Bioresource Technology Reports 16 (2021) 100840

Contents lists available at ScienceDirect

Bioresource Technology Reports

journal homepage: www.sciencedirect.com/journal/bioresource-technology-reports

Assessment of the diversity and abundance of bacterial population and its correlation with medium chain fatty acids production from fermentation of two leachate qualities

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ARTICLE INFO

Keywords:
Bacterial community diversity
High-throughput sequencing
Leachate
Medium chain fatty acids
Anaerobic
Africa
Circular bioeconomy

ABSTRACT

The potential of medium chain fatty acids (MCFA) production from two seasonal leachates along with their bacterial community structure were investigated using GC-FID and Illumina high-throughput sequencing during the acid chain elongation process. Significant amounts of MCFA (C6-10) were produced during the experiment, especially with rainy season leachate. The amounts ranged from 1646 mg/l to 22,000 mg/l for C6-C8 and reached 959.65 mg/l for C9 and C10, with optimum values obtained on day 21. Bacterial communities were also different among seasonal samples, with *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* being the most abundant phyla. The relative abundance of *Lactobacillus*, *Clostridium*, and *Pseudomonas* increased during fermentation and showed positive correlations with MCFA with longer chains (C6-C10), demonstrating their probable involvement in the chain elongation process. This study enhances our understanding of the composition, structure and contribution of the bacterial community during the chain elongation process and gives value to leachate from solid waste in sub-Saharan countries.

1. Introduction

The world's population increased over the past decades, resulting in overexploitation of fossil resources and a sharp increase in greenhouse emissions, which are the main contributors to global warming. It is projected that the mentioned population will rise from 7.4 to 9.1 billion in 2040, which would lead to the depletion of fossil resources by more than 80% (IEA, 2017). It is recognized that fossil resources utilization has to be rationalized even though more energy is needed to ensure adequate living standard for the entire world's people. Therefore, the development of alternative solutions to various fossil resource products is important to slow down the depletion of fossil fuels and mitigate their adverse effects on economic development, especially in developing countries. To this end, a substantial part of the chemicals and fuels in use could be substituted with suitable substances produced from biomass sources.

According to "What a waste 2.0", a report published by the World Bank (2018), Sub-Saharan Africa generates significant amounts of waste, representing about 5% of global production (Kaza et al., 2018). The management of waste in these countries, particularly in Côte d'Ivoire, is critical. Waste recovery is more than necessary since it is known that Ivorians produce 0.64 kg of waste per capita per day on average, which is higher than the average for Sub-Saharan Africa (0.46 kg per day), and all Ivoirian waste is landfilled. Meanwhile, waste decomposition produces large amounts of leachate. Owing to the presence of various contaminants (e.g., pesticide, antibiotics, heavy metals, and chlorinated organic and inorganic salts), efficient and cost-effective treatment of leachate is challenging (O'Kelly et al., 2021).

Nevertheless, with regard to their high emission load characteristics (Côte d'Ivoire is a net sink of GHG) and their recycling value (consisting

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https://doi.org/10.1016/j.biteb.2021.100840
Received 22 September 2021; Accepted 22 September 2021
Available online 30 September 2021

of high chemical oxygen demand, polysaccharides, carboxylic acids, etc.), these generated leachates could be an appropriate feedstock for value-added products. Using them for this purpose has the advantage of settling the crucial issue of environmental pollution and human health caused by their penetration through the soil, resulting in contamination of groundwater and surface water. Also, their use as feedstock has the advantage of providing an added value for the Ivorian economy. Recent developments in waste management in the country have led to the closure of the old open-air landfill and the creation of a sanitary landfill with provision for recycling waste to biogas. Anaerobic digestion is the most widely used technology for resource recovery from waste all over the world (Raheem et al., 2017). During this process, waste will be hydrolyzed, acidified and finally converted to methane. However, methane is a product with relatively low monetary value. Thus, more recently, the utility of anaerobic fermentation in the production of organic acids (e.g. MCFA) and alcohols instead of biogas is considered as a more viable alternative in terms of circularity and valorization. MCFAs production can be achieved through a chain elongation process using various substrates and an externally supplied electron donor (Agler et al., 2012). In addition, leachate has been largely used as a substrate for MCFA production in various countries of the world, mainly in Europe, with chain length limited to 6 to 8 carbons and production disparities of 0.74–12.8 g/l (Kannengiesser et al., 2015; Menon and Lyng, 2021; Saadoun et al., 2021). However, the potential of MCFAs production from solid waste leachate in most Sub-Saharan African countries is not documented yet. Observations made in Côte d'Ivoire during leachate collection over the months showed differences in quality with regard to the seasons (rainy and dry). Leachates collected during dry season appeared darker with higher density and a more pronounced odor, suggesting a variable ecological system that harbors changing microbial assemblages, degradation activities and ability, and ecological features due to unique adaption and evolution of microbiome under external pressures. These leachates could be converted to bioproducts such as MCFAs through anaerobic digestion (AD) by consortia of microbial communities. In this sense, particular attention is put on these microbial communities, and different culture-dependent and culture-independent techniques have been used for their characterization and to study their potential functions in leachate (Song et al., 2015; Zhao et al., 2021). However, based on the literature, microbial population structure and diversity and their involvement in MCFA production are far from being extensively characterized; even so, understanding the MCFA production potential and bacterial community structure seems to be important and critical for the anaerobic digestion process improvement and management.

Therefore, by considering the quality variation of leachates according to climate and season (rainy or dry), which could influence the production of MCFA and, consequently, the production yield, we use different approaches to profile MCFA production and the associated bacterial community in bioreactors and further examine the interactions between this microbiota and the carboxylic acid chain elongation process. Subsequently, this study explores the potential of MCFA production from different qualities of leachates (rainy season leachate « LRS » and dry season leachate «LDS»), and the results identify global relationships and highlight potential bacteria that could synthesize these fatty acids. In the end, the valorization of MCFA from leachate and the resulting production of biobased products from MCFA can significantly contribute to a circular bioeconomy (Stegmann et al., 2020).

