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The core microbiome is responsible for volatile silicon and organic compounds degradation during anoxic lab scale biotrickling filter performance

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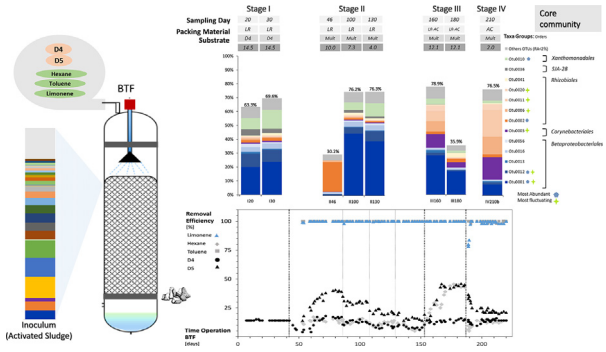
HIGHLIGHTS

- Elimination capacity of silicon in the BTF ranged from 296 to 350 mg m^{-3} h^{-1}.
- D4 removal in multicomponent operation was similar than with D4 as carbon source.
- Microbial community showed a relative low diversity with a defined core community.
- Changes in packing material were determinant in shaping the structure of biofilm.

GRAPHICAL ABSTRACT



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ABSTRACT

Volatile silicon compounds present in the biogas of anaerobic digesters can cause severe problems in the energy recovery systems, inducing costly damages. Herein, the microbial community of a lab-scale biotrickling filter (BTF) was studied while testing its biodegradation capacity on octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), in the presence of toluene, limonene and hexane. The reactor performance was tested at different empty bed residence times (EBRT) and packing materials. Community structure was analysed by bar-coded amplicon sequencing of the 16S rRNA gene. Microbial diversity and richness were higher in the inoculum and progressively decreased during BTF operation (Simpson's diversity index changing from 0.98–0.90 and Richness from 900 to 200 OTUs). Minimum diversity was found when reactor was operated at relatively low EBRT (7.3 min) using a multicomponent feed. The core community was composed of 36 OTUs (accounting for 55% of total sequences). Packing material played a key role in the community structure. *Betaproteobacteriales* were dominant in the presence of lava rock and were partially substituted by *Corynebacteriales* and *Rhizobiales* when activated carbon was added to the BTF. Despite these changes, a stable and resilient core microbiome was selected defining a set of potentially degrading bacteria for siloxane bioremoval as a complementary alternative to non-regenerative adsorption onto activated carbon.

1. Introduction

The energy and climate policies in the EU and the introduction of support schemes for promoting the utilization of renewable resources have encouraged the biogas collection and energy recovery from

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anaerobic digesters in wastewater treatment plants and urban landfills. However, the energetic utilization of biogas is severely compromised by the presence of volatile organic silicon compounds, so-called siloxanes, arising from the degradation and volatilization of organosilicon materials disposed in waste and wastewater (Papurello et al., 2012; Shen et al., 2018). Siloxanes cause severe problems in the pipelines, potentially degrading the device performance, inducing costly problems during biogas combustion, and reducing the energetic quality of the biogas (Li et al., 2016; Tansel and Surita, 2017). Thus, removal of siloxanes is a mandatory step before biogas could be used as renewable fuel (Santos-Clotas et al., 2019b; Slupek et al., 2020; Wasajja et al., 2020). Commercially available technologies are based on physicochemical separation processes (Santos-Clotas et al., 2019d), where non-regenerative adsorption onto activated carbon (AC) is the most widely used (Cabrera-Codony et al., 2021; Ruiling et al., 2017). Despite the high adsorption capacity of activated carbon towards siloxanes, the competence with other biogas impurities, such as volatile organic compounds (VOCs) usually present at higher concentration, reduces the lifespan of activated carbon filters (Cabrera-Codony et al., 2014), which implies high operational costs and environmental impact of waste disposal (Liu et al., 2019). In order to overcome these limitations, the use of biotechnologies for siloxane removal in biogas upgrading processes has been proposed (Santos-Clotas et al., 2020).

Bio-treatment of gases containing volatile compounds includes the concept of bio-filtration, such as biotrickling filters (BTFs), bioscrubbers and membrane bioreactors (Berenjian et al., 2012; Garner and Barton, 2002; Gospodarek et al., 2019; Miller and Allen, 2004; Wu et al., 2017, 2018). The effectivity of these systems is restricted to the low bioavailability of hydrophobic compounds which has been improved by means of developing models to characterize mass transfer in order to optimize BTF design and scale up (Lebrero et al., 2012) and by the addition of silicone oil or polymers (Montes et al., 2010) as well as the use of polyurethane foam as packing material to enhance the homogeneous substrate distribution (Salamanca et al., 2017). Wu and his co-authors (Wu et al., 2017) investigated the responses of microbial communities and metabolic profiles to volatile organic compounds biodegradation in bioscrubbers by using metagenomics and evaluated compounds other than siloxanes with different characteristics, i.e. hydrophilic, hydrophobic, biodegradable or recalcitrant, and identified specific microbial populations pointing to a selectivity of degrading bacteria towards the organic substrate used. In biotrickling filters, *Pseudomonas* spp. were found to be predominant in communities degrading silicon compounds from landfill and digester gases, and led to removal efficiencies up to 20% of octamethylcyclotetrasiloxane (D4) compared to a control in sterile conditions (Accettola et al., 2008; Popat and Deshusses, 2008). In other experiments, *P. aeruginosa* strain S240 exhibited a relatively high removal efficiency (74%) when D4 was used as carbon source in a lab-scale aerobic BTF (Li et al., 2014). In addition, Wang et al. (2014) proposed the degradation pathways and mechanism for D4 degradation by *Phyllobacterium myrsinacearum* isolated from the effluent of an organic silicon manufacturer.

The above cited studies show that D4 can be biodegraded by microorganisms that have been previously isolated, but thorough analyses of how the bacterial community composition changes in relation to siloxane removal in laboratory scale reactors has not been investigated in detail. Moreover, the use of molecular tools to elucidate composition and structure of microbial communities in a time basis after a bioreactor has been inoculated has led to interesting data on the selection process of the most suitable bacteria for many metabolic processes (Gonzalez-Martinez et al., 2015a, 2015b; Liu et al., 2018) but studies focused on siloxane bioremoval are still scarce. Only recently, few publications have studied the microbial communities involved in siloxane removal. More specifically, Y. Wang et al. (2019) reported an effective removal of polydimethylsiloxanes in batch experiments and identified several bacterial genera with a potential role in their biodegradation. Pascual and co-authors (Pascual et al., 2020) reported that two-phase partitioning BTF showed higher L2, L3, D4 and D5 abatement compared to conventional BTF and more than 30% of the bacterial genera at the end of both BTF comprised the genera *Reynarella*, *Chitinophaga* and the uncultured genus KBMC-112 of the family *Acidithiobacillaceae*. Moreover, Zhang et al. (2020) described the simultaneous removal of siloxane and H_2S from biogas in a biotrickling filter under acidic conditions where the microbial community was mainly composed by the genera *Ferroplasma* (*Archaea*) and *Acidiphilus* (*Bacteria*). However, the above cited studies may not represent degradation in real conditions since siloxanes are always encountered with other volatile compounds in the biogas. These additional C sources could be selected as preferred carbon sources, and alter effective siloxane degradation. In addition, these studies do not identify the core microbial community since they do not consistently asses the evolution of the microbial communities all along the reactor performance. As far as we could check in the literature, no tests had been carried out in BTF using gas stream closer to the real biogas, i.e. the simultaneous addition of siloxanes and representative VOC. Moreover, using a complex microbial community for biodegradation enables the coexistence of distinct metabolisms and promotes co-degradation. Codegradation can affect highly different compounds, such as hydrogen sulphide, siloxanes and a range of volatile organic compounds (Yang et al., 2018). Therefore, the implementation of hybrid technologies that combine absorption technologies and biological technologies is an viable alternative (Santos-Clotas et al., 2019b; G. Wang et al., 2019). Previous studies conducted in our research group (Santos-Clotas et al., 2019a, 2019b, 2020) reported interesting results obtained in scenarios of application of hybrid technologies that support this claim. Accordingly, in this study we aimed to elucidate the changes of a core microbial community involved in siloxane removal from synthetic biogas in the presence of other organic volatile compounds simulating realistic conditions during the BTF performance in order to infer putative metabolic capabilities from the molecular identification of the relevant members of the community during the siloxane degradation process. To do so, the composition of the microbial community was determined in an anoxic BTF supplemented with a mixture of siloxanes (D4 and D5) and volatile organic compounds usually found in biogas (i.e., toluene, limonene and hexane), during several operational conditions including changes in the carbon source, the empty bed residence time (EBRT) and the type of solid packing material.

