Journal Of Hazardous Materials
January 2021, Volume 402 Pages 123477 (10p.)
https://doi.org/10.1016/j.jhazmat.2020.123477
https://archimer.ifremer.fr/doc/00804/91647/

Microbial degradation of hydrophobic emerging contaminants from marine sediment slurries (Capbreton Canyon) to pure bacterial strain

Azaroff Alyssa [1], Monperrus Mathilde [1], Miossec Carole [1], Gassie Claire [2], Guyoneaud Rémy [2, *]

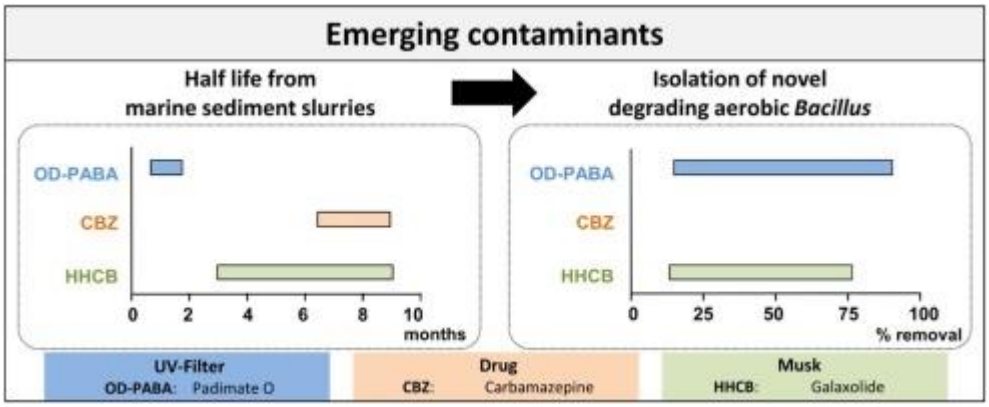
[1] Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM-MIRA, UMR 5254, 64600, Anglet, France
[2] Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM-MIRA, Environmental Microbiology, UMR 5254, 64000, Pau, France

* Corresponding author : Rémy Guyoneaud, email address : remy.guyoneaud@univ-pau.fr

Abstract :

Despite emerging contaminants (ECs) are more and more monitored in environmental matrices, there is still lack of data in marine ecosystems, especially on their fate and degradation potentials. In this work, for the first time, the degradation potential of synthetic musks (galaxolide and tonalide), UV filters (padimate O and octo-crylene) and a pharmaceutical compound (carbamazepine) was studied in marine sediment samples, under laboratory conditions using sediment slurry incubations under biotic and abiotic conditions. Minimum half life times under biotic conditions were found at 21 days, 129 days and 199 days for padimate O, galaxolide and carbamazepine, respectively. Enrichments conducted under anoxic and oxic conditions demonstrated that degradations after one month of incubation either under both biotic and abiotic conditions were limited under anoxic conditions compared to oxic conditions for all the contaminants. Novel aerobic bacteria, able to degrade synthetic musks and UV filters have been isolated. These novel strains were mainly related to the Genus Bacillus. Based on these results, the isolated strains able to degrade such ECs, can have a strong implication in the natural resilience in marine environment, and could be used in remediation processes.

Graphical abstract



Highlights

► (Bio)-degradation of hydrophobic emerging contaminants in marine sediment slurries is slow. ► Emerging contaminants are recalcitrant under anaerobic conditions. ► Emerging contaminant degrading aerobic bacteria were isolated from marine sediments. ► Isolated strains showed ability for UV filters and synthetical musks biodegradation. ► These isolated strains are promising for future studies (pathways, genetic determinism).

Keywords : Emerging contaminants, Musks, UV filters, Pharmaceuticals, Degradation, Marine sediments, Pure bacterial strains

1. Introduction

As a result of the last century, Man created and is still creating new synthetic substances. Although well known persistent organic contaminants, such as organochlorine pesticides or polycyclic aromatic hydrocarbons, are regulated in industrialized countries (European Commission, US, Japan) for several decades, regulation for emerging contaminants (ECs) arised only in the early of the 21th century and their update is still ongoing in Europe (Directive 2008/105/EC and Commission implementing decision 2018/840). Indeed, the scarce information available of their occurrence, reactivity and impact have led to a rising interest in identifying and screening these new compounds in the environment [1]. Among those ECs, pharmaceuticals and personal care products (PPCPs) are substances widely consumed and continuously released in the environment, mainly through wastewater, both treated and untreated [2,3].

Marine ecosystem is the final receptor for these organic ECs. They were found in marine sediments, mainly close to the high density population coast nearby the main estuaries [4–7].

Marine sediments act as integrative matrices reflecting the pollution state in a given area [8,9]. The affinities of contaminants with the suspended particulate matter (SPM) lead them to be readily scavenged from the water column and to be deposited in the sediments. Submarine canyons are known to act as transfer zones of suspended particulate matter and contaminants between the continent and the open ocean where organic pollutants can be accumulated [7,10,11] into these productive ecosystems containing important stocks of commercially important fishes. Capbreton Canyon is located nearby the coast, with important urban and agricultural activities.

Indeed, since the Capbreton canyon is connected to the Adour estuary, its geomorphology and the local currents lead to transfer particles and micropollutants further, amplified during storm events (e.i. turbidic currents)[12–14]. For instance, in the Capbreton canyon sediments collected in the first 25 km from the coast, UV filters and synthetical musks have been

detected where the highest concentrations were observed in sediment collected at 25 km from the coast [7]. This trend was also observed for mercury compounds [15]. Additionally, wastewater treatment plants (WWTPs) are known to be an important pathway for introduction of ECs both through the treated effluents and particles released in aquatic environment [16,17]. In coastal areas, those WWTPs might be the main source of ECs in coastal and submarine sediments [7].

Microorganisms play a key role in ecological processes such as biogeochemical cycling and among them the carbon cycle and the organic compounds degradation. This bioremediation provides an important ecosystem service for the maintenance of the environment quality. The physicochemical properties of the sediment, such as organic carbon, grain size or pH, drive most of its interactions with the contaminants [18]. The bioavailability for the dwelling benthic organisms of these ECs is dependent on the adsorption, desorption and transformation processes which are themselves under control of the biogeochemical parameters. Even at low concentrations, those ECs can be hazardous for the sediment-dwelling benthic organisms. It is widely accepted that their microbial co-metabolization could be the main degradation pathway leading to limited structural changes and incomplete mineralization (e.g. formation of degradation products). Consequently, the fates of ECs in submarine canyon sediments depend on physicochemical properties, and on the presence and activity of microorganisms.

Several studies were performed to explore bioremediation by microorganisms of priority contaminants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyl (PCBs) [19,20]. Few studies have shown the potential of bacteria and microbial consortia involvement in the ECs remediation [21]. Biotransformation potential of ECs in natural and oceanic environments is still unknown and it is urgent to better understand their fate in marine ecosystems [22].

The aim of this present work was to study the degradation potentials of ECs such as synthetic musks (galaxolide, HHCB; tonalide, AHTN), UV filters (padimate O, OD-PABA; octocrylene, OC) and an antiepileptic (carmabazepine, CBZ) in marine sediments in order to highlight, for the first time their natural resilience in a submarine canyon sediments. Moreover, a specific focus was put on the isolation of pure strains exposed to these hazardous substances in order to estimate their capacities in biotransformation of these contaminants in natural ecosystems and more particularly in marine sediments

2. Material and methods

2.1 Study area and sampling strategy

The Capbreton Canyon (South-Eastern Bay of Biscay, NE Atlantic) begins 250 m from the coastline and reaches up to 3000 m water depth. In this study, we focused on two surface sediments (G10 and G14) sampled in the Capbreton Canyon area during the oceanographic cruise HAPOGE organized in July 2017. Surface sediments (0–10 cm) were sampled with Shipeck sampler grab. First sampling station was into the canyon (G10) and the second one was on the adjacent continental shelf (G14) at 4.4 and 9 km from the coast, and at 70 and 120 meters of depth, respectively (Fig. 1). After collection, the sediment samples were placed into sterile polyethylene bags sealed and stored in the dark at 4°C until slurry incubations in the laboratory (described further) within 48 h. In parallel, for each sample, contaminants analysis and geochemical parameters were analysed [7,15]. Briefly, G10 sediments were more muddy than those in G14 and were characterized with particulate organic matter of 1.60 % and 0.92 %, respectively and fine grain size (<63 µm) of 76 % and 46%, respectively .

2.2. Chemicals

Ethyl acetate (EtOAc) and methanol were of analytical grade and supplied by Sigma Aldrich (Saint-Louis, USA). Acetone (laboratory reagent. 99.5%) used for cleaning the glassware was supplied by Fisher (Hampton, USA). Ultrapure water was obtained with a PURELAB Classic water purification system from Veolia (Paris, France). Reference standards of galaxolide (HHCB), octocrylene (OC), padimate O (OD-PABA) and carbamazepine (CBZ) were purchased from Sigma–Aldrich. Tonalide (AHTN) was purchased from LGC Standards (Molsheim, France). Internal standards musk xylene-d15 (MX-d15) (100 ng μL^{-1} in acetone) was purchased from LGC Standards and carbamazepine-d10 (CBZ-d10) (100 ng μL^{-1} in methanol) was purchased from Sigma Aldrich.

2.3 Determination of half life: sediment slurry incubations

In the laboratory slurry incubations spiked with HHCB, OD-PABA and CBZ were performed for 110 days in order to estimate the degradation potential (Fig. 2). Briefly, for each station (G10 and G14), a slurry was prepared by mixing fresh sediment with underlying water (50:50, w:w). Incubation experiments were performed in 10 mL glass tubes sealed with PTFE stoppers filled with 10 g of slurry. For all assays under abiotic and biotic conditions, HHCB, OD-PABA and CBZ were added independently, to have a final concentration at 100 µg g^{-1}. Initial time assays were stopped immediately by storing samples at -80°C whereas incubated assays were placed in the dark at 14°C (*in situ* temperature) and stopped at different elapsed times until 110 days. Slurries under abiotic condition (control), were twice sterilized for 20 min at 120 °C. All assays were performed in triplicate.

