain elongation studies indicate ethanol and lactate as the most suitable electron donors. However, they still exhibit their respective short-comings, which largely limit the substrate utilization ratio and MCFA productivity (Magdalena et al., 2020; Wu et al., 2018). According to the reviews of Agler et al. (2011) and De Groof et al. (2019), many other parameters affect the chain elongation process, such as temperature, retention time, pH and the microbial community. Only few studies have evaluated the effect of using co-electron donors (Nzeteu et al., 2018; Wu et al., 2018). Wu et al. (2018) achieved a cooperative relationship for enhancing MCFA production by using ethanol and lactate as co-electron donor. Nonetheless, there are still gaps in literature concerning research on methanol as electron donor (Chen et al., 2017, 2016) as well as co-electron donor with ethanol. Leachate has been studied as a substrate for producing MCFAs, but studies investigating the potential of producing MCFAs from leachate of different ages are lacking.

Based on these gaps, the specific research question under investigation is as follows:

(1) *What influences do the use of different electron donors and the age of substrates have on MCFA production?*

Therefore, the aim of this study is to investigate the feasibility of using a co-electron donor (ethanol and methanol) in batch experiments for producing MCFAs from different leachates. Subsequently, this study explores the potential of MCFA production from different leachates according to their age (old leachate, young leachate and fresh leachate) by the use of different electron donors.

2. Material and methods

To answer the research question, the following methodological approach is chosen. In the first step, leachate samples from different locations in Marrakech (Morocco) are collected for analysis. After determining the quality of the samples, they are subjected to the chain elongation process. GC-FID analysis is performed to analyze the produced MCFAs. A statistical analysis using R software enables an easy visualization of the distribution of MCFA concentration results regarding the investigated variables: age and electron donor. In the following chapter, the sampling areas as well as the conducted physical, chemical and statistical analyses are described.

2.1. Description of the sampling areas

Three spots for leachate sampling are chosen based on the factor of age. The first sample, which is the old leachate, was collected from the closed uncontrolled landfill Al Azzouzia. The second sample, the young leachate, was collected from the active controlled landfill El Mnabha and, finally, the third sample consists of fresh leachate, which was directly collected from the tank of a waste collection truck.

2.2. Old leachate (OL)

The old leachate was collected from the closed uncontrolled landfill Al Azzouzia located 15 km north of Marrakech (Morocco). The coordinates of the sampling area in this landfill are x = 2,46723; y = 126,918; z = 385 (Google earth). In addition, this landfill was operational from 1987 to 2014 (Hakkou et al., 2001). However, it was rehabilitated in 2014 but finally closed in 2016 due to negative sanitary, environmental and economic effects. Also, the basin built on the landfill site for leachate collection is neither exploited in the valorization area nor in the treatment field. Evaporation is the actual treatment of leachate here. The leachate sample was taken in 2019, i.e., after the closure of the landfill.

2.3. Young leachate (YL)

The young leachate was collected from the controlled landfill El Mnabha located 42 km from Marrakech. Since 2015, the landfill has been operating as part of the delegated management adopted by Marrakech after the rehabilitation and closure of the uncontrolled landfill Al Azzouzia. In addition, the coordinates of the sampling area of this landfill are: x = 31,913; y = 8,0811 (Google earth).

The detailed waste compositions of Al Azzouzia and El Mnabha landfills are presented in Table 1.

2.4. Fresh leachate (FL)

The fresh leachate is collected from the tanks of the waste collection trucks in the transfer station coordinated 31°38′31.4″N 7°59′04.1″W (Google earth). The liquid is generated when the waste is pressed inside the truck. The truck collects around 1,000 to 3,500 kg of waste per day, which is composed of 72% of organic waste and produces 6 to 8 m^3 of leachate (Ouchen, 2018). For the FL collection, five samples are taken from the tanks of five different waste collection trucks. Before further analysis, the five samples are preliminary analyzed (see section 2.2) with the result showing that their free fatty acid concentrations are approximately equal. The results of this characterization analysis can be found in Table A1 (see Appendix).

2.5. Preliminary characterization of samples

Characterization of leachate from the three sampling spots is the first step before starting the chain elongation process in order to determine the quality of the samples. The characterization consists of measuring the pH and electrical conductivity by using a multi-parameter probe (Electro photometric Multiparameter HI 2829); chemical oxygen demand (COD). Sulfate, nitrite, ammonium, total phosphorus and orthophosphate were analyzed in accordance with standards methods (AFNOR., 1997, APHA., 2005; Rodier, 2009). The free fatty acids (FFA) titration was carried out according to ISO 660:2009 EN.

2.6. Chain elongation process experiments

After defining the quality of the leachate samples, the chain elongation process is launched (see flow chart Fig. A2 in the Appendix). According to Kannengiesser et al. (2015 and 2018), the chain elongation process consists of the production of MCFAs under anaerobic conditions by adding an electron donor, such as ethanol or methanol, to the liquid samples to initiate the elongation of the short chain fatty acids. About 3 L of each sample (OL, YL and FL) is stored in canisters at mesophilic temperature (30 ± 3 °C) after adding the three electron donors (methanol (M), ethanol (E) and a mixture of ethanol and methanol (E/M)), and the ratios of alcohol to leachate (v:v) are 1:100, 1:100 and 1:50:50, respectively. To each liter of leachate, 10 ml of ethanol or methanol is added, while for the mixture, 5 ml of each alcohol is added. Nonetheless, the experiment is performed under uncontrolled pH system in batch test reactors in duplicates. However, according to some researchers, a pH adjustment to between 5.5 and 6 using HCl or NaOH is required before the addition of the electron donors, because it is the optimal condition for microorganisms to elongate carboxylic acid chains (De Groof et al., 2019; Ge et al., 2015; Kenealy and Waselefsky, 1985; Vasudevan et al., 2014). Under similar conditions, the blanks of the chain elongation experiment are conducted without adding E, M and E/M to study the efficiency of the chain elongation using an external electron donor for MCFA production. During one week of experiments, samples of 12 ml are taken every two days for further analysis.