2. Materials and methods

2.1. Sampling site and leachate collection

The leachate used in this study was collected from the biggest waste transfer station of Abidjan district, located in Abobo-Dokoui (5°22'43.94"N 4°0'22.84"W). This station is the most modern in Abidjan, and it receives waste from three main municipalities housing populations of different social levels, in particular the most upscale areas, the most populated areas, and the less well-off areas. Before the leachate collection, waste from this station was sorted on a three-day interval basis according to the method described by Kannengiesser et al. (2015), and then different spots of leachate were identified throughout the station and characterized in situ (pH and electrical conductivity measurement). The climate in Côte d'Ivoire is humid, with a very high rainfall. The year is characterized by major and small rainy seasons that last 7-8 months and major and small dry seasons that last 4-5 months. An average of 1761.04 mm of rainfall per year is recorded (SODEXAM, 2017), and it can reach 600 mm per month during the rainy seasons and slightly more than 100 mm per month during the dry seasons (Kouadio et al., 2011), thus ensuring a viable flow of leachate for MCFA production. The collection of leachate was first done during the rainy season (September to November 2018) and repeated in the dry season (February to April 2019). For the collection, 10-liter sterile flasks were placed at the base of the waste collection containers to collect leachate. The collected leachate was then stored in a cooler and transported to the laboratory for analysis.

2.2. Preliminary characterization of samples

The characterization of leachates from the collecting periods was achieved to determine the quality of samples to be used for the chain elongation process. The characterization consists of determining the pH by using a multi-parameter probe (Electro photometric Multi-parameter HI2829), chemical organic demand (COD), and total organic acids using respectively the LCK 514 and LCK 365 test cuvettes following the manufacturer's instructions, with a HACH Lange DR 2800 photometer for readings.

2.3. Bioreactor set up for chain elongation process experiments

Carboxylic acid chain elongation was performed in bioreactors built according to Kannengiesser et al. (2015 and 2018). About 8 l of each type of leachate (LDS and LRS) were transferred into 10-l sterile canisters after pH adjustment to about 5.5-6.5 and kept for 24 h at ambient temperature (32-35 °C) to ensure pH stability. Once pH was stabilized, an electron donor (absolute ethanol in this case) at a rate of 1% (*v*/v) was added to the leachate, and the recipients were sealed and left at ambient temperature for 7 days. Then, 800 ml of an extraction solvent (biodiesel), prepared following the method described by Ma and Hanna (1999), was added. The biodiesel creates anaerobic condition in the bioreactors, and its hydrophobicity allows the extraction of the fatty acids produced by a liquid-liquid extraction phenomenon. Then, the non-polar MCFAs produced are extracted from the percolate in order to avoid an accumulation that could inhibit the activity of the bacteria responsible for the chain elongation process. The whole process lasted for 5 weeks, and samples (50 ml of leachate and 30 ml of biodiesel) were taken weekly for organic acids, COD, MCFA, and bacterial community analyses. The experiment was performed in triplicates under a controlled pH system in batch test reactors.

2.4. MCFAs analysis

The MCFA in the percolate and biodiesel were analyzed using a gas chromatograph (Agilent 7890B) connected with a flame ionization detector (FID) and mounted with a liquid injection autosampler (PAL3 CTC Analytics AG, Swiss). Before the GC-FID analysis, the substrate samples were diluted at a ratio of 1:10 with Milli-Q water, and the pH was adjusted to between 1.5 and 2.0 by adding 1 N HCl solution, while those from the extraction solvent were diluted at 1:10 with n-Hexane (n-Hexane >99%, Roth). Both sample types were then filtered using a microfilter (0.45 µm Polyethersulfone, VWR International, USA) before the GC-FID analysis. TG WAXMS-A (30 m; i.d. 0.32 mm; thickness: 0.50 µm; stationary phase: polyethylene glycol; Thermo Scientifics, Dreieich) was the column used for this study. For each measurement, 0.5 µl of

samples were injected into a split/splitless injector heated at 260 °C and analyzed at a split ratio of 1:10. The GC oven program was set as follows for substrate samples: initial temp of 60 °C for 2 min; 10 °C/min till 80 °C; 21.665 °C/min till 145 °C; 6.1 °C/min till 205 °C; 205 °C for 10 min. For extraction solvent samples, it was set as follows: initial temp of 80 °C for 1 min; 20 °C/min till 120 °C; 6.1 °C/min till 175 °C; 4 °C/min till 185 °C; 6.1 °C/min till 205 °C; 10 °C/min till 220 °C; 220 °C for 23.5 min.

2.5. Bacterial community analysis

2.5.1. Extraction, amplification of DNA, and illumina MiSeQ library preparation

Three leachate samples from each collection period and the triplicates of each elongation process collection time were extracted and pooled to obtain the complete bacterial community information. An aliquot of 2 ml of each leachate pool was centrifuged at 8000 rpm for 15 min, and metagenomic DNA was extracted from the resulting pellets using E.Z.N.A soil DNA kit (OMEGA bio-tek, Georgia, USA). The DNA obtained was then quantified with a NanoDrop 2000 (Thermo Scientific, USA) and stored at −20 °C until further processing. The amplification of the 16S rRNA gene V4-V5 hypervariable region was carried out with 0.2 μM of each forward 515F (5′- GTGYCAGCMGCCGCGGTAA-3) and reverse 926R (5- CCGYCAATTYMTTTRAGTTT-3′) primers according to Parada et al. (2016). The reaction mix contained 1× Phusion HF buffer, 0.2 nM dNTPs, 3% DMSO, 0.5 Phusion Hot Start II DNA pol. (Biolabs, New England), 30 ng of DNA and nuclease-free water to make up a total volume of 25 μl. PCR conditions consisted of initial denaturation at 94 °C for 3 min followed by 35 cycles of 94 °C for 45 s, 50 °C for 60 s, 72 °C for 90 s and a final extension at 72 °C for 10 min. The equimolar DNA concentration of each purified amplicon sample was sent for Illumina MiSeq PE 300 sequencing to the genomics platform of CERMO FC (Montreal, Quebec, Canada).

2.5.2. Data processing and analysis

Analysis of raw sequences from the leachate samples was done using QIIME 2 2020.8 (Bolyen et al., 2019). These sequences were quality filtered and denoised using the q2-dada2 plugin from DADA2. To do this, raw FASTA files were trimmed of adapter and barcode sequences, and then poor quality ends were truncated, with a truncation length of 280 nts for forward reads and 260 nts for reverse reads. The resulting sequences were aligned to the MAFFT (Katoh and Standley, 2013), used to infer a phylogenetic tree and subsequently rooted at the midpoint using fasttree2 (Price et al., 2010) via q2-phylogeny methods. Taxonomy assignments were associated with OTUs based on the taxonomy from the Greengenes 13_8 99% OTU's as reference sequences using the q2-feature-classifier (Bokulich et al., 2018) based on the classify-sklearn naïve Bayes taxonomy classifier.