Based on the assumption that degradation reaction of these contaminants are of pseudo-first-order (1),biodegradation rate constants (k) were estimated with the following equation (2) :

(1) $Ln(C) = f(t)$

(2) $k = Ln(C_0/C_t)_t$

Where C_t is the concentration of the contaminant at the kinetic time t; C_0 is the initial contaminant concentration; k is the biodegradation rate constant and t is the time. Then, half time reactions were also determined by the following equation (3):

(3) $t_{1/2} = Ln(2)/k$

2.4. Enrichment experiments in liquid medium

In order to do a selective enrichment of microbial populations potentially involved in the ECs degradation, 0.5 grams of oxic (surface, 0.1 cm, clear yellow color) and 0.5 grams of anoxic (dark color) sediments (Fig. 2) were subsamples from the last days of sediment slurry incubations and were diluted with modified multipurpose medium [23] at 20 g L^{-1} of NaCl, called MM_{20} . The medium contained : 1 L of MilliQ water, 1 ml of SL_{12}, 1 mL SeTg, HEPES 10 mM, yeast extract 0.1 g L^{-1}, 20 g NaCl, 3 g $MgCl_26H_2O$, 0.15 g $CaCl_2\,H_2O$, 0.25 g NH_4Cl, 0.5 g KCl. pH was ajusted at pH= 7.5 before sterilization and autoclave (20 min at 120°C). After autoclaving, 1 mL of solution V7 (vitamins) and KH_2PO_4 (final concentration of 0.2 g L^{-1}) were added with a syringe and a cellulose nitrate 0.2 µm filter (Fisher).

While we determined the half life for 3 ECs, the enrichment experiments in liquid medium were performed for 5 ECs, HHCB, AHTN, OD-PABA, OC and CBZ. Since AHTN has the same molecular mass than HHCB but with a different chemical structure (Table 1), we performed the enrichment experiment for both from the HHCB sediment slurry incubation. Then, OC was added to this list due to its occurrence in coastal sediments [24,25]. ECs were added independently at 100 µM final concentration (corresponding to 25, 25, 28, 36 and 24 ppm, respectively) according previous experiments [26–29]. These ECs have low solubility in water (Table 1), and they were added first into the tube with organic solvent (EtOAc, or methanol for CBZ) and evaporated at ambient temperature, under microbiological safety cabinet (MSC). This step was performed in order to release organic solvent, a potential carbon source [30]. Enrichment was performed in an agitator (120 rpm) [26] under oxic and anoxic conditions, at 27°C for one month. This enrichment was repeated three times, with

each time a dilution at approximatively 1/11 of the previous assay. Briefly, under MSC, micropollutant was added in a falcon tube (50 mL) or penicillin tube (100 mL) (for oxic and anoxic conditions, respectively). Then 5.5 mL of the MM_{20} were added before the addition of 0.5 g of anoxic and oxic sediments from the slurry incubations (1st step, Fig 2). After one month in the agitator, new tubes were prepared with ECs by using the same protocol. Then 5 ml of MM_{20} were added and inoculated with 0.5 ml of the previous enrichment, corresponding to a 1/11 dilution (2nd and 3rd steps, Fig. 2).

2.5 Strains isolation experiments

After the third enrichment step, in order to isolate the strains previously selected and enriched, solid medium of MM_{20} (with 20 g L^{-1} of agar bacteriologic, previously rinsed 3 times with ultrapure water) were prepared in Petri plates. Under MSC, micropollutants were added (274, 274, 306, 498 and 254 µl of HHCB, AHTN, OD-PABA, OC and CBZ at 1000 ppm, respectively) at the surface of the solid medium and organic solvent was evaporated. Then, inocula of the last enrichments were spread at the surface of the solid medium with sterile inoculator and sealed with parafilm before incubation for 1 month, at 27°C to favour the growth of colonies. After one month, colonies were selected and isolated by streak plates as described (A, B, C steps, Fig. 2). Then all strains isolated were stored at -80°C in sterile LB (20 g L) supplemented with NaCl (20 g L^{-1}), and glycerol 30% (v:v).

2.6 Identification of isolated strains: DNA extraction, 16s rDNA gene amplification, sequencing and phylogenetic analysis

DNA was amplified from isolated strains after growth in LB Lennox medium. Amplification of the 16S rRNA gene was done with the universal primers 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387R (5'-GGG CGG WGT GTA CAA GGC-3')[31]. PCR amplification was performed using ampliTaq Gold® 360 master mix (Applied Biosystems, CA, USA), 0.2 µM of each primer and 1 µL of strain. PCR cycling was as following : after 10 min of initial denaturation at 95 °C (lysis of cells), 35 cycles of 40 s denaturation at 95 °C, 40 s annealing

at 58 °C and 60 s elongation at 72 °C with 7 min final elongation at 72 °C. Amplicons were sequenced by SANGER sequencing at GATC (Köln, Germany). Sequences were trimmed with ChromasPro (Technelysium software) and were aligned with MUSCLE [32].A tree was generated using MEGA X software [33], with the Maximum Likelihood method and Tamura-Nei model [34] (with n replication boostraps = 500). Phylogenetic analysis were processed with NCBI (https://www.ncbi.nlm.nih.gov/) and corresponding reference type strains as defined by the bacteriological code of nomenclature for Prokaryotes (http://www.bacterio.net/-classifphyla.html). Sequences are archived in GenBank under accession numbers MT658667 to MT658707.

In parallel, in order to compare with the isolated strains, DNA of *in situ* samples and from the final kinetic time of the slurry incubations were studied to determine the global diversity. DNA was extracted from frozen sediments with the QIAGEN DNeasy Powersoil kits (Qiagen Inc., Netherlands) according to the manufacturer's instructions. Diversity of the 16S rDNA were determined by sequencing the V4-V5 hypervariable regions of the 16S rDNA with universal primers V4-515F (5' GTGYCAGCMGCCGCGGTA 3') and V5-928R (5′-ACTYAAAKGAATTGRCGGGG 3') [35–37]. PCR was performed using ampliTaq Gold® 360 master mix (Applied Biosystems, CA, USA), 0.5 µM of each primer and 3 ng of extracted DNA. PCR cycling was as following : after 10 min of initial denaturation at 95 °C, 30 cycles of 30 s denaturation at 95 °C, 30 s annealing at 60 °C and 40 s elongation at 72 °C with 7 min final elongation at 72 °C. Amplicons were sequenced using MiSeq 250-pair-end technology (Illumina, CA, USA) with V3 kit version, in Get-plage sequencing platform (INRA, Toulouse, France). Data were preprocessed using Galaxy FROGS pipeline [38]. Chimera and PhiX reads were removed, Operational Taxonomic Units (OTUs) clustering, after a de-noising step allows building fine clusters with minimal differences, with an aggregation distance equal or above 3. Data were normalized with the minimum number of reads. Taxonomic affiliation was performed using the Silva database v.128 [39]. Sequences data in situ and from the final

kinetic time of the slurry incubations have been deposited in Genbank under the accession number PRJNA608532 and PRJNA640934, respectively.

2.7 Experimental ECs degradation test

Isolated strains were re-cultivated in medium MM_{20}-PYG (MM_{20} medium supplemented with peptone, 5 g L^{-1}, yeast extracts, 2.5 g L^{-1} and glucose at 5 g L^{-1}) à 37 °C for 90 hours [40]. Optical density (DO) at 600 nm was measured during the growth until the maximum growth using spectrophotometer (Spectronic 20). Degradation tests were performed in glass tubes with contaminants (HHCB, AHTN, OD-PABA, OC and CBZ) evaporated (Delgado-Moreno et al 2019) to be at 1 ppm final concentration, with 1 ml of the cultivated strains, 9 ml of MM_{20}-PYG at 27°C in agitator (120 rpm) [26,40]. 10 µL of micropollutant at 1000 ppm were added with sterile syringe and PTFE filter 0.2 µm (Fisher). Degradation was stopped when DO_{600} reached the maximum value. For initial and final times, chemical analysis were performed (in triplicates) to evaluate the ECs degradation capacity of the isolated strains under controlled conditions (described below).

2.8 ECs analysis

HHCB, AHTN, OD-PABA, OC and CBZ were extracted by a liquid:liquid extraction, for both the second enrichment in liquid medium (3) and the ECs degradation test (Fig.2) at the initial and final time of each experiment. Briefly, 10 and 2 mL of EtOAc were added directly into the essay tube before to vortex for 1 min, respectively. Then, 20µL of the supernatant was diluted with EtOAc (960 µL) in GC-vial and spiked with 20 µL of an internal standard mixture at 10 µL ng^{-1} (CBZ-d10 and MX-d15) before analysis. Extracts were analysed by 7890A gas chromatograph coupled with 5975C mass spectrometer (GC/MS) with an Electron Ionization (EI) source using a Large Volume Injection (LVI) (Agilent Technologies). The GC/MS system was equipped with a single taper ultra-inert liner with glass wool and a HP-5MS UI capillary column (30 m length × 0.25 mm diameter and 0.25 µm film thickness). Carrier gas was helium (He) with a purity greater than 99.999% (Linde). Separation was performed at a

constant He flow of 1.5 mL min^{-1}, and the GC oven temperature was programmed to hold at 50 °C for 3 min, then increase at 25 °C min^{-1} until 195 °C (hold 1.5 min), then 8 °C min^{-1} until 265 °C (hold 1 min) and 20 °C min^{-1} until 310 °C (hold 5 min). Injection volume was 20 µL in splitless mode. Instrument control, data acquisition and data treatment were performed using Agilent Chemstation software. Quantification was carried out in Selected Ion Monitoring (SIM) mode, selecting two characteristics fragments ions for each compound. Calibrations with the target analytes (HHCB, AHTN, OD-PABA, OC and CBZ) were used to quantify target compounds. A six-point calibration curve was performed in EtOAc spiked with increasing pollutants concentration levels ranging from 0 to 500 µg L^{-1} as well as internal standards at 50 µg L^{-1} transferred into GC vials (analytical performances are compiled in Table S1, supporting information). Recoveries achieved for all target ECs in MM_{20} medium spiked at 100 µg L^{-1} ranged from 91% to 102% for musk and UV-filter compound and was at 74% for CBZ (Supplementary information, Table S1).