2. Materials and methods

2.1. BTF design and operation conditions

A biotrickling filter (BTF) was operated as described in Santos-Clotas et al. (2019a) (Supplementary information 1, Fig. S1). Briefly, a cylindrical column made in Plexiglas (inner diameter: 6 cm, height: 46 cm) was constructed and operated in a counter-current configuration. Inside the column the packing material was distributed homogenously. Two types of packing material were combined along the operational conditions in order to get benefit from the properties of both of them. More precisely, lava rock was initially used as inert packing material (particle size: 8–12 mm) to promote biofilm growth since some studies have reported a high affinity to biofilm formation with this type of material (Campos et al., 2009; Gorbushina, 2007; Lu et al., 2018; Singh et al., 2006; Watnick and Kolter, 2000). In a subsequent stage, a layer of granular activated carbon (particle size: 2–3 mm) was added with the aim of improving siloxane bioavailability through siloxane adsorption to the packing material and/or silanediol formation via siloxane hydrolysis promoted by the surface of activated carbons (Cabrera-Codony et al., 2018). Finally, the operation was tested using activated carbon as sole packing material once biofilm was effectively developed on this material. A trickling solution of synthetic mineral medium (composition detail in next section) was continuously recirculated from an external reservoir by a peristaltic pump at a rate of 47 cm h^{-1}. The synthetic

solution was sprayed through the top of the bed column, and was renewed every 72 h.

The feed gas was generated by a syringe pump (11 Elite, Harvard Apparatus) used for infusing the target compounds to a N_2 gas stream regulated by means of a mass flow controller (MC Series, Alicat Scientific). The inlet and outlet composition of the gas stream was continuously monitored and analysed by GC-FID (7890B Agilent Technologies) in order to calculate both the removal efficiency (RE) and the elimination capacity (EC) following the procedures described in our previous work (Santos-Clotas et al., 2019a).

The bioreactor was initially operated under abiotic conditions (non-inoculated) for a period of 72 h in order to rule out physicochemical removal of siloxanes. After the inoculation, four stages of operation were stablished, whose main operational conditions are gathered in Table 1. The BTF was operated for a short period (42 days, stage I) at constant empty bed residence time of 14.5 min with single D4 as the sole carbon substrate. During the second stage (operation days: 43 to 152), the BTF was fed with a multicomponent mixture and the EBRT was progressively decreased from 14.5 to 4 min. On the third stage, from 153 to 186 operation days, the packing media was supplemented with 35 g of activated carbon (PhAC-1), particle size 8–12 mm and fully characterized in Cabrera-Codony et al. (2018), which accounted for a 5 cm high layer at the top of the lava rock. The activated carbon added had a pellet size of 2–3 mm and it was free of bacteria when added to the BTF. Thus, the EBRT was increased up to 12 min. Finally, in the fourth stage, lava rock was removed from the bottom of the cylinder and the activated carbon layer constituted the only packing media, decreasing the EBRT to 2 min.

2.2. BTF inoculum and medium composition

The BTF was inoculated with 320 mL of activated sludge obtained from an anaerobic digester of an urban wastewater treatment plant. Previously, the activated sludge was processed with four cycles of centrifugation (2 × 6000 rpm, 10 min; 2 × 8000 rpm, 100 min) and resuspension in synthetic mineral media with the aim to decrease the content of soluble organic matter in the inoculum. The synthetic mineral media contained ($g\,L^{-1}$): NaCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.1; $CaCl_2 \cdot 2H_2O$, 0.01; NH_4Cl, 0.02; $NaNO_3$, 1.0; $KH_2PO_4 \cdot H_20$, 0.58; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2.38. The pH was adjusted to 6.9 with NaOH 1 M. The mineral solution was boiled in a microwave to reduce oxygen content and cooled to room temperature under a nitrogen gas flow. Finally, it was autoclaved (121 °C, 20 min) and cooled under nitrogen gas. Once cooled, 10 mL of a solution of 10 vitamins (Bartscht et al., 1999) and 1 mL trace element solution SL-10 (Widdel, 1983) were added per litre.

Density of the initial inoculum was set to an $OD_{550} > 0.2$. In order to test for the stability of selected siloxane degraders in the system, approximately every 50 days, the BTF was re-inoculated using 250 mL of a bacterial mixed culture (cell density: 4.06×10^6 cell mL^{-1} measured by cell counting) of isolates previously obtained in our group all showing the capacity to grow with D4 as sole carbon and energy source (Boada et al., 2020). A mixture of *Alicycliphilus denitrificans* (iso02); *Pseudomonas aeruginosa* (iso03 and iso07), *Ciceribacter lividus* (iso05), *Pseudomonas citronellolis* (iso22); *Nocardiodes* sp. (iso40), *Gordonia polyisoprenivorans* (iso45), *Rhodococcus erythropolis* (iso52), *Microbacterium foliorum* (iso55) and *Methylibium* sp. (iso58) at equal densities was used. Except the stage III and IV which used *P. aeruginosa* (iso03 and iso07) as unique inoculum. Before every re-inoculation the mix culture was previously adapted to the presence of D4 (20 mg L^{-1}, 99%) by maintaining the cultures in closed 250 mL serum bottles. Immediately before inoculation those cultures were washed by centrifugation and resuspended with mineral media to remove residual organic compounds.

Table 1
Operational conditions by each operation stage of the BTF. AC, activated carbon.

Stage	Operation period [days]	EBRT [min]	Heigh [cm]	Operational conditions	
				Substrate	Packing material
I	0–42	14.5	36	D4 (62 ± mg m^{-3})	Lava rock
II-1	43–85	14.5	36	Multicomponent[a]	Lava rock
II-2	86–106	10.1	25	Multicomponent[a]	Lava rock
II-3	107–127	7.3	18	Multicomponent[a]	Lava rock
II-4	127–152	4.0	10	Multicomponent[a]	Lava rock
III	153–186	12.0	30	Multicomponent[a]	Lava rock + AC
IV	187–220	2.0	5	Multicomponent[a]	AC

[a] Composition of multicomponent substrate: Hexane (375 ± 18 mg m^{-3}), Toluene (24 ± 2 mg m^{-3}), Limonene (220 ± 11 mg m^{-3}), D4 (54 ± 3 mg m^{-3}), D5 (102 ± 4 mg m^{-3}).

2.3. Biomass sampling and analysis

Samples for the determination of microbial communities were collected from activated sludge used as inoculum, and from the bottom section of the BTF. For activated sludge, 500 μL of a homogenized sample were transferred to 2 mL sterile centrifuge tubes and immediately stored at −20 °C. For BTF samples, 5 g of granular packing material (lava rock, activated carbon, or both) were periodically collected from the BTF and stored at −20 °C.

Before DNA extraction, packing material was crushed using a sterile mortar and pestle to obtain a homogeneous solid sample. When a combination of activated carbon and lava rock were used as packing support the sample was crushed for each support and an equally mass mix of both was used in next analyses.

DNA was extracted from 500 mg of crushed material or 500 μL of activated sludge using the FastDNA® Spin kit for soil (MP Biomedicals, Solon, OH, USA) in accordance with the manufacturer's instructions. DNA concentration and purity were determined in a Nanodrop ND-1000 UV–Vis spectrophotometer (Nanodrop, Wilmington, DE, USA).

2.4. Quantitative PCR (qPCR) of bacterial gene 16S rRNA

Gene abundances were determined using quantitative PCR (qPCR). The qPCR amplification was performed for the bacterial 16S rRNA gene and used as a proxy for total abundance. All reactions were performed in a Lightcycler 96 Real-Time PCR system using the LightCycler® 480 SYBR Green I Master (Roche Life Science, Basel, Switzerland). The reactions were performed with a final volume of 20 μL containing 1× LightCycler ® 480 SYBR Green I Master, 35–40 ng of DNA, and 1 μM of each primer. Primers and thermal cycling conditions were used as described earlier (Bru et al., 2008; López-Gutiérrez et al., 2004) with minor modifications: 95 °C for 15 s, 60 °C for 30 s, 72 °C for 30 s, 35 cycles with primers forward 341F (5′-CCTACGGGAGGCAGCAG-3′) and 534R (5′-ATTACCGCGGCTGCTGGCA-3′). Standard curves were obtained using serial dilutions from 10^2 to 10^8 copies of linearized plasmids containing a copy of a 16S rRNA gene. PCR efficiency was 89% (r^2 0.98) and controls without template gave null or negligible values.