Table 1
Waste composition of Al Azzouzia (Hakkou et al., 2001) and El Mnabha (Ouchen, 2018).

Waste fractions [in weight %]	Al Azzouzia landfill	El Mnabha landfill
Organics	70	72
Paper	14.30	14
Plastics	7.10	7
Textile	4.80	4
Glass	1.50	2
Metal	1.00	0.20
Leather	0.13	0.16
Rubber	0.06	
Wood	0.20	0.14

2.7. GC-FID analysis

Samples taken from the reactors are diluted in a ratio of 1:10 with Milli-Q water, and the pH is adjusted to a range of 1.75 to 2.25 by adding 1 N HCl solution. Samples are filtered using a micro filter (0.45 μm Polyethersulfone, VWR, North America) before the GC-FID analysis. The column used for this study is TG WAXMS-A (30 m; i.d. 0.32 mm; thickness 0.50 μm; stationary phase: polyethylene glycol; Thermo Scientifics, Dreieich). For each measurement, 1 μL of the sample is injected into a split/splitless injector heated to 260 °C and analyzed at a split ratio of 1:10. The GC oven program is set as follows: at 80 °C for 1 min, 20 °C/min till 120 °C, 6.1 °C/min till 205 °C and at 205 °C for 10 min.

2.8. Statistical analysis

For the statistical analysis, the open source R software package from FactoMiner is applied. In order to visualize the distribution of the different MCFA concentrations used for the different leachate ages and different electron donors, the data are subjected to principal component analysis (PCA). The result of PCA is shown with individual factor maps and cluster Dendrogram. Then, one-way ANOVA is used to verify the PCA analysis.

3. Results and discussion

In this chapter, the results of the experiments are described and discussed. The first part concerns the quality of the leachate sample, which is assessed using the preliminary characterization results (see Section 3.1). The second part of the results focuses on the influencing factors regarding the chain elongation process and MCFA production: effect of time and pH (see Section 3.2), COD evolution (see Section 3.3), impact of electron donors (see Section 3.4) and the efficiency of the process of chain elongation through comparison with the blanks (see Section 3.5). The two last sections, 3.6 and 3.7, are devoted to the results of the statistics analysis.

3.1. Leachate preliminary characterization

The preliminary characterization parameters of the three investigated leachate samples regarding the chain elongation process are presented in Table A3 (see Appendix).

Table A3 shows big differences regarding the preliminary characterization of the analyzed leachate samples. The OL and YL are both alkaline, while the FL is acidic, with a pH of around 4. This lower pH-value can be mainly explained by the accumulation of volatile fatty acids through the dominant presence of hydrolytic and acidogenic bacteria in FL, which work in the first steps of organic waste decomposition (Sun et al., 2011). In the OL and YL, higher pH values appear, which might be due to the process of biodegradation of macromolecular organics in the leachate to produce organic acids, which increases the conductivity (Sivula et al., 2012).

For all three samples, a negative and low redox potential is observed, which indicates anaerobic conditions, especially for OL and YL (-254.35 ± 24.36 mV and −398.81 ± 14.56 mV, respectively). For FL, the redox potential indicates the start of anoxic

conditions (>−200 mV). While the concentrations of COD and FFA in the OL are low, they are high for YL and FL samples. These results confirm Ehrig and Stegmann (2018) thoughts that during the first year of operation of a landfill, as is the case for YL, the COD is more than 125,000 mgO_2/L. It can be explained by the presence of mainly easy degradable organic acids in YL and FL. The result of the sulphate concentration assessment shows that FL contains the highest concentration, around 5,194.80 ± 56.25 mg/L, which decreases with age to less than 100 mg/L due to the reduction of sulphate to sulphide under anaerobic conditions. In addition, the results of nitrogen compounds' (NO_2^-, NH_4^+ and total nitrogen) assessment show slight variations between the three samples; the concentrations are high, more than 300 mg/L and 1,000 mg/L, respectively, for NH_4^+ and total nitrogen. The landfill age and the associated biodegradation lead to a decrease in the concentration of biodegradable organic compounds and waste mass; simultaneously, the concentration of ammonia increases (Ren et al., 2017). Nevertheless, nitrite, which is produced from nitrate reduction reaction or the incomplete oxidation of ammonium ions, shows a high value 38.21 ± 1.47 mg/L for FL contrarily to YL and OL which could be explained by the low value of red/ox potential (AE et al., 2014). Contrarily, the three leachate samples have low concentrations of less than 10 mg/L concerning phosphorous compounds (total phosphorus and orthophosphate). These low concentrations can be explained either by the waste composition (e.g. organic waste and paper are rich in phosphorus) or the adsorption of the phosphorus by the mineral compounds (such as clay) used for covering the waste during the land filling process. Even if the concentrations of all nitrogen and phosphorous compounds are high, they do not inhibit the microbiological reactions. Moreover, the chloride results show low concentrations for all leachates samples. However, a high concentration of cations salts (potassium, sodium and calcium) has been characterized for all three samples.

Briefly, the YL and FL have promising characteristics for elongating carboxylic acid chain. In contrast, the OL has low concentrations of COD and FFA, which should be further investigated to study the feasibility of chain elongation.