3. Results and discussion

3.1 Degradation of HHCB, OD-PABA and CBZ in sediment slurry incubations

Based on kinetic degradation for 110 days under both abiotic and biotic conditions (Supplementary information, Fig. S1), degradation rate constants, k, were estimated from slurry incubations under biotic condition (Table 2). While CBZ in both sediment samples exhibited similar rates ($0_{\overline{7}}.0035 \pm 0_{\overline{7}}.0004$ and $0_{\overline{7}}.0029 \pm 0_{\overline{7}}.0001$), HHCB and OD-PABA exhibited different rates of degradation according the location (e.g. G10 and G14) with a factor around 2 (Table 2). This suggested that ECs degradations were depending on the geochemical parameters of the sediments (e.i. grain size, organic matter content). For instance, a previous study demonstrated that ECs have a strong affinity for particles [41], as observed with sludge in wastewater treatment plants [42], suggesting those parameters and as well as environmental factors have to be taken in consideration to understand the fate of ECs in the ocean. Estimation of the half life time of reaction for these ECs indicated that the UV filter OD-PABA was between 21±1 and 44±4 days (Table 2). These results are similar

255 with another UV filter degradation (i.e. EH-DPAB) estimated in marine sediments microcosm
256 with a $t_{1/2}$ ranging from18 to 50 days [43]. Nevertheless, half life reaction for synthetic musk
257 HHCB and anti-epileptic CBZ reached around 8 months and 7 months, respectively,
258 indicated the high persistence of these contaminants in natural marine sediments compare to
259 the UV filter OD-PABA (Table 2). Lack of data on HHCB and CBZ degradation potentials in
260 marine sediments does not allow comparison with these present data. Nevertheless, these
261 preliminary results demonstrate the high persistence of these ECs in natural environment. In
262 sediments, ECs may be associated to particulate matter, establishing equilibrium relations in
263 the water-sediment interface, limiting their bioavailability. ECs remobilisation depends both of
264 geochemical characteristics variations of the sediment, the overlying water column and pore
265 water. Thus, ECs persistence in environment depend on coupling of abiotic and biotic factors
266 [44,45], such as physicochemical properties of the ECs, environmental factors (e.i.
267 temperature [46], pH [19], redox processes[47] photolysis process [48,49]) and the presence
268 of microbial communities able to degrade these ECs [50]. It was also demonstrated that
269 bioturbation or resuspension improved oxidation of anoxic sediments leading an increase of
270 lability of ECs which can be more available for degradation processes [51].
271 Additionally, chemical characterization of the collected sediments revealed that the three-
272 targeted ECs were not found at concentration detectable with the analytical method used [7].
273 The removal potential was observed under both abiotic and biotic conditions with percentage
274 of removal for each compounds, HHCB, OD-PABA and CBZ, ranging from 37.5% to 51.4%,
275 from 50.1% to 98.5% and from 11.6% to 49.6%, respectively (Fig. 3). Results of HHCB and
276 CBZ slurry incubations suggested that the degradation was mainly driven by an abiotic
277 process (except for G10 exposed with CBZ). Removal potential of OD-PABA under biotic
278 condition was 2 times higher compared to the abiotic condition, suggesting that micro-
279 organisms in these sediments improved the degradation of this UV filter compound (Fig. 3).

280 **3.2 Enrichment in liquid medium**

Results of the second enrichment in liquid medium (Fig. 4) demonstrated a higher removal in oxic conditions than under anoxic conditions for the five ECs studies (e.i. HHCB, AHTN, OD-PABA and CBZ). These ECs are well known to be observed in WWTP, where CBZ is frequently detected in the liquid effluents while the other contaminants, due to the very hydrophobicity, occur in sludges or remain through membrane post-treatment [52]. Although new advanced treatment processes, such as activated carbon adsorption, advanced oxidation processes, reverse osmosis or membrane bioreactors, are developed to increase the micropollutant removal of the WWTPs to struggle against the spread of micropollutants in aquatic environment, they are still observed in the WWTP effluents [53]. Those results suggested that oxic processes could improve the ECs removal and limit their spread from the WWTP sewage. Moreover, higher removals under abiotic anoxic conditions than biotic were observed. It is likely due to a higher ECs adsorption on cells present in biotic condition leading to trap ECs and reduce the quality of the liquid extraction [54,55]. Additionally, the high concentrations (ppm level) used for the selection of strains in these enrichments could inhibit microbial activity, thus could have mask their ECs removal capacities under anoxic condition [56].

Under oxic condition, similar removal potentials were observed for the abiotic and biotic condition, except for OC and CBZ (Fig. 4). That may indicate a potential involvement of microorganisms in the OC removal. Contrary to OC, CBZ removal was observed only in abiotic anoxic condition, likely due to the same explanations suggested previously for the removal potential under anoxic condition (e.i. adsorption on cells and/or high exposition concentrations). These results led to continue the study of ECs degradation with the isolated strains under oxic conditions.

Nevertheless, even though an efficient removal of ECs under oxic condition was observed, metabolites were not studied. Indeed, degradation of ECs to metabolites have been observed for CBZ [28], HHCB [26] and also for others UV filters [43]. These results highlighted the complexity of ECs removal under experimental conditions as well as in

WWTPs, where their efficiency are dependant of both physical, chemical and biological processes but also to identify metabolites in further studies.

3.3 Isolated strains involved in ECs degradation

While the degradation of complex organic substances can occurs both under anaerobic and aerobic conditions, it is well known that their degradation rates can be higher under aerobic condition [57,58]. In accordance with this observation, isolation of strains and degradation test were consequently performed under aerobic condition. Twenty seven strains were isolated with the current method with 6, 8, 4, 4 and 5 strains constrained with HHCB, AHTN, OD-PABA, OC and CBZ, respectively (Supplementary information, Fig. S3). These strains were related to two families, dominated by the *Firmicutes,* followed by the *Actinobacteria* (Fig.5 A B).

Within the *Actinobacteria*, *Rhodococcus* was the main bacteria genus isolated followed by *Streptomyces, Mycobacterium and Micrococcus*. Within the *Firmicutes*, only *Bacillus*-related strains were isolated (Fig. 5 A, B). Studies have shown that *Actinobacteria* were involved in degradation of some organic and inorganic compounds. For instance, *Rhodococcus* strains are involved in the removal of pharmaceutical compounds [59], tetrabromobisphenol A (TBBPA) [60] and dominant in naphthalene degrading enrichments [61]. It has also been demonstrated that the members of the genus *Streptomyces* produce laccase (i.e. ligninolytic enzymes) that can be involved in the removal of recalcitrant and ECs [62]. It has also been demonstrated that members of the genus *Streptomyces* were involved in the CBZ biodegradation [40,63]. Finally, experiments performed from marine sediments demonstrated that *Bacillus thuringiensis* was a novel group participating to the removal of PAHs and pesticides [64].

Within these isolated strains, only eight exhibited a capacity to degrade contaminants in the conditions used (1 ppm, 5 days, 37 degrees in rich organic medium) with 1, 2, 3 and 2 strains for HHCB, AHTN, OD-PABA and OC, respectively (Table 3). Seven were related to the genus *Bacillus* and one was related to the genus *Rhodoccocus* (23 AHTN G14). While

studies have demonstrated high bioremediation capacities by *Actinobacteria*, this study demonstrated high capacity of marine *bacillus* to degrade ECs. These results suggested that the isolated *bacillus* were able to remove OD-PABA with a capacity ranging between 34% ± 19% and 71% ± 19% (Table 3). These strains could be involved in the biotic degradation observed in the slurry incubation experiment (Fig. 3). Although CBZ slurry incubations experiments (G10) demonstrated a possible biotic degradation (Fig. 3), isolated strains did not exhibit a remediation for this pollutant under the conditions tested (Supplementary information, Fig. S3). Moreover, the $t_{1/2}$ of CBZ was estimated around 7 months, suggesting that the time should be expanded to observe a remediation capacity. Moreover, it is well known that in nature, the microbial consortia have to be considered to estimate biodegradation pollutant removal process [65].Moreover, global microbial communities analysed *in situ*, in G10 and G14 demonstrated a relative abundance of the main phyla similar in both sediments (and similar to the final kinetic time of the slurry incubations, Supplementary information Fig. S2), with a dominance of *Proteobacteria* (relative abundance ranged between 47.9 and 49.4%), followed by *Bacteriodetes*, *Planctomycetes* and *Acidobacteria* (Fig. 6). Although the *Actinobacteria* and *Firmicutes* were minors *in situ* (Fig. 6), representing less than 1% of the total abundance, phylogenic trees based the sequences of the isolated strains (Fig. 5 A,B) demonstrated that these isolated strains were related to the *Actinobacteria* and *Firmicutes* families. This suggests a good selection process of these bacterial groups in the presence of these ECs exposition with the isolation method used.In accordance with previous studies, the isolated bacterial strains from the Capbreton Canyon sediments could be good candidate to degrade ECs. Nevertheless, in order to estimate the natural resilience of those ECs, it is crucial also to take account both the biological (e.i. biodegradation, biosorption/adsorption), physical and chemical processes, highlighting further studies are needed to decipher their fate in oceans. It is difficult to extrapolate results based on pure strains to the field. The culture conditions used (medium, temperature, …) enhance some microorganisms and inhibit others. The role of co-metabolisms in microbial consortia is extremely important as well as the role of the uncultivated prokaryotes. But the

363 objective of this study *in fine* to isolate strains on which we will be able to work on. The
364 isolated strains can be a fantastic tool since they can be used to study the degradation
365 products, to study the isotopic fractionation due to micro-organisms, or to study the genetic
366 determinism of this pathway. These data could then be used as new tools for new
367 environmental studies (compound specific isotopic fractionation, functional genes, ...).