2.5. Analysis of microbial community structure

Analysis of microbial community was carried out in DNA extracted from all samples. The bacterial 16S rRNA V4 region was amplified using dual indexed Illumina compatible primers Pro515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and Pro806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011; Walters et al., 2016). Raw sequence data from this study was deposited via the Biosample Submission Portal (National Center for Biotechnology Information) under the accession number **PRJNA554091**. Sequencing of the 16S rRNA gene was performed at MSU Genomics Core (Michigan, USA) using an Illumina MiSeq platform 2 × 250 bp paired end format using a v2 500 cycle reagent cartridge (Mardis, 2008). Raw sequences were quality-filtered using a maximum expected error of 0.25 (90.4% merged sequences) and a minimum

sequence length of 250 bp using Usearch v.9.1 (Edgar and Flyvbjerg, 2015). Paired reads of filtered sequences were joined and checked for the presence of chimera before being clustered into Operational Taxonomic Units (OTUs) (97% cut-off). Singletons and doubletons were removed to avoid spurious diversity. Mothur v1.35.1 was used for taxonomic classification of the representative sequences of each OTU using the SILVA release 132 reference alignment and taxonomy database (Quast et al., 2013; Schloss et al., 2009). To deeply analyse the microbial community diversity, Simpson's index of diversity (1-D), as well as Good's coverage were calculated. A "composition approach" was used to define the core community of the siloxane degrading BTF (Shade and Handelsman, 2012). Members of the core community were identified as those OTUs being present in all BTF samples that occurred at a relative abundance (according to number of sequences) above 2%.

2.6. Statistical analyses

Statistical analyses were performed using SPSS Windows 25.0 (IBM SPSS, Inc) and Primer-e v6 (PRIMER-E, Ltd). Differences in gene abundances among samples from BTF (inoculum of activated sludge included) were tested for significance with Kruskal-Wallis test. Differences in diversity indices from BTF and inoculum were tested with Kruskal-Wallis test or U-Mann-Whitney tests. Moreover, Kruskal-Wallis test was used when more than two BTF operation stages were compared, while U-Mann-Whitney test was chosen to determine the differences between diversity indices of inoculum and the operation of the BTF.

Differences among whole bacterial community compositions from the BTF were analysed using the non-parametric multivariate statistical test permutational analysis of variance (PERMANOVA) tests using primer-e v6. Differences between relative abundances of each core community member during the operation stages in the BTF (e.g., absence vs. presence of activated carbon) were tested with U-Mann-Whitney tests. Significant differences were declared when $P < 0.05$.

3. Results and discussion

3.1. Operation of BTF

The experimental set-up was initially operated abiotically to assess the physical-chemical processes underlying D4 removal from the synthetic gas emission. After 72 h of abiotic operation, the substrate (D4) concentration at the outlet of the BTF matched the inlet substrate concentration, which ruled out the occurrence of significant abiotic siloxane removal mechanisms. After that, the inoculated system was run for 220 days to study bioremoval of siloxanes and the other volatile organic compounds. During the whole experimental process, the substrate concentrations at the gas outlet and inlet of the BTF were measured by GC-FID.

Fig. 1 shows the removal efficiency (RE) of each substrate in the course of the BTF operation and Fig. 2 the elimination capacity (EC) as the silicon equivalent from the removal of siloxanes and the carbon equivalent from the removal of VOCs per volume of BTF and time. Details on biodegradation by-products and mass balance are provided in our previous work (Santos-Clotas et al., 2019a).

After the inoculation with sewage active sludge, the BTF was initially run for 42 days (Stage I) with a feed gas containing D4 (62 mg m^{-3}). This period was devoted to the biomass grow and fixation on the lava rock used as packing material and acclimation to the use of siloxane D4 as sole carbon source, with a load corresponding to 260 mg m^{-3} h^{-1} provided by the feed gas. At the steady state, the D4 removal efficiency achieved was 14% (36 mg m^{-3} h^{-1}), the ECs per carbon and silicon were closely to 0 (2.5 and 3.0 mg m^{-3} h^{-1}, respectively).

Aiming at reproducing more realistic conditions for biogas treatment, the feed gas was changed (Stage II) to a multicomponent mixture

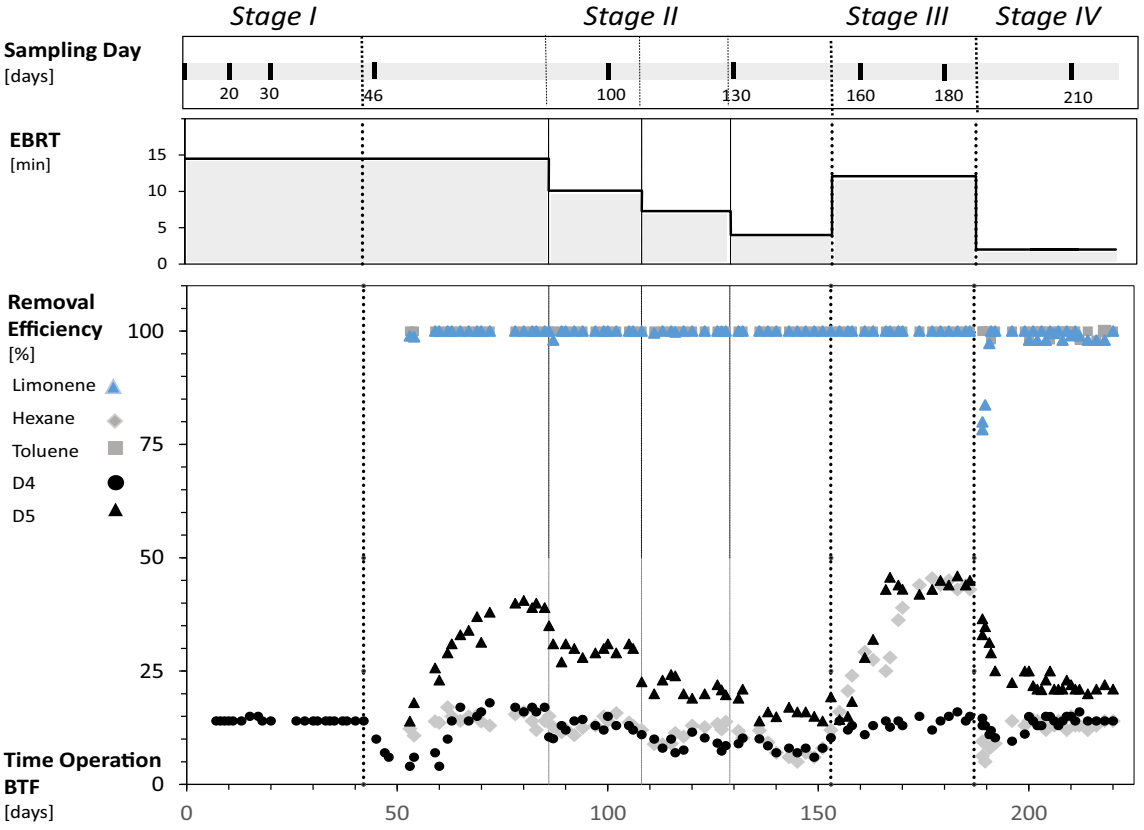

Fig. 1. Time-course of the EBRT [min], removal efficiency (RE [%]) of evaluated substrates and sampling day in the BTF. Vertical dashed lines represent operational stages.