2.6. Statistical analysis

Data from QIIME 2 were imported into R version 3.6.3 software to carry out statistical analysis using various packages. The phyloseq object was used to calculate relative abundances, α diversity (Chao1 and Shannon index), β diversity and to perform statistical tests after rarefication with a depth of 90% of the minimum sample depth in the dataset. Relative taxa abundances were plotted from the microbiome package (Lahti et al., 2017). To assess the significance of α diversity, the Kruskal–Willis test was performed. The Bray-Curtis dissimilarity matrix (Legendre and Legendre, 1998) was used to perform the principal coordinate analysis (PCoA) of the samples and the visualization using the vegan package (Oksanen et al., 2019). The statistical significance of clusters obtained from PCoA was tested with stratified permutational multivariate analysis of variance (PERMANOVA) and analysis of similarities (ANOSIM) with 999 permutations using the vegan package. The *p*-values for multiple comparisons were adjusted according to the Benjamini and Hochberg method of controlling false discovery rate (Benjamini and Hochberg, 1995) to assess the statistical significance ($P < 0.05$). Heatmap analysis was performed from the top 10 OTUs after log-transformation (log xi +1) of data, and the plot was done using a microbiome package (Lahti et al., 2017). The strengths of correlations between bacterial species and MCFAs were estimated using Spearman correlation coefficients and visualized using a microbiome package (Lahti et al., 2017).

2.7. Sequence data accession number

All sequence data in this study have been deposited in Sequence Read Archive (SRA) of NCBI database (https://www.ncbi.nlm.nih.gov/) as Bio project under the accession number PRJNA602130.

3. Results and discussion

3.1. Waste composition

The largest waste transfer station in Abidjan, Abobo-Dokoui transfer station, receives mostly biological wastes mainly composed of putrescibles (kitchen waste, fruits, etc.) and green waste at average rates of 51% and 6% respectively, which is consistent with previous waste sorting results in Akouedo landfill (Abidjan) (Cyril et al., 2018) (see also Table A1 in Appendix A). The waste sorting revealed relatively the same amounts of waste fractions in both sampling seasons (Table A1). The heterogeneity due to the lack of upstream sorting and the composition of these wastes make them appropriate for use as a raw material in bioconversion processes. The high biodegradable rate could serve as a guide for bioconversion programs, such as MCFA production.

3.2. Preliminary characteristics of the leachates under study

Table 1 shows significant differences regarding the preliminary characteristics of the collected leachate samples.

The leachates collected from this waste transfer station in the rainy and dry seasons were acidic, with a lower pH (3.69 ± 0.01 against 4.16 ± 0.03) in the dry season. This acidic feature could initially be explained by the accumulation of volatile fatty acids resulting from the predominance of hydrolytic and acidogenic bacteria, which colonize the first steps of organic waste decomposition, as reported by Sun et al. (2011). High-throughput Illumina MiSeq Amplicon sequencing of bacteria communities associated with these leachates revealed a high dominance of *Lactobacillus* genus, which are recognized to have high acidifying power by the production of various organic acids, such as lactic and acetic acids. Moreover, the difference in pH within seasons was also reported by Rafizul and Alamgi (2012) and Zhao et al. (2013), and it may be due to the variation of the temperature as it has been reported that high temperature can result in a lower pH (Rafizul and Alamgi, 2012). With average values of 185,000 mgO_2/l and 57,600 mg/l respectively for COD and organic acids, leachates collected during the dry season were approximately eight times more concentrated than those collected in the rainy season and even more concentrated than leachate in previous studies from other countries (Saadoun et al., 2021).

Table 1
pH and organic content of the collected leachate in dry and rainy season at the transfer station (Abobo-Dokoui) of Abidjan.

Parameter	Collection seasons	
	Dry season	Rainy season
pH	3.69 ± 0.01[a]	4.16 ± 0.03[b]
COD (mg/l)	185,000[a]	21,900[b]
Organic acids (mg/l)	57,600[a]	7920[b]

Values are expressed as mean ± sd. In a same line. Means with the same letter are not significantly different (α = 0.05).

The high difference in COD and organic acid contents between seasons seems to derive from dilution due to rain. However, these values indicate a very high organic content, showing that these samples are under reducing conditions (Mejraoua and Zine, 2017) and could constitute valuable feedstock for bioconversion processes, notably MCFA production, as the chain elongation process is highly dependent on acetic acid (Spirito et al., 2014). However, this was not the case with LDS, probably due to the toxic effects of the very high acetic acid content on certain bacterial communities.

3.3. Changes in COD, organic acid contents, and MCFA production during the chain elongation process

The changes in COD and total organic acid contents during the chain elongation process of MCFA production for both types of leachates (LDS and LRS) are presented in Fig. 1. After the addition of ethanol as an electron donor to initiate the chain elongation process, an increase in COD and organic acid contents were observed for both leachate types in the earlier stages of the process. This increase was more noticeable in the bioreactor with leachate collected in rainy season, in which organic acids contents (initially 7920 mg/l) doubled in just one week and reached 18,240 mg/l after 14 days before decreasing. Meanwhile, COD increased continuously and reached 78,100 mgO_2/l (more than three times the initial value) after 21 days; it decreased thereafter. During fermentation of LDS, both parameters also increased but at a slower rate. Investigations carried out in previous studies showed that both parameters could serve as indicators of the operating state of bioreactors as well as chain elongation (Roghair et al., 2018). The increase in the

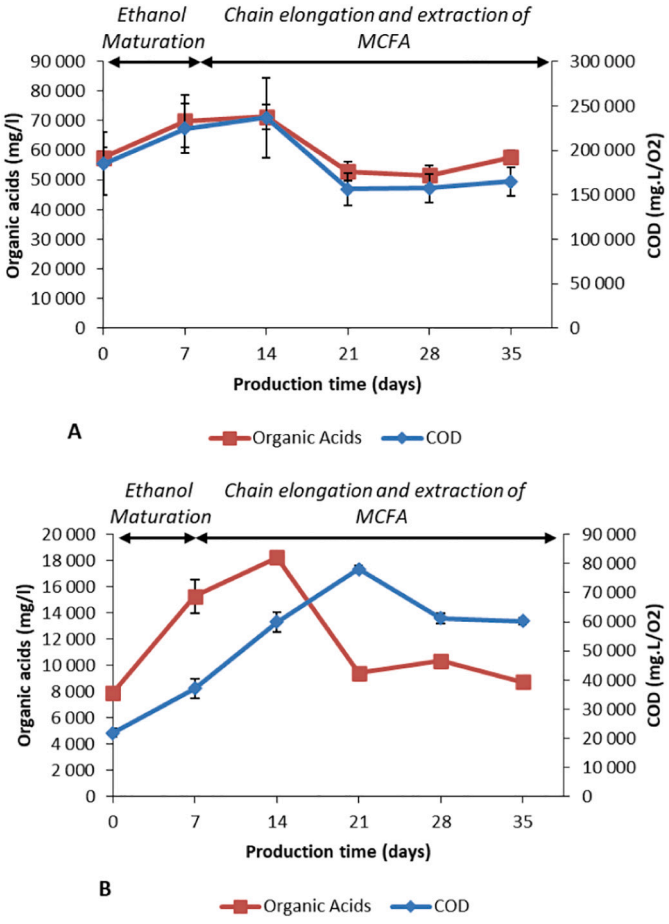

Fig. 1. Change in COD and total organic acids contents during MCFA production from leachates. A: production with dry season leachate; B: production with rainy season leachate.