368

369 **4. Conclusion**

370 This work characterized the degradation kinetics for ECs (HHCB, OD-PABA and CBZ) from
371 marine sediments under *in situ* conditions (biotic, anaerobic and temperature) thought slurry
372 sediment incubations. The half life times obtained demonstrated that half time live of OD-
373 PABA was lower than HHCB and CBZ, with $t_{1/2}$ ranging from few weeks to 8 months. This
374 underlies that the ECs degradation in marine sediment is a long process and could be a high
375 concern for the environment considering a chronical contamination from the coastal human
376 activities (e.i. WWTPs sewage, agriculture…). Additionally, contrary to CBZ, different half
377 lives were observed between muddy (G10) and sandy (G14) sediments for HHCB and OD-
378 PABA. That indicates ECs degradation depends on the physicochemical properties of both
379 chemicals and sediment.

380 Moreover, novel synthetic musks and UV filters degrading bacteria were isolated from marine
381 sediment. Based on phylogenetic characteristics, microorganisms were mainly identified as
382 *Bacillus sp*. dominated by *Bacillus megaterium* followed by *Bacillus aquimaris* and *Bacillus*
383 *vietnamensis*. One other isolated strain which was able to degrade musk was related to
384 *Rhodococcus ruber*. The isolated bacteria here, could have a strong potential to degrade a
385 wide variety of organic pollutants (e.g. priority and emerging), such as synthetic musks and
386 UV filters. Improving the knowledge about the microbial diversity in marine environment may
387 lead to the development of technical tools with degradation abilities and high tolerance in the

388 future but also highlight how microbial remediation contributes to the nature resilience in
389 marine environment.

390

391

392

393 **Acknowledgements**

394 This work was supported by the European Union and the Adour Garonne Water Agency
395 (Micropolit Project). Europe commits the New Aquitaine with the European Regional
396 Development Fund.

397 **Compliance with ethical standards**

398 **Conflict of interest** The authors declare that they have no conflict of interest.

399 **References**

400 [1] J.A. Field, C.A. Johnson, J.B. Rose, What is “emerging”?, Environ. Sci. Technol. 40
401 (2006) 7105–7105. https://doi.org/10.1021/es062982z.
402 [2] W.C. Li, Occurrence, sources, and fate of pharmaceuticals in aquatic environment and
403 soil, Environ. Pollut. 187 (2014) 193–201. https://doi.org/10.1016/j.envpol.2014.01.015.
404 [3] S. Rainieri, A. Barranco, M. Primec, T. Langerholc, Occurrence and toxicity of musks
405 and UV filters in the marine environment, Food Chem. Toxicol. 104 (2017) 57–68.
406 https://doi.org/10.1016/j.fct.2016.11.012.
407 [4] M. Beretta, V. Britto, T.M. Tavares, S.M.T. da Silva, A.L. Pletsch, Occurrence of
408 pharmaceutical and personal care products (PPCPs) in marine sediments in the Todos
409 os Santos Bay and the north coast of Salvador, Bahia, Brazil, J. Soils Sediments. 14
410 (2014) 1278–1286. https://doi.org/10.1007/s11368-014-0884-6.
411 [5] W. Huang, Z. Xie, W. Yan, W. Mi, W. Xu, Occurrence and distribution of synthetic
412 musks and organic UV filters from riverine and coastal sediments in the Pearl River

estuary of China, Mar. Pollut. Bull. 111 (2016) 153–159. https://doi.org/10.1016/j.marpolbul.2016.07.018.

[6] M.G. Pintado-Herrera, C. Wang, J. Lu, Y.-P. Chang, W. Chen, X. Li, P.A. Lara-Martín, Distribution, mass inventories, and ecological risk assessment of legacy and emerging contaminants in sediments from the Pearl River Estuary in China, J. Hazard. Mater. 323 (2017) 128–138. https://doi.org/10.1016/j.jhazmat.2016.02.046.

[7] A. Azaroff, C. Miossec, L. Lanceleur, R. Guyoneaud, M. Monperrus, Priority and emerging micropollutants distribution from coastal to continental slope sediments: A case study of Capbreton Submarine Canyon (North Atlantic Ocean), Sci. Total Environ. 703 (2020) 135057. https://doi.org/10.1016/j.scitotenv.2019.135057.

[8] J. Bellas, Ó. Nieto, R. Beiras, Integrative assessment of coastal pollution: Development and evaluation of sediment quality criteria from chemical contamination and ecotoxicological data, Cont. Shelf Res. 31 (2011) 448–456. https://doi.org/10.1016/j.csr.2010.04.012.

[9] D.A. Roberts, Causes and ecological effects of resuspended contaminated sediments (RCS) in marine environments, Environ. Int. 40 (2012) 230–243. https://doi.org/10.1016/j.envint.2011.11.013.

[10] C.K. Paull, H.G. Greene, W. Ussler, P.J. Mitts, Pesticides as tracers of sediment transport through Monterey Canyon, Geo-Mar. Lett. 22 (2002) 121–126. https://doi.org/10.1007/s00367-002-0110-1.

[11] A.M. Costa, M. Mil-Homens, S.M. Lebreiro, T.O. Richter, H. de Stigter, W. Boer, M.A. Trancoso, Z. Melo, F. Mouro, M. Mateus, J. Canário, V. Branco, M. Caetano, Origin and transport of trace metals deposited in the canyons off Lisboa and adjacent slopes (Portuguese Margin) in the last century, Mar. Geol. 282 (2011) 169–177. https://doi.org/10.1016/j.margeo.2011.02.007.

[12] T. Mulder, O. Weber, P. Anschutz, F. Jorissen, J.-M. Jouanneau, A few months-old storm-generated turbidite deposited in the Capbreton Canyon (Bay of Biscay, SW France), Geo-Mar. Lett. 21 (2001) 149–156. https://doi.org/10.1007/s003670100077.

[13] T. Salles, T. Mulder, M. Gaudin, M.C. Cacas, S. Lopez, P. Cirac, Simulating the 1999 Capbreton canyon turbidity current with a Cellular Automata model, Geomorphology. 97 (2008) 516–537. https://doi.org/10.1016/j.geomorph.2007.09.005.

[14] S. Brocheray, M. Cremer, S. Zaragosi, S. Schmidt, F. Eynaud, L. Rossignol, H. Gillet, 2000years of frequent turbidite activity in the Capbreton Canyon (Bay of Biscay), Mar. Geol. 347 (2014) 136–152. https://doi.org/10.1016/j.margeo.2013.11.009.

[15] A. Azaroff, E. Tessier, J. Deborde, R. Guyoneaud, M. Monperrus, Mercury and methylmercury concentrations, sources and distribution in submarine canyon sediments (Capbreton, SW France): Implications for the net methylmercury production, Sci. Total Environ. 673 (2019) 511–521. https://doi.org/10.1016/j.scitotenv.2019.04.111.

[16] C. Miège, J.M. Choubert, L. Ribeiro, M. Eusèbe, M. Coquery, Fate of pharmaceuticals and personal care products in wastewater treatment plants – Conception of a database and first results, Environ. Pollut. 157 (2009) 1721–1726. https://doi.org/10.1016/j.envpol.2008.11.045.

[17] P. Verlicchi, M. Al Aukidy, E. Zambello, Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review, Sci. Total Environ. 429 (2012) 123–155. https://doi.org/10.1016/j.scitotenv.2012.04.028.

[18] L.H. Nowell, P.W. Moran, R.J. Gilliom, D.L. Calhoun, C.G. Ingersoll, N.E. Kemble, K.M. Kuivila, P.J. Phillips, Contaminants in Stream Sediments From Seven United States Metropolitan Areas: Part I: Distribution in Relation to Urbanization, Arch. Environ. Contam. Toxicol. 64 (2013) 32–51. https://doi.org/10.1007/s00244-012-9813-0.

[19] L.W. Perelo, Review: In situ and bioremediation of organic pollutants in aquatic sediments, J. Hazard. Mater. 177 (2010) 81–89. https://doi.org/10.1016/j.jhazmat.2009.12.090.

[20] R. Duran, C. Cravo-Laureau, Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment, FEMS Microbiol. Rev. 40 (2016) 814–830. https://doi.org/10.1093/femsre/fuw031.

[21] M.C. Ncibi, B. Mahjoub, O. Mahjoub, M. Sillanpää, Remediation of Emerging Pollutants in Contaminated Wastewater and Aquatic Environments: Biomass-Based Technologies, CLEAN – Soil Air Water. 45 (2017) 1700101. https://doi.org/10.1002/clen.201700101.

[22] M. Gavrilescu, K. Demnerová, J. Aamand, S. Agathos, F. Fava, Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation, New Biotechnol. 32 (2015) 147–156. https://doi.org/10.1016/j.nbt.2014.01.001.

[23] F. Widdel, F. Bak, Gram-Negative Mesophilic Sulfate-Reducing Bacteria, in: A. Balows, H.G. Trüper, M. Dworkin, W. Harder, K.-H. Schleifer (Eds.), Prokaryotes Handb. Biol. Bact. Ecophysiol. Isol. Identif. Appl., Springer New York, New York, NY, 1992: pp. 3352–3378. https://doi.org/10.1007/978-1-4757-2191-1_21.

[24] T. Combi, M.G. Pintado-Herrera, P.A. Lara-Martin, S. Miserocchi, L. Langone, R. Guerra, Distribution and fate of legacy and emerging contaminants along the Adriatic Sea: A comparative study, Environ. Pollut. 218 (2016) 1055–1064. https://doi.org/10.1016/j.envpol.2016.08.057.

[25] C.L. Mitchelmore, K. He, M. Gonsior, E. Hain, A. Heyes, C. Clark, R. Younger, P. Schmitt-Kopplin, A. Feerick, A. Conway, L. Blaney, Occurrence and distribution of UV-filters and other anthropogenic contaminants in coastal surface water, sediment, and coral tissue from Hawaii, Sci. Total Environ. 670 (2019) 398–410. https://doi.org/10.1016/j.scitotenv.2019.03.034.