3.2. MCFA production during chain elongation process: Time effect and pH-evolution

The amounts of MCFA produced from leachate with pH-value fluctuations for the three samples with different electron donors during the chain elongation process are shown in the Figs. 1–3. The results of the chain elongation with E, M and E/M show a change in the production of MCFA in relation to the retention time and pH-value. Fig. 1 shows stability in the MCFA composition for OL during the chain elongation process, and acetic acid is the dominant product. However, the concentration of acetic acid slightly increases by one percent after the second experimental day, but the pH-value is stable from the beginning of the process until the fifth day when it starts decreasing from 5.5 to less than 4; this could be explained by the excessive ethanol oxidation, which leads to an increment of acetic acid production (Roghair et al., 2018). The other MCFAs concentrations remain stable during the whole experiment of OL. Reasons for that might be the degradation of all degradable matters and their transformation to biogas. This would also explain why the concentration of the carboxylic acids was low at the start of the chain elongation process.

Moreover, the results obtained from YL experiment (Fig. 2) are highly different. During the first day of the process, butanoic acid is the leading product, with more than 32% for the three experiments of YL, followed by acetic acid. However, a minor concentration is observed for heptanoic and octanoic acids. From the second experimental day, the butanoic acid concentration drops for all three electron donors. However, the concentration of the other acids

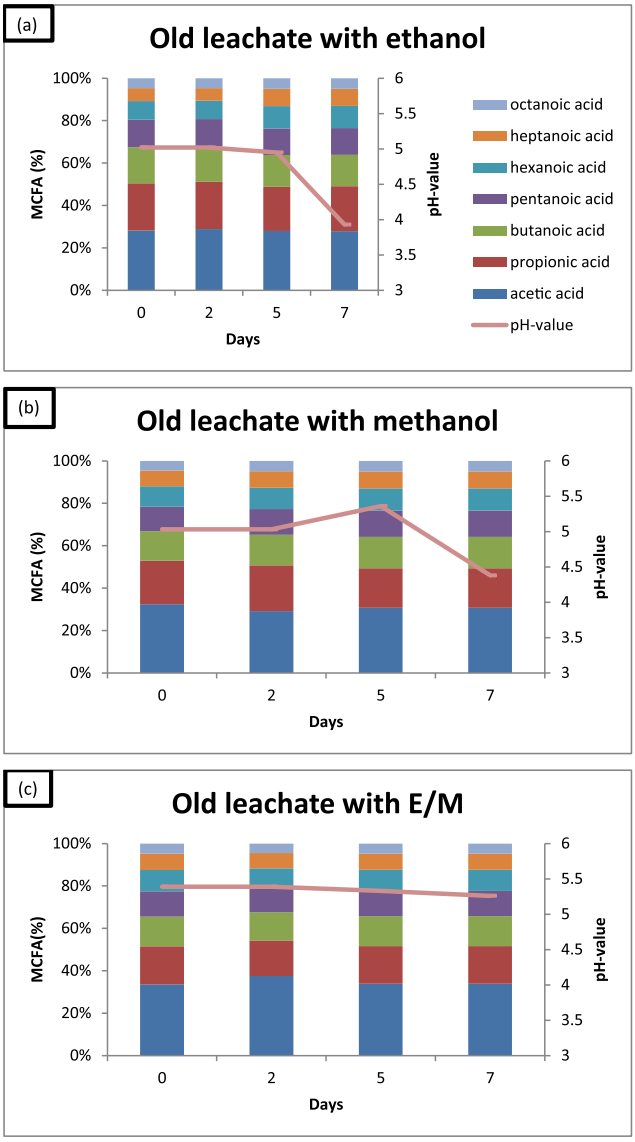

Fig. 1. Retention time effect and pH-value evolution of different electron donors (E, M and E/M) for OL (a, b, c).

with longer carboxylic chains increases, such as hexanoic acid. These results could be explained by the cycle of butanoic acid elongation to hexanoic acid (Wu et al., 2019). The pH-value slightly increases from the start to the fifth day. Afterwards, the pH-value drops (from 5.78 to 5.23; from 5.78 to 5.08 and from 5.08 to 4.37, respectively for E, M and E/M), which is related to the accumulation of acetate.

For all tested electron donors of FL (Fig. 3), acetic acid is the dominating product (98%, 85%, 84%, respectively) at the start of the experiment, but its concentration slightly decreases from the second day of the experiment; the concentrations of acids with longer chains increased: propionic acid (1 to 8%), butanoic acid (1 to 4%), pentanoic and hexanoic acids (each 0 to 2%). In all experiments, minor concentrations of heptanoic and octanoic acids (less 2%) are observed during the whole process. Also, acidic pH-value in the range of 4 to 5.9 is shown during the chain elongation with different electron donors. This can be explained by a precocious step of the AD and the high concentration of acetic acid.

The results of the Figs. 1–3 coincide with Jankowska et al.'s (2018) results on the significant impact of time and pH-value on the potential of MCFA production and its composition.