concentration of organic elements in the earlier stages in both fermenting substrates may be due to the addition of ethanol, which is also an organic compound. However, the low increase rate observed in the LDS bioreactor during the first stages of the process may be equivalent to a dysfunction during the elongation process. The most plausible explanation for this dysfunction is the initially high level of organic acid acting as an inhibitor or limiting factor for microbial activities. Warnecke and Gill (2005) reported the inhibition of microbial growth by acetate and other organic acids as one of the major problems of fermentation processes in biotechnology.

The profile of MCFAs in controlled pH conditions, as well as the amounts produced during the experiment, are shown in Fig. 2. Fig. 2 shows that the pH remained stable at about 5.7 until the end of the process in the LDS and LRS bioreactors, while various fatty acids were produced and accumulated either in the percolate or in the biodiesel. The results showed changes in the production of MCFA based on the type of leachate (LDS and LRS) and over time. Acetic acid was the major product in the crude leachates, representing about 88% and 27% of the total fatty acids respectively in LDS and LRS, equivalent to 8763.55 mg/l and 2234.74 mg/l. The abundance of acetic acid in both leachates is obvious (the third stage of anaerobic digestion is acetogenesis, which leads to the formation of acetic acid and hydrogen from the degradation products of the two previous stages), as it is frequently encountered in hydrolyzed and acidified organic waste (Arslan et al., 2017) and is also reported by a previous study on the same substrate (Saadoun et al., 2021). Moreover, under anaerobic condition and in the presence of ethanol, an excessive oxidation reaction of ethanol to form acetate and hydrogen can occur. Excessive oxidation of ethanol produces acetate (Roghair et al., 2018) and results in a large increase in the acetic acid content of the substrate. During the elongation process, acetic acid remained the major component (at least 85%) in the LDS bioreactor, while it was surpassed by other types of fatty acids, notably pentanoic, hexanoic, heptanoic, and octanoic acids, in the LRS bioreactor. Their amounts continuously increased, reaching the maximum after 21 days (Fig. 2B). The amounts ranged from 1646 mg/l for octanoic to more than 22,000 mg/l for hexanoic and heptanoic acids. Substantial amounts of nonanoic and decanoic acids were also produced in this bioreactor, with the highest contents (959.65 mg/l and 305.81 mg/l respectively) recorded on the 21st day. This finding demonstrates the particularity of Abidjan leachates since previous thoughts limited the ability to elongate fatty acid chains to just 8 carbon atoms, as reported by Kannengiesser et al. (2018). Furthermore, the presence of a sufficiently high concentration of butyric acid (1644.47 mg/l), as observed in the crude LRS, seems to be essential for the formation of isobutyrate and, therefore, is potentially beneficial for the production of hexanoic and octanoic acids, as reported by Chen et al. (2020). Overall, the elongation process with LRS led to the production from the short chain fatty acids (acetic, propanoic, and butanoic) in raw leachate of large amounts of MCFA, which ultimately represented more than 90% of total fatty acids (Fig. 3B). Furthermore, the amounts of MCFA obtained with this type of leachate were quite considerable and far greater than most of those reported by Menon and Lyng (2021). In the LDS bioreactor, MCFA production remained low or even negligible (387.4 mg/l of hexanoic, 88.5 mg/l of heptanoic, 128.5 mg/l of octanoic, 67.9 mg/l of nonanoic, and 30.4 mg/l of decanoic acid at the end of the process), confirming the dysfunction of the production process, which may be due to an excess of waste hydrolysis products, like acetic acid, hydrogen, and carbon dioxide. At very high concentrations, as measured in LDS, acetic acid becomes toxic to microorganisms by causing damage to their cell membrane elongation (Pinhal et al., 2019). Therefore, this inhibits the microorganisms responsible for chain elongation, explaining the low rate of MCFA with this type of leachate. This low production of MCFA could also be related to hydrogen partial pressure in the anaerobic digestion process, which has been reported to inhibit the chain elongation process (Ge et al., 2015; Zhou et al., 2017).