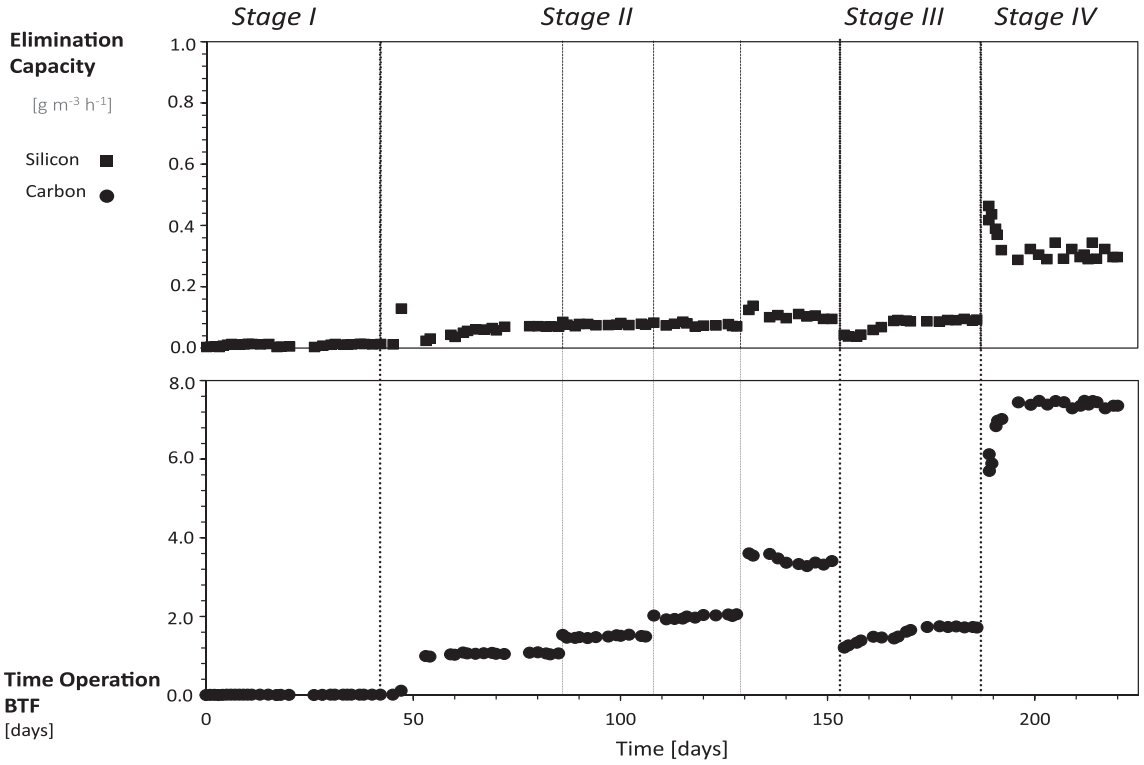

Fig. 2. Time-course of elimination capacity (EC) of evaluated substrates in terms of removed C and Si (g m^{-3} h^{-1}). Vertical dashed lines represent operational stages.

including siloxanes D4 (54 + −3 mg·m^{-3}) and D5 (102 + −4 mg·m^{-3}) and volatile organic compounds usually found in biogas such as toluene (24 + −2 mg·m^{-3}), limonene (220 + −11 mg·m^{-3}) and hexane (375 + −18 mg·m^{-3}) at concentrations similar to those reported in anaerobic digestion biogas, as described in a former publication (Santos-Clotas et al., 2019a). This stage was subdivided into four shorter periods where the EBRT was sequentially reduced aiming at minimize the bioreactor size required for biogas treatment.

Both limonene and toluene were completely removed (RE 100%) shortly after the beginning of the multicomponent gas operation. Operating at EBRT of 14.5 min, limonene and toluene EC were 830 mg m^{-3} h^{-1} and 100 mg m^{-3} h^{-1}, respectively, and increased to 3000 and 350 mg m^{-3} h^{-1} at the shortest EBRT tested (4 min). 210 mg m^{-3} h^{-1} of hexane was initially removed, which corresponds to a RE of 14%. With the reduction on the EBRT, hexane's EC subsequently increased up to 290, 340 and 450 mg m^{-3} h^{-1}. Regarding siloxane removal, the elimination capacities reported for D4 and D5 were 30 mg m^{-3} h^{-1} (13%) and 140 mg m^{-3} h^{-1} (37%), respectively at the longest contact time. Thus, the removal of D4 in the multicomponent operation was similar to those with D4 as single carbon source, while D5 was more effectively removed from the beginning of the operation. At the shortest contact time, the performance recorded for D4 and D5 were 60 mg m^{-3} h^{-1} (8%) and 230 mg m^{-3} h^{-1} (15%), respectively.

Overall, in this stage the ECs increased from 990 to 3500 mg m^{-3} h^{-1} and from 42.8 to 138 mg m^{-3} h^{-1} for carbon and silicon, respectively. The performance of the BTF towards the most hydrophobic compounds, i.e. D4, D5 and hexane, was more limited than limonene of toluene. The combination of packing materials has been proved to enhance the removal efficiency of pollutants in BTF (Tu et al., 2019). Accordingly, in order to improve the mass transfer of the target compounds and thus their availability for the biofilm bacteria, an activated carbon layer was supplemented (Stage III). This layer accounted for and extra EBRT of 2 min besides 10 min provided by the lava rock. After 20 days of operation, the steady state was reached, where the performance of the BTF towards D5 and hexane abatament drastically increased. The catalytic activity of activated carbon towards siloxane ring-opening hydrolysis reactions has been previously described (Santos-Clotas et al., 2019c), which leads to the formation of more water soluble compounds (Table 1). This effected increases the availability of the molecules to the biofilm thus increasing the biodegradation of gaseous compounds. Additionally, the higher EBRTs increased the contact time of the pollutants with the biofilm enhancing its bioavailability and therefore stimulating the growth of some microbial species as discussed in Section 3.3. However, such effect was not noticeable for D4 which still maintained at previous level. Therefore, assuming a higher mass transfer rate, the removal of D4 was mainly limited either by the biodegrading ability of microorganisms or due to their preference for limonene, hexane and toluene which show higher solubility in water. However, D4 removal in the BTF was higher since batch growth assays of 10 mixed isolated strains using activated carbon showed that D4 removal increased up to 28,98% when D5, toluene, hexane and limonene were present compared to 12,59% when D4 was the unique carbon source (Boada et al., 2020).

At the light of the successful colonization of activated carbon as biofilm support, lava rock was removed activated carbon added to the BTF. Partial removal of the packing material was also used to test the effect of the reduction of reactor size, which may facilitate future scale-up applications. In this sense, activated carbons possess a larger surface area and provide a better possibilities for biofilm formation. Thus, the EBRT during this operation period was only 2 min. Limonene and toluene were still completely removed, corresponding to elimination capacities of 5999 and 693 mg m^{-3} h^{-1}, respectively and 1445 mg m^{-3} h^{-1} of hexane (13%), 200 mg m^{-3} h^{-1} of D4 (14%) and 616 mg m^{-3} h^{-1} of D5 (22%) were removed in such conditions. Global elimination capacity of carbon was ranged from 7000 to 7500 mg m^{-3} h^{-1} and from 296 to 350 mg m^{-3} h^{-1} for silicon. Carbon samples were analysed at the end of Stage IV to determine the compounds adsorption or retention in the biofilm. The siloxane retained in the solid phase was in a concentration

below the quantification limit proving that both physicochemical adsorption and biosorption of siloxane removal could be ruled out.

3.2. Abundance of 16S rRNA genes

Quantitative PCR of the 16S rRNA gene was used as a proxy for bacterial density in the reactor biofilm. Table 2 shows the concentration of bacterial 16S rRNA gene of the samples taken during the BTF operation, which ranged from $4.2 \cdot 10^5$ to $8.5 \cdot 10^7$ copies mg^{-1} of packing material.

The Kruskal-Wallis test resulted in $p > 0.05$, meaning that the differences in the abundance of total bacteria were not statistically significant between samples thus indicating a rather stable bacterial density as a consequence of an effective bacterial colonization throughout the whole BTF. Similar bacterial abundances are reported in other BTF for the removal of organic pollutants according to the inoculum used (Sun et al., 2013; Zhou et al., 2016). Slight variations were due to the changes in the operational conditions. In this sense, the sample II_{46}, taken during the early days of operation with multicomponent feed gas, presented the lowest value ($4.2 \cdot 10^5$) although the BTF was re-inoculated at day 40 using a mixed culture of bacterial isolates, suggesting a transient negative effect in the cell density caused by the perturbation of substrate modification with the addition of the volatile organic compounds at concentrations present in the biogas which could be toxic for some bacteria.

On the other hand, the analysis during Stage III (day 160) revealed a rapid colonization of activated carbon, which increased from $2.1 \cdot 10^7$ to $8.5 \cdot 10^7$ copies mg^{-1} of activated carbon in 20 days. Moreover, samples taken during the last stage, when activated carbon was used as sole packing material, presented a density of biomass similar or slightly higher (in the top of the BTF) than the samples taken from sole lava rock (Stage II), suggesting a good affinity of the bacterial community for this material. There are several physicochemical factors that explain the higher bacterial abundance when activated carbon is present, namely its lower particle size (2–3 mm) compared to lava rock (8–12 mm) and the higher surface/volume ratio due to its larger pore volume (Cabrera-Codony et al., 2014; Oshita et al., 2010). Rivera-Utrilla et al. (2001) showed *E. coli* adsorption on activate carbons increased with hydrophobicity and macropore volume. In addition, phosphoric acid-activated carbons contribute to the uptake of all types of siloxanes increasing their bioavailability by forming α-ω-silanediols. Similarly, oxygen-functional groups in the surface of the activated carbons complex many types VOCs which can be used as a carbon source.