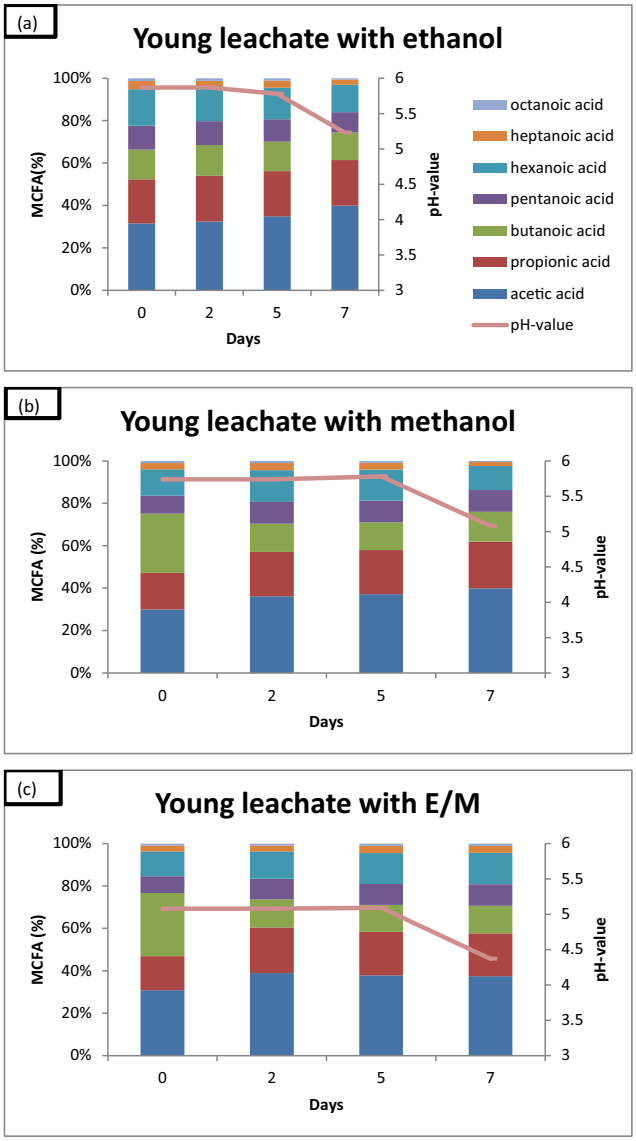

Fig. 2. Retention time effect and pH-value evolution of different electron donors (E, M and E/M) for YL (a, b, c).

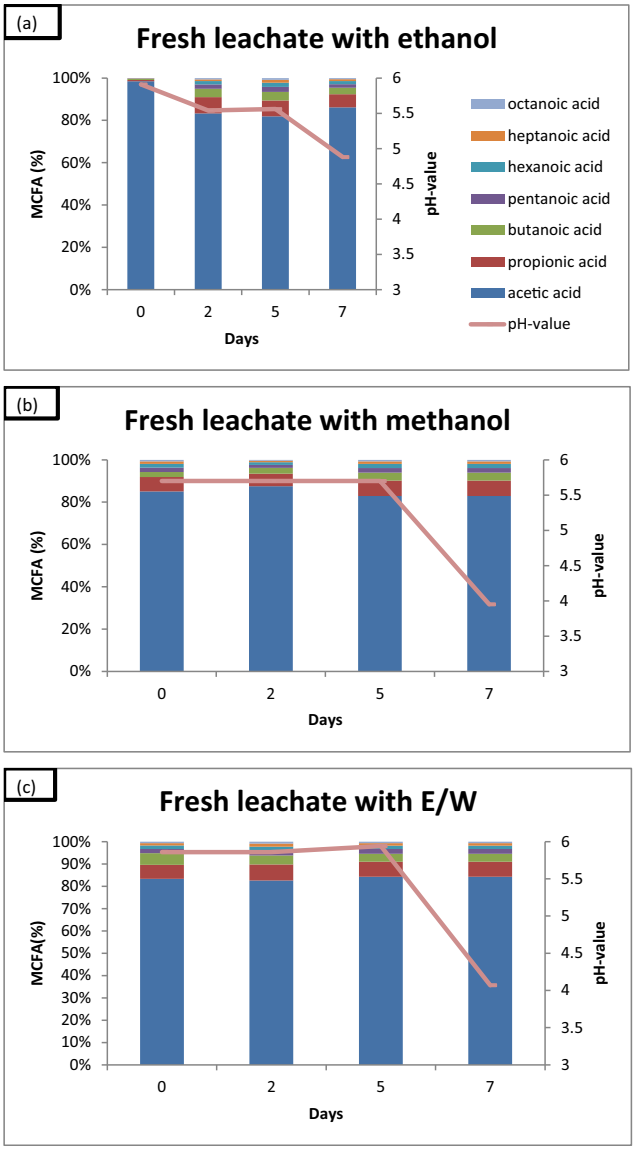

Fig. 3. Retention time effect and pH-value evolution of different electron donors (E, M and E/M) for FL (a, b, c).

For example, during the experiments involving OL, a stable quantity or a slight shift in the quantity of produced acid is observed when the pH-value remains stable. However, the retention time positively influences MCFA production and accumulation. Also, the results of the Figs. 1–3 support the results of different authors (Bengtsson et al., 2008; Jankowska et al., 2018; Kim et al., 2016), who reported acetic and butanoic acids as being the dominant acids when the pH is around 5. Moreover, several researchers (Chen et al., 2020, 2016; De Groof et al., 2019; Roghair et al., 2018) reported the positive effect of controlling the pH during chain elongation process for increasing the MCFA productivity. According to De Groof et al. (2019), "[...] the pH might need optimization depending on other operational parameters and fermentation characteristics, such as other factors influencing competitive reactions or substrate composition".

3.3. Chain elongation process: COD evolution

Figure A4 (see Appendix) shows the variation of COD at the start and the end of the chain elongation process for the leachate samples using the different external electron donors. Globally, for the whole experiments of the investigated leachate samples, COD increases at the end of all experiments. While, the highest COD value is shown for YL experiment (from 100,000 mg O2/L to 300,000 mg O2/L). However, OL has the lowest evolution of COD concentration. These results converge with the results of Section 3.2, which reveal an increment of MCFA production at the end of the experiment, especially for YL. This result could be explained by the low organic carbon source available in the OL in contrast to YL and FL. In addition, the low value of COD for YL-E could be explained by the excessive ethanol oxidation (Roghair et al., 2018).