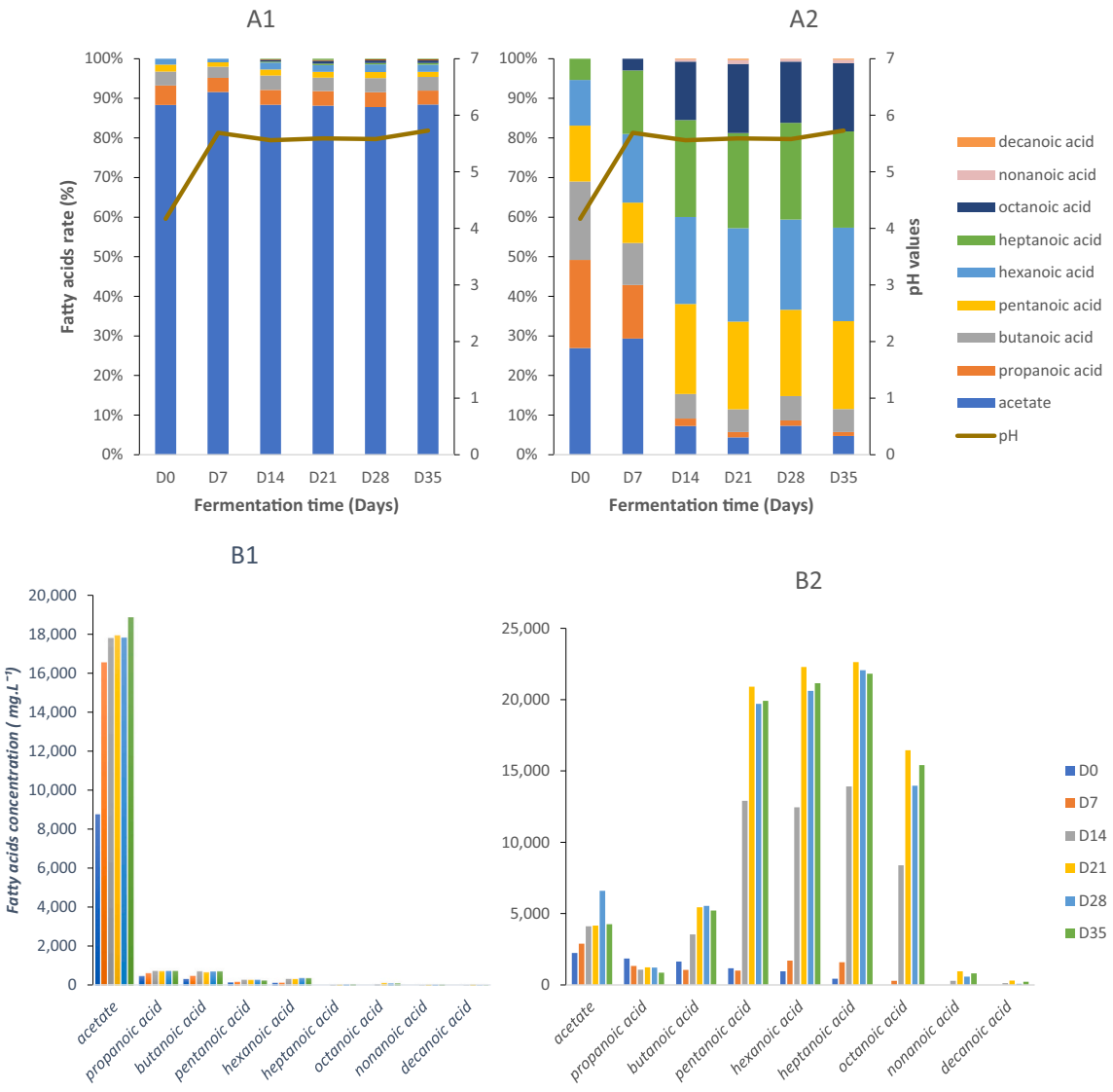

Fig. 2. Relative abundance (A) and concentration of MCFA produced (B) from two leachates quality over the course of 5 weeks. 1: production with dry season leachate; 2: production with rain season leachate.

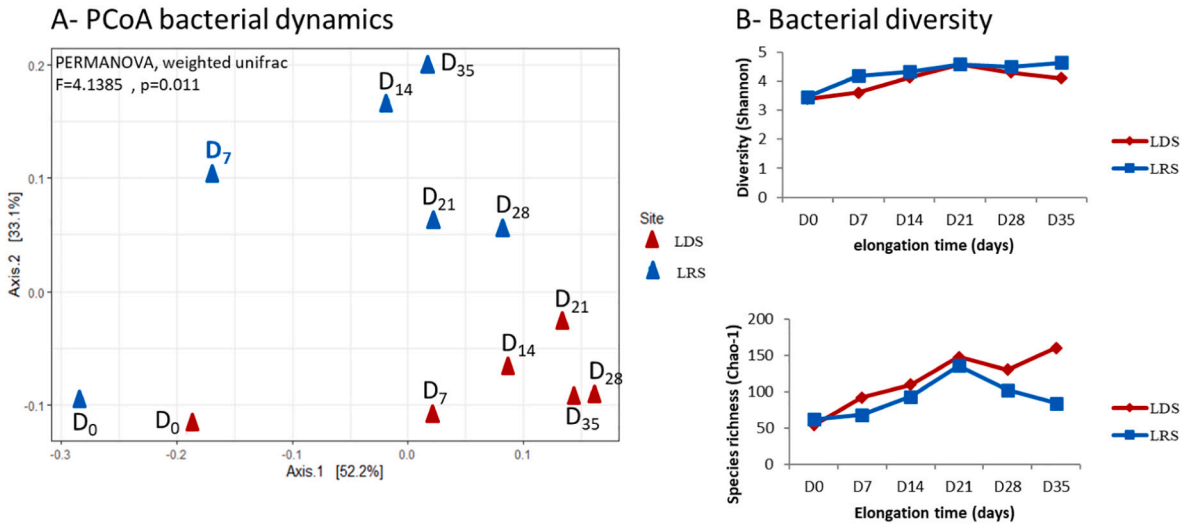

Fig. 3. Principal coordinate analysis (PCoA) plot based on the calculated distances in a weighted UniFrac matrix showing the dissimilarity of bacterial communities over the course of MCFA production (A) and the shift in bacterial diversity represented by Shannon index and Chao 1 richness (B) in both qualities of leachate.

3.4. Diversity and dynamics of bacterial community and their correlation with MCFA production

3.4.1. Diversity and dynamics of bacterial community structure

The V4-V5 region of 16S rRNA gene high throughput sequencing has been used for the first time to characterize and determine the changes in the bacterial community structure and the diversity of leachates from a sub-Saharan country as a substrate for MCFA production. The overall bacterial community structure and dynamics during the fermentation of leachates collected during both seasons (rainy and dry seasons) were analyzed by PCoA using Bray–Curtis dissimilarity based on the species-level OTUs relative abundances over the course of 35 days. Even the addition of ethanol, as an electron donor, seems to act as a modulator of the bacterial community structure by the elimination of sensitive flora (seen in the great proximity between the starting variables D0 on the plot). The bacterial community present during the chain elongation process significantly differed between both types of fermenting leachates ($p = 0.011$, $F = 4.138$, PERMANOVA) (Fig. 3). The PCoA score plot shows that the bacterial community during the rainy season leachate (LRS) fermentation progressed rapidly. As shown in Fig. 3A, the first fast score plot shift occurring in the earlier stages (first to 14th day) in the LRS bioreactor and its maintenance along axes 1 and 2 with positive