3.3. Changes in the microbial community structure during operation

The BTF community structure was determined by sequencing the V4 region of 16S rRNA gene by means of Illumina MiSeq sequencing (Table 3). The diversity of the microbial community was estimated by Simpson's diversity index, which showed the highest value in the activated sludge samples used for the inoculum (0.98). After 20 days of operation, in Stage I, the Simpson's diversity index of the packing material samples was 0.94, and progressively decreased during the operation in Stage II. However, richness of the community, pointed out by the number of sequences and OTUs, drastically increased on the first analysis of the multicomponent gas operation respect the operation with D4 as single carbon source. The system was run with lava rock and multicomponent gas at different EBRT for ca. 3.5 months. During this period the number of sequences and OTUs decreased successively and the Simpson's index stabilized around 0.8, until the addition of activated carbon (Stage III) when the diversity index raised again up to 0.92 together with the microbial richness. Besides the diversity, richness (number of OTUs) pointed out the microbiome specialization in the BTF reactor. Taking into account that the inoculum accounted for 944-975 OTUs, the number of OTUS in the BTF samples was halved at the beginning of the operation and successively reduced to ca. 200.

Fig. 3a shows the relative abundance of taxa (*Order* level) found at relative abundances >1%. The use of a restrictive substrate (i.e. siloxane and other organic compounds) in the system produced the decrease of many bacterial groups present in the inoculum that were progressively replaced by a specialized bacterial community at early stages of operation (mainly Stage I). Wu et al. (2017) reported that the bioreactor operation has a strong influence in the development of microbial communities, suggesting that succession processes (including microbial acclimations to changing operation conditions), promote the isolation of specialized microbes in engineered microbial ecosystems.

Betaproteobacteriales, *Rhizobiales* and *Corynebacteriales* appeared as dominant members of the community. Some bacteria within these taxa have been described as ubiquitous and have been frequently found in bioreactors (Liu et al., 2018). Moreover, reports on bacteria from these groups as potential degraders of recalcitrant compounds (i.e. hydrocarbons, volatile organic compounds, etc.) also exist (Gonzalez-Martinez et al., 2015a, 2015b; Wang et al., 2018).

In order to study if differences in bacterial composition between different parts of the BTF column existed, samples IV_{210top} and $IV_{210bottom}$ were taken from the upper and lower sections and compared. Results obtained indicated that microbial community structures were almost identical in these two samples (U-Mann-Whitney tests, $p > 0.05$, Supplementary information 2), suggesting that sampling at one point was enough for the characterization of the whole BTF biofilm.

The effects of the use of activated carbon as biomass support on the variability of microbial communities of the BTF were analysed using a Principal Coordinate Analysis (PCoA) based on the resemblance Bray-Curtis similarity matrix (Fig. 3b). Samples were grouped according to presence or absence of activated carbon. The sample taken in stage II at 46 operation days, i.e. when the substrate was changed at day 43 from D4 to multicomponent substrate, significantly differed from the

Table 2
Mean abundance values of 16S rRNA gene at the studied samples from BTF and inoculum.

Sample code	Number of copies mg^{-1} of crushed packing material	Crushed packing material
0_a	$1.8 \cdot 10^6$	Activated sludge
0_b	$1.2 \cdot 10^6$	Activated sludge
I_{20}	$1.6 \cdot 10^7$	Lava rock
I_{30}	$2.2 \cdot 10^7$	Lava rock
II_{46}	$4.2 \cdot 10^5$	Lava rock
II_{100}	$2.9 \cdot 10^7$	Lava rock
II_{130}	$2.3 \cdot 10^7$	Lava rock
III_{160}	$2.1 \cdot 10^7$	Lava rock and AC
III_{180}	$8.5 \cdot 10^7$	Lava rock and AC
IV_{210top}	$4.4 \cdot 10^7$	AC
$IV_{210bottom}$	$2.2 \cdot 10^7$	AC

0: inoculum sample; I, II, III, IV: operation stage. Subscript number: sampling day; AC: activated carbon.

Table 3
Diversity analyses of microbial communities.

Stage	Sample information		Diversity sequences analyses			
	Sample code	Sampling day	Number of sequences	Number of OTUs[b]	Good's coverage	Simpson's Index of diversity (1-D)
0[a]	0_a	0	130,010	944	0.992	0.98
	0_b	0	219,819	975	0.992	0.98
I	I_{20}	20	54,527	471	0.997	0.94
	I_{30}	30	24,465	385	0.996	0.91
II	II_{46}	46	156,093	556	0.996	0.89
	II_{100}	100	26,313	376	0.997	0.78
	II_{130}	130	4332	190	0.992	0.83
III	III_{160}	160	82,264	365	0.996	0.89
	III_{180}	180	20,503	193	0.998	0.81
IV	IV_{210top}	210	19,923	224	0.998	0.92
	$IV_{210bottom}$	210	21,643	230	0.998	0.91

[a] Stage 0: Inoculum.
[b] Total number of OTUs: 1410 OTUs.

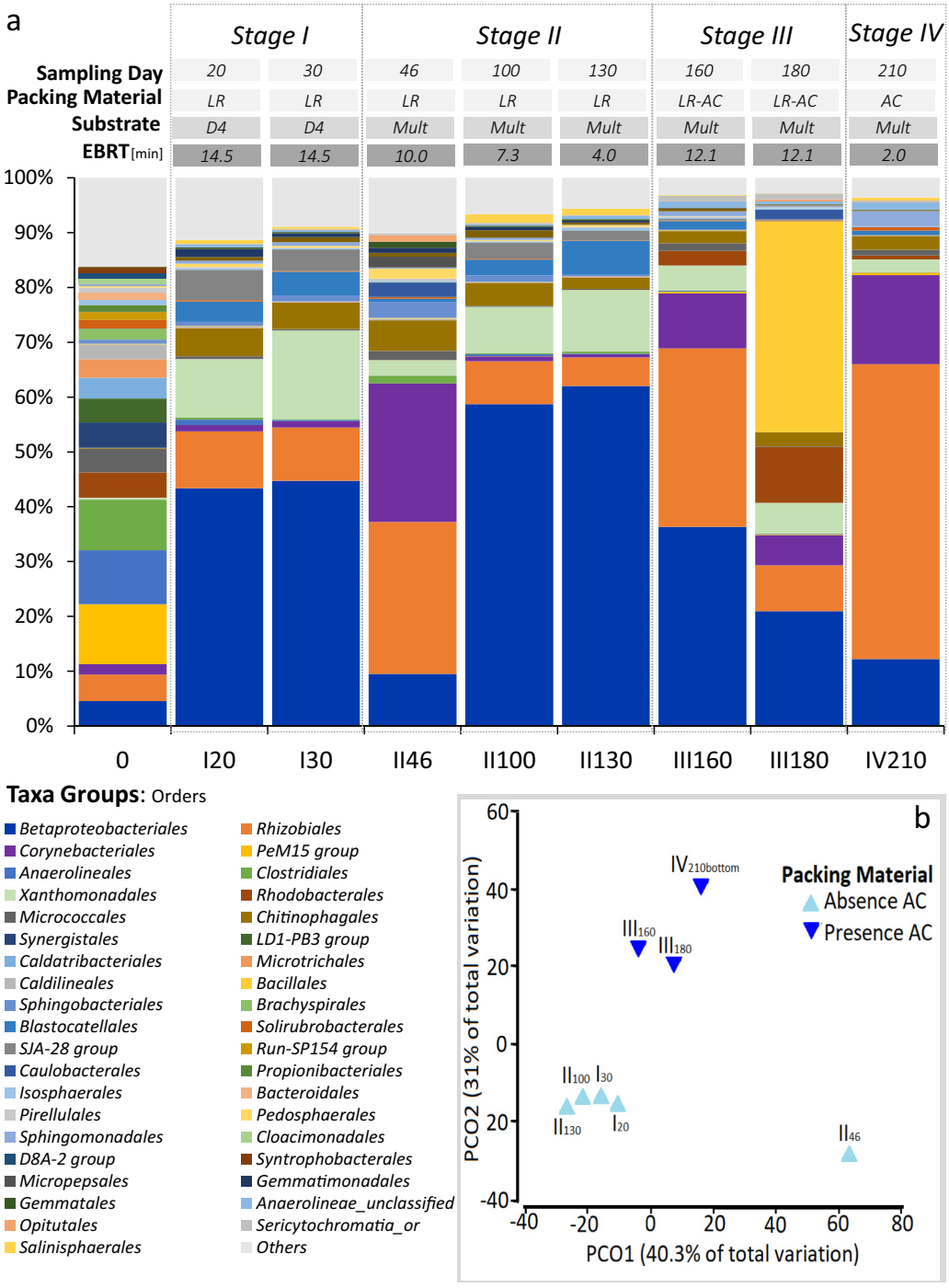

Fig. 3. a. Relative abundance of main orders (found >1% of sequences) in each sample in the studied phases and inoculum. Bars are grouped according to the operation phase of the BTF. "Others" refers to orders that represented <1% of total sequences in all samples. b. PCoA distribution of OTU-based microbial community composition according to packing material (Absence and presence of activated carbon). Resemblance Bray-Curtis similarity.