[26] L. Vallecillos, Y. Sadef, F. Borrull, E. Pocurull, K. Bester, Degradation of synthetic fragrances by laccase-mediated system, J. Hazard. Mater. 334 (2017) 233–243. https://doi.org/10.1016/j.jhazmat.2017.04.003.

[27] F. Balk, R.A. Ford, Environmental risk assessment for the polycyclic musks AHTN and HHCB in the EU: I. Fate and exposure assessment, Toxicol. Lett. 111 (1999) 57–79. https://doi.org/10.1016/S0378-4274(99)00169-1.

[28] A. König, C. Weidauer, B. Seiwert, T. Reemtsma, T. Unger, M. Jekel, Reductive transformation of carbamazepine by abiotic and biotic processes, Water Res. 101 (2016) 272–280. https://doi.org/10.1016/j.watres.2016.05.084.

[29] A. Sauvêtre, R. May, R. Harpaintner, C. Poschenrieder, P. Schröder, Metabolism of carbamazepine in plant roots and endophytic rhizobacteria isolated from Phragmites australis, J. Hazard. Mater. 342 (2018) 85–95. https://doi.org/10.1016/j.jhazmat.2017.08.006.

[30] L. Delgado-Moreno, S. Bazhari, R. Nogales, E. Romero, Innovative application of biobed bioremediation systems to remove emerging contaminants: Adsorption, degradation and bioaccesibility, Sci. Total Environ. 651 (2019) 990–997. https://doi.org/10.1016/j.scitotenv.2018.09.268.

[31] J.R. Marchesi, T. Sato, A.J. Weightman, T.A. Martin, J.C. Fry, S.J. Hiom, W.G. Wade, Design and Evaluation of Useful Bacterium-Specific PCR Primers That Amplify Genes Coding for Bacterial 16S rRNA, Appl Env. Microbiol. 64 (1998) 795–799.

[32] R.C. Edgar, MUSCLE: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Res. 32 (2004) 1792–1797. https://doi.org/10.1093/nar/gkh340.

[33] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms, Mol. Biol. Evol. 35 (2018) 1547–1549. https://doi.org/10.1093/molbev/msy096.

[34] K. Tamura, M. Nei, Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees, Mol. Biol. Evol. 10 (1993) 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023.

[35] A.E. Parada, D.M. Needham, J.A. Fuhrman, Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples, Environ. Microbiol. 18 (2016) 1403–1414. https://doi.org/10.1111/1462-2920.13023.

[36] W. Walters, E.R. Hyde, D. Berg-Lyons, G. Ackermann, G. Humphrey, A. Parada, J.A. Gilbert, J.K. Jansson, J.G. Caporaso, J.A. Fuhrman, A. Apprill, R. Knight, Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys, MSystems. 1 (2015). https://doi.org/10.1128/mSystems.00009-15.

[37] Y. Wang, P.-Y. Qian, Conservative Fragments in Bacterial 16S rRNA Genes and Primer Design for 16S Ribosomal DNA Amplicons in Metagenomic Studies, PLOS ONE. 4 (2009) e7401. https://doi.org/10.1371/journal.pone.0007401.

[38] F. Escudié, L. Auer, M. Bernard, M. Mariadassou, L. Cauquil, K. Vidal, S. Maman, G. Hernandez-Raquet, S. Combes, G. Pascal, FROGS: Find, Rapidly, OTUs with Galaxy Solution, Bioinforma. Oxf. Engl. 34 (2018) 1287–1294. https://doi.org/10.1093/bioinformatics/btx791.

[39] E. Pruesse, C. Quast, K. Knittel, B.M. Fuchs, W. Ludwig, J. Peplies, F.O. Glöckner, SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB, Nucleic Acids Res. 35 (2007) 7188–7196. https://doi.org/10.1093/nar/gkm864.

[40] C. Popa, L. Favier, R. Dinica, S. Semrany, H. Djelal, A. Amrane, G. Bahrim, Potential of newly isolated wild Streptomyces strains as agents for the biodegradation of a recalcitrant pharmaceutical, carbamazepine, Environ. Technol. 35 (2014) 3082–3091. https://doi.org/10.1080/09593330.2014.931468.

[41] S. Li, G. Lu, Z. Xie, J. Ding, J. Liu, Y. Li, Sorption and degradation of selected organic UV filters (BM-DBM, 4-MBC, and OD-PABA) in laboratory water-sediment systems, Environ. Sci. Pollut. Res. 23 (2016) 9679–9689. https://doi.org/10.1007/s11356-016-6126-2.

[42] Y.-S. Liu, G.-G. Ying, A. Shareef, R.S. Kookana, Occurrence and removal of benzotriazoles and ultraviolet filters in a municipal wastewater treatment plant, Environ. Pollut. Barking Essex 1987. 165 (2012) 225–232. https://doi.org/10.1016/j.envpol.2011.10.009.

550 [43] A. Volpe, M. Pagano, G. Mascolo, P. Grenni, S. Rossetti, Biodegradation of UV-filters in
551 marine sediments, Sci. Total Environ. 575 (2017) 448–457.
552 https://doi.org/10.1016/j.scitotenv.2016.10.001.
553 [44] N. Lee Wolfe, D.L. Macalady, New perspectives in aquatic redox chemistry: Abiotic
554 transformations of pollutants in groundwater and sediments, J. Contam. Hydrol. 9
555 (1992) 17–34. https://doi.org/10.1016/0169-7722(92)90048-J.
556 [45] J.-R. Jeon, K. Murugesan, P. Baldrian, S. Schmidt, Y.-S. Chang, Aerobic bacterial
557 catabolism of persistent organic pollutants — potential impact of biotic and abiotic
558 interaction, Curr. Opin. Biotechnol. 38 (2016) 71–78.
559 https://doi.org/10.1016/j.copbio.2015.12.016.
560 [46] C. Lange, B. Kuch, J.W. Metzger, Occurrence and fate of synthetic musk fragrances in
561 a small German river, J. Hazard. Mater. 282 (2015) 34–40.
562 https://doi.org/10.1016/j.jhazmat.2014.06.027.
563 [47] J.-R. Jeon, K. Murugesan, I.-H. Nam, Y.-S. Chang, Coupling microbial catabolic actions
564 with abiotic redox processes: A new recipe for persistent organic pollutant (POP)
565 removal, Biotechnol. Adv. 31 (2013) 246–256.
566 https://doi.org/10.1016/j.biotechadv.2012.11.002.
567 [48] S. Chiron, C. Minero, D. Vione, Photodegradation Processes of the Antiepileptic Drug
568 Carbamazepine, Relevant To Estuarine Waters, Environ. Sci. Technol. 40 (2006) 5977–
569 5983. https://doi.org/10.1021/es060502y.
570 [49] D. Vogna, R. Marotta, R. Andreozzi, A. Napolitano, M. d'Ischia, Kinetic and chemical
571 assessment of the UV/H2O2 treatment of antiepileptic drug carbamazepine,
572 Chemosphere. 54 (2004) 497–505. https://doi.org/10.1016/S0045-6535(03)00757-4.
573 [50] K.M. Onesios, J.T. Yu, E.J. Bouwer, Biodegradation and removal of pharmaceuticals
574 and personal care products in treatment systems: a review, Biodegradation. 20 (2009)
575 441–466. https://doi.org/10.1007/s10532-008-9237-8.

[51] K. Booij, E.P. Achterberg, B. Sundby, Release rates of chlorinated hydrocarbons from contaminated sediments, Neth. J. Sea Res. 29 (1992) 297–310. https://doi.org/10.1016/0077-7579(92)90070-U.

[52] P. Krzeminski, C. Schwermer, A. Wennberg, K. Langford, C. Vogelsang, Occurrence of UV filters, fragrances and organophosphate flame retardants in municipal WWTP effluents and their removal during membrane post-treatment, J. Hazard. Mater. 323 (2017) 166–176. https://doi.org/10.1016/j.jhazmat.2016.08.001.

[53] Y. Luo, W. Guo, H.H. Ngo, L.D. Nghiem, F.I. Hai, J. Zhang, S. Liang, X.C. Wang, A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment, Sci. Total Environ. 473–474 (2014) 619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065.

[54] J.P. Bell, M. Tsezos, Removal of Hazardous Organic Pollutants by Biomass Adsorption, J. Water Pollut. Control Fed. 59 (1987) 191–198.

[55] M. Tsezos, J.P. Bell, Comparison of the biosorption and desorption of hazardous organic pollutants by live and dead biomass, Water Res. 23 (1989) 561–568. https://doi.org/10.1016/0043-1354(89)90022-5.

[56] A. Barra Caracciolo, E. Topp, P. Grenni, Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities. A review, J. Pharm. Biomed. Anal. 106 (2015) 25–36. https://doi.org/10.1016/j.jpba.2014.11.040.

[57] M. Biel-Maeso, C. González-González, P.A. Lara-Martín, C. Corada-Fernández, Sorption and degradation of contaminants of emerging concern in soils under aerobic and anaerobic conditions, Sci. Total Environ. 666 (2019) 662–671. https://doi.org/10.1016/j.scitotenv.2019.02.279.

[58] G.-G. Ying, X.-Y. Yu, R.S. Kookana, Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling, Environ. Pollut. 150 (2007) 300–305. https://doi.org/10.1016/j.envpol.2007.02.013.