3.4. MCFA potential using different external electron donors

The results of Fig. 4 show the fluctuations of the MCFA composition for the different external electron donors at the start and end of the experiment.

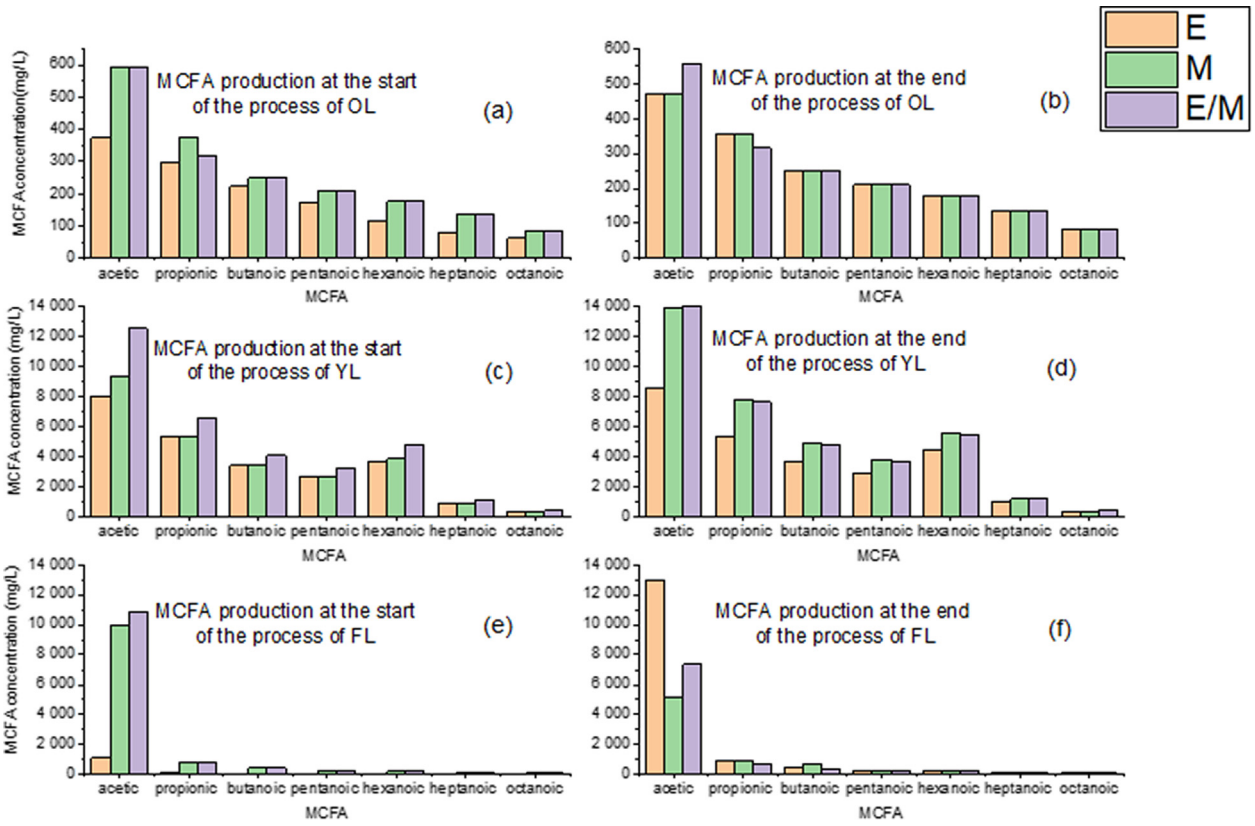

Fig. 4. Fluctuations of MCFA composition from the start to the end of the chain elongation process with different electron donors (E, M and E/M) for OL (a, b), YL (c, d) and FL (e, f).

Fig. 4 (a, b) shows a slight increment in the MCFA concentration of OL, especially for acetic acid, by the end of the ethanol chain elongation (from 373 mg/L to 470 mg/L). Also, propionic acid concentration rises with the use of E as electron donor (from 295 mg/L to 358 mg/L). However, the other acids with longer chains remained stable for the different electron donors used. In contrast, the results obtained by YL chain elongation (Fig. 4 (c, d)) show significant differences concerning MCFA composition and concentration between the investigated electron donors from the start to the end of the experiment. The comparison of the three alcohols indicates that E has the smallest production of different MCFA compounds by the end of the experiment. By using E as electron donor for YL samples, the concentrations of the even- numbered chain carboxylic acids increase between 600 and 1,000 mg/L from the start to the end of the process. While, odd-numbered chains, such as propionic, pentanoic and heptanoic acids, rise by around 100 mg/L each by the end of the experiment. Though, higher concentrations of the different MCFAs can be observed utilizing M or E/M. Propionic acid is the most abundant product at the end of the chain elongation process after acetic acid, with a value more than 7,600 mg/L. Also, high concentrations of hexanoic acid (1,200 and 1,300 mg/L for M and E/M, respectively) are detected, and a slight increment in acids with longer carbons chains, such as heptanoic and octanoic acids, is observed. Furthermore, the chain elongation process of YL with M is characterized by a high concentration of odd-numbered acids, such as propionic and pentanoic acids. Nevertheless, the current results stand in contrast to the results of Chen et al. (2017 and 2016), who reported that the leading products of methanol chain elongation are butanoic and *iso*-butanoic acids as well as even-numbered carbon chain fatty acids. Also, Chen et al. (2016) stated that hexanoic acid could not be detected using M as electron donor in batch tests. In contrast to this, our study indicated that hexanoic acid is produced with a significant concentration (5.517 mg/L) during methanol chain elongation. The chain elongation using the co-electron donor E/M shows high MCFA concentrations. It seems that cooperation enhances MCFA production, e.g. the effect of the co-electron donor ethanol and lactate (Wu et al., 2018). Also, the continuous use of E and M as a co-electron donor in the recent study of Chen et al. (2020) revealed an improvement in MCFA (butanoic and hexanoic acids) production, which is in line with the results of our investigation, but in the batch reactor. Conversely, they can also be competitive and may inhibit the production of some MCFAs, such as *iso*-butanoic acid, when the ethanol feed rate increases (Chen et al., 2020).