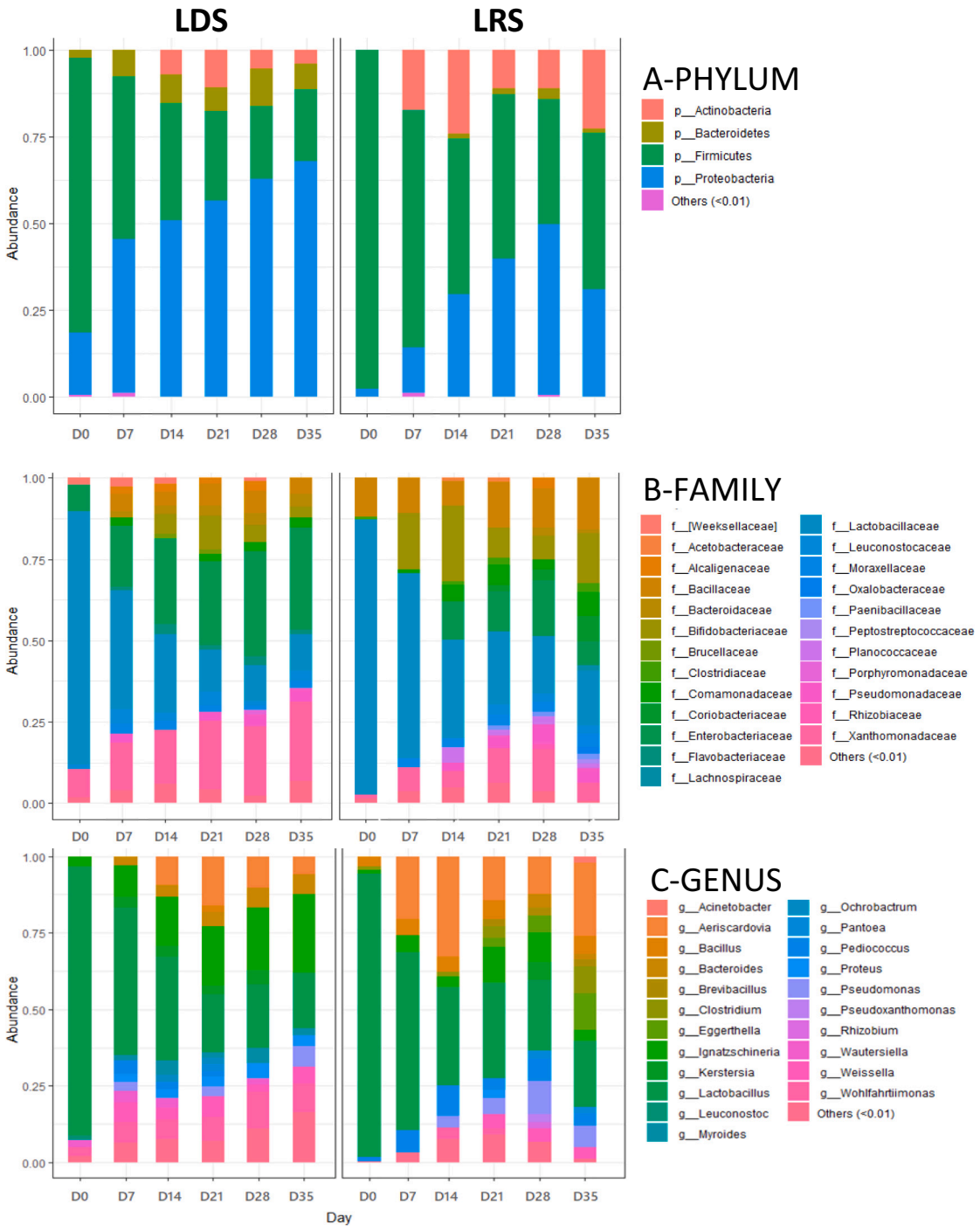

Fig. 4. The composition and relative abundance of bacterial community at phylum (A), family (B) and genus (C) levels, over the course of 5 weeks of MCFA production from two qualities of leachates (LDS and LRS).

correlation is in accordance with an intense chain elongation activity leading to important production of MCFAs (Fig. 2B). In parallel, the score plot shifted in the LDS bioreactor along axis 2 with a negative correlation, which might corroborate the hypothesis of the dysfunction of the chain elongation process in this bioreactor. However, the comparison of diversity and richness throughout the entire elongation process, estimated by the Shannon index and Chao-1, show similar trends of increase for both types of leachates (Fig. 3B). The findings suggest that community richness and diversity differ at each chain elongation step with different patterns. Thus, each sampling season presents a unique feature due to various factors, resulting in the separation of fermenting leachate samples from both seasons in the PCoA plot. Song et al. (2015) points out that the separation or clustering between samples depends on the unique or shared abundant OTUs in different samples and considered heterogeneous physicochemical properties and geographical locations of landfills as the main factors affecting the microbial community structure in landfill leachate. In this study, the factors contributing to the differences may derive from climatic conditions, waste composition, and collection duration. These factors can result, to some extent, in leachate chemical parameters' variations and may influence the distribution of the bacterial communities, as previously observed by Zhao et al. (2021).

The differences in the bacterial community composition at the different times points of MCFA production were also observed by comparing their relative abundances at different taxonomic levels, notably phylum, family, and genus levels (Fig. 4). The results show that 99% of the total OTUs are represented by 12 phyla, among which *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *and Bacteroidetes* are the four most dominant groups, accounting for more than 99% of the totals.

At the beginning of the process, after the addition of ethanol, the medium was dominated by *Firmicutes* in the LDS and LRS bioreactors, but with a difference in their relative abundance (78.12% and 97.63% respectively in LDS and LRS). This phylum was followed in the LDS bioreactor by *Proteobacteria* (14.76%) and *Bacteroidetes* (2.37%), whose relative abundance was negligible in LRS samples. Throughout the fermentation process, the relative abundance of *Proteobacteria* continuously increased in LDS and reached about 70%, thus becoming the predominant phylum by surpassing *Firmicutes*. *Bacteroidetes* also increased from 2.37 to reach about 10% after 28 days. In the LRS bioreactor, the relative abundance of *Firmicutes* gradually decreased, but it remained the dominant phylum. We also noticed a meteoric appearance of *Actinobacteria*, whose relative abundance varied between 10% and 25% throughout the process (Fig. 4A). The variation of bacterial community composition suggests that microbial succession occurred during the chain elongation process, depending on the leachate type. The dominance of proteobacteria in leachate samples was previously reported by D'Costa et al. (2006) and Song et al. (2015), and it has been suggested that these groups of bacteria may play important roles in the degradation of aromatic oils (Vukanti et al., 2009) and denitrification (Hu et al., 2012). According to Eichorst et al. (2013), *Firmicutes* and *Bacteroides* are respectively responsible for the hydrolysis of cellulose into sugars and the metabolization of these sugars into carboxylic acids, alcohols, carbon dioxide, and hydrogen for the production of methane in the waste decomposition process. The phylum *Firmicutes* is frequently associated with antibiotic resistance, and *Actinobacteria* is a group of antibiotic-producing bacteria that is well-known for being often detected with multi-resistance (D'Costa et al., 2006). Thus, interactions between both phyla may occur to the detriment of others in the LRS bioreactor, which could justify the high chain elongation activity, as it is known that *Actinobacteria* could perform various functions, including degradation/decomposition of all kinds of organic substances, such as cellulose, polysaccharides, protein fats, and organic acids, while producing a variety of secondary metabolites of high interest to the pharmacological and commercial industries. (Anandan et al., 2016). *Firmicutes* and *Actinobacteria* are cited as the most abundant microbial community on the switchgrass stillage during MCFA production and listed as harboring microorganisms with potential roles in the synthesis of MCFA (e.g. *Clostridium Kluyveri*, *Megasphaera elsdenii*, etc.) (Scarborough et al., 2018b).