603 [59] I. Ivshina, E. Tyumina, E. Vikhareva, Biodegradation of emerging pollutants: focus on
604 pharmaceuticals, Microbiol. Aust. 39 (2018) 117–122. https://doi.org/10.1071/MA18037.
605 [60] S. Xu, Y.-F. Wang, L.-Y. Yang, R. Ji, A.-J. Miao, Transformation of tetrabromobisphenol
606 A by Rhodococcus jostii RHA1: Effects of heavy metals, Chemosphere. 196 (2018)
607 206–213. https://doi.org/10.1016/j.chemosphere.2017.12.173.
608 [61] S.-K. Rhee, X. Liu, L. Wu, S.C. Chong, X. Wan, J. Zhou, Detection of Genes Involved in
609 Biodegradation and Biotransformation in Microbial Communities by Using 50-Mer
610 Oligonucleotide Microarrays, Appl. Environ. Microbiol. 70 (2004) 4303–4317.
611 https://doi.org/10.1128/AEM.70.7.4303-4317.2004.
612 [62] J.-A. Majeau, S.K. Brar, R.D. Tyagi, Laccases for removal of recalcitrant and emerging
613 pollutants, Bioresour. Technol. 101 (2010) 2331–2350.
614 https://doi.org/10.1016/j.biortech.2009.10.087.
615 [63] C. Popa Ungureanu, L. Favier, G. Bahrim, A. Amrane, Response surface optimization
616 of experimental conditions for carbamazepine biodegradation by Streptomyces MIUG
617 4.89, New Biotechnol. 32 (2015) 347–357. https://doi.org/10.1016/j.nbt.2014.12.005.
618 [64] L. Ferreira, E. Rosales, A.S. Danko, M.A. Sanromán, M.M. Pazos, Bacillus thuringiensis
619 a promising bacterium for degrading emerging pollutants, Process Saf. Environ. Prot.
620 101 (2016) 19–26. https://doi.org/10.1016/j.psep.2015.05.003.
621 [65] Y. Wu, J. He, L. Yang, Evaluating Adsorption and Biodegradation Mechanisms during
622 the Removal of Microcystin-RR by Periphyton, Environ. Sci. Technol. 44 (2010) 6319–
623 6324. https://doi.org/10.1021/es903761y.
624
625

Figures

Microbial degradation of hydrophobic emerging contaminants from marine sediment slurries (Capbreton Canyon) to pure bacterial strain

Fig. 1 Sediment sampling sites in the Capbreton Canyon (dark circle) and on the adjacent continental shelf (white circle). Isobath data from SEDIMAQ3 (Gillet 2012).

Fig. 2 – Design of experiments to determine natural resilience and microbial strains involved in the degradation of emerging contaminants (ECs): Galaxolide (HHCB), Padimate O (OD-PABA), Carbamazepine (CBZ), Tonalide (AHTN) and Octocrylen (OC). First step was the determination of *in situ* degradation constant rates from slurry sediments spiked with three ECs under biotic and abiotic conditions at 100 ppb (AHTN and OC were not monitored). Second step was the selection of strains from the last slurry sediments incubation (day 110) through enrichments performed under oxic/anoxic and biotic/abiotic conditions at 25, 25, 28, 36 and 24 ppm of HHCB, AHTN, OD-PABA, OC and CBZ, respectively (corresponding to 100 µM). These enrichments were repeated three times (1, 2, 3) with liquid multipurpose medium at 20 g L^{-1} of NaCl, called MM_{20}. Third step was strains isolation from the last enrichment (3) where microbial consortia were separated (spread plates) on agar MM_{20} at same ECs exposition concentrations than previously (A). After one month of growth, colonies were twice isolated by streak plating (B, C). The last step was the characterisation of isolated strains through both their identification and the determination of their ECs degradation capacities. Isolated strains were identified by 16s rDNA amplification and Sanger sequencing. ECs degradation tests were performed at 1 ppm of ECs using liquid MM_{20} suppplemented with peptone (5 g L^{-1}), yeast extract (2.5 g L^{-1}) and glucose (5 g L^{-1}), called MM_{20}-PYG. * indicates that results are presented in the figures.

Fig. 3 - HHCB, OD-PABA and CBZ removal potential (%) after 110 days of sediment incubation ()under biotic and abiotic conditions from Capbreton Canyon sediments (G10, and G14). Initial exposure concentration was 100 ppb. Data are mean ± SD of three replicates.

Fig. 4 – HHCB, AHTN, OD-PABA, OC and CBZ removal potential (%) after one month on the second liquid enrichment under anoxic and oxic conditions with inoculum from G10 and G14 stations (biotic). Abiotic control was achieved without innoculum.

Fig. 5 Phylogenic trees of strains obtained after the aerobic isolation in solid medium with different ECs (AHTN, HHCB, OC, OD-PABA and CBZ) from sediments collected in the submarine Canyon of Capbreton (stations G10 and G14). Fig.5A and Fig.5B correspond to *Actinobacteria* and *Firmicutes*, respectively. Isolated strains are in bold, reference strains are in italic. The evolutionary history was

inferred by using the Maximum Likelihood method and Tamura-Nei model. The bootstrap values areabove 50% are shown next to the branches. The scale indicates nucleotides substitution. Groups 1, 2, 3, 4 and 5 included 10 (20, 21 AHTN-G10; 22, 27 AHTN-G14 and 4, 6, 7, 8, 11, 12 HHCB- G14), 3 (33, 35, 36 ODPABA-G14), 4 (17, 18, 19 AHTN-G10 and 24 AHTN-G14), 6 (45, 46, 47, 48 CBZ-G14 and 50, 52 CBZ-G10) and 3 (41, 42, 43 OC-G10) isolated strains, respectively.

Fig. 6 –Relative abundance of prokaryotic 16S rDNA gene sequences (MiSEQ) from sediments of G10 and G14. Data are average percentages (± SE). Categories representing > 1% are shown. Relative abundances of phyla from which the isolated strains belonged are also shown.

Fig1.

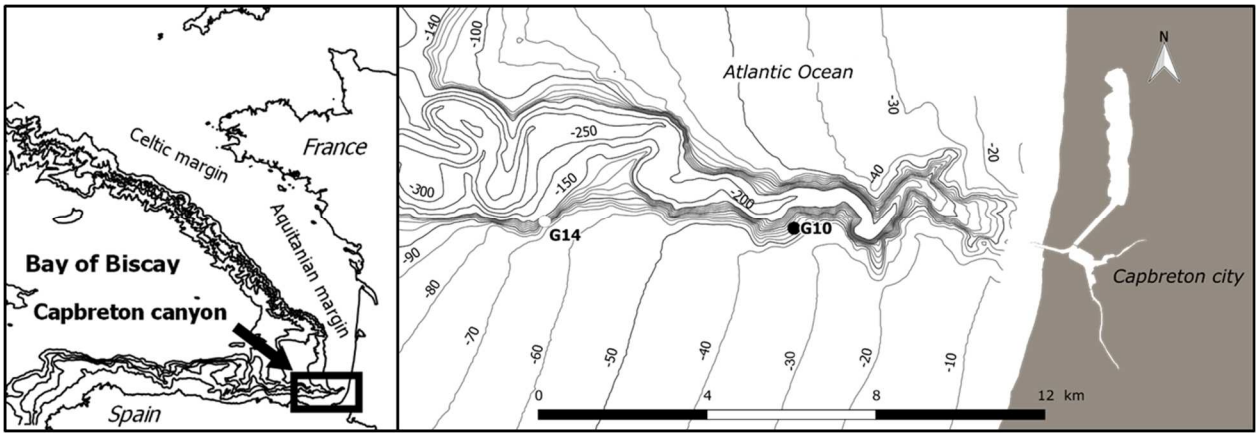

Celtic margin
France
Aquitanian margin
Bay of Biscay
Capbreton canyon
Spain
Atlantic Ocean
G14
G10
Capbreton city
N
0
4
8
12 km