The results of the chain elongation with ethanol for FL are presented in Fig. 4 (e, f). They show a significant increment in all the MCFAs from the start to the end of the process, with a predominance of acetic acid. In contrast, for M and E/M chain elongation, acetic acid concentration drops, while other acids show a slight increment in their concentrations. However, among the three electron donors for chain elongation process, acetic acid is the main produced acid. Moreover, chain elongation with M for FL leads to a production of odd-numbered MCFAs, such as propionic and pentanoic acids. Other carboxylic acids with longer chains start their production at the end of the experiment.

Based on all experiments, the use of pure methanol and the mixture of methanol and ethanol show a dominant production of odd-numbered carboxylic acids, such as pentanoic acid, which corresponds with the result of Ganigué et al. (2018). The odd-numbered chain acids predominate when the reactor is fed with an odd chain electron donor, such as methanol (Ganigué et al., 2018).

3.5. Process efficiency: Blanks and chain elongation samples comparison in MCFA production

Fig. 5 presents the efficiency of the chain elongation process for the three investigated electron donors in comparison with their blanks relating to MCFA composition and concentration. In general, the blanks of the investigated leachate samples have the smallest total MCFA (pentanoic to octanoic acid) concentrations (3,360, 42,880 and 3,970 mg/L, respectively for BL-OL, BL-YL and BL-FL). Therefore, this result proves the positive effect of adding external electron donors for enhancing MCFA production. Using M and E/M−mixture as electron donors have the highest effect concerning MCFA production for YL and FL. This is in contrast to OL samples, where E has the highest total MCFA production due to the minor increment in acids production during the chain elongation process, as mentioned above (section 3.4). In addition, in terms of MCFA composition and concentration, OL shows a slight increase in acetic acid concentration compared with BL-OL when E is used as electron donor. Meanwhile, the other MCFA components have similar concentrations. Conversely, M and E/M chain elongation processes for YL lead to a high concentration of acetic acid and odd-numbered chain fatty acids. However, BL-YL produces butanoic and hexanoic acids, which confirms the explanations of Ganigué et al. (2018) and Grootscholten (2013) that the reason for even-numbered chain acids predominance over odd-numbered chain acids is related to the substrate transport and its availability. The effect of adding electron donors is much more obvious for FL due to the high concentrations of the different MCFAs compared with the BL-FL, which is in line with the results of Kannengiesser et al. (2018). Also, incomplete anaerobic condition degrades organic components and produces many intermediates, such as carboxylic acid with longer chains and alcohols that could be consumed by bacteria during the chain elongation process. Generally, in comparison with the blanks of the experiments, the production of the MCFAs for YL increases by 58%, 68% and 69%, respectively, for E, M and E/M. However, FL shows an increment of over 90% for the three electron donors compared with the BL-FL. In contrast, OL does not show any increment in the total MCFA production.

3.6. PCA analysis: Effect and interaction between age and electron donors in MCFA production

Principal component analysis (PCA) is conducted on the GC-FID data and provides individual factor maps (see Fig. 6 A and B), which highlight the relationships between the produced MCFA concentration obtained from the GC-FID analysis and the distribution within the samples regarding different ages (OL, YL and FL) (Fig. 6 A) as well as the different external electron donors (E, M and E/M) (Fig. 6 B).

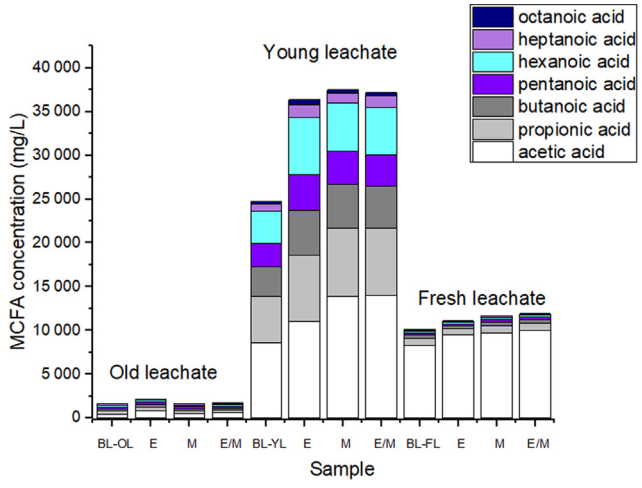

Fig. 5. Comparison of MCFA concentration between blanks (BL-OL, BL-YL and BL-FL) and chain elongation electron donors (E, M and E/M) for the three investigated substrates.