In this study, the dominant bacterial genera were affiliated with the dominant phyla, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*. These genera included *Lactobacillus*, *Bacillus*, *Clostridium*, *Pediococcus*, *Weissella* (affiliated to *Firmicutes*), *Eggerthella*, *Aeriscardovia* (affiliated to *Actinobacteria*), *Ignatzschineri*, *Kerstersia*, and *Pseudomonas* (affiliated to *Proteobacteria*) (Fig. 4C). A clustered heatmap based on the relative abundance of significantly differing predominant bacterial species shows a distinct and dynamic bacterial community profile in both types of fermenting leachates, with unique species such as *Proteus myxofaciens*, *Myroides odoratimimus*, and *kersterisia gyiorum* present in LDS and *Aeriscardovia aeriphila*, and *Bacillus megaterium* found in LRS (Fig. 5).

The genera *Bacillus* and *Clostridium* show increasing relative abundances over time, reaching a maximum of 14.80% and 22% respectively on day 35 in the LRS bioreactor. Previous studies using shotgun metagenomic analysis reported the supremacy of *Clostridium* and its dominance in the major metabolic pathways of the chain elongation process (Agler et al., 2012). *Kerstersia* was observed only on days 21 and 28 (with respective relative abundances of 1.66% and 10.06%). *Weissella* with an abundance of 8.53% on day 21 reached an abundance of 10% on day 35, and *Eggerthella* appeared only on day 35 with an abundance of 30%. Fluctuations were also observed in the relative abundance of *Ignatzschineria* and *Pediococcus* during the 35 days of the experiment ($P < 0.05$), with maximum values of 21.58% and 18.67% obtained on day 21 and 14 respectively.

The increase over time of *Bacillus* and *Clostridium* and the strong predominance of *Lactobacillus* coincide and were correlated with increase in the content of MCFA (C6 to C10). This relationship is similar to those previously suggested (Andersen et al., 2017; Scarborough et al., 2018a, 2018b), where *Megasphaera* and *Pseudoramibacter* used lactate generated by *Lactobacillus* to produce MCFA.

3.4.2. Correlation between bacterial communities and MCFA production

The Spearman correlation coefficients used to establish relationships between MCFA and bacterial communities showed that some of the taxa identified at the genus level were significantly correlated with the various MCFA produced during the chain elongation process (Fig. 6). This implies that these genera may be responsible for the synthesis of particular MCFA or may be intermediates in the synthesis. This behavior was especially notable for the genus *Lactobacillus*, which displayed significant correlations with a large variety of MCFA, from C4 to C8 (Fig. 6), with the highest correlations with C7 and C8 ($r = 0.7$). At the same time, *Pseudomonas* preferentially showed a high correlation ($r = 0.8$) with nonanoic acid (C9) and a lesser correlation with decanoic acid (C10). Similarly, *Clostridium* had a good correlation with nonanoic acid ($r = 0.6$) and a small correlation with decanoic acid ($r = 0.2$). The other genera, namely *Aeriscardovia*, *Bacteroides*, *Pantoe*, and *Weissela*, had a positive low correlation with C9 (r between 2 and 3).

The genera *Lactobacillus* and *Clostridium* (*Firmicutes*) have often been associated with the production of MCFA. Species of *Lactobacillus* and other lactic acid bacteria, notably *Leuconostoc* and *Weissella*, were also detected in this study, and were recognized to produce only lactic acid by homofermentation or lactic acid, acetic acid, and ethanol by heterofermentation (Hwang and Lee, 2019; Cruz Ramos et al., 2000). The lactic acid produced can be used as a substrate for MCFA production, as already demonstrated by Kucek et al. (2016) and Scarborough et al. (2018a), either by species of this genus or by other groups such as *Clostridium*, whose species *C. kluyveri* has been largely documented as an MCFA producer (Reddy et al., 2017). The genera of *Lactobacillus* and *Bacillus* can ferment sugars into lactic acid, which can be used by species of *Clostridium* and any related genus during chain elongation. Since *Pseudomonas* has already been cited among microorganisms degrading waste, its involvement in MCFA biosynthesis has to be elucidated. Thus, its strong correlation with higher MCFA is a finding that should open up perspectives for studying its actual involvement in fatty acid chain