rest of samples indicating that the microbial community composition was mainly influenced by the short-term exposure to VOCs, probably due to a toxic effect. Interestingly, the bacterial abundance determined by qPCR was slightly lower in this sample compared to the others as mentioned above (Section 3.2), which was also attributed to the sudden exposure to VOCs. Despite sample distribution on the PCoA, differences due to the packing material replacement could not be fully confirmed by a PERMANOVA at the 0.05 significance level (pseudo-*F* value 1.875; *p* value = 0.068, when sample II46 was excluded from the analysis pseudo-*F* value 1.8063, *p* value = 0.088). According to the procedure used in the experimentation, little differences in the community structure were expected. Biofilms were initially established on lava rock for a period of 153 days, constituting a selected defined seed for the latterly added activated carbon. We considered microorganisms colonizing activated carbon mostly came from the previously colonized lava rock surface and therefore communities stablished in the two materials would be rather similar. At day 187 the packing material was completely replaced by activated carbon and the bioreactor operated along 33 days which would not be sufficient for the development of a significantly different community from that developed on lava rock.

3.4. The establishment of a core microbial community

In order to infer the main taxonomical groups responsible for siloxane-degradation in the BTF, a core microbial community was defined at the OTU level. Taxonomically defined, the core microbiome is composed of consistent groups (taxa) of bacteria that are present at all tested conditions and are likely to participate in basic metabolic processes that are taking place in a microbial assemblage (Shade and Handelsman, 2012). This concept has been widely applied to microbial communities involved in metabolic processes in different types of bioreactors (Mei et al., 2016; Seo et al., 2019; Zhou et al., 2016). In our work, we defined the core microbiome as those OTU appearing at all operational conditions tested with a minimum relative abundance of 1%. The core community was composed of 36 OTUs from a total of 1410 accounting for 2.55% of bacterial OTUs and comprised 14 orders: *Anaerolineales*, *Betaproteobacteriales*, *Chitinophagales*, *Corynebacteriales*, *Flavobacteriales*, *Ignavibacteriales*, *Isosphaerales*, *Micrococcales*, *Opitutales*, *Planctomycetales*, *Rhizobiales*, *Rhodospirillales*, *Salinisphaerales* and *Xanthomonadales*. Specific changes in the community were detected between Stages I and II and at the end of Stages III (gas retention times), like for instance, the increased of orders *Rhizobiales* and *Corynebacteriales* at the Stages III and IV. Apparently, the microbial community recovered shortly after the substrate changes (Stage II), and a composition similar to the one found in Stage I was resumed. On the contrary, changes of packing material, the addition of the activated carbon layer on top (Stage III) and the further removal of the lava rock (Stage IV), showed a particular effect on the bacterial community that was also evident in the core community (Fig. 4).

Members of the *Corynebacteriales* (OTU005) and *Rhizobiales* (OTU006, OTU011 and OTU020) progressively increased their abundance upon the addition of activated carbon in the system, while *Betaproteobacteriales* (OTU001 and OTU012) and *Xanthomonadales* (OTU010) decreased. This indicated that packing material affinity played an important role in selecting microbial populations (Lameiras et al., 2008).

Chemical and metabolic profiles of taxa groups present in the core community suggest common traits among members during the degradation process of siloxane (D4 and D5) and volatile compounds (toluene, limonene and hexane). More precisely, the most abundant members of the core community (OTU001, OTU002, OTU005, OTU006, OTU010, OTU011 and OTU012) are close related to bacteria known to have a metabolism specialized in single-carbon or multi-carbon compounds some of which that contain no carbon-carbon bonds as Si-bond (Boada et al., 2020; Liu et al., 2013; Lu et al., 2007; Pérez et al., 2016; Puentes-Cala et al., 2018). The OTU002 (tentatively identified as *Labrys* sp. according to BLAST refseq search, percentage similarity (PE) 100%) has been reported as isolated from a methylamine enrichment culture, and representing a novel species of facultative methylotrophic bacteria, suggesting that the core community could have potential bacteria with methylotrophic capacities, and could use one-carbon compounds or multi-carbon compounds that contain no carbon-carbon bonds, such as methylsiloxanes (Miller et al., 2005). The OTU001 (*Castellaniella defragrans*, PE 100%), OTU010 (*Dokdonella soli*, PE 99.8%) and OTU012 (*Methylibium* sp. PE 100%) have an interesting background of aromatic and organic microbial degraders. Puentes-Cala et al. (2018) reported a limonene dehydrogenase that was purified from the facultative anaerobic betaproteobacterium *Castellaniella defragrans* strain 65Phen grown on monoterpenes under denitrifying conditions in the absence of molecular oxygen. While the *Dokdonella soli* has been isolated from activated sludge in a sequencing batch reactor used for the treatment of triphenylmethane dye effluent (Liu et al., 2013). We have previously reported *Methylibium* sp. as a D4-degrading bacteria able to grow in a BTF on lava rock in previous works (Boada et al., 2020; Santos-Clotas et al., 2019a). Remarkably, strains of *Afipia felis* (OTU006) isolated from different environments have been described to oxidize methanesulphonate (Moosvi et al., 2005) while members of the genus *Hypomicrobium* (OTU011) are reported to biodegrade single carbon compounds like dimethylsulfone (Borodina et al., 2000), the pesticide methamidophos (Wang et al., 2010) and dichloromethane (Hayoun et al., 2020). Even, the capacity for methane oxidation has been identified in the genome of *Ensifer mexicanus* (OTU020) according to KEGG metabolic pathways https://www.genome.jp/kegg/ (Kanehisa, 2000). Overall, the most abundant members of the core community (OTU001, OTU002, OTU005, OTU006, OTU010, OTU011 and OTU012)

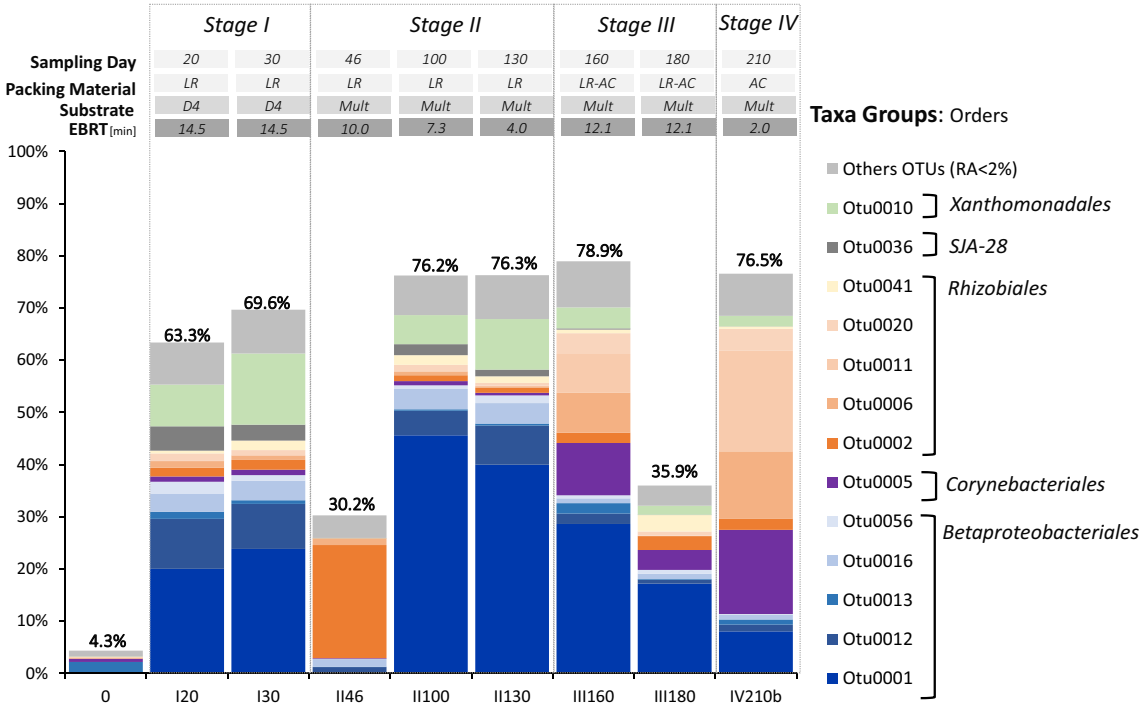

Fig. 4. Percentage value of OTUs present in the core community in each sample of the studied stages and inoculum. Bars are grouped according to the operation stage of the BTF. OTUs are grouped by square bracket according to the taxa group: Order. "Others" refers to OTUs that represented <2% of total sequences.

were selected as the set of bacteria most suitable for the removal of siloxanes within the core community. Taking all together, the core community harbour both phylogenetic and functional redundancy of the bacterial populations with the potential biodegradation of siloxanes D4 and D5 using the methyl groups as carbon source as well as the capacity to use other volatile simple carbon compounds pointing to a functional robust community which could buffer disturbance responses (Shade and Handelsman, 2012).