Individual factor maps, as presented in Fig. 6, are used to determine whether there are statistical differences between each variable of MCFAs for visible representation of the degree of separation between the variables of age (Fig. 6 A) and electron donors (Fig. 6 B). In other words, if there is any relationship between MCFA production and the variables of age and electron donors. Dimensions 1 and 2 (denoted as Dim1 and Dim2) represent 98.6% of the total variability of the produced MCFA concentration in leachate samples during the whole chain elongation experiment. The individual factor-map A identified three end-members, which represented and compared the degree of separation by age of the mean values of the MCFA concentrations. The first one, YL, is positively correlated with Dim1 and yields high positive loadings for the major production of MCFAs. From this plot, it is inferred that there is a strong correlation between the variables of MCFAs and YL, which means that a high production of MCFAs is found in YL samples. The second one, FL, yields positive values for MCFAs, but is negatively correlated with Dim1 and positively correlated with Dim2. This result is defined as partial correlation between MCFAs and FL samples. As a consequence, this pole yields slightly positive values for MCFAs in FL, which is interpreted as a small effect on MCFAs production (or the produced concentration). The third one, OL, is negatively correlated with Dim1 and Dim2. Therefore, it is inferred as an absence of effect on MCFA production. The results show a high apparent significant separation by age factor. Generally, the age of a sample affects MCFA production. Conversely, the individual factor-map B (Fig. 6 B) highlights the specificity of each electron donor used in the experiments. Generally, the effects of the three electron donors are not well distinguishable from this map because we could not define a separated end member as in map A. However, most of the variables from M and E/M are either positively correlated with one dimension or both (Dim 1 and Dim2). As a consequence, a slight effect is shown on MCFA production.

The cluster dendrogram (Fig. 7 A) gives a hierarchical and a graphical representation of a large set of data where all individual values are plotted in color regarding their production potential of MCFAs. YL samples (plots in blue) are ranked first because they have the highest MCFAs potential, followed by FL samples (plots in yellow) and lastly by the samples of OL (plots in grey), for which the lowest production of MCFA could be verified. Using PCA, a factor map is generated, which segregates the MCFAs and volatile fatty acids' variables (from acetic acid to octanoic acid) of the different sample blends into three different clusters (Fig. 7 B). Cluster I represents samples from the experiment of OL. This group is characterized by low values for all variables (from acetic acid to octanoic acid). Cluster II is composed of samples from FL experiment. It is characterized by strong values for the variable acetic acid and low values for the variables C5, C6, C7, C8, C3 and C4 (from most extreme to least extreme). Cluster III represents samples from YL experiment. This cluster is characterized by strong values for variables C7, C6, C8, C5, C3, C4 and C2 (from the most extreme to the least extreme). In few words, the larger the cluster is, the greater the production potential. Therefore, the large grey cluster III can be described as the lead producer of MCFAs, while the smallest blue cluster I is the least producer of the different MCFAs.

3.7. ANOVA results

ANOVA (Analysis of variance) test is carried out to investigate the differences associated with the leachate age and the electron

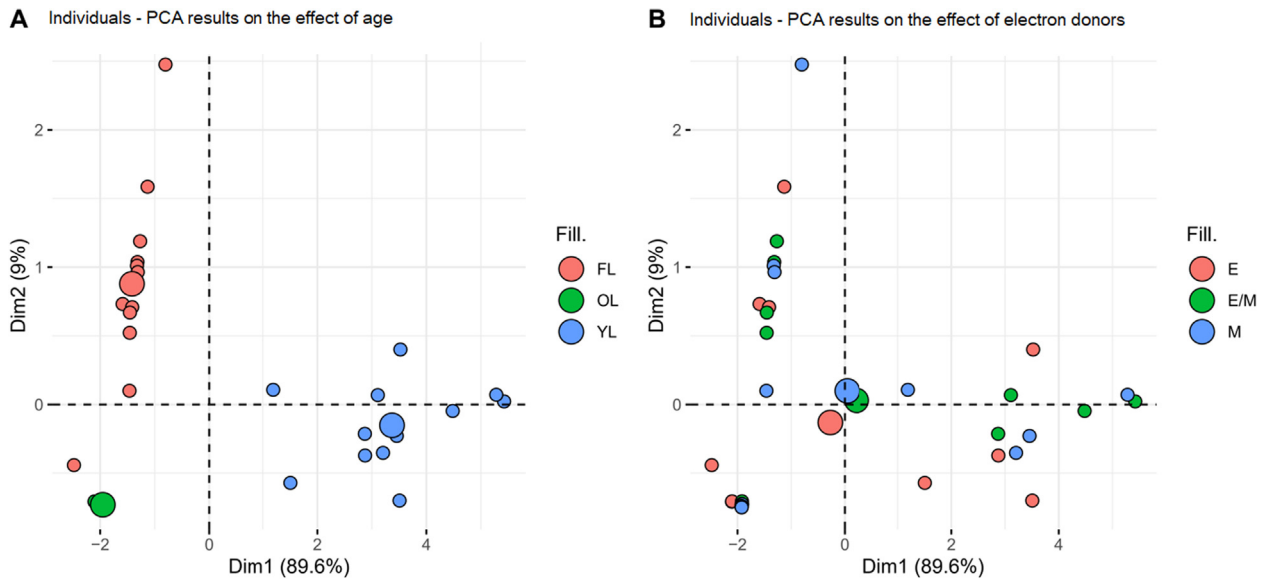

Fig. 6. PCA results on the effect of age (A) and electron donors (B) on MCFA production.