Fig. 2

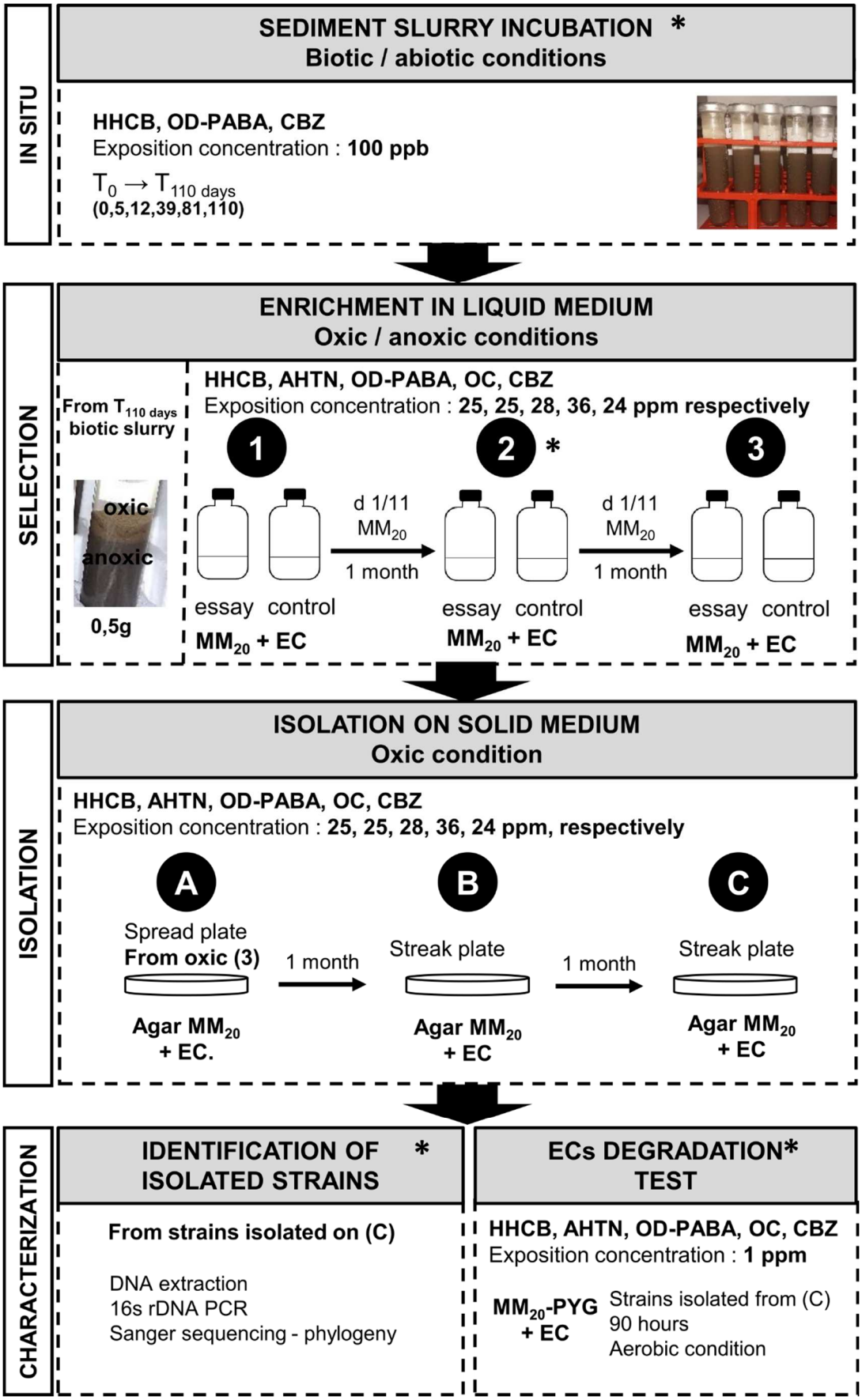

IN SITU
SEDIMENT SLURRY INCUBATION *
Biotic / abiotic conditions
HHCB, OD-PABA, CBZ
Exposition concentration : 100 ppb
T0 → T110 days
(0,5,12,39,81,110)
SELECTION
ENRICHMENT IN LIQUID MEDIUM
Oxic / anoxic conditions
From T110 days biotic slurry
oxic
anoxic
0,5g
HHCB, AHTN, OD-PABA, OC, CBZ
Exposition concentration : 25, 25, 28, 36, 24 ppm respectively
1
2 *
3
d 1/11 MM20
1 month
d 1/11 MM20
1 month
essay control
MM20 + EC
essay control
MM20 + EC
essay control
MM20 + EC
ISOLATION
ISOLATION ON SOLID MEDIUM
Oxic condition
HHCB, AHTN, OD-PABA, OC, CBZ
Exposition concentration : 25, 25, 28, 36, 24 ppm, respectively
A
B
C
Spread plate
From oxic (3)
1 month
Streak plate
1 month
Streak plate
Agar MM20 + EC.
Agar MM20 + EC
Agar MM20 + EC
CHARACTERIZATION
IDENTIFICATION OF ISOLATED STRAINS *
From strains isolated on (C)
DNA extraction
16s rDNA PCR
Sanger sequencing - phylogeny
ECs DEGRADATION* TEST
HHCB, AHTN, OD-PABA, OC, CBZ
Exposition concentration : 1 ppm
MM20-PYG + EC
Strains isolated from (C)
90 hours
Aerobic condition

Fig. 3

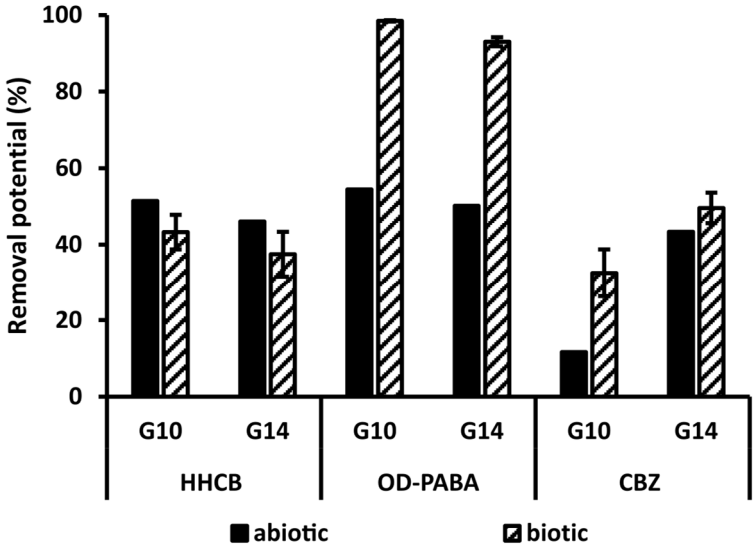

Removal potential (%)
100
80
60
40
20
0
G10
G14
G10
G14
G10
G14
HHCB
OD-PABA
CBZ
abiotic
biotic

Fig. 4

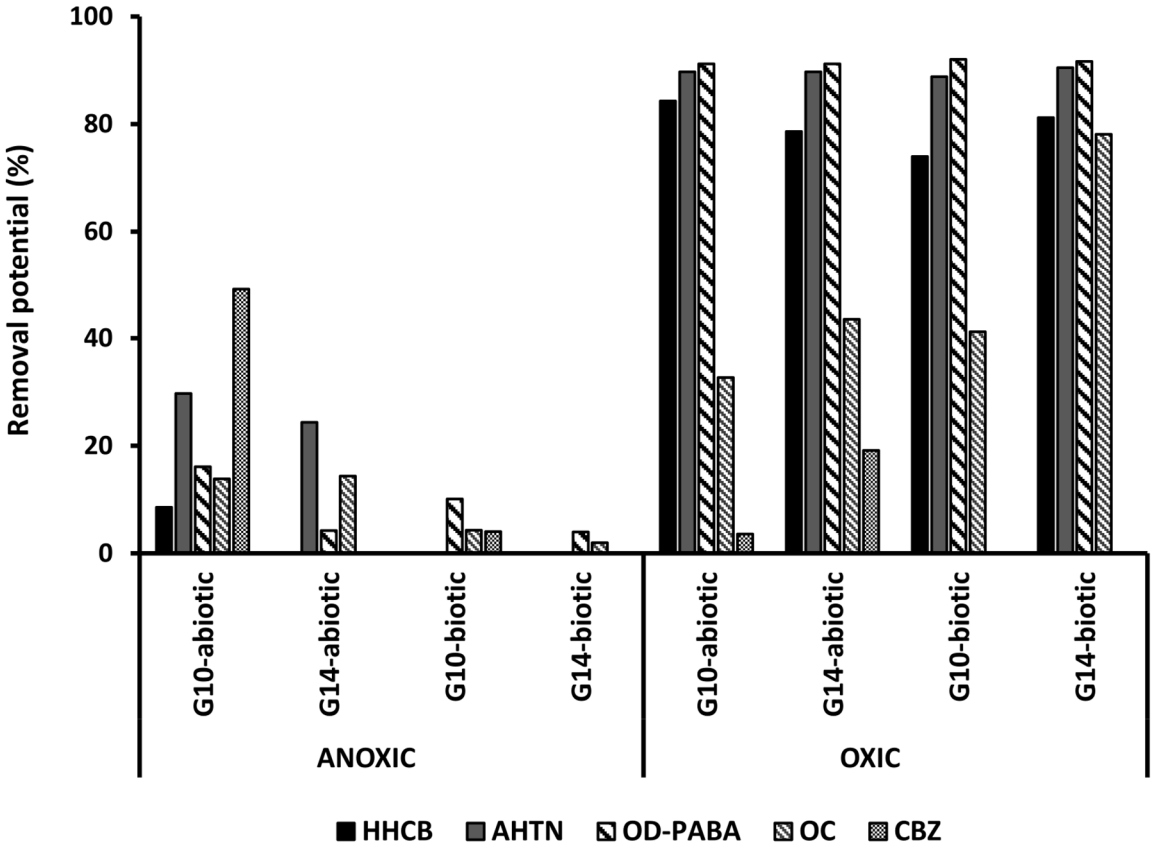

Removal potential (%)
100
80
60
40
20
0
G10-abiotic
G14-abiotic
G10-biotic
G14-biotic
G10-abiotic
G14-abiotic
G10-biotic
G14-biotic
ANOXIC
OXIC
HHCB
AHTN
OD-PABA
OC
CBZ