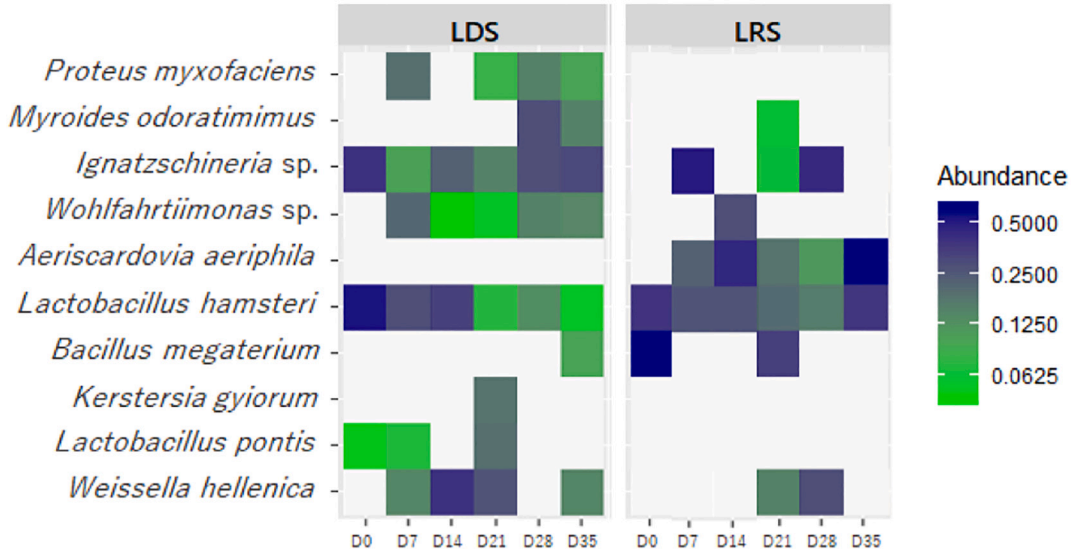

Fig. 5. A hierarchically clustered heat map shows the microbial species-level differential abundance in both types of leachates. The significantly differencing 10 bacterial species ($q < 0.0001$, Wilcoxon test, BH corrected) between both leachate qualities (LDS and LRS) are clustered here. The abundance difference is shown as a color key with green and blue color gradient. The heat map was generated using R version 3.6.3 software (https://cran.r-project.org/bin/windows/base/old/3.6.3/) after normalization [log10 (xi +1)-transformed] of The ASV relative abundance data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

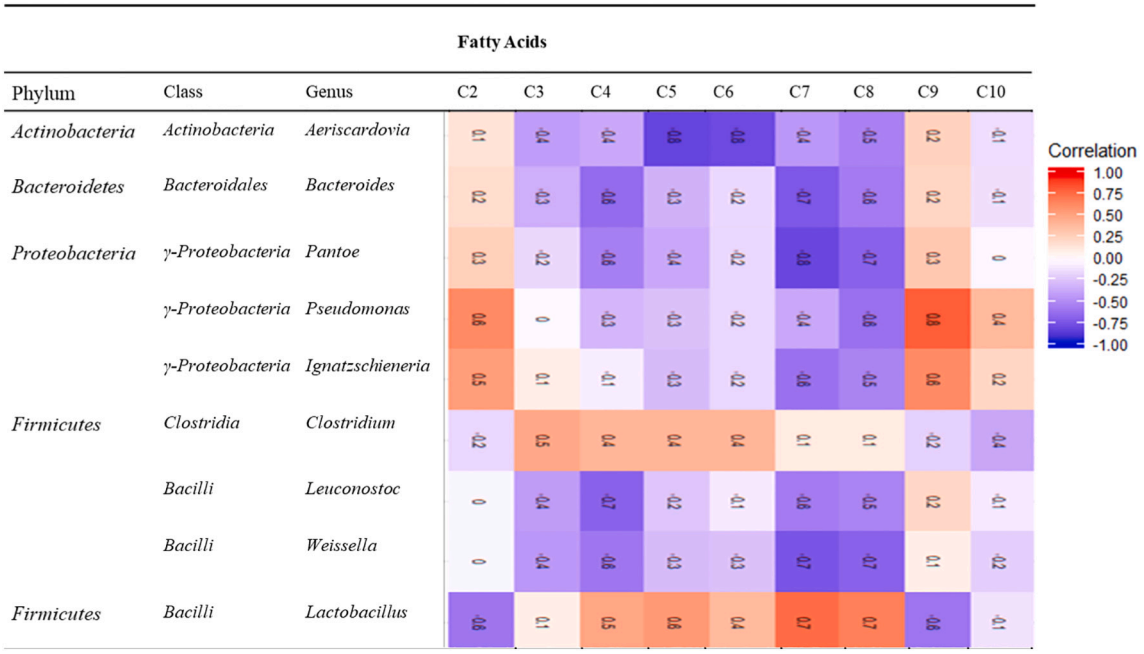

Fig. 6. Spearman correlation coefficients showing relationships between bacterial communities and MCFAs contents produced from household waste leachates. The significance of the correlation is shown as a color key with blue and red color gradient. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

elongation processes. Previous authors have highlighted the presence of the fatty acid synthesis (FAS) initiating enzyme in *Pseudomonas aeruginosa*, which may be involved in the extension of a key intermediate (β-keto decanoyl-ACP) to supply the cellular fatty acid needs (Yuan et al., 2012).

4. Conclusion

This study shows that the characteristic variation of leachates according to seasons (rainy or dry) has a high influence on the production of MCFAs. The highest MCFA contents were obtained from leachates collected in rainy season, whose volatile fatty acids (C2 to C4) were elongated to carboxylic acids with longer chains (C6 to C10), with C7 and C8 being the most abundant. The microbial diversity and structure exhibited a clear difference and a seasonal distribution pattern with *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* emerging as the main phyla. Genera *Lactobacillus*, *Clostridium*, and *Pseudomonas* displayed high correlations with the longest chain carboxylic acids, demonstrating their involvement in the chain elongation process.

Funding

The research work is part of the "Trans4Biotec" project: Know-how transfer in waste management for developing new biotechnology applications in developing countries. It is financially supported by the BMBF (German Federal Ministry of Education and Research) under grant number 01DG17011 and the DAAD (German Academic Exchange Service) under grant number 57357946.

Declaration of competing interest

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

Acknowledgments

We would like to thank all the project partners for the good collaboration spirit, particularly the IWAR Institute of the Technical University of Darmstadt, where substantial parts of the research activities were achieved. We would also like to express our gratitude to the anonymous reviewers from Bioresource Technology for their valuable and constructive feedback and for the opportunity to transfer our work to Bioresource Technology Reports.

Appendix A

Table A1
Composition of household waste sorted at Abobo-Dokoui (Abidjan) transfer station in dry and rainy season.

Type of materials		Collection seasons	
Waste	Fractions (%)	Dry season	Rainy season
	Organic matter	49.18 ± 0.06[a]	53.03 ± 0.02[b]
	Plastics	11.61 ± 0.14[a]	9.03 ± 0.11[a]
	Paper and cardboard	16.86 ± 0.23[a]	17.41 ± 0.19[a]
	Green cut	4.31 ± 0.03[a]	5.23 ± 0.05[a]
	Textile	2.88 ± 0.01[a]	3.08 ± 0.04[a]
	Glass	2.54 ± 0.12[a]	2.19 ± 0.09[a]
	Metal	1.83 ± 0.09[a]	1.94 ± 0.06[a]
	Electronics	2.43 ± 0.17[a]	2.45 ± 0.21[a]
	Complex*	0.95 ± 0.21[a]	0.98 ± 0.15[a]
	Fine material	7.41 ± 0.04[a]	5.32 ± 0.01[b]

Values are expressed as mean ± sd. In a same line. Means with the same letter are not significantly different (α = 0.05).
* Complex means waste that is heterogeneously assembled and therefore cannot be assigned to a group in the sorting table.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2021.100840.

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