As previously stated, sample II46 presented a particular microbial community probably resulting from a toxic effect from a sudden exposure to the multicomponent mixture (Section 3.3). Accordingly, the analysis of the relative abundance of each member within the core community was calculated excluding sample II46. Table 4 shows the relative abundances of each member according to the absence or presence of activated carbon as a packing material. Most members of the defined core community presented not significant differences in relation to the relative abundance in presence of activated carbon (U de Mann-Whitney test, $p > 0.05$), except *Dokdonella soli*. Interestingly, *Dokdonella* sp. was previously isolated in our group from a BTF for siloxane and other volatile compounds removal (Boada et al., 2020) and other representatives of this genus have been reported as important organisms in studies about biological removal treatments and biofilm formation (Li et al., 2013; Rodrigues et al., 2010; Yoo et al., 2009). Therefore, the combination of packing materials would significantly favour the growth of some members of the community as well as the removal of the target compounds as previously described in Section 3.1. Similarly, Tu et al. (2019) showed that the combination of packing materials enhanced the removal of the target compounds and remarkably, it favoured the interconnections among the members of the core community, as revealed the analysis of co-occurrence networks. Besides, Huang et al. (2019) showed that packing carriers favoured the growth of some members of the community belonging to the *Actinobacteria*. Thus, future studies should aim at evaluating the growth and the siloxane degradation capacity of these groups in BTF operated with the most favourable packing materials for biogas purification.

3.5. Analysis of bioaugmentation on the performance and microbial communities of the BTF

In order to test for the stability (growth and steadiness) of selected D4-degraders in the system, approximately every 50 days (44, 106, 150, 196 days), the BTF was re-inoculated using a mixed culture of bacterial isolates previously obtained in our group (Boada et al., 2020). Up to $8 \cdot 10^8$ cells equivalently distributed for all ten isolates were inoculated. Assuming all inoculated cells would be able to attach to the packing material (200 g) and integrate into the biofilm this would represent a maximum of 4×10^6 cells g^{-1} of material, slightly below the cells density in the operative biofilm (10^7 cells). Estimated figures indicated that for the bioaugmentation to be effective in contaminant removal, growth of inoculated isolates should occur in the BTF. A phylogenetic inference of the partial 16S rRNA gene sequence of core microbiome OTUs and amended isolates was performed in order test for the presence of these isolates as members of the core BTF community (Fig. 5).

Five OTU sequences, OTU012, OTU013, OTU050, OTU152, and OTU005, presented an almost complete sequence similarity to isolates *Methylibium* sp. iso58, *Alicycliphilus denitrificans* iso02, *Microbacterium*

Table 4
Relative abundances of members of the core community in the two operation states in the BTF (absence of AC and presence of AC). Sequence species identification was performed using GenBank's BLAST algorithm.

Taxonomic OTU information			% Rel. Abund.		−AC vs. AC
Order	Most probable species identification	OTU ID	−AC	+AC	*p* value u-m
Betaproteobacteriales	*Methylibium* sp.	Otu0012	7.59 ± 2.11	1.35 ± 0.64	0.057
	Aquabacterium limnoticum	Otu0056	1.40 ± 0.71	0.50 ± 0.29	0.114
	Simplicispira psychrophila	Otu0016	3.73 ± 0.23	0.92 ± 0.08	0.057
	Acidovorax caeni	Otu0013	0.69 ± 0.49	1.05 ± 0.09	1.000
	Castellaniella defragrans	Otu0001	32.3 ± 12.3	17.9 ± 10.3	0.229
	Burkholderiales bacterium	Otu0049	0.74 ± 0.41	0.39 ± 0.25	0.229
	Zoogloea ramigera	Otu0058	1.14 ± 0.37	0.29 ± 0.09	0.057
Salinisphaerales	*Solimonas soli*	Otu0115	0.88 ± 0.52	0.16 ± 0.16	0.114
Opitutales	*Opitutus* sp.	Otu0331	0.06 ± 0.03	0.17 ± 0.15	0.114
Xanthomonadales	*Dokdonella soli*	Otu0010	9.23 ± 3.38	2.41 ± 1.09	0.029*
	Pseudoxanthomonas sp.	Otu0158	0.07 ± 0.11	0.59 ± 0.46	0.057
Anaerolineales	*Ornatilinea apprima*	Otu0069	0.47 ± 0.15	1.03 ± 0.49	0.229
Planctomycetales	*Planctomyces* sp.	Otu0259	0.21 ± 0.06	0.06 ± 0.05	0.057
Isosphaerales	*Isosphaera* sp.	Otu0233	0.19 ± 0.13	0.12 ± 0.09	0.857
	Uncultured Isosphaeraceae	Otu0457	0.07 ± 0.03	0.04 ± 0.03	0.229
Chitinophagales	*Ferruginibacter* sp.	Otu0025	1.02 ± 0.27	0.87 ± 0.62	1.000
	Chitinophaga sp.	Otu0180	0.41 ± 0.28	0.14 ± 0.08	0.229
	Terrimonas sp.	Otu0302	0.14 ± 0.07	0.04 ± 0.02	0.114
Flavobacteriales	*Uncultured Flavobacteria*	Otu0147	0.36 ± 0.19	0.22 ± 0.22	0.629
Rhodospirillales	*Rhodovibrio* sp.	Otu0107	0.68 ± 0.38	0.24 ± 0.19	0.114
Rhizobiales	*Afipia felis*	Otu0006	0.79 ± 0.43	6.85 ± 6.45	0.629
	Rhodopseudomonas sp.	Otu0074	0.13 ± 0.04	0.72 ± 0.59	0.629
	Pseudolabrys sp.	Otu0222	0.05 ± 0.02	0.05 ± 0.04	1.000
	Afipia genosp	Otu0108	0.17 ± 0.04	0.22 ± 0.15	0.629
	Bosea thiooxidans	Otu0041	1.37 ± 0.62	1.20 ± 1.30	0.486
	Labrys sp.	Otu0002	1.42 ± 0.41	2.27 ± 0.34	0.057
	Bauldia consociate	Otu0198	0.19 ± 0.08	0.03 ± 0.03	0.057
	Hyphomicrobium sp.	Otu0011	0.05 ± 0.05	8.96 ± 9.74	0.114
	Ensifer mexicanus	Otu0020	0.83 ± 0.57	2.97 ± 1.90	0.250
	Mesorhizobium sp.	Otu0072	0.38 ± 0.10	0.41 ± 0.19	1.000
	Sinorhizobium sp.	Otu0152	0.14 ± 0.28	0.20 ± 0.18	0.629
Ignavibacteriales	*Unclass. Ignavibacteriaceae*	Otu0151	0.33 ± 0.11	0.08 ± 0.07	0.057
	Melioribacter roseus	Otu0036	2.77 ± 1.44	0.08 ± 0.09	0.057
Micrococcales	*Leifsonia kafniensis*	Otu0050	0.20 ± 0.14	0.34 ± 0.20	0.400
	Salinibacterium sp.	Otu0070	0.07 ± 0.01	0.49 ± 0.45	0.629
Corynebacteriales	*Mycobacterium* sp.	Otu0005	0.83 ± 0.27	9.99 ± 6.15	0.057

* $p < 0.05$.