Fig.5

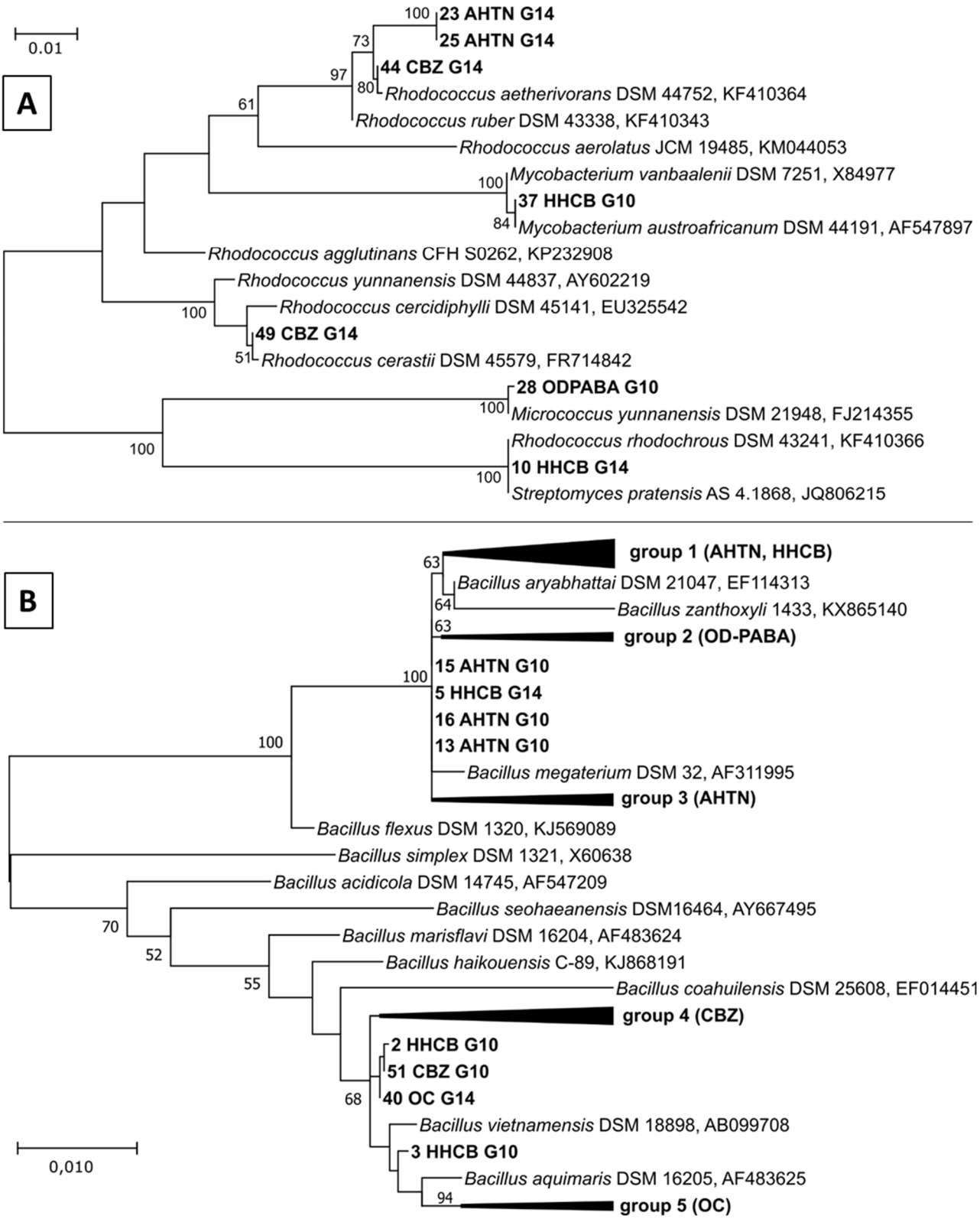
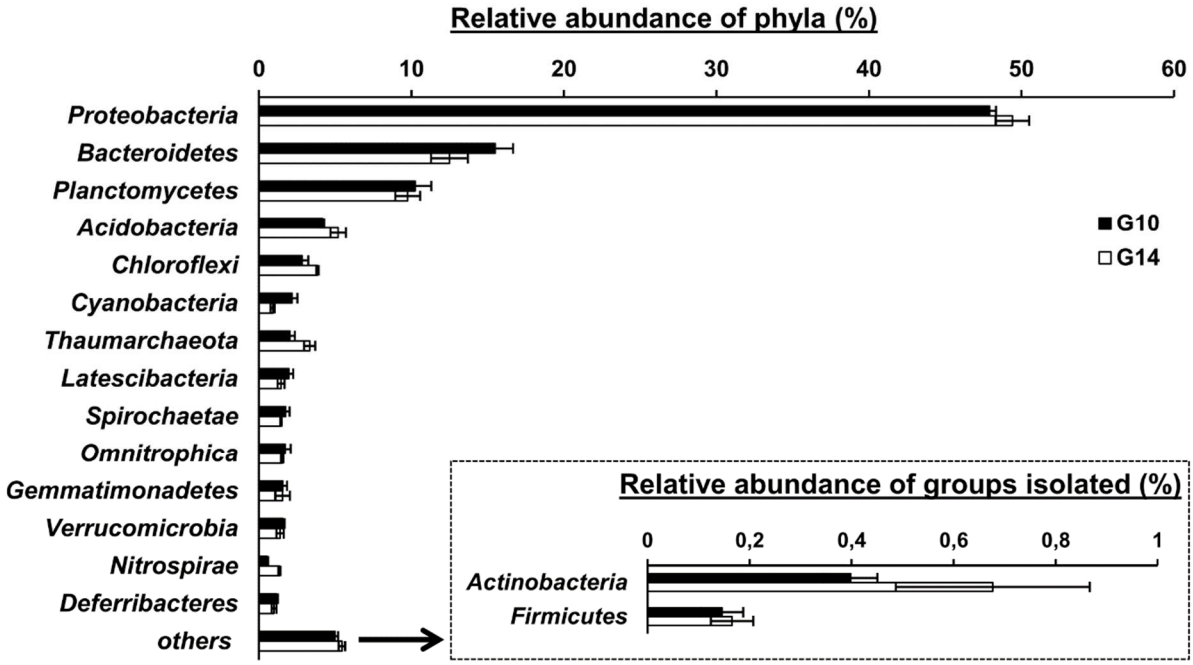

A
0.01
100
23 AHTN G14
25 AHTN G14
73
44 CBZ G14
97
80
Rhodococcus aetherivorans DSM 44752, KF410364
61
Rhodococcus ruber DSM 43338, KF410343
Rhodococcus aerolatus JCM 19485, KM044053
100
Mycobacterium vanbaalenii DSM 7251, X84977
37 HHCB G10
84
Mycobacterium austroafricanum DSM 44191, AF547897
Rhodococcus agglutinans CFH S0262, KP232908
Rhodococcus yunnanensis DSM 44837, AY602219
100
Rhodococcus cercidiphylli DSM 45141, EU325542
49 CBZ G14
51
Rhodococcus cerastii DSM 45579, FR714842
28 ODPABA G10
100
Micrococcus yunnanensis DSM 21948, FJ214355
100
Rhodococcus rhodochrous DSM 43241, KF410366
10 HHCB G14
100
Streptomyces pratensis AS 4.1868, JQ806215
B
group 1 (AHTN, HHCB)
63
Bacillus aryabhattai DSM 21047, EF114313
64
Bacillus zanthoxyli 1433, KX865140
63
group 2 (OD-PABA)
15 AHTN G10
100
5 HHCB G14
16 AHTN G10
100
13 AHTN G10
Bacillus megaterium DSM 32, AF311995
group 3 (AHTN)
Bacillus flexus DSM 1320, KJ569089
Bacillus simplex DSM 1321, X60638
Bacillus acidicola DSM 14745, AF547209
Bacillus seohaeanensis DSM16464, AY667495
70
Bacillus marisflavi DSM 16204, AF483624
52
Bacillus haikouensis C-89, KJ868191
55
Bacillus coahuilensis DSM 25608, EF014451
group 4 (CBZ)
2 HHCB G10
51 CBZ G10
68
40 OC G14
Bacillus vietnamensis DSM 18898, AB099708
0,010
3 HHCB G10
Bacillus aquimaris DSM 16205, AF483625
94
group 5 (OC)

Fig.6


Relative abundance of phyla (%)
0
10
20
30
40
50
60
Proteobacteria
Bacteroidetes
Planctomycetes
Acidobacteria
Chloroflexi
Cyanobacteria
Thaumarchaeota
Latescibacteria
Spirochaetae
Omnitrophica
Gemmatimonadetes
Verrucomicrobia
Nitrospirae
Deferribacteres
others
G10
G14
Relative abundance of groups isolated (%)
0
0,2
0,4
0,6
0,8
1
Actinobacteria
Firmicutes

Tables

Microbial degradation of hydrophobic emerging contaminants from marine sediment slurries (Capbreton Canyon) to pure bacterial strain

Table 1 – Characteristics of the emerging contaminants used in this study. Log K_{ow} is octanol/water partition coefficient.

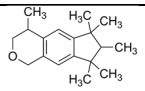
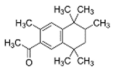
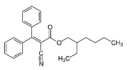
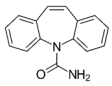
Compound	Family	Formula		Molar mass g mol^{-1}	Solubility in water mg L^{-1}	log K_{ow}	References
Galaxolide (HHCB)	Musk	$C_{18}H_{26}O$		258.4	1.75	5.70	[1]
Tonalide (AHTN)	Musk	$C_{18}H_{26}O$		258.4	1.25	5.90	[1]
Padimate O (OD-PABA)	UV filter	$C_{17}H_{27}NO_2$		277.4	0.54	6.15	[2]
Octocrylen (OC)	UV filter	$C_{24}H_{27}NO_2$		361.5	0.36	6.88	[3]
Carbamazepine (CBZ)	Pharmaceutical	$C_{15}H_{12}N_2O$		236.3	17.7	2.45	EPA's EPI Suite™

Table 2 – First-order rate constants k (in $days^{-1}$) for degradation of HHCB, OD-PABA and CBZ in the slurry sediments incubations under biotic condition (in triplicate). Pearson determination coefficients ranged from 0.76 to 0.98 and p-values <0.05 for estimation of k. The corresponding half-lives ($t_{1/2}$, in days) were calculated as ln(2)/k. G10 and G14 correspond to canyon surface sediment and continental shelf sediment, respectively

Emerging contaminants	Slurry incub.	k ± SD ($days^{-1}$)	$t_{1/2}$ ± SD (days)
HHCB	G10	0.0032 ± 0.0005	224 ± 41
	G14	0.0065 ± 0.0029	129 ± 44
OD-PABA	G10	0.0324 ± 0.0014	21 ± 1
	G14	0.0158 ± 0.0014	44 ± 4
CBZ	G10	0.0035 ± 0.0004	199 ± 19
	G14	0.0029 ± 0.0001	231 ± 17

Table 3 – Removal capacity of isolated strain and their related affiliation based on phylogenetic tree (Fig. 5). % of removal is calculated relatively to the control removal and in triplicate, after 120 hours of experimentation at 1 ppm exposition concentration for each emerging contaminants. The strains for which degradation were not determined are not shown (Fig. 5)

Isolated strains	***Phyla***	***Family***	**% of removal ± SD**
2 HHCB G10	*Firmicutes*	*Bacillus*	44 ± 31
17 AHTN G10	*Firmicutes*	*Bacillus*	68 ± 9
23 AHTN G14	*Actinobacteria*	*Rhodoccocus*	20 ± 7
33 ODPABA G14	*Firmicutes*	*Bacillus*	34 ± 19
35 ODPABA G14	*Firmicutes*	*Bacillus*	45 ± 9
36 ODPABA G14	*Firmicutes*	*Bacillus*	71 ± 19
40 OC G14	*Firmicutes*	*Bacillus*	17 ± 17
43 OC G10	*Firmicutes*	*Bacillus*	14 ± 13

Graphical abstract

Microbial degradation of hydrophobic emerging contaminants from marine sediment slurries (Capbreton Canyon) to pure bacterial strain