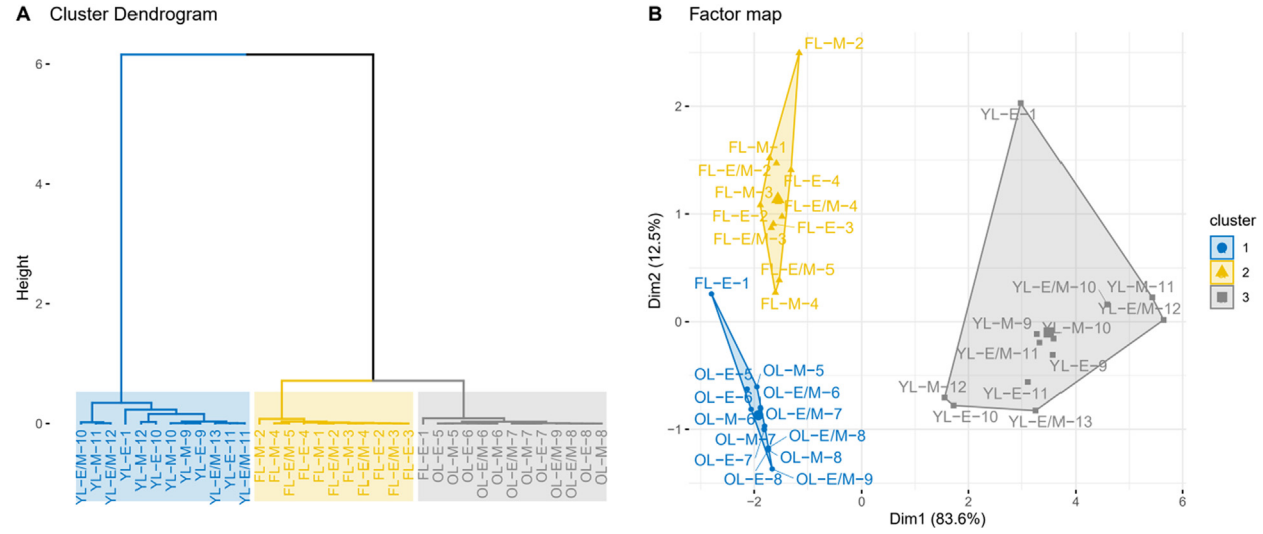

Fig. 7. Schematic overview of the PCA results of the whole experimental samples according to the MCFA concentrations.

donors used. The significance probability associated with F statistic, labeled "Pr > F," is calculated by one-way ANOVA procedure of the R software. The goal of this analysis is to identify the differences in MCFA (acetic acid to octanoic acid) production between three leachate samples of different ages and between three electron donors. Table A5 (see Appendix) shows how the factors of age and electron donor correlate with MCFA production using the results of one-way ANOVA. The result of the ANOVA showed a highly significant difference regarding age with respect to MCFA production ($F = 78.15$; $Pr > F = 2.10^{-16}$). In contrast, one-way ANOVA revealed no significant difference concerning electron donors with respect to MCFA production ($F = 0.192$; $Pr > F = 0.862$). Therefore, ANOVA confirmed the result of PCA regarding the major effect of age on global MCFA production.

4. Conclusion and outlook

In this study, we investigated the effect of the age of leachate samples and the use of different electron donors on MCFA production. Three types of leachate (OL, YL and FL) were used as feedstock in the chain elongation process by adding three different external electron donors (E, M, E/M) for the duration of one week. The investigation on MCFA production potential showed the significant positive effect of longer retention time on the accumulation of the different carboxylic acids. In addition, age is relevant to leachate composition and characterization, which explains its high influence on the production of MCFAs and their composition. OL showed poor MCFA production (less than 700 mg/L for the total MCFA) during the whole performed experiments due to the advanced stage of degradation of the sample and the exhausted carbon source, which is basically needed for the chain elongation process. However, the highest MCFA concentration was observed for the substrate YL. In addition, the FL was characterized by the predominance of acetic acid production in all the three experiments carried out, with the initiation of the production of carboxylic acids with longer chains. Among all the external electron donors, M and E/M experiments showed high MCFA production rates with a prevalence of odd-numbered fatty acids production.

Nonetheless, FL needs to be pre-treated, treated by hydrolysis or acidification or in an advanced step of fermentation, to be involved in the chain elongation process. Consequently, YL is a promising substrate to conduct chain elongation process for two reasons: firstly, we detected huge production rates of MCFA on the output side; secondly, on the input side, this substrate is produced in large amounts in the investigated controlled landfill. Young leachate can be a suitable resource to produce bio-based products, so this waste product can be valorized into a secondary raw material. Moreover, the use of the different external electron donors, especially methanol and the co-electron donor E/M, showed an encouraging potential for MCFA production. Finally, the results of this paper suggest further research regarding the investigation of several ratios of external electron donors as well as the integration of an extractive digestion process for enhancing MCFA productivity and eliminating their toxicity.

In order to optimize the process of producing MCFAs and to integrate it into waste treatment facilities, more investigations are required. Firstly, it is essential to control conditions such as pH and temperature to optimize the chain elongation process. Secondly, the external electron donors used in the experiments showed very promising results, which require much more investigations regarding the optimal ratio. Thirdly, the high demand for MCFAs in the market requires researchers to find an efficient way to extract and purify it from aqueous polar phase (leachate). Due to the economic and environmentally friendly values of MCFAs, several techniques of separation have been applied. However, in order to fulfill the abovementioned values, a liquid–liquid extraction will be tested in future experiments using green solvents (non-polar phase) as the extracting solvent. Lastly, the saturation of leachate with MCFAs or their overproduction could be toxic for the microbial community. Therefore, an extractive digestion process would be a valuable pathway for investigation at the laboratory scale.

Declaration of Competing Interest

None.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2020.10.013.

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