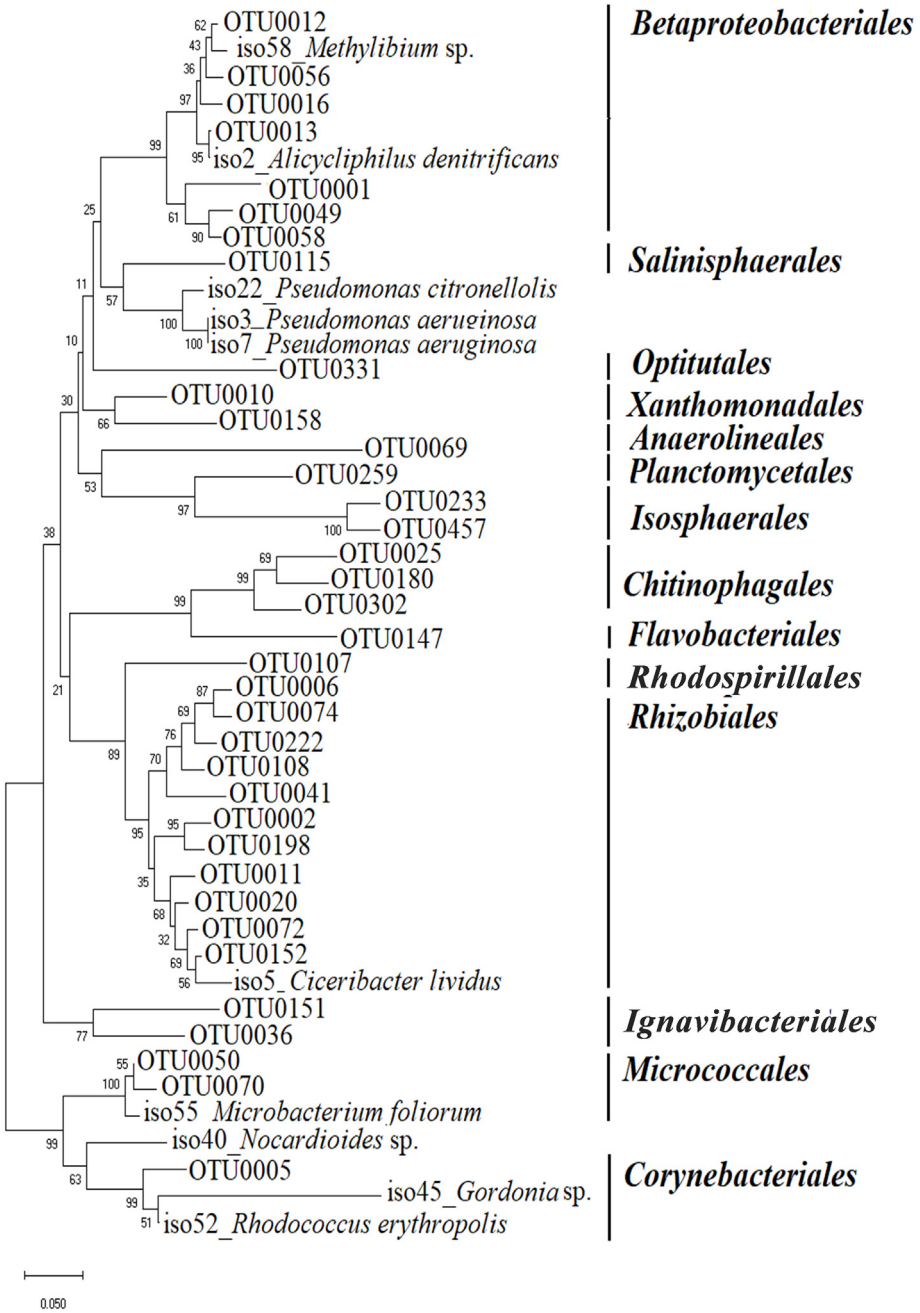

Fig. 5. Maximum likelihood phylogenetic tree calculated for 16S rRNA gene sequences of bacterial isolates and OTUs of core communities. Numbers indicate bootstrap values. OTUs and isolates are grouped by square bracket according to the taxa group: order. Bar, 5 nucleotide substitutions per 100 nucleotides.

foliorum iso55, *Ciceribacter lividus* iso05, and *Rhodococcus erythropolis* iso52, respectively. Whereas sequences similar to isolates *Pseudomonas aeruginosa* iso03 and iso07, *Pseudomonas citronellolis* iso22, *Nocardiodes* sp. iso40, and *Gordonia polyisoprenivorans* iso45, could not be detected at reasonable densities or were only transiently detected in the core community. These results indicate that not all organisms added in the bioaugmentation experiment can effectively remain as members of the biofilm community and are washed out from the system.

Interestingly, *Methylibium* and *Alicycliphilus* related OTUs, appeared to be abundant members of the core community during operational stages when lava rock was used as packing material indicating that some of the bioaugmented strains showed a preference for adhering to this type of material as previously described (Boada et al., 2020). Remarkably, the detection of OTU012 and OTU013 in stage I previous the inoculation of strains *Methylibium* sp. iso58 and *Alicycliphilus denitrificans* iso02 in stage II confirms that the conditions applied to the BTF select for defined bacterial groups present in the anaerobic activated sludge used as inoculum. *Methylibium* sp. iso58 and *Alicycliphilus denitrificans* iso02 were isolated from a previous BTF operated at similar conditions and from a batch culture enrichment, respectively, both inoculated with the same anaerobic activated sludge (Boada, PhD thesis). Similarly, bacterial species close related to all bacterial strains previously isolated used as reinforcement inocula were detected (Fig. 5). According to the high cell density present in the packing material ($\sim 2.4 \cdot 10^7$ copies mg^{-1}) and considering a total amount of 200 g of packing material, the total cell abundance in the BTF was in the range $4{,}8 \cdot 10^{12}$ copies. In consequence the inoculated $8 \cdot 10^8$ cells of equivalently distributed ten isolates represented $8 \cdot 10^7$ cells per isolate which was five orders of magnitude below the total cell abundance established in the packing material. Considering that the BTF selects bacteria

present in the anaerobic activated sludge with similar metabolisms to those of the isolates, it would be plausible to not identify some of the isolates inoculated like *Pseudomonas aeruginosa* iso03 and iso07, *Pseudomonas citronellolis* iso22, *Nocardiodes* sp. iso40, and *Gordonia polyisoprenivorans* iso45, which could not succeed in colonize the packing material since a mature biofilm was already formed harbouring other abundant acclimated bacteria.

In contrast, among the most abundant members in the presence of activated carbon (OTU001, OTU002, OTU005, OTU006, OTU10 and OTU11), only one (OTU005), was closely related to *Gordonia* sp. iso45 and *Rhodococcus erythropolis* iso52 isolates. Altogether, the analysis of the bioaugmentation reinforce the key role of the packing material and affinities of different bacterial species to adsorb and form a stable biofilm, in defining bacterial communities in the BTF harbouring bacterial representatives close related to previously isolated strains, and therefore their performance for contaminant removal. Examples of bioaugmented BTF using selected pure cultures are scarce and, with few exceptions, usually lead to limited increases in reactor performance (Popat et al., 2012; Sercu et al., 2005). Tu and co-workers (Tu et al., 2019) also concluded that using combinations of different packing materials with selected physical properties was mandatory for increasing the performance of a BTF for the removal of sulfur compounds. As stated in our work, those changes were funnelled through changes in the microbial community composition.

4. Conclusions

Observed changes in the microbial community structure were rather limited, pointing at a high resilience of bacteria involved in silicon compounds degradation. The core community showed both phylogenetic and functional redundancy for the potential biodegradation of siloxanes and single-carbon compounds suggesting to a functional robust community. Packing material played an important role in shaping the composition of the bacterial community and the effectiveness of bioaugmentation events. The relative stability of the microbial community allowed defining a core community of 36 members within the BTF, putatively participating in degradation of the organic compounds. Core members of the community rarely belonged to bioaugmented strains and only five of them could be detected at lower relative abundances, thus suggesting that growth of those strains was prevented in the system by the competition with other bacteria already stablished from the inoculated anaerobic activated sludge, causing a selection. Transient changes in the microbial community composition and activity due to large operation times in BTF should be further investigated in order to predict and evaluate the occurrence of undesirable activities, such as lower degradation rates in siloxane removal treatment process.

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Data availability statement

Data of the study are available from the authors.

Sample availability

Samples of the compounds are available from the authors.

CRediT authorship contribution statement

Ellana Boada: Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Eric Santos-Clotas:** Conceptualization, Investigation, Methodology, Data curation, Visualization, Writing – review & editing. **Alba Cabrera-Codony:** Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft, Supervision. **Maria J. Martín:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Lluís Bañeras:** Data curation, Formal analysis, Writing – review & editing, Supervision. **Frederic Gich:** Conceptualization, Validation, Writing – original draft, Supervision.

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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